Valerio Causin

Polymers on the Crime Scene

Forensic Analysis of Polymeric Trace Evidence

Springer

Polymers on the Crime Scene

Valerio Causin

Polymers on the Crime Scene

Forensic Analysis of Polymeric Trace Evidence

Springer

Valerio Causin
Università di Padova
Padova, Italy

ISBN 978-3-319-15493-0 ISBN 978-3-319-15494-7 (eBook)
DOI 10.1007/978-3-319-15494-7

Library of Congress Control Number: 2015935432

Springer Cham Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer International Publishing AG Switzerland is part of Springer Science+Business Media (www.springer.com)

Acknowledgements

I am grateful to many people who, in different ways, helped me in the challenge of writing this book.

First of all thank you Ania Levinson, Editor at Springer Business+Media, who supported me from the preparation of the proposal until the final submission of the manuscript. Thank you for all the patience and understanding, even when I missed some deadlines.

Thank you Abira Sengupta, Assistant Editor at Springer Business+Media, for all the suggestions and the assistance on the editorial process.

A big thank you is due to all my former colleagues in Carabinieri, for the fruitful discussion, for providing research ideas, for sharing casework, and for teaching me something on forensic science each time I visit them.

Financial support for this book was provided by Università di Padova by Project CPDA128143 "Polymer characterization in a forensic intelligence perspective."

A final thank you is due to Gioia for her patience and encouragement throughout this project.

Contents

1 Introduction 1
References 7

2 Polymers: An Overview 9
2.1 Why Polymers Need Their Own Science? 9
2.2 A Short History of Polymers 11
2.3 The Fundamentals of Polymer Science: Some Definitions 12
2.4 Polymerisation Reactions 13
2.5 Average Molecular Weight and Molecular Weight Distribution 16
2.6 Classification of Polymers 21
2.6.1 Source-Based Classification 21
2.6.2 Organic and Inorganic Polymers 22
2.6.3 Thermoplastic and Thermosetting Polymers 22
2.6.4 Application-Based Classification 23
2.7 Describing Polymers: Constitution, Configuration, Conformation 23
2.7.1 Constitution 24
2.7.2 Configuration 26
2.7.3 Conformation 29
2.8 Commercial Polymers: A Round Up 30
2.8.1 Polyethylene 30
2.8.2 Polypropylene 31
2.8.3 Polystyrene 32
2.8.4 Polyacrylonitrile 33
2.8.5 Polyacrylates 33
2.8.6 Polyesters 34
2.8.7 Polyoxymethylene, Polyoxyethylene, Poly(ethylene glycol) and Poly(propylene glycol) 36
2.8.8 Polycarbonates 37
2.8.9 Polysulphone 37

2.8.10 Polyamides 37
2.8.11 Polyurethanes 38
2.8.12 Poly(vinyl acetate) and Poly(vinyl alcohol) 39
2.8.13 Poly(vinyl chloride) and Other Chlorinated Polymers 40
2.8.14 Fluorinated Polymers 41
2.8.15 Polyisobutylene 42
2.8.16 Polyisoprene: Natural Rubber and Gutta-Percha 43
2.8.17 Elastomers Derived from Butadiene and Its Derivatives 44
2.8.18 Polychloroprene 44
2.8.19 Formaldehyde-Based Resins 45
2.8.20 Epoxy Polymers 47
2.8.21 Silicone Polymers 48
2.8.22 Cellulose and Derivatives of Cellulose 48
2.8.23 Rayon 49
2.8.24 Cellulose Acetate 50
2.8.25 Other Cellulose Derivatives 51
2.8.26 Proteins 51
References 52
Suggested Reading 52

3 Polymeric Traces: Transfer, Persistence, Recovery, Analysis and Interpretation of Analytical Data 53
3.1 Polymers as Sources of Evidence: Transfer, Persistence, Recovery 54
3.2 The Development of an Analytical Method 63
3.3 The Statistical Treatment of Analytical Data 72
3.3.1 Comparisons 72
3.3.2 Principal Component Analysis: The Basics 75
3.3.3 Principal Component Analysis: Some Examples 78
3.3.4 Clustering 80
3.4 The Interpretation of Evidence 86
3.4.1 The Frequency of Traces: Population and Target Studies 87
3.4.2 The Levels of Proposition [78–80] 88
3.4.3 The Bayesian Analysis of the Results 89
References 100

4 Polymers on the Crime Scene 105
4.1 Textile Fibres 105
4.1.1 A Classification of Fibres 106
4.1.2 Technological Requirements for Textile Fibres 107
4.1.3 Transfer and Persistence of Fibres 108
4.1.4 Recovery of Fibres 109
4.1.5 The Characterisation of Textile Fibres 111
4.2 Paints 113
4.2.1 The Composition of Paints 114

4.2.2 Automotive Paints 118
4.2.3 Recovery and Sampling of Paints 119
4.2.4 The Characterisation of Paint Evidence 121
4.3 Adhesive Tapes 122
4.3.1 The Composition of Adhesive Tapes 123
4.3.2 Recovery and Sampling of Adhesive Tapes 124
4.3.3 The Characterisation of Adhesive Tape 125
4.4 Rubber Gloves 128
4.4.1 The Manufacturing of Rubber Gloves 129
4.4.2 Recovery and Handling of Gloves 130
4.4.3 Characterisation of Latex Gloves 131
4.5 Tyre Rubber Traces 132
4.5.1 Manufacturing of Tyres 132
4.5.2 Sampling of Tyre Rubber Traces 134
4.5.3 Characterisation of Tyre Rubber Traces 135
4.6 Condom Lubricants 136
4.6.1 The Composition of Condoms and Lubricants 137
4.6.2 Collection and Handling of Condom-Related Traces 138
4.6.3 The Characterisation of Condom-Related Traces 138
4.7 Foam Fragments 141
4.7.1 The Composition of Foams 142
4.7.2 Collection and Handling of Foam Traces 144
4.7.3 Characterisation of Foam Traces 144
4.8 Plastic Bags 147
4.8.1 Composition of Plastic Bags 148
4.8.2 Sampling and Handling of Plastic Films 148
4.8.3 Characterisation of Plastic Films 148
4.9 Polymers in Documents: Paper and Toner 149
4.9.1 Paper 150
4.9.2 Toner 152
4.10 Post-fire Traces 153
4.10.1 The Identification of Pyrolysis Products in Fire Debris 153
4.10.2 Post-fire Materials Identification 155
References 157

5 Formulation: Polymer Matrix, Fillers, Dyes, Pigments and Other Additives 167
5.1 The Techniques: Spectroscopy 170
5.1.1 UV–Visible Spectroscopy 173
5.1.2 Infrared Spectroscopy 175
5.1.3 Raman Spectroscopy 178
5.2 The Techniques: Atomic Spectrometry 180
5.2.1 Optical Atomic Spectrometry 181
5.2.2 Atomic Mass Spectrometry 185
5.2.3 X-ray Fluorescence 187

5.3 The Techniques: Chromatography 189
5.4 The Techniques: Pyrolysis 196
5.5 The Techniques: Mass Spectrometry 198
5.6 The Techniques: Thermogravimetric Analysis 206
5.7 The Characterisation of the Matrix 209
5.8 The Characterisation of the Fillers 217
5.9 The Characterisation of Dyes and Pigments 224
5.10 Characterisation of Other Additives 238
References 258

6 Synthesis-Dependent Parameters: Molecular Weight, Constitution and Configuration 265
6.1 Average Molecular Weight and Molecular Weight Distribution 266
6.1.1 The Techniques: Size Exclusion Chromatography 269
6.1.2 Size Exclusion Chromatography: The Forensic Applications 273
6.1.3 The Techniques: Mass Spectrometry for Measuring High Molecular Weights 277
6.1.4 Other Methods for Molecular Weight Determination 280
6.2 Comonomers 281
6.3 Isotacticity 290
6.4 Stereoregularity 295
References 296

7 Processing-Dependent Parameters: Structure and Morphology of Polymeric Materials 301
7.1 Morphology 302
7.2 Semicrystallinity 307
7.2.1 The Techniques: X-Ray Diffraction 311
7.2.2 The Techniques: Thermal Analysis—Differential Scanning Calorimetry 320
7.2.3 Other Methods for the Determination of the Degree of Crystallinity 325
7.3 The Mobility of Macromolecules 327
7.3.1 The Glass Transition Temperature 328
7.3.2 The Techniques: Time Domain Nuclear Magnetic Resonance 329
7.4 Orientation 335
References 341

Index 345

About the Author

Born in 1975, Valerio Causin received a Ph.D. in Chemical Sciences at the University of Padova in 2004. From 2001 to 2003, he was First Lieutenant at the Chemistry, Explosives, and Flammables section of the Forensic Branch of Carabinieri in Rome. Since 2015, he has been working as a researcher at the Department of Chemical Sciences at the University of Padova. His research interests are focused on the application of polymer characterization techniques to forensic sciences in collaboration with Carabinieri in Rome, Messina, and Verona. He served as a forensic consultant for the Court, for the Prosecutor, and for the Defense in over 100 civil and criminal cases. In 2009, he was awarded the AIM prize for young researchers in polymer science, and in 2011 he was elected a member of the Scientific Committee of POLYCHAR World Forum on Advanced Materials. An author of 75 papers in international journals, he was invited as a keynote speaker at a number of international conferences and in universities and research centres worldwide.

Chapter 1
Introduction

> Criminalistics is an occupation that has all of the responsibilities of medicine, the intricacy of the law, and the universality of sciences—Paul L. Kirk, Ph.D. (1902–1970)

The words of Paul Kirk, one of the most eminent criminalists of the twentieth century, summarise the reason why forensic science elicits so much fascination. Criminalistics (or in its broadest sense forensic science) combines all those analytical and characterization techniques that support all the stakeholders in the judicial system in the investigation or explanation of crimes. As such, a vast array of scientific disciplines can be incorporated within it and, indeed, this comprehensiveness is its greatest strength.

The arsenal of forensic science includes fields as diverse as meteorology, botany, entomology, molecular biology, chemistry, physics, psychology, and many more. The scientist, or more often a team of scientists, borrows strengths and capabilities of these various disciplines to analyse and interpret all the information contained in the remnants of a criminal act. The importance of science in apprehending suspects, adjudicating cases, and applying judgments is unparalleled among the various tools available to modern law enforcement.

The general definition of forensic science is that it is 'science applied to the administration of justice'. However, this definition does not entirely reflect the peculiarity of this discipline. An emerging view within the scientific community identifies traces as the elements on which forensic science is based, leading to a definition of 'forensic science as the study of traces' [1]. Traces are the remnants of an activity [2], and they can be considered as the most basic 'material or physical' information on crime. This 'trace-centred' definition more clearly portrays the distinguishing features and implications of this discipline.

The possibility of identifying the source of a trace or to reconstruct how that trace was shed, and therefore the dynamics of a crime, are not only dependent on how the analytical process is carried out, but on a variety of information and circumstances that need to be assessed on a case-by-case basis. It is nevertheless out of question that the quality of analytical data obtained in the laboratory plays a crucial

V. Causin, *Polymers on the Crime Scene,*
DOI 10.1007/978-3-319-15494-7_1

part in the whole process of attribution of significance to forensic evidence. Despite the contribution of criminalistics to critical legal matters and the perceptions of popular culture, there are branches of forensic science that are still in need of a considerable research effort, in order to allow a full exploitation of their potential. The characterization of polymeric traces is among these latter areas of knowledge. The peculiarities that make each case unique highlight the difficulties in achieving a standardisation of this discipline.

While forensic science has a long and rich history dating back hundreds of years, it is just recently that the field received proper attention. The advent of new technologies such as DNA typing, the weight of scientific evidence in criminal trials of widespread publicity, and the proliferation of fictional and non-fictional popular media have contributed to making forensic science well known, although perhaps not as well understood, by the general public.

The casework of a chemical crime laboratory may be divided into four main categories. The first includes the use of chemical and biochemical methods to detect and track the illegal use of drugs, the second deals with items connected to flammables and explosives, and the third category includes the forensic identification and comparison of trace evidence such as fibres, glass, paint etc.

A fourth category can be added, which includes the analysis of biological evidence, with always new or improved methods for DNA amplification and analysis. These kinds of tests are in fact soundly based on biochemistry, especially in the sample extraction, amplification and detection steps. However, the organisation chart of a typical forensic laboratory devotes the DNA typing to a biology section.

The first two categories are surely the ones that involve most of the casework of a forensic laboratory, and as such many procedures and analytical methods were developed and are available. The same is not true for polymeric traces, for which academic research and published protocols are rather scarce, mainly due to the vastness of possible samples and thus to the difficulty in obtaining general methods useful for all the items possibly encountered in actual investigations.

A very large number of the objects that surround us in our daily lives are polymeric, so it is highly likely that polymers may be connected to the commission of crimes also. Among the many possible examples, we can cite textile fibres that are shed during a struggle, paint chips transferred or reflectors broken in a car accident, or adhesive tape that is often used in kidnappings. These can be considered the most common items, but also latex gloves, bits of polyurethane foam, resins contained in inks, and plastic bags are materials that a forensic scientist could be called to deal with.

Moreover polymers related to the commission of crimes are usually present in objects which are not directly correlated to the core of the illegal action. Two examples will clearly demonstrate this concept. In drug trafficking, polymers are used for the packaging of the illegal substances. In the manufacture of explosive devices, polymers are present in the adhesive tape or in the insulation of the electrical wires. In both these applications, polymers are rather innocuous substances, if compared to drugs or to explosives. Obviously, a criminal who wants to prevent his identification and capture will try to change his modus operandi, with the aim of confusing

investigators, who on the other hand look for recurrent features to link cases together. Normally, this effort for changing modus operandi will be focused on the most illicit aspects of the activity, e.g. changing the formulation or the exterior aspect of the drug (this is very common in ecstasy trafficking, for example), or changing the type of explosive used in bombs. Packaging or other ancillary materials such as adhesive tape or electrical wires will attract less attention, because they are perceived as mass-produced materials which are less prone to individualisation by forensic scientists. Therefore, odds are high that, for example, a serial bomber will change the source of his explosive, however, using in all his devices the same adhesive tape or kind of cabling. This yields a relevant potential: if the forensic scientist is able to thoroughly characterize all the traces and materials left by the offender, a large amount of data will become available, which, if properly handled, will offer a profile of his modus operandi, thus improving the effectiveness of the investigation.

One of the major reasons that delayed the exploitation of polymeric items in forensic science is their 'commonness'. Even though most plastic objects are industrial products very similar in structure and properties, a certain degree of differentiation between them occurs. This possibility of discrimination lies in the influence that slightly different production processes have on the structure of the polymer obtained. For example, most of the plastic bags on the market are made of polyethylene. A large number of manufacturers exist, though, each one of them uses different raw materials, additives and processing procedures. A scrupulous characterisation approach, targeted to the individuation of the manufacturer's profile, is fundamental. Polymers, as other traces, are class evidence, and as such they acquire evidential value only if they can be placed in as small a class as possible. Sound analytical procedures capable of yielding the largest amount of information are therefore necessary to achieve this aim and in order to do so familiarity with polymer science is a must.

Polymers are peculiar chemicals indeed and much information is contained within their complexity and heterogeneity. Synthesis and manufacturing processes modify the material on the molecular scale, tuning its microstructure, solid state structure, morphology and physical-mechanical properties. Understanding this will improve the efficacy of the work of a forensic practitioner under a number of aspects.

A scientist conversant with polymers will be more efficient in developing an analytical strategy for polymeric items: the more is known about a sample, the better characterisation methods can be devised for it. Experience plays a significant role in this regard, but it must be built on the foundations of knowledge of the chemical and physical features which make polymers a special class of materials deserving its own science.

Familiarity with polymer science will lead to a more conscious application of existing protocols. An operator should not be a mere executor of a sequence of operations, but should be aware of the reason why such operations were devised. Understanding the rationale of an analytical strategy helps detecting errors and problems at an earlier stage, for example.

The work of forensic scientists also involves communication of the analytical results to an audience with no scientific background. A deep knowledge of polymer

science will improve the ability to convey complex information with a simple yet rigorous language. Needless to say, expertise is a key asset for improving cross-examination skills.

The scope of this book goes in this direction, in order to give the reader a complete picture of the state-of-the-art and of the tools available for the forensic analysis of polymeric items. Collating a number of examples and analytical approaches, regarding many different items and materials, in one single text, the reader is given a comprehensive overview of the features of existing protocols and of the challenges related to the development of new ones.

Practically no plastic object is made of 100 % polymer. Most polymers in fact do not possess adequate aesthetical and/or functional properties to fit common end uses, so additives are extensively used in industrial practice.

Plastic objects are therefore usually more aptly described as composites. As such, their characterization can be made, on a first level, of the formulation of the matrix polymer and of the inorganic and organic additives (Fig. 1.1). Also contaminants, such as for example monomer or initiator residues, should be considered as possible components of a finished polymer item. This may be especially the case in adhesives or paints, even though modern manufacturing processes usually limit to the minimum such species. A second level, though, is much more underrepresented in the forensic science literature, and makes use of methods and characterization techniques which are typically applied by polymer scientists. In this approach, focus is posed on the polymer matrix. Features such as the average molecular

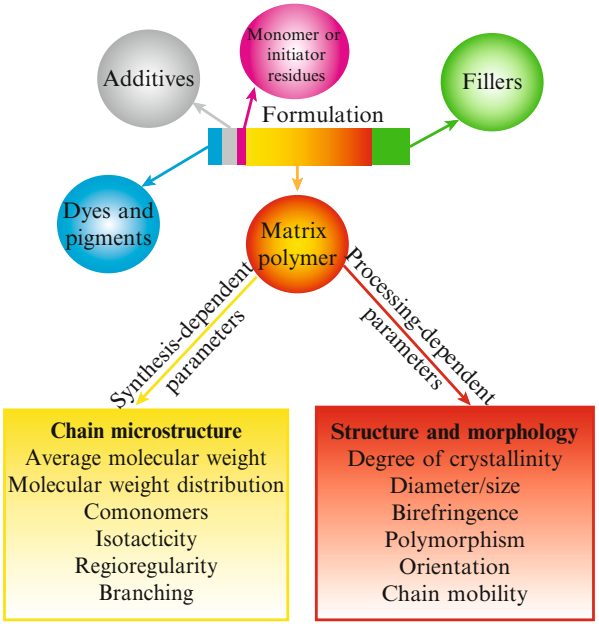


Fig. 1.1 Characterisation scheme for a polymeric item of forensic interest. Adapted from Ref. [3]

weight, molecular weight distribution, isotacticity, or presence of co-monomers, differentiate macromolecules from ordinary small molecules, and are strictly dependent on the synthetic pathway chosen to produce the polymer (Fig. 1.1). Investigating the structure and morphology of the semicrystalline framework can shed light on the processing imposed during manufacturing (Fig. 1.1). This information, dependent on the synthesis, and the manufacturing process of the item may allow to restrict the class in which it can be placed, dramatically increasing its evidential value.

As introduced above, the aim of this book is that of merging polymer science with forensic science, in order to promote a point of view, in the design of a characterization procedure for polymeric items of forensic interest, which may contribute to maximise the significance of this kind of trace evidence.

Chapter 2 will be devoted to an introduction to polymer science, highlighting the distinguishing features of polymers, to understand the key concepts which are the foundations of any analytical determination on these materials.

Polymeric materials, as any other material indeed, become items of evidence just if a correlation between them and a criminal activity can be established. The concepts of transfer and persistence are fundamental for understanding how traces are shed on the crime scene and they will be presented in Chap. 3. Provided that a trace was shed on the crime scene and that it persisted until the arrival of investigators, other steps are necessary for bringing it into Court. It must be retrieved, preserving its integrity, and taken to the laboratory, it must be properly analysed, the data must be rigorously interpreted and its evidential value must be evaluated. The second part of Chap. 3 will be focused on these aspects of forensic investigation.

Chapter 4 contains the state-of-the-art on the forensic analysis of polymeric items. The most common types of polymeric evidence are presented, reviewing the analytical protocols which were published in the literature. Particular attention is given to the technological processes for manufacturing polymeric items, because that is the basis for identifying the analytical parameters most discriminant for a forensic characterisation. As said above, in fact, the most distinguishing features useful for a discrimination of mass-produced items are consequences of the variations in processes adopted by the different manufacturers in the market.

The next chapters of this text will be organised according to the type of information that can be gathered on the item, i.e. formulation, synthesis-dependent features and processing-dependent features.

It may appear that the organisation of the book is mostly beneficial to forensic practitioners interested in better understanding and improving analytical protocols which were already developed for polymeric items. In such cases in fact the general composition of the material is known, and the analytical strategy can be focused on specific components of the formulation, e.g. dyes, pigments or fillers. This is the case of fibres or paints, for example, which have been widely used in casework for a long time, and for which excellent monographs have been published [4, 5].

However, as discussed earlier, familiarity with polymers will help in developing efficient analytical methods also for unknown materials. In this instance the topics covered in this book should be used as a toolbox, for assembling a protocol which

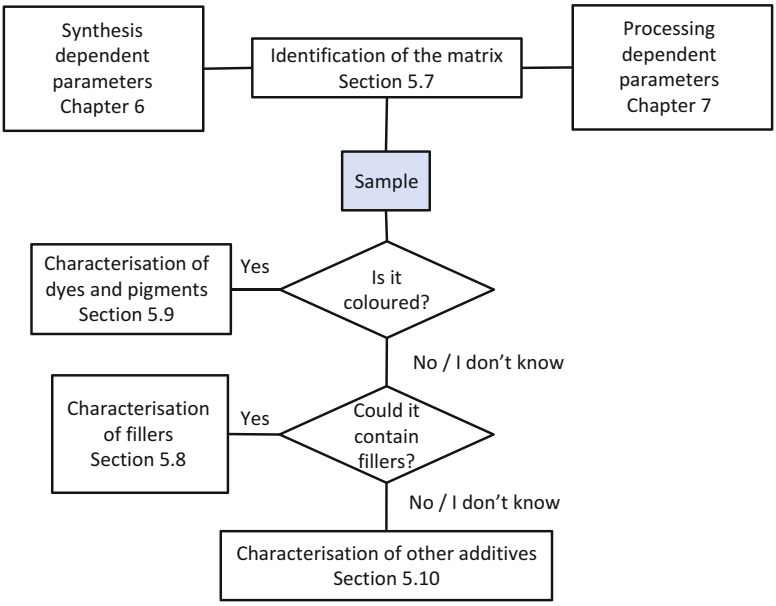


Fig. 1.2 Flowchart for the development of an analytical method for unknown polymeric items

is at the same time informative and compatible with the most stringent requirements of forensic casework. The flowchart in Fig. 1.2 can be a guide in this activity.

The first step is necessarily the identification of the polymer matrix, which normally is carried out by infrared spectroscopy. Once the base polymer is known, the analyst will educately choose among the techniques described in Chaps. 6 and 7, for a more thorough characterisation of this material. For example, if the polymer is insoluble, the determination of molecular weight by size exclusion chromatography will be ruled out. If the polymer is able to crystallise, then a measurement of the degree of crystallinity could be feasible. Sample size will be a good criterion for choosing the methods among those contained in this book: some techniques are applicable to extremely small items such as single fibres; some require a more substantial sample size. This is where the expertise of the forensic scientist has the most relevant role, because the knowledge on the different analytical techniques and on the peculiar features of each polymeric material must be merged in the design of an analytical strategy. For example, in the case of poly(vinyl chloride) (PVC), it would be useless to measure the degree of isotacticity, since the polymer is produced in atactic form, but the determination of the plasticiser content will be more likely significant, since this material is very often treated with this additive. The method chosen will depend on the type of item: very flexible PVC objects will contain big amounts of plasticiser, which will be detectable simply by infrared spectroscopy, whereas in rigid PVC the content of this additive will be lower and will require more specific and sensitive techniques, for example chromatography after extraction with a solvent.

The same may be said for the lower portion of the flowchart in Fig. 1.2, which is focused on formulation. The more is known about the sample, the easier it will be to devise an analytical protocol. One striking characteristic, colour, can partly indicate the way. If the object is coloured, it will contain dyes or pigments, which can be characterised according to the techniques described in Sect. 5.9. It is less straightforward to verify the presence of fillers. However, these additives are usually present in large amounts, and should be detectable in the infrared spectrum acquired for the preliminary identification of the polymeric matrix. If this analysis suggests that fillers may be contained in the item, it is worth applying some of the approaches presented in Sect. 5.8. Section 5.10 reviews a number of methods which are not focused at a particular additive, but which are able to give a broad informative picture on the formulation of the material. These are the techniques which should be applied in cases where so little information is available on the item that it is impossible to devise a protocol aimed at a particular class of additives.

Wherever possible, tips and suggestions were given on how to carry out the analyses. The huge variety of polymers and of polymeric items does not permit a generalisation, and often the choice of the best experimental conditions absorbs much of the time dedicated to casework. However, many examples and references from the literature are presented, and hopefully the reader will find inspiration for translating them into his or her specific cases.

References

1. Ribaux O, Baylon A, Lock E et al (2010) Intelligence-led crime scene processing. Part II: intelligence and crime scene examination. Forensic Sci Int 199:63
2. Locard E (1920) L'enquète criminelle et les méthodes scientifiques. Flammarion, Paris
3. Causin V (2010) Polymers on the crime scene: how can analytical chemistry help to exploit the information from these mute witnesses? Anal Methods 2:792
4. Robertson JR, Grieve M (eds) (1999) Forensic examination of fibres. Taylor & Francis, London
5. Caddy B (ed) (2001) Forensic examination of glass and paint: analysis and interpretation. Taylor & Francis, London

Chapter 2
Polymers: An Overview

2.1 Why Polymers Need Their Own Science?

What are polymers, one of the most pervasive class of materials in our everyday life? The most immediate answer defines polymers as complex and giant molecules which, due to their large size, are starkly different from low molecular weight compounds. To put such difference in a numerical context, the molecular mass of table salt is 58 g/mol, that of aspirin is about 180 g/mol, that of nitroglycerin is 227 g/mol, whereas the typical molecular weight of a polymer ranges from the tens of thousands up to millions of grams per mole. A high molecular weight, though, is not enough to have a polymer. Another important prerequisite is that the giant chemical species is composed of smaller moieties, called repeat units, which recur along the molecule. Such nature is reflected in the name: the word polymer comes in fact from the Greek πολύς, which means 'many' and μέρος which means 'part'. The small molecules, which combine to form a big molecule, can share the same chemical nature or be different. To illustrate, imagine a train, which is a big vehicle composed of a sequence of basic units, railroad cars (Fig. 2.1). In some trains all the cars are of the same type (for example they are all similar passenger cars), in some others there are mixed types of cars (for example passenger and sleeping cars). Analogously, polymers can be composed of repeating units which are all equal, or they can contain two or more different building blocks. The former species are called homopolymers, the latter are defined as copolymers.

As introduced above, the feature that makes polymers special within the universe of chemical species, is their giant molecular weight. Further comparisons with small molecules will clarify this point. If one heats solid benzene, for instance, phase transitions at precisely defined temperatures will be observed. The solid will become liquid at 5.5 °C and the liquid will subsequently boil at 80 °C, going in the vapour phase. If the process is inverted, i.e. cooling a vapour instead of heating a solid, the phase transitions will occur at the same temperatures. The vapour will condensate at 80 °C, and the liquid so obtained will become a solid at 5.5 °C.

V. Causin, *Polymers on the Crime Scene,*
DOI 10.1007/978-3-319-15494-7_2

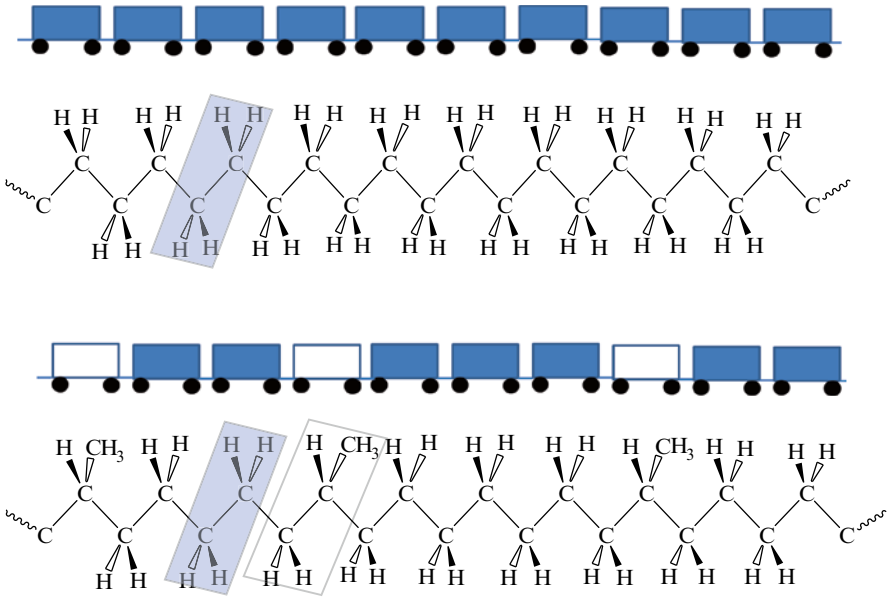


Fig. 2.1 Like a train is composed by a sequence of cars, polymers are composed by a sequence of molecular repeating units. Exactly like in trains, the repeat units in polymers can be all similar or they can be different. The *boxes* on the chemical structures identify the repeat units

The same does not happen if the same experiment is repeated using a polymer as a sample. Heating a solid polymer sample will induce melting (or softening in amorphous, i.e. non-crystalline, polymers), however this transition will not happen at a precisely defined temperature, but over a range of temperatures, which can be as wide as some tens of degrees. Another stark difference is that if heating is prolonged beyond the melting point, eventually formation of gaseous products will be obtained. However this is due to the decomposition of the material, not to evaporation. In other words, if the obtained gas is cooled down, the polymer is not recovered.

A further evident difference between polymers and small molecular weight species is found in their solubility pattern. If we add sodium chloride to water, the compound readily dissolves and its concentration can be increased up to a point where no more solute will dissolve in the solvent, i.e. up to saturation of the solution. Moreover, the viscosity of the solution is not much different from that of the pure solvent. If one uses poly(vinyl alcohol) as a solute in water, dissolution takes much more time than table salt. The process is longer because it proceeds along several steps: first the polymer pellets absorb water, swelling and gradually distancing the macromolecules up to a point that their intermolecular interactions are overcome and they are eventually dissolved. If the polymer is continuously added, no saturation point is reached, but rather the viscosity of the solution steadily increases, until a semisolid dough is obtained. No point in which poly(vinyl alcohol) settles

down at the bottom of the solution, as is the case of a small molecule reaching the saturation limit, is observed.

These and other peculiarities which will be encountered in this text make polymers a special class of compounds. These materials of course must follow the laws of chemistry and physics, and they do indeed. However, due to their large size, they deviate from the ideality of small molecular weight species, and thus deserve a separate treatment to catch and exploit their uniqueness.

2.2 A Short History of Polymers

As often happened in the course of the history of chemistry, man started using polymers long before understanding their nature.

Indeed polymerisation is one of the reactions that allowed the development of life, when the combination of simple compounds such as methane, ammonia, or carbon dioxide yielded amino acids, which subsequently connected together in large molecules, proteins.

The evolution of several biological species from these first biochemical steps brought about the amazing diversity of Earth. The structure of plants developed around a polymer, cellulose. This soon became the basis material which aided the survival of the first humans, providing protection, in the form of cellulosic fibres for clothing or wood for building shelters or weapons. Collagen, keratin and other proteins were the constituents of leather and animal hairs, other popular materials for manufacturing clothing, containers and accessories.

Polymers aided also the enjoyment of humans: the balls used by pre-Colombian populations of Central and South America were made with the rubber extracted by a local tree.

Since the 1800s, the advancement of scientific knowledge, and some lucky accidents, began to shed light on polymers. In a Swiss laboratory, Christian Schonbein unfortunately spilled a mixture of nitric and sulphuric acid, and promptly cleaned the floor with his wife's apron. In an effort to cancel the evidence of such an act, he washed the apron and dried it near a fireplace, just to see it catch fire and disappear. Nitrocellulose, or gun cotton, had just been discovered.

Experimenting in a Massachusetts rubber factory, Charles Goodyear accidentally dropped a mixture of rubber and sulphur on a hot stove. The rubber did not melt but rather improved its properties, becoming an elastomer with many remarkable virtues. He had discovered vulcanisation, the secret that was going to make rubber a commercial success.

The peculiarity of polymers was still unclear at that time, the idea of molecules with a molecular weight of the order of hundreds of thousands was simply unacceptable at that time. Herman Staudinger, who started studying polymerisation as early as 1920, got this advice from a fellow chemist: 'Dear colleague, leave the concept of large molecules well alone…. There can be no such thing as a macromolecule'.

However, the potential of these materials did not go unnoticed. John Wesley Hyatt developed celluloid for proposing a substitute for ivory in the manufacturing of billiard balls, and Leo Baekeland invented a resin which could be synthesised from phenol and formaldehyde and could be moulded into hard and durable items. He was probably the first scientist who adopted a 'bottom-up' approach, recognising that the functionality of the reagents was the key to control the final properties of the material. Since the beginning of the 1900s, Staudinger, Herman Mark and Linus Pauling struggled, and eventually succeeded to create an academic and scientific ecosystem where polymer science could grow. Still a division existed, though, between the industry who used polymers without really being interested in their intimate nature, and scientists, who mainly studied existing natural polymers to understand how atoms were connected and what their properties were. The figure who connected these two worlds, becoming the father of synthetic polymer science, was Wallace Hume Carothers. In 1927 DuPont created a division dedicated to fundamental research. Carothers, then an instructor in organic chemistry at Harvard, was hired to work in this newly established laboratory. From then on, he discovered how to manufacture polyamides and put the basis for the comprehension of polymerisation reactions. His colleague Paul Flory was a prominent figure in the understanding of the relationship between structure and properties. Merging applied and fundamental research was the key factor that led to a fast advancement of polymer science, such that by the 1930s the base concepts that characterise polymers were accepted by the scientific community. Polymers played a critical role during World War II, when shortage of materials such as silk or rubber spurred an intense research and the invention of several polymeric materials with analogous or even superior properties. Since then, the development of polymer industry continued until today, with the introduction in the market of hundreds of polymeric solutions for the big and small problems of everyday life.

2.3 The Fundamentals of Polymer Science: Some Definitions

Figure 2.2 shows the chemical equation for the synthesis of poly(ethylene), and gives the opportunity to define some key terms in polymer science. The basic molecular moiety which is repeated along the polymer is the repeat unit (or constitutive unit): it is the structure represented on the product side of Fig. 2.2. On the other hand, the small molecule which is the precursor of the polymer is the monomer, ethylene in this example. Even though they may contain exactly the same number and type of atoms, monomer and repeating unit have a fundamental difference.

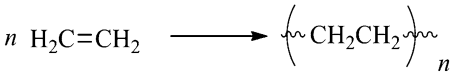


Fig. 2.2 Chemical equation of the polymerisation of ethylene which yields poly(ethylene)

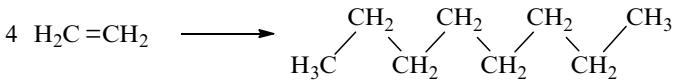


Fig. 2.3 The formation of an oligomeric chain of polyethylene formed by 4 repeat units

The monomer can exist as an individual chemical species, stable and separable. On the other hand, the repeating unit does not exist as a separate entity, but it is only observable within a polymer chain.

Polymerisation is the process by which the monomer molecules are connected to form a big polymer molecule. For a polymerisation reaction to succeed, a prerequisite is that the monomer has two or more reactive sites. For understanding this necessity, one can think that in order to form a line of people holding hands, each of the participants must have both hands available for connecting with his neighbours, otherwise the continuity of the line is interrupted.

Ethylene has a double bond, for example, which, when broken, locates on each of the two carbon atoms one lone electron that is available for reaction. The mechanism is more complex than just reported, but the aim in this case is to explain where the double functionality resides in olefinic monomers. Other typical examples of bifunctional monomers are molecules containing two aminic groups, two hydroxyl groups, or two carboxylic acid functionalities.

The size of a single polymer chain is simply determined by the number of repeat units that are included in it, which is called degree of polymerisation.

For example, the product of the process schematized in Fig. 2.3 is a polymer with degree of polymerisation 4, because four monomers reacted and were included in the chain, which has thus four repeat units.

The molecular weight of a single polymer chain will of course be the product of the weight of each repeat unit by the degree of polymerisation of that chain.[1] The concept of molecular weight of a polymer sample is more complex and deserves a deeper discussion (Sect. 2.5).

2.4 Polymerisation Reactions

The synthetic procedure which is used for the conversion of monomers into polymers is called polymerisation. Two major mechanisms of this family of reactions can be identified: step-growth and addition polymerisation. Step-growth polymerisation is suitable for monomers with functional groups, such as –OH, $–NH_2$, or –COOH, able to react with each other, usually (but not always) in a sequence of condensation reactions. Each reacting step, in the case of condensation reactions, brings about a

[1] Rigorously, the molecular weight of the groups at the end of the chain should be considered in the calculation of the molecular weight of the whole macromolecule. However, due to the large number of repeat units normally present in a polymer, the contribution of end groups to the total molecular weight is almost always negligible.

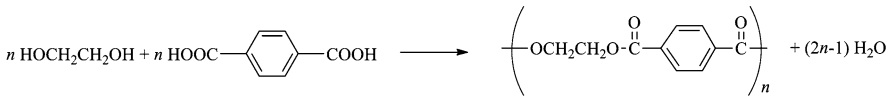


Fig. 2.4 Synthesis of poly(ethylene terephthalate) by condensation polymerisation

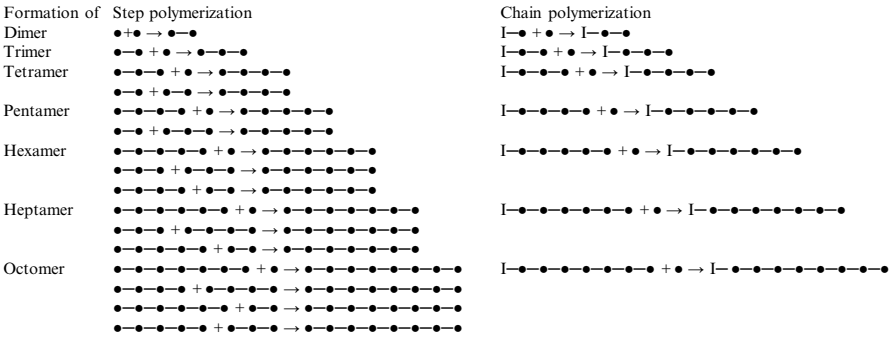


Fig. 2.5 Scheme representing the different mechanisms of chain lengthening in polymerisation. ●: repetitive unit, —: bond, I: initiator residue

bigger molecule and the expulsion of a small molecule, such as H_2O, HCl, CH_3OH, etc. The outcome is that not all the atoms of the monomer end up into the polymer chain. A typical example of step-growth polymerisation proceeding through condensation reactions is poly(ethylene terephthalate), commonly abbreviated as PET and widely used for the manufacturing of fibres and plastic containers (Fig. 2.4).

Polyamides are another common class of polymers synthesised by this mechanism. As anticipated above, all the molecular species present in a polymerisation mixture of the step-growth type are ready to react with each other. The consequence of this reactivity is that, since the early stages of the reaction, the monomer is almost entirely incorporated in a bigger molecule. The growth of the polymer does not happen by addition of one repetitive unit at a time, but rather through a sequential aggregation of increasingly big molecular fragments. In other words monomers combine with monomers giving dimers, which then react with other dimers yielding tetramers, or with other monomers giving trimers. Trimers can react with dimers giving pentamers, or with other trimers giving hexamers, or with other tetramers giving heptamers, etc. The scheme in Fig. 2.5 illustrates how a polymer grows according to this mechanism.

The alternative major type of polymerisation is the chain reaction, which progresses most often by addition reactions. In this case, the monomer usually contains at least one degree of unsaturation (double bonds, triple bonds or cyclical structures) which is activated by a radical or ionic initiator. Since no expulsion of small molecules happens in this mechanism, all the atoms of the monomer are included in the polymer chains. An example of polymer obtained by a chain addition polymerisation is polyvinylchloride (PVC), a very versatile material applied in diverse fields, ranging from watering hoses to building pipes to flooring material (Fig. 2.6).

Fig. 2.6 Synthesis of poly(vinyl chloride) by addition polymerisation

Reaction step	Chain polymerization
Initiation	$I \rightarrow I^*$ $I^* + \bullet \rightarrow I{-}\bullet^*$
Propagation	$I{-}\bullet^* + \bullet \rightarrow I{-}\bullet{-}\bullet^*$ .... $I{-}[-\bullet-]_n{-}\bullet^* + \bullet \rightarrow I{-}[-\bullet-]_{n+1}{-}\bullet^*$
Termination	$I{-}[-\bullet-]_n{-}\bullet^* + {}^*\bullet{-}[-\bullet-]_m{-}I \rightarrow I{-}[-\bullet-]_n{-}\bullet{-}\bullet{-}[-\bullet-]_m{-}I$ $I{-}[-\bullet-]_n{-}\bullet^* + {}^*\bullet{-}[-\bullet-]_m{-}I \rightarrow I{-}[-\bullet-]_n{-}\bullet \quad \bullet{-}[-\bullet-]_m{-}I$
Chain transfer	$I{-}[-\bullet-]_n{-}\bullet^* + X \rightarrow I{-}[-\bullet-]_n{-}\bullet + X^*$

Fig. 2.7 Scheme representing the different steps of the chain polymerisation mechanism. ●: monomer, —: bond, I: initiator, X: species present in the reaction system (e.g. monomer, solvent, additives, polymer chains). The * indicates that the species is activated

Differently from the case of step-growth polymerisations, in chain addition polymerisation the monomers are not ready to react, but must be activated by a suitable initiator, which can be of radical or ionic type. This initiation step induces the formation of a tiny portion of activated monomers which are able to react with other (inactive) monomers in the propagation step: this is the event which brings about the formation of long sequences of repetitive units and thus of polymer chains. In the propagation step the active site of one monomer is transferred to another monomer which becomes the growing end of the polymer chain, able to link with yet another monomer, transferring to it the active site and so on. The growth of the chain happens, in chain reactions, sequentially one repeating unit after the other (Fig. 2.5). This way, the active site on the reagent side of the reaction is maintained on the product side of the chemical equation representing the propagation step. Termination occurs when active sites are inactivated, for example, in the case of a free radical mechanism, by combination of two growing chains. Different termination reactions can happen, depending on the chemistry of the system. Events such as chain transfer, when the active site is transferred to other chains or to other species present in solution, complicate the control of the polymerisation.

The scheme in Fig. 2.7 summarises the main steps of a chain polymerisation.

This schematic introduction to the chemistry of polymerisations highlights the complexity of polymerisation systems and should help the reader to understand why it is impossible to achieve a perfect control of the synthesis of macromolecules. Many different reactions occur simultaneously, generating a statistical distribution of molecular sizes (Sect. 2.5) and introducing a number of defects in the product polymer.

A further complication is introduced by the problems associated to the practical realisation of polymerisations. The synthesis of polymers involves the transformation of a low molecular weight substance such as the monomer, which is typically a liquid

or a gas, into a macromolecule, which depending on its molecular weight is a viscous liquid and eventually a solid. This transformation is thus associated to a transition from an easily processable liquid- or gas-phase system to a solid product, through phases with increasing viscosity. The radically changing physical state of the reaction mixtures brings about significant problems when industrial production is involved. Polymerisation processes are mainly carried out industrially in the bulk, in emulsion, in suspension or, less commonly, in solution. Irrespectively of the details of each process, which are beyond the scope of this book, suffice here to say that different reaction conditions have a very significant influence on the final product. Polymers with very different microstructures can be synthesised just by changing the process by which they are made. This variability is a resource for the forensic scientist, who is often interested in detecting features which discriminate apparently similar items.

2.5 Average Molecular Weight and Molecular Weight Distribution

As introduced in Sect. 2.4, polymerisation processes are impossible to control in every single detail, because they consist of several simultaneous reactions, happening in a medium with continuously changing physical properties (the growing chains become larger and larger and thus their motion becomes slower and slower). The net result of this complexity is that the product of polymerisation is not a set of perfectly equal species, but rather a distribution of molecules which share the same chemical nature and differ by number of repeat units that they contain. Small molecular weight species are typically monodisperse, i.e. each molecule has exactly the same molecular mass. Polymers, on the other hand, are markedly polydispersed in nature: each molecule is of different size than the others (Fig. 2.8).

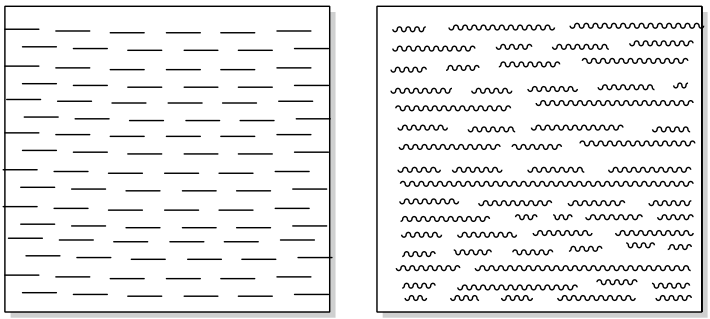


Fig. 2.8 Schematic of a monodisperse system and of a polydisperse system. The length of the line represents the size of the molecule

In polymers, molecular weight becomes a statistical concept, and must be expressed in terms of a distribution quantitatively identified by an average and by the width of the distribution curve.

All the matter in a litre of pure water is made of individual molecules, all composed by two hydrogen atoms and one oxygen atom. Excluding the effect of different isotopes, all the molecules which form that amount (or any amount, for that matter) all have a molecular weight of 18 g/mol. The same does not happen in the case of a polymer sample. The molecules contained in a polymer item will appear as a rather heterogeneous mix of macromolecules with different sizes, therefore it is impossible to assign an exact molecular mass to a polymer. This descends from the fact that, as summarised in Sect. 2.4, in polymerisation reactions the chain length is a consequence of a mix of simultaneous reactions governed by largely random events. Among the other factors which influence the molecular weight of a condensation polymer are the reaction time and the availability of reactive groups (stoichiometry of the system and reactivity of the groups). In the case of chain reactions, the molecular weight of the chains is the outcome of a delicate balance between the rates of all the steps involved. Such complexity in the chemical activity is thus the reason why the product of any polymerisation is a mixture of chains of differing lengths. The characterization of the molecular mass of a polymer must therefore be quantified in terms of average molecular mass and distribution of molecular masses, rather than by a single molar mass such as in the case of small molecular weight substances.

Figure 2.9 shows the typical distribution of molar masses for a synthetic polymer. As may be seen, molecules weighing 50,000 g/mol and chains with a molecular mass of 200,000 g/mol coexist in the same sample.

There is not a universal definition of average molecular weight. Different methods for measuring the molar masses of polymers are based on different averaging approaches, therefore yielding different estimates of the average molecular weight.

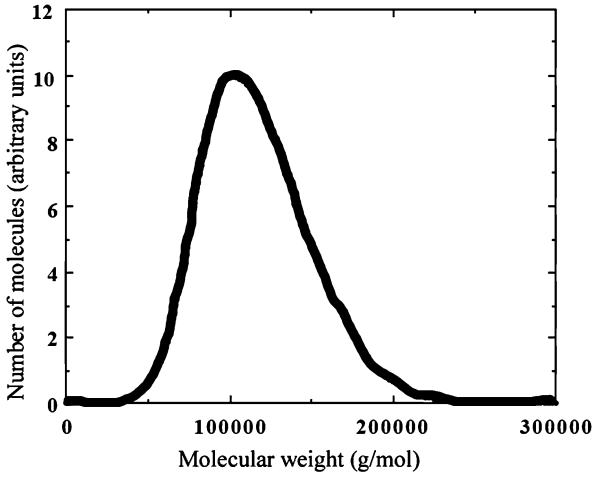


Fig. 2.9 Typical molecular mass distribution of a synthetic polymer

For example, the measurement of colligative properties, such as the osmotic pressure, depends on the number of molecules in solution and thus provides a number-average molar mass, $\overline{M_n}$:

$$\overline{M_n} = \frac{\sum_i N_i M_i}{\sum_i N_i} \tag{2.1}$$

Where *Ni* indicates the number of molecules, which have a molecular mass *Mi*. Let's imagine that a polyethylene sample is composed by 5 linear chains, 3 with 2,000 repeat units and 2 with 4,000 repeat units. Since one repeat unit of polyethylene contains two carbon atoms and four hydrogen atoms, its molecular weight $M_{\text{repeat unit}}$ will be:

$$M_{\text{repeat unit}} = \left(2 \times M_{\text{C}}\right) + \left(4 \times M_{\text{H}}\right) \tag{2.2}$$

Where M_{C} and M_{H} are the molecular weights of carbon and hydrogen, respectively, which are equal to 12 and 1 Da, respectively.[2] Therefore, one repeat unit of polyethylene has a $M_{\text{repeat unit}} = 28$ Da.

As a consequence, the molecular weight of the chains with 2,000 repeat units will be $2{,}000 \times M_{\text{repeat unit}}$ and that of the chains with 4,000 repeat units will be $4{,}000 \times M_{\text{repeat unit}}$, i.e. 56,000 and 112,000 Da, respectively.

Inclusion of these numbers into (2.1) results in:

$$\overline{M_n} = \frac{\sum_i N_i M_i}{\sum_i N_i} = \frac{3 \times 56{,}000 + 2 \times 112{,}000}{3 + 2} = \frac{392{,}000}{5} = 78{,}400\,\text{Da} \tag{2.3}$$

Light scattering is a technique sensitive to the size, rather than to the number, of the molecules in the system, and therefore it allows to estimate the weight-average molar mass, $\overline{M_w}$:

$$\overline{M_w} = \frac{\sum_i N_i M_i^2}{\sum_i N_i M_i} = \frac{\sum_i w_i M_i}{\sum_i w_i} \tag{2.4}$$

Where w_i is the weight of the fraction of polymer with molecular mass M_i. In the case of the polyethylene sample introduced above, each of the 3 chains with 2,000 repeat units weighs 56,000 Da, for a total weight of the fraction with this molecular weight equal to $3 \times 56{,}000 = 168{,}000$ Da. The two chains with 4,000 repeat units

[2] Da is the symbol of Dalton, a common unit for expressing masses on an atomic or molecular scale. 1 Da = 1 atomic mass unit (uma) = 1 g/mol and is equal to one twelfth of the mass of an unbound neutral atom of carbon-12 in its nuclear and electronic ground state. It is equivalent to $1.660538921(73) \times 10^{-27}$ kg.

weigh 112,000 Da each, and $2 \times 112{,}000 = 224{,}000$ Da in total. Introducing these numbers in (2.4) yields

$$\overline{M_w} = \frac{\sum_i w_i M_i}{\sum_i w_i} = \frac{168{,}000 \times 56{,}000 + 224{,}000 \times 112{,}000}{168{,}000 + 224{,}000} = \frac{3.45 \times 10^{10}}{3.92 \times 10^{5}} = 88{,}000\,\text{Da} \tag{2.5}$$

The example shows also that $\overline{M_n} < \overline{M_w}$, a relationship which is universally valid for all polymers.

Other definitions of molar mass exist, as a function of the method used for their measurement, such as for example the *z*-average molar mass, assessed by ultracentrifuging, or the viscosimetric molar mass, quantified by measurements of the viscosity of polymer solutions.

Whenever it is not possible to use a technique able to draw a curve like that in Fig. 2.9, a single parameter which allows to quantify the width of the molar mass distribution is the ratio between weight-average and number-average molar masses, called polydispersity index ($\overline{M_w} / \overline{M_n}$). Such parameter is always larger than 1: if it were equal to one, the sample would be monodisperse and all the chains would have the same length. Synthetic approaches exist to obtain polymers with a polydispersity index (PI) extremely close to 1, however such materials are mainly confined to laboratory use and lack practical commercial applications which could be of interest to the forensic scientist. The PI of commercial polymers is usually much larger than 1. Values in the 2–5 range are usual, even though materials with PI up to 25 are not rare at all [1, 2].

The need to thoroughly understand and control the polymer average molecular weight and its distribution has a relevant practical significance.

The key parameter examined in industrial practice for choosing polymeric materials is essentially their performance. Their selection is done on the basis of their physical-mechanical properties, which are typically directly dependent on the molecular weight of the polymer or its degree of polymerisation.

Figure 2.10 reports the typical trend of mechanical properties, in this case tensile strength, as a function of the degree of polymerisation. Qualitatively, the shape of this curve is the same for all polymers; different materials will differ just by the numerical values. As may be seen, every polymer has a threshold value for its degree of polymerisation below which the material does not have any significant mechanical strength, existing as a brittle powder or as a viscous liquid. The threshold value of the degree of polymerisation of course depends on the chemical nature of the polymer, being around 60 for cellulose and in the vicinity of 100 for vinyl polymers.

Another critical point of the graph in Fig. 2.10 is the degree of polymerisation at which the curve levels off, corresponding to the average size of the chains at which the material attains more or less full strength. Also this value depends on the type of polymer, ranging from 400 for vinyl polymers, to 250 for cellulose and to about 150 for polyamides.

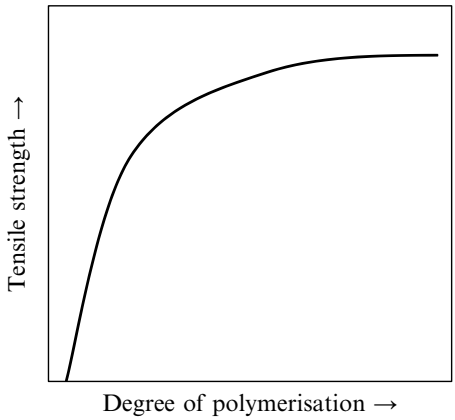


Fig. 2.10 Typical trend of the tensile strength of a polymer as a function of its average degree of polymerisation

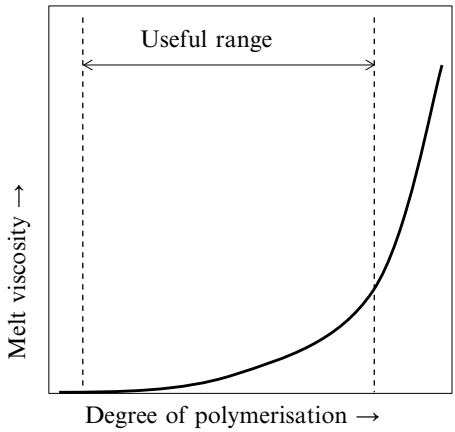


Fig. 2.11 Typical trend of the melt viscosity of a polymer as a function of its average degree of polymerisation

Another important variable to consider when selecting a polymer for industrial manufacturing is its workability. Here the key parameter is melt viscosity, which shows the typical trend, as a function of the degree of polymerisation, depicted in Fig. 2.11.

The increase is in this case slower for polymers of small molecular size, whereas it becomes much steeper at high molecular weights.

Merging the two diagrams of Figs. 2.10 and 2.11, it is possible to optimise the range of molecular weights for polymers, which allows an easy processing due to a sufficiently low melt viscosity and at the same time guarantees a good mechanical performance.

For a forensic scientist, this aspect of polymer science and technology is of great interest, because it shows that there is not a single solution for attaining the desired performance. The same result can be obtained with polymers of several different average molecular weights, provided that they fall within the optimal range of melt

viscosity and mechanical properties. Therefore, for producing the same item, with a particular polymer (e.g. polyethylene), one can choose materials with different average molecular weights, yet obtaining the same net result. This introduces a powerful strategy for differentiating mass produced items, which appear at first sight as all the same and indistinguishable, especially to an audience of non-scientists such as that involved in the judicial process. In reality, even mass produced objects contain chemical or physical signatures that are ascribable to the particular manufacturing process applied to produce it.

2.6 Classification of Polymers

The term polymer comprises a large number of materials with a high molecular weight. Polymers can have very different chemical nature, physical properties, mechanical performance, thermal behaviour, etc. For simplifying the treatment and the investigation of this complex class of materials, several different categorization strategies can be chosen, as a function of the particular aspect which is of interest.

2.6.1 Source-Based Classification

According to their origin, polymers can be categorised as natural, artificial or synthetic.

Natural polymers are those that occur in nature. Polymers derived from animals include wool, the hair of various animals, silk. Collagen, a protein, is the main component of leather. Polymers of vegetal origin are primarily based on cellulose, among which are all cellulosic fibres or wood (which is a composite of cellulose and lignin).

Artificial polymers are produced by man employing naturally occurring polymers as raw materials. In other words, Nature takes care of the polymerisation reaction, whereas man intervenes in the functionalisation or modification of the naturally occurring material. The physical-mechanical properties of the artificial polymer are always different from those of the original natural material. In some cases, when a chemical functionalization step is involved in the process, such as in the manufacturing of cellulose acetate, also the chemical properties of the artificial polymer are changed.

Synthetic polymers are produced from simple starting material, such as coal or oil. The whole synthetic process, from monomer to polymer, is entirely carried out by man. A wide variety of polymers are employed by the industry, in order to meet all the requirements, in terms of mechanical characteristics, morphology and, broadly speaking, performance, posed by the customers. The synthesis and processing of each material put on the market is engineered with an end-use-oriented point of view.

2.6.2 Organic and Inorganic Polymers

An organic polymer is one which has a backbone essentially made of carbon-based moieties. As common in organic chemistry, other atoms in addition to carbon and hydrogen can be present in the molecule, for example nitrogen or oxygen. The majority of polymers in common everyday use are organic. The class of inorganic polymers, i.e. those that have a backbone not based on a sequence of carbon atoms, is much less populated. Within this family, though, a very important group of polymers appear: silicones. These materials are chemically based on molecules whose skeleton is formed by a sequence of Si–O bonds, and are very common in the building industry and in other businesses for sealing, moulding or for the preparation of resins with variable applications.

2.6.3 Thermoplastic and Thermosetting Polymers

An important and useful classification of polymers is based on their behaviour when heated. Thermoplastic polymers soften on heating and can be moulded into any shape, which is retained upon cooling. This process of heating and reshaping can be in principle repeated innumerable times, even though at each cycle some degradation can occur, which eventually makes the material not usable anymore. Most polyolefins and vinyl polymers, polyesters such as poly(ethylene terephthalate) (PET) or polyamides like nylons are thermoplastic polymers. Among the advantages of thermoplastics are recyclability and ease of processing. The drawbacks are that these materials can be used up to a ceiling temperature, when they soften and lose their shape. They are more prone to chemical attacks also, because they can be swollen and eventually dissolved by solvents. However, these limitations are not enough to offset the virtues of these polymers, which are currently used for most mass market applications, and especially for the manufacturing of moulded items.

As opposed to thermoplastics, thermosetting polymers, when heated, undergo a series of chemical cross-linking reactions which transform the precursor material into an insoluble and infusible solid mass. As an analogy, one can think of the yolk of an egg, which when raw is a quite viscous liquid that, after heating, becomes a solid mass that cannot be reshaped upon further heating. The extensive cross-linking which connects the individual polymer chains into a single, giant molecule extended over the whole volume of the sample makes thermosetting materials very durable and impervious to the effect of chemical agents and of temperature. However, they are not recyclable and are quite difficult to shape. There is only a short window in which the material is mouldable and, if any error occurs, the item must be discarded, since there is no possibility to melt it and repeat the process again.

2.6.4 Application-Based Classification

According to its final intended use, polymers can be broadly categorized into plastics, elastomers, fibres or resins. This is a particularly useful classification for forensic scientists, since they deal with finished items, in an effort to identify their source or to compare them.

When a polymer is moulded into hard and tough items by application of heat and pressure, it is used as a plastic. This term typically applies to thermoplastic commodity polymers such as polypropylene, polystyrene, polyvinylchloride, etc. Elastomers are materials which display big elongations and a very wide elastic range, i.e. the range of elongations where the material returns to the original shape after the tensile stress is released. Rubbers derivated from dienes are the typical elastomers, even though polyurethanes or poly(ethylene-*co*-propylene) copolymers can also be engineered to have an elastomeric behaviour. Fibres represent a primary application of polymers. Fibres are defined as long and filament-like materials with a diameter less than about 100 times their length. Fibres also represent maybe the kind of polymer most widely encountered in forensic casework, and will therefore be discussed more in depth in other sections of this book. Resins are polymers used in liquid form as adhesives, paints, sealants, etc. and that are subsequently cross-linked in solid form. They are usually, but not always, thermosetting materials.

Some polymers are preferentially applied in just one of the previous main applications. This is for example the case of natural rubber which is the typical elastomer, or of acrylonitrile which is mostly applied in the fibre industry. However, many other materials are more versatile and can be used in several different industries. For instance poly(ethylene terephthalate) is used as a plastic for moulding bottles for soft drinks, but also as a fibre for polyester textiles. The same can be said of polypropylene, which has an even wider range of applications: plastic for moulding a variety of objects, raw material for fibres in industrial textiles, and when copolymerised with ethylene it can be even used as an elastomer.

2.7 Describing Polymers: Constitution, Configuration, Conformation

Polymers are complex materials, and their properties, which are ultimately what is of interest for their applications, are hierarchically determined by their chemical nature and by their three-dimensional structure.

Constitution defines the nature of the atoms and of the bonds in the molecule, irrespective of their spatial arrangement. In the case of macromolecules, describing the constitution consists in identifying the molecular skeleton, the type and the consequentiality of the repeat units, the kind of terminal groups and the presence of possible defects.

Configuration is a description of the spatial arrangement of atoms in the molecule, which considers the bond length and the angle between bonds, but ignores the rotation around single bonds. Finally, conformation completes the picture of the geometry of the molecule taking into consideration also the arrangement of atoms which is a consequence of rotation around single bonds. Configuration and conformation are studied within the discipline of stereochemistry.

2.7.1 Constitution

The first information useful for defining the constitution of a polymer is obviously defining its repeat unit. Polypropylene is different from polyethylene first and foremost because it contains a different constitutive unit (Fig. 2.12).

Section 2.8 of this book will be devoted at summarising the chemical features and the main properties of the most commonly encountered classes of polymers.

When a polymer contains just one type of repeat unit, it is defined homopolymer. As anticipated above, though, not all polymers are composed by the same repeat unit. A common approach which is followed when all the requirements for a particular application can be met by two or more different polymers consists in including their repeat units in a single molecule. This hopefully allows to take advantage of the positive properties of all the component repeat units, merging them in a single material. When two or more repeat units are present within the same polymer, this is called copolymer. Copolymers are synthesised by polymerising different comonomers. Further classifications can be made of copolymers on the basis of how the different repeat units are distributed within the molecules (Fig. 2.13).

A further approach for merging the positive properties of different polymers consists in mixing them together, obtaining a blend. Differently from the case of a

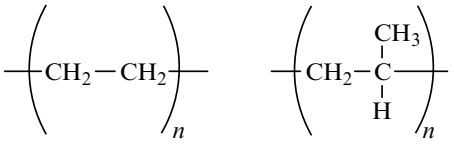


Fig. 2.12 Repeat units of polyethylene (*left*) and of polypropylene (*right*)

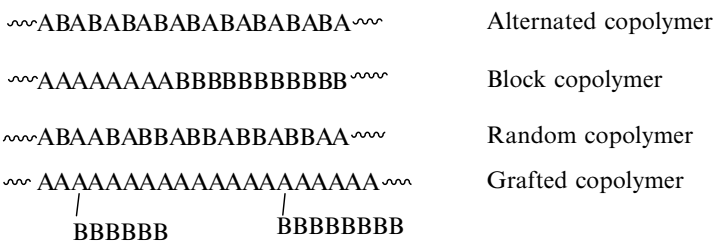


Fig. 2.13 Different types of copolymers. A and B represent the different kinds of repeat units

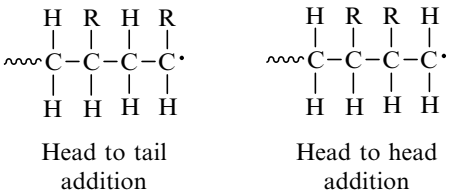


Fig. 2.14 Different types of addition of substituted olefins. In head-to-tail addition the substituent groups R are alternated and as far as possible along the chain, in head-to-head addition the substituent groups are adjacent along the chain

copolymer, in which the different repeat units are held together by covalent bonds, in a blend polymers with different repeat units are held together by intermolecular interactions. The kind and entity of such interactions will define how much advantage could be taken of the respective properties of the two components of the blend.

Also how the repeat units are connected together is a matter of constitution. In the polymerisation of olefinic monomers with a general formula CH_2=CHR, two modalities of addition are possible: head-to-head and head-to-tail addition (Fig. 2.14).

Head-to-tail addition is usually preferred due to steric hindrance reasons. However, in some cases it is not easy to avoid the occurrence of head-to-head addition, which is normally seen as a defect. Examples are poly(ethylene-*co*-propylene) or polyvinylidenefluoride, which show non-negligible fractions of head-to-head repeat units.

When discussing polymers, the word 'chain' is often used as a synonym of 'molecule', but this is not necessarily accurate.

Linear polymers, i.e. those in which the repeat units are concatenated to yield linear chains, are only a subset of all the possible synthesizable polymers. Branched polymers are also possible, and the number, distribution and length of the branches is an important parameter with serious repercussions on the physical-mechanical performance. The presence of branches, in fact, dramatically alters the relative mobility of the polymer molecules and their ability to form ordered structures and crystalline frameworks in the solid state.

Figure 2.15 reports some examples of the different kinds of branching that can be observed in a polymer.

Short chain branching is observed when the side chains are short with respect to the main backbone of the polymer, and are usually just a few carbon atoms long. In star polymers all the branches have comparable size and are connected in a single point. An intermediate situation is represented by long-chain-branched polymers, in which a main backbone can be identified, but where the branches are much longer than in the short branching case.

A cross-linked (or network) polymer is a further extreme case of constitution, in which the chains are connected together by side polymer segments. In this case, this interlinking brings about the formation of a single giant molecule, in which a reticulated network of chain segments is extended in all three dimensions. In a cross-linked polymer it is possible to go from any atom to any other atom of the sample through a sequence of covalent bonds.

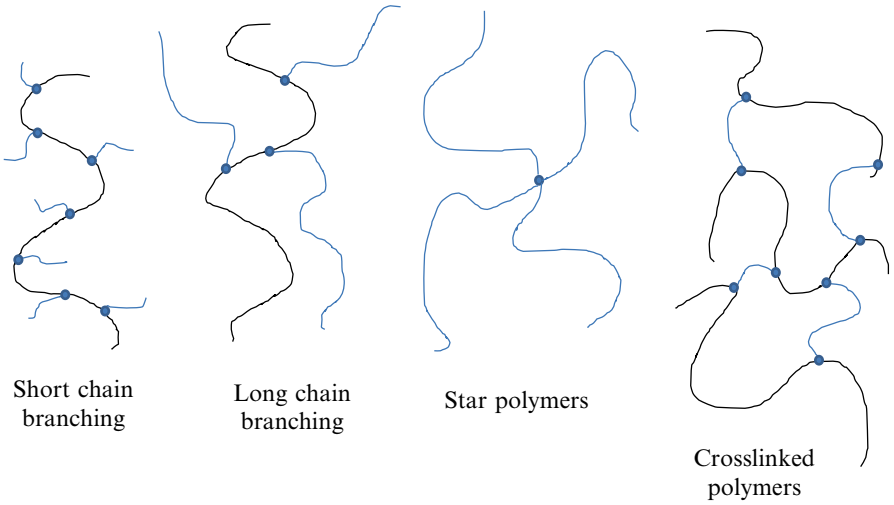
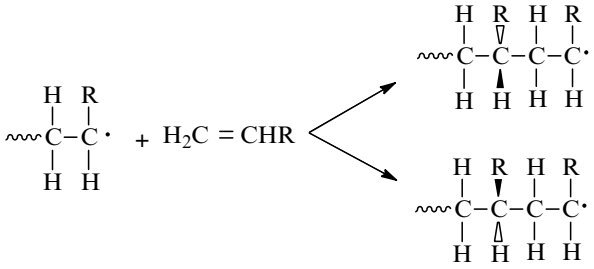


Fig. 2.15 The possible branching schemes which can be found in a polymer. For reasons of clarity, the main chain is drawn in *black*, whereas the branches are drawn in a *lighter colour*. Star polymers are composed by several branches, without a main chain

Fig. 2.16 Stereochemistry of the addition polymerisation of a monosubstituted olefin



2.7.2 *Configuration*

Analogously to the stereochemical definition, configuration defines the arrangement of atoms in a molecule. Configuration remains unaltered, unless chemical bonds are broken and reformed.

The example in Fig. 2.16 shows the polymerisation of an olefin bearing one R substituent group.

As may be seen, when such a monomer is included in a polymer chain, one of the carbons in the repeat units is linked to four different substituents: H, R, and two portions of the chain of different length. Every other carbon atom is thus asymmetric and its substituents can be arranged in two different configurations. Each of these carbon atoms will provide a site for optical isomerism, and the regularity of the configuration adopted by the successive asymmetric carbons has very important

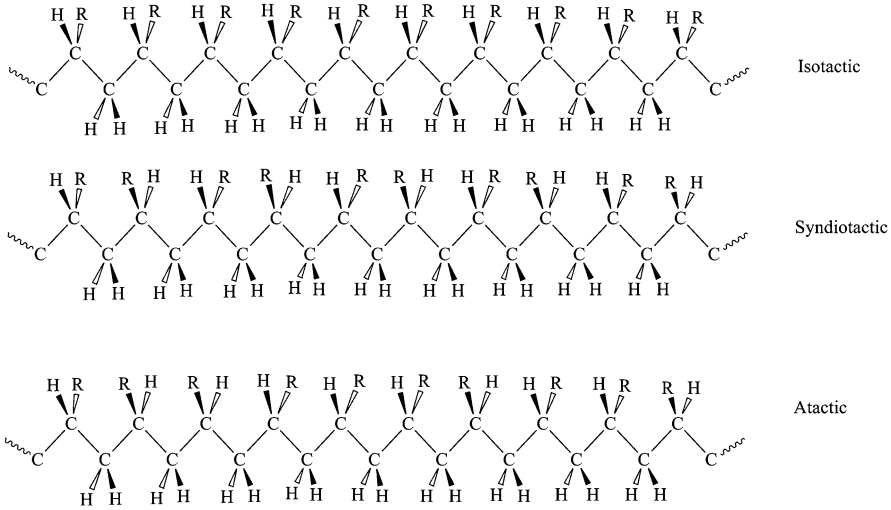


Fig. 2.17 Isotactic, syndiotactic and atactic polymers

repercussions on the properties of the whole material. For simplicity,[3] the polyolefin shown above can be represented with the backbone chain laying on a plane, and with the H and R substituents pointing upwards or downwards with respect to that plane. A polymer is defined as isotactic if the configurations of all the asymmetric carbon atoms are the same, i.e. if in the scheme of Fig. 2.17 all the R substituents are all located below or above the plane. Syndiotactic polymers show an alternation in the configuration of successive asymmetric carbon atoms. In syndiotactic polymers, R groups are alternatively above and below the plane of the backbone chain. When the configurations of the asymmetric carbons are randomly arranged, the polymer is atactic.

To understand the importance of tacticity for the practical application of polymers, suffice it to say that if it were not possible to polymerise polypropylene in isotactic form, this material would not have practical applications. Atactic polypropylene, in fact, is an amorphous, sticky material, with very poor physical-mechanical performance. On the other hand, isotactic polypropylene is a semicrystalline polymer, which has a huge array of applications, due to the versatility of its properties and to its very low cost. The astonishing repercussion that the discovery of a catalyst for isotactic polypropylene had in the polymer industry was recognised in 1963, when Giulio Natta was awarded the Nobel Prize for Chemistry, together with Karl Ziegler.

Another kind of isomerism, geometrical isomerism, is observed when dienes are polymerised (Fig. 2.18).

[3] It should be remarked that this is a simplification: most polyolefin molecules are not planar and not in an extended chain conformation when they are in the solid or liquid state.

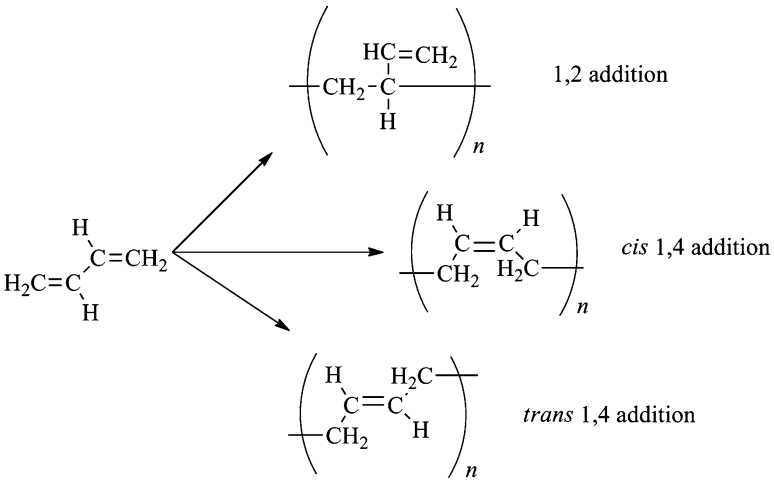


Fig. 2.18 Different modes of polymerisation of a diene

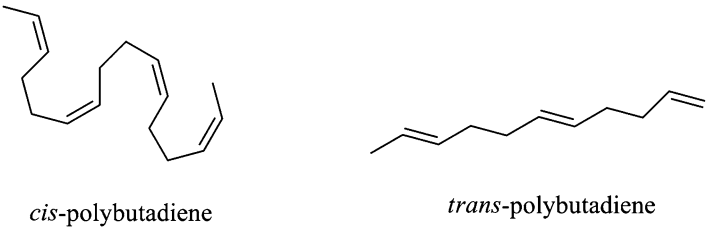


Fig. 2.19 Most favoured conformations of a *cis*- and *trans*-polybutadiene

In optical isomerism single bonds are involved, whereas geometrical isomerism is generated by the presence, in the repeat unit, of a double bond. The *cis* configuration arises when both the sections of the backbone of the chain lay on the same side of the double bond. *Trans* configuration is observed when the chain sections are located on opposite sides of the double bond. Note that in the 1,2 vinyl configuration no geometrical isomerism exists, but indeed the formation of isotactic, syndiotactic or atactic polymers is possible.

A *trans* or *cis* configuration, if consistently repeated along the whole molecule, has a strong effect on the macroscopic properties of the material. Figure 2.19 shows that a polydiene with a *cis* configuration has a tendency to form curled molecules, whereas when it is in *trans* configuration, chains can attain a straight and elongated conformation.

Due to this microstructural difference, *cis*-1,4-polyisoprene, i.e. natural rubber, has dramatically different properties than *trans*-1,4-polyisoprene, i.e. gutta-percha. Natural rubber can be elongated to a large extent because its curled molecules unfold upon traction, but promptly return to the original conformation when stress is relieved. Gutta-perca, on the other hand, is composed of molecules which assume a

straight and stiff rod-like structure, and behaves as a rigid resin, with a much lower elongation and without rubber-like properties.

When derivatives of butadiene are polymerised, the control of the *cis* or *trans* configuration of the repeat units is indeed a way to tune the properties of the whole material, especially its elongation.

2.7.3 Conformation

Conformation is the arrangement which results from the rotation of chain segments around single bonds. This movement does not involve any breaking or reforming of chemical bonds, but is the result of a complex set of intramolecular and intermolecular interactions which favour a particular shape of the molecule over an alternative one. The conformations attained by a macromolecule are mainly a function of the flexibility of the polymer. In a flexible polymer, the chain segments can easily rotate one with respect to the other, with a relevant degree of freedom. On the contrary, a rigid chain polymer is one in which the rotation of the chain segments is hindered by steric factors (for example bulky side groups or aromatic moieties in the backbone) or by strong intermolecular interactions (for example hydrogen bonding in polyamides or cellulose). As a consequence of the chemical nature and of the shape of the macromolecules, the possible conformations vary from linear rigid-rods to flexible random coils.

Rotation around single bonds is a phenomenon that does not usually require high energy, and so molecules in a fluid state, e.g. in the melt or in solution, can easily shift their conformation as a consequence of simple thermal motion. However, some notable conformations can be identified. When the polymeric chains are completely disordered they favour a random coil conformation. More ordinate and regular conformations are found when the intramolecular interactions favour the arrangement of the macromolecules into helices or extended chains (Fig. 2.20).

Polymer chains in ordered conformations can be the building blocks of crystalline domains within the material. This introduces a critical and very distinctive feature of polymers: semicrystallinity.

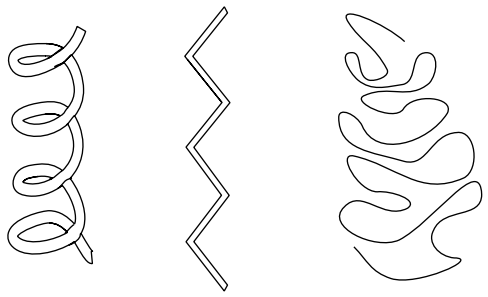


Fig. 2.20 Some conformations commonly attained by polymers

The very large size of polymer molecules precludes, for thermodynamic reasons, a complete and thorough ordering in the solid state of the macromolecules in their entirety. A crystallisation such as that observed in small molecular weight species, where all the atoms have a specific position within the crystalline lattice cannot be achieved in polymers. The entropy change associated to the transition from the totally disordered conformations which are observed in the molten polymer to a situation in which all such molecules attain a regular conformation and arrange themselves in a perfectly ordered framework would be simply too large to allow a spontaneous process. This thermodynamic limitation is coupled with the intrinsic heterogeneity linked to the existence of a distribution of the molecular weight, and thus to the coexistence of several chains within the same material, with the same chemical nature, but differing by size.

The consequence of this is that the crystallisation of polymers cannot be extended to the whole material. Chains too defective, too short, or too less mobile to fit into the crystalline framework will remain in the amorphous phase. On the other hand, if the molecules have the right features, they can give rise to crystalline domains. The structure and morphology of polymers is therefore better described as an alternation of crystalline and amorphous domains, which coexist in the same material. This stark element of distinction between polymers and small molecular weight species will be discussed more in detail in Chap. 7.

2.8 Commercial Polymers: A Round Up

This section will briefly describe the main features of the polymers most commonly encountered in forensic casework, due to the fact that they find several practical industrial applications. Polymers rarely found outside the laboratory or just employed in niche and very rare applications, such as polyimides, poly(vinyl carbazoles), or poly(vinyl pyrrolidone) will not be covered in this section.

2.8.1 Polyethylene

Polyethylene is probably the simplest polymer and has the repeat unit shown in Fig. 2.21.

There are different varieties of polyethylene, mainly distinguished on the basis of their density. Low density polypropylene (LDPE) is produced by high pressure and high temperature polymerisation of ethylene. The very harsh conditions of this

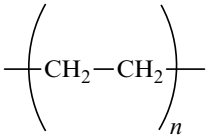


Fig. 2.21 Repeat unit of polyethylene

process bring about the formation of extensive branches along the macromolecules, whose location, size and frequency cannot be controlled. Since they form through different mechanisms, branches as long as the main chain can appear along the molecule, together with much shorter branches. The low density of the material is due to such branching, which prevents an ordinate and tight packing of the molecules.

High density polyethylene (HDPE) is obtained by a Ziegler-Natta catalytic process, which produces perfectly linear molecules, without any branching. In such instance, the packing of the macromolecules is much more efficient, yielding a higher degree of crystallinity and as a consequence a higher density. This neat and ordinate structure brings about a material which is stiffer, stronger and harder with respect to LDPE.

Whenever it is necessary to tune the crystallinity, density or properties of polyethylene, linear low density polyethylene (LLDPE) is a viable alternative to the materials produced by the hardly controllable LDPE process. LLDPE is obtained by using a catalytic synthetic process in which a α-olefin is copolymerised with ethylene. This way, branches of controllable size and frequency can be included into the chains. The scheme in Fig. 2.22 shows an example of the materials which can be obtained by this approach: using 1-octene as a comonomer, branches with six carbon atoms can be formed.

The tunability of the structure brings about a wide array of polyethylenes with different mechanical performances. Low density and low crystallinity materials are used mainly in film form for packaging or wrappings. An ideal combination of low density, high tear resistance, flexibility and chemical resistance can be obtained, often without using additives like plasticisers. The flexibility and resilience of HDPE or LLDPE, along with their chemical inertness, are exploited for manufacturing containers and pipes, for a variety of applications from agriculture to household products to the building industry. The apolar nature of these materials makes them suitable for electrical applications. Whenever higher stiffness, tensile strength and lower gas permeability are required, HDPE is the material of choice.

2.8.2 *Polypropylene*

Polypropylene has the repeat unit shown in Fig. 2.23.

Propylene can in principle be polymerised in atactic, syndiotactic and isotactic form, even though the only material with a significant commercial importance is isotactic polypropylene. The possibility to synthesise polypropylene in a stereospecific manner is given by Ziegler-Natta catalysts and provided a major breakthrough in the

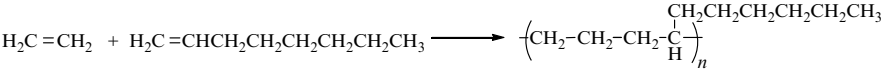


Fig. 2.22 Synthesis of LLDPE: copolymerisation of ethylene with α-olefins

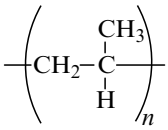


Fig. 2.23 Repeat unit of polypropylene

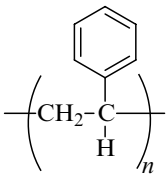


Fig. 2.24 Repeat unit of polystyrene

history of polymer science. In fact it made available to the general public a cheap and performing material useful for manufacturing a number of everyday items. Polypropylene is a semicrystalline, stiff and hard material with a remarkable tensile strength. It melts around 160 °C, so it can be sterilised, allowing its use in the biomedical field. It is very impervious to solvents and to chemical agents, but it is relatively sensitive to oxidation. However, a variety of additives can easily amend this limitation, making polypropylene a material useful for the manufacturing of moulded items, of films for packaging, of pipes, tanks and other containers and of fibres.

2.8.3 Polystyrene

The repeat of polystyrene is shown in Fig. 2.24.

Styrene has a very high tendency to polymerise, so it is not a surprise that the first description of polystyrene dates back to mid-1800s. Its commercial exploitation, though, began many years later, around the 1930s. Polystyrene can be obtained with a number of different mechanisms, but industrially it is mainly produced by radical polymerisation in suspension or in bulk. Commercial polystyrene is atactic, and therefore it is amorphous. The chemical nature of the repeat unit, with an aromatic moiety which is able to scavenge radicals or charges, makes the material remarkably inert to acids, bases, oxidising and reducing agents. Moreover, this polymer is transparent. On the other hand, polystyrene is soluble in a number of organic solvents; it is brittle and has poor resistance to weathering. Despite these disadvantages, polystyrene is currently employed for manufacturing a wide variety of objects, such as moulded containers, lids, jars, bottles, toys and disposable household items.

The drawbacks of polystyrene, in particular its brittleness, can be partly amended by copolymerisation. Poly(styrene-*co*-butadiene) (SBR) is a very common elastomer (Sect. 2.8.17). Poly(styrene-*co*-acrylonitrile) is a transparent plastic with good impact strength for example. Another commercially important material is the terpolymer poly(acrylonitrile-*co*-butadiene-*co*-styrene) (ABS), which efficiently

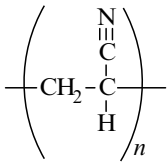


Fig. 2.25 Repeat unit of polyacrylonitrile

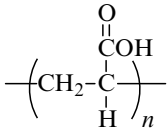


Fig. 2.26 Repeat unit of poly(acrylic acid)

merges the properties of all the component monomers delivering excellent strength and toughness. ABS is used for moulding automotive parts and for shock resistance equipment, such as motorbike helmets.

2.8.4 Polyacrylonitrile

Polyacrylonitrile (PAN), whose formula of the repeat unit is illustrated in Fig. 2.25, is produced by acrylonitrile by radical polymerisation.

PAN can be dissolved in dimethylformamide or dimethylsulphoxide. This polymer's main application is in the textile industry, for the manufacturing of fibres. However, as more deeply elaborated in Sect. 6.2, homopolymer PAN is rather difficult to dye and has poor mechanical properties. Copolymerisation is therefore widely employed: introduction of comonomers such as vinyl acetate, methyl acrylate and methyl methacrylate in quantities above 15 % allows to optimise the quality of the obtained fibres.

2.8.5 Polyacrylates

Polyacrylates derive from the polymerisation of derivatives of acrylic acid (Fig. 2.26).

The polymer obtained from this monomer behaves as a polyelectrolyte, since the COOH moiety can be ionised, attaining the structure shown in Fig. 2.27.

Poly(acrylic acid) in this form is extremely viscous in solution, and is therefore used as a thickening agent in adhesives, but it does not find relevant applications as a structural material.

Another derivative of acrylic acid with notable applications in the polymer industry is methacrylic acid (Fig. 2.28).

The properties of poly(methacrylic acid) are similar to those of poly(acrylic acid), and as such it has similar applications. Methyl acrylate and methyl methacrylate are also used comonomers of acrylonitrile in the manufacturing of acrylic fibres (see Sect. 6.2).

Fig. 2.27 Repeat unit of poly(acrylate)

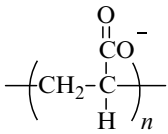


Fig. 2.28 Methacrylic acid

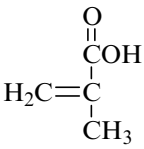


Fig. 2.29 Repeat unit of poly(methyl methacrylate)

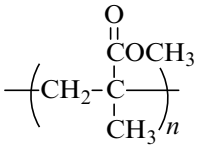
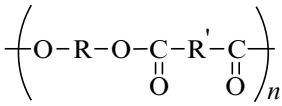


Fig. 2.30 General formula of a polyester



Poly(methyl methacrylate) (PMMA) is definitely the most important acrylic polymer, and has the repeat unit shown in Fig. 2.29.

Methyl methacrylate is polymerised by a radicalic chain mechanism. The most notable property of PMMA is its transparency. It also has good mechanical properties and a remarkable resistance to weathering, although it is soluble in many organic solvents. It is an amorphous polymer, unable to crystallise due to the bulkiness of its side groups. PMMA has an optical clarity comparable to that of glass, even though with a lower hardness and scratch resistance. This makes PMMA a very widely employed substitute for glass, especially in automotive lighting or in the building industry.

2.8.6 Polyesters

Polyesters share the general formula shown in Fig. 2.30.

They are obtained by polycondensation between a carboxylic acid (or one of its derivatives) and a diol. R and R′ can be either aliphatic or aromatic. Aliphatic polyesters, such as poly(lactic acid), poly(glycolic acid), polycaprolactone or polyhydroxyalkanoates, are biodegradable and biocompatible (Fig. 2.31). A growing number of items in the biomedical industry, such as prostheses or resorbable sutures, are being manufactured with these materials.

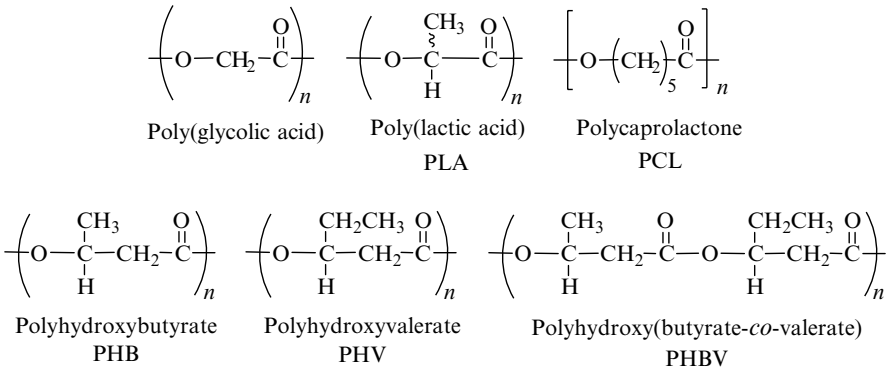


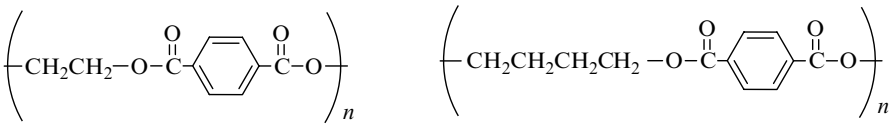
Fig. 2.31 Structures of the repeat unit of the most common aliphatic polyesters

Poly(lactic acid) is a material increasingly used for disposable and compostable household items, despite its cost, which is much higher than other polymers traditionally used, like polystyrene. Another interesting feature of aliphatic polyesters is that many of them can be obtained by biosynthetic techniques (some bacteria for example are able to synthesise polyhydroxyalkanoates) or by renewable sources (the monomer lactic acid can be obtained by the fermentation of corn, sugar cane, sugar beet, etc. [3])

Despite these virtues, aliphatic polyesters are still very underrepresented in the volume of industrial production. In fact, the vast majority of the polyesters of commercial importance are those which contain aromatic rings in the polymer chain. Poly(ethylene terephthalate) (PET) is surely the most common polyester found in normal forensic casework. Its properties include a high melting point, which is around 260 °C, a remarkable resistance to chemicals and mechanical performance. Polyester garments and textiles are invariably based on this material. Another possible polymer used for manufacturing fibres, which however are not applied for the production of textiles, but for niche products such as brushes, is poly(butylene terephthalate) (PBT). The repeat units of PET and PBT are shown in Fig. 2.32.

Textile fibres are not the only application of PET, which is one of the most commonly encountered polymers in daily life. The use of PET for the production of plastic bottles is widely known, but PET is also used in the form of films and of non-woven tissues, especially for scaffolds for roofing or packaging or in the building industry.

PET, PBT, and the other mentioned aliphatic polyesters are thermoplastics. When units of unsaturation are included in the polymer backbone, for example by using maleic acid as the bifunctional acid, usually in conjunction with phthalic acid derivatives, the polymer produced is a thermoset. Radical initiators can in fact be added, spurring the cross-linking of the macromolecular chains, through the reaction of the double bonds included in them. Polyester paints and coatings exploit this mechanism.



poly(ethylene terephthalate), PET poly(butylene terephthalate), PBT

Fig. 2.32 Repeat units of poly(ethylene terephthalate) and of poly(butylene terephthalate)

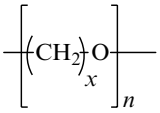


Fig. 2.33 General formula of polyoxymethylene, polyoxyethylene, poly(ethylene glycol), poly(propylene glycol)

2.8.7 Polyoxymethylene, Polyoxyethylene, Poly(ethylene glycol) and Poly(propylene glycol)

This class of polymers actually share a similar repeat unit (Fig. 2.33).

When $x=1$ the polymer is called polyoxymethylene (POM), when $x=2$ the material is polyoxyethylene or poly(ethylene glycol) (PEG). Poly(propylene glycol) has $x=3$.

Polyoxymethylene (POM) is an engineering polymer. It can be obtained by cationic polymerisation of formaldehyde or trioxane, and it is prone to unzipping, i.e. to a depolymerisation reaction which yields the monomer from the polymer, so it should be stabilised by reacting its hydroxyl end-groups with acetic acid or pyridine. Its high crystallinity and relatively high melting point around 185 °C make this material very hard and rigid, suitable for high-end structural applications.

When $n=2$ in the formula above, we are dealing with a material which is called polyoxyethylene if its molecular weight is high enough that it behaves as a solid plastic, suitable for thermoforming and for moulding objects. The properties of polyoxyethylene are not dissimilar from those of POM. On the other hand, when the molecular mass of the polymer is low and its physical state is that of a viscous liquid, the name used for the material is poly(ethylene glycol) (PEG). These liquids can solidify into waxy materials, and are soluble in many organic solvents and in water as well. PEGs are widely employed in the pharmaceutical and cosmetic industry.

Poly(propylene glycol) (PPG) is normally synthesised in relatively low molecular weight, and it displays properties similar to those of PEG. Its main application is as the diol in polyurethane formulations (Sect. 2.8.11).

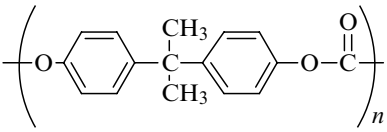


Fig. 2.34 Repeat unit of the polycarbonate derived from bisphenol A

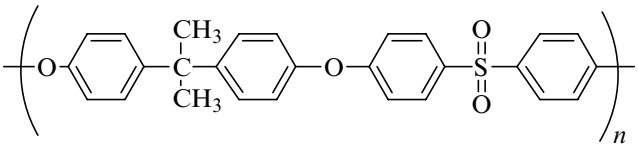


Fig. 2.35 Repeat unit of a polysulphone

2.8.8 Polycarbonates

When carbonic acid reacts in an esterification reaction with a diol, polycarbonates are obtained. A typical material of this class is obtained using, as the bifunctional alcohol, bisphenol A (Fig. 2.34).

They are characterised by a high melting point, beyond 250 °C, and by good chemical inertness, except with alkalis, which attack them with a slow hydrolysis process. Polycarbonates merge a very high resistance to impact with an excellent transparency, making them suitable as substitutes for glass. Transparent items which must be resilient to shocks, such as safety goggles, shields for motorcycle helmets, airplane cockpit covers, spectacle lenses and transparent parts of machinery are often manufactured with this material. One drawback of polycarbonate is that it is subject to a progressive yellowing, which must be avoided through suitable additives.

2.8.9 Polysulphone

Polysulphone is the product of polycondensation of bisphenol A and dichlorodiphenyl sulphone (Fig. 2.35).

It is a thermoplastic, and its main advantage is the very high thermal stability, which brings about good mechanical properties over a range of temperatures spanning from −180 to +140 °C. It is transformed by injection moulding into items which reflect the outstanding characteristics of this material.

2.8.10 Polyamides

Polyamides have the general formula shown in Fig. 2.36.

They are obtained by polycondensation between a carboxylic acid (or one of its derivatives) and a diamine. As in the case of polyesters, R and R′ can be either

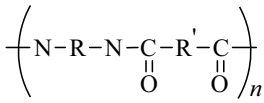


Fig. 2.36 General repeat unit of a polyamide

aliphatic or aromatic. However, differently from polyesters, aliphatic polyamines are more widely diffused and are collectively known as nylons. The designation of nylons is usually made with one or two numbers. When two numbers are used, the first one indicates the number of carbon atoms in the precursor diamine, and the second one is the number of carbon atoms in the dicarboxylic acid. For example, nylon 6,10 derives from the polycondensation of hexamethylenediamine and the diacid with 10 carbon atoms, i.e. sebacic acid.

When only one number is present in the name of the material, such as in nylon 6, just one monomer was used for its synthesis. In the case of nylon 6, such monomer is caprolactam, a cyclic amide which, when the ring is opened, is a chain of six carbon atoms with a carboxylic acid at one end and an amine group at the other end.

Nylon 6 and nylon 6,6 are surely the most commonly found nylons, especially in fibres. Separately or in mixtures, they are the raw material for the production of fibres which then are woven into textiles for 'polyamide' garments. Nylon 11 is also used for textiles, but is less common than nylon 6 and nylon 6,6. Other applications of the nylon 6,6, nylon 6 and nylon 6,10 are tyre cords, brushes and bristles. Due to their excellent mechanical properties, especially high tensile strength, toughness, and abrasion resistance, nylon fibres are well suited for making textiles for heavy duty applications, from carpets to technical mountain gear.

Due to their toughness, nylons are good plastics, used for moulding mechanical parts such as gears or bearings, or in general for manufacturing objects which are employed in demanding situations. Nylons are resistant to most solvents, however, they are attacked by phenols, formic acid or cresols. At room temperature they resist well to water, but at high temperature they can be degraded by hydrolysis if water is present. As a consequence, they must be thoroughly dried and contact with water must be avoided in the moulding process.

A further improvement of the mechanical properties of nylons can be achieved using aromatic diacids and diamines in the polymerisation. In this case, the class of material is that of aramides. The most widely known aramides are Kevlar® and Nomex®, which derive from the polymerisation of a phenylenediamine and a phthalic acid.

Aramides have a very high melting point (>400 °C) and extremely high moduli. These make them suitable for reinforcing polymer matrices in all-polymer composites or for manufacturing specialty textiles for bulletproof garments or for safety apparatus, such as gloves for the manipulation of hot objects.

2.8.11 Polyurethanes

The chemical feature which defines polyurethanes is the presence of the urethane linkage. Their repeat unit has the general formula illustrated in Fig. 2.37.

Fig. 2.37 Repeat unit of a polyurethane

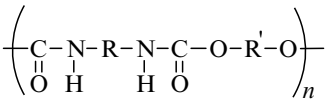
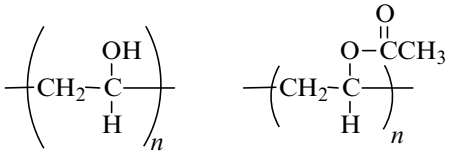


Fig. 2.38 Repeat units of poly(vinyl alcohol) and of poly(vinyl acetate)



poly(vinyl alcohol) poly(vinyl acetate)

They derive from the reaction of a species containing two or more isocyanate groups (–N=C=O), and a diol. Polyurethanes are probably the clearest representation of the versatility of polymer science: from the same chemical base structure, a huge amount of materials with extremely different properties can be obtained. Polyurethanes can be in fact used for making soft stuffings for car seats, couches or pillows, for elastomeric materials with rubber-like properties, or for hard and durable coatings, paints and adhesives. The reason of such tunability in the properties lies in the possibility of acting on the flexibility of the macromolecular chain, by changing the nature and length of the R and R′ moieties. When R and R′ correspond to very short carbon–carbon chain or to aromatic rings, the resulting macromolecule will be very stiff, yielding a hard and rigid material. On the contrary, when for example long and flexible chains of poly(propylene glycol) are used as the diol, the flexibility of the obtained polymer is transferred to the macroscopic properties of the material, which will be very soft and will display elastomeric properties. Lycra® is a renowned material, consisting of elastomeric polyurethane fibres, which are added to textiles to make them more elastic. Polyurethanes are very well suited for being expanded by the injection of a gas during the polymerisation step, therefore obtaining foams with a wide variety of softness and compressibility. As in the case of polyesters, multiple functionalities can be included into the polyurethane chain, allowing a subsequent cross-linking stage. This approach is used when coatings and adhesives are made.

2.8.12 Poly(vinyl acetate) and Poly(vinyl alcohol)

These two polymers, whose repeat units are shown in Fig. 2.38, are often considered together because poly(vinyl alcohol) (PVA) is produced from the hydrolysis of poly(vinyl acetate) (PVAc). Actually most of the poly(vinyl acetate) which is produced industrially goes into the manufacture of PVA. Other applications of PVAc are in the adhesive field.

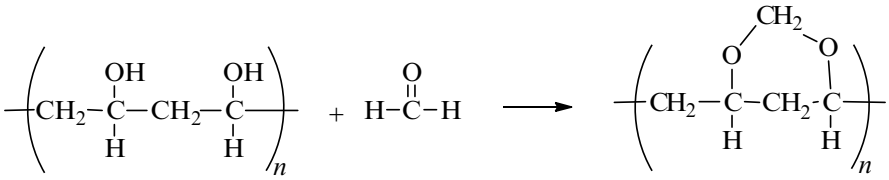


Fig. 2.39 Reaction for the production of poly(vinyl formal)

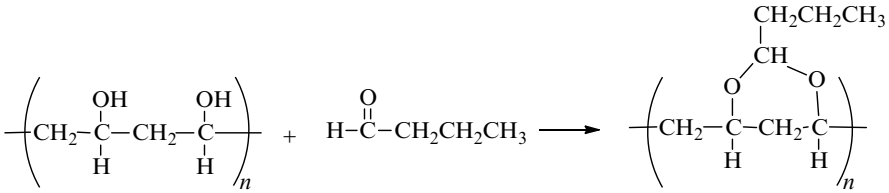


Fig. 2.40 Reaction for the production of poly(vinyl butyral)

As introduced before, PVA derives from the hydrolysis of PVAc. Such hydrolysis can be prolonged until just about 1 or 2 mol% of acetyl groups remain, or it can be stopped, in order to create a PVA-PVAc copolymer. The most remarkable property of PVA is that it is one of a few water-soluble polymers. This is at the basis of its applications, especially as a stabiliser of emulsions and suspensions. It is also used as an adhesive and in the textile industry, as a sizing agent. If it is reacted with formaldehyde in a sodium sulphate solution with sulphuric acid, it forms poly(vinyl formal) (Fig. 2.39), used in wire insulation, due to its excellent dielectric properties.

A similar reaction can be carried out with butyraldehyde, which yields poly(vinyl butyral) (Fig. 2.40).

This polymer is used in the formulation of adhesives, and also for the manufacturing of shatter-proof glass. For this purpose, a thin film of poly(vinyl butyral) is placed between two glass sheets and adhesion between the components of this system is favoured by application of heat and pressure. If, due to some impact, the glass is broken, the splinters remain stuck to the polymeric film and do not harm anybody or provoke any damage to any object in the vicinity.

2.8.13 Poly(vinyl chloride) and Other Chlorinated Polymers

Poly(vinyl chloride) (PVC) is a polymer used for a large number of applications. It has the repeat unit shown in Fig. 2.41.

It is obtained from the emulsion or suspension polymerisation of vinyl chloride, a very hazardous monomer. PVC contains a stereogenic carbon, but ordinarily the

Fig. 2.41 Repeat unit of poly(vinyl chloride)

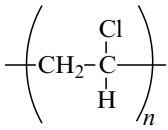
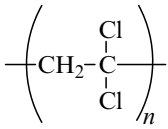


Fig. 2.42 Repeat unit of poly(vinylidene chloride)



polymer is obtained in atactic form, with linear or slightly branched chains. It is impervious to most solvents, being dissolved just by ketones or chlorinated hydrocarbons. It degrades beyond 200 °C with evolution of HCl, but it can be easily stabilised by additives. Pure PVC is a quite stiff and brittle material at room temperature, and as such its applications would be very limited. However, it may be easily plasticised, making processing easier and tuning its properties from fully flexible to fully rigid. As such it is suited for very diverse applications, ranging from watering hoses to cable insulation, from pipes to fibres, from laminated materials to machinery parts. PVC can be used in the form of plastisols or organosols, which are dispersions of PVC in plasticisers or organic solvents. These paste-like formulations can be applied onto surfaces, or otherwise shaped, and then, when treated at a temperature higher than 150 °C, cross-linked obtaining the final item. Coating textiles with a PVC layer applied with this method is one of the ways to give them a leather-like finish, or to otherwise change their aspect.

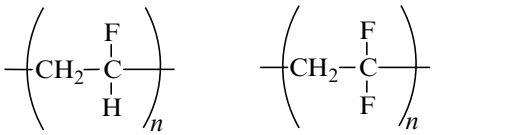
The amount of chlorine in PVC can be increased by exposing it to a solvent such as chlorobenzene at a high temperature (around 100 °C). The product which is obtained by this treatment is called chlorinated PVC. It is thought that in this process some hydrogen atoms are substituted by chlorine atoms. Chlorinated PVC has a higher resistance to acids and bases, but a lower stability to light and heat. It is used in the adhesive, coating and fibre industry.

Another example of chlorine-containing polymer of industrial interest is poly(vinylidene chloride) (Fig. 2.42).

The homopolymer, or the copolymer with vinyl chloride, vinyl acetate or acrylonitrile, is a member of a special member of halogen-containing fibres which, due to the peculiar chemistry of halogens, have useful anti-flame properties.

2.8.14 Fluorinated Polymers

The fluorinated polymers industrially exploited can be imagined as derivatives of polyethylene in which one or more hydrogen atoms were substituted with fluorine atoms. The presence of fluorine introduces in these materials a very high resistance



poly(vinyl fluoride) poly(vinylidene fluoride)

Fig. 2.43 Repeat units of poly(vinyl fluoride) and poly(vinylidene fluoride)

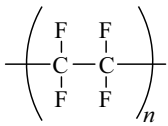


Fig. 2.44 Repeat unit of polytetrafluoroethylene

to chemicals and to solvents. As such, the simplest members of this family, poly(vinyl fluoride) and poly(vinylidene fluoride) (Fig. 2.43) find applications in coating compositions.

In addition to their chemical inertness, these materials often display remarkable structural integrity and thermal stability, which are however offset by a very high cost, compared to other polymers.

Within this family, the most important polymer is polytetrafluoroethylene (PTFE) (Fig. 2.44), which comes from the emulsion polymerisation of tetrafluoroethylene.

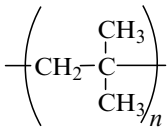
PTFE has very linear molecules, because branching would require the breakage of the stable C–F bonds. Due to this regularity of the microstructure, PTFE is a highly crystalline polymer, which melts at more than 300 °C. Its remarkable mechanical strength remains unaltered in a very wide temperature range, from −100 to +300 °C, and it has a very low dielectric constant. It is insoluble in all solvents, and in the molten state its viscosity is extremely high, so its workability is quite problematic: items made of PTFE are produced by sintering the polymer powder into moulds. Despite these difficulties and its high cost, PTFE has so impressive properties that it is used in high-end applications such as specialty equipment, e.g. pipes or valves, for the chemical industry where resistance to aggressive agents is required, but also in the coating of non-sticking pans. Other applications include films for sealing in plumbing and the realisation of moving parts in mechanical devices, because of the self-lubricating characteristic of this material.

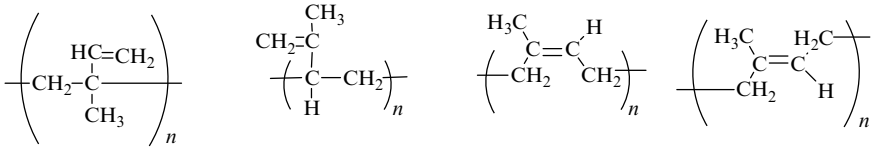
2.8.15 Polyisobutylene

Polyisobutylene (Fig. 2.45) is the result of cationic polymerisation of isobutylene. It dissolves in many hydrocarbons, but it is remarkably resilient to attacks from a number of chemical species, such as acids, alkalis and water, which have no effect on it.

As a homopolymer, it is used as an insulating material in electrical applications, or as an additive to motor oil for tuning its viscosity as a function of temperature.

Fig. 2.45 Repeat unit of polyisobutylene





1,2-polyisoprene 3,4-polyisoprene *trans*-1,4-polyisoprene *cis*-1,4-polyisoprene

Fig. 2.46 The different repeat units obtainable from the polymerisation of isoprene

The copolymer of isobutylene and 2–5 % isoprene is known as butyl rubber and is an important elastomer. The double bonds introduced in the chain by isoprene allow the cross-linking of the macromolecules.

2.8.16 Polyisoprene: Natural Rubber and Gutta-Percha

Polyisoprene is probably the best example of the consequences of configuration on the physical-mechanical macroscopic properties of a material.

Polymerisation of isoprene can yield four different types of repeat units (Fig. 2.46).

1,2- and 3,4-polymerisation have not a significant industrial interest, and the only important kinds of polymer derived from isoprene are the 1,4-polymerised ones. In particular, *cis*-1,4-polyisoprene is commonly known as natural rubber, a soft and elastic material, still unsurpassed for heavy duty applications such as truck tyres. If the configuration of the double bond in the repeat unit changes into *trans*, the properties of the material drastically change. Gutta-percha is *trans*-1,4-polyisoprene, and differently from its *cis* counterpart, it is a hard thermoplastic solid, which currently has no significant industrial application.

Natural rubber is extracted from the latex produced by the rubber tree (*Hevea brasiliensis*). From the latex, through a number of manufacturing steps, rubber sheets can be obtained, which are treated by smoking, to prevent attack from micro-organisms and to improve shelf-life. The material so prepared is not ready for trans-formation though, because it would yield a sticky matter without elastomeric properties. To obtain the material commonly associated to rubber, the product of the processing of latex must be formulated with sulphur, accelerants and antioxidants, and vulcanised. This procedure creates sulphur bridges between the macromolecules which prevent the material from suffering permanent deformation upon tensile or compressive strength, at the same time allowing an efficient instantaneous,

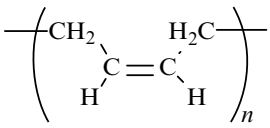


Fig. 2.47 Repeat unit of *cis*-1,4-polybutadiene

reversible deformation. Since after vulcanisation still some unreacted double bonds remain, natural rubber, and also elastomers based on butadiene and chloroprene, remain quite reactive and prone to oxidative stress. This calls for the use, in the formulation of all these materials, of proper antioxidants, such as carbon black which acts as a ultraviolet light scavenger. The mix of cost, elastomeric properties, and workability made natural rubber the material of choice for tyres and for sealing applications. For this latter use, it must be remembered that natural rubber has a tendency to swell in hydrocarbons.

2.8.17 Elastomers Derived from Butadiene and Its Derivatives

Polybutadiene is synthesised by the polymerisation of butadiene. As in the case of the polymerisation of isoprene, four types of polybutadienes can be obtained. Among these, *cis*-1,4-polybutadiene, whose repeat unit is shown in Fig. 2.47, is the most desired due to its elastomeric properties after cross-linking and to its resistance to abrasion.

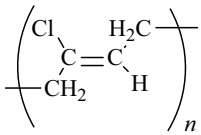
The amount of *cis*-1,4-polybutadiene obtained in the polymerisation of butadiene is maximised by the use of Ziegler-Natta catalysts. However, as a homopolymer, polybutadiene does not find many applications. Low molecular weight liquid polybutadienes, terminated with carboxylic acid or hydroxyl groups are used as adhesives.

More industrially important materials are copolymers of butadiene and styrene (styrene-butadiene rubber, SBR) or of butadiene and acrylonitrile (nitrile rubber). These polymers are obtained in emulsion by radical polymerisation, obtaining more than 80 % of 1,4 concatenated units. The output of this process is a latex, which can be worked up similarly to natural rubber. SBR blends well with natural rubber and thus these two materials can substitute each other well. Nitrile rubber does not swell when in contact with hydrocarbons or oils, so it is suitable for seals or other applications in which resistance to oil is a requirement.

2.8.18 Polychloroprene

Polychloroprene (Fig. 2.48) is another kind of elastomer, commonly known as neoprene. It derives from the emulsion polymerisation of chloroprene.

Fig. 2.48 Repeat unit of *trans*-1,4-polychloroprene



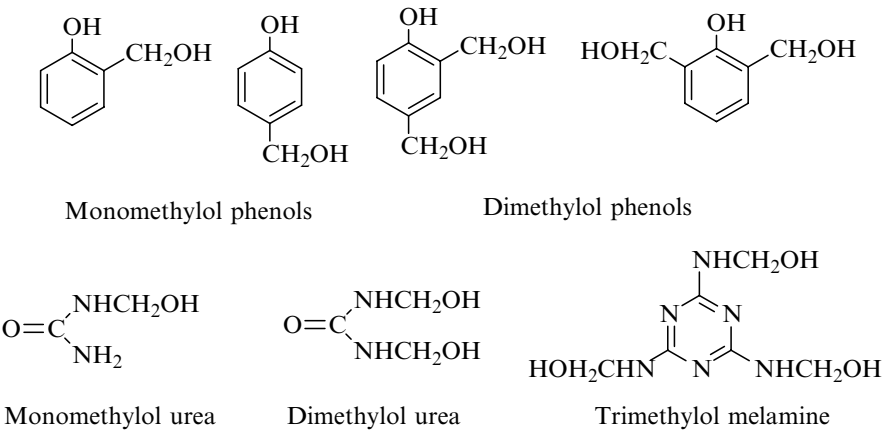


Fig. 2.49 Examples of methylolated species formed on the synthesis of formaldehyde-based resins

The major product of the synthesis is *trans*-1,4-polychloroprene, so neoprene is able to crystallise and, after vulcanisation, has an excellent tensile strength. It is more resistant than nitrile rubber to oil and hydrocarbons. Its main applications are insulating coatings of wires and cables, shoe soles, solid tyres, gloves, industrial hoses and wetsuits for scuba diving.

2.8.19 Formaldehyde-Based Resins

Formaldehyde can easily condense with phenol, urea or melamine, yielding thermoset materials. The mechanism of such condensation comprises two main steps. In the first step methylolated species are formed (Fig. 2.49).

Then, condensation between methylolated species and the monomer happens, connecting the different moieties of the system together (Fig. 2.50).

The three dimensional network obtained by the condensation of the methylol melamine derivatives is shown in Fig. 2.51.

The practical realisation of such reactions as well proceeds in two or more stages. In a first step oligomeric species are formed, which are in the form of a viscous fluid. In subsequent steps, condensation is completed, cross-linking happens and a solid thermoset material is obtained.

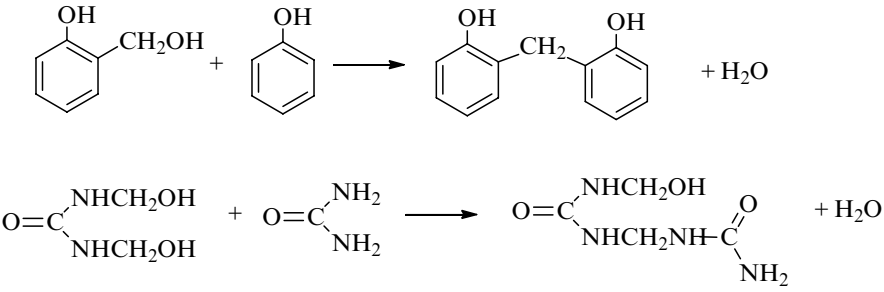
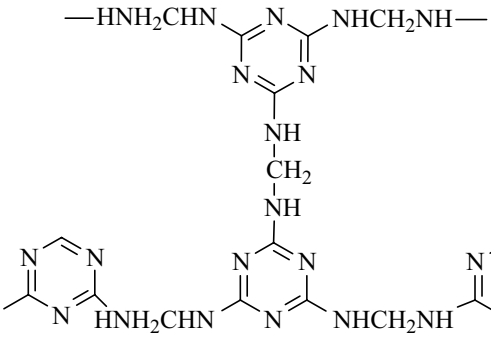


Fig. 2.50 Examples of condensation reactions in phenol-formaldehyde resins (*top*) and urea-formaldehyde (*bottom*) resins

Fig. 2.51 Structure of the condensation product of melamine and formaldehyde



Phenol-formaldehyde resins can be prepared by two approaches. In the first one formaldeyde is reacted in acid conditions with an excess of phenol, forming novolac, i.e. a set of linear molecules, which can be stored for a long time without the risk of spontaneous hardening. Addition of an excess of formaldehyde and increase in temperature will trigger the cross-linking which brings to bakelite, the final material. The alternative method of production of phenol-formaldehyde resins is through a reaction in basic conditions of equimolar quantities of phenol and formaldehyde. The reaction conditions are carefully controlled in order to obtain a material, called resol, containing just linear oligomeric species. Resol already contains all the functional groups which are sufficient to complete the cross-linking of the resin. This has two consequences. The first is that the preparation of the final resin can be achieved by a mere heating of resol. The second consequence is that resol has a limited shelf-life. Even in the absence of heating, a slow cross-linking proceeds, which eventually brings to the unintentional hardening of the resin on storage. Bakelite can be used to mould items whose main requirement is durability. Electrical insulators are an example of such application. Resols or novolacs can be used to impregnate textiles or other substrates, which are subsequently layered one on top of the other to assemble laminates.

Similar processes are involved in urea formaldehyde (UF) and melamine formaldehyde (MF) resins. In the cases of these materials, collectively known as

amine resins, the first stage consists in the formation of the methylolated species, and the second stage is their condensation. Before cross-linking, UF and MF are water-soluble, making them useful as sizing and finishing agents in the textile industry. They are very efficient adhesives as well, still unsurpassed, when the cost-benefit ratio is involved, in the plywood industry. Amino resins are used in the manufacture of paints and coating, because they are colourless and hard. MF are harder and resist to moisture and heat better than UF. On the other hand, phenolic resins are superior in terms of heat and moisture resistance with respect to amino resins.

2.8.20 Epoxy Polymers

Epoxy resins are polyethers obtained by the ring opening of epoxide rings. Such reaction happens when an hydroxide-containing monomer attacks the epoxide. An example of the mechanism of the reaction between epichlorohydrin and bisphenol A, is shown in Fig. 2.52.

The same action of bisphenol A can be carried out by other species such as glycols, glycerols, novolacs, aromatic amides, aliphatic alcohols or polyols, and aromatic amines. The products of these reactions are usually viscous liquids or solids with a low melting point. The subsequent mixing with a cross-linker based on polyfunctional amines, acid anhydrides, phenols, or thiols, hardens the material

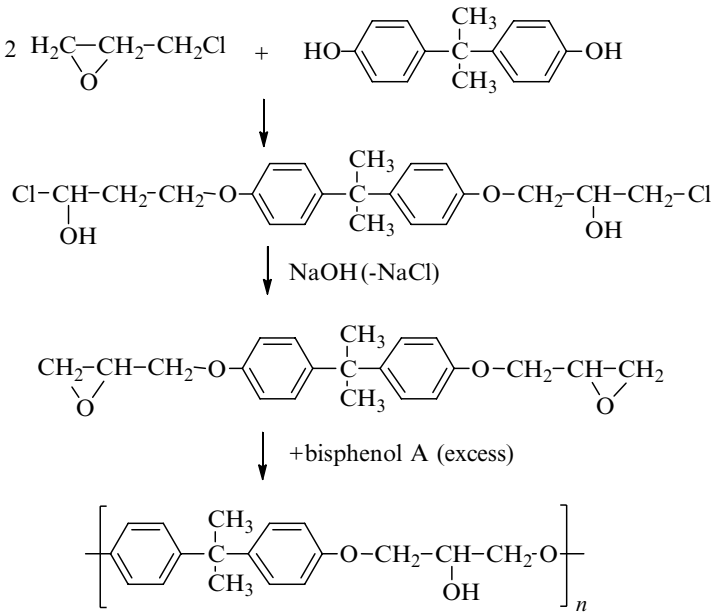


Fig. 2.52 Reaction sequence for the formation of epichlorihydrin-bisphenol A epoxide resin

into a thermoset solid. The most distinguishing features of epoxy resins are a remarkable chemical resistance and very good adhesion. These make epoxy resins very efficient adhesives, but also performing materials for floorings, for the manufacture of electrical insulators and as matrices for fibre-reinforced composites.

2.8.21 Silicone Polymers

This class of polymers shows a radical difference from the other materials discussed so far, because it is not based on a concatenation of carbon atoms. Despite the chemical similarity between carbon and silicon, which are both in group 6 of the periodic table, the Si–Si bond is not stable enough to form large molecules. As a consequence, the framework of the macromolecular chain in silicone polymers is based on a sequence of –Si–O–Si– bonds. These materials are obtained by condensation polymerisation of silanols, species derived from the hydrolysation of alkyl chlorosilanes, aryl chlorosilanes or esters of substituted orthosilicic acid.

The repeat unit of silicon polymers is shown in Fig. 2.53.

Silicone polymers are very versatile materials, available in liquid, waxy or rubbery form. They display a high resistance to heat, remarkable water repellency and resistance to chemicals. Silicone coatings, sealings or foams are used anytime these properties are required by the specific application.

2.8.22 Cellulose and Derivatives of Cellulose

Cellulose is the most abundant polymer on Earth. It is a naturally occurring, linear and stereoregular polysaccharide composed of β-D-glucose residues (Fig. 2.54).

Due to the possibility of formation of several hydrogen bonds along the cellulose chains, this material does not melt (it actually degrades before melting), it does not dissolve in most solvents, and has a remarkably high mechanical performance.

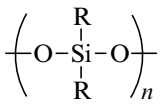


Fig. 2.53 General repeat unit of a silicone polymer

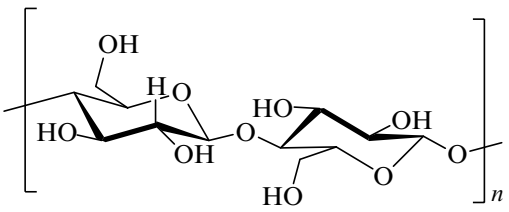


Fig. 2.54 Repeat unit of cellulose

This positive mix of properties made cellulose the material chosen by Nature for providing the structure to vegetable living matter. As such, a number of cellulosic fibres, with similar chemical composition, but with different morphology and properties, can be found, ranging from cotton to flax, from jute to hemp. These fibres were among the first materials available to human beings for covering their bodies and protecting themselves from the elements. Cotton fabrics display superior properties to many synthetic textiles. They resist well to laundering because they retain their tensile strength in wet conditions as well as in the dry state and also after many folding–unfolding cycles. Their moisture absorption capability and their dyeability complete the picture, showing why cotton is still now an ubiquitous material for the textile industry. Other cellulosic fibres have properties which are similar to those of cotton, because they share a similar chemical composition. The different morphology of the fibres confers to the textiles a different aesthetical appearance and a different hand.[4]

The applications of naturally-occurring cellulose are not limited to the textile industry. Wood is a composite of cellulose and lignin, and has been used since the dawn of times as a structural material for a huge variety of purposes. The cellulose contained in wood can also be extracted and treated, to provide pulp, the base raw material for the manufacturing of paper.

Due to the tight structure favoured by the extended formation of hydrogen bonds, cellulose is insoluble and quite resistant to chemicals attacks. However, a few chemical reactions were identified which are able to break hydrogen bonds and to separate the macromolecules. By this approach cellulose can be dissolved. This way, it is possible to purify, modify or functionalise the polymer chains, and to subsequently reprecipitate them. The product of such treatment is regenerated cellulose. Cotton is probably the best raw material for carrying out this process, because it has the highest content of cellulose, but on the other hand it is the most expensive.

2.8.23 Rayon

Rayon is regenerated cellulose, mainly used for spinning fibres. The process for manufacturing rayon starts with the treatment of cellulose from wood pulp, linters or other sources with strong bases. The alkali cellulose so obtained is reacted with carbon disulfide, to form cellulose xanthate, as shown in the scheme of Fig. 2.55.

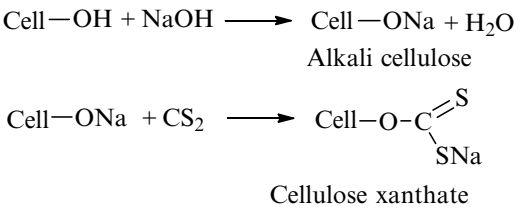


Fig. 2.55 Process of production of cellulose xanthate

[4] In the textile industry, the hand is the feeling experienced by touching a particular cloth.

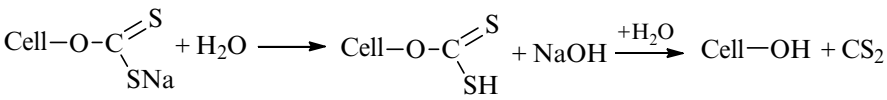


Fig. 2.56 Hydrolysis of cellulose xanthate

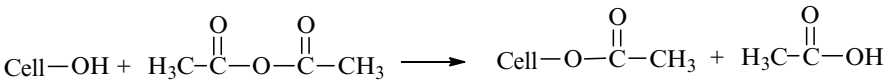


Fig. 2.57 Synthesis of cellulose acetate

Cellulose xanthate is soluble in a sodium hydroxide solution, yielding the so-called 'viscose solution'. This allows to separate cellulose from the other unwanted chemicals present in the raw materials used, e.g. from lignin in wood or from proteins or pectins in cotton. Cellulose xanthate can be hydrolyzed and cellulose can be regenerated by treating the viscose solution with a dilute aqueous solution containing sulphuric acid and a sulphate salt (Fig. 2.56).

This allows to obtain fibres with a desired and controlled morphology, and to tune their chemical composition.

Rayon is a material associated to the fibre industry. However, regenerated cellulose can be used to manufacture films. In this latter case, the material is commonly referred to as cellophane. The process for cellophane is the same as that outlined above. A thin film of the viscose solution is extruded into a thin film which passes through a regeneration bath analogous to that described before. Differently from the case of fibres, however, glycerol is added as a plasticiser, by immersion in a final glycerol solution bath. Cellophane is now much less used than some years ago, but it still finds application in the wrapping and packaging field.

2.8.24 Cellulose Acetate

Cellulose acetate is another important derivative of cellulose used in the fibre industry. It is produced reacting cellulose with acetic anhydride, with a strong acid as a catalyst (Fig. 2.57).

The degree of acetylation of the hydroxyls available along the cellulose chain is a critical factor for tuning the properties of the final material. When all the hydroxyl groups (three per repeat unit) are acetylated, the resulting material is called cellulose triacetate and is used for fibres. Partial hydrolysis of the acetylated groups can lead to secondary cellulose acetate, which has 2.2–2.5 acetylated groups per repeat unit of cellulose. This material has a wider range of applications, because beside the production of fibres, it can be used also for moulding items. Optimisation of the properties of cellulose acetates can be attained by esterification with other agents

in addition to the acetate moiety, such as butyrate or propionate. This is equivalent to obtaining a copolymer. Cellulose(acetate-*co*-butyrate) or cellulose(acetate-*co*-propionate) are excellent plastics with better dimensional stability and impact strength than cellulose acetate.

2.8.25 Other Cellulose Derivatives

Cellulose nitrate (or nitrocellulose) is an ester of cellulose and nitric acid. Nitration of cellulose is achieved by reacting it with a mixture of nitric acid and sulphuric acid, followed by washing with water and dilute alkali and by bleaching. The degree of nitration controls the final properties of the material. With a percent of nitrogen larger than 12.3 % an explosive is obtained, nitrolaquer has a nitrogen content between 11 and 12 %, and celluloid plastic has an even lower nitrogen content. Celluloid is still used for niche application like spectacle frames or fountain pens. It used to be the material on which films for cinema projectors were printed. Nitrolaquer is applied in the paint and coating industry.

Ethyl and methyl cellulose are derivatives of cellulose, in which the hydroxyl groups of cellulose form ethyl and methyl ethers, respectively. They are applied as finishing additives for fibres and as paint binders.

Carboxymethyl cellulose is produced according to the process shown in Fig. 2.58.

The availability of carboxyl groups on the macromolecules allows to tune their solubility. Carboxymethyl cellulose thus finds application as a thickening agent in many different fields, such as in the cosmetics, food and textile industry.

2.8.26 Proteins

Proteins are found in all living organisms and are the base materials of all natural fibres of animal origin. Silk and wool are proteic fibres. Albeit not a fibre, leather as well is an artificial proteic material. The monomers from which proteins derive are α-amino acids, i.e. carboxylic acids which contain an amine group on the α carbon atom. The α carbon atom is in this case the atom adjacent to the carboxylic acid group (Fig. 2.59).

Many α-amino acids are known or were synthesised. However, among this large number of known molecules, 20 α-amino acids were identified as the building blocks which are the base materials of most of the proteins naturally occurring in the animal and vegetable organisms.

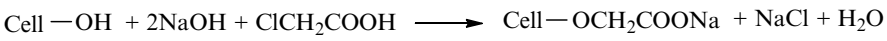
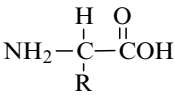


Fig. 2.58 Synthesis of carboxymethyl cellulose

Fig. 2.59 General formula of an α-amino acid



Amino acids can be linked by a condensation reaction, with elimination of water molecules and the formation of peptide bonds. The sequence of the amino acids that form the macromolecular chains of the proteins, along with the three-dimensional structure that they attain as a consequence of intermolecular and intramolecular forces, determine the properties of the proteic material. Proteins have a very wide array of biological functions, from providing the structure of several tissues, to catalysing chemical reactions, to controlling the exchange of chemicals between and within the cells. In the context of this book, the most interesting proteins are those which constitute animal hairs (such as fur or wool) or animal secretions (such as silk), used as natural fibres. There is not much variability in the chemical composition of these proteins. Animal fibres are mainly based on keratin. Probably the most remarkable progress in the analysis of proteic fibres or material is the application of DNA typing for determining the species of the animal which shed that material [4].

References

1. Strazielle C (1975) Molecular characterization of polyethylene. Pure Appl Chem 42:613
2. Strazielle C, Benoît H (1971) Molecular characterization of commercial polymers. Pure Appl Chem 26:451
3. Ray SS, Okamoto M (2003) Biodegradable polylactide and its nanocomposites: opening a new dimension for plastics and composites. Macromol Rapid Commun 24:815
4. Ji W, Bai L, Ji M et al (2011) A method for quantifying mixed goat cashmere and sheep wool. Forensic Sci Int 208:139

Suggested Reading

Bower DI (2002) An introduction to polymer physics. Cambridge University Press, Cambridge
Brandrup J, Immergut EH, Grulke EA (2003) Polymer handbook. Wiley, Hoboken
Carraher CE (2013) Carraher's polymer chemistry, 9th edn. CRC, Boca Raton
Carraher CE (2012) Introduction to polymer chemistry, 3rd edn. CRC, Boca Raton
Cowie JMG, Arrighi V (2007) Polymers: chemistry and physics of modern materials. CRC, Boca Raton
Ravve A (2012) Principles of polymer chemistry. Springer, New York
Young RJ, Lovell PA (2011) Introduction to polymers. CRC, Boca Raton

Chapter 3
Polymeric Traces: Transfer, Persistence, Recovery, Analysis and Interpretation of Analytical Data

Polymers are ubiquitous in our everyday life, so it is highly likely that they may be somewhat involved in the commission of crimes. Fibres will be shed by the garments of a burglar, paint smears and plastic fragments can be left in car accidents, adhesive tape can be used for the packaging of illegal drugs, wires insulated by a polymeric coating are part of many bombs, etc.

However, exploitation of the information carried by polymers on the dynamics of crimes is not straightforward. It is indeed the result of a quite complex set of requirements and of analytical and statistical treatments, which is the main topic of this chapter.

Since traces are the remnants of an activity, it is first of all necessary to understand how polymeric traces can end up on a crime scene. The concepts of transfer and persistence are paramount on this regard.

After traces are shed, though, they must be found and recovered. They then must be properly analysed and the experimental data must be suitably treated, with the final aim of assessing the evidential value of these items, ultimately interpreting the amount of information contained in them.

This chapter will start with a discussion on the theoretical basis for the exploitation of polymeric traces for forensic purposes. It will present then how to devise an efficient process to bring evidence from the crime scene to the laboratory. The second part of this chapter will present the basics of analytical data treatment and the most widespread approaches for the interpretation of evidence, in order to efficiently and reliably convey to the Court the significance of the information contained by the traces.

All these issues regard any type of forensic evidence, and are not restricted to polymeric traces. Therefore, most of the topics discussed in this chapter can be applied with relatively minor modifications, to other kinds of trace evidence.

V. Causin, *Polymers on the Crime Scene*,
DOI 10.1007/978-3-319-15494-7_3

3.1 Polymers as Sources of Evidence: Transfer, Persistence, Recovery

Differently from biological traces or fingerprints, polymers cannot be used for the personal identification of the individual who committed a crime. However, if properly analysed and characterised, they can yield significant information on the dynamics of crime and on the circumstances related to it.

Figure 3.1 depicts a hashish block, seized during a Police operation against drug trafficking.

Cling film was used for wrapping the drug, and the outer layer was sealed with abundant adhesive tape. The mode of packaging and the materials used, if properly analysed, can yield precious investigative information on the modus operandi of the organisation which traffics and commercialises the illegal substance. If further hashish is seized with a packaging consistent to the one showed in Fig. 3.1, it could be evidence that the same group of people were responsible for repeated shippings.

Figure 3.2 regards a double homicide, in which a man and the infant he was holding were killed. During the autopsy, just one bullet was retrieved from the man's body, and it was wrapped by a bundle of red and light blue fibres.

The child's clothes were pierced actually in red and blue areas of his garments' motif (Fig. 3.3). In order to reconstruct the events, it was of interest to understand if the bullet trajectory first hit the child and then the man, killing both.

The textile fragment was separated into its constituting fibres, which were then compared with the child's clothes. Figure 3.4 shows the micrographs taken at the comparison microscope.

The remarkable morphological similarity is evident. Spectroscopic data confirmed that the fibres were indistinguishable also for chemical composition and colour, corroborating the hypothesis that the bullet had first killed the child and then penetrated into the man's body, killing him as well.

Fig. 3.1 Hashish block, before (*left*) and after (*right*) opening its packaging

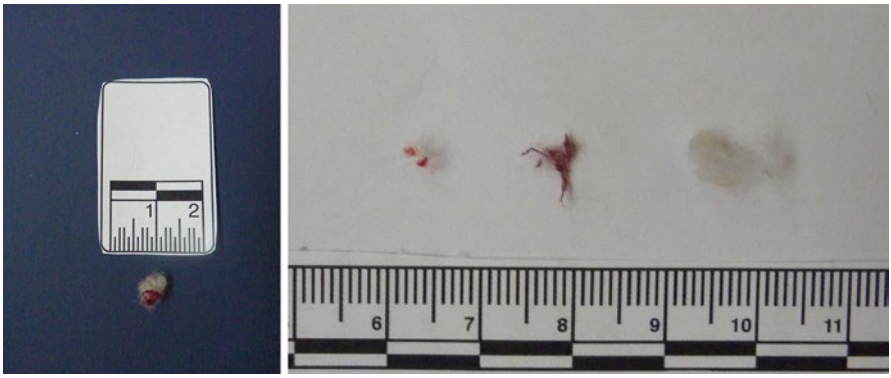


Fig. 3.2 Bundle of textile fibres found wrapped around the bullet, during the autopsy of one of the victims of a double homicide. The *left* image shows the bundle as extracted, the *right* image shows the same item, after separating the bundle of fibres

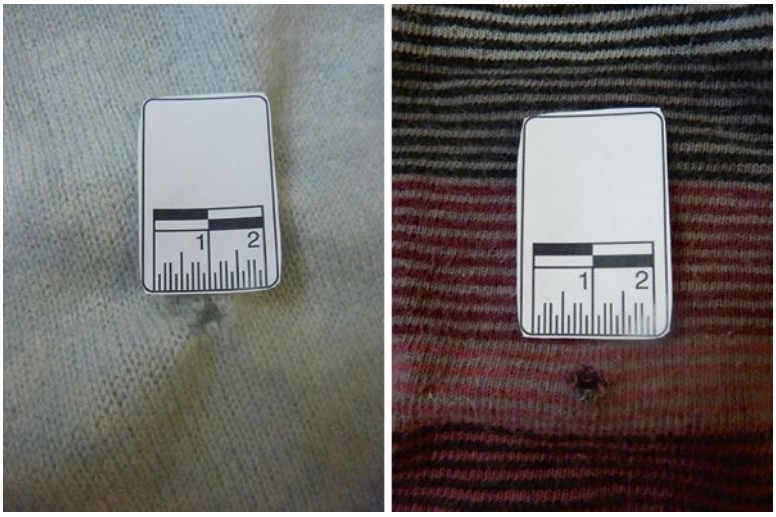


Fig. 3.3 The child's clothes were pierced in *red* and *blue* areas

A third example [1] regards the case of a 60-year-old woman, found stabbed to death in the store where she was substituting for her son-in-law, who that day was busy in bureaucratic matters. According to his statements, when the man returned to the shop, he saw the elderly lady lying on the ground in a pool of blood and panicked. He threw himself on the body trying to assist her. After having realised that there was nothing he could do, he called the police.

In the meantime he tried to clean himself of the blood that had soaked his clothes and his hands. He used a roll of paper towels wetted with detergent for windows: the

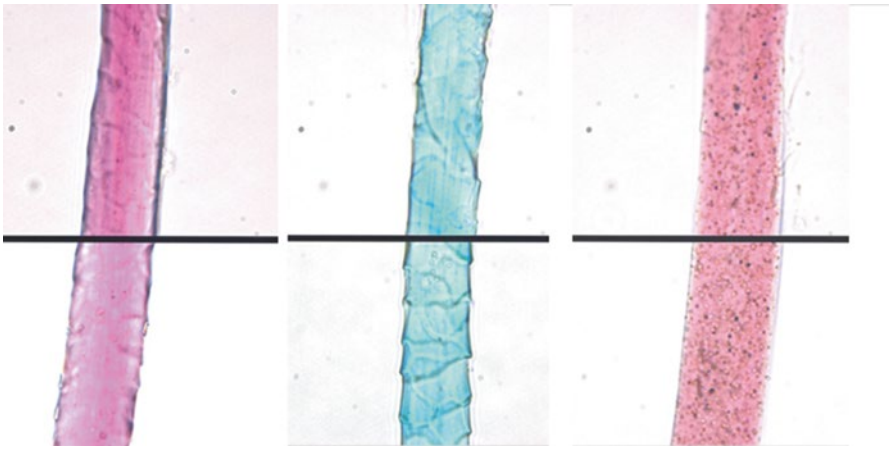


Fig. 3.4 Morphological comparison of the fibres found around the bullet (*top panels*) and the fibres composing the child's garments (*bottom panels*)

first things he saw as suited for the purpose. Seeing that the effort was useless for the garments, he rubbed his hands and threw the towels in the trash bin.

Police investigating the case inspected the crime scene, looking for evidence that could help in reconstructing the events. No fingerprints or biological trace pertaining to extraneous persons was found and the suspicions immediately fell on the victim's son-in-law. The weapon used to kill the woman was never recovered.

In order to assess the depositions made by the man, the paper towels he said to have used to clean his hands were seized and brought to the laboratory for examinations. One of the towels, in particular, was extensively damaged and it was of interest to determine if the lacerations observed were cuts or tears. An attempt to infer what instrument could have produced the damage was also done, by performing some simulations on the same paper as that found on the scene.

The exhibit consisted in the bloodstained and lacerated towel depicted in Fig. 3.5.

Simulations were performed in order to determine if the lacerations observed could be produced by a rubbing action of the hands or if use of some sharp object was necessary to explain their presence. A cutter, a scalpel and two scissors of different sizes were stained with blood and used to provoke lacerations on paper either dry or wet with the same window detergent that the suspect declared to have used. The implements were employed in order to simulate every possible modus operandi to remove the bloodstains. The same actions were performed with the hands, wearing bloodstained latex gloves. In this case holes and tears were made by the fingers and a rubbing action was simulated. The outcome of such experiments was that the lacerations observed on the towel of Fig. 3.5 were produced by the rubbing and cleaning of a pointed and sharp tool, like scissors for example. Many damages observed on the exhibit, in fact, belonged to the category of cuts, and some of them could be considered characteristic marks of scissors, such as for example the crown-shaped and the triangular holes illustrated in Figs. 3.6 and 3.7.

Fig. 3.5 Damaged paper towel collected on the crime scene. *Numbers* denote the 12 lacerations detected on the exhibit. The metric *bar* measures 20 cm. Reprinted from Ref. [1], copyright 2005, with permission from Elsevier

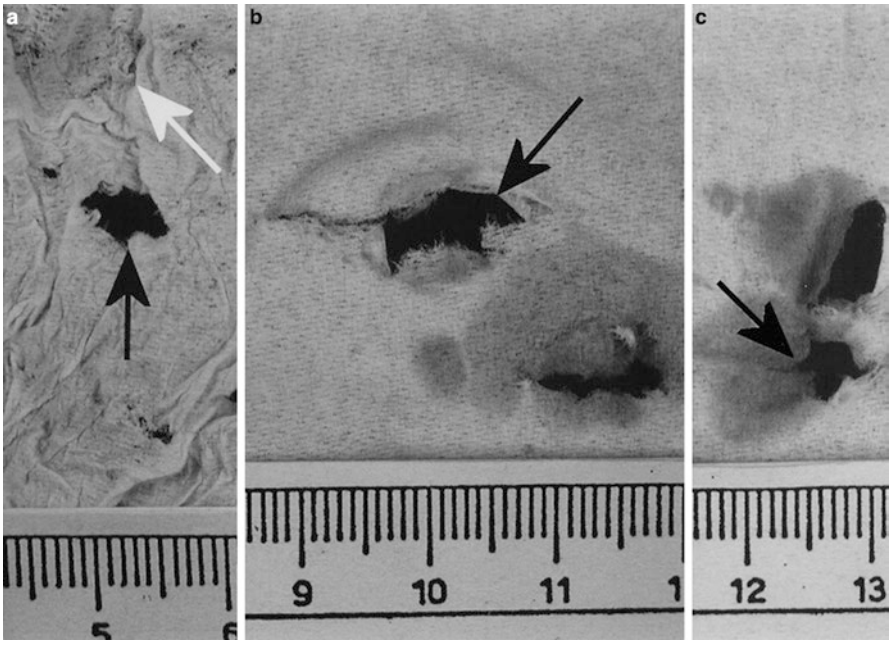


Fig. 3.6 (**a**) Laceration nr. 5 in the towel found on the crime scene. The crown-shaped damage is indicated by the *black arrow*. The *white arrow* points to a damage due to rubbing; (**b**) crown-shaped damage produced by punching wet paper with the open blades of the scissors; (**c**) crown-shaped damage obtained by stab action on wet paper with the open blades of scissors, followed by rubbing. Reprinted from Ref. [1], copyright 2005, with permission from Elsevier

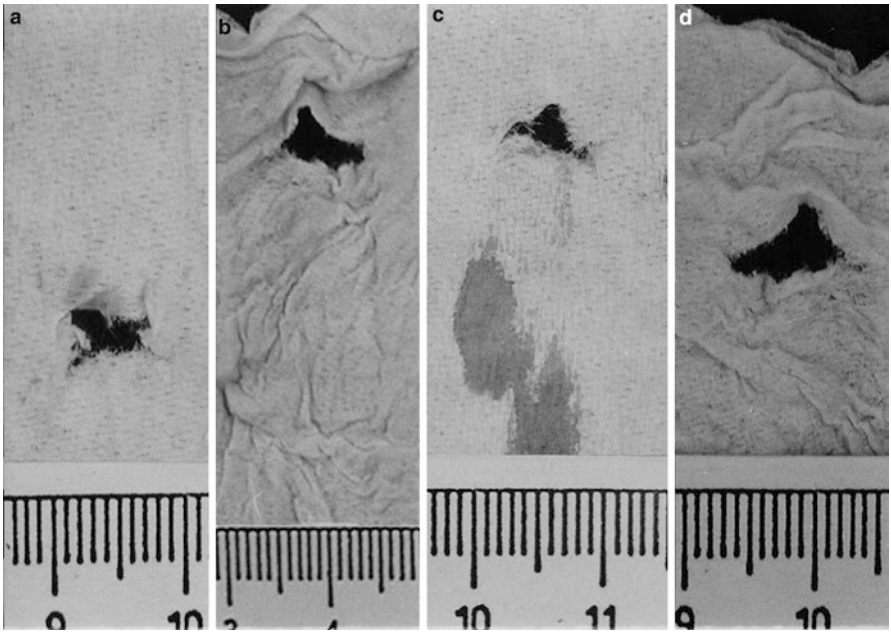


Fig. 3.7 (**a**) Laceration produced by a stabbing action with scissors with closed blades on wet paper; (**b**) laceration nr. 7 in the towel found on the crime scene; (**c**) damage obtained by stabbing of dry paper with the closed blades of scissors; (**d**) laceration nr. 8 in the towel found on the crime scene. Reprinted from Ref. [1], copyright 2005, with permission from Elsevier

All these examples share, as a common denominator, the presence of polymeric items as pieces of evidence carrying some information on the modus operandi of a criminal or on the dynamics of a crime. Broadly speaking, they are traces: they represent the remnants of a criminal activity.

The usefulness of a polymeric trace (or of any trace, for that matter) strictly depends on the circumstances of the specific case, and it is thus very difficult to generalise or identify traces 'worth considering' as opposed to 'worthless' ones.

If no other drug packaging like the one shown in Fig. 3.1 was found, an accurate information about the chemical nature of all the polymeric layers would be surely interesting under a scientific point of view, but it would carry no value for the investigators. The same would be even more true, of course, if the packaging of Fig. 3.1 did not contain illegal drugs at all. On the contrary, if different persons in different locations were found with packages corresponding for chemical nature and design to the one of Fig. 3.1, even if they contained different illegal substances, this would be an important connection between apparently unrelated cases, useful to investigators to reconstruct the organisation of a criminal cartel which commercialises drugs.

The analyses on the fibres of Fig. 3.2 carry relevant information just if, for some reason, it is of interest to reconstruct how many shots were fired against the man and his child. Conflicting testimony could be clarified by these forensic results.

Otherwise, the presence of fibres from the child's garments unfortunately does not add much to our previous knowledge, i.e. that the child was killed by a gun shot.

If the towel of Fig. 3.5 was not recognised by the suspect as the one he had used to clean his hands, it would have lost its evidential value, because not anymore useful to corroborate one of the reconstructions of the facts.

More on the evaluation of the evidential value will be said in a later section of this chapter.

A particularly relevant kind of traces are contact traces. The basic concept which underlies the concept of contact trace is the so called Locard's principle. This principle has been popularised with the sentence 'every contact leaves a trace' even though Locard himself never formulated such an expression. He rather expressed the principle by saying that:

> it is impossible for a criminal to act, and especially to act with the intensity that a crime requires, without leaving traces of his presence [2].

He later elaborated the concept by stating that such traces can be evidence left by the felon on the crime scene, but also, for a reverse action, that the felon can collect on himself or on his clothes evidence of where he has been or what he has done [3]. A famous quotation by Kirk further clarifies the concept:

> wherever he steps, whatever he touches, whatever he leaves, even unconsciously, will serve as a silent witness against him. Not only his fingerprints or his footprints, but his hair, the fibers from his clothes, the glass he breaks, the tool mark he leaves, the paint he scratches, the blood or semen he deposits or collects. All of these and more, bear mute witness against him. This is evidence that does not forget. It is not confused by the excitement of the moment. It is not absent because human witnesses are. It is factual evidence. Physical evidence cannot be wrong, it cannot perjure itself, it cannot be wholly absent. Only human failure to find it, study and understand it, can diminish its value [4].

Locard's principle is a fundamental cornerstone of forensic science. If contact is always accompanied by the transfer of some material, then the analysis and characterisation of such material can allow the forensic scientist to prove or verify the contact that originated it. Of course the success of such logical path depends on a number of non-negligible factors. Transfer, persistence and recovery are the three main processes that, if successful, allow the trace, with the information it contains, to reach the laboratory and eventually the Courtroom. These concepts have been elaborated particularly well for glass and fibres [5–8], but they hold true also for several other types of evidence, among which are foam particles [9], hairs [10], soil [11] or particulate matter [12]. The details strictly regarding fibres will be presented in Sect. 4.2.3.

Transfer consists in the loss of trace material from a *donor* object, to an individual, a surface, an object, which is called *recipient*. The entity of transfer depends on the intensity of the contact, on the duration of the contact, and on the physical characteristics of the trace itself, of the donor and of the recipient. An old and rugged wool sweater will shed many more fibres than a smooth silk cloth. The curly and long wool fibres, exposed by the worn out textile will be less firmly attached to the donor garment, and will be more liberally transferred in response to the slightest

contact. A car will likely leave many more paint traces if it hits a wall at high speed, rather than at a slow speed. If a person sits for a long time on a torn armchair, he will likely collect on his clothes many more foam flakes than someone who just sat for a few seconds. If a piece of textile rubs a smooth steel surface, the friction will be much lower than if the same cloth rubs a rough brick wall, and therefore less fibres will be transferred in the former case than in the latter.

Traces can be transferred multiple times. *Primary transfer* happens when the trace passes directly from the donor to the recipient. However, the same trace can end up from the initial donor to the final recipient through several intermediate passages. For example, while a person drives his car, some fibres are shed from his garments to the seat. If a thief steals that car, it is probable that he will collect on himself some of the fibres that were on the seat. Although no direct contact between the owner of the vehicle and the thief has taken place, the latter will bear traces of the former. This is an instance of *secondary transfer*, that can be very helpful in reconstructing complicated cases. Several studies have found traces of illegal drugs, especially cocaine, in banknotes [13]. If a drug user contaminates his money with cocaine, and then spends it in a shop, the contaminated note will be stored in the cash register of the shop. The same contaminated note will be then transferred to the next customer as change, at the same time transferring traces of drug to a person totally unrelated to the original drug user.

Even if a trace is transferred from a donor during a contact associated to a crime, it would be useless if it didn't remain on the recipient for a time long enough to be recovered.

Persistence represents the tenacity with which traces adhere to the recipient. Any study regarding contact traces should be preceded by an assessment of the persistence of such trace. Knowledge of the persistence of a particular item is important for the assessment of the feasibility of the analysis. Persistence influences the probability of finding traces on the recipient after a significant time has passed, and the possibility of multiple transfers (secondary, tertiary, etc.) to occur. This property depends mainly on the nature of the trace and on the physical properties of the recipient. Assessing persistence typically consists in quantifying the number of traces which remain on the recipient as a function of time. The loss of particles normally follows an exponential process. Figure 3.8 shows an example of such trends for the persistence of hairs on different kinds of fabrics.

Even though most of the traces are lost in the first few hours after transfer, this does not mean that they will totally disappear after so short a time. Actually persistence studies gave particularly surprising results. In a recent investigation [14], wool and cotton fibres were transferred onto pig skin which was subsequently buried for 14 days. Some of the fibres persisted on the skin even after the latter had been brushed to remove any soil adhering to it. None of the replicate experiments resulted in a total loss of fibres. This shows that the persistence of apparently labile traces can be surprisingly tough.

The third necessary step for traces to be useful in criminal investigations is to recover them. If they are transferred during the crime, and they persisted on the recipient item until the arrival of crime scene investigation units, it is necessary to

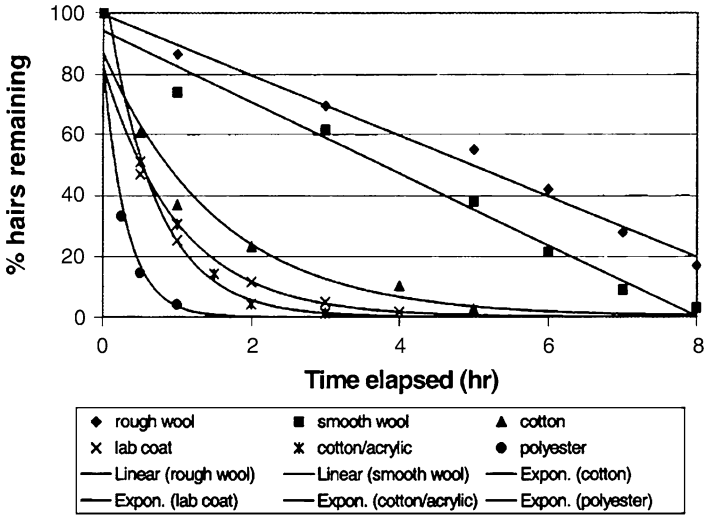


Fig. 3.8 The different rates at which hairs were lost from six different garments. Each non-wool point is the average of 30 replicates, each wool point is the average of 20 replicates. Reprinted from Ref. [10], copyright 2003, with permission from Elsevier

retrieve them in the most efficient way. *Recovery* is a part of the wider activity of crime scene examination, an activity separated from the analyses in the laboratory, usually accomplished by different persons with different expertise. On this regard, polymeric traces do not deserve a special treatment. The techniques of retrieval of polymeric evidence are not different from those used in the case of other types of particulate matter, e.g. glass or soil. If on one hand recovery of evidence does not require specific technical skills, on the other hand, training of crime scene investigators is crucial. They should know and follow protocols for reducing the risk of contamination and destruction of evidence. Perhaps more importantly, the personnel intervening on crime scenes must be aware of all the possible analyses and tests which can be executed on the items present on the crime scene. This knowledge will guide the crime scene investigators in deciding what should be recovered and taken to the laboratory, what should be tested on site and what is irrelevant. If they did not know, for example, what information can be acquired by the examination of fibres, they would leave fibres unnoticed and such information would be lost. The support of a forensic practitioner should always be sought in case of doubt, and if this were not available, it is always suggested to acquire any item that could possibly yield information, leaving to the forensic laboratory analyst the choice of what can or cannot be analysed. Inspecting many times the crime scene should be avoided: the GIFT (get it first time) principle should be always obeyed, since the risk of alteration or contamination increases for each successive inspection. The collection of the traces which are the topic of this book must not interfere with the search and retrieval of other types of evidence, such as fingerprints or biological traces. Contamination of the crime scene by the investigator should be avoided by wearing suitable Tyvec® overalls.

The first step in any examination is an accurate visual inspection, in which any trace that could be seen by the naked eye or under low magnification can be directly lifted with tweezers. In that case the area should be photographed before the removal of evidence and the item put into a suitable sealable container. Paper or plastic envelopes, plastic test tubes closable with screw caps, plastic or cardboard boxes are all suitable for evidence collection. Polymeric evidence is very resilient to degradation or alteration in normal ambient conditions, but if the item is wet it should be put in a breathable pouch, to prevent the growth of bacteria or moulds which can complicate the analyses. Other obvious requirements for the packaging material are that it is of suitable size to contain the item without any deformation and that it can be sealed and marked for guaranteeing the integrity of the chain of custody. Envelopes exist on the market with special tamperproof seals and their use is encouraged for an improved case management.

When cross contamination can be an issue, such as when evidence is collected on the crime scene and at a suspect's premises, different operators should be employed. Cross contamination consists in secondary transfer which happens when the crime scene investigator inadvertently transports and transfers the suspect's traces to the crime scene and vice-versa. The mere hint of cross contamination can jeopardise and make void the best of the analytical work. Cross contamination in fact cancels the direct and clear connection between the analysed item and the location where it was found, which is the real foundation of the exploitation of contact traces.

After all the visible material has been retrieved, there are two other approaches for the collection of particulate traces. The shaking method is suitable for recovering coarse material such as glass fragments, but also foam fragments [15], especially on garments. It consists in putting a large piece of parcel or filter paper onto the work surface and then shaking vigorously the recipient object over such paper for several seconds. The debris leaves the recipient object, and falls on the paper, from where it is transferred in a smaller container such as a Petri dish or a plastic sample holder. This procedure must be performed carefully avoiding any risk of contamination. For smaller items, the shaking can be also performed inside a paper bag. The particulate remains on the bottom of the bag and can be easily retrieved. Some traces will not be removed by shaking the item, therefore brushing or vacuuming may be necessary. Special vacuum cleaners with extractable filters where the evidence is collected are available in the market.

Before and after the examination of each recipient, the work surface must be cleaned and wiped, the paper must be always changed and the operator should change his gloves. A Tyvec® overcoat should be used in the procedure, in order to avoid contamination from the operator and to quickly detect if some fragments were shed on the operator instead of the paper. For this reason, it is suggested to perform the shaking method in the laboratory, on individually packaged material acquired by the crime scene investigation team.

Probably the most commonly used method for recovering transferred traces from the recipient object is by tape lifting. This technique can be employed both directly on the crime scene or in the laboratory. Pieces of transparent adhesive tape are pressed on the surface of the object being examined, as shown in Fig. 3.9, and

Fig. 3.9 The tape lifting procedure

afterwards are posed on an acetate sheet for overhead projectors. The stickier the tape, the more material is picked up, even that tightly bound to the item.

The location of each tape lifting must be accurately recorded, and possibly pictures or video should be taken to document the activity. Transfer during contact is a two-way phenomenon. This should be remembered while collecting evidence, so traces from the crime scene or the victim should be searched for on the suspect as well. More details on the tape lifting procedure can be found in Sect. 4.1.4.

Recovery techniques especially suited for particular items will be described when discussing the different classes of polymeric evidence (Chap. 4).

3.2 The Development of an Analytical Method

Some branches of forensic chemistry have been quite comprehensively systematised, primarily because they rely on a few instrumental methods applied on samples very similar to one another. The large amount of casework associated with drugs, explosives or fires, allowed to spur an intense research and regulatory activity aimed at standardising the procedures. Moreover, a rich literature exists, focused at the several interpretational problems associated to this kind of evidence.

The case of trace evidence is much more complicated. When dealing with this kind of items, the criminalist often has to design a novel protocol to characterise them. In many cases the analyst has never worked on a material analogous to that under scrutiny, so he has to adapt techniques ideated for applications in which the sample size is much greater than usual in casework. In other cases the operator should resort to his experience to adequately gather the information that is requested.

Academic research and published protocols are rather scarce, primarily due to the vastness of possible samples and thus to the difficulty of obtaining general methods useful for all the items possibly encountered in actual investigations.

In the case of the types of trace evidence most commonly encountered in forensic casework, standard procedures are available. ASTM published, for fibres, four standard guides: E2228 (Standard Guide for Microscopic Examination of Textile Fibers), E2225 (Standard Guide for Forensic Examination of Fabrics and Cordage), E2224 (Standard Guide for Forensic Analysis of Fibers by Infrared Spectroscopy)

and E2227 (Standard Guide for Forensic Examination of Non-Reactive Dyes in Textile Fibers by Thin-Layer Chromatography). For paints, two standard guides are available: ASTM E1610 (Standard Guide for Forensic Paint Analysis and Comparison) and ASTM E2808 (Standard Guide for Microspectrophotometry and Color Measurement in Forensic Paint Analysis).

Guidelines and procedures have been devised by collaborative working groups, such as the Scientific Working Group for Materials Analysis (SWGMAT) in North America or the groups established within the European Network of Forensic Science Institutes (ENFSI) in Europe. In particular, guidelines, standard guides and best practice manuals produced by the SWGMAT are freely available on the association's website (http://www.swgmat.org) for paints, glasses, fibres, adhesive tapes, and hair.

If on one hand there is a considerable amount of technical literature on the most common polymeric traces found in casework, the necessity often arises to develop a method for peculiar items, related to a specific case, for which little forensic literature exist.

The development of an analytical method for forensic purposes is not very different from performing the same activity for other purposes. The steps in an analysis can be summarised according to the flow chart of Fig. 3.10.

The definition of the problem is the first step in the development or choice of an analytical method but its importance is often underevaluated. The aim of a forensic analysis is not, as popular culture would suggest, to 'catch the killer' or to 'solve the case'. The role of the forensic scientist is to analyse the items related to the commission of a crime, the traces, to obtain the information that they contain about the dynamics of such crime and about who committed it. The purpose of the analysis is limited by several factors, some of which are technical. Polymers are not fingerprints or DNA, they do not identify persons. Polymeric traces can be critical for clarifying the dynamics of a crime, to verify the reconstruction of the events given

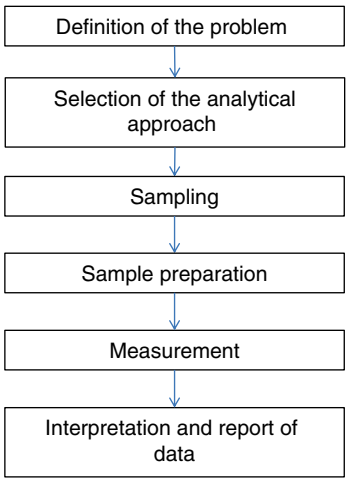


Fig. 3.10 The steps of the analytical process

by the persons involved, and to give investigators information on which they can base their further activity. The circumstances of the case, the conditions of the crime scene, the advancement of investigation, the identification of suspects are all fundamental aspects needed to circumscribe which analyses can or cannot be performed.

Another important limitation to be considered is that the law system under which the forensic scientist works sets the perimeter of his degree of freedom. In other words, the activity of the analyst is always subject to the coordination and control of some higher authority such as the Court, the Police, a Judge or an attorney. These are the players who are in charge of asking the questions, which in turn define the purpose of the analyses. A preliminary assessment of the case between the forensic scientist and the person in charge of the investigation is vital for a fruitful analysis. The answer to a question posed in the wrong way could be detrimental for an entire investigation.

The purpose of forensic science can be interpreted in two ways: justice driven forensic science and intelligence driven forensic science. The justice driven approach consists in the use of forensic case data to assist the evaluation of evidence in the perspective of the court trial.

It involves all the analyses which are carried out to verify a particular reconstruction of the criminal event. In other words, the forensic examinations in the justice driven approach are used to corroborate the thesis either of the prosecutor or of the defendant.

Intelligence driven forensic science consists in the development of models by which forensic case data can be used to contribute to decision making in the context of traditional investigation, with the aim of increasing the efficiency of the process of identification of the felon and allowing to better interfere with the criminal action. Forensic intelligence is much more challenging than justice driven forensic science, because it involves correlating heterogeneous data coming from different sources, expressed in different ways, and with various meaningfulness. Forensic intelligence is therefore the new frontier in the exploitation of forensic expertise in the context of a global investigative action, and shows its most promising potential in serial and organised crime. The work of Margot and Ribaux is very informative and is focused on the analysis of the inference processes at the basis of investigation, and on how to include forensic information in such a process [16–23]. The key issue in this field is the ability to identify details of the modus operandi of the felon which allow to connect together a series of illegal activities and to trace back all of them to a single individual or organisation.

Irrespective of the purpose or driving force which motivates the analyses, they can be divided into two categories: aimed at comparison or identification.

In the first case, traces found on or related to a crime scene must be compared with the object from which they are supposed to come, in order to assess their compatibility. For example, if during a robbery the felons used a car in which textile fibres were found, and the suspects wore garments of the same colour of those fibres when they were arrested, one recurs to this type of analysis to determine how likely it is that the traces in the car were left by the persons apprehended. It is paramount

that the followed protocol be as thorough as possible, in order to reduce to a minimum the risk for false positives (that would expose an innocent to an unjust charge) and for false negatives (which would lift a guilty felon from the charges) and, catching the subtle compositional and structural differences between apparently similar products, to enhance the evidential weight of these exhibits.

The second type of investigation, whose purpose is identification, is applied in cases in which one wants to trace back something found on the crime scene to the object where it came from. An example could be the finding of fibres with a peculiar cross-sectional shape on the soles of the shoes of a homicide victim. It is known that nylon multilobal fibres are used for the manufacturing of carpets, so if items of this type are recovered, they could contribute to a general description of a piece of furniture of the place where the killing possibly happened, offering a lead to investigators. A scrupulous and targeted characterisation is fundamental in these cases, that for their successful resolution require a tight collaboration between the analyst and the industries producing the objects that each time are of interest.

The next step in the flowchart of Fig. 3.10 regards the choice of the analytical method most suitable for answering the questions identified in the previous stage.

This will be the topic of most of the rest of this book, and it is indeed its main purpose. A knowledge of the available techniques, along with information on their cost, on their advantages and of their limitations, will help identify the best analytical strategy for the case.

Such activity should follow a precise hierarchy:

$$\text{Technique} \rightarrow \text{method} \rightarrow \text{procedure} \rightarrow \text{protocol}$$

The technique is the general scientific principle which can provide the required analytical information. Examples of techniques are infrared spectroscopy, or fluorimetry.

The method is the adjustment of the technique for the purpose of measuring a particular analyte. An example of the method can be the colourimetric detection of nickel by complexation with dimethylglyoxime.

A procedure is a list of operations which guides whoever wants to apply a method. ASTM standard test methods are examples of procedures: they describe all the steps that the operator must take to perform an analysis properly. They are the result of a collaborative work among scientists and technicians which, by a continuous reviewing process, optimise and standardise a method.

A protocol is a set of instructions more stringent than a procedure. It contains prescriptions that must be followed scrupulously and without modification, to make the result acceptable for particular purposes. Protocols are similar, to this extent, to law prescriptions: they are the standard methodologies which are acknowledged for giving a definitive answer to controversies. Protocols are common in the environmental chemistry field, e.g. EPA reference methods, or in the analysis of goods for the definition of the custom code according to the combined nomenclature.

Since the context in which the forensic scientist works is that of the administration of justice, a fundamental prerequisite of any analytical method must be its acceptability in Court. Under this aspect, the Daubert opinion offers some useful guidelines.

In its 1993 Daubert v. Merrell Dow opinion, the United States Supreme Court articulated a new set of criteria for the admissibility of scientific expert testimony, displacing the vague Frye standard that admitted those techniques that were 'generally accepted'.

In the Daubert opinion, the Supreme Court stated that:

> Ordinarily, a key question to be answered in determining whether a theory or technique is scientific knowledge that will assist the trier of fact will be whether it can be (and has been) tested. Scientific methodology today is based on generating hypotheses and testing them to see if they can be falsified; indeed, this methodology is what distinguishes science from other fields of human inquiry. [Daubert vs. Merrell Dow Pharmaceuticals (1993) 509 U.S.]

The Daubert criteria for evaluating the admissibility of expert testimony are: (1) whether the method used is based upon a testable hypothesis; (2) the known or potential rate of error associated with the method; (3) the existence and maintenance of standards controlling the technique's operation; (4) whether the method has been subject to peer review; and (5) whether the method is generally accepted in the relevant scientific community.

It can be appropriate to point out that virtually no expert testimony will satisfy the last two factors unless it satisfies the first three.

The SWGMAT website (http://www.swgmat.org) contains, for each of the trace evidence classes for which guidelines are proposed, also a thorough evaluation of how the Daubert requirements are satisfied. These are very useful guides for any forensic scientist wishing to tackle the issue of admissibility of his expert testimony.

Further precious information when preparing expert testimony in Court on the acceptability of the forensic data, can be found in a book regarding questioned document examinations [24], which nevertheless contains ideas and concepts useful for any forensic scientist.

According to the hierarchy indicated above, a protocol or a standard procedure by definition have already met all the Daubert requirements. The most established techniques described in this text and in analytical chemistry textbooks are perfectly acceptable under the Daubert criteria. The most critical point in the hierarchy above is the method. When no standard procedures or protocols are available, the circumstance of the use of an established technique according to a non validated method can be encountered. The utmost caution must be exercised in this case, in order to preserve the rigour and the significance of the analytical data which is a non negotiable asset in forensic science.

An example, the microspectrophotometric characterisation of paints, will help in understanding how the reliability of a method can be assessed.

Forensic trace examination starts by developing a hypothesis (Sect. 3.4.2), for example that the questioned source item and the known source item did not originate from the same source. This hypothesis is then scientifically tested, subjecting the samples to a series of tests that provide a high degree of discrimination in order to prove that they are different. For example, if the UV/visible spectra of the two items being compared are different, it can be positively concluded that the original hypothesis was correct, i.e. the two samples did not originate from the same source.

If no differences are observed between the spectra of the analysed items, this does not necessarily prove that the two samples originated from the same source, but only that this possibility cannot be eliminated. If this approach is followed, the first Daubert criterion is fulfilled.

The determination of the known or potential rate of error associated with the method is more complex. For example, in the case of paints, this requires an evaluation of the variability of commercial paint formulations, and of the ability of the chosen analytical methods to distinguish these different formulations. Studies aimed at the quantification of the discriminating power of a technique, or of a sequence of techniques, are fundamental under this point of view. Discriminating power (DP) can be defined, according to Smalldon and Moffat, as the ratio between the number of differentiated pairs over the number of possible pairs in a sample population [25]:

$$\mathrm{DP} = \left(\text{number of discriminated sample pairs}\right) / \left(\text{number of possible sample pairs}\right)$$

The number of possible sample pairs in a population of n samples is $[n \cdot (n-1)]/2$.

Discriminating power studies consist in characterising, by the same analytical method, a large number of samples pertaining to the same population, and determining how many of such samples can be successfully discriminated. Gothard, for example, reported one of the first studies of this kind for automotive paints [26]. He acquired a population of 500 different paint samples, thus in Gothard's population 124,750 sample pairs were possible. Pairwise comparison of these paints by a full analytical characterisation showed that just two of the possible pairs could not be discriminated, i.e. that 99.8 % was correctly discriminated. Analogous data were reported by Ryland and Edmondstone [27, 28], showing on one hand that a large variety of paints exist in the market, and on the other hand that the analytical strategies proposed by the authors, mainly based on a mix of IR spectroscopy, UV-visible microspectrophotometry, and physical features such as the number of layers and their thickness, were able to successfully describe this variety. Specific studies limited to the discrimination of paints based on colour showed that the discrimination potential is in the range 90–95 % [29, 30]. It should be highlighted here that the acceptability criterion does not require a *low* error rate, but a *known* error rate. It is necessary to be able to assess such error rate, because on the basis of its value, the significance of evidence may change and can be quantified. Of course the lower the error the more significant the evidence, as will be further discussed in Sect. 3.4. Discrimination potential studies are thus a necessary first step for the assessment of the viability of a technique for forensic applications. For their success, the sample population must be as representative as possible of the actual assortment of materials and items present in the market where the forensic laboratory operates. It must be remarked that the discrimination potential of the technique is just a component for the determination of the total error rate associated to a method. Other important contributors are related to the figures of merit of the techniques, which will be described more in detail in the following of this section. However, these features are much easier to assess, because they are usually already available in the literature. Moreover, quality control measures are currently enacted in most laboratories,

which minimise the potential of errors related to an uncorrect application of the method, such as using uncalibrated instruments, or performing a wrong sample preparation procedure. These good laboratory practices include the verification of the performance of the analyst and of the instruments by running suitable standard materials, the continuous training of personnel and auditing processes for checking that all the procedures are correctly enforced. These latter measures fulfill the third Daubert requirement, which calls for measures which guarantee flawless operation of the technique. Availability of standard protocols and of standard reference materials allows to minimise procedural and instrumental analytical errors and to maximise discrimination potential. In the case of the measurement of colour of paints, for example, peer-reviewed published guidelines are available from SWGMAT, which specify acceptable procedures. Suitable commercial standard materials moreover exist, for checking the overall operation of the microspectrophotometer.

The last two Daubert parameters regard the communication and diffusion of the chosen analytical approach within the scientific community. The existence of peer-reviewed literature on the method is easily and objectively verified, whereas the level of acceptance within the relevant scientific community can be sometimes debatable. Generally, it is necessary to prove that the method is well established and applied since a long time. If the method is described in monographies or review papers dedicated to forensic science, this is further evidence of its widespread use and acceptance. Inclusion in standard protocols or guidelines by national or international forensic associations corroborates this point. In the case of microspectrophotometric determination of colour of paint chips, there is no doubt that it is a method accepted by the forensic scientific community. All the above requirements are in fact fulfilled, since it is included as a method in all forensic science books, it is included in all guidelines for forensic paint characterisation, it has been used in thousands of cases worldwide, and it is widespread not only in the forensic field, but also in the paint industry.

Other factors are key for a successful choice of the analytical strategy. Usually the major issues defining the optimal technique for carrying out the analysis are what accuracy is required, what is the acceptable error, what is the sample size available, what is the physical state of the sample, what is the concentration range expected for the analyte, if there are any interfering species in the sample and how many samples should be analysed. All these features are relevant in forensic science as well, of course.

Accuracy, precision, sensitivity, detection limit, concentration range and selectivity are also called figures of merit of an analytical technique, because they can be used to quantify its performance. Accuracy defines how close the result of the analysis is to the true value for the sample. It is usually verified by analysing standard reference material of known composition and chemical nature. In microspectrophotometry, substances with a known UV-visible absorption spectrum are used, for example. Unfortunately, the variability of trace evidence does not always allow to have suitable reference material available. In such instance, validation should be performed on a sample as similar as possible to the item of interest, whose composition is known as much in detail as possible. Collaboration with the industry is

usually very useful under this aspect. Manufacturers can in fact provide quite well-characterised materials which can serve as standards for calibration and quality assurance purposes.

Precision quantifies how much replicate determinations made with the same method on the same sample mutually agree. It assesses the entity of random errors, and it is usually determined by repeating measurements on a same standard sample and by calculating the standard deviation. Knowing the precision of a method is very important in forensic science, because most of the times the available sample size does not allow to replicate the measurements on the item of interest. The precision is therefore in these cases the error to be associated to the experimental data. The sensitivity is the ability of a method to distinguish between small differences in the analyte concentration. The detection limit is the minimum amount of analyte which can be detected at a given confidence level. The limit of quantitation is the lowest concentration of an analyte which can be quantified with acceptable precision and accuracy. Another defining feature of an analytical method is the range of concentrations which can be quantified with acceptable precision and accuracy. It is comprised between the limit of quantitation and the limit of linearity, i.e. the concentration at which the calibration curve departs from linearity.

These latter figures of merit are fundamental especially because they determine the minimum sample size analyzable with the correspondent method. As can be imagined, the quantity of sample available in casework is extremely limited, so much so that many very common and informative techniques in analytical science, such as for example nuclear magnetic resonance, cannot be used. The working range of the method defines also the maximum sample size, because it identifies the maximum concentration which can be quantified. This is very seldom a problem in forensic science, when big sample sizes are the exception rather than the norm.

Selectivity is an important feature of an analytical method, since it describes how sensitive the result is from matrix effects, i.e. from the presence of other species, in addition to the analyte of interest. This is quite important in forensic science, because in most of the cases the composition of the item is unknown, but it is almost always complex. Polymeric objects contain many additives, sometimes in remarkable amounts. A selective method will provide significant results also for composite materials, on the contrary a strong matrix effect could lessen the reliability of the analytical determinations. However, in forensic casework the quantification of a particular analyte is always carried out after a screening step, where the items have been grouped according to their polymeric matrix. For example, the determination of the elemental composition of a paint sample is surely dependent on the type of polymeric binder used in its formulation. On the other hand, though, in every protocol the acquisition of the elemental profile of paint samples is preceded by an IR spectroscopy assessment of the binder: only samples which have the same kind of binder will be compared on the basis of their elemental composition, therefore reducing the detrimental consequences of the matrix effect.

Some procedures, for example those developed for fibres or paints, are established and recognised worldwide. They are applied often and statistical quality assurance can be implemented to check the proficiency of the forensic laboratory

and operators. Control charts and interlaboratory collaborative studies may help in quantitatively assessing the reliability of the analyses carried out, in addition to the robustness (the sensitivity of the method to changes in the test conditions) and the reproducibility (the variability of results obtained when the same test is conducted in different laboratories) of the procedures.

Other important aspects should be kept in mind when devising a method aimed at forensic applications.

The method should be preferentially non-destructive and non-alterative of the sample. In other words, it is desirable to apply techniques in which the sample can be recovered unmodified after the analyses. This allows on one hand to perform multiple analyses on the same item, obtaining from it a larger amount of information. On the other hand, if the evidence is preserved unaltered, further analyses can be made in the successive stages of the judicial process, allowing to clarify aspects of the dynamics of the crime which were not apparent at the time of the first examination, or to repeat the analyses in cases of controversies.

Simplicity should be sought after. Even though there is currently a strong drive towards the employment of highly professional operators in forensic laboratories, in some countries and in some legal systems the execution of forensic analyses is demanded to the police. The personnel in these laboratories do not always have a degree in chemistry or the theoretical preparation to master the more complicated techniques available to the chemist nowadays. The procedures developed should be carried out by a well-trained technician, supervised by someone with a science degree and a more thorough preparation in the characterisation of materials. Another factor which influences the choice of the analytical strategy is cost. Under this point of view, the less expensive instruments are needed, the better.

Another aspect that should be taken into account is that the audience of these analyses are lawyers and judges, who often don't have the technical bases to completely understand the results. In order to adequately employ this discipline at the full extent of its social usefulness, it is thus necessary to consider the need for a clear communication of the outcome of the characterisations to the final users. On this regard, a beautiful example was given by Flynn and coworkers [31]. These authors proposed IR mapping as a powerful method for the characterisation of paint chips. By this approach they were able to obtain IR spectra of each layer of the paint assembly, not much differently from what is normally done by traditional IR microspectrophotometry. However, these authors proposed a very efficient method to convey the spectroscopic information. They represented the section of the paint chip by thousands of IR images, each one representing with colours the intensity of the absorption of a particular wavelength. Then, the images were assembled in a 'movie', which showed all of them in a full sequence across the spectral range. The 'movies' relative to a paint chip from a hit-and-run scene and one from a suspect vehicle could be viewed side-by-side. At this point anybody, even without any notion on spectroscopy, can simply compare the colours appearing during the video: if the two paint chips are from the same source, each frame of the movie shows the corresponding layers of the two chips behaving identically [31].

After the choice of the method, Fig. 3.10 shows that the next step is the obtainment of a representative sample. Sampling is a luxury seldom granted to a trace

analyst. The sampling stage is often an easy one to deal with, because it consists in taking all the traces found on the crime scene and analysing it.[1] Forensic science rarely deals with samples, but with specimens. Specimens are traces whose representativity of the whole source is unknown and can only be supposed, just as the homogeneity of the source has to be supposed. Tracing back a specimen to the alleged source is most of the time the purpose of the analysis, and it will be therefore more thoroughly discussed in the next section, which deals with the interpretation of evidence.

Sample preparation is a very controversial issue in the development of a forensic analytical method. Any manipulation of the item is not desirable because it could increase the risk of contamination of the sample, and of legal controversies on whether the obtained data faithfully represent the item, or whether this was altered or modified by the operator in the preparation step. Moreover, since sample preparation modifies the sample, further analyses with other methods are not possible. Therefore, in order to exclude the possibility of altering important sources of evidence, emphasis should be always posed in minimising sample preparation.

3.3 The Statistical Treatment of Analytical Data

The previous section was concerned with the issues underlying the choice and the development of an analytical method. This is fundamental in order to obtain reliable experimental data. Once such data are acquired, though, they must be properly treated, both for drafting meaningful conclusions and to form a solid foundation for the assessment of the evidential value. Statistics is a very important asset for every forensic scientist, and many excellent resources exist on the subject [32–34], to which the reader is directed for a treatment more comprehensive and complete than that proposed in this section.

3.3.1 Comparisons

If two samples are characterised on the basis of a single property, for example their diameter, the values of this feature can be compared and a decision can be made. If a graphical representation is needed (and it is usually desirable for an effective presentation of the results in Court), the samples can be plotted on a number line.

In the case of univariate data, i.e. when the items are described by a single variable x, the comparison of two samples consists in assessing if both can be ascribed to the same population or if they come from different populations. This in most cases is equal to stating that they came from the same source. The best estimates of x for each sample will be the averages $\bar{x}_1$ and $\bar{x}_2$, based on n_1 and n_2 measurements,

[1] This is the reason why in this book the terms item, specimen and sample will be used as synonyms, even if they rigorously represent different concepts.

respectively (the subscripts 1 and 2 represent sample 1 and 2, respectively). The samples will be considered similar if $\bar{x}_1$ and $\bar{x}_2$ are statistically indistinguishable.

The size of forensic items rarely allows to perform many replicate measurements, so the best option when comparing univariate data in this contest is to apply a *t* test, which was devised specifically for small data sets. The first step consists in calculating the following statistic:

$$t = \frac{\left|\bar{x}_1 - \bar{x}_2\right|}{s_p \sqrt{1/n_1 + 1/n_2}} \tag{3.1}$$

where s_p is the pooled estimator of the standard deviation [33, 34]:

$$s_p^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2} \tag{3.2}$$

where s_1 and s_2 are the standard deviations of samples 1 and 2, respectively.

This statistic can then be used to test the hypothesis that the means of the two sample populations are equal. A threshold value $t_{\alpha/2,n_1+n_2-2}$ can be found in *t* distribution charts, which depend on the level of significance (or level of confidence) α and on the degrees of freedom (n_1+n_2-2). α is the probability of type I errors, i.e. of rejecting the hypothesis even if it is true, and it is often expressed in tables as a percentage: $100(1-\alpha)\,\%$. When *t*, calculated from experimental data, is larger than $t_{\alpha/2,n_1+n_2-2}$, there is sufficient evidence to reject the proposition that the two samples came from the same source. In other words, when this happens, the conclusion must be drawn that the samples are statistically different.

Example

A forensic analyst must compare the diameter of a fibre retrieved on the crime scene (Q) with the fibres coming from a garment seized to a suspect (K). Four measurements are made on the Q fibre, ten measurements are made on the K sample, obtaining the following results:

$\bar{x}_1 = 21.3\,\mu m$, $s_1 = 0.8\ \mu m$, $n_1 = 4$

$\bar{x}_2 = 22.1\,\mu m$, $s_2 = 0.3\ \mu m$, $n_2 = 10$

By (3.2), $s_p = 0.48\ \mu m$, and by (3.1), the *t* value is 2.83.

The degrees of freedom of this problem are $n_1+n_2-2=12$ and a commonly used level of confidence is 95 %, i.e. $\alpha = 0.05$. For these parameters $t_{\alpha/2,n_1+n_2-2} = 2.179$. This value can be found in *t*-distribution tables in any statistics handbook, or calculated by widely available software such as Microsoft Excel.

Since $t > t_{\alpha/2,n_1+n_2-2}$, the hypothesis that the fibres come from the same population can be rejected. In fact, the result of the *t* test indicates that, at the 0.05 level of significance, we have strong evidence that the two samples have significantly different diameters.

Nature is complex, though, and not always a single property is enough to grasp all the various aspects that make samples different. An item is therefore usually characterised according to a multitude of analytical data. For example, it was proposed that the amounts of eleven elements are good descriptors for office paper sheets [35]. Textile fibres are described by the peaks in their IR spectra, by their UV-visible spectrum, by the spots in a TLC chromatogram, by their morphological features, etc. The problem is not univariate anymore, but it becomes multivariate. In this case, each set of measurements on a sample is described as a vector, $\mathbf{x}$. Analogously to the univariate case, when more than one measurement are available for the same sample, the mean of such n measurements can be calculated. It will be in this case a vector, which is denoted as $\bar{\mathbf{x}}$. The problem of comparison of multivariate data consists in assessing if the vectors $\bar{\mathbf{x}}_1$ and $\bar{\mathbf{x}}_2$ which describe the two samples 1 and 2 are equal or different. Two vectors are equal when each of their components is the same.

The multivariate version of the t test is Hotelling's T^2 test, for which the test statistic is [32]:

$$T^2 = \left(\bar{\mathbf{x}}_1 - \bar{\mathbf{x}}_2\right)^T \left[\left(\frac{1}{n_1} + \frac{1}{n_2}\right)\mathbf{U}\right]^{-1} \left(\bar{\mathbf{x}}_1 - \bar{\mathbf{x}}_2\right) \tag{3.3}$$

where the subscripts 1 and 2 represent samples 1 and 2, respectively, and $\mathbf{U}$ is the within-group[2] covariance matrix, considered equal for the two populations.

The evaluation of the results of the test is very similar to the univariate case. A threshold value can be found in the F distribution charts for the desired confidence level: $F_{p,\, n_1+n_2-p-1}$ where p is the number of variables which describe each sample, i.e. the components of the vectors $\mathbf{x}_i$. When T^2, calculated from experimental data, exceeds $F_{p,\, n_1+n_2-p-1}$, there is sufficient evidence to reject the proposition that the two samples came from the same source. In other words, when this happens, the conclusion must be drawn that the samples are statistically different.

A word of caution is necessary. Multivariate data treatment implies the estimation of a large number of parameters (averages, variances and covariances). To do so with a sufficient precision, very large data sets are necessary, a requirement met with difficulty in casework. This approach should be then restricted, as much as possible, to a limited number of variables [32].

Another important aspect to keep in mind is that the multiple variables which can be determined are not necessarily independent, and this would have two significant consequences. The first is that determining non-independent variables, in other words features which are correlated together, is a waste of time and money. They in fact replicate the same information. The second consequence is that the significance of a comparison made on the basis of non-independent variables is greatly diminished with respect to the same comparison based on independent features.

[2] Within-group variation refers to replicates within the same group or source, between-group variation is that between groups or sources [32].

Principal component analysis (PCA), described below, is a common method for simplifying data and for identifying the independent variables which are more efficient for comparison analyses. Once such independent variables are identified, they can be compared, with the methods just seen.

3.3.2 Principal Component Analysis: The Basics

The complexity of analytical data in forensic analyses is definitely an issue.

When samples are described by more than two or three variables, they cannot be represented as data points in a plane or in a three-dimensional space. In such case, common operations like visual inspection for outliers, grouping of data points or trend analysis become very difficult.

An useful statistical method of data treatment, Principal Component Analysis (PCA), has been developed to be able to represent data points that are characterised by a large number of variables, in two or three dimensions, while retaining much of the information about their variance. In other words, the parameters that contribute to a more efficient discrimination between the samples are weighted more than those that don't vary much in the population. A further advantage of this new frame of reference is that it provides uncorrelated and linearly independent descriptors.

PCA is a multivariate analysis technique for pattern recognition that has been proposed by Karl Pearson in 1901 [36] and was developed in its current form by Harold Hotelling in 1933 [37]. A sample, characterised by n variables, represents a point in an n-dimensional space. PCA operates a rotation of all the axes of that space, so that the first new axis corresponds to the direction of maximum variance in the data, and each remaining axis represents the maximum residual variance. Without changing the data structure, the purpose that is sought after is that of finding orthogonal axes representing the directions of maximum variance. The outcome of this process is that the variables describing the samples are transformed into new variables, called principal components, that are linear combinations of the original variables and whose major characteristic is that of being orthogonal one with respect to each other.

The principal assumption at the basis of the method is that the samples, on which the variables are measured, be 'similar' or at least 'not very dissimilar'. The case of polymeric items is ideal, because they are industrial products, manufactured with processes that are only slightly differing one from another.

The data set should be as homogeneous as possible, so before the application of PCA, data must be preprocessed, transforming the original variables to become *features*. The extent to which preprocessing is to be applied is very context-dependent: the questions involved, chemical and physical factors, if data are from a single (gathered on just one instrument) or multiple-source (obtained by a variety of instruments). Treatment of missing data and autoscaling are the main steps in preprocessing. Of course the best strategy concerning missing data is… that they shouldn't exist! Each measurement corresponds to a coordinate on an axis of an

n-dimensional space, so if a variable is for any reason missing, the representation of the corresponding data point won't be accurate. Nevertheless it can happen that for some samples it wasn't possible to measure some property, so a method is needed to fill in the blanks.

Two procedures are quite widespread: the mean fill and the random fill techniques. In the former, a missing datum is given the mean value of that variable within the remaining sample population. In the latter, data are completed assigning random values from the measurements of the appropriate category.

The purpose of scaling is that of putting all variables on an equal basis in terms of their variance. For example, for acrylic fibres typical values of degree of crystallinity in polymers are on the order of 60–80 %, whereas their refractive index varies in the range 1.50–1.52. The variation of these two variables are then on the order of 20 for the degree of crystallinity and just 0.02 for the refractive index. A method is necessary to harmonise the variance of two different features, in order not to have an incorrect weighting of those with larger values. Autoscaling to unit variance, that consists in mean centring followed by a division by the standard deviation, s_k, on a variable-by-variable basis, is a common approach.

$$x'_{ik} = \frac{x_{ik} - \overline{x}_k}{s_k} \tag{3.4}$$

where x_{ik} is the datum associated with the *k*th measurement on the *i*th sample and $\overline{x}_k$ is the mean of the *k*th measurement over all the samples.

The features obtained by this autoscaling procedure will form a population with mean 0 and variance 1, for each of the measured quantities.

After preprocessing, and after having created a data set as homogeneous and comparable as possible, PCA can be finally applied.

Figure 3.11 shows a simple example on a two-dimensional case. The samples have been characterised on the basis of two variables: *X* and *Y*. The variance of the data set is scattered across the whole *X*–*Y* plane. The application of PCA leads to the definition of two principal components (PC1 and PC2) and thus to a new frame of reference for the representation of the samples.

As can be observed, the first principal component, PC1, is in the direction of maximum variance, and its origin is centred on the mean value of the variable, as a result of autoscaling. Most of the items can now be discriminated on the basis of just one variable: PC1. The residual variance is represented by the second principal component, PC2, in a direction perpendicular to the first. Since in this case just two variables were originally measured, the two principal components entirely describe the original data, in other words 100 % of the variance is explained. In the more common case, in which the samples are characterised by more than three variables, the first three principal components won't explain 100 % of the total variance, but will account for a smaller percentage, albeit usually quite high. It is quite common that the first three principal components describe more than 75 % of the total variance associated to the samples.

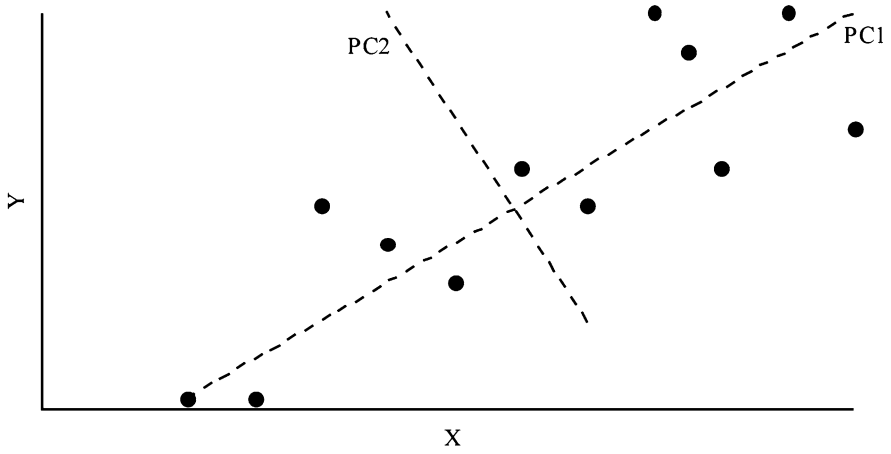


Fig. 3.11 Extraction of two principal components in a two-dimensional case

In the simple example depicted in Fig. 3.11, each of two principal components, PC1 and PC2, is a linear combination of the original variables, *X* and *Y*.

More generally, if a sample is represented by the vector **X** associated to its *n* measured properties:

$$\mathbf{X} = \left| x_1 \quad x_2 \quad . \quad x_j \quad . \quad x_n \right| \tag{3.5}$$

the PCA procedure, will extract *n* principal components, defined as:

$$\begin{aligned}
\text{PC1} &= a_{11}x_1 + a_{12}x_2 + \cdots + a_{1i}x_i + \cdots + a_{1n}x_n \\
\text{PC2} &= a_{21}x_1 + a_{22}x_2 + \cdots + a_{2i}x_i + \cdots + a_{2n}x_n \\
&\cdots \\
\text{PC}j &= a_{j1}x_1 + a_{j2}x_2 + \cdots + a_{ji}x_i + \cdots + a_{jn}x_n \\
&\cdots \\
\text{PC}n &= a_{n1}x_1 + a_{n2}x_2 + \cdots + a_{ni}x_i + \cdots + a_{nn}x_n
\end{aligned} \tag{3.6}$$

PC1, PC2, etc. are the new coordinates of the sample in the new frame of reference and are defined as *scores*.

The coefficients of the linear combinations, a_{pq} in (3.6), are called *loadings* and represent the weight of the *q*th variable in the *p*th principal component. Each component owes most of its value to the variables with the largest loadings. Consequently, variables that are highly correlated tend to concentrate their weight (either in positive or negative, depending on the sign of their coefficient) on one or a few principal components. An examination of the loadings will then allow to identify non-independent variables.

Each component is associated to a variance, which is a fraction of the total variability of the samples, it is reported in percentage and is called *explained variance*.

The variance explained by the first principal component is the largest, and it tends to rapidly decrease from one component to the next.

Practically, very frequently the problem can be reduced to the analysis of the first few principal components, because the remaining ones will represent just 'noise' and won't carry useful information for the differentiation of the samples.

3.3.3 Principal Component Analysis: Some Examples

Thirty-six samples of colourless acrylic fibres were subjected to pyrolysis, to study if their degradation behaviour could be a distinguishing feature useful for forensic comparisons [38]. PCA was used to interpret the data coming from integration of the intensities of the peaks in the pyrograms.

The amounts of the following degradation products were designated as suitable descriptors for the samples: hydrocyanic acid, acetonitrile, acrylonitrile (AN), acetic acid, methacrylonitrile (MAN), 1,3-dicyanopropene, 1,3-dicyanobutene and 1,3,5-tricyanohexane. When PCA was applied to the fibres and these eight variables were used, the first three PCs accounted for 88.3 % of the total variance. Figure 3.12

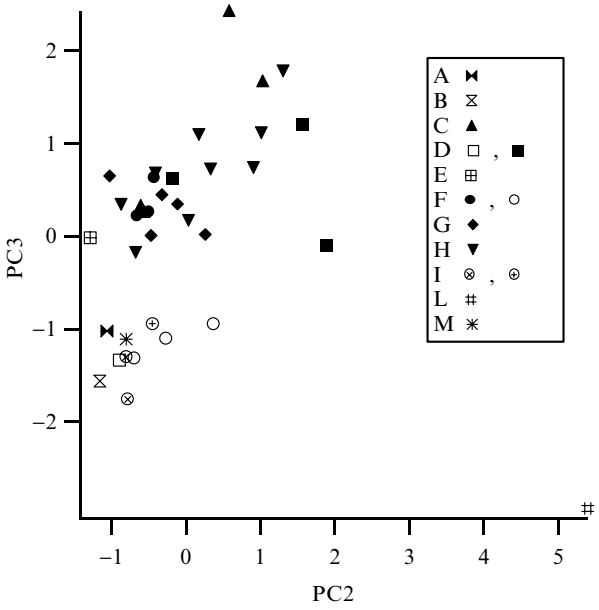


Fig. 3.12 Score–score plot of the samples, represented on the plane individuated by PC2 and PC3. *Solid symbols* identify polyacrylonitrile/vinyl acetate copolymers, *open symbols* identify the acrylic fibres containing other comonomers. # represents the homopolymer acrylic fibre. Reprinted from Ref. [38], copyright 2006, with permission from Elsevier

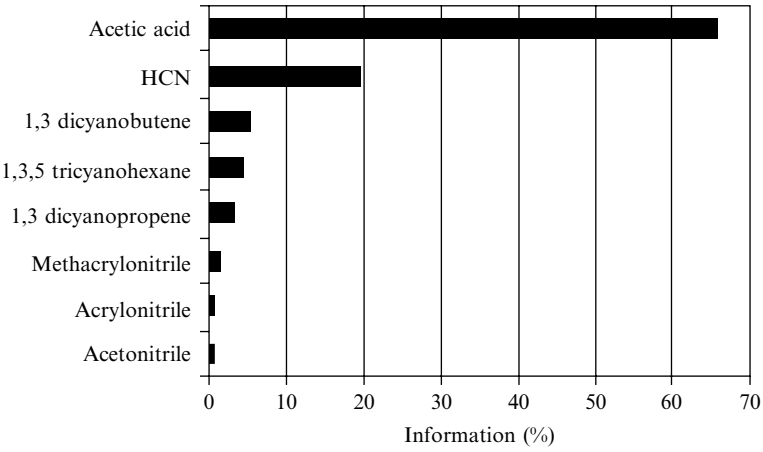


Fig. 3.13 Histogram of the loading of each variable on PC3. Reprinted from Ref. [38], copyright 2006, with permission from Elsevier

shows a representation of the samples in a two-dimensional plane identified by the principal components PC2 and PC3.

Figure 3.12 shows that clustering of the samples was obtained when they were represented on the PC2–PC3 plane. The homopolymeric acrylic fibre was dramatically separated from the other items that appeared to form two groups. In particular, the acrylic fibres containing the vinyl acetate comonomer had positive PC3 values, whereas those which contained other comonomers were located on the negative portion of the PC3 axis. The chemical interpretation of this behaviour can be obtained examining the loadings of PC3 (Fig. 3.13). PCs are linear combinations of the original variables. The loadings are coefficients that indicate how much a single variable weighs in the PC.

PC3 allows for a qualitative identification of the copolymer employed in the production of the fibres. This principal component is mainly constituted by acetic acid. This compound is produced if polyacrylonitrile/vinyl acetate (PAN/VA) is degraded, and it is absent in the pyrograms of the other samples. The value of PC3 is the basis for the formation of the two clusters evident in Fig. 3.12: PAN/VA polymers have a $PC3 > -0.2$, the others are characterised by $PC3 < -0.2$.

Other examples of applications of PCA in a forensic context were reported on adhesive tapes [39], on plastic bags [40], on paper [41] or on silicone elastomers [42]. Lewicki and colleagues aimed at identifying, through an analysis of the degradation products, silicone materials formulated via differing cure chemistries. PCA applied on pyrolysis data allowed to identify these unique signatures in a rapid and reliable fashion. As in the example above, PCA also helps a chemical interpretation of the data, with the concurrent advantage of improving the clarity and rigour of the presentation of data to the Court.

3.3.4 Clustering

Polymers, as other traces, are class evidence, and as such they acquire evidential value only if they can be placed in as small a class as possible. Of course the evidential value of the statement 'the two fibres come from the same garment because they are both red acrylic fibres' is much lower than 'the two fibres come from the same garment because they are poly(acrylonitrile-*co*-vinyl acetate) acrylic fibres with a superimposable UV-visible spectrum, with the same dye formulation, with the same morphology'. The population of red acrylic fibres is obviously much larger than that of the fibres which share the characteristics described in the second statement.

The ultimate purpose of a forensic characterisation is in most cases the detection of similarity between objects. This concept is quantified on the basis of geometrical distance. An object described by p analytical variables can be represented as a point in a p-dimensional space. The more distant two objects are within this p-dimensional space, the more different they will be. On the contrary, if two objects appear as points close to each other in the p-dimensional space, they will be similar.

Each classification method starts from a series of samples whose class is known, called the *training set*. This collection is necessary for identifying the rules which will be used to classify unknown samples. Each object of the training set is a priori assigned to a particular class.

An example can clarify the method or reasoning on the basis of classification. Let's imagine that our problem is to classify a horse, a car and a motorbike. We can choose many criteria (the analytical variables) for describing each of these objects: 'is it a means of transportation?', 'is it an animal?', 'does it have wheels?', 'how many wheels does it have?', 'what colour is it?'. The kind of variable we will choose will depend on the purpose of our classification and on its efficiency in discriminating within the population. 'Is it a means of transportation' is not a good criterium, because all the objects are indeed means of transportation. 'Is it an animal' or 'does it have wheels' differentiate between the horse and the car and motorbike. The number of wheels discriminates car and motorbike. Answering this sequence of questions will allow us to correctly assign any horse, motorbike and car to their relevant class.

The colour allows to discriminate between similar objects in the same class: a white horse is obviously different from a black horse, even if they belong to the same 'horse' class.

Clustering methods do not require a training set and are used when no prior knowledge on the data are available. In this case, the purpose is to verify if the points that describe a population of samples are homogenously dispersed or if they form groups, or clusters. If at the end of the analysis it is possible to assign a physical, chemical or logical meaning to such groups, we can define them as classes. Clustering problems never have a single answer and they rarely have a right answer. The best clustering approach depends on the purpose for which this technique is applied.

As anticipated above, distance is the quantitative proxy of similarity. Many different formalisations of distance have been proposed, Euclidean, Manhattan, Pearson, Canberra, but for its intuitive and easy application the Euclidean is often favoured. The Euclidean distance d_{12} between two objects, 1 and 2, described by p variables is:

$$d_{12} = \sqrt{\sum_j \left(x_{1j} - x_{2j}\right)^2} \tag{3.7}$$

where x_{1j} and x_{2j} are the values of the jth variable for samples 1 and 2, respectively. Similarity is inversely correlated to distance: the larger the distance the lower the similarity between two objects. Similarity between the same objects 1 and 2 mentioned above may be quantified as:

$$s_{12} = 1 - d_{12} / d_{\max} \quad \text{or} \quad s_{12} = 1 / \left(1 + 1 d_{12}\right) \tag{3.8}$$

In the algorithms which govern clustering calculations, the first step consists in determining the distance matrix. For a set of n objects, the distance matrix is a $n \times n$ matrix which contains in the ith row the distances of the i-th object from all the other objects. It is a symmetric matrix, and all the elements on the diagonal are equal to 0 (each object is located at a zero distance from itself). Some methods use the similarity matrix, which is analogous to the distance matrix, but it contains in the i-th row the similarities (calculated according to (3.8)) of the i-th object from all the other objects.

Clustering methods can be hierarchical or non-hierarchical [43, 44]. Hierarchical clustering can be agglomerative or divisive. In agglomerative hierarchical clustering the distance matrix is calculated. The two closest objects are identified and they are then agglomerated into a new cluster. The distance of this new cluster from the other elements is then measured and a new distance matrix is calculated. The process is then repeated, identifying the two closest clusters or objects in this new population, which are agglomerated, etc. The distance or similarity of the new clusters coming from this fusion process can be calculated in several ways. The centroid linkage describes the cluster with the average value of the coordinates of the elements which compose the cluster itself. It identifies the cluster with its centroid, and thus measures the distances from this point to the other objects or clusters of the considered population. Other methods still consider the individual elements within the cluster for the determination of the distance or similarity with the rest of the population. In the complete or in the single linkage methods, the distances of each element of the cluster with all the other objects in the population are measured. In the complete distance method, the distance between clusters equals the distance between those two elements (one in each cluster) that are farthest away from each other. The opposite happens in the single linkage method: the distance between clusters equals the distance between those two elements (one in each cluster) that are closest to each other. An example of these three different approaches is represented in

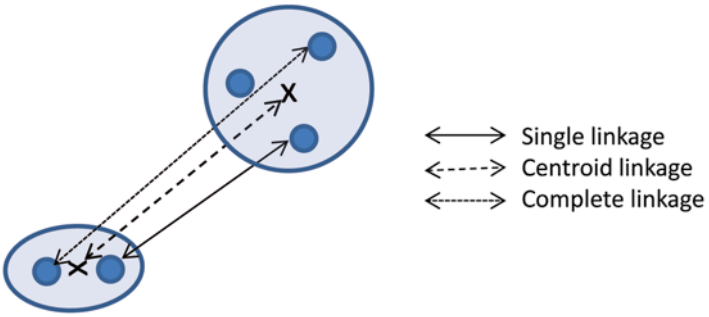


Fig. 3.14 Calculation of the similarity between clusters according to three approaches

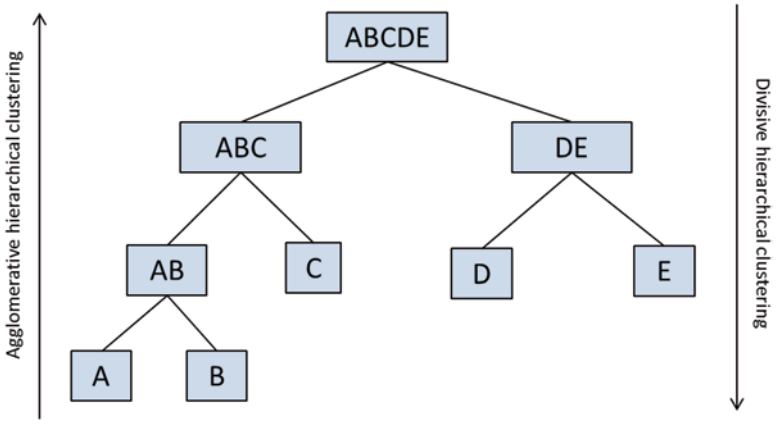


Fig. 3.15 The two different approaches to hierarchical clustering

Fig. 3.14. Many other procedures for assessing the similarity between clusters have been devised [43, 44].

At the beginning of the agglomerative clustering process, each object is in a cluster of its own. The opposite starting point is assumed in divisive clustering processes: all the objects are initially considered as part of the same cluster. By a procedure analogous to that presented above, on the basis of the distances between all the objects, the population is divided into two subsets. These subsets are in turn divided again, and this process continues until every subset contains just a single object.

Figure 3.15 summarises the two approaches of hierarchical clustering.

Example

Five samples, A, B, C, D and E, were characterised according to four analytical parameters *α, β, γ* and *δ* (Table 3.1).

Using (3.7), the distance between samples A and B is calculated as follows:

$$d_{AB} = \sqrt{\sum_j \left(x_{Aj} - x_{Bj}\right)^2} = \sqrt{(100-80)^2 + (80-60)^2 + (70-50)^2 + (60-40)^2}$$
$$= \sqrt{1600} = 40$$

Repeating the same calculation for all the other sample pairs, the distance table can be constructed (Table 3.2).

According to the agglomerative method, the most similar samples are D and E, because they are separated by the shortest distance, 14.1. Therefore, they are fused into a new cluster, which is designated as D*, i.e. with the lowest of the two letters and a superscript *. The distance of cluster D* from the other samples is calculated by the centroid linkage approach. For example, the distance between D* and A is the average of the distance between D and A and E and A:

$$d_{AD^*} = \frac{1}{2}\left(d_{AD} + d_{AE}\right) = \frac{1}{2}(110.4 + 11.4) = 110.9$$

A new distance table can be calculated (Table 3.3).

From this new table the couple of samples closest to each other are B and C, so a new cluster, B*, will be created. The distance between B* and the

Table 3.1 Characterisation data of samples A, B, C, D, and E

		Analytical parameters			
		α	*β*	*γ*	*δ*
Samples	A	100	80	70	60
	B	80	60	50	40
	C	80	70	40	50
	D	40	20	20	10
	E	50	10	20	10

Table 3.2 Distance table for samples A, B, C, D, and E

	A	B	C	D	E
A	0				
B	40.0	0			
C	38.7	17.3	0		
D	110.4	70.7	78.1	0	
E	111.4	72.1	80.6	14.1	0

(continued)

Table 3.3 Distance table for samples A, B, C, and the cluster D*

	A	B	C	D*
A	0			
B	40.0	0		
C	38.7	17.3	0	
D*	110.9	71.4	79.3	0

other objects is calculated as above. For example, the distance between B* and D* is:

$$d_{B^*D^*} = \frac{1}{2}\left(d_{BD^*} + d_{CD^*}\right) = \frac{1}{2}(71.4 + 79.3) = 75.3$$

A new distance table is then calculated, the most similar objects are identified, a new cluster is created and so on, until just one cluster contains all the samples.

The results of this process can be summarised in the diagram shown in Fig. 3.16, called dendrogram.

The similarities of the objects can be evaluated moving from the bottom to the top of the graph. D and E are the most similar, because they join at the earliest stage, then B and C become part of the same cluster. Successively A is included in the cluster previously containing B and C. The last step is the connection of all the samples in a single cluster.

There is no theoretical rule about the level at which the tree is cut, i.e. the similarity level when the number of identified clusters is suitable for the solution of the classification problem. This choice carries a relevant amount of subjectivity, but a practical criterion can be that of including in the calculation replicates of the same sample [45]. The tree can be cut at a level in which all the replicates from that sample are included in the same cluster. For example, let's imagine that A and C are replicate measurements of the same object. Logically, it is expected that they are part of the same cluster. If the tree were cut at the level indicated by the dashed line in Fig. 3.16, A and C would fall into two separate clusters, a clearly unacceptable result. A reasonable similarity level could be the one represented by the dotted line, which divides the sample set into two clusters: ABC and DE.

The same objects represented in Table 3.1 can be treated according to a divisive clustering method. From the distances in Table 3.2, the sum of each object from all the others is calculated (Table 3.4).

The most dissimilar object, i.e. the one farthest from all the others, is A. It is therefore isolated from the others, and the distance of each object from A is compared with the average distance from each object remaining in the original cluster (Table 3.5). For example, for object B, the distance d_{AB} is compared with the average of distances d_{BC}, d_{BD}, and d_{BE}, i.e. to $\left(d_{BC} + d_{BD} + d_{BE}\right)/3 = (17.3 + 70.7 + 72.1)/3 = 53.4$

(continued)

Table 3.4 Sum of the distances of each sample from all the others

	Sum of the distances	Total
A	40.0 + 38.7 + 110.4 + 111.4	300.5
B	40.0 + 17.3 + 70.7 + 72.1	200.1
C	38.7 + 17.3 + 78.1 + 80.6	214.7
D	110.4 + 70.7 + 78.1 + 14.1	273.3
E	111.4 + 72.1 + 80.6 + 14.1	278.2

Table 3.5 Comparison of the distances of each object from A and from all the others

	A	Others
B	40.0	53.4
C	38.7	58.7
D	110.4	54.3
E	111.4	55.6

Table 3.6 Comparison of the distances of each object from cluster A* and from all the others

	A*	Others
B	28.6	71.4
D	94.3	42.4
E	96.0	43.1

Table 3.7 Comparison of the distances of each object from cluster A* and from all the others

	A*	Others
D	86.4	14.1
E	88.0	14.1

C is the most similar to A, so it is included into the A* cluster. The table is updated (Table 3.6), calculating the distances related to the cluster A* as the average of the distances from to its individual components A and C. So, the distance between B and A* will be $d_{A^*B} = \frac{1}{2}\left(d_{AB} + d_{BC}\right) = \frac{1}{2}\left(40.0 + 17.3\right) = 28.6$

On the other hand, the distance between B and the other samples will be equal to the average of the distance of B with the objects remaining in the original cluster, i.e. to $\frac{1}{2}\left(d_{BD} + d_{BE}\right) = \frac{1}{2}\left(70.7 + 72.1\right) = 71.4$.

B is included in cluster A*, which now contains A, B and C, and the distances are recalculated (Table 3.7).

Since the distance between D and E is shorter than that between D and A* or between E and A*, D and E are clustered together. The initial set ABCDE was divided into two subsets: ABC and DE. The method proceeds now separately for each cluster, until a total separation of the objects is achieved. The dendrogram obtained at the end of this divisive process is similar to that of Fig. 3.16.

(continued)

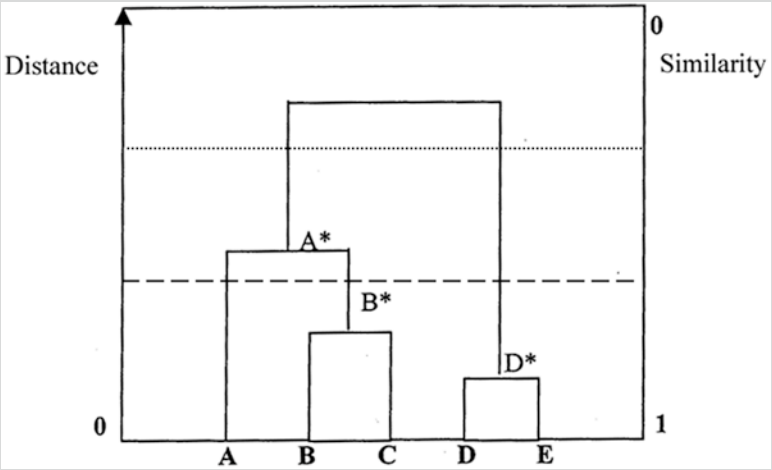


Fig. 3.16 Dendrogram obtained from the data in Table 3.1. The meaning of the *dashed* and *dotted horizontal lines* are explained in the text

Non-hierarchical clustering methods are much less easy to summarise, and will not be considered here. The reader is referred to more specialised texts for details [43, 44, 46].

A recent example of hierarchical clustering in a forensic context was reported by Kumooka, who worked on a population of adhesive tapes, representative of the Japanese market [45]. He acquired the IR spectra of 46 different tapes, divided in two big families: acrylic-based and rubber-based. In his treatment he measured the Euclidean distance between the spectra at each wavenumber on the IR spectra, and the subsequent clustering process showed that it was possible to classify rubber-based adhesives according to the distributor.

3.4 The Interpretation of Evidence

Analysis in the laboratory is just a part of a much wider process that relies on proper detection, selection, collection and observation of specimens at the crime scene.

An excellent book on the interpretation of evidence was published by Aitken and Taroni [32]. Once detected and collected, such traces follow a complex set of inter-related processes that ultimately compose the whole information system [20]. They are collated and interpreted in order to provide knowledge that is used to make decisions at various levels of security systems and criminal justice [20]. This relates to some key issues in the process of interpretation of the information.

The most accurate and precise analysis of trace evidence would be meaningless if it weren't accompanied by an assessment of its value in the context of the particular case it is involved in. Two main approaches have been developed: the frequentist and the Bayesian. In cases involving comparisons, the frequentist approach involves the assessment of the probability that the two sets of traces, the ones of known origin and the ones of unknown origin, can be found to be similar, even though they come from different sources. If this probability is very low, then this hypothesis can be rejected. The Bayesian approach is more complete (even though more complicated) because it puts all the evidence in the context of two opposing reconstructions of the events, roughly that of the prosecution and that of the defense. As will be elaborated in the rest of this section, Bayesian interpretation is useful when dealing with contact traces. All the examples and the discussion will focus on the case of transfer of fibres, because it is a common instance, for which many statistical data are available. However, the concept can be extended to any contact trace, such as glass fragments, paint or polyurethane foam [6, 32, 47, 48].

3.4.1 The Frequency of Traces: Population and Target Studies

When evaluating the evidential value of the presence of a particular trace on the crime scene or on a suspect, the availability of data about the frequency (*f*) of such trace is critical. In other words, to be able to assess if the presence on a homicide victim of a purple fibre similar to that of a suspect's shirt is significant or not, we must know how common such purple fibres are and how likely it is to find them by chance in a room like the one where the victim was.

Fibres are surely the traces for which are available the largest number of such statistical studies. Population [49–61] and target fibre [49, 62–72] studies have been published and, even though many others are still necessary to reflect every possible circumstance that could be encountered in casework, they can help the forensic scientist in the assessment of the variable *f*. In population studies [49–61], the tapings taken on a large number of items (for example on garments, car and cinema seats, …) are inspected, all the foreign fibres are characterised and statistics are produced on the composition of the population of foreign fibres. These studies are very time consuming and their relevance relies on an accurate choice of a significant population of items that are to be inspected. Surveys have been made on garments [50, 53, 58, 59], cars [49, 54, 55], outdoor surfaces [57], cinema seats [56], washing machines [52], skin [51] and bus seats [60, 61].

Target fibre studies [49, 62–72] start from a different approach and are a much easier and faster way to gather frequency data. In these works, a fibre is chosen and characterised. This target fibre is afterwards searched for in a large number of tapings taken from a population of items (for example cinema seats, car seats, garments, …). The number of fibres that will be found matching the target one will give an assessment of how rare the latter is. A few target fibre studies are available in the literature. The substrates surveyed are garments [62, 66, 69–71], cars [49, 67, 68],

hair combings [63–65], seats in cinemas [68] and pubs [72]. The target fibres chosen were wool [62–67, 72], nylon [49, 66], acrylic [62–66, 68, 71], polyester [49, 62, 67] and cotton [69, 70], variedly coloured and with different shapes. The most recent target fibre study by Palmer and colleagues, which is focused on black acrylic and blue polyester, also summarises the results of target fibre investigations carried out in the last 30 years [73].

Brüschweiler and Grieve, for example, examined 435 different garments, looking for a particular red acrylic fibre [71]. Just two target red acrylic fibres were found, showing that the chances of finding significant numbers of red acrylic fibres on a random garment is less than 0.5 %. Other authors confirmed these results [66]. It should be noted that red acrylic is perceived as a rather common fibre type on crime scenes [74]. This stresses the importance of a thorough characterisation of each item found on the crime scene. Even though industrial products like fibres 'all look like the same', a great degree of differentiation among them is possible. Two items that both pertain to the general group 'red acrylic' can be further subclassified and there is a very low probability that two fibres coming from different sources match just by chance.

Roux and Margot's study [55] on the population of fibres on car seats showed that the frequency of most fibres is far below 0.1 %.

Even though available data show frequencies often lower than 1 %, in the evaluation of actual cases a reasonable approach can be taken, choosing larger values for f, usually 0.01 (or 1 %), in order to be more conservative and to avoid any accusation of prejudice against the defendant.

In addition to fibres, population studies are available also for polyurethane foam fragments, for architectural paints [75, 76], for paint flakes [77] and for automotive paints [29, 30]. Moreover, about the latter class of items, several projects have been developed since the 1970s to promote the accumulation of data on automotive paints. Currently, a database such as the PDQ (Paint Data Query) contains analytical information on thousands of vehicles worldwide, and is a solid base for identifying the number of car models which were treated with a particular kind of paint layer. More details on paint databases can be found in Sect. 4.2.4. Crossing the statistics extractable from these databases with the diffusion of car models within the relevant market, the frequency of paint evidence can be assessed, and as a consequence its evidential value.

3.4.2 The Levels of Proposition [78–80]

The assessment of evidence by the forensic scientist consists in determining which of two alternatives, either that of prosecution or that of defence, is the best in explaining the presence and nature of the analysed items. These alternatives are formalised representations of the framework of circumstances. The formulation of these propositions is crucial and depends on the context of the case, the observations that have been made and the available background data. Three levels can be addressed.

If for example, on a broken window in a burglary scene some red extraneous fibres were found and a suspect wearing a red sweater had been apprehended a few hours later, three categories of propositions could be suggested:

(a) Mr X's sweater is the source of the red fibres recovered
Mr X's sweater is not the source of the red fibres
(b) Mr X passed through the broken window
Mr X did not pass through the broken window
(c) Mr X committed burglary
Mr X did not commit burglary

If the propositions (a) are chosen, the case is addressed at the *source* level. The probability of the evidence under the first of these two propositions is assessed by an accurate comparison between the sweater and the fibres found on the crime scene. The probability of the evidence under the second sentence is estimated comparing the fibres on the crime scene with some other population of alternative sources.

The propositions of group (b) represent the *activity* level. The assessment of the case at this level implies not only a thorough characterisation, as that necessary for the source level, but also an evaluation of the transfer and persistence associated to each of the two opposing reconstructions. In other words the forensic scientist must determine not only that the red fibres found on the crime scene are compatible with the red sweater, but also that that group of items is consistent with the transfer associated to a man passing through that window and the persistence expected in the period of time elapsed between the burglary and the inspection of the crime scene. Much more information on the context of the events is needed to answer these questions, but the significance of the evidence treated at the activity level would be much greater than that considered only under the source point of view.

Finally, formulating propositions (c) is equivalent to addressing the case at the *offence* or *crime* level. This level is that of the uttermost importance to the jury, but it is rarely within the grasp of the forensic scientist. To effectively determine the probabilities of each of the two sentences in group (c), the scientist should be aware of a significant body of circumstantial information, either of scientific and non-scientific (i.e. eyewitness depositions) nature. Moreover, in some judicial systems the scientist can't express opinions which are not strictly related to his qualification. It's not allowed, for example, that a single criminalist puts together the evidence coming from chemical analysis of traces, DNA typing and fingerprint examinations. It is usually a duty strictly limited to judges to examine propositions at the crime level, and the forensic scientist's role is to give the judge the tools and information necessary to do so.

3.4.3 The Bayesian Analysis of the Results

The Bayesian approach for the interpretation of the data is very dependent on the context, so it could contribute in a much more efficient way to a clear and significant reconstruction of the events.

Once a choice has been made about the level at which the case should be addressed, two opposing propositions are formulated. The Bayesian theory operates on the probabilities associated to each of these two propositions, revising them on the basis of new information, for example scientific evidence.

Given the two competing propositions (H_1 and H_2), formally, Bayes' theorem can be stated as:

$$\frac{P(H_1 \mid E)}{P(H_2 \mid E)} = \underbrace{\frac{P(H_1)}{P(H_2)}}_{\text{prior odds}} \cdot \underbrace{\frac{P(E \mid H_1)}{P(E \mid H_2)}}_{\text{LR}} \tag{3.9}$$

where E represents the new information coming from forensic analyses, $P(H_i)$ indicates the probability of the proposition H_i and $P(y|x)$ is the probability of the event y, given x is true. This formula means that the ratio of the final probabilities is equal to the product of the two ratios: prior odds and Likelihood Ratio (LR).

Prior odds are then updated by the LR to give posterior odds which are conditioned to E, in addition to preliminary information (which were omitted from (3.9) for the sake of simplicity).

Prior odds are the ratio between the probability that hypothesis H_1 is true and the probability that proposition H_2 is the right representation of the facts. This ratio is necessarily evaluated in an approximate way, because it depends on non-measurable variables, like for example the reliability of an eyewitness. Posterior odds represent the same ratio, estimated according to the new information coming from forensic analyses also.

The estimation of prior and posterior odds, even though usually not as a real number but as a qualitative assessment, is the Court's role. In the Doheny and Adams case, in 1997 (1 Cr. App. R. 369) it was ruled that:

> the scientist should not be asked his opinion on the likelihood that it was the defendant who left the crime stain.

In other words, the scientist can't assess prior or posterior odds. This is because if the scientist had to give conclusions such as these, he would need much more information besides the strictly scientific aspect of the case, and there would be a significant overlap between the two roles, his and the judge's. The forensic scientist's duty is that of developing the LR, in order to give the right weight to the results he presents.

As may be seen in (3.9), the LR is composed by a numerator and a denominator. The numerator represents the probability of the evidence E, given the hypothesis H_1 favourable to the prosecution.

The denominator is the probability of the same evidence E, but under the proposition H_2 developed on the basis of the explanation proposed by the defense.

In other words, the LR says how much more likely it is that evidence E is on the crime scene because of explanation H_1, rather than on the basis of reconstruction H_2.

The LR depends on many variables, some of which can be estimated on the basis of literature data, some others being much more subjective.

Fig. 3.17 The bodies of two victims were found wrapped in an inner coloured blanket and in an outer beige-red bedspread. The bed spread was thorn in two locations, indicated by *arrows*, from where the coloured fibres of the inner blanket could be shed. Reprinted from Ref. [81], copyright 2004, with permission from Elsevier

A formalisation of the LR can quantify the role that such subjective variables play. Two examples will be presented in which this approach was applied.

The first case is that of a double murder [81] in which it was of interest to determine if the car of a suspect was used for the transportation of two bodies wrapped inside a blanket and a bedspread (Fig. 3.17).

The textiles of the blanket and of the bedspread were characterised, and the analyses showed that six groups of fibres recovered in the trunk of the car matched those of the blankets, with hundreds of compatible items. The evidence in this case was overwhelmingly against the suspect, but it was nevertheless of interest to determine which of the variables involved (the probability of transfer in a context like that of the case, the rareness of the fibres, the number of groups recovered, the version of the facts proposed by the defense, …) weighted more in the assessment of the significance.

The two competing hypotheses were:

H_1: the pool of fibres recovered on the trunk was originated by the transportation of the wrapping which contained the corpses.
H_2: the pool of fibres recovered on the trunk came from some other source.

Under the hierarchy of propositions of Cook and Evett [80], the case has been addressed at an activity level.

The formulation of proposition H_2 was made according to the declarations of the suspect, who denied having transported the victims in his car, but couldn't identify an alternative source for the fibres found in the trunk.

To evaluate the LR, it is necessary to give an analytical form to its numerator and denominator.

To simplify the problem of evaluating these two probabilities, the recovered fibres were classified according to their generic colour, obtaining four groups that together represent the evidence E.

For the numerator, at least five possible explanations for the evidence E must be considered [6, 82]:

- The four groups were transferred, persisted and were recovered in connection to the transportation of the corpses.
- Three groups originating from the wrapping were transferred, persisted and were recovered, while one was present in the trunk beforehand and has no association with the blankets.
- Two groups are associated with the bodies' wrapping, while the other two were not originated by the bedspread and the blanket.
- One group was transferred from either the bedspread or the blanket, persisted and was recovered, the remaining three were present beforehand in the trunk and were originated by other sources.
- The four groups are not associated with the victims' wrapping, but have different origins.

The numerator is then the sum of five terms:

$$P(\mathrm{E}\,|\,\mathrm{H}_1) = b_0 t^4 + \binom{4}{3} t^3 (1-t) b_1 f + \binom{4}{2} t^2 (1-t)^2 b_2 f^2 + \binom{4}{1} t (1-t)^3 b_3 f^3 + b_4 f^4 (1-t)^4 \qquad (3.10)$$

where:

t is the probability that a group of fibres comparable to that of the case has been transferred from the wrapping of the corpses, has persisted and has been recovered. The hypothesis under which this probability must be estimated is H_1.

f is the frequency of the fibres with the same characteristics as those recovered on the suspected vehicle's trunk, estimated on the population of extraneous fibres found on car trunks.

b_n is the background probability of the presence by chance of n groups composed of extraneous fibres in numbers compatible with those of the case. The term 'extraneous fibres' denotes those distinguishable from textiles owned by the habitual users of the vehicle.

For the sake of simplicity it may be assumed that probability t was the same for each of the four groups and that all the fibres had the same frequency $f = 0.01$ among the population. Of course, in making these assumptions, the common values of t and f must be chosen with a conservative and pro-defendant approach, to guarantee an appropriate application of his right to be presumed innocent.

Each term in (3.10) represents one of the five possible explanations presented above.

If n is the total number of groups of interest in casework and k is the number of groups that have been transferred, have persisted and have been recovered in association with the offence (being $n-k$ the groups that were present beforehand), a general term can be written as:

$$\binom{n}{k} t^k b_{n-k} f^{n-k} (1-t)^{n-k} \tag{3.11}$$

The binomial coefficient is needed to consider all the possible combinations of crime related and non-crime related groups. For example, if the groups of fibres compatible with those of the suspect were 2 ($n=2$) and we consider the case where just one ($k=1$) is crime related, while the other is present by chance, $\binom{n}{k} = \binom{2}{1} = 2$.

This means that there are two ways in which this situation can occur: group A is crime related and B was present beforehand, or group B is associated with the offence, while A is due to chance.

Term t_k in (3.11) is associated with the probability that k groups of fibres compatible with those selected as targets may have been transferred, persisted and have been recovered simultaneously. Combination $b_{n-k} f^{n-k}(1-t)^{n-k}$ takes into account the presence, by chance, of $n-k$ groups. $(1-t)$ is the probability of the complementary event with respect to t, i.e. that the group has not been transferred, has not persisted or has not been recovered, while $b_{n-k} f^{n-k}$ represents the probability that the $n-k$ recovered groups that resulted compatible were there by chance alone.

The expression for the denominator is analytically analogous to (3.10):

$$P(\mathrm{E}\,|\,\mathrm{H}_2) = b_0 t_1^4 + \binom{4}{3} t_1^3 (1-t_1) b_1 f + \binom{4}{2} t_1^2 (1-t_1)^2 b_2 f^2 + \binom{4}{1} t_1 (1-t_1)^3 b_3 f^3 + b_4 f^4 (1-t_1)^4 \tag{3.12}$$

where t_1 is the probability that a group of fibres comparable to that of the case has been transferred from the wrapping of the corpses, has persisted and has been recovered. The hypothesis under which this probability must be estimated is H_2. In this particular case, H_2 asserts that the fibres came from some other source than the victims, it follows that $t_1=0$ and the denominator of the LR reduces to:

$$P(\mathrm{E}\,|\,\mathrm{H}_2) = b_4 f^4 \tag{3.13}$$

So the LR for a case like that exposed can be calculated by:

$$\mathrm{LR} = \frac{P(\mathrm{E}\,|\,\mathrm{H}_1)}{P(\mathrm{E}\,|\,\mathrm{H}_2)} = \frac{b_0 t^4 + \binom{4}{3} t^3 (1-t) b_1 f + \binom{4}{2} t^2 (1-t)^2 b_2 f^2 + \binom{4}{1} t (1-t)^3 b_3 f^3 + b_4 f^4 (1-t)^4}{b_4 f^4} \tag{3.14}$$

A generalisation to the case of n-recovered groups can be proposed remembering (3.11):

$$\mathrm{LR} = \frac{P\left(\mathrm{E}\,|\,\mathrm{H}_1\right)}{P\left(\mathrm{E}\,|\,\mathrm{H}_2\right)} = \frac{\sum_{k=0}^{n}\binom{n}{k} t^k b_{n-k} f^{n-k} \left(1-t\right)^{n-k}}{b_n f^n} \tag{3.15}$$

In the case of $n=1$, the LR is similar to those proposed by a number of authors [32, 82, 83].

If proposition H_2 included some sort of explanation other than invoking the presence of the fibres just by chance, the denominator should be defined by (3.12). If, for example, the defendant declared that the fibres in the trunks had been shed by two blankets he had used for a picnic a few weekends before, the correct form of the likelihood ratio would have been:

$$\mathrm{LR} = \frac{P\left(\mathrm{E}\,|\,\mathrm{H}_1\right)}{P\left(\mathrm{E}\,|\,\mathrm{H}_2\right)} = \frac{b_0 t^4 + \binom{4}{3} t^3 \left(1-t\right) b_1 f + \binom{4}{2} t^2 \left(1-t\right)^2 b_2 f^2 + \binom{4}{1} t \left(1-t\right)^3 b_3 f^3 + b_4 f^4 \left(1-t\right)^4}{b_0 t_1^4 + \binom{4}{3} t_1^3 \left(1-t_1\right) b_1 f + \binom{4}{2} t_1^2 \left(1-t_1\right)^2 b_2 f^2 + \binom{4}{1} t_1 \left(1-t_1\right)^3 b_3 f^3 + b_4 f^4 \left(1-t_1\right)^4} \tag{3.16}$$

The frequencies f and the background probabilities b_i can in some instances be somewhat dependent on the circumstances, but they may generally be assessed as the same under both propositions, for reasons of clarity and simplicity, without losing accuracy in the determination of the significance of the evidence.

Again, (3.16) can be generalised according to (3.11):

$$\mathrm{LR} = \frac{P\left(\mathrm{E}\,|\,\mathrm{H}_1\right)}{P\left(\mathrm{E}\,|\,\mathrm{H}_2\right)} = \frac{\sum_{k=0}^{n}\binom{n}{k} t^k b_{n-k} f^{n-k} \left(1-t\right)^{n-k}}{\sum_{k=0}^{n}\binom{n}{k} t_1^k b_{n-k} f^{n-k} \left(1-t_1\right)^{n-k}} \tag{3.17}$$

While f, on the basis of literature data (see Sect. 3.4.1), can be conservatively estimated, in this case, as equal to 0.01, the quantities t and b_i in (3.14) and (3.16) are not precisely measurable, but carry a big deal of subjectivity.

The analyst should give to the Court an indication of what is the meaning of subjective parameters and how their variation influences the LR, giving at the same time background information useful for a wise choice of these variables.

Background probabilities b_i should, in this case, be evaluated by performing a survey on the presence of extraneous groups of fibres in car trunks. Such information hardly exists in the literature, though. Even when the choice of b_i values is based on published data, their use must be carefully considered. In some cases in fact the surface of interest heavily departs from the average identified in surveys: it is the case, for example, of vehicles that are scrupulously and regularly cleaned

by the owner or user. In these instances, literature b_i's must be varied in order to reproduce in the most accurate manner the real background present on the surface.

It is recommendable to explore two or three different background scenarios, so the judge or lawyers, considering all the elements of the case, can choose the one most adhering to reality. The three situations may correspond to a low expected number of foreign fibres' group, i.e. a low background (accurately cleaned surface), a medium background and a high background (dirty and messy trunk).

It has been shown [82] that b_i tend to follow a Poisson distribution:

$$b_i = \frac{e^{-\lambda} \cdot \lambda^i}{i!} \tag{3.18}$$

where $\lambda = -\ln b_0$. The last term can be determined as the complement to 1 of the sum $b_0 + b_1 + \cdots + b_{n-1}$ and represents the probability of finding by chance n or more foreign fibres' groups.

In the case presented as an example, three scenarios were designed for the background probabilities b_i and in each case the LR was calculated as a function of the probability of transfer t (being $t_1 = 0$).

The evaluation of transfer probabilities t and t_1 can be attained by performing simulations on surfaces analogous to those of the case. The experiments should be carefully designed in order to reconstruct the circumstances of the events. Simulations of this kind are not always feasible and their reliability depends on the reproduction of the quantity and complexity of the variables involved in transfer, persistence and recovery mechanisms. When these experiments are not feasible, for example due to the availability of only small quantities of material, studies on the general mechanisms of transfer and persistence of fibres are very helpful for proposing realistic t values.

Once that the background scenarios have been defined, it is very useful to plot the LR as a function of probabilities t and t_1. In the proposed case the only transfer variable was t, so it was very easy to represent the dependence of the LR on this quantity. It has been seen [81] that even for very low probabilities of transfer, persistence and recovery, the LR assumed very large values (Fig. 3.18).

In this instance, the large number of recovered and matching groups of fibres led to an increase of the LR, because chance becomes a less probable explanation of the facts, as the number of matching fibres becomes greater. The effect of the number of recovered groups was in fact investigated and Fig. 3.19 shows the LRs calculated vs. the probability t under the hypothesis of medium background using (3.14), with n (total number of groups compatible with the chosen target fibres) values of 1, 2, 3 and 4.

This result formalises an intuitive concept. The more corresponding features are found in a comparison, the less likely is the hypothesis that such correspondence happens just by coincidence. In the examination of paints, for example, it has been noted that if two paint chips correspond by six or more layers, the chances that those samples came from different sources are extremely remote [29].

It can be seen that the addition of one recovered group enhances the LR of about two orders of magnitude.

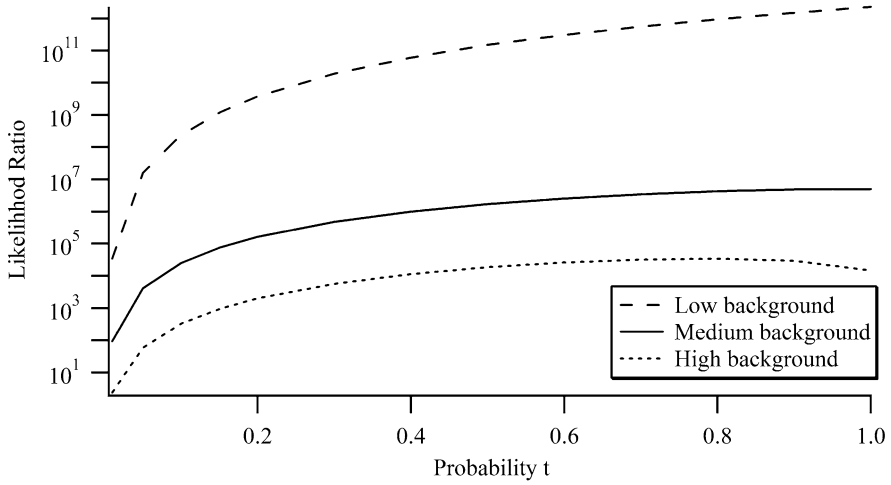


Fig. 3.18 Likelihood ratio vs. *t*, the probability of transfer, persistence and recovery, under the three different background scenarios considered in the case example. Reprinted from Ref. [81], copyright 2004, with permission from Elsevier

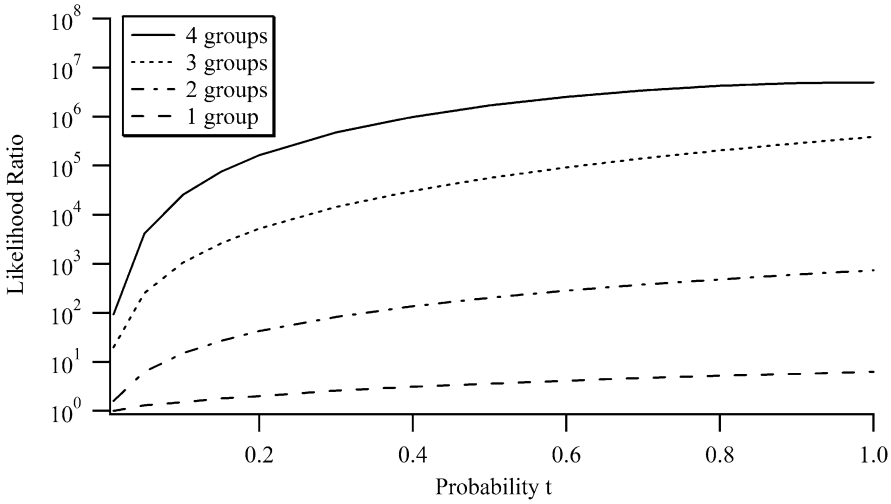


Fig. 3.19 Likelihood ratio vs. the probability of transfer, persistence and recovery *t*, in the case of 1, 2, 3 and 4 extraneous fibres' groups compatible with those chosen as targets, in the case example. Reprinted from Ref. [81], copyright 2004, with permission from Elsevier

The influence of background on the LR is that of enhancing the evidential value when less chance of occurrence of foreign fibres' groups is expected (Fig. 3.18). Finding matching fibres on a perfectly clean surface would be much more significant than finding them in a messy environment, among tens of other items.

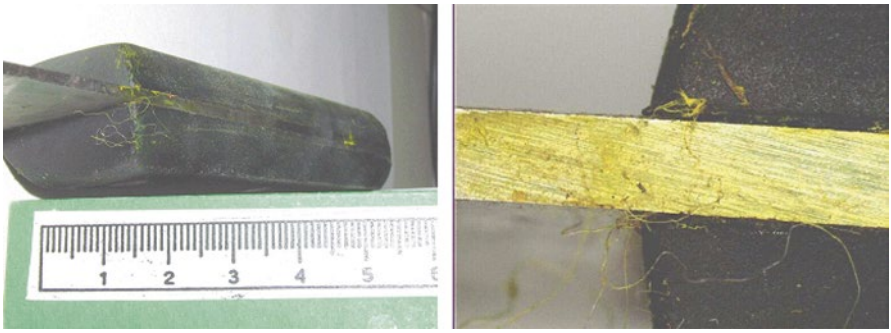


Fig. 3.20 The fibres recovered on the knife

If the defense presented a more complex reconstruction of the events other than 'the defendant never committed the crime and states that the fibres found on his premises are there coincidentally', (3.16) should have been used. Figures analogous to Fig. 3.18 would have been plotted, each one for a fixed t_1 value. The analyst, considering the circumstances of the case, would have the responsibility to guide the judge in the attribution of the most realistic t and t_1 values and to the selection of the background scenario that best describes the recipient item.

Another case which could be taken as an example of application of a Bayesian approach to the interpretation of evidence is that of a homicide by stabbing. The alleged weapon was a knife that presented on the base of its blade a few fibres (Fig. 3.20), positively compared with those of the polyester wadding of the victim's windcheater. No DNA or fingerprints were available, since such traces were carefully cancelled.

This was quite a routine case, but it could be of interest analysing how the LR would change depending on the different explanations that the defense could propose.

The formulation of H_1 could be made at the action level: 'the knife was used for stabbing the victim'.

Three very different versions for H_2 were taken into account:

1. The knife was not used for stabbing the victim and the fibres come from some other source
2. The knife was not used for stabbing the victim and the fibres were transferred on it when the owner accidentally cut his own windcheater
3. The knife was not used for stabbing the victim and the fibres were transferred on it when the owner stabbed a burglar that entered his house a week before

Three different types of matching fibres were recovered, for which f can be estimated [55] as 0.01. The presence of fibres on the blade of a knife is a highly unlikely eventuality, so the background factors b_i were estimated according to (3.18) with a b_0 equal to 0.9, viz. a 90 % probability that no group of fibres would be present on the blade purely by chance.

Equation (3.18) yielded the following b_i's: $b_0 = 0.9000$, $b_1 = 0.0948$, $b_2 = 0.0050$, $b_3 = 0.0002$.

The analytical expression of the LR is:

$$\mathrm{LR} = \frac{P(\mathrm{E}\,|\,\mathrm{H}_1)}{P(\mathrm{E}\,|\,\mathrm{H}_2)} = \frac{b_0 t^3 + \binom{3}{2} t^2 (1-t) b_1 f + \binom{3}{1} t (1-t)^2 b_2 f^2 + b_3 f^3 (1-t)^3}{b_0 t_1^3 + \binom{3}{2} t_1^2 (1-t_1) b_1 f + \binom{3}{1} t_1 (1-t_1)^2 b_2 f^2 + b_3 f^3 (1-t_1)^3} \tag{3.19}$$

Under the first hypothesis of the defense, the stabbing is negated and the presence of the fibres is due to chance. t_1 is thus 0, so (3.19) becomes:

$$\mathrm{LR} = \frac{P(\mathrm{E}\,|\,\mathrm{H}_1)}{P(\mathrm{E}\,|\,\mathrm{H}_2)} = \frac{b_0 t^3 + \binom{3}{2} t^2 (1-t) b_1 f + \binom{3}{1} t (1-t)^2 b_2 f^2 + b_3 f^3 (1-t)^3}{b_3 f^3} \tag{3.20}$$

This is a case perfectly analogous to that seen before (Fig. 3.21), yielding a LR of the order of magnitude of 10^9 (this large value is also due to the background scenario, for which no foreign fibre is to be expected). It is over five billion times more likely that the fibres found on the knife were there because it had been used for the stabbing, rather than by chance.

The formal treatments of the second and third hypothesis are similar. In both cases (3.19) must be used. Figure 3.21 shows a plot of the LR as a function of t, the probability that the fibres were transferred, persisted and were recovered as a

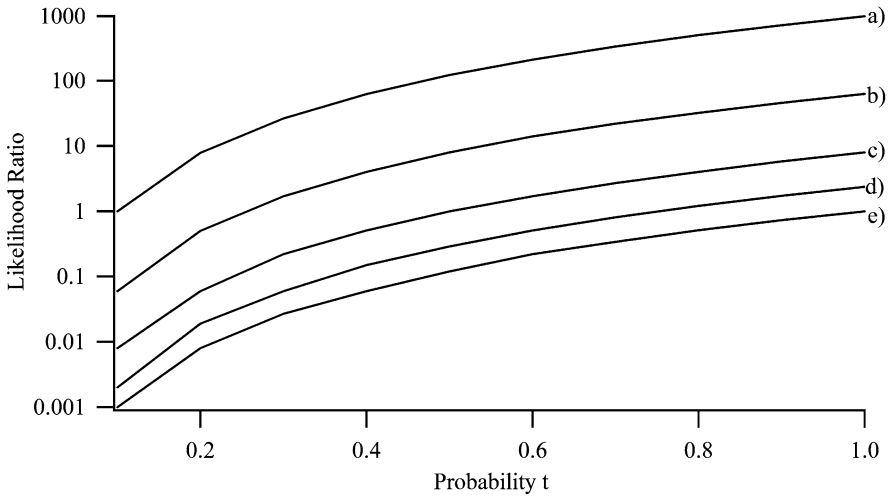


Fig. 3.21 Likelihood ratio vs. the probability of transfer, persistence and recovery under the prosecution hypothesis, t, for different values of the probability of transfer, persistence and recovery according to the defense proposition, t_1: (*a*) $t_1 = 0.1$, (*b*) $t_1 = 0.25$, (*c*) $t_1 = 0.5$, (*d*) $t_1 = 0.75$, (*e*) $t_1 = 1$

Table 3.8 Qualitative scale for reporting the support of evidence for H_1 against H_2

LR value	Formulation for reporting the results
$1 < LR \leq 10$	Limited evidence to support
$10 < LR \leq 100$	Moderate evidence to support
$100 < LR \leq 1{,}000$	Moderately strong evidence to support
$1{,}000 < LR \leq 10{,}000$	Strong evidence to support
$10{,}000 < LR$	Very strong evidence to support

consequence of the stabbing, for different values of t_1, viz. the probability that the fibres were transferred, persisted and were recovered in the context of each of the defense hypotheses.

If the knife was the weapon used to stab the victim (as proposition H_1 says), there would be a high probability that fibres were transferred on it, so according to proposition H_1, t should be estimated as approaching the value of 1.

The t_1 value associated to hypotheses 2 and 3 set forth by the defense is different. It is not very probable that a simple accidental cut of a windcheater could bring about the transfer of material on the whole area of the blade and on the handle. The presence of fibres between blade and handle implies that the knife was deeply and forcefully plunged in the fabric, a fact that is inconsistent with an accidental action. Under hypothesis 2 a low value for t_1 should be thus chosen, not larger than 0.25, yielding a LR between 60 and 1,000.

Evett and coworkers [84] proposed a qualitative scale for reporting the value of evidence. Table 3.8 gives these interpretational guidelines.

According to Evett's verbal scale for reporting LRs, under hypothesis 2, the evidence supplies moderate to moderately strong support to the proposition that the knife was used in the stabbing.

If hypothesis 3 is taken into account, that the knife was used for stabbing, but not against the victim, but against another person, t_1 should have a large value, approximately as that of t. The LR approaches 1 and the analyses turn out to be inconclusive in the determination if the knife was the weapon used in this specific crime. Fibre analysis can tell investigators just if the weapon was used to pierce someone's garments, but it is not a means of personal identification.

From the examples above, it has been shown how the defendant's version can greatly diminish the evidential value of fibres if only the LR is considered. In the case of hypothesis 3 the usefulness of these traces seems to have been cancelled. This is because the LR has been calculated using just the variables strictly pertaining to the comparison of fibres. Hypothesis 3 implies, though, that the suspect stabbed a burglar, a week before the murder which was allegedly committed with his knife. According to his version, the burglar should have worn a windcheater exactly analogous to that of the victim. How likely is that? The forensic scientist should accompany his results and his calculation of the LR with a market research on how common that specific windcheater is. With this information, along with a confirmation from the police records that a burglary actually occurred in the suspect's house as he declares, the judge will be able to decide if the defendant's version is acceptable and likely, or if it is a lie.

References

1. Causin V, Marega C, Schiavone S (2005) Cuts and tears on a paper towel: a case report on an unusual examination of damage. Forensic Sci Int 148:157
2. Locard E (1920) L'enquète criminelle et les méthodes scientifiques. Flammarion, Paris
3. Locard E (1923) Manuel de technique policière. Payot, Paris
4. Kirk PL (1953) Crime investigation: physical evidence and the police laboratory. Interscience, New York
5. Robertson JR, Grieve M (eds) (1999) Forensic examination of fibres. Taylor and Francis, London
6. Curran JM, Hicks TN, Buckleton JS (eds) (2000) Forensic interpretation of glass evidence. CRC Press, Boca Raton
7. Pounds CA, Smalldon KW (1975) The transfer of fibres between clothing materials during simulated contacts and their persistence during wear. Part 1: fibre transference. J Forensic Sci Soc 15:17
8. Pounds CA, Smalldon KW (1975) The transfer of fibres between clothing materials during simulated contacts and their persistence during wear. Part 2: fibre persistence. J Forensic Sci Soc 15:29
9. Wiggins KG, Emes A, Brackley LH (2002) The transfer and persistence of small fragments of polyurethane foam onto clothing. Sci Justice 42:105
10. Dachs J, McNaught IJ, Robertson J (2003) The persistence of human scalp hair on clothing fabrics. Forensic Sci Int 138:27
11. Dawson LA, Hillier S (2010) Measurement of soil characteristics for forensic applications. Surf Interface Anal 42:363
12. Bull PA, Morgan RM, Sagovsky A et al (2006) The transfer and persistence of trace particulates: experimental studies using clothing fabrics. Sci Justice 46:185
13. Sleeman R, Burton IFA, Carter JF et al (1999) Rapid screening of banknotes for the presence of controlled substances by thermal desorption atmospheric pressure chemical ionisation tandem mass spectrometry. Analyst 124:103
14. DeBattista R, Tidy H, Thompson TJU et al (2014) An investigation into the persistence of textile fibres on buried carcasses. Sci Justice 54:288
15. Reed G, Lofts C, Coyle T (2010) A population study of polyurethane foam fragments recovered from the surface of 100 outer-garments. Sci Justice 50:127
16. Ribaux O, Margot P (1999) Inference structures for crime analysis and intelligence: the example of burglary using forensic science data. Forensic Sci Int 100:193
17. Ribaux O, Walsh SJ, Margot P (2006) The contribution of forensic science to crime analysis and investigation: forensic intelligence. Forensic Sci Int 156:171
18. Esseiva P, Ioset S, Anglada F et al (2007) Forensic drug intelligence: an important tool in law enforcement. Forensic Sci Int 167:247
19. Ribaux O, Baylon A, Roux C et al (2010) Intelligence-led crime scene processing. Part I: forensic intelligence. Forensic Sci Int 195:10
20. Ribaux O, Baylon A, Lock E et al (2010) Intelligence-led crime scene processing. Part II: intelligence and crime scene examination. Forensic Sci Int 199:63
21. Lociciro S, Esseiva P, Hayoz P et al (2008) Cocaine profiling for strategic intelligence, a cross-border project between France and Switzerland: part II. Validation of the statistical methodology for the profiling of cocaine. Forensic Sci Int 177:199
22. Lociciro S, Hayoz P, Esseiva P et al (2007) Cocaine profiling for strategic intelligence purposes, a cross-border project between France and Switzerland: part I. Optimisation and harmonisation of the profiling method. Forensic Sci Int 167:220
23. Esseiva P, Dujourdy L, Anglada F et al (2003) A methodology for illicit heroin seizures comparison in a drug intelligence perspective using large databases. Forensic Sci Int 132:139
24. Kelly JS, Lindblom BS (eds) (2006) Scientific examination of questioned documents, 2nd edn, Taylor and Francis. Boca Raton, FL

25. Smalldon KW, Moffat AC (1973) The calculation of discrimination power for a series of correlated attributes. J Forensic Sci Soc 13:291
26. Gothard JA (1976) Evaluation of automobile paint flakes as evidence. J Forensic Sci 21:636
27. Edmondstone G, Hellman J, Legate K et al (2004) An assessment of the evidential value of automotive paint comparisons. Can Soc Forensic Sci J 37:147
28. Ryland SG, Kopec RJ (1979) The evidential value of automobile paint chips. J Forensic Sci 24:140
29. Somerville PN, Ryland S, Kopec RJ (1981) The evidential value of automotive paint. Part II: frequency of occurrence of topcoat colours. J Forensic Sci 26:1
30. Buckle J, Fung T, Ohashi K (1987) Automotive topcoat colour occurrence frequencies in Canada. Can Soc Forensic Sci J 20:45
31. Flynn K, O'Leary R, Lennard C et al (2005) Forensic applications of infrared chemical imaging: multi-layered paint chips. J Forensic Sci 50:832
32. Aitken C, Taroni F (2004) Statistics and the evaluation of evidence for forensic scientists. Wiley, Chichester
33. Montgomery DC (2001) Introduction to statistical quality control. Wiley, New York
34. Morgan SL, Bartick EG (2007) Discrimination of forensic analytical chemical data using multivariate statistics in Forensic analysis on the cutting edge, ed. RD Blackledge. Wiley, Hoboken
35. Trejos T, Flores A, Almirall JR (2010) Micro-spectrochemical analysis of document paper and gel inks by laser ablation inductively coupled plasma mass spectrometry and laser induced breakdown spectroscopy. Spectrochim Acta B 65:884
36. Pearson K (1901) On lines and planes of closest fit to systems of points in space. Lond Edinb Dubl Philos Mag J Sci 2:559
37. Hotelling H (1933) Analysis of a complex of statistical variables into principal components. J Educ Psychol 24:417
38. Causin V, Marega C, Schiavone S et al (2006) Forensic analysis of acrylic fibers by pyrolysis-gas chromatography/mass spectrometry. J Anal Appl Pyrolysis 75:43
39. Kumooka Y (2011) Classification of OPP adhesive tapes according to pyrogram of adhesives. Forensic Sci Int 206:136
40. Yang L, Thomas P, Stuart B (2012) Discrimination of thermally treated low density polyethylenes using DSC and principal component analysis. J Therm Anal Calorim 108:445
41. Kher A, Stewart S, Mulholland M (2005) Forensic classification of paper with infrared spectroscopy and principal component analysis. J Near Infrared Spectrosc 13:225
42. Lewicki JP, Albo RLF, Alviso CT et al (2013) Pyrolysis-gas chromatography/mass spectrometry for the forensic fingerprinting of silicone engineering elastomers. J Anal Appl Pyrolysis 99:85
43. Deming SN, Michotte Y, Massart DL et al (1988) Chemometrics: a textbook. Elsevier, Amsterdam
44. Breretron RG (ed) (1992) Multivariate pattern recognition in chemometrics. Elsevier, Amsterdam
45. Kumooka Y (2009) Hierarchical cluster analysis as a tool for preliminary discrimination of ATR-FT-IR spectra of OPP acrylic and rubber-based adhesives. Forensic Sci Int 189:104
46. Everitt BS, Landau S, Leese M et al (2010) Cluster analysis. Wiley, Chichester
47. Parsons NS, Mountain C (2007) Investigating polyurethane foam as a form of trace evidence. Sci Justice 47:24
48. Wiggins KG, Emes A, Brackley LH (2002) The transfer and persistence of small fragments of polyurethane foam on clothing. Sci Justice 42:105
49. Jones J, Coyle T (2011) Synthetic flock fibres: a population and target fibre study. Sci Justice 51:68
50. Marnane RN, Elliot DA, Coulson SA (2006) A pilot study to determine the background population of foreign fibre groups on a cotton/polyester T-shirt. Sci Justice 46:215
51. Palmer R, Burch HJ (2009) The population, transfer and persistence of fibres on the skin of living subjects. Sci Justice 49:259

52. Watt R, Roux C, Robertson J (2005) The population of coloured textile fibres in domestic washing machines. Sci Justice 45:75
53. Fong W, Inami SH (1986) Results of a study to determine the probability of a chance match occurrence between fibers known to be from different sources. J Forensic Sci 31:65
54. Roux C, Margot P (1997) The population of textile fibres on car seats. Sci Justice 37:25
55. Roux C, Margot P (1997) An attempt to assess the relevance of textile fibres recovered from car seats. Sci Justice 37:225
56. Cantrell S, Roux C (1999) The population of textile fibres on cinema seats in the Sydney region. In: Proceedings of the 7th European fibers group meeting, Zurich
57. Grieve MC, Biermann T (1997) The population of coloured textile fibres on outdoor surfaces. Sci Justice 37:231
58. Rothe M (1997) Examination of foreign fibre populations. In: Proceedings of the 5th European fibers group meeting, Berlin
59. Massonnet G, Schiesser M, Champod C (1998) Population of textile fibres on white T-shirts. In: Proceedings of the 6th European fibers group meeting, Dundee
60. Was-Gubala J, Chochól A (2000) Population study of fibres found on bus seats in Cracow. In: Proceedings of the 8th European fibers group meeting
61. Pukanszky B, Mudra I, Staniek P (1997) Relation of crystalline structure and mechanical properties of nucleated polypropylene. J Vinyl Add Tech 3:53
62. Wiggins K, Drummond P, Hicks Champod T (2004) A study in relation to the random distribution of four fibre types on clothing (incorporating a review of previous target fibre studies). Sci Justice 44:141
63. Cook R, Webb-Salter M, Marshall L (1997) The significance of fibres found in human head hair. In: Proceedings of the 5th European fibers group meeting, Berlin
64. Yan Z, Chen S, Wang H et al (2008) Biosynthesis of bacterial cellulose/multi-walled carbon nanotubes in agitated culture. Carbohydr Res 74:659
65. Cook R, Webb-Salter MT, Marshall L (1997) The significance of fibres found in head hair. Forensic Sci Int 87:155
66. Cook R, Wilson C (1986) The significance of finding extraneous fibres in contact cases. Forensic Sci Int 32:267
67. Jackson G, Cook R (1986) The significance of fibres found on car seats. Forensic Sci Int 32:275
68. Palmer R, Chinherende V (1996) A target fiber study using cinema and car seats as recipient items. J Forensic Sci 41:802
69. Grieve MC, Biermann T, Davignon M (2003) The occurrence and individuality of orange and green cotton fibres. Sci Justice 43:5
70. Alwattari AA, Lloyd DR (1998) Isothermal kinetics of isotactic polypropylene-hexamethylbenzene blends: kinetic analysis. Polymer 39:1129
71. Brüschweiler W, Grieve MC (1997) A study on the random distribution of a red acrylic target fibre. Sci Justice 37:85
72. Kelly E, Griffin RME (1998) A target fibre study on seats in public houses. Sci Justice 38:39
73. Palmer R, Burnett E, Luff N et al (2015) The prevalence of two 'commonly' encountered synthetic target fibres within a large urban environment. Sci Justice. doi:10.1016/j.scijus.2015.01.001
74. Grieve MC (2000) A survey on the evidential value of fibres and on the interpretation of the findings in fibre transfer cases. Part 1—fibre frequencies. Sci Justice 40:189
75. Buzzini P, Massonnet G (2004) A market study of green spray paints by Fourier transform infrared (FTIR) and Raman spectroscopy. Sci Justice 44:123
76. Wright DM, Bradley MJ, Mehltretter AH (2011) Analysis and discrimination of architectural paint samples via a population study. Forensic Sci Int 209:86
77. Moore R, Kingsbury D, Bunford J et al (2012) A survey of paint flakes on the clothing of persons suspected of involvement in crime. Sci Justice 52:96

78. Cook R, Evett IW, Jackson G et al (1999) Case pre-assessment and review in a two-way transfer case. Sci Justice 39:103
79. Cook R, Evett IW, Jackson G et al (1998) A model for case assessment and interpretation. Sci Justice 38:151
80. Cook R, Evett IW, Jackson G et al (1998) A hierarchy of propositions: deciding which level to address in casework. Sci Justice 38:231
81. Causin V, Schiavone S, Marigo A et al (2004) Bayesian framework for the evaluation of fiber evidence in a double murder—a case report. Forensic Sci Int 141:159
82. Champod C, Taroni F (1997) Bayesian framework for the evaluation of fibre transfer evidence. Sci Justice 37:75
83. Evett IW (1986) A bayesian approach to the problem of interpreting glass evidence in forensic science casework. J Forensic Sci Soc 26:3
84. Evett IW, Jackson G, Lambert JA et al (2000) The impact of the principles of evidence interpretation on the structure and content of statements. Sci Justice 40:233

Chapter 4
Polymers on the Crime Scene

Some polymers are more common than others, and so they were more deeply studied, researched and reported by forensic scientists. For these kinds of items, protocols and analytical guidelines are available, and in some cases abundant literature was published. The aim of this chapter is presenting a summary of the types of polymeric items most commonly encountered in casework, in order to show the reader the state-of-the-art in this branch of forensic analysis. An examination of the existing literature on these materials is also informative to understand the issues and the features of well-established analytical strategies, and to catch how the many concerns and requirements discussed in Sect. 3.2 can be satisfied. For each kind of evidence, the chemical composition, the manufacturing process, the issues regarding recovery and sample preparation and the techniques useful for its characterisation will be presented.

4.1 Textile Fibres

Single textile fibres are quite frequently encountered on crime scenes and can be very useful contact traces for the reconstruction of the events.

Analyses carried out on fibres can be aimed at comparison or identification. However, most of the times the focus is on comparison, because just in a few cases it is possible to identify features which lead to a particular manufacturer or application. An example of this latter possibility are fibres used for carpeting, which have frequently a multilobal cross-sectional shape, useful for responding to compressive stress. When a suspect has not been identified yet, fibres of peculiar colour or uncommon chemical nature can also offer leads to investigators, indicating that they should look for individuals owning clothes of a particular colour or made with a rare textile or for places where furniture may contain a certain kind of fibres.

Textile fibres are one of the commodities that from the most ancient times have been employed by mankind for a multitude of purposes, such as protection and adornment of bodies or objects. In a wide sense, the term can indicate all those materials

V. Causin, *Polymers on the Crime Scene*,
DOI 10.1007/978-3-319-15494-7_4

that, irrespective of the fact that they are apt to constitute yarns or threads, have a length which is much larger than their diameter or cross-sectional size.

4.1.1 A Classification of Fibres

Fibres, according to their origin, can be categorised as natural, artificial or synthetic.

Natural fibres are those that occur in nature. They can be divided into three subclasses: animal, vegetal and mineral.

Animal fibres include ovine wool, the hair of various animals (goat, cashmere goat, angora goat and rabbit, alpaca, vicuña, llama, camel, etc.) and silk. Wool and fur are mainly composed of cheratin, a sulphur-containing protein.

Vegetal fibres are cellulose-based materials that are named after the part of the plant which is the source of the fibre: they can be seed (cotton, kapok), bast (flax, hemp, jute) or leaf fibres (sisal).

The only naturally occurring mineral fibres are asbestos, hydrate magnesium silicate minerals that readily separate into long flexible fibres, that have been implicated as causes of certain cancers, and that have been formerly used especially as fireproof insulating materials. Differently from those listed above and below, asbestos are not polymeric, but are rather very big crystals with a fibrillar shape.

Artificial fibres are produced by man employing naturally occurring or synthetic polymers as raw materials. The natural starting matter from which artificial fibres are produced can be animal or vegetal fibres. The most important artificial fibres are derivatives of cellulose. Their production sequence includes dissolution and depolymerisation of cellulose to obtain a cellulose derivative (cellulose xanthate, acetate ester of cellulose, etc.) that is extruded, spun and solidified by evaporation of the solvent or coagulation. During the final solidification stage, the filaments may be regenerated back to cellulose, as happens in viscose, modal or rayon cupro, or remain as derivatised cellulose, as in the case of cellulose esters, acetate or triacetate.

Further items of this category are carbon fibres. Although not used in the textile industry and very rarely found on crime scenes, they are noteworthy because they are increasingly being employed in the manufacture of plastic composite materials, due to the optimum performance they confer. They are prepared from synthetic polymers, mainly polyacrylonitrile, by pyrolysis and carbonisation.

Synthetic fibres are produced directly from the monomer, which is in turn usually derived from raw materials such as coal or oil. A wide variety of polymers is employed by the industry, in order to meet all the requirements, in terms of dynamical mechanical characteristics, morphology and, broadly speaking, performance, posed by the customers. The synthesis and processing of each fibre put on the market is engineered with an end-use-oriented point of view.

The most common synthetic fibres found on crime scenes [1–4], are polyesters (mostly poly(ethylene terephthalate)), acrylics (predominantly copolymers of polyacrylonitrile with vinyl acetate, methyl acrylate and methyl methacrylate) and polyamides (mainly nylon 6 and 66), because they are widely used in garments and textiles. Polyolefins, chloro- and fluoropolymers and polyurethanes are limited

to niche-usages like ropes, cinema seats and bathing suits. These not ordinarily encountered fibres have the advantage, from the forensic scientist's standpoint, of having a larger evidential value.

The industrial production of man-made synthetic fibres is essentially divided into two stages: polymerisation and spinning.

Spinning is the process by which the polymer, in a viscous form, either in solution or in the molten state, is forced through the holes of a spinneret. Three different spinning technologies exist: wet spinning, dry spinning and melt spinning.

In both wet and dry spinning, solutions of the polymer are used. In the wet process, the solvents are either aqueous or organic and the filaments are immediately precipitated in a coagulation bath after extrusion. The dry spinning technique makes use of volatile solvents that are removed by evaporation. Finally, in the melt spinning procedure the polymer is melted, extruded and solidified just by cooling. It is a very simple and convenient process, applicable to thermoplastics that don't degrade with temperature.

Natural fibres tend to be quite affected by unpredictable changes in morphology (so no fibre can be deemed completely similar to another) and they are very uniform in terms of composition. Synthetic fibres, on the other hand, carry a much larger load of information. Depending on the production process, the microstructure of the polymer changes and thus its properties vary. The exploitation of this 'predictable variability' is very useful if the polymer characterisation is performed for forensic science purposes. If two cotton fibres coming from the same garment are compared, chances that they are different are quite high. As a natural product, cotton cannot be as homogeneous as an industrially produced acrylic, for example. The evidential weight of such an item would then be inevitably low. In fact, in case of a comparison, if differences are reported, they could be attributed to natural variations, while in case of compatibility, a simple coincidence could not be ruled out. This difficulty in correctly assessing the value of the evidence eventually turns out to be detrimental to the criminal trial, because it induces doubt and offers the possibility for the parties to the case to stretch the facts in their own interests.

4.1.2 Technological Requirements for Textile Fibres

Textiles are made of yarns (or more generically threads), that are composed of twisted fibres or filaments.

According to the end-use for which they were intended, fibres must have specific mechanical performances. Tenacity, resistance to traction, hygroscopicity, flexibility and elasticity are just a few of all the parameters that must be taken into account to manufacture an optimal product.

An accurate choice of the most suitable polymer can greatly help in defining a good starting point to create a fibre with the desired characteristics. Dynamic-mechanical properties can be conferred to fibres in the drawing stage that immediately follows spinning. The filaments are mechanically stretched in order to orient the polymer chains in the direction of the axis of the fibre. This causes an increase in polymer crystallinity and in fibre strength. Polymers for fibres are mostly semi-

crystalline, this means that their structure is composed of crystalline regions and amorphous zones. The overall properties of the fibre are heavily dependent on the extent to which either region predominates over the other within the structural framework.

Another quite important technological parameter is the titre, the linear density of fibre. It is a quantity that indicates how thin a fibre is and it is defined as the mass corresponding to a yarn of a certain length. The more commonly used units are the count denier (den), the mass in grams of a yarn 9,000 m long, and the basic tex unit (tex), the mass in grams of a yarn 1,000 m long, especially with its subunit dtex, which is the mass referred to a yarn of 10,000 m.

A further way of modifying the performance of a fibre is by changing its cross-sectional shape. This feature can vary the mechanical qualities of filaments, as well as different aspects like water uptake or the thermal properties of a cloth. For instance, if the shapes of fibres are engineered in such a way as to capture more air than with the usual circular cross-section, the cloth obtained will have better insulating properties.

4.1.3 Transfer and Persistence of Fibres

The mechanisms of transfer of fibres have been extensively studied in the past years [5–14]. Transfer can be defined as the loss of a fibre from a garment (donor), to an individual, a surface, an object, another item of clothing (recipient). The processes by which transfer of fibres occurs are essentially three [6]:

- Transfer of loose fibre fragments that are already on the surface of the donor.
- Extraction by friction of fibres that are weakly held by the weave of the fabric.
- Diffusion of fragments of fibres, broken by mechanical stress associated to contact.

The extent to which each of these mechanisms takes place is strictly dependent on the intensity of the contact and on the type of fibres that are transferred. The most important factors affecting the efficiency of transfer are [5]:

- The spatial extension, the strength and duration of the contact: more fibres will be transferred if a high pressure is applied for a long time on a large area.
- The number of contacts: repeated contacts can transfer back fibres from the recipient to the donor.
- The nature of the donor textile and its wear status: a lacerated and coarse cloth will release far more fibres than a smooth and intact fabric.
- The nature of the recipient textile: a roughly textured weave will be more efficient in holding transferred fibres.
- The morphological features of the fibres, shorter fragments being transferred in larger numbers than long ones.

Pressure of contact is important: it increases the number of transferred fibres, but up to a certain point, beyond which the effect of further augmentations of the pressure is negligible.

The fibre types that are more efficiently transferred are cotton, acrylic and wool, with respect to viscose or polyester.

Moreover, the differential shedding phenomenon could take place. If the donor item is a mixed fabric, fibres will not necessarily be transferred with the same proportion to the textile composition.

Persistence depends on the nature of the fibres and of the recipient object [6, 7, 13, 15–17]. Generally speaking, four main factors decrease the persistence of transferred fibres:

- The wearing of the recipient garment: if the item is worn or moved, many of the transferred fibres will be lost to the surrounding environment.
- The covering of the recipient with other garments: this would enhance the possibility of secondary transfer.
- The area on which fibres have been transferred: elbows and back have numerous contacts each time a person sits down or works on a desk.
- If the initial contact has occurred with low pressure.

It has been proposed [5] to divide transferred fibres into three categories: loosely bound, bound and strongly bound. After transfer, the first items to be lost will be the loosely bound, followed by the bound ones. Strongly bound fibres, which are physically constrained inside the weave of the textile, are the last to be dispersed.

Short and curly fibres persist for longer times on the recipient, because they more easily adapt into the framework of the fabric's threads. Persistence is increased on recipient items with wet or adhesive surfaces.

Simulation experiments [18] showed that about 80 % of the transferred fibres are lost within the first 4 h after contact, with just 5–10 % persisting after 24 h. On the other hand, it is not uncommon that fibres have resisted on the recipient garment after it has been worn for ordinary activities, washed, or even subject to harsh conditions. Transferred fibres resisted after 14 days on buried carcasses, or fibres determinant for the resolution of a case were retrieved after 29 days since the crime [17, 19].

4.1.4 Recovery of Fibres

Contamination is a very critical issue in fibre casework. Different operators should be in charge of examining the crime scene and the suspect's belongings, in order to avoid cross contamination. The same principle holds when clothing or textiles from both the victim and the suspect are available for inspection. In such case they should be examined in different rooms and by different operators.

The conditions of the donor cloths should also be noted, in order to be able to estimate their shedding capability or their aptness for transfer or persistence.

As broadly described in Sect. 3.1, an accurate visual inspection is the first step of the examination of every item or location on the crime scene, in order to detect if there are any traces that could be directly lifted with tweezers. After all the visible material has been retrieved, the most commonly used method for recovering transferred fibres from the recipient object is by tape lifting (Sect. 3.1).

Adhesive sheets should be preferred to rolls, because the former can be cut in whatever size and shape is necessary. Moreover, a very functional aspect of adhesive sheets is that the protecting paper can be detached at the moment of usage. The sides of traditional adhesive tape rolls, on the contrary, expose some glue, so there is the risk that some airborne fibres or debris remain attached and thus contaminate the evidence.

The retrieval of the evidential items trapped between the adhesive tape and the acetate sheet is easily done by cutting a small window with a scalpel and by dissolving the glue with a drop of solvent. The kind of solvent depends on the kind of tape, and can be as simple and harmless as a water/ethanol solution, or an organic solvent requiring more safety precautions such as toluene.

The approach normally chosen during routine crime scene inspections is that of covering, with the same piece of adhesive paper, the widest possible area, in order to minimise the number of substrates to be analysed in the lab. In certain cases, though, it can be important to maintain information on the exact position of the fibres on the crime scene, so '1:1 taping' is employed. This is a technique where one area of taping exactly represents the same area on the surface from which fibres were removed [20]. It is not a frequently used method, because even small areas can yield a very large quantity of recovered material to be analysed: For example the 1:1 taping of a 1 m^2 surface implies using (and successively analysing) 1 m^2 of adhesive paper. 'Pseudo 1:1 taping' can be a good alternative when it is possible to divide the area to examine into smaller regions [21]. In such case, the same piece of adhesive sheet is used for sampling an area somewhat larger than its size. Figure 4.1 shows an example of how the trunk of a car was divided into 14 regions, about 20×20 cm^2

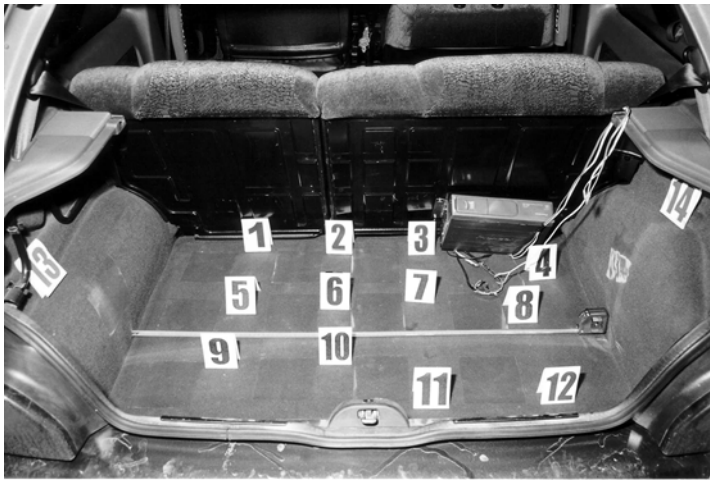


Fig. 4.1 The trunk of this car was divided into 14 areas, each one about 20×20 cm^2 in size, which were sampled by repeatedly using 10×10 cm^2 pieces of adhesive tape. Reprinted from [21], copyright 2004, with permission from Elsevier

in size, which were sampled by 10×10 cm^2 of adhesive paper. It was therefore possible to retain information on the position of the traces, while keeping the number of items recovered at an acceptable level.

When both the garments of the suspect and the victim are available, tape lift on these latter textiles is recommended as well. It should not be forgotten that transfer due to contact is a two-way phenomenon: fibres can pass from the assailant to the victim, but also vice versa.

4.1.5 The Characterisation of Textile Fibres

Typically, in fibre-related cases the aim is to examine a sample of unknown origin for determining if it could share a common origin with fibres from a known source. To make an example, the fibres found on a mattress in the suspect's basement (pool of known source) are compared with the extraneous fibres found on the clothes of the victim of a kidnapping (pool of unknown source).

A typical flowchart which can be followed as a protocol for the forensic examination of fibres is reported in a fundamental reference for the forensic scientist interested in fibre analysis [22].

The typical comparative forensic analysis of textile fibres starts with a thorough characterisation of the items of known origin, i.e. garments, beddings or furniture pertaining to either a suspect or the victim or to the crime scene. Among these, target fibres are identified, to be searched within the pool of the fibres of unknown source.

A few golden rules assist the operator in choosing the most suitable target fibres from those involved in the case. It is usually better to avoid:

- Very common fibres. White cotton and blue cotton coming from denim are everywhere, it is not particularly significant to find them on the crime scene. However, it should be noted that the concept of commonness must always be interpreted in the perspective of the case. For example, the fibres of a military uniform are a very rare item in a school, but they become ubiquitous in an army base.
- Fibres that come from garments with poor sheddability.
- Colourless fibres, because an important term of comparison like colour would be missing in their characterisation.
- Fibres with very variable and hardly comparable characteristics (some natural fibres have this problem).

As introduced in Chap. 3, fibres are the forensic items for which the widest array of statistical data exist. Population studies can be used to assess what is the frequency of a particular kind of fibre in environments similar to those relevant for the case [1, 4, 13, 23–31]. Target fibre studies are further tools which help to estimate the evidential value of a particular kind of fibre in a particular environment [12, 25, 26, 32–38].

Points to be considered in the choice of target fibres are:

- Darkly hued fibres are to be preferred because the more intense the colour, the more reliable microspectrophotometry can be in contributing to the comparison.
- The more uncommon fibres are, the more useful they are, because the possibility that they were present by pure chance on the crime scene is very unlikely.
- Damaged garments will be much more prone to transferring fibres.
- Fibres which are fluorescent under UV illumination would be very easy to search for.
- Fibres which starkly differ from those of the garments of the other persons involved in the facts are to be preferred, not only because they would stand out during searching, but also because their presence could not be attributed to chance.

The aim that should be kept in mind is that of being able to exclude, at the end of the analysis, that the recovered fibres where on the crime scene because of coincidence or chance, thus helping in outlining, corroborating or confirming the reconstruction of the events.

Target fibres will be searched for among the items recovered on the crime scene (or more generally among the fibres whose origin is not known).

The analysis of the material obtained by tape lifting is a very tedious and demanding job. In order to facilitate and make more objective the activity of the analyst, automatic fibre finding systems were developed [39, 40]. These devices are composed by a microscope, interfaced with a digital camera connected to a computer, with a movable sample holder. The first operation consists in defining the colour of the target fibre in terms of its colourimetric coordinates. Then, the adhesive sheet used for tape lifting is posed on the movable sample holder and the instrument automatically scans all the area of the sampling, identifying any object with a colour compatible to that of the target fibre. At the end of the search, a 1:1 map of the sheet is available, and it is possible to quickly retrieve the position of the potentially compatible fibres, on the basis of colour, with the targets. Such potentially compatible items can be separated from the adhesive by a solvent and subsequently analysed in more detail by an array of analytical methods.

The analytical procedure used for the forensic characterisation of fibres starts with an observation under a microscope, first at low magnification through a stereomicroscope or a fibre finder, and then at a higher magnification at the comparative microscope. Fibres potentially compatible with the target fibres on a morphological basis will be more deeply characterised by instrumental analytical techniques.

IR spectroscopy, usually IR microspectrophotometry, is often the first step, because it allows to identify the chemical nature of the fibre [41–52]. Raman spectroscopy is another vibrational spectroscopy, able to complement IR spectra [53–60]. If the fibres are coloured, chromatographic or spectroscopic techniques are extremely useful for obtaining significant and very discriminative data [34, 35, 38, 54–57, 59–76]. Complementary data such as elemental analysis, pyrolysis/gas chromatography/mass spectrometry, the measurement of refractive index and others can complete the picture, especially in the case of undyed fibres [77–82].

Case reports will be useful guides to understand the peculiarities of casework on fibres [17, 21, 83, 84].

Many examples and details on the individual techniques mentioned above will be singularly introduced in the rest of this book, presenting their potential for the analysis of polymers of forensic interest.

4.2 Paints

Paints are another very important class of polymeric items often involved in forensic casework.

A relatively recent statistical report indicated that, just in the 6 months between January and June 2010, in Italy, 249 hit-and-run accidents happened which killed 38 and injured 346 persons. However, investigations led to the identification of the offenders in 78 % of the cases. These positive data can definitely be ascribed to the contribution of forensic science to the traditional investigative action. Paints are contact traces. Paint fragments or smears are found in almost every car accident scene. The collisions which may generate transfer of paint from one object to another are collisions between vehicles, or between vehicles and other objects present on the road (Fig. 4.2).

It is not infrequent that paint smears may be found on the garments of pedestrian, cyclist and motorcyclist victims of accidents with cars (Fig. 4.3).

Fig. 4.2 A paint smear on a piece of marble, allegedly left by a van hitting a wall

Fig. 4.3 (*Left*) Garment of the victim of a car accident. The *circles* show the location of paint smears left by the car hitting the pedestrian. (*Right*) A detail of one of the paint traces

Transfer of paint is not necessarily associated just to car accidents and hit-and-run episodes, but it can be linked to any crime which is committed using a car. Cars or other vehicles are used to escape after robberies, to transport illicit drugs, victims of homicides or of kidnappings, to break through doors or gates when committing theft or robberies. In many of these instances collisions of the vehicle with objects on the road can lead to the shedding of a paint fragment, which in turn can offer useful investigative leads on the kind of vehicle used by the felons.

Architectural paints [85–90] can be found on objects used to break into houses or building, and then can be used to connect to the committed burglary the suspect holding the implements with these traces.

4.2.1 The Composition of Paints

The ultimate scope of any paint is to protect a surface from damages due to weathering, corrosion or contact with chemicals. In addition to this functional role, paints often have also the aim of improving the aesthetic appearance of the objects they coat. Paints can be commercialised in powder, paste or liquid form, but they can be broadly described as mixtures of pigments and other additives together with a binder. In the case of liquid paints, a further important component is a solvent, which tunes the viscosity and the density of the product. The paints most frequently encountered in forensic casework are definitely car paints, which are mostly powder coatings in which the binder is present in amounts ranging from 50 to 60 %; the rest of the formulation is pigments (about 40 %) and inerts and additives. The qualitative and quantitative composition of the paint determines the properties of the final coating, the conditions of applications and the suitability for the surface that one intends to cover [91–93].

Binders are polymers with variable molecular weight whose scope is to form a continuous film which is able to coat the recipient surface and to retain on such surface all the other components of the paint, such as pigments and additives.

Sometimes binders with a large molecular weight are used, among which we find polyacrylates, copolymers of polyvinylchloride (PVC) and nitrocellulose. However, more commonly, oligomeric precursors are used, which are then crosslinked yielding a continuous film, covering in a durable manner the recipient surface. Among these precursors with a relatively low molecular weight, natural or synthetic resins can be found:

- Natural resins such as rosin (a resin extracted from conifers).
- Alkyd resins. These thermoset polyesters are produced, as other polymers of the same class, from acids or anhydrides and alcohols with multiple hydroxyl groups. Further modification of the obtained resin is achieved by adding fatty acids, oils or other resins. When these compounds are added, the result is a branched polyester containing fatty-acid side groups. Subsequently, the oil portion of the polyester undergoes a crosslinking reaction in the presence of oxygen from air as it dries, yielding a tack-free film. The most commonly employed acids are phthalic or fumaric acids, and among the alcohols there are glycerol, propylene glycol, pentaerythrol, etc. Common fatty modifiers are tung oil, linseed oil or dehydrated castor oil.
- Epoxy resins. These polyethers are obtained from a crosslinking reaction which exploits the ring opening of epoxide moieties contained in prepolymers. Such precursors are usually obtained by introducing epoxide rings in species such as bisphenol-A, novolacs (condensation products of phenol and formaldehyde), aromatic amides, aliphatic alcohols or polyols and aromatic amines. The products of these reactions are usually viscous liquids, which, when mixed with a crosslinker based on polyfunctional amines, acid anhydrides, phenols or thiols harden into a thermoset solid. These resins, used as glues as well, have a remarkable mechanical performance and are quite resistant to heat and chemical attacks. However, they tend to deteriorate upon exposure to UV radiation. Due to their remarkable mechanical properties and durability, epoxy resins are becoming a viable alternative to tiles and marbles for flooring, especially because they can be formulated in any colour and in very aesthetically pleasant patterns.
- Acrylic resins. Thermoset acrylic resins find their main industrial application as paints, either as water-borne emulsion paints or as solvent-borne paints. Their tough protective finish makes them suitable as topcoats, for example on car bodies. These copolymers of monomers derived from acrylic and methacrylic acids bear, both within the polymer chain and in its ends, functional groups such as hydroxyls, carboxylic acids or amides, to name just a few. In addition to tuning the chemical nature of the molecule, and thus its properties, these groups allow crosslinking with different species, such as alkyd, aminic, polyurethane or epoxy resins. This flexibility in the chemical design makes this class of materials suitable for adhesion on a variety of surfaces.
- Polyurethane resins are prepared reacting a prepolymer with isocyanate endgroups with hydroxyl-rich resins, such as polyesters, polyols, alkyd or acrylics. The flexibility of these latter species impart to the polymer its macroscopic properties. The requirements of a good coating are hardness and shape stability,

so the molecules composing the paint will preferentially be more rigid than flexible and contain several crosslinking points, in order to guarantee a thorough hardening of the resin.

- Aminic resins. These resins are obtained as condensation products of urea or melamine. The second reagent, which makes the crosslinking reaction possible in these cases can be as simple as formaldehyde, or, more frequently in the paint industry, polyester, epoxy, alkyd or acrylic resins.

The most commonly used binders are synthetic resins such as acrylic, alkyd, polyester or epoxy. The type of binder determines many final properties of the paint, such as the method of application, its hardness, its adhesive strength to the substrate, its mechanical properties and its resistance to chemicals or weathering.

The essential requirement that a binder has to satisfy is the filmogenic power, i.e. the capacity to provide a continuous film on top of the surface, which prevents penetration of agents like oxidising gases, water or other chemicals. The importance of the binder for the formulation of paints is reflected by the fact that commercial paints are classified on the basis of the type of binder they contain.

Pigments are materials, insoluble in the matrix where they are dispersed, in this case the binder, which are responsible for the colour and the covering power of paint. Sometimes pigments are used to support the functionality of paints, especially in its role as an anticorrosive. They are generally crystalline solids, finely dispersed, and they represent a significant portion of paints, typically between 10 and 35 % in weight. An extremely common white pigment is titanium dioxide (TiO_2), which is often used also in conjunction with pigments of other colours to yield opacity to the coating. The covering power and depth of the colour depend on the particle size of the pigments. A general range of sizes can be between 0.1 and 2.0 μm. To be thoroughly dispersed, pigments must be wettable as efficiently as possible by the binder. If this happens, the macroscopic result on the coating will be an optimal stability, brightness and resistance under working conditions. Every paint has a typical concentration of pigment in volume (critical pigment volume concentration, CPVC) at which the binder is able to occupy all the available space between the pigment particles. Larger concentrations of pigments correspond to an inefficient wetting and dispersion, with a consequent decrease in properties and a faster deterioration of the coating. Pigments can be organic, inorganic, organometallic or metallic. Inorganic pigments are usually oxides or insoluble salts, such as, for example, the white pigments TiO_2, ZnO and $BaSO_4$. Inorganic pigments have a particularly high resistance to weathering, an excellent resistance to high temperature and light, but on the other hand they are not suitable for bright hues and they cannot reproduce any possible colour. Moreover, when they contain heavy metals they are subject to strict environmental and regulatory limitations, due to their potential toxicity. Pigments can have an organic nature as well. Many different classes of such materials exist, but they are mainly composed of highly conjugated systems: the most common categories are azo compounds, organometallic complexes or polycyclic molecules (anthraquinones, naphthoquinones, phthalocyanines, etc.). Their advantage is that they allow to render an extremely wide array of

hues, either by direct synthesis of new compounds, by functionalisation of existing pigments or by mixing of different pigments. The colours that can be obtained by organic pigments are brilliant, bright and very definite.

The disadvantage of organic pigments is their higher cost, with respect to inorganic ones, and this can limit their applicability in less added-value applications.

Additives are auxiliary chemicals which are added, in small amounts, to confer to the product particular properties, such as, for example, brightness, opacity, resistance to weathering or UV light, or to improve its mechanical performance. Usually they are present in percentages ranging from 0.1 to about 1 %. Additives can be classified as a function of the role they play in the formulation:

- Fillers. These additives are usually inorganic species such as talc, calcium sulphate, calcium carbonate, calcium carbide, aluminium and magnesium oxides, which are insoluble as pigments but are not coloured. They are used to modify the physical–mechanical performance of paints and they can increase the solid residue of the paint, and therefore the thickness of the coating.
- Driers accelerate the drying of the paints by catalysing the crosslinking of the binder. Their nature obviously depends on the chemistry of the coating system. In the case of binders which dry by exposure to air, they accelerate the decomposition of peroxides and hydroperoxides which are formed during the process, allowing the radicalic polymerisation of the binder. They are often inorganic salts of magnesium, zinc, calcium, barium or zirconium.
- Texturisers promote the formation of textures to the surface. If a smooth surface is desired, they are high-boiling point solvents such as butylether, ethylene glycol, cyclohexane or other aliphatic or aromatic hydrocarbons. Low molecular weight resins such as silicones can be employed as well.
- Filmogens are agents which reduce the temperature necessary for the formation of a film of coating, leading to a surface without pores and as uniform as possible. They are often high-boiling point esters, combined with hydrocarbons.
- Wetting agents, emulsifiers, stability enhancers are often the most important assets in the paint industry, because they allow to increase the shelf life of the product and to deliver a product which is easy to apply and that gives defect-free coatings. Their aim is to improve the wettability of the pigment by the binder, avoiding flocculation, aggregation or sedimentation phenomena, which would in turn bring about an inhomogeneous and flawed film.
- Protectors such as fungicides, biocides and insecticides prevent attacks from fungi, bacteria and insects. The role of corrosion inhibitors is to slow down the corrosion rate of the substrate. UV stabilisers protect the paints from degradation due to ultraviolet light irradiation.
- Plasticisers, flow control agents and defoamers tune the physical properties of the paint either after crosslinking (plasticisers increase the flexibility of the coating) and during the application phase. Defoaming is necessary to decrease the tendency of the paint to absorb gases from the atmosphere, and thus to trap bubbles within the hardened film.

Solvents are used to dissolve the binders and to disperse the pigments and other additives, improving the homogeneity and stability of the mixture. The application on the surface is facilitated by the decrease in viscosity due to the solvent, which subsequently evaporates leaving behind a thin film of solid coating. Ketones are the most commonly used between the solvents. Organic solvents, in the drying step of the coating, are dispersed in the atmosphere, causing pollution. For this reason, there is currently a strong drive in the paint industry to progress towards water-based coating systems.

4.2.2 Automotive Paints

Automotive paints are surely the most common coatings encountered in forensic casework. They are very complex and technologically sophisticated systems, which must guarantee a number of prerequisites, such as maximum resistance to weathering and to oxidation, coupled to an aesthetically pleasant effect, with brilliant, durable and homogenous colours. A single layer does not satisfy all these requirements, so automotive paints are in fact layered systems composed by at least four components (Fig. 4.4).

Together, the first two components of an automotive paint, electrocoat and primer, form a base coating, applied directly on the metal frame of the car, whose main roles are to protect from rust, to favour a strong and durable adhesion of the paint to the car, and to level and smooth the surface. The next two layers have mainly aesthetical functions, conferring to the car its colour and appearance.

- *Electrocoat*. Preliminary to the application of any coating, the metal car body is thoroughly cleaned, in order to remove any trace of dirt, oil or grease which would not allow an optimal adhesion of the primer. After a pretreatment with

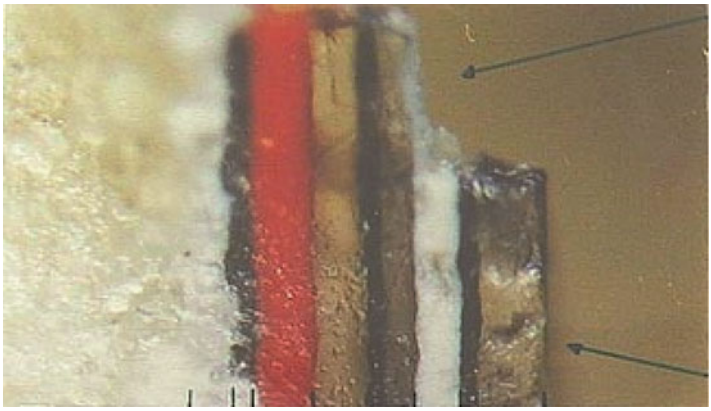


Fig. 4.4 Cross-section of an automotive paint chip

zinc phosphate, the electrocoat is applied. The car frame is dipped in a bath with a waterborne dispersion of crosslinking agents and binders, followed by the application of a direct voltage to the car, while a counter electrode is located in the bath. Electrically charged paint particles accumulate on the metal, forming a coating with a thickness of about 20 μm.

- *Primer*. This layer, approximately 30 μm thick, is composed of epoxy, polyester or polyurethane-based binders, applied by electrodeposition. It forms a matte and very smooth base which prepares the car body for the successive deposition of the coloured layer. It is highly pigmented and it contains high amounts of inorganic fillers such as kaolin, talc or calcium carbonate, in order to reduce the contrast between the primer and the next layer. Other important functions of the primer are the improvement of adhesion, a synergistic action with the electrocoat towards resistance to corrosion, and a protection from mechanical stress. The balance between hardness, rigidity and flexibility of this layer allows to dissipate the energy of impact with small stones or other objects which the car may encounter in its functioning, preventing the detachment of paint flakes or chips as a consequence of these common mechanical stresses.
- *Base coat* (or *topcoat*, or *colour coat*). It is the layer which yields the final colour to the car. The application stage of this component of automotive paint is critical: pigment particles must be suitably oriented, in order to guarantee the final metallic or pearl effect. It is applied in two phases, the first one by an electrolytic bath, the second by spraying. The resulting coating is treated at high temperature in an oven, where a continuous paint layer is formed, which is subsequently smoothed by sanding. The binder is normally polyurethane, and a variety of organic, inorganic and metallic pigments are used to confer to the car a pleasant look. This layer is the thinner in the paint sequence, with a thickness below 20 μm.
- *Clearcoat*. It is a transparent, acrylic- or polyurethane-based layer, with a double role: decorative and functional. With its glossy appearance, it improves the aesthetic properties of the basecoat. Moreover, it is intended to protect the coating from the aggression of UV radiation, from weathering and from other physical and chemical attacks. Its thickness is about 40 μm.

4.2.3 Recovery and Sampling of Paints

Two types of paint traces can be found on crime scenes: chips and smears. Chips can detach from the vehicle as a consequence of impact and be transferred on the object which was hit. They surely are the most effective kind of paint evidence. In fact, they deliver the largest amount of information on the donor vehicle because they contain all the layers used for the coating. They are usually big enough to be picked up by tweezers. Caution must be exercised, because they are quite fragile items. The Crime Scene Investigator must be careful not to break the chip during handling. To prevent them from breaking during storage or transport to the laboratory, they should be put into rigid plastic sample holders.

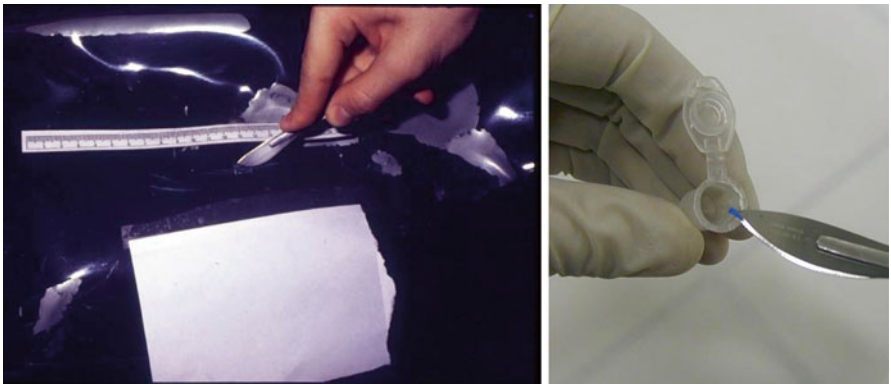


Fig. 4.5 Recovery of a paint sample

Transfer of paint smears happens due to shear stress, to friction between the vehicle and other objects. They are less informative items, with a lower evidential value, because knowledge about the composition of each of the layers of the paint is lost. When the accident involves two vehicles, difficulties in interpretation may arise, because the two paints can mix together and complicate the separation of the trace evidence from the background. The optimal approach to collect smears is to recover them as they are found on the crime scene, including the support where they were transferred to. However, this is not always possible or practical, so smears are removed from the surface where they are by incision with a scalpel. Care must be taken in this case to cut the substrate deep enough to collect the whole smear and the underlying layer without fragmenting the evidence (Fig. 4.5).

The sampling of paint from the vehicle which is allegedly the source of the traces must be done on a non-damaged portion of the vehicle, but as close as possible to the part which participated to the impact. In fact, if the sampling were done right where the collision happened, some contamination from the paint of other vehicles could hamper the analysis. On the other hand, collection of the paint should be done not far from the location where the hit occurred, to avoid analysing parts of the body which were repainted or repaired with other kinds of coatings or by adding additional paint layers, not originally present in the rest of the vehicle. In this case also, the sampling should be done with a scalpel, cutting deep enough to recover all the layers which compose the paint.

As with other evidence recovery activities, sampling of paint must be preceded by an accurate photographic documentation of the site and of the vehicle, both before and after the collection of the item. Crime scenes containing paints are often car accident sites, which are exposed to weathering and to traffic. This should prompt those who investigate on such scenes to attentively note the aspect and the conservation conditions of the traces found. This will help the interpretation of the results, because any contamination of evidence will severely complicate the outcome of the analysis.

4.2.4 The Characterisation of Paint Evidence

As outlined above, paints are complex heterogeneous systems: each layer can be in turn a mixture of different materials. For this reason, a complete and thorough analysis of paints requires the combined use of various methods.

As in other kinds of polymeric traces, the characterisation can be aimed at the identification of the vehicle which left the trace found on the accident scene, or at a comparison with a suspect source vehicle.

In the former case, the forensic scientist exploits the fact that the composition of the coating of a vehicle varies as a function of its manufacturer, of its model and of its construction year. Databases were developed for allowing an identification, on the basis of analytical data, of the kind of vehicle on the basis of its coating. Such databases contain information on the composition, IR spectra, Raman spectra, chromatograms, etc. of tens of thousands of car models [94–96]. The RCMP (Royal Canadian Mounted Police) was the first to create, in 1975, a database, obviously mostly focused on the North American car market. However, in 1998, data related to European and Japanese car models were added. The EUCAP (European Car Paint) is a database on the European production, the fruit of the collaboration, within ENFSI (European Network of Forensic Science Institutes), of 42 laboratories from 21 countries. In 2000, the Federal Bureau of Investigation in the United States implemented the NAPF (National Automotive Paint File) database, which contains samples provided by the manufacturers. Probably the most comprehensive database of paints, from primer to topcoat, containing information on 50,000 layers coming from 15,000 car models is PDQ (Paint data query). It is maintained by the RCMP, and it includes data from car models worldwide. In the context of a growing international collaboration, EUCAP, NAPF and PDQ exchange samples and information, in order to improve the reliability and completeness of each database.

Identification of the model of a car from a paint trace found on the crime scene is a challenging task, aided by the circumstance of recovering complete paint chips shedded in the collision.

When this is not possible, the forensic analysis of paint is strictly associated to traditional investigation, in which a set of suspect vehicles is singled out, and comparative analyses are performed to determine if some of these could be the origin of the traces left on the accident site.

The starting point of any investigation is a microscopic observation of the trace by optical microscopy. This allows to determine the physical characteristics of the paint, qualitatively assessing the colour and, in case the whole chip were available, also the thickness of each layer.

This preliminary stage, albeit simple, allows to rule out suspect vehicles manifestly incompatible with the traces of unknown origin. Successive analyses are focused on a quantitative determination of the chemical and physical characteristics of the constituents of the paint. In the majority of cases, not all the possible analytical methods are applicable, due to the small available sample size, or to the contamination of the item. The sample conditions are the feature which determines the most suitable sequence of tests to carry out.

The characterisation of paint is necessarily focused on two key aspects: colour and formulation. Colour is quantitatively measured by ultraviolet-visible spectroscopy [75, 97–99]. The small size of the average paint trace involves the use of microspectrophotometry, i.e. of a spectrometer hyphened to a microscope.

Formulation of each layer of a paint system can be carried out by a variety of techniques, such as infrared and Raman spectroscopy [85, 86, 88, 89, 100–117], elemental analysis [104, 110–113, 118–124], and gas chromatography, hyphened to pyrolysis [87, 125–132]. All these latter techniques have advantages and disadvantages which will be discussed in the next chapters of this book. However, the analysis of colour may be carried out on every paint trace, both in smears or chips. On the contrary, all the techniques focused at elucidating the formulation of paint are very difficult to apply on smears, because smears cannot be efficiently separated from the support where they were transferred to.

4.3 Adhesive Tapes

In 2001 a girl left her home to go to see a doctor for routine examination. She never returned home and was later found dead in a field nearby, tied with adhesive tape. Close to the body the roll of the adhesive tape was found. Searches immediately started to identify, from the few inscriptions on the cardboard cylinder inside the roll, who was the manufacturer and where that adhesive tape could be bought. The purpose was of course that of acquiring information which could bring about the individuation of a suspect. The search did not bring to any significant result, mainly due to the fact that the adhesive tape market tends to be pulverised into a multitude of small companies which assemble and commercialise products of many producers.

This unsuccessful case history shows how variegate the world of adhesive tape can be, with a large variety of products in the market, with a large variety of end uses and thus of chemical compositions, optimised for each of them.

Adhesive tape is an extremely common item which can be found in most houses, offices and workplaces and which has a wide range of legitimate applications. However, adhesive tape becomes useful in the commission of a number of crimes, such as kidnappings, to tie up victims of several kinds of aggressions, in the manufacturing of improvised explosive devices and for the packaging of illegal drugs.

The initial case history also shows, one more time, how knowledge of the relevant market of an item of interest in casework is fundamental for a successful outcome of the investigation. In this case, the distribution of tapes differs quite starkly from one country to the other. For example duct tape, which is extremely common in North America for both legitimate and criminal uses, is quite seldom used in Italy, where it is considered a ‘specialty’ product for particular exceptional applications, rather than a ‘commodity’ for the solution of everyday problems.

4.3.1 The Composition of Adhesive Tapes

Adhesive tape basically consists of a backing film which is covered with a pressure-sensitive adhesive. By application of a slight pressure, the tape can bond to the surface, usually reversibly, so that it can be removed by a not too intense force and without any substantial damage to the surface itself.

As many other objects in practical use, tapes must satisfy a number of requirements: they must have a long service life, they must resist to weathering and environmental factors such as temperature or exposure to UV light, they must be mechanically strong and their adhesion should remain stable with time.

A very detailed resource which describes the structure and forensic analysis of adhesive tapes is an excellent book chapter written by Jenny M. Smith [133].

The *backing* is the support material for the adhesive. It may be a fabric, or a polymeric film. Fabrics are used in medical tape and in duct tape. In the former case, the fabric is the backing itself and it is a tightly woven, resistant material. In the latter case, a loose open weave fabric is added to a polyolefin backing, usually polyethylene, to reinforce it. In duct tape, the fibrous reinforcement is an additional layer sandwiched between backing and adhesive, and it provides a framework which strengthens the mechanical properties of the whole assembly, improving the resistance to traction and to tear. Synthetic fibres like nylon and poly(ethylene terephthalate) are commonly used materials for the reinforcement of duct tape, even though glass fibres are also found.

In most of the commonly used tapes the backing is a polymeric film, and a quite large variety of materials are employed in the industry: polypropylene or cellulose acetate for office tape, polypropylene for packaging tape, poly(vinyl chloride) for insulating electrical tape, paper for masking tape, polyethylene for duct tape. Despite this apparent wide variability in materials, similar materials are used for similar applications. Practically all the packaging tapes will have a polypropylene backing. Since it is very easy to visually distinguish packaging tape from other types of tape, a simple identification of the material used for its backing rarely yields very useful information. When comparing two pieces of packaging tape, it is of little significance to conclude that they both have a polypropylene backing, because most packaging tapes share this characteristic. A deeper characterisation of the backing is necessary, focused on the structural, morphological and constitutional properties of the polymer, or on the formulation of the material. A number of additives are in fact used in the backing [133]: inorganic pigments (aluminium powder, TiO_2, iron oxides) and carbon black, plasticisers (especially in the case of PVC) and inorganic fillers such as calcium carbonate or talc are those present in larger amounts. Additives can be present in very relevant quantities, for example the amount of plasticiser in PVC backings can be of the order of 40 %. Minor additives, which are also more difficult to characterise, are UV stabilisers, fire retardants or crosslinkers.

The second main component of an adhesive tape is the *pressure-sensitive adhesive* layer, which is the mixture of an elastomer with a tackifier. The elastomer can be natural rubber, styrene-butadiene rubber, block copolymers of styrene with isoprene or butadiene or other rarer kinds of synthetic rubbers. Acrylate polymers are very common. Typical monomers are 2-ethylhexyl acrylate, *n*-butyl acrylate, methyl acrylate and *t*-butyl methacrylate. The tackifier is a plasticiser which makes the elastomer more fluid and sticky. Small hydrocarbons or terpenic compounds are used as tackifiers. The adhesive contains additives as well. These can be inorganic fillers, analogous to those cited in the case of backings, for improving the mechanical performance of the material. Surfactants are used to tune the surface tension of the adhesive, and to improve and control wettability and adhesion. Other compounds such as chain extenders or crosslinkers are used to regulate the entanglement of the polymer chains and to tune the adhesive strength. Obviously most of these latter additives are industrial secrets difficult to penetrate and to de-formulate. Common types of packaging tape are tan coloured or white. Most of the times the colour is imparted by pigments such as iron oxides or TiO_2 added to the adhesive mixture.

To this basic product design, several additional layers can be added, to adapt the tape to any possible practical application. A release liner is coated on top of the backing to prevent that it sticks to the adhesive while wound in the roll. A primer can be used to mediate the chemical compatibility of adhesive and backing. These minor components are surely much more difficult to characterise than the main ones, i.e. adhesive and backing.

4.3.2 Recovery and Sampling of Adhesive Tapes

Casework on adhesive tapes does not usually suffer from the small sample size limitation which characterises other types of evidence. Normally large quantities of tape are available for analysis. The only thing to carefully consider is that, due to their nature, tape items can contain a number of other potential pieces of evidence. Saliva traces can be found at the edges, where the tape could have been torn with the teeth, and fingerprints could have been shed during its manipulation [134, 135]. Since these kind of traces can lead to the personal identification of the individual who committed the crime, their retrieval and analysis must be prioritised. However, the treatments done for developing fingerprints, for example cyanacrylate fuming, greatly modify the chemistry of the items, jeopardising the accuracy of their characterisation [136]. It is therefore necessary to remove a small piece of tape, enough to perform a full set of chemical analyses, before beginning the biological and fingerprint examinations. This preserves the integrity of the material and the significance of the work of the chemist, at the same time leaving untouched most of the surface of the item for the other professionals working on different traces. Adhesive tape, due to its sticky nature, is a natural repository for other trace evidence [135]. Fibres, particulate, soil, pollen and any other small object present

in the environment where the tape was manipulated is likely to be caught by the adhesive. After all adhesive tape is the material used for the actual collection of trace evidence from the crime scene!

4.3.3 The Characterisation of Adhesive Tape

As said in the previous section, sample size is rarely a problem in casework where adhesive tapes are involved. This allows to carry out a large number of analyses and to perform a very detailed characterisation.

Adhesive tape characterisation always starts from a thorough examination of the morphology [137, 138]. If two pieces of adhesive tape are different for their morphological features, no chemical analysis is necessary, with significant savings in terms of time and money. Features such as the width and the thickness are very rapidly determined and, in comparison cases, they can provide a very quick first screening. Many replicates should be done when measuring thickness because, due to the softness of the material, the experimental values can be quite dispersed. Other morphological features which must be noted are the surface texture, observed in direct and oblique lighting.

Fluorescence using different light sources should be assessed, not only in the ultraviolet range, but also in the visible, because samples fluorescing at 450, 505, 530 and 555 nm were reported [139]. Fluorescence is principally due to the adhesive. The birefringence of the backing should be also checked, observing it through crossed polarisers [139]. Comparison may be, on a first instance, made on the basis of the macroscopic colour. If the samples appear different by such comparison, the result can be considered conclusive. However, a deeper characterisation of colour is needed in case of apparent compatibility, through UV–visible spectrophotometry in reflection mode [139].

It was shown that scanning electron microscopy, even though more labour intensive and expensive than a simple optical microscopical examination, offers the potential for a remarkable discrimination of electrical tape, based on the surface texture and simultaneously also on the elemental profile [140].

A typical analysis for adhesive tapes is the physical end-matching [141, 142]. This consists in determining if one end of a piece of tape from the crime scene, or otherwise related to the crime, matches the roll from which it allegedly came. When this happens, it is often considered conclusive and sufficient proof that that piece of tape indeed came from that roll. However, tapes are not solid and rigid materials, and as such deformations and distortions related to the mechanical stress implied by the tearing action must be taken into account when interpreting the results (Fig. 4.6) [141]. As a consequence, it may be possible to associate tape ends that do not actually match. This is why the Federal Bureau of Investigation Laboratory modified its tape comparison protocol in 2003, imposing to proceed to a full physical and chemical comparison even after a successful end match, even confirmed by a second qualified individual.

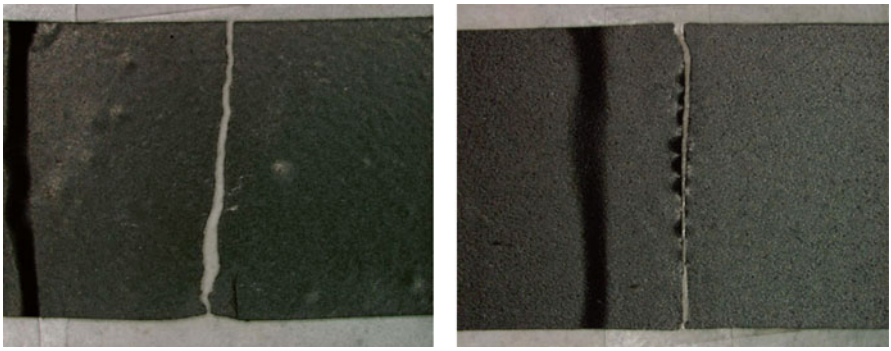


Fig. 4.6 Two examples of end-matching tests. The image on the *left* represents two extremities of a torn tape, which due to permanent deformation do not perfectly match and are difficult to correctly assess. The image on the *right* represents two pieces of tape which were not torn from each other, even though they appear to match. Reproduced with permission from [141]. Copyright (c) 2011 American Academy of Forensic Sciences

The step which follows the observation of the morphology is the separation of the components of the tape, namely of the backing from the adhesive and, if present, from the reinforcing gauze of fibres used in duct tape. Chloroform is an effective solvent, especially if coupled with sonication. Hexane [133], acetone [143] and tetrahydrofuran (THF) [144] were also cited as suitable solvents for such separation procedure. In addition to separating the two main components of adhesive tape, exposure to a solvent may also extract some of the additives from the backing. If the same tape is exposed to different solvents, slightly different results could arise. It is then important that a laboratory standardises this step, in order to obtain significant and consistent results. Part of the sample should be always preserved unaltered, to allow for repetition of the analyses.

After separation of the various components of the tape, the repetition of the thickness measurement of the backing is suggested, since the interference of the adhesive is now absent. After the separation step, it is possible to proceed with a chemical characterisation of the components of the tape. As with many other kinds of traces, IR spectroscopy is a good starting point [137–139, 145–147]. If an ATR accessory is available, separation is not necessary for carrying out such analyses because it just samples the surface of the item positioned on the crystal [146]. Possible inconveniences can arise in electrical tapes which are usually highly filled with additives which intensely absorb IR radiation. Carbon black saturates the IR absorption signal, covering the peaks due to the other components. Plasticisers, especially if the tape is of a low quality or old, tend to migrate towards the surface of the backing: ATR will then detect just the spectrum of the additive, and the information on the rest of the material will be lost. In cases like these, a suitable solution consists in repeating the analyses after different treatments with different solvents. Chloroform does not dissolve polyvinylchloride, but it can extract organic additives [148]. THF dissolves the polymer and most of the organic additives, but not carbon black.

The already cited work by Smith [133] reports a collection of spectra which may guide the analyst in the interpretation of IR data. However, the forensic scientist who approaches the field of adhesive tapes analysis is urged to create his own spectral database, on a population of tapes collected in his geographical region of influence. This is of course also valid for all the other items which are presented in this chapter. In addition to giving a picture of the variability of the products diffused in the region of interest, building a database of the population of the adhesive tapes will contribute to the establishment of a practical expertise in the various steps of sample preparation and analysis of adhesive tapes. Some of the actions involved in the analysis of tape are less trivial than one may think, especially because dealing with a sticky substance like the adhesive makes the collection of contaminants from the laboratory environment an issue. Moreover, repeating the separation steps on several products of the same kind which reflect the relevant local market may allow to optimise the solvent system and to identify other distinguishing features: sometimes the adhesive will detach from the backing forming balls or aggregates, sometimes it will appear as a semisolid fluid or paste.

Elemental analysis and pyrolysis are two very useful complements to IR spectroscopy, which yield information on the additivation of the tape components [133]. The formulations of both the backing and of the adhesive of tapes frequently contain inorganic additives. Pigments and fillers are those present in the largest quantities. Any technique of elemental analysis, such as an energy dispersive X-ray detector in a scanning electron microscope [137, 138, 140, 147], X-ray fluorescence [149], atomic spectroscopy [150] can be used for characterising such aspect of the material. X-ray diffraction was used as well, as a method able to identify the inorganic species used as fillers, and not only the general elemental composition of the material [140]. Of course each of these techniques have their advantages and disadvantages, especially regarding sensitivity and detection limit, but it is worth noting that they all can be performed in a non-destructive way. Isotope ratio mass spectrometry (IRMS) was suggested by several authors as an effective way to refine the elemental characterisation of adhesive tapes [148, 151, 152]. The isotopic signature of carbon and hydrogen depend on the source from which a particular material was manufactured, so it is a very sensitive way to individualise similar mass-produced items. In particular, the reports on IRMS of tapes showed that a particularly effective strategy consists in performing this analysis on the pristine sample, on the tape after the separation of the adhesive from the backing, and also after the extraction of additives from the backing with a solvent [148, 151]. Both IRMS and elemental analyses were shown to be resilient to exposure to weathering and also to exposure to explosions [147, 148, 151].

Pyrolysis, coupled to gas chromatography with mass spectrometry detection, is on the other hand a suitable and efficient technique for characterising the organic additives [137–139, 145, 153]. This technique is indeed discriminatory, because it often allows to distinguish tapes which were indistinguishable by IR spectroscopy [139]. By merging IR spectroscopy and pyrolysis/gas chromatography/mass spectrometry, Sakayanagi and colleagues reported that colourless, transparent PP tapes could be distinguished in terms of manufacturer [145]. The identification of the

Fig. 4.7 (*Left*) Gloves found outside the robbed shop; (*right*) box of gloves seized in the suspect's house

fragments produced by the pyrolysis step can be a cumbersome task, albeit usually not necessary. When comparisons are performed, the presence or absence of a particular fragment in the chromatogram will be significant, provided that it is consistent, irrespective of its nature or of the fact that it is difficult to understand by what species it was originated. A drawback of pyrolysis is that it is destructive. However, the sample size required is amply lower than that normally available in tape casework.

Mass spectrometry with desorption ionisation methods was proposed as an alternative for the characterisation of the organic components of the adhesives [154, 155].

Adhesive tapes are one of the few items of forensic interest where size exclusion chromatography, a characterisation technique typical of polymers, was applied and reported [144].

A final word should be dedicated to the characterisation of duct tapes which, in addition to the features mentioned above, have a further element to analyse: the reinforcement fibres. However, the approach to these items is not different from that described for textile fibres. The sample size allows to apply all the techniques mentioned in Sect. 4.1.5, so a very clear description of these fibres can be obtained.

4.4 Rubber Gloves

A shop was robbed by a man wearing a motorbike helmet and latex gloves. The weapon used by the offender was a cardboard cutter. The police, called immediately after the aggressor left, investigated in the vicinities and apprehended a man who matched the description given by the shop owner. The cutter was not found, but a pair of latex gloves were located a few metres from the shop. Surveillance cameras confirmed that the clothing and the design of the helmet were the same as the suspect arrested, and that the robber indeed threw the gloves in the location where they were found. The house of the suspect was searched and the box of latex gloves depicted in Fig. 4.7 was found.

The gloves in the box appeared visually similar to those discarded by the felon, and they were compared in the laboratory to assess if indeed the latter could be of the same type and brand found in the suspect's premises. Thermal analysis (Sect. 5.6) confirmed that they were compatible.

The diffusion of forensic science in fictional and non-fictional media made available a large amount of information, which can unluckily be exploited by felons for optimising the commission of their crimes. Fingerprints and biological traces are surely the first types of evidence everybody designing the 'perfect crime' will want to avoid. This is why an increasingly large number of felonies are committed wearing gloves, not to shed fingerprints on the crime scene. Just crimes committed without premeditation, under the drive of emotion, are associated to a reduced attention to prevent leaving traces. Although it is sometimes possible to detect DNA traces or latent prints on the gloves, the forensic awareness of some crime committers is limited and gloves themselves can be found discarded at the crime scene or nearby. In some instances, as in the case reported above, it has been proved useful to compare the gloves found at the crime scene with those seized at the suspect's premises.

4.4.1 The Manufacturing of Rubber Gloves

There are many types of rubber gloves, with many applications, which go from gardening to dishwashing to the use in the laboratory or for medical purposes. The design of gloves is strictly dependent on the end use and on the degree of protection required. Heavy duty gloves are usually cloth gloves covered by a layer of rubber to protect them from water or solvents. The gloves commonly used for dishwashing or other house care purposes are mainly composed of rather thick rubber, sometimes lined with an inside layer for more comfort of use. These two types of gloves are not common at all in forensic practice and are not involved in cases like those introducing this section. The kind of disposable gloves usually chosen by felons for avoiding the shedding of fingerprints is the thin type used in laboratory or in medical practice. The flexibility, tightness and mechanical resistance of these items allow to perform any action with a mobility and grip comparable to those of naked hands. Moreover, latex or vinyl gloves have a neuter colour (vinyl gloves are almost transparent) and they can go unnoticed for example when the offender enters the shop or bank that he intends to rob, or when a burglar approaches the house he is planning to break in.

Three main types of laboratory/medical gloves exist: latex, nitrile, and vinyl. Latex gloves are made of natural rubber, nitrile rubber is a copolymer of butadiene and acrylonitrile. Vinyl gloves are made of polyvinylchloride. Neoprene gloves exist, but they are less common, because they are typically used for surgery, they are commercialised in sterile conditions and are therefore much more expensive and less readily available. Gloves are relatively simple items: in addition to the polymer, the only other additive present in significant amounts can be some starch powder added in the inside to lubricate them and make their wearing easier.

Other commercial specifications regard their quality, which can be exam or surgical grade. Surgical gloves must meet higher standards, in terms both of quality of the material and of the sensitivity guaranteed by the design. Moreover, gloves can be sterile or non-sterile, with surgical gloves which are generally sterile.

Natural rubber is produced by a tree (*Hevea brasiliensis*) which grows in tropical regions. When the bark of this plant is cut with a suitable hook-shaped knife, the latex vesicles of the tree are broken and latex starts running down the trunk and can be collected in a cup. The flow lasts a few hours, before spontaneous clogs of latex plug the vesicles. The fluid latex to be used for manufacturing gloves is compounded with ammonia and other chemicals useful for vulcanisation, and then stored in holding tanks for a maturing stage.

Gloves are produced by a dipping process. Hand-shaped metal moulds pass through a cleaning bath, they are rinsed and then dipped into a bath with a coagulating agent, such as calcium nitrate. Subsequently, the mould, covered with a thin layer of coagulant, is immersed in chilled latex. The latex bath is kept at a low temperature to prevent a spontaneous pre-vulcanisation. The coagulant induces latex to deposit on the mould and to form a regular film. Some rinsing processes then follow, aimed at removing any excess of coagulant. The mould is then introduced into an oven for the vulcanisation stage. In this step the fluid latex crosslinks and it becomes a solid material able to retain its shape, while affording a considerable deformability. Vulcanised rubber is able to elongate, without being permanently deformed. The vulcanisation rate and efficiency is optimised by the accelerants present in the compound latex bath. Since the proteins present in latex are well-known allergens, one or more further washing steps with hot water and detergent are applied, to extract most of them. Powdering then follows, if applicable, otherwise the inside of the glove is coated with lubricants which help to wear it. The glove is then released from its mould and the quality control and packaging steps complete the process.

The manufacture of nitrile, vinyl or neoprene gloves is analogous to the one just described, just with a different starting material, which is synthetic and not extracted from a tree like natural rubber.

4.4.2 Recovery and Handling of Gloves

The sample size in rubber gloves casework is usually large. At least one full glove is recovered on the crime scene, whereas usually many items are available for comparison. No significant precaution should be taken in their recovery or handling for what regards the chemical characterisation. Obviously, priority should be given to the fingerprint or DNA typing analyses, because chances are high that traces of this type are present in the inside of the gloves. When no such evidence can be found, the chemical characterisation becomes a viable option for gathering investigative information. Powdered gloves contain a particulate that allows an easier wearing. In order to assess if it is worth focusing some analyses on such powder, it is important

to have information on the conditions of the crime scene. If it is too prone for sample contamination, for example by dust or environmental pollutants, the characterisation of the powder on the gloves could be inconclusive because too influenced by the background.

4.4.3 Characterisation of Latex Gloves

The process of glove manufacturing is relatively easy and does not involve a significant use of additives. Accelerants and coagulants are related to the vulcanisation process, and in some cases starch particles are added in powdered gloves.

The literature on the forensic analysis of latex items is extremely limited.

Latex, a natural rubber, is a highly regular *cis*-1,4-polyisoprene produced by more than 400 different species of plants. The majority of the most common techniques for the characterisation of polymers do not yield interesting information for the forensic differentiation of similar latex products. This material is amorphous, so X-ray diffraction will not be very useful [156]. The analysis of the inorganic content [157, 158] could be in principle a viable technique. However, since gloves are almost invariably found discarded on the ground, the elemental analysis does not allow to single out the additives from the contaminants, yielding possibly contradictory results.

Infrared spectroscopy is not very informative because latex gloves all yield extremely similar spectra, except in the case of chlorinated, powderless gloves that show some additional and potentially useful signals [159]. Physical mechanical characterisation cannot be applied in the majority of real cases because it requires samples of relatively large size and of standardised shape. Most of the times, there is not enough material to perform the replicate measurements necessary to obtain a reliable result.

An approach which proved efficient for the discrimination of latex gloves was thermal analysis, and notably thermogravimetry (TGA) and differential scanning calorimetry (DSC), which detect the variations in sample mass and the heat exchanged as a function of temperature as a consequence of degradation, oxidation or other reactions. Thermal degradation of polymer materials depends in fact on their formulation and slight variations in additivation can bring about measurable changes in their behaviour at high temperatures.

A population of 28 latex gloves was studied. By application of basic procedures such as visual examination or thickness measurements, all the samples were indistinguishable. Simultaneous TGA/DSC was performed in two conditions: in N_2 atmosphere or in air [160]. Several different degradation patterns were identified, both in the number of steps and in the entity of the steps.

These features are in fact dependent not only on which organic components are present in the rubber matrix but also to the degree of crosslinking, and finally to the possible occurrence of some inorganic additive.

More details on these techniques will be presented in Sects. 5.6 and 7.2.2, suffice here to say that by these techniques nearly all the latex gloves examined could be discriminated. Sample preparation was avoided, but the methods are partially destructive. However, they require sample sizes of the order of less than 10 mg, much less than the amount normally available in casework.

A further characterisation method which was useful for differentiating apparently similar latex gloves was ^{1}H time domain nuclear magnetic resonance (TD-NMR) (Sect. 7.3.2) [161]. This technique focuses on the mobility and on the composition of rubber. Two parameters can be quantified by this technique: the proton weight fraction, which is correlated to the chemical nature and formulation of the material, and the relaxation time T_2, which is a measure of polymer chain dynamics. Both these features can be ultimately associated to differences in raw materials, formulation and processing of the gloves, thereby offering a means to discriminate items which are indistinguishable by visual examination. TD-NMR, without sample preparation and preserving the integrity of the specimen, allowed to differentiate 88 % of the possible pairs of samples in a population of 20 latex gloves.

4.5 Tyre Rubber Traces

Tyres are the family of rubber items most commonly encountered in forensic casework. Typical tyre evidence is in the form of small rubber particles left on the road when a car brakes or skids. The purpose of the analyses is the identification of a vehicle connected to the commission of a crime, or the reconstruction of the dynamics of an accident. Under this aspect, rubber traces left by skidding vehicles are similar to paint smears or chips. Together with the pieces of plastic automotive parts, such as fragments of bumpers or of reflectors, they represent most of the debris found at car accident scenes and which yield considerable information on the vehicles involved and on the events which occurred.

4.5.1 Manufacturing of Tyres

A tyre is a quite complex object, made by several layers assembled to achieve the multiple requirements which the tyre must meet: it has to allow a comfortable and safe drive, to be durable, to resist from wearing under the friction and the hits which may happen during use.

The tyre parts which are of most interest to the forensic science are the tread and the sidewalls. They are in fact the only parts of the assembly which are in contact with the external environment, namely the road, whereas innerliner, body plies and belts are confined in the inside of the tyre.

The major ingredients in rubber compounds for tyres are the rubber itself and the filler. The formulation depends on the desired end-use, because it will be different in high performance, fuel economy or mud and snow models.

In general, four major rubbers types are used: natural rubber, styrene-butadiene rubber (SBR), polybutadiene rubber and butyl rubber (along with halogenated butyl rubber). The first three are primarily used as tread and sidewall compounds, while butyl rubber and halogenated butyl rubber are primarily used for the innerliner, or the inside portion that holds the compressed air inside the tyre.

Differently from the case of latex gloves, the raw material for tyres is not latex, but rubber sheets. These sheets are prepared treating the latex extracted from the rubber tree with a dilute acid such as formic acid. The coagulated rubber obtained is then rolled to remove excess water and to shape the material into rubber sheets. These sheets are dried and smoked, in order to avoid the attack from microorganisms, and they are ready for export and commercialisation.

The most abundant additives in the formulation of rubber compound for tyres are fillers, which are carbon black and silica. Their main function is to improve the tribological properties of rubber, i.e. resistance to friction and abrasion. Carbon black has the additional advantage of scavenging UV radiation, protecting the rubber from degradation. The average particle size and its dispersion is very variable, several products exist in the market. The kind of filler used depends on the performance requirements, and on the tyre part which must be manufactured. Different relative amounts and morphologies of the fillers will be used for producing the tread or the sidewall. As introduced in Sect. 2.8.16, the abundance of residual double bonds in rubbers derived from the polymerisation of dienes makes these materials very sensitive to oxidation and ageing. Important additives in the compounding of rubber are therefore antioxidants, antiozonants, UV protectors and radical scavengers. Finally, curatives and accelerators, i.e. the cure package, are added to the rubber, for regulating and triggering the vulcanisation reaction during the manufacture process of the different parts of the tyre.

The mixing of the additives, fillers and rubber matrix to obtain a homogeneous compound is carried out in an industrial mixer, a heavy machine capable of treating batches of more than 200 kg of rubber compound in less than 3–5 min. The components of the mixture are added in a two-stage process, with fillers and stabilisers included since the beginning of the process, and the cure package introduced at a later stage. The temperature must be carefully controlled, especially after the addition of the curatives and accelerants, to prevent scorching and a premature vulcanisation. The compound so obtained is unloaded from the mixer and a continuous sheet is formed, which goes through the next steps of the process.

Several layers of the tyres are then constructed from this sheet of compound. The first is the belt, in which the rubber compound is connected to the fabric or steel cord. This element is necessary for reinforcing the rubber compound and providing strength to the material. For fabric cords the most commonly used fibres are cotton, rayon, polyester, steel and aramid. Steel cords are usually made with steel wires coated with brass. The fabric or steel cord must be tightly bonded to the rubber, so the connection is obtained by a calendering process, an operation in which the rubber compound is forced on and into cords through the pressure exerted by revolving rolls. The innerliner is the innermost layer of the tyre and it serves to retain the compressed air inside the tyre and maintain tyre pressure. It is made with butyl rubber or halogenated butyl rubber by calendering. The bead component of the tyre

is a non-extensible composite loop that anchors the body plies and locks the tyre onto the wheel assembly. It is made with a steel wire loop coated with rubber and with a cross-sectional profile that guarantees a stable mounting of the tyre on the rim.

As said before, the most interesting parts of a tyre for a forensic scientist are the tread and the sidewall. These components are manufactured by extrusion. The rubber compound is fed into the extruder barrel where it is subject to heat, mixing and pressure. Then, the rubber compound exits through the extruder head where it is given the desired cross-sectional shape.

The tyre tread is the part of the tyre which comes in direct contact with the road, and consists of the tread itself, of the tread shoulder and of the tread base. These three parts require rubber compounds with different formulations, so the assembly of the tread must be carried out by a complex extrusion system. Three rubber compounds are extruded simultaneously from different extruders and are then merged into a shared extruder head. The product of extrusion is transferred to a die plate which has the final shape of the tyre tread. A slow cooling allows to control the size and the shape of the product, which is subsequently cut according to the specific length and weight for the tyre being built. At this stage, the tread does not have its pattern yet, which will be given after the assembly and the curing of the tyre.

The tyre sidewall is even more complex than the tyre tread, because four different rubber compounds are required. Four extruders working simultaneously are therefore used, with a process very similar to that of the tyre tread.

Once all the components have been separately manufactured, they are finally assembled. The process starts with the innerliner, then the bead, which is connected to the sidewall and finally the belts and the tread. At this point, the assembly is put in a mould, where it is subject to vulcanisation. The mould will give the tyre its shape and its external pattern, which is fundamental for a safe drive. In the mould, the high-temperature and high-pressure conditions activate the cure package and trigger vulcanisation, which consists in the crosslinking of the rubber molecules, to give a solid material, which retains its shape even after considerable deformation and which has an elastomeric behaviour.

4.5.2 Sampling of Tyre Rubber Traces

Sampling of tyre rubber traces from the road does not normally present relevant difficulties. If they cannot be directly lifted with tweezers, they can be scraped with a scalpel or a cutter. Since rubber traces were in contact with the road, they could have collected some contaminants. This should be kept in mind, and it is suggested to pick several samples to be able to assess the extent of such contamination. Actually, most of the expected extraneous substances should be inorganic, and so they do not interfere with the characterisation of the rubber traces, which is focused on the organic portion of the traces.

4.5.3 Characterisation of Tyre Rubber Traces

Casework in tyre rubber traces is normally of a comparative nature: the hypothesis that a certain vehicle was the source of the traces found on the road must be verified.

The formulation of tyre rubber compounds contains both inorganic components, i.e. silica, and organic components, i.e. the rubber matrix, the cure package and the stabilising additives. In principle, then, elemental analyses can be applied for characterising the formulation of tyre rubber traces. However, as introduced in the previous section, contamination from the inorganic substances present on the road is always present, and such analytical strategy is thus precluded. It is not possible in fact to identify whether the elements identified in the analysis come from the rubber formulation or from the road surface.

The literature on the forensic characterisation of tyre rubber traces is limited, but all the reports agree in indicating pyrolysis/gas chromatography/mass spectrometry as the most suitable method [162–166]. Lachowicz and colleagues identified limonene, styrene and isoprene as the most significant pyrolysis products for the discrimination of these traces [166]. These authors worked on a collection of 42 different samples of car tyres. According to a very simple hierarchical process, which started with a classification of the traces into four main classes according to the relative intensities of the three pyrolysis products mentioned above, and then continued comparing the samples on the basis of the minor peaks appearing in pyrograms, they were able to discriminate all the samples in their population. Pyrolysis was performed at 550 °C for 15 s. Interestingly, in order to increase the ability to differentiate between samples, an online derivatisation technique, flash methylation, was used. A 10 % aqueous solution of tetramethylammonium hydroxide as a methylating agent was added to the rubber sample in the quartz tube of the pyrolyser. With the same pyrolysis parameters above, derivatisation allowed to detect fatty acids, improving the discrimination potential of the process. After derivatisation, all the samples in the population could be distinguished [166].

Another interesting recent study on the pyrolysis of rubber traces was published by Gueissaz and Massonnet [163]. They worked on a smaller sample population than the study previously discussed, analysing the residues of ten kinds of tyres differing for manufacturer and country of production. With experimental design criteria, they first optimised the pyrolysis program, identifying a temperature of 650 °C for 15 s as the best parameters. They found that the effect of pyrolysis temperature was much more relevant than that of time on the quality and reproducibility of pyrograms. In these conditions, 86 pyrolysis products were consistently obtained, most of them with a variability within the same tyre much lower than that between different tyres. This confirmed that the products of pyrolysis can indeed be useful for the discrimination of the tyre rubber traces. In particular, ten compounds had a particularly high variability between the samples, thus showing the highest discrimination potential. These substances were isoprene, *o*/*p*-xylene, 1-methyelthylbenzene, α-methylstyrene, α-limonene, indene and 1,1′-(1-methyl-1,2-ethandiyil)bis-benzene and three other unidentified compounds.

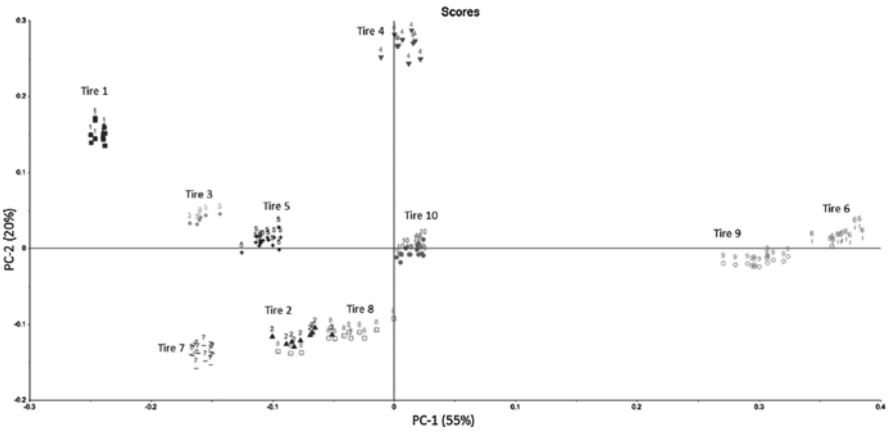


Fig. 4.8 Representation of the pyrolysis data on ten tyres on the plane identified by the first and second principal component after application of PCA. Reprinted from [163], copyright 2013, with permission from Elsevier

Application of principal component analysis (Sect. 3.3.2) confirmed that such ten substances were the most effective for the discrimination of the samples. Figure 4.8 shows the representation of the samples in the plane identified by the first two principal components. All the replicates for each tyre formed well-defined clusters, which in turn were all very well separated from each other (Tyre Nos. 2 and 8, which almost overlap in Fig. 4.8, were discriminated by considering the three first principal components.)

The loadings showed that the main contribution to the first three principal components came from the ten substances which showed the highest intersample variability. Clustering methods applied to the peaks of these ten compounds further confirmed the validity of pyrolysis/gas chromatography/mass spectrometry for discriminating and classifying tyre rubber traces. If such results were confirmed on a wider population of samples, the possibility of identifying the tyre brand from the traces left on the road could become a viable option.

4.6 Condom Lubricants

Sexual assaults often involve exchange of biological fluids between the offender and the victim, with the subsequent possibility of identifying the felon by DNA typing. With recent increasing public awareness, cases in which the assailant wore a condom to avoid shedding biological traces are becoming significant in number [167–170]. However, on the surfaces of condoms there are substances that, if found in swabs taken on the persons involved, could provide valuable information on the dynamics of the crime. The ideal case is when swabs are available both from the

victim and the suspect. When this happens, a link can be established between the two, if substances related to the same kind of condom are found in both the swabs. It is more often the case that this type of evidence is used to corroborate the description of the events made by the victim or by the assailant. In many instances it is important to determine if a condom was used during intercourse, also to understand if it was consensual. Moreover, knowing the extent of intercourse, for example whether or not it happened with penetration, is a piece of information useful to the Court for determining the severity of the penalty. Few studies exist on the persistence of condom-related traces, but it has been reported that starch powder particles could be detected in vaginal swabs up to 4 days after intercourse, and on penile swabs after 1–2 days [168, 171]. Traces of lubricant have been shown to resist up to 2 days after protected intercourse, with PDMS lubricants more persistent than water-base lubricants [169, 171].

4.6.1 The Composition of Condoms and Lubricants

The traces associated with the use of a condom can originate from the condom itself or be due to the use of additional lubricants.

The vast majority of condoms are manufactured with a process very similar to that described for gloves (Sect. 4.4.1), from natural rubber, *cis*-1,4-polyisoprene, latex. Latex contains about 2 or 3 % proteins, which are allergenic, and thus products made with sheep caecum, polyurethane or polyisoprene were introduced in the market.

No traces are shed from the bulk polymer material used for producing condoms, which is thus quite irrelevant from a forensic point of view. For this purpose, the ancillary substances which are involved in condom manufacturing and use are much more significant. These will be the analytes which will be searched in swabs or in evidence regarding sexual assaults.

Keil compiled a quite informative picture of the composition of the substances involved in condom production [168].

Some of these additives are added in the production of the condom by vulcanisation of rubber. It is the case of accelerants for vulcanisation, such as dithiocarbamates or nitrosamines, which are usually present in very small amounts since they are almost completely consumed in the vulcanisation process. Moreover, dithiocarbamates and nitrosamines are not univocally ascribable to condom use, and so they are not so significant in forensic casework [168]. The additives related to the subsequent steps of condom production are more abundant and prone to transfer. After vulcanisation, the condom is treated with silicone oil, powdered to make unrolling and wearing easier, and finally lubricated.

Starch and polyethylene or even mixtures of the two, are commonly used for powdering. Together with the powder, antioxidants and preservatives can be added, to avoid the degradation of latex. Even though they are present in a very small amount, they can nevertheless be detected.

In addition to their obvious main function, lubricants aid unrolling and protect the latex from degradation damage which may happen when it is excessively dry. They are usually siliconic polymers, such as polydimethylsiloxane (PDMS), but also poly(ethylene glycol) (PEG) is employed.

The lubricant's formulation is completed by other additives which yield particular functional features such as spermicides (nonoxynol-9 or benzalkonium chloride, even if the former is overwhelmingly the most common), flavourings or aromas, or lidocaine or benzocaine, anesthetics with a retardant action [172, 173].

In some cases, personal lubricants, in addition to those already present on the condom, are used, which are mostly water-based mixtures of glycerine and PEG with other additives. Another class of personal lubricants exist, which is oil-based and incompatible with the use of condoms, usually based on vaseline or petrolatum oil [169].

4.6.2 Collection and Handling of Condom-Related Traces

Condom-related traces are searched within swabs taken from the victim and/or the suspect of a sexual aggression. The collection of such swabs is never performed by the forensic chemist, but by medical staff, according to a defined protocol [174]. Most countries have first response teams which are trained to collect all the necessary information and traces which can be useful to reconstruct the events, at the same time helping the victims of this kind of crime to deal with the emotional shock. Any biological trace should of course be preliminarily examined in order to extract DNA profiles. A portion of the swab should be set aside, or one extra swab could be collected, expressly for chemical examinations of the type described below [169, 174]. Swabs already examined for biological traces should not be analysed for chemical traces, since the extraction procedure used in the DNA typing process removes also lubricants [169]. The precautions to be observed in the handling of swabs are those normally valid for potentially biohazardous samples. Contamination of the results of chemical analyses is not a significant risk, whereas any measure should obviously be taken to avoid contamination by the operator of the DNA traces.

4.6.3 The Characterisation of Condom-Related Traces

The components of the lubricants or additives used on condoms available in different brands and markets are considerably diverse [172].

The examination of genital swabs should start from a microscopical examination, aimed at identifying the presence of residues from the powder coating of the condoms [168]. Staining the slides with hematoxylene-eosine, starch particles appear as small dark spots of micrometric diameter. By polarised light microscopy, starch is identified by the maltese cross patterns typical of birefringent particles.

The other material significantly used in the industry for the formulation of the powder, polyethylene, is extremely difficult to identify in swabs.

The search of chemical traces due to condoms is mainly focused on the detection of lubricants and spermicides [167, 169, 172, 173, 175, 176].

As a first precaution, it is advisable to examine just one half of the swab, keeping the other half for possible further confirmation analyses, performed later in the course of the judicial process.

The analytes are extracted from the swab with a suitable solvent. Many procedures were proposed. The swab is exposed to a volume of some millilitres of a solvent, for a time of the order of magnitude of 1 min. Swirling or sonication will help maximise the efficiency of the extraction process.

Blackledge and Vincenti applied an extraction with dichloromethane, followed by IR spectroscopy [167]. PDMS could be easily identified. IR spectroscopy offers a quite relevant potential for a precise description of the particular lubricant used [167, 169, 177, 178]. For example, the possibility to assess the viscosity of a PDMS lubricant by the analysis of its IR spectrum was reported [177]. The viability of vibrational spectroscopy in this kind of casework was confirmed also by reports which proved the ability of Raman to provide valuable information [179, 180].

However, a common feature of most proposed protocols for condom-related traces is that a complementary method should be applied to confirm the analyses. Blackledge proposed desorption chemical ionisation mass spectrometry [167]. In this technique, an amount of diluted sample solution, corresponding to less than 200 ng of solute, is loaded on a rhenium wire mounted on a probe. The wire is then placed in the mass spectrometer ion source, where it is heated in the presence of a reagent gas. This produces a fast vaporisation of the sample molecules, which undergo multiple collisions in the gas phase with the reagent gas, being thermally stabilised and subsequently ionised. This allows to obtain mass spectra of thermally labile and high molecular weight analytes, such as PDMS, without incurring in their thermal decomposition [167]. Mass spectrometry allowed to obtain further information on the kind of lubricant used, because it showed the possibility to differentiate between lubricants of different origin. Moreover, by this second technique the identification of the spermicide nonoxynol was feasible.

Maynard and colleagues proposed a double extraction procedure: first with hexane, followed by IR spectroscopy for identifying the presence of PDMS or petrolatum oil, then (after having dried the swab for at least 3 h) with methanol, to detect nonoxynol, glycerol and/or PEG [169]. Chromatographic analyses, pyrolysis–gas chromatography on the hexane extracts and liquid chromatography on the methanol extract, allow to confirm and refine the characterisation. These authors showed that the observation of fluorescence under several excitation light sources is a viable technique for a screening of the different classes of lubricants [169].

Nuclear magnetic resonance (NMR) was also reported as a technique for distinguishing between sexual lubricants used in condoms by different manufacturers [175, 181]. After an extraction with hexane or carbon tetrachloride, NMR allowed to detect and identify the lubricant traces present in the swab. However, despite its advantages, the sample preparation, the quite high detection limit and the high cost of the apparatus limit the application of NMR in forensic trace analysis.

Even though most reports reported that biological fluids do not significantly interfere with the analysis of condom-related traces, still the extracts from swabs are potentially complex mixtures. As such, probably the most logical analytical approach for their analysis is chromatography. Keil confirmed that lubricants with a silicon base can be detected by gas chromatography/mass spectrometry [168]. The swabs must be washed, and then a basic extraction using a buffer at pH 8.9 and a ether/ethylacetate mixture must be performed. Derivatisation with *N*,*O*-*bis*(trimethylsilyl)-trifluoroacetamide (BTSFA) and trimethylchlorosilane (TMCS) then follows, to favour the volatilisation of the compounds. The sample so obtained can be injected in a gas chromatographer, obtaining patterns which allow to differentiate lubricants of different brands and type. More importantly, Keil showed that this technique allows the detection of lubricant traces in real vaginal and anal swabs, up to 12 h after intercourse, even though not in all cases [168]. Campbell and Gordon as well applied gas chromatography to the analysis of condom lubricants, using pyrolysis/gas chromatography/mass spectrometry on hexane extracts of PDMS-based products and just gas chromatography/mass spectrometry on methanol extracts for PEG-based lubricants [171].

Mass spectrometry has lately emerged as a very performing technique for the analysis of condom-related traces [170, 173]. A particularly significant contribution was given by Spencer and colleagues, who developed a method for the matrix-assisted laser desorption/ionisation–time-of-flight mass spectrometry (MALDI–TOF–MS) profiling of trace constituents of condom lubricants [173]. These authors showed that MALDI–TOF–MS is ideal for detecting traces of condom lubricants and additives, mixed with biological fluids. They identified a sample preparation method which gave the most reproducible results with the lowest limit of detection [173]. One microlitre of a sample/bradykinin acetate mixture (bradykinin is used as an internal standard) was spotted onto a steel MALDI plate and allowed to air-dry. Next, 0.5 μL of 0.5 M sodium chloride (the cationisation reagent) in 50/50 HPLC-grade water/methanol was spotted on top of this and the solvent was allowed to evaporate. Finally, 1.0 μL of 2,5-dihydroxybenzoic acid (75 mg/mL in acetone) was spotted and allowed to dry. Other preparation procedures were proposed by Hollenbeck et al. [182], and by Bradshaw et al. [183].

For the extremely low sample size required, MALDI/TOF/MS is a very appealing technique for sexual assault-related cases, since it leaves much of the extract from the swabs available for confirmation analyses.

By this method, a very good differentiation between lubricants was obtained, which could be classified according to their main polymeric base and also on the content of additives, such as benzocaine or nonoxynol. Five separate classes of lubricants were identified by MALDI–MS, with detection limits between 0.5 and 0.003 % lubricant in biological fluid. Figure 4.9 shows the validity of MALDI/TOF/MS data, after PCA, in clustering the different samples.

The main segregation was achieved according to the polymeric base, whether PDMS or PEG. The PDMS cluster appears to be quite concentrated, but the samples could be further differentiated by IR spectroscopy. Indeed, lubricant formulas with hydroxyl-containing additives detectable by ATR–IR (Class A3) are somewhat

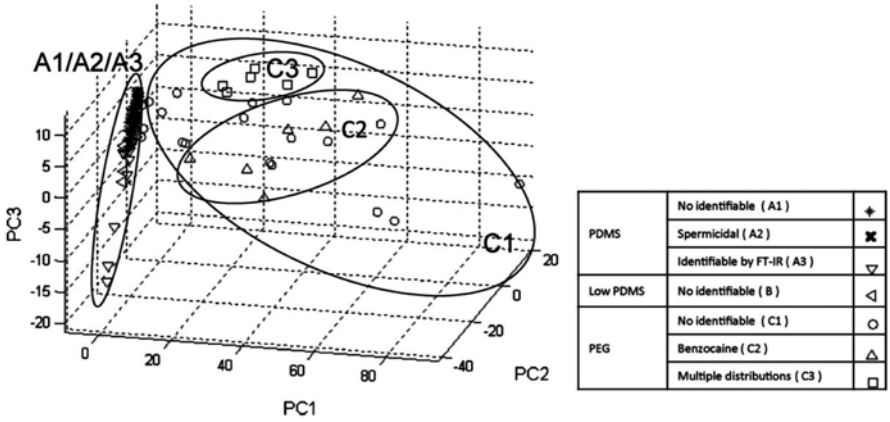


Fig. 4.9 3D PCA score plot of seven different condom lubricant types, four of which are easily distinguished. Data was generated using MALDI-MS data. Reprinted from [173], copyright 2011, with permission from Elsevier

separated also in the representation of Fig. 4.9 from the other two lubricant types (A1 and A2). On the other hand, PEG-based lubricants showed some separation between those with multiple PEG distributions (Class C3), and PEG-based benzocaine-containing lubricants (Class C2).

4.7 Foam Fragments

In Sect. 3.1, the tape lifting technique was presented, to show how the collection of small traces transferred to surfaces and objects can be done. Traditionally, the tape lifting technique is always presented as the way to recover fibre evidence. However, it is not a selective collection technique. In addition to fibres, many other particles remain attached to the tape. Within this particulate matter, there are some traces which often go unnoticed, but which could provide useful information on the circumstances of crimes. Wiggins, Emens and Brackley were the first who recognised the potential of these traces [184]. The interest in this kind of evidence was initiated by a murder case happened in 1979. On the victim, 15 small fragments of polyurethane foam were recovered, which at the time were not recognised as significative pieces of evidence. In a revision of the case 20 years later, the foam fragments were connected to an old mattress found in the suspect's lorry or to a seat of the vehicle. This motivated a study to assess the probability of transfer and the persistence of foam fragments, in order to understand if these could be treated as contact traces, in analogy to fibres, glass, paint or other better known pieces of evidence. The authors devised a number of tests which showed that indeed transfer from a donor of polyurethane fragments could happen with a pressure comparable to sitting on a car seat or lying on a bed [184]. The persistence studies indicated that the fragments rapidly

left the recipient object. The polyurethane fragments retained on blue denim after 4 h, on average, were 20 % of the original. However on the black and white cotton sample only 2.5 %, on average, of the original number were retained. Of course foam fragments adhered to the coarser surface of denim far better than the smoother surface of the black and white cotton. Even though the persistence results may seem poor, they are nevertheless comparable to those found for fibres [5, 18].

Reed and colleagues carried out a population study which further validated the evidential potential of foam fragments [185]. They examined 100 outer-garments of different kinds. They found foam fragments on 84 % of the considered items. Forty-eight percent of the garments carried 0–1 fragments, 26 % carried 2–4 fragments, 14 % carried 5–9 fragments and 12 % of the garments carried 10 or more fragments [185]. These authors classified the recovered fragments according to their colour, finding that 76 % of the items were amber, pale yellow or black. Other 15 colours were detected in the population, but with much lower abundances [185].

4.7.1 The Composition of Foams

Foams are materials in a lightweight cellular form resulting from the introduction of gas bubbles during the manufacturing process. They can be interpreted as a heterophasic material in which there are solid domains, in most of the common cases polymeric, which are called struts, and void regions, called cells or pores. Cells are closed when they are completely delimited by solid walls and when no interconnection between them exists. Cells are open when the pores are interconnected and the polymeric framework is much lighter, composed by filaments, rather than surfaces (Fig. 4.10).

The mechanical characteristics of a foam depend on its density, i.e. mass of struts per unit volume, and porosity, i.e. percentage of pores per unit volume. The size of the pores is an important parameter as well, in addition to the nature of the structural material used to manufacture the foam. Ceramic and metallic foams can exist, which of course will have very different mechanical performances than polymeric foams, such as those of interest in this section. The features mentioned above have a macroscopic effect on the appearance of the final product and on its applications. Flexible foams have an open-cell structure and can be produced in both high and low densities. Applications include cushioning for furniture and automobiles, mattresses and pillows, sponges, packaging and shoe soling. Rigid foams are highly crosslinked polymers with a closed cell structure that prevents gas movement. Their main application is as insulation for buildings, refrigerators and refrigerated transport vehicles.

The foam fragments with the most forensic interest are those coming from objects common in everyday life, and especially from cushioning, mattresses or pillows. The base material for these applications is polyurethane. As introduced in Sect. 2.8.11, polyurethane comes from the reaction of a prepolymer terminated with isocyanate groups and a hydroxyl-terminated species. This latter component can

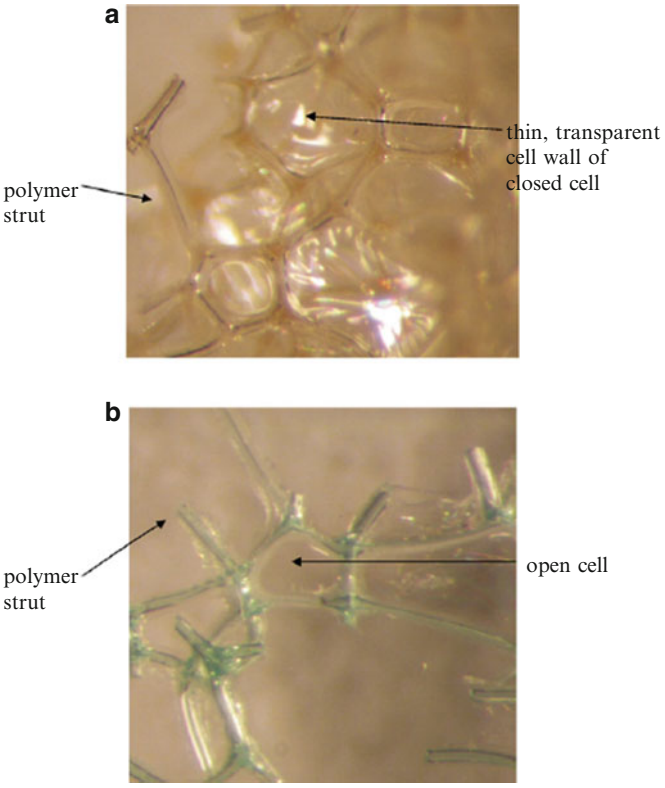


Fig. 4.10 (**a**) Foam taken from an eyewash station and (**b**) foam taken from a scourer, showing structural differences between closed cell and open-cell foam at ×200 magnification. Reprinted from [186], copyright 2007, with permission from Forensic Science Society

have several chemical natures and several structures, and it is normally an oligomeric or polymeric chain, whose flexibility and molecular weight will strongly influence the rigidity of the final material. In the polyurethane industry, two main families of hydroxyl-terminated compounds are used: polyethers or aliphatic polyesters.

Foaming of polyurethanes is obtained in the crosslinking step, in which the isocyanate component reacts with the hydroxyl-terminated species. If in this reactant mixture a foaming agent is added, gas bubbles will be trapped inside the solidified material, yielding the desired cellular structure. The more foaming agent, the larger and more abundant cells will be obtained within the foam. The simplest foaming agent is water, which reacts with isocyanate producing gaseous CO_2. More frequently, a separate foaming agent is added. Freon used to be the most common, but due to environmental regulations it has been substituted by pentane or heptane.

Other possible sources of foam fragments can be sponges for household cleaning. In this case, cellulose-based foams are often employed. Cellulose foams are produced mixing together cellulose, pore-forming agent, reinforcement fibres and the desired additives (fillers, dyes, etc.). This mixture is then transferred to a mould,

where it is heated, to crosslink the fibres and the cellulose matrix, while at the same time melting the pore-forming agent which drains away through openings in the bottom of the mould, leaving behind empty spaces, i.e. the pores of the foam.

The additivation of both kinds of foams is optimised as a function of the intended end use. Fillers are, as in many other polymeric items, the most abundant of the additives, and are used to improve the mechanical properties of the material. Dyes and pigments are other important components.

4.7.2 Collection and Handling of Foam Traces

As introduced above, no specific procedure of recovery of foam traces is applied in crime scene examination. Foam traces are collected together with other particulate traces and fibres by tape lifting. The same precautions and suggestions on the sample handling of fibre traces are valid also for foam fragments.

The presence of voids within the structure of foam fragments calls for some attention in the mounting of the samples for microscopy. Large fragments can be opaque to the observation in transmittance microscopy, so cross-sections as thin as possible should be cut with a scalpel, holding the fragment with tweezers. This can be a challenging task in the most flexible samples. The section so obtained is put on a microscope slide and a mounting medium is added. A cover slide is then added and carefully positioned. In order to expel any air bubble, it is suggested to wait about a minute between the addition of the mounting medium and the positioning of the cover slide. Moreover, the application of some pressure on the cover slide helps to expel air trapped in the sample.

4.7.3 Characterisation of Foam Traces

To the knowledge of the author, just two papers exist, regarding the forensic characterisation of foam fragment [186, 187]. A viable protocol for foam fragments starts with an observation under low and high-power microscopy for a first screening. Considerable morphological differences can exist in the morphology of the foam when observed at high magnification, as shown in Fig. 4.11. A number of features can be assessed, such as the morphology of the struts, the density of the cells, the kind of colourant, whether dye or pigment, which was used.

The technique used for colouring the material can be easily detected just by observing the sample. Figure 4.12 shows two foam particles, coloured by a dye and a pigment, respectively. The presence of particulate material in the pigmented item is very evident. On the other hand, the dyed foam displays an homogeneously coloured surface, without evidence of heterogeneity in the formulation.

The use of different light sources, such as transmitted white light, light reflected through a blue filter or light reflected through a UV filter proved to be useful for refining the observation of foam fragments [186].

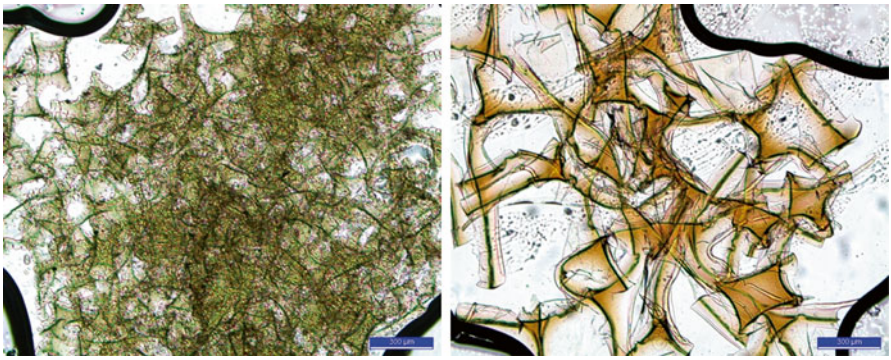


Fig. 4.11 Micrographs comparing the morphology of two different foam fragments

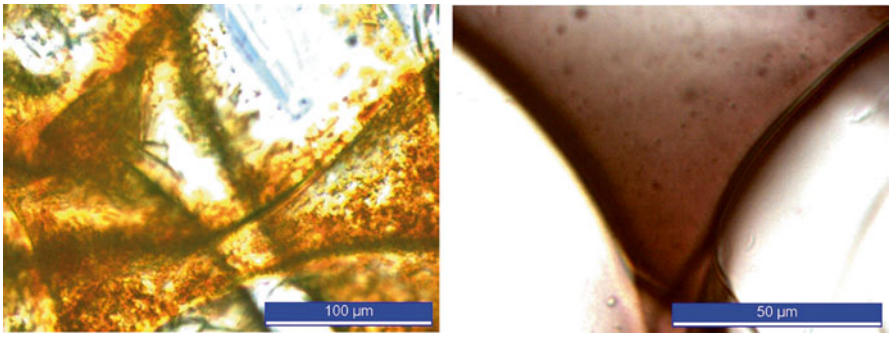


Fig. 4.12 Micrographs comparing the morphology of a (*left*) pigmented foam fragment and of (*right*) a dyed foam fragment

Foam fragments which are microscopically indistinguishable can be efficiently discriminated by microspectrophotometry in the visible range. This simple procedure allowed to distinguish foam fragments from different sources, whereas foam fragments from the same source did not display microscopical or chemical variations. It was reported that a more efficient distinction between the spectra can be obtained by calculating their first derivative [186].

According to Parsons, IR spectroscopy was not a viable method for the characterisation of foam fragments. This was due to difficulties in sample mounting in a diamond cell, which had a poor spectral quality as a consequence. Actually, an unpublished work by this author showed that ATR sampling allows to obtain clean and significant IR spectra. This indeed allowed to differentiate among a population of 48 foam samples. Such samples were part of a collection of foams from different origins, mainly from cushioning and stuffings for furniture and cars, but also from sponges. All the samples came from the North East of Italy. Most of the analysed foam fragments in this work were composed of polyurethane. IR spectroscopy allowed to differentiate between the samples based on poly(ether urethane) and those based on poly(ester urethane) (Fig. 4.13).

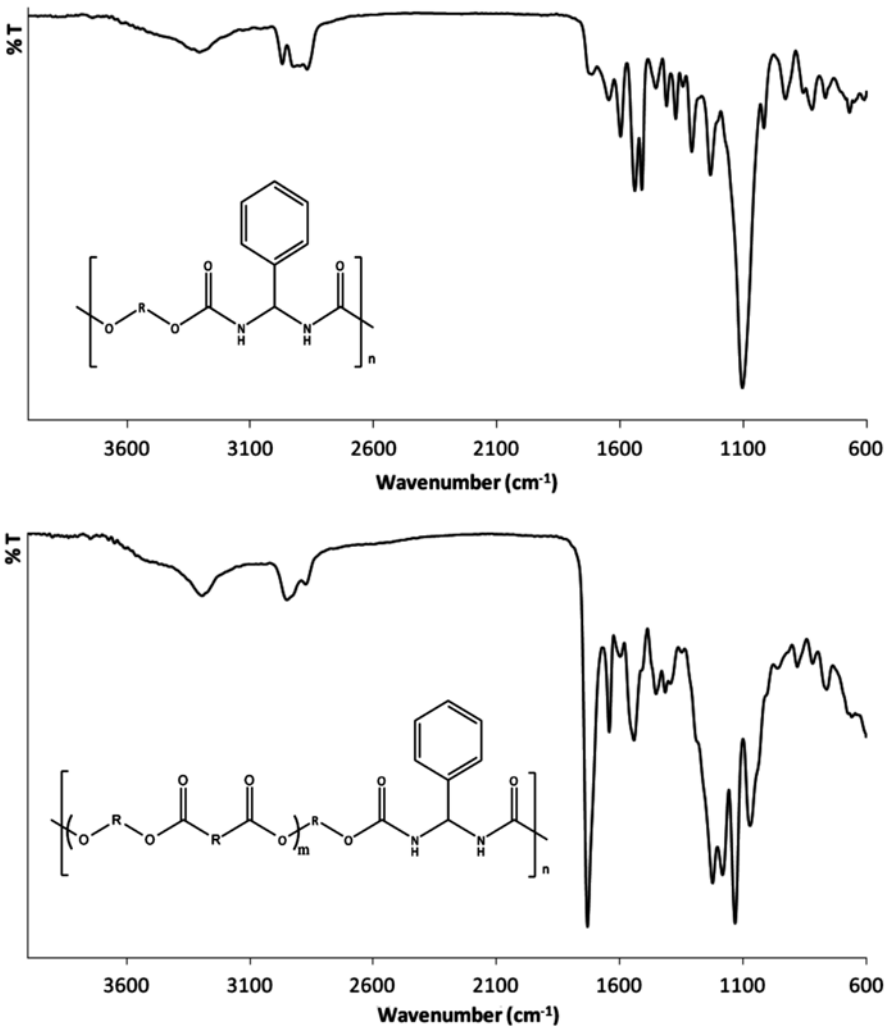


Fig. 4.13 IR spectra of a foam based on poly(ether-urethane) (*top*) and of a foam based on poly (ester-urethane) (*bottom*)

In addition to this, within the considered population of foams two other base polymers were identified: cellulose, and polyethylene. The preponderant polymer base was poly(ether urethane), which represented 79 % of the total population, followed by poly(ester urethane) with 8 % of the samples. Six percent of the samples was composed by cellulose, 4 % by polyethylene. Moreover, in some cases signals due to fillers such as talc or silica were detectable in the IR spectra of the foam fragments.

Parsons proposed two further techniques for refining the characterisation of foam fragments [187]. The first consists in the gas chromatographic analysis of a dichloromethane extract. The sample size used by these authors was about 3 mg/mL of solvent. A remarkably detailed picture of the formulation of the material could be obtained if mass spectrometry was used as a detection method, because stabilisers, catalysts, antibacterials, dyes, fragrances and other additives could be isolated in the chromatograms. All the seven samples considered by the authors yielded different chromatographic patterns [187].

As a second complementary approach to the microscopic observation and to spectroscopical techniques, elemental analysis was proposed. In particular, the content of tin was deemed particularly diagnostic, because it is contained in the catalysts used in the manufacturing of polyurethane. Contents ranging between 20 and 350 mg/kg were measured by atomic emission absorption spectroscopy, on samples digested in a two-step process using nitric acid and H_2O_2 [187]. This quantity of analyte is not excessively low, so it is in principle accessible with other techniques of elemental analysis. Micro-X-ray fluorescence could be a feasible alternative. Experiments would be necessary to assess if scanning electron microscopy with energy dispersive X-ray detectors could be a viable option.

4.8 Plastic Bags

The characterisation of plastic packaging material may be a problem of relevant importance in forensic science. Plastic bags are used to conceal body parts of a victim, other crime-related materials or illicit drugs. As with adhesive tapes, plastic films can be used for the packaging and manufacturing of explosive or fire igniting devices. Shopping bags, in particular, are frequently used for preparing, from a larger amount, single doses of illicit drugs to be sold to the final user. The typical case which involves plastic bags is one in which a smuggler uses them to prepare small one-dose packages, which are subsequently hidden in a public location such as a park or in a communal area of a building. The more aware drug smugglers tend in fact to avoid keeping illicit substances in the premises they own or where they live. If hidden packages such as those depicted in Fig. 4.14 are found or seized, it

Fig. 4.14 (*Left*) Single doses of heroin found in the common garden of a condominium; (*right*) torn plastic bags found in the garbage bin of a suspect drug smuggler living in that condominium

becomes important from a probatory point of view to prove a connection between these items and the person who is suspected to have prepared them.

Searching the house of an alleged drug smuggler, chances are that torn plastic bags may be found in the trash bin. In order to connect the suspect with the drug, a thorough examination of the packaging used to contain the illicit substance is necessary.

4.8.1 Composition of Plastic Bags

Plastic bags are experiencing a period of rapid change. Regulations in the European Union and elsewhere in the world are moving towards a ban of the previously employed materials, mainly polyethylene, in favour of biodegradable materials. The performance of these substituting materials is still inferior to that of polyethylene, which can still be found in some shops and can still be bought. Garbage bags which are sold in supermarkets are still made with polyethylene and have the mechanical properties and durability expected for packaging purposes. Biodegradable polymers used to substitute traditional disposable shopping bags are patented materials, which are mostly composed of a mixture of polyesters and plasticised starch.

In addition to the polymer matrix, the additives present in larger amount are fillers. Calcium carbonate and talc are the most common. Additional stabilisers and UV protectors, which are normally used in polyethylene, are moreover present.

4.8.2 Sampling and Handling of Plastic Films

The sample size in casework involving plastic packaging is usually large enough to allow the application of several techniques, still preserving enough material for repeating the analyses. When plastic films are used for manufacturing large drug packages (Fig. 3.1) or explosive devices, fingerprints can be present. Before proceeding to the development of these traces, a portion of the material should be collected, because cyanacrylate fuming modifies the chemistry of the films [136]. This allows one to perform chemical analyses on a pristine amount of sample, while leaving untouched most of the surface of the item for the fingerprint specialist.

4.8.3 Characterisation of Plastic Films

The packaging market is very diffused, with a huge number of small producers. Analyses aimed at the identification of the manufacturer are almost unthinkable, because of the difficulties associated in reaching all the possible companies

producing packaging and plastic bags. The aim in this casework is therefore comparative.

The characterisation of films for plastic packaging is quite straightforward. Many similarities exist with the characterisation of the backing of adhesive tapes. First of all, an observation of the morphology must be done, measuring the thickness of the film and noting the response of the material to illumination with light of different wavelengths and also the birefringence through crossed polarisers.

The chemical characterisation of these materials must be designed choosing the analytical techniques which are able to catch the subtle differences linked to the slightly different production processes and formulations used by the multitude of manufacturers present in the field. A number of methods have been proposed for the analysis of plastic packaging films, among which are UV-Visible [188] and Infrared (IR) spectroscopy [188–190], Differential Scanning Calorimetry (DSC) [190, 191], X-ray diffraction [192] and elemental analysis [193, 194].

Like in the case of adhesive tapes, IR spectroscopy is a very useful first instance technique, which allows a first screening of the base polymer which composes the material and of the major filler. More details on the IR spectroscopy of plastic films for packaging will be presented in Sect. 5.8.

Analogously to the case of paper (Sect. 4.9.1), XRD is an effective technique to yield, in a non-destructive way, information on the structure of the polymer and on its formulation. The X-ray diffractogram of plastic bags presents signals which are due to all the crystalline species within the material: the crystalline domains of the polymer and the fillers. As is more completely elaborated in Chap. 7, the crystallinity of a polymer is strictly dependent on the processing used to transform it, therefore it is a very indicative parameter for discriminating plastic bags [192].

Similar and complementary information on the structure of a polymer can be obtained by DSC [190, 191]. This technique is much more widespread and less expensive than XRD, but it is sensitive just to the polymer matrix, not to the inorganic fillers, and it is destructive. The required sample size of DSC is though in the order of 5 mg, so perfectly compatible with the amount of evidence available in normal casework.

4.9 Polymers in Documents: Paper and Toner

Even in the modern world, dominated by digital technologies, paper documents are still necessary for attributing legitimacy to agreements, transactions, wills or other acts between persons. When the validity of a document is challenged or otherwise disputed, any information on how it was formed can be useful to assess if it was modified, altered or forged.

Pen inks are obviously the most important substances which are of interest to a questioned document examiner, because in many jurisdictions a handwritten signature or a stamp are the fundamental features which signify the formal acceptance of

what is written. Inks contain polymers, in the form or resins which are used to optimise the viscosity and to fix the dyes and pigments to the paper. However, their small amount, with respect to the other components of the formulation, prevents the detection of resins in inks on documents.

The two polymeric items which can be analysed on documents and which in some cases can give useful information are paper and toner.

4.9.1 Paper

The possibility to discriminate between sheets of paper can be of considerable importance in questioned document examinations. A series of anonymous letters can be connected to the same author by the use of a common type of paper. The analysis of paper can help to connect or relate different documents, or to identify that a particular document was written on the paper seized in the suspect's premises. Document frauds can be carried out substituting a page of an agreement without the consent of all parties. In this instance, the different or common nature of the paper of the questioned page with respect to that of the rest of the document would be a strong point to support or exclude forgery.

The evidential value of paper examination is very highly dependent on the circumstances of the case, both referred and inferred. There are no laws prescribing that a multipage document must be all printed on the same kind of paper. If a page in the middle of a five-page contract is different from the others, this surely raises some suspicions, but it is rarely a conclusive proof sufficient to deem that document a fraud. All the other elements available to the Court will therefore be key for deciding if the mode of formation of the document, reconstructed from the experimental data, is consistent with the reconstruction given by the parties to the case.

The main component of paper is cellulose. Papermaking consists in connecting fibres in a durable two dimensional network with mechanical properties which are actually remarkable, if the small thickness of a paper sheet is taken into account. The raw material for obtaining the cellulose fibres depends on the type of desired paper. High-quality papers are obtained from cloth rags or from cotton and linen fibres. Normally, most of the cellulose is extracted from trees. Coniferous trees, classified as softwood in the paper industry, have longer fibres which allow to make a stronger paper. Deciduous trees are called hardwood in the industry. They provide less valuable fibres but they grow faster and so they are very widely cultivated. Wood can be converted in a pulp composed of cellulose fibres by a mechanical process, in which a progressively finer grinding is applied, or by a chemical process, in which wood chips are digested, boiling at high pressure in a solution of sodium hydroxide and sodium sulphide. The pulp is then filtered and bleached or coloured. Lower quality pulp derives from the recycling of paper. Papermaking can be shortly summarised as a process in which cellulose fibres are mixed and then cooked in a hot basic aqueous solution until the fibres are soft but not dissolved. Water is subsequently removed squeezing it out through a screen by application of pressure.

The formulation of paper is therefore quite simple, since the other additives present in this material are fillers (calcium carbonate is the most common, and since the 1990s it is the only filler used in normal office paper; other fillers used in specialty papers are kaolin or clays), dyes or pigments (TiO_2), bleaches and optical brighteners, and sizings such as rosin, gum and starch. Sizing controls how paper will interact with inks. Without any sizing, paper will be too absorbent. Starch is a good sizing for making the paper resistant to water-based ink. Fillers and sizings are added in the beating step of the process, which is the first that the pulp experiences. After the beating step, a homogenous pulp is fed onto a moving belt of fine mesh screening. The pulp is squeezed through a series of rollers, while suction devices below the belt drain off water. Watermarks can be created in this stage, pressing a so-called dandy on the sheet in order to produce thinner portions of the paper with a particular design. The residual water is absorbed through a series of rollers lined with wool felt and with large heated cylinders. A final calendering step imparts to dried paper the desired finish, whether soft, dull or shiny. Sometimes a superficial coating is added. The rolls of paper so produced are then cut in the desired format.

A number of techniques have been proposed to characterise paper, among which X-ray diffraction (XRD) [195–199], elemental analysis [200, 201], IR spectroscopy [199, 202–204], Raman spectroscopy [205], image analysis [206], UV–visible spectroscopy [207] and pyrolysis gas chromatography [208]. Physical tests, such as the measurement of density or of mechanical performance, are less viable since they require special apparatus and imply destructive procedures. Moreover, they often yield not significant results, given the high standardisation of the market and of production procedures, e.g. all common photocopy paper will weight 80 g/m^2.

Probably the most performing techniques for an effective analysis of paper are XRD and IR spectroscopy. The data obtained from both these approaches allow to acquire information simultaneously on cellulose and on the additives present in the formulation, especially of fillers. By XRD it is possible on one hand to assess the degree of order, i.e. the degree of crystallinity, attained by cellulose as a consequence of the nature of raw materials and of the processing parameters adopted in its manufacturing [198, 199]. On the other hand, it allows also the detection of peaks due to the crystalline structure of inorganic fillers and therefore yields information on the additive profile of the paper sample [199]. XRD offers the significant advantage of being non-destructive. Diffractometers are usually big instruments, which can accommodate in the sample holder normal size documents, without the need to collect or remove samples.

IR spectroscopy is another useful technique for the examination of paper because semiquantitative information can be obtained on its formulation. This can be done by measuring the ratio between the intensity of the peaks due to cellulose and the intensity of the peaks due to inorganic fillers such as calcium carbonate (Sect. 5.8).

The combined application of these two techniques allowed to discriminate all the samples in a set of 19 kinds of office paper differing by manufacturer and brand [199].

4.9.2 Toner

Until a few years ago, the only documents printed with toner were photocopies. With the increasing availability of affordable laser printers, the quantity of documents printed with this material is expanding.

Different technologies exist for printing with toner, but they basically consist in distributing the fine particles (of a diameter in the range of less than 20 μm) of this material selectively in certain area of the paper, i.e. in correspondence to the letters or drawings to be reproduced. Toner is subsequently fused on the paper and fixed on it.

Even though toner appears like soot, it is actually a very complex material, which has to satisfy several requirements to make the printing process as smooth and as precise as possible. This brings about the necessity, within this industry, of controlling particle shape and size distribution, and the formulation.

The polymeric binder is the major ingredient in toner formulation, and it may represent between 40 and 95 % of its total mass. The polymers most commonly used are copolymers of styrene and acrylic monomers, such as poly(styrene-c*o*-methyl methacrylate). Copolymers of styrene and butadiene, polyesters or epoxide resins are also encountered. Iron oxide is the second most abundant component of toner, accounting for up to 50 % of its composition, even though it is not always present. The development step of the printing process exploits a magnetic field for its functioning. Some printing technologies using coloured dual component non-magnetic toners in fact do not require magnetite as an additive. In dual component toners, the magnetic properties are due to a ferromagnetic carrier which is added to the toner. On the contrary, in single component toners, iron oxide imparts intrinsic ferromagnetic characteristics to this material.

Pigments represent about 5 % of the toner formulation. Black toner is pigmented with carbon black. Colour laser printing is obtained by using a combination of four toners, black, cyano, magenta and yellow. Surface additives (fumed silica, metal stearates, fluoropolymer powders, magnetite, cerium oxide, carbon black) are used for tuning flow properties, charge, conductivity and to optimise the cleaning process of the machine. Waxes and internal charge control agents complete the formulation.

The most efficient analytical approach tackles the double nature of toner, as a mainly polymeric item which however contains a significant amount and variety of inorganic substances. IR spectroscopy is surely the technique which was most used for the characterisation of toner ([209, 210] and references therein). It allows to classify toners within a limited number of categories, and with IR microscopes the analysis can be performed in a non-destructive way, and without suffering from the interference of paper, which is a major problem in the in situ IR spectroscopy of inks on documents. As a complementary technique for the analysis of toner, elemental analysis allows to exploit the rich additivation with inorganic compounds

which characterises toner. X-ray fluorescence, with its non-destructiveness and possibility to probe microscopic areas of the document, is the technique of choice for this problem [210, 211].

4.10 Post-fire Traces

The debris recovered on the scene of a fire is a different kind of forensic evidence than those examined thus far. In fire investigation, the focus is understanding how a fire started and how it proceeded, and especially to verify if it was intentionally set or if it occurred for some random cause, not related to the commission of a crime.

For example Roberts and colleagues showed that the spectral changes to paint samples allowed to estimate the temperature it had reached [108].

Polymers are involved in two possible ways in such investigational activity. On one hand, they share a remarkable chemical similarity with the accelerants commonly used to start fires, such as gasoline, kerosene and diesel fuel. The residues after combustion of accelerants and polymers can be difficult to distinguish. Any method of detection of accelerants should be therefore tested according to its selectivity and to its ability to prevent the misinterpretation of pyrolysis products of polymers as traces of accelerants. The other role that polymers play in fire investigation is in the reconstruction of the device which started the fire. The perfect way to commit arson is to invent an ignition device which, after having originated the fire, is destroyed by it and disappears. However, sometimes this is not possible and the remnants of the contrivance are detected. A reconstruction of the mechanism of such device is often the best way to prove arson and knowledge of the materials used is part of such investigation.

4.10.1 The Identification of Pyrolysis Products in Fire Debris

Because the most common fire accelerants consist of mixtures of volatile organic compounds (VOCs), accurate and sensitive determinations of the VOCs found at the scenes of fires is the method of choice in arson investigations. For determining fire-related VOCs, the analysis in gas chromatography (GC) is the one predominantly used, mainly due to its selectivity, sensitivity, low detection limit and wide availability. Since fires are associated to high temperatures, it is to be expected that most of the VOCs in accelerants will evaporate, so some kind of sample preconcentration process is necessary before analyses are performed. Usually passive head space concentration on activated carbon or Tenax resin is applied. The use of solid-phase microextraction is increasingly common. The material preconcentrated on such

solid supports is subsequently extracted (in the case of carbon or Tenax) or desorbed (in solid-phase microextraction) and injected in a gas chromatograph.

If the chromatogram shows the presence of substances characteristic of an accelerating liquid, then this is in principle significant evidence that the fire was set on purpose. However, this apparently linear and simple analytical strategy is considerably complicated by the fact that, in the scene of fires, many polymeric items are usually present. Since polymers share molecular similarities with many ignitable liquids, when exposed to heat, they can decompose, producing several VOCs which can interfere with the identification of the chromatographic markers of accelerants. Interference from background materials falls into two basic categories [212]. The first consists in actual petroleum products present in the substrate material. For example, lubricant oil can be present on the floor of a parking garage. The second class of interfering substances which can be detected are due to substrate materials that pyrolyse to form VOCs in the range of common ignitable liquids. Many studies were focused on understanding the consequences on the significance and on the interpretation of forensic data that the presence of volatile pyrolysates have on the identification of ignitable liquids.

It has been reported by many authors that many of the target compounds used as markers of ignitable liquids are present in a number of other common items. For example, toluene and xylenes, which are used for the identification of gasoline, were identified in adhesives, shoes, clothing and other substrates containing polymers [212]. Normal and branched alkanes, and aromatic products such as 2-methylnaphthalene are found within the pyrolysis products of carpets or of plastic objects like bottles or bags [213]. When carpets burn, they can generate large amounts of alkyl-benzenes, benzene, toluene, ethylbenzene, styrene and alkyl-styrenes, naphthalene and methylnaphthalenes and other hydrocarbons usually interpreted as diagnostic components of petrol [214–216].

It is beyond the purpose of this book to discuss the implications of pyrolysis of polymers on arson investigation. The understanding of this topic requires a study of the mechanisms of degradation at high temperature of each polymer, and represents a whole field of knowledge on its own [217, 218].

Studies like these allow to select target compounds to identify petroleum distillates even in the presence of pyrolysis products. Keto proposed a method to distinguish the very similar pattern of the pyrolysis products of polyethylene from the VOCs of kerosene [219]. Alkenes are in fact present only in the polymer pyrolysate, and branched alkanes are present only in burnt kerosene.

The presence of alkenes in polyethylene pyrolysates is due to the particular degradation of this material, which undergoes a random scission process which results in the formation of alkadienes, alkenes and alkanes [217, 218]. Extracting from the chromatogram the species with ions with a m/z 83 is further informative, because it represents the alkenes in the pyrolysed polyethylene and the alkylcyclohexanes in kerosene. These two families of compounds appear at different retention times, and so they can be distinguished. The distinction of polyethylene pyrolysis

products from diesel was proposed by Lentini, who based the discrimination on the fact that the pyrolisate of diesel is dominated by the normal alkanes, and it contains pristane and phytane [220]. The product of pyrolysis of polyethylene does not contain pristane and phytane, but it contains alkadienes (*m*/*z* 57) and is dominated by alkanes [220].

Recent chemometric and multivariate statistics methods allowed to further refine these discrimination capabilities [221].

4.10.2 Post-fire Materials Identification

The identification of the materials which composed the burned remains of an object can be of considerable interest during fire investigation. The information which can be obtained by such analysis are as diverse as acquiring insight on how and how fast the fire spread across the room or the building, if and which toxic gases were evolved, or how ignition happened.

This kind of identificative analysis is not easy, since fire can considerably modify the chemical nature of the materials, and so knowledge on their degradation and pyrolysis patterns is desired, as discussed above in the case of the identification of pyrolysis products in fire debris. Pyrolysis/gas chromatography/mass spectrometry was proposed as a suitable technique, but its practical application in cases like these is quite time-consuming [222, 223]. The application of IR spectroscopy [52] is an option, but the interpretation of spectra can be quite difficult. Three recent works proposed convincing results with Raman spectroscopy coupled with chemometric data analysis [224–226]. Chemometry is necessary to extract most of the possible information from spectra which can be either very poor and almost featureless or on the contrary they can contain the contribution from several irrelevant contaminants.

The authors of these studies proceeded collecting commonly found polymeric items such as carpets, stockings or packagings and burned them with different ignitable liquids. Raman spectra were acquired before and after burning.

Figure 4.15 shows an example of how the Raman and IR spectra are modified by exposure to fire.

Fire severely modified the spectra. However, principal component analysis was applied to the Raman spectra of the burned materials, and their positive identification by Raman spectroscopy was possible. The method was successful irrespective of the different fuels used for setting fire and also after the chemical and structural identity of the plastic had been altered in the fire [224].

Raman spectroscopy can be coupled to ATR-IR spectroscopy, thus improving even further the ability to identify burned materials. As can be verified in Fig. 4.15, in fact, the limitations associated with Raman spectroscopy in post-fire identification can be offset by IR spectroscopy data, and vice versa [226].

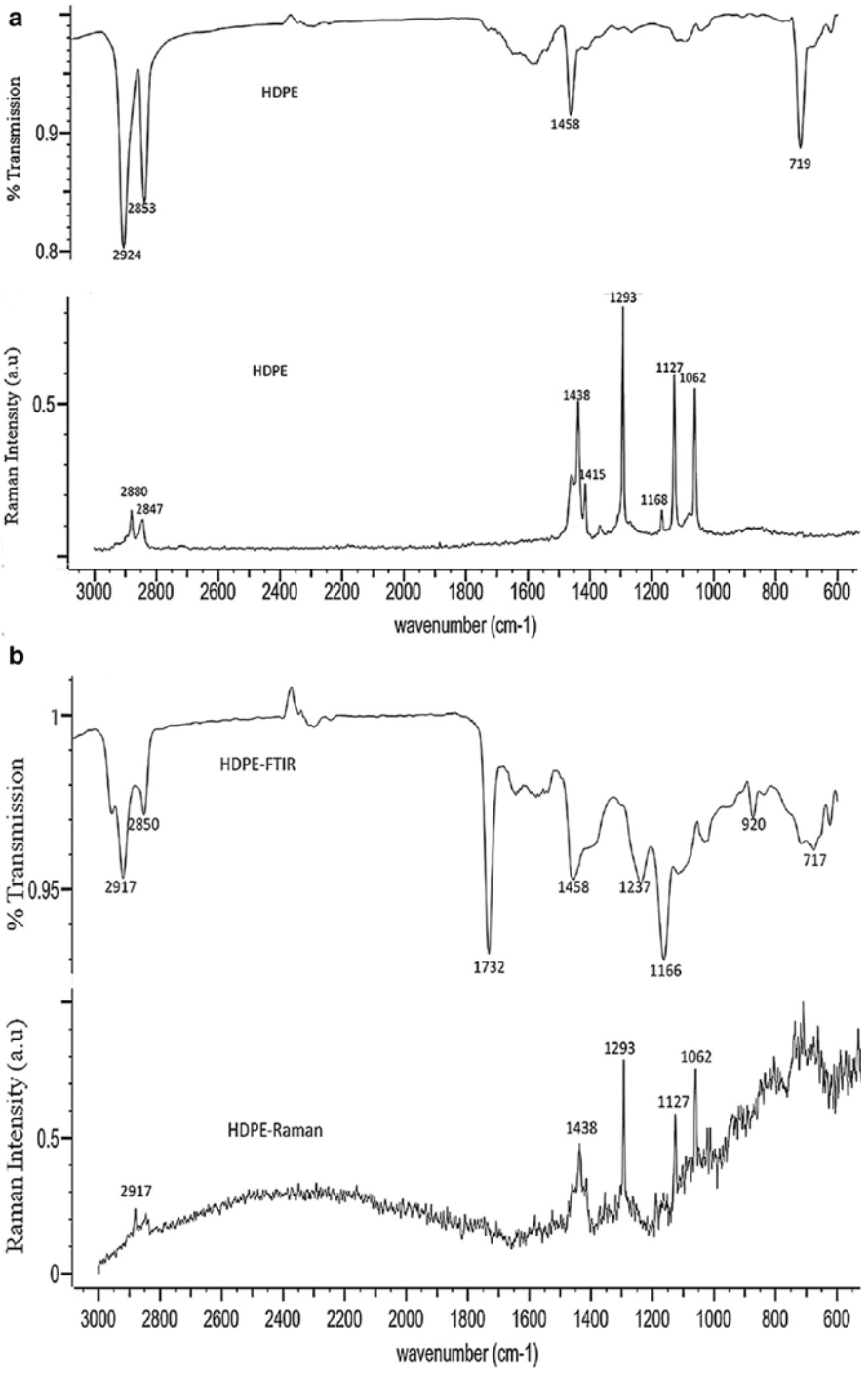


Fig. 4.15 IR and Raman spectra of polyethylene (**a**) before and (**b**) after exposure to fire. Reprinted with permission from [226], copyright 2013, with permission from Elsevier

References

1. Biermann TW, Grieve MC (1998) A computerized data base of mail order garments—a contribution toward estimating the frequency of fibre types found in clothing. Part 3: The content of the data bank—is it representative? Forensic Sci Int 95:117
2. Biermann TW, Grieve MC (1996) A computerized data base of mail order garments: a contribution toward estimating the frequency of fibre types found in clothing. Part 1: the system and its operation. Forensic Sci Int 77:65
3. Biermann TW, Grieve MC (1996) A computerized data base of mail order garments: a contribution toward estimating the frequency of fibre types found in clothing. Part 2: the content of the data bank and its statistical evaluation. Forensic Sci Int 77:75
4. Grieve MC (2000) A survey on the evidential value of fibres and on the interpretation of the findings in fibre transfer cases. Part 1—fibre frequencies. Sci Justice 40:189
5. Pounds CA, Smalldon KW (1975) The transfer of fibres between clothing materials during simulated contacts and their persistence during wear—Part 1: fibre transference. J Forensic Sci Soc 15:17
6. Pounds CA, Smalldon KW (1975) The transfer of fibres between clothing materials during simulated contacts and their persistence during wear. Part III—a preliminary investigation of the mechanisms involved. J Forensic Sci Soc 15:197
7. Roux C, Langdon S, Waight D et al (1999) The transfer and persistence of automotive carpet fibres on shoe soles. Sci Justice 39:239
8. Kidd CB, Robertson J (1982) The transfer of textile fibres during simulated contacts. J Forensic Sci Soc 22:301
9. Grieve MC, Dunlop J, Haddock PS (1989) Transfer experiments with acrylic fibres. Forensic Sci Int 40:267
10. Salter MT, Cook R (1996) Transfer of fibres to head hair, their persistence and retrieval. Forensic Sci Int 81:211
11. De Wael K, Lepot L, Lunstroot K et al (2010) Evaluation of the shedding potential of textile materials. Sci Justice 50:192
12. Grieve MC, Biermann TW (1997) Wool fibres—transfer to vinyl and leather vehicle seats and some observations on their secondary transfer. Sci Justice 37:31
13. Palmer R, Burch HJ (2009) The population, transfer and persistence of fibres on the skin of living subjects. Sci Justice 49:259
14. Roux C, Chable J, Margot P (1996) Fibre transfer experiments onto car seats. Sci Justice 36:143
15. Robertson J, Kidd CB, Parkinson HM (1982) The persistence of textile fibres transferred during simulated contacts. J Forensic Sci Soc 22:353
16. Palmer R, Polwarth G (2011) The persistence of fibres on skin in an outdoor deposition crime scene scenario. Sci Justice 51:187
17. Spencer R (1994) Significant fiber evidence recovered from the clothing of a homicide victim after exposure to the elements for 29 days. J Forensic Sci 39:854
18. Pounds CA, Smalldon KW (1975) The transfer of fibres between clothing materials during simulated contacts and their persistence during wear—Part 2: fibre persistence. J Forensic Sci Soc 15:29
19. DeBattista R, Tidy H, Thompson TJU et al (2014) An investigation into the persistence of textile fibres on buried carcasses. Sci Justice 54:288
20. Biermann T (1998) The advantages and disadvantages of 1:1 taping. In: Proceedings of the 6th European fibres group meeting, Dundee, 1998
21. Causin V, Schiavone S, Marigo A et al (2004) Bayesian framework for the evaluation of fiber evidence in a double murder—a case report. Forensic Sci Int 141:159
22. Robertson JR, Grieve M (eds) (1999) Forensic examination of fibres. Taylor & Francis, London
23. Cantrell S, Roux C, Maynard P et al (2001) A textile fibre survey as an aid to the interpretation of fibre evidence in the Sydney region. Forensic Sci Int 123:48

24. Grieve MC, Biermann T (1997) The population of coloured textile fibres on outdoor surfaces. Sci Justice 37:231
25. Jones J, Coyle T (2011) Synthetic flock fibres: a population and target fibre study. Sci Justice 51:68
26. Kelly E, Griffin RME (1998) A target fibre study on seats in public houses. Sci Justice 38:39
27. Marnane RN, Elliot DA, Coulson SA (2006) A pilot study to determine the background population of foreign fibre groups on a cotton/polyester T-shirt. Sci Justice 46:215
28. Palmer R, Oliver S (2004) The population of coloured fibres in human head hair. Sci Justice 44:83
29. Roux C, Margot P (1997) An attempt to assess the relevance of textile fibres recovered from car seats. Sci Justice 37:225
30. Roux C, Margot P (1997) The population of textile fibres on car seats. Sci Justice 37:25
31. Watt R, Roux C, Robertson J (2005) The population of coloured textile fibres in domestic washing machines. Sci Justice 45:75
32. Grieve MC, Biermann T, Davignon M (2003) The occurrence and individuality of orange and green cotton fibres. Sci Justice 43:5
33. Grieve MC, Biermann TW, Davignon M (2001) The evidential value of black cotton fibres. Sci Justice 41:245
34. Grieve MC, Biermann TW, Schaub K (2005) The individuality of fibres used to provide forensic evidence—not all blue polyesters are the same. Sci Justice 45:13
35. Palmer R, Hutchinson W, Fryer V (2009) The discrimination of (non-denim) blue cotton. Sci Justice 49:12
36. Biermann TW (2007) Blocks of colour IV: the evidential value of blue and red cotton fibres. Sci Justice 47:68
37. De Wael K, Baes C, Lepot L et al (2011) On the frequency of occurrence of a peculiar polyester fibre type found in blue denim textiles. Sci Justice 51:154
38. Eng M, Martin P, Bhagwandin C (2009) The analysis of metameric blue fibers and their forensic significance. J Forensic Sci 54:841
39. Wiggins KG, Turner YJ, Miles JH (1999) The use of the Foster & Freeman Fx5 Fibre Finder in forensic textile examinations. Sci Justice 39:19
40. Paulsson N, Stocklassa B (1999) A real-time color image processing system for forensic fiber investigations. Forensic Sci Int 103:37
41. Grieve MC, Griffin RME (1999) Is it a modacrylic fibre? Sci Justice 39:151
42. Causin V, Marega C, Guzzini G et al (2005) The effect of exposure to the elements on the forensic characterization by infrared spectroscopy of poly(ethylene terephthalate) fibers. J Forensic Sci 50:887
43. Causin V, Marega C, Guzzini G et al (2004) Forensic analysis of poly(ethylene terephthalate) fibers by infrared spectroscopy. Appl Spectrosc 58:1272
44. Cho LL, Reffner JA, Gatewood BM et al (2001) Single fiber analysis by internal reflection infrared microspectroscopy. J Forensic Sci 46:1309
45. Cho LL, Reffner JA, Gatewood BM et al (1999) A new method for fiber comparison using polarized infrared microspectroscopy. J Forensic Sci 44:275
46. Espinoza E, Przybyla J, Cox R (2006) Analysis of fiber blends using horizontal attenuated total reflection Fourier transform infrared and discriminant analysis. Appl Spectrosc 60:386
47. Farah S, Tsach T, Bentolila A et al (2014) Morphological, spectral and chromatography analysis and forensic comparison of PET fibers. Talanta 123:54
48. Fox RH, Schuetzman HI (1968) The infrared identification of microscopic samples of man-made fibers. J Forensic Sci 13:397
49. Grieve MC, Griffin RME, Malone R (1998) Characteristic dye absorption peaks found in the FTIR spectra of coloured acrylic fibres. Sci Justice 38:27
50. Nayak RK, Padhye R, Fergusson S (2012) Identification of natural textile fibres. In: Kozlowski R (ed) Handbook of natural fibres, vol 1, Types, properties and factors affecting breeding and cultivation. Woodhead, Cambridge, p 314

51. Siroka B, Siroky J, Bechtold T (2011) Application of ATR-FT-IR single-fiber analysis for the identification of a foreign polymer in textile matrix. Int J Polymer Anal Char 16:259
52. Was J, Knittel D, Schollmeyer E (1996) The use of FTIR microspectroscopy for the identification of thermally changed fibers. J Forensic Sci 41:1005
53. Yu MML, Sandercock PML (2012) Principal component analysis and analysis of variance on the effects of Entellan new on the Raman spectra of fibers. J Forensic Sci 57:70
54. Thomas J, Buzzini P, Massonnet G et al (2005) Raman spectroscopy and the forensic analysis of black/grey and blue cotton fibres—Part 1. Investigation of the effects of varying laser wavelength. Forensic Sci Int 152:189
55. Was-Gubala J, Machnowski W (2014) Application of Raman spectroscopy for differentiation among cotton and viscose fibers dyed with several dye classes. Spectros Lett 47:527
56. White PC, Munro CH, Smith WE (1996) In situ surface enhanced resonance Raman scattering analysis of a reactive dye covalently bound to cotton. Analyst 121:835
57. Jochem G, Lehnert RJ (2002) On the potential of Raman microscopy for the forensic analysis of coloured textile fibres. Sci Justice 42:215
58. Lepot L, De Wael K, Gason F et al (2008) Application of Raman spectroscopy to forensic fibre cases. Sci Justice 48:109
59. Abbott LC, Batchelor SN, Smith JRL et al (2010) Resonance Raman and UV-visible spectroscopy of black dyes on textiles. Forensic Sci Int 202:54
60. Buzzini P, Massonnet G (2013) The discrimination of colored acrylic, cotton, and wool textile fibers using micro-Raman spectroscopy. Part 1: in situ detection and characterization of dyes. J Forensic Sci 58:1593
61. Goodpaster JV, Liszewski EA (2009) Forensic analysis of dyed textile fibers. Anal Bioanal Chem 394:2009
62. Cochran KH, Barry JA, Muddiman DC et al (2013) Direct analysis of textile fabrics and dyes using infrared matrix-assisted laser desorption electrospray ionization mass spectrometry. Anal Chem 85:831
63. Chen CS, Brown CW, Bide MJ (1997) Non-destructive near-infra-red analysis for the identification of dyes on textiles. J Soc Dyers Colourists 113:51
64. Carey A, Rodewijk N, Xu X et al (2013) Identification of dyes on single textile fibers by HPLC-DAD-MS. Anal Chem 85:11335
65. Huang M, Russo R, Fookes BG et al (2005) Analysis of fiber dyes by liquid chromatography mass spectrometry (LC-MS) with electrospray ionization: discriminating between dyes with indistinguishable UV-Visible absorption spectra. J Forensic Sci 50:526
66. Petrick LM, Wilson TA, Fawcett WR (2006) High-performance liquid chromatography-ultraviolet-visible spectroscopy-electrospray ionization mass spectrometry method for acrylic and polyester forensic fiber dye analysis. J Forensic Sci 51:771
67. Soltzberg LJ, Hagar A, Kridaratikorn S et al (2007) MALDI-TOF mass spectrometric identification of dyes and pigments. J Am Soc Mass Spectrom 18:2001
68. Stefan AR, Dockery CR, Baguley BM et al (2009) Microextraction, capillary electrophoresis, and mass spectrometry for forensic analysis of azo and methine basic dyes from acrylic fibers. Anal Bioanal Chem 394:2087
69. Stefan AR, Dockery CR, Nieuwland AA et al (2009) Forensic analysis of anthraquinone, azo, and metal complex acid dyes from nylon fibers by micro-extraction and capillary electrophoresis. Anal Bioanal Chem 394:2077
70. Suzuki S, Suzuki Y, Ohta H et al (2001) Microspectrophotometric discrimination of single fibres dyed by indigo and its derivatives using ultraviolet-visible transmittance spectra. Sci Justice 41:107
71. Was-Gubala J (2009) The kinetics of colour change in textiles and fibres treated with detergent solutions. Part I—colour perception and fluorescence microscopy analysis. Sci Justice 49:165
72. Was-Gubala J, Grzesiak E (2010) The kinetics of colour change in textiles and fibres treated with detergent solutions. Part II—spectrophotometric measurements. Sci Justice 50:55

73. Wiggins K, Holness JA (2005) A further study of dye batch variation in textile and carpet fibres. Sci Justice 45:93
74. Zhou C, Li M, Garcia R et al (2012) Time-of-flight-secondary ion mass spectrometry method development for high-sensitivity analysis of acid dyes in nylon fibers. Anal Chem 84:10085
75. Zieba-Palus J, Trzcinska BM (2012) Comparing the color of forensic traces. Anal Lett 45:1333
76. Wiggins K, Palmer R, Hutchinson W et al (2007) An investigation into the use of calculating the first derivative of absorbance spectra as a tool for forensic fibre analysis. Sci Justice 47:9
77. Prange A, Reus U, Boddeker H et al (1995) Microanalysis in forensic-science—characterization of single textile fibers by total-reflection X-ray-fluorescence. Anal Sci 11:483
78. Hiroma Y, Hokura A, Nakai I (2010) Forensic identification of Trunk Mat by trace element analysis of single fiber with laser ablation ICP-MS. Bunseki Kagaku 59:759
79. Armitage S, Saywell S, Roux C et al (2001) The analysis of forensic samples using laser micro-pyrolysis gas chromatography mass spectrometry. J Forensic Sci 46:1043
80. Daeid NN, Meier-Augenstein W, Kemp HF (2011) Investigating the provenance of un-dyed spun cotton fibre using multi-isotope profiles and chemometric analysis. Rapid Commun Mass Spectrom 25:1812
81. Causin V, Marega C, Schiavone S et al (2005) Employing glass refractive index measurement (GRIM) in fiber analysis: a simple method for evaluating the crystallinity of acrylics. Forensic Sci Int 149:193
82. Causin V, Marega C, Schiavone S et al (2006) Forensic analysis of acrylic fibers by pyrolysis-gas chromatography/mass spectrometry. J Anal Appl Pyrolysis 75:43
83. Suzuki S, Higashikawa Y, Sugita R et al (2009) Guilty by his fibers: suspect confession versus textile fibers reconstructed simulation. Forensic Sci Int 189
84. Was-Gubala J (2009) Selected aspects of the forensic examination of textile traces. Fibr Text East Eur 17:26
85. Bell SEJ, Fido LA, Speers SJ et al (2005) Forensic analysis of architectural finishes using Fourier transform infrared and Raman spectroscopy, Part I: the resin bases. Appl Spectrosc 59:1333
86. Bell SEJ, Fido LA, Speers SJ et al (2005) Forensic analysis of architectural finishes using Fourier transform infrared and Raman spectroscopy, Part II: white paint. Appl Spectrosc 59:1340
87. Milczarek JM, Zieba-Palus J (2009) Examination of spray paints on plasters by the use of pyrolysis-gas chromatography/mass spectrometry for forensic purposes. J Anal Appl Pyrolysis 86:252
88. Buzzini P, Massonnet G (2004) A market study of green spray paints by Fourier transform infrared (FTIR) and Raman spectroscopy. Sci Justice 44:123
89. Wright DM, Bradley MJ, Mehltretter AH (2013) Analysis and discrimination of single-layer white architectural paint samples. J Forensic Sci 58:358
90. Wright DM, Bradley MJ, Mehltretter AH (2011) Analysis and discrimination of architectural paint samples via a population study. Forensic Sci Int 209:86
91. Siegel JA, Mirakovits K (2006) Forensic science: the basics. CRC, Boca Raton
92. Ullmann (1998) Ullmann's encyclopedia of industrial chemistry. Wiley, Weinheim
93. Fisher BAJ, Tilstone WJ, Woytowicz C (2009) Introduction to criminalistics: the foundation of forensic science. Academic, San Diego
94. Lavine BK, Fasasi A, Mirjankar N et al (2014) Search prefilters for mid-infrared absorbance spectra of clear coat automotive paint smears using stacked and linear classifiers. J Chemometr 28:385
95. Lavine BK, Fasasi A, Mirjankar N et al (2014) Development of search prefilters for infrared library searching of clear coat paint smears. Talanta 119:331
96. Lavine BK, Fasasi A, Sandercock M (2014) Improving PDQ database search strategies to enhance investigative lead information for automotive paints. Microchem J 117:133
97. Zieba-Palus J, Trzcinska B, Koscielniak P (2010) Comparative analysis of car paint traces in terms of color by vis microspectrometry for forensic needs. Anal Lett 43:436

98. Trzcinska B, Zieba-Palus J, Koscielniak P (2013) Examination of car paint samples using visible microspectrometry for forensic purposes. Anal Lett 46:1267
99. Liszewski EA, Lewis SW, Siegel JA et al (2010) Characterization of automotive paint clear coats by ultraviolet absorption microspectrophotometry with subsequent chemometric analysis. Appl Spectrosc 64:1122
100. Muehlethaler C, Massonnet G, Esseiva P (2011) The application of chemometrics on Infrared and Raman spectra as a tool for the forensic analysis of paints. Forensic Sci Int 209:173
101. Maric M, van Bronswijk W, Lewis SW et al (2014) Synchrotron FTIR characterisation of automotive primer surfacer paint coatings for forensic purposes. Talanta 118:156
102. Lv J, Feng J, Liu Y et al (2012) Discriminating amino resin paints from alkyd resin paints with two kinds of pigments in automotive coatings in forensic analysis by FTIR spectroscopy. Anal Lett 45:1079
103. Lv J, Feng J, Liu Y et al (2012) Discriminating paints with different clay additives in forensic analysis of automotive coatings by FT-IR and Raman spectroscopy. Spectroscopy 27:36
104. He J, Lv J, Ji Y et al (2013) Multiple characterizations of automotive coatings in forensic analysis. Spectros Lett 46:555
105. De Gelder J, Vandenabeele P, Govaert F et al (2005) Forensic analysis of automotive paints by Raman spectroscopy. J Raman Spectrosc 36:1059
106. Buzzini P, Massonnet G, Sermier FM (2006) The micro Raman analysis of paint evidence in criminalistics: case studies. J Raman Spectrosc 37:922
107. Otieno-Alego V (2009) Some forensic applications of a combined micro-Raman and scanning electron microscopy system. J Raman Spectrosc 40:948
108. Roberts K, Almond MJ, Bond JW (2013) Using paint to investigate fires: an ATR-IR study of the degradation of paint samples upon heating. J Forensic Sci 58:495
109. Stewart SP, Bell SEJ, Armstrong WJ et al (2012) Forensic examination of multilayer white paint by lateral scanning Raman spectroscopy. J Raman Spectrosc 43:131
110. Suzuki EM, McDermot MX (2006) Infrared spectra of US automobile original finishes. VII. Extended range FT-IR and XRF analyses of inorganic pigments in situ-Nickel Titanate and Chrome Titanate. J Forensic Sci 51:532
111. Suzuki EM (2014) Infrared spectra of U.S. automobile original finishes (1998–2000). IX. Identification of bismuth oxychloride and silver/white mica pearlescent pigments using extended range FT-IR spectroscopy, XRF spectrometry, and SEM/EDS analysis. J Forensic Sci 59:1205
112. Suzuki EM (2014) Infrared spectra of U. S. automobile original finishes (Post-1989). VIII: in situ identification of bismuth vanadate using extended range FT-IR spectroscopy, Raman spectroscopy, and X-ray fluorescence spectrometry. J Forensic Sci 59:344
113. Zieba-Palus J, Borusiewicz R, Kunicki M (2008) PRAXIS—combined μ-Raman and μ-XRF spectrometers in the examination of forensic samples. Forensic Sci Int 175:1
114. Zieba-Palus J, Michalska A (2014) Photobleaching as a useful technique in reducing of fluorescence in Raman spectra of blue automobile paint samples. Vib Spectrosc 74:6
115. Zieba-Palus J, Michalska A (2014) Characterization of blue pigments used in automotive paints by Raman Spectroscopy. J Forensic Sci 59:943
116. Zieba-Palus J, Trzcinska BM (2013) Application of infrared and Raman spectroscopy in paint trace examination. J Forensic Sci 58:1359
117. Flynn K, O'Leary R, Lennard C et al (2005) Forensic applications of infrared chemical imaging: multi-layered paint chips. J Forensic Sci 50:832
118. Alamilla Orellana F, Gonzalez Galvez C, Torre Roldan M et al (2013) Applications of laser-ablation-inductively-coupled plasma-mass spectrometry in chemical analysis of forensic evidence. Trends Anal Chem 42:1
119. Asfaw A, Wibetoe G, Beauchemin D (2012) Solid sampling electrothermal vaporization inductively coupled plasma optical emission spectrometry for discrimination of automotive paint samples in forensic analysis. J Anal Atom Spectrom 27:1928

120. Deconinck I, Latkoczy C, Gunther D et al (2006) Capabilities of laser ablation-inductively coupled plasma mass spectrometry for (trace) element analysis of car paints for forensic purposes. J Anal Atom Spectrom 21:279
121. McIntee E, Viglino E, Rinke C et al (2010) Comparative analysis of automotive paints by laser induced breakdown spectroscopy and nonparametric permutation tests. Spectrochim Acta B Atom Spectrosc 65:542
122. Mukai T, Nakazumi H, Kawabata S et al (2008) Direct identification of various copper phthalocyanine pigments in automotive paints and paint smears by laser desorption ionization mass spectrometry. J Forensic Sci 53:107
123. Nakai I, Ono Y, Li Q et al (2010) Capability of aTES microcalorimeter SEM-EDS system for forensic analysis of automotive paint and gunshot residue. Surf Interface Anal 42:402
124. Nakano K, Nishi C, Otsuki K et al (2011) Depth elemental imaging of forensic samples by confocal micro-XRF method. Anal Chem 83:3477
125. Zieba-Palus J, Zadora G, Milczarek JM et al (2008) Pyrolysis-gas chromatography/mass spectrometry analysis as a useful tool in forensic examination of automotive paint traces. J Chromatogr A 1179:41
126. Zieba-Palus J, Milczarek JM, Koscielniak P (2008) Application of infrared spectroscopy and pyrolysis-gas chromatography-mass spectrometry to the analysis of automobile paint samples. Chemia Analityczna 53:109
127. Yang S, Shen JY, Chang MS et al (2012) Quantification of vehicle paint components containing polystyrene using pyrolysis-gas chromatography/mass spectrometry. Anal Methods 4:1989
128. Burns DT, Doolan KP (2000) A comparison of pyrolysis-gas chromatography-mass spectrometry and Fourier transform infrared spectroscopy for the analysis of a series of modified alkyd paint resins. Anal Chim Acta 422:217
129. Burns DT, Doolan KP (2005) The discrimination of automotive clear coat paints indistinguishable by Fourier transform infrared spectroscopy via pyrolysis–gas chromatography–mass spectrometry. Anal Chim Acta 539:157
130. Burns DT, Doolan KP (2005) A comparison of pyrolysis–gas chromatography–mass spectrometry and Fourier transform infrared spectroscopy for the characterisation of automative paint samples. Anal Chim Acta 539:145
131. Burns DT, Doolan KP (2006) The automation of the acquisition and evaluation of pyrolysis-gas chromatography–mass spectrometry data for paint samples. Anal Chim Acta 571:25
132. Smalldon KW (1969) The identification of paint resins and other polymeric materials from the infra red spectra of their pyrolysis products. J Forensic Sci Soc 9:135
133. Smith JM (2007) Forensic examination of pressure sensitive tape. In: Blackledge RD (ed) Forensic analysis on the cutting edge. Wiley, Hoboken
134. Zech W, Malik N, Thali M (2012) Applicability of DNA analysis on adhesive tape in forensic casework. J Forensic Sci 57:1036
135. Choudhry MY, Whritenour RD (1990) A new approach to unraveling tangled adhesive tape for potential detection of latent prints and recovery of trace evidence. J Forensic Sci 1373
136. Koester CJ, Blankenship JF, Grant PM (2013) Effects of superglue fuming on materials characterization of zip-lock polyethylene bags for route forensic analyses. J Radioanal Nucl Chem 295:2015
137. Mehltretter AH, Bradley MJ, Wright DM (2011) Analysis and discrimination of electrical tapes: Part I. Adhesives. J Forensic Sci 56:82
138. Mehltretter AH, Bradley MJ, Wright DM (2011) Analysis and discrimination of electrical tapes: Part II. Backings. J Forensic Sci 56:1493
139. Maynard P, Gates K, Roux C et al (2001) Adhesive tape analysis: establishing the evidential value of specific techniques. J Forensic Sci 46:280
140. Goodpaster JV, Sturdevant AB, Andrews KL et al (2007) Identification and comparison of electrical tapes using instrumental and statistical techniques: I. Microscopic surface texture and elemental composition. J Forensic Sci 52:610
141. Bradley MJ, Gauntt JM, Mehltretter AH et al (2011) A validation study for vinyl electrical tape end matches. J Forensic Sci 56:606

142. Bradley MJ, Keagy RL, Lowe PC et al (2006) A validation study for duct tape end matches. J Forensic Sci 51:504
143. Kumooka Y (2011) Classification of OPP adhesive tapes according to pyrogram of adhesives. Forensic Sci Int 206:136
144. Kumooka Y (2007) Discrimination of rubber-based pressure sensitive adhesives by size exclusion chromatography. Forensic Sci Int 171:5
145. Sakayanagi M, Konda Y, Watanabe K et al (2003) Identification of pressure-sensitive adhesive polypropylene tape. J Forensic Sci 48:68
146. Merrill RA, Bartick EG (2000) Analysis of pressure sensitive adhesive tape: I. Evaluation of infrared ATR accessory advances. J Forensic Sci 45:93
147. Goodpaster JV, Sturdevant AB, Andrews KL et al (2009) Identification and comparison of electrical tapes using instrumental and statistical techniques: II. Organic composition of the tape backing and adhesive. J Forensic Sci 54:328
148. Dietz ME, Stern LA, Mehltretter AH et al (2012) Forensic utility of carbon isotope ratio variations in PVC tape backings. Sci Justice 52:25
149. Ninomiya T, Nomura K, Taniguchi K et al (1995) Applications of a glazing incidence X-ray fluorescence analysis to forensic samples. Anal Sci 11:489
150. Dobney AM, Wiarda W, de Joode P et al (2002) Sector field ICP-MS applied to the forensic analysis of commercially available adhesive packaging tapes. J Anal Atom Spectrosc 17:478
151. Carter JF, Grundy PL, Hill JC et al (2004) Forensic isotope ratio mass spectrometry of packaging tapes. Analyst 129:1206
152. Horacek M, Min J, Heo S et al (2008) The application of isotope ratio mass spectrometry for discrimination and comparison of adhesive tapes. Rapid Commun Mass Spectrom 22:1763
153. Williams ER, Munson TO (1988) The comparison of black polyvinylchloride (PVC) tapes by pyrolysis gas chromatography. J Forensic Sci 33:1163
154. Kumooka Y (2008) Analysis of rosin and modified rosin esters in adhesives by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Forensic Sci Int 176:111
155. Kumooka Y (2010) Classification of OPP adhesive tapes according to MALDI mass spectra of adhesives. Forensic Sci Int 197:75
156. Causin V (2010) Wide-angle X-ray diffraction and small-angle X-ray scattering studies of rubber nanocomposites in Rubber nanocomposites. In: Thomas S, Stephens R (eds) Preparation, properties and applications. Wiley, Singapore
157. Green RL, Watling RJ (2007) Trace element fingerprinting of Australian ocher using laser ablation inductively coupled plasma-mass spectrometry (LA-ICP-MS) for the provenance establishment and authentication of indigenous art. J Forensic Sci 52:851
158. Denman JA, Kempson IM, Skinner WM et al (2008) Discrimination of pencil markings on paper using elemental analysis: an initial investigation. Forensic Sci Int 175:123
159. Sen S, Mabuni C, Walsh D (2001) Development of a methodology for characterizing commercial chlorinated latex gloves. J Appl Polym Sci 82:672
160. Causin V, Marega C, Marigo A et al (2009) A method based on thermogravimetry/differential scanning calorimetry for the forensic differentiation of latex gloves. Forensic Sci Int 188:57
161. Mauri M, Mauri L, Causin V et al (2011) A method based on time domain nuclear magnetic resonance for the forensic differentiation of latex gloves. Anal Methods 3:1802
162. Ding JK, Liu HS (1989) A study of identification of trace rubber residues in marks from rubber-soled shoes and tyres by Py-GC. Forensic Sci Int 43:45
163. Gueissaz L, Massonnet G (2013) Tire traces—discrimination and classification of pyrolysis-GC/MS profiles. Forensic Sci Int 230:46
164. Sarkissian G (2007) The analysis of tire rubber traces collected after braking incidents using pyrolysis-gas chromatography/mass spectrometry. J Forensic Sci 1050
165. Sarkissian G, Keegan J, Du Pasquier E et al (2004) The analysis of tires and tire traces using FTIR and Py-GC/MS. J Can Soc Forensic Sci 37:19
166. Lachowicz T, Zieba-Palus J, Kościelniak P (2013) Chromatographic analysis of tire rubber samples as the basis of their differentiation and classification for forensic purposes. Anal Lett 46:2332

167. Blackledge RD, Vincenti M (1994) Identification of polydimethylsiloxane lubricant traces from latex condoms in cases of sexual assault. J Forensic Sci 34:245
168. Keil W (2007) Condom trace evidence in sexual assaults: recovery and characterization. In: Blackledge RD (ed) Forensic analysis of the cutting edge. Wiley, Hoboken
169. Maynard P, Allwell K, Roux C et al (2001) A protocol for the forensic analysis of condom and personal lubricants found in sexual assault cases. Forensic Sci Int 124:140
170. Shen ZX, Thomas JJ, Siuzdak G et al (2004) A case study on forensic polymer analysis by DIOS-MS: the suspect who gave us the SLIP (R). J Forensic Sci 49:1028
171. Campbell GP, Gordon AL (2007) Analysis of condom lubricants for forensic casework. J Forensic Sci 52:630
172. Burger F, Dawson M, Roux C et al (2005) Forensic analysis of condom and personal lubricants by capillary electrophoresis. Talanta 67:368
173. Spencer SE, Kim SY, Kim SB et al (2011) Matrix assisted laser desorption/ionization-time of flight mass spectrometry profiling of trace constituents of condom lubricants in the presence of biological fluids. Forensic Sci Int 207:19
174. Blackledge RD (1996) Condom trace evidence: a new factor in sexual assault investigations. FBI Bulletin, May
175. Lee GSH, Brinch KM, Kannangara K et al (2001) A methodology based on NMR spectroscopy for the forensic analysis of condoms. J Forensic Sci 46:808
176. Thomas JJ, Shen Z, Blackledge R et al (2001) Desorption-ionization on silicon mass spectrometry: an application in forensics. Anal Chim Acta 442:183
177. Blackledge RD (1995) Viscosity comparisons of polydimethylsiloxane lubricants in latex condom brands via Fourier self-deconvolution of their FT-IR spectra. J Forensic Sci 40:467
178. Cho LL, Huang KB (2012) Identification of condom lubricants by FT-IR spectroscopy. Forensic Sci J 11:33
179. Wolfe J, Exline DL (2003) Characterization of condom lubricant components using Raman spectroscopy and Raman chemical imaging. J Forensic Sci 48:1065
180. Coyle T, Anwar N (2009) A novel approach to condom lubricant analysis: in-situ analysis of swabs by FT-IR Raman spectroscopy and its effects on DNA analysis. Sci Justice 49:32
181. Conti S, Dezzi S, Bianco A (1995) Traces of polydimethylsiloxane in case histories of rape: technique for detection. Forensic Sci Int 76:121
182. Hollenbeck TPE, Siuzdak G, Blackledge RD (1999) Electrospray and MALDI mass spectrometry in the identification of spermicides in criminal investigations. J Forensic Sci 44:783
183. Bradshaw R, Wolstenholme R, Blackledge RD et al (2011) A novel matrix-assisted laser desorption/ionisation mass spectrometry imaging based methodology for the identification of sexual assault suspects. Rapid Commun Mass Spectrom 25:415
184. Wiggins KG, Emes A, Brackley LH (2002) The transfer and persistence of small fragments of polyurethane foam on clothing. Sci Justice 42:105
185. Reed G, Lofts C, Coyle T (2010) A population study of polyurethane foam fragments recovered from the surface of 100 outer-garments. Sci Justice 50:127
186. Parsons NS, Mountain C (2007) Investigating polyurethane foam as a form of trace evidence. Sci Justice 47:24
187. Parsons NS, Lam MHW, Hamilton SE et al (2010) A preliminary investigation into the comparison of dissolution/digestion techniques for the chemical characterization of polyurethane foam. Sci Justice 50:177
188. Roux C, Bull S, Goulding J et al (2000) Tracing the source of illicit drugs through plastic packaging—a database. J Forensic Sci 45:94
189. Chan KW, Tan GH, Wong RCS (2011) Forensic applications of IR spectral data at macro and micro levels: a study on plastic packages. Spectrosc Lett 44:440
190. Causin V, Marega C, Carresi P et al (2006) A quantitative differentiation method for plastic bags by infrared spectroscopy, thickness measurement and differential scanning calorimetry for tracing the source of illegal drugs. Forensic Sci Int 164:148
191. Tsukame T, Kutsuzawa M, Sekine H et al (1999) Identification of polyethylene by differential scanning calorimetry—application to forensic science. J Therm Anal Calorim 57:847

192. Causin V, Marega C, Carresi P et al (2007) A quantitative differentiation method for plastic bags by wide angle X-ray diffraction for tracing the source of illegal drugs. Forensic Sci Int 168:37
193. Simmross U, Fischer R, Duwel F et al (1997) Quantitative determination of cadmium in polyethylene using total reflection X-ray fluorescence (TXRF) spectroscopy. Fresenius J Anal Chem 358:541
194. Nissen KE, Keegan JT, Byrne JP (1998) Characterization of plastic films from trace element analysis using inductively coupled plasma-mass spectrometry. Can J Anal Sci Spectrosc 43:122
195. Kelly JS, Lindblom BS (eds) (2006) Scientific examination of questioned documents, 2nd edn. Taylor & Francis, Boca Raton
196. Levinson J (2001) Questioned documents: a lawyer's handbook. Academic, London
197. Ellen D (1997) The scientific examination of questioned documents. Methods and techniques. Taylor & Francis, London
198. Foner HA, Adan N (1983) The characterization of papers by X-ray diffraction (XRD): measurement of cellulose crystallinity and determination of mineral composition. J Forensic Sci Soc 23:313
199. Causin V, Marega C, Marigo A et al (2010) Forensic differentiation of paper by X-ray diffraction and infrared spectroscopy. Forensic Sci Int 197:70
200. Spence LD, Francis RB, Tinggi U (2002) Comparison of elemental composition of office document paper: evidence in a homicide case. J Forensic Sci 47:648
201. Spence LD, Baker AT, Byrne JP (2000) Characterization of document paper using elemental compositions determined by inductively coupled plasma mass spectrometry. J Anal Atom Spectrom 15:813
202. Andrasko J (1996) Microreflectance FTIR technique applied to materials encountered in forensic examination of documents. J Forensic Sci 41:812
203. Kher A, Stewart S, Mulholland M (2005) Forensic classification of paper with infrared spectroscopy and principal component analysis. J Near Infrared Spectrosc 13:225
204. Kher A, Mulholland M, Reedy B et al (2001) Classification of document papers by infrared spectroscopy and multivariate statistical techniques. Appl Spectrosc 55:1192
205. Kuptsov AH (1994) Applications of Raman spectroscopy in forensic science. J Forensic Sci 39:305
206. Miyata H, Shinozaki M, Nakayama T et al (2002) A discrimination method for paper by Fourier transform and cross correlation. J Forensic Sci 47:1125
207. Causin V, Casamassima R, Marruncheddu G et al (2012) The discrimination potential of diffuse-reflectance ultraviolet-visible-near infrared spectrophotometry for the forensic analysis of paper. Forensic Sci Int 216:163
208. Ebara H, Kondo A, Nishida S (1982) Analysis of coated and non-coated papers by pyrolysis gas-chromatography. Rep Natl Res Inst Police Sci 2:88
209. Merrill RA, Bartick EG, Taylor JH (2003) Forensic discrimination of photocopy and printer toners. I. The development of an infrared spectral library. Anal Bioanal Chem 376:1272
210. Trzcinska BM (2006) Analytical differentiation of black powder toners of similar polymer composition for criminalistic purposes. Chemia Analityczna 51:147
211. Egan WJ, Galipo RC, Kochanowski BK et al (2003) Forensic discrimination of photocopy and printer toners. III. Multivariate statistics applied to scanning electron microscopy and pyrolysis gas chromatography/mass spectrometry. Anal Bioanal Chem 376:1286
212. Lentini JJ, Dolan JA, Cherry C (2000) The petroleum-laced background. J Forensic Sci 45:968
213. Almirall JR, Furton KG (2004) Characterization of background and pyrolysis products that may interfere with the forensic analysis of fire debris. J Anal Appl Pyrolysis 71:51
214. DeHaan JD, Bonarius K (1988) Pyrolysis products of structure fires. J Forensic Sci 28:299
215. Bertsch W (1994) Volatiles from carpet: a source of frequent misinterpretation in arson analysis. J Chromatogr A 674:329
216. Cavanagh K, Du Pasquier E, Lennard C (2000) Background interference from car carpets—the evidential value of petrol residues in cases of suspected vehicle arson. Forensic Sci Int 125:22

217. Stauffer E (2003) Concept of pyrolysis for fire debris analysts. Sci Justice 43:29
218. Ballice L, Reimert R (2002) Classification of volatile products from the temperature-programmed pyrolysis of polypropylene (PP), atactic-polypropylene (APP) and thermogravimetrically derived kinetics of pyrolysis. Chem Eng Process 41:289
219. Keto RO (1995) GC/MS data interpretation for petroleum distillate identification in contaminated arson debris. J Forensic Sci 40:412
220. Lentini JJ (1998) Differentiation of asphalt and smoke condensates from liquid petroleum distillates using GC/MS. J Forensic Sci 43:97
221. Prather KR, Towner SE, McGuffin VL et al (2014) Effect of substrate interferences from high-density polyethylene on association of simulated ignitable liquid residues with the corresponding liquid. J Forensic Sci 59:52
222. Birky MM, Voorhees KJ (1989) The use of soot analysis as an investigative tool in aircraft fires. Aviat Space Environ Med 60:B72
223. Tsao R, Voorhees KJ (1984) Analysis of smoke aerosols from nonflaming combustion by pyrolysis/mass spectrometry pattern recognition. Anal Chem 56:368
224. Gonzalez-Rodriguez J, Sissons N, Robinson S (2011) Fire debris analysis by Raman spectroscopy and chemometrics. J Anal Appl Pyrolysis 91:210
225. Kerr T, Duncan K, Myers L (2013) Post fire materials identification by micro-Raman spectroscopy and principal components analysis. J Anal Appl Pyrolysis 102:103
226. Kerr TJ, Duncan KL, Myers L (2013) Application of vibrational spectroscopy techniques for material identification from fire debris. Vib Spectrosc 68:225

Chapter 5
Formulation: Polymer Matrix, Fillers, Dyes, Pigments and Other Additives

Polymers are extremely rarely used as pure materials. Many applications have a large number of functional and structural requirements. Probably the most graphic example of the complex requisites which materials must meet is found in tissue engineering. In this branch of science, the aim is that of devising materials which can act as scaffolds for the substitution, regeneration or healing of damaged organic tissues. If a polymer is intended for such biomedical applications, it must be compatible with certain tissues or cells, but on the other hand it must be unreactive with other molecules present in the organism, for instance it must not be considered an extraneous substance by the immune system. Such materials must have mechanical properties compatible with the intended use, not too stiff, but not too soft either. They must be degradable in the body, yielding harmless residues which can be easily excreted, in a time span compatible with the concurrent growth of the tissue, which is regenerated by the organism. This is definitely an extreme example of a material very demanding for properties and performance. A similar amount of complexity can however be found in much more mundane objects, such as car tyres, textile fibres or paper sheets. A single polymeric material usually does not have the right mix of properties to be ready to use for practical applications.

However, a very appealing characteristic of polymers is the possibility to modulate their characteristics and functionality by additivation.

Additivation allows to very efficiently tune the properties of a polymer matrix, in some instances changing radically its behaviour. For example, polyvinylchloride can be used as a tough and rigid material for flooring applications, and its resistance to abrasion is improved by inorganic fillers. On the other hand, polyvinylchloride can be used for manufacturing water hoses, whose flexibility and softness is achieved by addition of plastifiers. Additives can have multiple roles. It is the case of carbon black in vehicle tyres, which has a reinforcing effect on the rubber, and it also protects the material from ultraviolet radiation and prevents deterioration during service.

The industry of additives for polymers is one with a very high added value, because it aims at providing substances which yield, when added in small amounts,

V. Causin, *Polymers on the Crime Scene*,
DOI 10.1007/978-3-319-15494-7_5

a remarkable improvement to the final properties of the material. This industry strongly invests in research and development, to provide additives with the highest possible performance. Due to its research-intensive nature, intellectual protection is a primary issue in this field, so it is not easy to find information on the chemical nature of the additives in the open literature. This, in addition to the variety of compounds in the market, complicates the activity of the analyst.

Many different categories of additives exist, divided according to their function. The most important and common ones are:

- Fillers
- Dyes and pigments
- Plastifiers
- Stabilisers
- Fire retardant additives

Fillers are additives aimed at improving the mechanical performance of the polymeric matrix, increasing features such as modulus, tensile strength and/or resistance to impact. Fillers are predominantly inorganic in nature–common species are calcium carbonate, talc, silica, titanium dioxide, different kinds of clays–but organic materials can be added as well, among which wood flour is the most encountered. Since it is used in considerable amounts, which can easily exceed 30 % in weight, the choice of filler is done on the basis of economical and functional considerations. Its cost must not be too high, but at the same time the improvement in performance must be efficient. Silica, under this point of view, is a good example. Its higher cost with respect to wood flour is justified by the ability to improve the hardness and thermal stability of the polymeric composite, in addition to its modulus and mechanical resistance.

The inorganic fillers mentioned above tend to be particulate in shape, but also fibrous fillers are very popular in the plastic industry. As a function of the application, long or short, natural or synthetic fibres can be used. Fibres have a high aspect ratio, and they optimise the surface area of interaction between matrix and filler. They can also be dispersed along a particular direction, therefore yielding the material anisotropic properties.

The reasons why *dyes* and *pigments* are employed in the plastic industry are obviously those of conferring to the materials a pleasant appearance. The specifications of the molecules used for these purposes are very stringent, because the consumers are very demanding under this aspect, and the human eye is able to catch very subtle differences in colour. The homogeneous dispersion of dyes and pigments is a critical issue, otherwise the colour of the item would appear irregular. Homogeneity is important also to prevent that, during service life, friction or abrasion could alter the hue of the object, as would happen if the coloured species were just concentrated on its surface layers. The difference between dye and pigment is due to their solubility in the matrix where they are dispersed. Dyes are soluble in the polymer, whereas pigments are not, and they constitute a separate phase within the material. Dyes are generally organic, whereas pigments can be both organic and inorganic in nature. Organic colourants are more expensive, but on the other hand

they allow to attain brighter hues, with the possibility to tune quite precisely the colour. An important requirement for dyes and pigments is that they are resistant to oxidation and ultraviolet light, because otherwise the appearance of the material would change during its use and its life.

Plastifiers are additives used for tuning the flexibility of polymeric materials, by increasing the mobility of their macromolecules. Plastifiers act as lubricants, favouring the relative motion of the polymer molecules. The use of these additives allows to transform a rigid polymer such as pure polyvinylchloride in a flexible, rubber like material, useful for manufacturing impermeable coatings, water hoses or fake leather. Important requirements for plastifiers are that they have a high boiling point and that they are insoluble in as many solvents as possible. The main risk to be avoided is in fact the migration of the additive out of the polymer, with the consequence of a stiffening and an increase in brittleness of the material, which lacks the plastifying effect. This problem can also be avoided by a careful choice of the nature of the matrix and of the polymer. If they are as compatible as possible, on one hand a detrimental aggregation of the additive is circumvented, and on the other hand the driving force for migration out of the matrix will be mitigated. Small molecular weight plastifiers such as phthalates are still very commonly used. However, due to their small size, they are quite mobile species, which have a tendency for migration. Oligomeric plasticisers have been successfully introduced in the market, because they are efficient in improving the flexibility of the polymer matrix, at the same time, due to their larger size than phthalates, migrating with much less difficulty out of the material.

Stabilisers have the role of protecting polymers from degradation and oxidation. Degradation is normally triggered by exposure to heat or ultraviolet radiation. In these conditions, chemical reactions may start, which segment the chain into smaller chain portions, with a net decrease in molecular weight and in properties. Carbon black is a very commonly used stabiliser agent, because it absorbs radiation before it can interact and degrade the polymer. As said before, carbon black acts also as a filler, so it is used in quite large amounts, up to 50 % in weight in rubbers. Oxidation happens when the atmospheric oxygen reacts with the polymer chain, appending reactive sites on it. These sites, in turn, are able to participate in other reactions, such as crosslinking or degradation. Anyway, the result of oxidation is a decrease in properties and it must be presented by suitable antioxidant additives.

Flammability can be a problem in the application of polymers. Some matrices, especially those containing halogen atoms, are intrinsically more resistant to fire. Chlorine or fluorine provide fire resistance due to their incapacity of participating to the combustion reaction, at the same time hindering the exchange of air and combustion gases in the combustion site, and finally by hindering the mechanism of the reaction, by scavenging radicals. A similar approach is exploited by additives aimed at improving resistance to fire. Compounds rich in antimony, chlorine or bromine have been used for a long time, but health concerns suggested to phase out these additives.

The assessment of the formulation of a polymeric item of forensic interest is probably the first step to be taken in an analytical protocol. It is not a surprise, then,

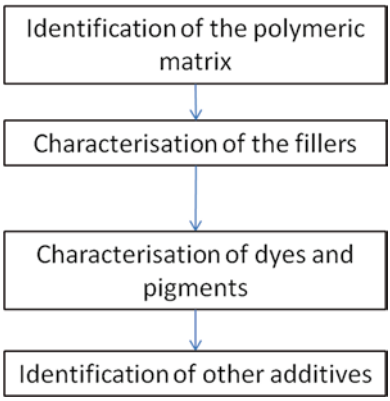


Fig. 5.1 A scheme for the characterisation of the formulation of polymers

that this is a field quite well covered in the literature, also because the analysis of the formulation is a common activity in industrial quality control and in research and development [1]. Since every manufacturer will use its own recipe of raw materials for the production of a certain object, knowledge of the formulation of an item in some way connected to a crime is a very important piece of information. This aim can be achieved in several ways, some allowing a deeper and more complete characterisation, however at the expense of some degree of destruction of the sample (Fig. 5.1).

The first step in such a procedure is obviously the identification of the polymer matrix, principally by infrared spectroscopy.

Next, the additives present in larger amount, such as fillers, can be determined even with the tiny items usually available in forensic casework.

Dyes and pigments, even though they are present in smaller concentrations than fillers, can be characterised on the basis of their main function: the colour that they confer to the material. If the sample size allows, such additives can be quantified more thoroughly by separation techniques.

In some cases, a precisely aimed analytical strategy is not feasible, mainly because few information is available on the sample. In such instances, approaches which give a more general picture of the formulation of the material can be applied.

This chapter will present an overview of the techniques which may be used for analyses targeted at the formulation of the sample, followed by a presentation of the various levels of information that can be gathered in a forensic context.

5.1 The Techniques: Spectroscopy

Spectroscopic methods investigate how a sample interacts with electromagnetic radiation.

Electromagnetic radiation is radiant energy which is propagated as a wave, vibrating in a direction perpendicular to the direction of propagation. Such waves

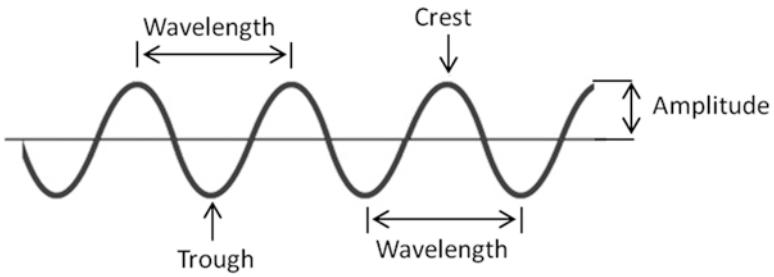


Fig. 5.2 Describing features of a wave

can be described in terms of wavelength, i.e. the distance between adjacent crests or troughs (Fig. 5.2), or of frequency, i.e. the number of cycles passing by a fixed point per unit time.

Wavelength and frequency are related as follows:

$$\lambda = \frac{c}{\nu} \tag{5.1}$$

where λ is the wavelength in centimetres, c is the speed of light (3×10^8 cm/s) and ν is the frequency, in cycles/second or hertz (Hz).

The frequency is a very important parameter, also because the energy of the electromagnetic radiation depends on its frequency:

$$E = h\nu \tag{5.2}$$

where E is the energy of the photon and h is the Planck constant, 6.62×10^{-34} J s. Equation (5.2) shows that the greater the frequency (or the shorter the wavelength) the greater the energy of the radiation. Even though in principle electromagnetic radiation does not change its main properties as a function of frequency or energy, the electromagnetic spectrum, i.e. the set of all the possible radiations, has been broken down in several regions according to wavelength (Fig. 5.3).

The visible range, i.e. the subset of electromagnetic radiation that can be detected by human eye, is a tiny portion of the whole electromagnetic spectrum, comprising light with wavelengths between 380 and 780 nm. Below the high energy limit of the visible range, the ultraviolet spectrum starts. The analytically useful region in the ultraviolet range is the near ultraviolet, with wavelengths from 200 to 380 nm. Beyond the low energy limit of the visible spectrum we find the infrared region, which contains two analytically useful sets: near infrared (NIR) ($\lambda = 0.78$–2.5 μm) and middle infrared ($\lambda = 2.5$–15 μm).

The mechanism of absorption of visible light can help in understanding how this very diverse amount of electromagnetic radiations can be exploited for analytical purposes. When light containing all the visible wavelengths (white light) is passed through an object, the object will absorb only certain wavelengths, whereas

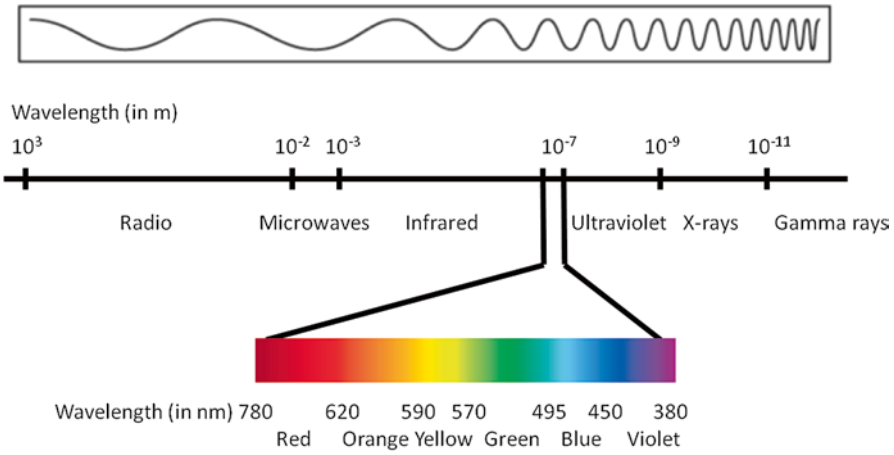


Fig. 5.3 The electromagnetic spectrum

unabsorbed wavelengths will be transmitted. These latter wavelengths will be perceived as a colour. The colour seen will be complementary to the absorbed colours. In other words, absorption by the sample molecule 'sequesters' some of the colours contained in white light, allowing only the unabsorbed hues to reach the observer. The above description deals with an experiment devised in transmission mode (light passes through the object) but the same considerations hold true for reflection from opaque objects. In such a case, the wavelengths which are not absorbed will be reflected back to the eye of the observer, and detected. Absorption of visible light and the vision of colours is a phenomenon ubiquitous in our everyday life, but the same happens when impinging light is electromagnetic radiation pertaining to different regions of the spectrum.

If on one hand the general framework of the absorption process is common, irrespective of the wavelength of impinging light, the underlying mechanism changes as a function of the energy involved. The process by which molecules can absorb radiation involve excitation, i.e. raising the energy of the molecular species by an amount equal to the energy of absorbed light ($h\nu$). As the energy of incoming light is increased, several different transitions can be initiated. Microwaves or radiation in the far infrared region, which are quite less energetic, can promote the rotation of the molecules, radiation in the mid-infrared range spurs vibrations of the bonds, and the more energetic ultraviolet and visible waves allow to raise the energy of electrons to higher levels.

Such transitions are quantised, meaning that they will happen just with light of definite wavelengths, which are in turn dependent on the chemical nature of the sample molecule. In other words, the unique arrangement of atoms and bonds of each molecule will allow a unique set of transitions of rotational, vibrational and electronic type, which is reflected by a unique set of absorbed wavelengths that can be detected by spectroscopy. The potential of this latter approach for identification of substances is therefore clear.

The investigation of rotational transitions by excitation with microwaves is a niche application, with a very limited diffusion in analytical laboratories. Much more commonly used is spectroscopy in the UV–visible and infrared range, and as such these two techniques will be discussed in more detail in the following paragraphs.

5.1.1 UV–Visible Spectroscopy

What is UV–visible spectroscopy?
UV–visible spectroscopy is a technique which can objectively characterise the colour of a sample.

Why use this technique?
Colour is one of the most intuitive parameters that can be used for the comparison of items. Textile fibres, car paints or inks are examples of materials in which colour is a key specification. For example white cotton fibres are so common and ubiquitous that their evidential value is usually very low. The characterisation of the polymer nature of the morphology of the fibre and of the structure of the material seldom yields significant results, given the large variability of cotton being a natural fibre. This picture radically changes when coloured cotton fibres are involved in a case. In this instance, the significance of the fibre item does not lay in the polymer matrix (cellulose in the case of cotton), but in the rarity of the mixture of dyes and pigments used for yielding the colour to the fibre.

As such, it is paramount to have an objective way to characterise colour, in order to portray to the Court meaningful results, not based on the subjectivity that is usually involved when describing hues by words.

Where can this technique be found?
UV–visible spectroscopy is extremely widely available, and such analyses are very cheap, with prices of the order of some euros. UV–visible spectrophotometers can be found in all chemistry departments of all universities, in all analytical laboratories and in practically all governmental forensic science laboratories. The most commonly found instruments, though, operate in solution and require quite large sample sizes, not approachable in routine fibre casework, for example. In cases where very small items must be analysed, microspectrophotometers must be used, i.e. spectrophotometers which are hyphenated to a microscope and which allow to gather the absorption/reflection spectrum of very small items. The diffusion of such instruments is much more limited. The institutions which are usually equipped with these facilities are the largest forensic science laboratories and some university laboratories, especially those specialised in the field of artistic heritage conservation.

Fig. 5.4 Block diagram of a spectrophotometer

The complex mix of properties required to a polymeric material is not limited to those related to mechanical or functional performance. In many applications aesthetical characteristics play an important role, and thus the white colour of polymers is usually not acceptable. Dyes and/or pigments are extremely widely used in the polymer industry. Similar colours can be obtained by different mixes of such additives. In other words, for example, a red colour can be given to a material by several different molecules, each one with its own distinctive shade. A thorough analysis of colour which goes beyond the subjective evaluation is an important tool for the forensic scientist for two reasons. First of all colour is a quality very hard to define: what appears to be blue to one person can be perceived as green to another one. Relying on words for describing the colour of an item exposes to misunderstandings and inaccuracies in the characterisation. Secondly, the human eye is not sensitive enough to be able to discriminate between similar colours produced by different mixes of pigments and/or dyes. Spectrophotometry in the ultraviolet and visible spectral region (UV–vis spectrophotometry) gives the possibility to objectively and numerically describe colours.

As introduced in Sect. 5.1 the use of ultraviolet and visible radiation promotes electronic transitions in the sample molecules.

The scheme in Fig. 5.4 describes a spectrophotometer, an instrument which allows to study how much each wavelength of the light produced from a source is absorbed by a sample.

All spectrometers are constituted by a source, which provides the electromagnetic radiation, a monochromator, which resolves the polychromatic emission of the source into radiation of single wavelength, a sample holder, a detector, which transduces light into an electrical signal, and finally a data storage and analysis system.

The assembly of these components can be aimed at realising the spectroscopic analysis in different geometries and on different kinds of samples. Transmission measurements are preferred in the case of transparent samples. In this geometry, light passes through the specimen and the amount of radiation absorbed by it is quantified. This kind of analytical setup is the one most commonly found in a chemical laboratory. For opaque objects, the reflection geometry is preferred: light impinges on the sample and the radiation reflected by it is detected. The area (or volume) of sample illuminated by light and involved in the spectroscopic experiment is, in normal spectrometers, quite big, and of the order of magnitude of at least some millimetres. However, most items of forensic interest are much smaller and require a microspectrophotometer. These instruments consist of an optical microscope which focuses the incoming light on a small area through a set of lenses. After interacting with the sample, either in transmission or reflection, depending on the necessity and on the instrumental design, light is conveyed to a spectrophotometer,

which quantifies it and determines how much of the incoming radiation was absorbed by the sample. Microspectrophotometers acquired an increasingly important spot in forensic laboratories, due to the possibility they offer to selectively analyse even the smallest items retrieved on the crime scene.

5.1.2 Infrared Spectroscopy

What is IR spectroscopy?
Infrared (IR) spectroscopy is an analytical method ideal for obtaining qualitative information about molecules.

Why use this technique?
Most of the times, the first question which the analyst has to face is 'what is this material?' Use of IR spectroscopy is the best approach for answering this question. IR spectroscopy, as other spectroscopic techniques, studies how a sample interacts with light. Of course in this case the light used is in the infrared range, and the transitions investigated are the vibrations of the bonds constituting the molecules. Molecules can be considered assemblies of atoms kept together by bonds. The position of the atoms is not perfectly fixed, but they fluctuate around average equilibrium positions. If the correct amount of energy is transferred to the molecules, vibration of the atoms can be initiated, altering the mode in which they oscillate. Two basic categories exist for vibrations: stretching and bending. Stretching involves a change in the interatomic distance, and so in the length of the bonds, whereas bending implies a modification of the angle between bonds. Figure 5.5 shows examples of some of the possible vibrations which can be induced by infrared light.

Each of these vibrations requires light of particular energy, i.e. light of particular wavelength and frequency, to be started. Such wavelength depends on the neighbouring groups surrounding the relevant atoms which are vibrating.

If one considers that molecules, and especially polymers, are composed by a large number of atoms and bonds, which all experience a different chemical environment around them, it can be understood that the array of absorption bands in the IR spectrum is rich and varied. Moreover, IR absorption peaks are much sharper than those detected in the ultraviolet or visible region. These two facts determine the peculiarity that each molecule has a unique IR absorption spectrum, which can be used as a 'fingerprint' for the identification of that molecule. The creation of databases of infrared spectra for comparison purposes is therefore possible and provides a very powerful tool for the characterisation of the chemical nature of an unknown sample. In addition to these advantages, the IR spectrum can be also interpreted, reconstructing which functional groups are present in an unknown molecule, and ultimately its structure. This is particularly useful when mixtures must be analysed, for

(continued)

example additivated polymers. In such cases, the spectrum is dominated by the characteristic absorptions of the matrix, i.e. of the substance present in the bigger amount, whereas solely the most intense additive's signals will emerge and will be detectable, due to the small concentration of this species and also on the likely overlap with the matrix spectrum. In this case, the full spectrum of the additive is not available, and so its identification must be made according to a chemical interpretation of its detectable peaks. Examples of this procedure can be found for filled polymers such as paper containing calcium carbonate, or polyethylene films additivated with talc. A further example is found in acrylic fibres, where in addition to the peaks due to the polymeric matrix, the signals related to solvent residues can shed light on the manufacturing process and so enhance the significance of the comparison between items coming from the crime scene and the suspect.

In the forensic engineering field, a thorough interpretation of IR spectra often allows to identify the cause of failures or defects. For instance, the presence of a peak due to a C=O stretching, a particularly intense signal, reflects the fact that oxidative processes occurred in polyolefins, which can bring about weakening of their mechanical performance and a yellowing with aesthetical consequences.

Where can this technique be found?

IR spectroscopy is a very accessible technique, with low analysis costs. Prices are in the few tens of euros region. IR spectrophotometers are ubiquitous in chemistry departments of all universities, in many analytical and industrial laboratories and in governmental forensic science laboratories. If sample sizes are too small for conventional IR spectroscopy and alteration of the item is to be avoided, non-destructive sampling techniques can be applied, such as attenuated total reflection, also widely available, at approximately the same cost.

Some casework may require the use of a IR microspectrophotometer, through which IR spectra can be obtained in absorption or reflection mode of extremely small items. Such instruments are rarer, but still not extremely difficult to access. This technique is quite popular in the most equipped industrial laboratories because it allows an efficient identification of contaminations and of process defects. Other institutions where an IR microspectrophotometer can be found are large forensic science laboratories and some university laboratories, especially those engaged in research in materials science or in the conservation of artistic heritage.

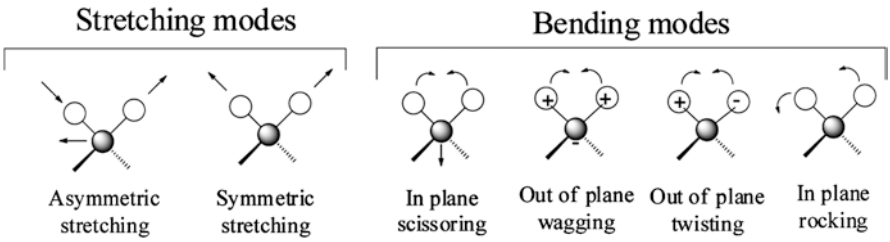


Fig. 5.5 Vibrational modes of a molecule. *Plus* indicates the movement of the atom out of the plane of paper, towards the reader, *Minus* indicates the movement of the atom into the plane of paper

Similar objects can be made with different materials, and such difference is not always evident to the naked eye. A clear example of this case is textile fibres. The identification of the type of fibre by morphological observation is effectively achievable for natural fibres, whereas it is much more cumbersome in the case of synthetic fibres. In such cases, IR spectroscopy is the method of choice, because it allows a quick and precise recognition of the polymer used for manufacturing that fibre. A further advantage of this method is also the possibility to operate in a non-destructive manner.

In their original design, IR spectrophotometers worked according to the block diagram shown for UV–visible spectrometry in Fig. 5.4. As in that case, in fact, the experiment consists in shining light on the sample and detecting which wavelengths of the impinging light are absorbed by the sample and in what amount. Modern spectrophotometers operate according to a different technical solution. The vast majority of these kinds of instruments use a Michelson interferometer to avoid the use of a monochromator and to gather all the experimental data simultaneously in a non-dispersive way. Data points are acquired in the time domain, and the obtained signal, called interferogram, is subsequently subjected to Fourier transform to obtain a spectrum in the frequency domain.

The traditional method for mounting samples for IR spectroscopy consists in mixing the material with KBr. This mixture is subsequently subject to pressure, to make a pellet which is introduced in the instrument and analysed in the transmission mode. When items are very small, the preparation of a KBr pellet is not easy, because there are serious risks of losing the sample. In such cases, the diamond anvil cell becomes a valid alternative. The sample is sandwiched between two flat diamond windows and pressure is applied, creating a thin film of material, which can be analysed by transmission IR spectroscopy.

A further handy technique for sampling in IR spectroscopy consists in using an attenuated total reflection (ATR) accessory. The most common geometry is the horizontal one. In these devices, the sample is positioned on a crystal and pressed against it. Common materials suitable for such crystals are ZnSe, Ge and diamond. An IR beam is then directed into the crystal, with an angle which allows total internal reflection: the beam will be reflected between the upper and lower surfaces of the crystal, until it exits and is directed to the detector. Every time the IR beam impinges the surface of the crystal, an evanescent wave is projected outside the crystal and

inside the sample. The penetration depth into the sample is of the order of 0.5–2 μm, as a function of the refractive index of the crystal and of the sample and of the geometry of the system. The interaction of the sample with the evanescent wave will bring about the absorption of the wavelengths which are able to excite molecular vibrations. In other words, each time the IR beam impinges the surface of the crystal which contacts the sample, some of its wavelengths will be absorbed, and when it finally reaches the detector such attenuated wavelengths will be revealed and a spectrum will be obtained. IR spectra acquired by preparing KBr pellets or on a diamond anvil cell will be equal to those acquired by ATR, as far as the position of the absorption peaks are concerned. The relative intensities will be, on the contrary, different, because in ATR spectra the intensity of the peaks at larger wavenumbers tends to be suppressed. However, a very simple correction procedure is available in any software for IR spectroscopy, so ATR-IR spectra absolutely equal to those acquired by the more traditional sample preparation techniques can be obtained.

A very brilliant development of IR spectroscopy, which made this technique even more suited to forensic science, was the invention of IR microspectrophotometry, which consists in the hyphening of microscopy with IR spectroscopy. The infrared source is the same as that of a normal spectrometer, whereas the microscope is equipped with a Schwartzchild objective. This particular piece of optics is constituted by two spherical mirrors which decrease the size of the light beam to a spot of the diameter of a few microns. Microspectrophotometers can merge examination and IR analysis in a single step, allowing to acquire the IR spectrum in particular locations of the sample and therefore yielding a very detailed picture of its composition. These advantages made IR microspectrophotometry a very popular characterisation technique in forensic science, applied to many different fields, such as the analysis of fibres and other trace evidence, of explosives, of illegal drugs etc.

5.1.3 Raman Spectroscopy

What is Raman spectroscopy?
Raman spectroscopy is a complementary technique to IR spectroscopy which allows to obtain qualitative information about molecules.

Why use this technique?
Raman spectroscopy is a kind of vibrational spectroscopy, and as such it yields the same type of information obtainable by IR spectroscopy. Why then one should use Raman, which is without doubt less common and less easily applicable than the more standard IR spectroscopy? Because even though the physical meaning of the data obtained by these two methods is the same, in fact they both deal with the study of vibrations induced by light to the atoms in a molecule, the information conveyed by them is complementary. The reason lies in the different selection rules that govern the two techniques.

(continued)

In other words, without going into detail on the underlying physics of the two methods, some vibrations give rise to signals in IR spectroscopy, and some other vibrations will produce a Raman peak. Usually vibrational modes are referred as 'Raman active' and 'IR active'. For example, IR spectra of species in aqueous solutions will be dominated by the very intense IR-active signals of water. On the contrary, the Raman spectrum of water is featureless because the vibrations of the water bonds are Raman-inactive, allowing the sampling of molecules in aqueous solution.

The complementarity between Raman and IR spectroscopies can be fruitfully exploited by the forensic scientist for a more thorough, and non-destructive, characterisation of complex materials. For example, when examining automotive paints, Raman proved very useful for the analysis of pigments and did not give information on the polymeric binders, but the opposite happened for IR spectroscopy, which was well suited for studying the polymers, but failed to provide useful data on dyes or pigments [2].

Where can this technique be found?
Raman spectroscopy is becoming increasingly widespread in the forensic science community, and Raman spectrometers are easily found in chemistry departments of research universities. On the contrary, few commercial analytical laboratories are able to perform Raman spectroscopy. A big advantage of Raman, and one of the main reasons of its emerging importance in the forensic world, is that it is relatively easy to couple this kind of spectroscopy with a microscope, therefore allowing to examine very tiny samples. Due to the high cost of instrumentation and to the level of expertise required for a meaningful choice of experimental parameters, Raman analyses are more expensive than IR ones. A typical quotation can be around 100–200€.

When a sample is irradiated by laser light of visible or NIR wavelength, it can diffuse such light in the surrounding space. If a detector is positioned at 90° with respect to the incoming radiation, the diffused (or scattered) light can be detected, and it will be of three types: Stokes scattering, anti-Stokes scattering and Rayleigh scattering. Rayleigh scattering has a wavelength exactly equal to that of the excitation source, and represents the main fraction of scattered light. The wavelength of Stokes and anti-Stokes scattering, on the contrary, is different from that of the laser used for excitation, because some of the energy involved in the excitation has been used for vibrational transitions. Since the wavelength of the Stokes and anti-Stokes radiation is different from that of the incoming radiation, this kind of scattering is inelastic, i.e. diffused light has not the same energy of the light used for excitation. The energy difference between the laser source light and the Stokes radiation represents the energy involved in the vibrational transition.

Inelastically diffused radiation is extremely weak, representing just about 0.001 % of the intensity of the source. Raman spectrometers are therefore very complex apparati devised for separating the Stokes light from the preponderant

Rayleigh scattering. If this can be done, a Raman spectrum can be obtained, usually represented as the intensity of the scattered signal as a function of the frequency shift with respect to the frequency of the exciting light.

Fluorescence is often a problem in Raman measurements. When the sample fluoresces when illuminated by the impinging laser light, the Raman signals due to the vibrational transitions are completely hidden under a strong featureless fluorescence emission. However, fluorescence is a phenomenon strictly dependent on the sample and on the wavelength of the illuminating light. In order to avoid the detrimental effects on spectral quality due to fluorescence, multiple excitation laser sources should be used. Even though a universal source capable of cancelling fluorescence does not exist, it has been observed that NIR lasers are more efficient in avoiding this problem than visible light sources.

The miniaturisation of laser sources allows portable Raman spectrometers to be assembled, also equipped with NIR lasers. Vitek and coworkers, for example, recently reported a handheld Raman spectrometer equipped with a 1,064 nm excitation source, which proved to be efficient in detecting a number of different samples in the forensic, pharmaceutical and art fields [3].

5.2 The Techniques: Atomic Spectrometry

Polymers always contain carbon and hydrogen atoms, and occasionally oxygen or nitrogen can be present as well. If polymers were used as pure materials, without any additive, knowledge of the elements present in polymeric items would be quite insignificant for identification or comparison purposes. However, as introduced in the beginning of this section, in practically all instances additivation is extensively used in the polymer industry for optimising the performance of materials. The composition of such additives is much more varied, and often atoms different from those listed above are found in their structures. Fillers are a class of additives where inorganic species are predominant, introducing in organic materials such as polymers elements like calcium, silicon, aluminium or titanium.

Catalysts are another family of species which are involved in the synthesis of most polymers and are typically based on transition metals. Some pigments as well contain atoms which are different from C, H, N or O. Information on the formulation of polymeric items can therefore be acquired by a measurement of their elemental profile.

There are three main families of techniques which allow to identify and quantify the elements present in samples: optical atomic spectrometry, atomic mass spectrometry and X-ray spectrometry [4–7].

Optical and mass atomic spectrometry share the same approach in the atomisation process, in which enough energy is transferred to the sample that the bonds and interactions which keep together the atoms are broken. The individual atoms are therefore brought into the gas phase and their presence detected in different ways. This is where optical and mass atomic spectroscopy separate, because in the former the elements are detected on the basis of their absorption, emission or fluorescence in the ultraviolet/visible range, and in the latter they are identified by a mass analyser.

X-ray spectrometry investigates the X-rays emitted by the atoms which relax from excitation due to the absorption of X radiation.

5.2.1 Optical Atomic Spectrometry

What is optical atomic spectrometry?
Optical atomic spectrometry is indeed a family of techniques which probe the absorption, emission or fluorescence of ultraviolet/visible light by atomic species which are brought in the gas phase by the process of atomisation. In other words, the sample is destroyed into its constituent individual atoms. In such process, part of the energy is transferred to the atoms. This has the consequence that the atomic species produced in the bond cleavage reactions are in excited states. When the process of excitation of the atoms from the ground state is investigated, absorption atomic spectrometry is applied. On the contrary emission or fluorescence atomic spectrometry focus on detecting the transitions associated with the return from the excited levels to the ground state.

Why use this technique?
Optical atomic spectrometry, analogously to other techniques of elemental analysis, yields information on which atoms are contained in a sample. This in turn is related to the nature and quality of additives, catalyst residues or other inorganic materials which are part of the formulation of polymeric items. In some cases, the inorganic species are added on purpose, to modify the performance of the whole material. However, it is worth noting that in some instances the inorganic compounds are present in the material as a consequence of contamination or as residues of the synthetic procedure, with no significant functional role. These are the most interesting data coming from elemental analysis, because they reflect the variability in the manufacturing process useful for discriminating between different production lots and for singling out the precise source of polymeric items.

Where can this technique be found?
This family of techniques is very widely available, and it can be found in the majority of private commercial laboratories. The cost of quantification of a single element is below 10€, so a focused analysis can be quite cheap. The price increases sharply when no preliminary guess of the atoms to be searched is available, and a complete survey of all the elements of the periodic table is required.

The wide availability of optical atomic spectroscopy equipment is true when the sample is in solution. Accessories that allow non-destructive sampling in the solid phase are much less common and are less apt for routine analyses, requiring a strong expertise for the obtainment of significant data. Academic research laboratories are probably the best option if this kind of analytical approach is requested.

The physical rationale behind optical atomic spectroscopy is that of exploiting the interactions between samples, energy and electromagnetic radiation. At room temperature, practically all the atoms in a sample are in the ground state. If energy is transferred to such atoms, for example by an increase in temperature, by a flame, by a plasma or by an electric arc, they can be promoted to an excited state. However, the lifetime of this excited state is short, and the atom tends to quickly return to the ground state, getting rid of the extra energy by emission of a photon. The energy of the photon, being equal to the difference in energy between the excited and the ground state, is strictly dependent on the energy level diagram of the species originating it. As a consequence, the emitted photons will have energies specific and indicative of the particular atom present in the sample. If the sample is composed of several different elements, the emission spectrum will show peaks at the characteristic wavelengths, i.e. energies, specific of all the individual atoms, allowing to identify qualitatively and quantitatively its elemental composition.

The inverse process can of course be investigated as well. When atoms are introduced in a highly energetic medium, such as a flame for example, they will absorb energy to pass from the ground state to the excited state. The wavelengths absorbed in this case, i.e. in absorption atomic spectrometry, are the same as those involved in emission atomic spectrometry of the same sample.

The third process which can be exploited in optical atomic spectrometry is atomic fluorescence. Fluorescence is produced by a sample which absorbs light of a particular wavelength, but which emits radiation of a longer wavelength. Typical fluorescence phenomena involve absorption of ultraviolet light and emission in the visible range. An example is sodium, which absorbs at 330.3 nm and emits at 589.0 and 589.6 nm. The difference between the energy of the absorbed photon and that of the emitted one is due to a radiationless deactivation step which occurs when the atom relaxes from the excited to the ground state. Normally fluorescence is probed at 90° with respect to the direction of the exciting beam.

Flame and electrothermal atomisation are the most common methods of atomisation employed in atomic absorption and atomic fluorescence spectrometry. In a flame atomiser, a solution of the sample is nebulised and sprayed, together with a gaseous fuel, into a flame where the temperature ranges between 1,700 and 3,500 °C.

In electrothermal atomisation, on the other hand, the sample solution is evaporated and then ashed in a small graphite tube or cup. Subsequently, the ash so formed is heated to a temperature around 2,000–3,000 °C in less than 1 s, dissociating the molecules into the individual atoms. The absorption or fluorescence of the atomised species is measured in the area above the heated surface. Electrothermal atomisation has been proposed also for solid samples, which can be directly introduced into the graphite furnace in special sample holders. This approach is associated to difficulties in calibration and to strong matrix effects, which call for standards approximating as much as possible the composition of the sample.

As emerges from the description above, these atomisation techniques are mostly useful for samples in solution, usually aqueous.

Dissolution of polymeric matrices in water is usually impossible, so a number of decomposition and sample preparation steps are necessary, such as digestion at high temperature or microwaving with mineral acids or ashing by combustion in sealed

reactors. This implies a concurrent risk of loss of analytes and sample contamination. If on one hand the quantity of fillers such as talc, calcium carbonate or clays, contained in a typical polymeric material can reach 20 % w/w, on the other hand catalyst residues or contaminations due to the process are at the trace level, and their analysis can be seriously jeopardised by a lengthy sample manipulation. Despite this problem, coupled with the necessity to control spectral and chemical interferences, the detection limits of absorption and fluorescence atomic spectrometry are very low, usually below the ppb range.

Even though the methods discussed above are still widespread in the practical everyday activity of analytical laboratories, atomic emission spectrometry has rapidly gained an increasing portion of the market share for elemental analysis instruments. The main advantage of the emission approach to atomic spectrometry is that it is feasible to simultaneously identify ideally all the elements in the periodic table contained in the sample. On the contrary, absorption and fluorescence techniques require a careful optimisation of experimental conditions as a function of the particular element of interest. Even though originally emission or absorption spectrometry were devised on the basis of flame or electric arc or spark atomisation, now atomisation in this kind of spectrometry is obtained almost always by plasma sources. Plasma is an electrically conductive mixture in the gas phase containing cations and anions. A very widely used type of plasma used in atomic spectrometry is the argon plasma, which contains argon cations and electrons. In inductively coupled plasma (ICP), an ionic flow is created by a fluctuating magnetic field produced by an induction coil, and in the motion so produced the resistance of the charged species increases the temperatures up to 4,000–8,000 °C. Direct current plasma (DCP) operates ionising argon by a plasma jet obtained by opportunely arranged electrodes. The ionisation generates a current which is able to induce the formation of further ions, which in turn sustains the current. Temperatures beyond 9,000 °C can be reached in this way.

Using plasma sources, several advantages can be achieved: chemical interference can be minimised, the majority of elements can be detected with the same set of experimental conditions, lower detection limits and wider concentration ranges can be obtained. This allows the detection, in the same analysis, of the presence and quantity of all the elements, comprising non-metals, which are inaccessible to other optical atomic spectrometry. These many virtues are balanced by an increased complexity in the instrumental hardware, drastically increasing the cost of plasma-based emission atomic spectrometry with respect to the simpler atomic spectrometry operating in absorption.

A very critical issue in this kind of analysis is sample introduction. The vast majority of the applications requires dissolution of the sample, which is then introduced into the plasma. This brings about the same set of problems highlighted previously in the case of absorption and fluorescence, i.e. an extensive sample preparation step which implies a high risk of sample loss and/or contamination. This is indeed a problem in forensic applications, because it is always associated to sample destruction, it requires quite relevant sample sizes and it can raise controversies around the possibility that manipulation of the item can modify the results of the analysis.

Direct solid analysis approaches have been devised to overcome this drawback. Electrothermal vaporisation in graphite furnaces can be used to vapourise the sample, which is then directed towards the plasma and atomised. Differently from

absorption atomic spectrometry, in this case electrothermal vaporisation is not used for atomisation, but just to introduce the sample in the atomisation section of the instrument. The most recent innovation for directly analysing solids are ablation devices, especially laser ablation, because arc and spark ablation is suitable for conducting materials, not so for polymers. In laser ablation, a laser beam impinges the sample, concentrating a big amount of energy in a very small area, so creating a mix of vapour phase and particulate which is sent to the atomiser for the downstream flow of the analysis.

Laser induced breakdown spectroscopy (LIBS) is an alternative sample introduction method which offers the advantages of being rapid, with multi-element and remote analysis capabilities with possibilities for spatial resolution, which requires minimal sample consumption, and virtually no sample preparation.

In LIBS, the sample is illuminated by pulses of laser light. If the energy of each pulse is high enough (about $1 GWcm^{-2}$), the ablation of the solid happens, with the formation of a plume, derived from the vaporisation of the material, which can be converted in an extremely luminous plasma. Right after the formation of the plasma, the vaporised material undergoes a breakdown in atoms and ions. At the end of the pulse, which is about 10 ns long, the plasma cools down and the excited ions and atoms emit their characteristic radiation. The signal is detected at intermittent intervals, in order to avoid the intense continuous radiation emitted during the formation and accretion of the plasma. Figure 5.6 schematises the sequence of events which happen during a LIBS analysis.

The portability of this technology is another plus. Optical fibres can in fact be used to transport the laser pulse to the sample and the emission signal to the spectrometer, permitting in-situ analysis.

Depth profiling is possible because, increasing the exposure time to the laser beam, the penetration within the sample is increased. This indeed brings about a destruction of the sample, which is however confined in an extremely small area.

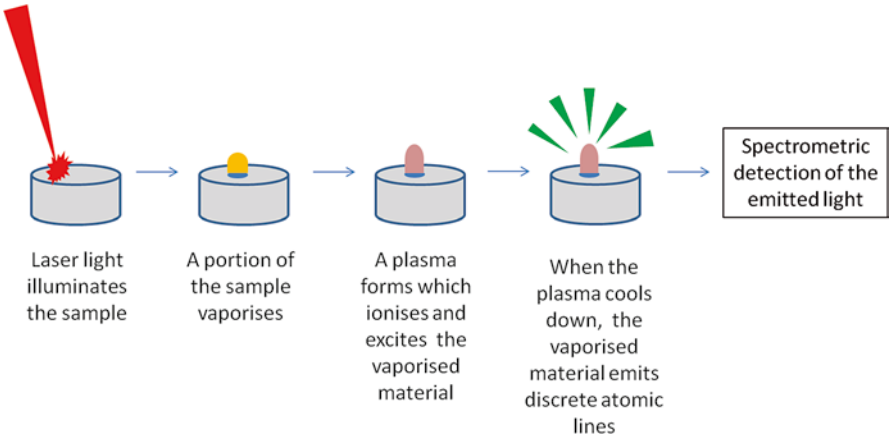


Fig. 5.6 The sequence of events which happens in a LIBS measurement

The item, after the analysis, will display just a tiny hole with the diameter of the impinging laser beam (Figs. 5.27 and 5.38). Variants with a double beam were developed as well, where the first laser pulse ablates the sample, and the second one produces the plasma [8].

5.2.2 Atomic Mass Spectrometry

What is atomic mass spectrometry?
Atomic mass spectrometry is in many aspects very similar to optical atomic spectrometry, especially emission atomic spectrometry. Analogously to this latter technique, in atomic mass spectrometry a sample is dissociated into its constituent atoms, which are ionised and then detected by a mass spectrometer (see Sect. 5.5).

Why use this technique?
By atomic mass spectrometry we can determine which elements are contained in a sample, and therefore gather relevant information on its formulation and on the remnants of the manufacturing process. Even though the cost of this kind of analyses is higher than that of optical methods, atomic mass spectrometry offers several advantages: very low detection limits, simpler spectra than atomic emission spectra, and the possibility to extend the analysis to isotopic ratios, a very powerful technique, especially for source determination and comparisons. Isotopes are atoms of the same element, differing by the content of neutrons in the nucleus, and therefore by atomic weight. Elements often have several different isotopes, which are present in nature in different relative quantities as a function of their source and especially of their geographical origin. Knowledge of the relative amounts of isotopes of the same element in the sample material can therefore be a source of precious information for comparative purposes, and if databases or previous research is available, also for source determination.

Where can this technique be found?
The cost of instruments for atomic mass spectrometry is quite high, making this technique less common than optical atomic spectrometry. However, atomic mass spectrometry is easily found in academic and governmental laboratories, in addition to the best equipped commercial analytical facilities.

Atomic mass spectrometry couples the atomisation system of a plasma-based atomic emission spectrometer with a mass analyser, such as a quadrupole or a time of flight detector [9]. Emission atomic spectrometry yields very complex spectra, because each atom gives rise to several emission lines, even more than a hundred, i.e. as many as the possible transitions from the ground state to the excited states. On the contrary, atomic mass spectrometry spectra are much simpler. In the low

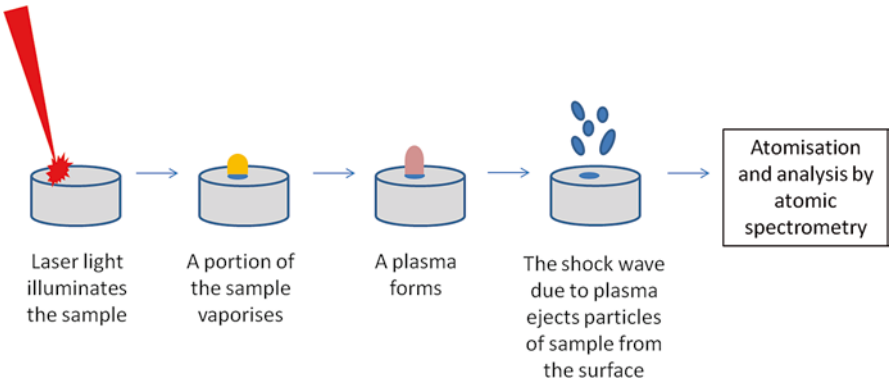


Fig. 5.7 Sequence of events associated to laser ablation

resolution mode just one major signal per element is expected, whereas if a more detailed analysis is desired, a few signals associated to the isotopes will be found, allowing isotope ratio analysis.

This spectral simplicity is coupled with remarkably low detection limits, which compete with the very sensitive optical atomic spectrometry with electrothermal atomisation.

These multiple advantages are even increased if atomic mass spectrometry is carried out in direct solid sample analysis mode. As introduced above, laser ablation consists in the removal of a small quantity of material from the surface of the sample by a laser beam. The laser pulse locally induces the formation of a high temperature plasma which is followed by ejection of the sample in the form of fine particles. Such particulate is subsequently introduced into the plasma torch of an ICP-mass spectrometer where atomisation takes place and the elemental ions are detected by a mass analyser (Fig. 5.7).

Orellana and colleagues recently reviewed the ever growing applications of laser ablation ICP-MS in forensic science [10]. The huge advantage of laser ablation is related to the very small region which can be sampled, which makes the technique globally non-destructive. In other words, if on one hand the part of sample which is illuminated by the laser is destroyed by the large energy transfer, on the other hand such region is very small and allows to save most of the item and to repeat the analysis several times. In addition, the small sampling region also allows to perform spatially resolved analyses. The absence of sample preparation steps avoids also contamination issues, which can be quite relevant in analyses such as these, involving analytes at the trace level. Laser ablation is also a more universal sampling system, because it allows to analyse also chemically inert elements (e.g. zirconium, hafnium, tantalum, niobium, titanium, thorium and uranium), which due to their rarity are extremely significant if found in a sample. Finally, since, different from traditional ICP-MS, no acid digestion step is needed, laser ablation is also safer.

Laser ablation has drawbacks as well, of course. The two main problems associated to this analytical approach are the rather time consuming optimisation of the experimental parameter and the difficulty in obtaining quantitative data. The optimisation of the laser characteristics depends on the matrix type, and it is necessary to maximise the amount and homogeneity of vaporised material which is directed to the ICP-MS instrument. Quantification, on the other hand, requires the use of an internal standard to correct for variations in mass ablation between replicates, it suffers from relevant matrix effects and it is limited by the impossibility of diluting the sample or of using the common calibration approaches typical of the analysis of aqueous solutions such as single standard addition or isotope dilution. A major issue which may prevent an accurate quantification is fractionation, which results in a non-faithful description of the elements present in the sample. Fractionation was defined as 'the occurrence of non-stoichiometric effects in the transient signals' during LA-ICP-MS analysis [11] and it depends simultaneously on the characteristics of the matrix, of the elements and of the parameters of ablation and atomisation. Ways to minimise fractionation were identified. A more detailed discussion of these issues can be found in the review from Orellana et al. [10].

Despite the difficulties listed above, quantification by atomic mass spectrometry is surely feasible with sufficient accuracy and precision, provided that interferences are considered and matrix effects are reduced. Since sets of standard materials which reproduce the matrix of interest rarely are available, in most of the cases solely a semiquantitative analysis is possible. In such cases, a solution with known concentrations of the elements to be measured is used as a standard, and the peak ion current or intensity is measured. The analyte concentration in the sample is obtained, assuming that the ion current is proportional to concentration. Albeit a bit simplistic, this approach is effective and allows a meaningful comparison of samples based on similar matrices, for example fibres or plastic automotive parts.

5.2.3 X-ray Fluorescence

What is X-ray fluorescence?
X-ray fluorescence (XRF) is a technique in which a sample is illuminated with X-rays. These excite the atoms contained in the sample, which in turn emit X-rays with characteristic energies. Analysis of the emitted X-rays is therefore a way to identify which elements are present in the sample, with the possibility also of quantifying them.

Why use this technique?
The aim of the use of XRF in forensic science is the same as that of the atomic spectrometry techniques previously presented. A big advantage of XRF is that it is completely non-destructive and that it does not require any sample preparation step. Moreover, the technique can be miniaturised under two aspects.

(continued)

On one hand, XRF portable spectrometers are commercially available, which allow an onsite screening of macroscopic samples, also on the crime scene. On the other hand, micro-XRF spectrometers were devised, by which the analysis of very small regions of the sample, or of very small samples, can be accomplished. All the emitted radiation is acquired at the same time, so a multi-element analysis can be completed in a very short time. A further advantage is that XRF spectra are rather simple, with a few emission lines for each element, and so interpretation is not so complex. Among the drawbacks, there is a reduced sensitivity with respect to the atomic spectroscopy techniques mentioned above. Elements present in amounts lower than 0.01 % become quite difficult to detect. The efficiency of detection is not equal for all elements: heavier elements have a much more intense fluorescence emission. The consequence of this is that the detection limit of XRF for light elements is much lower than that of the heavier ones, and that elements below carbon cannot be accurately determined.

Where can this technique be found?

The availability and cost of XRF analyses is comparable to that of atomic mass spectrometry. It can be therefore found in academic and governmental laboratories, in addition to the best equipped commercial analytical facilities.

When a material is exposed to an X-ray beam, it can absorb a portion of this radiation, producing excited ions which subsequently relax and return to their ground energy state. The energy involved in such disexcitation process is dissipated by emitting X-rays, whose wavelength will depend on the characteristic energy levels of the atoms composing the sample. XRF is therefore a powerful method for the identification of which elements are present in the sample.

A XRF instrument is composed by a source of X-rays, either a X-ray tube or a radioactive substance, by a sample holder and by an X-ray detector. The source shines X-rays on the sample, which emits radiation which in turn is collected by the detector. Two main families of instruments exist: wavelength dispersive and energy dispersive. In the former, a monochromator is used, and the emitted fluorescence light is analysed one wavelength at a time. In the latter, a semiconductor detector is employed, and all the emitted X-ray light is measured simultaneously. The detector is then able to classify the acquired photons according to their energy, allowing to associate them to the element which emitted them. The simpler design of energy dispersive instruments, which do not require moving parts and which do not have losses of intensity due to the use of monochromator, makes these apparati less expensive and more common. They are more sensitive, even though their resolution at wavelengths longer than 1 Å is lower than wavelength dispersive instruments.

Even though the mechanism is somewhat different, the emission of X-rays can be triggered also in a scanning electron microscope (SEM). In this case, a highly

collimated electron beam illuminates a small portion of the sample. This electron beam can extract some of the electrons closest to the nuclei of the atoms composing the samples. This produces excited ions, which can relax to the more stable ground state by transferring electrons from the more external electronic levels to the electron vacancy in the inner region of the atom. The extra energy of the excited ion is dissipated as X-rays with particular wavelengths. The X-rays emitted according to this mechanism by bombardment with electrons have the same wavelengths than those induced by shining X-ray light. Even though just the latter can be rigorously called XRF, it is not unusual that elemental analysis can be carried out examining the X-rays emitted under the electron beam of a SEM. In SEM, energy dispersive detectors are used for detecting X-ray, so the technique is normally referred to as SEM-EDX. SEM-EDX shares advantages and disadvantages of XRF, including the non-destructivity and the possibility of proceeding to the analysis without any sample preparation, if Environmental Scanning Electron Microscopy (ESEM) is used. ESEM in fact allows to avoid the metallisation step which is necessary in normal SEM if non-conductive samples must be analysed.

5.3 The Techniques: Chromatography

What is chromatography?
Chromatography is a separation technique. It is used whenever mixtures of different analytes are involved. Most of the analytical techniques yield complex sets of data when they are not applied to pure substances. Chromatography separates the components of mixtures, allowing to analyse them separately.

Why use this technique?
Polymeric items are complex systems, composed by a matrix and by a variety of additives, which can be organic and inorganic. This multifaceted nature is not approachable with a single analytical approach, but it requires a number of techniques.

Chromatographic techniques allow one to separate the components of a mixture, so they can be individually analysed. Usually, albeit not always, separation is carried out in a column, filled or lined with a suitable stationary phase. The samples are introduced from one end of the column and pushed towards the other end by a mobile phase, which can be a liquid or a gas. The analytes with more affinity to the stationary phase will interact with it more, and their exit from the column will be delayed. On the contrary, if the analytes have more affinity to the mobile phase, they will interact much less with the stationary column and they will pass through the column in a shorter time.

(continued)

When the analytes exit from the column they reach the detector and they are revealed. In some cases, such as when mass spectrometry is used for detection, it is possible to identify the chemical nature of each analyte, in some other cases, this is not possible and just the number of components in the mixture can be determined.

Many variants of chromatography exist, adapted for separating a very wide range of different mixtures, but two main types can be identified. Gas chromatography separates mixtures of gaseous compounds, or of compounds that can be volatilised. Liquid chromatography is applied on liquid mixtures, and it can be performed on columns (high performance liquid chromatography, HPLC) or on planar plates (thin layer chromatography, TLC).

Where can this technique be found?
Gas chromatography and liquid chromatography are very common techniques. Every commercial laboratory is equipped with at least a gas chromatographer with a simple flame ionisation detector, and often with a HPLC apparatus. Chromatography is a fundamental technique for the analysis of illicit drugs or of flammables or explosives, so this kind of technique will be accessible also in every forensic institution. Finally, these instruments for chromatography are ubiquitous in academic laboratories and in many technical high schools. As such, the cost of a chromatographic analysis is limited, provided that a method was already published. A single analysis can have a cost between 100 and 200€. However, the expense will significantly increase if no method is already available. The development of a method is a time consuming and complex activity, because a number of tests must be performed and several chromatographic runs must be replicated.

The International Union of Pure and Applied Chemistry (IUPAC) defined chromatography as

> a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction [12].

The mechanism of chromatographic separation may be better understood on the basis of an analogy. Three individuals have to go from one end to the other of a crowded corridor. In their intentions, they would go straight to the exit without interrupting their motion. However, if they meet somebody they know they have to stop and exchange a few words. The person who knows the most of the people in the crowd will proceed very slowly in the corridor and will take a long time to reach the other end. The person who does not know anybody will have no interaction with the other people and will quickly cover the distance towards the exit of the corridor. The remaining individual who knows just a fraction of the people in the crowd will exit in a time intermediate between those of the first and second persons.

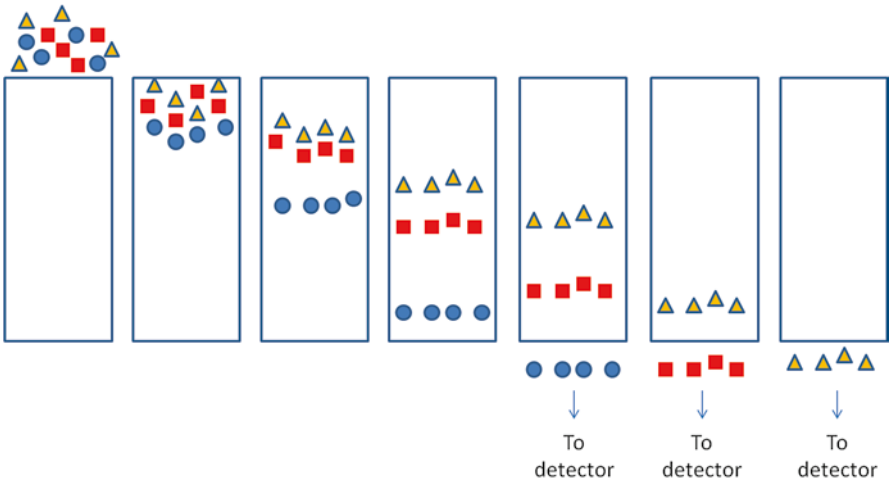


Fig. 5.8 Principle of chromatographic separation. The circles show the most affinity with the mobile phase and the least affinity with the stationary phase, and so they exit first. The opposite happens with the triangles, which interact more with the stationary phase than with the mobile phase, so they exit last. An intermediate situation characterises the squares

Chromatographic separations work exactly in the same way. The analytes are introduced all together, driven by a stream of liquid or of gas, the mobile phase, and they come into contact with the stationary phase. The analytes with more chemical affinity with the stationary phase will prefer to interact with the stationary phase, rather than to proceed with the liquid or gaseous flow. They will be *retained* more. The contrary happens with the analytes which are not particularly prone to interaction with the stationary phase, but they rather favour remaining in the mobile phase and travel quickly (Fig. 5.8).

The movement along the stationary phase is called elution. When the analytes exit the column they are said to elute. If we measure the concentration of the eluted molecules and we plot them as a function of time or of the volume of mobile phase passed through the column, a chromatogram is drawn. With time, and thanks to the efforts of thousands of analytical chemists, chromatography has grown as a very versatile technique able to tackle the problem of separating a wide range of different mixtures.

Different chromatographic techniques can be classified according to a number of criteria.

One classification can be made according to the type of interactions on which the mechanism of separation is based. In adsorption chromatography the stationary phase is a solid where the sample compounds are adsorbed. The components distribute between mobile and stationary phase by a combination of sorption and desorption processes. In modern partition chromatography, the stationary phase is usually a silicone-based polymer, functionalised with siloxanes. The polarity of the stationary phase can be tuned by modifying the kind of functional groups on the surface of such material. Separation is obtained through a balance between the

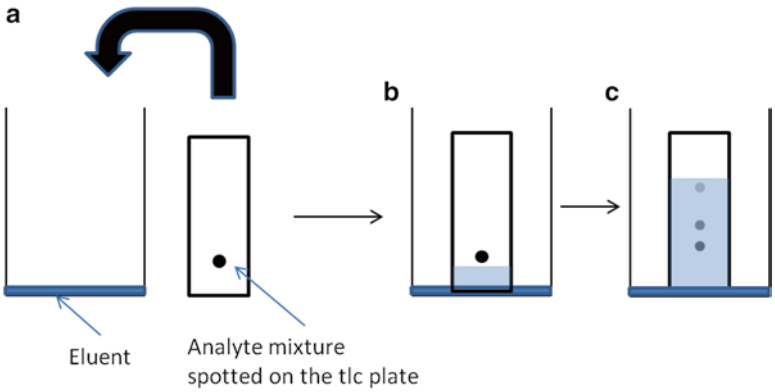


Fig. 5.9 The steps of a TLC analysis. (**a**) The analyte mixture is spotted on the stationary phase and a development tank is prepared with some millilitres of mobile phase (the eluent) on the bottom. (**b**) The plate is introduced in the tank, and the eluent starts rising on the silica gel due to capillarity. (**c**) As separation proceeds, as many spots as the components of the mixture are noted

intermolecular forces among solute, mobile phase and stationary phase. Ion exchange chromatography exploits an ion exchange resin as the stationary phase. Size exclusion chromatography separates the analyte molecules according to their size (Sect. 6.1.1).

Chromatography techniques can be divided into liquid chromatography and gas chromatography, according to the physical state of the mobile phase.

Finally, a further classification of chromatography can be made according to the form of the stationary phase: it can be either a column or a plane.

The only relevant kind of planar chromatography in the forensic analysis of trace evidence is TLC. In this technique, the stationary phase is a glass, plastic or aluminium plate coated with a thin layer of silica gel. The analyte is spotted close to the bottom edge of this plate, and developed immersing the bottom of the plate, but not the sample spot, in a suitable solvent or solvent mixture: the mobile phase, also called eluent. Capillary action draws the eluent upward, and this flow moves also the components of the sample up the plate (Fig. 5.9). The magnitude of the interactions between each component and the stationary phase will change the rate of migration. Since different compounds will interact differently with the silica gel, they will raise up the plate at different rates, and so their separation will be obtained. The resolution of the separation is improved if the spots in the sample seeding step are kept at a minimum size. Typically sample volumes of about 10 μL are used and sample spots of less than 2 mm in diameter can be obtained adding the sample dropwise, evaporating the solvent with an air dryer between each drop.

When the separated components of the mixture are coloured, they give rise to spots visible by the naked eye. Observation under ultraviolet light is often an efficient method for visualising colourless developed spots. Commercial TLC plates exist which are treated with a UV fluorescent dye. The presence of an analyte will quench the fluorescence, appearing black under ultraviolet light.

As an alternative, spots due to white compounds can be visualised forming a coloured derivative by treatment with suitable reagents. Exposing the plate to iodine vapours is a common staining method.

Another method consists in spraying the plate with sulphuric acid and subsequently heating it; organic compounds are charred and will appear on the TLC plate as black spots. Ninhydrin is another stain, popular for amino acids.

TLC, as other chromatographic methods, has the drawback of not allowing the identification of the chemical nature of the components of the mixture. Even if it is possible to scrape the solid layer containing the spot and separately analyse it, this is a cumbersome procedure rarely performed. Most of the times, TLC is used for comparative purposes. In such instances, it is therefore sufficient to compare the number, position and colour of the spots obtained from the samples. If they match, the samples contain the same substances.

Due to its simplicity and low cost, TLC is a very commonly used technique. Reproducibility is relatively difficult to achieve, so it is always advisable to seed all the samples on the same plate, developing all of them simultaneously, to guarantee that they are subject to exactly the same conditions.

Column chromatography is more sophisticated than TLC, and it allows a deeper investigation and in some cases an identification of the individual components of the mixture. Gas chromatography is column chromatography in which the mobile phase is a gas. The most common way to realise column chromatography with a liquid mobile phase is HPLC. Even though these two techniques obviously differ by instrumental design, they share the same basic scheme (Fig. 5.10).

In HPLC, the mobile phase is delivered by a pump. Very high pressures are used in this technique, from 50 to a few hundred bars. In gas chromatography, the driving force for the mobile phase is given by the pressure of the carrier gas cylinder. Helium is a very common carrier gas, but also nitrogen or hydrogen is used. The linear velocity of the gas is generally in the order of magnitude of 20–40 cm/s.

The sample is then injected in the mobile phase flow. A few microlitres of sample are introduced by a microsyringe, through suitable devices which allow to preserve the pressure of the mobile phase. In HPLC, the sample is a liquid or a liquid solution. Ideally, the solvent is the mobile phase itself. It is important in fact that the analytes in the sample do not precipitate in the eluent; otherwise they will remain in the column, hindering the analysis and plugging the apparatus. The sample is a liquid or a liquid solution in gas chromatography as well. However, a fundamental requirement in this case is that the analytes present in the sample can be volatilised at a reasonable temperature. The injection temperature must therefore be high enough to volatilise the sample component, at the same time avoiding its thermal degradation. Sometimes, prior to injection, the sample is treated with suitable

Fig. 5.10 Scheme of a column chromatography apparatus

derivatising agents, which react with the non-volatile compounds yielding more volatile products. For example, the non-volatile fatty acids can be converted to their volatile methyl esters.

The sample then enters the column, where separation occurs. The choice of the stationary phase contained within the column is one of the key factors on which the efficiency of a chromatographic method is based. The other one is the physicochemical nature of the mobile phase. In gas chromatography, column temperature is the parameter which can be tuned to optimise the separating power of the mobile phase. Its choice is a compromise between speed, sensitivity and resolution. At high column temperatures the sample components spend more time in the gas phase, improving the speed of the elution, but reducing resolution. At low temperatures, the opposite happens. Elution is slower, but the increased interaction of the analytes with the stationary phase of the column brings about a better resolution, albeit at the expense of a lower sensitivity due to a spreading of the peaks. Most gas chromatograpy methods involve a variation of the temperature during the chromatographic run. In HPLC, the polarity of the mobile phase can be changed mixing different solvents together. If the composition of the mobile phase does not change during the chromatographic run, the method has an isocratic elution. If the composition changes, gradient elution is used.

Different columns with different stationary phases are commercially available. In HPLC, adsorption or partition chromatography are the most common method of separation. Both these mechanisms of separation are mostly influenced by the polarity of the sample, of the stationary and of the mobile phase. Partition chromatography is sensitive to small differences in molecular weight and should be thus preferred for the separation of homologous series. It is more indicated for polar samples. Adsorption chromatography is more suited for the separation of isomers with different shapes, because it is sensitive to steric effects. It works better with non-polar samples.

Reversed phase chromatography is performed when the mobile phase is polar and the stationary phase is apolar. It is the favourite approach in modern liquid chromatography. The opposite situation, when the mobile phase is a non-polar solvent system and the stationary phase is a polar surface, is defined as normal phase chromatography. This definition stems from the fact that this was the combination of relative polarities first used in early work in liquid chromatography.

The choice of the column for gas chromatography is made on the basis of the polarity of the stationary phase as well. Capillary columns are by far the most common, with internal diameters of less than 1 and up to 100 m long. Many stationary phases are available, ranging from the non-polar 100 % dimethyl polysiloxane to the very polar poly(ethylene glycol) coating. A common general purpose column is coated with a diphenyl, dimethyl polysiloxane copolymer, containing 5 % diphenyl units and 95 % dimethylsiloxane units.

If the column and the mobile phase are the key parameters which define the efficiency of the separation process, the detector determines which information can be acquired on the separated components of the sample. As previously discussed, the result of chromatography is a graph called chromatogram (Fig. 5.11).

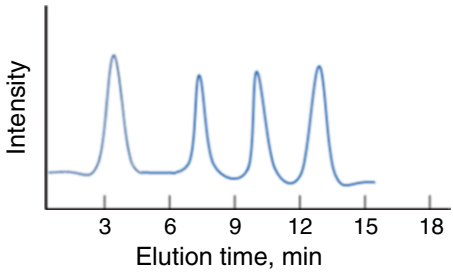


Fig. 5.11 Example of a chromatogram

The basic feature which characterises any peak in the chromatogram, irrespective of the detection method used, is the elution time (or retention time). Elution time is the time that an analyte takes for passing through the column, and can be also indicated in terms of elution volume. For a certain compound, elution time depends on the chromatographic conditions. The repeatability of column chromatography is much more than that of TLC so, with the same chromatographic conditions, a particular compound will exit the column always at the same elution time. If the analysis is aimed at identifying the presence of a certain substance in a mixture of unknown composition, a chromatographic analysis made on a standard reference sample of the target substance will be used to determine its particular elution time. Repeating the analysis in the same conditions on unknown mixtures will reveal if that substance is present: this happens if a peak appears in the chromatogram at the elution time of the target compound. This is the procedure followed in the analysis of illicit drugs. In order to verify if a sample seized to a suspect smuggler indeed contains drugs of abuse, for example cocaine, a portion of the sample is analysed by chromatography (normally gas chromatography). A method is used that has been previously validated in the same and other laboratories, analysing standard samples of cocaine, which must show a peak consistently appearing at a particular elution time, for example 10 min. If the sample seized to the suspect, analysed by such method, shows a peak at 10 min, this can be considered evidence that cocaine is present. However, a complication sets in. Many substances may exist which, in the same chromatographic conditions, may elute at the same time. In other words, in the example above the appearance of a peak at 10 min is necessary but not sufficient to prove that cocaine is indeed present in the seized sample. A further confirmatory analysis will be necessary if one wants to positively identify the compound contained in the mixture. However, it is rarely necessary, in the characterisation of the formulation of trace evidence, to identify its individual components. Usually in these cases comparisons are made, where samples can be deemed similar or different, if the number and position of each peak in their chromatograms match. This means that if two samples contain the same components, their chromatograms will show the same number of peaks, located at the same elution times.

The flame ionisation detector for gas chromatography and the refractive index detector for HPLC are detectors useful for this kind of analysis. They are universal detectors, meaning that they reveal a signal, irrespective of the chemical nature or

composition of the analyte. Even though they do not give any additional information on chemical or physical features useful for the identification of each compound, they nevertheless provide useful data for comparison purposes.

When identification of the analytes associated to each chromatographic peak is required or desired, more elaborate detectors are used. This is true of diode array UV–visible detectors used for HPLC, which are able to provide the full UV–visible absorption spectrum of the material which exits the column at any given elution time. Even though the UV–visible spectrum is not the most informative for the qualitative identification of compounds, it can indeed help in formulating at least some educated hypotheses. Surely the most indicated detection method for the identification of the analytes separated in chromatography is mass spectrometry (MS). Hyphenation of gas chromatography with mass spectrometry (GC–MS) is a mature technology which brought about affordable, easy to use and reliable instruments. Recently MS detection in HPLC has become more common as well. MS detectors in chromatography provide the mass spectrum of the material which in any given moment exits from the chromatograpic column. GC–MS usually is equipped with an electron impact ion source (see Sect. 5.5). The eluting material is bombarded by a highly energetic electron beam, which fragments the molecules, and favours a number of rearrangements of the molecular structures. The mass of each of these fragments is subsequently measured by the mass analyser/detection section of the mass spectrometer. Since the mechanism of fragmentation and the number and types of rearrangements which occur are highly reproducible and, at equal ionisation conditions, are uniquely dependent on the chemical structure of each molecule, the mass spectrum is a very efficient method for the univocal identification of chemical compounds. Even more sophisticated mass detectors are widespread in HPLC-MS, with the possibility to tune the amount of fragmentation of the molecule, and also allowing further fragmentation of the fragments themselves. This incredibly improves the accuracy of qualitative analysis.

5.4 The Techniques: Pyrolysis

What is pyrolysis?
Pyrolysis is hardly an analytical technique in its own right. It can be more aptly described as a preparation method. However, the importance of pyrolysis in forensic science is very relevant, and as such it deserves a separate discussion. Pyrolysis is the fragmentation of a substance in an inert atmosphere by the effect of high temperature. The analysis and identification of the fragments can yield information on the chemical identity of the materials present in the considered item and thus on its formulation. To do so, pyrolysis is normally associated to gas chromatography with mass spectrometry detection.

(continued)

This way, the fragments derived from the thermal degradation of the material are separated and individually identified by mass spectrometry.

Why use this technique?
Pyrolysis-gas chromatography-mass spectrometry (Pyr-GC–MS) is a technique which allows a reliable identification and comparison of the components of materials, which requires very small sample sizes and no sample preparation. Traditionally, pyrolysis-based methods have been considered to be less reproducible. However, if modern equipment is correctly used, this is not an issue anymore. The big advantage of Pyr-GC–MS is that it makes GC–MS accessible also for exquisitely non-volatile materials like polymers.

Where can this technique be found?
Pyr-GC–MS systems are not very commonly found in commercial laboratories. However, this technique should not be missing in any forensic trace laboratory. Universities and research centres are somewhat in the middle, with the largest institutions more likely to be equipped with such facilities. Sometimes protocols are already available but, when this is not the case, acceptable results can be obtained after a limited number of tests.

The cost of one analysis by this approach is not much different from that of a normal gas chromatography run.

As introduced above, pyrolysis consists in the rapid heating of a sample in an inert atmosphere. This treatment brings about a series of degradation reactions which fragment the molecules of the sample and which yield a number of low molecular weight gaseous species which are easy to analyse and characterise. As such, pyrolysis must necessarily be associated to an analytical technique capable of extracting information from the fragments produced. Even though infrared spectroscopy has been reported as a viable detection technique for identifying the products of pyrolysis [13], the vast majority of applications couples pyrolysis with gas chromatography. The best performing combination of techniques consists in adding mass spectrometry as a detection system for the column eluent. As mentioned in Sects. 5.3 and 5.5, in fact, the mass spectrum is a very efficient method to positively identify compounds.

Due to this common connection with chromatography, commercial pyrolysers are normally in the form of accessories which can be easily mounted on the injector of a gas chromatographer. Three main types of pyrolysing devices are the most diffused.

The filament type consists of a platinum coil or ribbon which can be heated by Joule effect by circulating an electrical current. Normally filament type pyrolysers allow to choose and change the most practical sample holder, so both the coil and the ribbon can be used in the same apparatus. When the coil is employed, the sample is

inserted in a small quartz tube which is subsequently plugged with quartz wool and introduced into the coil. The ribbon sample holder is used for liquids and it is basically a surface on which the sample is evaporated. Once the sample is mounted, the sample holder is introduced into an interface which connects it with the injector of the gas chromatograph. A pulse of current is given, the temperature of the filament increases rapidly to temperatures of the order of 700–1,000 °C, and at the same time a carrier gas pushes the degradation products into the gas chromatographer.

In Curie type pyrolysers, the sample is positioned on a flat wire made of a ferromagnetic material. This assembly is put inside a magnetic field and heated by induction. When the wire reaches its Curie point, the relative permeability of the sample carrier decreases suddenly, it instantaneously changes from ferromagnetic to paramagnetic, and the temperature rapidly increases. In contrast to filament type pyrolysers, which allow to use the same coil or ribbon for achieving any temperature up to around 1,000 °C, in Curie point ones, the pyrolysis temperature is controlled by the composition of the sample carrier. In other words, for any particular desired pyrolysis temperature a particular sample holder must be used. The introduction of the pyrolysis products into the GC is performed as in the filament type case.

Finally, furnace type pyrolysers exist. They are placed on top of the GC injector. The sample is introduced from the top of an oven, and is released in a vertical quartz furnace. With this design, it is more complex to clean and to prevent that residues from previous analyses contaminate the successive ones.

Carry over of analytes between subsequent analyses is a problem which is solved by accurate cleaning of the sample holders after each measurement. The reproducibility of Pyr-GC-MS is dependent on the performance of the pyrolyser and of the chromatography parameters. It is advisable to frequently check the efficiency of the apparatus by running standard specimens and checking the consistency of results.

5.5 The Techniques: Mass Spectrometry

What is mass spectrometry?
Mass spectrometry is an analytical method that measures the mass of molecules or of molecular moieties. Different technical approaches exist for performing mass spectrometry, but all of them share the need to put some charge on the molecules (ionisation) and to measure their mass.

Why use this technique?
Mass spectrometry is particularly efficient for the analysis of small molecular weight species. This technique is thus very well suited for the analysis of the formulation of polymers. Ionisation methods that allow analysis of the sample in the solid state, even if they do not volatilise the biggest macromolecules, are surely efficient with small molecules, and so can yield information on the

(continued)

small molecular weight additives present in the formulation of the material being analysed. An advantage of mass spectrometry over other techniques for determining molecular weight is the ability to identify the species. Using different ionisation approaches, the molecules can be fragmented according to different patterns, which can be eventually interpreted reconstructing the chemical nature of the original molecule.

Where can this technique be found?
Mass spectrometry is a family of techniques which comprises a number of different methods and instrumental apparati. Laboratories specialised in mass spectrometry are usually located at universities or research centres, where the expertise necessary for the set up of ad hoc experimental protocols can be found. Even though, in fact, some guidelines and experimental reports can be found in the literature, standardisation is difficult for heterogeneous and variable samples such as polymers, especially those involved in forensic cases. Each comparison must be preferentially conducted in the same experimental session, in order to minimise the random fluctuations which can jeopardise the quality of the results.

The cost of mass spectrometry analyses is very variable, because the optimisation of the experimental parameters is sometimes quite complex and the expertise needed is not very widespread.

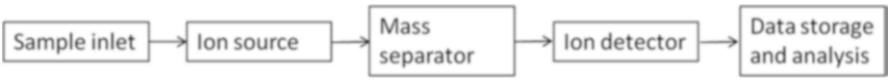


Fig. 5.12 Block diagram of a mass spectrometer

Mass spectrometry is without any doubt a sophisticated technique, however describing the general picture of how it works is a quite easy task. The basic components of a mass spectrometer are shown in Fig. 5.12.

Irrespective of the technical solutions chosen, mass spectrometry is an analytical technique which produces, separates and detects ions in the gas phase.

The sample inlet system introduces a very small amount of material in the instrument, where its components are converted, by the ion source, into gaseous ions. In the subsequent step, these ions are separated according to their mass, and they are individually revealed by an ion detector.

The array of data which can be gathered through mass spectrometry spans from the elemental composition of samples, mixtures or surfaces, to the structure of molecules, and further to the isotopic ratios of atoms in samples. This flexible and versatile applicative potential made mass spectrometry probably the most sparkling branch of modern analytical chemistry.

In this chapter, the focus is on the formulation of polymeric materials, and thus on the small molecules contained within commercial products based on macromolecular matrices. Section 6.1.3 will cover the issues related to the measurement of molecular weight of polymers. Under this aspect, mass spectrometry is very well suited, because a wide expertise and a rich literature exist on mass spectrometry applied to mixtures of small molecular weight species.

The most critical parts of a mass spectrometer are the mass analyser and the ion source.

The mass analyser is the part of the apparatus in charge of separating the ions produced in the ion source according to their mass.

Four main families of mass analysers exist.

The magnetic sector analysers use a magnetic and an electric field to direct the ions along a circular path. The radius of such trajectory will depend on the mass-to-charge (m/z) ratio of the ion, and on the intensities of the magnetic and electric field. When ions with different m/z ratio are introduced into the analyser, they will be deflected to different degrees, achieving their separation according to the mass.

The quadrupole mass filters consist of four parallel metal rods to which a direct current voltage and an oscillating radiofrequency voltage are simultaneously applied. Two opposite rods are positively charged and the other two are negatively charged. The trajectory of the ions along the path between the four poles will depend on their mass, charge and on the voltage. Only the ions with a certain m/z ratio will resonate and have a stable path through the detector, whereas all the others will follow an unstable path, they will be deflected and they will eventually collide with the rods. By varying the voltages, ions of one mass after the other will take a stable path and will be collected by the detector. The potential can be changed very rapidly and very efficiently, so this mass analyser is very quick, allowing to complete a mass scan in fractions of second. It is the most commonly used mass analyser in gas chromatography/mass spectrometry instruments.

In ion trap analysers, ions are confined by electrical and/or magnetic fields. All the ions from an ion source are introduced simultaneously in the ion trap, and then the electrical/magnetical field parameters are changed to selectively release just ions with a particular m/z ratio, which eventually reach the detector.

The time-of-flight (TOF) mass analyser is by far the most popular for the detection of ions of high molecular mass, since it has no real upper mass limit of detection. In this device, ions are accelerated by pulsating plates with a voltage of some kV. The pulsating mode is necessary to group together the ions in packets, which are subsequently introduced in a drift or flight tube with a constant kinetic energy. Due to this constancy of kinetic energy, the ions with larger mass will travel more slowly, and will therefore cover the distance of the flight tube in a time longer than that of lighter species.

The mass analyser is certainly important for the definition of the figures of merit of the mass spectrometry measurement. However, when considering mass spectrometry as a potential technique for an analytical protocol, the choice of the ion source is by far the most important. As described above, in fact, mass spectrometry works on ions in the gas phase. This implies that the sample must be volatilised, or

that at least gaseous ions can be extracted from it. As will be clearer in the following discussion, this step has tremendous effects on the significance and usefulness of the data that can be obtained by mass spectrometry. Some sources work on samples in all physical states, some only on gases or volatile liquids, for example. With some approaches it is possible to ionise the analytes preserving their structures, but in others fragmentation of the compounds occurs, complicating the interpretation. Some techniques require an extensive sample preparation, some others do not. Finally, some sources can be easily hyphenated with other instruments, such as for example chromatographers, some others perform better as stand-alone techniques.

In gas-phase sources–electron impact, chemical ionisation and field ionisation–the sample is first volatilised and then ionised. They can only be used on thermally stable samples, which volatilise below 500 °C.

Ionisation by electron impact was the first approach in mass spectrometry. It consists in bombarding a vapour of the sample with a highly energetic beam of electrons. In the impact, molecules are fragmented in several molecular moieties, because the energy transferred by the electrons to the analytes is dissipated by breaking bonds; for this reason, electron impact sources are classified as hard sources. It is worth noting that fragmentation is not necessarily a defect of the ionisation system. Fragmentation, in fact, obeys precise rules that govern the mechanisms of bond breaking and rearrangement. As a consequence, from the daughter ions (the fragments) it is normally possible to reconstruct the parent molecule. Moreover, often the molecular ion, i.e. the ion with a mass equal to that of the original molecule, is detectable as well, much helping the interpretation of the data. Indeed, electron ionisation is the most common ion source in gas chromatography/mass spectrometry. Very rich databases exist which allow to identify the mass spectrum of a large number of species from the fragments which are formed by electron ionisation of the analytes.

Chemical ionisation and field ionisation are softer ion sources, because they are associated with limited or no fragmentation of the analyte molecule. In chemical ionisation, ions are produced by electron bombardment of a suitable gas, for example CH_4. Species such as CH_4^+ or CH_3^+ are formed, which collide with the molecules of the samples, ionising them. Chemical ionisation, which is probably the second most common ion source, yields very simple mass spectra, often consisting just of a base peak closely related to the molecular ion accompanied by other minor peaks.

In field ionisation sources, ions are formed by application of an intense electric field. It is a soft source as well, yielding very simple mass spectra with little fragmentation.

The methods of ionisation just mentioned work best when it is easy to volatilise the molecules present in the mixture. In other words, they would be very efficient for creating ions if the additives in the polymer formulation were dissolved in a solution. However, such additives are included and trapped into a polymeric matrix, and therefore they do not volatilise easily. A preliminary step for applying these most basic mass spectrometric approaches consists in an extraction of the additives with suitable solvents.

This sample preparation step is not desirable in a forensic context, because it alters the items and can interfere with subsequent steps in the analytical procedure.

These drawbacks can be overcome by applying desorption techniques, which do not require a volatilisation of the sample prior to the ionisation step. A number of desorption sources have been developed over the last 20 years, however they all share the same rationale: energy is transferred to the sample in the condensed state, in such a way to promote the direct formation of gaseous ions. This kind of ionisation is much gentler (at least if compared with the techniques based on the bombing of the analyte with various kinds of particles such as electrons or ions), with the consequence that the resultant ions are not fragmented in the process and that ions extremely similar to the original molecule (molecular ion or protonated molecular ion) are detected. This greatly helps the interpretation of the mass spectra: in case of fragmentation, mixtures will yield a complicated array of signals, difficult to single out.

Another great advantage of desorption techniques is that they allow to study compounds with molecular masses much higher than those analysable with gas-phase sources. This makes desorption sources appealing also for the measurement of the molecular weight of polymers. This issue will be discussed in more detail in Sect. 6.1.3. Here the principles of each approach will be presented, and the focus will be on the analysis of the formulation of a polymeric item.

Probably the desorption methods with the largest diffusion in polymer science are field desorption and matrix-assisted laser desorption/ionisation (MALDI).

Field desorption requires a cumbersome sample preparation step, because a filament electrode is coated with a solution of the sample. A high voltage and sometimes an increase in temperature are then applied, causing the desorption of the sample and its concurrent ionisation.

In MALDI, the energy for ionisation is supplied by a laser, but it is mediated and attenuated by a suitable matrix. The sample preparation step of MALDI is the most critical one. The polymer is mixed, usually through a common solvent, with a material, called a matrix, such as nicotinic acid, 1,8,9-trihydroxyanthracene, *trans*-3-indoleacrylic acid, pyrazine-carboxylic acid, 3-nitrobenzylalcohol, 3-aminopyrazine-2-carboxylic acid or derivatives of benzoic acid or of cinnamic acid. Sometimes, the sample is sandwiched between layers of one or more matrix components, to promote a more efficient desorption process. The relative concentrations of sample and matrix are of the order of 1:1, and less than 1 μL of solution is necessary for spotting the sample on a suitable sampling plate. Laser light (the most typical wavelengths are 266, 337 and 355 nm) is shone on the mixture so prepared. It is important that the wavelength of the laser light is absorbed just by the matrix and not by the polymer, which otherwise would be damaged and fragmented by the large energy impulse. A chain of events must occur, where the energy of the laser is absorbed by the matrix, which attains an excited state and vapourises, at the same time transferring some of the energy to the polymer, which is desorbed into the gas phase. The mechanism of this process has not been completely understood yet, and this brings about a significant difficulty in identifying the most suitable matrix for the materials of interest. The know-how of an experienced specialist and

a trial-and-error procedure are still in many cases the foundations of the development of methods of sample preparation for MALDI mass spectrometry.

The difficulties associated to sample preparation, which are one of the drawbacks of MALDI, can be overcome by the most recent ambient ionisation techniques. DESI (desorption electrospray ionisation), DART (direct analysis in real time), and a number of other methods have been proposed in the last few years [14–17].

DESI is a combination of electrospray and desorption.

In electrospray, a solution of the sample is pumped through a capillary needle. This needle is maintained at high electrical potential with respect to a surrounding cylindrical counterelectrode. The charged spray then is directed through another capillary where the solvent evaporates. In this path, the size of the droplets decreases and the charge density increases up to a point that desorption of ions occurs. The ions so produced are introduced into a mass analyser and they are detected.

In DESI, a solvent or solvent mixture is sprayed onto the sample. Liquids such as methanol, water, acetonitrile or dichloromethane can be used for the spray. A good choice for polymers is methanol [18]. The impact with these highly charged microdroplets liquefies and desorbs microscopic regions of the sample surface. As a consequence, analyte ions are ejected from the sample surface, which are then directed towards an atmospheric interface of a mass spectrometer [19].

In DART, a gas such as helium is subjected to an electric discharge, which yields ions and helium atoms at the excited state. The ionic species are removed by a series of filtering electrodes, whereas the stream of excited-state atoms is directed towards the sample, through a path in the outside open space. In this trajectory, the excited species tend to return to their ground state, transferring their excess energy to the molecules present in the atmosphere, and the products of such reactions in turn interact with the sample, desorbing its components and ionising them. The polarity of the electrodes can be tuned in order to favour the formation of positively or negatively charged ions, which are subsequently introduced in a TOF mass analyser [15, 20]. The remarkable feature of DART is the possibility to analyse solid, liquid and gaseous samples, under ambient and solventless conditions. Moreover, the analysis is non-destructive and non-alterative, since it requires no sample preparation (Fig. 5.13).

Laramée and colleagues are probably the most active researchers in reporting the multifarious possible applications of DART [14]. Of particular interest is the possibility to execute DART experiments in which the temperature of the excited gas is varied. Operating at low gas temperature, it is possible to selectively ionise the low molecular weight species present in the formulation of a polymer, such as dyes or plasticisers. A higher gas temperature will make polymer ionisation possible. Figure 5.14 shows a comparison of two different brands of cyanoacrylate glue, showing the potential of this technique for discriminating two materials on the basis of their formulation.

Polymer desorption happens with concurrent fragmentation. This makes DART unsuitable for the measurement of the molecular mass of the sample. However, DART is a very useful technique for the identification of the polymer, since the

Fig. 5.13 Since DART-MS can sample solid material directly under ambient conditions, the sample need only be held with tweezers between the ion source (on *left*, in *blue*), and the cone of the mass spectrometer inlet (*silver cone* on *right*). Reproduced with permission from Ref. [21]. Copyright © 2012 John Wiley & Sons, Ltd

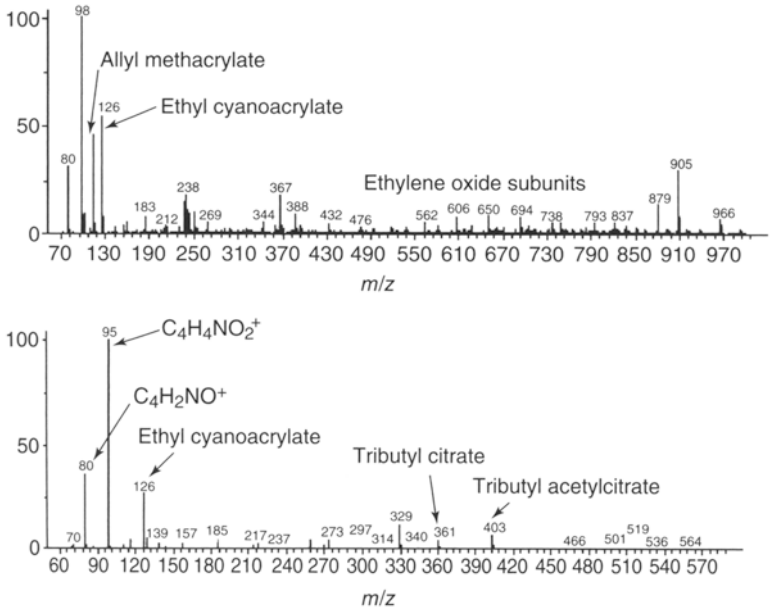


Fig. 5.14 DART mass spectra of two different brands of cyanoacrylate glue. Reprinted with permission from Ref. [14]. Copyright © 2007 John Wiley & Sons, Inc.

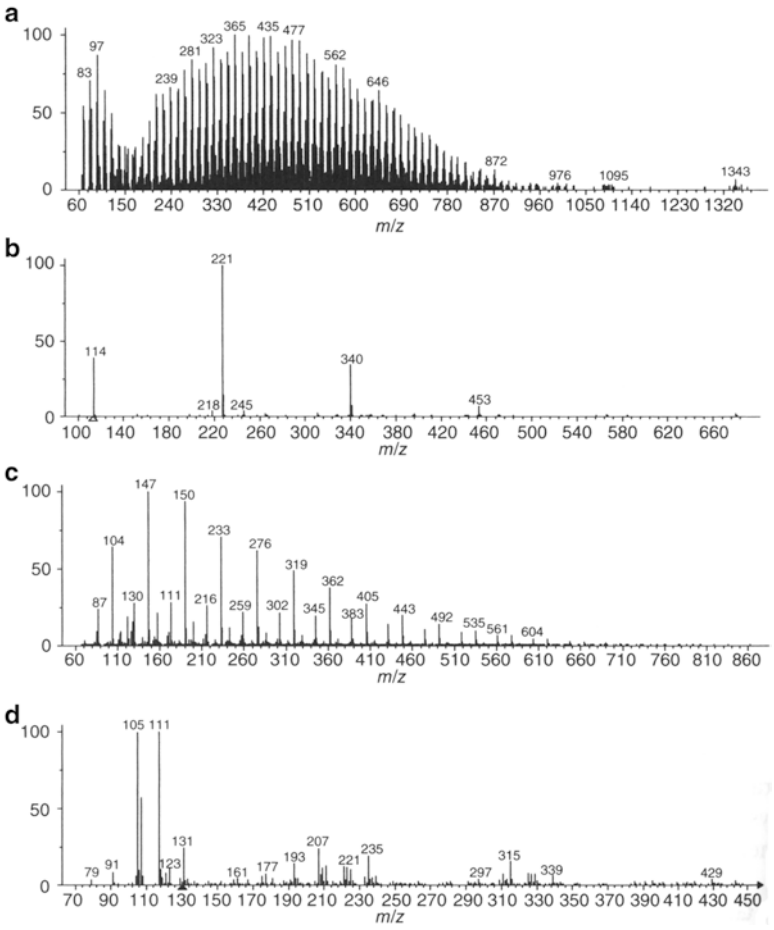


Fig. 5.15 DART mass spectra of polymers: (**a**) low-density polyethylene, (**b**) poly(ε-caprolactam) or nylon 6, (**c**) polyethyleneimine, (**d**) polystyrene divinylbenzene beads. Reprinted with permission from Ref. [14]. Copyright © 2007 John Wiley & Sons, Inc.

fragments will have a similar nature regardless of the size of the originating macromolecule. Figure 5.15 shows that different polymers yield radically different mass spectra.

In DART, fragmentation starts at much lower temperatures than those necessary for pyrolysis so DART-specific mechanisms must be invoked. DART spectra are simpler than those obtained by pyrolysis (Fig. 5.16).

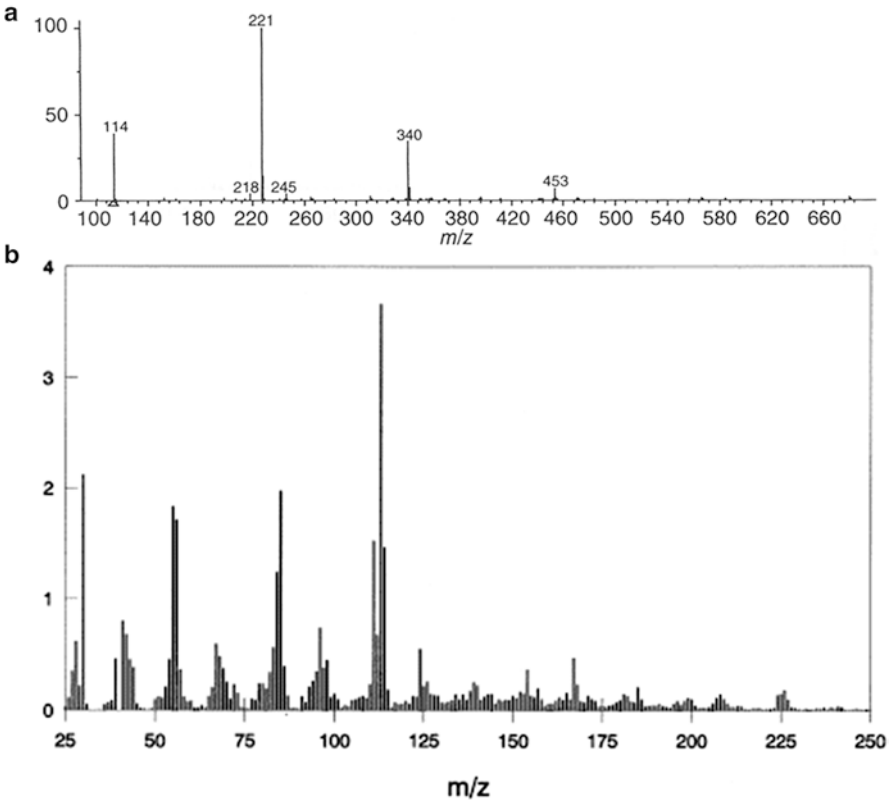


Fig. 5.16 (**a**) DART and (**b**) Pyr-GC-MS spectrum of nylon 6. Reprinted with permission (a) from Ref. [14]. Copyright © 2007 John Wiley & Sons, Inc.; and (**b**) from Ref. [22], copyright 1998 with permission from Elsevier

5.6 The Techniques: Thermogravimetric Analysis

What is thermogravimetry?
Thermogravimetric analysis (TGA) is a thermal analysis technique which monitors the mass of a substance as a function of temperature or time, while the sample is subjected to a controlled temperature program in a controlled atmosphere.

Why use this technique?
TGA is a very simple technique and the interpretation is rather undemanding as well. A TGA thermogram consists in a sequence of steps, corresponding to degradation or desorption processes, which are connected to the evolution of

(continued)

gas species, with the concurrent decrease in the mass of solid sample remaining in the sample holder. The degradation behaviour is strictly dependent on the components of the material, so different thermograms will be obtained from polymeric items with different formulations.

Where can this technique be found?
TGA is quite common in commercial and industrial laboratories because, by the thermal data obtained by this technique the formulation of a material can be assessed. Another reason why TGA is widespread is that it yields information on the mode and conditions of degradation of a material when exposed to heat. This is necessary for assessing the safety of the material and its working range. TGA is almost never applied in forensic science, so probabilities are high that it will not be found in forensic laboratories. Academic research groups in materials science and in polymer science are almost always equipped with TGA apparati. The cost of TGA analyses is on the order of 100€. Common temperature programs (usually a heating ramp at 10 °C/min) are normally suitable for analysing any polymeric or non-polymeric sample. Usually it is not necessary to develop an ad hoc method any time a new kind of sample is analysed.

TGA is a simple and versatile component of the big family of thermal analysis techniques [23, 24]. From an instrumental point of view it is basically a balance in an oven. TGA instruments can have a horizontal or vertical design. In horizontal TGAs there are two arms which terminate with two sample holders, connected to thermocouples. The sample is positioned inside an alumina or platinum crucible, which is in turn placed on one of the sample holders. The other sample holder is occupied by another crucible filled with a suitable standard material, e.g. alumina, or, more commonly, empty. A sliding cylindrical furnace is then moved to include the sample holders, and the temperature program can be started. The vertical design consists of two hooks to which platinum or aluminium sample holders are attached. As before, one of these pans will contain the sample, the other one will be empty or will contain alumina. The hooks are then set down into the furnace. The normal sample size is between 5 and 10 mg, but modern instruments allow to perform reliable measurements on just 1 or 2 mg. This sample size is indeed too large for fibre and most paint casework, but it can be reached in cases involving for example drug packaging or adhesive tapes.

Despite its simplicity, TGA is informative, especially for applicative purposes. Other advantages include the possibility to change the measurement atmosphere (the most common are N_2, which is inert, and air, which is oxidative), the wide temperature range (commercial instruments can easily reach temperatures of 1,000 °C), the relatively low sample size (below 10 mg). As introduced above, knowing how a material degrades is key background knowledge for a wider assessment of its safety,

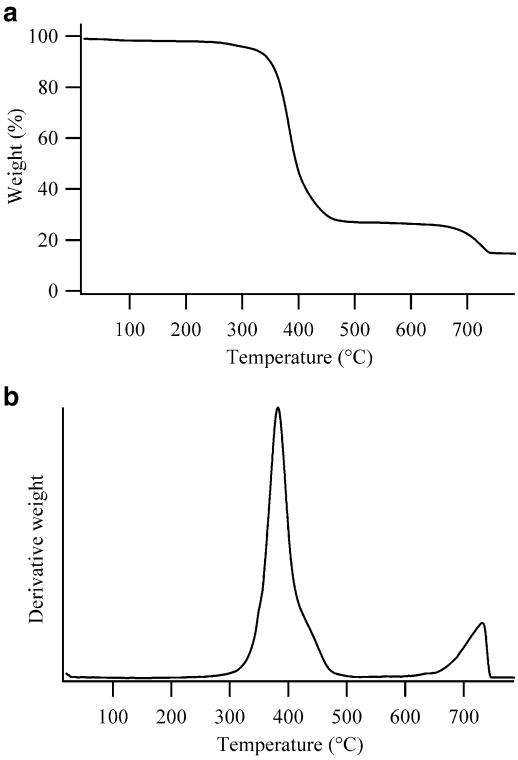


Fig. 5.17 (**a**) TGA thermogram of a rubber sample; (**b**) first derivative curve with respect to temperature of the thermogram in part a

of the optimal parameters for processing and of the acceptable conditions for operation. From a forensic point of view, the degradation behaviour probed by TGA is of interest because it is very dependent on the formulation of the material. The presence of stabilising additives or of flame retardants will increase the degradation temperature and will tend to slow down the degradation rate. A typical TGA thermogram is illustrated in Fig. 5.17. Two modes of representation of TGA thermograms are usually employed. The first consists in plotting the mass as a function of temperature. Usually the ordinate axis is expressed as a percentage of the initial mass of the sample. Figure 5.17b shows the first derivative of the mass with respect to temperature, as a function of temperature. In the derivative curve, the steps of Fig. 5.17a become peaks, and so it becomes easier to quantitatively measure a value of temperature which identifies each degradation step. This representation is very useful for aiding comparisons.

Every step in Fig. 5.17a corresponds to a mass loss. This event can be due to desorption of moisture or of other adsorbed species, or to degradation reactions which yield gaseous products. All these processes bring about a partial volatilisation of the sample. When the gaseous species leave the sample, the mass measured by the instrument decreases and a step is observed in the thermogram. The notable

features in a TGA thermogram are the number of mass loss steps, the entity of mass loss corresponding to each step, the temperatures at which the step starts and finishes, the temperature of maximum rate of mass loss (given by the maxima in the derivative curve) and the residual mass at the end of the experiment. The number of mass loss steps and their characteristic temperatures are related to the degradation mechanism of the material. This is in turn dependent on the type of polymer and on the additives in its formulation. The entity of the steps offers, in some cases, quantitative information on the formulation. When the different steps in the thermogram are due to the degradation reactions of separate components of the material, the mass lost in each step will correspond to the amount of each of these components. Since practically all polymers degrade completely beyond 700–800 °C, when a residue mass resists at the end of a TGA run, the remaining material corresponds to the amount of refractory inorganic filler contained in the material. As may be seen in Fig. 5.17a, filler contents, and therefore mass residues, of the order of 20 % are not unusual. A word of caution is necessary here. TGA is not a selective technique. If the sample introduced in the instrument is contaminated by soil or other inorganic environmental impurities, these will be detected as a mass residue at the end of the analysis. The same is true if the sample is wet. A mass loss around 100 °C will be detected, due to the evaporation of the water contained in the sample. If the hypothesis of such kind of contamination exists, either the sample is thoroughly cleaned and dried, or the amount of contaminant is assessed with other methods, otherwise TGA results will not be reliable enough to take significant conclusions.

Another aspect to consider carefully is the calibration of the instrument. The balance is calibrated by standard weights. The vast majority of modern instruments are simultaneously able to acquire the differential scanning calorimetry signal. In other words, a thermocouple is placed in contact or very close to the sample. Temperature calibration (and the enthalpy change, if desired) can be calibrated using high purity metals as standards.

5.7 The Characterisation of the Matrix

In the investigation following a homicide case, in which the victim was stabbed to death, a knife was recovered in the flat of a suspect. The blade of the knife was burned and cleaned to cancel any biological trace, and in fact no useful DNA evidence could be acquired (Fig. 5.18). However, on the blade itself some fragments of molten polymeric matter were found and retrieved, which, when analysed by IR spectroscopy, showed that they were composed of nylon, spectroscopically indistinguishable from the material forming the outer layer of the windcheater of the victim.

(continued)

Further investigation corroborated the identification of the suspect as the actual murderer.

Another case which shows the power of polymer identification for providing investigative information regarded the theft of copper from electric cables. Between 2010 and 2011, the price of copper skyrocketed, attracting the appetites mainly of Eastern European criminal organisations. During that period, episodes of individuals stealing copper wires and cables from building sites, or cutting electric cables or interrupting the railway supply lines multiplied. On one occasion, in the course of regular controls, two people were questioned, because cutting implements were found in their car. These individuals were not able to explain the reason why they had such materials in their car, so they were connected to some copper thefts that had happened in the region in that period of time. In particular, on a cutting tool found in the car, plastic fragments were found (Fig. 5.19).

Five fragments of polymeric matter were collected, which were compared to the outer insulator layer of the cables from which copper was stolen. The small size, the degree of contamination and the colourless nature of the fragments did not allow to perform a large number of analyses. However, IR spectroscopy confirmed that the composition of the fragments, PVC filled with carbonates, matched that of at least two of the cut cables. This evidence, in addition to close circuit camera footage, allowed investigators to connect the suspects to the alleged theft.

Fig. 5.18 The alleged weapon used for murdering the victim

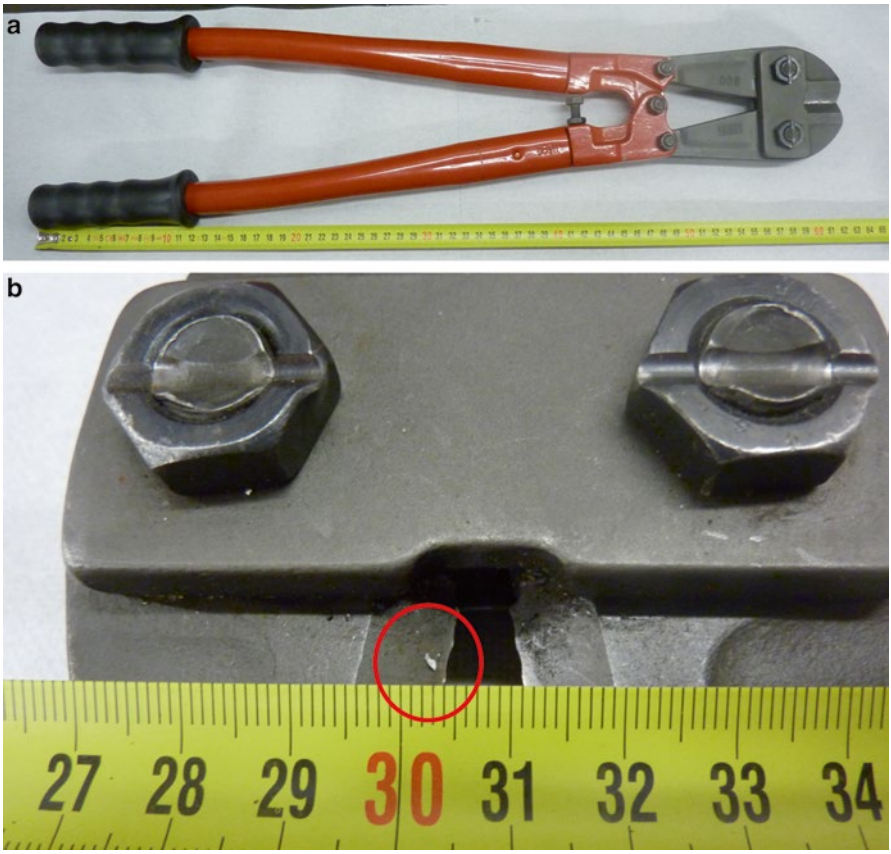


Fig. 5.19 (**a**) One of the implements seized to the suspects. (**b**) Detail of the blades of the cutter shown in part (**a**). The location of one of the plastic fragments is shown by the *circle*

The first step in the characterisation of the formulation of a polymeric item is without any doubt the identification of the polymer matrix. To this aim, IR spectroscopy with attenuated total reflection acquisition is unsurpassed because it is a rapid, non-destructive and inexpensive technique. The analysis can be extended to different levels of investigation. In this section, focus will be posed on the identification step, whereas in the following chapters information will be given on the potential of IR spectroscopy for elucidating structural and morphological details related to the processing or synthesis steps.

The fundamental feature of IR spectroscopy which is exploited in the identification of polymers is that each substance, due to its unique chemical structure, has a unique spectrum which is different to that of any other substance. This characteristic is so significant that a particular region of the IR spectrum is called 'fingerprint region' due to its ability to differentiate each substance in the huge population of chemical compounds.

Sometimes substances with a similar structure exhibit spectra differing just by subtle spectral details. This prevents in most actual casework, for example, to distinguish the different types of nylon.

Figure 5.20 shows the signals which in principle could be used for distinguishing the different kinds of nylon. These differences are so subtle that rarely a positive attribution can be made.

Another example of how polymers of the same class can appear similar is the case of biodegradable polymers. Although still quite uncommon, these materials are increasingly widening their range of application, and they will be likely encoun-

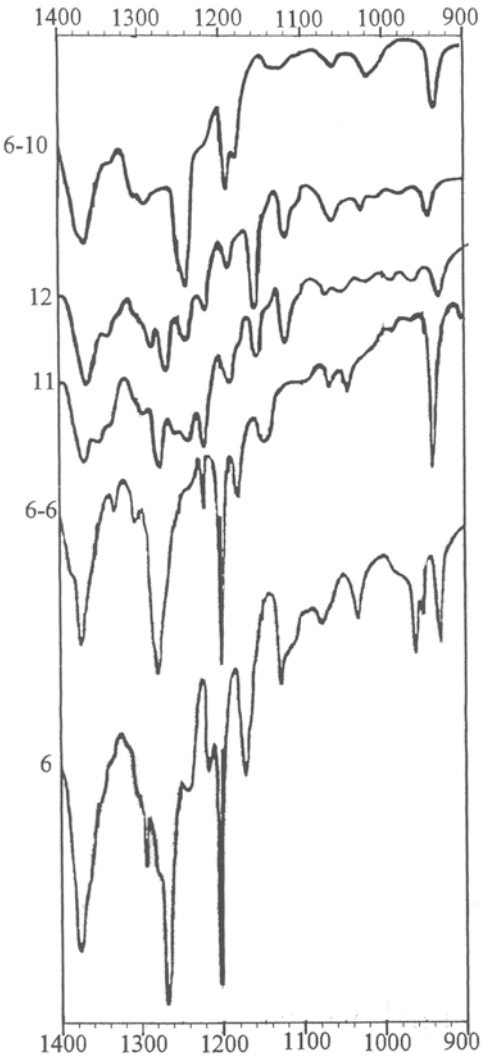


Fig. 5.20 IR spectra of (from *top* to *bottom*) nylon 6-10, nylon 12, nylon 11, nylon 6-6 and nylon 6

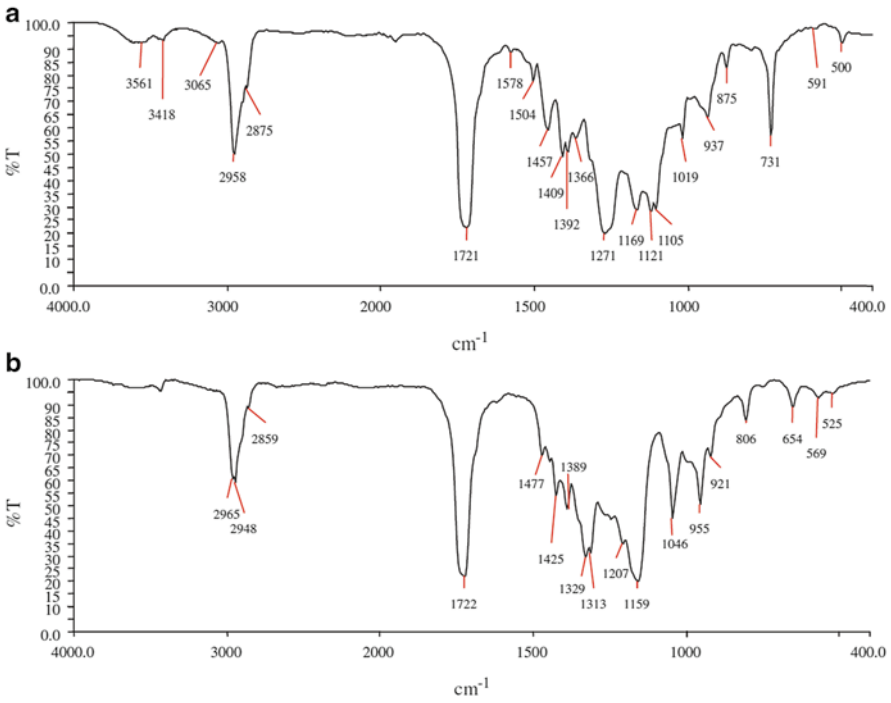


Fig. 5.21 IR spectra of (**a**) poly (butylene adipate-co-terephthalate) and of (**b**) poly (butylene succinate). Reprinted from Ref. [25] with kind permission from Springer Science + Business Media

tered more and more in daily life. Cai et al. [25, 26] recently discussed the issue of spectral characterisation of the biodegradable polyesters poly (lactic acid), poly (butylene adipate-*co*-terephthalate), poly (hydroxybutyrate-*co*-hydroxyvalerate), poly (butylene succinate) and poly(ethylene terephthalate).

Figure 5.21 compares for example the spectra of poly (butylene adipate-*co*-terephthalate) (PBAT) and of poly (butylene succinate) (PBS). The shape of the spectra can appear, at a first superficial glance, similar, but the absorptions of these compounds are indeed different, in particular in the spectral range between 1,000 and 1,500 cm^{-1}.

The spectra shown in Fig. 5.21 were obtained on pure samples, without any additive or dye. However, in the analysis of a real sample additional peaks could be introduced in those spectra, due to the other components of the formulation, or due to contaminants which were gathered from the exposure to the crime scene or to the usage of the source item. Many inorganic materials, such as commonly used fillers or minerals found in soil, display strong absorptions right in the region between 1,000 and 1,500 cm^{-1} which is the most important for the discrimination of these polymers.

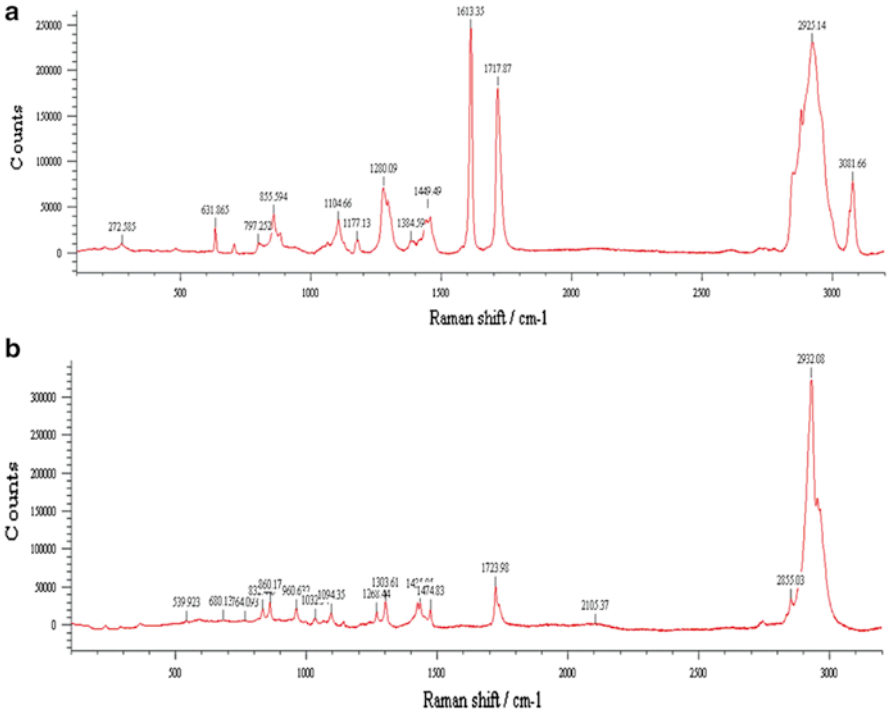


Fig. 5.22 Raman spectra of (**a**) poly (butylene adipate-*co*-terephthalate) and of (**b**) poly (butylene succinate). Reprinted from Ref. [25] with kind permission from Springer Science + Business Media

Of course, the quality of a spectrum depends on the conditions of the analysed specimen: any contamination is detected as additional signals, and any degradation or reaction that happened while the item was on the crime scene is reflected by a modification of the spectrum. Care must therefore be taken, because IR spectra strictly depend on the chemical history experienced by the material.

In other words, the quite easy task of the identification of two polymers with similar appearance, properties and application, but with different absorptions in their IR spectrum, can be complicated by the circumstances of casework. In cases like these, when the identification is not straightforward, Raman is of great help, because it offers complementary information, yet at the same time focusing on the same features of the molecule, i.e. on its vibrational transitions. Raman is much more efficient in discriminating the polymers cited above: the spectra in Fig. 5.22 are, since the first glance, starkly different.

Figure 5.21 introduces another issue. A tool of considerable help in the qualitative analysis by IR spectroscopy is given by commercially available databases, which compare the spectrum of the unknown compound with large collections of spectral data, providing a short list of candidate substances. These instruments

should be used with care, though, and they should not be considered as a system to automatically identify a material. The short list indicated by the software must be interpreted by an expert analyst, to verify if the proposed options are indeed acceptable. Databases do not obviously contain all the known chemical species, and it can happen that some materials were not included in the collection of spectra. In such cases, the output of the search could be a species with a spectrum similar to that of the unknown sample. If one used a database containing just PBS and not PBAT to identify a PBAT sample, chances are high that among the hypotheses with the highest score the software would propose PBS. If the analyst does not carefully compare the unknown spectrum and the proposed hypotheses, misattributions are likely. The problem of completeness is a common one to all databases. Even though the population of commodity polymers does not change much, specialty materials are from time to time introduced in the market. A forensic scientist working in trace analysis should always be updated on the commercialisation of new materials, in the diffusion of known polymers and on the phase out of old products. When possible, physical collections of the materials should be built and the databases should be updated with data on such new substances.

IR spectroscopy is the method of choice for the identification of the chemical nature of single fibres, as proved by decades of experiences in the laboratory and in casework [27–31].

However, the amount of information that can be acquired varies greatly. IR spectroscopy does not differentiate among cellulosic fibres, such as cotton, rayon, jute or flax, and it is much more useful in the analysis of synthetic fibres. For example, in the case of acrylic fibres, data on the composition of their macromolecular chains and also on the process of their manufacture can be acquired (Sect. 6.2). Another example of the power of this analytical approach was given by Grieve and Griffin on modacrylic fibres [28]. Both acrylic and modacrylic fibres are copolymers, usually with just another comonomer, but sometimes even terpolimers are made, of acrylic acid. Acrylic fibres contain at least 85 % of acrylic acid repeat units, whereas modacrylics have an acrylic acid content below 85 %. These authors reported and detailed, presenting spectral data on more than 80 samples from 15 trade names, how IR spectroscopy, coupled with microscopy, can provide information about the comonomer, termonomers added to produced dye sites, the presence of solvent residue, dyes, and additives, helping to distinguish modacrylic from acrylics, a somewhat challenging problem due to the similarities existing between these classes of fibres. It should be stressed one more time that being able to correctly classify fibres in a group is fundamental for assessing their evidential value. Modacrylics are much rarer than acrylics, and therefore their evidential value is extremely large.

IR spectroscopy, and in particular IR microspectrophotometry, are an indispensable step for the analysis of paints [32–35].

Ryland proposed a very useful flowchart for the identification of the type of binder [36].

As described in Sect. 4.2, paints rarely consist of a single coating, but especially automotive paints are composed of several stacked layers, each one with its own functional role. A significant disadvantage of traditional IR spectroscopy applied to paint is that, if it is applied to a paint chip, it will provide a superposition of all the layers simultaneously, giving a complex spectra, very difficult to interpret. This problem can of course be overcome by using an IR microspectrophotometer, which allows to analyse each layer individually, mapping the composition of a paint chip along its whole thickness. By combining IR microspectrophotometry with a motorised sample stage, IR mapping (Sect. 5.10) can be performed, obtaining very effective chemical images of the layers in the paint chip [37].

Paint smears are challenging samples to analyse. Since smears involve the surface of an object or of a substrate, the sampling method of choice is attenuated total reflection (ATR). However, since the thickness of smears is often lower than the depth of penetration of the IR beam through the surface of the sample, their IR spectrum is often contaminated by the support material, introducing difficulties in the interpretation. In principle, this problem could be circumvented by taking a spectrum of a clean region of the support material, and then subtracting this from the spectrum of the smeared support. In practice, this is not so straightforward. A number of problems arise when a simple spectral subtraction is attempted [38]. The first one is that the sample quantity is unknown and usually small with respect to the support. This determines a very large absorption of the base, compared to that of the sample. When such large absorption is subtracted from the spectrum of the sample transferred on the surface, actually serious oversubtraction happens, which in turn brings about the loss of relevant spectral information on the sample. Another risk factor is due to the fact that the measure and the collection of the spectrum of the sample does not happen in the same conditions as those of the base spectrum. Heterogeneities both in the surface and in the smear do not allow to easily obtain a suitable blank spectrum, causing many problems in subtraction procedure.

The conclusion is that the mathematical approach separating the paint and base spectra effectively has to include some additional procedures in addition to the simple subtraction process. Szafarska and colleagues [38] tackled this problem proposing a subtraction and normalisation procedure which allowed to extract mathematically the pure paint spectrum from the spectrum of a smeared surface. Figure 5.23 shows the remarkable performance of this treatment, which is able to extract a spectrum of paint eliminating the interference of the support, in this case, cotton fabric.

The obvious advantage of procedures like this is related to the collection of the IR spectrum of the smear, depurated from the absorptions due to its substrate. Moreover, algorithms like those proposed by Szafarska and colleagues [38] limit the subjectivity on the treatment of data, leading to less challengeable results.

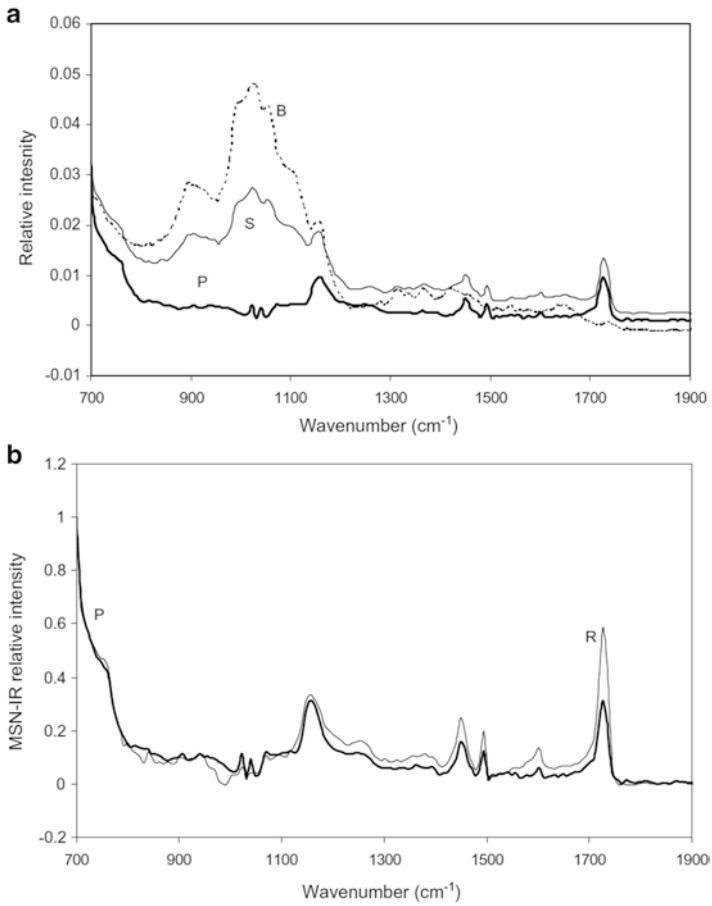


Fig. 5.23 ATR spectra: (**a**) of pure SparVar paint (*P*), of the base (cotton fabric) (*B*), of SparVar paint on the cotton fabric (*S*), and (**b**) of pure SparVar paint normalised (*P*), obtained by the MSN-IR method (*R*). Reprinted from Ref. [38], copyright 2009, with permission from Elsevier

5.8 The Characterisation of the Fillers

Plastic bags or cling films, commonly used for the wrapping of drugs of abuse, are polymeric pieces of evidence that are occasionally analysed by the forensic scientist. As indicated in Fig. 5.1, the first step in the analysis of these items is IR spectroscopy, for identifying of which polymer they are composed. These films are a good example of the importance of evaluating the extent of chemical variation in a relevant reference population prior to undertaking casework. This is obviously fundamental for assessing the evidential value of a match between a known and a questioned piece of cling film. The variability in polymer composition and in the formulation of plastic items is extremely dependent on the geographical context

relevant for casework. For example, a study was conducted in Malaysia in 2011 on the packaging of 311 heroin seizures [39]. Ninety-three percent of the films were made of polypropylene, 5 % of unfilled polyethylene, and the rest of variously formulated polyethylene or polypropylene. In Malaysia, heroin packaged in a polyethylene film filled with calcium carbonate is an extremely rare item, which in turn has a very high evidential value attached.

The European situation is the opposite. In this geographical context, most of the plastic packaging film is manufactured with polyethylene, which is therefore the most common material encountered in casework on illicit drug packaging.

Holman and colleagues [40] recently reported an investigation aimed at identifying the level of chemical variation both within and between a population of samples. They examined three samples of cling film, acquired in supermarkets in the United Kingdom. They focused their attention on the spectral region between 1,560 and 1,200 cm^{-1}, deconvoluting and fitting the weak and overlapping absorption bands in this region. The outcome of such treatment was that, in the sample population identified by the authors, no between-sample chemical variation could be found. Moreover, they found that intrasample chemical variation was of the same order of magnitude of the intersample variability. It is interesting to note that the materials examined by these authors consisted of practically unfilled, pure polyethylene. The IR spectra of the cling films analysed were those of polyethylene, with no detectable extra absorption.

Another study, carried out on a population of 50 plastic bags gathered in the North-East of Italy [41], showed, on the contrary, that in that geographical region, IR spectroscopy was a suitable method for discriminating such items. Also in this case the polymeric matrix of all the items in the population was polyethylene. However, in some films the presence of inorganic fillers enriched the polyethylene spectrum with additional peaks. Figure 5.24 shows that the relative quantities of

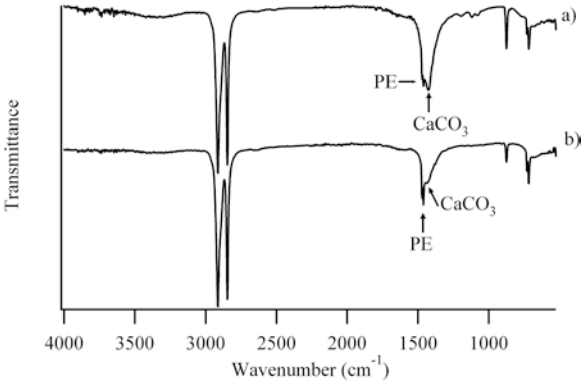


Fig. 5.24 IR spectrum of two different plastic bags. *Arrows* show the characteristic peaks of PE and calcium carbonate useful for differentiation, according to the different relative intensity between polyethylene peaks at 1,461 and 1,471 cm^{-1} and that of calcium carbonate at 1,430 cm^{-1}. Reprinted from Ref. [41], copyright 2006, with permission from Elsevier

matrix and filler could be assessed on the basis of the relative intensities of the absorption peaks related to these components.

The discriminating power (DP) (Sect. 3.2) found in this study was 0.60, meaning that 60 % of the possible pairs of samples could be distinguished by IR spectroscopy alone. This DP is lower than that reported by Roux et al. [42], who studied samples coming from Australia and Asia, though.

In fields of application where just one type of matrix is mainly employed, the characterisation of the material must be more deeply investigated. The second most abundant component of the formulation of a typical polymeric item is the filler, which can be present in amounts reaching 50 %. As seen in the discussion above, this large concentration allows to detect absorptions due to the filler in addition to those due to the matrix polymer.

Paper is another example of this instance. Of course, the main component of paper is cellulose, so an analysis of this material by IR spectroscopy should not focus on the signals due to this base polymer, but rather on the fillers used by the pulp and paper industry. Calcium carbonate is a particularly common filler added to office paper, and it significantly contributes to the IR spectrum of paper.

Figure 5.25 compares the IR spectra of plain cellulose, of pure $CaCO_3$ and of a typical sheet of office paper. The latter is the sum of the two former components, and so the relative contents of cellulose and filler can be quantified according to the relative intensity of their characteristic signals [43]. In selecting such peaks, care should be taken that they do not overlap with other absorptions or with each other. As a measure of cellulose content, the intensity of the bands between 915 and 1,200 cm^{-1} can be integrated (A^{Cell}). The amount of $CaCO_3$ can be quantified by integrating the area $A_{880}^{CaCO_3}$ of the peak at 880 cm^{-1} (symmetric C–O stretching mode) and the area $A_{1420}^{CaCO_3}$ of the peak at 1,420 cm^{-1} (asymmetric C–O stretching mode). A ratio can then be computed dividing the sum of the peak areas due to $CaCO_3$ by the area of the cellulose, i.e.:

$$R = \frac{A_{880}^{CaCO_3} + A_{1420}^{CaCO_3}}{A^{Cell}}$$

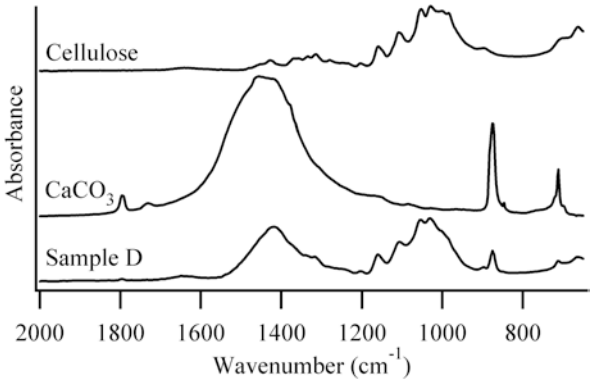


Fig. 5.25 IR spectra of an office paper sample, of cellulose and of $CaCO_3$. Reprinted from Ref. [43], copyright 2010, with permission from Elsevier

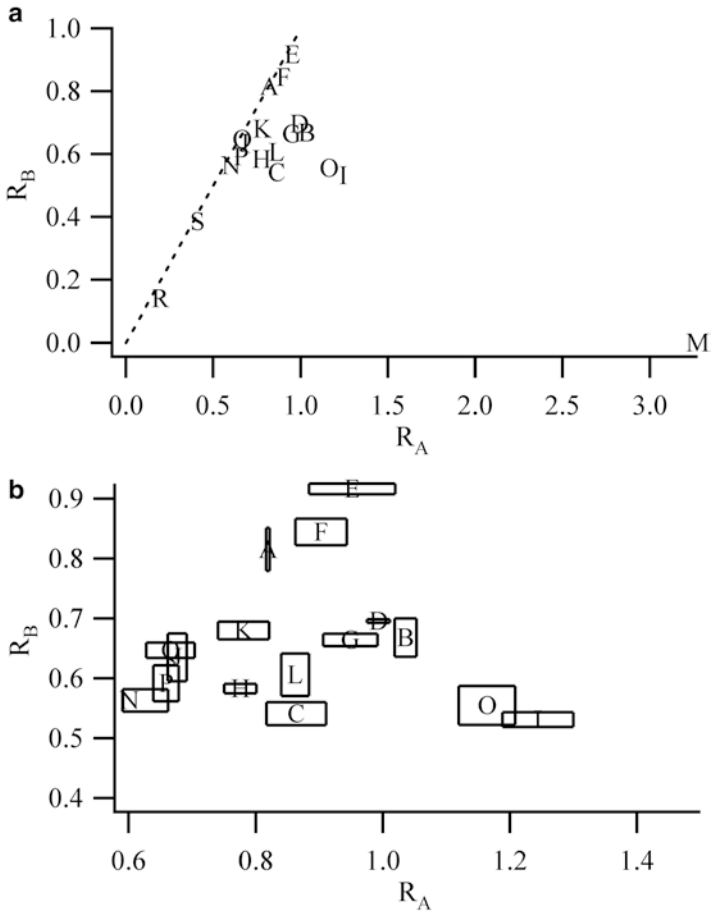


Fig. 5.26 Plot of R_B vs. R_A for (**a**) 19 paper samples. Part (**b**) is a magnified view of the cluster where most of the samples are concentrated. The *dotted line* is a line of unit slope, intended to show the behaviour of sheets with the same R on both sides. R_A and R_B are the ratios R, described in the text, calculated on one side and on the other side of the sheets of each sample. Reprinted from Ref. [43], copyright 2010, with permission from Elsevier

The parameter R was calculated for a population of 19 samples of office paper, coming from different manufacturers [43]. The distribution of the filler was assessed by repeating the measurement of R on both sides of the paper sheets considered in that work. The results, shown in Fig. 5.26, indicated that such quantity varies between different samples, reflecting the fact that different manufacturers use different amounts of filler. In addition, Fig. 5.26 shows that the filler is not homogeneously dispersed in the whole paper sheet, but it is unevenly accumulated on one side rather than on the other. This is probably due to the effect of the process of production of the paper, and it is indeed a further element of analytical discrimination.

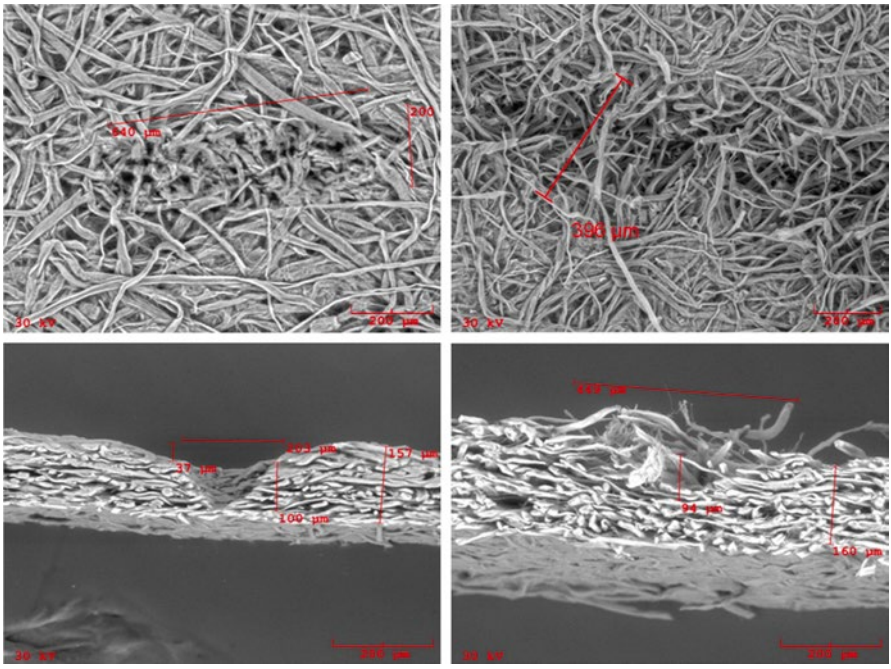


Fig. 5.27 SEM image of the ablation crater on a paper document by LA-ICP-MS (*left*) and LIBS (*right*), 200× magnifications. The *top panel* shows a view from the *top*, the *bottom panel* shows the cross-section of the sheet. Reprinted from Ref. [44], copyright 2010, with permission from Elsevier

Since fillers are inorganic, the technique which most logically lends itself to their characterisation is atomic spectroscopy [44–46]. Trejos and colleagues published a paper in 2010 in which they used, for the characterisation of a collection of papers and inks, non-destructive sampling techniques such as Laser Induced Breakdown Spectroscopy and Laser Ablation ICP-MS [44]. Non-destructivity is, in the questioned document field, an issue even more stringent than in other branches of forensic science. The integrity of a document is in fact connected, in many legal systems, to its validity, so it is desirable that examination alters its ink and paper as little as possible. Laser ablation and LIBS allow to sample portions of paper and ink suitable for obtaining significant elemental analyses, but from a very small region of sample, without macroscopically altering the aspect of the document. Figure 5.27 reports SEM pictures which show that the crater produced by the incoming laser beam both in laser ablation and in LIBS are negligible.

The preliminary assessment which must be done in any elemental analysis procedure is the choice of the elements to consider as good descriptors of the sample. Trejos and coworkers formalised such requirements [44]: these elements must be quantified with a good precision between replicates, they must have a homogeneous distribution within the samples, they must be present above the limit of detection in the sample population, they must be detectable with a low chance of false positives

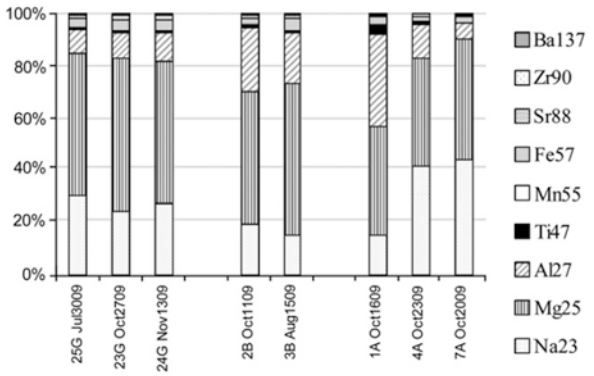


Fig. 5.28 Normalised elemental profile from document paper collected from three different brands, processed in different mills and production batches. Reprinted from Ref. [44], copyright 2010, with permission from Elsevier

and false negatives, and finally their intrasample variability must be significantly smaller than their intersample variability. According to the rules, the authors identified the following elements as good discriminators for paper by LA-ICP-MS analysis: ^{23}Na, ^{24,25}Mg, ^{27}Al, ^{47}Ti, ^{55}Mn, ^{57}Fe, ^{63,65}Cu, ^{64,66}Zn, ^{88}Sr, ^{90}Zr and ^{137}Ba. As an internal standard, which is necessary in laser ablation analyses for correcting for differences in mass ablated between replicates, a low abundance carbon isotope was selected (^{13}C, 1.1 %). LIBS gave results with a lower repeatability and so just five elements could be quantified with acceptable precision: Na, Mg, Al, Ca and Sr.

Figure 5.28 shows the elemental profile of different batches of paper. The three columns on the left are related to papers produced in the same mill at different times, and significant differences in composition can be found between samples manufactured 3 months apart. The two samples represented by the central bars in Fig. 5.28 are batches which were manufactured by the same company, in the same month/year, using similar pulp and raw materials but processed at different mills. The differences in composition are evident. Finally, differences in the elemental profile were observed from samples that were manufactured in the same mill, produced just days apart (columns on the right of Fig. 5.28), because of a change, in that short period, of the raw material with the introduction of different quantities of recycled material in the pulp.

An alternative to the use of these elemental analysis techniques for the assessment of the fillers can be X-ray diffraction (XRD) [43, 47]. This non-destructive method, which will be discussed more in detail in Sect. 7.2.1, is sensitive to the presence of crystalline material within the sample. Since fillers are inorganic species which most commonly have a crystalline form, they will yield diffraction signals which will be separated and easily distinguishable from the major reflections of cellulose, the principal component of paper. The relatively large amount of fillers within polymeric items are often above the detection limit of XRD. Figures 5.29 and 5.30 report two examples of the potential of XRD in revealing the presence of inorganic crystalline fillers in a polymeric matrix. In some cases, if the intensity of the diffraction signals is high enough and if proper databases are available, the nature of the fillers can be also identified. However, when several fillers are simultaneously

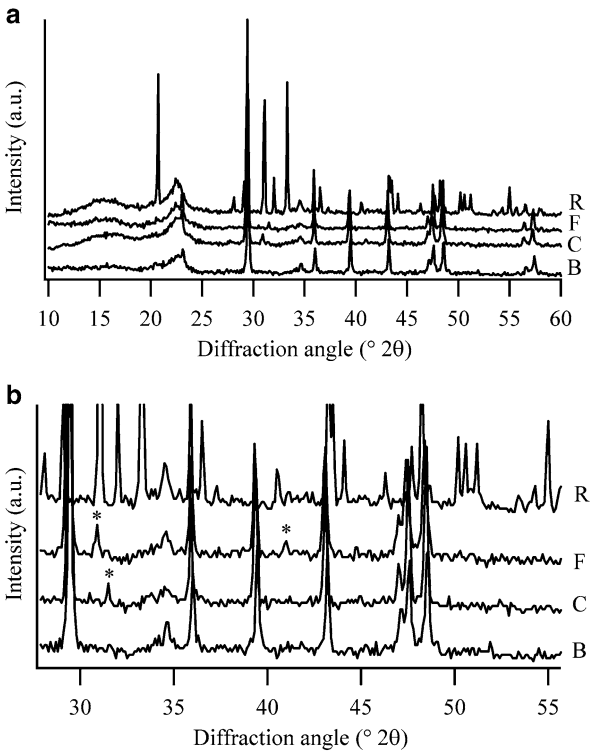


Fig. 5.29 XRD spectra of different paper samples (**a**) in the whole angular range and (**b**) magnifying the region of the inorganic peaks. Minor peaks useful for differentiation of the samples are indicated by *asterisk*. All the samples were mounted in the perpendicular direction. Reprinted from Ref. [43], copyright 2010, with permission from Elsevier

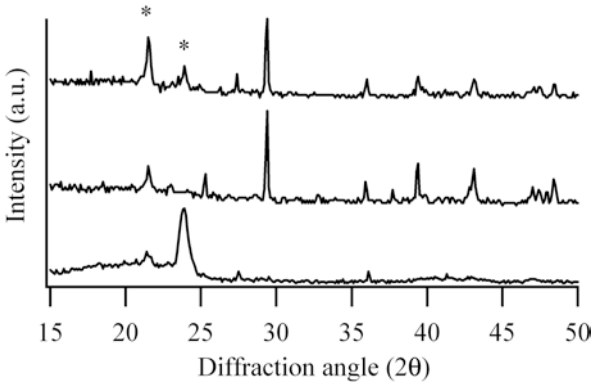


Fig. 5.30 XRD spectra of different plastic bags. The *asterisks* indicate the position of the reflections due to the polyethylene matrix. The other peaks are due to inorganic fillers

present in the formulation, it may become difficult to separate the signals due to the different crystals, and so the identification can be a complex, if not impossible task. A positive identification of the additives, though, is rarely a necessity, since most of the times analyses are comparative. A difference in the diffraction profile reflects a difference in the formulation (Fig. 5.31).

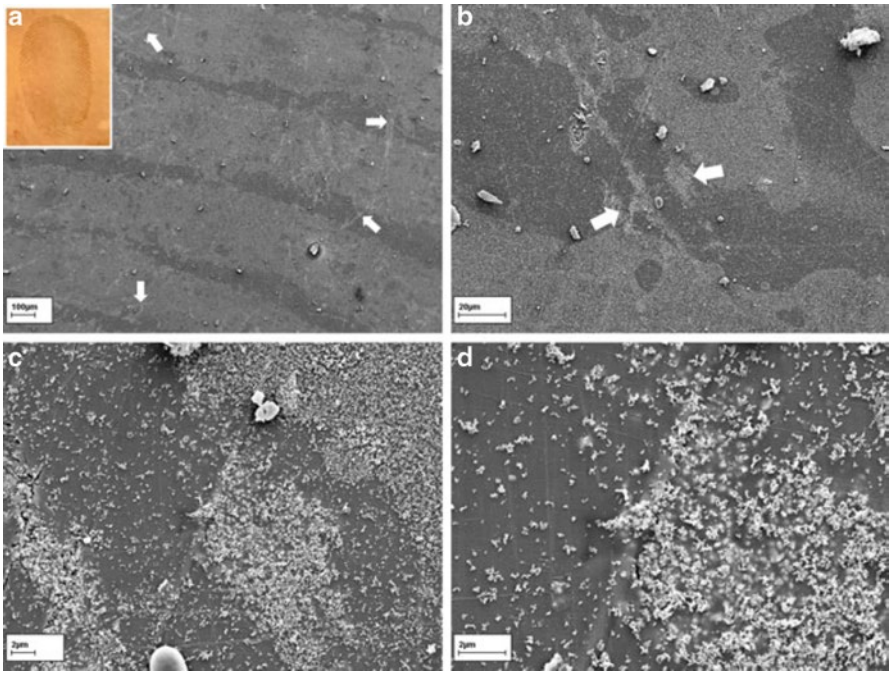


Fig. 5.31 (**a**) Low magnification SEM image of four fingerprint ridges developed with carbon powder suspension on a Formica surface. White patches of overdevelopment are visible on and off ridge. (**b**) Increased magnification SEM image from the centre of (**a**). (**c**, **d**) Two more increases in magnification, revealing a feature within the Formica surface and its association with overdevelopment. Reprinted with permission from Ref. [48]. Copyright © 2013 American Academy of Forensic Sciences

5.9 The Characterisation of Dyes and Pigments

As mentioned in the introduction to this chapter, dyes and pigments are almost invariably employed by the polymer industry to improve the aesthetic properties of these materials.

Pigment characterisation is not necessarily always aimed at the elucidation of the formulation of an unknown item. In a forensic context, knowing how inorganic species are distributed on a surface can also be of interest for optimising fingermark development. Bacon and coworkers recently reported that the efficiency of carbon powder suspension development of fingerprints on polymers is detrimentally affected by the presence of a common white pigment, titanium dioxide [48].

Scanning electron microscopy is in this case the best method for preliminarily characterising the surface where the fingermarks must be researched, in order to assess the best method of development as a function of the detected contaminants [48–50].

The difference between dyes and pigments is based upon their solubility within the polymer matrix. Dyes are soluble in the matrix, whereas pigments are not only

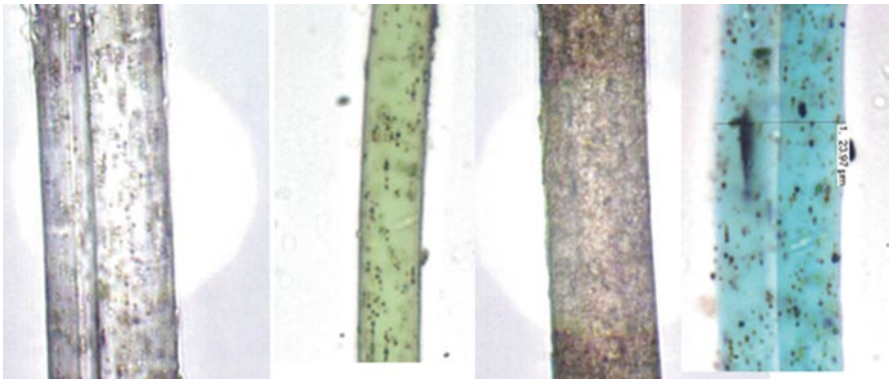


Fig. 5.32 Polyester fibres with different amounts of TiO_2 delusterant

insoluble in the polymer matrix, but also they are difficult to solubilise even in the most common solvents. This dictates the analytical strategy to be chosen in their characterisation.

The different materials used for colouring polymeric items can sometimes be distinguished by a mere observation under an optical microscope. Figure 4.12 shows two foam fragments, one coloured with pigments, and the other with dyes. In the pigmented material, discrete particles can be individuated, whereas the dyed one is evenly coloured, without observable heterogeneities.

Not only the presence or absence of pigments can be detected by optical microscopy, but also the different amounts of pigments can be assessed. Figure 5.32 compares four polyester fibres containing different amounts of TiO_2, a white pigment used as a delusterant.

A fascinating and still underinvestigated approach for the investigation of inorganic additives in polymers is the use of cathodoluminescence (CL). CL consists in the emission of visible radiation from a sample that has been impinged by an electron beam. This technique is not new–its discovery dates back to 1879–and it is routinely applied in geology for provenance studies based on the content of trace elements or compounds, and in physics and material science to detect the presence of inorganic contaminants. Palenik and Buscaglia offered an excellent review of the potential of CL in which every instrumental and technical detail is well described [51]. The electron beam can be produced by a cold cathode, which avoids the necessity of coating the sample with a conductive layer, and allows operation in low vacuum with a beam of large dimension, up to 2.5 cm. The drawback is that the obtained cathodoluminescence is low in intensity and quantitative measurements are not very reliable. Using a hot cathode source, such limitations are overcome and higher resolution is possible by a finer beam, but coating of the sample and high vacuum are necessary. CL is a surface technique, therefore, if information on the bulk of the material is desired, a cross-section should be prepared. CL has been shown to be extremely effective in the comparison of paint and duct tape (Fig. 5.33) [51].

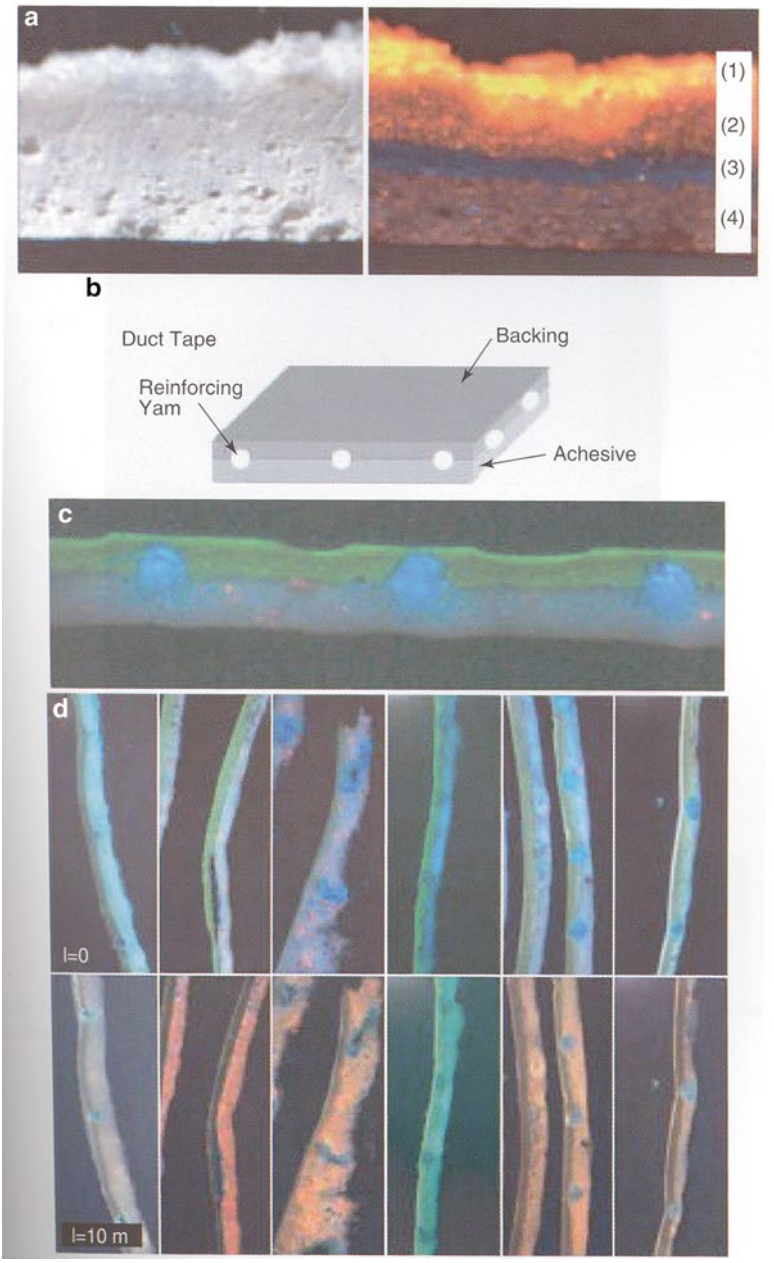


Fig. 5.33 Luminescence of paint and duct tape. (**a**) By reflected light (*left*) a paint chip appears homogeneous, whereas CL shows the presence of four layers differing by inorganic content. (**b**) Schematic of duct tape construction and (**c**) a CL image of duct tape, which shows that each layer contains a luminescent component. (**d**) Six duct tapes can be differentiated by CL, the *top row* shows CL immediately after exposure to the electron beam, the *lower row* shows the same samples after 10 min of electron beam exposure. Reprinted from Ref. [51].

The advantage of CL is that of complementing elemental analysis with information on the phase of the minerals present in the sample. For example, the two phases of $CaCO_3$, calcite and aragonite, luminesce orange–red and yellow–green, respectively. Trace elements such as Mn or Fe, which can be related to the geologic origin of the mineral, or can derive from its anthropogenic synthesis, further modify the CL spectrum [51]. In case of complex samples it can be very hard to identify the various phases of the mineral additives by traditional methods such as X-ray diffraction. Associating CL with elemental analysis can be a much more immediate approach, also improved by the possibility to visualise the dispersion and location of the fillers.

Dyes can be extracted and then separately analysed, mostly by chromatographic techniques. On the contrary, the extraction of pigments is much more complex to achieve, and an in situ characterisation must be preferred.

The most obvious approach for analysing how a polymer item was coloured, is actually the spectroscopic characterisation of its colour. This is a relatively easy task for samples with a macroscopic size, whereas it can become a problem for tiny specimens such as single fibres or the individual layers of a paint chip. In these cases, the use of a microspectrophotometer is mandatory. The power of spectrophotometry is its ability to quantify colour, making it an objective quantity, as opposed to subjective verbal definitions.

Figure 5.34 illustrates a comparison of four different ballpoint black inks. Ink is, after all, a dye which is absorbed by the cellulose fibres of paper. A sheet of paper with handwriting is therefore a, maybe unusual, example of dyed polymer. All the inks whose spectrum is shown in Fig. 5.34 look indistinguishable by the naked eye, but UV–visible spectroscopy allows to catch the subtle differences in their colour.

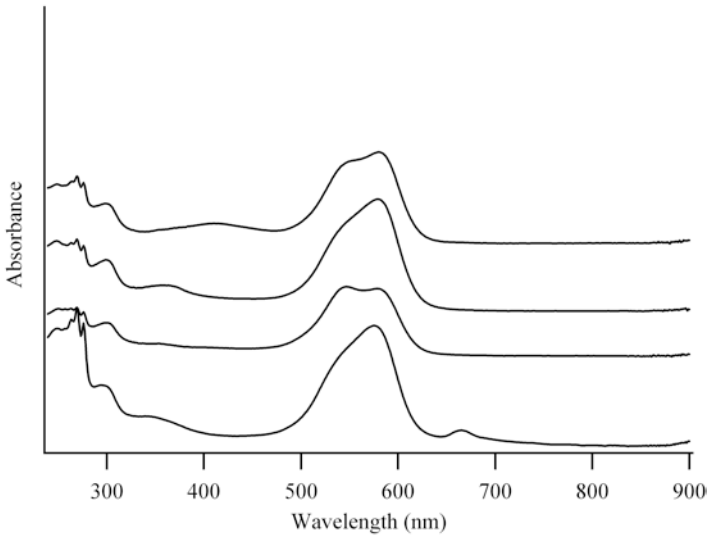


Fig. 5.34 UV/VIS absorbance spectra of four ballpoint black ink samples

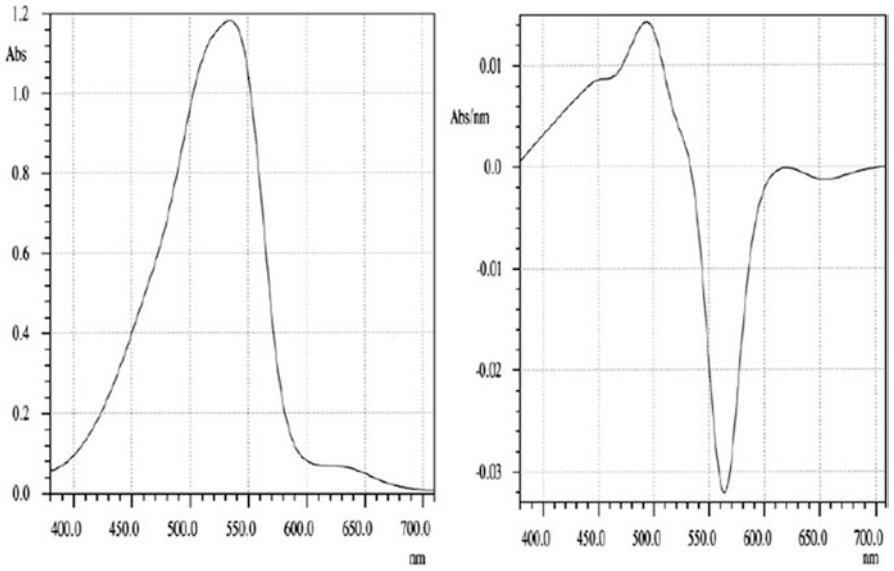


Fig. 5.35 Absorbance spectrum (*left*) and associated first derivative spectrum (*right*) of a red acrylic fibre. Reproduced from [53], copyright 2007, with permission from Forensic Science Society

Figure 5.34 also suggests opportunities to further quantify spectral data. In many instances, to render a particular hue, mixtures of dyes or of pigments are used in the industry. In such instances the UV–visible spectrum is the superposition of the absorptions due to each component of this mixture. This is particularly true for inks. Most probably the double peak between 500 and 600 of Fig. 5.34 is due to the co-presence of different coloured species in the same ink. Deconvoluting such absorption with two Gaussian or Lorentzian functions allows to quantify the relative amounts of such individual components [52].

When absorption spectra are very simple, their comparison becomes difficult, because of the lack of enough suitable elements of characterisation such as troughs, shoulders or peaks. In these cases, use of the first derivative of the absorption spectrum can be a feasible approach [53, 54]. Figure 5.35 shows how a spectrum can change when its first derivative is computed. The original absorbance spectrum shows just a peak maximum at around 535 nm, with a further small peak at about 630 nm. The first derivative plot, on the contrary, is very rich in details, with four maxima and minima whose position can be quantified, with the consequence of a more distinctive pattern.

Even though this approach can be indeed be useful, as demonstrated by Parsons and colleagues on polyurethane foams [54], caution should be exercised. Wiggins and colleagues, working on fibres, warned that the first derivative spectra could show differences even between items that match. This happens when the absorbance spectra of the compared items have a significant intrasample variation, due for

example to an uneven colouring or to a gradient in the colouration. These differences in colour between regions of the same sample translate into large differences in the first derivative spectra, and so in erroneous discriminations. This can be avoided by taking many replicates in different areas of the items, and considering always first derivative spectra together with the original absorption spectra, to assess the homogeneity of spectral data.

The extension of spectroscopic data outside the visible region can be especially significant for non coloured items. For example, in white objects very often optical brighteners are used, which absorb light in the UV range, with a concurrent re-emission of blue visible radiation. This cancels possible yellowing effects and allows to obtain an off-white hue [55, 56]. The presence of these additives is a good reason for extending spectroscopy to the UV frequencies.

Some spectrometers allow one to probe also the NIR spectral region. Even though the NIR region is less informative than the UV–visible range, the NIR can provide some useful data [57, 58]. For example, it was shown that, measuring the average reflectivity of paper in the range 680–900 nm, data useful for the comparison of office paper sheets could be obtained (Fig. 5.36) [57]. The average reflectivity was assessed in the range of the visible red and NIR because in that range the spectrum was featureless and so it was possible to measure a reproducible mean value.

Another spectroscopic approach to the characterisation of dyes and pigments exploits IR spectroscopy. Grieve and coworkers in 1998, for example, published a very complete paper on the IR identification of the characteristic dye absorption peaks found in the FTIR spectra of coloured acrylic fibres [31]. The amount of dye in the formulation of a polymeric item is very low, at most few percent. As a consequence,

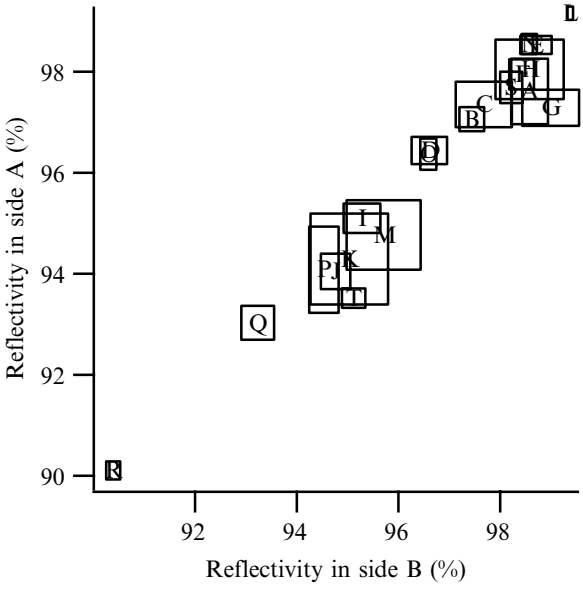


Fig. 5.36 Average reflectance measured on one side of the sheets of paper vs. average reflectance measured on the other side of the sheets of paper for a population of 20 reams of white, 80 g/m^2 office paper from different manufacturers, randomly collected from stationery shops in Italy. The reflectance values plotted are average values measured using a spectrophotometer equipped with an integrating sphere in the range 680–900 nm. Reprinted from Ref. [57], copyright 2012, with permission from Elsevier

the intensity of the dye peaks in an IR spectrum is usually very low, difficult to isolate from the other absorptions due to the polymer matrix. For these reasons, only one or two of the most intense dye or pigment peaks can be identified within the spectrum of a typical coloured fibre, making the identification of a dye in textile fibres an extremely challenging task. However, such positive identification is very rarely necessary in fibre casework, which in most cases involve comparisons. The presence of a consistent peak in the spectrum of a fibre can be, irrespective of the chemical nature of the species that originated it, an element of distinction or of similarity between two compared items.

Characterisation and quantification of colouring additives in fibres can be more easily achieved with a particular kind of pigment. Titanium dioxide, or titania (TiO_2), is a delustering agent, added in filler-like amounts to fibres to make them more opaque and to improve their appearance. In this case, the large quantity of this additive allows an identification and quantification approach based on vibrational spectroscopy, such as that presented above in the case of fillers. Vann and colleagues [59] exploited the potential of Raman spectroscopy to distinguish between the rutile and anatase form of the inorganic pigment titanium dioxide (TiO_2), also making quantitative measurements of the titania loading. The easy hyphenation of Raman spectroscopy with a microscope makes this approach a very promising one when a thorough characterisation of fibres is necessary. Titania can in fact be heterogeneously distributed along the fibre, but the possibility to sample just selected portions of the sample allows to overcome this problem. The amount of titanium dioxide in single delustered polyamide fibres was quantitated at concentration levels ranging from 0 to 7.1 % TiO_2 [59].

Vibrational spectroscopy of dyes and pigments can be carried out with satisfying results by employing Raman. It is true for many kinds of samples that IR spectra are dominated by signals due to the polymer matrix, whereas Raman is more sensitive to the transitions of dyes and pigments [2]. . This has been reported for example in the case of textile fibres by Minceva-Sukarova and colleagues [60]. These authors analysed by micro-Raman and micro-IR spectroscopy colourless and three dyed acrylic fibres. Dyed fibres gave Raman spectra with bands characteristic of both the polymer and the dyes, whereas the IR spectra displayed just the bands due to the matrix polymer. The relative intensities of the signals due to the dye and to the polymer matrix could be used for the quantification of the dye content [60].

Similar results were reported for cellulosic fibres by Was-Gubala and Machnowski [61]. Cotton and regenerated cellulose, such as viscose, have the same chemical composition. This common chemical nature of cotton and viscose fibres means that the same dye classes, most often direct and reactive dyes, may be used for dyeing the textile products from these fibres. However, the differences between natural and regenerated cellulose fibres are practically just in the degree of polymerisation and on the supramolecular structure. This brings about different adsorption properties of dyes within the fibre. Raman allows to differentiate the two different types of cellulose fibres, dyed with the same dye, at the same or very similar concentrations. The presence of strong bands in the Raman spectrum, namely at 898 and at 350 cm^{-1}, allowed to differentiate viscose from cotton [61]. The differentiation between these

two classes of fibres is indeed possible just by observation under a microscope, so the usefulness of this result could appear to be limited. However, it could be valuable in casework involving very damaged fibres or post-fire debris. In such instances, the morphology of the fibres can be so spoiled that a positive identification could be impossible: the use of a spectrometric approach would confirm a tentative attribution made through microscopy.

The same complementarity between Raman and IR spectroscopy is found in the analysis of paint. Analogous to the case of fibres, IR spectroscopy does not allow to easily extract information on the pigment used in a particular layer of paint. The spectrum is in fact dominated by the binder absorptions. Moreover, the pigment formulation in paints, especially in the automotive field, is very complex, with several different species mixed together to obtain the desired hue and effect. A series of eight papers by Suzuki and coworkers ([62] and references therein) is iconic in describing the endeavour of obtaining a systematic characterisation of pigments in paint.

Organic pigments lend themselves to a successful identification by Raman spectroscopy because they are usually good scattering species, due to the fact that they tend to contain many aromatic rings and several conjugated systems, with a high symmetry. The main disadvantage is that they can be fluorescent, forcing to tune the experimental conditions using an excitation wavelength which minimises this phenomenon. Figure 5.37 shows the IR and Raman spectra of the basecoats of three cars, differing by make and colour [63]. The three models cannot be distinguished on the basis of their IR spectra, because they appear identical. However, the Raman spectra show substantial differences, and also allow to identify which pigments were used in each paint. The Raman spectrum of the Citroën car shows the co-presence of purple dioxazine PV23 and copper phthalocyanine PB15 pigments (curves (d) and (e) in Fig. 5.37). The Renault basecoat contains indanthrone (curve (f) in Fig. 5.37) and a small amount of phthalocyanine. Finally, the Raman spectrum of the Opel Astra basecoat coincides with that of chlorinated copper phthalocyanine PG7 (curves (g) in Fig. 5.37).

Sometimes the particular formulation of samples does not allow to obtain a sufficient intensity and quality of Raman spectra. In such cases, surface-enhanced Raman scattering (SERS) can be an efficient approach [64]. This involves a sample preparation step which, however, does not imply a macroscopic modification of the item. In SERS, a bead of a polymer hydrogel loaded with a solution containing water, an organic solvent, and a chelating agent is used to extract a tiny amount of dye from the examined item. The use of a gel allows to selectively confine the action of this solution just to the desired region of the specimen. After this microextraction procedure is terminated, the gel bead is removed, covered with a drop of Ag colloid, and examined with a Raman microscope. Due to the small amount of extracting agent and to the limited size of the gel bead used, SERS is not alterative of the item, and can be considered a non-destructive technique. For this reason, it is particularly suited for the analysis of questioned documents, works of art and items of historical and archaeological interest. The drawback of SERS, however, is that no universal solution for the microextraction process exists, and some tests must be carried out to optimise such sample preparation step for the particular item of interest.

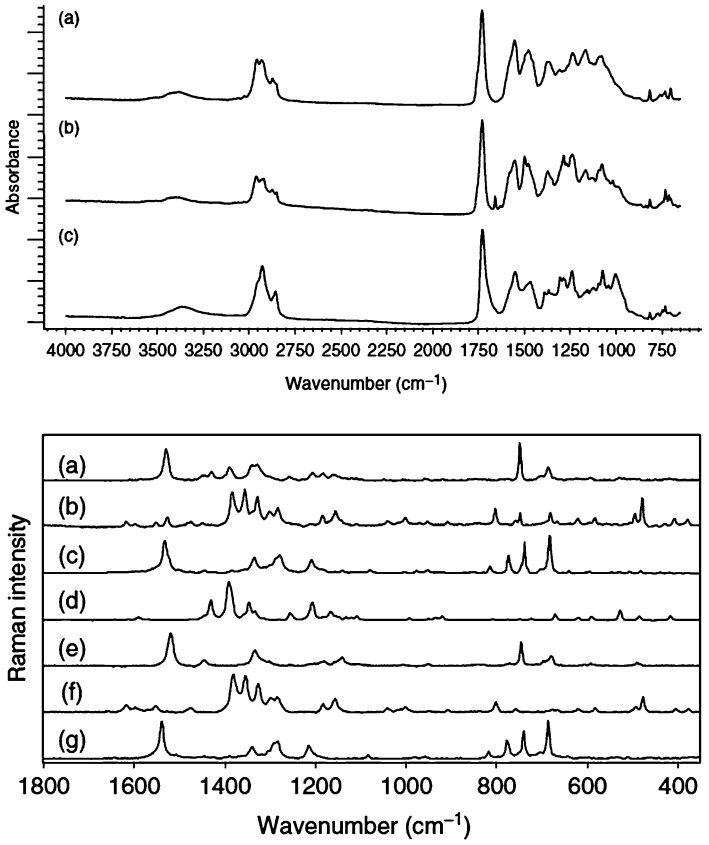


Fig. 5.37 *Top panel*: IR spectra of the cross-section of the basecoat of (**a**) a dark metal blue Citroën, (**b**) a dark metal blue Renault, (**c**) a green Opel Astra. *Bottom panel*: Raman spectra of the cross-section of the basecoat of (**a**) a dark metal blue Citroën, (**b**) a dark metal blue Renault, (**c**) a green Opel Astra, (**d**) purple dioxazine PV23 pigment, (**e**) copper phthalocyanine PB15 pigment, (**f**) indanthrone, (**g**) chlorinated copper phthalocyanine PG7 pigment. Reprinted with permission from Ref. [63] copyright © 2005 John Wiley & Sons, Ltd

Mass spectrometry with desorption ion sources allows an in-situ direct analysis of dyes in fabrics and fibres. Cochran and colleagues reported the possibility to do so by infrared matrix-assisted laser desorption electrospray ionisation-mass spectrometry (MALDESI) [65]. In MALDESI, a laser is used to desorb the analytes from the sample with the aid of a matrix. The desorbed dye molecules are then post-ionised by electrospray ionisation (ESI). This work is particularly appealing because the matrix used to absorb the laser energy and to favour the desorption of dye molecules was water. Nylon, polyester, and acetate textiles were analysed, successfully obtaining the mass spectrum of the dye, which could be easily identified [65]. No damage was inflicted to the fabric. However promising, this study is currently limited as a proof of concept, because it has been applied to macroscopic pieces of

textile and not on the single fibres which are common in casework. Nevertheless, the potential of modern desorption methods and the continuous development of mass spectrometry promise that in the future more and more forensic protocols will include this technique.

Pigments are often inorganic, and sometimes they are not visible in IR or Raman spectra. Therefore, the determination of the elemental profile of paint is a very meaningful approach for its characterisation. In the interest of the reproducibility of the examination in the several steps of the judiciary process, non-destructive technique should be preferred. Among these, XRF is surely the best performing. These analyses can be carried out in a dedicated X-ray fluorimeter or by an Energy Dispersive X-ray detector mounted on a Scanning Electron Microscope. Zieba-Palus and colleagues assembled a multipurpose instrument, named Praxis, able to carry out both micro-Raman and micro-XRF measurements [34]. The usefulness of XRF measurements for the characterisation of paints is well established, especially if data are acquired in a small area by using a microscopic electron beam. Ti, Fe, Ca, Pb and Zn are among the elements which can most easily be detected and which are more discriminating. Lighter elements are more difficult to accurately reveal with XRF equipment, especially operating in microscopic mode. Because of this, the contribution of elements such as magnesium, aluminium and silicon, which are usually present in the internal layers of paint coats, could be lost. Moreover, when using XRF techniques, an overlap of the lines of elements commonly present in car paints (Ba and Ti, S and Pb) impairs interpretation of the results [10]. Another problem that can be associated to micro-XRF of paint chips is that the diameter of the incoming X-ray beam is around 30 μm, bigger than the thickness of some of the thinner layers (15–20 μm). Prior separation is thus necessary if one wants to obtain separate information regarding such layers.

Laser ablation inductively coupled plasma-mass spectrometry (ICP-MS) is a valid alternative for the analysis of paint chips [10, 66, 67]. Laser ablation is a sample treatment technique which consists in illuminating a solid sample with a beam of laser light. The energy transferred to the material disgregates the bonds and intermolecular forces holding the atoms, which are in turn brought in the vapour phase and introduced in an ICP-MS apparatus, to be ionised and detected by mass spectrometry. This approach can be considered essentially non-destructive, since the laser beam, which leaves a crater about 100 μm in diameter and 100 μm deep (Fig. 5.38), removes an extremely small amount of material (~500 ng) [67]. No sample preparation step is required.

The possibility of sequential analysis of all the paint layers without the need for extensive sample preparation is another reason which makes laser ablation very useful in paint analysis. The depth of penetration of the laser beam within the sample depends on the time of exposure. If time-dependent measurements are performed, it is then possible to obtain the elemental profile as a function of the penetration inside the paint chip. In other words, it is possible to analyse the sequence of layers of a typical automotive paint chip, without physically separating them. This capability is shown in Fig. 5.39, where the composition of the three layers of a paint system is obtained by a time-resolved laser ablation ICP-MS measurement.

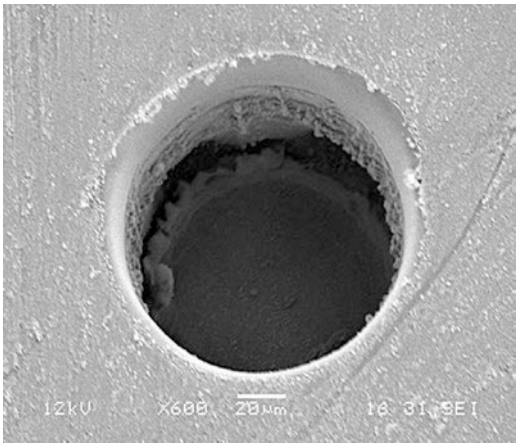


Fig. 5.38 SEM image of a typical crater obtained under typical laser ablation conditions on automotive paint. Reprinted with permission from Ref. [67] with kind permission from Springer Science + Business Media

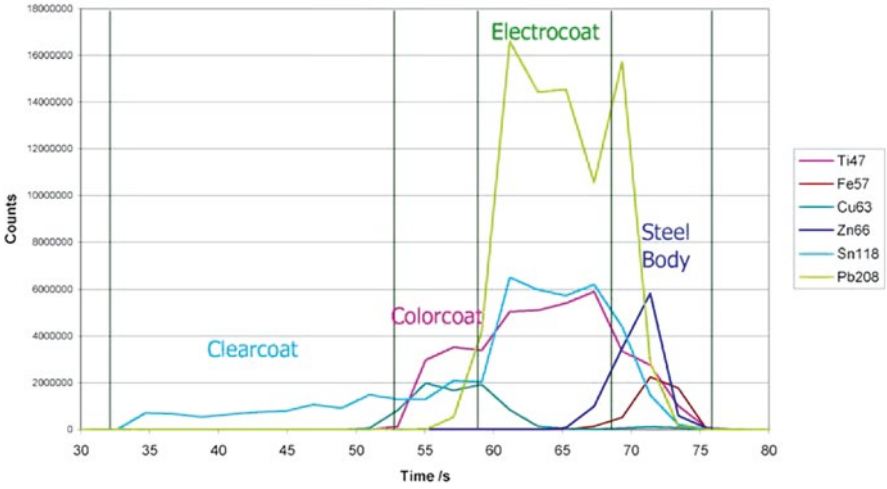


Fig. 5.39 A time-resolved plot of the ablation of a three-layer paint chip. The separation of the layers is marked by the vertical lines. Reprinted with permission from Ref. [67] with kind permission from Springer Science + Business Media

One problem which could arise when performing laser ablation ICP-MS measurements on paint chips is that, when the laser is ablating from top to bottom, the elemental intensities of the deepest layers begin to be mixed together, as can be seen also in Fig. 5.39. An easy solution to overcome this issue consists in turning the paint chip upside down and to analyse it again [10].

Confocal micro-XRF is another way to obtain, in a non-destructive way, 3 dimensional information on the elemental composition of a sample [68–70]. The basic principle of confocal micro-XRF was proposed by Gibson and Kumakhov in

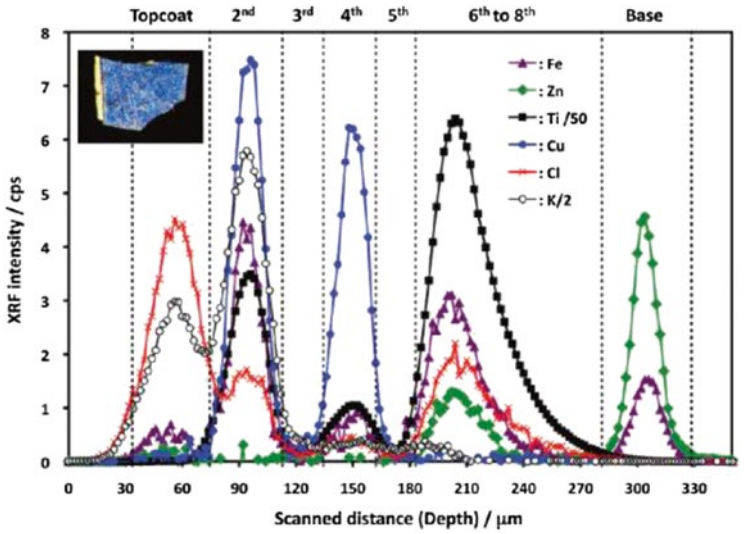


Fig. 5.40 Elemental depth profiles of Fe, Zn, Ti, Cu, Cl and K in a blue paint fragment (*inset*) measured by confocal micro-XRF. Reprinted with permission from Ref. [70]. Copyright (2011) American Chemical Society

1992 [68]. By the combined action of two very highly collimated X-ray beams, the focusing spots for both excitation and detection of XRF are adjusted to be at the same location, i.e. in the confocal point, which has a micrometric size. Orienting the two X-ray beams, it is thus possible to probe the material in all three dimensions, obtaining 3D elemental mapping images. This apparatus, albeit complex and available just in a few specialised laboratories, allows acquiring the XRF spectra in a non-destructive manner, along the depth of a paint chip, without the need for physical separation of the layers. Nakano and coworkers reported examples of the capabilities of confocal micro-XRF [70]. On one hand it allows one to visualise the depth profile of the concentration of elements from the topcoat to the basecoat, analogously to Fig. 5.39 (Fig. 5.40). On the other hand, the analytical results can be represented as false colour images (Fig. 5.41), so obtaining an image of the cross-section, without the need to actually cut the samples, i.e. in a totally non-destructive mode.

The elemental profiles obtained by confocal micro-XRF were compared with the results of conventional micro-XRF of the cross-section of the same paint chips. Two sets of similar data were obtained, confirming that the confocal micro-XRF has accuracy and precision comparable to more established XRF approaches [70].

All the mentioned methods allow an analysis which does not modify the integrity of the piece of evidence, and therefore they are desirable as first techniques in any forensic protocol. Sometimes, a deeper characterisation is required, and so referral can be made to methods which imply a sample preparation step and which in some way alter the item. In the case of the analysis of dyes, surely the most indicated

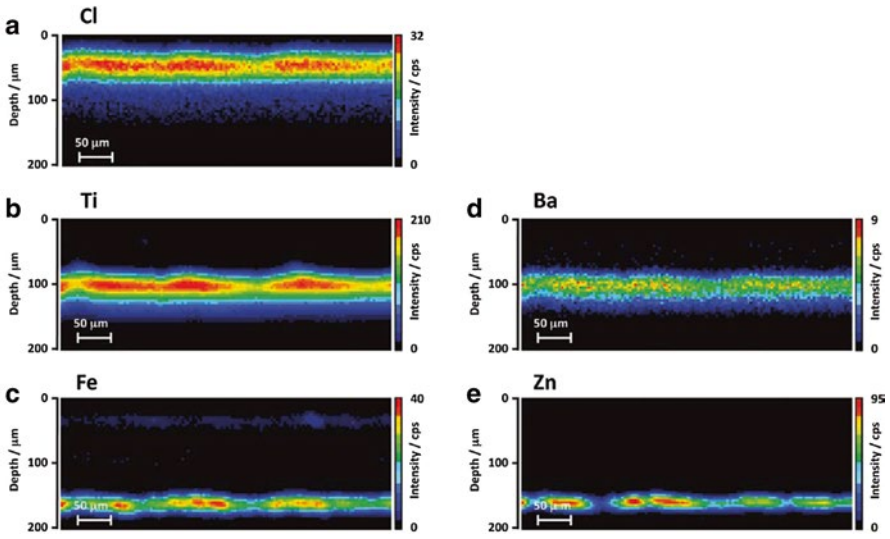


Fig. 5.41 Non-destructive elemental depth imaging of Cl, Ti, Fe, Ba and Zn in different areas of a black paint fragment measured by confocal micro-XRF. Scanning area of the analysis was 200×500 µm. The minimum step size was 4×4 µm and the measurement time was 30s per pixel. Reprinted with permission from Ref. [70]. Copyright (2011) American Chemical Society

approach is the application of chromatography. The literature on this topic mainly regards the chromatographic analysis of dyes in textile fibres,. The reader interested in a detailed discussion of the chromatographic analysis of dyes in fibres is directed to two excellent book chapters by Wiggins [71] and by Griffin and Speers [72]. Two types of chromatography can be used in this case: TLC and HPLC. Even though they significantly differ in the type and cost of the necessary facilities, they both share the same sequence of operations for performing the analysis. The dye mixture must be first of all extracted from the fibres, and the solution so obtained must be chromatographed. The most relevant feature which is of interest when devising an extraction procedure is whether the fibres are dyed with a reactive or non-reactive system. In the case of reactive dyes, covalent bonds are formed between the dye and the polymer. Solubilisation of reactive dyed polymers implies fragmentation of the macromolecules and analysis of the fragments so obtained. Wool can be disrupted by treatment with sodium hydroxide and citric acid, cotton by the action of sodium hydroxide, acetic acid and the enzyme cellulase [71]. Non-reactive dyes are easier to treat, because they interact with intermolecular forces with the polymer molecule, and so they can be separated from the matrix simply by solvent extraction. The solvent used depends on the type of dye system used and on the analytical technique which one intends to employ. In TLC, pyridine/water (4:3 v/v), formic acid/water (1:1 v/v) and oxalic acid/water (0.2:10 m/v) have been suggested [71]. In addition to the ones just listed, chlorobenzene, dimethylformamide (DMF) and DMF/

acetonitrile have been proposed for HPLC [72]. Since the most commonly used HPLC columns for this type of analysis are made with an octadecylsilane (C18) stationary phase, care should be taken that the extraction solvent does not interfere with the chromatographic separation. Solvents such as chlorobenzene should be evaporated after extraction, because they can disturb the analysis.

The easiest and most economical technique is surely TLC, which indeed offers often a sufficient sensitivity and detection limit to carry out satisfying analyses even on single fibres. It is advisable, in the case of small or pale items, to test a similar object with comparable size and hue, in order to determine if there is enough material for carrying out a TLC analysis. HPLC is more sensitive, and diode array detectors allow to simultaneously acquire the UV–visible spectrum of the dyes. In any case, to validate and corroborate the quality of chromatographic data, both TLC and HPLC, it is advisable to check the eluent system by running a standard dye mixture. Of course, HPLC grade solvents must be used in all stages of the chromatographic analyses.

Electrophoresis is a separation method based on the fact that different charged species migrate with different rates in a buffer solution across which a DC electric field has been applied. Capillary electrophoresis is an analytical technique which exploits electrophoresis, allowing high speed, high sensitivity, very low detection limits, very high resolution and extremely small sample volume (0.1–1 nL). Due to these advantages, capillary electrophoresis found many applications in forensic analysis, especially for the analysis of drugs [73–76]. In this technique, a small volume of sample is injected into an aqueous buffer solution contained in a fused silica capillary. A high potential is applied at the extremes of the capillary, which causes the ions of the sample to migrate toward one of the electrodes. The rate of migration of each analyte depends on its size-to-charge ratio: the larger this ratio, the faster its migration.

In the field of trace evidence, very few reports exist on the applications of capillary electrophoresis. The main problem associated to this technique is that most additives are organic, and they require organic solvents for extraction. However, organic solvents are not compatible with the aqueous buffers used in capillary electrophoresis. As a result, the performance is not up to the expectations and use of capillary electrophoresis in this branch of forensic science is not widespread [77]. Stefan and coworkers reported good results for the analysis of acid dyes in nylon and of basic dyes in acrylic fibres [78, 79]. They identified, by a combinatorial approach, a pyridine/ammonia (1:1) mixture and a formic acid/water mixture (88 % formic acid) as the best extraction systems for acid and basic dyes, respectively. Capillary electrophoresis proved to be a promising technique for the analysis of dyes in fibres, especially if the instrument is equipped with a diode array detector. In this case in fact, spectra can be obtained even for dyes of similar colour, introducing a further element of characterisation (Fig. 5.42).

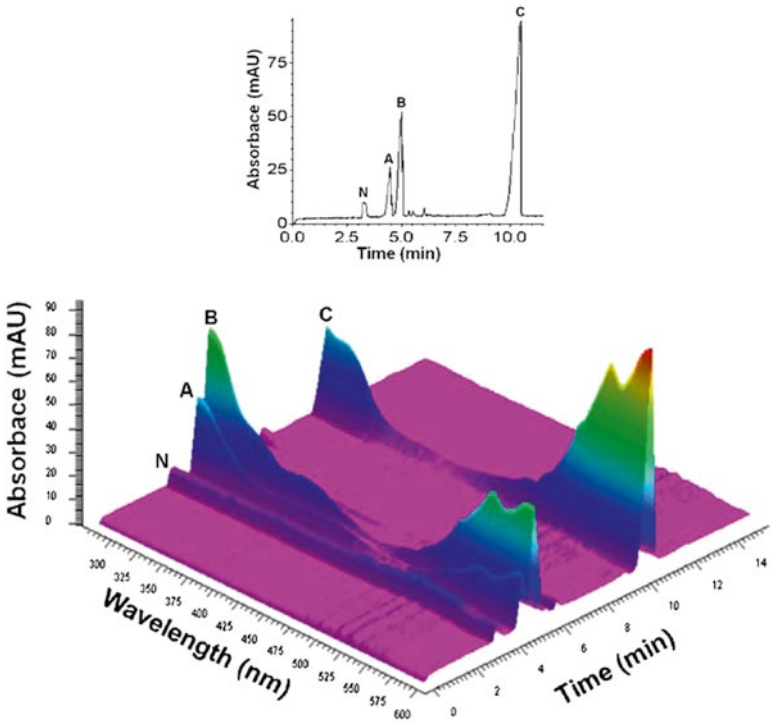


Fig. 5.42 *Top*: electropherogram at 600 nm of acid dyes extracted from nylon 6,6 fibre; peak N represents neutral components. *Bottom*: electropherogram showing UV/visible absorbance spectra. Reprinted with permission from Ref. [78]. With kind permission from Springer Science + Business Media

5.10 Characterisation of Other Additives

Figure 5.43 shows an example of the potential of IR spectroscopy for the elucidation of the formulation of paints [34]. As may be seen, from the analysis of the spectra the binder, the fillers and the pigments present in the coating can be identified. This result is not the one commonly achieved in forensic casework. An experienced scientist can obtain so extensive a characterisation just for a few class of items, paints and some synthetic fibres, which are commonly encountered. The main industrial processes for the manufacturing of these materials are known, and the chemical nature or composition of many additives used in these industries is available. Moreover, for these materials spectral and forensic databases were created.

However, in most of the forensic cases involving polymers, the formulation of the item of interest is not known. In some unfortunate instances so little information is available in the open literature that no previous hypothesis on the formulation can be made. In such instances, it becomes difficult to devise an analytical protocol for

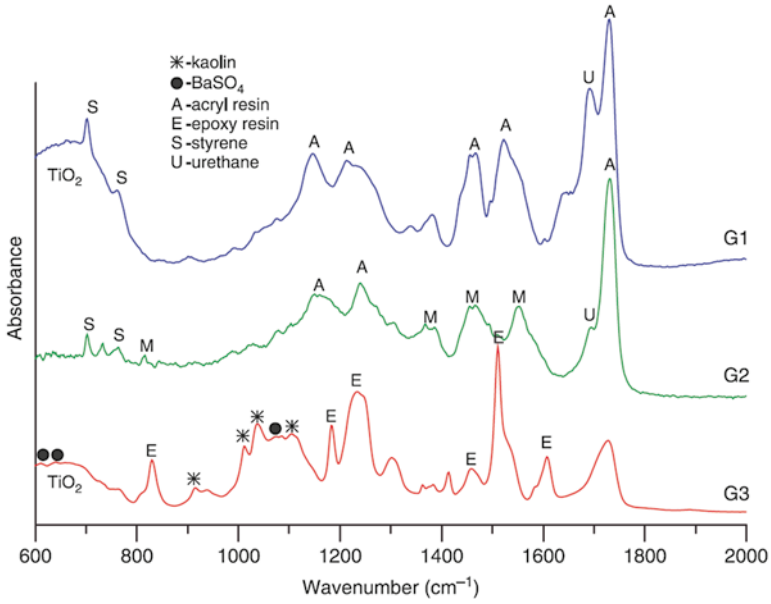


Fig. 5.43 IR spectra of three paint samples of different colours. Reprinted from Ref. [34], copyright 2006, with permission from Elsevier

individually characterising each class of additive, as described above. However, the starting point can still be the same as seen in the preceding section. Spectroscopic methods allow a preliminary assessment of the material, which can subsequently guide the analyst towards the following steps of the procedure.

In comparative analyses, when the spectra of the two items being compared show different signals, it is not even necessary to positively identify the additives which originate such peaks. The consistent presence of different features is a sufficient indication that the formulation of the two materials is not the same, and thus they cannot originate from the same source. An interesting application of this concept was recently given, in the proposal of IR spectroscopy as a way to identify counterfeit banknotes [80, 81]. Sonnex and colleagues, for example, obtained from the police a set of high quality forged British £20 banknotes and they acquired the IR spectra in three different areas, namely the holographic strip, a blank and a printed region. Comparing the results with genuine samples, they recorded significant spectral differences, as shown in Fig. 5.44.

The origin of such differences could not be positively identified, nevertheless they were consistently found in all the forged and genuine banknotes which were examined, and were significant.

Even more interestingly, these same authors made IR maps of portions of the banknotes at their disposal. IR mapping consists in the sequential acquisition of multiple infrared spectra from different spatially resolved points on the same sample.

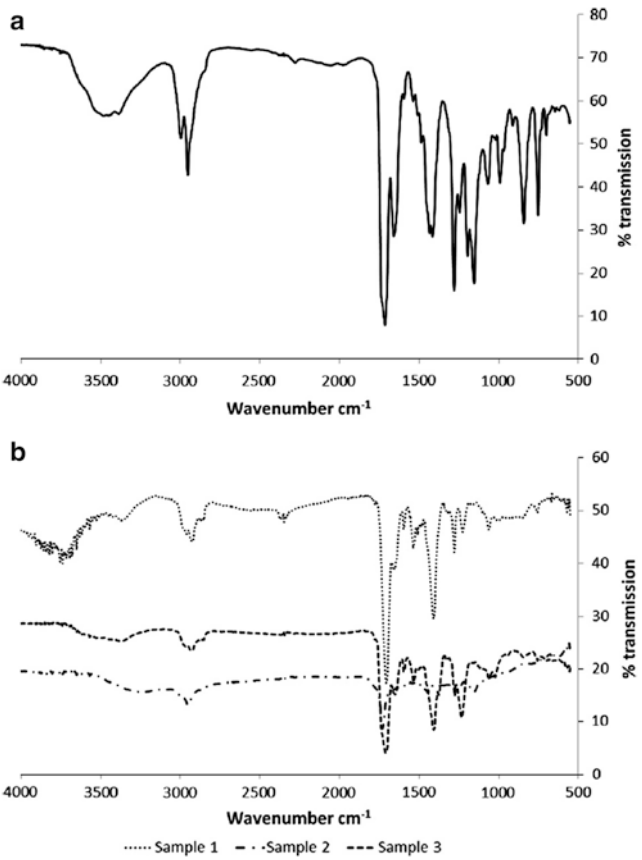


Fig. 5.44 IR spectra of the holographic strip of (**a**) a genuine £20 banknote and (**b**) of three counterfeit notes. Reprinted from Ref. [81], copyright (2014), with permission from Elsevier

It allows to simultaneously store both spectral and spatial information, thereby making the characterisation of the chemical heterogeneity possible. It is an extremely versatile technique by which visualisation of a vast array of data is facilitated [69, 82–85]. Application of IR mapping on banknotes showed that genuine banknotes displayed a significant spectral difference between the printed and non-printed areas, as shown in Fig. 5.45.

On the contrary, no contrast was observed in forged banknotes, which showed insignificant spectral differences between the printed and non-printed areas. The same result was reported by other authors who analysed Euro currency [80].

The IR map on the top right quadrant of Figs. 5.45 and 5.46 is particularly efficient in exhibiting the difference in composition between the two materials.

Another iconic example of the usefulness of IR imaging was given by Flynn and coworkers at the University of Technology of Sydney and of the Australian Federal Police [37], who acquired IR maps on microtomed sections of paint chips included

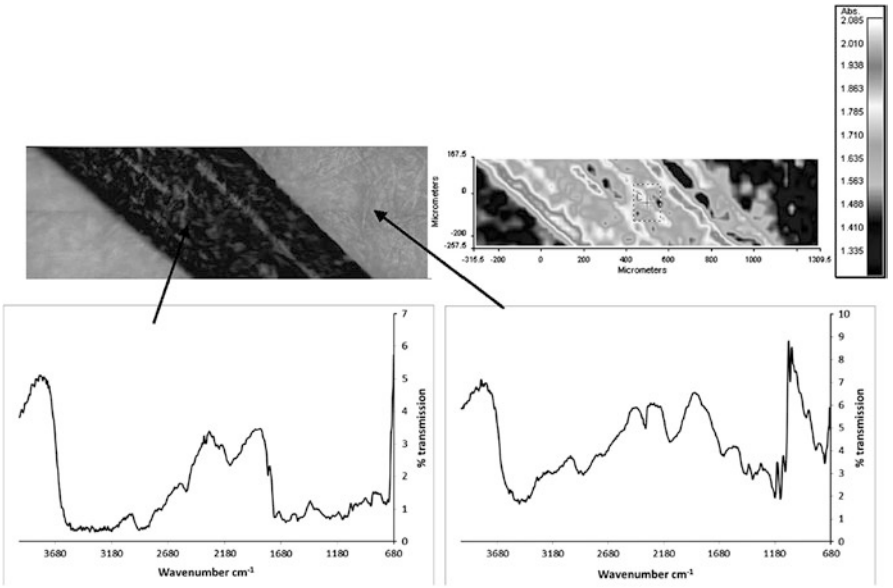


Fig. 5.45 Visual image and infrared spectral map of the Bank of England writing on a genuine 20£ note, with the corresponding IR spectra. Reprinted from Ref. [81], copyright (2014), with permission from Elsevier

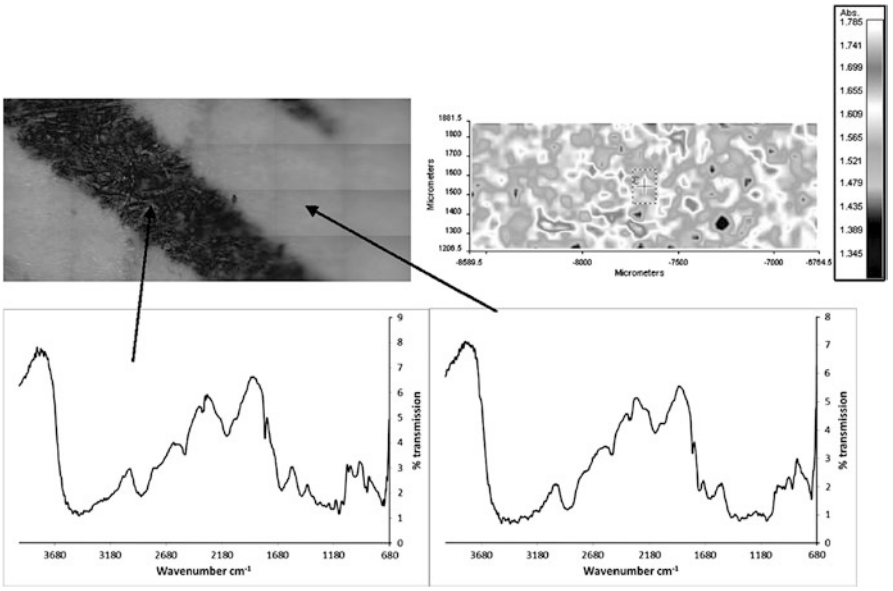


Fig. 5.46 Visual image and infrared spectral map of the Bank of England writing on a forged 20£ note, with the corresponding IR spectra. Reprinted from Ref. [81], copyright (2014), with permission from Elsevier

into KBr pellets. Different chemical images could be obtained by representing the integrated spectral intensity at a particular wavelength by a colour scale, obtaining a result very similar to the false colour images in Fig. 5.41. The different layers composing the paint chip could thus be very effectively visualised. Moreover, creating a sequence of chemical images across the spectral range a 'movie' could be obtained, which was especially useful for conveying chemical information to laypersons (Sect. 3.2) [37].

As said in the introduction of this section, most of the times vibrational spectroscopy, either IR or Raman, is but the first step of a more complete characterisation of the formulation of a sample. It is the case of the analysis of toner [86, 87]. Powder toner resembles paint in its composition, because it contains binders, dyes and/or pigments, and additives. The formulation of toner is optimised to obtain particles with an appropriate mass, electrical charge, and magnetic properties. The formulation of commercial toners is protected by patents, so not much is known about its chemical details; however some key components are carbon black as a pigment, and iron-containing inorganic compounds as additives yielding the necessary magnetic properties.

Of course the forensic interest of the toner analysis is that of identifying the machine which was used to produce a certain document.

Several works have shown that IR spectroscopy is a very good method for narrowing down the potential candidates ([86, 87] and references therein). Spectral libraries were also created. In one case, regarding a database with 807 toner samples, 98 groups could be identified on the basis of the IR spectral features [86]. However, IR spectroscopy does not have a sufficient discrimination potential to be able to stand alone in a characterisation protocol. Some further investigation must be made, focused on the other additives contained in this material. The most commonly and abundantly used pigment, carbon black, does not have enough chemical variability to be useful for discrimination of toner. Fortunately, the inorganic additives used for tuning the magnetic properties of toner offer an efficient strategy for gathering further information on the formulation of this material. XRF is the method of choice in this case. These measurements can be made in a dedicated spectrometer, or more practically in a scanning electron microscope, through an energy dispersive X-ray detector. This latter solution has the advantage of allowing to selectively identify the location where the measurement is acquired, a key aspect in this case, because toner is found just on the tiny printed parts of the questioned document.

Studies made by XRF on different kinds of toners showed that the elemental profile of these materials is quite rich, with about ten elements found in measurable amounts [87, 88]. In their study using SEM-EDX, Egan and coworkers detected two main kinds of toners: ferrite-based single component toners displayed high levels of iron, combined with other elements and dual-component toners had a low silicon content and high levels of organic components [88]. They identified ten elements as the most useful for toner characterisation: Al, Si, S, Cl, Ca, Ti, Cr, Mn, Fe and Zn. Other studies indicate different elements [87] but this should not be a major concern, since XRF allows the simultaneous detection of all the elements contained in

a sample. No previous knowledge of a tentative composition is required for setting up an analysis. An indication of the discriminative power of the combination of IR spectroscopy and XRF was given by Trzcinska and coworkers who, by sequentially applying these two techniques to 34 kinds of toners, were able to successfully differentiate 558 out of the possible 561 pairs of samples [87].

As introduced above, elemental analysis is often an analytical approach capable of integrating spectroscopic data for an increased discrimination potential and an improved and deeper investigation of the formulation. XRF, with its non-destructivity, is surely an appealing option, but it suffers some drawbacks, among which a lower sensitivity and limit of detection, a stronger dependence from matrix effects and a poor potential for accurate quantitation. When these factors are of interest, partially destructive approaches become attractive and can be introduced in the analytical protocol. Of course, care should be taken to turn to elemental analysis after having acquired all the necessary data from non-destructive techniques, in cases where sample size does not allow to save a portion of the material for further investigation. In polymers, the presence of elements different from carbon, hydrogen, nitrogen and oxygen is related to the use, in the formulation, of inorganic additives. The use of such additives strictly depends on the intended final application of the material and on the geographical region where the item was fabricated. A quite dated study from Nissen and coworkers clarifies this point, highlighting that elemental analysis is not always useful for discrimination [89]. These authors measured trace element concentrations in two types of polyethylene film, colourless cling film and coloured garbage bags, using inductively coupled plasma-mass spectrometry (ICP-MS). Garbage bags of identical colour, but different manufacturing origins could be successfully discriminated by the differences in concentrations of some elements (Mg, Al, Fe and Ba in the case of black bags, and Mg, Cr, Mn, Fe and Pb for orange bags). On the contrary, colourless cling films could not be discriminated by these methods because of the low concentrations of trace elements present and their high variability within a single sample. Cling film is intended for contact with food, and it therefore cannot contain any metallic contaminant which could be harmful for human health. For these reasons, as confirmed also by IR studies [40], cling film is predominantly made of pure and unfilled poyethylene.

Surely the most promising and interesting application of atomic mass spectrometry is isotope ratio mass spectrometry (IRMS) [90]. This technique is commonly exploited in provenance studies in diverse fields such as environmental, biological and geological analysis. The utility of this approach has been proven in these disciplines for accurately singling out the individual features that characterise different samples with a similar nature (for example the same kind of mineral coming from different geographical areas, or the same kind of vegetable material grown in different plantations). It is therefore surprising that the application of IRMS measurements in trace evidence is very underrepresented [91]. Only a few reports exist in the literature on post blast debris [92, 93], architectural paints [94], plastic bags [95], and adhesive tapes [6, 96, 97].

IRMS is based on the principle that, despite the fact that the average isotopic abundance of each element is globally determined by the geological and physical

events occurred since the time of the formation of Earth, such average value is locally modified by a number of natural and artificial fractionation processes [91]. Use of raw materials of different origin, processed in different ways by different manufacturers will therefore result in different stable isotope ratios, and therefore in an unique opportunity for a deep forensic discrimination of otherwise similar items.

Obtaining significant and reliable IRMS data is not easy, though. In 2008, Blessing and coworkers pointed out the several pitfalls associated to this analysis [98]. Even though this paper is focused on forensic environmental chemistry, i.e. on the detection of the source of pollution or of contamination of the environment, it gives a clear idea of the complexity involved in carrying out IRMS. A fundamental aspect which must be considered when devising a IRMS protocol is that all sample treatment procedures can potentially lead to isotope fractionation. Hence, prior to application in real casework, every step of the sample preparation protocol must be thoroughly evaluated by means of standards with known isotopic composition that are treated identically to the samples [98].

IRMS focuses on the tiny variations in stable isotope composition, therefore yielding information on the source and origin of a given material, which no other analytical method can provide. The isotope ratios are presented as a shift (e.g. $\delta^{13}C$), expressed in ‰, of the ratio R between two isotopes, relative to an appropriate standard. For example, in the case of the ratio $^{13}C/^{12}C$:

$$\delta^{13}C = \left(\frac{R_{\text{sample}}}{R_{\text{std}}} - 1 \right) \times 1{,}000$$

The sensitivity of IRMS allows one to enrich the data by sampling different zones of the samples that may be isotopically distinct from each other. For example grip-seal plastic bags are manufactured assembling the seal to the plastic bags, each component having a different origin [95], or adhesive tapes are composite materials that can be schematically described as glue on a plastic backing, each coming from a different source as well [6] Analysing each component of the object greatly increases the significance of the comparison.

An interesting application of IRMS was proposed for the characterisation of adhesive tapes [6, 96, 97, 99]. The motivation starts from the premise that conventional adhesive tapes share many common components. In particular, the variability in the materials used for producing the backing of the tape is very limited, being polypropylene and poly(vinyl chloride) overwhelmingly used. Moreover, few inorganic additives are used, making elemental analysis less discriminative than in other fields. IRMS, with its capability of differentiating if similar materials came from the same or different sources seems an ideal approach for a detailed characterisation of a class of items with a small variability. The carbon isotopic signature, derived from substrate polymer, associated additives and adhesive is highly characteristic of a particular tape and allows samples from different sources to be readily distinguished.

Figure 5.47 shows the $\delta^{13}C$ data relative to a set of adhesive tapes analysed as received (untreated samples) and after removal of the glue (treated samples).

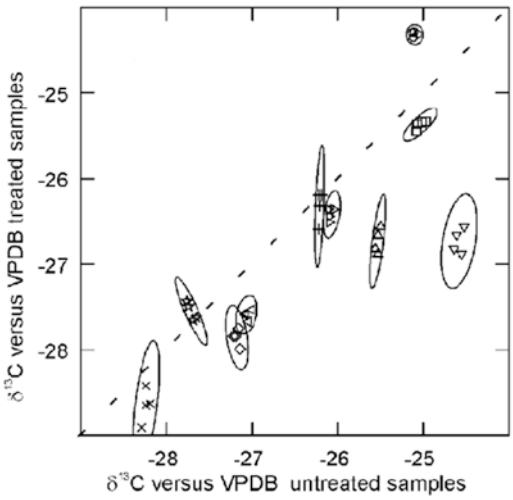


Fig. 5.47 $\delta^{13}C$ data for complete adhesive tape samples (untreated samples) versus tape samples where the glue was removed (treated samples). VPDB indicates the standard used as a reference for the $^{13}C/^{12}C$ isotopic ratio: Vienna PeeDee Belemnite. Reproduced from Ref. [6] with permission of The Royal Society of Chemistry

If the isotopic ratio of glue and backing were the same, $\delta^{13}C$ should not change, and the data should lie on the diagonal of the diagram. However, experimental data show that a change in isotopic ratio indeed happens after removal of the glue. The data points tend to lie below the diagonal, meaning that after the removal of the adhesive, the backing contains a higher proportion of ^{12}C. In other words, the adhesive is enriched in ^{13}C. Decoupling of the backing and the adhesive turns out to be very useful for the forensic characterisation of adhesive tape, because it allows to compare samples on the basis of multiple parameters instead of just one.

A similar approach was proposed by Dietz and colleagues as well [99], who applied a separation process to extract the plasticiser from electrical tapes. The results were similar to those reported by other authors: separation of the different components has a strong influence on the isotopic signature. This on one hand allows to refine the discriminatory power of IRMS because it allows to decouple their isotopic abundance. However, on the other hand this may be also a source of uncertainty in the interpretation of data. For example, when significant quantities of plasticisers could have been lost from a tape backing, as in the case of exposure to heat, the difference in the amount of plasticiser could be due to the circumstances of the case, and not to a different source. In such instances, it is better to exclude the influence of the plasticiser, extracting it before carbon isotope analysis [99].

Since all polymers contain carbon and hydrogen, and most of them also oxygen atoms, these elements are those that deserve more focus. Further discrimination may be achieved by the incorporation of deuterium and oxygen isotopic data. Figure 5.48 confirms this, and reflects the results of an IRMS survey made on a population of adhesive tapes from the South Korean market. The data reported in this figure confirm what was reported by Carter et al. [6], i.e. that since backing and glue come from different sources, they also have different isotope ratios, both for carbon and for hydrogen. Interestingly, the Korean samples analysed by Horacek et al. [97] display a higher amount of ^{12}C in the glue, than in the backing of the

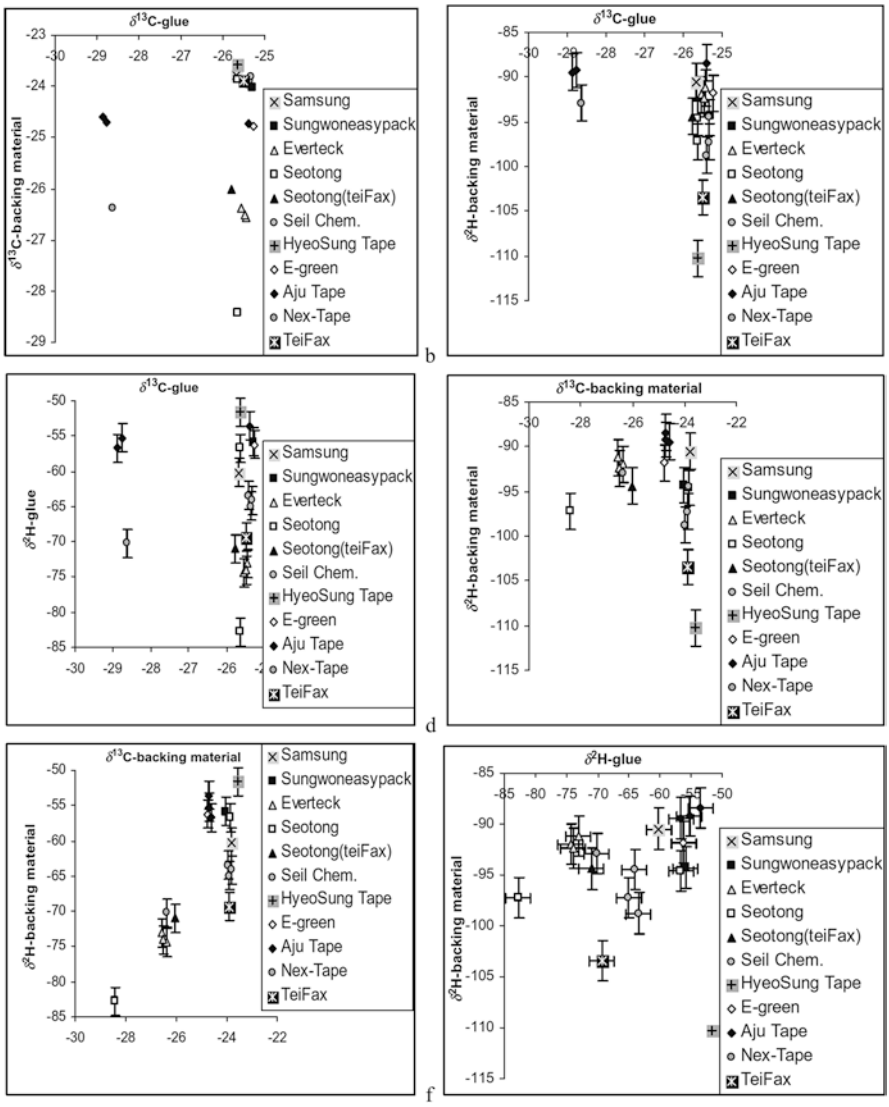


Fig. 5.48 Carbon and hydrogen isotope data of glue and backing material. All data expressed in ‰ versus Vienna PeeDee Belemnite for $\delta^{13}C$ and Vienna Standard Mean Ocean Water for $\delta^{2}H$. Most samples can be unequivocally discriminated from other samples. Standard deviation for $\delta^{13}C$ is smaller than symbol size, for $\delta^{2}H$ it is smaller than whiskers shown. Reprinted with permission from [97]. Copyright © 2008 John Wiley and Sons, Ltd

tapes, an opposite situation than that revealed by Carter on British samples. This one more time calls for the necessity to perform an accurate population study on the relevant market, before applying any technique to actual casework. Even in a globalised world, significant differences exist in the production processes of common

objects, which depend on a variety of geographical reasons, such as the local economy or the availability of raw materials.

In particular cases of highly filled samples, e.g. fibres containing large quantities of TiO_2, it can be worth extending IRMS to other elements too.

A key issue in assessing the reliability of a proposed analytical approach is the dependence of results from the weathering of the sample. To be actually useful, a technique must be able to relate samples of adhesive tape to a common origin following a period of use or storage. Carter et al. showed that the isotopic signature of the tape is not affected by adverse storage conditions, such as immersion under water for several weeks [6]. This situation reflects the common practice of concealing packages of drugs in water tanks or cisterns. On the same note, Dietz and colleagues showed that the isotope signature of tapes does not significantly change after exposure to an explosion, displaying IRMS as a very robust characterisation technique [99].

Manufacturing variation requires to sample several locations of the item, before concluding an association. As an example, Quirk et al. published a work in which they used IRMS to associate post-blast residues of two-way radios with the undamaged radio used to initiate explosive devices. They found that apparati with sequential serial numbers were not necessarily made from all of the same components [92].

With its capability of separating complex mixtures, chromatography would seem the ideal technique for the characterisation of the formulation of polymeric items. However, its use is somewhat limited and not many examples were reported in the literature. The difficulties associated to this approach are mainly related to the development of extraction and analysis methods which are universal and reliable under a variety of circumstances. This aim is very difficult to achieve, and so preference is given, as far as the analysis of formulation is concerned, to elemental analysis for obtaining data on inorganic additives and to pyrolysis for gaining information on the organic additives. However, some good examples in which separation techniques were used in polymeric traces of forensic interest have been reported. Parsons and colleagues explored the potential of gas chromatography for the characterisation of seven foam fragments [100]. They treated the fragments with dichloromethane and injected the extracted mixture in a gas chromatogram. They worked both with a flame ionisation detector (FID), which is universal and more sensitive, but it does not allow one to identify the chemical structure of the eluted compounds, and with a mass spectrometry (MS) detector, which at the cost of a lower sensitivity and a higher detection limit permits also a qualitative analysis of the components of the sample. In particular, using GC-MS they were able to identify a large number of additives. Plasticisers, catalysts, stabilisers, protectors from degradation, antibacterials, dyes, insecticidals, fragrances, anti-ageing agents and flame retardants were found [100]. Moreover, residues of the original isocyanate monomers used for the manufacturing of foams were detected [100]. A very detailed description of the several additives present in the formulation could thus be achieved, with an obvious advantage in terms of interpretation of the significance of evidence.

Another example which highlighted the potential of separation techniques for obtaining discriminative and significant data was reported by Burger and colleagues,

who applied capillary electrophoresis to condom and personal lubricants [101]. These authors analysed 68 different condom and personal lubricant samples by micellar electrokinetic capillary chromatography with ultraviolet absorbance detection. A very good discrimination of the samples was achieved, also with the possibility of correctly classifying them into homogeneous groups. This confirmed that capillary electrophoresis is in principle a rapid and efficient means for characterising condom and personal lubricant. The main limitation in this case was this technique does not yet have the sensitivity required for sexual assault swab analysis. However, this work is a good example of how data from separation techniques should be used and interpreted in the context of the development of a forensic method [101].

Despite its nature of destructive technique, Pyr-GC is an extremely efficient approach for the characterisation of the organic components of polymeric materials. The pyrolysis patterns obtained from two or more samples can be compared visually on the basis of presence or absence of peaks, of their retention times and their relative intensities. Mass spectrometry detection allows also to identify the pyrolysis products, with the concurrent possibility of chemically interpreting the possible differences between the samples. A series of papers by Wang illustrated the potential of Pyr-GC-MS to identify a wide variety of additives: plasticisers [102], lubricants [103], antioxidants [104], flame retardants [105].

It is not a surprise then that Pyr-GC, and especially Pyr-GC-MS, are used for many applications in forensic science.

Without a doubt the majority of the reports on the forensic use of Pyr-GC was centred on the analysis of paints, where it is a very performing complementary technique to IR spectroscopy and elemental analysis [33, 106–116]. When integrative data to these techniques were necessary, Pyr-GC could yield precious information on the organic species present in the formulation. The main profile of the pyrogram is dictated by the polymer matrix, and as such Pyr-GC is surely a suitable technique for identifying the polymeric material which composes the analysed item. However, IR spectroscopy is equally suitable for this aim, with the advantage of being applicable in a non-destructive manner, and more easily, due to the existence of rich databases for a fast qualitative analysis. The real advantage of pyrolysis is that the smaller features in a pyrogram, due to the components present in low concentrations, can be essential for differentiation between the samples. These peaks inform about the presence of secondary components of the binder, which cannot be detected by infrared spectroscopy [33].

Figure 5.49 shows the pyrograms of two acrylic styrene melamine paint samples. The pyrolysis conditions indicated in the caption are those typically employed for paint samples: pyrolysis temperature 700–750 °C for 2–10s [33, 106–112, 114]. As may be seen, the main peaks are the same for both materials, and they are ascribable to the degradation products of the styrene-derived component of the binder. Differences between the paints were only in the occurrence of various acrylic compounds. 2-hydroxyethyl acrylate, 2-hydroxypropyl methacrylate, 3-hydroxypropyl methacrylate, dodecyl acrylate, 2-hydroxyethyl methacrylate and 2-ethylhexyl acrylate were the compounds useful for differentiation [112].

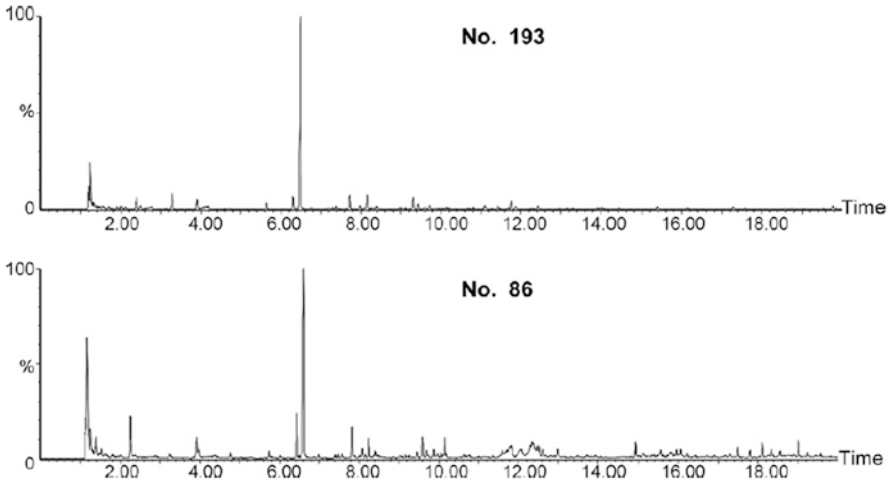


Fig. 5.49 Pyrograms of two acrylic styrene melamine paint samples. Pyrolysis conditions: 750 °C for 10 s without derivatisation. Chromatographic conditions: 40 °C held for 2.5 min; ramped 10.5 °C min^{-1} to 320 °C; 320 °C maintained for 5 min. RTx-35MS chromatographic capillary column (30 m × 0.25 mm, 0.25 µm) (stationary phase 35 % diphenylpolysiloxane and 65 % dimethylpolysiloxane). Carrier gas: helium, 70 kPa. Reprinted with permission from Ref. [112], copyright (2008), with permission from Elsevier

In the conditions chosen for the analysis of the samples shown in Fig. 5.49, no degradation product due to melamine was detected. In order to do so, precolumn derivatisation with tetramethylammonium hydroxide (TMAH) or tetrabutylammonium hydroxide (TBAH) should have been applied [112]. Derivatisation is an appealing strategy for improving the discrimination potential of Pyr-GC [110, 117]. Figure 5.50 shows two pyrograms obtained without derivatisation. At first sight, the patterns are not very different, except in the region at high elution time, between 20 and 25 min.

Figure 5.51 shows two pyrograms after derivatisation at 400 °C with TMAH. The mix of degradation products changes dramatically, discriminating the samples beyond any doubt [110].

The discriminating power of Pyr-GC is remarkable, and its application is suggested wherever possible. Zięba-Palus and coworkers, by a careful examination of the minor peaks in a pyrogram, were able to differentiate two acrylic styrene urethane paint samples taken from cars of the same model, originating from the same manufacturer but produced in different years [112]. The plasticiser phthalate anhydride was one of the additives contained in one of the paint samples, which was absent in the other.

In comparisons, it is often difficult to assess whether a minor feature in a chromatogram, a spectrum or, in this case, in a pyrogram are significant for forensic characterisation. Zięba-Palus and coworkers assessed, with an approach based on a likelihood ratio model, the reliability of a Pyr-GC characterisation of styrene acrylic

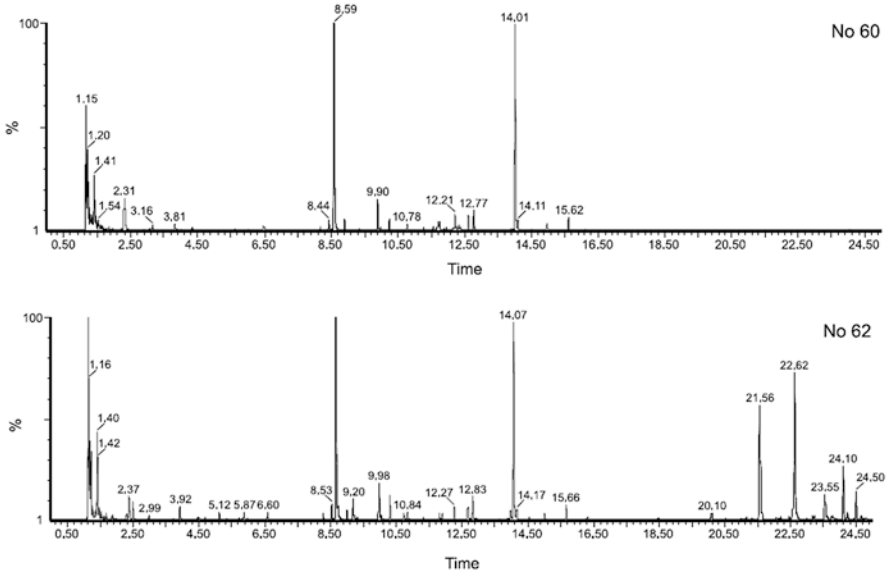


Fig. 5.50 Pyrograms of two different paint samples, without derivatisation. Pyrolysis conditions: 750 °C without derivatisation. Chromatographic conditions: 40 °C held for 2.5 min; heating at 10.5 to 320 °C min^{-1}; 320 °C maintained for 5 min. RTx-35MS chromatographic capillary column (30 m × 0.25 mm, 0.25 µm) (stationary phase 35 % diphenylpolysiloxane and 65 % dimethylpolysiloxane). Carrier gas: helium, 70 kPa. Reprinted from [110] with permission from Jakub Milczarek, Ph.D

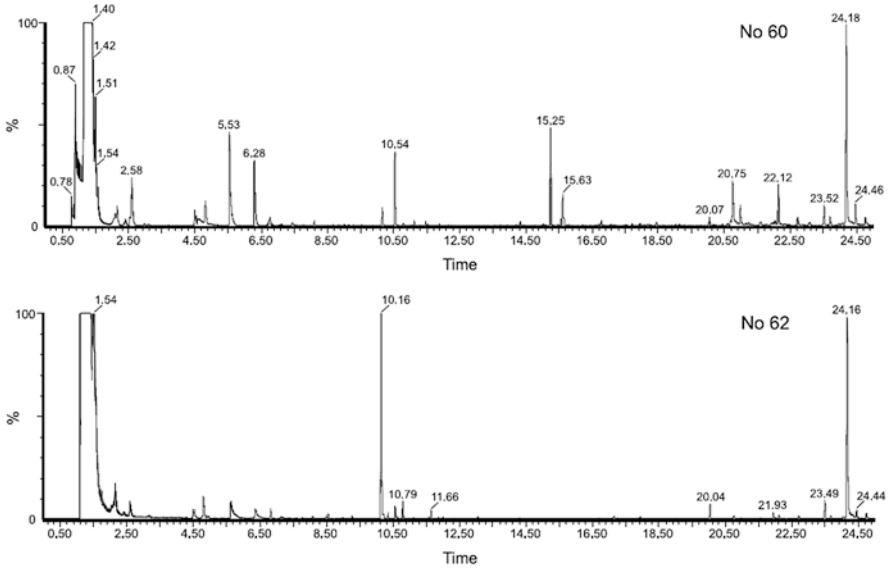


Fig. 5.51 Pyrograms of the same paint samples of Fig. 5.50, after derivatisation with TMAH at 400 °C. Pyrolysis conditions: 400 °C. Chromatographic conditions: 40 °C held for 2.5 min; heating at 10.5–320 °C; 320 °C maintained for 5 min. RTx-35MS chromatographic capillary column (30 m × 0.25 mm, 0.25 µm) (stationary phase 35 % diphenylpolysiloxane and 65 % dimethylpolysiloxane). Carrier gas: helium, 70 kPa. Reprinted from [110] with permission from Jakub Milczarek, Ph.D

urethane topcoat car paints, on the basis of the quantification of seven degradation products [111]. 36 paint samples of different origin, which were indistinguishable on the basis of their IR spectra and elemental composition were considered. Pyr-GC-MS allowed researchers to differentiate all of them with low false positive and false negative rates: 2.7 % and 5.5 %, respectively [111].

Pyrograms can be used for qualitative comparisons, based on the presence/absence of peaks. More quantitative approaches are of course desired, and several techniques for examining the pyrograms have been proposed. Zięba-Palus and coworkers for example, define three categories of peaks, as a function of the intensity relative to the largest peak height. Those higher than 10 % with respect to the largest peak are located in class A, those between 5 and 10 are part of class B, and the peaks with a relative height from 2 to 5 % are classified as C. Peaks smaller than 2 % in most cases are skipped because their reproducibility is unsatisfactory [112]. The peaks are then examined not only on a presence/absence criterion, but they also have to be in the same class for a positive comparison.

Yang and coworkers quantified the amount of styrene in paint samples and showed that this can be a relevant discriminating feature [114]. This paper is a good example of how an analytic parameter should be used for the characterisation of forensic items. In fact, possible interferences from the matrix, from external contaminations and from molecular weight were explored, concluding that they have little influence on the results.

The applications of Pyr-GC are by no means limited to the analysis of paints. This technique was shown to be reliable also for the classification of pressure-sensitive adhesives (Fig. 6.12) [118] and siliconic polymers [119].

In this latter class of materials, it was demonstrated that silicone materials formulated via differing cure chemistries have distinct degradation fingerprints, allowing the chemical origins of the degradation fingerprints to be relatively easily assessed. In this same work, Lewicki and coworkers showed that thermal history or irradiation had little effect on the pyrolysis fingerprint of silicone elastomers. Replicate data points were more scattered, but no significant modification of the pyrogram was observed [119].

It is not possible to generalise universal pyrolysis parameters valid for any polymeric sample, but it is safe to say that the temperature can be comprised between 600 and 1,000 °C, and the pyrolysis time is between 2 and 10 s, for a sample size of the order of 10–50 μg. The temperature which yields the most reliable and reproducible results must be assessed with preliminary tests on materials similar to those of interest in particular casework.

Mass spectrometry is surely the technique which is currently attracting the largest attention from the community of analytical chemists. However, this field is still somewhat underrepresented in the literature on the forensic analysis of traces. This is surprising, because sample introduction and ionisation approaches based on desorption allow to extract organic additives and small molecules from polymeric matrices without creating a significant damage and sometimes without the need of treating it with solvents. The very low level of fragmentation associated to desorption mass spectrometry allows to obtain an array of peaks in the mass spectra, each related to an individual component of the formulation.

Matrix-assisted desorption methods are the most commonly used. Kumooka proposed to use MALDI as a complementary technique for adhesive tapes [118, 120]. He was able to identify acrylate oligomers, emulsifiers, aliphatic resins, an aliphatic aromatic copolymer and other additives. This author performed MALDI after extracting the adhesive with solvents, tetrahydrofuran for acrylic-based adhesives and 2-butanone for rubber-based adhesives [120]. The extracts were then mixed with a matrix of dithranol and sodium trifluoroacetate and examined by MALDI. Figure 5.52 shows three examples of MALDI spectra acquired on three different rubber-based adhesives.

Three families of peaks can be identified in Fig. 5.52. There is a background of equally spaced peaks, related to fragments of polymer matrix, each one differing by one repeat unit. Other peaks, designated with the letters from 'a' to 'f', are due to additives of small molecular weight. Since MALDI ionises with little fragmentation, each of these peaks is a molecular ion or a base peak ion closely related to the

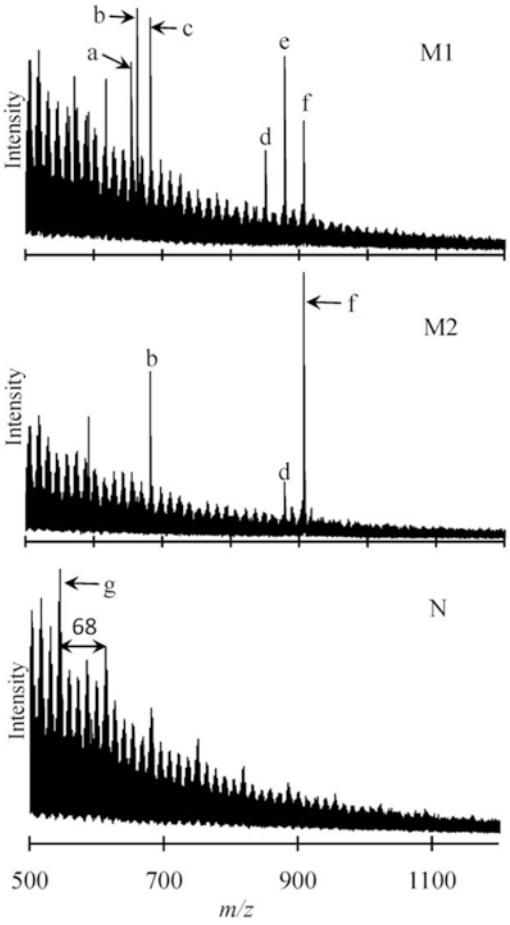


Fig. 5.52 MALDI spectra of the 2-butanone extracts of three different rubber-based adhesives. The *lowercase letters* indicate the signals due to additives present in the material. *Capital letters* indicate the classification of the tape. Reprinted from Ref. [120], copyright 2010, with permission from Elsevier

intact analyte molecule. From the *m*/*z* ratio associated to each of these peaks, the molecular weight of the corresponding additive can be calculated and the compound can possibly be identified. Finally a third class of signals that appears in Fig. 5.51 is the family of 'g' peaks. This is a homologue series of peaks, each separated by 68 g/mol, which is due to a polymeric additive, C5 petroleum resin. A number of additives are thus detectable. This result is similar to those obtainable by HPLC, i.e. the identification of additives, without the extensive method optimisation, sample size and long analysis time required by chromatography.

MALDI proved useful also for the analysis of inks on documents [121] and of very small amounts of fibres, even though conclusive evidence is missing that it is suitable for the single fibres common in casework [122]. A digestion or extraction step was suggested as necessary for carrying out MALDI on fibres. A matrix which works well on cationic and anionic dyes is a saturated methanol solution of 9-aminoacridine. This compound produces abundant analyte ions for both anionic acid dyes and cationic basic dyes, with the mass spectrometer operating in negative ion and positive ion mode, respectively. This gives the opportunity to quickly identify a dye as acid or basic by comparing the intensity of its signal in the negative and positive ion MALDI spectra.

Condom-related traces are optimal analytes for mass spectrometry. MALDI is very efficient, both on pure samples and on extracts from vaginal swabs. Extraction of the condom-related traces can be carried out with dichloromethane, followed by drying and re-solubilisation with methanol. Suitable matrices are 2,5-dihydroxybenzoic acid [123], dithranol in acetonitrile/dichloromethane/tetrahydrofuran (45:45:15 v/v) [124] and 2,5-dihydroxybenzoic acid and sodium chloride (75 mg/mL in acetone) [125]. Lubricants, additives and spermicides can be detected by this approach. MALDI can be used for imaging purposes as well. The very small diameter of the laser allows to acquire images with resolutions of the order of 100 μm × 100 μm. The laser is moved across the surface and in each point a MALDI spectrum is acquired. The data are collated, elaborated by a computer, and then images showing the distribution of a particular diagnostic ion on the probed surface can be created. Figure 5.53 shows how MALDI imaging evidences the presence of condom-related traces on the fingerprints [124].

Bradshaw and colleagues reported a viability study which demonstrated that MALDI imaging is suitable for detecting and documenting the presence of particular target chemicals in the fingerprints of a person who manipulated a condom.

Samples were prepared with a volunteer rubbing his fingers on the outer surface of a freshly opened condom a number of times, mimicking a real usage. The fingers were then rubbed together to form an even coating on the fingers and the index finger was pressed lightly against an aluminium surface, which was then sprayed with the matrix dithranol (10 mg/mL in a solution acetonitrile/dichloromethane/tetrahydrofuran 45:45:15 v/v).

The distribution maps of the ions originated by the spermicides nonoxynol-9 and octoxynol-9 clearly reproduces the ridge details of the fingerprint of the person who manipulated the condom.

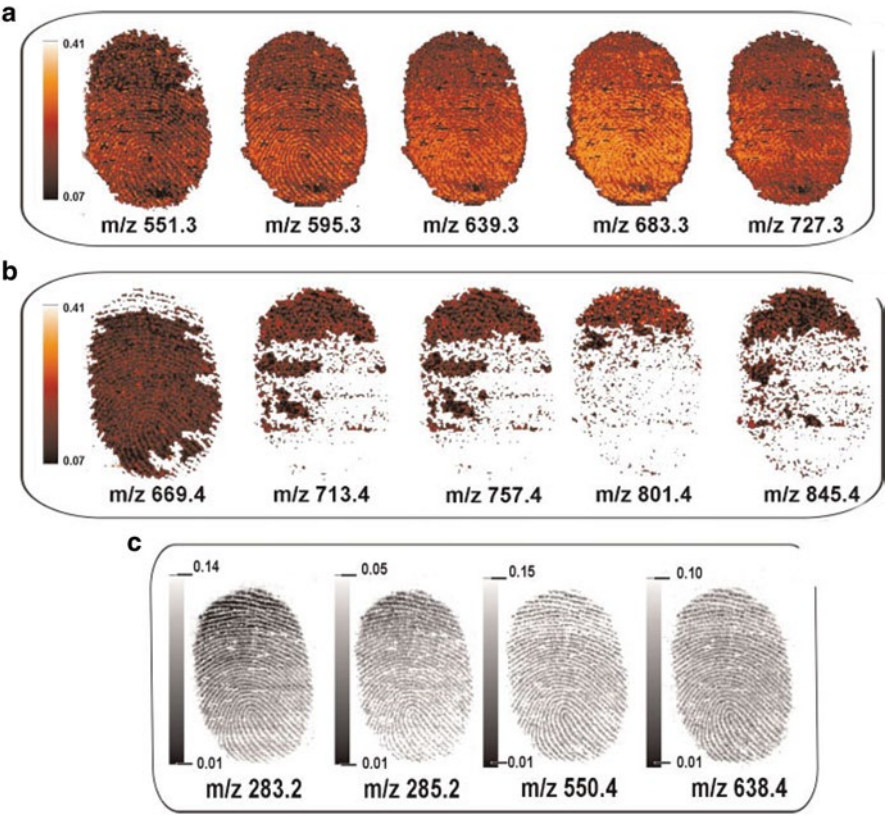


Fig. 5.53 MALDI MS images of a fingermark contaminated by condom traces. (**a**, **b**) Mass images of sodiated nonoxynol-9 ion series (from *m/z* 551.3 to 727.3) and sodiated octoxynol-9 ion series (from *m/z* 669.4 to 845.4). (**c**) Distribution of two endogenous fatty acids (oleic acid at *m/z* 283.2 and stearic acid at *m/z* 285.2), a phospholipid at *m/z* 638.4 and the exogenous dimethyldioctadecylammonium ion at *m/z* 550.4. Reprinted with permission from Ref.[124]. Copyright © 2011 John Wiley & Sons, Ltd

As a term of comparison, Fig. 5.53 shows the distributions of fatty acids which are produced by the sudoriparous glands. These faithfully reproduce the minutiae of fingerprints, and corroborate the viability of the use of MALDI imaging for the detection of latent fingermarks [124].

Desorption-Ionisation on silicon (DIOS) is an interesting alternative to MALDI. In this case, the sample is positioned on a special silicon surface [126, 127]. Such surface collects the laser energy, and releases part of it to the sample, which is desorbed and ionised and can be analysed by a mass analyser. Differently from MALDI, DIOS does not require to mix the sample with a matrix. Most samples are simply applied to the porous silicon surface, allowed to dry, and mass analysed. This greatly simplifies the sample preparation step and also the optimisation

of the method. Generally, MALDI works better than DIOS for analytes at high concentration (millimolar). The suitability of DIOS for analysing) condom-related traces was reported [127] and this technique was also applied to practical cases [126]. The spermicides nonoxynol-9 and octoxynol-9 and the common water-soluble lubricant poly(ethylene glycol) are very clearly detected, even in biological swabs [127].

Desorption electrospray-ionisation mass spectrometry (DESI-MS) was proposed as well for detecting condom-related traces. Compounds such as nonoxynol-9, polyethylene glycol, and polydimethylsiloxane, as well as small molecules such as *N*-methylmorpholine, *N*-octylamine, and *N,N*-dibutyl formamide are among the compounds that can be detected by DESI-MS [18]. Mirabelli and coworkers showed that DESI-MS can be used to acquire images of fingerprint such as those in Fig. 5.52, by which the contamination of hands which manipulated condoms can be detected [18]. The great advantage of DESI-MS imaging with respect to MALDI-MS imaging is of course the possibility of examining the surface of the item without any preliminary sample preparation step.

A further ambient mass spectrometry technique which showed its value in forensic science applications is direct analysis in real time (DART). The advantages of DART have been presented in Sect. 5.5 earlier in this chapter, along with some applications. Since DART does not expose neither the sample nor the operator to high voltage, to radiations or to sprays of solvents, this technique lends itself for the non-destructive and non-intrusive detection of contaminants or of illicit substances on a variety of substrates, such as the fingertips of a suspect. Laramée and colleagues reported many examples of DART application in forensic science [14]. Those of particular interest for the topics covered in this book are the detection of condom-related traces, of arson accelerants and of glues and other resins. The example regarding arson accelerants is especially valuable, because it was shown that residues of several solvents, of diesel fuel or of stove fuel could be detected on the burnt residues of a carpet, without any interfering signal due to the carpet fibres [14]. The difficulties associated to the disturbing effects due to the pyrolysis products of plastic objects were highlighted in Sect. 4.10.

Adams' work is an outstanding example of how the capabilities of DART can be exploited [128]. She characterised a population of 16 paper samples, differing by composition and production method. The manufacturing process of paper can be identified by the products in DART-MS spectra. Products derived from the thermal degradation of lignin characterise chemithermomechanical pulp paper: syringyl-lignin units are produced by cellulosic raw materials from hardwood, and lignin derivatives with the guaiacyl and coumaryl moiety result from the thermolysis of chemithermomechanical pulp paper from softwood. The kraft process implies an extensive delignification step, so no derivatives of lignin are detected in DART spectra of paper made by this process. Phytosteroids are volatilised from bleached hardwood kraft but not from bleached softwood kraft papers. Stone groundwood papers are obtained by the mechanical grinding of wood chips or of recycled paper, and they can be differentiated from the more refined papers listed above by the presence of two pinosylvin methyl ethers (*m*/*z* 227 and *m*/*z* 241), of hexadecanoic acid

(*m*/*z* 257), of three resin acids (*m*/*z* 301.2, 303.2, and 315); and four derivatives of β-sitosterol (*m*/*z* 397, 411, 413, and 429) [128].

Another destructive technique which can be applied, when sample size allows, for the characterisation of the formulation is thermogravimetry (TGA). Very few forensic applications of this method were reported [129, 130]. Ihms and Brinkman were the first to explore the potential of TGA for the forensic discrimination of polymeric items [129]. Materials such as polyvinyl chloride, chlorinated polyvinyl chloride, polystyrene, polypropylene, nitrile rubber, and nylon could be efficiently distinguished, even when samples were contaminated with silicates, organic matter, moisture and char. These authors had the analysis of blast fragments and arson debris in mind, but in principle the technique can be applied whenever necessary. A few years later, TGA was applied to the discrimination of latex gloves [130].

A population of 28 samples of latex gloves of different manufacturers was collected from different stores, either small hardware stores or large supermarkets. All the collected samples were similar and indistinguishable by visual observation. TGA revealed considerable differences between the different items. Measurements were performed with a temperature ramp extended from room temperature to 800 °C at 15 °C/min, in an inert atmosphere, N_2, or in air. This latter condition was the one yielding the richest and more complicated thermal behaviour. Degradation in nitrogen occurred in two steps, whereas heating in air triggered a degradation mechanism with at least three steps. The first two are due to the decomposition of the main poly-*cis*-1,4-isoprene and other minor organic substances present in the formulation. The step between 600 and 800 °C is associated with the pyrolysis of carbonaceous residues and ashes either already present in the latex or deriving from the thermo-oxidation of organic additives [131]. Figure 5.54 illustrates how useful the derivative curve of the thermogram can be for visualising distinguishing features between samples.

Quantification of the weight loss associated to each of the degradation steps is a reliable and efficient approach for the characterisation of the degradation behaviour of a material. In fact, the relative amount of material which is volatilised depends on the balance of the microstructure of the polymeric matrix (e.g. degree of branching, molecular weight etc.) and of the additives present in the formulation. These features are strictly related to the particular settings of the manufacturing process which every producer has, and so they are likely to be useful for discriminating mass-produced items of different origins (Fig. 5.55).

Latex gloves frequently contain a powder which facilitates their wearing. As such, a quantification of the residue could in principle be useful. However, they are very frequently found discarded on the ground, where they can collect impurities and contaminants which could decrease the reliability of such determination. This must of course be assessed on a case-by-case basis, because sometimes the conditions of the gloves recovered on the crime scene can be good and clean, and so a measurement of the residue would definitely become significant.

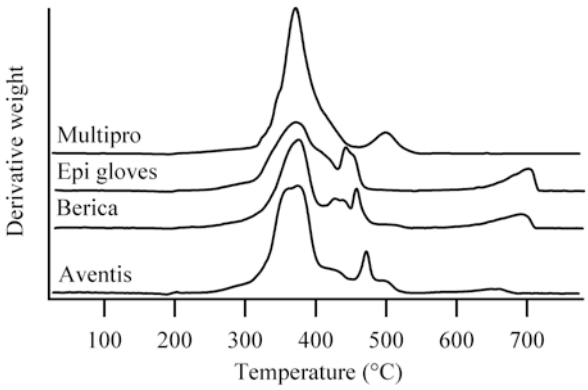


Fig. 5.54 Differential thermogravimetric curve of different latex gloves in air. Reprinted with permission from Ref. [130], copyright (2009), with permission from Elsevier

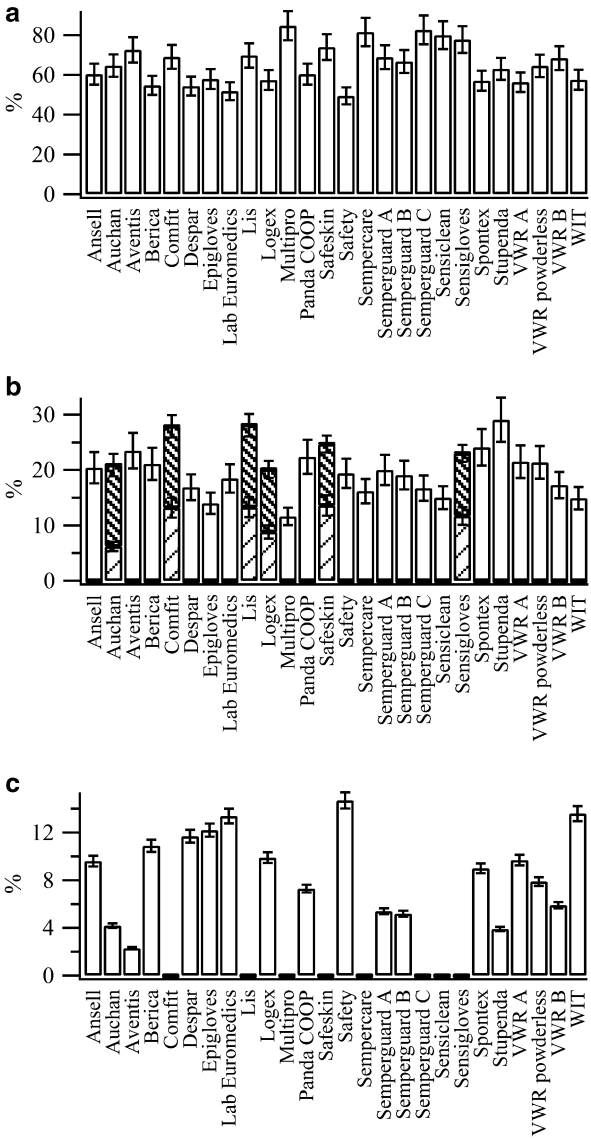


Fig. 5.55 Weight losses of a collection of latex gloves analysed in air: (**a**) between 200 and 400 °C, (**b**) between 400 and 500 °C, *hatched bars* are used for samples that degrade in two steps in this temperature interval, and (**c**) between 500 and 800 °C. Reprinted with permission from Ref. [130], copyright (2009), with permission from Elsevier

References

1. Cao X, Masatani P, Torraca G et al (2010) Identification of a mixed microparticle by combined microspectroscopic techniques: a real forensic case study in the biopharmaceutical industry. Appl Spectrosc 64:895
2. Jochem G, Lehnert RJ (2002) On the potential of Raman microscopy for the forensic analysis of coloured textile fibres. Sci Justice 42:215
3. Vitek P, Ali EMA, Edwards HGM et al (2012) Evaluation of portable Raman spectrometer with 1064 nm excitation for geological and forensic applications. Spectrochim Acta A Mol Biomol Spectrosc 86:320
4. West M, Ellis AT, Kregsamer P et al (2008) Atomic spectrometry update. X-ray fluorescence spectrometry. J Anal At Spectrom 23:1409
5. Crighton JS, Fairman B, Haines J et al (1997) Industrial analysis: metals, chemicals and advanced materials. J Anal At Spectrom 12:509R
6. Carter JF, Grundy PL, Hill JC et al (2004) Forensic isotope ratio mass spectrometry of packaging tapes. Analyst 129:1206
7. Fisher A, Goodall P, Hinds MW et al (2004) Atomic spectrometry update. Industrial analysis: metals, chemicals and advanced materials. J Anal At Spectrom 19:1567
8. McIntee E, Viglino E, Rinke C et al (2010) Comparative analysis of automotive paints by laser induced breakdown spectroscopy and nonparametric permutation tests. Spectrochim Acta B Atom Spectrosc 65:542
9. Ammann AA (2007) Inductively coupled plasma mass spectrometry (ICP MS): a versatile tool. J Mass Spectrom 42:419
10. Alamilla Orellana F, Gonzalez Galvez C, Torre Roldan M et al (2013) Applications of laser-ablation-inductively-coupled plasma-mass spectrometry in chemical analysis of forensic evidence. Trends Anal Chem 42:1
11. Fernandez B, Claverie F, Pecheyran C et al (2007) Direct analysis of solid samples by fs-LA-ICP-MS. Trends Anal Chem 26:951
12. Ettre LS (1993) Nomenclature for chromatography. Pure Appl Chem 65:819
13. Challinor JM (1999) Fibre identification by pyrolysis techniques. In: Robertson J, Grieve M (eds) Forensic examination of fibres. Taylor & Francis, London
14. Laramée JA, Cody RB, Nilles JM et al (2007) Forensic application of DART mass spectrometry. In: Blackledge RD (ed) Forensic analysis on the cutting edge. Wiley, Hoboken
15. Cody RB, Laramée JA, Nilles JM et al (2005) Direct analysis in real time (DART) mass spectrometry. JEOL News 40:8
16. Wiley JS, Shelley JT, Cooks RG (2013) Handheld Low-temperature plasma probe for portable "point-and-shoot" ambient ionization mass spectrometry. Anal Chem 85:6545
17. Ifa DR, Jackson AU, Paglia G et al (2009) Forensic applications of ambient ionization mass spectrometry. Anal Bioanal Chem 394:1995
18. Mirabelli MF, Chramow A, Cabral EC et al (2013) Analysis of sexual assault evidence by desorption electrospray ionization mass spectrometry. J Mass Spectrom 48:774
19. Takats Z, Wiseman JM, Gologan B et al (2004) Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. Science 306:471
20. Cody RB, Laramée JA, Durst HD (2005) Versatile new ion source for the analysis of materials in open air under ambient conditions. Anal Chem 77:2297
21. Musah RA, Domin MA, Walling MA et al (2012) Rapid identification of synthetic cannabinoids in herbal samples via direct analysis in real time mass spectrometry. Rapid Commun Mass Spectrom 26:1109
22. Czernik S, Elam CC, Evans RJ et al (1998) Catalytic pyrolysis of nylon-6 to recover caprolactam. J Anal Appl Pyrolysis 46:51
23. Menczel JD, Prime RB (eds) (2009) Thermal analysis of polymers: fundamentals and applications. Wiley, Hoboken

24. Hatakeyama T, Quinn FX (1994) Thermal analysis. Fundamentals and applications to polymer science. Wiley, Chichester
25. Cai Y, Lv J, Feng J (2013) Spectral characterization of four kinds of biodegradable plastics: poly (lactic acid), poly (butylenes adipate-co-terephthalate), poly (hydroxybutyrate-co-hydroxyvalerate) and poly (butylenes succinate) with FTIR and Raman spectroscopy. J Polym Environ 21:108
26. Cai Y, Lv J, Feng J et al (2012) Discrimination of poly(butylenes adipate-co-terephthalate) and poly(ethylene terephthalate) with Fourier transform infrared microscope and Raman spectroscope. Spectrosc Lett 45:280
27. Siroka B, Siroky J, Bechtold T (2011) Application of ATR-FT-IR single-fiber analysis for the identification of a foreign polymer in textile matrix. Int J Polym Anal Charact 16:259
28. Grieve MC, Griffin RME (1999) Is it a modacrylic fibre? Sci Justice 39:151
29. Robertson JR, Grieve M (eds) (1999) Forensic examination of fibres. Taylor & Francis, London
30. Grieve MC (1995) Another look at the classification of acrylic fibres, using FTIR microscopy. Sci Justice 35:179
31. Grieve MC, Griffin RME, Malone R (1998) Characteristic dye absorption peaks found in the FTIR spectra of coloured acrylic fibres. Sci Justice 38:27
32. Lv J, Feng J, Liu Y et al (2012) Discriminating amino resin paints from alkyd resin paints with two kinds of pigments in automotive coatings in forensic analysis by FTIR spectroscopy. Anal Lett 45:1079
33. Zieba-Palus J, Milczarek JM, Koscielniak P (2008) Application of infrared spectroscopy and pyrolysis-gas chromatography-mass spectrometry to the analysis of automobile paint samples. Chem Analityczna 53:109
34. Zieba-Palus J, Borusiewicz R (2006) Examination of multilayer paint coats by the use of infrared, Raman and XRF spectroscopy for forensic purposes. J Mol Struct 792:286
35. Caddy B (ed) (2001) Forensic examination of glass and paint: analysis and interpretation. Taylor & Francis, London
36. Ryland SG (1995) Infrared microspectroscopy of forensic paint. In: Humecki HJ (ed) Evidence in practical guide to infrared microspectroscopy. Marcel Dekker, New York
37. Flynn K, O'Leary R, Lennard C et al (2005) Forensic applications of infrared chemical imaging: multi-layered paint chips. J Forensic Sci 50:832
38. Szafarska M, Wozniakiewicz M, Pilch M et al (2009) Computer analysis of ATR-FTIR spectra of paint samples for forensic purposes. J Mol Struct 924–26:504
39. Chan KW, Tan GH, Wong RCS (2011) Forensic applications of IR spectral data at macro and micro levels: a study on plastic packages. Spectrosc Lett 44:440
40. Holman SW, Emmett TF, Cole MD (2012) A quantitative assessment of the chemical variation in food grade polyethylene cling film, a common wrapping material for illicit drugs, using attenuated total reflection-Fourier transform infrared spectroscopy. Anal Methods 4:1667
41. Causin V, Marega C, Carresi P et al (2006) A quantitative differentiation method for plastic bags by infrared spectroscopy, thickness measurement and differential scanning calorimetry for tracing the source of illegal drugs. Forensic Sci Int 164:148
42. Roux C, Bull S, Goulding J et al (2000) Tracing the source of illicit drugs through plastic packaging—a database. J Forensic Sci 45:94
43. Causin V, Marega C, Marigo A et al (2010) Forensic differentiation of paper by X-ray diffraction and infrared spectroscopy. Forensic Sci Int 197:70
44. Trejos T, Flores A, Almirall JR (2010) Micro-spectrochemical analysis of document paper and gel inks by laser ablation inductively coupled plasma mass spectrometry and laser induced breakdown spectroscopy. Spectrochim Acta B Atom Spectrosc 65:884
45. Spence LD, Baker AT, Byrne JP (2000) Characterization of document paper using elemental compositions determined by inductively coupled plasma mass spectrometry. J Anal At Spectrom 15:813

46. Spence LD, Francis RB, Tinggi U (2002) Comparison of elemental composition of office document paper: evidence in a homicide case. J Forensic Sci 47:648
47. Causin V, Marega C, Carresi P et al (2007) A quantitative differentiation method for plastic bags by wide angle X-ray diffraction for tracing the source of illegal drugs. Forensic Sci Int 168:37
48. Bacon SR, Ojeda JJ, Downham R et al (2013) The effects of polymer pigmentation on fingermark development techniques. J Forensic Sci 58:1486
49. Jones N, Mansour D, Stoilovic M et al (2001) The influence of polymer type, print donor and age on the quality of fingerprints developed on plastic substrates using vacuum metal deposition. Forensic Sci Int 124:167
50. Jones BJ, Downham R, Sears VG (2010) Effect of substrate surface topography on forensic development of latent fingerprints with iron oxide powder suspension. Surf Interface Anal 42:438
51. Palenik CS, Buscaglia JA (2007) Chemiluminescence in forensic analysis on the cutting edge. In: Blackledge RD (ed) New methods for trace evidence analysis. Wiley, Hoboken
52. Causin V, Casamassima R, Marega C et al (2008) The discrimination potential of ultraviolet-visible spectrophotometry, thin layer chromatography, and Fourier transform infrared spectroscopy for the forensic analysis of black and blue ballpoint inks. J Forensic Sci 53:1468
53. Wiggins K, Palmer R, Hutchinson W et al (2007) An investigation into the use of calculating the first derivative of absorbance spectra as a tool for forensic fibre analysis. Sci Justice 47:9
54. Parsons NS, Mountain C (2007) Investigating polyurethane foam as a form of trace evidence. Sci Justice 47:24
55. Hussain M, Khan KM, Ali SI et al (2009) Synthesis and properties of symmetrically substituted 4,4′-bis(1,3,5-triazinyl)-diamino stilbene-2,2′-disulfonic acid derivatives as UV absorbing and fluorescent whitening agents. Fibers Polym 10:407
56. Koskinen M, Wilen CE (2009) Preparation of core-shell latexes for paper coatings. J Appl Polym Sci 112:1265
57. Causin V, Casamassima R, Marruncheddu G et al (2012) The discrimination potential of diffuse-reflectance ultraviolet-visible-near infrared spectrophotometry for the forensic analysis of paper. Forensic Sci Int 216:163
58. Kher A, Stewart S, Mulholland M (2005) Forensic classification of paper with infrared spectroscopy and principal component analysis. J Near Infrared Spectrosc 13:225
59. Vann BC, Angel SM, Hendrix JE et al (2009) Analysis of titanium dioxide in synthetic fibers using Raman microspectroscopy. Appl Spectrosc 63:407
60. Minceva-Sukarova B, Mangovska B, Bogoeva-Gaceva G et al (2012) Micro-Raman and micro-FT-IR spectroscopic investigation of raw and dyed pan fibers. Croat Chem Acta 85:63
61. Was-Gubala J, Machnowski W (2014) Application of Raman spectroscopy for differentiation among cotton and viscose fibers dyed with several dye classes. Spectrosc Lett 47:527
62. Suzuki EM (2014) Infrared spectra of U.S. automobile original finishes (post-1989). VIII: in situ identification of bismuth vanadate using extended range FT-IR spectroscopy, Raman spectroscopy, and X-ray fluorescence spectrometry. J Forensic Sci 59:344
63. De Gelder J, Vandenabeele P, Govaert F et al (2005) Forensic analysis of automotive paints by Raman spectroscopy. J Raman Spectrosc 36:1059
64. Leona M, Decuzzi P, Kubic TA et al (2011) Nondestructive identification of natural and synthetic organic colorants in works of Art by surface enhanced Raman scattering. Anal Chem 83:3990
65. Cochran KH, Barry JA, Muddiman DC et al (2013) Direct analysis of textile fabrics and dyes using infrared matrix-assisted laser desorption electrospray ionization mass spectrometry. Anal Chem 85:831
66. Deconinck I, Latkoczy C, Gunther D et al (2006) Capabilities of laser ablation-inductively coupled plasma mass spectrometry for (trace) element analysis of car paints for forensic purposes. J Anal Atom Spectrom 21:279
67. Hobbs AL, Almirall JR (2003) Trace elemental analysis of automotive paints by laser ablation–inductively coupled plasma–mass spectrometry (LA–ICP–MS). Anal Bioanal Chem 376:1265

68. Gibson, WM, Kumakhov, MA (1992) Application of X-ray and neutron optics. Proc. SPIE 172
69. Patterson BM, Havrilla GJ (2006) Three-dimensional elemental imaging using a confocal X-ray fluorescence microscope. Am Lab 38:15
70. Nakano K, Nishi C, Otsuki K et al (2011) Depth elemental imaging of forensic samples by confocal micro-XRF method. Anal Chem 83:3477
71. Wiggins K (1999) Thin layer chromatographic analysis for fibre dyes. In: Robertson J, Grieve M (eds) Forensic examination of fibres. Taylor & Francis, London
72. Griffin R, Speers J (1999) High performance liquid chromatography. In: Robertson J, Grieve M (eds) Forensic examination of fibres. Taylor & Francis, London
73. Tagliaro F, Pascali JP, Lewis SW (2013) Capillary electrophoresis in forensic chemistry. In: Siegel JA, Saukko PJ, Houck MM (eds) Encyclopedia of forensic sciences. Academic, Waltham
74. Anastos N, Barnett NW, Lewis SW (2005) Capillary electrophoresis for forensic drug analysis: a review. Talanta 67:269
75. Issaq HJ (2002) Thirty-five years of capillary electrophoresis: advances and perspectives. J Liq Chromatogr Relat Technol 25:1153
76. Issaq HJ (2000) A decade of capillary electrophoresis. Electrophoresis 21:1921
77. Robertson J (1999) Capillary electrophoresis. In: Robertson J, Grieve M (eds) Forensic examination of fibres. Taylor & Francis, London
78. Stefan AR, Dockery CR, Nieuwland AA et al (2009) Forensic analysis of anthraquinone, azo, and metal complex acid dyes from nylon fibers by micro-extraction and capillary electrophoresis. Anal Bioanal Chem 394:2077
79. Stefan AR, Dockery CR, Baguley BM et al (2009) Microextraction, capillary electrophoresis, and mass spectrometry for forensic analysis of azo and methane basic dyes from acrylic fibers. Anal Bioanal Chem 394:2087
80. Vila A, Ferrer N, Mantecón J et al (2006) Development of a fast and non-destructive procedure for characterizing and distinguishing original and fake euro notes. Anal Chim Acta 559:257
81. Sonnex E, Almond MJ, Baum JV et al (2014) Identification of forged Bank of England 20 pound banknotes using IR spectroscopy. Spectrochim Acta A Mol Biomol Spectrosc 118:1158
82. Kazarian SG, Chan KLA (2010) Micro- and macro-attenuated total reflection Fourier transform infrared spectroscopic imaging. Appl Spectrosc 64:135A
83. Ricci C, Chan KLA, Kazarian SG (2006) Combining the tape-lift method and Fourier transform infrared spectroscopic imaging for forensic applications. Appl Spectrosc 60:1013
84. Lewis LL, Sommer AJ (2000) Attenuated total internal reflection infrared mapping microspectroscopy of soft materials. Appl Spectrosc 54:324
85. Wolfe J, Exline DL (2003) Characterization of condom lubricant components using Raman spectroscopy and Raman chemical imaging. J Forensic Sci 48:1065
86. Merrill RA, Bartick EG, Taylor JH (2003) Forensic discrimination of photocopy and printer toners. I. The development of an infrared spectral library. Anal Bioanal Chem 376:1272
87. Trzcinska BM (2006) Analytical differentiation of black powder toners of similar polymer composition for criminalistic purposes. Chem Analityczna 51:147
88. Egan WJ, Galipo RC, Kochanowski BK et al (2003) Forensic discrimination of photocopy and printer toners. III. Multivariate statistics applied to scanning electron microscopy and pyrolysis gas chromatography/mass spectrometry. Anal Bioanal Chem 376:1286
89. Nissen KE, Keegan JT, Byrne JP (1998) Characterization of plastic films from trace element analysis using inductively coupled plasma-mass spectrometry. Can J Anal Sci Spectrosc 43:122
90. Lichtfouse E (2000) Compound-specific isotope analysis. Application to archaelogy, biomedical sciences, biosynthesis, environment, extraterrestrial chemistry, food science, forensic science, humic substances, microbiology, organic geochemistry, soil science and sport. Rapid Commun Mass Spectrom 14:1337

91. Benson S, Lennard C, Maynard P et al (2006) Forensic applications of isotope ratio mass spectrometry—A review. Forensic Sci Int 157:1
92. Quirk AT, Bellerby JM, Carter JF et al (2009) An initial evaluation of stable isotopic characterisation of post-blast plastic debris from improvised explosive devices. Sci Justice 49:87
93. Benson SJ, Lennard CJ, Maynard P et al (2010) Forensic analysis of explosives using isotope ratio mass spectrometry (IRMS) - Part 2: Forensic inter-laboratory trial: Bulk carbon and nitrogen stable isotopes in a range of chemical compounds (Australia and New Zealand). J Forensic Sci 55:205
94. Reidy LJ, Meier-Augenstein W, Kalin RM (2005) 13C-Isotope ratio mass spectrometry as a potential tool for the forensic analysis of white architectural paint: a preliminary study. Rapid Commun Mass Spectrom 19:1899
95. Taylor E, Carter JF, Hill JC et al (2008) Stable isotope ratio mass spectrometry and physical comparison for the forensic examination of grip-seal plastic bags. Forensic Sci Int 177:214
96. Aziz N, Greenwood PF, Grice K et al (2008) Chemical Fingerprinting of Adhesive Tapes by GCMS Detection of Petroleum Hydrocarbon Products. J Forensic Sci 53:1130
97. Horacek M, Min J, Heo S et al (2008) The application of isotope ratio mass spectrometry for discrimination and comparison of adhesive tapes. Rapid Commun Mass Spectrom 22:1763
98. Blessing M, Jochmann MA, Schmidt TC (2008) Pitfalls in compound-specific isotope analysis of environmental samples. Anal Bioanal Chem 390:591
99. Dietz ME, Stern LA, Mehltretter AH et al (2012) Forensic utility of carbon isotope ratio variations in PVC tape backings. Sci Justice 52:25
100. Parsons NS, Lam MHW, Hamilton SE et al (2010) A preliminary investigation into the comparison of dissolution/digestion techniques for the chemical characterization of polyurethane foam. Sci Justice 50:177
101. Burger F, Dawson M, Roux C et al (2005) Forensic analysis of condom and personal lubricants by capillary electrophoresis. Talanta 67:368
102. Wang FC (2000) Polymer additive analysis by pyrolysis–gas chromatography: I. Plasticizers. J Chromatogr A 883:199
103. Wang FC, Buzanowski WC (2000) Polymer additive analysis by pyrolysis–gas chromatography: III. Lubricants. J Chromatogr A 891:313
104. Wang FC (2000) Polymer additive analysis by pyrolysis–gas chromatography: IV. Antioxidants. J Chromatogr A 891:325
105. Wang FC (2000) Polymer additive analysis by pyrolysis–gas chromatography: II. Flame retardants. J Chromatogr A 886:225
106. Burns DT, Doolan KP (2000) A comparison of pyrolysis-gas chromatography-mass spectrometry and Fourier transform infrared spectroscopy for the analysis of a series of modified alkyd paint resins. Anal Chim Acta 422:217
107. Burns DT, Doolan KP (2005) The discrimination of automotive clear coat paints indistinguishable by Fourier transform infrared spectroscopy via pyrolysis–gas chromatography–mass spectrometry. Anal Chim Acta 539:157
108. Burns DT, Doolan KP (2005) A comparison of pyrolysis–gas chromatography–mass spectrometry and fourier transform infrared spectroscopy for the characterisation of automotive paint samples. Anal Chim Acta 539:145
109. Burns DT, Doolan KP (2006) The automation of the acquisition and evaluation of pyrolysis-gas chromatography–mass spectrometry data for paint samples. Anal Chim Acta 571:25
110. Milczarek JM, Dziadosz M, Zieba-Palus J (2009) Way to distinguish Car paint traces based on epoxy primer layers analysis by pyrolysis-gas chromatography-mass spectrometry. Chem Analityczna 54:173
111. Zieba-Palus J, Zadora G, Milczarek JM (2008) Differentiation and evaluation of evidence value of styrene acrylic urethane topcoat car paints analysed by pyrolysis-gas chromatography. J Chromatogr A 1179:47
112. Zieba-Palus J, Zadora G, Milczarek JM et al (2008) Pyrolysis-gas chromatography/mass spectrometry analysis as a useful tool in forensic examination of automotive paint traces. J Chromatogr A 1179:41

113. Smalldon KW (1969) The identification of paint resins and other polymeric materials from the infra red spectra of their pyrolysis products. J Forensic Sci Soc 9:135
114. Yang S, Shen JY, Chang MS et al (2012) Quantification of vehicle paint components containing polystyrene using pyrolysis-gas chromatography/mass spectrometry. Anal Methods 4:1989
115. Wampler TP (2005) Analysis of automobile paints by pyrolysis-GC-MS. Lc Gc North Am 79
116. Wampler TP, Bishea GA, Simonsick WJ (1997) Recent changes in automotive paint formulation using pyrolysis gas chromatography mass spectrometry for identification. J Anal Appl Pyrolysis 40–1:79
117. Challinor JM (1995) Examination of forensic evidence. In: Wampler TP (ed) Applied pyrolysis handbook. Marcel Dekker, New York
118. Kumooka Y (2011) Classification of OPP adhesive tapes according to pyrogram of adhesives. Forensic Sci Int 206:136
119. Lewicki JP, Albo RLF, Alviso CT et al (2013) Pyrolysis-gas chromatography/mass spectrometry for the forensic fingerprinting of silicone engineering elastomers. J Anal Appl Pyrolysis 99:85
120. Kumooka Y (2010) Classification of OPP adhesive tapes according to MALDI mass spectra of adhesives. Forensic Sci Int 197:75
121. Heudt L, Debois D, Zimmerman TA et al (2012) Raman spectroscopy and laser desorption mass spectrometry for minimal destructive forensic analysis of black and color inkjet printed documents. Forensic Sci Int 219:64
122. Soltzberg LJ, Hagar A, Kridaratikorn S et al (2007) MALDI-TOF mass spectrometric identification of dyes and pigments. J Am Soc Mass Spectrom 18:2001
123. Hollenbeck TPE, Siuzdak G, Blackledge RD (1999) Electrospray and MALDI mass spectrometry in the identification of spermicides in criminal investigations. J Forensic Sci 44:783
124. Bradshaw R, Wolstenholme R, Blackledge RD et al (2011) A novel matrix-assisted laser desorption/ionisation mass spectrometry imaging based methodology for the identification of sexual assault suspects. Rapid Commun Mass Spectrom 25:415
125. Spencer SE, Kim SY, Kim SB et al (2011) Matrix assisted laser desorption/ionization-time of flight mass spectrometry profiling of trace constituents of condom lubricants in the presence of biological fluids. Forensic Sci Int 207:19
126. Shen ZX, Thomas JJ, Siuzdak G et al (2004) A case study on forensic polymer analysis by DIOS-MS: the suspect who gave us the SLIP (R). J Forensic Sci 49:1028
127. Thomas JJ, Shen Z, Blackledge R et al (2001) Desorption-ionization on silicon mass spectrometry: an application in forensics. Anal Chim Acta 442:183
128. Adams J (2011) Analysis of printing and writing papers by using direct analysis in real time mass spectrometry. Int J Mass Spectrom 301:109
129. Ihms EC, Brinkman DW (2004) Thermogravimetric analysis as a polymer identification technique in forensic applications. J Forensic Sci 49:505
130. Causin V, Marega C, Marigo A et al (2009) A method based on thermogravimetry/differential scanning calorimetry for the forensic differentiation of latex gloves. Forensic Sci Int 188:57
131. de Oliveira LCS, de Arruda EJ, Favaro SP et al (2006) Evaluation of thermal behavior of latex membranes from genetically improved rubber tree (Hevea brasiliensis). Thermochim Acta 445:27

Chapter 6
Synthesis-Dependent Parameters: Molecular Weight, Constitution and Configuration

As outlined in Chap. 1, any polymeric object is actually a composite, containing a number of ingredients with different functions. Of course, the matrix is the fundamental component because it constitutes the largest portion of the material. The scheme in Fig. 1.1 shows that the characterisation of the polymer matrix can be organised along two main directions. One is focused on the molecular features directly related to the polymerisation process, the other is centred upon the description of the structure and morphology attained by the material. In the former approach, the constitution and the configuration (Sect. 2.7) of the polymer are investigated. Constitution defines which repeat units are present in the macromolecules and how they are connected together. Section 5.7 introduced the issue of the identification of the polymer matrix, mostly with the aim of broadly classifying the type of material within the families described in Sect. 2.8. However, much more information can be obtained, in order to discriminate between materials pertaining to the same class. Molecular weight, the presence of comonomers or the regularity of the sequence of repeat units are constitutional features which can be exploited for this purpose. The tacticity of the polymeric chains, i.e. the evenness of the succession of the configuration of the repeat units, is another important attribute which is directly dependent on the ability of the polymerisation process to control the stereoregularity of the synthesis. All these characteristics cannot be modified without breaking chemical bonds, and are therefore the result of the reactions involved in the synthesis of the material. On the other hand, processing has a very negligible role in influencing them.

This chapter discusses how information regarding the constitution and the configuration of polymers can be acquired and how it can be useful in a forensic context.

V. Causin, *Polymers on the Crime Scene,*
DOI 10.1007/978-3-319-15494-7_6

6.1 Average Molecular Weight and Molecular Weight Distribution

Many years after the homicide of a usurer, his tomb was violated and his coffin was stolen. The casket was found a few hours later on the bank of a river, not far from the graveyard. The police immediately directed their investigations towards the person who was, at the time of the homicide, suspected as the killer of the usurer but who was later acquitted of this crime. Searches were conducted at the suspect's premises and a van with damaged rear lights was found (Fig. 6.1).

In the vicinity of the broken tombstone, small pieces of red and transparent plastic were retrieved (Fig. 6.2), which were soon identified as fragments of rear reflectors.

The shapes of the fragments and of the broken van's taillights perfectly matched, showing that the van had entered the graveyard and hit the marble wall where the usurer's tomb was located.

Paint traces left on the same marble wall were as well positively compared with the paint of the van, therefore further corroborating the conclusion. Moreover, fragments of the metal decorations of the coffin were found in the storage space of the van. Even though conclusive evidence was available, this case spurred interest on a more in-depth analysis of the materials used for manufacturing car and other vehicles' taillights. A very limited chemical variability

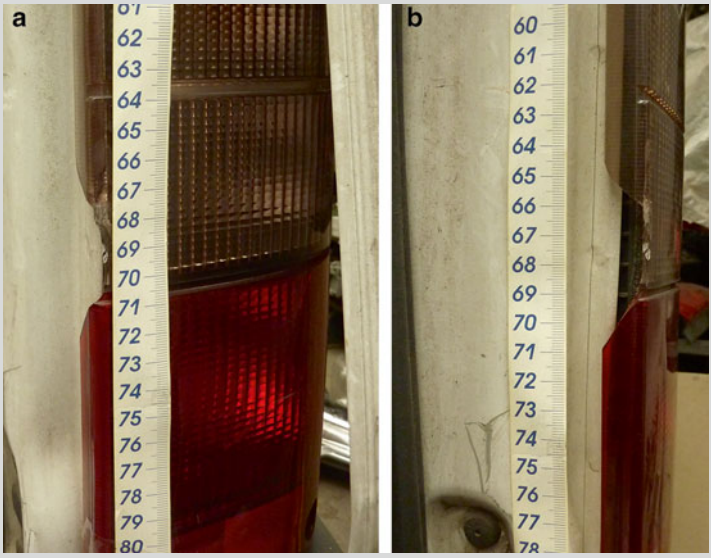


Fig. 6.1 Taillight of the suspect's van. (**a**) Front view, (**b**) Lateral view

(continued)

Fig. 6.2 Plastic items found in the vicinity of the wall where the broken tomb was. The picture on the *right* shows the perfect correspondence of the items with the broken part of the tail reflector

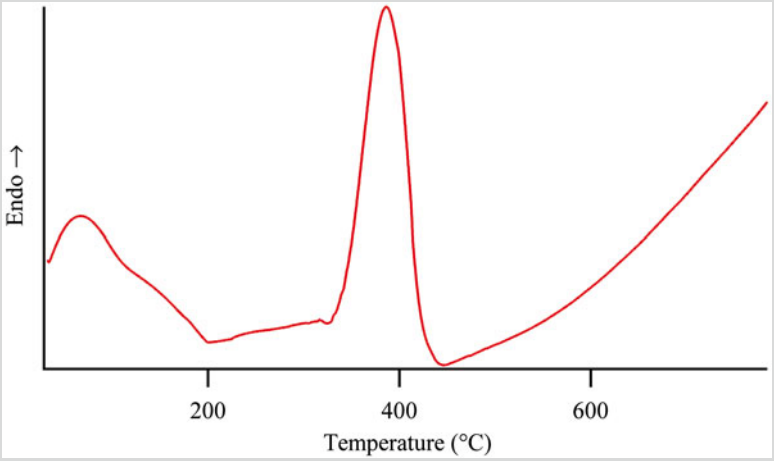


Fig. 6.3 DSC thermogram of a polymethylmethacrylate taillight

was found, with poly(methyl methacrylate) (PMMA) as the most common material used. The analysis of colour through different approaches allowed for a good degree of differentiation of the items in the population at disposal. Further information was related to the molecular weight of the polymers used for manufacturing these car parts. By thermal analysis techniques, namely by differential scanning calorimetry (DSC), the heat of degradation of PMMA was measured (Fig. 6.3). More information on DSC can be found in Sect. 7.2.2, but suffices here to say that this is a technique which can be used to measure the heat associated with transitions between physical states or to chemical

(continued)

reactions. PMMA degrades by depolymerisation, i.e. by the 'unzipping' of the polymer chain yielding back the monomers [1]. Since depolymerisation consists in the deconstruction of the macromolecules, the heat evolved in this process is logically related to the quantity of monomers which on average are contained in the polymer chain.

Applying this approach to a collection of 27 taillights, it was found that the heat of depolymerisation ranged from 466 to 672 J/g, with relative uncertainties of the order of 3–5 %. This allowed to identify three clusters of samples in the population. More interestingly, this allowed to verify the validity of an approach based on an indirect measurement of molecular mass for the forensic characterisation of materials.

Molecular weight is what makes polymers unique substances deserving a special and individual treatment in the discipline of chemistry. The multifaceted nature of the concept of molecular mass of polymers offers a very rich array of analytical data that can be exploited by the forensic scientist for obtaining a detailed and thorough characterisation of the items of interest.

Many properties of plastics, especially those related to mechanical performance, derive from the interlocking of macromolecules. Of course, the longer the polymer chains, the more entangled they will be, and thus the harder and tougher the material will be. A fundamental role is also played by the distribution of molecular weights, because the availability of short chains within the sample 'lubricates' the relative motion of the macromolecules, tuning the viscous and elastic properties of the material. It is worth noting that, by changing the value and/or the distribution of molecular weight, it is possible to tune the physical–mechanical properties of a material without changing its chemistry. Moreover, the sensitivity of performance from molecular weight is quite high, therefore slight variations in the latter feature brings about quite drastic alterations to physical properties. Obviously, different manufacturers of the same product, for example polyester fibres or polyethylene garbage bags, will use different raw materials, synthesised differently and specified according to the properties required for the end product. Since molecular weight is one of the variables in the structure–property equation, its potential for being one of the distinguishing features for discriminating or tracing the source of plastic items seems clear.

Due to the critical implications that the knowledge of the size of polymer molecules has on the industrial applications of these materials, many different techniques exist in polymer science for characterising the average molecular weight and the distribution of molecular weight of a polymer. However, due to the extensive sample mass required or the intrusive sample preparation, some methods of determination of the polymer average molecular weight, such as those based on measures of colligative properties, light scattering or viscosity, are not likely to find any application in forensic science, and thus deserve much less attention.

One final word of caution, before proceeding towards the discussion of the techniques for measuring the molecular mass of polymers, should be given. Molecular weight can be altered by possible degradation pathways likely to decrease the size of polymer chains. This is especially relevant for items coming from a crime scene which has been exposed for long times to the elements, UV light especially. When setting up an analytical protocol, the sensitivity of the results to such chemical processes induced by the exposure to the elements must be tested.

6.1.1 The Techniques: Size Exclusion Chromatography

What is size exclusion chromatography?
Size exclusion chromatography (SEC) is an analytical method that is used to determine the size of polymer molecules.

Why use this technique?
The molecular weight and the distribution of molecular weights are features directly dependent on the synthetic procedure followed for manufacturing the material. This information is very useful especially in case of comparisons of items with a very simple formulation. A typical example is polyester fibres, which are less variable in composition, being almost invariably composed by poly(ethylene terephthalate) (PET) [2]. Given this extremely small variation in composition, the problem of classification of polyester fibres, especially undyed ones, is very relevant in the forensic science field.

Another instance of application of SEC is on polymeric materials which are easily soluble but which are, due to their heterogeneity, difficult to analyse. An example is pressure-sensitive tapes. These sticky materials easily tend to attract contaminants from the surrounding environment, therefore preventing reliable analyses of the content of organic and inorganic species. Information on the average molecular mass and on its distribution can well complement simple qualitative identification with quantitative data.

Where can this technique be found?
It is not overly difficult to find a laboratory equipped with a SEC instrument. Polymer science laboratories in any university, in addition to middle-big sized commercial analytical laboratories will make this technique available. Due to its very rare application in routine forensic cases and the scarcity of scientific forensic literature, the diffusion of SEC into governmental or police forensic facilities is not widespread. The cost of SEC analyses is in the range of 300 € per sample.

SEC, sometimes also called gel permeation chromatography (GPC), is a type of chromatography where separation is achieved on the basis of the size and shape of the molecules.

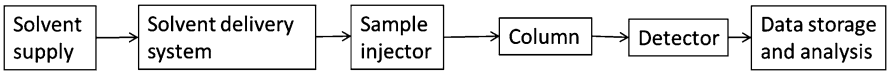


Fig. 6.4 Schematic of a SEC apparatus

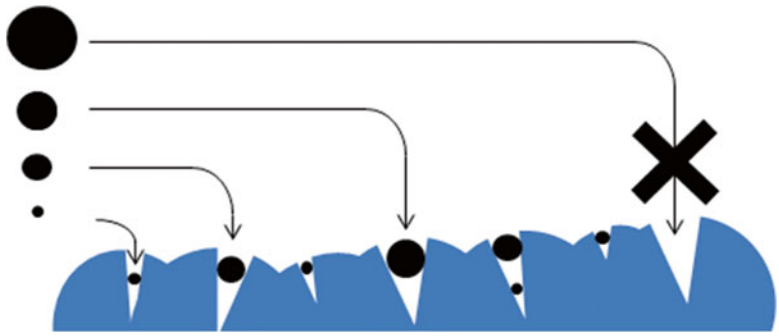


Fig. 6.5 Scheme of the mechanism of size selection by a SEC resin. Small molecules can penetrate any pore and so they spend a long time interacting with the column, whereas big molecules are excluded from the pores due to their large size and so they pass through the column without significant interaction with the resin

As may be seen from Fig. 6.4, which shows a schematic of a SEC apparatus, the instrumentation needed for performing SEC is not different from a typical liquid chromatography setup. Once the sample has been suitably dissolved, it is introduced via an injection mechanism into a set of columns. Each substance contained in the sample will be subject to a different mix of interactions between mobile and stationary phase, and thus will elute (exit the column) in different times, therefore obtaining the separation of the sample mixture. In other words, all the components of the sample enter the column together and exit the column and reach the detector separately.

The heart of a SEC system is the column, in which the stationary phase is a molecular sieve. The most common technical solution for manufacturing such sieves is using a cross-linked polymeric open network, which behaves as a porous gel. A very common material used for manufacturing SEC columns is a styrene-divinylbenzene copolymer. Polymer molecules larger than the largest pores cannot penetrate the gel particles and so they will pass through the column, without any interaction with the stationary phase (Fig. 6.5). Such big molecules will exit the column before all other analytes. No fractionation can be performed for molecules larger than the largest pore, whose size is defined as the exclusion limit (Fig. 6.6).

On the other hand, molecules smaller than the smallest pores will be able to access every cavity in the network (Fig. 6.5), and so they will spend the longest time within the stationary phase, exiting the column after all the other species present in the system. In this case, fractionation is not possible for molecules smaller than the smallest pore (the size of this pore is called the permeation limit), because they are all subject to the maximum interaction with the stationary phase (Fig. 6.6).

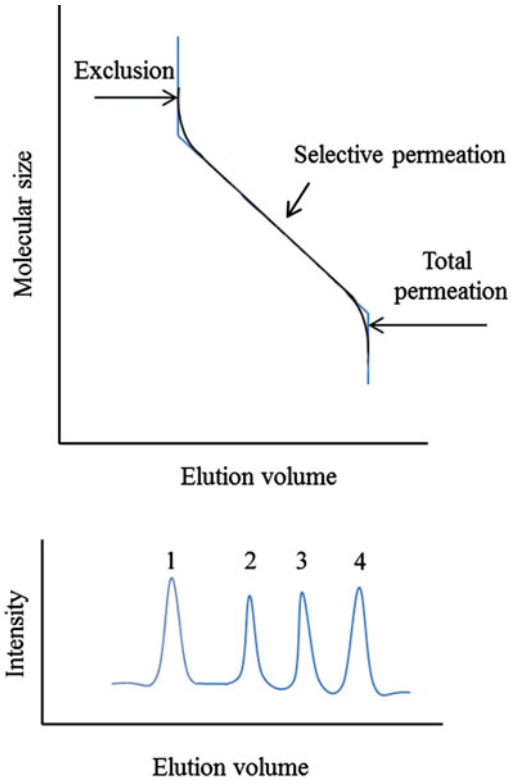


Fig. 6.6 Elution curve schematically showing the range of elution volumes that are valid for a particular column. Molecules larger than the exclusion limit are totally excluded and eluted without discrimination, molecules smaller than the total permeation limit are able to permeate all the pores, and so they are eluted without discrimination as well. The bottom diagram is a chromatogram showing an example of GPC analysis of four samples with different average molecular weight. Sample 1 has an average molecular weight larger than the exclusion limit, sample 4 has an average molecular weight smaller than the total permeation limit, samples 2 and 3 have average molecular weights comprised within the selective permeation range, and so they can be effectively separated on the basis of their molecular size

Molecules with a size ranging from the permeation to the exclusion limit can penetrate the gel network to different degrees, therefore being retarded in their path through the column by different degrees.

Macromolecules will be eluted in order of decreasing molecular size. For the sake of rigour, it should be emphasised that actual fractionation happens on the basis of the size and shape of the molecules, rather than strictly of molecular weight. This is relevant, because the size and shape of molecules depend on the solvent in which they are dissolved, whereas molecular weight is not dependent on such condition. However, correlation through a universal calibration curve allows to obtain molecular weight values from a SEC measurement [3]. Calibration is essential, and can be done using a set of standards with a very narrow distribution of molecular weights.

Table 6.1 Correlation between pore size and molecular weight range of typical SEC columns with a styrene/divinylbenzene packing

Molecular weight range	Pore size
100–1,000	50 Å
1,000–18,000	500 Å
5,000–40,000	10^3 Å
10,000–200,000	10^4 Å
50,000–1,000,000	10^5 Å
200,000 to >5,000,000	10^6 Å
5,000,000 to ~20,000,000	10^7 Å
~1,000–10,000,000	Mixed bed—high
~100–100,000	Mixed bed—low

Commercially available sets of standards suitable for SEC include polystyrene or polymethylmethacrylate samples.

The most important specifications to take into account when choosing SEC columns for the experimental setup are the pore size and the temperature/solvent compatibility.

The pore size is measured in Angstroms (1 Å = 10^{-10} m). Table 6.1, which is compiled according to the values reported in application notes and in catalogues of SEC columns manufacturers, shows the correlation between the molecular weight and the pore size of styrene/divinylbenzene packings based on polystyrene chain length exclusion limits (in Angstroms). These values are just approximate, the actual molecular weight range analysable by a particular column depends on its packing, and it is specified by the manufacturer.

In contrast to HPLC, where the use of a single column is the norm, SEC almost always requires multiple columns to cover the whole range of molecular masses of the polymer sample. Columns that separate a wide range of molecular sizes are available. These are called ‘mixed bed’, ‘linear’ or ‘extended range’ and their stationary phase contains blends of different pore sizes, covering a broader molecular weight range than a column with a narrower pore size. However convenient and practical to use, these mixed bed columns suffer the drawback of a lower resolution than individual pore size columns.

The second important issue to be considered when choosing a column is its suggested operating range of temperatures and its compatibility with solvents. In SEC analyses, the columns are almost always heated to increase the resolution of the separation, to enhance the permeation process, to tune the viscosity of the solvent, and to reduce backpressure across the column. The compatibility with the solvent to be used for elution or for dissolving the sample is also a very important issue. Chemical interaction between the gel which lines the column must be avoided, to preserve the integrity of the material. De-swelling of the stationary phase must be prevented because this would bring about a modification of the pore size and pore size distribution, which would alter the separation efficiency and reliability.

As in all other HPLC instruments, the detector should be sensitive and, in principle, it should have a wide linear range, in order to respond to both trace amounts and large quantities of material if necessary. Forensic casework seldom allows for

big sample sizes, therefore sensitivity and low detection limit are the most important features, rather than the possibility of analysing large quantities of material. In forensic applications it may be also desirable to have a detector non-destructive to eluting components, therefore allowing, if desired, to collect them and store them for further analysis.

The most common detector in GPC is the differential refractometer, a 'universal' detector, since all compounds refract light. The refractive index of polymers is constant above approximately 1,000 MW, making the detector signal directly proportional to concentration.

UV absorbance detectors are much more sensitive, however at the expense of the universality of detection: only the species giving absorbance at a certain wavelength will be revealed. Additional advantages can be obtained by combining these two 'traditional' detectors with online light scattering detectors and viscometers. In such cases absolute molar masses and distributions can be measured, without the need for column calibration. A multiple detector setup is important for analysing branched polymer systems.

A fundamental issue when choosing an analytical method for forensic applications is sample size. A typical concentration suitable for SEC is 0.10 % (w/v). Injection volumes are of the order of some microliters. The solubility of polymers is often limited and not many good solvents exist for some materials, making the sample preparation step not straightforward at times. For example, some polyolefins need temperatures greater than 120 °C to dissolve, and are typically run in 1,2,4-trichlorobenzene at 140 °C.

6.1.2 Size Exclusion Chromatography: The Forensic Applications

The use of SEC for forensic applications was first suggested in 1979 by McGee and colleagues [4]. However, since that pioneering intuition, the examples of SEC applications in the forensic field are still extremely limited. Yoshio Kumooka, of the National Research Institute of Police Science in Japan was the first to successfully apply SEC to differentiate rubber-based pressure-sensitive adhesive used in adhesive tapes [5]. Although the main methods for characterising pressure-sensitive adhesives are elemental analysis, optical microscopy, pyrolysis-gas chromatography–mass spectrometry (Pyr-GC-MS) and infrared spectroscopy (IR) [6], sometimes the discrimination obtained by such techniques is not sufficient. In his report, Kumooka applied SEC to distinguish couples of adhesives which had the same Pyr-GC-MS pyrograms and IR spectra (Fig. 6.7). As can be seen in Fig. 6.8, the UV-detector (at 254 nm) of SEC was able to reveal differences in the lower molecular weight fractions of the two compared samples, evidenced by the appearance of different peaks at higher elution volume, around 6 mL. RI was not equally efficient for showing the differences between the samples. The chromatogram obtained by this detector showed the same pattern in both adhesives, albeit with slight

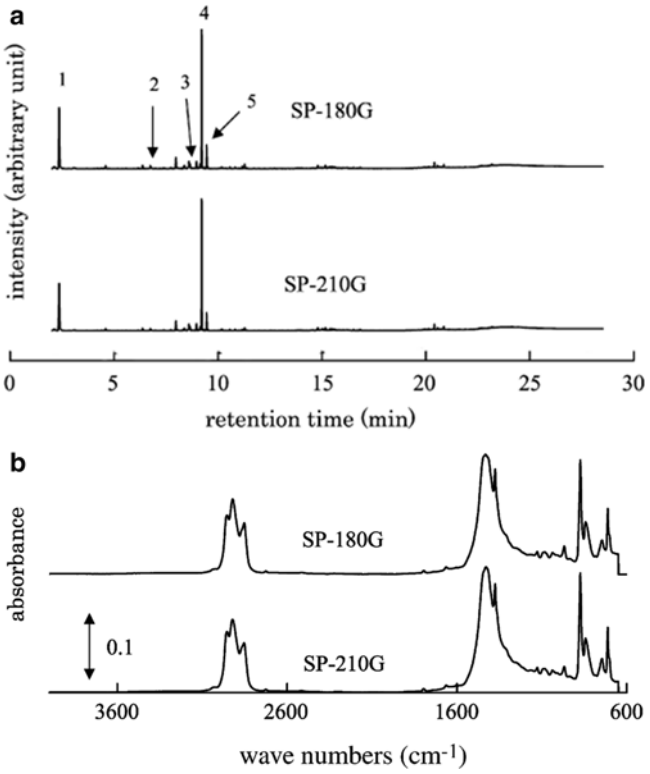


Fig. 6.7 Comparison of the characterisation of two pressure-sensitive adhesives: SP-180G and SP-210G. (**a**) Total ion pyrograms of SP-180G, SP-210G. Peak identities: (1) isoprene; (2) styrene; (3) methylstyrene isomers; (4) limonene; (5) indene; (**b**) ATR/IR spectra. Reproduced from Ref. [5], copyright 2007, with permission from Elsevier

differences in the relative intensity of peaks, not sufficient for objectively concluding for a negative comparison.

The sample size used by Kumooka is not small, because he analysed pieces of adhesive tape 1 cm long and 2 cm wide. However, such quantities are normally available in casework involving adhesive tape. Moreover, this approach is applicable in cases in which relatively large plastic items are present, e.g. car accident scenes, failure analysis or investigations on improvised explosive devices.

Very recently Domb et al. in Israel proposed SEC for a thorough characterisation of polyester fibres (namely poly(ethylene terephthalate), PET) indistinguishable by microscopical observation, thermal analysis and IR spectroscopy [7].

To perform SEC, remarkable problems were faced in finding a suitable solvent for PET and for the polystyrene samples needed for calibration. The optimal system identified by the authors was hexafluoropropanol in chloroform (4 % v/v). RI and UV (254 nm) detectors were used, but the sensitivity of the RI was not high enough,

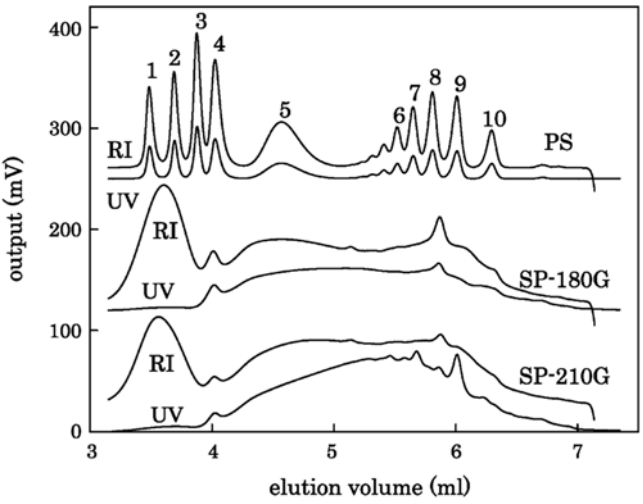


Fig. 6.8 SEC chromatograms of standard polystyrenes (PS), and of two pressure-sensitive adhesives: SP-180G and SP-210G. The chromatograms obtained using the detectors based on the variation in refraction index (RI) and the absorbance at 254 nm (UV) are shown for each sample. The numbers on the peaks in the top chromatograms indicate PS standards with the following average molecular weight: (1) 7.1×10^5 Da; (2) 1.9×10^5 Da; (3) 3.8×10^4 Da; (4) 1.0×10^4 Da; (5) 2.6×10^3 Da; (6) 682 Da; (7) 578 Da; (8) 474 Da; (9) 370 Da; (10) 266 Da. Reproduced from Ref. [5], copyright 2007, with permission from Elsevier

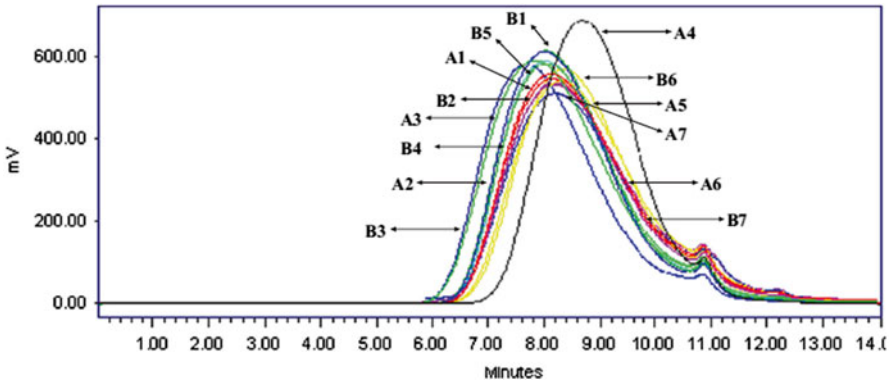


Fig. 6.9 SEC chromatograms of 14 different PET fibre samples collected from various commercial producers. Fibres were analysed by GPC using UV at 254 nm, mobile phase 2 % v/v HFIP in chloroform at a flow rate of 1 mL min^{-1} and 30 µL injection volume of 1 mg mL^{-1} fibre solution. Reproduced from Ref. [7], copyright 2014, with permission from Elsevier

and the only significant results were obtained by UV detection. Figure 6.9 shows the discrimination achieved on 14 PET fibres.

Interestingly, the authors of this report calculated the limit of detection of their SEC method, finding that measurable peaks could be obtained for concentrations as low as 1 µg mL^{-1} with an injection volume of 30 µL (Fig. 6.10).

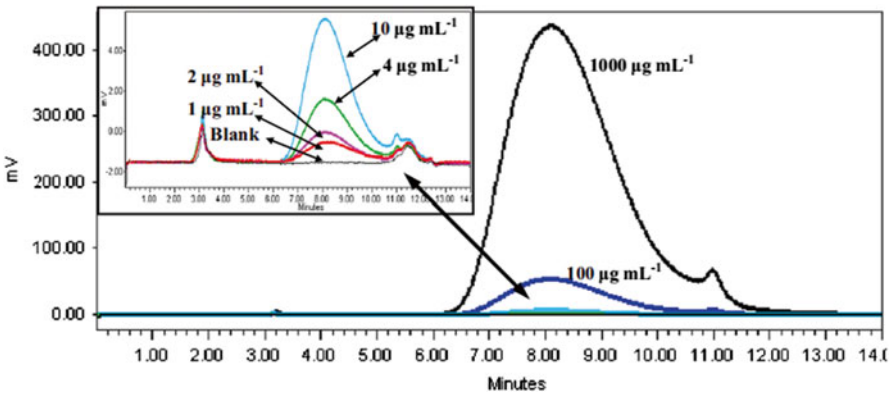


Fig. 6.10 GPC chromatograms for PET fibres at a concentration range of 1–1,000 µg mL^{-1}. GPC conditions are the same as in Fig. 6.9. Reproduced from Ref. [7], copyright 2014, with permission from Elsevier

Fig. 6.11 Fibre bundle found on a metallic fence, and later attributed to a suspect of burglary

Single fibre cases are obviously inaccessible for this approach, but if larger quantities of fibres are found on the crime scene it should be definitely taken into account. Figure 6.11 shows an example of a fibre bundle found on a fence which could be attributed to a burglar who penetrated private premises. The weight of this bundle is in the range of the 0.1–1 mg, large enough to perform SEC.

6.1.3 *The Techniques: Mass Spectrometry for Measuring High Molecular Weights*

What is mass spectrometry?
Mass spectrometry was presented in detail in Sect. 5.5. Mass spectrometry was indeed invented for measuring the mass of molecules or of chemical species, and so its natural application will be the assessment of the average molecular weight of polymers as well.

Why use this technique?
Mass spectrometry can in principle yield an absolute measurement of the average molecular weight and of the distribution of molecular weights of polymers. This information is available by SEC as well, but mass spectrometry has some advantages (and of course disadvantages). It is a direct method, therefore not needing any calibration. Moreover, samples can be analysed in any physical state, and especially in the solid state. As a consequence, there is no need to solubilise the sample and the integrity of the item is not jeopardised, since a negligible quantity of the material is enough for obtaining significant data. Among the drawbacks, difficulties associated to the ionisation step limit the maximum molecular weight which can be analysed. Macromolecules with a molecular mass beyond 50,000 g/mol are quite difficult to ionise and are therefore not analysable.

Where can this technique be found?
Mass spectrometry is a family of techniques which comprises a number of different methods and instrumental apparati. Laboratories specialised in mass spectrometry are usually located at universities or research centres, where the expertise necessary for the set up of ad hoc experimental protocols can be found.

Low molecular weight polymers, such as those found in lubricants or adhesives, are easier to analyse than larger macromolecules. Under this aspect, of course, the cost of the analyses and the time needed to optimise the parameters for a significant quantitation will be relatively lower for oligomers and small polymers than for higher polymers.

As described in Sect. 5.5, mass spectrometry produces, separates and detects ions in the gas phase. One of the requirements for the functioning of the technique is thus that the ions are gaseous. This is the point where the difficulties arise in the case of the materials covered in this book. Polymers are in fact exquisitely solid and non-volatile materials, so the majority of the traditional methods for sample inlet and ionisation (e.g. electron impact, chemical ionisation or field ionisation sources) are not applicable, if the mass of the macromolecules is of interest. In order to tackle this issue, and in order to increase the maximum analyzable mass beyond the 1,000 g/mol typical of traditional methods, the paradigm in ionisation was shifted

towards the exploitation of various desorption mechanisms. These allow to overcome the objective impossibility to volatilise polymers. Ionisation by desorption has also the advantage of avoiding the fragmentation of the molecules. Fragmentation is not a problem when mass spectrometry is used for identification purposes. As a function of the ionisation approach, the molecules will fragment following precise and repeatable rules. It is therefore possible, even though not always easy, to reconstruct the structure of the analyte from the fragments detected. However, fragmentation jeopardises the significance of mass spectrometry data if they are used for measuring the molecular weight of a sample. If one polymer chain with molecular weight 10,000 g/mol can be ionised without fragmentation, just one signal corresponding to 10,000 g/mol will be detected, with the result of an accurate representation of the original molecular weight of the analyte. However, if fragmentation occurs, the same polymer chain will produce several fragments, with at best the possibility to infer the original molecular weight of the analyte chain (in this example by summing all the weights of the individual fragments). This can be done if just one chain is analysed at a time, but becomes an impossible challenge in the real situation, in which many polymer molecules, with a distribution of sizes, coexist in the sample.

Probably the methods with the largest diffusion in the polymer science field are Field Desorption and Matrix-Assisted Laser Desorption/Ionisation (MALDI). Field Desorption has a maximum limit of measurable molecular weight around 10,000 g/mol [8–10], due to the onset of thermal decomposition of the polymer, quite low for practical forensic applications.

The mass range analysable by MALDI is much wider, so it is the most promising technique for performing mass spectrometry on polymers of forensic relevance. Other advantages are the ability to provide an absolute measurement of molecular mass and molecular mass distribution, in addition to information on the composition of the repetitive units and the nature of end groups. The sample preparation and the mechanism of functioning of MALDI were discussed in Sect. 5.5.

MALDI is now the method of choice for the determination of the molecular mass of proteins, even though it is well suited also for the synthetic polymers, more common in the casework of a forensic analyst [8, 11]. Differently from proteins, the ionisation of polymers happens by cationisation and not by protonation. Polar polymers such as (polymethylmethacrylate) [12, 13] or others [12, 14, 15] readily form addition products, i.e. adducts, with alkali metal ions. Non-polar polymers, such as polystyrene or polyethylene can be cationised using salts of transition metals like Ag or Cu [16–19].

Figure 6.12 shows that MALDI is able to yield information on the molecular weight of polymers with a remarkably high molar mass [20], even though most of the reports in the literature deal with polymers with a molecular weight below about 50,000 g/mol.

MALDI is particularly accurate for yielding the average molecular mass and the molecular mass distribution of samples with a polydispersity below 1.2, but the data become increasingly dependent on sample preparation and mass-dependent effects on the desorption/ionisation mechanism when the polydispersity increases above 1.6. No applications of MALDI for the measurement of the molecular weight of

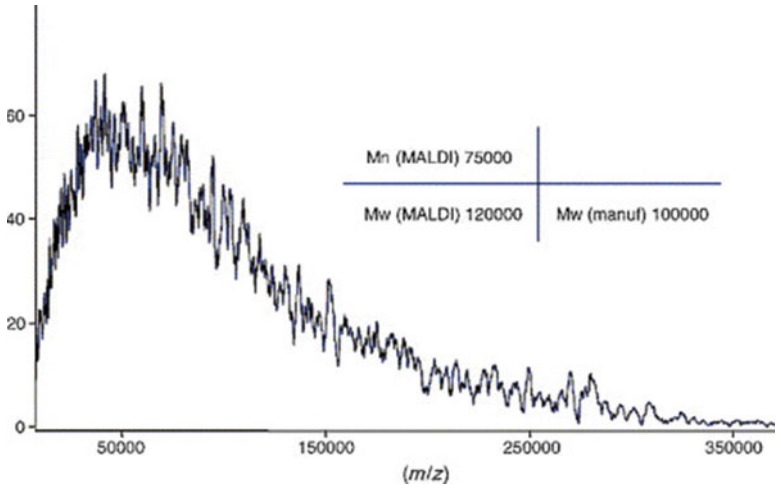


Fig. 6.12 MALDI-TOF MS spectrum of PEO 100,000. Reproduced with permission from Ref. [20]. Copyright © 2002 John Wiley and Sons, Ltd

synthetic polymers in the forensic field have been reported mainly because of the difficulty in identifying suitable matrices for the most commonly found commercial polymers with a high molecular weight.

Among the most recent ambient ionisation techniques, Direct Analysis in Real Time (DART) is the only one for which desorption of high polymers was reported [21]. However, in order to achieve such an aim, the temperature of the excited gas must be increased, with the consequence of desorption with fragmentation of the macromolecules. This brings about a loss of the knowledge on the pristine molecular weight of the chains. Moreover, the low energy involved in the technique is not high enough to desorb and ionise the biggest chains, and will be just enough to break the smaller molecules, with the risk of underevaluating the global molecular weight of the polymer. Therefore, as pointed out in Sect. 5.5, DART is a viable technique for the identification of the polymer, not for the measurement of the mass of high molecular weight matrices.

Even though from this short review the applicability of mass spectrometry to the characterisation of high polymers appears to be limited, there is indeed big scope for mass spectrometry in the forensic characterisation of polymers. This is especially true for low molecular weight synthetic polymers such as resins in inks [22], or in pressure-sensitive adhesives [23], or lubricants for condoms [24]. It should be moreover kept in mind that many small molecular weight species exist in the formulation of polymeric materials. Under this aspect, gentle ionisation sources such as those based on desorption processes are ideal, since they yield, in a single step, information on all the small molecules which coexist in these materials. Further insights on the issues related to the characterisation of the formulation of a polymeric item, are contained in Chap. 5.

6.1.4 *Other Methods for Molecular Weight Determination*

The methods just described for the determination of the molecular weight of a polymer are applicable to a rather large interval of molecular sizes. SEC allows the characterisation of macromolecules weighting from 100 to 10 million grams per mole. Mass spectrometry is much more limited but still capable to yield useful information for forensic purposes. The same can be said of the estimation of the number average molecular weight of a polymer sample by an end-group analysis. End groups are the extremities of the polymer chains which, usually, have a different chemical nature than the repetitive units which make up the most of the polymer. The rationale of this molecular mass measurement approach is that if, in a sample of given mass, there are fewer very long chains, the number of end-group moieties will be less than those present in the case the same samples were composed of a larger number of small chains. The scheme in Fig. 6.13 illustrates the two competing situations.

This technique can only be applied to polymers with suitable end groups, i.e. groups which can be detected in some way. An example are carboxyls that can be titrated [25]. Titrations usually require relatively big sample sizes, at least of the order of magnitude of the milligrams. However, smaller samples can be investigated by IR spectroscopy, by ratioing signals due to the end groups with respect to those related to atoms or bonds located in the backbone of the chain (NMR as well is suitable, however at the expense of a sample size comparable to that of titrations) [26–29]. The larger the signals due to end groups with respect to those related to the unit backbone segment, the lower the average molecular weight.

The sensitivity of the detection method used for quantifying end-groups limits the maximum measurable molar mass. A reasonable upper limit for the number average molecular weight that can be measured by end-group analysis is 15,000 g mol^{-1}. This value is lower than that of the majority of commercial structural plastic materials. However, this technique can still be useful in selected circumstances. Oligomers are more widespread than one may expect, because they are at the basis of the formulation

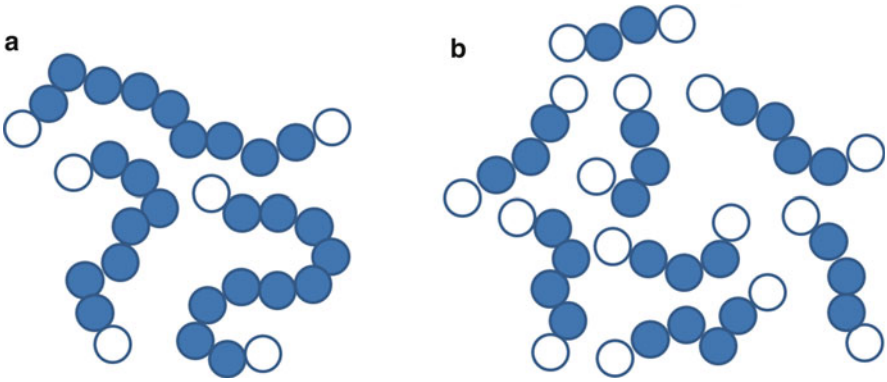


Fig. 6.13 Schematic of the difference in the amount of end groups when (**a**) the average molecular weight of the chains is large and (**b**) when it is small. The amount of repetitive units (*coloured circles*) is the same in both situations, but due to the fact that each chain contains two end groups (*white circles*) the ratio between end groups and repetitive units is much larger in case (**b**) than in case (**a**)

of lubricants, additives, paints or adhesives. A particular subset of end-group analysis is the determination of the acid number (or acid value or neutralisation value) of paints or adhesives. In such materials, adhesion is attained by reacting the end groups on prepolymers or oligomers in a crosslinking reaction which involves also the molecules located at the surface of the substrate. The number and availability of such reactive moieties on the precursors of the paint or adhesive resins can be evaluated by titration. Since these are key parameters for the functionality of these commercial products, their knowledge is very important in forensic engineering cases, when it is requested to understand the reasons for the failure of materials and machinery.

6.2 Comonomers

The partially decomposed body of a prostitute was found in a wood in central Italy. The doctor who performed the autopsy determined that she had been killed by suffocation, and in the general examination of the body, samples were taken of the material in the mouth of the victim.

Examination under the stereomicroscope revealed the presence of several red fibres which were identified, separated and washed (Fig. 6.14). IR spectroscopy allowed to classify the fibres as acrylic.

In parallel, the police focused on verifying if aggressions to other prostitutes had happened in the same period. The level of awareness was increased. Eventually a suspect who was harrassing a prostitute in the vicinities of the location where the corpse was found was apprehended.

His house was searched and all the red garments were collected. All the seized textiles were characterised and a preliminary compatibility emerged

Fig. 6.14 Fibres found in the mouth of the victim

(continued)

between the fibres found on the victim and those of the red sweater illustrated in Fig. 6.15.

The morphology and the UV/visible spectra were indistinguishable. However, the IR spectra were significantly different (Fig. 6.16). Even though both sets of fibres were within the class of acrylics, the sweater was made with poly(acrylonitrile-*co*-methyl methacrylate), whereas the fibres found on the victim were poly(acrylonitrile-*co*-styrene sulphonate)

The suspect was released from the charges. The culprit was later identified in another man, who was sentenced for the homicide of the woman.

Fig. 6.15 The sweater of the suspect which exhibited a preliminary compatibility with the fibres found in the mouth of the victim

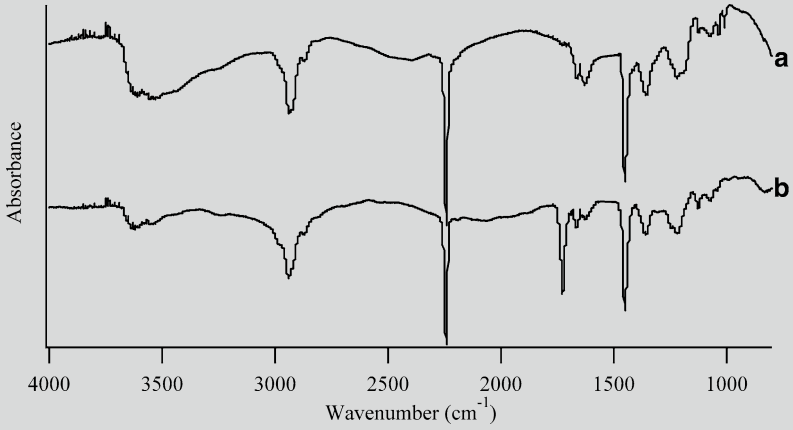


Fig. 6.16 IR spectra of (**a**) the fibres found in the mouth of the victim and (**b**) the fibres of the suspect's sweater

In most of the examples examined so far, we considered macromolecules all composed of a single repeating unit, and therefore they were homopolymers. Another important class of materials are industrially used though: copolymers, which are polymers containing different repeating units within the same molecule. Just as monomers are the precursors of the repeating unit of a homopolymer, a comonomer is a precursor of one of the different repeating units in the copolymer.

Copolymerisation is much more common in the industry than one may expect. Quite frequently in fact, when applications have complex requirements that cannot be met by a single homopolymer, it may be desirable to merge the qualities of different materials in a single one. This is where copolymerisation is done. The introduction of different monomers in the same chain is intended for yielding the resulting copolymer all the positive features of the individual building blocks, ideally minimising their defects.

The raw materials used for acrylic fibres are the ideal example explaining this concept.

As a homopolymer, polyacrylonitrile is rather difficult to dye and has a poor mechanical performance, making the textiles obtained thereof unsuitable for application in the clothing industry. The textile industry therefore implemented copolymerisation as a solution to this issue, introducing comonomer quantities which can reach 15 % [30]. Current legislation actually defines acrylic fibres as those containing no less than 85 % acrylonitrile moieties. The function of comonomers, mainly vinyl acetate (VA), methyl acrylate (MA) and methyl methacrylate (MMA), or less frequently polyvinylpyrrolidone (PVP) or styrene sulphonate (SS), is that of making the material more compatible to dyes and to enhance elasticity and in general physical–mechanical properties.

Figure 6.17 reports the chemical formula of such comonomers.

Obviously, manufacturers tune the nature and quantity of comonomer as a function of the characteristics requested.

The work of Grieve is still an unsurpassed guide for the characterisation of acrylics [31, 32], which can be carried out in an extremely deep level of details, to some degree even unexpected, by IR spectroscopy (Sect. 5.1.2).

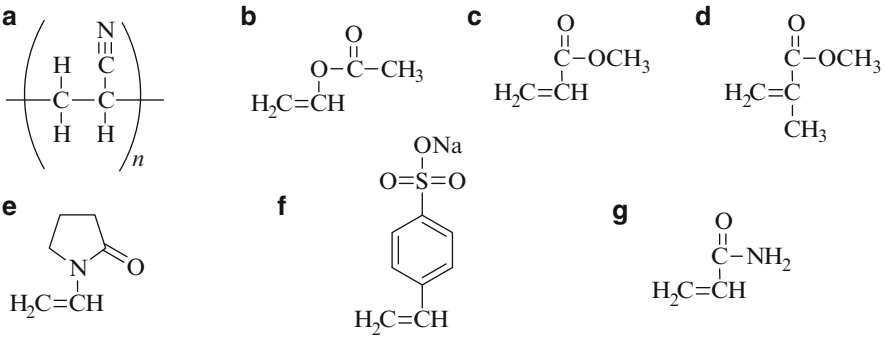


Fig. 6.17 Chemical formulas of the repetitive unit of (**a**) poliacrylonitrile, (**b**) vinyl acetate, (**c**) methyl acrylate, (**d**) methyl methacrylate, (**e**) vinylpyrrolidone, (**f**) styrene sulphonate, (**g**) acrylamide

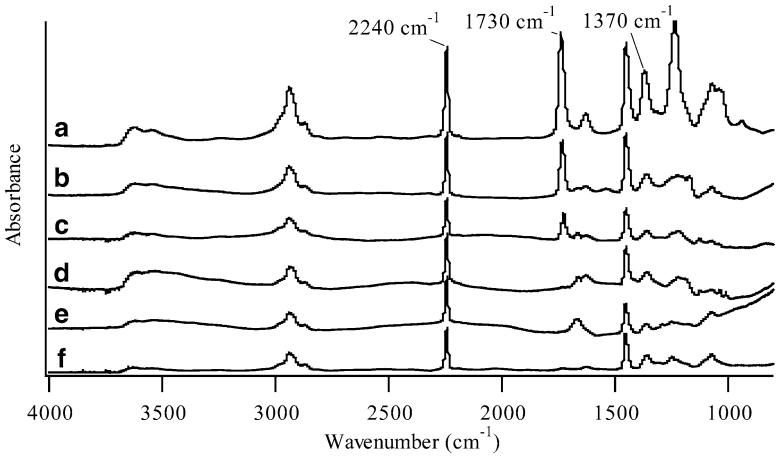


Fig. 6.18 IR spectra of (**a**) PAN/VA, (**b**) PAN/MA, (**c**) PAN/MMA, (**d**) PAN/SS, (**e**) PAN/PVP and (**f**) PAN homopolymer. Reprinted from Ref. [33], copyright 2005, with permission from Elsevier

An inspection of the IR spectra of acrylic fibres allows to identify the nature of the polymer. The peculiar peak characterising acrylic fibres is located at 2,244 cm^{-1}, attributed to the C≡N group in the polyacrylonitrile repetitive unit.

A strong IR absorption at 1,730 cm^{-1} is associated to a C=O bond, and in this case is related to the presence of a comonomer containing an ester group. An analysis of the spectral region around 1,200 cm^{-1} allows to identify its nature. A peak at 1,170 cm^{-1}, with smaller signals at 1,204, 1,229 and 1,250 cm^{-1} is indicative of methyl acrylate, an absorption at 1,240 cm^{-1} means that the ester is vinyl acetate, and bands at 1,220 and 1,130 cm^{-1} are due to methylmethacrylate (Fig. 6.18).

The absence of the signal at 1,730 cm^{-1} reveals that either no comonomer was employed (a quite unusual case, since homopolymer acrylic fibres are mainly for industrial use) or that alternatives to the more common ester comonomers were used. The latter is the case of styrene sulphonate with characteristic peaks at 1,036 and 1,011 cm^{-1}, polyvinylpyrrolidone with a major peak at 1,670 cm^{-1}, or acrylamide, with a strong absorption at 1,684 cm^{-1}.

A further level of investigation can be introduced by a quantification approach. A method has been reported by Tungol and coworkers [34], in which the ratio between the IR signals at 2,240 and 1,730 cm^{-1} was computed. The nitrile (C≡N) band at 2,240 cm^{-1} is originated from the AN comonomer, while the carbonyl (C=O) absorption at 1,730 cm^{-1} is due to carbonyl-containing comonomers or additives. The relative standard deviations found for repeated measurements on the same location of the fibre was below 0.2 %, along the length of the fibre was about 1.5 %, and among different fibres from the same sample was below 6 %.

An effect of fibre diameter was recorded on the precision of measurements, with a larger standard deviation on thinner fibres. This should not be considered a big issue, since IR spectroscopy would come, in an analytical protocol, later than observation

of the morphology under the microscope. Consequently, normally fibres with the same diameter would be compared by this quantitative method, with similar errors.

Albeit the efficacy of such approach was shown to be very satisfying, it suffered from the limitation of not being universally applicable. As can be seen in Fig. 6.18, the carbonyl band is significant only in the PAN/VA, PAN/MA and PAN/MMA copolymers, while absent in the others.

In order to overcome this shortcoming, another work [33] introduced a further parameter for the characterisation of the comonomer content, using the IR peak at 1,370 cm^{-1}, related to the C–H bonds of the macromolecular backbone [35], and so present in any acrylic fibre.

The potential of this approach for the classification of acrylic fibres is summarised in Fig. 6.19.

Many different production processes exist for acrylic fibres. The variations appear as early as in the polymerisation step, which can be carried out in suspension or in solution. Suspension polymerised materials can then be dry spun using dimethylformamide (DMF) as a solvent for re-dissolution, or wet spun with dimethylacetamide (DMA).

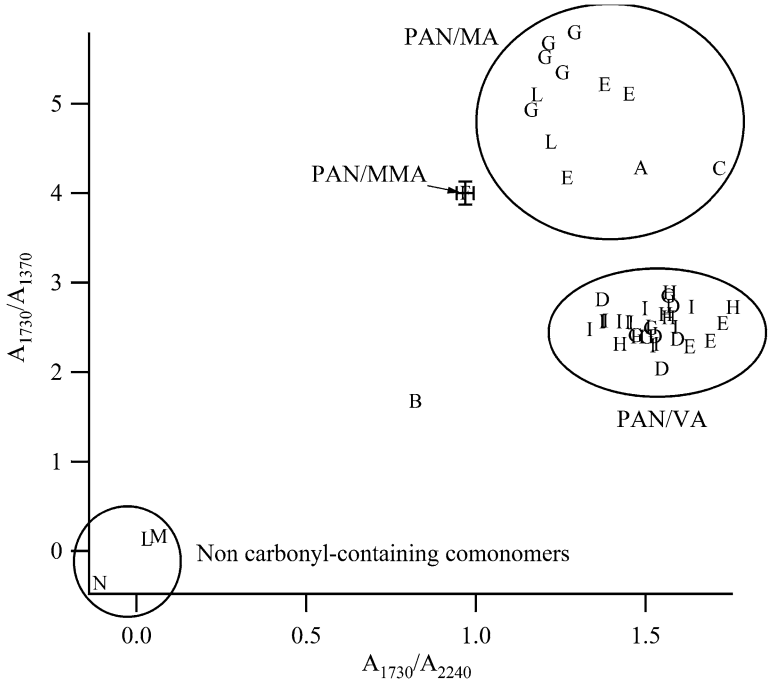


Fig. 6.19 Representation of 48 acrylic samples on the plane formed by ratios A_{1730}/A_{2240} and A_{1730}/A_{1370}. A_x indicates the absorbance of the IR peak located at x wavenumber. Data points are designated by a letter associated to the manufacturer of the fibre. The error bars are shown only for sample F to avoid confusion. Reprinted from Ref. [33], copyright 2005, with permission from Elsevier

Further information attainable by IR spectroscopy is the identification of solvent residues which can reveal which process was used for the production of the fibres being analysed. Very indicative under this point of view is a peak at 1,670 cm^{-1}, which is due to DMF residues. The presence or absence of this peak allows to divide acrylic fibres copolymerised with methylmethacrylate or methylacrylate in two groups, according to whether or not DMF was used in this spinning. It should be pointed out, though, that since processes using heat or water tend to reduce the amount of DMF, its search is reliable only on undyed fibres.

To improve and optimise even more the properties of their fibres, many producers introduced in the chain a third comonomer, in addition to the main ones discussed above. This third species is usually specifically chosen to provide the macromolecule with sites for the bonding of dyes. The small quantity of such components and the confidentiality with which they are treated by manufacturers makes their identification much more complex than that of the major comonomers. However, it is worth checking for a peak at 1,040 cm^{-1}, reflecting the presence of SO_3Na groups, which have proven to be very efficient in promoting the interaction with dyes.

Pyrolysis-based methods can be extremely useful in complementing IR spectroscopy for a thorough characterisation of acrylic fibres.

Almer [36] proposed a method based on (Pyr-GC-MS) (see Sect. 5.4 for a description of the technique) for the qualitative identification of acrylic fibres. Figure 6.20 shows a pyrogram obtained from an acrylic fibre, and Table 6.2 reports the peak assignments [37, 38]. The qualitative pattern for a typical acrylic fibre follows the basic profile of Fig. 6.20, irrespective of the copolymer formulation. The only significant difference is the appearance within the pyrolysis products of acetic acid, which is the degradation product of fibres containing vinyl acetate.

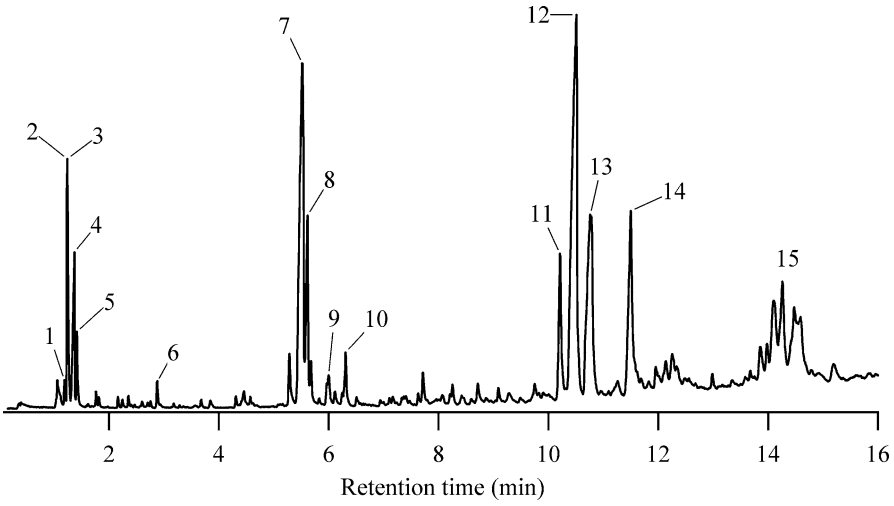


Fig. 6.20 Pyrogram of an acrylic fibre. Assignments of the labelled peaks are shown in Table 6.2. Reprinted from Ref. [39], copyright 2006, with permission from Elsevier

Table 6.2 Assignments of the peaks indicated in Fig. 6.25

Peak	Retention time (min)	Assignment
1	1.06	Hydrocyanic acid
2	1.19	Acetonitrile
3	1.24	Acrylonitrile
4	1.36	Acetic acid
5	1.42	Methacrylonitrile
6	2.88	1,3-Dicyanopropene
7	5.52	2,4-Dicyanobutene
8	5.61	1,3-Dicyanobutene
9	6.00	1,3-Dicyanopentadiene
10	6.11	1,3-Dicyanohexene
11	10.21	2,4,6-Tricyanohexene
12	10.51	1,3,5-Tricyanohexane
13	10.76	1,3,5-Tricyanopentane
14	11.50	1,3,5-Tricyanohexene
15	~14	Tetramers

Reprinted from Ref. [39], copyright 2006, with permission from Elsevier

Interestingly, acrylics containing styrene sulphonate and vinylpyrrolidone do not yield signals that can be associated with the degradation of the minor comonomers, making their pyrograms qualitatively indistinguishable from the more common fibres copolymerised with methylacrylate or methylmethacrylate.

As better discussed in Sect. 3.3, treatment of Pyr-GC-MS data of acrylics by PCA allowed to obtain a better discrimination of undyed fibres.

Another quite common copolymer encountered in daily life is poly(ethylene-*co*-vinyl acetate) (EVA), in which ethylene is copolymerised with up to about 40 % vinyl acetate.

The main applications of EVA are hot melt adhesives and in the packaging field, especially in food wrappings, in multi-layered films with other materials, such as polyethylene. EVA is a very popular material for the manufacturing of padding in equipment for various sports, but also used as a shock absorber in sports shoes. EVA is one of the materials of choice for slippers and sandals, due to its light weight, easy mouldability, good aesthetic properties, lack of odour, and especially because it is cheaper with respect to natural rubber.

Other applications of EVA, yet irrelevant for forensic casework, are in the biomedical engineering field, as a drug delivery device, or in the photovoltaics industry as an encapsulation material for crystalline silicon solar cells in photovoltaic modules.

The vast array of applications is linked to the versatile properties of this material, which can be tuned modifying the relative contents of the comonomers contained in the polymer chains.

Due to this wide availability of items produced with this material, it is possible that EVA traces are encountered on some crime scenes. This, coupled with the variability

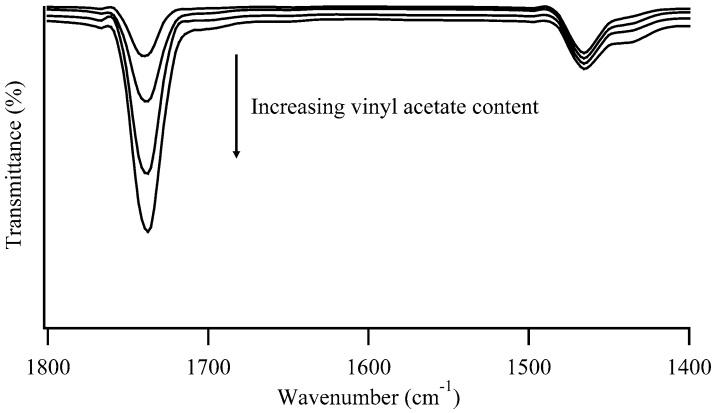


Fig. 6.21 IR spectra of EVA sample with an increasing vinyl acetate content (from top to bottom, 9 %, 16 %, 23 % and 30 %, respectively). The spectra were shifted along the *y* axis for clarity of representation

in composition of commercial EVA mentioned above, indicates that a quantitative approach for its characterisation can be desired. IR spectroscopy is indeed a promising technique, because it allows one to determine the relative quantities of each comonomer in the material. The IR spectrum is mainly dominated by the signal of polyethylene, with the addition of strong bands due to the vinyl acetate moiety at 1,240 and 1,738 cm^{-1}. It is worth reminding that both these peaks must be present for a positive identification of EVA, because only a peak at 1,738 cm^{-1} could be ascribed to carbonyl-containing additives, such as glycerol monooleate, used for polyethylene. The composition of the copolymer can be assessed ratioing the areas of two signals, one due to a functional group contained in vinyl acetate, and the other related to some bond characteristic of the polyethylene moiety. Particularly useful for this purpose is the ratio between the absorption signal of the C=O functional group of vinyl acetate at 1,738 cm^{-1} and that of the methylene bending vibrations at 1,466 cm^{-1} (Fig. 6.21). Alternatives are the acetate bending band at 610 cm^{-1} and the methylene rocking peak at 722 cm^{-1}.

The glue industry exploits quite deeply the potential of copolymers, since for achieving a good result, the adhesive must have properties which mediate those of the surfaces to be connected. Poly(styrene-*co*-butyl acrylate) is an example, along with several other types of copolymers containing acrylate-based comonomers, of this kind of approach. Styrene yields to the material resistance to alkali and to water, whereas butyl acrylate is responsible for flexibility and adhesive properties. Varying the relative quantities of these two components, it is feasible to tune the performance of the glue. Also in this case, given the structural difference between the comonomers, it is easy to identify IR signals due to functional groups present in styrene and in the acrylate moiety, namely the signal due to the deformation of the phenyl ring at 698 cm^{-1} and the C=O stretching absorption at about 1,700 cm^{-1}, respectively (Fig. 6.22).

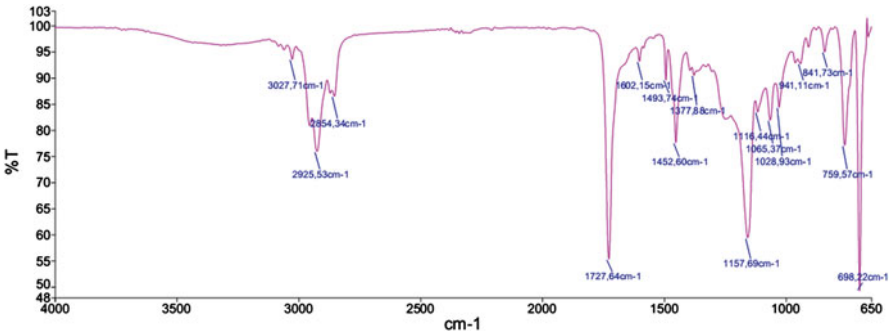


Fig. 6.22 IR spectrum of a poly(styrene-*co*-butyl acrylate) adhesive

Further interesting examples of copolymers whose structure can be elucidated by IR spectroscopy are ethylene–propylene copolymers. There are two major types of ethylene–propylene copolymers: those made with the two monomers alone (ethylene–propylene monomer, or EPM) and those made with amounts of the order of 5 % of a diene (ethylene–propylene–diene monomer, EPDM). EPDM, with the presence of a double bond covalently connected to the main chain, allows crosslinking of the macromolecules.

Polyethylene and polypropylene are not mutually soluble, so the closer this copolymer is to a block copolymer, the stronger the tendency to phase segregation that will be observed. The relative quantity of each comonomer, the distribution of the two building blocks along the chain and the technology used for the synthesis are fundamental parameters through which the properties of the material can be tuned. Operating on these process parameters, it is possible to obtain a large number of copolymers differing by chemical composition and morphology, with properties ranging from hard and stiff plastics to soft rubbery materials. Due to a very low cost and flexibility in tuning their properties, ethylene–propylene copolymers find their main application in the automotive field, but also for sealing and insulating equipment, in the tire industry, for shoes and for roofing panels. Mixed with polypropylene, EPM is used as an impact modifier, whereas EPDM can make a thermoplastic elastomer. These rubbery solids have several advantages over traditional elastomers like vulcanised rubber: the thermoplasticity of polypropylene allows to process, reprocess and recycle the materials, which are also more resistant to oxidation, ozone attack, and weathering.

The diffusion of ethylene–propylene copolymers in the automotive industry makes these materials likely to be encountered in forensic trace analysis casework.

In the previous examples, it emerged that the nature and relative quantity of comonomers are important parameters which can be quite easily characterised by IR spectroscopy. In ethylene–propylene copolymers, this technique allows to obtain further important information on the monomer sequences.

In the IR spectra of these materials, there are two regions especially sensitive to the different repeat units comprised in the copolymer. The 720–820 cm^{-1} wavenumber

range is due to the methylene rocking modes, and the 930–980 cm^{-1} is associated to the rocking of CH_3 groups [40, 41].

A band at 730 cm^{-1} is due to the insertion of an ethylene unit within a polypropylene sequence [40, 41]. If more than two ethylene monomers are connected together, an absorption at 720 cm^{-1} is observed. Calculating the ratio of the absorbances at 720 cm^{-1} and at 730 cm^{-1}, the copolymer structure can be characterised [40, 41]. A high value of this ratio reflects a copolymer which has a block-like structure, in which ethylene units tend to stick together in homogeneous sequences. On the other hand, a lower value of this ratio will be found in copolymers where the different repeat units are more intimately mixed together.

A further refinement of this characterisation can be focused on the relative intensities of the absorptions at 752, 733 and 722 cm^{-1}, originated by sequences of two, three and more than five methylene groups, respectively [42]. A large relative intensity of the 722 cm^{-1} signal is related to long polyethylene segments within the copolymer. On the contrary, if the polyethylene sequences are interrupted by propylene units, the relative intensity of the peaks at 752 and 733 cm^{-1} increases and becomes preponderant.

The peak at 972 cm^{-1} is attributed to a sequence of two or more propylene units, whereas isolated propylene units absorb at 1,155 cm^{-1} [43, 44]. The ratio between these peaks can therefore be a useful indication of the relative length of polypropylene segments within the polymer. A higher intensity of the absorption at 972 cm^{-1} will be associated to a copolymer with long blocks of propylene units, whereas if the peak at 1,155 cm^{-1} predominates, it is an indication that propylene repeat units are alternated and separated by ethylene segments.

6.3 Isotacticity

Some polymers owe their commercial importance to isotacticity. It is the case of polypropylene, which became a useful material for a large number of applications, just when catalysts became available for an industrial production of the purely isotactic polymer. Atactic polypropylene is a tacky substance, unsuitable for any application and useless. In principle, syndiotactic polypropylene is a material with properties comparable to its isotactic counterpart. Even though it has electrical insulation properties which are superior to those of the isotactic material [45, 46], syndiotactic polypropylene is basically confined within the realm of academic and fundamental research.

Tacticity is in principle an issue in many other polymers derived from a monosubstituted olefin. However, usually polymers of this latter kind, such as polystyrene, poly(vinyl chloride), polyacrylonitrile and others are atactic and they display their best properties in atactic form. An example is polystyrene, which is appreciated for many applications because it is transparent. Isotactic polystyrene can indeed be synthesised with suitable catalysts. Nonetheless, this is associated to a loss of transparency, without an appreciable increase in mechanical properties which may

justify the much higher price of the isotactic material with respect to the common atactic version.

Even though the concept of isotacticity is practically and industrially limited to polypropylene, this material is a very important and ubiquitous one, employed for a large number of applications as diverse as backings for adhesive tapes, packaging materials, moulded items or fibres. Chances are therefore high that a forensic scientist may encounter this material related to some case. A quantitation of the degree of isotacticity can be a fruitful analytical strategy for obtaining a very deep characterisation of a polypropylene item. It may be especially worth to explore this approach in forensic intelligence instances, provided that some preliminary survey on the population of polypropylenes with different isotactic contents is done. No study has in fact been reported so far about the discriminating power of a measurement of the degree of isotacticity within normal polypropylene items.

A significant drawback which limits the perspective value of this approach is that modern Ziegler-Natta catalysts reduce the atactic fraction to an almost insignificant portion of the total synthesised material. Degrees of isotacticity higher than 99.5 % are normal, and most of the times the isotacticity approximates 100 %. It seems nevertheless worthwhile to introduce this topic in this book because, as will be seen in the rest of this section, this fundamental feature of polypropylene can be assessed quite easily by IR spectroscopy, and with no extra effort with respect to what is usually done in any routine examination protocol. Moreover, as will be commented at the end of this section, the IR tacticity index is also interrelated to other structural features, useful for characterising the identifying features of the manufacturing process.

Two methods can be used when suitable quantities of material are available, to measure the isotacticity degree of polypropylene. One is the determination of the fraction of sample which is insoluble in xylene. This test consists in treating 3–4 g of polymer with about 100 mL of boiling xylene, until complete dissolution of the sample. The system is then allowed to cool down to room temperature: isotactic polypropylene is insoluble in cold xylene, whereas the atactic fraction remains in solution. Separation of the solid from the liquid, and determination of the mass of the two fractions with respect to the total sample mass will yield the percentage of material which is in isotactic form.

A more elaborate, accurate and precise method makes use of ^{13}C Nuclear Magnetic Resonance (NMR). This is not a routine quality control technique, because it requires time-consuming analyses and expensive equipment. Due to the extremely limited applications of NMR in the analyses of contact traces, this technique will not be extensively described in this text. Suffice here to say that it is a technique capable of revealing the subtle differences in chemical environment which exist in a molecule. In ^{13}C NMR the signals in the spectrum will be originated by the carbon atoms present in the molecule. Difference in chemical environment means that if a carbon atom is surrounded by hydrogen atoms, it will behave, as far as NMR is concerned, differently from a carbon atom linked to a chlorine atom, or surrounded by other carbon atoms.

^{13}C NMR is unsurpassed among all the analytical methods for isotacticity determination. As described in Sect. 2.7.2, basically isotacticity depends on the configurations

of the asymmetric carbon atoms along the polymeric chain. Different sequences of configurations of adjacent asymmetric carbons will be reflected by slight changes in the chemical environment around these carbons. In other words, for each methyl group the particular arrangement of two adjacent methyl groups gives rise to a signal at a specific and resolvable chemical shift. In particular, at the resolutions typically attainable with ^{13}C NMR, it is possible to differentiate the pentads, i.e. sequences of five consecutive asymmetric carbons. Figure 6.23 shows the NMR signals related to all the possible pentads [47]. In Fig. 6.23, the letter 'm' represents the situation in which two adjacent carbons maintain the same configuration, the letter 'r' represents the situation in which an inversion in configuration happens between two adjacent asymmetric carbon atoms.

The signal at about 21.7 ppm is related to a sequence of carbon atoms which share all the same configuration. The peak at 20.2 is due to syndiotactic sequences. Integrating such signals, with respect to the total area of all the peaks, will yield the fractions of isotactic and syndiotactic material. Figure 6.24 shows the typical ^{13}C NMR spectra of a syndiotactic and of an isotactic polypropylene.

^{13}C NMR is the technique which allows to obtain the most reliable quantitative data on the fraction of isotactic material in a polypropylene sample. However, its detection limit is very low: quite concentrated solutions, and long acquisition times, are required for recording a spectrum. This drawback, added to the limited accessibility of ^{13}C NMR instruments, stimulated research aimed at devising a quantitative determination of isotacticity by IR spectroscopy [41]. Absorption bands at 809, 841, 900, 998, 1,168 and 1,220 cm^{-1} have been associated with isotactic helices [48].

The IR band at 975 cm^{-1} was identified as deriving from at least five contiguous isotactic sequences, the signal at 998 cm^{-1} as due to 11–12 isotactic units, and the absorption at 841 cm^{-1} as originated from 13 to 15 consecutive isotactic units [41]. Sundell and coworkers tested several ratios between the IR peaks [49]. They found that the one between the peak at 998 cm^{-1} and the peak at 973 cm^{-1} was the ratio maximising precision and accuracy of the measurement of the isotacticity index. In this case, the peak at 998 cm^{-1} represents very long isotactic sequences, whereas the short ones which originate the peak at 975 cm^{-1}, are used as internal standards. The use of the signal at 841 cm^{-1}, which is originated by even longer sequences of isotactic units, did not yield any improvement in the calibration, so the best definition of the isotacticity index measured by IR spectroscopy (IR tacticity) is A_{998}/A_{973}, where A_{998} and A_{973} are the areas of the peaks at 998 cm^{-1} and at 973 cm^{-1}, respectively.

A remarkable correlation between IR tacticity and the same parameter measured by ^{13}C NMR could be obtained (Fig. 6.25)

The curve in Fig. 6.25 allows to measure tacticity with an error of less than 1 %, with respect to the more accurate value determined by ^{13}C NMR.

Not only IR spectroscopy is a suitable approach for the assessment of tacticity in polypropylene. Also Raman spectroscopy was reported to yield data which are very well correlated to the degree of isotacticity measured by ^{13}C NMR spectroscopy [49]. The most diagnostic Raman peak reflecting the content of isotactic helices is located at 809 cm^{-1}. Also in this case, the reference peak is the CH_3 rocking signal at 974 cm^{-1}.

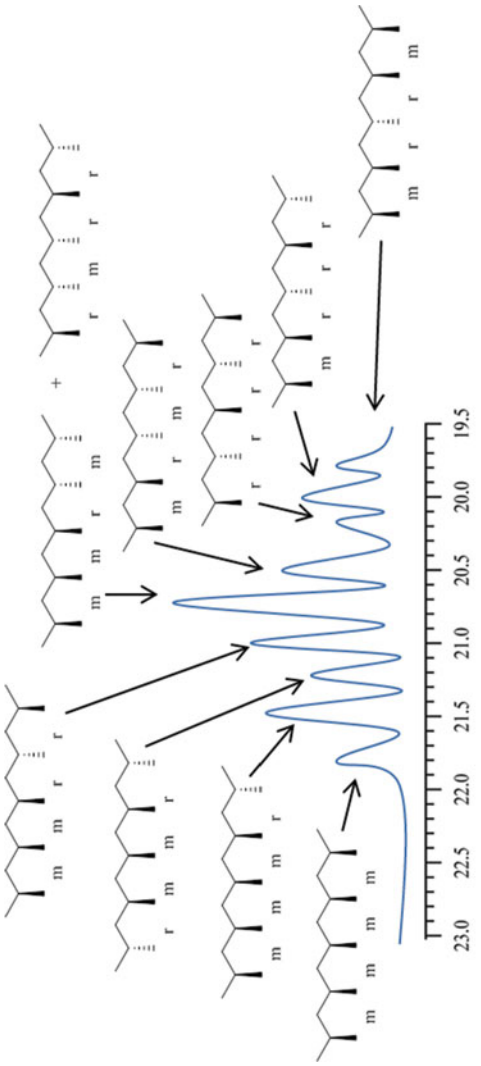


Fig. 6.23 Chemical shifts of the possible pentads in polypropylene

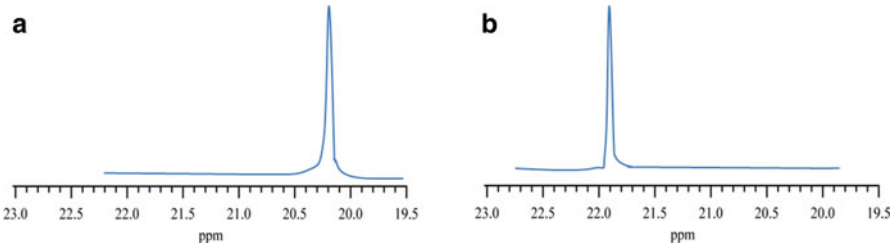


Fig. 6.24 ^{13}C NMR of (**a**) a completely syndiotactic polypropylene and of (**b**) a completely isotactic polypropylene

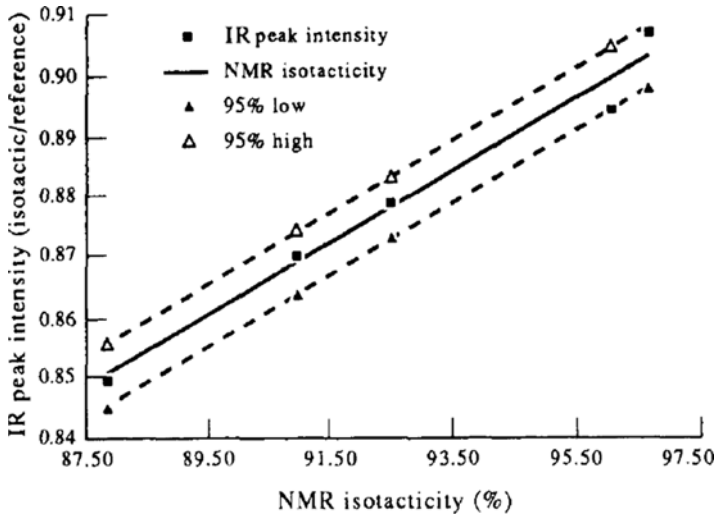


Fig. 6.25 IR tacticity versus ^{13}C NMR tacticity for polypropylene. Reprinted from Ref. [49], copyright 1996, with permission from Elsevier

A very good correlation between Raman and ^{13}C NMR spectroscopy data can be obtained (Fig. 6.26), and the isotacticity can be assessed by Raman with a 0.75 % error [49].

It is worth remarking that the bands mentioned above are actually sensitive to the formation of regular isotactic helices, rather than strictly on the configuration of asymmetric carbons [49]. This is confirmed by the strict correlation between isotacticity and structural framework: the IR absorptions at 809, 841, and 998 cm^{-1} have been also linearly correlated with the degree of crystallinity [50–52].

Sundell and colleagues warned about the dependence of IR or Raman isotacticity index on the thermal history. If the polymer is slowly cooled from the melt, its A_{998}/A_{973} ratio is more than 25 % higher than that detected on the same material, quickly cooled. For obtaining correlations like those shown in Figs. 6.25 and 6.26, the thermal history of the samples must be erased, by annealing them at a temperature

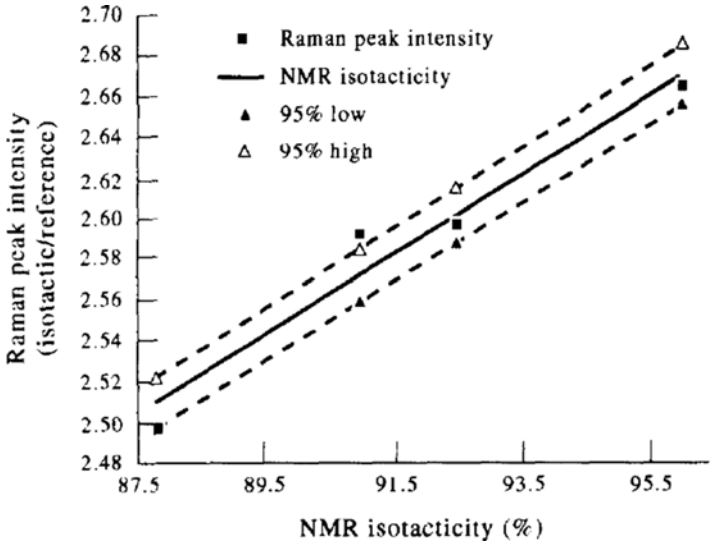


Fig. 6.26 Raman tacticity versus ^{13}C NMR tacticity for polypropylene. Reprinted from Ref. [49], copyright 1996, with permission from Elsevier

between 160 and 165 °C for at least 15 min [41, 49]. This interrelation between crystallinity, thermal history and isotacticity indeed decreases the reliability of a measurement strictly aimed at determining the degree of isotacticity. However, the forensic scientist is not actually interested on the verification of the success of a polymerisation approach. The focus in forensic characterisation of polymeric items is that of catching the subtle differences existing within mass-produced items made by different manufacturers, in different plants, or pertaining to different lots. Under this point of view, parameters like those indicated above depend on the configuration of the material, which is due to the polymerisation process used to synthesise it, and on its structure, which is a function of the transformation and processing steps imposed to the material for producing the finished items. All these features are strictly related to the industrial process that brings from the monomer to the finished item, and so they are appealing for the discrimination or the identification of mass-produced items.

6.4 Stereoregularity

As schematised in Fig. 2.14 in Sect. 2.7.1, when polymerising monosubstituted olefins the growing chain can add monomers in a head-to-tail or head-to-head fashion. The steric hindrance of the substituting group usually drives the addition towards head-to-tail sequences. However, occasionally some repeat units can connect in a head-to-head mode. These are usually interpreted as defects in the polymerisation process,

because they interrupt the regularity of the concatenation of the building blocks of the macromolecule. This has repercussions for example on the crystallisability and in general on the attainment of an ordinate solid structure. Because of this, head-to-head sequences should be kept below a certain quantity to guarantee a good performance of the material. The fact that stereoregularity is maximised in industrial practice makes this feature analogous to isotacticity. The scope for the inclusion in a forensic characterisation protocol is limited, because the presence of head-to-head defects is expected to be very low and no study has been reported so far about the discriminating power of such measurement. However, in a limited number of cases it is known that the presence of head-to-head defects is non-negligible. The materials which display a measurable amount of such defects are ethylene–propylene copolymers and poly(vinylidene fluoride) (PVDF). Moreover, exactly like in the case of isotacticity discussed above, even though the ideal technique would be NMR, the assessment of the stereoregularity can be made by IR spectroscopy. Some selected IR absorption peaks are in fact related to the occurrence of sequence defects. This allows to assess this feature of the material, without requiring any significant extra effort with respect to the operations normally performed in routine examinations of any polymeric item. When the materials mentioned above are encountered in casework, it is therefore worth considering estimating the amount of head-to-head defects for a more complete characterisation.

In the IR spectra of ethylene–propylene copolymers, the bands related to the stereoregularity of the propylene sequences are located at 752 and at 972 cm^{-1}. A band at 752 cm^{-1} is originated by two contiguous methylene groups, coming from the head-to-head addition of propylene monomers [40, 41]. On the other hand, the peak at 972 cm^{-1} is due to a sequence of two or more units connected in a head-to-tail fashion [43, 44]. The ratio between the two signals will be indicative of the amount of defective head-to-head sequences, with respect to the regular head-to-tail ones.

A very similar approach can be followed in the case of PVDF, a polymer mainly used for coatings and in electrical appliances. In commercial PVDF, quantities of head-to-head defects of the order of 5–10 % are not unusual [53, 54]. Also in the case of this material, peaks in the IR spectrum can be identified, which are sensitive to the concatenation of the repeat units. The appearance of peaks located at 1,450, 1,320–1,330 and 678 cm^{-1} was associated to the presence of head-to-head defects [53, 55–57]. The intensities of the mentioned peaks are usually weak, rarely sufficient for a reliable quantitation. Therefore, these absorptions will be useful in the characterisation of PVDF on a presence/absence basis.

References

1. Salam LA, Matthews RD, Robertson H (2000) Pyrolysis of poly-methyl methacrylate (PMMA) binder in thermoelectric green tapes made by the tape casting method. J Eur Ceram Soc 20:335
2. Tungol MW, Bartick EG, Montaser A (1990) The development of a spectral data base for the identification of fibers by infrared microscopy. Appl Spectrosc 44:543

3. Grubisic Z, Rempp P, Benoit H (1967) A universal calibration for gel permeation chromatography. J Polym Sci B Polym Phys 5:753
4. McGee WW, Coraine K, Strimaitis J (1979) Use of gel permeation chromatography in the crime laboratory. J Liq Chromatogr 2:287
5. Kumooka Y (2007) Discrimination of rubber-based pressure sensitive adhesives by size exclusion chromatography. Forensic Sci Int 171:5
6. Smith JM (2007) Forensic examination of pressure sensitive tape. In: Blackledge RD (ed) Forensic analysis on the cutting edge. Wiley, Hoboken
7. Farah S, Tsach T, Bentolila A et al (2014) Morphological, spectral and chromatography analysis and forensic comparison of PET fibers. Talanta 123:54
8. Gross JH (2011) Mass spectrometry: a textbook, 2nd edn. Springer, Heidelberg
9. Matsuo T, Matsuda H, Katakuse I (1979) Use of field desorption mass spectra of polystyrene and polypropylene glycol as mass references up to mass 10000. Anal Chem 51:1329
10. Lattimer RP (2001) Field ionization and field desorption. In: Montaudo G, Lattimer RP (eds) Mass spectrometry of polymers. CRC Press, Boca Raton
11. Montaudo G, Samperi F, Montaudo MS (2006) Characterization of synthetic polymers by MALDI-MS. Prog Polym Sci 31:277
12. Jackson AT, Yates HT, Scrivens JH et al (1996) The application of matrix-assisted laser desorption/ionization combined with collision-induced dissociation to the analysis of synthetic polymers. Rapid Commun Mass Spectrom 10:1668
13. Kassis CM, DeSimone JM, Linton RW et al (1997) An investigation into the importance of polymer–matrix miscibility using surfactant modified matrix-assisted laser desorption/ionization mass spectrometry. Rapid Commun Mass Spectrom 11:1462
14. Montaudo G, Montaudo MS, Puglisi C et al (1994) Self-calibrating property of matrix-assisted laser desorption/ionization time-of-flight spectra of polymeric materials. Rapid Commun Mass Spectrom 8:981
15. Tang X, Dreifuss PA, Vertes A (1995) New matrices and accelerating voltage effects in matrix-assisted laser desorption/ionization of synthetic polymers. Rapid Commun Mass Spectrom 9:1141
16. Rashidezadeh H, Baochuan G (1998) Investigation of metal attachment to polystyrenes in matrix-assisted laser desorption ionization. J Am Soc Mass Spectrom 9:724
17. Rashidezadeh H, Hung K, Baochuan G (1998) Probing polystyrene cationization in matrix-assisted laser/desorption ionization. Eur J Mass Spectrom 4:429
18. Goldschmitt RJ, Wetzel SJ, Blair WR et al (2000) Post-source decay in the analysis of polystyrene by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J Am Soc Mass Spectrom 11:1095
19. Kéki S, Deak G, Zsuga M (2001) Copper(I) chloride: a simple salt for enhancement of polystyrene cationization in matrix-assisted laser desorption/ionization mass spectrometry. Rapid Commun Mass Spectrom 15:675
20. Malvagna P, Impallomeni G, Cozzolino R et al (2002) New results on matrix-assisted laser desorption/ionization mass spectrometry of widely polydisperse hydrosoluble polymers. Rapid Commun Mass Spectrom 16(16):1599–1603
21. Laramée JA, Cody RB, Nilles JM et al (2007) Forensic application of dart mass spectrometry. In: Blackledge RD (ed) Forensic analysis on the cutting edge. Wiley, Hoboken
22. Bugler JH, Buchner H, Dallmayer A (2005) Characterization of ballpoint pen inks by thermal desorption and gas chromatography-mass spectrometry. J Forensic Sci 50:1209
23. Kumooka Y (2008) Analysis of rosin and modified rosin esters in adhesives by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Forensic Sci Int 176:111
24. Thomas JJ, Shen ZX, Blackledge R et al (2001) Desorption-ionization on silicon mass spectrometry: an application in forensics. Anal Chim Acta 442:183
25. Bikiaris D, Karavelidis V, Karavas E (2009) Novel biodegradable polyesters. Synthesis and application as drug carriers for the preparation of raloxifene hcl loaded nanoparticles. Molecules 14:2410

26. Al-AbdulRazzak S, Lofgren EA, Jabarin SA (2002) End-group determination in poly(ethylene terephthalate) by infrared spectroscopy. Polym Int 51:174
27. Koenig JL (1992) Spectroscopy of polymers. American Chemical Society, Washington, DC
28. Lu DD, Yuan JC, Lei ZQ (2009) High molecular weight biodegraded poly(lactic acid-glycolic acid-ε-caprolactam) copolymer: direct polycondensation of lactic acid, glycolic acid and ε-caprolactam using Sn(II)-organic anhydride as catalysts. Polym Adv Technol 20:536
29. Campbell D, Pethrick RA, White JR (2000) Polymer characterization: physical techniques. Stanley Thornes, Cheltenham
30. Sağlam M (1986) Qualitative and quantitative analysis of methyl acrylate or methyl methacrylate of acrylonitrile fibers by pyrolysis gas chromatography. J Appl Polym Sci 32:5719
31. Grieve MC (1995) Another look at the classification of acrylic fibres, using FTIR microscopy. Sci Justice 35:179
32. Grieve MC, Griffin RME (1999) Is it a modacrylic fibre? Sci Justice 39:151
33. Causin V, Marega C, Schiavone S et al (2005) A quantitative differentiation method for acrylic fibers by infrared spectroscopy. Forensic Sci Int 151:125
34. Tungol MW, Bartick EG, Montaser A (1993) Forensic analysis of acrylic copolymer fibers by Infrared Microscopy. Appl Spectrosc 47:1655
35. Kakida H, Tashiro K (1998) Mechanism and kinetics of stabilization reactions of polyacrylonitrile and related copolymers IV. Effects of atmosphere on isothermal dsc thermograms and ft-ir spectral changes during stabilization reaction of acrylonitrile/methacrylic acid copolymer. Polym J 30:463
36. Almer J (1991) Subclassification of polyacrylonitrile fibres by pyrolysis capillary gas chromatography. J Can Soc Forensic Sci 24:51
37. Minagawa M, Onuma H, Ogita T et al (2001) Pyrolysis gas chromatographic analysis of polyacrylonitrile. J Appl Polym Sci 29:473
38. Surianarayanan M, Vijayaraghavan R, Raghavan KV (1998) Spectroscopic investigations of polyacrylonitrile thermal degradation. J Polym Sci A Polym Chem 36:2503
39. Causin V, Marega C, Schiavone S et al (2006) Forensic analysis of acrylic fibers by pyrolysis-gas chromatography/mass spectrometry. J Anal Appl Pyrolysis 75:43
40. Veerkamp TA, Veermans A (1961) On the structure of copolymers of ethylene and propylene. Die Makromol Chem 50:147
41. Karger-Kocsis J (1995) Polypropylene: structure and morphology, vol 1. Springer, Berlin
42. Bucci G, Simonazzi T (1961) Contribution to the study of ethylene-propylene copolymers by infrared spectroscopy. Distribution of the monomeric units. J Polym Sci C Polym Symp 7:203
43. Ciampelli F, Valvassori A (1967) Infrared study on the distribution of propylene units in ethylene—propylene copolymers. J Polym Sci C Polym Symp 16:377
44. Natta G, Valvassori A, Ciampelli F et al (1965) Some remarks on amorphous and atactic α-olefin polymers and random ethylene–propylene copolymers. J Polym Sci A 3:1
45. Kurahashi K, Matsuda Y, Miyashita Y et al (2006) The application of novel polypropylene to the insulation of electric power cable (3). Electr Eng Jpn 155:1
46. Kim KW, Yoshino K, Inoue T, Abe M, Uchikawa N (1999) Influence of morphology on electrical properties of syndiotactic polypropylene compared with those of isotactic polypropylene. Jpn J Appl Phys 38:3580
47. Malpass DB, Band E (2012) Introduction to industrial polypropylene: properties, catalysts processes. Scrivener, Beverly
48. Burfield DR, Loi PST (1988) The use of infrared spectroscopy for determination of polypropylene stereoregularity. J Appl Polym Sci 36:279
49. Sundell T, Fagerholm H, Crozier H (1996) Isotacticity determination of polypropylene using FT-Raman spectroscopy. Polymer 37:3227
50. Kissin YV, Rishina LA (1976) Regularity bands in the i.r. spectra of C3H6-C3D6 copolymers. Eur Polym J 12:757
51. Kissin YV, Tsvetkova VI, Chirkov NM (1972) The stereoregularity of polypropylene from i.r. and nmr data. Eur Polym J 8:529

52. Quynn RG, Riley JL, Young DA et al (1959) Density, crystallinity, and heptane insolubility in isotactic polypropylene. J Appl Polym Sci 2:166
53. Kobayashi M, Tashiro K, Tadokoro H (1975) Molecular vibrations of three crystal forms of poly(vinylidene fluoride). Macromolecules 8:158
54. Maccone P, Brunati G, Arcella V (2000) Environmental stress cracking of poly(vinylidenefluoride) in sodium hydroxide. Effect of chain regularity. Polym Eng Sci 40:761
55. da Conceicao TF, Scharnagl N, Dietzel W et al (2011) Study on the interface of PVDF coatings and HF-treated AZ31 magnesium alloy: determination of interfacial interactions and reactions with self-healing properties. Corros Sci 53:712
56. Bormashenko Y, Pogreb R, Stanevsky O et al (2004) Vibrational spectrum of PVDF and its interpretation. Polym Test 23:791
57. Boccaccio T, Bottino A, Capannelli G et al (2002) Characterization of PVDF membranes by vibrational spectroscopy. J Membr Sci 210:315

Chapter 7
Processing-Dependent Parameters: Structure and Morphology of Polymeric Materials

Processing is the mix of operations which are applied to materials when objects are made. A whole branch of engineering deals with the design of viable solutions to all the problems which can arise in the transformation of polymers. With a maybe rough approximation, it can be said that processing consists in the transfer of mechanical or thermal energy to the polymer, with the aim of mixing it with other substances and of shaping it in the desired final form. Processing is rarely mild, because the quest for maximisation of industrial production imposes the conditions which minimise the manufacturing time and which often correspond to high temperatures and shear forces. The polymer thus suffers a relevant mechanical and thermal stress, which is the main driving force determining the structure of the material in the solid state. The reason why this should be of interest to the forensic scientist is that processing-dependent parameters contain information extremely indicative of the manufacturer.

In the previous chapters, focus was placed on the parameters and on the information related to the composition of the polymeric items. The techniques described in the previous chapters are the necessary starting point when the identification of a common source for two objects is sought after. As a consequence of a thorough characterisation of formulation and of synthesis-dependent parameters, one can usually determine if two compared items are made with the same material. However, not always this allows to conclude that the items come from the same source. Transformation of the same material under different conditions brings about a different structure in the solid state. In other words, it could happen that two items sharing the same formulation and composition do not actually originate from the same source. Especially when the formulation and composition are very simple, for example when very few additives are present, or when one deals either with very common polymers or with items predominantly manufactured with the same raw materials (e.g. paper, plastic bags or automotive parts), the information coming from the structure of the material can be critical for achieving a meaningful forensic conclusion.

Most of the time, in fact, the forensic scientist deals with fragments of items of mass-produced objects. The aim of the industry is producing articles made with a

V. Causin, *Polymers on the Crime Scene,*
DOI 10.1007/978-3-319-15494-7_7

particular material and displaying particular mechanical, chemical or physical performances. This reflects in an accurate standardisation of the raw materials and of the formulation and of the performance. Although each manufacturer will use a basic and recurrent set of processing parameters, these can be slightly adjusted in response to the random changes in the boundary conditions that can happen during production. Structure is very sensitive in catching such slight variations in the process, and can therefore be an extremely useful proxy for similarities and differences between items pertaining to the same or different production lots, or for similarities and differences between objects made of materials with a very simple and extremely standardised composition, such as for example paper.

7.1 Morphology

The villa of an entrepreneur was attacked by three masked men, who penetrated through the garden, entered in the building and threatened the family members, coercing them to open the safe and hand over jewellery and cash. In a common area of a building in a town nearby, a pair of heavily soiled shoes (Fig. 7.1), and a series of other items such as a holster, gloves and stockings cut into a mask were found. Three persons living in that building were identified as possible suspects, and their DNA was found on the retrieved material.

Fig. 7.1 One of the heavily soiled shoes attributed to the suspects

(continued)

Fig. 7.2 Cross-sections of the fibres found on the shoes of the suspect

In order to understand if the shoes, garments and other accessories could be those worn by the robbers, the soil present on them was analysed and compared with that of the garden of the victim.

The analysis of soil traces implies a preliminary sieving procedure, to divide the material in fractions differing by granulometry. This is an occasion to search for other traces, different from soil, but equally useful to understand the type of surface treaded on by the person wearing the shoes. An analysis by the stereomicroscope evidenced the presence in the mud of colourless fibres with a peculiar shape. The cross-sectional shape of these fibres was trilobal, as shown in Fig. 7.2.

These fibres were compared with those of the carpets present in the robbed house, and they matched not only by morphology, but also by chemical nature, because they were both nylon. This, associated to the positive comparison of the soil beneath the shoes with the ground of the victim's garden, was considered evidence that these were the garments and accessories worn by the robbers.

It may appear trivial, but the exterior appearance of an object is the most macroscopic result of the process which was used to transform the raw materials into the finished item. The observation of the sequence of layers in a paint chip found on the

scene of an accident can be determinant for obtaining a quick assessment of the compatibility with the paint of a suspect vehicle (Fig. 4.4).

Of course the significance of an approach like this requires that the shape is consistently the same among objects coming from the same source. Natural fibres do not satisfy this requirement, for example. Cotton and wool fibres, even though they come from the same plant or the same animal, have a quite wide distribution of sizes and diameters. Moreover, in the natural fibre market, each batch comes from grouping together fibres from different plants and animals. Just average values of diameter and fibre length can be identified in this case, and this should be clearly kept in mind when performing comparisons. The situation for synthetic fibres is of course very different. These items are composed by starting from the monomer, by polymerising it and then by spinning the polymer. However, since the manufacturer regulates all the conditions of the whole process, the morphology of the fibres can be controlled in detail, and it is especially consistent within the same batch. Therefore, the diameter or cross-section of a synthetic fibre become features which allow to quickly assess the compatibility between two items. Of course, the comparison on the basis of morphology must be considered just the first step of a characterisation procedure, and cannot be sufficient to take conclusions. At least IR spectroscopy must be applied to assess the chemical composition of the item, and in case of coloured items also spectrophotometric measurements should be done.

In addition to the more evident features related to the size and shape of items, there are further details that are worth considering when examining plastic items of forensic interest. The transformation of polymers into finished items can be carried out basically by two processes. The first is extrusion, where the polymer melt is forced through an orifice of suitable cross-sectional shape. If a fibre or a strand is needed, the orifice will have a circular shape, if a tube is desired it will be a circular crown, if a film or plate is wanted, the polymer will be forced through a slit. Complex shapes can also be obtained, provided that their cross-section is constant. The other way to process polymers consists in moulding. The molten polymer mass (or a fluid precursor for thermoset materials) is transferred into a mould, and by application of pressure and heat it is shaped in the desired form. Subsequent cooling allows to open the mould and retrieve the finished item. All these processes involve transfer of mass steps, in which the polymer passes through machinery parts, which can leave some distinguishing mark on the produced object. Very clear evidence of these traces in the production process can be found in plastic bags, or films, used for the packaging of illegal drugs [1–3]. These are products which are produced by extrusion, either through a slit-shaped orifice or through a circular crown. If extrusion is done through a slit, the plate so obtained is tensioned by rolls proceeding at different speeds, with a consequent reduction of thickness up to fractions of millimetre. Another approach consists in extrusion through a circular crown, producing a tube. Air is blown inside the tube, expanding its diameter and decreasing the thickness of its walls. In any case, the process is associated to high shear forces, and if scratches or imperfections exist in the inner surface of the orifice of the extruder, or in the tensioning rolls section of the equipment, they are reproduced in the finished item.

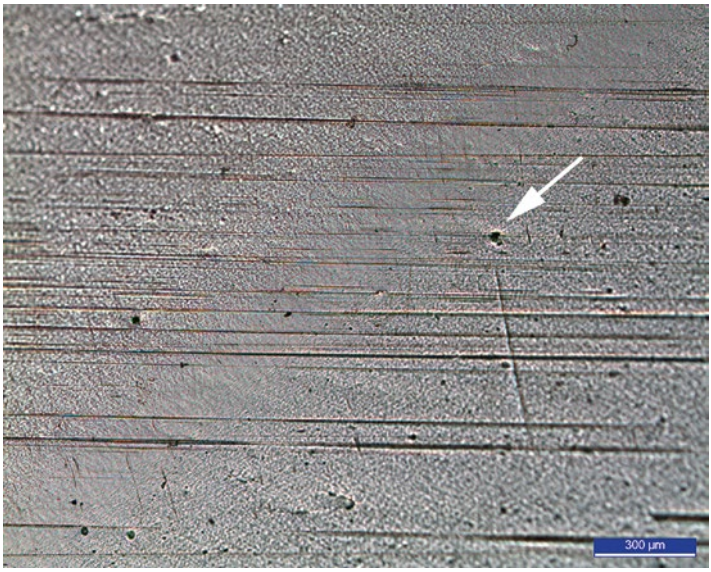


Fig. 7.3 Striation marks in a plastic film. The *arrow* indicates an inclusion of particulate matter, which caused a 'fish eye' defect. Observation conditions: optical microscope, objective 5×, transmitted white light

Figure 7.3 illustrates an example of the striation marks which are very frequently found on plastic films. In this picture, an inclusion of an impurity in the polymer is visible as well. These defects can be called, according to their shape, 'fisheyes' or 'arrowheads' and appear as dark or light spots with respect to the surrounding matrix. The appearance of such defects is due to the molten polymer flowing around a contaminant and interrupting the homogeneity of the film. If they consistently occur in the material and a recurrence in their location can be found, these characteristics are useful for identifying items manufactured in lots close to each other in the manufacturing sequence.

Other notable features which can be detected in plastic film are pigment bands, which are due to a defective mixing of dyes or pigments with the molten resin (Fig. 7.4). They generally run in the direction of film production.

Other marks can be due to the pinching or piercing which the plastic film underwent both in their production process and in their usage history. Figure 7.5 shows an example of such features.

The size of defects is such that they can be often detected by the naked eye, or with a low magnification stereomicroscope. Transmitted, incident and oblique light should all be tested as illumination strategies for optimising the visualisation of the defects, and their documentation by taking pictures. Transmitted light is useful to portray pigment bands and inclusions. Oblique transmitted light is suited for the inspection of transparent films. Incident light is useful to evidence features on the surface of the item, such as scratches and dents. Since most processes bring about

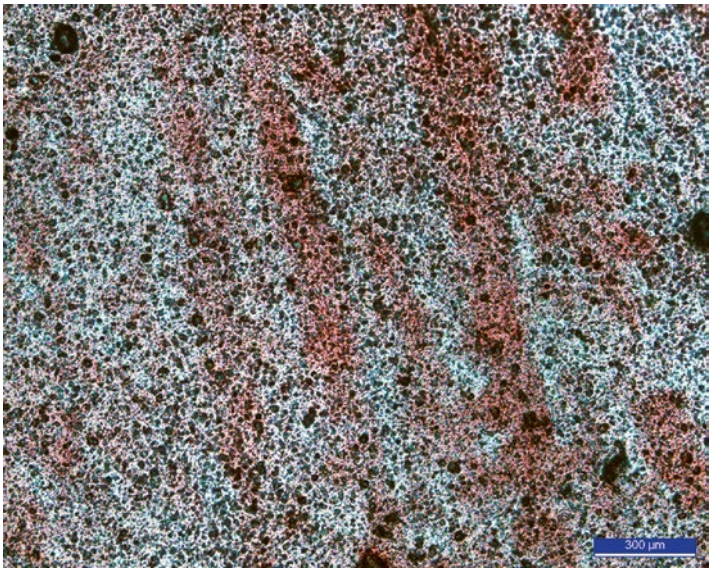


Fig. 7.4 Pigment bands in a plastic film. Observation conditions: optical microscope, objective 5×, transmitted white light

Fig. 7.5 Traces due to the use of pinching devices on a plastic film. The *scale bar* in the *bottom right corner* is equivalent to 5 mm. Observation conditions: oblique transmitted white light

an orientation of the polymer molecules, observation under crossed polarising filters is useful. The sample is positioned between two linear polarising filters, placed at 90° one with respect to another. If one of the filters is rotated and coloured patterns appear, the plastic is birefringent, indicating orientation of the molecules. Crossed polarisers allow to more clearly visualise machining marks, such as scratches and roller marks.

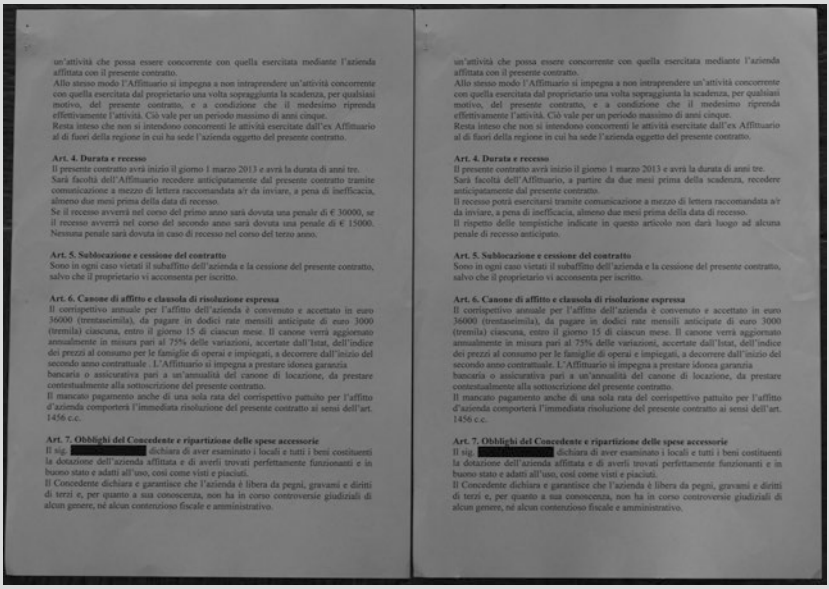
Rarely scanning electron microscopy (SEM) is necessary for the observation of the morphology of polymeric items, because the magnification offered by stereomicroscopes or optical microscopes is usually enough. SEM has the disadvantage of requiring metallisation, i.e. the coating of the surface of the sample with a very thin layer of gold or carbon, to make insulating items conductive. If this is not done, accumulation of charge on the surface due to the impinging electron beam would not allow the functioning of the instrument. Of course metallisation brings about a modification of the sample and the impossibility to proceed with further analyses. Environmental SEM (ESEM) allows to work on insulating samples such as polymers without preliminary metallisation, so the observation can be done in a non-destructive fashion [4]. SEM, and especially ESEM become appealing as characterisation techniques when elemental analysis is planned. Most of these instruments are in fact equipped with an energy dispersive detector of X-rays which reveals the X-rays emitted by the atoms of the elements in the sample, due to excitation by the scanning electron beam.

7.2 Semicrystallinity

Firms A and B signed a contract, where firm A agreed to rent a portion of a warehouse to the other, allowing storage space and sharing of office facilities, in exchange for a certain amount of money. The rent was indicated in the second page of the contract and was equal to 3,000 € a month that firm B had to pay on the 15th day of each month to firm A.

When firm B decided to move into a new building and to terminate the contract, they communicated to firm A their determination, and as a reply they received an injunction to pay 30,000 € of severance fees. The contracts were compared: the document produced by firm A contained, at article Nr. 4 regarding severance, the indication of fees for 30,000 €, whereas the contract presented by firm B, at the same article, contained the agreement that severance fees were waived if a 2-month notice was given. Due to this relevant difference, the Court asked to assess which of the two documents was genuine and which one was altered by substituting a page (Fig. 7.6).

(continued)



un'attività che possa essere concorrente con quella esercitata mediante l'azienda affittata con il presente contratto.
Allo stesso modo l'Affittuario si impegna a non intraprendere un'attività concorrente con quella esercitata dal proprietario una volta sopraggiunta la scadenza, per qualsiasi motivo, del presente contratto, e a condizione che il medesimo riprenda effettivamente l'attività. Ciò vale per un periodo massimo di anni cinque.
Resta inteso che non si intendono concorrenti le attività esercitate dall'ex Affittuario al di fuori della regione in cui ha sede l'azienda oggetto del presente contratto.

Art. 4. Durata e recesso
Il presente contratto avrà inizio il giorno 1 marzo 2013 e avrà la durata di anni tre.
Sarà facoltà dell'Affittuario recedere anticipatamente dal presente contratto tramite comunicazione a mezzo di lettera raccomandata a/r da inviare, a pena di inefficacia, almeno due mesi prima della data di recesso.
Se il recesso avverrà nel corso del primo anno sarà dovuta una penale di € 30000, se il recesso avverrà nel corso del secondo anno sarà dovuta una penale di € 15000.
Nessuna penale sarà dovuta in caso di recesso nel corso del terzo anno.

Art. 5. Sublocazione e cessione del contratto
Sono in ogni caso vietati il subaffitto dell'azienda e la cessione del presente contratto, salvo che il proprietario vi acconsenta per iscritto.

Art. 6. Canone di affitto e clausola di risoluzione espressa
Il corrispettivo annuale per l'affitto dell'azienda è convenuto e accettato in euro 36000 (trentaseimila), da pagare in dodici rate mensili anticipate di euro 3000 (tremila) ciascuna, entro il giorno 15 di ciascun mese. Il canone verrà aggiornato annualmente in misura pari al 75% delle variazioni, accertate dall'Istat, dell'indice dei prezzi al consumo per le famiglie di operai e impiegati, a decorrere dall'inizio del secondo anno contrattuale . L'Affittuario si impegna a prestare idonea garanzia bancaria o assicurativa pari a un'annualità del canone di locazione, da prestare contestualmente alla sottoscrizione del presente contratto.
Il mancato pagamento anche di una sola rata del corrispettivo pattuito per l'affitto d'azienda comporterà l'immediata risoluzione del presente contratto ai sensi dell'art. 1456 c.c.

Art. 7. Obblighi del Concedente e ripartizione delle spese accessorie
Il sig. [redacted] dichiara di aver esaminato i locali e tutti i beni costituenti la dotazione dell'azienda affittata e di averli trovati perfettamente funzionanti e in buono stato e adatti all'uso, così come visti e piaciuti.
Il Concedente dichiara e garantisce che l'azienda è libera da pegni, gravami e diritti di terzi e, per quanto a sua conoscenza, non ha in corso controversie giudiziali di alcun genere, né alcun contenzioso fiscale e amministrativo.

un'attività che possa essere concorrente con quella esercitata mediante l'azienda affittata con il presente contratto.
Allo stesso modo l'Affittuario si impegna a non intraprendere un'attività concorrente con quella esercitata dal proprietario una volta sopraggiunta la scadenza, per qualsiasi motivo, del presente contratto, e a condizione che il medesimo riprenda effettivamente l'attività. Ciò vale per un periodo massimo di anni cinque.
Resta inteso che non si intendono concorrenti le attività esercitate dall'ex Affittuario al di fuori della regione in cui ha sede l'azienda oggetto del presente contratto.

Art. 4. Durata e recesso
Il presente contratto avrà inizio il giorno 1 marzo 2013 e avrà la durata di anni tre.
Sarà facoltà dell'Affittuario, a partire da due mesi prima della scadenza, recedere anticipatamente dal presente contratto.
Il recesso potrà esercitarsi tramite comunicazione a mezzo di lettera raccomandata a/r da inviare, a pena di inefficacia, almeno due mesi prima della data di recesso.
Il rispetto delle tempistiche indicate in questo articolo non darà luogo ad alcuna penale di recesso anticipato.

Art. 5. Sublocazione e cessione del contratto
Sono in ogni caso vietati il subaffitto dell'azienda e la cessione del presente contratto, salvo che il proprietario vi acconsenta per iscritto.

Art. 6. Canone di affitto e clausola di risoluzione espressa
Il corrispettivo annuale per l'affitto dell'azienda è convenuto e accettato in euro 36000 (trentaseimila), da pagare in dodici rate mensili anticipate di euro 3000 (tremila) ciascuna, entro il giorno 15 di ciascun mese. Il canone verrà aggiornato annualmente in misura pari al 75% delle variazioni, accertate dall'Istat, dell'indice dei prezzi al consumo per le famiglie di operai e impiegati, a decorrere dall'inizio del secondo anno contrattuale. L'Affittuario si impegna a prestare idonea garanzia bancaria o assicurativa pari a un'annualità del canone di locazione, da prestare contestualmente alla sottoscrizione del presente contratto.
Il mancato pagamento anche di una sola rata del corrispettivo pattuito per l'affitto d'azienda comporterà l'immediata risoluzione del presente contratto ai sensi dell'art. 1456 c.c.

Art. 7. Obblighi del Concedente e ripartizione delle spese accessorie
Il sig. [redacted] dichiara di aver esaminato i locali e tutti i beni costituenti la dotazione dell'azienda affittata e di averli trovati perfettamente funzionanti e in buono stato e adatti all'uso, così come visti e piaciuti.
Il Concedente dichiara e garantisce che l'azienda è libera da pegni, gravami e diritti di terzi e, per quanto a sua conoscenza, non ha in corso controversie giudiziali di alcun genere, né alcun contenzioso fiscale e amministrativo.

Fig. 7.6 The questioned pages of the contracts produced by firm A (*left*) and B (*right*)

The font type and size, the alignment and the general features of the printed text were indistinguishable between the two documents. The identification of the authenticity of the signatures was not conclusive, due to their simplicity. A morphological comparison of the toner did not display significant differences. An analysis of toner did not show significant differences among all the pages of all the contracts, and they matched the toner of the printer of firm B, which was originally used for preparing the documents before the conclusion of the agreement. Equally, no difference was found on the inks of the signatures of all the pages: they were all written with a common BIC crystal pen. So an analysis of paper was performed.

The diffractogram of the questioned page from contract B was starkly different from that of the other pages of the contract, which showed a pattern like that of trace 'Ref.' in Fig. 7.7. All the non-questioned pages of both contracts showed the same XRD pattern, which matched that of the questioned page of contract A. It could be thus concluded that firm B had forged their copy of the contract, including a favourable clause, which was not present in the original contract.

(continued)

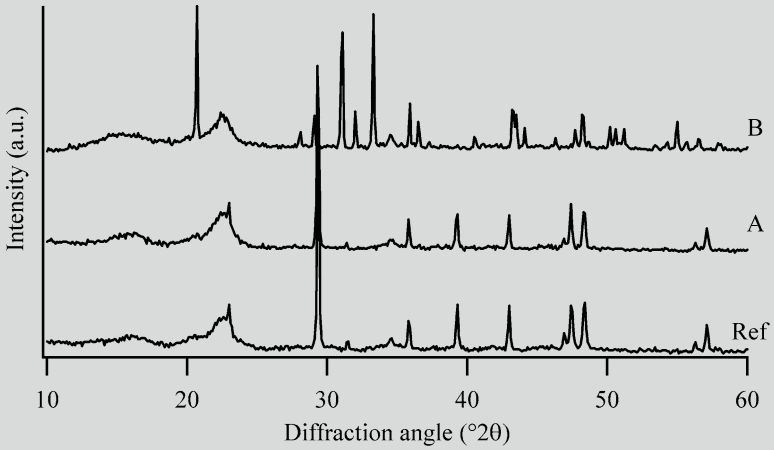


Fig. 7.7 Comparison of the XRD spectra of one of the genuine pages from the contracts (*trace Ref.*), the questioned page from contract A (*trace A*), the questioned page from contract B (*trace B*)

A very distinctive feature of polymers is related to their structure in the solid state. When dealing with low molecular weight substances, usually just two extreme cases can be observed: the amorphous and the crystalline state. The amorphous state is characterised by a completely disordered framework, with no long-range order between the molecules. On the other hand, in a crystal all the atoms of all the molecules are arranged in a very ordinate way within elementary cells which repeat themselves in three dimensions throughout the whole material. In low molecular weight substances, usually all the molecules or atoms of the material conform to the degree of order dictated by the amorphous or crystalline state. In other words, all the molecules and atoms in low molecular mass substances are disorderly arranged if the material is amorphous, and on the contrary all of them occupy very ordered positions in crystals.

The case is very different in polymers which are able to crystallise. In such instance, in fact, the concept of semicrystallinity must be introduced. As said in Chap. 2, polymers are very heterogenous mixes of molecules with different sizes, sometimes with different amounts of microstructural defects. If the difficulty in promoting the motion of big and intermingled macromolecules is added, it is easy to understand how unlikely it is that they arrange themselves in perfectly ordered structures. Figure 7.8 schematises the crystallisation process.

As a further factor that does not allow the formation of 100 % crystalline structures in polymers there is the thermodynamic issue that the decrease in entropy

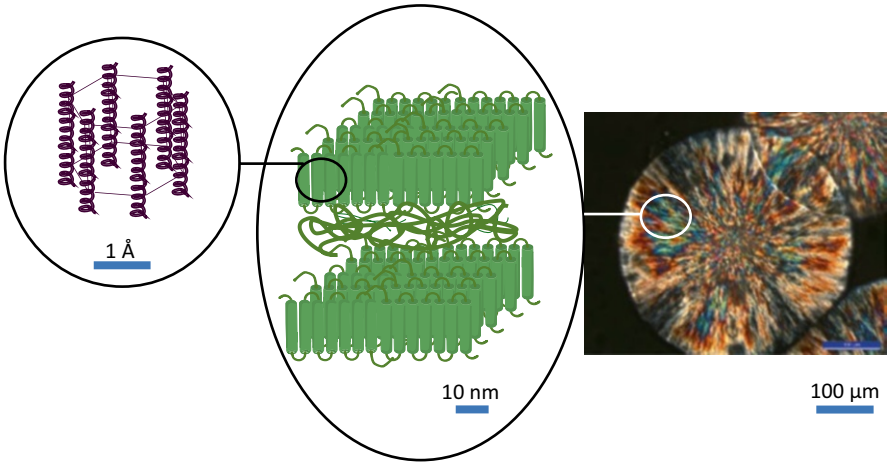


Fig. 7.8 The hierarchy of polymer crystallisation. *Left*: portions of polymer chains arrange themselves into crystalline cells. The distance between such elementary constituents of the crystals is of the order of 1 Å. *Centre*: to be able to crystallise, polymer chains must fold, so they can align several molecular segments in an ordered way, according to the crystalline cell, without the need to fully extend the macromolecules, which is an extremely unfavourable process because of the large entropy associated to it. This folding creates crystalline lamellae, which have a thickness of the order of magnitude of 10 nm and a width up to several microns. Between each lamella, randomly arranged macromolecules form an amorphous layer. The same chain can participate in the formation of the crystalline lamellae, but with some portions in the amorphous layer. Some chains can be included in one lamella, then extend in the amorphous layer and then enter another lamella. Such chains, called tie molecules, are very important in physically connecting the polymeric framework. The alternation of lamellae and amorphous layers is called lamellar stack. *Right*: lamellar stacks arrange themselves in a radial shape, departing from a central nucleus outwards. This forms spherulites, i.e. macroscopic crystals with a size of hundreds of microns up to millimetres

going from a disordered random coil configuration to a perfect crystal would be too large to justify spontaneity of the process. In other words, thermodynamic, and particularly entropic, constraints make 100 % crystallinity in polymers impossible, rather than unlikely.

As a consequence of the concept of semicrystallinity, the structure and morphology of polymers is therefore better described as an alternation of crystalline and amorphous domains, which coexist in the same material. Figure 7.9 shows a schematic of the morphology of a semicrystalline polymer.

The degree of crystallinity is the fraction (in mass or volume) of sample which is arranged in the crystalline domains, and it is a critical parameter from which many of the physical mechanical properties of the material depend. From the point of view of the forensic scientist, the degree of crystallinity is appealing because it strictly depends on the processing imposed to the polymer, and so in many cases it reflects a distinctive mark of a particular manufacturer of a mass-produced item.

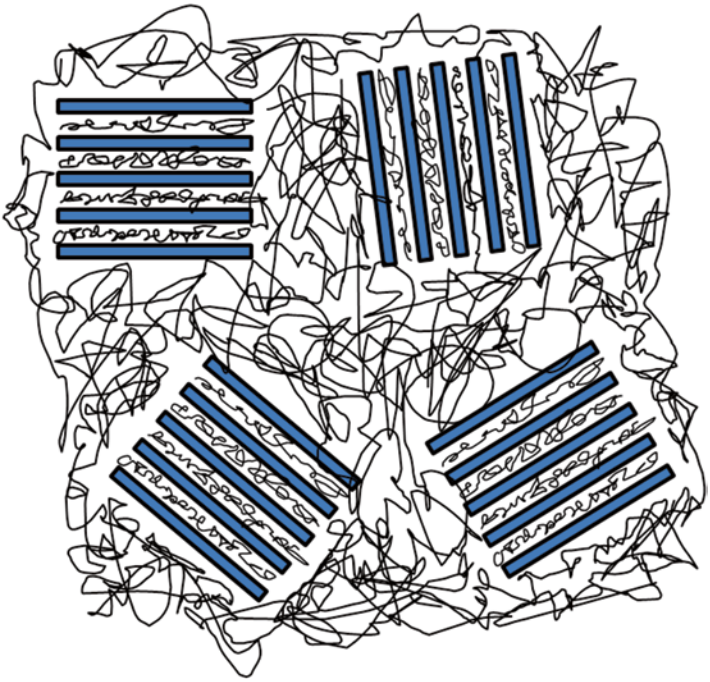


Fig. 7.9 Scheme of the solid-state structure of a semicrystalline polymer. Stacks of crystalline lamellae (*rectangles*) coexist with polymer in the amorphous phase

7.2.1 The Techniques: X-Ray Diffraction

What is X-ray diffraction?
X-ray diffraction (XRD) is the physical phenomenon which is observed when a beam of X-rays is scattered by an ordered array of atoms. When this happens, interference takes place among scattered rays because the wavelength of X-ray radiation is of the same order of magnitude of interatomic distances. Diffraction consists in the reinforcement due to constructive interference just along certain directions, governed by the geometry of the crystal lattice, whereas in all other directions destructive interference occurs.

Why use this technique?
XRD is the main technique for the investigation of the crystalline structure of materials. The directions along which constructive interference occurs are dictated by the arrangement of atoms within the crystal lattice, so the structure

(continued)

of the material can be calculated by the position of the diffraction peaks in a diffractogram. Actually, this is a very complicated endeavour, very useful for a fundamental understanding of matter, but not really relevant in a forensic science context. XRD is indeed useful in the forensic analysis of polymers, though. Since these are semicrystalline materials, XRD is the most suitable method for the determination of the degree of crystallinity. In addition to this important quantitative information, XRD is capable of elucidating also the polymorphism of the material. Differences in the location of the peaks in the diffractogram of two materials mean that they adopted a different crystalline structure, i.e. a different phase. Since this is a consequence of differences in the processing conditions, it can be exploited as further relevant information in forensic comparisons.

Where can this technique be found?
The XRD of polymers is carried out by powder diffractometers. Geology and chemistry departments of any university are equipped with these instruments. They can be found also in some commercial laboratories, mainly those which are specialised in the characterisation of materials for the building industry. XRD is used in the forensic analysis of soil traces, so these instruments can be possibly found in forensic laboratories which offer this kind of expertise. XRD analyses are not expensive, the cost per sample is below 100 €.

XRD can be interpreted and better understood using the Bragg law. This law was derived by considering the diffraction phenomenon as equivalent to the simultaneous reflection of a X-ray beam by a family of parallel crystal planes passing through the points of a crystal lattice.

In Fig. 7.10, a beam of X-ray light strikes, with an incident angle θ, an assembly of two crystal planes, which are at an interplanar distance d one with respect to the other. Constructive interference occurs just when the beams reflected by the two planes are in phase with each other, or in other words when the difference in their optical path is equal to an integer number of wavelengths. As may be also qualitatively inferred by the scheme in Fig. 7.10, the light impinging the bottom crystal plane will cover a longer path to emerge from the crystal, with respect to the top plane. Such difference in optical path is equal to the sum of segments BC + BD, which due to the geometry of the phenomenon is equal to $2d\sin\theta$. The Bragg law can thus be enunciated as:

$$2d\sin\theta = n\lambda \tag{7.1}$$

where λ is the wavelength of the radiation and n is an integer number. When (7.1) is satisfied, an intense X-ray reflection emerges at an angle θ with respect to the impinging radiation. When (7.1) is not obeyed, destructive interference will occur, and no intensity will be detected.

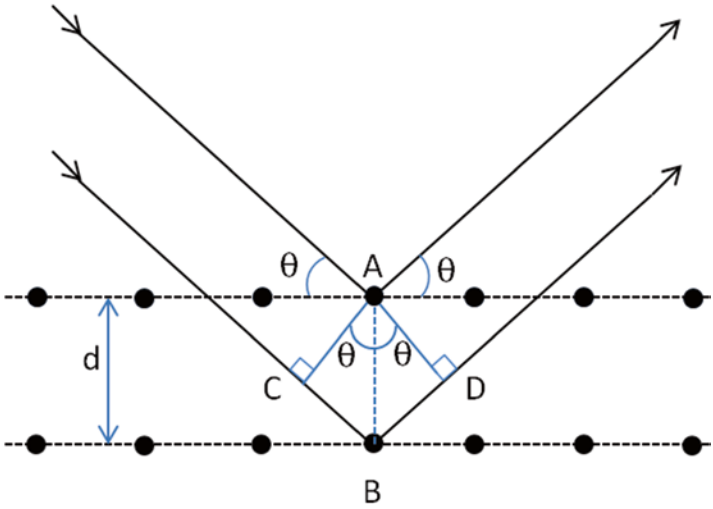


Fig. 7.10 The geometry of the Bragg law

It is worth remarking that the planes represented in Fig. 7.10 are imaginary planes. According to the geometry of the crystal, many parallel planes can be identified, each one with its characterising interplanar distance *d*, which will diffract at different angles. The designation of each of these planes is done with three bracketed numbers, e.g. (100), (010), (021), called the Miller indices. The procedure to assign Miller indices to crystal planes is part of the vaster discipline of crystallography and will not be covered in this text because, for the use of XRD which is done in the context of forensic science, it is not a requirement. The interested reader can refer for this and other details of X-ray crystallography, to more specialised books [5–8]. Figure 7.11 shows some examples of the different crystal planes which can be identified in a two-dimensional array of crystal points.

The number of such different planes, and thus the number of diffraction signals which should be expected, depends on the geometry and on the symmetry of the lattice.

XRD of polymers must be studied with powder diffractometers, which can operate either in transmission or in reflection geometry. Some of the most modern equipment are capable of performing analyses in both geometries, however the majority are reflection mode instruments. In this latter case, the sample is mounted horizontally. A monochromatic X-ray beam impinges on its upper surface, and a detector rotates around it, acquiring the diffracted radiation (Fig. 7.12).

The incoming X-ray beam is generated in a X-ray tube, a device where a focused electron beam is directed towards a metal target, called anticathode. The impact with the metal expels electrons closest to the nucleus leaving a vacant orbital. When an electron leaves an outer electronic level, at higher energy, to fill the vacancy, energy is released as radiation in the X-ray range. This process will therefore emit a discrete series of lines, characteristic of the metal used as a target. The most commonly

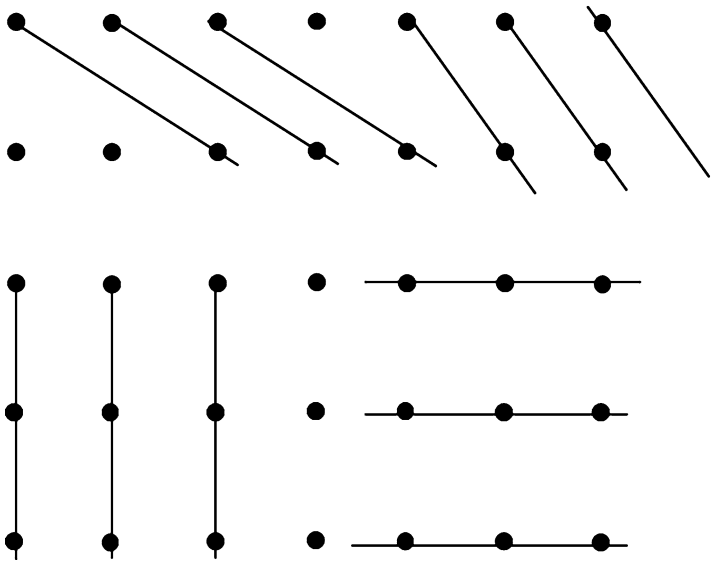


Fig. 7.11 Different families of crystal planes which can be identified in an ordered lattice

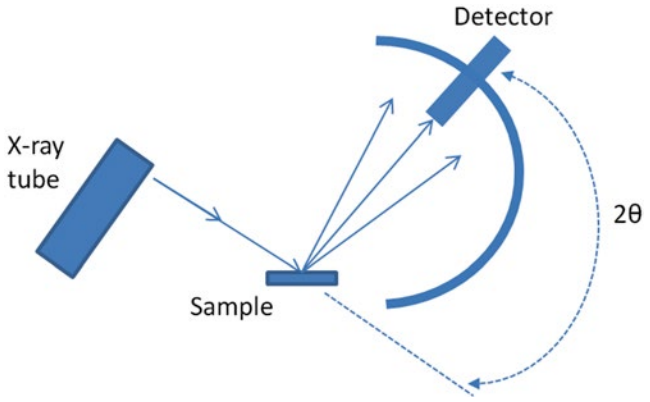


Fig. 7.12 Schematic of a diffractometer in reflection, θ–θ geometry. X-rays are generated by a X-ray tube, they are filtered and monochromatised (this section is not shown in the scheme) and directed towards the sample. The detector revolves around the sample, acquiring the radiation scattered around it

employed X-ray tubes in powder XRD have a copper anticathode and among the emitted wavelengths the CuKα line, with a wavelength of 1.5406 Å, is selected. CuKα designates the electron transition which originates the emitted radiation, i.e. the one from a 2*p* orbital of the second electronic level to the innermost shell (principal quantum number 1). Selection and collimation of X-ray light is obtained by filters and monochromators. X-ray monochromators are crystals, oriented in such a way that they deflect in the desired direction just the desired wavelength. Quartz or graphite are common materials for monochromatising X-rays.

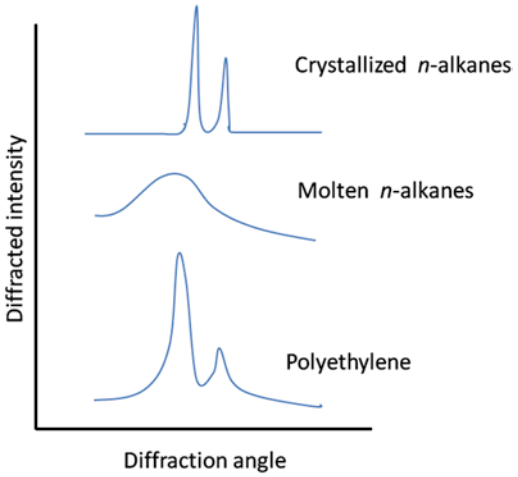


Fig. 7.13 Diffraction patterns of (*top*) a completely crystalline substance (crystallised low molecular weight *n*-alkane), (*centre*) an amorphous substance (a molten *n*-alkane) and (*bottom*) of a semicrystalline polymer (polyethylene)

Modern X-ray detectors are scintillation or semiconductor counters. Scintillation counters contain a crystal which emits a photon of visible light every time X-rays impinge on it. These visible photons are detected by a photomultiplier and transformed in an electric signal. Semiconductor transducers have emerged as X-ray detectors of major importance. When they absorb a X-ray photon, they promote electrons to the conduction band, and thus a marked increase in conductivity results, with a concurrent pulse of electrical current.

Diffractograms are represented as diagrams in which the intensity of the scattered X-ray radiation is plotted as a function of the angle between the detector and the impinging X-ray beam. In 100 % crystalline substances, diffracted intensity is detected just at angles where Bragg law is satisfied, whereas a flat background signal is present where only destructive interference happens (Fig. 7.13). In an amorphous material, there is not an ordered lattice, so no crystal plane can be clearly identified within the material. The scattering bodies (atoms and molecules) are randomly distributed in the three-dimensional space. Constructive and destructive interference are in this case related by the mutual distances between them. The diffractogram of an amorphous material, e.g. glass or molten wax, is a broad and rather featureless halo, such as that shown in the centre trace of Fig. 7.13. The most likely distance between the chains can be calculated applying (7.1) to the angle where this halo has a maximum. Semicrystalline polymers are a mixture of crystalline and amorphous domains, so their diffraction pattern will be the superposition of the two types of diffractograms: narrow and intense reflections emerge from a broad amorphous halo.

The structure attained by a polymer is strictly dependent on the processing it was subjected to. The same material, fastly or slowly cooled, or precipitated from a solution, will organise itself in different manners. Most polymers are able to form different kinds of crystalline cells, i.e. different crystalline phases, as a function of the crystallisation conditions. This ability is called polymorphism. A qualitative inspection is informative for elucidating the structure of the sample, because

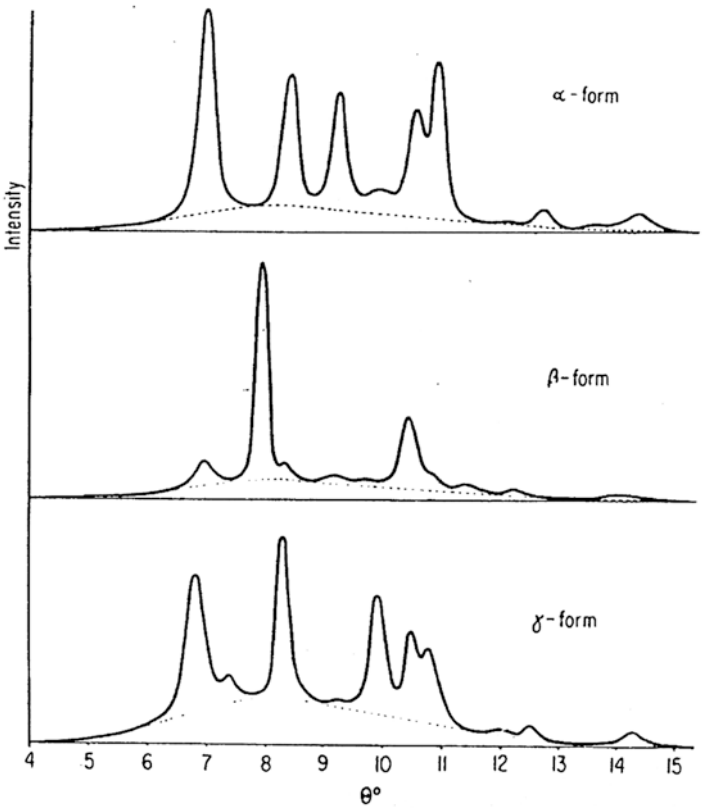


Fig. 7.14 Diffractograms of three polymorphs of polypropylene. Reprinted with permission from Ref. [9] Copyright © 1964 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

different crystalline phases, or polymorphs, can be distinguished by the shape of their diffractograms (Fig. 7.14).

Of course, if in a comparison between two samples, differences in the polymorphism of the polymer matrix are detected, this can be considered conclusive evidence that they come from a different source. The identification of the polymorph adopted by the polymer should be done just on the basis of the presence or absence of peaks, and not to their relative intensity, because the latter feature is also dependent on the orientation of the macromolecules due to processing. This should be studied more in depth with specific sets of measurements, as will be discussed later in this chapter. A further comment on polymorphism regards the possibility that more than one crystal phase are simultaneously present in the same sample. Polypropylene is particularly prone to this behaviour. It predominantly crystallises in α form, but often γ peaks, and more rarely β peaks, can coexist in the diffractogram (Fig. 7.15).

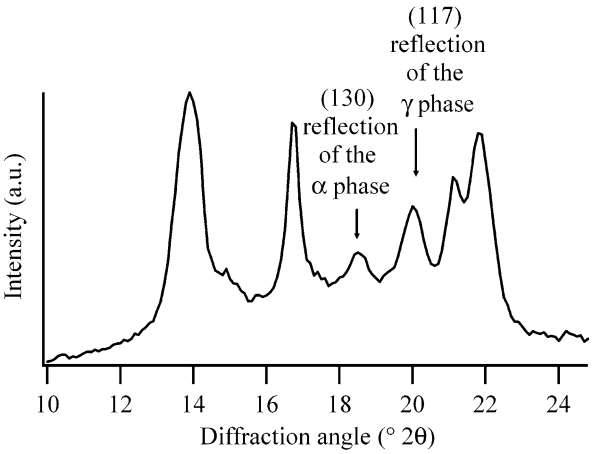


Fig. 7.15 Diffractogram of a polypropylene sample with two coexisting crystalline phases, α and γ

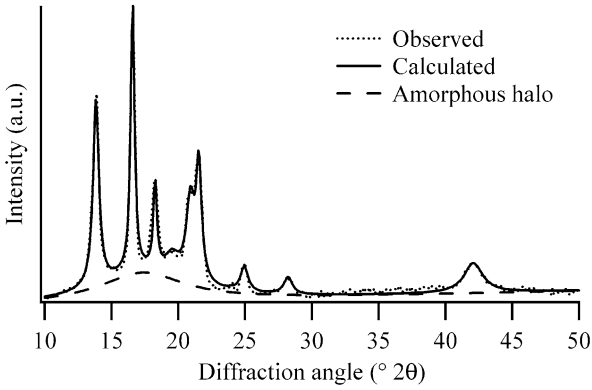


Fig. 7.16 Example of the deconvolution procedure which allows to calculate the degree of crystallinity of a polymer. This example shows the diffractogram of polypropylene

A quantitative treatment of XRD data allows to measure the degree of crystallinity of the polymer. A fitting procedure of the experimental patterns can be performed, by which the contributions of the crystalline and amorphous domains are deconvoluted [10] (Fig. 7.16).

A certain degree of subjectivity exists in such data treatment, especially in the choice of the fitting functions (usually Gaussians or Lorentzians) and of the width and position of the amorphous halo. In the comparison of samples, always the same width and position of the amorphous halo, changing just its intensity, and the same kind of fitting functions should be used. This is important, because this makes the results more objective and reproducible. Crystallinity can thus be evaluated as the ratio between the area of crystalline peaks (A_c) over the total area of the diffractogram ($A_c + A_a$):

$$X_c = \frac{A_c}{A_c + A_a} \tag{7.2}$$

The sample size required by XRD is quite large, and it is compatible with classes of items available in relevant quantity, for example plastic packaging material, adhesive tape and paper. The geometry of XRD instruments (Fig. 7.12) implies that the sample is illuminated by a collimated X-ray beam, usually of rectangular shape. To be sure that the sample is actually reached by this X-ray beam, it must have a minimum area of about 3 cm × 0.5 cm. Smaller items can be analysed with a point-collimated X-ray beam or with a synchrotron source. However, these instruments are much less easily available and are rarely employed outside academic research.

The measurement of the degree of crystallinity was reported as a discriminating parameter for plastic bags and paper [11, 12]. Application of a quantitative treatment of XRD data allowed to obtain a discriminating power of the order of 80 % of the possible pairs of sample in the relevant sample population [11, 12].

The XRD study of cellulosic materials is a very good example of how this technique can give information on the manufacturing process of a finished item. Cellulose is found in a number of different varieties, generally named cellulose I, II, III and IV. Of these, the most commonly encountered in nature is cellulose I [13]. In turn, two variations of cellulose I are known: triclinic Iα and monoclinic Iβ [14]. The proportion of these two polymorphs varies in the different plants: higher plants like cotton have predominantly Iβ phase, on the contrary, the lower forms of cellulose are dominated by the metastable Iα form [14]. In textiles, cotton is usually subject to treatment with strong alkali, the so-called mercerisation process, which triggers a transition from cellulose I to cellulose II. Mercerisation is applied to correct some undesirable properties of cellulose I such as bad elasticity or low dyeability, due to the high crystallinity and orientation of cellulose I [15].

A cellulosic material which can be encountered in forensic casework is paper, because cellulosic textile fibres are usually available in too small amounts for allowing the acquisition of diffraction spectra. The XRD spectrum of paper is very broad and diffused, and consists of a main peak located at about 22.7 °2θ with a shoulder at around 22 °2θ and of three secondary maxima at 14.8, 16.3 and 20.3 °2θ (Fig. 7.17).

The characteristic peaks of cellulose I are located at 14.8, 16.2 and 22.7 °2θ [13, 15, 16]. Cellulose II yields XRD peaks at 20.2 and 21.8 °2θ [15, 16]. The characteristic reflections of cellulose Iα and Iβ are overlapped [17, 18]. Figure 7.17 shows a coexistence of both cellulose I and II. Strong alkali such as NaOH are in fact used in the manufacturing process of pulp and paper, and it is known that a cellulose I → cellulose II transition is induced at high pH values. Moreover, Foner and Adan [13] showed that mechanical treatments, normally employed in the pulp and paper industry, such as grinding or beating, bring about severe modifications of the WAXD profile, i.e. cellulose peaks are much smoothed and broadened. The relative intensities of the peaks due to cellulose I and II can thus serve as diagnostic features which reflect differences in the severity of the mechanical beating and grinding steps in the process of paper production.

Of course, a quantitative evaluation of the degree of crystallinity is suggested every time a diffractogram of a polymer is available. Figure 7.17 shows the calculated patterns and the shape of the amorphous halo on the considered paper samples. This makes available quantitative data for a more objective and reliable comparison of the samples.

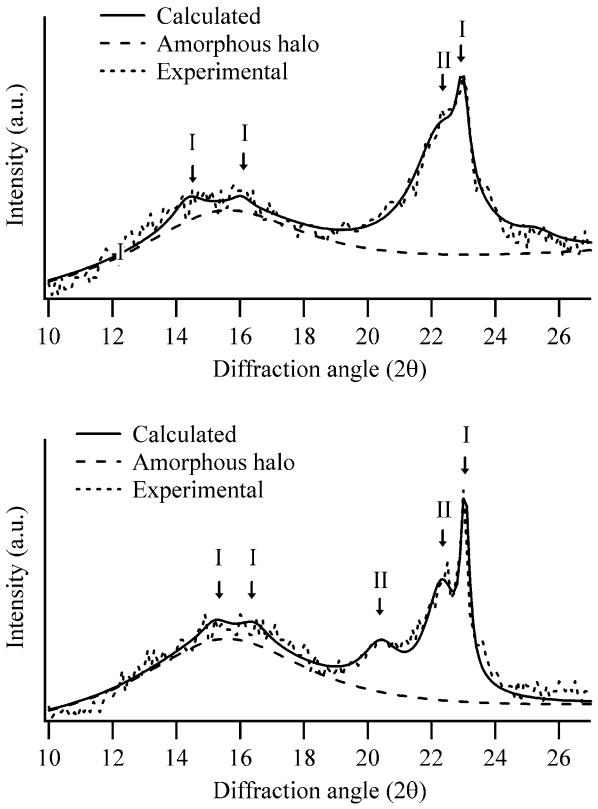


Fig. 7.17 WAXD spectra of two paper samples in the angular range relative to cellulose. All the samples were mounted in the same direction. The indexes of the major reflections of cellulose I and II are indicated. Calculated patterns and the amorphous halo after deconvolution are also shown. Reprinted from Ref. [12], copyright 2010, with permission from Elsevier

As a further level of exploitation of XRD data, the examination of peaks due to fillers is worth mentioning. Since this aspect of XRD regards the characterisation of formulation, it was more thoroughly presented in Chap. 5. It is not always trivial to identify which peaks in a diffractogram are due to the polymer matrix and which to the inorganic fillers. As a rule of thumb, polymers give rather broad reflections. Their major peaks are normally located at angular ranges below 30 °2θ. On the contrary, inorganic crystalline compounds give very sharp signals, located at diffraction angles larger than 30–40 °2θ. Figures 5.29 and 5.30 show examples of the coexistence of polymeric and inorganic crystalline peaks. In both sets of patterns, the polymer peaks are located between 20 and 25 °2θ, whereas the signals due to inorganic additives appear from 25 to 30 °2θ upwards. It is interesting to focus interest on trace 'R' of Fig. 5.29a, where around 20 °2θ a very sharp reflection occurs close to a much broader signal at about 22–23 °2θ. The former has the characteristic features of a diffraction signal due to a very ordered inorganic crystal, the latter is typical of a semicrystalline material, where the coexistence of the crystalline and amorphous domains greatly decreases the sharpness of the peak.

7.2.2 *The Techniques: Thermal Analysis—Differential Scanning Calorimetry*

What is differential scanning calorimetry?
Differential Scanning Calorimetry (DSC) is a thermal analysis technique in which the heat flow through a sample is measured as a function of temperature, while it is subjected to a controlled temperature program. It is a method which allows to monitor how the sample exchanges thermal energy with the surroundings as a function of temperature.

Why use this technique?
DSC is the most widespread technique of thermal analysis, because it is able to give reliable information on the physical transitions and on the possible chemical reactions which happen in different thermal conditions. This has a relevant scope in a forensic science context. In polymers, the temperature and latent heat of transitions between physical states depend on the structure of the material and on how that structure was achieved. The presence of additives in the formulation can be detected in a thermogram because they can degrade or evaporate when heated, or because they modify the thermal behaviour of the matrix, changing its glass transition temperature.

Where can this technique be found?
DSC is very common in industrial and academic laboratories in which polymers are studied, produced or transformed. The array of data that DSC yield on a very small sample size make this technique ideal for performing failure analysis, quality control, to investigate the structure of polymeric materials and to study their thermal behaviour.

The use of DSC in forensic science is much rarer, and thus this technique is not commonly available in forensic science institutions. The cost of DSC analyses is in the order of 100 € per sample. Standard temperature programs (usually a heating ramp up to the melting temperature at 10 °C/min, followed by cooling to room temperature and a subsequent melting step at the same rate) are normally suitable for analysing any polymeric material. Usually it is not necessary to develop an ad hoc method any time a new kind of sample is analysed.

Due to its ability to yield information for solving a variety of problems, DSC is the workhorse of thermal analysis [19, 20], which found also some applications in forensic science [21–25]. The instrumental design of DSC was firstly designed upon the power compensation concept. In this kind of instrument, there are two small furnaces, inserted in a large temperature-controlled heat sink. The sample is positioned

inside an aluminium crucible, which is in turn placed into one of the furnaces. The other furnace contains another crucible filled with a suitable standard material, e.g. alumina, or, more commonly, empty. Each furnace is equipped with a thermometer. During a measurement run, power is independently given to the two furnaces, in order to change the temperature of sample and reference according to the desired temperature program. Throughout the experiment, sample and reference must be kept at the same temperature. If exothermal transitions occur in the sample, the sample furnace will require less power input, because the extra heat evolved by the sample can be used. On the contrary, when endothermal transitions occur in the sample, more power input will be required to the sample furnace because the latent heat to complete the transition must be provided. A signal proportional to the difference in power input to the two furnaces is related to the heat absorbed or evolved by the sample as a function of temperature. Power compensated instruments are currently produced by the manufacturer who originally invented DSC. All other major producers of instruments for thermal analysis have turned to the heat flux design. In this kind of instruments, the sample and reference crucibles are placed on two sample holders located inside a furnace. A complex series of thermocouples allows to directly measure the heat which the sample absorbs or evolves with respect to the reference. The simpler instrumental design allowed heat flux apparati to gain an increasing market share, despite their comparatively poorer performance in heating and cooling rate.

Regardless of the kind of DSC instrument used, sample size is between 2 and 5 mg. As already noted in the case of thermogravimetry, this sample size is too large for contact trace casework, but it is not prohibitive in many cases involving larger pieces of evidence. Even though DSC experiments can in principle be carried out changing the measurement atmosphere, practically always they are performed in nitrogen. The temperature range of DSC instruments goes from about −200 to 350 °C.

DSC is extremely useful in an industrial context for quality control and especially for failure analysis, because it allows to quickly assess if the reason for poor mechanical performance is an abnormal formulation or processing. This is done by comparing the thermograms of the defective item with the thermogram of a well performing object. The typically used program in this case consists in a heating ramp up to melting, followed by a cooling and by another melting. If the traces are different just in the first heating step, but not in the subsequent cooling and reheating, this means that the problem is due to a defective processing. The features of a thermogram in fact depend, as will be more clearly elaborated in the following pages, on the structure of the material, which in turn depends on the processing it was subjected to. The first heating thermogram reflects the structure of the sample imposed by the manufacturing process. Melting, though, erases the previous thermal history, so in the cooling and second heating steps both the defective and well performing samples are subject to exactly the same processing. If they do not display significant differences in the thermogram this means that they both have the same formulation. Materials with the same formulation, treated by the same processing, will attain the same structure and have the same thermal behaviour as detected by DSC.

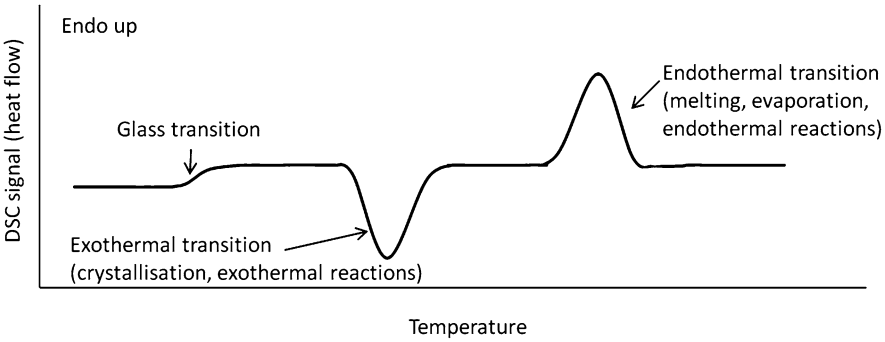


Fig. 7.18 Example of a DSC thermogram

DSC is useful for identifying problems in thermosets, because it allows to check if the reticulation was optimal [26]. When in fact crosslinking in thermosets is only partial, the heat provided during the DSC measurement can complete it, and a reaction exotherm is observed in the thermogram.

A DSC thermogram is a plot of the heat flow through the sample as a function of temperature (Fig. 7.18). Peaks and steps are the most notable features that can appear in the trace. Peaks are due to endothermal or exothermal transitions. There is not an universal convention on the direction of the peaks: endothermic signals can be oriented upward and exothermal downwards, or vice versa. The chosen mode of representation must be always indicated. In Fig. 7.18, for example, the 'endo up' label clarifies that endothermal peaks are oriented upwards.

Normally, in polymeric systems no reaction occurs in the typical temperature range, especially because experiments are usually carried out in an inert atmosphere. So in the vast majority of the cases, in a DSC thermogram of a polymer, endothermal peaks will correspond to melting of the matrix and exothermal peaks to its crystallisation. Occasionally endothermal signals can be related to the evaporation of some volatile additive or solvent residue.

The melting endotherm of a polymer is particularly informative on the structure of the material. Melting is in fact the phase transition from the crystalline state to the liquid state. Just crystalline solids have a definite melting temperature, whereas amorphous materials gradually soften from the solid to a fluid state, without a sharp transition between the two states. In a semicrystalline material such as a polymer, then, the contribution to the observed melting peak will be just given by the crystalline component of the material, whereas the amorphous phase will not produce any observable peak in the thermogram. Two features can be quantified in a melting peak: temperature and enthalpy. Of course in DSC as well the issue of calibration is fundamental for a reliable measurement. Temperature and enthalpy are calibrated using high purity metals as standards. Indium and tin are the most commonly used.

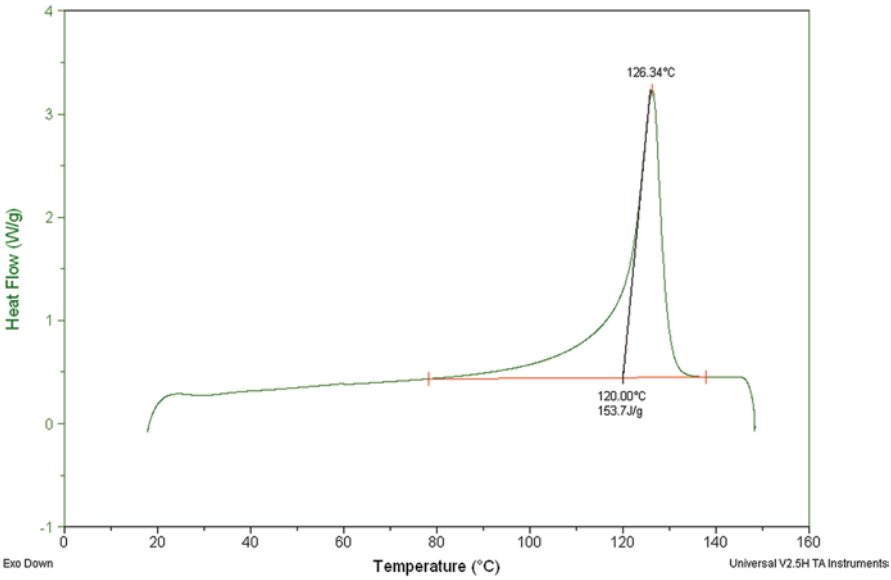


Fig. 7.19 An example of integration of a DSC melting peak. Endothermal signals are oriented upwards. Heating rate 10 °C/min

The transition temperature is normally taken as the maximum of the melting peak, less often as the onset temperature, which corresponds to the intersection between the tangent in the point of maximum slope and the baseline. The maximum is easier to assess, but on the other hand it is somewhat dependent on the sample mass. Care should be taken then that the same amount of material is probed in comparison analyses. The melting enthalpy is calculated from the thermogram by integrating the melting peak. Since the melting peaks for polymers are broad, they can span a temperature range of several tens of degrees. It is advisable, when comparing items on the basis of their enthalpy, to always use the same integration limits. These should in turn be chosen so that they identify a baseline which smoothly joins the instrumental baseline (Fig. 7.19).

The melting temperature is a viable parameter for a first screening of the compatibility between two items. However, it is not so sensitive upon the structure or the processing parameters to be sufficient for a significant comparison [25]. The melting enthalpy is much more useful for this purpose, because it is proportional to the degree of crystallinity, thus DSC is still a method alternative to XRD for the measurement of this feature [22, 24]. A quantification of the degree of crystallinity ($\%C$) can be done, by calculating the following ratio:

$$\%C = \frac{\Delta H_{\mathrm{m}}^{\mathrm{sample}}}{\Delta H_{\mathrm{m}}^{100}} \times 100$$

Where ΔH_m^{sample} is the melting enthalpy measured by integrating the melting peak of the sample, and ΔH_m^{100} is the melting enthalpy of a sample of the same material with 100 % degree of crystallinity. Of course this value must be extrapolated because no totally crystalline polymer can be prepared; ΔH_m^{100} data are available in the literature for many common polymers. However, since ΔH_m^{100} are estimated values, a significant degree of uncertainty can exist in the reported values for a specific polymer. Therefore, in casework, it is suggested to merely use the ΔH_m^{sample} as a feature describing the polymer, without attempting to calculate a degree of crystallinity. The physical meaning of the comparisons made according to ΔH_m^{sample} values will still be that of comparing the samples on the basis of their structure, because ΔH_m^{sample} is directly proportional to the degree of crystallinity.

As introduced above in this section, a triple ramp is usually applied as a thermal program in DSC analysis. This is done to decouple the effects of processing and formulation on the obtained data, and so to more thoroughly characterise the material. The temperatures and enthalpies acquired in the first heating ramp are surely the most important when investigating a sample found on the crime scene, because they depend on the processing and formulation applied by the manufacturer, and can thus give information on the source of that object. However, also the temperature and enthalpy data coming from a cooling step followed by re-heating, which are done on the second and third segments of the typical DSC thermal method, are useful for a deeper study of the sample because they operate on a material whose previous thermal history has been erased and thus which is processed in a controllable and reproducible manner. If two samples give a similar thermogram in all three ramps of this standard thermal method, they can be considered made by the same material, equally formulated and processed in the same way [24, 25].

The shape of the melting peak is significant, also. Multiple melting peaks are associated to multiple populations of crystallites, and thus can be directly connected to the thermal history experienced by the sample during processing [21, 24]. DSC data are very informative, but their overinterpretation should be avoided. The melting and crystallisation behaviour depend on a number of factors, among which formulation, microstructure of the macromolecular chain and crystallisation conditions are the most significant. Different combinations of these features can bring about similar crystalline structures in different materials. DSC, and more widely all the techniques that focus on the structure of the polymer semicrystalline framework, are not ideal techniques for the qualitative identification of polymers. IR spectroscopy is much more suited for this purpose and in general an array of techniques must be employed to attain a general description of the item. One analytical method, or more analytical methods which focus on the same aspects of a material, are rarely sufficient to gain knowledge for taking useful and diriment information.

If the melting or crystallisation peaks are the features in a thermogram which most naturally lend themselves to an exploitation for forensic comparison purposes, the glass transition temperature (T_g) is another polymer-specific parameter that can easily, reliably and accurately be measured by DSC. Since it is more strictly related to the mobility of the macromolecules, it will be discussed in Sect. 7.3.1.

7.2.3 *Other Methods for the Determination of the Degree of Crystallinity*

IR spectroscopy and Raman are also suitable methods for the quantification of the degree of crystallinity of some kinds of polymers. For example vibrational bands ascribable to crystalline and amorphous domains have been identified for poly(ethylene terephthalate) [27, 28], polypropylene [29, 30] and polyethylene [31]. The relative intensities of such bands allow to assess the relative quantities of material which is ordered in the crystalline domains and of material which is disordered in amorphous zones.

In the IR spectrum of poly(ethylene terephthalate), which is a very commonly encountered component of textile fibres, bands indicative of *trans* and *gauche* conformations can be identified. In the crystalline phase of PET, the conformation of the molecules is *trans*, while in the amorphous phase both conformers are present. *Trans* conformers give rise to the bands at about 846, 973 and 1,340 cm^{-1}, while *gauche* ones originate the peaks at about 896 and 1,370 cm^{-1}. A *gauche*/*trans* ratio which proved useful for differentiation of colourless polyester fibres was obtained ratioing the absorbancies of the peaks at 1,370 and 846 cm^{-1} ($A_{1,370}/A_{846}$) [27, 28]. Another ratio was calculated, between the absorbancies of the peaks at 3,440 and 874 cm^{-1} ($A_{3,440}/A_{874}$), which was related to the molecular weight of the polymer. Even though the correlation of the degree of crystallinity with $A_{1,370}/A_{846}$ was not completely satisfying because it was non-linear and with some outliers, its coupling with the $A_{3,440}/A_{874}$ ratio allowed to successfully discriminate many of the possible sample pairs in a population of 17 colourless polyester fibres, indistinguishable by morphology [27]. The validity of this approach was confirmed also after treating the same fibres in conditions commonly found in casework, such as immersion in water or exposure to sunlight for periods up to 3 months [28].

In the case of polypropylene, the ratio between the absorbances of the peaks at 998 cm^{-1} and at about 975 cm^{-1} was shown to be linearly dependent on the crystallinity of the polymer (Fig. 7.20) [32].

An analogous method was proposed for estimating the degree of crystallinity in isotactic polypropylene by using the integrated intensities of the Raman bands at 808 and 841 cm^{-1} [33].

Raman proved useful to evaluate the crystallinity of polyethylene as well. In this case, a band at 1,418 cm^{-1} was reported to be proportional to the fraction of orthorhombic crystalline component in this polymer [31]. As an internal standard band, the integrated intensity between 1,250 and 1,350 cm^{-1} was suggested [31].

The degree of crystallinity affects many properties of the material, which can in turn be exploited as features to assess the crystallinity degree by an indirect way.

One of these is refractive index (RI), i.e. the ratio between light's velocity in a vacuum and the light's velocity in the material of interest. Correlations between RI and density are known for example for isotactic polypropylene films [34] and glass [35]. The density of a semicrystalline polymer is known to be proportional to its degree of crystallinity. In a forensic science context, it was shown that a measurement

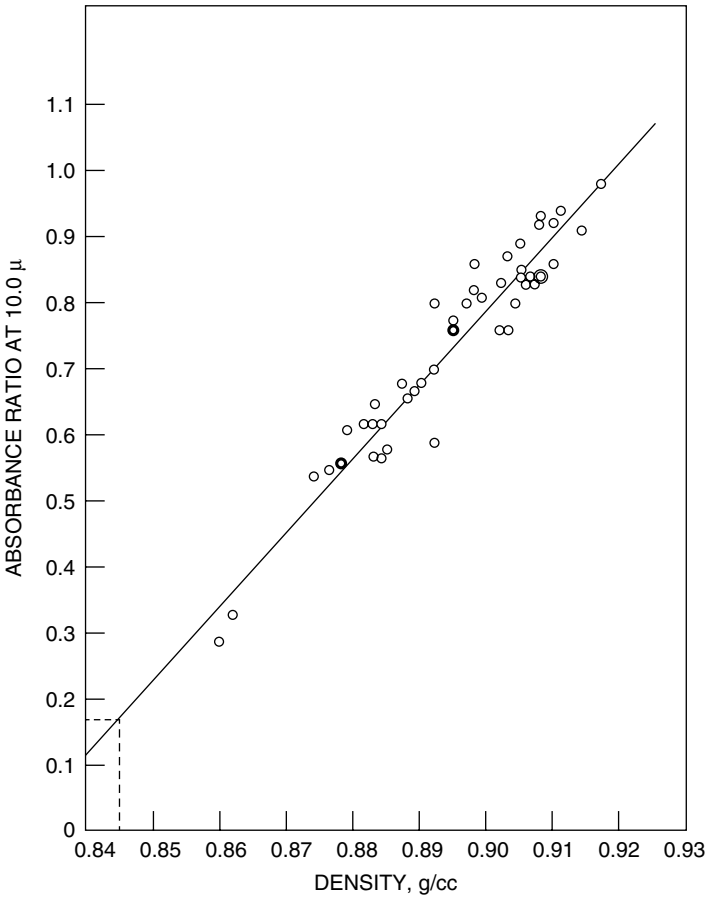


Fig. 7.20 Correlation between the ratio of the IR absorbances of the peaks at 998 cm^{-1} and at about 975 cm^{-1} and the density of polypropylene. The density is directly proportional to the degree of crystallinity. Reprinted with permission from [32]. Copyright © 1959 John Wiley and Sons, Ltd

of RI was a viable method to quantify the degree of crystallinity of colourless acrylic fibres (Fig. 7.21) [36].

The determination of RI is not unusual in the forensic characterisation of synthetic fibres. The most widespread technique is the immersion method. The fibre is mounted in oils with a different index of refraction until no solid–liquid interface is visible upon observation under a phase contrast microscope. If temperature correction is applied and monochromatic light is used, the RI can be measured to the third decimal place [37]. A more convenient and quicker way makes use of a technique which is standard in the measurement of RI in glass fragments [38]. This technique, called 'immersion oil and hot stage method' [39], has become very popular because it was efficiently automatised in a system called GRIM by Foster + Freeman. The adaptation of GRIM to the measurement of RI of acrylic fibres is very simply fea-

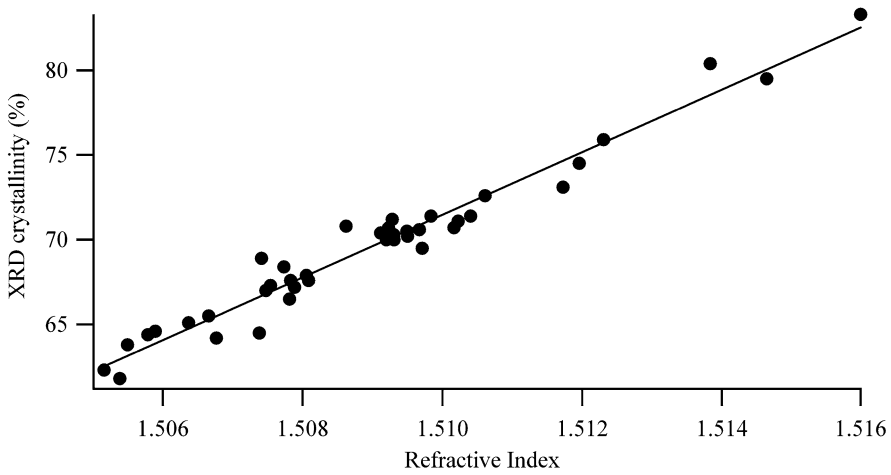


Fig. 7.21 Correlation between crystallinity degree measured by XRD and refractive index of 40 colourless acrylic fibres. The *line* shown was obtained by linear regression of the data. Reprinted from Ref. [36], copyright 2005, with permission from Elsevier

sible because polyacrylonitrile copolymers used in the textile industry are characterised by a RI range very similar to that of glass. This instrument provides a rapid way to acquire RI data on samples of that type, with a lower error than the established method [36]. Adaptation of GRIM to other polymers is likely to be a bit more complex, especially when the refractive index of these materials is different from that of glass. In such case, immersion oils different from those bundled to the GRIM apparatus should be used, and the development of a specific method would be necessary. This is however feasible with relatively little effort, since there are many sets of immersion oils for microscopy, covering a very wide range of refractive indexes.

7.3 The Mobility of Macromolecules

The ability of the macromolecules contained in a polymeric item to move and to change their position one with respect to the other has relevant repercussion on the macroscopic mechanical behaviour of the whole material. Impacts or mechanical stresses of whichever nature or geometry are basically a transfer of energy from the surrounding to an object. If the molecules of the material are able, through microscopic displacements, to disperse the extra energy, the object will deform but it will not break. If dissipation of the extra energy is not possible due to the rigidity of the molecules, such extra energy will be used for breaking bonds or for overcoming intermolecular forces, bringing about the breakdown of the material.

The important aspect of the mobility of macromolecules, due to its relevant repercussions on the mechanical performance of the whole material, is tuned by changing its formulation and structure in the solid state.

7.3.1 The Glass Transition Temperature

The thermogram in Fig. 7.18 shows that, in addition to the peaks which are associated to endothermal or exothermal processes, also a step transition can be detected. This is associated to the glass transition temperature (T_g) of the material. The T_g is a very important feature from the manufacturer's point of view. In fact, it defines the temperature range where a polymer can be used and where it performs at its best for a particular application. The T_g is a boundary where the properties of a polymer drastically change. Below T_g, the material behaves as a glassy solid, beyond T_g the polymer acquires plastic properties. On a microscopic scale, this is due to the fact that below T_g the molecules do not have a sufficient energy to allow for a relative translational motion able to dissipate the extra energy introduced by external mechanical stress. On the contrary, beyond T_g the molecules are mobile enough to respond with a cooperative translational motion to mechanical stress, dissipating the extra energy and resisting without breaking. Below T_g just vibrational motions are allowed, whereas long-range micro-Brownian motions are forbidden.

The glass transition is a prerogative of the amorphous phase, so it takes place in amorphous polymers or in the amorphous fraction of semicrystalline polymers. In this latter case, then, the glass transition step will characterise the amorphous phase and the melting peak will characterise the crystalline phase which coexist within the same material. As said above, the glass transition is revealed by a step in the baseline of the DSC thermogram. The height of this step is proportional to the amount of sample which is in the amorphous phase. Normally the T_g is quantified as the flex point of the step. In totally amorphous polymers, where the step due to T_g is of relevant height because it is originated by the whole sample, such quantification is normally straightforward. Not the same can be said of semicrystalline polymers, in which, if the degree of crystallinity is high, the step due to the glass transition can be so weak that it is difficult to identify and assess. Increasing the sample size up to 10 mg can be useful for improving the quality of the signal in these cases. As a further element of complication, often the glass transition step is followed and partially hidden by a peak, the so-called enthalpic relaxation or hysteresis peak. This feature complicates an accurate assessment of T_g and can be amended by using modulated DSC. This is a sophisticated mode of operation of DSC which allows to decouple the DSC signal into two traces, isolating the glass transition step from the enthalpic relaxation peak. However, this requires a quite extensive method development on a sample-by-sample basis, making this approach unsuitable for forensic applications.

The T_g is primarily dictated by the polymer used for manufacturing the item of interest. Polyethylene and polypropylene have T_g well below room temperature, at about −36 and −14 °C, respectively. Their high crystallinity normally hinders a clear detection of the glass transition step. Rubbers have very low T_g, often below the lower temperature attainable by normal instruments. Other polymers have T_gs at higher temperatures, like PET (79 °C), nylons (around 50 °C), polyacrylonitrile (80–95 °C), polystyrene (100 °C) and PVC (81 °C) [19], well within the optimal operating range of most commercial DSC apparati. However, in the plastics industry, T_g is often modified and tuned by addition of plastifiers, and by control of

the structure and morphology. Polyvinylchloride (PVC) is a classical example of polymer whose T_g is varied much, especially by the use of plastifiers, as a function of the intended application. By this strategy, PVC can be used both for producing hard and stiff pipes for the building industry and soft and flexible coatings for fake leather manufacturing. T_g is therefore an indicator of the manufacturing process and as such it may be of help to the forensic scientist.

7.3.2 The Techniques: Time Domain Nuclear Magnetic Resonance

What is Time Domain Nuclear Magnetic Resonance?
When a nucleus with a spin quantum number of ½ (e.g. ^{1}H, ^{13}C, ^{19}F or ^{31}P) is immersed in an external magnetic field, its magnetic moment becomes oriented along the field. However, an amount of energy corresponding to radio-wave radiation is enough to flip these nuclei, orienting them against the direction of the field. If the sample is illuminated by a radio-wave impulse, while immersed in an external magnetic field, firstly its nuclei will orient in such a way that their magnetic moment is opposite to the magnetic field, but when the impulse will stop they will tend to return to the more favourable orientation along the field. The extra energy of the excited state will be dissipated by emission of a radio frequency signal, called free induction decay (FID). This signal is in the time domain, and represents the rate with which the molecules are able to relax the excitation brought about by the radio-wave pulse. Time Domain Nuclear Magnetic Resonance (TD-NMR) consists in studying the FID signal, to extract information on the mobility of the material's molecules.

Why use this technique?
TD-NMR investigates how the excitation due to a radio-wave pulse is relaxed in a material. This study in the time domain elucidates how mobile and homogeneous is a material. If for example the whole sample is composed of rigidly arranged molecules, the time necessary for its relaxation will be shorter than in a sample where molecules are flexible and free to move one with respect to the other. If the sample is a blend of a rigid and of a rubbery polymer, the two components in the material will relax according to different mechanisms and on different time scales, so the relative composition can be assessed by deconvoluting the two contributions to the FID signal. Since the mobility of a polymer is influenced by the microstructure of its macromolecules, by the presence of plasticisers and by its degree of crystallinity, TD-NMR data can be useful for detecting differences in items which were manufactured according to different processes.

(continued)

Where can this technique be found?
TD-NMR is a niche technique. It is emerging as a quick and relatively cheap approach for the determination of water in food or in cosmetics, for example, so it can be found in some industrial laboratories and in the best equipped commercial laboratories. It is usually present in academic laboratories specialised in nuclear magnetic resonance. Applications in forensic science have been limited so far, so it is very unlikely that TD-NMR will be found in a forensic laboratory. Since TD-NMR is not an analytical method applied in routine protocols, the cost must be assessed on a case-to-case basis, but due to the relatively low cost of the instrument (around 40,000 €), the quick analysis time and the absence of sample preparation steps, it is not expected to exceed 150 € per sample.

TD-NMR offers a number of advantages with respect to traditional NMR instruments. A TD-NMR apparatus does not require a massive superconductive magnet, but just a simple electromagnet, it does not need a liquid helium cooling system, it has a small footprint in the laboratory since it is desktop size, it does not have moving parts, it does not expose the operator to intense magnetic fields, and it costs about 40,000 €, in comparison to traditional NMR whose cost is in the 500,000 € range.

The theoretical basis of NMR [40] is that when nuclei with nonzero spin S, such as ^{1}H, ^{13}C, ^{19}F, and many others, are immersed in a constant magnetic field B_0, their energy levels split. Electrons will preferentially populate the low-energy levels, therefore, a net magnetic moment M parallel to B_0 develops. However, through the absorption and emission of electromagnetic radiation, it is possible to trigger transitions between these energy levels. If the majority of electrons are promoted into the higher energy levels, the net magnetic moment can be inverted and it can have a direction opposite to B_0. In NMR experiments, a sample subjected to a static magnetic field is irradiated with electromagnetic pulses in order to perturb thermodynamic equilibrium, i.e. to promote electrons to higher levels, flipping the orientation of the net magnetic moment of the nuclei. The nucleus most widely studied is surely the proton, ^{1}H, followed by carbon, which is sampled through its isotope ^{13}C. The recovery of equilibrium (relaxation) of the system is associated to the dissipation of the extra energy of the system by emission of radiation in the radio-wave range, i.e. the FID signal. The process through which nuclear spins return to the equilibrium with the whole sample at the temperature of the experiment is called spin–lattice or longitudinal relaxation. High density of nuclear spins in bulk materials allows energy exchanges between neighbour spins and a thermal equilibrium within the spin system may develop at a temperature different from the lattice temperature. This process is described by a characteristic time constant T_2 called spin–spin or transverse relaxation time.

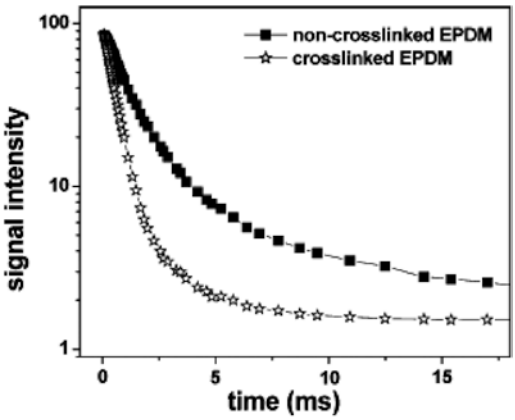


Fig. 7.22 ^{1}H NMR T_2 relaxation decay for crosslinked and non-crosslinked ethylene–propylene–diene–monomer rubber samples. Reprinted with permission from Ref. [41]. Copyright 2007 American Chemical Society

In conventional NMR the FID signal is Fourier-transformed in order to obtain information on the small shift in the energy levels due to the influence of the neighbourhood (chemical shift). In fact this shift, which corresponds to frequencies in the radio-wave range, is strongly affected by the chemical environment of each nucleus, i.e. on the electrons and atoms which surround it, so much information can be acquired on the structure of a molecule from its NMR spectrum. In order to obtain such detailed information on the molecular structure usually expected from NMR, very intense magnetic fields must be created, with a consequent complexity in the instrumental design. If the field is not intense enough, application of Fourier Transform to the FID will result in a very low resolution and wide spectral lines, which do not allow to catch the fine structure due to chemical environment.

In TD-NMR, relaxation signals are analysed directly, so much less intense magnetic fields are required with a substantial simplification of the instrument design. The information on the chemical environment of each nucleus is neglected, but the analysis provides information on the mobility of different nuclei. In the case of the protons in polymers, this can be related to the chain mobility, to the presence and quantity of rigid and mobile phases and to the interaction between different chains. Solid matter damps oscillations and produces a fast signal decay. In a liquid, the more mobile surroundings cause less damping and thus give a slower decay of the signal. Semicrystalline polymers often have within their bulk both stiff and hard regions, e.g. crystalline phase, and softer regions, e.g. amorphous zones. Some materials, such as polyurethanes, contain hard and soft segments in their macromolecular chains. Polymer chains in rubbers, elastomers and crosslinked materials have a different mobility if they are close or far from a crosslinking point.

A structural and morphological characterisation of such materials can be attained by analysis of the FID signal shape, which depends on the relative quantities of the solid, i.e. stiff or hard regions, and liquid, i.e. soft, components of the sample (Fig. 7.22) [41, 42].

TD-NMR is emerging as a viable technique for many applications in different industries: examples were reported in the analysis of fat and water content in fish chops [43], in the determination of hydrogen content in aviation fuels [44] or in the measurement of mobile fractions in semicrystallyine polymers and blends [45]. More refined applications are still in the process of being optimised for the industry but appear extremely promising. They include the measurement of crosslink density and distribution in rubbers and the measurement of nanometric domains in multiphase materials [46].

A particularly appealing feature of TD-NMR is its non-destructivity. The sample must simply be positioned inside a NMR tube and can be retrieved absolutely unaltered after the analysis. The sample size is of the order of magnitude of 35–45 mg. In cases in which not plenty of sample is available, as typical in forensic casework, preliminary tests should be performed, in order to identify the optimal position of the sample within the tube. This should locate the sample in the NMR cavity, in order to guarantee the most homogeneous static magnetic field and radiofrequency pulses.

Recently, two applications of TD-NMR to forensic science were proposed, for the characterisation of latex gloves and of polyurethane foams [47, 48]. In these two works, the potential of TD-NMR was exploited for acquiring two kinds of information: the proton quantity, which is correlated to the chemical nature and formulation of the material which composes the polymeric item, and the relaxation time T_2, which is a measure of chain mobility, and is thus dependent on the arrangement and degree of crosslinking of the macromolecular chains.

Each of these data sets required a specific pulse sequence, which is based on the assumption that if an electromagnetic pulse of a particular frequency (called resonance frequency and depending on B_0 and on the considered nuclear species) is applied to the sample, the net magnetic moment (M) of the sample is rotated from its equilibrium position by an angle that depends on the duration of the pulse [40].

In a first experiment, called Bloch Decay (BD), a single pulse is applied whose length is set in order to rotate the proton magnetisation M by 90° maximising the initial signal. The intensity of the FID signal just after the pulse is proportional to the proton magnetisation M induced by the static field. Since M is proportional to the number of proton spins, provided that all the instrumental parameters are accurately kept constant in all experiments, a measurement of the FID signal intensity just after the pulse at a fixed temperature will give an estimate of the number of protons in the sample. As an indication, a good estimate of the initial FID signal intensity can be obtained by measuring the first 0.03 ms of the real component of the BD curve. In other words, the initial intensity of the FID allows the quantification of the number of protons contained in the sample. In turn, the number of protons contained in the sample is proportional to its mass w, so the ratio I_0/w should be calculated. The relationship between I_0/w and the percentage of hydrogen content H (i.e. the weight of the hydrogen content over the total weight w of the sample) can be calibrated with standard substances for which the weight fraction of protons is known. Figure 7.23 shows the calibration curve obtained from 1,2-dichlorobenzene, thiophene, formamide, toluene, ethyl acetate, xylene, polybutadiene, and ethylene–propylene glycol.

On the basis of the calibration curve shown in Fig. 7.23, the hydrogen content in an unknown material can be assessed. Such compositional information is the

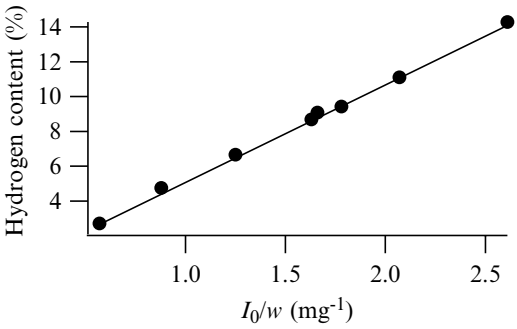


Fig. 7.23 Linear relationship between hydrogen content H and I_0/w obtained with eight standards. Reproduced from Ref. [48] by permission of The Royal Society of Chemistry

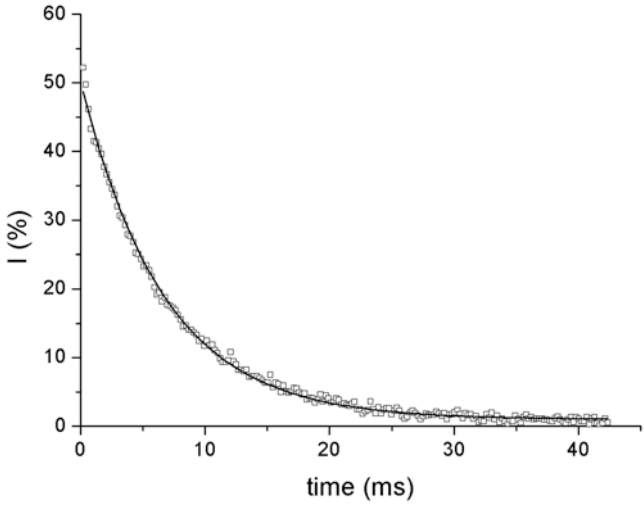


Fig. 7.24 FID signal obtained in response to a CPMG excitation sequence and exponential fitting for a latex glove sample. Reproduced from Ref. [48] by permission of The Royal Society of Chemistry

weighted average of the hydrogen content of all the substances present in the sample. This becomes an analytical data which summarises the formulation of a material, and it showed its value in discriminating between samples of latex gloves and polyurethane fragments indistinguishable by other more routine techniques [47, 48].

A second set of experiments which proved useful for a forensic characterisation of polymeric items was aimed at the estimation of T_2. The proton T_2 value is used by several authors in polymer science as indicator of the degree of freedom of polymer chain motion in a given phase. The characteristic features of the TD-NMR signal are thus informative of the microstructure and dynamics of a wide array of matrices, among which elastomeric materials [41, 42]. Different pulse sequences, such as the Carr-Purcell-Meiboom-Gill (CPMG) [49], the magic sandwich echo (MSE) or solid echo [45, 46, 50] can be used. Relaxation curves such as those displayed in Fig. 7.24 are obtained, which can be fitted by the function $I(t) = A \exp(-t/T_2)$, where I is the

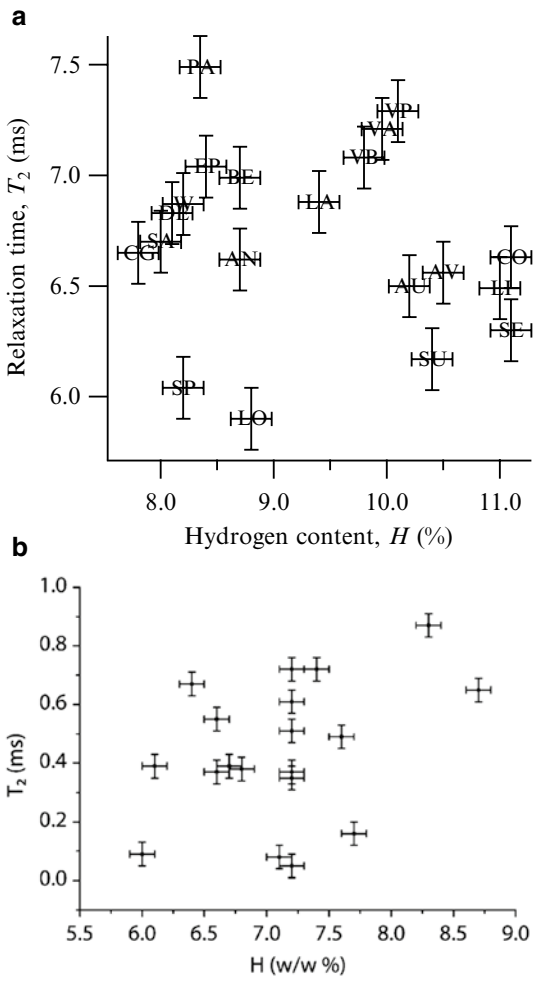


Fig. 7.25 Relationship between relaxation time, T_2, and hydrogen content, H for a population of 20 latex gloves (*top panel*) and of 19 polyurethane foam samples (*bottom panel*). Reproduced from Refs. [47, 48] by permission of The Royal Society of Chemistry

intensity, A is a pre-exponential factor, t is the time and T_2 is the transversal relaxation time. The obtained T_2 relaxation time is a parameter linked to the mobility of chains, and so to the overall morphological framework of the polymer. The frequency of crosslinks, the size of rubber chains, the presence of small molecular weight compounds, and the relative quantities of the solid, i.e. stiff or hard regions, and soft components of the sample all influence the shape of the relaxation signal, and thus the value of T_2 [41, 42].

T_2 is inherently non-local, and thus much different and not related to the measurement of proton quantity. Therefore, T_2 and the hydrogen content illustrated above, being not correlated, allowed to efficiently discriminate a population of 20 latex gloves (Fig. 7.25a) and of polyurethane fragments (Fig. 7.25b). TD-NMR allows also to assess the quantity of rigid and mobile fraction within the material, applying fitting models to the FID signal obtained by specific pulse sequences such

as the MSE [45, 47] and references therein]. Interestingly, this approach did not prove equally useful for the discrimination of a population of polyurethane fragments, because it was partially correlated to hydrogen content. It is well known that in most polyurethanes, the rigid part is due to condensed aromatic stems, while the softer part is more aliphatic. Since aromatic isocyanate precursors have a lower hydrogen content than the polyols used in the soft segments of polyurethanes, the correlation between rigid phase content and hydrogen percentage can be explained [47]. The same does not happen for T_2 measurements, which are only partly due to the composition of the polymer, but which strongly depend also on the structure and morphology imposed by processing.

7.4 Orientation

Manufacturing processes are not always isotropic, and thus they impart to the polymer different structures along different directions. A typical example is found in polypropylene backings for adhesive tapes. In the production of monoaxially oriented polypropylene (MOPP), the polymer film is stretched along a preferential direction, causing the chains to line up anisotropically. This causes the material to exhibit different properties if stress is applied along the direction of stretch or perpendicularly to it. MOPP is used in hand-tearable adhesive tapes because it is resistant along the axis of the tape but it can be easily broken if torn perpendicularly to it [51]. If the polymer is stretched in two directions during the cooling step, a biaxially oriented polymer is obtained. In this case, the tape cannot be broken just by tearing it. Among the properties which become anisotropic with orientation there is refractive index. Therefore, observation under plane polarised light allows to assess the orientation of a polymeric film. A magnification of about 100× is enough. The optimal instrumental setup is a polarising microscope with a rotating stage. However, a microscope with a normal stage, but with one of the polarising filters which can be rotated is equally effective. Polarisation of the light is obtained inserting a polarising filter before and after the sample. The filter before the sample is called polariser, the one after the sample is called analyser. When the direction of polarisation of polariser and analyser are perpendicular one with respect to the other, the observation is defined ‘under crossed polars’. A sample which is completely dark under crossed polars is isotropic. This normally means that the polymer is amorphous, even though isotropic semicrystalline polymers with very small crystal size exist, which could give a similar result. On the contrary, if the film is still visible under crossed polars, it means that some anisotropy is present. In polymers this is generally consistent with the presence of crystalline material.

A particularly informative mode of observation consists in rotating the sample by about 5° off the extinction position, i.e. the position where it appears darker under crossed polars. If a crisscross pattern emerges, this shows that the film is biaxially oriented. If the interference colours are oriented in just one direction, the film is monoaxially oriented (Fig. 7.26).

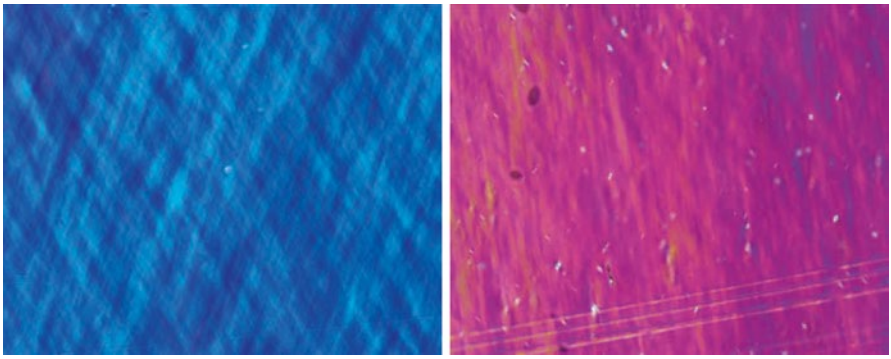


Fig. 7.26 (*Left*) Biaxially oriented polypropylene film and (*right*) monoaxially oriented polypropylene film observed in the conditions described in the text. Reprinted with permission from [51].

If the interference colours do not show any orientation, the film is unoriented.

Comparison of the edges of two films in the conditions where the interference colours are brighter allows to easily visualise small differences in the relative thicknesses [51]. Not always the orientation of the macromolecules is perfectly equal to the machine direction. In adhesive tape backings, the machine direction is along the length of the tape. In other types of films, it is not so straightforward to identify it. However, when the machine direction is detectable, by observation under polarised light with a rotating stage it is possible to assess the deviation of the orientation from the machine direction, if applicable. Under crossed polars, the machine edge of the film is oriented vertically in the view field. The stage position is noted. Then the stage is rotated to the extinction position, and the stage position is noted. The difference between the two stage positions is a measure of the disalignment of orientation with machine direction [51].

Spectroscopic measurements are a good approach for a more quantitative assessment of the orientation of macromolecules. Dichroism consists in the different absorption of light according to the direction of its polarisation. The study of this phenomenon is particularly suited to textile fibres, as well described by De Wael and colleagues in a series of papers [52–57]. In particular, De Wael's work was focused on spectrophotometry in the visible range of the electromagnetic spectrum. In the case of fibres, dichroism measurements consist in illuminating a sample with light of variable wavelengths, polarised in a direction parallel or perpendicular to the fibre axis, and in acquiring an absorption spectrum. The linear dichroism spectrum (LD) is obtained by subtracting, for each wavelength, the absorption under light polarised in the parallel and in the perpendicular position ($A_{\parallel}$ and $A_{\perp}$, respectively):

$$\mathrm{LD}(\lambda) = A_{\parallel}(\lambda) - A_{\perp}(\lambda)$$

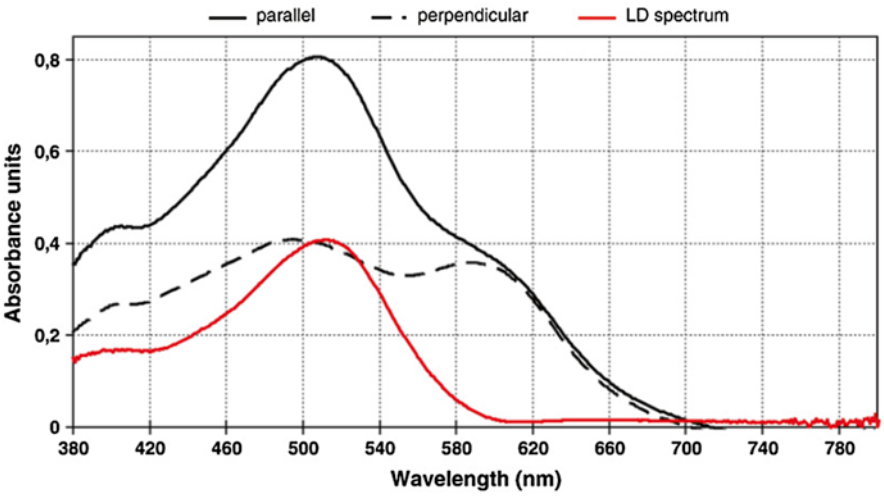


Fig. 7.27 Example of strong positive dichroism in a brown cotton fibre. Reprinted from Ref. [55], copyright 2012, with permission from Forensic Science Society

LD is positive for the wavelengths which are absorbed more when light is polarised along the parallel direction than when it is polarised along the perpendicular direction, and negative for the spectral regions for which the opposite occurs.

Figure 7.27 shows an example of strong positive dichroism.

This kind of approach suffers some limitations [54]. Very deeply coloured items can produce a saturation of the signal. Complex and overlapping bands, such as those encountered in polyamides, polyesters and other fibres coloured with mixtures of colourants [53, 56, 57], should be preferentially compared on the basis of their full LD spectrum, rather than on account of punctual values relative to a specific wavelength. Finally, these measurements are significant just when the dichroism of the considered items is relevant. Very weakly dichroic samples were shown to greatly decrease the reliability of the information obtained [54].

Despite these drawbacks, the study of dichroism in the visible range is a highly specific method, which requires no sample preparation, and a not too sophisticated equipment. Since dichroism depends both on the formulation of the fibre and on the orientation of the macromolecules, it is actually an informative technique for the characterisation of many oriented and coloured items. Actually, sometimes dyes with a strong dichroic effect are chosen expressly because they confer a peculiar appearance to the fibre and to the textile.

Consistent measurements require an accurate reproducibility of the positioning of the sample. Use of a microscope equipped with a rotating stage facilitates the probing of exactly the same region of the sample in the two successive stages of the measurement, i.e. when parallely and perpendicularly polarised light is used. De Wael and coworkers reported that the best spectral range for comparisons is between 425 and 725 nm [54]. They also proposed a criterion of comparison.

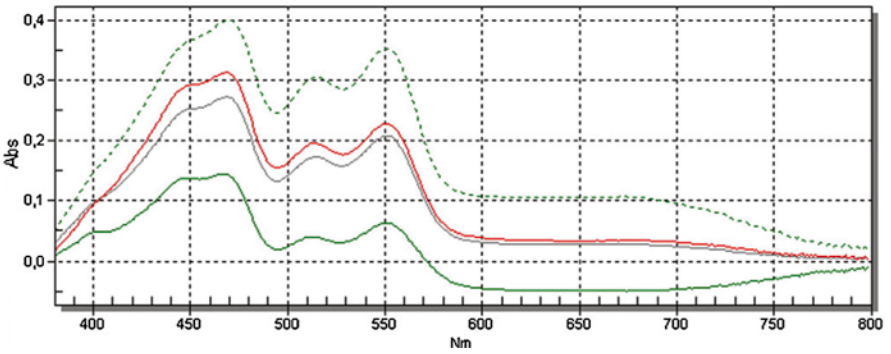


Fig. 7.28 The comparison of the spectra performed on control fibres and on a fibre trace. The *top* and *bottom* spectra are the limits defined by 2.5 times the standard deviation at each point, the gray trace is the mean LD spectrum of the control, and the red trace is the LD spectrum of the fibre trace. Reprinted from Ref. [54], copyright 2012, with permission from Forensic Science Society

First of all at least ten replicates on the samples of known origin should be acquired, in order to assess the homogeneity of the material, to compute an average LD spectrum and to calculate the standard deviation of the LD value for each wavelength. An unknown fibre will positively compare with the known ones if its LD spectrum lies within 2.5 standard deviations from the average LD spectrum obtained above (Fig. 7.28). This means that the LD spectrum of the questioned item lies within the natural variation of the control sample.

The examples presented so far involved comparisons on the basis of a full LD spectrum. However, the dichroic effect can be assessed for a particular wavelength or absorption peak by calculating the dichroic ratio, which is the quotient of the absorptions in parallel and perpendicular positions:

$$R(\lambda) = A_{\parallel}(\lambda) / A_{\perp}(\lambda)$$

When $R > 1$, the absorption is stronger for parallel polarised light than for perpendicular polarised light and this is called hypochromic effect. When $R < 1$ the opposite happens, and the effect is termed hyperchromic. Use of dichroic ratios in a forensic context was proposed in the visible range for textile fibres [52–57]. However, it is probably with IR spectroscopy that the measurement of dichroic ratios yields the most informative data [58–62]. Increasing draw ratios in the spinning process of fibres or in the manufacturing of films induces the orientation of the macromolecular chains (and thus of their functional groups) along the axis of the fibre. Figure 7.29 schematises the potential of polarised FTIR in acquiring information on the structure and orientation of polyester chains. In the unoriented state, C=O groups in a polyester are randomly distributed, and so the same amount of functional groups will be excited into a vibration by incoming IR light, regardless

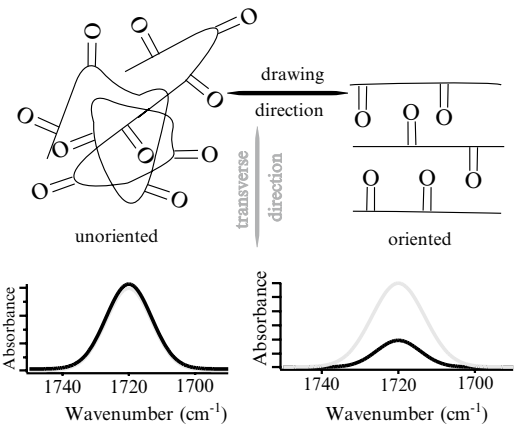


Fig. 7.29 Schematic showing the different orientation of carbonyl groups in an unoriented and in an oriented polyester. Below each cartoon, the correspondent expected polarised infrared absorption spectra are drawn (*grey* and *black traces* correspond to an impinging IR beam polarised along the transverse and drawing direction, respectively). Reproduced from [63] by permission of The Royal Society of Chemistry

of its polarisation. On the contrary, when the macromolecular chains are oriented, the C=O groups will be arranged along a preferential direction. Therefore, when light impinges the sample along the transverse direction, many more groups will be parallel to the light and will vibrate. Absorption of light will be strong in this case. IR light polarised along the drawing direction will encounter much less C=O groups which are arranged parallel to the direction of its polarisation, so the absorption of light will be much lower.

The possibility of identifying the functional groups which originate the peaks in an IR spectrum allows to chemically interpret the dichroism, eventually being able to understand the solid-state structure of the sample. For example, Wetzel and Cho investigated the dichroism of polyester fibres and found that, for the peaks at 973, 1,505 and 1,579 cm^{-1}, the dichroic ratio increased as a consequence of drawing (Fig. 7.30) [62]. This was consistent with the orientation of the terephthalate groups perpendicular to the chain axis.

Figure 7.30 clearly shows that no large spectral differences are detected when the unstretched sample is probed with IR light polarised in different directions: the two spectra are almost coincident. However, the absorption of the peaks starkly change in the case of the stretched sample, whether the polarisation is parallel or perpendicular to the fibre axis.

An elucidation of the structure of a sample is seldom necessary in forensic science, but dichroic ratios are more useful as diagnostic features for the classification or discrimination of items of a similar composition. For example, by a combination of IR dichroic ratio data and examination of the fibre morphology, Cho and colleagues were able to classify 32 polyester fibre samples into 22 unique individual fibre groups and five paired fibre groups [61]. Other examples of calculation of

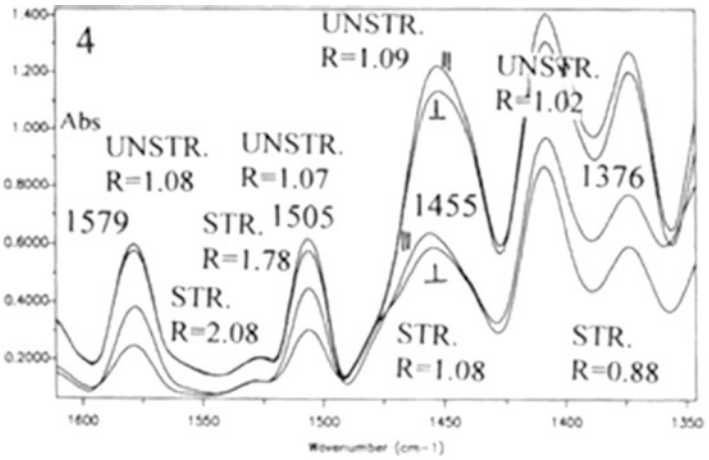


Fig. 7.30 Detail of the spectral region between 1,650 and 1,200 cm^{-1} of stretched and unstretched PET. Reprinted from Ref. [62], with kind permission from Springer Science+Business Media

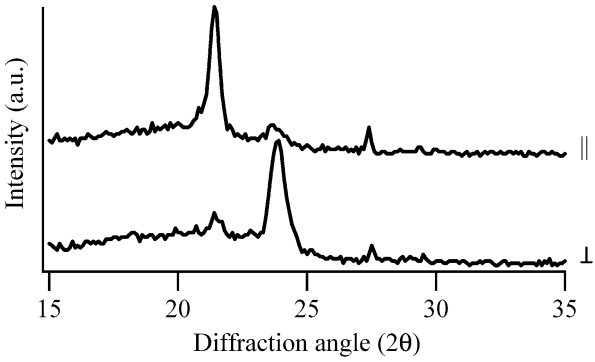


Fig. 7.31 XRD traces of the same plastic bag mounted in two directions, perpendicular to one another. The XRD patterns radically change, reflecting preferential orientation of the polymer crystallites. Reproduced by permission from Ref. [63] by permission of The Royal Society of Chemistry

dichroic ratios for assessing the effect of processing on the orientation of polymers were published on acrylics in a forensic context [59] and on polypropylene in a more fundamental framework [58].

Orientation can be detected and quantified also by XRD, by mounting an item along two different directions perpendicular to one another. If different XRD patterns are obtained, this shows that the structure is indeed anisotropic. Figure 7.31 shows how the relative intensity of the peaks changes when a sample of plastic bag is oriented in two perpendicular directions. The intensity of a diffraction reflection depends on a particular family of crystallographic planes. If such planes are isotropically distributed within the sample, an equal number of planes will be illuminated, irrespective of the direction of the incoming X-ray beam, which is also called primary beam. Samples with an anisotropic structure will have crystal domains oriented along a preferential direction. Therefore, if the X-ray beam illuminates the

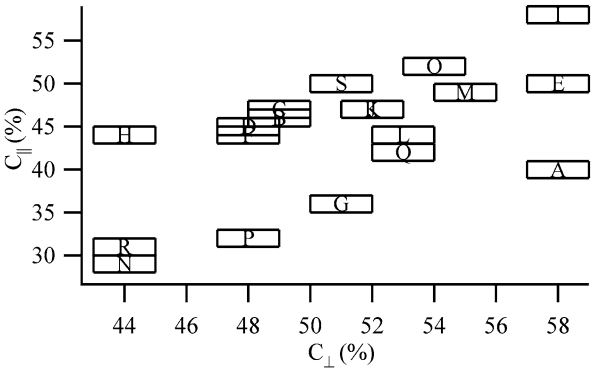


Fig. 7.32 Plot of the degree of crystallinity of a population of office papers, measured on XRD patterns obtained mounting the samples in two directions, perpendicular to one another. *Boxes* are used to represent *error bars*. Reprinted from Ref. [12], copyright 2010, with permission from Elsevier

sample from different directions it will encounter more or less crystallographic planes of a particular family. As a consequence, the intensity of the diffraction peak due to each family of peaks will change, as a function of the orientation of the sample relative to the primary beam.

The difference in relative intensity of particularly diagnostic peaks can be exploited as a discriminating parameter for the characterisation of polymeric items, such as plastic bags [11]. In this case the ratio between the peaks at 21.6 and 24.0 °2θ (Fig. 7.31) was computed. A further level of quantification of anisotropy by XRD can be carried out evaluating the degree of crystallinity on the XRD patterns from each direction of sample mounting. This was done on plastic bags [11] and on paper [12]. In both cases, severely different degrees of crystallinity were found mounting the samples along perpendicular directions, as shown in Fig. 7.32.

References

1. Sugita R, Sasagawa K, Suzuki S (2009) Illegal route estimation of the seized illicit drug, methamphetamine, by the comparison of striation marks on plastic packaging films. J Forensic Sci 54:1341
2. Berx V, De Kinder J (2002) 3D measurements on extrusion marks in plastic bags. J Forensic Sci 47:976
3. Denton S (1981) Extrusion marks in polythene film. J Forensic Sci Soc 21:259
4. Stokes DJ (2003) Recent advances in electron imaging, image interpretation and applications: environmental scanning electron microscopy. Philos Trans R Soc London Ser A 361:2771
5. Klug HP, Alexander LE (1974) X-ray diffraction procedures. Wiley, New York
6. Alexander LE (1969) X-ray diffraction procedures in polymer science. Wiley, New York
7. Ladd M, Palmer R (eds) (2013) Structure determination by X-ray crystallography: analysis by X-rays and neutrons. Springer, New York
8. De Rosa C, Auriemma F (eds) (2014) Crystals and crystallinity in polymers: diffraction analysis of ordered and disordered crystals. Wiley, Hoboken
9. Turner Jones A, Aizlewood JM, Beckett DR (1964) Crystalline forms of isotactic polypropylene. Die Makromol Chem 75:134
10. Hindeleh AM, Johnson DJ (1971) The resolution of multipeak data in fiber science. J Phys D Appl Phys 4:259

11. Causin V, Marega C, Carresi P et al (2007) A quantitative differentiation method for plastic bags by wide angle X-ray diffraction for tracing the source of illegal drugs. Forensic Sci Int 168:37
12. Causin V, Marega C, Marigo A et al (2010) Forensic differentiation of paper by X-ray diffraction and infrared spectroscopy. Forensic Sci Int 197:70
13. Foner HA, Adan N (1983) The characterization of papers by X-ray diffraction (XRD): measurement of cellulose crystallinity and determination of mineral composition. J Forensic Sci Soc 23:313
14. Chen R, Jakes KA, Foreman DW (2004) Peak-fitting analysis of cotton fiber powder X-ray diffraction spectra. J Appl Polym Sci 93:2019
15. Liu Y, Hu H (2008) X-ray diffraction study of bamboo fibers treated with NaOH. Fibers Polym 9:735
16. Moharram MA, Mahmoud OM (2007) X-ray diffraction methods in the study of the effect of microwave heating on the transformation of cellulose I into cellulose II during mercerization. J Appl Polym Sci 105:2978
17. Wada M, Okano T (2001) Localization of Iα and Iβ phases in algal cellulose revealed by acid treatments. Cellulose 8:183
18. Yan Z, Chen S, Wang H et al (2008) Biosynthesis of bacterial cellulose/multi-walled carbon nanotubes in agitated culture. Carbohydr Res 74:659
19. Menczel JD, Prime RB (eds) (2009) Thermal analysis of polymers: fundamentals and applications. Wiley, Hoboken
20. Hatakeyama T, Quinn FX (1994) Thermal analysis: fundamentals and applications to polymer science. Wiley, Chichester
21. Tsukame T, Kutsuzawa M, Sekine H et al (1999) Identification of polyethylene by differential scanning calorimetry: application to forensic science. J Therm Anal Calorim 57:847
22. Sajwan M, Aggarwal S, Singh RB (2008) Forensic characterization of HDPE pipes by DSC. Forensic Sci Int 175:130
23. Causin V, Marega C, Marigo A et al (2009) A method based on thermogravimetry/differential scanning calorimetry for the forensic differentiation of latex gloves. Forensic Sci Int 188:57
24. Causin V, Marega C, Carresi P et al (2006) A quantitative differentiation method for plastic bags by infrared spectroscopy, thickness measurement and differential scanning calorimetry for tracing the source of illegal drugs. Forensic Sci Int 164:148
25. Yang L, Thomas P, Stuart B (2012) Discrimination of thermally treated low density polyethylenes using DSC and principal component analysis. J Therm Anal Calorim 108:445
26. Causin V, Marega C, Marigo A (2007) When polymers fail: a case report on a defective epoxy resin flooring. Eng Fail Anal 14:1394
27. Causin V, Marega C, Guzzini G et al (2004) Forensic analysis of poly(ethylene terephthalate) fibers by infrared spectroscopy. Appl Spectrosc 58:1272
28. Causin V, Marega C, Guzzini G et al (2005) The effect of exposure to the elements on the forensic characterization by infrared spectroscopy of poly(ethylene terephthalate) fibers. J Forensic Sci 50:887
29. Rajakumar K, Sarasvathy V, Thamarai Chelvan A et al (2009) Natural weathering studies of polypropylene. J Polym Environ 17:191
30. Horrocks AR, Mwila J, Miraftab M et al (1999) The influence of carbon black on properties of orientated polypropylene 2. Thermal and photodegradation. Polym Degrad Stab 65:25
31. Cherukupalli SS, Ogale AA (2004) Online measurements of crystallinity using Raman spectroscopy during blown film extrusion of a linear low-density polyethylene. Polym Eng Sci 44:1484
32. Quynn RG, Riley JL, Young DA et al (1959) Density, crystallinity, and heptane insolubility in isotactic polypropylene. J Appl Polym Sci 2:166
33. Nielsen AS, Batchelder DN, Pyrz R (2002) Estimation of crystallinity of isotactic polypropylene using Raman spectroscopy. Polymer 43:2671
34. Samuels RJ (1981) Application of refractive index measurements to polymer analysis. J Appl Polym Sci 26:1383

35. Dabbs MDG, Pearson EF (1972) Some physical properties of a large number of window glass specimens. J Forensic Sci 17:70
36. Causin V, Marega C, Schiavone S et al (2005) Employing glass refractive index measurement (GRIM) in fiber analysis: a simple method for evaluating the crystallinity of acrylics. Forensic Sci Int 149:193
37. Palenik SJ (1999) Microscopical examination of fibres. In: Robertson J, Grieve M (eds) Forensic examination of fibres. Taylor and Francis, London
38. Curran JM, Hicks TN, Buckleton JS (eds) (2000) Forensic interpretation of glass evidence. CRC Press, Boca Raton
39. Ojena SM, DeForest PR (1972) Precise refractive index determination by the immersion method, using phase contrast microscopy and the Mettler hot stage. J Forensic Sci 12:315
40. Abragam A (1983) Principles of nuclear magnetism. Oxford University Press, Oxford
41. Orza RA, Magusin PCMM, Litvinov VM et al (2007) Solid-state 1H NMR study on chemical cross-links, chain entanglements, and network heterogeneity in peroxide-cured EPDM rubbers. Macromolecules 40:8999
42. Chinn SC, Cook-Tendulkar A, Maxwell R et al (2007) Qualification of automated low-field NMR relaxometry for quality control of polymers in a production setting. Polym Test 26:1015
43. Jensen SM, Pedersen HT, Engelsen SB (1999) Application of chemometrics to low-field ^{1}H NMR relaxation data of intact fish flesh. J Sci Food Agric 79:1793
44. Standard test method for hydrogen content of middle distillate petroleum products by low-resolution pulsed nuclear magnetic resonance spectroscopy (2005) ASTM International, 2003, West Conshohocken
45. Maus A, Hertlein C, Saalwaechter K (2006) A robust proton NMR method to investigate hard/soft ratios, crystallinity, and component mobility in polymers. Macromol Chem Phys 207:1150
46. Mauri M, Thomann Y, Schneider H et al (2008) Spin-diffusion NMR at low field for the study of multiphase solids. Solid State Nucl Magn Reson 34:125
47. Mauri M, Dibbanti MK, Calzavara M et al (2013) Time domain nuclear magnetic resonance: a key complementary technique for the forensic differentiation of foam traces. Anal Methods 5:4336
48. Mauri M, Mauri L, Causin V et al (2011) A method based on time domain nuclear magnetic resonance for the forensic differentiation of latex gloves. Anal Methods 3:1802
49. Carr HY, Purcell EM (1954) Effects of diffusion on free precession in nuclear magnetic resonance experiments. Phys Rev 94:630
50. Schmidt-Rohr K, Spiess HW (1999) Multidimensional solid-state NMR and polymers. Academic, San Diego
51. Smith JM (2007) Forensic examination of pressure sensitive tape. In: Blackledge RD (ed) Forensic analysis on the cutting edge. Wiley, Hoboken
52. De Wael K (2012) Dichroism measurements in forensic fibre examination. Part 4—dyed acrylic and acetate fibres. Sci Justice 52:81
53. De Wael K, Lepot L (2012) Dichroism measurements in forensic fibre examination: part 5—pigmented fibres. Sci Justice 52:161
54. De Wael K, Lepot L, Lunstroot K (2012) The use of linear dichroism in forensic fibre examinations—part 6. Validation and practical aspects of MSP-PPL. Sci Justice 52:249
55. De Wael K, Lepot L (2011) Dichroism measurements in forensic fibre examination part 3—dyed cotton and viscose fibres. Sci Justice 51:173
56. De Wael K, Vanden Driessche T (2011) Dichroism measurements in forensic fibre examination. Part 2—dyed polyamide, wool and silk fibres. Sci Justice 51:163
57. De Wael K, Vanden Driessche T (2011) Dichroism measurements in forensic fibre examination part 1—dyed polyester fibres. Sci Justice 51:57
58. Hedesiu C, Demco DE, Remerie K et al (2008) Study of uniaxially stretched isotactic poly(propylene) by ^{1}H solid-state NMR and IR spectroscopy. Macromol Chem Phys 209:734
59. Cho LL, Reffner JA, Gatewood BM et al (1999) A new method for fiber comparison using polarized infrared microspectroscopy. J Forensic Sci 44:275

60. Boulet-Audet M, Lefevre T, Buffeteau T et al (2008) Attenuated total reflection infrared spectroscopy: an efficient technique to quantitatively determine the orientation and conformation of proteins in single silk fibers. Appl Spectrosc 62:956
61. Cho LL, Reffner JA, Wetzel DL (1999) Forensic classification of polyester fibers by infrared dichroic ratio pattern recognition. J Forensic Sci 44:283
62. Wetzel DL, Cho LL (1997) Single fiber characterization by polarization FT-IR microspectroscopy. Mikrochim Acta Suppl 14:349
63. Causin V (2010) Polymers on the crime scene: how can analytical chemistry help to exploit the information from these mute witnesses? Anal Methods 2:792

Index

A
ABS. *See* poly(acrylonitrile-co-butadiene-co-styrene)
Absorption atomic spectrometry, 181, 182
Absorption process, 172
Acceptability in Court, 66
Acceptance within the relevant scientific community, 69
Accuracy, 69
Acetate
  MALDESI, 232
Acetate ester of cellulose, 106
Acrylic acid, 33
Acrylic fibres, 215, 283, 286
  dichroic ratios, 340
  IR spectroscopy, 215, 229
  refractive index, 326
Acrylic resins, 115
Acrylics, 106
Acrylic styrene melamine paint pyrogram, 248, 249
Activity level, 89
Addition polymerisation, 13
Addition reactions, 14
Additives, 167
  adhesive tapes, 123
  condoms, 137
  gas chromatography, 247
  paints, 117
  plastic bags, 149
  Pyr-GC–MS, 248
  thermogravimetry, 208
  tyres, 133
Adhesives, 279
Adhesive tapes, 273
  additional layers, 124
  characterisation, 125
  composition, 123
  IRMS, 243–245
  MALDI, 252
  Pyr-GC–MS, 251
  XRD, 318
Adsorption chromatography, 191, 194
Agglomerative hierarchical clustering, 81
Alkyd resins, 115
Ambient ionisation techniques, 203
Amine resins, 47
Aminic resins, 116
Amino acids, 51
Amorphous halo, 317
Amorphous phase, 30
Amorphous state, 309
Analyser, 335
Analytical method, 64
Animal fibres, 106
Anisotropy, 335
Anti-Stokes scattering, 179
Aramides, 38
Architectural paints, 114
  IRMS, 243
Arrowheads, 305
Arson accelerants, DART, 255
Arson debris, TGA, 256
Artificial fibres, 106
Artificial polymers, 21
Ashing, 182
ASTM standard guides, 63
Asymmetric carbon, 26

V. Causin, *Polymers on the Crime Scene,*
DOI 10.1007/978-3-319-15494-7

Atactic, 27
Atactic polypropylene, 27, 290
Atomic fluorescence, 182
Atomic mass spectrometry, 185
Atomic spectrometry, 180
  atomic mass spectrometry, 185
  optical, 181
Atomic spectroscopy
  adhesive tapes, 127
  fillers, 221
  paper, 221
Atomisation, 180, 181
Attenuated total reflection (ATR), 177
Automotive paints, 118
Autoscaling to unit variance, 76

B
Background probabilit(ies), 92, 94
Background scenarios, 95
Backing, adhesive tapes, 123, 335
Baekeland, L., 12
Banknotes
  infrared mapping, 240
  infrared spectroscopy, 239
Basecoat(s), 119
  IR and Raman spectra, 231
Bast fibres, 106
Bayesian approach, 87, 89
Bayes' theorem, 90
Bead, 133
Belt, 133
Bending, 175
Biaxially oriented polypropylene film, 336
Binder(s)
  paints, 114
  in paints, IR spectroscopy, 215
  toner, 152
Biodegradable polymers, IR spectrum, 212
Birefringence
  adhesive tapes, 125
  plastic bags, 149
Birefringent, 307
Bisphenol A, 37
Blend, 25
Bloch Decay (BD), 332
Bragg law, 312
Branched polymers, 25
Bulk polymerisation, 16
Butyl rubber, 43

C
Calcium carbonate, IR spectrum, 219
Calibration
  DSC, 322
  thermogravimetry, 209
Capillary electrophoresis, 237
  condom-related traces, 247
  dyes in fibres, 237
Caprolactam, 38
Carbon black, 133
Carbon fibres, 106
Carboxymethyl cellulose, 51
Carothers, W.H., 12
Car paints, 114
Carr-Purcell-Meiboom-Gill (CPMG), 333
Case reports, fibres, 113
Catalysts, 180
Cathodoluminescence (CL), 225
  duct tape, 225
  paints, 225
Cellophane, 50
Cells, 142
Celluloid, 51
Cellulose, 11, 21, 48, 151
  IR spectrum, 219
  XRD, 222, 318
Cellulose(acetate-co-butyrate), 51
Cellulose(acetate-co-propionate), 51
Cellulose acetate, 50
Cellulose nitrate, 51
Cellulose triacetate, 50
Cellulose xanthate, 49
Centroid linkage, 81
Chain mobility, 332
Chain of custody, 62
Chain polymerisation, 14
Chemical ionisation, 201
Chemical variation, 217
Chlorinated PVC, 41
Chromatogram, 191, 194
Chromatography, 189, 247
  condom-related traces, 139
  definition, 190
  dyes, 227, 235
  fibres, 112, 236
Cis configuration, 28
Cis-1,4-polyisoprene, 28, 43
Classes, 80
Clearcoat, 119
Cling films, 217
Closed cell foams, 142
Clustering, condom-related traces, 140
Clustering methods, 80
  tyres, 136
Clusters, 80
  tyres, 136

Coating, paper, 151
Collagen, 21
Collections of materials, 215
Colligative properties, 18, 268
Colour, 172
  adhesive tapes, 124
Colour coat, 119
Column chromatography, 193
Commodity polymers, 23
Communication, 71
Comonomer, 24, 283
Comonomer quantification, infrared spectroscopy, 284
Comonomers, 283
Comparison, 65
Complementarity, 179, 231
  Raman and infrared spectroscopy, 178, 230, 231
Complementary, IR and Raman spectroscopy, 214
Complete linkage, 81
Condensation, 45, 52
Condensation reactions, 13
Condom-related traces, 279
  capillary electrophoresis, 247
  DART, 255
  DESI-MS, 255
  DIOS, 255
  MALDI, 253
Configuration, 24, 26
Confocal micro-XRF, 234
Conformation, 24, 29
Constitution, 23, 24
Constitutive unit. *See* Repeat units
Constructive interference, 312
Contact traces, 59, 87
Contamination, 61, 62, 109
Copolymer, 24
Copolymerisation, acrylic fibres, 283
Copolymers, 283
Corrosion inhibitors, paints, 117
Cotton, 49, 106
Count denier (den), 108
Crime level, 89
Critical pigment volume concentration (CPVC), 116
Cross contamination, 62, 109
Crossed polars, 335
Crosslink density rubbers, 332
Cross-linked polymers, 25
Crystalline cells, 309
Crystalline lamellae, 309
Crystalline phases, 30, 316
Crystalline state, 309
Crystallinity, 150
Cure package, 133
Curie point, 198
Curie type pyrolyser, 198

D
Database(s), 88
  adhesive tapes, 127
  of infrared spectra, 175
  IR spectroscopy, 214
  paint, 121
  toner, 242
Daubert criteria, 67
Daubert opinion, 66
Daughter ions, 201
Definition of the problem, 64
Defoamers, 117
Degree of crystallinity, 310, 317, 318, 323
  differential scanning calorimetry, 323
  infrared spectroscopy, 325
  Raman spectroscopy, 325
  refractive index, 325
  X-ray diffraction, 317
Degree of polymerisation, 13
Degree of unsaturation, 14
Delustering agent, 230
Dependence of results from the weathering, 247
Depolymerisation, 268
Derivatisation, 249
Derivatising agents, gas chromatography, 193
Derivative curve
  thermogravimetry, 208
  UV/visible spectra, 228
  UV/visible spectra polyurethane foams, 228
DESI-MS imaging, 255
Desorption chemical ionisation mass spectrometry, condom-related traces, 139
Desorption electrospray ionisation (DESI), 203
Desorption electrospray ionisation mass spectrometry (DESI-MS), condom-related traces, 255
Desorption ionisation methods, adhesive tapes, 128
Desorption-Ionisation on silicon (DIOS), 254
  condom-related traces, 255
Desorption sources, rationale, 202
Destructive interference, 312
De-swelling, 272
Detection limit, 70
Diamond anvil cell, 177
Dichroic ratios, 338
  acrylic fibres, 340
  infrared spectroscopy, 338, 339
  polyester fibres, 339

Dichroic ratios (*cont.*)
visible spectroscopy, 338
Dichroism, 336
fibres, 336, 338
Differential refractometer detector, 273
Differential scanning calorimetry (DSC), 320
data overinterpretation, 324
plastic bags, 149
rubber gloves, 131
standard temperature program, 320
thermogram, 322
Differential shedding, 109
Differentiation of viscose from cotton, Raman spectroscopy, 230
Diffractograms, 315
Digestion, 182
Diode array UV-visible detectors, 196
Dipping process, gloves, 130
Direct analysis in real time (DART), 203, 255, 279
arson accelerants, 255
condom-related traces, 255
glues, 255
paper, 255
resins, 255
Direct solid analysis, 183, 186
Disalignement of orientation, 336
Discriminating power, 68, 219
Distance, in clustering, 81
Distance matrix, 81
Distance table, 83
Distribution of molecular weights, 268
See also Molecular weight distribution
Distribution of the filler, 220
Divisive hierarchical clustering, 82
Donor, 59, 108
Driers, paints, 117
Dry spinning, 107
Dtex, 108
Duct tape, cathodoluminescence (CL), 225
DuPont, 12
Dye(s), 144, 168, 224
chromatography, 227, 235
foam fragments, 144
IR spectroscopy, 229
microspectrophotometry, 227
paper, 151

E

Ectromagnetic radiation
energy, 171
Elastomers, 23
Electrical tapes, 245
Electrocoat, 118
Electromagnetic radiation, 170
Electromagnetic spectrum, 171
Electronic transitions, 174
Electron impact ionisation, 201
Electrophoresis, 237
Electrospray, 203
Electrothermal atomisation, 182
Electrothermal vaporisation, 183
Elemental analysis, 221, 243
adhesive tapes, 127
fibres, 112
foam fragments, 147
paints, 122
paper, 151
plastic bags, 149, 243
toner, 153
Elemental profile, 180
Elementary cells, 309
Eluent, 192
Elution, 191
Elution time, 195
Emission atomic spectrometry, 181
advantages, 183
Emission spectrum, atomic spectroscopy, 182
Emulsifiers, 117
Emulsion polymerisation, 16
End group analysis, 280
Endothermal peaks, 322
Energy dispersive X-ray fluorescence, 188
Enthalpic relaxation, 328
Enthalpy, 209, 322–324
DSC, 322
Environmental scanning electron microscopy (ESEM), 189, 307
Epoxy resins, 47, 115
Ethyl cellulose, 51
Ethylene-propylene copolymers, 289
Ethylene propylene diene monomer (EPDM), 289
Ethylene propylene monomer (EPM), 289
Euclidean distance, 81
European Car Paint (EUCAP), 121
European Network of Forensic Science Institutes (ENFSI), 64, 121
Evanescent wave, 177
Evett's verbal scale, 99
Evidential value, 218
Excitation process, 172
Exclusion limit, 270
Exothermal peaks, 322
Explained variance, 77
Extended chain conformation, 29
Extended range SEC columns, 272
Extrusion, 304

F
Fabric cords, 133
F distribution, 74
Fibre finding systems, 112
Fibres, 23, 105
  capillary electrophoresis, 237
  chromatography, 236
  dichroism, 336, 338
  TLC, 236
Fibrous fillers, 168
Field desorption, 202, 278
Field ionisation, 201
Figures of merit, 69
Filaments, 107
Filament type pyrolyser, 197
Filler(s), 168, 180, 218
  adhesive tapes, 123, 124
  atomic spectroscopy, 221
  foam fragments, 144, 146
  infrared spectroscopy, 219
  paints, 117
  paper, 151
  plastic bags, 149
  quantitation by IR spectroscopy, 219
  thermogravimetry, 209
  tyres, 133
  X-ray diffraction, 222
  XRD, 319
Filmogenic power, 116
Filmogens, 117
Fingerprint region, IR spectroscopy, 211
Fire resistance, 169
Fisheyes, 305
Flame atomisation, 182
Flame ionisation detector, 195
Flame retardants, thermogravimetry, 208
Flax, 49, 106
Flory, P., 12
Flow control agents, 117
Fluorescence, 180
  adhesive tapes, 125
Fluorescence atomic spectrometry, 181
Foam fragments
  gas chromatography, 247
  TD-NMR, 332, 334
Forensic analysis, aim of, 64
Formaldehyde-based resins, 45
Formulation, 217
  scheme for characterisation, 170
Fourier transform, 177
Fractionation, 187
Fragmentation, 201, 278
Free induction decay (FID), 329, 330
  signal shape, 331
Frequency, 171
Frequentist approach, 87
Frye standard, 67
Functional groups, 175
Furnace type pyrolyser, 198

G
Gas chromatography, 190, 192, 193
  additives, 247
  foam fragments, 147, 247
  post-fire traces, 154
Gas chromatography/mass spectrometry,
    condom-related traces, 140
Gas-phase sources, 201
Gel permeation chromatography (GPC). *See*
    Size exclusion chromatography (SEC)
Geometrical isomerism, 28
Get it first time (GIFT) principle, 61
Glass refractive index measurement (GRIM), 326
Glass transition temperature (T_g), 328
Glues
  DART, 255
  poly(styrene-*co*-butyl acrylate), 288
Good laboratory practices, 69
Goodyear, C., 11
Gradient elution, 194
Graphite furnaces, 183
Gun cotton, 11
Gutta-percha, 28, 43

H
Hard sources, 201
Hardwood, 151, 255
HDPE. *See* High density polyethylene (HDPE)
Head space concentration, post-fire traces, 153
Head-to-head addition, 25, 295
Head-to-tail addition, 25, 295
Heat flux DSC, 321
Helix conformation, 29
Hemp, 49, 106
Hevea brasiliensis, 43, 129
Hierarchical clustering, 81
High density polyethylene (HDPE), 31
High performance liquid chromatography
    (HPLC), 193
  fibres, 236
Hit-and-run accidents, 113
Homopolymers, 24, 283
Hotelling, H., 75
Hotelling's T^2 test, 74
HPLC. *See* High performance liquid
    chromatography (HPLC)

Hyatt, J.W., 12
Hydrogen content, 332
Hyperchromic effect, 338
Hypochromic effect, 338
Hypothesis, 67
Hysteresis peak, 328

I
ICP. *See* Inductively coupled plasma (ICP)
ICP-MS. *See* Inductively coupled plasma-mass spectrometry (ICP-MS), plastic bags
Identification, 65, 66
Inductively coupled plasma (ICP), 183
Inductively coupled plasma-mass spectrometry (ICP-MS), plastic bags, 243
Inelastic scattering, 179
Infrared, 171
Infrared mapping, 239
  banknotes, 240
  paints, 242
Infrared spectroscopy, 175, 280, 284, 286, 288, 289, 292
  adhesive tapes, 126
  banknotes, 239
  condom-related traces, 139
  dyes, 229
  fibres, 215
  fillers, 219
  foam fragments, 145
  paints, 122, 215, 238
  paper, 151, 152, 219
  pigments, 229
  plastic bags, 149
  polymer matrix, 211
  post-fire traces, 156
  rubber gloves, 131
  sample preparation, 177
  toner, 242, 243
Initiation, 15
Initiatior, 14
Inks
  MALDI, 253
  UV/vis spectra, 228
Innerliner, 133
Inorganic pigments
  adhesive tapes, 123
  paints, 116
Inorganic polymers, 22
Intelligence driven forensic science, 65
Interference colours, 336
Interferogram, 177
Internal standard, ICP-MS, 187
International Union of Pure and Applied Chemistry (IUPAC), 190
Interpretation of evidence, 86
Ion exchange chromatography, 192
Ionisation mass spectrometry, 198
Ion source, 199, 200
Ion trap analyser, 200
IR absorption spectrum, 175
IR fingerprint, 175
Iron oxide, 152
IR spectra, 284, 288
IR spectroscopy, 215
  fibres, 112
  toner, 153
Isocratic elution, 194
Isocyanate groups, 39
Isotactic, 27
Isotacticity, 290
Isotactic polypropylene, 27, 31, 290
Isotope fractionation, 244
Isotope ratio mass spectrometry (IRMS), 243
  adhesive tapes, 127, 243–245
  architectural paints, 243
  plastic bags, 243
  post blast debris, 243, 247
Isotope ratios, 244
Isotopes, 185
Isotopic ratios, 185
Isotropic, 335

J
Justice driven forensic science, 65
Jute, 49, 106

K
Kapok, 106
KBr pellet, 177
Keratin, 52
Kevlar®, 38
Known or potential rate of error, 68

L
Laser ablation, 184, 186, 221
  paints, 233
Laser ablation ICP-MS, paints, 233
Laser induced breakdown spectroscopy (LIBS), 84, 221
Laser induced breakdown spectroscopy and laser ablation ICP-MS, 221
Latex, 43, 44
  condoms, 137

Latex gloves, 129
characterisation, 130
TD-NMR, 332, 334
TGA, 256
LDPE. *See* Low density polypropylene (LDPE)
Leaf fibres, 106
Leather, 51
Levels of proposition, 88
LIBS. *See* Laser induced breakdown spectroscopy (LIBS)
Light scattering, 268
Lignin, 49
Likelihood ratio, 90, 249
Linear dichroism spectrum, 336
Linearity, limit of, 70
Linear low density polyethylene (LLDPE), 31
Linear polymers, 25
Linear SEC columns, 272
Liquid chromatography, 190, 192
LLDPE. *See* Linear low density polyethylene (LLDPE)
Loadings, 77
Locard's principle, 59
Long chain branching, 25
Low density polypropylene (LDPE), 30
Lubricants, condoms, 137
Lycra®, 39

M
Magic sandwich echo (MSE), 333
Magnetic sector analysers, 200
MALDESI. *See* Matrix-assisted laser desorption electrospray ionisation mass spectrometry (MALDESI)
MALDI. *See* Matrix-assisted laser desorption/ionisation (MALDI)
Man-made synthetic fibres, 107
Markers of ignitable liquids, 154
Mark, H., 12
Mass analyser, 200
Mass atomic spectrometry, 180
Mass spectrometry, 198, 199, 251, 279
adhesive tapes, 128
condom-related traces, 140
detection method for chromatography, 196
Mass spectrometry with desorption ion sources, dyes in fabrics and fibres, 232
Matrix-assisted laser desorption electrospray ionisation mass spectrometry (MALDESI), 232
Matrix-assisted laser desorption/ionisation (MALDI), 202, 278
adhesive tapes, 252
condom-related traces, 253
imaging, 253
inks, 253
matrix, 202
sample preparation, 140, 202
Maximum variance, 75
Mean fill, 76
Melamine formaldehyde (MF) resins, 46
Melting enthalpy, 323
Melting temperature, 323
Melt spinning, 107
Mercerisation, 318
Methacrylic acid, 33
Method, 66
Methyl acrylate, 34
Methyl cellulose, 51
Methyl methacrylate, 34
Methylolated species, 45
MF. *See* Melamine formaldehyde (MF) resins
Michelson interferometer, 177
Microscopy, 225
condom-related traces, 138
fibres, 112
foam fragments, 144
pigments, 225
Microspectrophotometry, 173, 174
dyes, 227
IR, 176, 178
paints, 122
Micro-XRF spectrometers, 188
Miller indices, 313
Mineral fibres, 106
Missing data, 75
Mixed bed SEC columns, 272
Mobile phase, 189, 191, 192
Modacrylic fibres, IR spectroscopy, 215
Modal, 106
Modulated DSC, 328
Molecular weight, 268
Molecular weight distribution, 17
Monoaxially oriented polypropylene (MOPP), 335
film, 336
Monochromator, 174
Monodisperse system, 16
Monomers, 12, 283
Moulding, 304
Multiple melting peaks, 324
Multivariate, 74

N
National Automotive Paint File (NAPF), 121
Natta, G., 27

Natural fibres, 106, 304
Natural polymers, 21
Natural rubber, 28, 43, 129
Near infrared (NIR), 229
Neoprene, 44
Neoprene gloves, 129
Network polymers, 25
Nitrile gloves, 129
Nitrile rubber, 44
Nitrocellulose, 11, 51
Nitrolaquer, 51
Nomex®, 38
Non reactive dyes, 236
Normal phase chromatography, 194
Novolacs, 46, 115
Nuclear magnetic resonance (NMR), 291
  condom-related traces, 139
Number-average molar mass, 18
Nylons, 38
  designation of, 38
  IR spectrum, 212
  MALDESI, 232

O
Observation under crossed polarising filters, 307
Offence level, 89
1:1 taping, 110
Open cell foams, 142
Optical atomic spectrometry, 180, 181
Optical atomic spectroscopy
  physical rationale, 182
  sample preparation, 182
Optical brighteners, 229
  paper, 151
Optical isomerism, 26
Organic pigments
  paints, 116
  Raman spectroscopy, 231
Organic polymers, 22
Organosols, 41
Osmotic pressure, 18

P
Packaging, poly(ethylene-*co*-vinyl acetate) (EVA), 287
Paint chips, 119
Paint data query (PDQ), 88, 121
Paint fragments, 113
Paints, 113
  CL, 225
  composition, 114
  infrared mapping, 242
  infrared spectroscopy, 238
  IR spectroscopy, 215
  Pyr-GC–MS, 248
  XRF, 233
Paint smears, 113, 119
  IR spectroscopy, 216
PAN. *See* Polyacrylonitrile (PAN)
Paper
  atomic spectroscopy, 221
  DART, 255
  IR spectrum, 219
  NIR, 229
  XRD, 318
Papermaking, 151
  chemical process, 151
  mechanical process, 151
Parent molecule, 201
Partition chromatography, 191, 194
Pauling, L., 12
PBT. *See* Poly(butylene terephthalate) (PBT)
PCA. *See* Principal component analysis (PCA)
Peaks DSC, 322
Pearson, K., 75
PEG. *See* Poly(ethylene glycol) (PEG)
Pentads, 292
Peptide bonds, 52
Permeation limit, 270
Persistence, 59, 60
  condom-related traces, 137
  foam fragments, 141
Persistence of fibres, 109
Personal lubricants, 138
PET. *See* Poly(ethylene terephthalate) (PET)
Phenol-formaldehyde resins, 46
Phthalates, 169, 249
Piercing, 306
Pigment bands, 305
Pigments, 144, 168, 180, 224
  foam fragments, 144
  IR spectroscopy, 229
  microscopy, 225
  paints, 116
  paper, 151
  toner, 153
  XRF, 233
Pinching, 306
Planar chromatography, 192
Plasma, 183
Plastic, 23
Plastic bags, 217
  composition, 149
  elemental analysis, 243
  ICP-MS, 243

IRMS, 243
XRD, 223, 318
Plasticisers, 245, 249
adhesive tapes, 123, 124
paints, 117
Plasticisers, oligomeric, 169
Plastic packaging material, XRD, 318
Plastifiers, 169, 328
Plastisols, 41
PMMA. *See* Poly(methyl methacrylate) (PMMA)
Poisson distribution, 95
Polarisation of light, 335
Polariser, 335
Poly(acrylic acid), 33
Poly(acrylonitrile-co-butadiene-co-styrene), 32
Poly(butylene terephthalate) (PBT), 35
Poly(ethylene glycol) (PEG), 36
Poly(ethylene terephthalate) (PET), 14, 35
degree of crystallinity, 325
IR spectrum, 325
Poly(ethylene-co-vinyl acetate) (EVA), 287, 288
Poly(glycolic acid), 34
Poly(lactic acid), 34
Poly(methacrylic acid), 34
Poly(methyl methacrylate) (PMMA), 34
Poly(propylene glycol) (PPG), 36
Poly(styrene-co-acrylonitrile), 32
Poly(styrene-co-butadiene). *See* Styrene-butadiene rubber
Poly(styrene-co-butyl acrylate), 288
Poly(vinyl acetate) (PVAc), 39
Poly(vinyl alcohol) (PVA), 39
Poly(vinyl butyral), 40
Poly(vinyl chloride) (PVC), 40
Poly(vinyl fluoride), 42
Poly(vinyl formal), 40
Poly(vinylidene chloride), 41
Poly(vinylidene fluoride) (PVDF), 42
Polyacrylates, 33
Polyacrylonitrile (PAN), 33
Polyamides, 37, 106
Polybutadiene, 44
Polycaprolactone, 34
Polycarbonates, 37
Polychloroprene, 44
Poly-cis-1,4-isoprene, 256
Polycondensation, 34, 37
Polydisperse system, 16
Polydispersity index, 19
Polyelectrolyte, 33
Polyester(s), 34
MALDESI, 232
paints and coatings, 35
thermoplastic, 35
thermoset, 35
Polyester fibres, 269, 274
dichroic ratios, 339
Polyester polyurethane
foam fragments, 143
Polyesters, 106
Polyether polyurethane
foam fragments, 143
Polyethylene, 30
Polyhydroxyalkanoates, 34
Poly(ester urethane), IR spectrum, 145
Poly(ether urethane), IR spectrum, 145
Polyisobutylene, 42
Polyisoprene, 43
Polymerisation, 13
Polymer matrix, identification, 211
Polymorphism, 312, 315
Polymorphs, 316
cellulose, 318
Polyoxyethylene, 36
Polyoxymethylene (POM), 36
Polypropylene, 31, 290
degree of crystallinity, 325
infrared spectroscopy, 325
Raman spectroscopy, 325
Polystyrene, 32
Polysulphone, 37
Polytetrafluoroethylene (PTFE), 42
Polyurethane, foam fragments, 142
Polyurethane foams, 228, 332
derivative curve UV/visible spectra, 228
Polyurethane resins, 115
Polyurethanes, 38
Polyvinylchloride (PVC), 14
plastifiers, 329
POM. *See* Polyoxymethylene (POM)
Pooled estimator of the standard deviation, 73
Population fibre studies, 87
Population stud(ies), 88, 246
fibres, 111
foam fragments, 142
Pores, 142
Pore size, 272
Post blast debris, IRMS, 243, 247
Posterior odds, 90
Post-fire debris, 231
Powder diffractometers, 312, 313
Power compensation DSC, 320
PPG. *See* Poly(propylene glycol) (PPG)
Precision, 70
Preliminary assessment of the case, 65
Pressure sensitive adhesive, 123
Primary transfer, 60

Primer, 119
adhesive tapes, 124
Principal component analysis (PCA)
condom-related traces, 140
preprocessing, 75
tyres, 136
Principal components, 75
Prior odds, 90
Procedure, 66
Processing, 301
Propagation, 15
Protectors, paints, 117
Proteins, 51
Protocol, 66
Protocol for fibres, 111
Proton quantity, 332
Pseudo 1:1 taping, 110
PTFE. *See* Polytetrafluoroethylene (PTFE)
Pulp, 49
PVA. *See* Poly(vinyl alcohol) (PVA)
PVAc. *See* Poly(vinyl acetate) (PVAc)
PVC. *See* Poly(vinyl chloride) (PVC)
Pyr-GC-MS. *See* Pyrolysis-gas chromatography-mass spectrometry (Pyr-GC-MS)
Pyrolyis/gas chromatography/mass spectrometry
tyres, 135
Pyrolysis, 196, 197
Pyrolysis-gas chromatography, condom-related traces, 139
Pyrolysis-gas chromatography-mass spectrometry (Pyr-GC-MS), 197, 248, 251, 286
additives, 248
adhesive tapes, 127, 251
condom-related traces, 140
fibres, 112
paints, 122, 248
paper, 151
post-fire traces, 156
quantitative, 251
siliconic polymers, 251
Pyrolysis parameters, 251
Pyrolysis products, post-fire traces, 153

Q

Quadrupole mass filter, 200
Quality control, 68
Quality control, DSC, 321
Quantitation, limit of, 70

R

Raman spectrometers, portable, 180
Raman spectroscopy, 178, 292
condom-related traces, 139
fibres, 112
organic pigments, 231
paints, 122
paper, 151
post-fire traces, 156
Random coil configuration, 310
Random coil conformation, 29
Random fill, 76
Rayleigh scattering, 179
Rayon, 49
Rayon cupro, 106
Reactive dyes, 236
Rear lights, 266
Recipient, 59, 108
Recovery, 59, 61
Recyclability, 22
Refractive index, 273
fibres, 112
measurement, 326
Refractive index detector, 195
Regenerated cellulose, 49
Regenerated cellulose fibers, Raman spectroscopy, 230
Relaxation, 330
Relaxation time T_2, 332
Release liner, 124
Repeat units, 9, 12, 24
Reproducibility, 71
Residual variance, 75
Resins, 23
DART, 255
Resins in inks, 279
Resol, 46
Retention, 191
Retention time, 195
Reversed phase chromatography, 194
Robustness, 71
Royal Canadian Mounted Police (RCMP), 121
Rubber compounds, 132–135
tyres, 132
Rubber gloves, 128–132
manufacturing, 129
Rubbers, 23
Rubber tree, 43

S

Sample preparation, 72
MALDI, 202
Sampling, 72
SBR. *See* Styrene-butadiene rubber (SBR)
Scanning electron microscopy (SEM), 188, 224, 307
adhesive tapes, 125

Schonbein, C., 11
Schwartzchild objective, 178
Scientific working group for materials analysis (SWGMAT), 64, 67
Scores, 77
SEC. *See* Size exclusion chromatography (SEC)
Secondary transfer, 60, 109
Seed fibres, 106
Selectivity, 70, 153
  thermogravimetry, 209
SEM. *See* Scanning electron microscopy (SEM)
SEM-EDX, 189, 242
  adhesive tapes, 127
Semicrystalline, 108
Semicrystallinity, 29, 307, 309
Sensitivity, 70
Separation of the components, adhesive tapes, 126
Shaking method, 62
Short chain branching, 25
Sidewalls, 132, 134
Sieving, soil, 303
Silanols, 48
Silicone polymers, 48
Siliconic polymers, Pyr-GC–MS, 251
Silk, 51
Similarity, 80, 81
Similarity matrix, 81
Single linkage, 81
Sisal, 106
Size exclusion chromatography (SEC), 192, 269, 271, 273, 274
  adhesive tapes, 128
  columns, 272
Sizing, paper, 151
Soft sources, 201, 202
Softwood, 151, 255
Solid echo, 333
Solid-phase microextraction, post-fire traces, 154
Solubility in xylene, 291
Solution polymerisation, 16
Solvent-borne paints, 115
Solvent residues, 286
Solvents, paints, 118
Source level, 89
Specifications, SEC columns, 270
Specimens, 72
Spectral subtraction, 216
Spectrophotometer, 174
Spectroscopy, 170
Spermicides, 138
Spherulites, 309
Spinning, 107
Stability enhancers, 117
Stabilizers, 169
Staining, TLC, 193
Standard dye mixture, 237
Star polymers, 25
Stationary phase, 189, 191
Statistics, 72
Step-growth polymerisation, 13
Stereochemistry, 24
Stereoregularity, 296
Stokes light, 179
Stokes scattering, 179
Stretching, 175
Striation marks, 305
Struts, 142
Styrene-butadiene rubber (SBR), 44
Styrene-divinylbenzene copolymer, 270
Surface-enhanced Raman scattering (SERS), 231
Suspension polymerisation, 16
Syndiotactic, 27
Syndiotactic polypropylene, 290
Synthetic fibres, 106, 304
Synthetic polymers, 21

T
Tackifier, 123
Tacticity, 27
Taillights, 268
Tape lifting, 62, 109, 141
1:1 taping, 110
Target fibres, 111, 112
Target fibre studies, 87, 111
t-distribution, 73
Technique, 66
Temperature, DSC, 322
Tensile strength, 19
Termination, 15
Texturisers, paints, 117
Tex unit (tex), 108
Thermal analysis, 207
Thermal history, 321
Thermogram, thermogravimetry, 206, 208
Thermogravimetric analysis (TGA), 206
Thermogravimetry (TG), 206, 256
  arson debris, 256
  latex gloves, 256
  quantitative, 256
  rubber gloves, 131
Thermoplastic polymers, 22
Thermosets, DSC, 322
Thermosetting polymers, 22
Thickness
  adhesive tapes, 125
  plastic bags, 149
Thin layer chromatography (TLC), 192
  fibres, 236
Threads, 107

Tie molecules, 309
Time domain nuclear magnetic resonance
rubber gloves, 132
Time Domain Nuclear Magnetic Resonance (TD-NMR), 329
foam fragments, 332, 334
latex gloves, 332, 334
Time-of-flight, 185, 200
Titanium dioxide, 116, 230
Raman spectroscopy, 230
Titre, 108
Toner
infrared spectroscopy, 242, 243
XRF, 242, 243
Topcoat, 119
Training set, 80
Trans configuration, 28
Transfer, 59
foam fragments, 141
Transfer of fibres, 108
Transformation of polymers, 304
Transition temperature, 323
Trans-1,4-polyisoprene, 28, 43
Tread, 132, 134
Triple ramp DSC, 324
t test, 73
T_2 test, 74
Tyre
characterisation, 134
parts, 132, 133

U
UF. *See* Urea formaldehyde (UF) resins
Ultraviolet, 171
Univariate data, 72
Universal calibration curve, 271
Unzipping, 268
Urea formaldehyde (UF) resins, 46
UV absorbance detectors, 273
UV stabilizers, paints, 117
UV/visible microspectrophotometry, foam fragments, 145
UV-visible spectrophotometry, adhesive tapes, 125
UV-visible spectroscopy, 173
fibres, 112
paints, 121
paper, 151
plastic bags, 149

V
Variability, 217
Vegetal fibres, 106
Vibrational modes, 175
Vibrational spectroscopy, 178, 230
dyes and pigments, 230
Vibrational transitions, 175
Vinyl gloves, 129
Viscose, 106
Viscose solution, 50
Viscosity, 268
Visible, 171
Vulcanisation, 11, 43, 134

W
Water-borne emulsion paints, 115
Wavelength, 171
Wavelength dispersive X-ray fluorescence, 188
Weight-average molar mass, 18
Wet spinning, 107
Wetting agents, paints, 117
Wood, 49
Wool, 51
Working range, 70

X
Xanthate, 106
X-ray detectors, 315
X-ray diffraction (XRD), 222, 311
adhesive tape, 318
adhesive tapes, 127
cellulose, 222
fillers, 222
paper, 151, 152, 222, 318
plastic bags, 149, 223, 318
plastic packaging material, 318
sample size, 318
X-ray fluorescence (XRF), 187
adhesive tapes, 127
paints, 233
pigments, 233
toner, 242, 243
X-ray monochromators, 314
X-ray spectrometry, 181
X-ray tube, 313
XRF. *See* X-ray fluorescence (XRF)

Y
Yarns, 107

Z
Ziegler, K., 27