# Quality and Reliability in Analytical Chemistry

# Analytical Chemistry Series

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***Quality and Reliability in Analytical Chemistry***
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# *Dedication*

*to Analytical Science*

*— George-Emil Baiulescu*

*to my parents Valeria and Ion Stefan for their continuous support and inspiration*

*— Raluca-Iona Stefan*

*to my parents, Nagla, Youssef, Faisal, and Basil for their constant encouragement and enlightenment*

*— Hassan Y. Aboul-Enein*

# *Preface*

Quality and reliability are two very important parameters in analytical chemistry. High-quality analytical information alone is not enough, as the information must also be reliable. Reliability is defined as the maintenance of quality through time. Although reliable analytical information is characterized by quality, not all analytical information that has quality properties is reliable.

To obtain reliable analytical data it is essential to examine the reliability of all the steps involved in an analytical process — sampling, black box, data processing — as well as the reliability of the instruments used. The complexity of the sample is key to reliable analytical information as the complexity of the sample influences the selection of the analytical process and the instrument used for analysis. In addition to its complexity, the history of the sample must also be considered. Usually, the sampling process is a most critical aspect, as its reliability affects the results considerably. The quality and reliability of analytical information cannot be guaranteed unless standards are used for measurements. Conversely, only reliable methods can be considered for standardization.

As we enter the new millennium, we hope that this book will offer the reader information regarding various aspects affecting the quality and reliability of chemical analysis. Further, we hope that this book may be used fruitfully by graduate students, researchers, clinical and analytical chemists, and workers at both meteorological and routine laboratories. It should also be beneficial to consultants and regulators who make evaluations and quality control decisions. The book offers a general view with regard to standards, sampling, methods, and instrument selection, with the goal of obtaining analytical information of high quality and reliability.

Thanks are extended to Miss Shelly Lynde for her excellent secretarial assistance during the preparation of the manuscript of this book. We are also grateful to Professor Charles H. Lochmüller, Department of Chemistry, Duke University, Durham, North Carolina, for his support, and to CRC Press for consideration and pleasant cooperation in the production of this book.

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Riyadh, Saudi Arabia — Hassan Y. Aboul-Enein

# *Contents*

# *Abbreviations Used in Text*

| | |
|---|---|
| AAS | atomic absorption spectroscopy |
| A/D | analog-to-digital |
| AES | Auger electron spectroscopy |
| AFM | atomic force microscopy |
| ASV | anodic stripping voltammetry |
| BIPM | Bureau International des Poids et Mésures |
| BZ | benzodiazepines |
| CE | capillary electrophoresis |
| CEM | conventional transmission electron microscopy |
| CFA | continuous-flow analysis |
| CIA | chemiluminescence immunoassay |
| CGC | gas-solid chromatography |
| CL | chemiluminescent |
| CVP-AFS | cold vapor generation–atomic fluorescence spectroscopy |
| CZE | capillary zone electrophoresis |
| D-AAOD | D-amino acid oxidase |
| D/A | digital-to-analog |
| DE | dextrose equivalent |
| DMAA | dimethylarsonic acid |
| DPC | diphenylcarbazide |
| DPP | differential pulse polarography |
| DPV | differential pulse voltammetry |
| DSMS | direct sampling mass spectroscopy |
| EC | electrochromatography |
| ECD | electron capture detector |
| ECIA | electrochemiluminescence immunoassay |
| EDTA | ethylenediaminetetraacetic acid |
| EIA | enzyme immunoassay |
| ELISA | enzyme-linked immunosorbent assay |
| EMIT | enzyme-monitored immunotest |
| ES | emission spectroscopy |
| ESCA | electron spectroscopy for chemical analysis |
| ESEM | environmental scanning electron microscopy |
| ETA | electrothermal atomization |
| ETAAS | electrothermal atomic absorption spectroscopy |

| | |
|---|---|
| EXAFS | X-ray absorption fine structure, extended |
| FAAS | flame atomic absorption spectroscopy |
| FCV | fast cycle voltammetry |
| FIA | flow injection analysis |
| FIA | fluoroimmunoassay |
| FID | free induction decay |
| FT-IR | Fourier transform infrared |
| GC | gas chromatography |
| GPC | gel-permeation chromatography |
| HG | hydride generation |
| HG-AA | hydride generation–atomic absorption spectroscopy |
| HIV | human immunodeficiency virus |
| HPGC | high-performance gas chromatography |
| HPLC | high-performance liquid chromatography |
| HRGC | high-resolution gas chromatography |
| HS-SPME | head space solid-phase microextraction |
| ICMA | immunochemiluminescence immunoassay |
| ICP-AES | inductively coupled plasma–Auger electron spectroscopy |
| ICP-MS | inductively coupled plasma–mass spectroscopy |
| IEMA | immunoenzymometric assay |
| IFMA | immunofluorimetric assay |
| IL5Rα | interleukin-5 receptor alpha |
| IR | infrared |
| IRMA | immunoradiometric assay |
| ISME | ion-selective membrane electrode |
| ISO | International Organization for Standardization |
| ITD | ion-trap detection |
| LA | laser |
| L-AAOD | L-amino acid oxidase |
| LIBS | laser-induced breakdown spectroscopy |
| LIF | laser-induced fluorescence |
| LP | laser photofragmentation |
| MALDI | matrix-assisted laser desorption/ionization |
| MCN | microconcentric nebulizer |
| MEKC | micellar electrokinetic chromatography |
| MLR | multiple linear regression |
| MMAA | monomethylarsonic acid |
| MWD | microwave digestion |
| NAA | neutron activation analysis |
| NIR | near infrared |
| NMR | nuclear magnetic resonance |
| OTC | open tubular column |
| PLS | partial least squares |
| PVC | polyvinyl chloride |
| RIA | radioimmunoassay |
| RM | reference materials |

| | |
|---|---|
| RSD | relative standard deviation |
| SDS | sodium dodecyl sulfate |
| SEM | scanning electron microscopy |
| SFC | supercritical fluid extraction |
| SIM | selective ion monitoring |
| SIMS | secondary ion mass spectroscopy |
| SPA | scintillation proximity assay |
| SPE | solid-phase extraction |
| SPM | scanning probe microscopy |
| SPME | solid-phase microextraction |
| STM | scanning tunneling microscopy |
| TID | time interval difference |
| TR-FIA | time-resolved fluoroimmunoassay |
| UME | ultramicroelectrodes |
| USN | ultrasonic nebulizer |
| UV-Vis | ultraviolet–visible |
| VP-SEM | variable-pressure scanning electron microscopy |
| XPS | X-ray photoelectron spectroscopy |
| XR | X-ray diffraction |
| XRF | X-ray fluorescence |

# *chapter one*

# *Quality in chemical analysis*

> "Qualitative analysis is an art."[1,2]

Analytical chemistry as a science concerns quality, quantity, and structure,[3] hence the three branches of chemical analysis: qualitative analysis, quantitative analysis, and structural analysis. Qualitative analysis, the focus of this chapter, has great importance because it establishes the nature of the sample components, as well as the approximate reissue. It differs from "quality control" in that the latter embraces all three branches of chemical analysis.

Three basic attributes of analysts are essential to all aspects of chemical analysis: intelligence, imagination, and intuition (Figure 1.1). Intelligence presumes the existence of imagination and intuition.

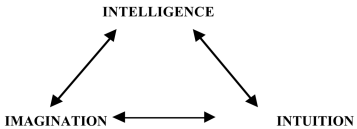



***Figure 1.1*** Basic elements of chemical analysis.

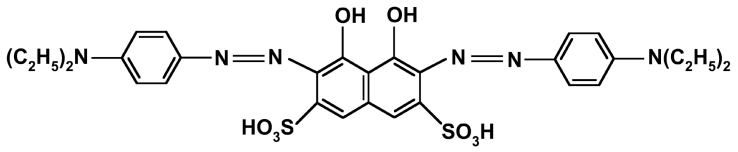
The first step for an analyst performing an analysis requires intuition, which acts at two levels: the first level refers to the sample components from the point of view of quantity and structures, and the second level is connected with the analysis itself. After these aspects have been established, the imagination of the analyst plays the important role of choosing the best conditions for the full qualitative analysis. The intelligence of the analyst is critical to developing experiments in good order. Further, the analyst must be flexible; flexibility includes intuition, imagination, and intelligence and assures the reliability of chemical analysis.

> To determine in what direction the analytical chemistry curriculum should go, it's important to look back at its origins.[4]

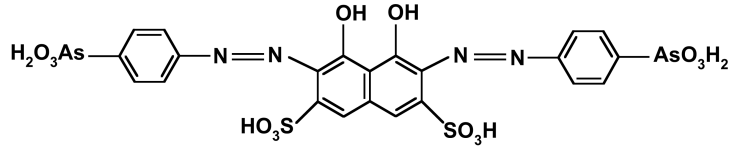
Analytical chemistry has an ancient origin. For example, the gallnut liquid recommended by Pliny for identification of iron is an organic reagent. According to Szabadváry,[5] several other organic reagents were in common use long before dimethylglyoxime was discovered, for example, oxalic acid, tartaric acid, succinic acid, and starch. The first organic reagents used were sufanilic acid and α-naphthylamine to identify nitrite ion (Griess, 1879; Ilosvay, 1889),* as well as α-nitroso-β-naphthol (Ilinski and Knorre, 1885).* Lowitz was the initiator of crystalochemics reactions at the end of the 18th century (1794–1798).

The first works dedicated to chemical analysis were very important to the development of analytical chemistry as a science. Books by Carl Remigus Fresenius, *Anleitung zur qualitativen chemischen Analise* (1841);* by Gaston Charlot, *Theorié et méthode nouvelle d'analise qualitative* (1942); and by F. Feigel, "*Chemistry of Specific, Selective and Sensitive Reactions*" (1949),* should be mentioned in appreciation of their foundational work.

The quality of chemical analysis can be characterized by sensitivity and selectivity. Generally, an increase in sensitivity results in a loss of selectivity. To obtain good results in chemical analysis, it is necessary to correlate these two parameters. Generally, to obtain good sensitivity it is necessary to introduce a chemical reagent with a large number of functional groups. Unfortunately, the selectivity of polyfunctional reagents is not so good. Therefore, it is necessary to establish rules for basic study for the synthesis of the organic reagents. As an example, consider the analytical chemistry of palladium. Popa et al.[6] made a systematic study of the analytical chemistry of palladium, and on the basis of this study Baiulescu et al.[7] proposed a bisazoic derivative of chromotropic acid for palladium determination:



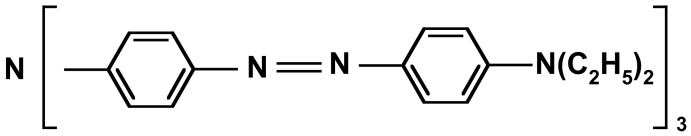

This reagent yields a very sensitive reaction with palladium, and competes with the palladiazo reagent proposed by Pérez-Bustamante and Burriel-Marti[8]:




By using these reagents, Pd(II) can be determined in the presence of Pt(IV).

* See Reference 5 for full citations.

A number of years later, Khalifa et al.[9] proposed pallatriazo as a very sensitive reagent for palladium determination:



Other methods to increase the performance of analytical reagents consist of modifying the operational parameters of the solution in which the chemical reaction takes place. In his book mentioned above, Charlot studied the influence of pH, ionic strength, and the dielectric constant on the operational parameters of the reactions. Masking agents play a very important role in increasing the selectivity of the reactions. An interesting chapter on masking and demasking of reactions is described by the Feigel textbook mentioned above. The introduction of complexants as analytical reagents also has an important role in chemical analysis in general. The abovementioned examples of the early reported organic reagents for palladium analysis indicate the importance of organic synthesis in improving the quality of organic analytical reagents. It is well known that oxin, introduced by Berg, is a good organic reagent but, unfortunately, is not very selective.[10,11] Yoe proposed another derivative of oxin named ferron, which yields a very sensitive reaction with Fe(III). As another example, one of the most famous analytical chemists, Ronald Belcher, described in 1958 the first reagent for the spectrometric determination of fluoride ion based on the formation of Ce(III) compounded with alizarine.[12]

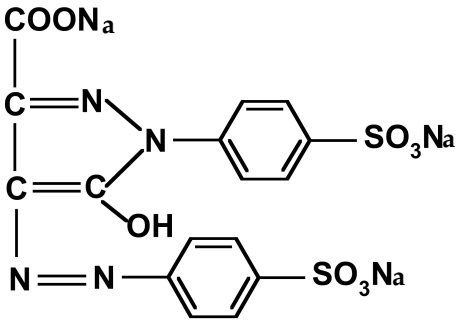
By using several organic reagents with good sensitivity and selectivity, qualitative analysis has become a very important step in characterization of sample compositions concerning major, minor, and trace components. In some cases, to increase the performance of chemical analysis it is necessary to concentrate the components of the samples using so-called nonselective solvent extraction by tandem reagents, such as oxin–dithizone and coupferrone–dithizone. These tandem reagents are called organic collectors.

A number of recent analytical techniques have been used to improve the reliability of chemical analysis from a qualitative and quantitative point of view. The work of Tubino et al.[13] should be mentioned as an example of using fiberoptic devices for spot tests of diffuse reflectance measurements.

The use of amplification reactions plays an important role in improving the sensitivity of some reactions, for example, the increase of phosphorus determination by the reduction of a heteropolycompound, ammonium phosphomolybidate. Another way to increase the sensitivity of a reaction from a qualitative and quantitative point of view is the use of radioactive isotopes in chemical analysis. However, this area of analytical chemistry was replaced by new, more sensitive, safer, and less hazardous techniques for qualitative and quantitative analysis (total analytical techniques), such as inductively

coupled plasma–Auger electron spectroscopy (ICP-AES) and especially ICP mass spectroscopy (ICP-MS). The last technique enables one to determine trace elements present in the level of ppt to ppm. New techniques for qualitative and quantitative analysis are very informative. However, the study of new types of reactions must be the great focus for researchers in analytical chemistry of the future.

It is of interest to mention here the contribution of Baiulescu and Turcu[14] for developing a tartrazine agent for zirconium determination:




This reagent reacts well with zirconium, obtaining a compound with a stoichiometric ratio $Zr_3Tz(OOH)_3$. It enables zirconium determination with good sensitivity and selectivity. Using this reagent, the authors demonstrated that it is possible to obtain stoichiometric compounds, with an ion that forms in solution hydrolysis, and polymeric compounds.

This short discussion about qualitative analysis demonstrates that qualitative analysis is, indeed, an art. This first step of chemical analysis plays a very important role in the knowledge and development of the full analytical process.

*chapter two*

# *Reliability in analytical chemistry*

The definition of *reliability* is the maintenance of quality through time. For analytical chemistry, reliability is the correspondence of results (analytical information) obtained using different apparata. Further, the reliability of a method is requisite to its automation, and also to its use in continuous-flow analysis (CFA).

Reliability in analytical chemistry requires a mathematical definition, which is given as a complex function of the sample reliability ($R_S$), method reliability ($R_M$), instrument reliability ($R_I$), and data-processing reliability ($R_{DP}$):

$$R_{AI} = f(R_S, R_M, R_I, R_{DP})$$

where $R_{AI}$ is the reliability of the analytical information.

The main determinant of the reliability of analytical information is the reliability of the sample. The sample acts as the "glue" between the method and the apparatus.[3] The method is chosen based only on the sample and on the components that must be determined. The apparata are chosen after the method, taking into account the sensitivity required.

Because computers are now used for data processing, the reliability of the interface between the apparatus and computer is also critical to the reliability of the analytical information. Thus, to obtain the best reliability for analytical information, it is essential to start with a reliable sample and to use a reliable sampling process, a reliable method connected with the type of analysis, reliable apparata, and the best software for data processing.

Pan[15] analyzed the reliability of the analytical process through the uncertainties of each step. He identified eight main sources of uncertainty, from sampling to reporting the results. These are the uncertainties concerned with homogeneity ($U_H$), recovery ($U_R$), analysis blank ($U_B$), measurement standard ($U_S$), calibration ($U_C$), matrix effect and interferences ($U_{MI}$), measuring instrument ($U_I$), and data processing ($U_{DP}$). It is necessary

to decrease these uncertainties as much as possible because only the lesser values of these uncertainties make the analytical process reliable. The reliability of the analytical information can be considered as a function of uncertainties proposed:

$$R_{AI} = f(U_H, U_R, U_B, U_S, U_C, U_{MI}, U_I, U_{DP})$$

Another way to express the reliability of analytical information is through the S/N ratio. This manner of expressing the reliability depends on coupling the reliability of the signal (S) with the uncertainty given by the noise (N). The main problem is to maintain the S/N ratio at a constant and maximum value. Because the signal is constant and the noise has a variable character, automation is necessary to maintain a constant S/N ratio.[16-18] Automation decreases many uncertainty values and increases the speed of the determinations.

The uncertainty inherent in the matrix effect and interferences causes flow injection analysis (FIA) to be used only for samples with simple matrices. More reliable analytical information is provided by CFA, which is used in quality control to assure the continuous control of very complex matrices. CFA also assures the sampling process by separation techniques, as the system is tandem: separation–analysis. For example, the chromatograph that also performs the separation is assured by using an adequate detection system for the analysis of components.

Maximum reliability can be assured only by robotics because of the maximum objectivity of robotics. Robots have been constructed for analytical use[19-22] that pick up the sample, prepare the sample, analyze the compounds, and perform the data processing. However, all automatic systems are coordinated by an operator who establishes the analytical parameters. The reliability of the analytical information obtained using an automatic system is assured only by a reliable operator, as shown in Figure 2.1.

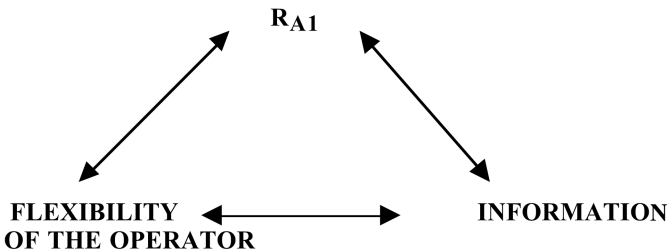



***Figure 2.1*** Reliability of analytical information scheme.

In order to obtain accurate and reliable analytical information, it is necessary for the system analyst to have three qualities: capability, correctness, and creativity, because the analyst is an essential part of the optimization of quality control of the analytical process.

Further, it is not enough to take into account only the reliability of the operator, the sample, method, instrument, and data processings, as well as the uncertainties values, to obtain reliable analytical information; the *connections* between sample and method, sample and instrument, and method and instrument must also be considered.

# chapter three

# Reliability of the sample

As is evident in the mathematical definition of reliability presented in Chapter 2, the reliability of the sample is the first factor to affect the reliability of analytical information because the analytical process begins with the sample. There are two main aspects that must be considered to obtain a reliable sample: the history of the sample and the homogeneity of the sample. Both are connected with knowledge of basic chemistry and with the flexibility of the system analyst.[23]

## 3.1 History of the sample

### 3.1.1 History of the sample in environmental analysis

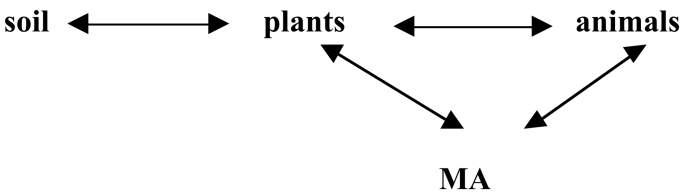
In environmental analysis, every sample is unique because of atmospheric reactions which take place in time, and because of circulation processes. Awareness of the problems associated with the chemistry of pollutants and their interactions is necessary. For example, for mineral water analysis it is necessary to have knowledge in the field of geochemistry, especially ore analysis. X-ray diffraction is usually used to determine ore composition[24,25] and this type of analysis requires very careful sample preparation. Morphological and crystal changes resulting from the sample preparation procedure have been characterized using techniques, such as scanning electron microscopy (SEM), infrared (IR), nuclear magnetic resonance (NMR), and ICP.[24]

For the fertilizer industry, the main toxic substances emitted are sulfur dioxide and nitrogen oxides $NO_x$. Measurements of these oxides do not represent the true value because their reactions take place in the atmosphere. The conversion of $SO_2$ to $H_2SO_4$ (acid rain) in the presence of metal catalyst and water is well known. To investigate the possibility of detecting the formation of sulfuric acid aerosols, laser photofragmentation (LP) and laser-induced fluorescence (LIF) may be used.[26] Also, formaldehyde (HCHO) in urban environments originates primarily from automotive traffic, but it is also present in rural and remote environments as an intermediate of the photochemistry of hydrocarbons. HCHO emission favors

hydroxy methanesulfonate ion formation from $SO_2$.[27] Because of the degradation process of organic acids and all mineral anions except sulfate and chloride, the rain samples must be preserved to obtain a representative analysis. Ferrari et al.[28] proposed two techniques consisting of freezing the samples to –18°C and of treating them with chloroform, respectively. These techniques assure the quality of the analytical information.

For analyzing water pollution it is necessary to know the area where the sample(s) were harvested. Among the primary water pollutants are pesticides, since they are slowly degraded and require dissemination. The chlorinated pesticides are lipophilic and are slowly accumulated in animals. The effect is due to metabolic system perturbation. Other water pollution sources are heavy metals, which are determined by the highly sensitive and selective analytical method ICP-AES.[29] The sampling process, in this case, consists of chromatographic separation techniques for pesticide separation.[30]

Soil is a complex matrix containing humic acids that have ion-exchange abilities with metals, such as lead, cadmium, bismuth, mercury, and zinc; which disturb the ecological system, taking into account the atmospheric circuit:




In the circuit, toxic substances are absorbed by plants from the soil. These substances enter the human body through ingestion of plants and animals that have, in turn, ingested plants and other animals, and so on. Accordingly, knowledge of the circulation process of toxic substances is also necessary for clinical analysis.

Soil represents the final destination for industrial residues and for biological and nonbiological processes. It is possible to obtain mathematical models for the transformation of chemical products in the soil, e.g., the fate of thiocarbamate herbicides in the system, the effect of atrazine on denitrification, and the effect of atrazine on the transformation of a nitrogen fertilizer (urea).[31] The chemical–physical model of the behavior of the herbicides in the air–water–solid system is presented in Figure 3.1.

The concentration of a toxic substance is critical; however, it is necessary to compare it with a reference "background pollution." The background pollution is specific to a zone free of toxic substances. It is then possible to make a diffusion map of toxic substances.

Plants and animals absorb toxic substances; however, their affinity may be higher or lower, and the substances may concentrate in various organs

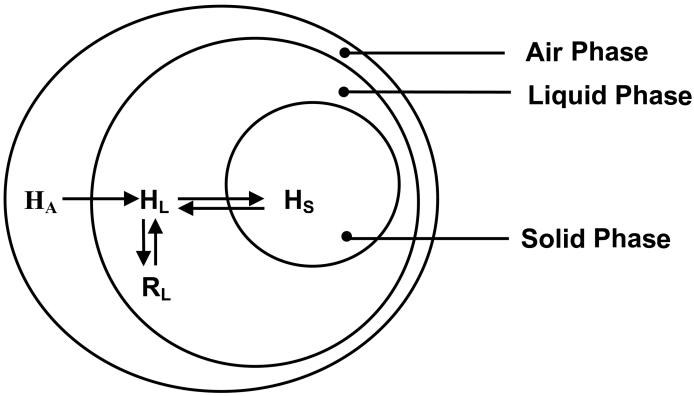



***Figure 3.1*** Chemical–physical model of the behavior of herbicides in the air–water–solid system. H is the herbicide, R the residue, and S, L, and A indicate the solid, liquid, and gaseous phases, respectively.[31] (From Cervelli, S. and Perret, D., *Ann. Chem.*, 86, 635, 1996. With permission.)

(e.g., heavy metals are assimilated by brain). Some toxic substances become dangerous only at certain well-known concentration levels. Therefore, it is necessary to detect this critical level.

### *3.1.2 History of the sample in food analysis*

Food is the main link between soil and humans. Good-quality food will be reflected in the good health of humans. Therefore, it is necessary to know both exactly what substances plants and animals derive from the soil and what methods are used during food manufacturing and processing. If one considers the pollution processes, there are three types of substances that affect food quality: heavy metals (e.g., lead), pesticides (the detection method that assures high reliability is gas chromatography–ms/ms, GC-MS/MS), and detergents.

To ensure the quality of analytical information, the International Organization for Standardization (ISO) established several standards. The most important and widely accepted international quality standard for testing laboratories is the ISO/IEC Guide 25: 1990 "General requirements for the competence of calibration and testing laboratories."[32] Samples for food analysis are often biological, and it must be ensured that they are protected against chemical, physical, and mechanical influences that may lead to changes during storage in well-sealed containers.[33]

The major problem in food analysis is the complexity of the matrices. For example, coffee has a complex matrix. Acids from coffee are important for the sensory quality of the coffee beverage. For every acid identification, it is necessary to perform an electrophoretic cleanup of all organic acids followed by the use of the GC–MS technique.[34]

### 3.1.3 History of the sample in clinical analysis

As in environmental analysis, for clinical analysis each sample is unique. A major problem for clinical analysis is sample collection, because immunoreactions can occur upon sample contamination. For this purpose, an automatic system for sample collection is required. In *in vivo* analysis the sample collection step is eliminated; however, there are problems with sterilization of instruments. Therefore, it is recommended that for the best reliability of the analytical information, microfabricated sensor arrays that give the best responses be applied.[35,36]

Because health depends on the environment and food quality,

**ENVIRONMENT ◄——► HEALTH ◄——► FOOD**

knowing the history of the samples in clinical analysis leads to reliable information for environmental and food analysis.

## 3.2 Homogeneity of the sample

> Ensuring the homogeneity of the sample is a sine qua non for obtaining reliable analytical information.[23]

The homogeneity problem is specific to solid samples, as liquid and gaseous samples are considered homogeneous by nature. Thus, for solid samples, an automatic sampling process is recommended to obtain reliable analytical information. To select the most adequate system to obtain a homogeneous sample it is necessary to take into account the sample complexity and the stability of the sample within a certain period of time.[37] Further, it is necessary to establish first the nature of analysis that will be used in sample control, especially when the limits of detection are low. One must also be wary of the many contamination risks from reagent impurities, laboratory vessels, laboratory climate, and the operator.

A nondestructive method may be applied to a sample with relatively simple composition, but for a more complex sample, digestion processes are recommended, which are destructive. Beam analysis is recommended as a nondestructive method for samples with simple composition. The reliability of the analytical information for the beam analysis technique is assured by reproducibility and homogeneity of surfaces. It is necessary to clean the surfaces before the analytical process. Also, there are mechanical steps used for sample preparation to assure surface homogeneity. Conventional scanning electron microscopy (SEM) is widely used as an analytical tool.[38-40] Variable pressure scanning electron microscopy (VP-SEM) opens new opportunities in the field of materials science. Samples such as liquids can be analyzed using the VP-SEM technique without any prior preparation method (e.g., the characterization of two-phase crude petroleum from the

oxidation of 1-decene into 2-decanone).[38] The main problem of these analytical techniques is comparability with standards because samples must be similar in composition to that of the standard.

A complex sample must be separated to assure the best reliability of the final analytical information. Smith and Sacks[41] proposed a "vector model of multiple separation." The proposed model facilitates the development of optimization strategies and associated algorithms for systems involving more than two stationary phases. Thus, it simplifies both the mathematics and the visualization of more complex multiphase separations. The parameters of chromatographic technique can be optimized with a computer. For a capillary zone electrophoresis (CZE) technique there are many theoretical models proposed for zone migration and dispersion. The computer program based on these models serves as the "integral part of a systematic optimization strategy" to search for the most favorable conditions for a separation.[42] Capillary zone electrophoresis assures the best sampling process[43] for electrospray mass spectrometry as well as for MS-MS techniques. Capillary zone electrophoresis technique further assures the best separation of isomers.[44]

There are many standard methods in sampling preparation that include a digestion step of solids by strong mineral acids,[45] or by flux. Usually these types of digestion are available for inorganic solids. Fusions with acidic or basic fluxes are used when acids do not digest the sample. Because of the acids and fluxes, the potential for contamination, especially in trace analysis, is great. To assure the best homogeneity for an organic material, a wet digestion process with a boiling oxidizing acid or a mixture of acids, or a dry ashing process at a high temperature (400 to 700°C) in a muffle furnace is essential.

To assure a noncontaminant dissolution process, microwave digestion[46] and ultrasonic processes[47] are applied. "Ultrasound is now an invaluable tool in sample preparation, for areas as diverse as geological surveying or pharmaceutical analysis"[47] (Table 3.1). Usually, the microwave digestion technique is one step of the sampling process used in ICP techniques. The microwave digestion method can be applied to environmental analysis, food analysis, and clinical analysis. Bordera et al.[48] proposed an optimization method using an automatic flow injection system that combines microwave digestion with atomic spectrometric detection flame atomic absorption spectroscopy, or FAAS (ICP-AES), for the determination of heavy metals in sewage sludge. The experiment includes two main steps:

1. A digestion step carried out in a closed-flow microwave heating system;
2. An elemental determination step by ICP-AES.

For determination of wool microelements, microwave digestion assures the best sampling process.[49-51]

*Table 3.1* Analytical Applications of Ultrasound

| | |
|---|---|
| Pharmaceutical quality control | Premix, disperse, and suspend samples; crack euteric coating on tablets for dissolution tests; degas samples prior to instrumental analysis; deagglomerate and dissolve powder in solution |
| Pharmaceutical and cosmetic research and development | Emulsify oils and water for creams and lotions; crystallization and promotion of crystal growth, extraction; formation of liposomes for microencapsulation of product |
| Biochemistry and molecular biology | Description of cells (bacteria, viruses, mammalian, tissue); breaking of hydrocarbons and nucleotides (DNA, RNA, proteins); extraction of cellular components; homogenization |
| Analytical chemistry | Breaking of bonds; formation of free radicals, polymerization and depolymerization of long-chain molecules; catalysis of reactions (e.g., reduction, alkylation, ester hydrolysis, acylation, or aromatics); preparation of catalyst; activation of catalyst |
| Food and drink industry | Degassing carbonated beverages (e.g., beer, soda, wine) before quality control analysis |
| Geology | Dispersal of sediments in liquids; suspension of solids |
| Environment | Analysis of soil samples (EP Test Method 3550) |

*Source*: Stanley, P., *Anal. Eur.*, 23, 1996. With permission.

Reliable analytical information can be obtained by using microwave digestion sampling for trace element determination in brain and liver. Krachler et al.[52] reported two microwave digestion systems (open-focused and closed-pressurized). They created a mineralization of human brain and bovine liver as dissolution steps prior to the determination of 16 trace elements (bismuth, cadmium, cobalt, cesium, copper, iron, mercury, manganese, molybdenum, lead, rubidium, antimony, tin, strontium, thallium, and zinc) by ICP-MS.

Because of the low detection limit assured by electrothermal atomic absorption spectrometry (ETAAS), the sampling process must be performed using a noncontaminant process. The best results were obtained using microwave digestion.[53] Figure 3.2 shows the distribution of 21 elements that have been determined by ETAAS in various matrices, such as biological, food, environmental, geological, and other materials, after their dissolution with microwave-assisted digestion.

To assure the most reliable information for voltammetric trace element analysis, it is necessary to use microwave digestion — especially when the matrix is organic. It was demonstrated that this technique can be successfully applied for decomposition of biological samples with a low fat content before the differential pulse anodic stripping voltammetry, using an $HNO_3$-$HClO_4$ mixture.[54] The digestion time is also almost half what it would be in lower-pressure vessels (CEM). In all cases, it is necessary to have the best power program that can be obtained by application of different power and different

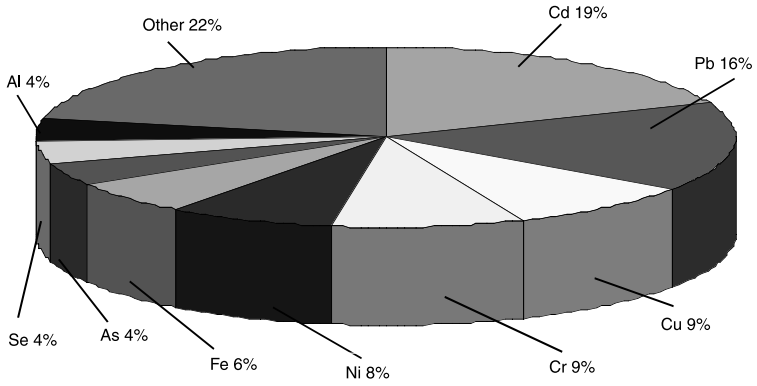



***Figure 3.2*** Distribution of elements determined by ETAAS after microwave-assisted treatment of various matrices. (From Chakraborty, A.K. et al., *Fresenius J. Anal. Chem.*, 355, 99, 1996. With permission.)

irradiation times up to their optimization. The best power program will assure the best sample digestion as well as the reliability of the sampling process.

There are many standard methods used for sampling process extraction.[55] This type of sampling process is usually used for ultraviolet–visible (UV-Vis) spectrometric methods. The assay of active substances from their pharmaceutical formulations requires an extraction process. For betamethasone assay, extraction with chloroform and benzene as solvents[56] is required, followed by formation of a charge transfer complex with benzocaprol red and/or acid ethyl blue for spectrometric determination. To improve the reliability of the sampling process for furosemide assay, isoamyl alcohol has been proposed as an extractant.[57]

There are numerous modern extraction techniques that improve the reliability of the sampling process; a silica bonded phase should be used to carry out a solid-phase microextraction of aromatic hydrocarbons such as benzene, toluene, *m*-xylene, and *o*-xylene.[58] A derivatization step has been proposed[59] to improve the separation process. The derivatization step assures the best reliability of fatty acids sampling (in water/air). Head space solid-phase microextraction (HS-SPME) has been employed for sampling of volatile components. This separation process can be successfully used for quality control of herbal medicines and other formulations containing herbal extracts.[60]

To improve the extraction process it is necessary to study the effect of modifiers on extraction–reextraction equilibria. The presence of the adsorption process also determines that both adsorption and extraction data are being modeled when self-modifier molecules and their mutual association and coabsorption are taken into account.[61]

Liu and Dasgupta[62] proposed a solvent extraction in a microdrop (≈1.3 μl), which is suspended inside a flowing aqueous drop from which the

analyte is extracted. The "drop-in drop" system, achieved with a multitube assembly, has two main advantages:

1. It is extremely efficient in consumption of organic solvent.
2. It facilitates automatic backwash.

A well-known extraction system is supercritical fluid extraction. It can be applied to matrices of different composition. This technique demonstrates the influence of matrix characteristics and common extraction variables on equilibrium analyte distribution.[63] These factors assure, for supercritical fluid extraction, the optimum conditions for efficient extraction of the matrix.

## 3.3 *Conclusion*

Thus, the reliability of the sampling process can be assured by careful attention to the sample and the analytical method that is to be used for determination. Practically all the sampling methods mentioned above can be performed automatically. Automation decreases contamination and increases the reliability of the analytical information.

It is impossible to say that one sampling method is, in general, more reliable than another because the complexity of the sample and the analytical method to be adopted for the analysis must be considered every time. Thus, one can compare various sampling processes only for the same sample using the same method. Using the best sampling process assures 90% of the reliability of the resulting analytical information since the sampling process plays the leading role in the sample assay, which is the first step of the analytical process. Further, the best sampling process assures the best assay of the sample if the best analytical method is chosen.

*chapter four*

# *Connection between reliability and the analytical method*

"No analysis is better than the sample itself."[23]

The dream of the analytical chemist is to be able to digest a sample in water or in an organic solvent and to measure the components without any prior separation or concentration processes. Of course, the chemist also wants to obtain accurate results, the best-quality analytical information, and the most reliable analytical information.

For the main fields of analytical chemistry, environmental analysis, food analysis, and clinical analysis, no single method can assure the best reliability of the analytical information in the same conditions for all fields. This is largely the consequence of the complexity of the matrix. For environmental analysis the complexity of the matrix is maximum; there are many possible interfering compounds that can affect the quality of the analytical information obtained. The best results are assured by using the best sampling process and adopting appropriate separation techniques. For food analysis, the separation process may or may not be necessary because the matrix is less complex than in environmental analysis. Clinical analysis is complicated. The matrix is not very complex, but the separation methods can affect the quality of the analytical information. This *in vivo* analysis assures the best quality of the analytical information for clinical analysis. To obtain reliable analytical information it is necessary to adopt a method suitable to the complexity of the sample matrix because, as always, the sample acts as a glue between method and instrument.

To assure the best quality of food requires improving the quality of the environment and assuring the best results in environmental analysis, since both food and the environment are essential to the health of humans. Water and air directly affect human health. The automatic determination of toxic

substances in air and water assures reliable analytical information, allowing the substances to be removed, thus improving human health.

Electrochemical analysis methods assure, generally, the most reliable analytical information because of the simplicity of the sampling process which includes (1) sample dissolution in water or in organic solvents and (2) the possibility of measuring directly and continuously the activity of the species present in the solutions. The preconcentration step is not necessary, because of the sensitivities and limits of detection that characterize the electrochemical methods. The determined species are not necessary to be converted to other measurable species. The electrochemical methods can be successfully used for *in vivo* monitoring. Spectrometric analysis methods, on the other hand, nearly always require a complex sampling process because of the presence of interfering species. Therapy is necessary to adopt the best separation techniques that can assure, for each analytical method, the most reliable analytical information. Nondestructive techniques are used especially for environmental analysis, and surface analysis assures the best reliability of the analytical information.

The proportionate use of the mentioned analytical methods for environmental analysis, food analysis, and clinical analysis is different for each picked because of the complexity of the matrix that is to be analyzed. Methods can be variously used analysis of different compounds of the matrix analyzed in each field. The methods used in each field are considered in turn in this chapter.

## *4.1 Environmental analysis*

The environment comprises air, water, and soil. Air, water, and soil all have complex matrices. For air and water, homogeneity is practically assured. However, soil samples require a more complex sampling process than air and water samples because of their nonhomogeneous nature. It is not easy to attain a reliable homogeneous sample for soil that can be analyzed by a well-established method.

To assure the best analytical information, separation steps are necessary, which are time-consuming and laborious. It is hard to determine each compound from the same class, but easy to determine the quantity that represents the sum of all compounds from the same class. However, occasionally it is very important to know the percentage of each compound. From this point of view the analysis of organic compounds in the environment is difficult to perform; inorganic compounds are usually easy to determine.

### *4.1.1 Air analysis*

To assure the reliability of the analytical information obtained by air analysis it is necessary to look at the photochemistry of the atmosphere. It is very important to know the catalytic influence of inorganic compounds on atmospheric processes. For studying atmospheric chemical reactions and aerosols,

laser photofragmentation (LP) and laser-induced fluorescence (LIF) techniques[26] have been proposed. These techniques were applied especially for detection of sulfuric acid aerosols, as well as for detection of gold and lead.

The most suitable technique used to characterize environmental aerosol particles collected on polyester substrates is atomic force microscopy[64] (AFM). This technique can be used with good results for small particles well below 100 nm since insulating substrates can be imaged readily without prior treatment, such as coating with a metal film, which hinders or disturbs subsequent imaging of ultrafine particles in electron microscopy. The accuracy of the analytical information is certified by the concordance between data obtained using the AFM technique and data obtained using light microscopy and scanning electgron microscopy (SEM) micrographs. The AFM technique assures good reliability of the analytical information for a natural aerosol sample from the micrometer range down to the molecular level.

For nitrous oxides as well as for sulfur dioxide assay there are well-known spectrometric methods. The magnitude of concentration of nitrous oxides and sulfur dioxide is ppm, which assures good accuracy of the analytical information connected to the best air sampling process.

The main problem of air analysis is the organic substances assay, including that of one of the main organic compound class, hydrocarbons. The best results can be assured by using infrared spectrometry.[65] This technique is not very selective, but it can determine the total hydrocarbon content of air. Only by coupling gas chromatography (GC) and infrared spectrometry can both selectivity and sensitivity of the hydrocarbon assay from the air be assured. Because the GC technique is a sampling process, its main role in hydrocarbon assay has been in the sampling process.

To improve the reliability of the analytical information for condensed ring aromatic hydrocarbon assay in air through the mass spectrometry (MS) technique, two different trapping systems are tested: a polytetrafluoroethylene filter coupled with XAD-2 sorbent, and a Carbotrap 150 cartridge.[66] These are used for quantitative sampling of exhaust gas and factory air before their desorption in the GC apparatus. For the sampling process, the experimental data show the best results by using a polytetrafluoroethylene filter coupled with XAD-2 sorbent because of the reliability of the sampling process that can be assured. A mass detector is used in this case that is programmed in scan acquisition to obtain the complete mass spectrum for each component. The information obtained from the mass spectrometer is compared with 32 commercial standards from a library suggested as being the probable compounds. The coupling between MS and a computer for data processing is necessary to assure the accuracy of the determination.

Halogenated methyl-phenyl ethers (anisoles) are ubiquitous organics in the environment. A number of compounds of the chloroanisoles, bromoanisoles, and bromo-chloroanisoles have been detected in the marine atmosphere,[67] in marine and freshwater fish,[68] in oysters,[69] in effluents of municipal wastewater treatment plants,[70] and in sediments.[71]

Halogenated anisols can generally be found in the marine environment in areas with high biological activity. The halogenated anisols represent a complex group of volatile biogenic organohalogenes in air. The high-resolution gas chromatography (HRGC) technique is the method of choice for the sampling process. Because of the complexity of the matrix, a high-resolution separation technique is necessary. Another problem is the separation of the halogenated anisols from the other classes of organic compounds (such as *n*-alkyl nitrates). This separation can be made by a high-performance liquid chromatography (HPLC) technique. The best reliability for halogenated anisols assay is assured by coupling an HRGC with an electron capture detection system (HRGC-ECD), or with mass-selective ion monitoring (HRGC-MS-SIM) based on the molecular ions system.[72] The magnitude for halogenated anisols assay is picogram per cubic meter.

Air analysis is very difficult to do because of the complexity of the matrix. To obtain reliable results one must choose the best sampling process. The selectivity of the method in the case of air analysis cannot assure the best reliability without any separation method. The development of chromatographic techniques have made them suitable for most separation processes utilized for air analysis.

Because for air analysis every sample is unique, it is very important to have a method that can be automated and that the air sample be monitored constantly. For that purpose, automation of all steps of the analytical process is necessary, beginning with sampling and finishing with data processing. It is important for the environmentalists to look at the concentration of different toxic compounds in the air in order to prevent their accumulation.

### *4.1.2 Water analysis*

The main sources of water contaminants are industries, pesticides, private sources at home, and public and private sewage disposal. There are many inorganic and organic compounds that are soluble in water, so it is very important to improve the sampling process — and the separation technique — to obtain reliable analytical information. The methods used for water analysis are based in many cases on spectrometric techniques. An increase in the use of potentiometric and amperometric techniques has been reported in the last few years.

Atomic spectrometry[73] is recommended for trace element analysis of surface water. It is well known that the sensitivity increases as follows: "flame-AAS < ICP-AES < ICP-USN (ICP-AES with ultrasonic nebulizer) < furnace-AAS < hydride-AAS < ICP-MS."[73] ICP-MS assures the best reliability of the analytical information. There are several standard methods used for water analysis that are based on graphite furnace-AAS. To improve the reliability of the analytical information, van der Jagt et al.[73] proposed the following:

(1) the use of pyrocoated graphite tubes (L'vov platforms); (2) rapid heating rates to prevent losses; (3) an element-specific matrix modification by adding chemicals; (4) dosage of reducing agents in order to convert the different valence states of an element into one valence; (5) addition of Triton X 100 (1%) as a detergent, especially when palladium nitrate is added as modifier; (6) the use of ultrasonic vibration prior to direct injection, for homogenizing the sample; (7) an element-specific background correction system by deuterium and/or Zeeman.

For speciation of arsenic in water it is necessary to look to the history of the sample because there are two main sources for arsenic: geological sources [$H_3AsO_4$ arsenic acid, As(V); $H_3AsO_3$, arsenous acid, As(III)] and industrial or agricultural sources (methylated arsenic compounds, such as monomethylarsonic acid, or MMAA, and dimethylarsonic acid, DMAA). The sampling process, especially the separation step, plays the main role in arsenic speciation because it is important to know the concentration of every compound that contains arsenic. For this purpose the HPLC technique is very useful. For routine speciation of arsenic the hydride generation-AAS method (HG-AA)[74] has been proposed. The main advantage over the ICP-MS method is the sensitivity and the fact that it is an economical procedure, being useful to detect arsenic at less than microgram-per-liter levels.

Arsenic speciation in saline waters can be made using a tubular membrane as a gas–liquid separator for HG-ICP-MS.[75] The detection limit achieved using this technique is at the picogram level. The separation step is very important during the sample process because of the presence of the chloride (estuarine and open ocean waters contain high levels of chloride). HCl is commonly used in the HG processes, which result in a matrix extremely high in chloride. For the separation step to be performed prior to the ICP-MS technique, some studies are carried out using capillary electrophoresis (CE) as a separation technique,[76] which includes the interface for CE and ICP-MS.[76,77]

There are several primary factors that affect the efficiency of separation through CE: the buffer system, the pH of the electrophoresis electrolyte, the voltages applied (higher voltages improve separation), and the interface between CE and ICP-MS, which reduces the ratio of forced flow to electroosmotic flow. The relative standard deviation values (RSD < 9%) obtained through the ICP-MS technique confirm the suitability for arsenic speciation in saline waters. In terms of reliability, one can say that the ICP-MS method is more reliable than HG-AAS for arsenic speciation in water.

It is well known that the maximum concentration of chromium (VI) permitted in potable water is 50 μg/l. Also, although chromium (III) is an essential nutrient for maintaining normal physiological functions, chromium (VI) has been demonstrated to produce toxic effects in animals and in the

human body. Therefore, sensitive analytical methods for chromium (VI) assay in water are necessary. Many analytical methods that cannot necessarily assure sensitivity require a preconcentration step in the sampling process. Manzori et al.[78] proposed a preconcentration step based on separation of chromium diphenylcarbazone on a sodium dodecyl sulfate (SDS)-coated alumina column. The sampling process includes the following steps: oxidation of Cr(III) to Cr(VI), using the $KMnO_4$ solution; reaction of Cr(VI) with diphenylcarbazide (DPC); adsorption of Cr–DPC complex quantitatively onto the SDS-coated alumina column from 800 ml of sample solution; and elution of the complex with an 8 ml mixture of methanol, acetone, and hydrochloric acid. The reliability of the proposed method depends especially on the sorption and elution parameters, such as the quantity of SDS vs. the quantity of alumina, the flow rate of complex solution over the coated alumina column, and the kind and ratio of solvents used for complex elution. The optimum parameters assure in practice the best reliability of analytical information. The precision for ten replicate measurements at the level of 10 μg/l of chromium (VI) was evaluated from the RSD value (3.5%). The limit of detection is a very important parameter for chromium (VI) assay in water. Through this proposed reliable method, the limit of detection is 0.040 μg/l, which assures its high selectivity and reflects the best quality of the analytical information. Because of the simplicity and suitability of this method, it can be used in every laboratory for chromium (VI) speciation.

Another well-known toxic metal is mercury (Hg), which acts as a poison in the body. The very low permissible level of Hg in drinking water of 2 ppb makes it essential to assure very sensitive methods for its assay. A good reliability for the Hg assay was obtained using a spectrometric method that follows an extractive process of a complex in toluene using *N*,*N*′-diphenylbenzamide[79] as the extraction agent. The recovery of 98.4% from the total Hg quantity after a single extraction process makes the method suitable for Hg assay in water. Only thallium and gold effectively interfere; it is of interest to note that some metals that are usually present in water (e.g., zinc, nickel, copper, cadmium, iron) do not remarkably interfere during the extraction of Hg. Therefore, the extraction of Hg is not only a separation step, but also a concentration step. These reasons confer reliability to the proposed method.

To obtain low detection levels (nanogram per liter), the cold vapor generation–atomic fluorescence spectrometry (CVP-AFS) method[80] was proposed. The rapidity and reliability assured by the AFS method make it suitable for the flow injection analysis (FIA) that incorporates an online bromide–bromate oxidation step. Because it is necessary to convert organic mercury into inorganic mercury (II) chloride, a heated reaction coil was incorporated into the FI manifold; this step is very important because only inorganic mercury can be detected. The FI manifold is presented schematically in Figure 4.1. Because of the low detection limit, a preconcentration step is not necessary for mercury assay. Good precision for mercury determination (RSD < 1.3%) is assured, and the obtained analytical information has the best reliability.

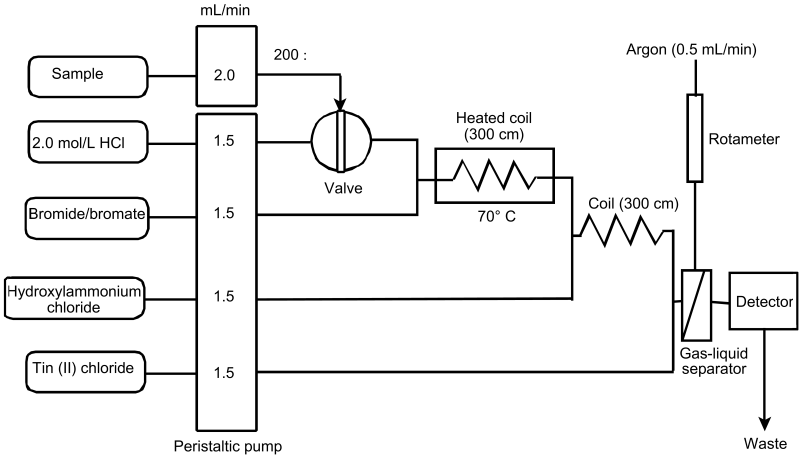



***Figure 4.1*** FI manifold for the determination of inorganic and organic mercury in seawater using CVG detection. (From Bloxham, S. J. et al., *J. Anal. At. Spectrom.*, 11, 511, 1996. With permission.)

One of the modern spectrometric methods used for water analysis is the chemiluminescence (CL) method. A flow injection CL sensor for the determination of free chlorine in tap water has been proposed.[81] CL sensor construction is based on the immobilization of luminol on an anion exchange resin column. By injection of sodium hydroxide through the column, luminol is eluted from the resin under basic conditions and then reacted with a sample stream to produce a CL signal. This sensor has a number of advantages, such as continuous monitoring of free chlorine in water, simplicity, rapidity, selectivity, and high sensitivity of the method. These sensor characteristics make the method reliable. The preconcentration step is not necessary due to the magnitude where free chlorine can be detected through this method: $10^{-7}$g/ml, for a RSD value less than 5%.

In conclusion, for determination of inorganic compounds in water, reliable sampling processes are necessary, followed by spectrometric techniques that assure low detection limits as well as good selectivity and reliability of analytical information. The rapidity and reliability of the spectrometric methods proposed for inorganic compound assay in water caused them to be used in a flow injection system with good results. Through utilization of these methods in flow injection systems, an increase in reliability of the analytical information was achieved.

Assay of organic substances in water is more difficult than assay of inorganic substances because of the difficulty in discriminating between organic compounds with similar structures. Although it is easy to assay for the total content of organic substances or the total content in phenolic compounds or pesticides, discrimination between the individual organic compounds that would improve the quality of analysis requires more specific analytical methods.

Aromatic compounds are highly toxic to the human body. There are a number of sources of aromatic compounds, and, because of the matrix complexity, a separation step is necessary. For aromatic compounds, such as benzene, toluene, ethylbenzene, *o*-xylene, *m*-xylene, *p*-xylene, 1-methyl-3-ethylbenzene, 1-methyl-4-ethylbenzene, 1,3,5-trimethylbenzene, 1,2,4-trimethylbenzene, 1-methylnaphthalene, and naphthalene, the separation step involves the application of solid-phase microextraction (SPME).[82,83] SPME assures a selective partition and preconcentration of the analyte.[5] A coupling of SPME and UV absorption spectrometry assures the reliability of the analytical information for discrimination of aromatic compounds in water. Coupling between the high selectivity of SPME with the high sensitivity and rapidity of UV absorption spectrometry is practical and useful. To improve the selectivity of the method further a second separation step is required which is assured by use of an HPLC technique. The detection limit (ppb magnitude) as well as the RSD values (RSD ≤ 10%) make the coupling SPME–HPLC–UV reliable for discrimination of aromatic compounds in water. Background absorbance due to optical scattering, cell fouling, and a variety of interfering species is suppressed by coupling the UV spectrometry with chemical reactions initiated by reactive species generated in a high-voltage corona discharge.[84] The reliability of the analytical information increases, in this case, for discrimination of aromantic compounds in water. The selectivity also increases. By using UV spectrometry with *in situ* corona reactions for assay of aromatic compounds in water, only a minimal level of the sample preparation[84] is necessary. This minimization of the sampling process assures the best reliability for discrimination of aromatic compounds in water.

Amines also have a toxic effect on the body. It is well known that the effect of secondary acyclic amines consists of the formation of extremely carcinogenic nitrosamines in the presence of nitrite. The discrimination of amines in water is one of the important problems to be solved by environmental analysis. A sampling process has been proposed that includes the use of GC and HPLC techniques as separation steps, and the use of MS and fluorescence detection techniques for detection.[85] Both methods complement one another — the HPLC/fluorescence detection method is better suited for highly polluted water samples, whereas the MS-GC method is essential for peak identification. A detection limit of 0.05 μg/l has been achieved. The reliability of the methods make them useful for online enrichment. Because of the detection limit and reliability, the methods can also be used to check drinking water treatment as well as to investigate the reactions of amines during selected technologies used in drinking water treatment, such as ozonation and biological degradation.

Frequently, the phenol class is found in water. Phenols are produced by a variety of sources such as industrial and mineral processes, municipal wastes, and the disinfection process with chlorine of wastewater and drinking water. Because of their toxicity at trace levels, it is very important to monitor these compounds in the water. The sampling process consists of

two main steps for discrimination of phenols in water: first, preconcentration; and second, separation. The preconcentration step is recommended to be done by a solid-phase extraction (SPE).[86] For the separation step, the GC technique is suitable.[86] The reliability of the sampling process determines the reliability of the analytical information. To test the reliability of the proposed sampling process, a water sample fortified with 39 phenols at 0.1 to 0.2 and 1.0 to 2.0 μg/l concentration levels was studied. The recoveries and repeatability obtained confirmed that the sampling process is reliable. To assure the reliability of the analytical determination, after sampling processes for the phenols, an electron capture detector (ECD) and ion-trap detector mass spectrometer (ITD-MS) were used. The MS detector was not sufficiently selective and sensitive for phenols discrimination in water samples. In sum, phenols can be reliably discriminated in water samples at microgram-per-liter levels using SPE-GC/ECD or SPE-GC/ITD-MS tandem techniques.[86]

Pesticides represent a universal contaminant in many sources of drinking water, groundwater, and soils. They are passed from water in vegetables, plants, and in food through animal fat. Therefore, it is important to detect the presence and the nature of the pesticides in water at low levels (nanogram per liter and lower). The reliability of the analytical information is very important for discrimination of pesticides because of their toxicity to the human body. There are many pesticide classes that can be detected in water: nitrogen-containing herbicides, organochlorine pesticides, and organophosphorus pesticide. These pesticide classes can be easily discriminated from each other, but it is very important to detect the content of every pesticide from within each class. For this reason, the sampling process must include a separation step. Performance of a preconcentration step by SPME is recommended.[87] The separation step can then be successfully accomplished with the GC technique. As a detection method, MS can be used for pesticide detection in water to assure the best selectivity and the best sensitivity of the analytical information. It is not necessary to use the ITD-MS. The reliability is assured for pesticide discrimination in water samples by the tandem system: SPME-GC/MS.[87]

Many papers have discussed discrimination of organic residues in water samples.[88-90] The main sources are industrial wastewaters and the agriculture industry. A wastewater analysis includes a laborious sampling process and the analysis of water contaminants. The sampling process generally contains two main steps: a preconcentration step and a separation step. The separation step must be made for organic substances to discriminate each class of organic compound, and then to discriminate every compound from each class. Sometimes it is very important to know the content of an organic compound in water because of its high toxicity. Also, it is sometimes necessary to know the total quantity of the organic compounds from a class — this is the case with compounds with low toxicity. There are essential methods that can easily detect at trace levels the content of organic contaminants in water samples.

The concentration step is usually done by using the supercritical fluid extraction (SFE) technique[88] or the well-known extraction technique with *n*-hexane.[90] Lepri et al.[90] described the concentration and separation of different classes of organic compounds, as shown in Figure 4.2. Fractions I, II, III, and IV contain the following organic compounds:

I. Aliphatic hydrocarbons and chloroderivatives;
II. Alkylbenzenes, polycyclic aromatic hydrocarbons;
III. Aldehydes, ketones, phthalates, fatty acid esters, alkyl phenoles and anilines, indole derivatives;
IV. Alcohols, acids, sterols, trialkyl phosphates, alkyl phenolethoxylates.

The next step after the separation of the different classes of organic compounds is the separation of each organic compound within each class. This step can be reliably accomplished using a chromatographic technique, GC or HPLC. For detection systems, the best reliability is assured by MS technique and sometimes by the coupling TID-MS. For cyclic siloxanes discrimination in wastewater samples, the free induction decay (FID) technique assures the best results.[89]

This kind of analysis is very laborious, with the main step separation. The reliability is very good but does not achieve maximum value because of losses of the organic substances during the multiple separation steps. Optimum values could be obtained if the separation step were excluded from the sampling process. Wise and Guerin[91] proposed a direct sampling mass spectrometric (DSMS) technique. The DSMS technique is defined as a "simple technique that provides real-time response, high sample throughput, and ppb detection limits at low costs."[91] The reliability of the analytical information obtained for environmental screening is due to the fact that the sample is introduced directly into a mass spectrometer using a simple interface with minimal sample preparation. It is possible to detect most types of volatile and semivolatile organics. The elimination of the chromatographic separation from the sampling process means that the mass spectrum is a composite spectrum of all the volatile or semivolatile components in a sample. The technique is more reliable than MS/MS coupling.

For discrimination of both organic and inorganic compounds in water with spectrometric techniques, a laborious sampling process is required because of matrix complexity. It is necessary to remember that the reliability of the analytical information depends first on the reliability of the sampling processes. Through the sampling process elimination, more reliable analytical information is obtained.

Activation analysis has been proposed to obtain more reliable analytical information.[73] The sample is irradiated with neutrons, photons (gamma radiation), or charged particles; the radioactivity generated is measured by sensing the entire gamma spectrum with a semiconductor detector. The most reliable variant is neutron activation analysis (NAA).[73]

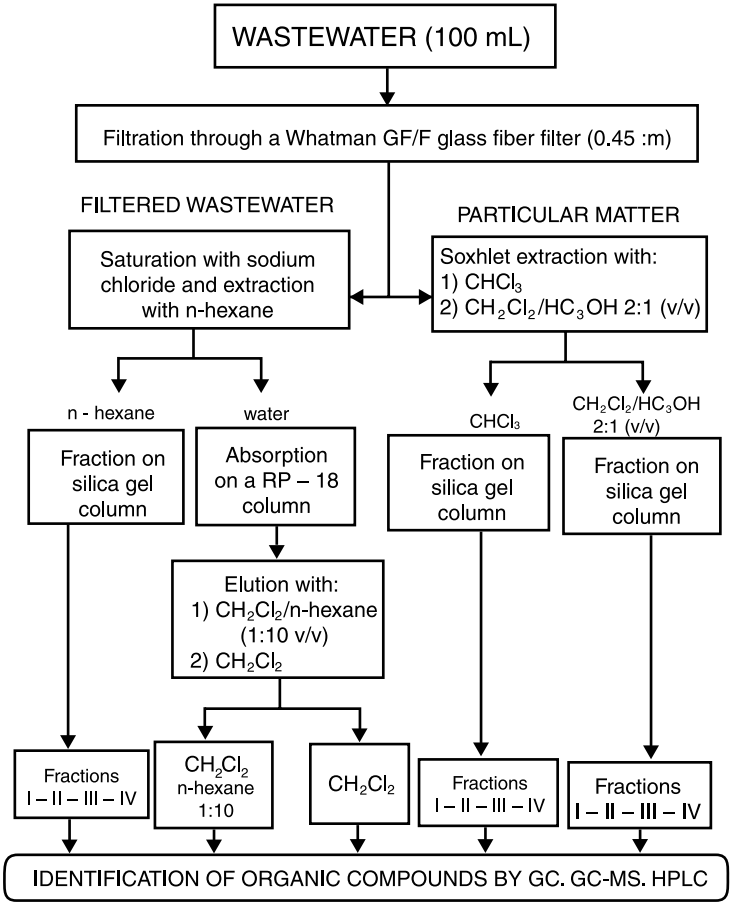



***Figure 4.2*** Complete scheme of sample treatment and separation of the different classes of organic compounds. (from Leprir, L. et al., *Ann. Chim.*, 87, 317, 1997. With permission.)

Electrometric methods have been used in the last few years for organic and inorganic compounds assay in water. However, they cannot be considered reliable for the assay of compounds in water because of the complexity of the matrix.

Potentiometric stripping analysis, as stated in one review,[92] "is not as general an analytical technique for the determination of metal traces as is graphite-furnace atomic absorption spectroscopy." It is used as a complementary technique for assay of some toxic metals in water (zinc, cadmium, lead, and copper in potable water and wastewater,[93,94] and lead and thallium in seawater.[95] The advantage of anodic stripping voltammetry (ASV) is summarized in two steps, which include electrolytic preconcentration and the stripping process. There are a number of interfering ions that can affect the

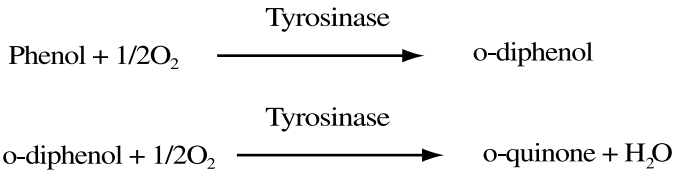
quality of the analytical information. ASV is recommended for arsenic assay in water,[96] but there are a number of interfering substances, such as cetyltrimethyl ammonium bromide, Triton X-100, sodium dodecyl sulfate, and copper, nickel, zinc, lead, and cadmium ions. The magnitude of the linear concentration range (microgram per liter) and the level of detection limit (microgram per liter) make this technique suitable for arsenic speciation at low magnitudes. To eliminate these interferences, the differential pulse-polarographic (DPP) method for arsenic assay is recommended.[97] For trace mercury assay in water, there are many papers that have proposed the ASV technique.[98] It provides good, reliable analytical information because of the selectivity and because of the possibility of detecting mercury at the ppb level. The differential pulse voltammetry (DPV) technique improves the detection limit to the ppt level for mercury assay.[99]

Ion-selective membrane electrodes as amperometric and potentiometric biosensors cannot be successfully used for ion monitoring in water. Their main characteristic is detection of an ion in the sample continuously and without any prior separation. The sampling process for a solid sample is reduced at its dissolution in distilled water. Due to the complexity of the matrix for wastewater or for seawater samples, there are a number of interfering inorganic and organic ions. Using biosensors for water analysis, one can obtain the total quantity of organic substances that are contained in a class; it is practically impossible to discriminate the content of every compound from within the same class.

An amperometric glucose biosensor[100] has been proposed for Hg(II), methylmercury and ethylmercury assay in water samples. The sensitivity is good (nanogram per milliliter level), and heavy metals do not interfere, but it is not in the range of pH within which the response of the biosensor has an invariable value. The lifetime of the biosensor is low.

Many papers present biosensors constructed for pesticide and insecticide assay in water. The constructed biosensors can detect the class of pesticides or insecticides, but they cannot discriminate every compound from within the same class. To assay the chlorinated hydrocarbons pesticides (DDT, endrin, lindane), an amperometric glucose biosensor has been proposed.[101] The limit of detection is 15 ppb. Organophosphorus insecticides can be assayed using an amperometric choline biosensor[102] at the same magnitude — ppb. It is very important to selectively detect organophosphorus insecticides in the water content because these compounds have progressively replaced organochlorine pesticides (DDT, aldrin, lindane). The main advantages of amperometric biosensors used for pesticide assay in water is the rapidity and simplicity assured for the analysis.

An enzyme sensor based on tyrosine can be used for assay of phenolic compounds at the millimole-per-liter level.[103] The method is based on the following reactions:




$O_2$ loss is detected with a Clark oxygen electrode. The proposed biosensor is free of interferences from other organic compounds. It offers reliable analytical information of total phenolic compounds in water, but it lacks the capability to discriminate every phenolic compound.

Despite their high cost and the long process involved in their development, immunochemical techniques may soon have broad applicability in the environmental field. Antibody production is the key step of any immunochemical technique. Immunosensors yield the best results for pesticides determination[104] in terms of both accuracy and reliability. The most reliable immunosensors are piezoelectric. They assure a good sensitivity and limit of detection.[105] The main problem for piezoelectric immunosensors utilization for water analysis is their low sensitivity, which results in a decrease in the S/N ratio because of the impurities present in water (from matrix or other sources).

### *4.1.3 Soil analysis*

Homogenization of the sample is considered an additional step in the sampling process for soil analysis. Following homogenation, the chemical compounds of interest can be extracted from soil samples through well-known extraction techniques. Spectrometric techniques are usually used for soil analysis because of the complexity of the matrix. Interferences are eliminated through various chromatographic, e.g., separation, techniques: GC and HPLC. For metal assay in soil samples the homogenization process must be accomplished with the minimum contamination. Microwave digestion[106] as well as laser[107] techniques are recommended.

The ICP-AES as well as ICP-MS coupling technique give reliable analytical information at low detection levels. The ICP-AES technique was used for cadmium, copper, lead, and zinc assay in soil samples at ppm levels.[106] The best reliability is assured by the laser-ICP-MS technique.[107] It was successfully used for magnesium, aluminum, calcium, chromium, manganese, iron, cobalt, nickel, copper, zinc, strontium, cadmium, barium, thallium, lead, bismith, and uranium assay from soil samples, with a relative standard deviation less than 7%. For improving the reliability of the analytical information it is necessary to use an internal standard. It is not easy to use the laser-ICP-MS technique, but it gives the best results for metals assay in soil samples.

The spectrometric assay of iron (II) with 1,10-phenantroline is used in a flow injection analysis (FIA) for iron determination in soil.[108] Iron (II) was extracted from soil samples by shaking the soil with an ammonium acetate solution (pH = 3) and the extract used for FIA determination. FIA manifold always contains a step of reduction of iron (III) to iron (II). The system can detect 60 iron samples per hour. It is a fast and reliable system for iron assay in soil. Also, it is very simple to use in every laboratory. The level of iron assay is only of milligram-per-liter magnitude, but a preconcentration step can solve this problem.

The systemic morpholine fungicide Corbel® with fenpropimorph as its active substance is frequently applied to cereal cropping. It is rapidly metabolized in soil to fenpropimorphic acid. For the assay of fenpropimorphic acid in soil, GC-MS coupling[109] has been proposed. The GC technique was used to assure the best separation for the fenpropimorphic acid from the complex soil matrix. The MS technique is very sensitive and assures the best reliability of the analytical information. The same reliability is also achieved when the HPLC-MS tandem system is used for sulfonylurea herbicide assay in soil.[110]

For triazine herbicides a very selective and sensitive immunological technique is recommended. The triazine herbicides are covalently bound to soil humic acids. A sandwich-immunoassay based on both polyclonal humic acid-antibodies and monoclonal triazine-antibodies[111] was used for triazine detection. The schematic view of sandwich immunoassay is presented in Figure 4.3. The technique is very selective and sensitive. It can assure the best reliability of the analytical information because it does not require a prior separation.

In conclusion, for soil analysis the most reliable information is obtained using the spectrometric techniques: ICP-MS, MS, or immunoassay techniques. The main advantage of the immunoassay technique is its specificity. The determination of compounds can be done without any prior separation. The more reliable the sampling process, the more reliable the analytical process.

Because of the complexity of the matrix, air, water, and soil samples require a very ardous sampling process, whose most important step is separation. The strong connection between the reliability of the sampling process and the reliability of the analytical process requires a reliable sampling process that obtains optimum reliability for the analytical information as long as the analytical method chosen is the best for the components that must be assayed. Thus, the reliability is also connected with the analytical method used for components assay. For air, water, and soil samples, the best reliability of the analytical information is obtained using spectrometric methods. The electrometric techniques, especially the ion-selective membrane electrodes or biosensors, cannot be used for determination of components in environmental samples with good results because of the low selectivity of the methods.

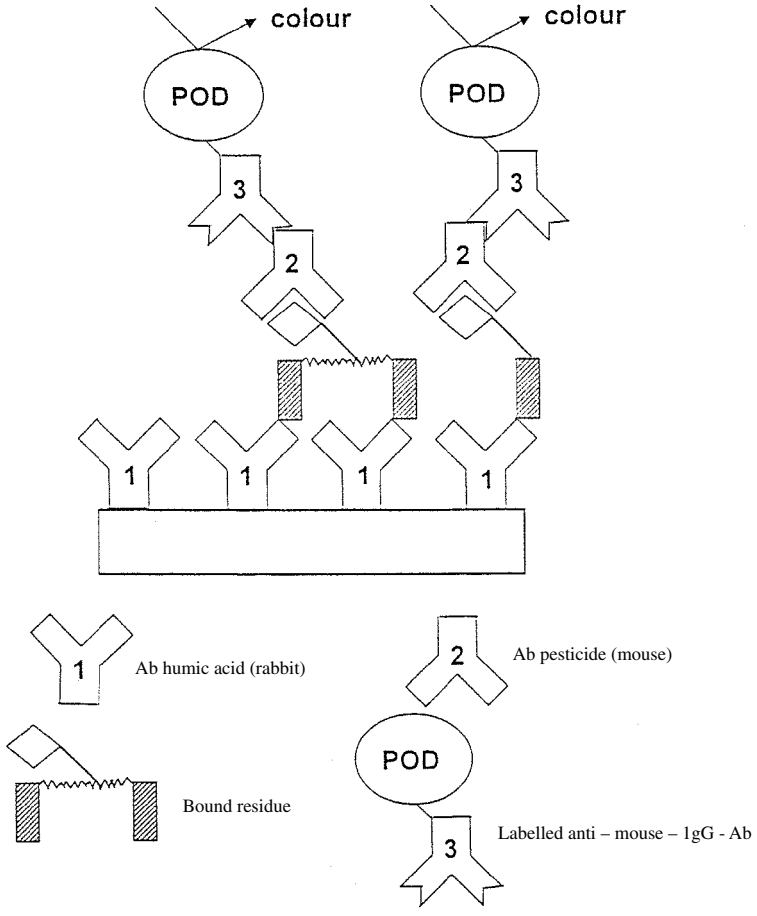



*Figure 4.3* Sandwich-immunoassay for the determination of bound pesticide residue. Schematic view. Antibody 1 (Ab 392) is immobilized on a polystyrene surface. Incubation of the analyte (bound residue). Addition of antibody 2 (Ab K1F4); detection by use of a third enzyme-labelled antibody 3. (From Ulrich, M.G., *J. Anal. Chem.*, 354, 352, 1996. With permission.)

The other problem for environmental analysis is assurance of a good connection between the sampling process and the determination of components step because it also plays an important role in establishing the reliability of the analytical information. For environmental analysis the most reliable analytical information is assured using tandem techniques. The best results in characterization of aerosol particles is obtained by scanning force microscopy.[112]

Monitorization in environmental analysis is necessary. Additional advantages are given by the dynamic integrated monitoring system design

used both to provide an alarm system to handle acute environmental incidents and to furnish longer-term data to indicate trends in waste parameters that could lead to future compliance problems.[113]

## 4.2 Food analysis

The quality of food is very important for health. The presence of heavy metals plays a large role in establishing food quality, and occasionally the presence of pesticides in food decreases the food quality. Thus, knowing the toxicity of adjuvants and their degradation products is very important.

Reliable methods are necessary for food analysis. The methods must assure both the best selectivity and the best sensitivity. As discussed earlier, spectrometric methods are used for environmental analysis because of the complexity of the matrix. In food analysis, the ratio between spectrometric methods and electrometric methods is 1:1 because the matrix is less complex.

Metallic ions can be assayed by ICP-AES or ICP-MS and by electrothermal atomization-AAS (FETA-AAS) as well as by polarographic methods. These methods assure reliable information for determination of metallic ions. For example, cadmium is analyzed in baby food with ETA-AAS with a tube and a L'vov pyrolytic graphite platform.[114] The results obtained for cadmium assay are then compared with the levels of a national standard. There are many standards containing quality requirements for laboratories, but the most important and widely accepted international quality standard for testing laboratories is the ISA/IEC Guide 25: 1990 "General requirements for the competence of calibration and testing laboratories."[32]

A reliable analytical method, cryogenic environmental SEM has been proposed for ice cream analysis[115] because of its four-phase structure: "ice crystal, air cells and small fat droplets embedded in a concentrated sugar solution matrix."[115] It is well known that the ice cream quality depends on its microstructural properties. Ice cream that is stored for a long time takes on a grainy texture because of the insoluble lactose from milk. It is necessary to change and refine the ice cream microstructure because this property is a measure of its quality. An environmental SEM (ESEM) is used for ice cream analysis due to its ability to image hydrate samples in their "native" state. ESEM specimens generally require no preparation techniques — even insulating samples can be looked at uncoated. As can be seen, the sampling process is not difficult. The cryogenic ESEM assures the best reliability for ice cream analysis.

Amines are very toxic if ingested in great quantities, and their toxic effect is increased in the presence of alcohol. For this reason it is very important to perform the determination of amines in wine. The main amines, which are products of amino acid decarboxylation processes, are tryptamine, tyramine, and ethanolamine. The wine matrix is complex and requires a separation step in the sampling process, for which HPLC assures the best reliability. The sampling process also requires an extraction step before the separation step.

2,2-Diphenyl-1-oxa-3-oxonia-2-boratanaphthalene (DOOB)[116] in diethyl ether has been proposed. UV spectrometry has been proposed as a detection technique. As always, the reliability of the analytical information depends on the reliability of the sampling process. The optimization of sampling parameters increases the sampling reliability.

The main advantage of the proposed wine analysis is its selectivity because only primary amines can be detected using this method. Also, by-products do not interfere with phenols or thiols. The quality of the wine and its organoleptic characteristics are well defined considering the effects of the malolactic fermentation process. The electrometric methods assure reliable results for the l-malic and l-lactic acids assay. The biosensors construction for l-malic and l-lactic acids assay in wine are based on malate dehydrogenase and lactate oxidase enzymes.[117] The reproducibility of the results as well as the selectivity make it reliable for establishing the quality of the wine.

To improve the selectivity for malate assay a bienzyme biosensor[118] based on malic enzyme has been proposed; the biosensor catalyzes the following reaction:

$$\text{L-malate} + \text{NADP} + \Leftrightarrow \text{pyruvate} + \text{NADPH} + CO_2 + H^+$$

and pyruvate oxidase catalyzes the following reaction:

$$\text{pyruvate} + HPO_4^{2-} + O_2 \Leftrightarrow \text{acetylphosphate} + H_2O_2 + CO_2$$

$H_2O_2$ is monitored at +650 mV applied potential vs. a silver/silver chloride cathode. Using the two enzyme biosensors results in an increase in the reliability because of the increase of selectivity and reproducibility of the results.

Microbial contamination of milk can be tested through determination of L- and D-lactate. Two amperometric biosensors based on the L- and D-lactate dehydrogenase enzymes have been proposed for L- and D-lactate assay.[119] They assure a continuous monitorizing of L- and D-lactate in milk, and they also assure good reliability of the analytical information.

The origin of coffee can be established by determining its volatile and semivolatile compounds. A reliable technique is the electronic nose technique,[120] which is presented in Figure 4.4. It has been established that the composition of volatile and semivolatile compounds depends on such factors as climate and soil temperature. The results obtained by the electronic nose technique are comparable with those obtained by spectrometric techniques. However, spectrometric techniques require a separation step in the sampling process, by GC technique, which results in a decrease of reliability of the analytical information by using the spectrometric techniques for assay of the volatile and semivolatile compounds in coffee.

The sensory quality of the coffee is conferred by its acids. It is very difficult to determine the acids in coffee in the sampling process because

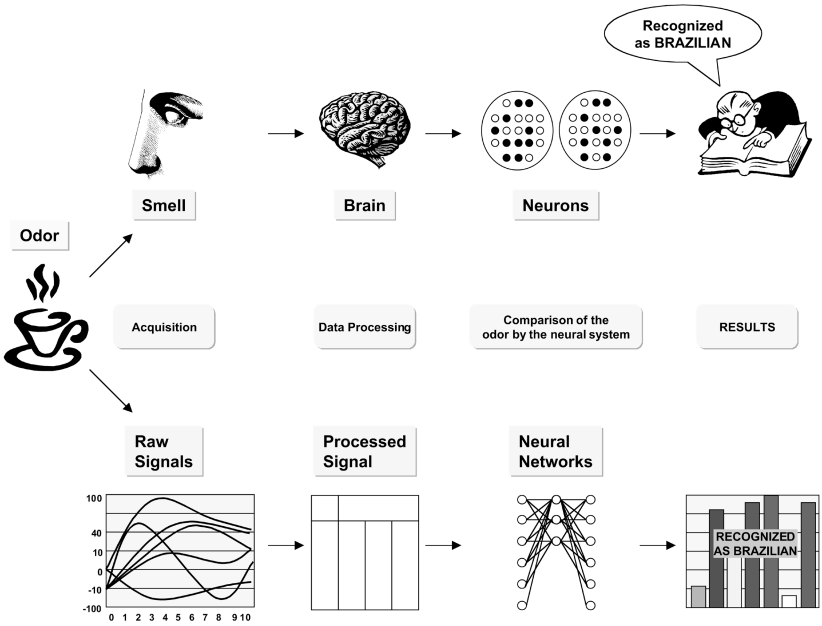



***Figure 4.4*** The schematic representation of electronique nose technique. (From M. Paz Carril, M. Soledad Corbilllón, J. Manuel Madariaga, *Mikrochim. Acta*, 124, 1, 1996.)

coffee has a very complex matrix. It is not possible to determine coffee composition with a spectrometric method without a prior separation step. To "clean-up" the organic acids from coffee a free-flow field step electrophoresis is required, followed by an ultrafiltration process.[34] Through this sampling process 31 acids could be "cleaned-up" from coffee. To increase the selectivity of the acids discrimination in coffee with the MS method, another separation step is necessary. The best reliability for this purpose is assured by a GC technique. In summary, using additional separation steps increases the selectivity, but decreases the quality of the analytical information. The reliability obtained is good, but for coffee analysis it is necessary to find more reliable methods and to simplify the sampling process.

It is very important to assay the essential amino acids in food and feeds for their nutritional value. To obtain the most reliable analytical information, an electrometric method based on amperometric biosensors[122,123] was adopted. Because of the reliability assured, the amperometric biosensors can be integrated in an amperometric FIA system.[123] For the assay of aminoacids it is very important to discriminate the L- and D-amino acids. Constructed amperometric biosensors based on L-aminoacid oxidase and D-amino acid oxidase can discriminate the L- and D-amino acids.[124,125] For this purpose, these electrometric methods assure the best reliability of the analytical information as a result of the simplicity of sampling process, and because of the possibility of assaying the enantiomers without prior separation from the matrix.

Determination of pesticides in food is very important to ensure human health. Pesticides are especially present in fatty food of animal origin. The sampling process must contain two main steps: a preconcentration step made by extraction of pesticides from the matrix, and a separation step performed by GC or HPLC technique. Choosing the optimum conditions for the first sampling step as well as the best chromatographic technique for discrimination of pesticides assures the best reliability of the analytical information.[126,127] The most reliable detection system for discrimination of pesticides is MS, which assures both the best selectivity and the best sensitivity for discrimination of pesticides.

In conclusion, for food analysis it is possible to use spectrometric methods as well as electrometric methods. To obtain the most reliable information the complexity of the matrix, the nature of compounds, and the selectivity and sensitivity of the method must be reconciled. There is no single, universal method used for food analysis. Using the same method for food analysis may result in both the best and the poorest analytical information for the same compound depending on the matrix in which the compound is found. Variant results are obtained because of interferences that affect the selectivity of the method as well as its sensitivity. Therefore, the quantity of the compound in the matrix, whether it is a major compound or if it is found in trace amount, is very important.

## *4.3 Clinical analysis*

Clinical analysis is one of the most important fields of analytical chemistry because of the importance to the health of the human body of the materials being analyzed. For example, because pharmaceutical products are meant to improve the health of the body, the analysis of pharmaceutical products *in vitro* as well as *in vivo* can be considered a branch of clinical analysis. Clinical analysis has two main branches: research clinical analysis and routine clinical analysis. As always, it is very important to obtain the optimum conditions for the methods applied in clinical analysis. First, the methods are applied by the researcher for *in vitro* tests. Then, it is very important to obtain biocompatible materials for *in vivo* determinations. For clinical analysis, methods with high sensitivity, a low detection limit, and high selectivity are necessary. The rapidity of assurance is one of the most important characteristics of the method that is applied.

Immunoassay plays an important role in clinical analysis. It is followed by electrometric techniques: polarography and electrometric sensors (ion-selective membrane electrodes, or ISME, and biosensors). Because of the complexity of the matrix, spectrometric techniques must precede the separation technique (extraction or chromatographic techniques). The best reliability for clinical analysis is achieved by using immunoassay methods and electrochemical sensors. Because of the possibility of assaying the activity of the ions continuously and without any prior separation, electrochemical

sensors (ISME and biosensors) are best for *in vivo* assay; they assure the best reliability of the analytical information.

Similar to the immunoassay techniques, radioimmunoassay, enzyme immunoassay, and chemiluminescence immunoassay are also applied and have proved useful. All techniques used for immunoassay represent coupling between the specificity of the antibody–antigen reaction and the sensitivity of the radiometric, electrometric, and chemiluminescence techniques. The limit of detection for immunoassay depends on the antibody affinity.[128-130] Materials such as silica,[131,132] latex,[133,134] and alkylamine films[135] are used for antibody absorption. SEM,[136] scanning tunneling microscopy,[137] and scanning force microscopy[138] have been employed as tools for visualization of the immobilized antibodies.

Methods based on radiolabels continue to hold an important place in routine analysis and in research related to clinical testing. The main techniques included in this group are radioimmunoassy (RIA), immunoradiometric assay (IRMA), and scintillation proximity assay (SPA). Many researchers in this field use short-lived radioisotopes and chelating agents in antibody labeling.[139] The most popular types of immunoassay are methods that use enzymatic labels: the enzyme-linked immunosorbent assay (ELISA), the enzyme-monitored immunotest (EMIT), the competitive binding enzyme immunoassay (EIA), and the immunoenzymometric assay (IEMA).

Benzodiazepines (Bzs) are an important class of pharmaceutical product used for their anticonvulsant, hypnotic, tranquilizing, and muscle relaxant properties. The most frequently used are diazepam, nitrazepam, and chlordiazepoxide. It is necessary to determine their quantity in urine, the metabolites as well as the quantities and nature of these drugs. Because the concentration of these drugs and metabolites in urine is low, the ELISA technique[140] is recommended for their assay. The technique assures good selectivity, sensitivity, and a low detection limit (0.3 μg/ml). The reliability of the analytical information as well as the rapidity of the analysis make the ELISA technique suitable for automation. It is simple and quick to use in the laboratory as a routine analytical technique.

Fluorescent immunoassay (FIA) employs a fluorescent signal for analyte detection. The following methods have been proposed: FIA, immunofluorimetric assay (IFMA), fluorescence polarization immunoassay, and time-resolved fluoroimmunoassay (TR-FIA). Lanthanide-loaded liposomes and lipid-tagged antibodies are used as reagents to improve the limit of detection in a TR-FIA method.[141] For routine clinical analysis FIA methods are especially recommended. The use of lipid-tagged antibodies in FIA opens a new field for the development of biosensors, which assure the best reliability of the analytical information for FIA.[142] The FIA technique assures the reliability of the analytical information for assay of drugs in urine. For example, it is very important to know the quantity of phenothiazines and thioxanthines (drugs used as neuroleptics) in urine. Among the most often recommended neuroleptic drugs are: chlorpromazine, thioridazine, fluphenazine, and flupenthixol.

For their assay in urine, FIA provides the best sensitivity, the best limit of detection, and the best selectivity.[143]

Many immunoassay methods are based on chemiluminescent labels; examples are the competitive binding chemiluminescence immunoassay (CIA), the immunochemiluminometric assay (ICMA), and the electrochemiluminescence immunoassay (ECIA). Aequorin is used as a label in these techniques and for bioluminescent signal production in immunoassay,[144-146] as well as for *in situ* analysis. CIA assures good sensitivity, a low detection limit, and good reliability. The best reliability using immunoassay is assured by using methods based on radiolabels; however, the easiest method to use in routine analysis is ELISA. In addition, when the generally accepted classical treatment of a condition is not sufficient, immunobiotherapy is often recommended. The maximum reliability for assay of drugs used in immunobiotherapy is obtained by using immunoassay techniques.

Electrochemical sensors have often been used in the last few years by virtue of the accuracy of the resulting analytical information. Sensors assure the best selectivity and sensitivity, and by the year 2001 electrochemical sensors–based clinical diagnostic testing products used for measuring electrolytes in whole blood and serum samples are expected to capture 25% of the clinical chemistry market and 11% of the microbiology market. Electrochemical sensors are very often used for assay of pharmaceutical products, both *in vitro* and *in vivo*. *In vivo* measurements are based on the following characteristics: the ability to measure the activity of species in the biological fluid continuously, and the ability to determine the species directly, without any prior separation from the matrix. Other very important reasons for using electrochemical sensors in clinical analysis are the simplicity and low cost of the analysis. All these advantages make the electrochemical sensors useful for routine clinical analysis, especially since they also offer the best reliability.

Ion-selective electrodes with a liquid membrane are more reliable than ion-selective electrodes with a solid membrane because of the uniformity of the active material partition in the membrane. For the construction of biosensors the maximum reliability is obtained by using graphite paste as the support. As of the present, for *in vivo* tests only sensors based on plastic membranes have been used. The main problem associated with using them for *in vivo* tests is the biocompatibility of the materials.[147-149] The membrane biocompatibility, the matrix biocompatibility, and the electroactive material biocompatibility are important factors. The matrix biocompatibility is assured by the biocompatibility of the polymer and by the biocompatibility of the plasticizer. The ratio between the quantity of polymer and quantity of plasticizer affects the response of electrochemical sensors because the matrix of the solid membrane electrodes plays the same role as does the solvent in liquid membrane electrodes.

The selectivity of electrochemical sensors is affected by the matrix,[150] and the selectivity must also be correlated with the response of the sensors. Because biological fluids are complex matrices, selectivity is one of the most important characteristics of the method applied in clinical analysis.

The role of some inorganic ions such as: $Ca^{2+}$, $K^{+}$, $Na^{+}$, $H^{+}$, $Li^{+}$, $Mg^{2+}$, $Cl^{-}$, and $CO_3^{2-}$, in the body is well known. For inorganic ions *in vivo* microfabricated sensors arrays[35,36] are proposed. The advantages of assay microfabricated selective sensor arrays over conventional ion-selective sensors based on polymeric membranes are their small dimensions, ease of sterilization for *in vivo* monitoring, relatively low source impedance, and suitability for mass production.[36] The main problem associated with microfabricated sensor arrays is design assurance. They are usually made of silicon and include integrated electronics. Their structure is two-dimensional planar. To increase their biocompatibility and to improve their use for electrode arrays, construction of a flexible polyimide substrate and a combination of thin-film, thick-film, and packaging technologies are used. The electrode array structure can be achieved through the use of a polyimide-patterned metal–polyimide sandwich with openings in the top layer of polyimide to define the electrode sites and bonding pads. Layers of silver, silver chloride, hydrogel, and polyvinyl chloride (PVC)-based ion-selective membrane are deposited over the site openings to form ion-selective electrodes.[36] This arrangement is shown in Figure 4.5. The main advantage of microfabricated selective sensor arrays is that they can be used for assay of ions directly in the body. The sampling process is eliminated, and thus the reliability of the analytical information is of the highest order. However, when electrochemical sensors are used for *in vivo* determinations, their selectivity is affected by their size[151] because of the antigen–antibody reaction. Nanometer-sized, glass-sealed metal ultramicroelectrodes (UMEs), prepared by using a laser-based micropipet puller, have solved this problem. The geometry of the UMEs is critical. The best reliability is assured by the conical UMEs.[151]

For assay of active substances both as raw material and from pharmaceutical dosage formulations, the best reliability is assured by using ISME. ISME can be successfully used for uniformity content tests as well as for *in vitro* and *in vivo* dissolution tests of tablets.[152-160] The ISME proposed for assay of pharmaceutical products is based on the ion pair complexes, but the responses of ISME are highly affected by the stability of ion pair complexes. The ISME run is explained by the multilayer configuration of the membrane.[161]

The most selective electrochemical sensors are amperometric and potentiometric biosensors. They couple the selectivity of enzymes with the sensitivity of an amperometric or potentiometric sensor. Because the best sensitivity is assured by amperometric sensors, it follows that the best reliability is obtained using amperometric biosensors.[162] A great deal of attention has been given to molecular-level modification of electrodes with glucose oxidase (GOx) for enhancing the performance of such devices.[163,164] The amperometric biosensors assure a very low limit of detection of micro- or nanogram magnitudes, thus the reliability of the analytical information is great. They can be used successfully for *in vivo* assay. For their construction, the molecular imprinting technique is a powerful method for preparing synthetic

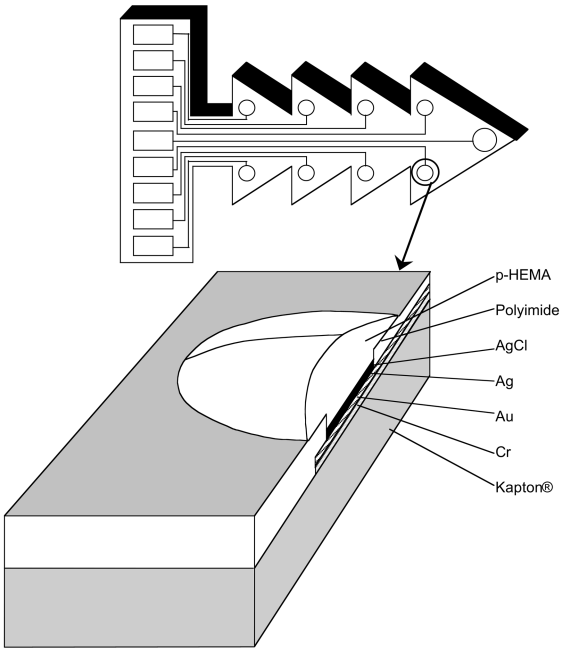



***Figure 4.5*** Nine-site Ag/AgCl electrode array used for sensors construction of $H^+/K^+$ and a cross section of a recording site. (From Cosofret, M. et al., *Anal. Chem.*, 67, 1647, 1995. With permssion.)

recognition sites.[165] Carbon paste enzyme electrodes can be reliably constructed[166] as a result of the uniformity of enzyme distribution in carbon paste.

Further, amperometric biosensors constructed using l-amino acid oxidase can enantioselectively assay l-amino acids.[125] For enantioselective analysis, amperometric biosensors assure the best reliability of the analytical information.

The "built-in" preconcentration step of stripping analysis has continued to attract considerable attention, particularly in connection with ultratrace measurements. The technique is used for monitoring inorganic substances as well as organic substances. One of the more interesting applications of the potentiometric stripping technique is nucleic acids assay.[167] A microfabricated thick-film electrochemical sensor has been proposed. The low detection limit (3.25 μg/l), the high sensitivity and selectivity, and the simplicity of the method unit make it suitable for this kind of analysis. The reliability of the analytical information is good.

Because the best reliability is assured by *in vivo* monitoring, differential pulse voltammetry (DPV) has been applied for *in vivo* assay of brain catecholamines.[168] The method was not very selective since ascorbic acid was the main interfering species. Furthermore, the implantation of an electrode

is traumatic to brain cells; and it is intuitively obvious that the smaller the electrode, the less damage occurs.[169] Therefore, modern carbon fiber microelectrodes, only ≈10 μm in diameter, must be used. The size of the electrode alters the detected species because of the antigen–antibody reaction. The low sensitivity of the method can be explained by the fact that the extracellular fluid contains protein that may be adsorbed onto electrode surfaces and impede electron transfer. To improve the quality of the analytical information, it has been proposed that fast cyclic voltammetry (FCV) be applied to detect dopamine[170] *in vivo*. The voltage scan rate was increased and, rather than lasting a couple of minutes, each scan took only 20 ms and thus could be repeated many times per second. The carbon fiber microelectrode used was 8 μm in diameter. The background current signals prior to a stimulation "event" were digitally subtracted from those obtained during a transmitter release event. It follows that only the faradaic current from the oxidized species remained. The FCV thus assures the best reliability for *in vivo* determinations. This technique was successfully used to obtain a "functional map of brain areas" by *in vivo* measurements.[169]

To obtain good analytical information using spectrometric techniques a sampling process that contains a separation step is essential. The best reliability can be obtained by electrophoretic techniques and by chromatographic techniques. The most frequent separation techniques used for diagnosis of metabolic disorders is capillary electrophoresis (CE),[171] which can also be used successfully for drug monitoring, protein analysis, and single-cell analysis.[171] The technique assures good selectivity of the separation and it has a low cost compared with liquid chromatography. CE-based immunoassay can be considered a specific separation technique used in clinical analysis based on the antibody–antigen reaction. The specificity of the antibody–antigen reaction is combined with the resolving power of CE for the determination of analytes in complex sample matrices.[172,173] Compared with the CE technique, the HPLC technique assures less reliability of the sampling process.

In competition with HPLC are two techniques: affinity chromatography and chiral separation. Affinity chromatography is usually used for the purification of specific antibodies or for the separation of individual antisera into fractions with different antigen-combining properties.[174] Chiral separation is very useful for the pharmaceutical industry because chiral active substances can have enantiomers with antagonist properties. The presence of antagonistic enantiomers in a pharmaceutical compound can affect the health of humans; therefore, a separation technique using a chiral stationary phase[175] is required. Other HPLC techniques for soluble grape proteins separation[176] and for purification of copper–zinc superoxide dismutase from bovine erythrocytes[177] have been described.

Immunochromatography is a relatively new chromatographic technique based on the specificity of the antigen–antibody reaction. It can be used for purification and determination of immunoglobulins.[178]

The determination of trace elements in the body is a very important field because of the effects of inorganic substances on the body. The most reliable information for determination of trace elements is assured by the ICP-MS technique. Because of the sensitivity of the technique, the sampling process must be done using the microwave digestion method. The technique MWD-ICP-MS has been used for trace element determination in brain, liver,[52] and dental tissues.[179] A laser technique is recommended for sample processing of dental tissues as it has proved more reliable. For urinary arsenic determination, an HPLC technique is indicated for the sampling process, followed by the ICP-MS technique.[180] Another way to determine urinary arsenic involves a sampling process using solvent extraction followed by atomic absorption spectrometry.[180] The best reliability is assured by the HPLC-ICP-MS technique. Because of the importance of accuracy of As determination in the body, the most reliable method must be chosen. For lead determination in whole blood, capacity-coupled microwave plasma atomic emission spectrometry is recommended.[181] The method is reliable, but the best reliability is assured using the ICP-MS technique.

Spectrometric methods can be applied with good results for monitoring organic compounds. The detection, number, and sequence location of sulfur-containing amino acids and disulfide bridges in peptides can be reliably achieved with ultrahigh-resolution matrix-assisted laser desorption/ionization (MALDI) FT-CR mass spectrometry because of the high sensitivity, high specificity, and low detection limit assured.[182] Disulfide bridge formation is a common posttranslational modification and a crucial step in achieving the correct three-dimensional structure of peptides and proteins. The proposed method is reliable, rapid, and can detect the proteins at femtomole magnitude both *in vitro* and *in vivo*.

The fluorescence technique can be coupled with the CE technique. Certain reliable analytical information can be achieved by CE-laser-induced fluorescence detection.[183] For example, restriction mapping is one of the essential steps in gene analysis and molecular biology studies. By using CE, a restriction map of a genomic λ phage clone of human interleukin-5 receptor α chain (IL5Rα) gene was constructed. The laser-induced fluorescence detection and a modified partial digestion mapping procedure were developed to map DNA fragments. The reliability of the analytical information is explained by the sensitivity of the fluorescence method coupled with the selectivity of the CE technique. The reliability assured by the fluorescence method was previously demonstrated for DNA assay.[184] Short noise is the dominant contribution to the resolution. The reliability is also demonstrated by the accuracy attended (RSD = 4.3%).

Chemiluminescence (CL) methods assure good results for analysis of body fluids and tissues. This is demonstrated by urinary iodide,[185] cholesterol,[186] and serum glucose[187] assay. The CL method has had a major impact on all forms of DNA diagnostic techniques.[188,189] NMR spectroscopy can also be used for *in vivo* measurements for diagnosis of illness. For example, the relationships between fiber composition and NMR assessment in human

skeletal muscle,[190] *in vivo* assessment of free magnesium concentration in the human brain,[191] and qualitative and quantitative characterization of $^{1}H$ NMR spectra of colon tumors, normal mucosa, and their perchloric acid extracts[192] represent some examples of applications of NMR spectroscopy in this field.

In conclusion, reproducible analytical information can be obtained through both electrometric and spectrometric methods and also through immunoassay techniques, but only electrochemical methods can be successfully used for *in vivo* assays because electrochemical methods assure the best reproducibility of the analytical information for clinical and pharmaceutical analysis.

*chapter five*

# *Connection between reliability and instruments*

> "The choice of analysis instrument is imposed by the sample and its matrix"[23]

As stated earlier, the sample acts as a "glue" between method and instrument. The use of more and more sophisticated methods and instruments is required as the matrix becomes more and more complex. Also, within the logical quality–quantity–structure chain, "the selection of analytical methods and of the corresponding instrumentation is a consequence of correct knowledge of the sample's history and homogeneity."[23]

The reliability of the instruments is connected with the "imagination, intelligence, creativity, and especially discerning power"[23] of the researcher. The researcher must connect the operational parameters of the method to the functional parameters of the instruments. Only the best connection results in reliable analytical information. There are a number of "intelligent" instruments, but "artificial" intelligence is not enough to obtain reliable information. The "intelligent" instrument must be used by human intelligence, creatively. As discussed, there is no one method that can be applied with good results for the determination of various components. Because of the method–instrument connection, it follows that it is impossible to maintain the same parameters of an instrument for the analysis of various compounds. The development of new fields of science and technology has mandated improvements in methods and instrument parameters because they have become outdated; to obtain reliable analytical information, the old method must be replaced by a new method which should be more competitive.

Instrument improvement bears high cost, so, although a more sophisticated instrument will assure the best reliability, it will cost more. The evolution of instruments in time is interconnected with the evolution of materials, sciences, and technology. This evolution was made possible only by the evolution of physics because the activity or concentration ($C$) is determined as its physical property ($P$) $P = f(c)$. Thus, it is necessary to assure the

reliability of the instruments used on sampling process, components determination, and data processing.

The weighing process has similar importance to the accuracy of analytical information as has the homogeneity and the history of the sample. Accurate weighing of a sample requires use of the appropriate balance: macro-, micro-, or ultramicrobalance. When ultramicrobalances are used for weighing, correction of temperature and pressure parameters is required. The analytical balance has evolved from a typical single-pan balance to an electronic analytical balance, which has improved the quality of the weighing process.[193] One of the parameters that affects the quality of the weighing process is the temperature of the object: hot or cold objects must be brought to ambient temperature before being weighed. There are two types of weighing done in analytical chemistry: rough and accurate. A rough weighing to two or three significant figures is normally used when the amount of substances to be weighed needs only be known to within a few percentage points — e.g., the reagents that are to be standardized later against a known standard. Accurate weighings are reserved for obtaining the weight of a sample to be analyzed; these are performed only on an analytical balance, usually to the nearest 0.1 mg, and they need the best reliability.

The quality of apparatus is crucial. The quality of the pipets directly affects the reliability of the analytical information. It is necessary to use measuring pipets of high quality for reliable measures of volume, e.g., clinical, serological pipets, micropipets, syringe pipets. Syringe pipets must be used for measurement of microliter volumes. In volumetric analysis more reliable burets are necessary. For small quantities of sample, micro- and ultramicro-micrometer burets are recommended. Only well-calibrated pipets and burets will assure the accuracy of volume determination.

The most critical sampling step is dissolution digestion of the sample. For less sensitive analytical methods, sample dissolution is accomplished by using mineral acids, by fusion with an acidic or basic flux, or by dry ashing processes. More sensitive methods require a dissolution process based on microwave digestion (MWD). MWD is often used in tandem with ICP-AES and ICP-MS techniques. MWD assures lower sample contamination, and ICP-AES and ICP-MS techniques are very sensitive and need special sample preparation. The best reliability is assured by using the MWD instruments. The use of MWD apparati also reduces the dissolution time from hours to minutes; and the blank level is low because of the reduced amounts of reagents required. Closed digestion vessels reduce contamination and analyte loss, and eliminate acid fumes.[194,195]

Another very important sampling process step is the separation step. The separation step plays an important role, especially for spectrometric methods, as it increases selectivity. By using adequate instruments that improve the separation process, reliability is assured by increasing the selectivity.

For the extraction process, the use of supercritical fluids extraction in adequate equipment improves the quality of the extraction. The most often used technique for separation is the chromatographic technique.[196] HPLC

enables the separation and analysis of highly complex samples with species of both neutral and ionic nature. Open tubular columns (OTCs) were first proposed for GC by Golay in 1958.[197] The main problem associated with chromatographic instrumentation is construction of the columns. If OTCs were to be used for the GC technique, the insufficient sensitivity and low mass loadability of OTCs would discourage instrument manufacturers from developing an OTC-LC instrument. The best alternative solutions for OTCs used in LC would drive a liquid through a capillary column either by application of a hydrostatic pressure difference or of a potential difference across the length of the column — the electroseparation technique. In 1980 the first electroseparation technique, capillary zone electrophoresis (CZE),[198,199] was introduced. With the CZE technique, charged species can be separated. For uncharged species separation by CZE, micelles[200] were applied and thus a new separation technique called micellar electrokinetic chromatography (MEKC),[200] based on the partitioning of the uncharged analytes between the electrolyte and the micelles that serve as a pseudostationary phase, was developed. The ability of the instrument to drive the eluent through an electro-osmotic flow created a new chromatographic technique: electrochromatography (EC).[199] Columns of 170 μm inside diameter, packed with particles of 10 μm, were used for the CE technique. In EC, the separation mechanism essentially originates at the interface between the mobile and stationary phases, and exchange kinetics between the two phases are very important. It follows that there are two sources of zone broadening, axial and radial diffusion. Radial diffusion determines the column diameter that can be used.[201] Because of the high resolution assured, as well as the small volumes that are required, the CZE and MEKC are in the forefront of chromatographic methods.

One may now ask what chromatographic technique assures, generally, the best reliability for the separation process and what apparati and columns are recommended for these purposes?

The answer is that there is no single chromatographic technique generally available for all types of separation. The selection of chromatographic technique must be made taking into account the nature of the compounds that will be separated, the complexity of the matrix, and the detection apparatus that is coupled with the chromatograph. The selection of the column is another very important issue when chromatography is used as the separation technique.

The best reliability will be assured, first by the best choice of the column and, second by the choice of a chromatographic technique that takes into account the detection system that is available. One of the important fields where HPLC, CZE, and MEKC can successfully be used is in resolution of racemic drugs to their corresponding enantiomers. The analysis of compounds requires a continuous development of the methods used. Because method development is connected with instrument development, this is followed by the introduction of new measuring instruments.[202]

Because the development of most spectrometric techniques is based on the flame, it is important to mention the contribution of Teclu for studying and understanding of the oldest "reagent," the flame.[203] Without a good understanding of the phenomena that occur in the flame as a reagent, it is impossible to construct a reliable apparatus for atomic emission and absorption spectrometry. The invention of the spectroscope was followed by application of spectroscopic methods to analytical devices. The instruments became more sophisticated because of developments in physics, the science that determines apparatus requirements.

The laser (LA) can be used as a source of monochromatic light. Ultratrace analysis requires very sensitive methods with very low detection limits. The use of a laser assures subfemtogram detection limits, thus improving the accuracy of the analytical information.[204] Because ICP-AES and ICP-MS techniques have low detection limits, the coupling between LA and ICP-AES (LA-ICP-AES) and between LA and ICP-MS (LA-ICP-MS) give the best reliability for the analytical information. The reliability of LA-ICP-AES and LA-ICP-MS techniques depends on the reliability of LA, the reliability of ICP, and on the reliability of the AES and MS apparati. Because the apparati used for LA, ICP, AES, and MS techniques are reliable, the resulting analytical information is at maximum reliability. The precision of the analytical information can be improved by optimization of method (LA-ICP-AES) parameters as well as by increasing the functional parameters of analytical instruments initially through improving the signal/noise (S/N) ratio.[205,206] The S/N ratio can be especially improved by developments in analytical instruments.[207] Using a digital system can decrease the noise, and the S/N ratio will increase. The S/N ratio value is especially important for techniques with low detection limits.

Fluorescence detection is often used in liquid chromatography. It has a low detection limit, but sometimes it does not cover the requirements of some compounds. To improve the detection limit, visible diode laser-induced fluorescence has been used as a detection system in liquid chromatography.[208] Spectra are automatically corrected for differences in excitation efficiency in the red region of the spectrum. The automation of all components of analytical instruments improves the reliability of the analytical information.

Different apparati with different characteristics can be used for the same analytical method. Sometimes apparati manufactured in the same factory register differences in characteristics. The differences can be due to construction modifications and to the ambient conditions where the apparatus functions, which explains the differences between results obtained using the same apparatus in different laboratories. An example was demonstrated for the Fourier transform infrared (FT-IR) spectrometry technique that was applied for the analysis of aqueous solution.[209] After the same aqueous solution determination in various laboratories by different workers with different instruments was produced by FT-IR technique, similar quality analytical information resulted using the same data processing: simple linear method calibration. The conclusion is that the FT-IR spectrometer can be unstable

and thought must be given to the collection of background spectra or the use of baseline correction. Further, data transfer between machines or between accessories on the same machine is proscribed without several conditions that must be respected when the data *must* be transferred between accessories on the same machine.

One can ask: What about the resolution and its connection with S/N ratio? Hobbs el al.[210] replies to this question: "Although the signal to noise ratio for an emission spectrum when properly evaluated is independent of resolution until the lines are actually resolved, there is a real gain to be made in the line to continuum ratio."[210] The noise is essentially an interfering signal, and it can be eliminated by modifying the parameters of the apparati and the working conditions. The S/N ratio is defined as the ratio between the mean value and the standard deviation.[211] As the standard deviation decreases, the S/N ratio and the reliability of the analytical information increase. The resolution cannot influence the standard deviation, as was demonstrated using different apparati for FT-UV/Vis techniques.[212] The maximum reliability for FT-UV/Vis techniques is assured by a low-pressure (e.g., minimum pressure broadening), low-temperature (e.g., minimum Doppler broadening) source with no continuum background (e.g., minimum shot noise) and no significant flicker noise contribution. By using these parameters, it is possible to perform isotopic analysis of a complex mixture.[212]

The new scanning probe microscopy, especially scanning tunneling microscopy (STM) and atomic force microscopy (AFM), have captured the interest of the science and engineering communities. The first apparatus for scanning probe microscopy (SPM) was available in 1986. Because of the quality assurance of SPM technique using this equipment, it can be reliably used as a quality control tool for environmental analysis.

The main factor in beam analysis that affects the reliability of the analytical information is the reproducibility of the surfaces. When using scanning electron microscopy (SEM), the apparati are connected to the computer, which makes it possible to obtain quite a bit of information about the sample, especially by X-ray and AES. However, the apparati cannot assure the same length for beam penetration on the surface, which means that the analytical information can be uncertain. Because the beam analysis is rapid, it requires very fast detectors, e.g., Ge/Li or Si/Li. The LA can be successfully used in surface analysis. An automated system has been constructed, laser-induced breakdown spectrometry (LIBS).[213] This is an alternative to other surface techniques — secondary ion mas spectroscopy (SIMS), SEM, X-ray photoelectron spectroscopy (XPS) — and it increases the lateral and depth resolution.

For characterization of thin-film electrochemiluminescent structures, some spectroscopic techniques, such as XRF, SIMS, AAS, and ES, as well as luminescence spectroscopy, FT-IR, XPS, EXAFS, and XR for structural information,[214,215] are essential. These kinds of measurements have been made possible by developments in physics and electronics.

The electrometrical methods have also experienced a high development rate because of the possibility of determining the analytes from solutions without a prior separation step. Altemouse[202] said, "The introduction of electrical methods allowed precise quantitation." The introduction of the automated buret and automated registration system improved the reliability of titration using electrodes (ISME, redox electrodes, biosensors).

Considering two electrometric methods, polarography and anodic stripping voltammetry (ASV), one can conclude that for trace analysis the polarographic technique does not provide reliable analytical information from a sensitivity point of view. On the other hand, the reliability of ASV for trace analysis is explained by two factors: the sampling process (preconcentration on mercury drops) and the pure analytical step consisting of registration of the redissolution wave. The problem for ASV apparatus reliability of the drop size was solved by connecting the apparatus with a computer; thus, one can choose the optimum size of the drop to obtain maximum reliability for the analytical information. The miniaturization of electrochemical devices has made them suitable for *in vivo* assay, with applications in clinical and pharmaceutical analysis.

When a chemiluminometer detector is used in the HPLC technique, it is necessary to take into account several instrumental parameters, such as reagent and eluent flows and concentrations, because many of these are not controlled (e.g., detector geometry and volume, pump pulsation, the mixing of the reagent and eluent flows, or background emission).[216] Because increases in detector volume increase the chemiluminescent signal, the detector parameters must be correlated with the HPLC parameters to obtain the best reliability.

The coupling between CZE and ICP techniques provides good reliabilities for analytical information. The interface plays a significant role in reliability assurance, especially when the coupling techniques are more sensitive. The interface between CZE and ICP was built outside and independent of the nebulizer and could be easily connected with a microconcentric nebulizer (MCN), as well as with conventional pneumatic nebulizers.[217] The interface decreases the limit of detection to the 1 μg/ml level.

In conclusion, the best interface and a good correlation of parameters of both the apparatus and the technique will assure the best reliability for analytical information. Automation of the apparatus not only improves the objectivity of the analysis, but is also necessary for the operator's protection. When radiochemical methods are used with automation, it is possible to obtain objective and reliable analytical information that is independent of the ambient conditions. For environmental analysis, automatic spectrometers are important to obtain continuous reliable analytical information, which is called environment monitorization. In cosmochemistry, automation of equipment and robotics is essential to assure the reliability of the information that is received by teleanalysis.[218]

# *chapter six*

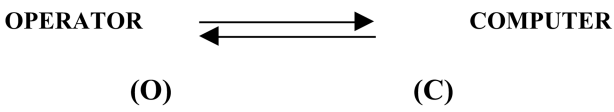
# *Reliability of data processing*

Data that are processed for chemical analysis consist essentially of the automated signal obtained from an analytical instrument as the instrument characterizes a sample. The reliability of the data is directly connected to the reliability of the analytical information, and reliability increases with the number of data bits obtained from the analytical instrument. Automation of the analytical instrument is then followed by automated data processing which requires a lengthy time because of its complexity.

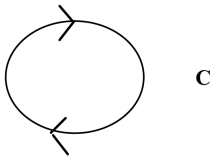
The term *chemometrics* has been introduced to represent chemical calculations. Chemometrics has become an independent and very complex interdisciplinary science which requires mathematical knowledge. Chemometrics entails not only statistics, but also parameters optimization strategy, neural networks, parameters estimation, and so on. The introduction of the computer into chemometrics has simplified the operator's work and has assured the maximum reliability of the information.

The role of the computer is to process the data, store the data, and transmit the data to the operator. The analytical instrument–computer interface is very important for the reliability of analytical information. Instruments record analog signals, whereas the computer is digital in nature and accepts only digital data. An analog-to-digital (A/D) converter accepts the data from the instrument and then converts it into language compatible with the computer to complete the interface. The computer has the ability to present the calculated results in digital form, or it may transfer them through a digital-to-analog (D/A) converter to present them in analog form.

The computer must be connected to the analytical instrument, and it must contain adequate software. The software is essentially the heart of a computer that is used for data processing, and its performance must be correlated with the number, speed, and complexity of the signals that are obtained from the analytical instrument. Another critical connection is that between the natural intelligence of the operator and the artificial intelligence of the machine used for data processing. This relationship is illustrated as follows:




which is essentially a closed circle:




Signal processing is defined as "a discipline of chemometrics that is concerned with the manipulation of analytical data to make the information contained in the data more accessible."[219]

The characteristics of electrodes used in electrometric analysis can be obtained using statistics. For example, the true detection limits are much smaller than those obtained using a simple linear calibration, and consideration should be given to the nonlinear response characteristics of these electrodes. The selectivity of ion-selective membrane electrodes can be determined using a statistical analysis of the effects of interfering species.[220] Because only a good determination of response characteristics and of the selectivity for ion-selective membrane electrodes allows their utilization for ion monitoring from solutions, their accurate and reliable determination using statistics or soft modeling is essential. Several determinations need to be performed since the accuracy increases with the number of determinations. The parameters that affect the potential measurements involve the soft modeling, which plays a major role as it involves the identification of a model from a data set rather than the fitting of an external model to the data set. Soft modeling allows the isolation of the sources of variation in a data set.

The utilization of ion-selective membrane electrodes involves their prior calibration. It is necessary to make a regression to obtain good reliability of the analytical information. Because a linear relation between the independent and dependent variables cannot forced, sometimes the nonlinear calibration method is successful. The ion-selective membrane electrode linearization and subsequent calibration use multiple linear regression (MLR) and/or partial least-squares (PLS) when limited calibration data are available.[219]

Anodic stripping voltammetry requires a computer for data processing because of the rapidity of signal emission from the analytical instrument. The computer is used to control the drop size, and also for signal registration and data processing. Therefore, it is possible to choose the parameters via the computer for all voltammetric techniques to register and process the signal obtained. Thus, computer utilization for voltammetric techniques causes analytical information to be more reliable.

Because of the complexity of the emission spectrum, it is very difficult to process it to obtain accurate analytical information. Only through automation and computer coupling is the reliability of the analytical information assured. Cuantometers have partially solved the problems associated with data processing for emission spectroscopy (ES). The XRF spectra are less complex than the ES spectra, but the X-ray cuantometers also assure good reliability of the analytical information. The introduction of a computer for data processing in these techniques assures an automated control of data processing.

Spectrometric methods require a prior sampling preparation containing a separation step. The separation step is necessary especially to eliminate interference. Nonspectral interferences in flame atomic absorption spectrometry can be overcome by using a calibration model.[221] The model uses two independent variables for analyte quantification (the amount of the sample and the amount of analyte added); the measured absorbance is the dependent variable. To control the matrix interferences without prior knowledge of the matrix composition, it is necessary to carry out nine calibration points to obtain accurate analytical information. This confers high reliability of the analytical information for determination of trace elements in complex matrices.

Spectrometric methods are usually recommended by pharmacopoeias for the assay of pharmaceutical products.[55] These require both a separation step and a calibration graph. The resolution of a spectrometric method used for assay of a ternary or quaternary mixture of drugs is not good when a certain, well-known calibration graph is used. To obtain the maximum reliability for data processing it is necessary to use the PLS methodology[222] because it eliminates the need for sample pretreatment. This model represents essentially an optimization method that can be applied to the problem of the experimental design.

A widely applied discipline of chemometrics is pattern recognition, which involves the classification and identification of samples. Its purpose is to develop a semiquantitative model that can be applied to the identification of unknown sample patterns. To assure the best reliability, pattern recognition requires the applications of a minimum of two analytical methods. The ammonium–azonium tautomerism in *N*,*N*-dialkylaminoazo dyes used as indicators requires three techniques: UV-Vis, IR, and Raman spectroscopy.[223] A study of the matrix composition concerning the ratio of the compounds is necessary as well. The application of pattern recognition analysis in geochemical techniques not only assures better reliability but is also quite useful in addressing real-world environmental problems.[224]

It is possible to map the toxicity potential areas of trace elements by obtaining classification models based on national-scale data synthesis programs. To obtain reliable analytical information, "the main methods of structural analysis may be coupled two–by–two from the point of view of correlating various sources of information"[1] (Figure 6.1).

Four analytical methods are required to obtain accuracy and precision in structure determination. Accordingly, the recommended improvements

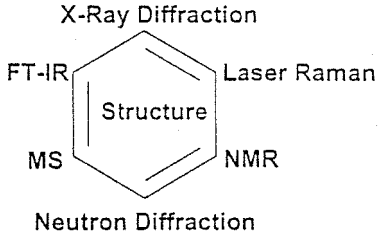



*Figure 6.1* The main methods of structural analysis.

of the analytical methods will increase the quality of the analytical information. If an IR technique is chosen, one must take into account the sensitivity required by the sample. Thus, an improvement in sensitivity is assured by working in emission rather than in absorption with high-temperature molecules, ions, and free radicals.[225] The instrument used is the high-resolution FT-IR spectrometer. For astronomical applications for which light levels are low, the FT-IR spectrometer is inferior in sensitivity compared to the cryogenic echelle spectrometer. X-ray absorbtion can be widely used for studying the complex structure.[226]

Laser Raman spectroscopy can be coupled with FT-IR spectrometry for establishing structure.[227] Raman spectroscopy reliability increases by using the laser for analyzing stimulated Raman scattering (SRS) and coherent anti-Stokes Raman scattering (CARS) in pure organic liquids. Nonlinear phenomena amplify the ordinarily weak Raman effect to the point where it is easily visible to the eye. The flexibility of SRS has made it useful in many analyses by production of coherent light of varying frequencies and the transfer of a signal from one laser source to another with a different frequency. One of the most important fields of Raman laser application is the identification and noninvasive monitoring of organic compounds.[228]

NMR selectivity is improved by coupling it with HPLC, supercritical fluid chromatography (SFC), gel–permeation chromatography (GPC), gas chromatography (GC), and capillary electrophoresis (CE).[229] The sensitivity of the NMR is low, but the sensitivity increases by using it in concert with chromatographic techniques because of the quality assured by the interfaces. The combined system has many applications: fingerprint determination of complex specimens (e.g., peptides, sugars, fuels)[230] and screening tests in environmental chemistry. The quality of the analytical information can be improved by using the combined system, HPLC-NMR-MS.[229]

Mass spectrometry can be improved by using the MS/MS tandem system that assures a high resolution for structure determination. The structural analysis cannot exist without MS, FT-IR, and NMR techniques accompanied by X-ray diffraction, neutron diffraction, and laser Raman techniques.

A very important and interesting field of chemometrics is the neural networks field, which can be successfully employed in nonlinear mapping, in nonlinear multivariate calibration, and in linear and nonlinear pattern classification. Also, confidence intervals are determined using calibration

with neural networks.[231] Good reliabilities can be obtained by using neural networks in classification of galaxies[232] and in identification of breast tumors.[233] Neural networks also assure good reliability when they are used to classify the clusters in a scatter diagram formed from images in a multispectral set.[234] Data processing is more reliable when neural networks are used.

Improvements in analytical methods as well as in chemometrics have allowed the redeterminations of the atomic mass values of some nuclides.[235] It is of interest to establish a true test of the reliability of data sets because of the differences obtained in successive published values of data. This reliability will be assured only by using competitive analytical methods that employ suitable computers for data processing.

Pattern recognition cannot be done well without a data bank, which can be defined as a storage system. However, it is necessary for a bank to accumulate data continuously and to be updated to operate efficiently. The incorporation of a data bank in a computer makes analytical instruments more reliable, especially if the data in the bank are kept updated. Teleanalysis needs a reliable data processing system. It automatically compares the data processing with the data contained in the computer data bank. Thus, when using the chemometrics and a computer with a data bank, the reliability of data processing can be of maximum value.

# *chapter seven*

# *Analytical process*

> "The analytical laboratory of the future will be highly automated, particularly in quality control"[236]

The analytical process comprises three main steps, which can be represented as shown in Figure 7.1. As can be seen, there are two primary time-consuming steps: the input and the output. The black box represents the measurement of the analytical information; it needs half the time that is required by the input or output steps.[3] The input step includes sample preparation and is a very difficult step to perform, especially when spectrometric methods are used. The output step includes data processing. The introduction of the computer for data processing improves the quality of the analytical information, as well as the rapidity and reliability of this step.

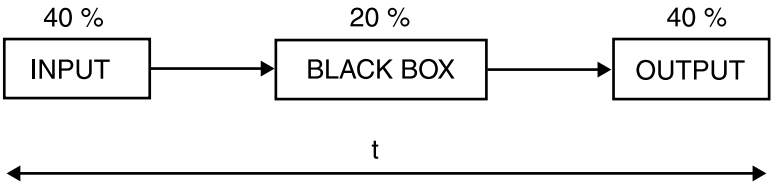



***Figure 7.1*** The analytical process.

An important role for the reliability of the analytical process is information. The information should have updated data about the sample, the sampling process, and the analytical method chosen for sample analysis. Using databases that contain abstracts of papers published in journals, books, and monographs provides useful information. The information must be obtained before the first step of the analytical process, which will assure correctness, capability, accuracy, and reliability.

## 7.1 Parameters of the analytical process

The main parameters of the analytical process are rapidity, reproducibility, and reliability. These parameters must be assured for every step of the analytical process. Assurance of reproducibility, rapidity, and reliability permits automation of the analytical process.

### 7.1.1 Rapidity

Rapidity is one of conditions for laboratory quality control. The time needed to develop an analytical process must be as short as possible. The length of time required influences the efficiency of analytical process since, as everyone is well aware, "time is money." The time an analytical process takes influences the cost of the analysis. It is also very important to consider the influence and effect of time on the aging of the analytical method. Automation of the analytical process, for all practical purposes, fulfulls the rapidity requirements. The rapidity of the analytical process is dependent on the reliability of the analytical method and that of the instrument. An increase in the reliability of an analytical process decreases the time required for analysis, and the use of computers for data processing decreases the time needed for analytical signal processing.

One of the most important fields in which the rapidity of the analytical process is necessary is *in vivo* clinical analysis. The use of chemical sensors (amperometric or potentiometric) as array sensors has solved the problem of time, sensitivity, and selectivity. Because of the selectivity and sensitivity assured by capillary electrophoresis, it can be successfully used for high-speed DNA genotyping, as in microfabricated capillary array electrophoresis chips.[237] Its capacity to analyze 12 different samples in parallel in less than 160 s has made it the method of choice for this type of analysis.

### 7.1.2 Reproducibility

Reproducibility is defined as "the closeness of agreement between independent test results obtained under stipulated conditions."[238] Reproducibility is indicated by the standard deviation; a small value of standard deviation assures the best reproducibility. The reproducibility of sampling directly affects the reproducibility of the analytical information. It is essential to secure homogeneity of the sample for both destructive and nondestructive analysis (surface analysis). The sampling process for destructive analysis must be reproducible. There are two important steps: concentration and separation. Usually, the techniques used are extraction and chromatography. Assurance of the best operational parameters guarantees that reproducibility will have the maximum value.

The matrix complexity and the nature and ratio of compounds that are to be determined require the best selection of the analytical method to be used in the black box. The reproducibility of this step is assured by the

sensitivity of the analytical methods. Every method has several parameters that must be chosen to obtain an accurate and reproducible signal. The sensitivity is influenced by the interface when chromatographic techniques are used in tandem with adequate detection systems.

Selectivity plans a very important role in analytical process reproducibility. However, selectivity is not assured totally through the analytical process. It can be increased by increasing the resolution of the analytical instruments. An adequate instrument should be chosen for the analytical method applied to assure the best reproducibility of the second step of the analytical process.

Data processing is reproducible using a computer. Adequate software for chemometrics presents the signal obtained from analytical instruments as reproducible analytical information. The importance of obtaining a reproducible analytical process is its potential for automation and miniaturization. Miniaturization of the analytical process for electrometric analysis has been achieved for *in vivo* measurements. The use of electrometric analysis provides good reproducibility of analytical information.

An increase in sensitivity can be achieved by coupling two well-known techniques. The determination of $Cu^{2+}$ using $[HgI_4][Cuen_2]$ ion pair complex can be done at a low level, $10^{-6}$ g, by weighing the precipitate. However, it is sometimes necessary to determine $Cu^{2+}$ ions in quantities smaller than $10^{-6}$ g. Then, the increase of sensitivity requires the use of a radiochemical method using iodine. Because radiochemical methods have a very low detection limit, sensitivity is improved. Reproducibility is assured by increasing the sensitivity and by automation, which is requisite to the radiochemical methods used.

### *7.1.3 Flexibility*

Flexibility is the characteristic that confers the ability to be adaptable to ambient conditions. For the analytical process, flexibility must be an attribute of the operator, the method, and the analytical instrument. Flexibility can be implemented through the ability of the operator to adapt the method to the sample, taking into account the complexity of the sample and the compounds that must be determined. The operator must adapt the method chosen to the analytical instrument used by correlation of the operational parameters of the method with the functional parameters of the analytical instrument. It is impossible to obtain accurate and reproducible analytical information without flexibility.

### *7.1.4 Reliability*

Reproducibility, rapidity, and flexibility of the analytical process together assure its reliability. The reliability of the analytical process permits its automation, which increases the quality and objectivity of the analytical information. Reliability of the analytical process is necessary in environmental

analysis, food analysis, and clinical analysis, as well as in cosmochemistry for teleanalysis. Automation is an integral part of teleanalysis in all steps of the analytical process, from the sample pickup and sampling process to data processing.

## 7.2 Automation and robotics

Within the analytical process, automation and robotics play very important roles in environmental analysis, food analysis, and clinical analysis. There are two basic types of automation equipment: automatic devices and automated devices.

### 7.2.1 Automatic devices

"Automatic devices perform specific operations at a given point in an analysis, frequently the measurement step."[65] The main characteristics of automation are objectivity, rapidity, flexibility, and reliability of the analytical instruments. With automation, the objectivity level increases. The reproducibility of the analytical information increases because analyst errors are eliminated.

*Objectivity.* Automation is necessary especially when objectivity of an analytical process used for a large number of samples is sought. Objectivity is assured by using a computer for optimization of parameters of the analytical method, as well as for data processing of the analytical signal.

*Rapidly.* Rapidity of the signal obtained through automatic devices allows one to obtain more analytical information in a very short time interval. The rapidity of automation devices is improved when automation is coupled with robotics. Robotics have a high degree of flexibility compared with continuous flow analysis (CFA). By using robotics, contamination is eliminated.

*Flexibility.* Flexibility results in the ability of the automatic devices to optimize the operational parameters and to work in optimum conditions for the assurance of the objectivity and quality of analytical information. Furthermore, the reliability of analytical information is assured by using reliable analytical instruments. Automation must be implemented beginning with the sampling process and ending with data processing.

*Reliability.* Because of the complexity of the sampling process, it is very difficult to make it reproducible, hence reliable, every time. Yet, this important characteristic can be assured by using automatic devices.

The determination of many compounds in food can be made only after a separation step from the matrix. For determination of aliphatic amines in food, a liquid–liquid microextraction unit[239] is applied. This kind of sampling

can be followed successfully by online monitoring of small organic plugs from which amines are extracted. The quantity of the organic solvents needed is fairly small, so the toxicity hazards are minimized. The flexibility of robotics allows its use for the extraction of ascorbic acid from food.[240]

The second step of the analytical process — the components analysis or black box — can be automated by using automatic devices. The samples automatically change the automation required for this step, decreasing the time needed for the analytical process. This has been used in radiochemical analysis for operator protection. Now, automatic devices are often used for RXF, AAS, and ESCA methods, as well as for chromatographic techniques, such as HPLC, to increase the speed of the analytical process. By using automatic titration, the reliability and quality of the analytical information increases, the objectivity increases and the time needed decreases dramatically.

FT-IR is a very sensitive technique and can be optimized by automation. It is successfully used for the determination of trichlorethylene.[241] Automatic detection of trichlorethylene by FT-IR is achieved at the instrumental limit of detection against a wide variety of IR backgrounds.

One of the most important fields in which automatic devices can be used successfully is clinical chemistry because of the complexity of the matrix, and because of the number of analyses that must be performed in a very short time. For example, an automatic device is recommended for automation of urea assay to monitor urea in hemodialysis fluids,[242] an important measure for human health. The urea analyzer consists of a flow injection system, a signal processing module, and an IBM-compatible PC. The flow system plays a major role in urea assay and can be schematically represented as shown in Figure 7.2. The quality, objectivity, and reliability of the analytical information obtained using this analyzer is good, and the analyzer has the added benefit of making it possible to assay urea without taking blood samples.

Immunoassay is a very sensitive technique that can be applied in environmental chemistry and food chemistry, and is very often used in clinical chemistry. By inclusion of immunoassay in the flow injection system, precise control of reaction times can be achieved; compatibility with any heterogeneous or homogeneous format, and detector, good accuracy, and reproducibility are assured; the analysis time decreases; and the analytical process becomes more flexible.[243]

The use of automatic flow system and sampling interfaces for solid complex matrices improves the analytical performance, particularly with respect to the overall analysis time.[244,245] To assure the best reliability of the output automation step, computers are used to ensure data processing proceeds at a good pace.

### 7.2.2 *Automated devices*

Automated devices can control and regulate a process without human intervention,[65] an attribute that causes them to be used in process control systems.

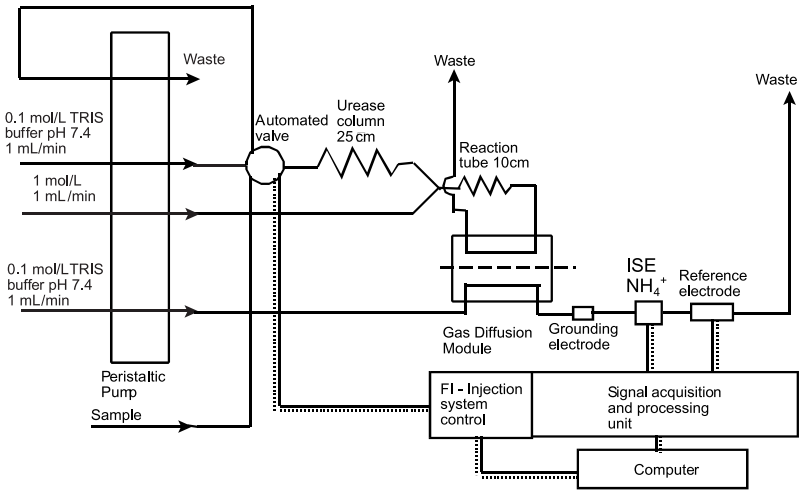



*Figure 7.2* Urea analyzer.

Automated devices are based on analytical methods with well-known parameters, which assures the best value of the analytical information. Before inserting an automated device into the process, it is necessary to know the history of the process.

A process can be defined as a sum of steps that assures obtaining a product. To obtain a quality product, by-products should be analyzed occasionally; therefore, automated devices are necessary in process control. Sometimes it is required to assay either the ratio between components or their quantity continuously, so electrometric methods based on electrochemical sensors would be adequate. However, to assure the most information in the shortest time, the computer used for data processing must contain a full data bank that is correlated with the history of the process into which the automated device is inserted.

Rapidity in analyzing samples is the most important characteristic of an automated device because the process is continuous, and the automated device must have enough time to make a decision in the case of nonobservance of composition. The analytical information obtained must be reliable. A nonreliable analytical process can modify the decision of the automated device, which results in a poor-quality product. By using standardization, the quality of the analytical information increases.

To automate all steps, it is important to use computer software tailored to conduct the analytical process, the data processing, and the optimization of the technological process automatically. The automated device for process control must only be constructed by a heterogeneous team. The team is composed of analysts, technologists, and electronic experts. The role of the team is to improve the quality and reliability of the automated device and, indirectly, of the product obtained through the technological process. The analytical method chosen for analysis of the components, as well as that

chosen for the analytical instrument, must take into account the S/N ratio. A correlation must be made to obtain the maximum value for the reliability of the analytical information. The reliability of automated devices and their speed increase the cost of products due to quality assurance.

CFA is very often used in process control. In CFA, the sampling process consists of samples flowing sequentially and continuously in a tube, where each sequentially mixes with reagents in the same tube at the same point downstream and then flows sequentially into a detector. Another automated device type is the discrete analyzer where the analysis is made by taking a batch sample at selected intervals and subjecting it to analysis. The main advantage of CFA is the objectivity assured by operator elimination at the sample pickup and sampling steps. However, when the complexity of the sample that is to be analyzed increases, CFA does not offer reliable analytical information; therefore, one should use discrete analyzers as an alternative.

*chapter eight*

# *The role of standards and standardization in reliability of analytical information*

Standards and standardization play a major role in reliability assurance. Only by using standard methods for product quality control can a product be introduced into the market. Quality standards are very important, especially for food analysis. Recommended standard (or *etalon* in French) matrices as well as methods are defined in terms of accuracy and precision.[246] The European Union has prescribed strict quality standards for laboratories and the methods of analysis to be used in laboratories when carrying out official food quality control.

*Standardization* refers to the compounds (standard compounds) and to analytical methods (standard methods). The *reference material* is defined as "material or substance one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials."[247]

*Primary standards* are recognized to have the highest metrological qualities for their specified fields of application.[248] The list of primary standards is established at the international level by the Bureau International des Poids et Mésures (BIPM), and every nation can take from this list primary standards, as well as add other primary standards, with the objective of obtaining a specific list of primary standards to suit the nation's needs.

It is very important to assure the quality of the standards themselves. The reliability of the analytical method and the reliability of the analytical process increases if standards are used. The time required for analysis is shorter, and the quality and reliability of the analytical information improves.

*Secondary standards* are defined as "standards whose value is assigned by comparison with a primary standard of the same quantity."[249] Other

terms very often used for quality determination are reference standard and certified reference material. *Reference standards* have the highest metrological quality available at a given location or in a given organization from which measurements are derived. *Certified reference materials* refers to the reference material accompanied by a certificate, one or more of whose property values are certified by a procedure that establishes traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by certainity at a stated level of confidence.

Reference materials for chemical composition are of two types: pure substance reference materials, intended to realize a specified chemical species, and composite substances reference materials, intended to realize a specified content of a specified chemical species in a specified matrix. One of the analytical fields where reference materials have great importance is clinical analysis.[250]

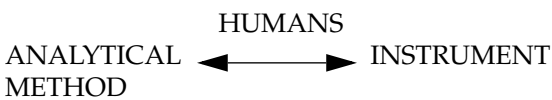
Standards are condensed from the full information about a material. The quality of a standard depends on the level of information available to the operator, and the reliability of a standard is a result of a high information level.

Standards act as a microdata bank. The maximum influence of a standard is for only one analytical method. There are many characteristics of a standard that ensure its reliability: it must be illustrative from a quality and quantity point of view; it must possess good stability, homogeneity, and flexibility; and it must have a composition similar to assay samples.

Standards are samples that are to be assayed. The correlation between the standard and the analytical standard methods is as follows:


It is necessary to correlate the standard method and the instrument via a standard to establish the best reliability:

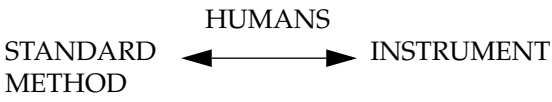

The reliability and flexibility of the standard method can be established by remembering that standards are produced by humans. The correlation between the analytical method and the instrument is also made by a human:




As always, the sample acts as a glue between the method and the instrument. However, by taking only this into account, a low reliability is obtained.

By considering that standards are also samples, which act as a glue between the standard methods and the instrument, reliability increases.

Humans choose the method and the instrument and then correlate the standard method with the instrument:




It follows that an increase in the flexibility of standards and of standard methods is desirable. Humans are the main factor that increases the flexibility of the standards and standard methods because of human creativity; thus, correlation is assured between standard methods and instruments.

A standard method is the method that assures maximum reliability by using a material characterization. The analytical methods used as standard methods must have the following characteristics: rapidity, reproducibility, flexibility, and reliability. These characteristics make a method suitable for automation by an automated device. Reliability refers to the aging of the analytical method used as a standard method (e.g., the use of ASV in some cases in place of polarography). If it is impossible to obtain a standard method for a particular material assay, it is necessary to use two or more analytical methods to certify the quality of the material. The methods used for this purpose must have similar analytical performance in terms of sensitivity, detection limit, and selectivity. Two analytical methods often used for the determination of the same material are ETAAS and ASV. In another example, in the GC/FT-IR/MS/C tandem technique, one method is a standard method for the other. The standard methods as well as the standards are simple to use only for simple samples. However, increasing the complexity of the matrix makes it difficult to use a standard method for material assay. Rapidity results from the use of fast instruments that assure a high speed of analysis. The signal-to-noise ratio can be increased by the instrument used. The digital system contributes to a decrease of noise. Because the analytical process plays an important role in components assay, the connection between standardization and the analytical process is very important. The analytical process increases the quality of quality control, and it is inserted in quality control. Account must be taken of the performance of the analytical process: flexibility, reliability, and quality.

The analytical process can be standardized if the analytical process also has good sensitivity, selectivity, low cost, is the best for the purpose selected, assures the reliability and accuracy of the analytical information, and has the ability to be used for other compositions. Sensitivity must consider the level of compounds in the matrix, and selectivity must be correlated with the complexity of the matrix. This occurs at a certain level for standardization of the analytical process. Above this level of complexity it is not possible to standardize.

One can ask, what is the best analytical process for quality control? The best analytical process is one that is perfectly adapted to the materials that must be assured. The aging of the method proposed must be taken into account.

The analytical process must assure the best quality of the analytical information, even when the ratio between components is not constant. The property can be translated through flexibility, so it must be flexible at the variable composition. By using standards and standard methods for quality control, the accuracy of the analytical information is assured.

The selectivity, sensitivity, and reproducibility of the standard method assure the main characteristic of analytical information for quality control: reliability. The reliability of the analytical information has been discussed in a number of books[251,252] on quality assurance and accreditation. The accreditation of the laboratory requires as a first step the utilization of standards and standard methods for quality control, indicating the importance of standards for this purpose. The standards, as well as the standard methods, must be more reliable. Only by using more reliable standards and standard methods it is possible to obtain reliable analytical information.

*chapter nine*

# *Sensitivity and selectivity in chemical analysis*

The quality of an analytical method is judged by its accuracy. A high accuracy assures reliability for the chemical analysis; and accuracy is directly dependent on the selectivity of the analytical method. The presence of interferences in a solution is a source of systematic errors.

The selectivity of an analytical method can be improved by modification of medium conditions for the measurement. A very important way to increase the selectivity is by masking the interfering ions. The most often utilized masking reagents are oxalate, sulfate, phosphate, and EDTA. The main characteristics of compounds resulting after the masking process are

1. They must be more stable than the compound formed by the masking agent with the analyte.
2. They must be more stable than the compound formed by the same ion with the reagent used in the proposed analytical method.
3. If the analytical method is a spectrometric one, it must not have any color.

Another way to improve the selectivity of an analytical method is by the selective extraction of the analyte from the matrix followed by its determination.

The precision of the method is a measure of its sensitivity. The most sensitive methods result in high precision. This property is very important for the quality and reliability of the analytical information. The high sensitivity property represents the main requirement for an analytical method to be used in trace analysis. For example, if a sample contains an ion at $10^{-8}$ mol/l concentration level, and it is therefore necessary to select between the electrometric methods of analysis, an amperometric technique is applied for its determination. The potentiometric methods cannot be applied in this case since their limit of detection is not less than $10^{-6}$ mol/l.

The interrelation between sensitivity and selectivity in chemical analysis plays a very important role for quality assurance, and furthermore in the reliability of the analytical information. This interrelation must be related to the complexity of the matrix from which the analyte must be determined. A good correlation must also be realized between the selectivity of the sampling and the selectivity of the analytical method, and between the sensitivity of the sampling and the sensitivity of the analytical method.

Currently, chemometrics favors treatment of analytical data in a selective way.[253-255] That is the reason nonselectivity is a problem for some analytical methods. A good example in this regard is given by the data processing for sensor arrays.[256] As of today, no *specific* analytical method has been found. In the past, it was believed that the fluoride-selective membrane electrode was specific, but recently it was found that it contains an interferent (hydroxyl ions); thus it is considered selective, not specific. The reason these sensors are not specific is that no specific reagent has been found, and their potential development is a result of the interface reaction. Enzymes can assure a group selectivity, but rarely group specificity.

Specificity is a property of monocomponent systems and it occurs when the method is free of interference. Selectivity is related to the complexity of the matrix and it occurs when not more than one ion (molecule) interferes in determination. *Enantioselectivity* is a relatively new term introduced for the assay of enantiomers.[257,258] An analytical method is enantioselective when it can discriminate between enantiomers. Enantiospecificity is an extreme case of enantioselectivity. It is possible to create the conditions for a highly enantioselective analysis, and in this case enantiospecificity can also occur. For example, a maltodextrin with dextrose equivalence (DE) of 4.0 to 7.0 was used in capillary zone electrophoresis as a stationary phase for the separation of the enantiomers,[259] and also in the design of a potentiometric, enantioselective membrane electrode.[260] The method for capillary zone electrophoresis is enantioselective, as it is for the potentiometric method.

Enantiospecificity occurs when the analytical method can determine only one of the enantiomers. In this regard, biosensors that used L or D type enzymes, e.g., L-amino acid oxidase or (L-AAOD, D-amino acid oxidase or D-AAOD), can be considered enantiospecific for classes of L or D enantiomers because the enzyme will be able to catalyze the reaction of only one particular enantiomer.

The chromatographic techniques are considered the most selective separation modalities. Their selectivity can be characterized through the separation factor.

The selectivity, enantioselectivity, and sensitivity of a method can be improved by using it as detection system in flow injection analysis and/or sequential injection analysis systems.[261]

## 9.1 Sensitivity vs. selectivity

By increasing the number of functional groups for a reagent, its sensitivity is increased, but the selectivity decreases due to the favoring of the reaction for many ions with the proposed reagent. Accordingly, the most sensitive reactions are not be the most selective ones. Most modern analytical methods are based on a chemical reaction between the analyte and the reagent. Therefore, the most sensitive methods are not the most selective ones (e.g., spectrometric methods of analysis are based on a chemical reaction; with increasing sensitivity of the reagent, the selectivity becomes very low).

For ion-selective membrane electrodes, the potential development is directly correlated with the stability of the compound formed at the membrane–solution interface.[262] Therefore, the selectivity of the electrodes is correlated with the stability of the compounds formed by the analyte and interference at the interface. Actually, the difference between their stabilities determines the selectivity of the electrodes.[263] The piezoelectric sensors are known as the most sensitive class of sensors, but their selectivity is very low.

The principle for biosensors and immunosensors is based on the biochemical reaction produced at the surface of the electrode followed by the detection of one of the products formed in the biochemical reaction by using a certain transducer. In this case, the selectivity of the biochemical reaction must be correlated with the sensitivity of the transducer.[264] The best correlation is accomplished when amperometric transducers are utilized. The potentiometric transducers in most cases are not suitable for biosensor design. For the design of immunosensors, the potentiometric transducer is not always able to measure the product obtained in the immunological reaction because it is known that the reaction between the antigen and the antibody is very sensitive. In certain cases the selectivity of transducer vs. different products of the biochemical reaction must also be considered. Because of the high sensitivity of the piezoelectric transducers, their selectivity is limited, and they cannot assure the best results when used in biosensor or immunosensor design.

Fluorescence and chemiluminescence sensors are considered the most sensitive in the class of optical sensors. They have a higher selectivity because the chemiluminescent and fluorescent reactions take place in certain medium conditions, and only a limited group of ions and molecules can be involved. When the selectivity of this type of sensor is not sufficient, the quality of analysis can be improved by using a biochemical reaction such as an enzymatic reaction (chemiluminescence- or fluorescence-based biosensors) or an immunoreaction (chemiluminescence- or fluorescence-based immunosensors). By using these types of optical sensors the chemical analysis becomes most sensitive and selective.[265]

It is well known that for assay of metals at low concentration levels, it is not easy to assure good selectivity of measurement. There are three analytical methods that can solve this problem: an electrochemical method, anodic stripping voltammetry (ASV); and two spectrometric methods, atomic absorption spectrometry (AAS);[266] and inductively coupled plasma (ICP).[267] The selectivity of ASV is related to the selectivity of the redox electrode utilized, and for the spectrometric techniques the selectivity is related to the wavelength where the elements are absorbed or emitted as light. This means that the selectivity of the measurement is given for the electrochemical method by the difference between redox potentials of the elements or ions from the solutions, whereas for the spectrometric methods it is given by the difference between the wavelength of absorption and emission.

The sensitivity of the spectrometric methods are given by the composition of the flame and plasma.[268] By coupling ICP with a mass spectrometer (MS) detector,[269] the selectivity and sensitivity of the optical measurement is increased considerably. For ASV, the low level of concentration is not a problem because of the steps followed by the technique: the first step is a concentration of the analyte on the surface of the electrode, and the second step is given by the redox reaction. As a result of the concentration step, the method can be used for very low concentration levels with high selectivity.

The best equilibrium between sensitivity and selectivity for a chemical analysis must always be found. Usually, a medium sensitivity is preferred for a good selectivity.

## 9.2 *Sensitivity, selectivity, and complexity of the matrix*

It has been proved that the selectivity of an analytical method is directly connected to the complexity of the matrix from which the analyte must be determined. As a result, the same method can be more selective or less selective, depending on the qualitative and quantitative composition of the matrix from which the analyte must be determined. For example, two types of electrochemical sensors are described for the assay of thyroid hormones: L-$T_3$ and L-$T_4$. The first is an amperometric biosensor based on L-amino acid oxidase (L-AAOD),[270] whereas the other is an amperometric immunosensor based on anti-L-$T_3$ and anti-L-T4.[271] If $T_3$ and $T_4$ have to be determined from phramaceutical products, both types of sensors have the necessary sensitivity and selectivity. When it is required to determine both hormones in biological fluids or in thyroid tissue, the proposed biosensors are not selective enough because L-AAOD catalyzes the reactions of both thyroid hormones. The amperometric biosensors can only make the discrimination between the two thyroid hormones, namely, L-$T_3$ and L-$T_4$, since the specific antibody reacts only with the specific antigen.

In chemical analysis, the complexity of the matrix decreases in the following order: environmental analysis, food analysis, and clinical analysis ("quality" of the body and quality of the pharmaceutical compounds). This

means that for environmental analysis, the use of the most selective methods is necessary. When it is impossible to find a method that can be selective enough for the environmental analysis, a selective separation technique must be employed. For example, for environmental analysis, only the utilization of tandem techniques achieves both selectivity and sensitivity.

Another example connected with the sensitivity, selectivity, and complexity of the matrix is illustrated by the utilization of amperometric biosensors in chemical analysis. It is well known that amperometric biosensors represent the best equilibrium between selectivity and sensitivity needed for an analytical method. Their selectivity can be highly variable in a very complex matrix such as the environment. By using amperometric sensors, the total amount of substances from a certain class are determined. That is the reason these amperometric biosensors cannot assure the accuracy of the analytical methods for analysis of analytes in complex matrices. In food analysis, the complexity of the matrix decreases considerably. Therefore, amperometric biosensors can be used with higher accuracy for the assay of certain compounds. The main field of applicability of amperometric biosensors is clinical analysis, since the matrices in clinical analyses assure for amperometric biosensors the maximum selectivity.

Ion-selective membrane electrodes are also not selective enough for complex matrices, but their selectivity can be improved by the utilization of high selective ligands and certain matrices for electrode membranes in electrode design,[272] or by changing the working conditions in the bulk solution. Surface analysis is not selective enough when is applied to very complex matrices. The best accuracy is obtained only for the simplest matrices. For this type of matrix the high selective and medium sensitive analytical methods must be considered. To achieve highly sensitive and selective analytical methods, especially for the analysis of analytes in complex matrices, a selective separation technique is required prior to performing the analytical method.

## 9.3 *Correlation between sensitivity, selectivity and sampling, and the black box in an analytical process*

To obtain reliable analytical information, the selectivity and sensitivity of the sampling must be correlated with the selectivity and sensitivity of the analytical method used in the black box. The sampling process plays a very important role in the quality and reliability of the analytical information because it is the result of the sample that is ready for measurement. If the sample is not reliable, the analytical information obtained will not be reliable.

The dissolution process can introduce interferences, especially if the matrix cannot be dissolved directly in water. To select the quality of the solvents and reagents used in a step of an analytical process, it is essential to look for the sensitivity and selectivity of all the steps that follow (e.g., if a sample must follow a separation step using a chromatographic technique, solvents and reagents of high chromatographic purity must be used). The

method that confers the best sensitivity and selectivity in sample dissolution is microwave digestion. A blank is utilized for this type of dissolution, and it is also measured by using the same analytical method as for the analyte, just to be able to find the real value of the analytical information.

The next step in the sampling process is the separation of the analytes. The most often utilized methods in this step are extraction and chromatography. There are only few selective extraction techniques because the selectivity of the reagents is low. Synergetic extraction gives a higher sensitivity for this step of analytical process. The chromatographic techniques are the best separation techniques, but, usually, they also depend on the selectivity and sensitivity of the extractions because extraction is a presampling techniques necessary in most analytical determinations that use chromatographic techniques for separation of the analyte(s). For a reagent, the conditions to be used in the derivatization process are as follows:

1. It must be selective for the main analyte,
2. The sensitivity of the reaction must be in concordance with the level of its concentration in the solution,
3. The compound formed must be easily separated through the selected chromatographic technique (or analyzed if the derivatization is post-column).

The selection of the type of chromatographic technique must take into account the selectivity and sensitivity of the detection system.[273-276] Universal detectors (e.g., ionization, UV detectors) are recommended when the analyst wants to know the total composition of a sample, and, alternatively, selective detectors (e.g., fluorescence, electrochemical, electron-capture, thermoionic specific detector, or nitrogen–phosphorus) are used in a particular trace analysis. The most selective detector is mass spectrometer (single-ion monitoring or selected-ion monitoring), which can operate in both the positive and the negative modes. Their sensitivity is in concordance with that given by the chromatographic technique. The utilization of the diode array as detector in capillary zone electrophoresis increases the selectivity of the measurement, but most of the time the sensitivity is not in good concordance with the one assured by the capillary zone electrophoresis technique. A good separation allows the possibility of using a most sensitive analytical method as a detector system for chromatography.

To be able to separate trace compounds, the stability of the compounds formed between the analyte and stationary phase must have the following properties: (1) they must be relatively high but low enough to be drawn by the mobile phase; (2) the differences between the stability of the compounds must be large enough to allow separation of the analytes.

Another often-used separation technique is membrane separation. The selectivity of this type of separation is good. The sensitivity is low, and also the recovery of the analyte low, less than 90. For this separation technique, the best results were recorded when liquid membranes were used.[277]

## 9.4 Enantioselectivity

Enantioselectivity was introduced especially for use in analysis of pharmaceuticals, where it was found that some pharmaceutical products have a chiral center and only one of the enantiomers exhibits the required pharmacological and pharmacokinetic behavior. The term was introduced first in relation to separation techniques,[278-281] and later sensor technology.[282]

The most sensitive enantioselective separation technique is capillary zone electrophoresis. Here, the detectors utilized are not sensitive enough to be able to detect the enantiomers. In the case of sensors, amperometric biosensors have been found to be most sensitive.[264] A better enantioselectivity was found for potentiometric, enantioselective membrane electrodes because a direct interaction between the chiral selector and enantiomer occurred.[282-285]

Immunoreactions have proved to exhibit good enantioselectivity. They can yield both a sensitivity higher these biosensors and an enantioselectivity higher than potentiometric, enantioselective membrane electrodes.

## 9.5 The role of flow systems in increasing the selectivity and sensitivity of an analytical method

Flow injection analysis and sequential injection analysis are utilized to improve the objectivity and rapidity of most reliable analytical methods.[286-290] The main conditions for use of analytical methods in flow systems are good sensitivity and good selectivity. Using flow systems, the limit of detection decreases and the selectivity and sensitivity increase.[290] Modification of the selectivity of method is a result of nonequilibrium conditions of measurement. The flow parameters can be manipulated in such a way that the selectivity increases to maximum value.[289] In this regard, improving the selectivity of ion-selective membrane electrodes is of high importance.[290-292] For example, the best selectivity of a calcium-selective membrane electrode (Orion type) was achieved by using it in a flow injection analysis system. It was possible also to use it reliably for assay of calcium in water samples (natural and borehole water). Even though the sensitivity of a method is decreased by using a detection system in flow analysis,[293] the best sensitivity and selectivity are achieved when sequential injection analysis is utilized. This technique has made possible the simultaneous determination of enantiomers with high sensitivity and selectivity.[293,295]

# chapter ten

# Uncertainty in chemical analysis

*Uncertainty* is a key topic of metrology in chemistry.[296] The term *chemical metrology* is discouraged since metrology operates on the same principles, almost independently of the field of application. The other terms used in describing quality of measurement, e.g., comparability, traceability, validation, can be defined in terms of uncertainty as the basic characteristic.

The importance of estimation of uncertainty in chemical analysis is due to its direct relationship with the quality of the analytical information, and with the reliability of the measurement. Taking into account the definitions given in a previous chapter of this book for the reliability of the analytical information — as a function of the reliabilities or uncertainties of different steps of the analytical process — it follows that the reliability of the analytical information ($R_{AI}$) is

$$R_{AI} = f(U_s, U_M, U_I, U_{DP})$$

where $U_s$ is the uncertainty of sampling process, $U_M$ is the uncertainty of the method, $U_I$ is the uncertainty of the instrument, and $U_{DP}$ is the uncertainty of data processing.

Comparing this relationship with the one given for reliability in Chapter 2 of this book, it is easy to see that reliabilities from the previous formula have been substituted with uncertainties. To increase the value of the reliability of the analytical information, it is necessary either to increase the reliability of each step of the analytical process or to decrease the values of uncertainties of each step of the analytical process. From a practical point of view, it is easier to work to decrease the values of uncertainties than it is to increase the values of reliabilities.

It has been demonstrated several times that the uncertainty arising from sampling is often large, indeed, much larger than that arising from method, instrument, and data processing.[297] The value of uncertainty in the sampling

process increases with the complexity of the matrix; therefore, special care must be taken in environmental analysis where the matrix has the maximum complexity, as discussed in previous chapters.

The uncertainty related to the method can be minimized by selecting the most sensitive and most selective method and by adapting it to the analysis requirements. One must keep in mind that there is no one analytical method that is good for each type of sample (analyte and matrix). In this regard, as always, the sample must be seen as the glue between method and instrument. The instrument must also be adapted to the method requirements, especially sensitivity. For the instrument to have the minimum value of uncertainty, it is necessary either to maximize the signal/noise ratio or to minimize the value of the noise. By using fully automatized instruments the uncertainty of the measurement decreases considerably.

Data processing is highly important in minimizing the uncertainty by virtue of its capacity for data comparison, elimination of interferences, and objectivity. Of course, all mathematical calculations have a certain level of uncertainty, and all approximations of values found create an uncertainty, but by using high-quality computers and programs these types of uncertainties can be easily minimized.

## *10.1 Estimation of uncertainties*

For estimation of uncertainty, all relevant sources must be taken into account.[298] Mathematical models proposed by different guides for uncertainty estimation are very complex, and sometimes not sufficiently relevant.

Knowledge concerning the history of a sample is the prime factor in estimations of uncertainty. According to GUM,[299] estimation of uncertainties is based on the identification and quantification of the effect of influence parameters, and requires an understanding of the measurement process, the factors influencing the result, and the uncertainties associated with those factors. Uncertainty can be estimated at different levels constituting global uncertainty, which is becoming a valuable concept.[300] Operations such as separation, clean-up, and homogenization are important in determining overall uncertainty.[301]

The choice of reference materials (RM) is a very important way to decrease uncertainty because RMs can be used to provide information on method performance, and because their certified uncertainties contribute to overall uncertainty.[301] However, it is often difficult to obtain an RM for environmental and clinical analysis. The calibration of instruments also plays an important role in decreasing uncertainty; the utilization of standards for calibration can reduce the uncertainty of analytical information.[302]

## 10.2 *The role of history of the sample in estimations of uncertainty*

The correct knowledge concerning the history of the sample is the only factor that assures the best selection of the method and instrument for analysis that will achieve a minimum value for uncertainty. In clinical and environmental analysis, knowledge concerning the sample plays a vital role in establishing the correct procedure for the analytical process, especially because each sample is unique. In food analysis, knowledge about the technological process in the food industry or the food source is very important in establishing the best analytical process and in decreasing uncertainty.

The history of the sample in environmental analysis provides an indication of the chemistry of the atmosphere, water, or soil that can greatly influence the composition of the sample, as well as the status of the analyte(s) to be determined. Of main importance for estimations of uncertainty is knowledge of the exact time, place, method of sample pickup, and the material of the container used for the sample.

To establish the "quality" of the human body, it is necessary to have correct information concerning its environment, and the food and pharmaceutical products administrated within less than 24 hours. With this knowledge, the medical practitioner will be able to request and establish the best correlation between analytical results and illness; also, the analyst can select the best method, free of interferences. The sample collection is of major importance in this case, especially when an immunological method is applied. It is well known that the human body is able to synthesize antibodies during the same period of time as sample pickup. It is possible for these antibodies to act as interfering species in analysis and to increase the uncertainty. As a result, an increased value of uncertainty may be recorded when a tourniquet is put on different places around the arm for blood sample collection. It is also important to know the exact time of day a sample has been taken.

Uncertainty in clinical analysis can be decreased by sterilizing the instruments, as well as by choosing the most biocompatible materials for their construction. Knowledge concerning the procedures used for sample pickup, the container used for the sample, and the time elapsed since sample pickup is always necessary to adopt the best method for sample analysis.

To establish the quality of pharmaceutical products by minimization of uncertainty, knowledge concerning the raw materials used in technological processes, by-products, possible degradation products, and metabolites are necessary. Also, when the uniformity content test must be performed, knowledge concerning the compression compounds is necessary. In this case, the uncertainty value will be smaller if a method free of interferences and with certain sensitivity is selected.

## 10.3 Estimation of uncertainty of different methods of analysis

To select the best analytical method for the analysis of a sample it is necessary to consider the complexity of the matrix, because the complexity affects the selectivity of the measurement and the limits of detection and sensitivities of the methods. Only by adapting the selectivity and sensitivity of the method to the matrix and analyte concentration can the value of uncertainty be as a minimum. In this regard, some methods have been found more suitable for a particular field of analysis (e.g., environmental analysis, food analysis, clinical analysis) than for another. The high complexity of some matrices make analysis difficult, if not impossible, without a separation step. One must keep in mind that each step in the analytical process introduces an uncertainty. It follows that the greater the number of steps in an analytical process, the greater the total value of uncertainty.

The methods that involve the minimum number of steps, with all the steps characterized by the minimum uncertainty, are the electrometrical methods. However, despite the high value of uncertainty obtained by using UV/Vis spectrometric methods, they are preferred over electrometrical methods in most cases.

### 10.3.1 Estimation of uncertainty of the spectrometric methods

The analysis of inorganic cations at trace and ultratrace levels in the environment, food, and biological fluids and drugs requires highly sensitive and selective methods. A high value for uncertainty was obtained by utilization of AAS for Pb and Mn assay[303,304] because of the low values of analyte concentration. By using ICP-MS for trace elements assay in liver and kidney, an uncertainty value is obtained in the range of uncertainty of certified values.[305,306] Thus, ICP-MS is more suitable for clinical analysis than AAS.

For many inorganic ions, the UV/Vis techniques are still used for determination of inorganic and organic ions.[306] Because of the interferences of the matrix components, the analytes must be separated from the matrix using an extraction or a chromatographic technique, processes well known for high levels of uncertainty.[307] These processes cause the sampling process to have the highest level of uncertainty of all analytical process. The next step is ion transformation in a certain product able to be detected by the spectrometer. This is also a step with a high level of uncertainty because the transformation of the ion into such a product is not complete (100%).

Chromatographic methods also introduce a high level of uncertainty in qualitative and quantitative analysis, especially when low concentration levels are involved (e.g., CGC used for nanoliter samples of inorganic ions assay, with a limit of detection on the order of $10^{-9}$ mol/l, has an RSD larger than 5.0% [308]). Because of the impossibility of having an ideal standard or RM for the analysis of most samples, the uncertainty of the qualitative analysis or peaks identification is high in chromatography.[309]

The best results concerning the separation techniques are achieved by using capillary electrophoresis;[310] in this case, the problem is the sensitivity of the detecting system, which is unconnected with the sensitivity of the separation technique.

The utilization of the NIR technique in clinical analysis has made only qualitative diagnoses with "positive" or "negative" qualifications possible. This demonstrates, once again, the level of uncertainty obtained when a structural analysis technique is used alone. To minimize the uncertainty value, complementary structural techniques, MS and NMR,[311] are proposed in clinical analysis. NMR can be used alone for diagnosis in different variants, in clinical analysis. MS is usually used coupled with GC as a detection system. The uncertainty of GC-MS coupling is attributed mostly to the separation technique,[312] and not to the analysis itself.

### *10.3.2 Estimation of uncertainty of electrochemical methods*

For trace analysis of cations, the technique that introduces the minimum value of uncertainty is anodic stripping voltammetry. The reason is that the concentration and determination steps take place on the same electrode. The lower value of uncertainty is also a function of the use in the technique of a fully computerized instrument. In this case, the computerization of the instrument is of prime importance for decreasing the uncertainty because it assures a high reproducibility of the parameter characteristics for anodic stripping voltammetry.

The minimum uncertainty for simple matrices is achieved with use of electrochemical sensors, especially for the assay of organic cations and organic and inorganic anions in food and clinical analysis. The selectivity and sensitivity of these sensors are adequate to detect numerous pharmaceutical products, without any prior separation. The ability of electrochemical sensors to determine continuously the activity of an ion in solution has made their use possible in *in vitro* and *in vivo* dissolution tests of drugs.

For the analysis of ions in biological fluids, the uncertainty of sampling is related only to the sample pickup and transport to the laboratory, resulting in a minimum possible value for uncertainty. For pharmaceutical products the sampling process is reduced to their dissolution and/or dilution in water. To improve the selectivity as well as the sensitivity of the determinations with the aim of minimizing the uncertainty value, four categories of electrochemical sensors are used in clinical analysis: ion-selective membrane electrodes (ISME), biosensors, immunosensors, and enantioselective sensors. The fourth category, enantioselective sensors, includes amperometric biosensors and potentiometric, enantioselective membrane electrodes which are used for the assay of enantiomers and the enantiomeric purity of chiral drugs.

The best sensitivity and selectivity is assured by immunosensors when an amperometric device is selected as transducer. With the utilization of a piezoelectric device as a transducer, the uncertainty value increases because

of the high sensitivity of the transducer. Concerning the utilization of the transducer for biosensor construction, the utilization of a potentiometric transducer is not a good solution because the sensitivity is comparable with that obtained by using an ISME; thus, the best transducer is amperometric.

ISME, because of their high selectivity over by-products, degradation products, and metabolites, can be successfully used in the assay of pharmaceutical products.[313] The uncertainty values are at a minimum for this use. For clinical analysis they also assure the best results, especially in anions and organic cations assay (e.g., utilization of oxalate-selective membrane electrode for its assay in urine[314]).

Furthermore, there are currently biosensors for glucose on the market that can be successfuly used for control of glucose levels by individuals. Validation of this type of analysis is a result, once again, of the excellent uncertainty value provided by electrochemical sensors.

Since in most cases only one enantiomer possesses a desired pharmacological activity, it is necessary to construct enantioselective sensors to improve the quality of analysis due to the high uncertainty obtained in chiral separation by chromatographic techniques.[315] For this purpose, enantioselective amperometric biosensors and potentiometric, enantioselective membrane electrodes have been proposed.[264] The selection of one sensor from among the electrochemical sensor categories for clinical analysis depends on the complexity of the matrix because the complexity of different biological fluids is not the same. For example, for the determination of $T_3$ and $T_4$ thyroid hormones an amperometric biosensor and two immunosensors have been proposed. The immunosensors are more suitable (uncertainty has the minimum value) for direct determination of $T_3$ and $T_4$ thyroid hormones in thyroid than are amperometric biosensors. For the analysis of the same hormones in pharmaceutical products, the uncertainty values are comparable.

Miniaturization and utilization of biocompatible materials for construction have allowed the electrochemical sensors to be successfully used for *in vivo* measurements. Usually, they are arranged as sensor detection systems in an array.[316] Because spectrometric methods cannot be reliably applied for *in vivo* measurements, their uncertainty cannot be compared with that obtained by *in vivo* measurements using electrochemical sensors. The main problems for *in vivo* measurements are the sterilization of sensors, dimension of sensors (usually cannot exceed a nanometer magnitude order), and their geometric configuration. The calibration of sensors for *in vivo* measurements is also a problem because high-quality standards are necessary.

Sample contamination in *in vitro* analysis, as well as the period elapsed since sample pickup, increase the value of uncertainty. To assure the best reliability of analysis for *in vivo* assay, it is necessary to consider all the conditions for this type of measurement, especially in an emergency where the necessity to develop highly reliable electrochemical sensors for this purpose is a priority.

To perform *in vitro* as well *in vivo* dissolution tests of drugs with a minimum uncertainty, it is necessary to begin by validating electrochemical sensors and to include them in the standards methods of pharmacopoeias. The uncertainty values are smaller for *in vivo* measurements than for *in vitro* methods because the sampling step is totally eliminated.

### *10.3.3 Estimation of uncertainty of immunoassay techniques*

Immunoassay techniques are known for their high selectivity and sensitivity. Radioimmunoassay (RIA) as well as enzyme-linked immunosorbent assay (ELISA) are often used in clinical analysis. However, to use these techniques it is necessary that more attention should be given to sample pickup due to the possibility of producing an antigen-antibody reaction that can act as an interferent for the analyte that is to be assayed. When an immunoassay is used in clinical analysis, the utilization of laser techniques makes sample collection possible without any adverse reaction from the body.[317]

It was demonstrated that for HIV assay, an immunoassay technique assures the minimum level of uncertainty.[318] Immunoassay techniques have become popular also for assay of pharmaceutical products because of their higher selectivity.

### *10.3.4 Estimation of uncertainty of radiometric methods*

Radiometric techniques, among the most sensitive analytical techniques, are very well known in clinical analysis and diagnosis. For sodium assay a neutron activation analysis (NAA)[319] is proposed in the place of a spectrometric method. This reduces the uncertainty value considerably because of the high selectivity and sensitivity of NAA.

## *10.4 Estimation of uncertainty of data processing*

The utilization of computers for data processing minimizes the value of uncertainty for this step of the analytical process. The computer should contain a specific program required for the type of analysis that is done. For data processing, certain spreadsheet programs[320] that assure a minimum value of uncertainty in data processing have been reported.

## *10.5 Minimization of uncertainty by using flow systems*

It is well known that a flow system and automatization are not possible for a nonreliable system. The only problem with reliable methods of analysis is objectivity. Objectivity also depends on the correctness of the system operator. To eliminate the subjectivity of the system operator and to increase the objectivity, it is necessary to limit the involvement of the system operator to

a minimum. This can be realized by automatization of the method using flow systems. If one analyzes the two main methods used today in flow systems, flow injection analysis and sequential injection analysis, is easy to conclude that the minimum uncertainty results from sequential injection analysis.

Another field where the utilization of flow system decreases both the uncertainty and the degree of contamination by the operator is the analysis of radionucleids using bead injection.[321] The proposed method has a low level of uncertainty, high objectivity, and low contamination from both system operator and sample.

*chapter eleven*

# *Validation criteria for an analytical method*

To validate an analytical method, it is necessary to take into account the quality, reliability, selectivity, and sensitivity of the method, and also its suitability for the particular types of samples. It can happen that the same method may be validated for clinical analysis, but not for environmental analysis. Always one must keep in mind that the method validation is related to the type and complexity of the matrix. Each method must be validated in part for the determination of a specific analyte in a specific type of matrix.

Furthermore, the method must be tested by several laboratories to verify that it meets criteria for validation that were previously established.[322] The utilization of standard solutions and samples is a very important condition to accept the quality of the analytical information obtained and to validate the method. Comparison between the quality of the analytical information and the uncertainty of the proposed method with the quality and uncertainty of a standard method used for the same type of analyte from the the same type of matrix is necessary. Only a statistical evaluation of data obtained from both methods (standard and proposed methods) can conclude if the qualities obtained for the analytical information are in concordance with each other, and if the method can be validated from this point of view.

There are only a few general criteria of validation that can be given for an analytical method. Each method has some specific criteria for validation. The general criteria must be related to both method and sample (especially concerning the type of matrix and provenance). The criteria referring to the method are related to selectivity, sensitivity, uncertainty, and limit of detection.

## *11.1 Selectivity*

To be validated, the selectivity of the method must be measured using a mixed solutions method because this method reflects real-world conditions for sample measurement. The separate solutions method gives only the

behavior of each of the components: analyte and interferences. The comparison between the analytical information obtained for both analyte and interferent can be treated as a preselectivity measurement. Its value cannot be taken into account for the validation of any of the methods.

The selectivity assures the accuracy of the determination and the quality of the analysis. Nonselectivity will have uncertainty as a result. The selectivity of each analytical method is dependent on the ratio between analyte and interferent. It follows that the composition of the sample represents a criterion of validation regarding the selectivity of the method.

For UV/Vis spectrometry, selectivity is determined in relation to the ratio between the concentrations of the analyte and the interferent. Usually, the minimum value of this ratio is determined, and it is known that the method will be selective for every ratio higher than this ratio. The main problem for UV/Vis spectrometry is the nonselectivity of the reagents. The selectivity of these methods can be improved by utilizing different types of buffers of different pH. Selectivity is also based on whether sensitivity of the reaction between the interferent and reagent is higher or lower. If the concentration of the interferent is below the detection limit of the method based on the utilization of the same reagent, it will not influence the reaction of the analyte. In this regard, the polyfunctional reagents (e.g., Arsenazo III) are not selective enough for assay of trace quantities of an analyte in complex matrices because their sensitivity over numerous cations is very high. The selectivity of the spectrometric method can be improved by performing a separation step before analysis.

For atomic absorption spectrometry, the selectivity refers to the reaction that takes place in the flame. Every element absorbs at a specific wavelength. Interference can be the result of anions from the matrix, which can produce a flame background or which can just mask the ions of interest. At a certain ratio, the influence of the anions is not significant. Furthermore, the influence depends on the composition of the matrix.[323-325]

Mass spectrometry is characterized by a high degree of selectivity. However, it is necessary to use a separation method prior to MS to decrease the uncertainty of measurement to a minimum.

For polarographic methods of analysis, the difference between the redox potentials gives the selectivity of the measurement. Because the redox potentials are constant values that characterize an element valuable for certain conditions (certain redox equilibria), it should be noted that the validation of such a method is strongly related to the composition of the sample.

Ion-selective membrane electrodes have as a main characteristic their selectivity. They are constructed to be utilized to determine an analyte directly in the solution without any prior separation from the matrix. This is achieved assuming a high selectivity of the electrode vs. the possible interfering ions. The selectivity is characterized through the potentiometric selectivity coefficient. The values of the coefficients that can be taken into account for validation are those obtained through the mixed solutions method at a ratio between analyte and interferent of 1:10. The method is

considered selective if the value of coefficients is less than $10^{-4}$, low selective for a value around $10^{-3}$, and nonselective for a value higher than $10^{-3}$. For a certain type of matrix, only the electrodes that yield a potentiometric selectivity coefficient less than $10^{-3}$ will be considered for validation.

Amperometric selectivity coefficients are reported for the validation of amperometric sensors. The ratio between the analyte and interferent must be 1:10, and the mixed solution must be used in the determination of these selectivity coefficients. Only under these conditions will the results of the selectivity test be validated, and only then will the method attain the status of validated or not validated.

Immunoassay is based on the reaction between an antigen and an antibody. Recently it was found that the reaction between antigen and antibody is not specific, but has only a high selectivity.[326] In the environment, antibodies are not able to select between components of the same class of organic compounds. The most problematic analysis is the one where a sensor is utilized for *in vivo* analysis. Immediately after the insertion of the sensor into the body, an instantaneous immunoreaction takes place and the compound and/or the antibody formed will be a possible interferent of the electrode used for *in vivo* analysis. To be validated for this type of analysis, sensors must have certain geometries and certain sizes because these are the factors that minimize the immunoreaction.

Playing a special role in increasing of the selectivity of the method is the application of the tandem techniques.[327,328] For separation/analysis-coupled techniques to be validated, the selectivity of the separation method must be correlated with the selectivity of the analytical method used for the detection of the analyte(s).

## *11.2 Sensitivity*

The sensitivity of the method as a validation criterion must take into account the status of the analyte in the matrix — if it is a minor or a major component, if it is present in a trace amount, etc. Sometimes it is not possible to use the most sensitive methods such as ICP or AAS for assay of the cations that are the major components in the matrix because the utilization of these methods would result in a high uncertainty value for the analytical information. It follows also that this criterion is related to both the method and the analyte of interest.

A high sensitivity of the method creates low selectivity. The interrelationship between selectivity and sensitivity must be a strong consideration in the validation of an analytical method. As was shown in Chapter 9, the best quality and reliability for analytical information is obtained only when the sensitivity is at a medium level and the selectivity is high.

For trace analysis it is necessary to use the most sensitive methods. If the selectivity of the most sensitive method is not great enough it is necessary to validate using tandem techniques, which entail a separation step before the analysis itself.

Concerning sensitivity, of high importance is the validation of biosensors and immunosensors. These types of sensors are based on the immobilization of a biological organism (enzyme, antibody, cell, etc.) on a transducer. The first reaction that takes place is the biochemical reaction, which is very sensitive. One of the products that is obtained in the reaction is measured by the transducer. The principle of the measurement using these types of sensors requires a high sensitivity of the transducer vs. the compound that forms in the biological reaction. Usually, the compound formed in this reaction is at a micromol per liter concentration magnitude. To be able to measure the compound with high reliability, the transducer must be amperometric (if an electrochemical sensor is proposed) or fluorescent or chemiluminescent (if an optical sensor is proposed). Because these three types of transducer can offer the maximum quality and reliability of the measurement of the product resulting from the biochemical reaction, only biosensors and immunosensors based on these transducers can be validated.

It is also not a good idea to use an ion-selective membrane electrode to detect thyroid hormone in pharmaceutical compounds or in the thyroid because the concentration of the hormone in both matrices is very low (ng/l). Amperometric immunosensors are recommended for assay of thyroid hormones; the concentration of the hormone is within the linear range of the electrodes and their selectivity is high.

The relationship between the sensitivity of the method and the concentration of the analyte in the matrix in connection with the selectivity of the method and the composition of the matrix must be taken into account as validation criteria for an analytical method.

## *11.3 Limit of detection*

The limit of detection of an analytical method must be directly correlated with the concentration of the analytes in the sample.[329] Generally, the magnitude order for the limit of detection for different types of methods (e.g., anodic stripping voltammetry, potentiometry, atomic absorption spectrometry, UV/Vis, etc.) is known. Also, the approximate concentration range of the analyte in samples is known. For validation of the method for the analysis of an analyte from a specific sample, the limit of detection must be lower than the concentration of the analyte in the sample.

## *11.4 Uncertainty*

Usually, uncertainty is expressed by the value of relative standard deviation (RSD %) for each analytical method. The value of uncertainty determines the quality of the analytical information, as well as the precision of the method. The value of RSD % must be as low as possible. Also, for validation of the method for a specific type of analysis, the RSD % must be within a certain range (e.g., RSD % must not exceed 6 % for pharmaceutical analysis). Uncertainty is also correlated with the complexity of the method and with

the selectivity of the analytical method. To avoid confusion concerning the estimation of uncertainty, the method used to evaluate the uncertainty must itself be validated.[330] After the proper method has been selected, the value of uncertainty must be estimated. If the value of the uncertainty is within the range given for the type of sample, the method can be validated. The uncertainty value must also be compared with the uncertainty resulting from a standard method designed for the same analyte in the same matrix.

## 11.5 Special criteria of validation for different analytical methods

The special criteria of validation for an analytical method are directly related to the characteristics of the analytical method. For UV/Vis spectrometry, the reaction between the analyte and reagent must be fast, reproducible, and quantitative. The solution of the product resulting from the reaction must have at least 10,000 times the value of molar absorbtivity. In this regard, higher values can be obtained by using a polyfunctional reagent (e.g., Arsenazo III). For atomic absorption spectrometry and ICP, reproducibility of the flame and plasma, respectively, are essential for the quality and reliability of the analytical information, as well as for the validation criteria of the method.

For electrochemical sensors, the essence of validation of the method is their design. There are three main types of designs for the membranes: PVC-based membrane, liquid membranes, and carbon paste-based membranes. The most reproducible membranes are the liquid and carbon paste membranes. Because the size of the pores of the support used for liquid membranes is not large enough to accommodate larger molecules, it is recommended to validate the sensors based on graphite paste.[331] The reproducibility of electroactive material repartition in the membrane of the sensor results in reproducibility of the construction of the membrane, and also the quality and reliability of the response characteristics of the sensor and the quality and reliability of analytical information. For *in vivo* analysis, one should also consider the biocompatibility of the materials involved in the electrode design along with the previously mentioned characteristics. All the materials must assure reliable response characteristics for the sensors, high selectivity, and reliability of the analytical information.

High importance must also be placed on validation of biosensors and immunosensors from the transducer type point of view. Because the biochemical reaction is very sensitive, only the sensors based on an amperometric, fluorescent, or chemiluminescent transducer may be validated.

## 11.6 Data processing

Some specific requirements are necessary to validate data processing.[332] The validation of the method must take into account the type of correlation

between the physical property (P) determined and concentration. In certain cases the correlation between physical property and the logarithm from concentration is not acceptable; the correlation between physical property and concentration is preferred. The error introduced by the logarithm can be negligible if one compares it with the one given using another analytical method. The application of chemometrics as a science is recommended for this step, as are computers.

*chapter twelve*

# *Method development in chemical analysis*

The development of a method for the analysis of a sample must take into account that the analytical information must be characterized with quality and reliability. The quality and reliability are obtained only if the system analyst is flexible in choosing the best method for the sample and for the instrument that is used for determination. As always, in method development, the sample is the glue between method and instrument.

A method developed for the analysis of a specific sample cannot always be applied with the same reliability for the analysis of another type of sample. The provenance of the analyte and the type of the analyte influence the reliability of the method. To obtain maximum reliability, changing the values for some of the parameters is necessary.

The source of the sample — environment, food, or clinical medium — is of great importance since the source indicates the complexity of the matrix. Of the same importance for method development is the history of the sample. Only by having good knowledge concerning the history of the sample will it be possible to select the best method, because it will be a simple matter to establish the composition and complexity of the matrix. Establishing the complexity of the matrix eases detection of the possible interfering species in the matrix. If the matrix is too complex, then a selective separation technique must be involved. A good correlation between the selectivity of the separation and the selectivity of the analytical method must be realized. Also, a good correlation between the sensitivities of the separation and the analytical method must be achieved.

Choosing a very good instrument that exhibits the best sensitivity appropriate for the proposed method is another important step in method development. A good instrument should show a maximum ratio between signal and noise. At lower concentration levels, as well as for higher sensitivity, the noise can obscure the signal, and the measurement will be of the noise rather than of the signal. Such a result decreases the quality of the analytical information.

Using chemometrics in data processing further increases the quality and reliability of the analytical information. There are certain programs constructed for specific types of analytical methods. Data acquisition directly by the computer followed by data processing considerably decreases the uncertainty of the last main step in the analytical process (data processing).

Because the reliability of the analytical information is especially affected by the reliability of the sampling process, this step of the analytical process must first be optimized. As a result, sampling can be considered the most important step in assurance of high reliability for the analytical method. That is the reason method development must begin with the sampling process.

The first step in sampling is sample pickup. It is recommended that this step to be done by the analyst, because the quality of the results will be given by the repartition of the analyte in the sample (e.g., for a solid sample, it is recommended to take small amounts from different parts of the solid and to homogenize of the sample collected). For this step, the best instruments and containers must be used for sample collection and sample preservation. Automation of this step increases its quality and objectivity and decreases the possibility of contamination of the operator in the case of samples that are radioactive, toxic, or volatile with high toxicity.

The next step in method development is the solubilization of the sample. The first solvent that is always tried is distilled/or deionized water, although the type of solvent is determined by the analytical method that has been chosen. There are some solid substances that must first be desegregated. They cannot be dissolved in any other way in any of the solvents. The best quality and reliability can be obtained in this step by utilization of a microwave digestion system.[332] This system of desegregation produces the minimum contamination of the sample. Usually, the solvents and reagents used for desegregation of solids also constitute the blank for the analysis.

The next step in the sampling process is the separation technique, if necessary. Usually, for medium and complex matrices, even the most selective techniques are not able to determine the analyte(s) selectively. For a simple matrix, electrochemical methods, which do not need a laborious sample, are recommended.

The main techniques that can be reliably used in separation are extraction and chromatography. The best results are yielded by the chromatographic techniques, but in most cases these techniques also require extraction before the introduction of the sample in the chromatographic column, as well as a precolumn derivatization that can be reliably done using the extraction technique. To improve the sensitivity and reliability of the extraction technique, a synergetic extraction can be performed. The choice of the ligand and solvent must be in concordance with the requirements of the next steps of sampling and analysis, respectively. The extraction can be followed by the analysis itself, or can be followed by a chromatographic technique. When the extract is going directly to the detector, the selectivity of the extraction is the main parameter that must be considered. Extraction can also be reliably

used for the concentration of the analyte in the sample when its concentration is not large enough to be detected (e.g., in trace analysis).

The most often used chromatographic techniques are HPLC and capillary zone electrophoresis (CZE). For method development in HPLC[333-338] and CZE,[339,340] selection of the stationary and mobile phases must be the best for the mixture that is to be separated. The most reliable separation technique is CZE because it entails simple sample preparation, low operating expenses, and provides high resolution in separation. The main disadvantage of CZE is the limit of detection for the detectors at present available for the CZE technique. The optimization of the parameters for HPLC can be done by applying special chemometrics.[341] Some expert systems have been reported for HPLC for determination of optimum working conditions, selection of selectivity optimization criteria, and optimization of chromatographic parameters.[342,343] Repeatability expert system and ruggedness test expert systems have also been described for HPLC technique development.

Automatic method development is increasing the quality of the analytical information, but it cannot be applied for nonreliable systems. Such systems have been proposed for the HPLC/diode array when the data acquisition was done directly by the computer, compared with the analytical data from the database, and evaluated.[344] Automatic method development was also applied to improve the quality of the LC/MS system interface;[345] the method was able to do the setup function according to the type and quality of the sample. This automatic setup considerably improved the objectivity of measurement, and it accelerated method development.

In speciation analysis,[346] the most important techniques are atomic absorption spectrometry[347] and ICP-AES[348] or ICP/MS.[349] These techniques can assure the best selectivity and sensitivity. By using ICP/MS it is possible to carry out the total analysis of the sample in one run. Development of electrochemical methods has reached a higher level for the determination of the analytes in simple matrices.

The utilization of special software for instrument driving increases the reliability of the measurements because the parameters can always be established to a certain value. Furthermore, the computer also records the acquisition of data, and then processes the data using specific chemometrics according to the method utilized. Usually, the software that drives the instrument contains all the chemometrics necessary for the proposed technique. By utilizing special chemometrics, incorrect values can be eliminated.

By taking into account all these steps, and the uncertainty introduced by all of them, one will be able to develop the best method for a given analyte in a given sample. To increase the objectivity and rapidity of the measurement, flow injection systems (flow injection analysis or sequential injection analysis) have been proposed. The method development for these flow systems necessitates optimizing their working parameters in such a way that the best sensitivity and the maximum reliability can be obtained.[288-292]

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