Monographs in Electrochemistry

Series Editor: F. Scholz

Renato Seeber
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Functional Materials in Amperometric Sensing

Polymeric, Inorganic, and Nanocomposite Materials for Modified Electrodes

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Functional Materials in Amperometric Sensing

Polymeric, Inorganic, and Nanocomposite Materials for Modified Electrodes

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Preface of the Editor

In modern interfacial electrochemistry, one can find two opposite directions of research: one focused on the most well-defined and clean interfaces (e.g., single crystal metal surfaces), and another in which attempts are made to construct the most complicated architectures at the interfaces. In some cases, these are also highly defined (e.g., in cases of self-assembled monolayers), but in other cases they may lack a high order, or we do not yet know the order, but these interfaces WORK as desired for certain applications. The immense variety of modified electrode surfaces is difficult to survey, and anybody entering into research and development of electrochemical sensors will greatly appreciate the competent advice available from a monograph such as this, written by highly experienced scientists. Renato Seeber, the senior author, and his colleagues Fabio Terzi and Chiara Zanardi from the University of Modena and Reggio Emilia, Italy, have focussed their book on amperometric sensors. Although amperometric sensing of the endpoint of a titration was first described in 1897 in a PhD thesis by Ernst Salomon [1–3], a student of Walther Nernst in Göttingen, progress was rather slow in the first half of the twentieth century, and amperometric sensors remained mainly confined to indicating titration endpoints. Amperometric titrations were intensively developed by Songina et al. [4] in the former USSR; unfortunately, this detailed Russian work [5] has never been translated to English. Clark filed a patent for his famous oxygen sensor in 1959 and published it in 1962 [6, 7]. The second half of the last century saw a burst of innovations, especially in the area of biochemical sensors, most prominently the glucose sensor [8]. Now, electrochemists (and chemists using electrochemical measuring techniques) have no hesitation in putting whatever they believe could be useful on electrode surfaces to achieve their goals. This experimental entrepreneurship is a challenge for many scientists who try to understand how these systems work. The present monograph is an important step towards a systematic view of complex sensors for amperometric measurements. The authors have avoided aspiring to complete literature coverage, but instead present a selection of instructive examples in the framework of a systematic classification.

Greifswald, Germany Fritz Scholz
August 2014

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Contents

Chapter 1

Importance of Modified Electrodes in Amperometric Sensing

It seems advisable to spend little room to give the reader a basic guide, evidencing some points to hopefully clarify, as much as possible, the aim and the language of the present monograph.

First of all, as a general observation, this monograph does not aim to be a handbook of *all that has been achieved so far in the field of modified electrodes in measuring currents* or, even worse, *all you would like to know about modified electrodes* … . Rather, it is intended as a sort of reference to those who are interested in switching from conventional electrodes to more ‘complex’ systems in order to overcome problems presented by usual electrode materials in amperometry. It also aims to stimulate those who develop new systems to carefully consider the enormous progress made in the field of conductive materials, choosing among the wide number of those that are potentially of interest, and testing those that seem most promising.

To best effectiveness, purposefully designed and synthesized materials could emerge from synergic work between electroanalysts/electrochemists and chemists who are experts specifically in synthesis. The importance of gathering suitable expertise from many different scientific and technological fields should be emphasized. Competences as to the matrices on which their work should also contribute, in order to address the properties of the device developed to the goal pursued: amperometric sensing is definitely a deeply interdisciplinary field.

Those who are interested in the topics within this monograph are assumed to possess competencies in electrochemistry and analytical chemistry, i.e., the two components of the word electro-analysis. These disciplines should guide the choice of the best electrode system, and even address the material scientist to innovations in the field. The electrode material is linked, in fact, to what comes before and after it along the path from design to use of a sensing device in a real matrix. The hardware and software components of the device as a whole will enable extraction of the best information from the efforts made.

As a preliminary point in terms of the glossary, the word ‘amperometry’ appears in the title and throughout the text. Much misunderstanding has surrounded the

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terms ‘amperometry’ and ‘voltammetry’. Simple etymology, without any supposition or groundless assumption, is definitely helpful. Amperometry means ‘*measurement of a current*’ and simply indicates whatever technique is used to measure a current. This may be measured as a function of an independent variable, such as the electrode potential or time, but also, in principle, as a function of any experimental variable. Measuring a current as a function of temperature, pressure, or physical quantity identified on the basis of a foolish choice, all still constitute an amperometric measurement.

It is worth recalling that amperometric measurements were first employed to follow redox titrations [1] in which (1) either the reactant or the relevant reaction product or (2) either the titrant or the relevant reaction product are electroactive.[1] Although the titrant volume in amperometric titration measurements is an example of independent variable, it is obvious that time or potential of the working electrode constitute the most ‘useful’ and frequently encountered variables in amperometric sensing. The i vs. t or i vs. E plots, where i is the current flowing in the working plus auxiliary electrode circuit, constitute the signal to analyse in chronoamperometric and voltammetric measurements, respectively.

It is for a monograph in the field of electrochemical sensing to evidence the advantages of amperometric sensors with respect to other sensing systems as well as to ‘big’ instrumentation, such as chromatographic, mass spectrometric, nuclear magnetic resonance, or even hyphenated complex instruments. As to the advantages of sensing, whatever the transduction (i.e., electrochemical, optical, gravimetric, or other), it should be emphasized that, on-line, or even in-line, analyses are possible, allowing a high frequency of measurements, and possibly even continuous monitoring. Conversely, alternative instrumentation most often requires sampling and pre-treatment of the sample, together with more qualified personnel. Preservation and transport of the sample to the appropriately equipped laboratory, together with the often high cost of the equipment itself (including maintenance), make the necessarily off-line approach much more expensive.

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[1] The potential is chosen in order that additional currents, i.e., those due to other species present, are either absent or constant along the titration. The best conditions are those in which a rotating disk electrode is polarized at a fixed potential at which the charge transfer of the electroactive species is controlled by diffusion, i.e., in correspondence to the ‘limiting current’. The linear dependence of the diffusion-controlled current on the concentration of the monitored species in solution renders the titration diagram particularly effective with respect to the precision of the identification of the final point. The diagram is obtained by plotting the current flowing as a function of the titrant volume added and resembles those relative to conductimetric or spectrophotometric titrations. In other words, the plot is necessarily linear sufficiently far from the final titration point, where it exhibits more or less marked curvature, depending on the reaction constant value. The evaluation of the final volume is performed by extrapolation to the value of the background current of the linear portions of the plot. Lingane and Kolthoff [2] describe the technique in detail and with respect to the relevant experimental set-up; further developments, such as biamperometric titrations, are also dealt with.

1.1 From a Look at the Historical Background to Considerations for Effective Perspectives

Modification of the electrode started with ‘academic’ studies on physical and chemical adsorption, i.e., with the appearance of fundamental researches on adsorption of different species on electrode surfaces, both under polarization and at open circuit potential [3]. The properties of similar ‘chemically modified electrodes’, in which the modifier consists of a monolayer of a variety of chemical species with different characteristics, possessing (or not) particular properties, were initially studied in a purely electrochemical context, aimed at the collection of fundamental physico-chemical data. A small group of electrochemists were among those involved in these basic studies, envisioning the perspectives opened by the novel systems. In the first, really fascinating, work with similar monomolecular layers, cobalt porphyrin and phthalocyanine, as well as deliberately synthesized dicobalt face-to-face porphyrins were adsorbed on Pt or C surfaces to catalyze molecular oxygen reduction [4]. However, similar systems were not always used or adequately tested in proper amperometric sensing by researchers more interested in electroanalysis; dicobalt face-to-face porphirins still constitute a rare example of tailored materials for selective amperometric detection.

Once similar systems were tested with respect to electroactive species, it soon became evident that radical changes in the overall charge transfer processes could be induced. Similar studies started in the 1970s, so much so that Royce W. Murray, after authoring pioneering studies (to which Fred Anson gave comparable contribution; apologies for not listing the other outstanding scientists belonging to the club), published a first survey on the topic in 1980 [5]. Then, Murray himself was able to feel that the issue had been explored deeply and widely enough to make a first exhaustive critical point, in the frame of a chapter within the *Electroanalytical Chemistry* series edited by Allen J. Bard [6]. The stability of the first proposed molecules with respect to adhesion to proper substrates is based on the interaction of the extended π electron system of the porphyrin ring, which is often quite strong. Subsequently, functionalities were anchored to the substrate surface by silanization of hydroxyl groups from metals or metal oxide surfaces; however, the resulting systems [7] were poorly stable, resulting sensitive even to traces of water in the solution. Similarly, carboxylic residues on carbon substrates were also used as anchoring groups.

Subsequently, two main paths were followed. The first went back to physical adsorption, involving different kinds of polymers on the electrode substrate. The second went on with chemical adsorption, i.e., pursuing the formation of true chemical bonds with the substrate electrode: thiol groups are the most commonly used to anchor so-called self-assembled monolayers.

It seems correct to affirm that true applications of novel materials to amperometric sensing date to the 1990s, and, further, to conclude that progress has been and still is rather slow. Huge steps forward have been made by material chemistry in the last decades, in the whole field of new conducting or semiconducting, both inorganic and organic, both bulk and nanostructured materials. However, only a small fraction has been used in electrochemistry. Among these, most have been and are applied in energy storage and production, and only a smaller portion has been exploited in amperometric sensing. As a result, the number of systems that have found actual application in established and accepted analytical procedures, also defined in order to operate in real matrices, is rather low. This may be partly the result of the greater level of funding that encourage the efforts of researchers working on electrochemical systems in energetics. However, it is important to emphasize that many requirements to electrode systems are common to all these applicative fields: amperometric sensing could pick up a lot from what is realized in the field of electrochemical energy production.

When dealing with bare surfaces, attention is essentially oriented to what happens in the solution phase and strictly at the interface between the solution and the 'inert' electrode; the nature of the electrode surface, and even more of the electrode bulk, may be considered hardly meaningful. At the end, many aspects dealing with the charge transfer are actually similar for Pt, Au, or carbon-based electrodes. This notwithstanding, electrochemistry and, specifically, electroanalysis at bare electrodes are quite complex topics. The comprehension of the operative electrode mechanisms, also necessary in the frame of a 'simple' quantitative determination, stimulated a number of theoretical and experimental works. They were devoted to giving accounts of complicated electrode mechanisms in which homogeneous and non-homogeneous events are coupled to the charge transfer. Oddly enough, it often happens that theoretical elaborations have not always found a counterpart in experimental matches.

When shifting from bare to modified electrodes [8] everything becomes even more complex. In any case, kinetic studies in 'molecular electrochemistry' constitute the basis of the knowledge in all fields that, in one way or another, refer to 'controlled potential amperometry', independent of the fact that the working electrode is either bare or modified. In other words, the culture acquired in molecular electrochemistry has been and still is of basic importance for an electroanalytical approach that lies on a rational explanation of shape and trends of a given response or series of responses. It is also evident that expertise in basic molecular electrochemistry is mandatory for those researchers who realize a new electroanalytical system, including suitable procedures for effective and reliable measurements, explaining what happens and why. This was and is important for bare electrodes, and is even more urgent for modified electrodes, which require an awareness of the complexity of the system and of the processes induced by the charge transfer.

Looking closer at the evolution of amperometric sensing, it must be admitted that significant breakthrough has been due to the extensive introduction of electronics in managing the electroanalytical measurements. After the first pioneering works [9], the upsurge of electronics allowed electrochemical instrumentation to become effective, versatile, and even diffused widely enough to satisfy the requirements of electroanalysis. Conversely, these days, it seems unreasonable to expect further meaningful progress in the field of electrochemical instrumentation. This is also because the definition of a series of electroanalytical techniques has seemingly been concluded, and the theoretical elaboration/analysis of the relevant responses, limited to the unmodified electrodes, has essentially been fulfilled. Electronics in the frame of computers allowed the use of sophisticated simulation and data elaboration tools; the relevant development paralleled progress in experimental fields, offering novel instruments to acquire the highest information content from the results of the experiments. In this frame, sophisticated operations, such as convolution and de-convolution procedures, differentiation and integration of half orders, filtering of different natures, and recursive fitting procedures on different simulated responses have been elaborated, not always achieving huge success.

On the other hand, sophisticated theoretical analysis of responses obtained by modified electrodes is extremely risky, as will be highlighted later: every coating attached at the underlying conductive substrate is almost unique, which would require an ad hoc theoretical treatment. The attribution of a given electrochemical response to a specific structural characteristic of the modified electrode may be risky, even for experienced researchers. It follows that the implementation into commercial instrumentation of procedures devoted to fit experimental data to specific models is even more risky. The charge percolation inside a coating, and the transfer at the two interfaces that should at least be considered, most often cannot be accounted for by a simple equivalent electric circuit or by a corresponding relatively simple system of equations properly building up the so-called ‘boundary value problem’.

Rigorous models can be developed in the case of organic monolayers, whenever the electrode charge transfer may be considered a surface process or a process controlled by the diffusion in the solution, leading to amperometric responses that can be reliably analysed and compared with theoretical ones. As the thickness of the coating is increased, different situations are encountered that drastically condition the amperometric responses, as well as those of other diagnostic techniques, such as faradic impedance. The value of the thickness of the deposit in solution, i.e., where it has to work, is the first problem. The structure of the deposit (i.e., compact vs. porous nature, rigid vs. elastic characteristics, hydrophobicity vs. hydrophilicity – with the consequent ability to swell and shrink) at varying potentials constitute additional characteristics that are difficult to assess and quantitatively estimate, so that the results reported in the literature should often be considered with legitimate suspicion. A priori assumptions on the status of the system under study are often arbitrary, leading to risky and doubtful conclusions.

In order to avoid overestimating the information content of the responses, it is often necessary, and even honest, to give very rough quantitative estimations, or to warn that values of quantities calculated actually account for a number of concomitant physical events. In this respect, a good example is given by the estimate of the ‘apparent diffusion coefficient’ (D_{app}) of a species. Apart from the cases in which the geometric area, instead of the electrochemical, is erroneously considered, it is too often assumed that the use of a probe with a known diffusion coefficient allows the evaluation of the suitable area value. The differences in the behavior of the probe with respect to the analyte within the coating are not considered. Moreover, it is not always accounted for that the computed D_{app} actually accounts for every possible step preceding or in parallel with the slowest one. These steps consist at least of (1) diffusion in the solution phase; (2) diffusion inside the coating (which is a function of the relevant nature, and of the polarization potential); (3) electrostatic interactions of the electroactive species and migration of ions called to support electroneutrality within the coating, etc.

Caution is hence strongly recommended, considering that the better one knows a technique, the more clearly he/she can see the limits. Since the opposite also holds, incorrect conclusions are too often found, even in the specific literature. It is definitely preferable to stop at qualitative or semi-quantitative but reliable and safe conclusions, than to transmit dubious bases on which other researchers will faithfully work.

It is essential to consider how dramatically the availability of tools for synthesis and, especially, for characterization, has increased over the last 20 years. They may, and hence should, be used by any researcher who develops a novel analytical device and a set of effective relevant procedures. This also very strongly affects the approach adopted to the realization of an amperometric system: the number and power of non-electrochemical tools available to best solve problems arising along the whole pipeline (from development and characterization of the innovative material to the analytical tests) have significantly increased. The studies of what happens in solution and at the interface where the exchange of electrons takes place are no longer only faced through electrochemical techniques. The behavior of the systems is elucidated only once, in addition to the modifier|solution interface, the charge percolation from the substrate to the outermost interface is also considered. In addition to the interaction of the analyte with the modifier at its outermost surface, more or less deep penetration of the analyte into the deposit, to a certain extent, should be also taken into account. Furthermore, the degree of porosity or compactness, morphology, and even composition of soft or hard coatings are most often a function of the potential applied.

Despite the complexity of the characterisation, it is not contradictory to stress the necessity of making every effort to achieve the best possible knowledge of the system. The definition of the structure of the coating, in the widest sense, requires the use of a number of spectroscopic, microscopic, morphological, and compositional measurements to complement the electrochemical ones. However, it must be stressed that, in many cases, the deposits have been studied in the dry state, i.e., not really in the conditions under which they are actually used. As an example, only a few studies are found in which atomic force microscopic measurements are made in the solution phase, where the deposit is created and subsequently used. On the other hand, the Pt and Au substrate|polymeric deposit interface has been studied in situ by surface-enhanced Raman spectroscopy.

By considering in situ characterization of the deposits, hyphenated techniques can represent very important tools to enrich the picture of the coating when subjected to electrochemical polarization. In this case, the electrochemical stimulus, in terms of either potential or current applied to the system, is coupled to a 'direct observation' of the modification induced. UV-visible and Raman spectroelectrochemistry were and still are infrequently used to simultaneously tune (electrochemistry) and monitor (spectroscopy) the changes consequent to the polarization of the modifier. Furthermore, although they potentially lead to an enormous quantity of information concerning the electrochemical behavior of electrochromic coatings, spectroelectrochemical detections are, for the most part, confined to the study of intrinsically conducting polymers (ICPs) [10] modified electrodes. This happens despite that the class of spectroelectrochemical techniques has been well established for a long time. Similarly, electrochemical quartz crystal microgravimetry has been used by a small number of researchers to go deeper inside the process of electrochemically induced film growth, as well as of the charge/discharge process of electroactive coatings.

It must be admitted that the electrochemists have often been poorly willing to be open to techniques other than electrochemistry itself in order to achieve the best characterization of the electrochemical systems. The electrochemical data are often overestimated, taken as being very sound, even exhaustive, diagnostic tools, for defining a system that is actually too complex for electrochemistry to satisfactorily characterize. Conversely, although we do not presume to ignore the positive aspects of the involvement of chemists who are not electrochemists or even physicists and engineers in the realization of amperometric sensors, we must complain about their often excessive self-confidence in dealing with complex electrochemical issues. In connection with this observation, two different possible causes may be identified for the progressive underestimation of the pure electrochemical culture that negatively affects the quality of the proposal of electroanalytical devices and relevant methodologies: (1) the worldwide difficulty of building effective interdisciplinary staff, which often do not include high-level electrochemists; (2) the complexity of the systems, which induces researchers to be too often willing to drop the “how does it work?” in favor of the “it works!” minimal approach.

On the other hand, in terms of the necessary interdisciplinarity required for the realization and exploitation of novel amperometric systems, it is not so easy to convince chemists who are experienced in chemical synthesis to give support to realizing an ad hoc material possessing the physical and chemical characteristics suitable for best efficiency as an electrode modifier.

Furthermore, the development of electroanalytical devices and relevant measurement procedures often presents quite complex issues in terms of reliability, repeatability, and reproducibility of the responses obtained. This is a problem that, in the case of modified electrodes, presents particularly complex aspects. Similar systems often exhibit a behavior that is far from being accounted for by simple or known physical laws that would offer a previous reference to the decision about a good or bad way to work. Hence, a critical analytical exam of the results obtained is mandatory, being ready to adopt even non-trivial treatment of the data.

All these arguments, far from being a source of discouragement, should encourage acceptance of the huge number of challenging tasks opened in the field: much room exists for scientists to make huge progress in amperometric sensing. We believe that electrochemical/amperometric methods often suffer from scarce faith, added to by the crucial transition from mercury to other electrode materials. After the ‘glorious years’ of mercury, we had to become aware of the numerous drawbacks of the ‘history’ that a solid electrode bears and suffers from when working inside a solution.

Electrochemists have been carrying out fundamental work for a long time, devoted partly to identifying the way in which stable modifications can be realized, partly to defining the behavior of the electrode once coated by various modifiers differently glued to it, and partly to exploring, by ‘spot tests’, the supposed best direction along which to move in order to achieve the properties sought. Proper experimental design techniques or even combinatorial approaches for the realization of most effective systems are only rarely found in the literature.

Regardless, it is undoubted that proper modifications may be of great help in rendering electroanalytical methods either complementary or even alternatively competitive with other instrumental analytical techniques, specifically with the optical ones, in quantitative analyses. Electrochemical sensors are not blind in opaque matrices, microelectrodes can even work in viscous media, and the selectivity that optical probes acquire from the changing wavelength is imparted to electrochemical probes by the polarizing potential. Furthermore, activation of electrocatalytic processes may also ‘alter’ the potential scale, ascribing the system additional selectivity.

By the end of this introduction, we hope to have given the reader a number of reasons to reinforce the pioneering enthusiasm for modifications of electrodes that led to the very ‘beautiful’ and promising scientific work in the 1970s and 1980s. It is now up to electroanalysts to take advantage of the enormous progress made in material chemistry in the last 20 years. Obviously, as highlighted above, opening up to complementary expertise is a must, once the complexity of the materials and systems employed does not allow electrochemists alone to optimize the electrochemical performance. The evaluation of the performance obtained should constitute the final ‘mark’ to assign to the system realized and, in the intermediate stages, very meaningful feedback for suggesting improvements. When talking of ‘the system’, one should consider that device and measurement procedures for a given analyte/matrix should always be considered as a whole. Ideally, the feedback information content necessary to achieve the final goal through a sort of iterative process comprises the device response, the procedure adopted, and all suitable non-electrochemical characterizations.

1.2 Advantages Sought with Respect to Bare Electrodes

We like to cite the definition of a modified electrode given by Royce Murray in his chapter of Bard’s *Electroanalytical Chemistry* series [6], based on the goals pursued through the modification: “... one deliberately seeks in some hopefully rational fashion to immobilize a chemical on an electrode surface so that the electrode thereafter displays the chemical, electrochemical, optical, and other properties of the immobilized molecule(s) (...) one selects immobilized chemicals on the basis of known and desired properties (. . .) the electrode can be tailor-made to exhibit these properties." After so many years and the realization of different systems developed to this purpose, such a definition is still fully valid, constituting also a guide to the attitude that the researchers in the field should have towards the development of new systems. Unfortunately, in fact, Murray's final assumption is not always convincingly kept in mind. As a consequence, the systems are often realized on the basis of a generic idea of effectiveness, and are tested on a 'standard' series of typical analytes that are claimed to be 'particularly indicated to play the role of benchmark species'. Actually, this is not always true. Tailor-made modifications rarely constitute the guide to the realization of systems designed and realized 'on purpose'. The same analytes are most often used for testing systems that are quite different from one another. The tests are supposedly performed in order to define the general interest that a new material deserves. It is evident that this is a necessary step towards the proposal of actually effective systems; however, it now seems the right time to have a higher number of actually effective and even specific systems at our disposal.

It was clear, since the first attempts to exploit the initial basic studies in electroanalysis, that the principal scopes pursued by modification of an electrode are (1) activation of electrocatalytic processes, possibly selective in character; (2) prevention of fouling of the surface; (3) concentration of the analyte close to the electrode, before the occurrence of the charge transfer in order to improve the sensitivity. A choice based on rational arguments coming from the acquired knowledge of the properties of the material, often only restricts the number of materials that subsequently should be tested, necessarily, with as low as possible uncertainty degree about the success.

It is well known that the actual value of the potential at which an electrode charge transfer occurs is conditioned by many factors; in addition to the formal potential of the redox couple involved, different sources of overvoltage [11], from charge transfer to crystallization, from weak or strong adsorption of reactant or products, to different kinetic effects should be considered. In principle, suitable modification may specifically act on one or more of these factors, possibly resolving signals that are originally overlapped on the potential axis. In this respect, electrocatalysis must be intended in the widest sense, i.e., as referring to any process that lowers one of the different overvoltages affecting the electrode process. It is evident that many different actions can be activated in order to do that. The nature of the surface that comes directly in contact with the electroactive species in solution plays a major role in this respect. It affects the activation energy for those charge transfers that require intimate contact with the electroactive species, i.e., the so-called 'inner sphere' electron transfers, due to the different energies of the atomic or molecular orbitals involved in the activated complex. Surfaces structured in a peculiar way (nanostructured materials) may favor a different path or energetics of the charge transfer reaction. The activation energy of the charge transfer step

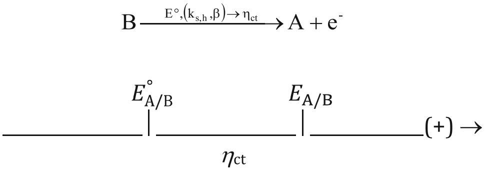


Fig. 1.1 Schematic representation of a generic irreversible oxidation from B to A

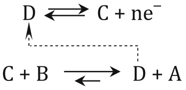
may be reduced, hence lowering the charge transfer overvoltage.[2] Furthermore, as discussed in the following, thermodynamic control can be restored, or at least approached, by calling a redox mediator to act as a sort of 'bridge' of charges between the electrode surface and the species in solution. Electrocatalysis is also involved in this case.

As an example aiming at giving an idea of what can be made, in one of the first reports on electrocatalysis at a chemically modified electrode, a pyrolitic graphite electrode was treated by radio frequency plasma in oxygen, with subsequent attachment of benzidine to this treated graphite. The species on the surface catalizes ascorbic acid oxidation, which was, at that time, a 'new entry', though very popular in the rest of the story . . . [14].

The occurrence of electrocatalysis by redox mediation is explained hereafter. Figure 1.1 sketches the irreversible one-electron anodic oxidation of the analyte B to A and the location of the relevant E° and of the actual oxidation potential, in the absence of redox mediation ($E = E^\circ_{A/B} + \eta_{ct}$).

The C/D reversible redox couple brought, for instance, by a redox polymer (RP) that fixes it at the electrode, acts according to the scheme illustrated in Fig. 1.2 in terms of the relevant cyclic voltammetric (CV) responses.

Let $E^\circ_{C/D}$ be less positive than $E^\circ_{A/B} + \eta_{ct}$, but more positive than $E^\circ_{A/B}$: the oxidized species C oxidizes B to A and is regenerated from D at the electrode, according to the scheme:



The process is possible thanks to the relative values of the thermodynamic potentials: C is the form largely predominant with respect to D at the potential at which the electrocatalysis is operative. In the case of a multilayer deposit, once the redox reaction between C and B occurs at the coating|solution interface, it generates

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[2] In linear sweep voltammetry, a maximum increase of ca. 27 % is compatible to the transition from a one electron totally irreversible to a reversible uncomplicated electron charge transfer, assuming the common value of 0.5 for the charge transfer coefficient, α, multiplied by n_a, the number of electrons exchanged in the slowest step [12, 13]. However, whenever complex mechanisms occur, electrocatalysis may act on the sources of different overvoltages. As a result, the catalytic effect may be operative in such an effective way to lead to a surprising increase of current.

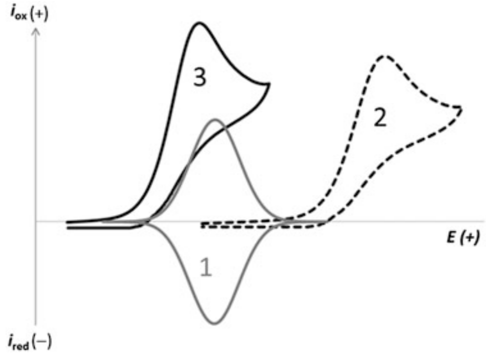

Fig. 1.2 Schematic representation of CV curves accounting for the electrocatalytic process induced by a species anchored at the electrode. *Peak 2*: irreversible oxidation of B to A at the bare electrode; *peak system 1*: reversible C/D system in the absence of B; *peak 3*: electrocatalysed oxidation of B to A by mediation of C. The shift of *peak 3* with respect to *peak 1* is due to the reaction of the charge transfer product, C. No backward peak due to C reduction is recorded, admitting that the redox reaction C + B is fast enough. The symmetric peak system is typical of surface processes, i.e., of a monolayer electroactive deposit

the relevant reduced and oxidized species, namely D and A, respectively. Inner redox reactions in charge of the C/D couple 'propagate' the charge into the coating to the underlying electrode that regenerates the form, C, stable at the imposed potential. The electrical circuit is closed and the process goes on. The oxidized species A is obtained as the unique actual final product of the overall electrocatalytic process. The oxidation of B to A hence occurs at the C/D sites fixed at the electrode and in contact with the solution at the same time, actually at a potential slightly less positive than that at which D is converted to C, due to the redox reaction subtracting the (oxidized) product, C, at the C/D equilibrium.

Summarizing, in the general case, the electrogenerated form of the mediator oxidizes or reduces the analyte, when thermodynamically unstable with respect to each other: an electrocatalysed (mediated) charge transfer occurs between the conductive substrate and the electroactive species in solution. The mediator in the coating acts as a 'charge bridge' that makes the charge transfer activation energy approach zero.

The advantages of anchoring the mediator at the electrode, in respect to dissolving it in solution, are evident. Let us highlight that the mediator is only present where it is useful, saving money if it is expensive but, more importantly from a merely scientific point of view, maximizing the yield of the use of the mediator, and avoiding possible pollution of the solution, such as the eventual occurrence of parallel undesired reactions.

In conclusion, in the frame of amperometric sensing, the activation of electrocatalytic charge transfers may offer definite advantages in (1) increasing resolution, i.e., by deconvolving partially or even totally overlapping responses; (2) allowing species to be monitored that are otherwise electroactive beyond the solvent discharge. On the other hand, electrocatalytic redox reactions may also be activated selectively, by coupling the catalytic site to functional groups suitable to only interact with given species.

Poisoning adsorptions of species, involved or not in the charge transfer, are often so difficult to rationalize that only the results of experimental tests give reasons for the actual effectiveness of a given modification. Far from being similar to spectroscopic measurements, although the voltammetric ones do not alter the overall composition of the solution, they actually modify the probe itself, i.e., the electrode. In this respect, it is evident that the definition of a suitable measurement procedure, e.g., in terms of electrode pre-conditioning, is of chief importance on the way to obtain reliable results. Of note, we are not always aware that, if the second response recorded within a series no longer overlaps with the first, due to the occurrence of fouling, this is in turn poorly reliable: the phenomena altering the second recorded trace actually take place in the course of the registration of the first, affecting it in dependence of very subtle differences in the experimental conditions. Unfortunately, literature reports do not always support the confidence of those who would like to apply what is reported to a practical problem; the repeatability and reproducibility issues are not so often dealt with, and even less frequently dealt with properly. Moreover, special attention should be paid to the specific characteristics of the matrix. All the critical issues outlined above, in fact, achieve the most weight when dealing with real matrices. The difficulties of working in complex matrices in the absence of a pre-treatment or filtering/separation are well known. It is just when the electrode is dipped into a complex matrix that fouling may constitute a significant drawback to reliable amperometric signals. However, the choice of suitable electrode modifications, together with the definition of proper methodologies based on carefully sought and checked cleaning procedures of the electrode surface, can definitely be of help in this respect. Mechanical polishing of the electrode surface is often not required, but rather a proper conditioning, typically polarizing the electrode at chosen potentials for a suitable time, eventually stirring the solution and bubbling inert gas through.

Relatively recently, the combination of two electrocatalysts has been proposed, ‘importing’ the idea and even the systems themselves from chemical heterogeneous catalysis; the mutual effects of two agents may act differently from the individual components. One of the possible effects lies in ‘cleaning’ off the electroactive catalytic site, removing intermediates or final products poisoning it. Remember here that the increasingly wide availability of disposable (screen printed) cells renders long-term repeatability less urgent, but emphasizing the need for reproducibility in the development of the electrode systems, as a condition to obtain reproducible responses.

Although adsorptions are rarely intentionally pursued, the adsorbed species does not always poison the electrode surface; in the simplest case adsorption involves the reactant or the product of the charge transfer. It is well known that adsorption of the electroactive species causes the response to occur at more extreme potentials, while adsorption of the product anticipates the response (see Evans et al. [15] for the relevant cyclic voltammetric responses). Similar effects may be intentionally sought by a suitable coating, for instance aiming at better resolution of responses.

A coating can mimic the mercury drop in concentrating the analyte in the proximity of the electrode. As an example, ionic polymers can be used for this purpose: the subsequent potential sweep to oxidize or reduce the electroactive species leads to a higher current intensity than that collected for direct monitoring of the species in solution.

As to modified electrodes in the field of amperometric biosensing, a distinction should be made between catalytic and affinity biosensors. In the former case, the biological element mainly consists of an enzyme or, more generally, of a protein. The coating is required (1) to fix a high level of biological species very close to the transducer and (2) to be sufficiently permeable to allow the diffusion of the analyte from the solution to the catalytic elements. For these reasons, hydrophilic thick electrode coatings including the active sites are generally preferred to monolayers. In many cases, the material itself may be suitable to exchange electrons with (1) species produced by the enzymatic reaction, generally consisting of NADH or H_2O_2, or (2) the enzyme. Second- and third-generation biosensors have been obtained in the two cases, respectively. In all cases, the overvoltage necessary to obtain the analytical signal is lowered.

Among the possible actions outlined, the quite recent development of nanostructured materials (see Chap. 6) has led to the most effort being directed toward the realization of third-generation biosensors: the nanostructure can affect the spatial orientation of the biological element, inducing a close contact with the transducer itself and allowing a direct charge transfer between enzyme and modifier, inducing very rapid electrode responses and high sensitivity of the biosensor.

Nowadays, nanostructured surfaces are also very frequently applied in the frame of affinity biosensors to assure stable immobilization of both oligonucleotides and antibodies at the electrode surface, for the development of genosensors and immunosensors, respectively. In these cases, the advantages arising from the use of a nanostructured surface with respect to a smooth one are not limited to the possibility of increasing the number of biological elements fixed on the transducer; the nanostructures also positively affect their spatial orientation, causing optimum steric conditions for interaction with the complementary species in solution. Different systems have been developed for the amperometric detection of the formation of the receptor-analyte adduct, due to the electrochemical inertness of antigens and of oligonucleotide chains. Although the marking with electroactive species or enzymes is mainly necessary when considering amperometric immunosensors, label-free approaches are also possible in the case of genosensors. Here, the simple addition of a redox active species to the solution, or even the inclusion into the coating itself, can give rise to a voltammetric signal that changes proportional to the amount of complementary oligonucleotide chains in solution. In any case, electrode coatings developed ad hoc for affinity biosensors must assure the strongest interaction between the biological element and the analyte in solution, so monolayers or very thin layers are generally preferred.

1.3 General Overview of the Modifying Materials

A first possible way to modify the surface is fairly physical, consisting in altering its morphology, by induction of roughness through various chemical or electrochemical etching procedures. Such a modification leads to disordered micro- and nanostructured surfaces that really are at the border of a truly modified system; it actually constitutes a very first step to electrode surface modification. A modification consisting of the deposition of nanoparticles (NPs) onto the surface of a substrate of the same metal is, for certain aspects, similar; however, it may lead to a more uniform nanostructured surface and is discussed in detail in Chap. 6.

Physi- or chemi-sorption constitute two alternative methods to anchor whatever is at the surface in a more or less stable way, depending on the nature of the substrate and deposit. The list of materials used to impart the sought-for characteristics of the electrode is long and can partly be deduced by the contents list of the present monograph. It is evident that the proposal of new material tools, most often independently of electrochemical science, constitutes the first stimulus for researchers to study their possible applications in electroanalysis. More rarely, the opposite direction is followed: the economic interests in different branches of the material sciences has constituted and still constitutes the actual driving force to the development of 'new entries' in the world of materials; electroanalysis has already been cited as not inducing a huge transfer of money in this respect.

Chapter 5 of this monograph is specifically devoted to monolayers modifying the electrode surface, under any of the forms possible. Attention was and still is devoted to organic monolayers that can be realized in the form of a more or less ordered assembly, by formation of bonds of more or less strong covalent characteristics with the metal atoms at the surface of the anchoring substrate. In the case of ordered deposits, we can appropriately talk of 'self-assembled monolayers'. It is worth noticing that it is often unnecessary to achieve a true order, as the overused 'self-assembled monolayer' denomination would suggest but, rather, only stable anchoring is mandatory. The difficulty in achieving order is due not only to the limited dimensions of the terraces in polycrystalline surfaces, which are not specifically developed for the highest co-planarity of the atoms, but also descends from the deliberate adoption of deposition procedures that are not suitable to impart the best order to the system. For example, the systems anchoring nano-objects, such as NPs or graphene at the electrode surfaces, are often far from being ordered, resulting however effective. In the case of NPs, the encapsulating agent chosen to stabilize the metal cores is often also suitable to link them at the underlying substrate in a structure that is quite far from being a proper self-assembled monolayer, despite the fact that such a denomination is often used in a free and easy way.

Multilayer mono- or bi-component disordered coatings are often encountered. There may be limited advantage in controlling thickness of the deposit, at least beyond a certain precision. However, one should be aware that a thick deposit implies the introduction into the system of a new phase, i.e., the bulk of the deposit, often also difficult to characterize with respect to a 'simple' monolayer. This makes

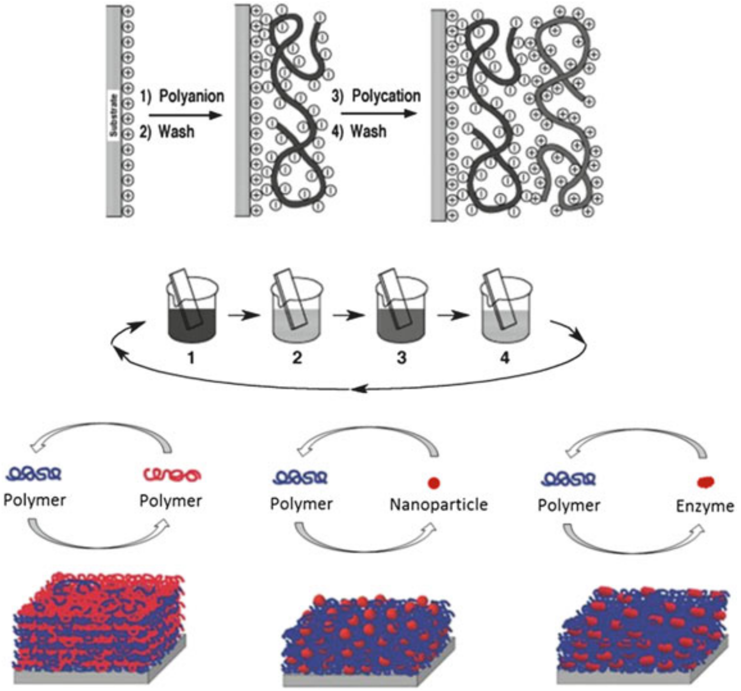

Fig. 1.3 Schematic representation of layer by layer deposition of thin films. Three different possible structures are shown (Adapted from Refs. [17, 18])

the system more complex and sensitive to structural changes, not easy to give a rationale. The link between the characteristics of the deposit and the performance of the amperometric system becomes less and less direct, i.e., increasingly complex.

Different approaches to obtaining stable layered structures have been proposed. One possibility consists of using a 'layer-by-layer' deposition method [16], schematically reported in Fig. 1.3. The growth of multilayer films is carried out by alternating the immersion of a solid conducting substrate into a cationic and then an anionic polyelectrolyte solution; the excess of weakly adsorbed molecules is washed out at the end of each deposition step and the coating is dried with nitrogen or air flow before the assembly of any new layer. The preparation method is simple and can be easily automatized.

In principle, the technique is very versatile, suitable for the deposition of a wide number of different materials, ranging from conducting and non-conducting polymers to biomolecules, provided they bear electrical charges, even to nanosized materials to which charged residues are linked. It often happens that only one of the two components is active in imparting the electrode system the properties sought; the other component is a necessary complement in furnishing the charges ascribing stability to the structure. It is well known that the 'layer on another layer' picture is only a simplified way to represent the situation; however, it is far from the physical reality. In particular, complex situations are proper of the interface between adjacent layers and interpenetration of layers, even relatively far from each other, is a rule.

As for pure inorganic materials, metal hexacyanoferrates are proposed as effective redox mediators; among them, Prussian blue, i.e., iron(III)/iron (II) hexacyanoferrate(II), still constitutes an often used material when pursuing the activation of electrocatalytic reductions, e.g., of hydrogen peroxide, and even oxidation. Materials containing silicon centers constitute particularly stable inorganic frameworks with very interesting properties. As better described in Chap. 7, montmorillonites, zeolites, and mesoporous silica-based materials possess characteristic functionalities that impart the electrode selective reactivity toward specific classes of analytes or allow the stable deposition of effective redox mediators. Hydrotalcites and hydrotalcite-like clays constitute the anionic counterpart of cationic clays; their part in electroanalysis has been extended thanks to their ability to be very simply electrogenerated, directly leading to the modified electrode starting from the relevant commercial salts. With respect to silica-based materials, sol-gel technology allows the inclusion of many chemical species, particles, and nanobjects in a very stable inorganic matrix possessing, on its own, specific reactivity and affinity toward given species in solution. The sol-gel material, when made conduct electricity adequately (e.g., with the addition of proper quantities of graphite), is also suitable to constitute the whole body of the electrode, presenting to the solution a surface that can be easily and ‘indefinitely’ renewed with good reproducibility. Finally, among inorganic materials, polyoxometalates are also proposed for electroanalytical purposes.

Polymer coatings probably constitute the most common modification of electrode surfaces in electroanalysis. Basically, three types of polymers can be identified: ICPs, RPs, and ion exchange polymers.

ICPs (see Chap. 2) have been relatively recently introduced and are still attracting enormous interest. They have found effective application in a number of fields, possessing potentialities that are far from being fully explored. They exhibit a variety of properties, from electrochromism to electrical conduction, also being used as sensing materials and as actuators. The intra-molecular conduction is due to delocalization of charge, made possible by the presence of delocalized π-electron systems on a high enough number of structural units, linked to each other in an oligomeric or polymeric chain. The terms p- and n-doping are used in analogy with inorganic semiconductors, indicating partially oxidized and partially reduced polymer chains, respectively; doping makes the polymer assume much higher conductivity, often passing from being semi-conductive to conductive. The intermolecular conduction occurs between adjacent molecules, thanks to redox reactions, or electron hopping events; it often constitutes the major source of resistance.

Polypyrroles, polyanilines, and polythiophenes are the most extensively used ICPs. Polypyrroles and polythiophenes currently attract the highest attention, thanks to the possibility of inserting different functionalities as substituents to the ring. Enormous room potentially exists for applications of these derivatives in amperometric sensing. It seems that the number of electroanalysts working in this field, as well as in others dealt with in this book, is simply too low.

RPs, extensively treated in Chap. 3, mainly consist of insulating polymer chains stably anchoring redox mediators, constituting an intrinsic part of the RP, at the electrode. Redox mediators typically consist of species reversibly switching from oxidized to reduced forms, and vice versa. Charge percolation throughout RPs, as well through ICPs, has been the object of many investigations. In particular, in RPs, it takes place by 'inner' redox reactions between adjacent similar redox couples, also called 'electron hopping' mechanism, coupled to ionic migration necessary to preserve electroneutrality. Organic (quinones and viologen residues) or inorganic redox sites (different transition metal ions, such as Rh, Ru, Ir, Os, Co, with a variety of ligand sets) are present within the polymer chains of RPs. Only a limited number of innovative molecules have been developed recently; on the other hand, those synthesized since the beginning (i.e., back to the 1980s) have not always been applied in electroanalysis: a very limited effort has been made in applicative studies on real non-trivial matrices. This happened, and still happens, despite the potential interest and variety of systems that modern coordination and organometallic chemistry on the one side, and polymer science on the other, do offer. Similar arguments hold for metallopolymers, in which the two electrical conduction mechanisms, proper of RPs and of ICPs, are more or less advantageously joined. They offer the potential possibility of stabilizing metals in oxidation states of interest for the induction of electrocatalytic charge transfers. Metallopolymers are also extensively dealt with in Chap. 3.

Ion exchange polymers, mainly anionic (e.g., Nafion®) but also cationic (e.g., Tosflex) in character, are used to concentrate ionic analytes close to the electrode, mimicking somehow what happens at Hg in the stripping methods of analysis. These polymers are also used simply to entrap and fix a variety of ionic species, enzymes included, acting as the proper redox mediator. They are specifically described in Chap. 4.

In the case of all these polymers, the adhesion forces to the substrate are strong enough to assure stability to the substrate|deposit system. In particular, electrogeneration of the ICP and, possibly, of RPs, directly on the substrate, realizing the electroanalytical device in a single step, constitutes the most spontaneous way to obtain the polymer-modified electrode. As a general consideration concerning electrochemical synthesis of modified electrodes, not strictly related to polymer coatings, it allows wide modulation of the properties of the coating by suitably choosing the electrochemical parameters. Furthermore, in many cases it is found to lead to quite reproducible electrode coatings.

Inside ICPs but, more frequently, inside more common, non-conductive polymer matrices, very highly selective centers may be present. 'Molecularly imprinted polymers' are generated around the species they are thought to host at the highest selectivity level. Molecularly imprinted polymers may be prepared by polymerization of functional and cross-linking monomers in the presence of the target molecule. They act as the best possible molecular templates for the analyte. Particularly in the case of molecularly imprinted polymers devoted to recognition by amperometric techniques, the problem of how to transmit the capture of the target species to the substrate electrochemical transducer is still open. It is evident that in principle

ICPs constitute the polymer matrix of choice in this respect. However, it is not currently as frequently used as it could be.

Nanochemistry, nanophysics, and nanotechnology have been the source of a number of 'nano' objects, inducing an increase of new proposals in the last 15 or 20 years. The peculiar properties ascribed to the dimension and shape, which is thoroughly discussed in the following chapters, specifically in Chap. 6, render them of outstanding interest in view of satisfaction of some of the most important peculiarities sought in a modifier. However, note that the term 'nano', so often found in the scientific literature, does not always actually refer to dimensions spontaneously quantified by nanometer units. This is definitely not a semantic issue, since only true nano dimensions ascribe the relevant objects peculiar properties.

Considering carbon-based nano-objects, C60 and C70 fullerenes were the first to be considered in the 1980s, fascinating the electrochemists with their capability of undergoing many subsequent reversible charge transfers: there was a true run to the driest possible solvent to allow the 'balls' to host one more electron. Single-wall and multi-wall carbon nanotubes were then on the rise. The 'idea' that the catalytic properties of these carbon nanotubes are actually ascribed to the residual metals from the synthesis is now a matter of intense discussion. Finally, graphene was introduced a few years ago as the last of the carbon nano-objects: without doubt, much effort is still required before the promising properties of such a material are exploited.

Among the different nanostructures based on metal and metal oxide, NPs have been synthesized and deeply studied with regard to their potential in electroanalysis. Most of the studies deal with metals such as Ag, Pt, and Au, which is far from taking full advantage of the available and potentially suitable possibilities. Furthermore, a partially discarded aspect regards the variable character of the encapsulating agent that is an often underestimated powerful portion of the NP system as a whole: the chemical cloud surrounding the NP metal core may act as a filter with respect to molecules approaching the nuclei.

In recent years, significant attention has been devoted to hybrids (see Chap. 6), or multi-component materials. Hybrids present a number of opportunities, thanks to the possibility of combining different materials within different structures. Apart from creating a structure with intimate contact between different metal oxides, and of metals with metal oxides, particularly interesting properties are ascribed not only to alloys, but also to mixtures of metal NPs. The resulting properties reflect, on one hand, those of metal NPs, typically ascribing catalytic properties. On the other hand, the close proximity of atoms of different natures may induce interesting co-catalytic effects, or even address the charge transfer toward a more favorable path, e.g., by preventing the occurrence of poisoning adsorptions. Incidentally, note that an important fraction of materials that are named 'hybrids' are actually 'composites' [19].

Thanks to the stability of the adhesion to the substrate and their conductive nature, ICPs often constitute the support of choice to anchor at the electrode substrate a number of further components committed to playing more specific roles, e.g., to activate electrocatalytic charge transfers. The additional component may simply be included; more intimate and stable contact may occur through coulombic interaction or even by the formation of true covalent bonds. The inclusion of nano-objects inside ICPs actually constitutes one of the possible effective ways to anchor the nano-objects at the substrate and, at the same time, to develop bi-component systems in which both peculiarities of the two components are simultaneously exploited. Once more, synergistic effects are also possibly pursued.

Different procedures have been proposed in order to include NPs inside ICPs; the method of choice depends on the nature of the specific ICP/NP couple. Pre-forming NPs is often preferable, due to the possibility of achieving the best control of the relevant characteristics, namely the dimension distribution and the nature of the encapsulating agent. The deposition of NPs from a solution onto the preformed ICP coating renders the ICP surface at the ICP|solution interface more densely coated by NPs. Conversely, electrodeposition of metals by cathodic reduction of relevant salts is also effective. However, such a procedure does not always lead to comparably narrow dimensional distribution of the resulting metal inclusions, which can sometimes hardly be assumed to be under nano dimensions.

1.4 Amperometric Sensing for Specific and Aspecific Analyses

In the majority of cases reported by the literature, modified electrodes are used in ‘classical situations’ in which the quantification of a target analyte is pursued. The efforts are spent finding a physical rationale, with a relevant mathematic function, relating the intensity of the current responses and the amount of analyte in solution. We want to cite here a different, particular approach to the analysis of complex samples that has been more rarely proposed. Assuming that the reader is familiar with multivariate analysis, aiming either to classification or to regression, he/she usually relates such a tool to compositional analysis on a series of samples. In the case in which the composition of the samples in respect to a number of chemical species is known, a bi-dimensional matrix is built in which, in different columns, the quantities of the different species chosen to represent the different samples (one row for each sample) are reported. On the other hand, it is well known that Near InfraRed (NIR) or even medium infrared spectra may be fruitfully used as ‘photographs’ (so-called ‘fingerprints’) of a sample: each sample is identified by the absorbance values at different wavelengths rather than by its own composition. Some call this approach ‘blind analysis’, referring to the peculiar aspects of coming to conclusions without knowing anything about the sample except for how it appears when observed with electromagnetic radiation, not relating the single absorptions to specific physical or chemical characteristics of the molecule.

Similarly, electronic noses were developed in order to ‘take a picture’ of the complex gaseous mixtures, typically on the basis to their effect on the electrical conductivity of semiconducting metal oxides. A similar approach is followed in the so-called electronic tongues, which are still much less established than noses. In particular, potentiometric and amperometric sensors for blind analysis of liquid samples have been proposed. The more easily controlled environment consisting of a solution could constitute a favorable element with respect to gaseous mixtures, even tested in natural environment, with so many uncontrolled variables.

Amperometric electronic tongues consist of a number of amperometric sensors, giving different voltammetric responses for the same sample, typically belonging to a complex matrix. This may be a food matrix, an environmental matrix, or other. The amperometric sensors composing the array are required only to be partially selective, characterized, however, by a different ‘interaction’ with the species in solution. In this respect, the variety offered by organic or inorganic modifiers, or even by composites, is definitely unprecedented. The signals coming from the different elements of the array are combined and treated with multivariate analysis techniques. The currents at different potentials parallel the absorbance values at different wavelengths in infrared blind analysis: an electrochemical picture of the sample is obtained. The goals pursued are typically (1) classification of the different matrices with respect to specific characteristics, such as the geographical origin or the varieties of the primary materials from which it comes, by using either simple ‘principal component analysis’ or ‘partial least square’ plus ‘discriminant analysis’; (2) multivariate regression, to estimate a parameter of the matrix that is of interest and difficult to determine directly. In this respect, note that the most correct approach relies in checking the information content, with respect to the classification or regression sought, of each of the sensors present in the array, in order to eventually remove those bringing noise, i.e., useless information with respect to what is being sought. Similarly, the most informative portion of the voltammograms can only be taken, avoiding the polarization of the electrode on a wider potential range than strictly necessary. Both choices correspond to the ‘feature selection’ operation in more classical similar data analyses. By following such an approach, it may even be that a single electrode is sufficient to lead to satisfactory results.

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Chapter 2
Intrinsically Conducting Polymers

Thanks to the presence of a π–electron conjugated system, a few organic polymers, such as polypyrrole (PPy), polyaniline (PANI), polythiophene (PTh), and relevant derivatives, are classified as semiconductors that become well conductive by partial oxidation or reduction [1–10]. For this peculiar characteristic, similar materials are called 'intrinsically conducting polymers' (ICPs). The structures of the most meaningful ICPs are displayed in Fig. 2.1.

Since the discovery, ICPs have been predicted to constitute the key materials of the next generation of electronic and optical devices. The beginning of research on ICPs goes back to the studies by Heeger, MacDiarmid, and Shirakawa: films of polyacetilene (PA) present a conductivity lower than 10^{-5} S cm^{-1}, but this value increases up to approximately 10^{2} S cm^{-1} when exposed to iodine vapor [11, 12] or to other oxidizing or reducing agents. Oxidation and reduction processes are called 'doping', in analogy with doping of inorganic semiconductors, though based on quite different events, i.e., true partial oxidation or reduction. Both these processes induce electron delocalization along the π−electron system, with consequent charge percolation within a single chain. This mechanism accounts for the intra-molecular conductivity. However, similarly to redox polymer discussed in Chap. 3, electron hopping between adjacent polymer chains, i.e., the inter-molecular conductivity, should also be considered in order to account for the actual capability of the material to carry electrical charge. Despite that, in many cases, the inter-molecular conductivity is supposed to be the rate-determining step of the conduction as a whole, much more effort has been devoted to studying intra-molecular charge percolation to which, within a certain limit, a rationale can be given. On the other hand, it must be admitted that inter-molecular conductivity involves many structural and conformational features of the specific polymer mass. As it happens in the case of redox polymers, a thoeretical approach is only possible once referred to 'typical' situations.

PA was the first reported polymer exhibiting high electrical conductivity. Thanks to this discovery and to their work on ICPs, Heeger, MacDiarmid, and Shirakawa were acknowledged with the Nobel Prize in Chemistry in the year 2000.

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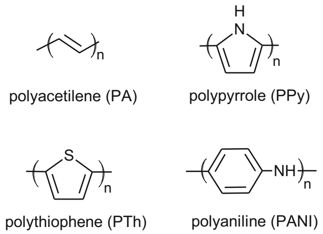


polyacetilene (PA)

polypyrrole (PPy)

polythiophene (PTh)

polyaniline (PANI)

Fig. 2.1 Chemical structure of main intrinsically conducting polymers (ICPs)

However, the environmental instability of PA constitutes a major obstacle to practical application: when this polymer is exposed to air, oxidation of the backbone by O_2 occurs, causing breakage of the C–C chemical bonds [13].

ICPs proposed later possess higher environmental stability, both in the neutral and in the p–doped state [14]; this aspect makes them more suitable for real applications. At the same time, the structural versatility of the ICPs allows the modulation of their physico-chemical properties, by suitably varying the monomer structures. This last aspect has given an enormous impulse to the synthesis and characterization of a very high number of new products, also envisaging the technical properties sought for a specific application. In line with the evolution of these materials in many technological fields, ranging from anti-corrosion coatings to flexible light-emitting diodes, amperometric sensing has also benefitted from the properties of ICPs. The main reason lies in the possibility of covering the electrode surface with a conductive material, capable of imparting antifouling and electrocatalytic properties, as well as activating possible selective interactions with the analyte. Furthermore, the development of nanostructured hybrid materials has opened exciting new perspectives to applications of ICPs in electroanalysis. In addition to constituting a conducting support to effective electrocatalytic species at the electrode surface, often also ascribing the electrode system antifouling properties, ICPs are claimed to activate co-catalytic processes, arising from a synergic action between the two components of the composite material. This Chapter deals with the properties and applications of ICPs as the single component of a coating, or even including biological elements or redox active metal complexes trapped inside. The properties of true composite materials, consisting of ICP and nano-objects of different natures, is treated in Chap. 6.

It is important to note that we do not want to mimic the excellent work of Inzelt in a recent monograph of this series [7]; the discussion here is focused on a critical examination of the most meaningful characteristics of ICP that have actually been or that are potentially suitable to be exploited in applications of ICPs in electroanalysis.

2.1 Synthesis and Deposition of ICPs onto Electrode Surfaces

ICPs can be prepared through different chemical or electrochemical routes. However, it is evident that, when aiming to obtain a homogeneous film covering a conductive support, i.e., of an electrochemical device, the electrochemical approach constitutes the most spontaneous and effective way to easily obtain the modified electrode. It also offers the advantage of a single-step process.

To this aim, the electrode is immersed in a solution containing the monomer and a suitable supporting electrolyte; it is then polarized at a suitable positive potential, where monomer oxidation occurs. The most widely accepted polymerization mechanism is represented in Fig. 2.2.

The first (electrochemical) step consists of the oxidation of the monomer to the corresponding radical cation. The second (chemical) step involves the coupling of two radicals to produce a di-hydro dimer dication that, in a further (chemical) step involving the loss of two protons, leads to a neutral dimer, with concomitant occurrence of re-aromatization. Thanks to the corresponding stabilization, this last event constitutes the driving force of the whole homogeneous process. Due to the higher electron delocalization, the dimer is more easily oxidized than the monomer, so it is in turn oxidized at the electrode to the relevant radical cationic form and undergoes a further coupling with a monomer or a dimer radical. Hence, electropolymerization proceeds through successive electrochemical and chemical steps according to an $E(CCE)_n$ mechanism until the oligomers become insoluble in the electrolytic medium. Since the homogeneous reactions are very fast, the relevant reaction layer is usually much smaller than the diffusion layer, so the yield in oligomers that precipitate onto the electrode is high. The further increase of film thickness is allowed, and even favored, by polymer p–doping occurring at a less positive potential with respect to monomer oxidation: the already formed ICP deposit is well conducting, and monomer oxidation experiences a lower charge transfer overvoltage than on the bare electrode.

Although many scientists have put enormous effort into studying how the electropolymerization–deposition process exactly occurs [2, 7, 9], some aspects

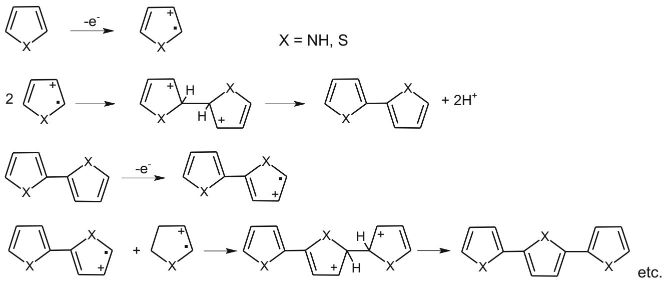


Fig. 2.2 Mechanism of oxidative polymerisation of intrinsically conducting polymers

are still unclear. As an example, in quite a recent review, Heinze et al. [9] still discuss the mechanism of polymer growth, occurring either by progressive addition of monomer units to the oligothiophene chain, as depicted in Fig. 2.2, or by a dimerization step of longer and longer radical cations generated. A further point debated about the polymerization mechanism concerns the site where polymer growth occurs, i.e., in solution before precipitation onto the electrode surface or on oligomeric chains already deposited on the electrode surface [15].

To preserve electroneutrality, anions from the supporting electrolyte enter during polymer p–doping, and a fraction of these are expelled in the de-doping step; however, not all of them ‘find the exit’, being included inside the polymer in excess with respect to the residual positive charges: cations are forced to enter to achieve bulk ICP electroneutrality. It was observed that a significant number of ions can be irreversibly trapped inside the electrode coating: the final situation consists of residual charges on the polymer chains, compensated by anions, and a certain amount of salt [16–18]. A higher level of anions are retained inside the film if they consist of bulky species. They can be suitably chosen in order to affect the electrochemical properties of the material, e.g., to induce filtering effects toward negatively charged interfering species from the solution, eventually improving the selectivity of the sensor [19].

By exploiting similar phenomena in doping and de-doping steps, many different redox active species, ranging from metal complexes to enzymes, can be included in ICP-based films (see hereafter). In comparison with inclusion of similar species in non-conducting polymers, the advantages offered by the improvement of the charge transmission throughout the film are obvious, at least in the case of amperometric sensors.

As can be easily inferred from what described, the nature of the solvent used for the electropolymerization process also plays a basic role in conditioning the properties of the resulting electrode coating. The primary role possibly requested of the solvent is to highly dissolve the monomer and very poorly dissolve oligomeric chains of the sought length at the same time. As a consequence, it strongly conditions the actual length of the polymer chains. However, solvation also affects the spatial disposition of the polymer chains on the electrode surface, as well as the hydration sphere of the supporting electrolyte that is included into the film.

In this scenario, it is evident that, when trying to obtain an electrode coating possessing specific characteristics for the electroanalytical application envisaged, quite a high number of variables should be considered in preparing the polymerization solution. One of the first choices to make is the electrochemical technique to use. In particular, electropolymerization can be carried out (1) by continuously cycling the electrode potential (potentiodynamic growth), (2) by polarizing the electrode at a fixed potential (potentiostatic growth) or (3) by applying a fixed current density (galvanostatic growth).

When using a cyclic voltammetric technique (Fig. 2.3a), the potential is varied linearly from a value at which no electrode reaction takes place to a value at which monomer oxidation occurs. When the highest potential values are reached, the series of charge transfers, radical–radical coupling reactions, and p-doping take

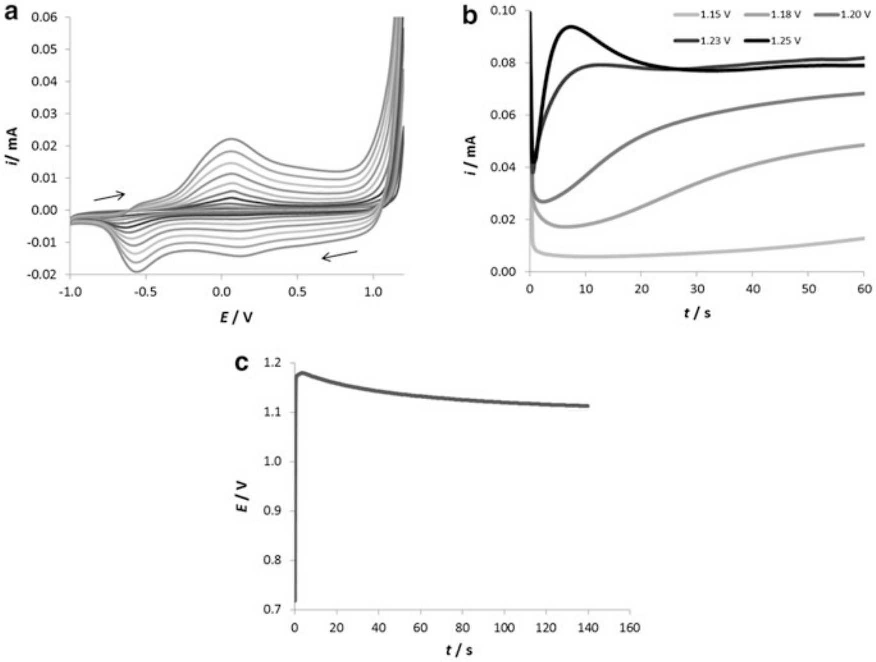


Fig. 2.3 (**a**) Potentiodynamic (ten scans from -1.0 to $+1.2$ V, 0.05 Vs^{-1} potential scan rate), (**b**) potentiostatic (60 s at different applied potentials) and (**c**) galvanostatic ($j = 0.4$ mA cm^{-2}, $Q_{\mathrm{tot}} = 2$ mC) electropolymerization of 10 mM 3,4–ethylenedioxythiophene, 0.1 M $\mathrm{LiClO_4}$, $\mathrm{CH_3CN}$ solution

place, as far as the chain is long enough to precipitate onto the electrode surface. Reversing the scan to less positive potentials, discharge (i.e., neutralization of the p–doped polymer deposited on the electrode) is observed in correspondence with a backward cathodic peak. In the following scans, the occurrence of an anodic/cathodic peak system at less anodic potentials than the monomer oxidation, growing in height scan after scan, is observed. It must be ascribed to the charge/discharge of the polymer coating progressively formed on the electrode surface, which represents the direct confirmation of actual polymer deposition. Concurrently, monomer oxidation occurs at less positive potentials, thanks to the electrocatalytic properties of the polymer coating.

Potentiostatic polymerization is carried out by polarizing the electrode at a fixed potential at which monomer oxidation occurs. The actual formation of a polymer coating on the electrode surface is confirmed by a characteristic chronoamperometric response, as shown in Fig. 2.3b. It is characterized by a current decay in the initial stage of the process, when radicals and oligomeric chains are formed, followed by a current increase that accounts for the additional p-doping process, consequent to precipitation of polymer onto the electrode surface [20]. As observed in Fig. 2.3b, the current increase occurs after an interval time that is lower the higher the applied potential. Moreover, the value of the applied potential can also strongly affect the morphology of the resulting polymer coating. The charge collected at a potential at which monomer oxidation occurs is, to a first approximation, directly proportional to the amount of polymer deposited. For this reason, fairly reproducible film coatings can be obtained by fixing the amount of charge spent during the electrogeneration process, at a given concentration of monomer in solution, once the polarizing potential is also fixed.

The electrogeneration of ICPs can be also performed through application of a constant current over a pre-fixed time length (Fig. 2.3c). It is well known that, in galvanostatic electrolysis, care should be taken about the potential assumed, in order to prevent reaching values at which over-oxidation of the deposited polymer occurs. This would imply irreversible polymer degradation. Also in this case, the total charge spent in the electrochemical process is directly related to the amount of polymer deposited, i.e., to the thickness of the resulting film, reasonably hypothesizing that the yield of the charge spent in polymer formation is constant over time.

Very careful attention should be paid to the experimental conditions followed (e.g., concentration of the monomer, parameters of the technique used, geometry of the electrochemical cell, etc.) in order to obtain reproducible deposits, in terms of thickness, size distribution of the macromolecules, and structure of the deposit. Once care is taken with respect to these key points, electropolymerization is definitely the most suitable tool to rapidly and directly modify the electrode surface. Rapidity in obtaining the modification is one of the most immediate features: the in situ growth of coatings with a thickness lower than a few hundreds of nanometers only requires a few seconds, which is much shorter than the several hours required by chemical methods. On the other hand, the chemical process permits the simultaneous synthesis of a large amount of polymer. This allows easy purification of the resulting material, e.g., through a Soxhlet extraction, which also leads to removal of short oligomeric chains. The attainment of a pure material permits a deeper characterization of the material through a variety of sensitive techniques, such as nuclear magnetic resonance (NMR) and mass spectrometry. Chemically synthesized polymers are suitable to be dispersed in the bulk of the electrode material, whenever appropriate, e.g., included in carbon paste [21] or in sol-gel [22], or anchored at the surface, e.g., by layer-by-layer deposition [23].

2.2 Polymer Doping and De-Doping

As already outlined, partial oxidation or reduction at suitable potentials converts the semiconducting neutral polymer to much more conductive cationic or anionic forms, respectively (see Fig. 2.4). As already outlined, according to a physically improper analogy with inorganic semiconductors, oxidation is said to lead to the p–doped polymer, while reduction to the n–doped form.

In analogy to inorganic semiconductors, the ‘band theory’ constitutes the generally accepted simplest model to give account for the conduction mechanisms in

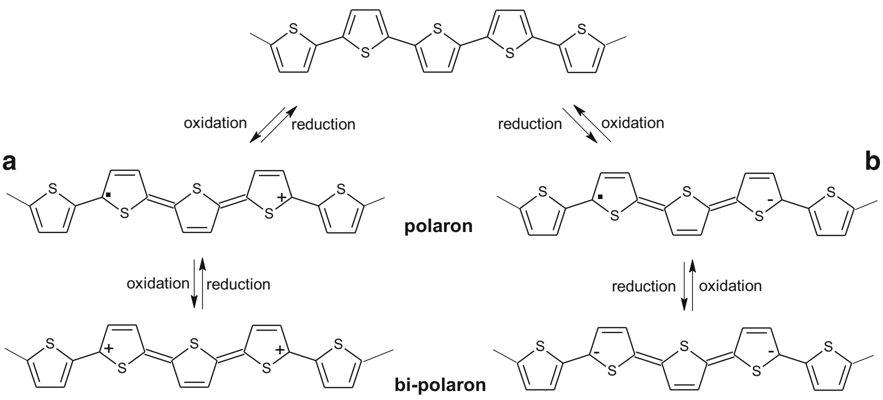


Fig. 2.4 Generation of (**a**) positively and (**b**) negatively charged polaron and bi-polaron in polythiophene, taken as prototypical example of conjugated organic polymers

ICPs [24]. Actually, the band model holds in the case of inorganic semi-conductors, i.e., materials where each atom satisfies the characteristic coordination number in a three-dimensional rigid structure. The model needs significant modifications in order to be applied to ICPs, because in this case the extraction or addition of an electron is coupled to ‘chain distortion’ that alters the energy level of the electron system. However, new proposals are far from being easy to access by those who do not need to go deeper into the issue.

In the frame of a rough though useful scheme, positive or negative polarons (unpaired electron possessing an electric charge) and positive or negative bipolarons (carbodications or carbodianions, respectively) are implied in the intra-chain conductivity mechanism in ICPs. In the case of neutral *trans*-PA [3, 24], neutral solitons, consisting of unpaired electrons without electric charge, are also present. Figure 2.5 schematically represents the different energy levels of the orbitals of a polymer chain in the undoped and doped form, respectively. The highest occupied electronic levels fix the energy of the valence band (VB), while the lowest unoccupied levels constitute the conducting band (CB). The energy difference between VB and CB is called band-gap and determines the intrinsic electric properties of the material: the band-gap of neutral ICPs is generally larger than 1.5 eV or even than 2 eV, which is too high a value for thermally activated conduction; ICPs in the neutral state can be considered as intrinsic semiconductors or even insulating.

The loss or acquisition of an electron generates a radical cation or a radical anion, respectively. They are called positive and negative polarons, respectively: polaronic states arise within the band-gap. Considering, for the sake of brevity, the case of p–doping, the arising of a polaron requires oxidation and induces distortion in a low number of structural units over which the polaron is delocalized. Further electron extraction requires that different polarons are confined in the same polymer chains, inducing a more marked chain distortion. As a result of distortion and

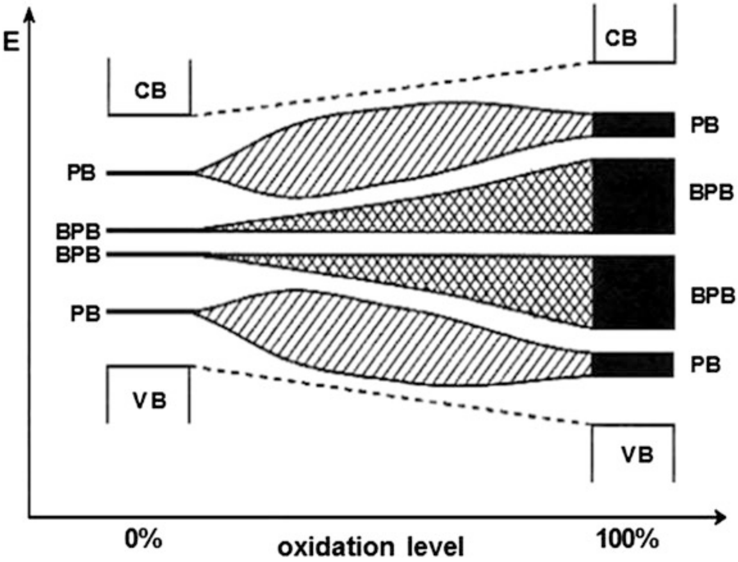


Fig. 2.5 Evolution of the population of polaron and bipolaron bands during polymer oxidation. *VB* valence band, *CB* conducting band, *PB* polaron bands, *BPB* bipolaron bands. The width of the polaron and bipolaron bands accounts for the relevant population (Reproduced from Ref. [25] with permission of Springer)

reciprocal interaction between adjacent polarons, the orbitals of the different polarons are not all at the same energy. Past a certain amount of charge injected, the closeness of similarly charged atoms in a π–electron delocalized system leads to loss of the discreteness within energetic levels and to consideration of the system in terms of continuous-energy bands: a polaron band arises within the band-gap, and polaronic conductivity is effective.

Forcing the oxidation further, bipolarons arise because they are more energetically favored than the presence of two polarons in the same oligomeric unit. The process evolves at increasing the applied potential, as far as the chain is also saturated in bipolarons. Actually, bipolarons may form when the chain is not yet ‘saturated’ by polarons: bipolarons are also effective in imparting intra-chain conduction.

At even higher potentials, no additional charge can be hosted by the chain without implying an irreversible breaking of the aromatic structure: over-oxidation occurs.

Cyclic voltammetry is the most widespread electrochemical technique used to investigate charge and discharge processes occurring at ICP-modified electrodes; chronocoulometry and double-step chronocoulometry are of comparable meaning, and are particularly suited to gaining information about the charge spent in charging/discharging processes. As is evident in Fig. 2.6, showing a PTh derivative as an example of the general behavior of ICPs, the formation of positively charged

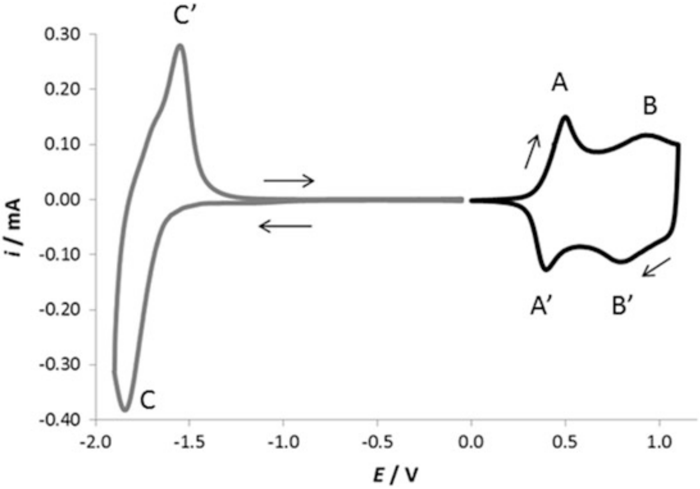


Fig. 2.6 Steady state cyclic voltammograms of poly(4,4′–bisbutylsulphanyl–2,2′–bithiophene) coated electrode in CH_3CN, 0.1 M $(C_4H_9)_4NPF_6$, limitedly to anodic (*black line*) or cathodic (*grey line*) potential region. 0.01 Vs^{-1} potential scan rate. A,A′ and B,B′ are peak systems relative to p-doping and de-doping processes for polaron and bipolaron, respectively; C,C′ is a peak system relative to n-doping and de-doping (Reproduced from Ref. [26] with the permission of Elsevier)

polaron and bi-polaron states results in a couple of well resolved anodic peaks (A and B, respectively). The width of these peaks reflects the presence of polymer chains possessing different band-gap values, as a result of the distribution of molecular weight, the variety of chain conformations, and the inter-chain chemical interactions; all these effects contribute to render a scheme of single energy levels, rather than of bands, unreliable for such a complex system. On the other hand, peaks C and C’ are relative to n–doping and corresponding de-doping, respectively.

The difference between the p– and n–doping potential values constitutes an estimation of the so-called ‘electrochemical band-gap’ of the polymer, often similar to the ‘spectroscopic band-gap’, consisting of the energy corresponding to the onset of the absorption of the polymer in the visible region. As already mentioned, the band-gap is an intrinsic property of basic importance in respect to many characteristics of the material. In particular, the lower the band-gap, the narrower the potential region within which the polymer is neutral, i.e., poorly conductive. The importance of these properties in terms of an application in the field of amperometric sensors is quite obvious.

As cited above, the occurrence of net positive charges during the p–doping process results in anions from the solution being included in the film to preserve the electroneutrality of the material. The occurrence of cathodic peaks in the backward scan (A’ and B’) indicates that p–doping is a reversible process. As a result of the processes described, the polymer coating acts as a sponge, swelling and shrinking during doping and corresponding neutralization processes, respectively;

the entrance and exit of counter-ions are coupled to the entrance and exit of tiny quantities of solution. Of note, the charge spent in de-doping is lower than that used in doping because of the occurrence of 'trapped anions', which also implies the occurrence of 'trapped charges', i.e., of incomplete neutralization of the polymer chains, as discussed previously.

Similar events occur at positive (p–doping) and negative (n–doping) potentials. We talk of positively or negatively charged polarons and bipolarons, respectively. However p–doping is possible for most ICPs, while n–doping may not be, because the solvent discharge often prevents the polymer reduction from occurring. It is worth highlighting that p–doping has been widely and deeply studied and even profitably exploited in electroanalytical applications. Conversely, n–doped ICPs, when even obtained, are much less frequently used due to the poor stability of the negative charges on the polymer at the rather negative potentials that are usually required for the reduction to take place. Obviously, the necessary removal of oxygen and water oxidants from the system is a further heavy limitation to the actual application of ICPs in the n–doped state.

Importantly, the presence of trapped charges results in the polymer being partially conductive, even at potential values lower than those of the de-doping process. This allows the use of the material in potential regions where it should be, in principle, neutral, i.e., poorly conductive. Furthermore, the occurrence of trapped charges can be exploited to irreversibly trap into the film redox-active species suitable for activating redox mediation in electrocatalytic processes.

As previously outlined, the inter-chain charge transfer most often constitutes the rate-limiting step. Polymers in which different chains are bridged with each other by conductive branches have been synthesized in order to improve the overall conduction [27, 28]. Peculiar examples of hybrids meeting with similar conditions are reported in Chap. 3, when discussing metallopolymers.

2.3 Principal ICPs Derivatives Used in Electroanalysis

2.3.1 Polypyrrole

PPy is one of the most frequently used ICPs in electroanalysis [29]. This is due both to the ease of electropolymerization (also possible in aqueous solutions and within a wide pH range) and to the relatively high stability of the p–doped polymer under ambient environmental conditions. Furthermore, many studies reveal that this material is biocompatible [30], which constitutes a fundamental feature when considering sensors for some clinical applications.

Among different ICPs, pristine PPy is characterized by very low band-gap values, resulting in the p–doping process occurring at relatively low potentials. The neutral polymer can be directly oxidized by oxygen present either in air or in aqueous solution. Although a majority of PPy applications in electroanalysis concern the pristine polymeric form, several derivatives have been synthesized, aiming at introducing specific functionalities to obtain selective interaction with the target analyte. To this aim, the reactivity of both β–positions of the aromatic ring, and more so of the N atom, have been exploited. At the same time, poly(3,4–alkylenedioxypyrroles) have also been proposed [31], similar to the wide diffusion of the analogous Th derivative (see hereafter), aiming at further reducing the potential at which p-doping of the material occurs, by introducing electron donor groups.

It is important to mention here that over-oxidised PPy (oPPy) has been also proposed in many electroanalytical applications, particularly in the frame of biosensors and coatings involving molecularly imprinted polymers (MIPs) [32–39]. Over-oxidation occurs by polarizing the polymer coating at potential values much higher than p–doping [40]. Depending on the experimental parameters chosen, over-oxidation can induce different degrees of polymer modification. When taken to the limit, the process leads to almost complete expulsion of anions from the coating and the formation of carbonyl groups in the β–positions of the PPy chains. Although over-oxidation drastically reduces the conductivity of material, it confers the polymer net negative charges. The coating finally possesses a remarkable permselectivity with respect to positively charged species from the solution, resembling the non-conducting polymers described in Chap. 4; on the other hand, it offers an additional advantage in terms of the control of the process of film electrogeneration. This aspect is not only fundamental in order to improve the reproducibility of film thickness and morphology, but also to insert a high amount of species of interest, e.g., enzymes, during the electrogeneration process (see hereafter). Moreover, depending on the conditions chosen for the electrogeneration of the precursor ICP and of the relevant further oxidation, the conductivity of the original material can be partially retained.

2.3.2 Polyaniline

As depicted in Fig. 2.7, PANI is found under a variety of forms differing in chemical and physical properties [41]. The structure consists of reduced (benzenoid diamine) and oxidized (quinoid diamine) repeated units. The form under which it is commercially available and stable under ambient conditions is Emeraldine Base (EB), possessing equal amounts of reduced and oxidized repeating units. The conductivity of this form is rather poor, but it can be easily converted into a more conductive form by lowering the pH of the solution to obtain the relevant fully protonated structure, namely Emeraldine Salt (ES). For this reason, PANI shows significant conduction in acidic solutions, namely below pH 2.5–3.0.

Conductivity of PANI can also be controlled by changing its oxidation state by either chemical or electrochemical treatment. In this way, the fully reduced or oxidized states can be reached, namely Leucoemeraldine (LE) and Pernigraniline (PG), respectively.

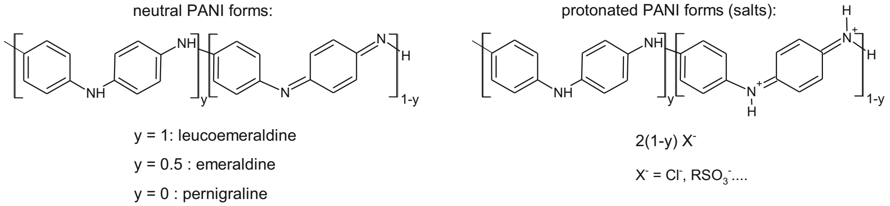


Fig. 2.7 Different structures of polyaniline (PANI): fully reduced form (leucoemeraldine, $y = 1$); partially reduced form (emeraldine, $y = 0.5$); fully oxidised form (pernigraniline, $y = 0$). PANI neutral forms may be converted in the relevant salts when treated with strong acids

2.3.3 Polythiophene

Despite the widespread use of PPy in electroanalysis, PTh derivatives may be considered the ICPs preferred nowadays for such an application. The main reason lies in the possibility to easily functionalize the Th ring in the β–positions in order to obtain a material characterized by specific physico-chemical properties, such as high conductivity within a wide potential window or particular interactions with species in solution.

Although the number of Th derivatives synthesized and studied from an electrochemical viewpoint is considerably higher with respect to Py derivatives, the majority of electroanalytical applications involves PTh compounds synthesized from the monomers reported in Fig. 2.8. It is worth highlighting again the big gap between organic chemists and those dealing with electroanalysis.

The use of a dimer, namely 2,2′–bithiophene (BTh), is necessary in unsubstituted PTh electrogeneration due to the very high potential values required to oxidize Th, which induces over-oxidation of the arising polymer chains. For this reason, the most popular Th derivative used in electroanalysis is 3–methylthiophene (MTh): the insertion of an alkyl chain that possesses electron donor inductive property induces a significant lowering of the potential value required for the monomer oxidation. On the other hand, regio-random polymers can be obtained in this case, inducing ‘defects’ in the charge delocalization, eventually lowering the conductivity of the material.

In order to significantly reduce the p-doping potential of the polymer material, oxoalkyl [42] or thioalky [43, 44] groups were inserted in the β–position of the Th ring, inducing strong mesomeric electron donating effects to the heteroaromatic π–system. Head-to-head and tail-to-tail dimers were synthesized in order to obtain regio-regular polymers, characterized by higher conductivity.

The research on PTh derivatives has, in recent years, been strongly oriented to exploiting the very appealing properties of 3,4–ethylenedioxythiophene (EDOT): the presence of two oxygen atoms directly connected to a single Th unit allows this monomer to be oxidized at particularly low potential values. Furthermore, the condensed ring confers the monomer a polarity suitable to make it soluble enough

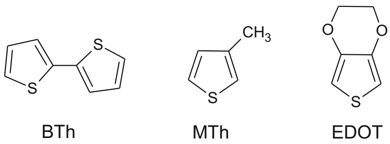


Fig. 2.8 Main thiophene (Th) derivatives used in electroanalysis: 2,2′–bithiophene (BTh), 3–methylthiophene (MTh), and 3,4–ethylendioxythiophene (EDOT)

in aqueous solution to allow polymerization to be carried out in the same medium in which it is subsequently most often employed in amperometric sensors. These aspects, combined with the symmetry of the molecule and the minimum in the steric hindrance of the oxygen-containing rings, which induces co-planarity of extended π–electron system, further favors effective delocalization of polarons and bipolarons. All these characteristics concur to the formation of a polymer, PEDOT, characterized by a very high conductivity and very low band-gap: both p– and n–doping processes are easily accessible [45–47].

2.3.4 Polyphenazines, Polyphenothiazines, and Polyphenoxazines

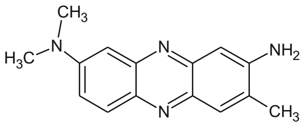
Although less frequently considered as electrode coatings, polyphenazines, polyphenothiazines, and polyphenoxazines [48, 49] are very attractive ICPs, exploitable in some electroanalytical applications. The main interest for these materials lies in their capability to simultaneously act as both conducting polymers and redox mediators [50, 51]. The applications as electrode coatings are particularly focused to the development of enzymatic sensors, both involving NAD(P)H and H_2O_2 detection [52, 53].

The monomers most frequently used in electroanalytical applications are reported in Fig. 2.9. Note that, unlike the monomers described so far, they all form nonlinear polymer chains. Although the exact nature of the material formed by electrochemical oxidation of these derivatives has not been clearly defined, the most plausible structures proposed consist of branched chains, only involving a few monomer units [50, 54].

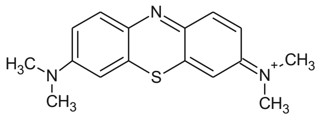
2.4 Applications of ICPs in Electroanalysis

2.4.1 Direct Detection of Organic Species

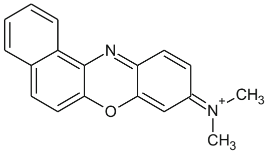
One of the most notable properties of ICP-modified electrodes lies in their capability to activate electrocatalytic redox processes: it has been experimentally



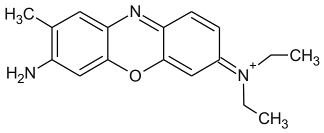
Neutral red



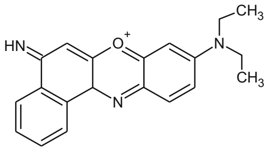
Methylene blue



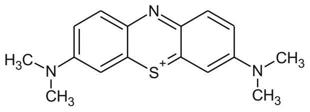
Meldola blue



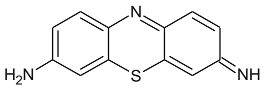
Brilliant cresyl blue



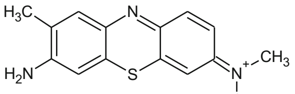
Nile blue



Methylene green



Thionine



Toluidine blue

Fig. 2.9 Chemical structures of polyphenazines, polyphenothiazines, and polyphenoxazines mostly used in electroanalytical applications

verified that the overvoltage that affects irreversible inner-sphere charge transfers at bare electrodes can be significantly lowered at polymer surfaces. Moreover, polymer surfaces are often found to prevent electrode poisoning, one of the main drawbacks of bare electrodes. As a result of both these effects, voltammetric traces recorded at ICP-modified electrodes are generally characterized by repeatable and well defined oxidation peaks, often located at less positive potentials with respect to bare electrodes. Obviously, their position depends on the potential at which the polymer becomes conductive; this is why, as evident from the examples reported in Table 2.1, ICP-modified electrodes are generally used in electrochemical oxidation processes and why PPy and PEDOT, which are characterized by particularly low p–doping potential, are preferred. On the other hand, it is also important to highlight that the applicability of similar electrode coatings is limited to the detection of the chemical species that can be oxidized at not too positive potentials, where polymer over-oxidation may occur.

Quite interestingly, the occurrence of the current signal at significantly less positive potentials makes it possible achieve good resolution between oxidation peaks ascribable to different species in solution. Typical examples are given by the case of solutions containing ascorbic acid and either dopamine [55, 56] or uric acid

Table 2.1 Examples of organic molecules detected at intrinsically conducting polymer (ICP)-modified electrodes

Analyte	Coating	Ref.
Ascorbic acid [80]	PANI	[81]
Ascorbic acid [80]	PEDOT	[82]
Ascorbic acid [80]	PPy/$[Fe(CN)_6]^{3-}$	[83]
Ascorbic acid [80]	PEDOT/$[Fe(CN)_6]^{3-}$	[74]
Hydroquinone	PANI	[84]
Dopamine, epinephrine, catechol, serotonin, ascorbic acid	PMTh	[85]
Dopamine, ascorbic acid	PPy/$[Fe(CN)_6]^{3-}$	[86]
Dopamine, ascorbic acid	PEDOT	[56]
Dopamine	PPy/Congo red	[59]
Dopamine, ascorbic acid, uric acid	PPy	[58]
Uric acid	PPy	[87]
Uric acid, ascorbic acid	PEDOT	[57]
Tyrosine, tryptophan, dopamine	PMTh	[88]
Cysteine	PEDOT	[65]
α–Tocopherol	PPy	[89]
Acetaminophen	PEDOT	[66]
Hydrazine	oPPy	[32]
Phenol	PEDOT/Polystyrene sulphonate	[63]
Phenol	PPy/o–aminobenzesulfonic acid	[61]
H_2O_2	PEDOT/poly(Neutral red)	[53]
2–Mercaptoethanol	oPPy/Co(II)phthalocyanine	[90]

[57]. An example of the effect of the electrocatalytic performance of PPy-modified electrodes in deconvolving voltammetric peaks due to different analytes is reported in Fig. 2.10.

As a further effect of the activation of electrocatalytic processes due to ICP coating, an increase of the current peak intensity can be obtained; this eventually increases the sensitivity of the sensor response and even improves the detection limit, compared with that of the bare electrodes. The current enhancement can be also ascribed to the intrinsic increase of the electroactive area, due to the porous nature of the polymer coatings, which allows diffusion of electroactive species inside the electrode coating. Obviously, this last effect is even more marked when the electrochemical process involves negatively charged analytes, thanks to the occurrence of electrostatic attractive forces, and hence of migration mass transfer, due to the positive charges occurring in the polymer coating during the p–doping process.

However, recalling the general comment already made for many electrode materials reported in this monograph, tests supporting the selectivity of the ICP coating are limited to only standard solutions or, at the most, to simple matrices

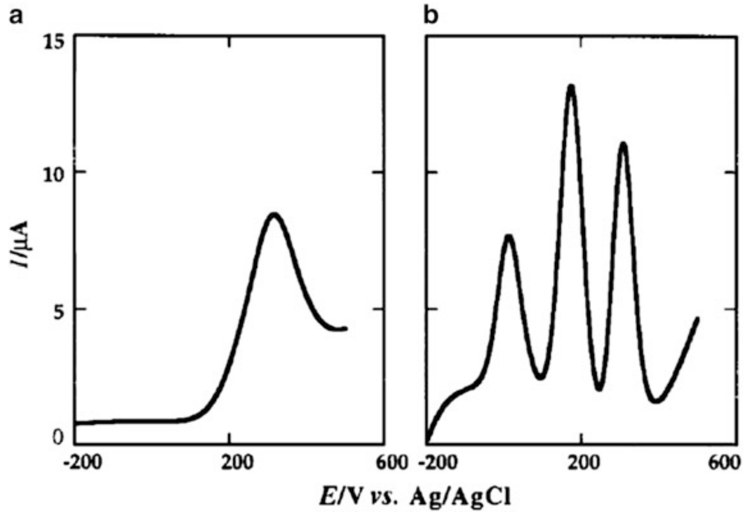


Fig. 2.10 Square wave voltammograms of 0.50 mM solutions of uric acid, dopamine, and ascorbic acid at a (**a**) bare and (**b**) polypyrrole/tetradecyl sulphate modified Au electrodes. 0.10 M phosphate buffer solution (pH 7.0) was the supporting electrolyte (Reproduced from Ref. [58] with the permission of Royal Society of Chemistry)

such as tap water or urine. The problems presented by untreated real matrices are discussed in Chap. 1 of this monograph.

The analyte-coating interaction can be modulated by the insertion of different organic surfactants inside the film during the polymer electrogeneration. As an example, negatively charged species can act as an electrostatic filter with respect to different molecules in solution, hindering or at least limiting the access of negatively charged species to the electroactive surface [19, 59, 60]. Figure 2.11 shows, as an example, the selective oxidation of dopamine in the presence of increasing amounts of ascorbic acid (Fig. 2.11a) and, vice versa, the oxidation of increasing amounts of dopamine in the presence of ascorbic acid (Fig. 2.11b).

As a particular example of the positive effect induced by the presence of bulky counter-ions inside the polymer film, it was observed that electrode poisoning occurring during phenol oxidation can be dramatically reduced when a surfactant containing SO_3^- groups is incorporated inside a polymer film: the film|solution interface is made hydrophilic by the presence of anionic charges exposed to the solution, and the electrode surface is thus protected from passivation induced, on bare electrodes, by the formation of an insulating polyphenol film [61–64].

Besides charged species, the inclusion of hydroxypropyl–β–cyclodextrin during polymer electrogeneration has been reported in a number of papers dealing with analytical applications, particularly involving PEDOT [56, 65–70]. Although the actual role of this species has not been well defined, the performance of similarly modified electrodes is found to be positively affected by its presence inside the film.

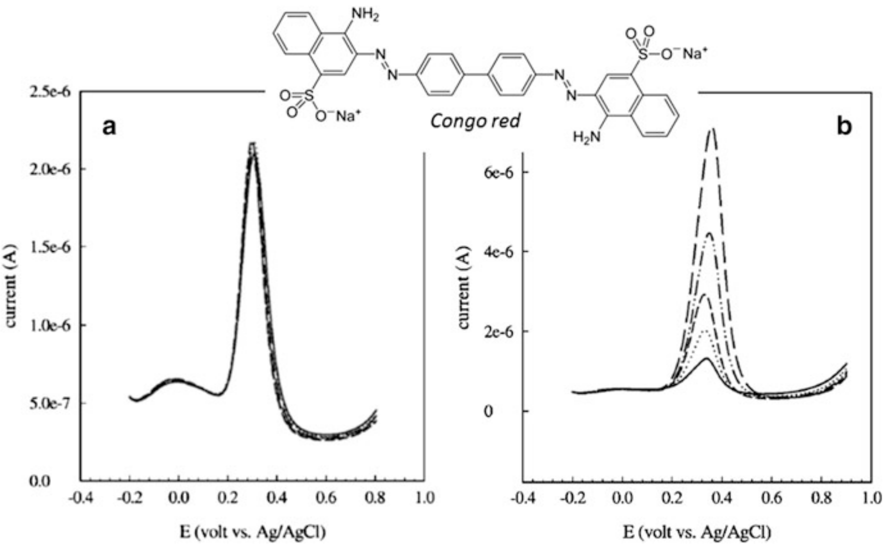


Fig. 2.11 Differential pulse voltammetric responses recorded at polypyrrole/Congo red modified electrodes in solutions containing (**a**) 10.0 μM dopamine (constant) and various concentrations of ascorbic acid (*down* to *up*): 4.0, 10.0, 20.0, 40.0 and 80.0 μM; (**b**) 10.0 μM solutions of ascorbic acid (constant) and various concentrations of dopamine (*down* to *up*): 1.0, 7.0, 15, 35 and 70.0 μM. 0.1 M acetate buffer (pH 5.0) was the supporting electrolyte (Reproduced from Ref. [60] with the permission of Wiley)

It influences the morphology of the polymer coating, so that well adherent and homogeneous deposits can be obtained [71]; the possibility of some selective host–guest complexation involving the analyte cannot be discounted.

By taking advantage of similar electrostatic interactions, negatively charged redox active species can also be included into the ICP matrix to stably support them as redox mediators. Anionic species most frequently involved consist of transition metal complexes; these are embedded into the coating either during film electrogeneration or in subsequent doping/de–doping electrochemical cycles, performed in a solution where the relevant monomer is absent and the anionic metal complex is present [72, 73]. The inclusion of similar anionic species can be very simply verified through voltammetric tests, thanks to the presence of current responses typical of the electroactive species incorporated. Although the approach can be considered very simple and versatile, the variety of polymer films actually tested is quite scarce. This is due to many constraints conditioning this synthetic approach: (1) it can only involve anionic species; (2) they should be redox active within the same potential window where the polymer is oxidized, hence conductive. Additionally, when the electroactive species are included during polymer electrogeneration, (3) they have to be soluble in the same solvent medium where the monomer is dissolved; (4) they should not directly oxidize the monomer. Due to the very low number of anionic-charged metal complexes possessing all the characteristics previously listed, the majority of the literature reports involve $[Fe(CN)_6]^{3-}$ [72–79]. Moreover, it must be highlighted that the effectiveness of the presence of this metal complex in activating electrocatalytic processes has not been fully convincingly demonstrated.

2.4.2 Molecularly Imprinted Polymers

MIPs are synthetic materials prepared by co-polymerization of a functional monomer in the presence of a template [91–96]. The species acting as the template can either be the target analyte itself or a chemical species very similar to it in terms of the shape, dimensions, and, possibly, functional groups brought, suitable to selectively interact with the hosting matrix. This implies that, after removal of the template from the coating, the material preserves cavities with specificities suitable to interact with the printing species, hence also with the target analyte. Figure 2.12 shows the formation of MIP–based coating, and the strategy to adopt in order to obtain a selective interaction with the analyte.

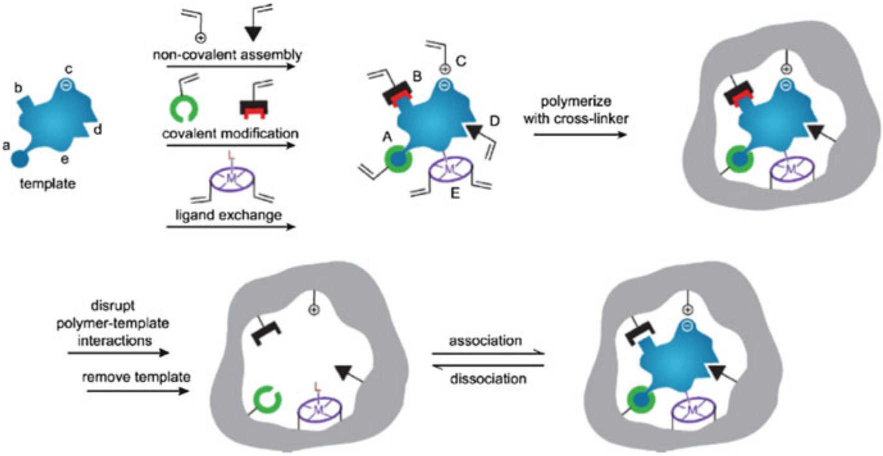


Fig. 2.12 Schematic representation of the molecularly imprinting process: the formation of reversible interactions between the template and polymerizable functionality may involve one or more of the following interactions: (**A**) reversible covalent bond, (**B**) covalently attached polymerizable binding groups that are activated for non-covalent interaction by template cleavage, (**C**) electrostatic interactions, (**D**) hydrophobic or Van der Waals interactions, (**E**) co-ordination with a metal center; each kind of interaction occurs with complementary functional groups or structural elements of the template, (**a**–**e**), respectively. A subsequent polymerization in the presence of crosslinker(s) results in the formation of an insoluble matrix in which the template sites reside. Template is then removed from the polymer through disruption of polymer-template interactions, and extraction from the matrix. (Reproduced from Ref. [97] with the permission of Wiley)

The system eventually recalls the ‘lock and key’ model of the enzymes, i.e., it is characterized by a particularly high degree of selectivity. In this regard, it is worth noting that MIPs may offer notable advantages with respect to natural enzymes that are stable only in quite limited experimental conditions, are quite expensive, and are only available for a limited number of possible analytes.

During MIP synthesis, the monomers bearing suitable functionalities and the template molecules are pre-organized in solution, eventually forming a complex based on either covalent or non-covalent interactions between complementarily binding functionalities. These complexes are preserved during the formation of a cross-linked polymer network, mostly prepared by light- or heat-induced polymerization, resulting in non-conducting polymers. Different techniques are then used to detect the actual occurrence of the inclusion of the target analyte, such as capacity measurements etc.

When considering the amperometric applications in electroanalysis, the possibility of dispersing MIPs in a conductive organic matrix appears promising in order to improve the conductivity between the transducer and the trapped electroactive target analyte [98] and to possibly activate electrocatalytic processes in charge of the analyte. Furthermore, the use of ICPs, replacing the most common non-conducting ones, can result in a strict control of the film thickness, constituting a very important parameter affecting the performance of MIP–based sensors. Finally, when using ICPs for the formation of MIP–based sensors, cavities offering best housing to the analyte can also be simply and rapidly obtained by electrogenerating the coating in the presence of the target molecules themselves [99]. Efficiency in respect to specific recognition can be favored by formation of hydrogen bonds between atoms of binding sites of the template and atoms of recognition sites of the polymer coating. These interacting functionalities should be removed by dipping the electrode in a proper solvent or, more simply, by polarizing the electrode material at fairly high potential values, suitable for polymer over-oxidation to occur. Thanks to the fact that this process is assumed not to completely cancel the conductivity of PPy, many applications involve the use of oPPy.

Some selected applications of ICP for the formation of amperometric sensors based on MIP strategy are reported in Table 2.2.

2.4.3 Detection of Metal Ions

Different metal ions can be detected on ICP-modified electrodes by anodic stripping voltammetry: metal ions from the solution are reduced, i.e., pre-concentrated onto the electrode surface, at a fixed negative potential, while their quantification is performed during the subsequent metal re-dissolution, by scanning the potential toward more positive values. It should be noted that, when considering this specific application, the polymer coating may be not in the doped state, at least when the reduction of the metal ions is carried out: thanks to the presence of trapped charges, the conductivity of the coating results adequate [108].

Table 2.2 Some examples of application of intrinsically conducting polymer modified electrodes for the development of molecularly imprinted polymers

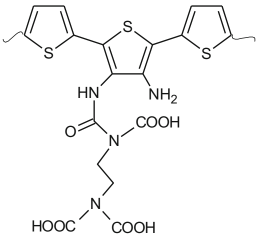
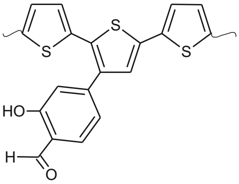
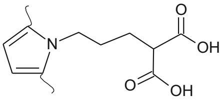
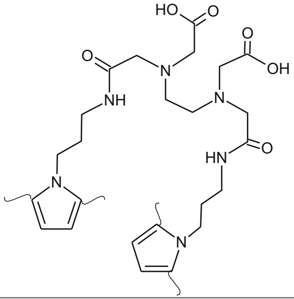
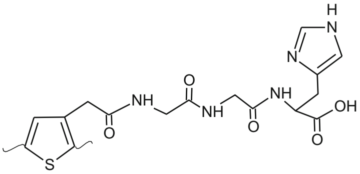
Analyte	Coating	Ref.
Dopamine	PPy	[100]
Morphine	PEDOT	[99]
Morphine	PEDOT, including pre-formed MIP	[101]
Sulfamethoxazole	oPPy	[34]
Caffeine	PPy	[102]
Paracetamol	oPPy	[33]
Ascorbic acid	PPy	[103]
Ascorbic acid	PANI	[104]
2,4–Dichlorophenoxyacetic acid	oPPy	[37]
L–tryptophan	oPPy	[38]
(−)–ephedrine	oPPy	[35]
Cathecol	PANI including pre-formed MIP	[98]
Atrazine	Poly(EDOT–co–Th–acetic acid)	[105]
Serotonin	oPPy/dodecylsulfonate	[106]
DNA	PPy	[107]

ICP films can constitute, as such, most favorable conductive materials for metal ion pre-concentration, not allowing the incidental formation of multi-metallic systems such as inter-metallic compounds that may occur at metal surfaces [109]. The selectivity of the sensor can be improved by employing ICPs possessing a certain degree of affinity toward the target metal ion. To this purpose, the presence of a specific heteroatom in the ICP chains can be exploited, e.g., by choosing PTh derivatives for the detection of Hg(II) [110, 111] or PPy for Ag(I) [112], thanks to the presence of S and N atoms in the heterocycle structural units, respectively. Alternatively, suitable counter-ions are inserted in the polymer coatings, aiming to form selective chemical bonds with metal ions in solution [113–117].

On the other hand, ICPs can be suitably functionalized with specific organic moieties. Typical functional groups employed to such a purpose are flexible organic chains containing N of O atoms, capable of selectively chelating a specific heavy metal ion, depending on the relevant formation constant. Some examples are reported in Table 2.3. The specific organic moiety can be inserted either in the monomer unit that is subsequently electropolymerized or in a step subsequent to the formation of the film, by employing monomer units with side chains possessing specific reactivity.

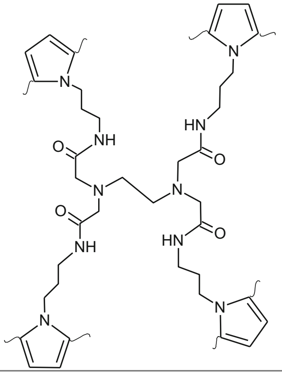
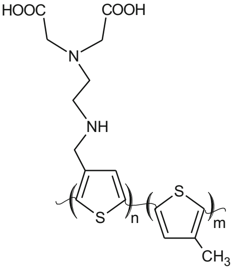
Functionalized ICP chains can be also used for the detection of non-electroactive alkaline and alkaline earth metals by exploiting quite a different approach: the coordination of the ICP molecules with the metal ions affects the physico-chemical properties of the polymer film, inducing a strong variation of the relevant voltammetric trace. In particular, the p–doping process results conditioned by the formation of more or less strong chemical interactions between the metal analyte

Table 2.3 Examples of intrinsically conducting polymers bearing organic moieties suitable for metal ion complexation

Analyte	Polymer coating	Ref.
Pb(II), Cu(II), Hg(II)		[118]
Al(III)		[119]
Cu(II), Pb(II), Cd(II), Hg(II)		[120]
Cu(II), Pb(II), Cd(II)		[121]
Cu(II)		[122]

(continued)

Table 2.3 (continued)

Analyte	Polymer coating	Ref.
Hg(II)		[123]
Cu(II)		[124]

and the organic moieties covalently bonded to the polymer chain. This leads to the recording of a current peak intensity that is lower, the higher the concentration of metal ion in solution [125, 126] as depicted in Fig. 2.13. Alternatively, a shift of the signal at progressively higher potential values, proportional to the concentration of the metal ion in the sample, has been observed [127, 128].

This effect can be ascribed to the accumulation of positive charges in the polymeric film when the suitable organic moiety is chemically bonded to metal ions: the further addition of positive charges to the polymer chains in the p–doping process is increasingly difficult. On the other hand, different events leading to a similar effect can also be invoked, e.g., the occurrence of chain twist induced by the complexation. Regardless, a direct electronic interaction between the organic moiety and the delocalized π–electron system must be present to obtain a signal variation depending on the analyte concentration in solution. Typical functional groups exploitable in this frame are calixarene [125] and crown moieties [6, 126–129]. The selectivity of the material is due to the dimension of the cavity, in its turn related to the number of O atoms in each organic moiety. However, we must highlight that, despite the fairly high number of papers dealing with the synthesis of this class of derivatives, none of them reports the actual analytical performance of similar sensors.

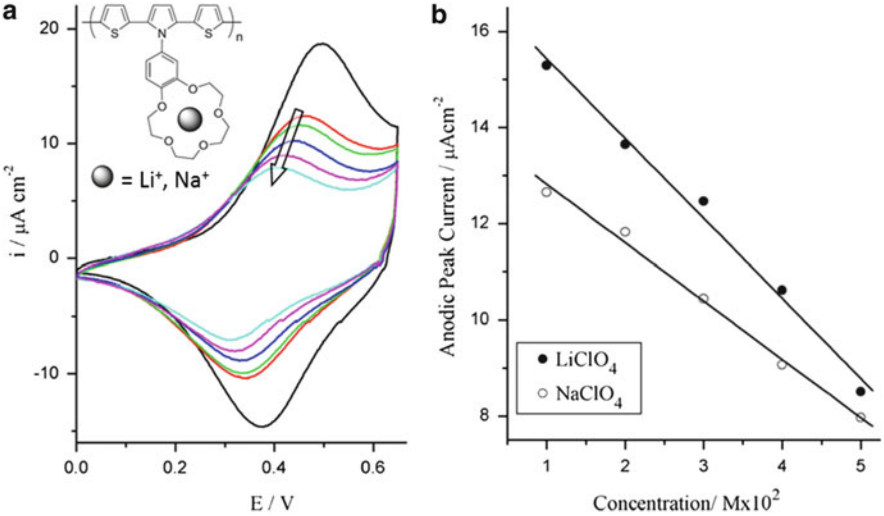


Fig. 2.13 (**a**) Cyclic voltammograms of indium tin oxide electrode coated with the crown derivative reported in the inset, recorded in ethanol solution containing 0.1 M $[CH_3(CH_2)_3]_4NClO_4$ and different amount of alkali metal salts (from 0 to 50 mM in the direction of the *arrow*); (**b**) relevant plot of the anodic peak currents as a function of the concentration of the alkali metal salt (Reproduced from Ref. [126] with the permission of Elsevier)

2.4.4 Electronic Tongues

ICP-modified electrodes have also found application in an electroanalytical frame that differs dramatically from the previous ones, i.e., as elements of the so-called electronic tongues (for general considerations, see Chap. 1). When considering similar sensor arrays, the characteristics of ICP-modified electrodes may make them preferable with respect to typical bare electrodes. The reasons are similar to those previously listed for amperometric sensors developed for the determination of a specific analyte, the main reasons being low passivation of the electrode surface, high reproducibility of the electrode coating, and occurrence of electrocatalytic processes. Moreover, the specific selectivity of the electrodes can very easily be changed by introducing different species in the same organic matrix, e.g., electroactive counter-ions [130–134] or metal nanoparticles [135].

Interestingly, fruit juices [136] and wines [135, 137] have been classified by considering not only the first voltammetric scan registered in the sample, but also the signal evolution in following scans. In these cases, the use of a single electrode is found to give enough information to obtain a proper classification of the different samples. This approach is made possible by the peculiar repeatability and reproducibility of ICP coatings in achieving quite reproducible interactions with species in the sample, giving rise to reproducible signal evolutions within a series of following scans.

Some further examples of sample classification from electronic tongues containing ICP-modified electrodes as at least one of the elements constituting the sensor array are reported in Table 2.4.

Finally, ICP-modified electrodes have also been proposed for the quantitative determination of some analytes by exploiting a multi-linear regression approach (see Chap. 1). By considering the entire voltammetric path registered in the sample it was possible to quantify meaningful parameters for quality control in the food industry, such as total polyphenol content [134, 139].

2.4.5 Enzymatic Biosensors

As often happens for the electrode coatings that are the subject of this monograph, enzymatic biosensors constitute an area in which ICP-modified electrodes have found the highest number of applications and, for this reason, the number of review articles written in this specific field is particularly high [140–145]. Table 2.5 reports a selection made from the very high number of amperometric biosensors found in the literature, aiming to give an idea of the applications proposed.

ICP films can merely constitute a conductive matrix to stably fix a high number of different enzymes: in comparison with different materials, the high conductivity constitutes a notable added value. Moreover, the possibility to synthesize the electrode coating by electrolysis allows the formation of reproducible coatings, possessing the correct thickness for the specific application. In this respect, it must also be noted that, at variance with non-conducting polymers, the film thickness does not need to be restricted to a few nanometers, allowing the deposition of a high number of biological elements inside: ICPs act as the transducer of the sensing elements. This aspect, combined with the possibility for the solution to penetrate into the film, improves the efficiency of bio-receptors on the transducer surface, both the outermost ones and those on pores. Moreover, electrogeneration allows the formation of homogenous coatings, independently of the electrode shape and dimension.

A further aspect to underline when considering ICP coatings in the frame of biosensors is that the manifold-cited electrocatalytic and antifouling properties of these materials can be profitably exploited to improve the performance of the sensor system. In particular, the use of ICP-modified electrodes leads to great advantages when considering biosensors involving enzymes producing H_2O_2 [52, 146] or nicotinamide adenine dinucleotide (NADH) [147–149]: both analytes are well known to present significant problems when reacting at usual bare electrodes. As an alternative to the electrocatalytic properties of pure ICP coatings, redox mediators may be also added to the solution [150–153] or included inside the film during polymer electrogeneration [154, 155]; the most diffused redox mediators used are water-soluble ferrocene derivatives when following the former approach and $[Fe(CN)_6]^{3-}$ in the latter case.

Table 2.4 Examples of application of intrinsically conducting polymer modified electrodes as elements of electronic tongues

Electrodes	Samples	Type of determination	Data processing algorithm*	Ref.
PMTh-, PANI-, and PPy-modified electrodes including different anions	Standard solutions	Discrimination between solutions containing chemical species representative of different tastes: sweet, bitter, acid, salty, and astringent	PCA	[130]
PPy-modified electrodes including different anions, bare carbon paste electrode, carbon paste electrodes modified with phtalocyanine, Au disk electrode	Red wines	Discrimination of wines possessing altered organoleptic characteristics	PLS, PCA	[138]
PPy-modified electrodes doped with different counterions; carbon paste electrodes modified with metallophthalocyanine complexes; carbon paste electrodes modified with perylene imide derivatives	Red wines	Discrimination between wine samples aged in oak barrels of different characteristics	PCA, SIMCA	[131]
PEDOT-modified electrode, Au and Pt electrodes	Fruit juices	Discrimination between fruit juices of different variety and bands	PLS	[136]
PEDOT-modified electrode and PEDOT-modified electrode including either Au or Pt nanoparticles	White wines	Discrimination with respect to grape varieties and geographical origins	PCA, PLS	[135]
PPy including different anions	Beers	Discrimination between dark and pale beers as well as between beers with and without alcohol	PCA, PLS	[133]

**PCA* Principal Component Analysis, *PLS* Partial Least Square Regression, *SIMCA* Soft Independent Modeling of Class Analogy

Thanks to the high degree of biocompatibility, the majority of papers dealing with biosensors consider the use of PPy. As previously discussed, in addition to the use of proper ICPs, oPPy has been also proposed: the presence of net negative charges at the solution|coating interface induces a filtering effect capable of rejecting negatively charged interfering species present in real samples, mainly consisting of ascorbic and uric acids [39, 156]. More recently, the use of PEDOT-modified electrodes has been also taken into account, thanks to the very high stability of the relevant polymer coating (allowing prolonged use of the sensor system [152, 157, 158]) and to the particularly low potential value at which

Table 2.5 Examples of applications of intrinsically conducting polymer modified electrodes in the frame of enzymatic biosensors

Analyte	ICPs	Enzyme	Immobilization method	Ref.
H_2O_2	PPy	Horseradish peroxidase	Entrapment during polymer electrogeneration	[170]
Glucose	PPy	Glucose oxidase	Entrapment during polymer electrogeneration	[159]
Glucose	PEDOT/polyethyleneglycol	Glucose oxidase	Entrapment during polymer electrogeneration	[150]
Glucose	PANI/polyvinyl–sulphonate	Glucose oxidase/ horseradish peroxidase	Adsorption at the p-doped polymer	[171]
Phenolic compounds and herbicides (atrazine and diuron)	PEDOT/ polyethyleneglycol	Tyrosinase	Entrapment during polymer electrogeneration	[172]
Phenol, catechol, *p*–cresol	Poly(N–3–aminopropyl pyrrole–co–Py)	Tyrosinase	Formation of imide bonds onto pre-formed polymer coating	[167]
Cholesterol	PANI	Cholesterol oxidase, cholesterol esterase, and peroxidase	Cross-linking onto the pre-formed polymer coating	[173]
Cholesterol	PPy/p–toluene sulphonate	Cholesterol esterase and cholesterol oxidase	Entrapment during polymer electrogeneration	[174]
Glycerol	PANI/$[Fe(CN)_6]^{3-}$	Glycerol dehydrogenase	Entrapment during polymer electrogeneration	[155]
Salicylate	PPy derivative functionalized with positively charged chains	Salicylate hydroxylase/polyphenol oxidase	Polymerization of the monomer-enzyme adduct	[161]
Organophosphate and organocarbamate pesticides	PPy	Acetylcholinesterase	Entrapment during polymer electrogeneration	[175]
NO	PTh derivative bearing –COOH moieties	Cytochrome c	Formation of imide bonds on the pre-formed polymer coating	[169]
L–lysine	oPPy	L–lysine–α–oxidase	Cross-linking onto the pre-formed polymer coating	[39]
Glucose	Poly(neutral–red)	Glucose oxidase	Cross-linking onto the pre-formed polymer coating	[52]

(continued)

Table 2.5 (continued)

Analyte	ICPs	Enzyme	Immobilization method	Ref.
Pyruvate	Poly(neutral–red)	Pyruvate oxidase	Cross-linking onto the pre-formed polymer coating	[52]
Glucose	Poly(brilliant cresyl blue)	Glucose oxidase	Cross-linking onto the pre-formed polymer coating	[146]
Urea	PPy/poly(5–amino–1–naphthol) bilayer	Urease	Direct adsorption, entrapment in cellulose acetate layer, cross-linking with glutaraldehyde, or covalent attachment to the pre-formed polymeric layer	[164]
Tyramine	PPy	Tyrosinase	Cross-linking onto the pre-formed polymer coating	[176]

p–doping occurs. Conversely, PANI is less frequently used because enzymes are usually less stable at the low pH values at which this polymer is conductive.

The approach most frequently followed to stably fix enzymes on and inside ICP-modified electrodes takes advantage of the positive charges on the growing polymer chains during the electrogeneration: they electrostatically interact with negatively charged enzymes present in the polymerization solution, leading to electrostatic inclusion [140, 159, 160]. The procedure requires that both the monomer and the biological element are soluble in aqueous solution, suitably buffered to control the nature of the enzyme. Moreover, the concentration of enzyme in the polymerization solution is required to be fairly high, which makes such a procedure not often applicable to expensive biological elements. One appreciable advantage of this procedure lies in the possibility of strictly controlling, and even modulating, the thickness of the polymer film and the concentration of enzyme inside the coating, accordingly to the performance required by the sensor system. Furthermore, a homogeneous distribution of the biomolecules inside the deposit is reasonably obtained.

According to the criticism advanced by several authors, merely electrostatic interactions with the growing or doped polymer chains lead to a low level of bio-receptors inside the coating. The same authors have proposed different strategies aiming to increase the amount of enzyme entrapped during polymer electrogeneration. On one hand, monomers functionalized with positive charges [161], and on the other hand, enzymes covalently bonded to anionic polyelectrolytes [162, 163] constitute two examples of the strategies adopted for this purpose. Alternatively, polar species, e.g., polyethylene glycol, are added to the polymerization solution, improving the chemical interaction of the arising polymer coating with amine groups present in lysine residues of the enzyme [150].

An alternative procedure for the deposition of enzymes onto the outermost surface of the polymer coating consists of a two-step approach [164]: polymer electrogeneration is carried out in the first step according to usual procedures, whereas the enzyme is allowed to react chemically with the polymer surface in a second step. Very weak electrostatic interactions [165] or proper covalent bonds [153, 154, 166–168] can be formed, depending on the chemical nature of the organic polymer. The procedure allows the use of monomers that can be polymerized at particularly high positive potentials or that are only soluble in organic media, since the contemporary presence of the enzyme in solution is not required. As an example, thiophene–3–acetic acid is one of the most frequently used monomers to obtain covalent deposition of biomolecules on the electrode surface; it possesses external carboxylic groups suitable to form amidic bonds with amine groups of the enzyme [153, 154, 168, 169]. Different effective functionalities in the polymer chains include amines, aldehydes, or carboxylic groups, once activated with N–hydroxysuccinimide, N–hydroxyphthalimide, or pentafluorophenyl (see Fig. 2.14). The critical point of this approach is that the formation of proper covalent bonds can lead to protein denaturation or affect the active site of the enzyme.

Adsorption processes between positively charged ICP films and negatively charged enzymes at pH above the isoelectric point are less risky in terms of preservation of the biological activity of the receptor. However, despite the simplicity of the process, the weakness of electrostatic interactions formed often limits the long-term stability of the sensor [165]. Moreover, the stability of the sensor is highly affected by the solution pH and by the doping state of the polymer coating. Finally, the amount of enzyme eventually fixed on the transducer is, reasonably, quite low.

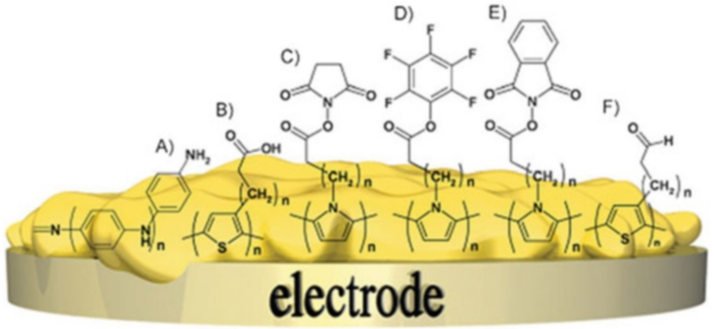


Fig. 2.14 Examples of functionalized polymer films bearing reactive groups for covalent anchoring of biomolecules. (*A*) amines (PANI), (*B*) carboxyls (PTh), (*C*) N–hydroxysuccinimide esters (PPy), (*D*) pentafluorophenyl esters (PPy), (*E*) N–hydroxy–phthalimide esters (PPy), (*F*) aldehydes (PTh) (Reprinted from Ref. [144] with the permission of Royal Society of Chemistry)

At the end of this exposition concerning enzymatic biosensors, it is also important to highlight that ICP electrode coatings discussed in this chapter are suitable for the development of second-generation enzymatic biosensors: the polymeric chains, as obtained from the electrochemical processes, are often too rigid to allow a direct charge transfer to the enzyme active site, which is a behavior proper for third-generation biosensors. A decisive step forward in this direction has been carried out with the use of nanosized materials. However, as better discussed in Chap. 6, the advantages discussed here as to the use of ICP-modified electrodes for the development of high-performance enzymatic biosensors can be transferred to those involving nanostructured surfaces.

As a final remark, it is worth noting that ICP-coated electrodes also find application in the development of highly efficient immunosensors that exploit an enzymatic detection for the quantification of the entity of the antibody-antigen adduct formation [177–182].

2.4.6 Genosensors

Genosensors constitute a third, very important, class of amperometric biosensors that may involve the use of ICP-modified electrodes [183]. In this case, the quantification of the target analyte is generally performed in an indirect way, due to the electrochemical inertness of oligonucleotide chains at low enough potentials. When considering ICP-based genosensors, the formation of DNA–DNA duplexes on the electrode surface is claimed to ascribe high rigidity to the polymeric structure, hindering the swelling of the polymer induced by p–doping [184]. On the other hand, one should also consider that the hybridization event enhances the negative charge density at the interface with the solution, due to the presence of the phosphoric groups of the oligonucleotide chain. Such a negatively charged cloud may hinder the access of anions from the supporting electrolyte into the polymer film, hence opposing the polymer p-doping. The result of the combination of both these effects, is that the doping process shifts at more positive potentials at increasing the concentration of DNA in the hybridization solution [185, 186]; concurrently, the current peak ascribable to the p-doping process results as lower, the higher the concentration of complementary DNA [186–188].

Since oligonucleotide chains possess a net negative charge, single-strand DNA chains can be enclosed into the electrode coating during polymer electrogeneration, in a way very similar to that previously described for the inclusion of enzymes [188–190]. On the other hand, polymer chains can be also suitably functionalized, aiming at linking oligonucleotide probes at the outermost zone of the electrode coating by a proper covalent bond. By following such a strategy, several PTh and PPy derivatives have been synthesized and tested (see, as an example, Fig. 2.15). Due to many constraints deriving from performing the polymerization of DNA functionalized monomers, the approach most frequently adopted consists in the post–functionalization of polymer coatings in a step following the polymerization

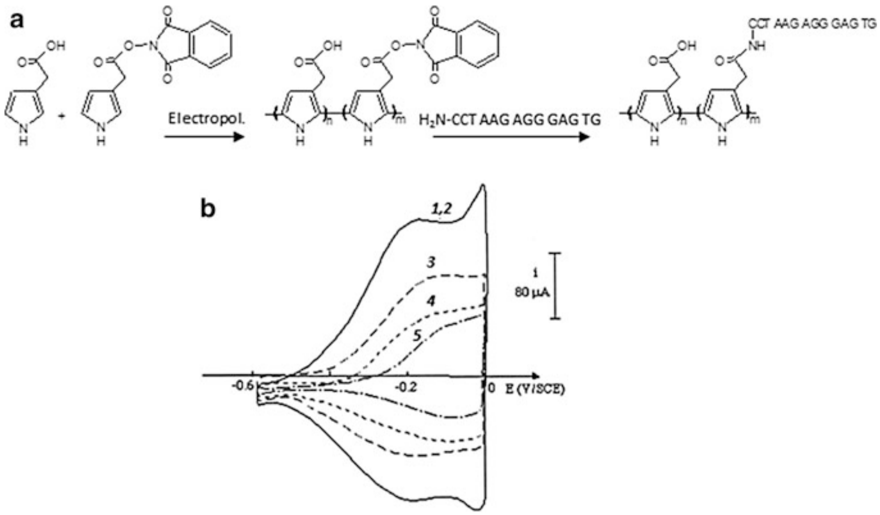


Fig. 2.15 (**a**) Strategy for the assembling of a DNA-modified PPy coating and (**b**) relevant cyclic voltammograms after 2 h incubation (37 °C) in buffered aqueous solution containing (**1**) NaCl 0.5 M, (**2**) non-complementary DNA and complementary DNA in concentrations of 66 nM (**3**), 165 nM (**4**), and 500 nM (**5**) (Reprinted from Ref. [186] with the permission of Elsevier)

process [184–187, 191]. Th and Py derivatives bearing functional groups capable of covalently linking the oligonucleotide probe, e.g., a carboxylic group, have been, in general, used for such a purpose.

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Chapter 3
Redox Polymers and Metallopolymers

3.1 Main Characteristics of Redox Polymers

Redox polymers (RPs) constitute a class of electrically conductive macromolecules: a polymer chain is covalently bound to a number of organic or inorganic redox centers typically equal to one another. Nitro-substituted styrene, quinone, viologen, and dopamine residues exhibit reversible redox behavior and should be considered pioneering organic redox centers in the frame of RPs. On the other hand, the redox centers of RPs more often consist of metal ions surrounded by different sets of co-ordinating ligands, thus opening, in principle, to a huge variety of derivatives: various complexes of Ir, Co, Re, Ru, and Os have been proposed. Significant examples of RPs used in electroanalytical applications are sketched in Fig. 3.1, which also reports the relevant usual abbreviations.

The redox centers are capable of reacting with each other when present in the different oxidation states of a single reversible redox couple, leading the charges to move along a preferred direction under the influence of an electric field. Redox reactions occur between close enough centers: so-called inner or self-exchange redox reactions are responsible for charge percolation through the polymer mass, by a so-called ‘electron hopping’ mechanism, as Fig. 3.2 depicts. At variance with ‘electronic conductivity’, characteristic of the intra-chain conduction in intrinsically conducting polymers (ICPs), dealt with in Chap. 2, RPs are characterized by ‘redox conductivity’.

The charge percolation by electron hopping is necessarily coupled to ion migration inside the coating, as required by fulfillment of electroneutrality. Some important conclusions concerning the conductivity of the coating can be drawn either from impedance spectra or by d.c. conductivity measurements (two-band electrode). Necessary condition for the material to be conductive is that it is polarized at potentials close to E° of the redox couple inside the RP. At similar potentials, in fact, meaningful concentration values of both redox forms are present in the RP. This restriction has been experimentally verified for a modified electrode

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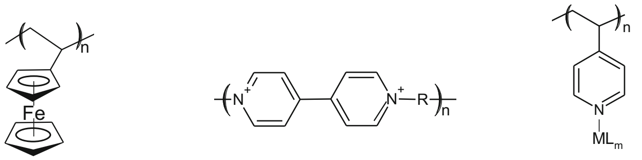
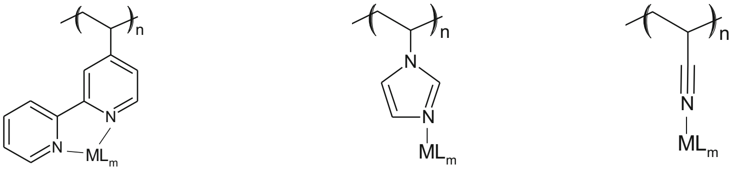


poly(bis(η5-cyclopentadienyl)iron(II)
poly(4-vinylferrocene)
PVFc

poly(4,4’-bypyridine) based RP
poly(viologen)
PV

poly(4-vinylpyridine) based RP
PVP



poly(4-vinyl-2,2’-bipyridine) based RP
PVbipy

poly(4-vinylimidazole) based RP
PVI

poly(acrylonitrile) based RP
PACN

Fig. 3.1 Examples of redox polymers used in electroanalytical applications. *M* and *L* represent a metal ion and a ligand, respectively

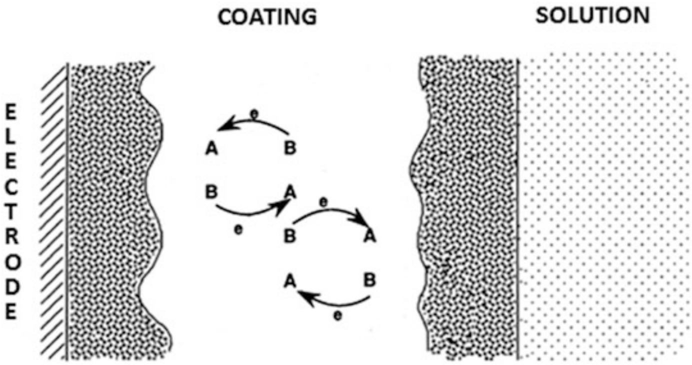


Fig. 3.2 Schematic representation of electron hopping process occurring inside a redox polymer coating (Reproduced from Ref. [1] with the permission of Wiley)

dipped inside a solution, in the absence of any oxidizable or reducible species. The relatively narrow potential range within which they are defined as ‘conductive’ renders RPs different from ICPs that, as better described in Chap. 2, are conductive at any potential that is either higher or lower than a given value. The aspects of the charge percolation in these two different cases have been extensively and critically discussed by Lyons [2].

Different results are gained when, for instance, an oxidizable species is present in the solution and the potential of the redox couple in the RP at the deposit|solution interface is higher than that of the couple in the solution: in this case, the solution is a potential source of electrons to the RP deposit. If the electrode is polarized at a suitable potential, higher than that of the RP at the opposite interface to an extent at least equal to the ohmic drop, the opposite interface may act as a sink of electrons injected into the deposit: the charge crosses the coating. From what has already been briefly exposed, the ohmic drop in the deposit depends on migration of the ion inside, necessary to assure electroneutrality, and on the energy required in order that electron hopping does occur.

Similarities exist between the situation depicted above and that consisting of an electrode modification in which molecules bearing redox centers are 'simply' dispersed inside an inert support. Obviously, stable anchoring of these species inside the coating, thanks to the formation of chemical bonds, is preferable; it makes the responses of the electrode system more repeatable. First attempts of stable fixation were made by anchoring charged metal complexes on substrate surfaces through electrostatic binding to polyelectrolytes [3]. Anionic (Nafion®) and cationic [4] polymers have been used for this purpose, as described in more detail in Chap. 4 of this monograph. Suitable strategies have also been developed to modify inorganic supports with organic molecules, creating organic–inorganic nanocomposites with particularly high sensitivity, e.g., in respect to Hg(II) probe species [5]. On the other hand, as described in detail in Chap. 7, in silica-based hybrid materials, the inorganic complexes can be fixed by adsorption, physical entrapment, or even proper covalent bonds.

Whatever choice is made to fix redox centers on an electrode surface through a polymer, i.e., proper covalent bonding or simple inclusion into an inert host matrix, the polymeric portion deserves attention with respect to: (1) length and flexibility of the individual chains and of the relevant ensemble, suitable to make the redox active centers prone to the electron exchange with the conducting substrate on the one side, with the species in the solution on the opposite side, and with each other in the bulk of the deposit; (2) energy required by the overall coating to swell and shrink in correspondence to charging and discharging processes, respectively; this conditions the permeability to the redox active species in solution, as well as to migrating and diffusing ions and solvent molecules. The deposits may be characterized as 'compact' or 'porous', capable to undergo swelling and shrinking more or less easily. These characteristics of the 'inert' supporting polymer chains are at least as meaningful as those of the redox centers themselves. In other words, polymer chains and redox centers play different roles, both with significant results in terms of the effective interaction of the coating with the electroactive species in solution. A huge variety of possible chemical and physical modifications also descends from these considerations in order to impart the actual electrode properties sought.

The density of redox centers within the polymer mass, i.e., the 'redox loading', is a further meaningful feature. This holds from the viewpoint both of the probability of actual occurrence of redox reaction with the species in solution that enter the deposit, and of the steric and flexibility conformational characteristics of the coating mass as a whole. Finally, it is evident that different behaviors, and consequently different properties, are exhibited in different solvent media, i.e., depending on the solvent characteristics, as well as on the nature and concentration of cations and anions dissolved therein.

3.2 Preparation of Electrode Modified by RPs

Since the beginning, procedures for assembling the electrode systems, i.e., for coating the conductive substrate with the RP chosen, have consisted of simple dip or spin coating or of drop casting. In other words, the modification was and still is often realized by pre-forming the polymer and subsequently somehow putting the solution into contact with the surface to modify, taking advantage of the poor solubility of the polymer in the solvent used and strong physical or even chemical adsorption on the surface of the underlying electrode substrate [6, 7]. It often happens that effective adhesion is induced by a proper 'mixing' of adsorption and insolubility in the medium in contact with the modified electrode.

However, it is evident that, whenever possible, direct electropolymerization at the electrode substrate [8] constitutes the method of choice when aiming at preparing a system for electroanalytical purposes. The conditions under which polymerization leads to deposition of the polymer formed onto the substrate are similar to those described above and also described in Chap. 2 for ICPs. This is actually the procedure followed nowadays in the large majority of the work reported in the present chapter.

Unlike ICPs, which have found application in a number of quite different fields, RPs are most used as electrode coatings. It is evident that, in similar situations, the in situ electrogeneration of the polymer *a fortiori* constitutes the synthetic solution that is first sought. Electropolymerization allows the deposit to grow where used: problems inherent to dissolving the polymer for dip coating or drop casting are circumvented, and the thickness of the deposit can, to a certain extent, be controlled. All the advantages already listed in Chap. 2 for electrogeneration of ICPs are gained. Electropolymerization and concomitant deposition onto the electrode substrate occur either by cathodic reduction or by anodic oxidation of the monomer and the growing oligomer units, depending on their nature. Similarly to ICPs, the polymerization–deposition is based on an electron transfer in charge of the monomer that generates a reactive species leading to chain propagation steps.

Different redox metal centers, such as Fe, and especially Ru, ions, under the form of ethylenediaminetetraacetic acid (EDTA) or ammonia complexes, anchored to PVP, PACN, or PVbipy [8–12], were among the first RP modifiers. In some of these cases, the most spontaneous synthetic approach was followed, consisting of the deposition of the preformed functionalized organic polymer onto the electrode substrate; the subsequent reaction with metal complexes, presenting weak ligands in correspondence to the sites devoted to coordinating the organic polymer, finally leads to the desired RP-modified electrode. However, alternative procedures have been followed, including metal complexation of the polymer prior to deposition and, most important, direct electropolymerization of the complex constituting the structural unit of the polymer.

In subsequent years, electropolymerization was definitely the established procedure. Polymerization by cathodic reduction, even exploiting redox mediation at the electrode, has been carried out on a wide series of Fe(II), Ru(II), and Os(II) vinyl-containing complexes based on differently substituted pyridine, and the relevant polymerization mechanism was extensively discussed in the same article [13]. An element of complexity of the RPs electrochemical growth lies in the presence, in the monomer, of additional electroactive groups.

3.3 Why Modifications with RPs?

Quite spontaneously and reasonably, the first studies on RP-modified electrodes dealt with the mechanisms on which charge conduction is based, rather than with applications, specifically in the field of electroanalysis. At the end of the 1970s, the restricted group of pioneers in electrode modifications identified RPs as effective tools to stably anchor redox couples at a conventional conductive surface, such as Pt, Au, or glassy carbon. In the late 1970s, years in which the so-called chemically modified electrodes (CMEs) started being investigated, simple alkyl chains were proposed to which redox centers are linked, namely Fc in PVFc [6] and nitrophenyl groups in poly(4-nitrostyrene) [7]. Attention primarily focused on the general electrochemical properties of the material coating the substrate; however, Van de Mark [7] also identified the electrocatalytic properties of the material, based on redox mediation by the organic functionality.

The first studies are excellently reviewed by R. W. Murray [14, 15], and progress in the field was marked by subsequent books [1, 2, 16].

We only cite here Andrieux and Saveant [17] as the start of a list of excellent theoretical studies carried out by the Paris school, paralleling the experimental work on the issue. They constitute a prosecution of the huge work made in previous years for ‘simpler’ systems that ‘only’ consider the solution phase and the unmodified electrode, the electrode surface eventually adsorbing species involved in the charge transfer process.

Electrode modifications by RPs are typically devoted to reducing the overvoltage, η_{ct}, affecting irreversible charge transfers occurring directly on the bare electrode, i.e., to activate electrocatalysis. This means that, in addition to redox conductivity inside the coating, a charge transfer, most often to ascribe to the same species involved in the conduction, should ‘close the circuit’ at the deposit|solution interface, activating electrocatalysis by redox mediation.

Figure 3.3 schematizes the different steps of the whole process, from the injection of charges at the substrate, to the conduction inside the coating, to the oxidation of a target species in solution.

Similarly to ICPs (see Chap. 2) and nanostructured surfaces (see Chap. 6), antifouling properties are also among the possible virtues of RPs; the change in

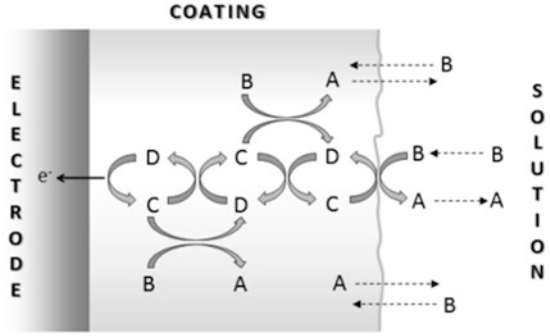


Fig. 3.3 The electrocatalytic events described in the text, for the case of a permeable deposit: the redox mediation also occurs 'inside' the coating bearing the mediator. The occurring net reaction is: B –> A + e$^-$

the nature of the actual electrode surface with respect to a bare one often promotes a lower tendency of the coating to adsorb species in solution. Any rationale for this behavior should take into account that each situation presents particular aspects, a huge variety of poisoning adsorptions on different surfaces being possible.

RPs have been proposed for a relatively long time, and nice pictures are found in many books on electroanalysis, in order to sketch the redox mediation in compact or swollen, differently structured, polymer systems. However, in our opinion, this class of conductive polymers has attracted insufficient attention from electroanalysts. The number of newly synthesized RPs actually used as electrode materials for electroanalysis is much lower than reasonable, on the basis of the appealing characteristics of similar materials. One reason for this can undoubtedly be found in the widespread use of ICPs that, once suitably functionalized, constitute valid and often preferable alternatives to RPs. It is for Chap. 2 to complain about a similar deficiency in the frame of ICPs, which induces doubts about the actual validity of the explanation given here. Moreover, when amperometric sensing is taken into consideration, common benchmark analytes such as ascorbic acid or uric acid, in aqueous buffered solution, are considered. Less often, the study is widened to different analytes, and, even less frequently, the effectiveness of the systems in real matrices is evaluated. It is evident that the last studies would be of basic importance, since they point to specific interfering species and deal with true 'matrix' effects, as to the presence of poisoning adsorbing species, possible speciation, etc.

3.4 RPs in Applications or Suitable for Applications: Much to Do in Electroanalysis

As already mentioned, poly(4-nitrostyrene) [6] constituted one of the first RP coatings exhibiting effective electrocatalytic properties; in particular, reduction of oxygen in acetonitrile was achieved at lower overvoltages with respect to the bare

Pt electrode. Though first proposed as simply a redox-conducting modifier, PVFc (quite a common polymer containing a typical reversible metallorganic redox couple, i.e., ferricinium ion/ferrocene (Fc^+/Fc) couple) has constituted since the very beginning a large portion of the story of RP-modified electrodes [6]. Almost half a century later, recent literature report a biosensor in which Fc^+ residues stably anchor the anionic form of lysine-oxidase enzyme. H_2O_2 generated by oxidation of lysine is oxidized by the Fc^+ residue. The resulting Fc is oxidized at the working potential, so that the Fc^+/Fc couple also simultaneously acts as the redox couple responsive of conduction. The performance of the developed biosensor is carefully evaluated by amperometric measurements at constant potential [18].

Similar Fc^+ residues in the frame of $PVFc^+$ have been used in genosensors, as cationic centers to fix DNA residues thanks to electrostatic interaction; the occurrence of DNA anchoring causes a decrease of the current due to Fc^+ reduction [19].

In Ref. [20], ascorbic acid is the object of the study of the effectiveness of Fc^+/Fc as a redox center responsible for conduction and electrocatalysis at the same time, when functionalizing poly(allylamine) or poly(ethyleneimine) chains fixed at the electrode via a Layer-by-Layer (LbL) technique in a bicomponent system complemented by poly(vinylsulphate) as the anionic polyelectrolyte.

Celebi et al. [21] follow a singular approach: the Cl^- counter-ions pre-concentrate Hg^{2+} from the solution. Reduction to Hg and subsequent anodic stripping allows effective determination of Hg^{2+} present in the solution.

N-based ligands are present in complexes most frequently encountered within the class of RPs. In particular, since the first electrode modifications with RPs, N-based ligands in Ru, Fe, and Os complexes are most frequently encountered: the reversible switch of both Ru and Os between II, III, and IV oxidation states, as well as of Fe between II and III, is exploited to electrocatalytic purposes. RPs based on similar metal complexes attracted the highest consideration [22], being also proposed as the active materials in amperometric sensing.

Within the general electrocatalytic scheme, in Ref. [23] PVP linking a 2,2′-bipyridine (bipy) complex, namely $[Os(bipy)_2]^{3+/2+}$ has also been characterized as to the behavior with respect to NO_3^- reduction. An analytical application to the evaluation of NO_3^- in river water was also proposed [24]. A similar polymer, cross-linked on purpose, is characterized and tested with respect to oxidation of uric acid present in urine [25]. The same $[Os(bipy)_2]^{3+/2+}$ group, coordinated to the imidazole residue of a PVI chain, deposited onto a microdisk electrode, has been characterized, and the behavior with respect to NADH oxidation has been defined as a preliminary step to the use in the frame of a biosensor [26]. Once more, an Os(III)/Os(II) complex constituted the functionalization of a non-conducting polymer chain of an RP used as an electrode modification in the determination of ascorbic acid in urine, also in the presence of uric acid and dopamine as interfering species [27].

The Ru(III)/Ru(II) couple may effectively substitute for Os(III)/Os(II) in similar RPs. $[Os(bipy)_2]^{3+/2+}$ linked to PVP acts as a redox mediator in the oxidation of oxalate, appearing to be suitable for an analytically effective determination [28]: coupled to the electrochemical characterization of the RP, an analytical study relative to the determination of oxalate in a flow injection analysis system is proposed. In Ref. [29], the effectiveness of an RP once more presenting the $[Ru(bipy)_2]^{3+/2+}$ residue as the active redox mediator is evaluated in respect to guanine oxidation; the statistics of the calibration line obtained by plotting the peak current intensity as a function of the concentration is elaborated, allowing the development of a complete analytical procedure.

Ru and Fe complexes in which two bipy ligands saturate the hexacoordination at PVP or PVbipy chains are among the most common residues: they were also obtained on the electrode surface by direct cathodic reduction of the relevant monomer, as reported in the already cited Ref. [8].

3.5 Different Species for Different Roles: RP-Based Composites

As evidenced above, RPs are, in principle, required to be conductive and, at the same time, suitable to exert redox mediation with respect to oxidation or reduction. Such a requirement to a single component is quite challenging, especially when best effectiveness is sought for both conduction and electrocatalysis. This is why, as also detailed in subsequent chapters, it is quite a common choice to ascribe the two different tasks to two different components, in more or less ordered micro-heterogeneous systems, viz., to use a composite electrode modifier.

Just in the frame of composites, RPs may find a new role. We report below a few examples of combinations with different kinds of nano-objects, such as carbon nanotubes (CNTs) and nanoparticles (NPs). However, note that such kinds of composite materials are specifically dealt with in another chapter of this monograph (see Chap. 6). In those briefly dealt with here, the RP plays the key role, and therefore is definitely the most meaningful component.

Examples of applications of composites in which RPs are deposited, through LbL technique, together with metal NPs, are found. A PV RP including Au NPs is proven to be effective with respect to H_2O_2 determination by cathodic reduction [30].

Qian et al. [31] deposited a similar polymer via the LbL technique, alternating with Au NPs, resulting in the ability to detect ascorbic acid; Havens et al. [32] linked a $[Os(bipy)_2]Cl$ residue to a PVP-polyvinylpyrimidine copolymer finally anchored, through an amidic bond, to a defect of a multiple-wall CNT, trapped inside a hydrogel. This is able to swell in aqueous solvent, becoming accessible to species in solution (see Fig. 3.4). Electrocatalytic action with respect to ascorbic acid was exploited in a complete analytic work, based on amperometric measurements at a fixed potential.

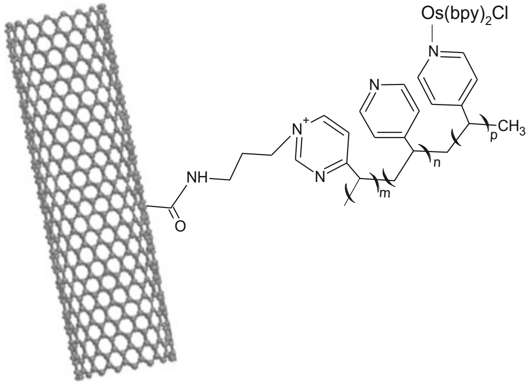


Fig. 3.4 Multiple-wall carbon nanotube (CNT) acting as an anchoring support for PVP[Os(bipy)$_2$]Cl (Reproduced from Ref. [32] with the permission of Elsevier)

In an article paying a great deal of attention to analytical issues, determination of dissolved NO has been carried out by employing a similar $[Os(bipy)_2]^{3+/2+}$ redox mediator system that saturates the co-ordination sphere thanks to the pyridine residues of a PVP polymer, supported by single-wall CNT on a glassy carbon electrode [33].

It is worth mentioning here that composites involving either an organic (ICPs) or an inorganic (silicate) host polymer have also been proposed. As for the first class of composites, applications in electroanalysis have, for the most part, dealt with the usual benchmark analytes and tests, such as ascorbic acid and dopamine determination, and resolution of the relevant responses. However, promising performance has been proven to be ascribed to the contemporary presence of two components. As an example, a composite consisting of RP- and ICP-conducting polymers, namely PVFc – poly(3,4-ethylendioxythiophene) (PEDOT) composite, has shown better performance in the oxidation process of ascorbic acid, with respect to the individual components, in exhaustive analytical studies [34]. Furthermore, as better described in Chap. 7, mesoporous silicate has been found to fix RPs on electrode surfaces in a very stable manner, leading to very efficient electrocatalytic coatings.

3.6 Prussian Blue and Analogous Transition Metal Hexacyanoferrates

Although metal hexacyanoferrates (HCFs) cannot be considered RPs, from both a structural and a compositional viewpoint, charge percolation occurs through electron-hopping mechanisms quite similar to those just described. For this reason, these pure inorganic materials are dealt with in the frame of this chapter.

Ferric HCF, called Prussian blue (PB), can be considered one of the very first materials used to coat bare electrodes with a redox-active film: in 1978, Neff [35] reported for the first time the electrochemical behavior of a thin layer of PB, deposited onto a Pt electrode from a solution of $FeCl_3$ and $K_3Fe(CN)_6$. In spite of the numerous literature articles reporting physico-chemical characterization of the material obtained, the exact chemical structure is still not well defined. On the other hand, it is recognized that it strongly depends on the experimental conditions adopted in the synthesis.

PB can be obtained in two different chemical structures that, according to an old definition [36], are called ‘soluble’ and ‘insoluble’ forms, respectively. The terms are actually misleading, because they are not referring to the more or less soluble nature of the two forms, which is very low in both cases. The denominations, most properly, point to a distinction between a form possessing peptized K^+ in the lattice structure, i.e., $KFe^{III}Fe^{II}(CN)_6$ (‘soluble’ PB), and a form where the excess of negative charge is fully compensated by Fe^{3+} ions, i.e., $Fe_4^{III}[Fe^{II}(CN)_6]_3$ (‘insoluble’ form). According to Ref. [36], ‘soluble’ PB is characterized by a basic cubic structure with cell dimensions of 10.2 Å, consisting of alternated Fe(II) and Fe(III) metal centers surrounded by C and N atoms, respectively. In the ‘insoluble’ form, K^+ ions in the interstitial sites are replaced by Fe^{3+}. However, further successive studies demonstrated that PB consists of a more complex structure with variable stoichiometry, also depending on the synthetic condition adopted [37]. This aspect makes the property–structure relationship quite tricky to define. The material also possesses a zeolite-like structure with channels possessing diameters of about 3.2 Å; as better discussed hereafter, it is this structure that confers the material the peculiar selectivity and sensitivity toward H_2O_2 detection.

The cyclic voltammogram of PB-modified electrodes shows the typical trend reported in Fig. 3.5.

According to the conclusions in Refs. [39, 40], the reversible reduction of PB to Prussian white occurs according to the following reaction:

$$\underset{\text{‘insoluble’ PB}}{Fe_4^{III}\left[Fe^{II}(CN)_6\right]_3} + 4K^+ + 4e^- \leftrightarrows \underset{\text{Prussian white (Everitt salt)}}{K_4Fe_4^{II}\left[Fe^{II}(CN)_6\right]_3}$$

The reduction is accompanied by the loss of the typical color of the pristine inorganic polymer and by necessary inclusion of K^+ into the film. Except for this cation, only $NH_4{}^+$, Cs^+, and Rb^+ were found to be able to penetrate the PB lattice, giving rise to the typical redox system shown in Fig. 3.5. Conversely, other monovalent cations, namely Na^+, Li^+, and H^+, as well as divalent cations, hinder the occurrence of the reduction process.

At fairly high positive potentials, PB converts to the fully oxidized form, namely Berlin green, according to the following reaction:

$$\underset{\text{‘insoluble’ PB}}{Fe_4^{III}\left[Fe^{II}(CN)_6\right]_3} + 3A^- - 3e^- \leftrightarrows \underset{\text{Berlin green}}{Fe_4^{III}\left[Fe^{III}(CN)_6A\right]_3}$$

where A^- is a generic anion present in the electrolyte solution that is necessarily included into the film to compensate for the arising positive charges. The

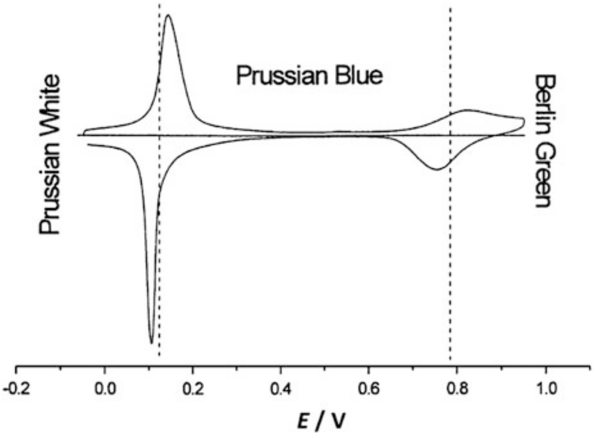


Fig. 3.5 Typical cyclic voltammogram of Prussian blue (PB) modified electrode in 0.1 M KCl (Reproduced from Ref. [38] with the permission of Wiley)

occurrence of this reaction is witnessed by the presence of a reversible anodic/cathodic peak system at *ca*. +0.8 V (Fig. 3.5).

Although we have previously mentioned that the exact nature of PB deposit is still not well defined, the reactions just discussed invoke the presence of the 'insoluble' form of PB, since appreciable amounts of K^+ are not found in the deposit obtained by the electrochemical procedure. On the other hand, 'soluble' PB has also been hypotesized to form by a chemical synthesis. For this purpose, different reactions have been proposed [41]. Obviously, the lack of an acknowledged, well defined nature of the electrochemical processes reflects the uncertainty concerning the PB structure.

The formation of a PB deposit on the electrode surface is carried out via either a chemical or an electrochemical approach [37, 38]. In both cases, aqueous solutions containing a mixture of Fe^{3+} and $[Fe(CN)_6]^{3-}$ have been used. The chemical procedure takes advantage of the spontaneous reaction between the two metal ions and results in a very stable PB layer [42, 43]; although the exact mechanism at the basis of Fe(III) reduction is not well understood, the high reactivity of $Fe^{III}[Fe^{II}(CN)_6]_3$ complex formed is invoked [39].

When considering the electrochemical approach, the reduction of Fe(III) is promoted by the polarization of the electrode at a suitable potential. It can be carried out either potentiostatically or galvanostatically; in any case, care must be paid to the choice of the potential values involved, in order to obtain a deposit possessing the characteristics sought [38].

Similarly to RPs discussed previously, PB can activate electrocatalytic reactions in charge of electroactive species present in the solution. In particular, it is proven to possess a marked electrocatalytic activity for the reduction, as well as for the oxidation, of H_2O_2 [44, 45] and some other organic compounds, such as catecholamines [46]. However, the fairly high potentials at which PB oxidation occurs prevent a real interest in this material as an electrocatalytic mediator for oxidation reactions. Conversely, the material has shown very interesting performance when used for H_2O_2 determination by reduction: the process can occur at a potential very close to 0 V (vs. Ag/AgCl) [43, 47], where the interference of different electroactive species is very low. Furthermore, the selectivity toward this analyte is increased by the previously described particular structure of PB, which only allows the access of low-molecular-weight molecules. This constitutes a main advantage at the level of selectivity, in the use of PB as the mediator for H_2O_2 reduction. Quite interestingly, Karyakin et al. [48, 49] found that H_2O_2 reduction occurs without the interference of O_2. Furthermore, by the careful choice of the experimental parameters used to obtain the PB film on the electrode substrate, the value of the rate constant for H_2O_2 reduction was found to be similar to that of peroxidase; this allows the obtainment of amperometric sensors characterized by very high sensitivity and low detection limits. For all these properties, PB has been defined as an “artificial peroxidase” [50, 51].

The outstanding properties of PB-modified electrodes in H_2O_2 detection have made this device a very good candidate for the development of oxidase-based amperometric biosensors. Among the different analytes proposed, the determination of glucose by the mediation of glucose oxidase still remains the primary application of PB-modified electrodes, even in a real context [52].

It is worth emphasizing that one of the main problems to overcome in order for PB to achieve wide popularity in the frame of biosensors is the very low stability of such a material in neutral and alkaline media [53], i.e., in conditions where enzymes show the highest catalytic activity. The reason for this behavior is probably to be ascribed to the interaction between Fe^{3+} and OH^- ions to form insoluble $Fe(OH)_3$. Fortunately, it was observed that chemically synthesized PB is characterized by a high long-term stability, even in slightly alkaline media [42, 43, 52]. Although the reasons for the different behavior of such a material are still not clarified, it again supports the conclusion that different PB structures are obtained by varying the synthetic parameters adopted.

Inspired by the success of PB-modified electrodes, different HCFs [54] have been more recently proposed for electroanalytical applications. Amongst others, Cu, Co, and Ni HCF have been the most frequently studied. In analogy to PB-modified electrodes, they have been proposed for the development of electrochemical sensors for H_2O_2 detection [55–57]. However, the performance of these materials was found to be definitely lower than that of PB or, as very recently demonstrated [58], only attributable to the presence of PB impurities in the structure of HCF films. On the other hand, non-iron HCF coatings, quite interestingly, demonstrate electrocatalytic properties in oxidation reactions involving, for instance, hydrazine [59], dopamine [60], and ascorbic acid [61, 62].

It is interesting to note that, besides the numerous studies concerning transition metal HCF, research has also recently been addressed, specifically to rare-earth derivatives [63–66].

3.7 From Pure Redox to Redox-Electronic Conduction in the Same Molecule: The Metallopolymers

From what has been written above about ICPs and RPs, it is evident that, in principle, best performance may be reached once the virtues of the two classes of differently conducting polymers are possessed by a single molecule that includes both redox centers and π-electron conjugated systems typical of ICP chains. Such a coupling is realized in so-called metallopolymers, where redox metal centers are co-ordinated to one or more ligands differently connected to the π-electron conducting organic backbone [67–79].

Pickup [68] authoritatively defined metallopolymers as materials in which “the metal is coordinated directly to the conjugated backbone, such that there is an electronic interaction between the electroactive metal centers and the electroactive polymer backbone.” Actually, not only the electrochemical, but also additional chemical, optical, and electronic properties may take advantage of such a combination within the same molecule. As a result, similar to ICPs, metallopolymers are of chief potential interest in many applicative fields of chemistry and physics. The structure of metallopolymers also presents advantages in terms of the feasibility of the synthesis directly at a bare electrode, since the π-electron conjugated chain usually makes the material conductive over a wide enough interval of potential values. The electropolymerization takes place at potentials at which the polymer coating is conducting, also presenting usually lower charge transfer overvoltages with respect to the bare electrode substrate (see Chap. 2).

However, just synthetic issues, namely those relative to the monomer from which to start, constitute a major drawback to the widespread use of similar materials as electrode modifiers. A recent review [80] collects a few results of efforts made in the field and new perspectives are envisioned, encouraging high consideration for such a potentially powerful class of electrode systems. The authors claim: “Recent developments in the field of synthesis and potential applications of metal-functionalized polymers obtained via electropolymerization are presented, highlighting the significant advances in this field of research.” At present, this class of conducting polymers is far from being exhaustively studied, both as to a general definition of the specific properties in respect to electrode electrochemistry and, even very much less, as to electroanalytical applications.

The properties of a specific metallopolymer are at first conditioned by the distance between metal center and π-electron organic backbone in terms of (1) space; (2) electronic energy. The differences in spatial locations and in electron energy levels of the metal redox center and of the π-electron organic backbone is related to the length and nature of the interposed chemical bonds, as well as to the intrinsic electronic characteristics of the organic chain and the metal complex. It is evident that similar differences condition both the nature and effectiveness of the charge transfer through the hybrid macromolecule.

Further possible effects of the presence of the metal adjacent to or inserted inside the π-electron chain must be taken into consideration. The conductivity is enhanced

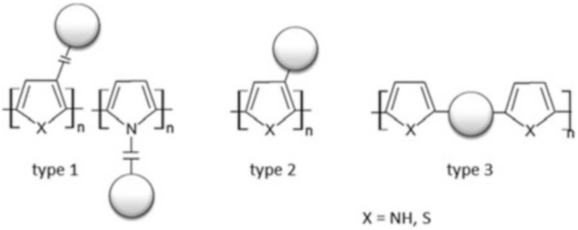


Fig. 3.6 Schematic representation of different types of metallopolymers, classified on the basis of the reciprocal position of intrinsically conducting polymer chain and metal complex (gray balls)

by the degree of conjugation, which is higher the lower the angle between the orbitals possessing π geometry. The characteristics of the complex may induce distortion in the π-electron system of the adjacent ICP segments: the intra-molecular conductivity may decrease. Moreover, the length of the fragments of ICP differently interposed between the sites on which the metal exerts influence condition both the doping potential and the electronic conductivity of the molecule as a whole.

It follows that, in principle, the interaction between the metal and the organic polymer backbone can be modulated; however, non-trivial chemical syntheses are necessary. On the one side, the interaction of redox and electronic conductivity may be made meaningless, so much so that the two conduction mechanisms are operative in parallel to each other. Conversely, the redox and the electronic conduction may be 'mixed' and affect each other to different extents, giving rise to peculiar conduction situations. Intra-molecular conduction is contemporarily affected by the presence of the redox centers and of polarons and bipolarons on the organic chain. Some reviews critically discuss different points of this basic aspect [68, 69]. The contemporary consideration of what happens in 'pure' ICP [81] (see also Chap. 2) is also a necessary complement to achieve full comprehension.

Independent of the specific nature of the metal and of the polymer chain, metallopolymers can be classified, to a first approximation, into categories, including roughly similar situations as to the reciprocal interaction between metal center and π-electron system (see Fig. 3.6) [69, 72].

In type 1 metallopolymers (see Figs. 3.7 and 3.8), the metal center is connected to the π-electron conjugated polymer backbone by a long enough, typically saturated, non-conducting organic chain, that makes the two conductive systems act independent of each other, in respect to the intra-molecular conduction.

Although similar polymers do not actually meet with the definition given above for 'metallopolymers', they represent an effective way to stably fix metal redox centers within a conducting matrix. They actually represent a limiting situation. In this respect, it should be noticed that most metallopolymers applied in electroanalysis do belong to this group. Figure 3.8 shows some examples of metallopolymers reported in the literature, chosen with the aim of illustrating the organic functionalities most frequently involved.

In type 1 metallopolymers there is no way for the d-π orbitals of the metal center to overlap the polymer π-orbitals to any extent. Obvious possible exceptions with

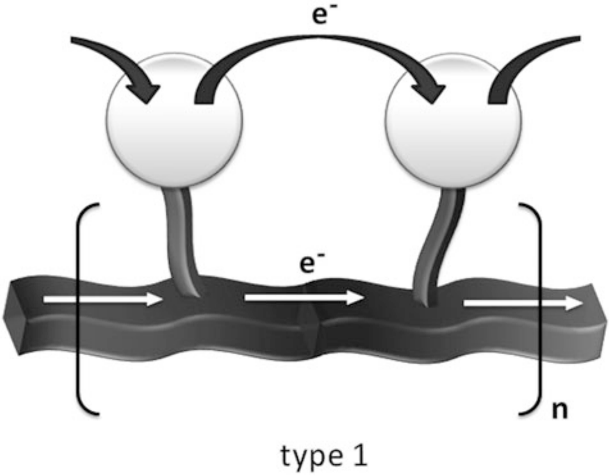


Fig. 3.7 Scheme of a typical unit of type 1 metallopolymer

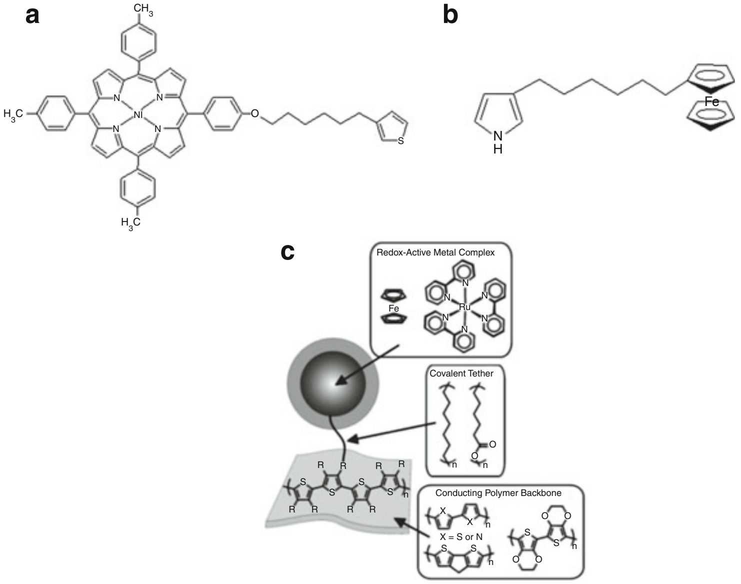


Fig. 3.8 Examples of monomers leading to metallopolymers belonging to type 1, (**a**) from Ref. [82] and (**b**) from Ref. [83]; **c** a scheme of different type 1 metallopolymers found in the literature [69] (Reproduced with the permission of Royal Society of Chemistry)

respect to reciprocal interaction lies in electron hopping, which may also be operative between analogous centers of different chains, as well as between a redox centre and a suitably oriented branch of the same or a different polymer

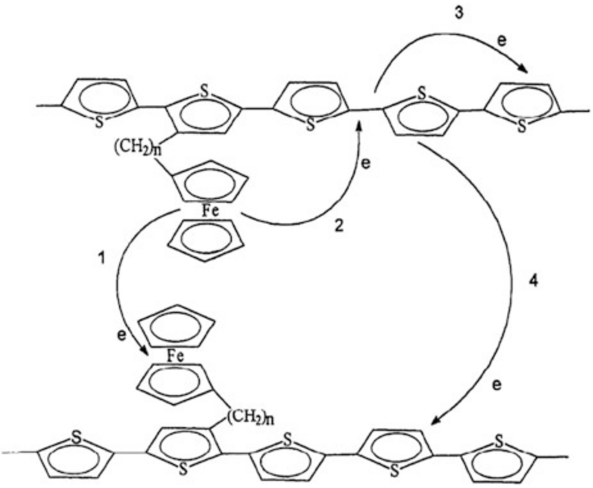


Fig. 3.9 Possible electron hopping processes occurring in a type 1 metallopolymer containing Fc centers (Reproduced from Ref. [84] with the permission of Elsevier)

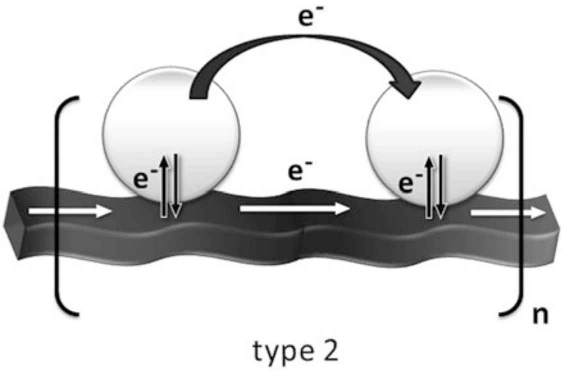


Fig. 3.10 Schematic drawing of possible ways of charge percolation in type 2 metallopolymers

chain [84], as schematized in Fig. 3.9. As to the charge transfer occurring by electron hopping between adjacent redox centers, similar percolation of charge is also indicated as an 'outer sphere mechanism'. The distinction between outer sphere charge transfer within a single chain and between adjacent chains is only a formal distinction. Moreover, these mechanisms are completely similar to the charge percolation in RPs.

Peculiar behavior, different from those exhibited individually by the metal complex and by the organic backbone, is proper of type 2 metallopolymers. Note that the presence of the complex does not usually render the flow of charge easier: on the contrary, the metal complex may constitute a sort of obstacle to the circulation along the π-electron system. Figure 3.10 shows the different paths followed by the charges along a chain.

It should be evidenced that, in type 2 as well as in type 1 metallopolymers, steric hindrance due to the substituents to the heterocyclic ring may require copolymerization, e.g., interposal of unsubstituted heterocycles. Alternatively, the group bearing the functionality should be linked to dimeric or trimeric rings.

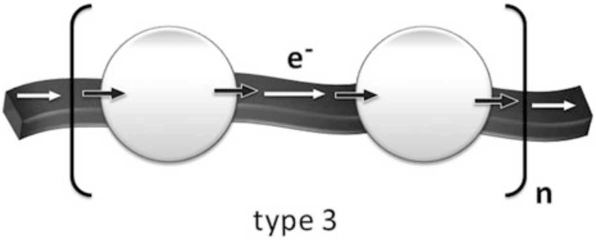


Fig. 3.11 Schematic drawing of charge percolation in type 3 metallopolymers

Even stronger interactions between the two entities, namely the oligomeric organic segments and the metal complex in between, affect the mechanism of charge percolation occurring in type 3 metallopolymers, sketched in Fig. 3.11. As to the electron flow along the whole chain, once again the metal usually constitutes an energy barrier to overcome, a possibly even higher barrier than for type 2 metallopolymers. Except for the situation in which so-called 'redox matching' occurs (discussed below), the redox center may act as a charge trap and lower the electronic conductivity of the π organic chain. Conversely, the organic fragments between two adjacent metal complexes should be long enough not to constitute a brake to the electron flow.

The energy barrier present in type 3 metallopolymers depends on the intrinsic nature of the inorganic and organic residues when passing from the π-electron organic system of the chain fragments to the metal complex, and vice versa. In other words, the higher the electronic energy difference between the polaronic/bipolaronic levels (see Chap. 2) in the p- or n-doped oligomeric organic branches and the lowest unoccupied molecular orbital (LUMO) or highest occupied molecular orbital (HOMO) levels in the complex, the higher the resistance induced by the presence of the metal complex. In type 3 metallopolymers, in fact, the flux of charge must occur across the ligand–metal adduct. Of note, in type 3 metallopolymers, since the length of the π-electron system is limited by the presence of the metal, the fragments are less easily oxidisable: it is more difficult to dope the ICP, i.e., to become actually conductive.

Figure 3.12 reports examples of different type 2 and 3 metallopolymers reported in the literature. 'Inner sphere mechanisms' are operative in these cases.

Integrating what is already exposed (see Fig. 3.7) for type 1 metallopolymers as to intra-chain charge percolation, summarizing the situations possible when considering type 2 and 3 metallopolymers, it is convenient to identify different paths [68, 69]. In a first path, the previously discussed outer sphere electron transfer (Fig. 3.13a) occurs between metal centers close to each other: this path definitely resembles the only possible path for purely RPs, also proper of type 1 metallopolymers. Alternatively, significant interaction between the metal-based d-π orbitals of the metal and the π or π* orbitals of the polymer occurs. This is the case of the previously mentioned inner sphere charge transfers (see Figs. 3.12, 3.13b and 3.13c), possible only for type 2 and 3 metallopolymers: both redox states of the metal should be accessible in order that it is involved, as an alternative to more or less altered electronic conduction only limited

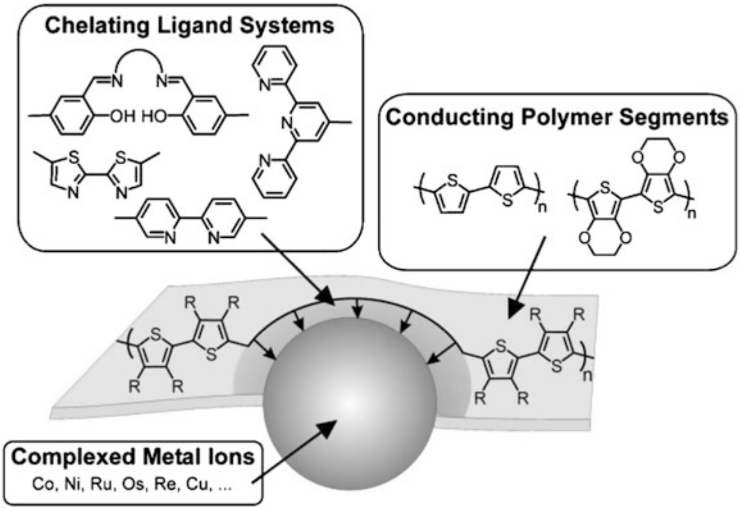


Fig. 3.12 Examples of type 2 and 3 metallopolymers (Reproduced from Ref. [69] with the permission of Royal Society of Chemistry)

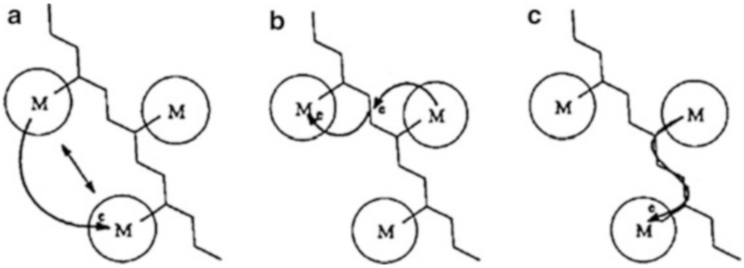


Fig. 3.13 Schematic representation of the three electron transfer mechanisms occurring between metal centers in metallopolymers: (**a**) outer sphere electron transfer; (**b**) polymer-mediated electron transfer; (**c**) superexchange pathway (Reproduced from Ref. [68] with the permission of Royal Society of Chemistry)

to the π-electron chain. Hence, inner sphere charge transfers may only occur at potentials at which both electronic and redox charge percolations are, in principle, possible. Two cases may be distinguished: in a first case, electron transfer from one to another metal center of the molecule occurs via two subsequent electron hopping steps, i.e., from the metal to a site of the chain and from this site to another metal center (‘polymer mediated pathway’; Fig. 3.13b). Similar electron transfers are only possible if electronic states of suitable energy are available: the fragment of polymer involved acts as a true redox center. Alternatively, electron transfer between two metal centers may occur through the polymer branch between them: a true electronic conduction is active within this organic fragment (Fig. 3.13c). This last type of intra-molecular charge percolation is also called ‘super-exchange pathway’ [68, 77]. In addition to

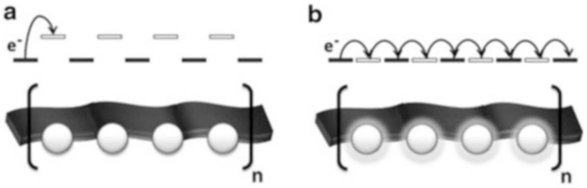


Fig. 3.14 Situation of low conductivity realized for imposed potentials far from E° of the metal redox couple: only one partner of the redox couple is present (**a**); redox matching situation (**b**)

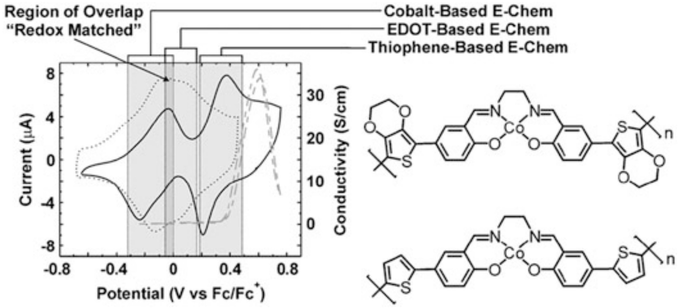


Fig. 3.15 Redox matching occurs for one metallopolymer, but not for the other (Reproduced from Ref. [69] with the permission of Royal Society of Chemistry)

these mechanisms, pure electronic conduction that does not directly involve the metal centers is possible in type 2 metallopolymers, although the effect of their presence on the electronic energy of the π-electron system of the organic backbone should be taken into account.

As discussed above, for type 2 and 3 metallopolymers the energy barrier induced by the presence of the metal complex lowers or may even vanish at the potentials at which significant concentrations of both forms of the metal redox couples are present, i.e., around the relevant *E*°, under the condition that the organic residues are doped. In this case, i.e., the so-called 'redox matching' conditions, the metal complex more or less smoothly allows the transfer of the charge between the two organic residues linked to it. Figure 3.14 illustrates the energy levels for a type 3 metallopolymer [69] at whatever potential is sufficiently far from *E*°, e.g., the open circuit potential (Fig. 3.14a), and at *E*° (Fig. 3.14b).

Figure 3.15 reports the formulation of two different type 3 metallopolymers, belonging to a class that is specifically discussed in the following, together with the relevant cyclic voltammetric curves [69]. The easier p-doping of the 3,4-ethylenedioxythiophene (EDOT) derivative with respect to the unsubstituted thiophene (Th) one allows overlap of potential region in which the polymer fragments are p-doped, hence conductive, with that in which the metal is present in two oxidation states, hence suitable for redox conduction: redox matching occurs in correspondence with this subset of potential values. On the other hand, no

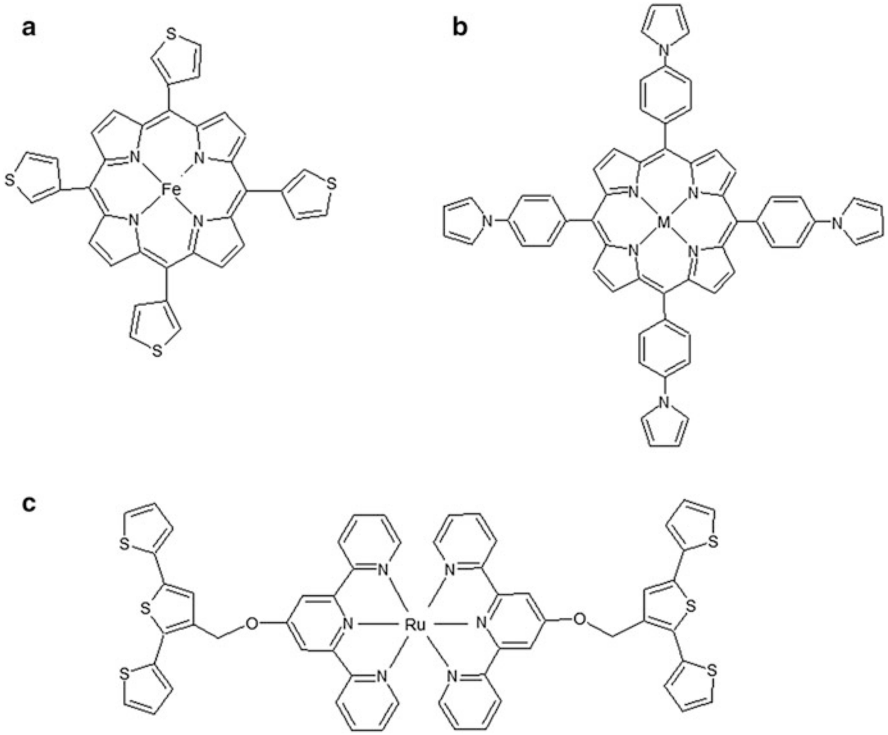


Fig. 3.16 Examples of monomers leading to type 4 metallopolymers, according to the denomination reported in Ref. [85]. (**a**) Adapted from Ref. [87]; (**b**) M = Co(II), Zn(II), Ni(II), Mn(III), and Fe(III), Adapted from Ref. [58] and Ref. [89]; (**c**) Adapted from Ref. [90]

overlap occurs with unsubstituted Th, the p-doping of which takes place at higher potentials, when the oxidized partner of the couple is the only stable form.

Differently formulated metallopolymers have been classified by different authors as type 4 metallopolymers. According to a first denomination [85], ICP chains may be linked by a bridge of various natures containing a metal complex, possibly aiming at increasing the inter-chain conductivity. Examples of monomers leading to similar metallopolymers are in Fig. 3.16. In metallopolymers such as those exemplified in Fig. 3.16a, b, the metal complex bridge does facilitate the inter-chain conduction only if the potential applied to the material is close to E° of the complex redox couple, which parallels, of course, what was written for type 3 metallopolymers as to the possibility of the charge to 'smoothly' cross the metal center [86]. Of note, the inter-chain 'bridge' in Fig. 3.16c does not account for meaningful increases in inter-chain conductivity: the -CH_2-O- bridge insulates electrically the complex from the polythiophene (PTh) chain.

According to different authors, metallopolymers containing metal–metal bonds are classified as type 4 metallopolymers [80]. Figure 3.17 reports an example of similar polymers, electro-synthesized on electrode surfaces.

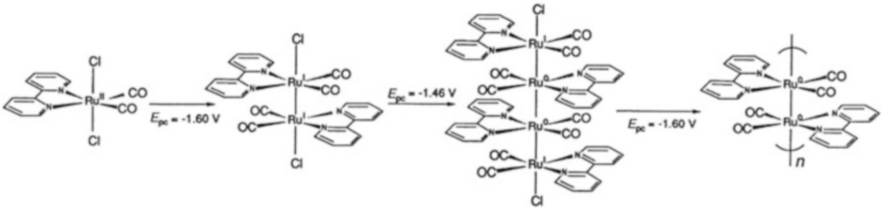


Fig. 3.17 Scheme of the synthesis of a type 4 metallopolymer exhibiting metal–metal bonds (Reproduced from Ref. [91] with the permission of Royal Society of Chemistry)

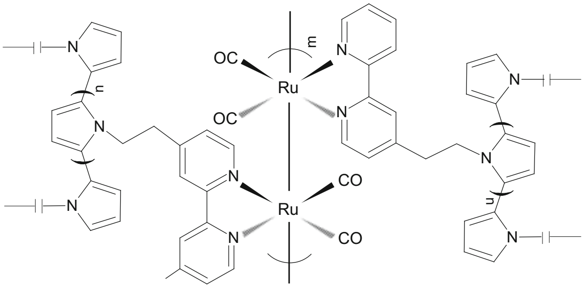


Fig. 3.18 Type 4 metallopolymer in which the inter-chain bridge includes a metal–metal bond [92]

Peculiar metallopolymers may involve ICP chains bridged by metal–metal chemical bonds. An example is reported in Fig. 3.18: two polypyrrole (PPy) chains are joined to each other by a third polymer chain consisting of a (-Ru-Ru-)$_n$ sequence; it constitutes a sort of type 4 metallopolymer [92].

Only in some cases data from conductivity measurements may give experimental account of what is predictable on the basis of the electronic interaction between metal and organic portions of the molecule (see, for instance, Ref. [84]). It is actually to emphasize that inter-molecular conduction, though of comparable importance in respect to the intra-molecular one, is particularly difficult to give a rationale. This is due to the dependence on many factors extraneous to the chemistry of the metallopolymer molecule as such. Not only the length of the chains, but also nature of the solvent and of included counter-ions and, generally, all the experimental conditions adopted for the polymer mass growth render any possible discussion largely speculative. It follows that experimental data can only be related to arguments dealing with properties of individual molecular entities in those cases in which intra-molecular charge percolation constitutes the rate-determining step for conduction. This conclusion is of chief importance, despite the very abundant work found in the literature about intra-molecular conduction.

As detailed in Chap. 2, the electronic conductivity is high either above or below a given potential, for p- and n-doping, respectively. As evidenced in the present chapter, a (parallel) redox conduction is only operative within a potential interval around E° of the redox couple. Of note, the redox center in similar metallopolymers, in analogy to what happens in RPs, may well exert the role of redox mediator in electrocatalytic oxidation or reduction processes, at potentials at which the oxidized or the reduced form is the only stable one, respectively. The charge necessary for the metal to be brought back to the 'useful' oxidation state, in fact, arrives at the polymer|solution interface thanks to two possible mechanisms: a former mechanism has been previously illustrated for single-component RPs, and a latter involves the electronic conduction due to the ICP discussed in Chap. 2.

Once reachable by the species in solution, the metallopolymer systems offer the advantage of presenting easy conductivity to the catalytic metal centers. The binding of the mediator to a properly conducting matrix adhering at the substrate constitutes a notable added value of metallopolymers with respect to composite materials in which conduction and redox mediation are in charge of different components. In addition, the metal centers are included inside a singular electronic energy environment, so that it may possibly assume otherwise less stable electronic energies, i.e., unusual oxidation states. The potentiality of similar systems in electrocatalytic processes, as well as in addressing electrode mechanisms to unusual paths, is far from being defined or even exploited.

The previously cited recent review by Friebe et al. [80] can be used by electroanalysts interested in testing a variety of similar metallopolymers in the frame of possible amperometric sensors. In particular, two tables in the review furnish practical addresses to original literature. Unfortunately, exhaustiveness in the field has become an unreachable goal due to the huge amount of publications.

3.8 Representative Metallopolymers

The aim of these pages is not only to hopefully pick up most meaningful examples among the materials proposed for analytical scope, but also to identify those that, for peculiar characteristic behavior, seem to be particularly appealing in respect to an application in electroanalysis.

A number of metal-containing systems, mainly including metallocenes and complexes with N-based ligands, have been linked to electronically conducting polymeric frameworks, principally consisting of PTh or PPy derivatives. 2,2′-bithiazole [93] and benzimidazole [94] have also been proposed as structural units of π-electron conjugated chains, acting, at the same time, as a ligand. Such a 'double role' is found in the polymeric chains in Fig. 3.19.

The metal centers are chosen from those that typically exhibit, in the frame of the complexes, reversible redox behavior, being stable in two oxidation states. Such a feature is required in order to sustain electrical conduction, either 'purely' redox in character or somehow 'hybridized' with the electronic one, proper of the π-electron chain fragments. Moreover, it also constitutes an evident necessity whenever one partner of the redox couple is called to play the role of redox mediator in

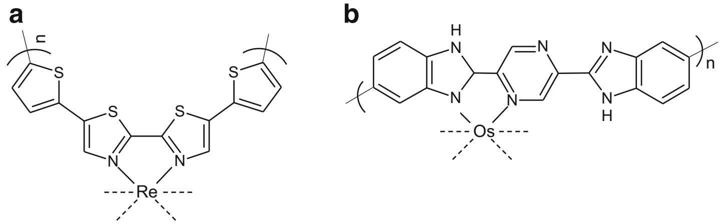


Fig. 3.19 Structural units of π-electron conjugated chains based on N-based ligands: (**a**) from Ref. [95]; (**b**) from Ref. [94]

electrocatalysed oxidations or reductions, with respect to a species in solution, at the polymer|solution interface. The claimed marked preference for N-based ligands meets with such a request, often giving rise to reversible redox couples with a variety of metal ions [85]. Among others, pyridyl complexes containing pyridine, bipy, 2,2′:5′,2″-terpyridine (terpy) ligands, are used particularly for the synthesis of metallopolymers with Ru(II), Fe(II), and Os(II) metal centers [80]. On the other hand, metalloporphyrins, metallophtalocyanines, and metal complexes with Schiff base ligands have also attracted lot of attention.

A number of type 1 metallopolymers based on pyrrole (Py) and Th units have been proposed, thanks to the easier synthesis of the relevant monomers. The reactivity of the N atom allows relatively easy functionalization of Py rings, leading to stable anchoring of groups or even complexes inside a conductive matrix: a more stable system than the simple inclusion of a specifically reactive molecule inside an ICP is obtained. Many systems have taken advantage of such a feature, biosensors included, as reported hereafter. Not only the reactivity of the N heteroatom has been used, but also that of the 3 and 4 positions in the Py ring. It should be highlighted that, on the other side, Th presents higher reactivity of the 3 and 4 positions, allowing specific functionalities suitable to fix a variety of metal complexes to be stably linked. Finally, note that Friebe et al. [80] give a partial albeit significant picture of electropolymerized metallopolymers. The relevant monomers consist of metal complexes with macrocyclic ligands such as porphyrins, phtalocyanines, and cyclam, which functionalize Th and Py structural units of the electron conductive organic backbone. A variety of metals are indicated as suitable to be inserted into the porphyrin ring. The delocalization of π electrons within the ring makes the interaction with the systems, constituting the backbone, particularly intriguing in the relevant type 2 and 3 metallopolymers.The reactivity in the 3 and 4 position of the Th ring has also been exploited to introduce organic functionalities suitable to tune the properties of the material. In particular, electron-donating groups can be inserted to reduce the potential value at which monomer oxidation, i.e., polymerization, occurs, as well as to lower the band gap value of the resulting metallopolymer. This aspect is particularly important when considering metallopolymers that can allow an electronic communication between the delocalized π-system of the organic moieties and the d-π orbitals of the metal center: as already evidenced, metallopolymers may exhibit redox matching by suitably choosing both the organic and the inorganic components.

It is evident that electropolymerization has been used, whenever possible, to synthesize the metallopolymers that have been envisaged as finding applications in electrochemistry, specifically in amperometric sensing. To this aim, two alternative synthetic strategies are possible: (1) the organic backbone, bearing part of the ligands set, is synthesized in advance and the complex is formed afterwards, by addition of metal ions under suitable form; (2) the starting monomer also bears the whole metal complex. In the majority of the cases reported by the literature, the latter approach constitutes the most often followed, also because the chemical structure of the metallopolymer can be more strictly controlled.

3.8.1 Metallocenes

A number of PTh, but also PPy, polyaniline, and additional ICPs based on π-electron delocalized systems, bearing different metallocenes of Fe, Ni, Co, Ru, or Os, either as a pendant group or as an inclusion complex, have been synthesized and even proposed as electrode coatings for amperometric sensing. They have been extensively and critically reviewed [86]. It was observed that good conductivity and accessible p-doping potentials are only exhibited either (1) by co-polymers of Fc-substituted Th and unsubstituted Th, or (2) by Th bearing only one Fc residue.

Biosensors have been developed exploiting Fc as a redox mediator or even as a redox probe exhibiting electroactivity that depends on variation in the environmental conformation as a consequence of the interaction of the analyte with the sensing element of the system. In Ref. [96], a PPy chain is randomly branched with side chains bearing Fc moieties or propanoic acid functionalities; the electrode coating has been proposed in the frame of an amperometric biosensor for glucose. Glucose oxidase enzyme is claimed to be covalently immobilized by formation of amine bonds through the acidic residue. In Ref. [97], genosensors are proposed in which the electrode is coated by electrogenerated PPy functionalized with Fc moieties bearing, in turn, DNA chains (see Fig. 3.20). Similar to what is already observed for pure ICP-modified electrodes (see Chap. 2), the occurrence of hybridization with the proper target molecule causes a decrease of the current and broadening of the anodic-cathodic redox system, due to increased difficulty of Fc to exchange electrons with the underlying surface and to increasing resistance inside the coating. This is ascribed to the increased steric hindrance that prevents easy entrance of counter-ions into the polymer, as required by neutralization of the p-doping centers. The system is also assembled on a microelectrode: changes in nucleation and growth of the PPy during electrogeneration, with respect to the conventional electrode, causes an increase in sensitivity [98].

Ion et al. [99–101] studied PPy bridged to an Fc unit through a crown ether suitable to complex alkaline and alkaline earth metal ions, such as Ca(II) or Ba(II), but also Cu(I), with respect to the modification of the relevant cyclic voltammetric

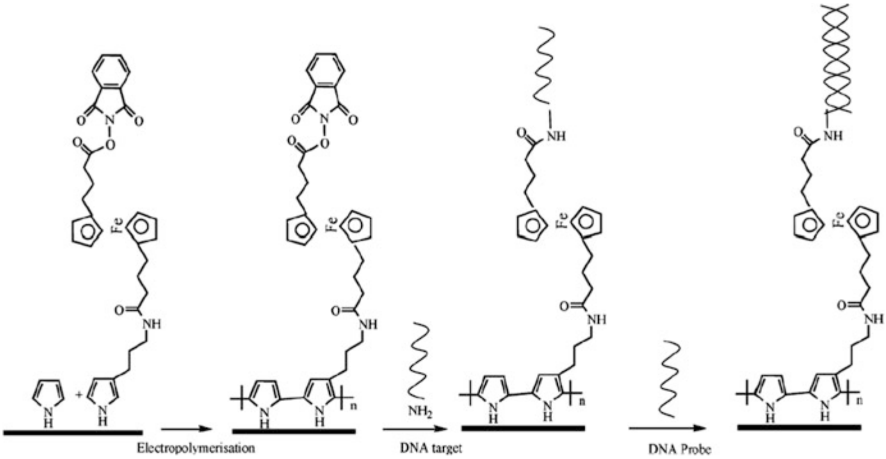


Fig. 3.20 Scheme of the synthesis and of the occurrence of coupling with the target DNA by the sensing element of a genosensor; the signal consists of a higher or lower availability of the Fc residue to reach the underlying electrode surface (Reproduced from Ref. [98] with the permission of Elsevier)

and impedance behavior in the absence and presence of crown-complexed ions. Impedance responses, specifically the change in the low-frequency pseudo-capacity measured on the polymeric deposit when passing from free to complexed crown residues, are identified as selective with respect to the nature of the metal. What is not good for a monograph on amperometry, the results from impedance are more promising for quantitative analysis than voltammetric measurements. As to these last ones, is well known that ether macrocycles are specific ligands for alkaline or alkaline–earth metal ions: the uptake of the proper ion increases the positive charge density close to the oxidisable Fc unit, and a split of the original Fc to Fc^+ ion oxidation peak is observed. The peak at more positive potentials increases in height increasing metal concentrations in solution, i.e., the fraction of complexed crown ligand.

Fc-substituted PPy derivatives have also been synthesized and characterized with the scope of quantitatively determining cytochrome *C* protein [102]. The Fc^+/Fc redox couple is responsible for the redox charge transfer inside the coating; as to redox mediation, only the polymer in which the Fc residue is linked to the PPy (neutral) chain by the shortest alkyl bridge, i.e., by three methylene groups, is capable of detecting the analyte. The necessity of an interaction with the PPy chain is hypothesized: the organic chain should play a role at a potential at which it is not conducting, so that the polymer acts as a purely redox one, thanks to the Fc residue.

Some examples are also reported in which metallocene different from the more diffused Fc are present. PTh derivatives have been synthesized that include Py units inside the π-electron chain; these aromatic rings act, at the same time, as the second π ligand to Fe, cyclopentadienyl anion being the other. Interesting modulation of the redox contribution to conductivity is induced by varying the number of Th units between subsequent azaferrocenes [103]. The conduction mechanism is hypothesized to possibly involve superexchange pathways, which means that both the metal complex and the chain, through 'localized' redox centers, are involved in the charge percolation mechanism.

3.8.2 Bipyridine and Terpyridine Ligands

Bipy and terpy are among the most frequently encountered N-based ligands in metallopolymers. Examples of applications in metallopolymers constituting electrode coatings for amperometric sensors are also reported. Furthermore, examples of networks consisting of more PTh or PPy chains interconnected by tris(bipy) metal complexes are reported. Typically, the alkyl chain linking the conducting π-electron system to the metal complex is long enough not to allow 'mixing' of redox and electronic conduction. Examples are in Fig. 3.21.

In the frame of enzyme-based biosensing, in Ref. [106], a glucose oxidase-based amperometric biosensor is realized in which the enzyme is entrapped within the mass of a metallopolymer, namely a PPy bounding $[Os(bipy)_2]^{2+}$ moieties, separated by an alkyl chain long enough to consider the PPy derivative a type 1 metallopolymer. Figure 3.22 reports the formulation of the polymer. The use of a glassy carbon substrate, on which H_2O_2 is not electroactive at the working potential, prevents interference of H_2O_2 eventually generated by the enzyme in presence of O_2, evidencing the direct charge transfer from the electrode to the enzyme through the Os complex.

The same Py structural unit functionalized with the Os complex (Fig. 3.22) has also been co-polymerized with Th and used in the frame of amperometric biosensing for pyruvate determination [107], taking advantage of the mediation

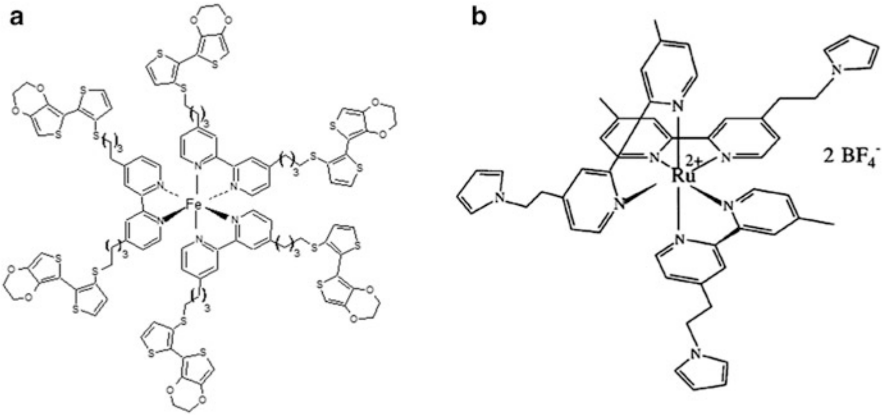


Fig. 3.21 Examples of monomers bearing bipy moieties. (**a**) From Ref. [104]; (**b**) reproduced from Ref. [105] with the permission of Wiley

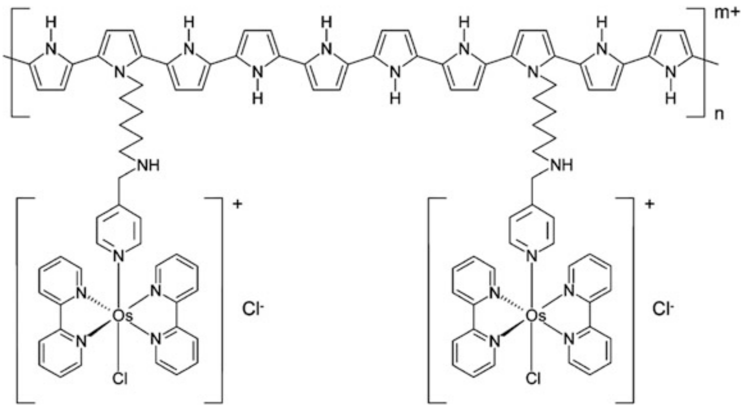


Fig. 3.22 PPy stably anchoring $[Os(bipy)_2]^{2+}$ complexes; the type 1 metallopolymer also acts as a redox mediator in enzyme biosensor (Reproduced from Ref. [106] with the permission of Elsevier)

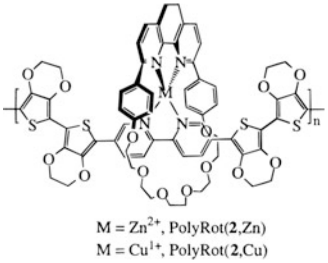


Fig. 3.23 Metallorotaxane inside oligoEDOT fragments; the behavior at varying the metal and substituting Th for EDOT is described in the text (Reproduced from Ref. [109] with permission of American Chemical Society)

of the Os(III)/Os(II) redox couple in the oxidation of $FADH_2$ residue of pyruvate oxidase, which is in turn adsorbed on the coated Pt black electrode. A similar copolymer with 3-methylthiophene has been previously characterized as to morphology and electrochemical behavior, as well as tested in the amperometric quantitative determination of ascorbic acid [108].

In Ref. [109], a particularly significant example is found of the potentiality of metallopolymers in detecting selectively different metal ions; bipy ligand is inserted inside an oligothienyl chain. Bipy reversibly captures Zn(II) or Cu(II)/Cu(I) metal ion and the coordination is completed by a rotaxane macrocycle; two additional N atoms 'satisfy' the tetracoordination tendency of both metals. Figure 3.23 represents the structural units of the two different systems.

Rationale is given to parallel experimental cyclic voltammetric and conductimetric tests in which the ions are either present or absent inside the coordination sphere: the results also depend on the nature of the metal and of the Th structural unit. In particular, reversible uptake of Cu(II) ions by macrocyclic ligands

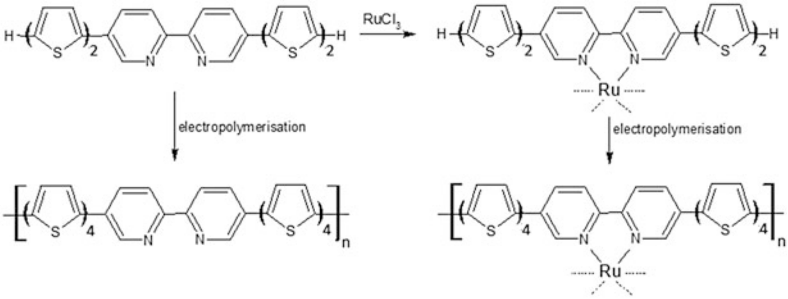


Fig. 3.24 Bipy inserted between PTh fragments, complexing (on the *right*) or non-complexing (on the *left*) Ru metal ions. (Adapted from Ref. [68])

interposed between four consecutive EDOT units, to give a polymetallorotaxane, on its own induces, by oxidation, p-doping of the neutral fragments of π-electron systems. The resulting system, including formal Cu(I) metal, exhibits an increase in conductivity of several orders of magnitude with respect to the pure organic system: redox matching is realized and the Cu(II)/Cu(I) metal centers effectively ‘connect’ the adjacent EDOT fragments. Completely different behavior is found by substituting Th for EDOT, due to the higher oxidation potential of four consecutive Th units. On the other hand, inclusion of Zn(II) induces redox conductivity. This last result confirms the data on pure PPy polymer: the bipy inside the π-electron chain is meaninglessly involved in p-doping, hence in electronic conduction. The presence of the metal renders the density of π electrons on the bipy even lower, correspondingly lowering the participation of the ligand to the delocalization of the charge carriers along the chain; no significant difference in electronic conductivity behavior is exhibited with respect to the metal-free organic polymer.

Peculiar metallopolymers, seemingly representative of situations intermediate between type 2 and 3 metallopolymers, present the metal co-ordinated to a ligand that is part of the π-electron chain and completes the co-ordination set with additional ‘external’ ligands. Similar metallopolymers have been synthesized and characterized, in which the Ru metal is co-ordinated to bipy belonging to the ICP backbone, consisting of bipy and Th basic structural units in a co-polymeric chain [68] (see Fig. 3.24):

Aware that the response of analytical meaning is, in the case depicted, in terms of conductivity, we firmly believe, however, that amperometric sensing could take advantage of similar effects, e.g., based on the behavior with respect to an electroactive probe in solution, or on some other mechanism.

Similar systems to those discussed above are found in Ref. [110] and Ref. [111]. In Ref. [111], Fe or Ru metal ions are co-ordinated to two bipy ligands, each one interposed between two EDOT units. Polymerization at the extreme EDOT residues occurs, the metal also acting as a bridge between two adjacent oligomeric chains. The shortness of the organic segments between adjacent metal complexes causes the redox conductivity character to largely predominate. A similar metallopolymer is electrosynthesized and characterized in Ref. [110]: a 2,6-bis(pyrazol-1-yl)pyridine ligand is linked to EDOT residues, constituting part of the π-electron system, and complexes a Ru(II) ion: the coordination sphere is completed by a terpy molecules.

Cosnier et al. [105] reported on a copolymer of Py functionalized with polypyridyl complex of Ru(II) and of Py functionalized with a biotin derivative. This peculiar metallopolymer is shown to be suitable to immobilize functionalized glucose oxidase and alkaline phosphatase and the realized biosensors to be effective in quantitative detection of the relevant analytes.

3.8.3 Phenanthroline Ligand

The use of 1,10-phenanthroline as the organic binder allows the synthesis of metallopolymers possessing different metal ions with respect to the most common Ru(II) and Os(II). Vidal et al. [112] synthesized and electropolymerized a monomer containing Cu(I) centers co-ordinated to two 1,10-phenanthrolines, each one bearing one, two, or three consecutive Th units in 2 and 9 positions (see Fig. 3.25 for the case of a 2,2′-bithiophene unit).

Polymers presenting two adjacent oligothiophene chains bridged by the 1:2 metal:phenanthroline complexes have been obtained. From a combination of voltammetric and conductivity measurements, it could be concluded that the Cu(II)/Cu(I) couples are responsible for the conduction when the metal ions are separated from each other by a short π-electron chain fragment, i.e., consisting of four Th rings in the same molecule. On the other hand, significant electronic interaction with the π-electron system is found when six Th units are interposed between the $[\mathrm{Cu(1,10\text{-}pheneathroline)_2}]^+$ complex along the chain. This finding is consistent with the often claimed occurrence of polaron delocalization over six Th units. Interesting structural peculiarities are predictable on the basis of the strong link between different chains, induced by Cu ions coordinated to four N atoms of phenanthroline moieties belonging to different chains or portions of one chain.

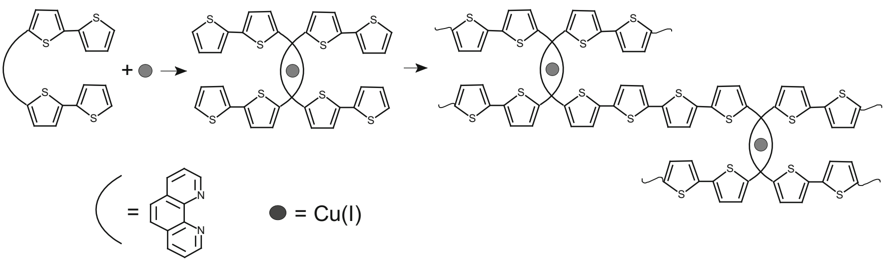


Fig. 3.25 Schematic representation of the synthetic strategy to obtain 1,10-phenantroline bithiophene-based metallopolymers (Reproduced from Ref. [85] with the permission of Nova Publishers)

3.8.4 Schiff Base Ligands

Metal complexes differently involving Schiff base ligands have been the object of a number of studies on metallopolymers, thanks to the highly delocalized π-electron system of such an organic ligand. In particular, Cu(II)salen and Ni(II)salen [salen = N,N′-ethylenebis(salicylimine)] complexes have been electrochemically polymerized, chain propagation occurring at the two outermost phenyl residues; the relevant mechanism has been investigated in Refs. [113–116]. Figure 3.26 shows a general scheme of a reaction leading to anodic polymerization of Cu(II) or Ni(II)-salen complexes.

A number of M-(salen) complexes, where M = Co(II), Fe(II), Cu(II), or Mn(II), have been polymerized onto a microelectrode that proved to be suitable for amperometric quantitative determination of dissolved NO [117]. Also, in view of the unusual nature of the metal center, polymers obtained from [Pd-(salen)]-type complexes, electrosynthesised and characterized in Ref. [118], deserve to be cited.

Abundant literature also exists about Schiff base ligands linked, by the N atoms, to the β positions of Th or Py rings, for the development of particular type 2 metallopolymers. Thanks to the versatility of the Schiff bases as ligands, different metal ions can be coordinated according to the scheme in Fig. 3.27a. Alternatively, the phenyl residue of the outermost portion of the Schiff base is bound to α-positions of the heterocycle (see Fig. 3.27b). By polymerization of monomers like that reported in Fig. 3.27a, metallopolymers are obtained. They show a structure that classifies them as type 2 metallopolymers, with possible interesting coupling of electronic and redox conduction, by redox matching. On the other hand, type 3 metallopolymers are obtained from monomers reported in Fig. 3.27b.

Starting from a series of monomers consisting of a Th ring linked in 3,3′ positions to N atoms of a Schiff base ligand complexing different metal ions (see Fig. 3.28), Reddinger and Reynolds [119] obtained PTh chains by 'blocking' the phenyl residues with methyl groups and minimizing the steric hindrance by using terthiophene as the unit constituting the π-electron organic portion of the monomer. On the other hand, polymers comprising phenylene or thienylene-linkages, or mixtures of the two, are achieved by suitably substituting the C atom to which the polymer chain grows (see Fig. 3.28).

Peculiar behavior has been observed for a Th derivative similar to those reported in Fig. 3.28, in which the two phenyl rings are connected to each other by a crown ether bridge comprising four oxygen atoms. The resulting modified electrode (see

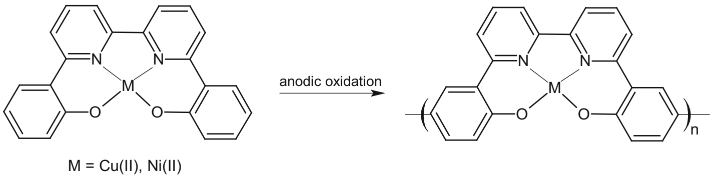


Fig. 3.26 Electropolymerization at the phenyl rings of a Cu or Ni-(salen) complex [113]

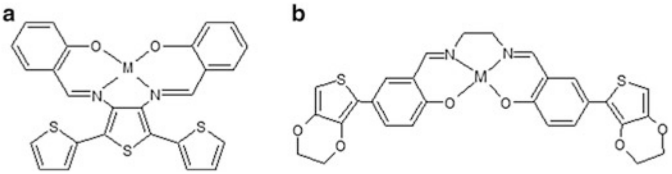
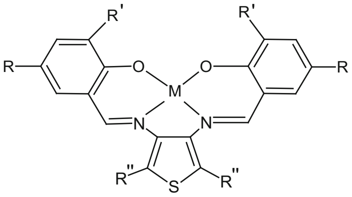


Fig. 3.27 Examples of formulations for Schiff base-based type 2 (**a**) and type 3 (**b**) metallopolymers



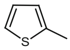
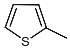
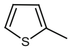
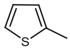
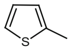
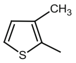
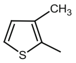
M	R	R′	R″	Polymer	Linkage
-	H	H	H	No	-
Ni	H	H	H	Yes	Phenylene
Cu	H	H	H	Yes	Phenylene
Cu	CH_3	H	H	No	-
Cu	H	H		Yes	Mixture
Cu	CH_3	H		Yes	Thienylene
Co	CH_3	H		Yes	Thienylene
Zn	CH_3	H		Yes	Thienylene
Cu	CH_3	CH_3		Yes	Thienylene
Cu	H	H		Yes	Phenylene
Cu	CH_3	H		No	-

Fig. 3.28 Differently substituted complexes of metals with Schiff base ligands, called SALOTH derivatives, suitable to polymerize differently (Reproduced from Ref. [119] with the permission of American Chemical Society)

Fig. 3.29) is suitable to detect, via a change in the electroactivity of the coating, the occurrence of complexation by alkaline or alkaline earth metal ions [120].

Salen and additional Schiff base ligands complexing Cu ions are functionalized by 'pseudo-crowns', according to the authors' definition. They are electropolymerized and identified as suitable to detect, by cyclic voltammetry, the occurrence of complexation with Ba(II) ions [121].

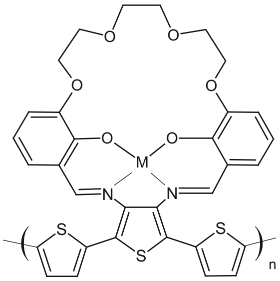


Fig. 3.29 SALOTH-based PTh bearing a complexing crown ether bridge [120]

3.8.5 Porphyrin Rings

Metalloporphyrin complexes constitute the monomers of a peculiar class of metallopolymers [122]. As such, they are used as catalysts in many reactions in the field of organic chemistry. Although the applications in electroanalysis are not correspondingly numerous, examples are found in the literature that once more envisage interesting developments for the relevant metallopolymers. Films consisting of poly(metalloporphyrins) or poly(metallotetraphenylporphyrin) with a variety of metals, e.g., Fe(II), Co(II), Ni(II), and Cu(II), on conductive substrates have been realized over time. Most of the metallotetraphenylporphyrins polymerize at the amino groups substituents to the phenyls [123–125] (see Fig. 3.30).

Moreover, in a monograph dealing particularly with sensing, it is important to highlight that metalloporphyrins, as well as metallophtalocyanines, are widely used under a monomeric form in gas analysis, e.g., in the frame of electronic noses; however, they do not use amperometric techniques...

Systems in which metalloporphyrin residues are anchored to ICP backbones, leading to the relevant metallopolymers, are much more diffused than poly-(metalloporphyrin). Due to the variety of reactive positions presented by differently substituted porphyrin and metalloporphyrin, a number of relevant polymers, presenting quite different properties, have been realized. Although examples are found of polymers in which meaningful interaction occurs between the π orbitals of the complex and those of the π chain [127, 128], most of the realized metallopolymers present the porphyrin ring far from the organic backbone, not leading to meaningful interactions between redox and electronic conductions [82, 88, 129, 130]. The high steric hindrance of the porphyrin ring forces the synthesizing of copolymers with unsubstituted bithiophene [82] or the use of terthiophene as the portion of the monomer on which polymerization occurs [88].

A behavior envisaging possible application in electroanalysis is found in Ref. [88]: the shift of the potential exhibited by the redox system due to the Fe-porphyrin complex in a metalloporphyrin functionalized PTh is shown to depend on the concentration of 2,4,5-trichlorophenol in solution. Interaction of the chlorinated phenol with the Fe centre is hypothesized to cause the modification of the electron

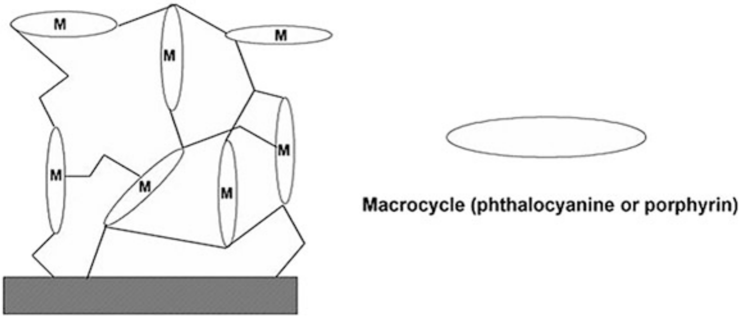


Fig. 3.30 Scheme of possible arrangement of metal poly(metalloporphirine) or poly (metallophthalocyanine) coatings on an electrode substrate (Reproduced from Ref. [126] with the permission of Royal Society of Chemistry)

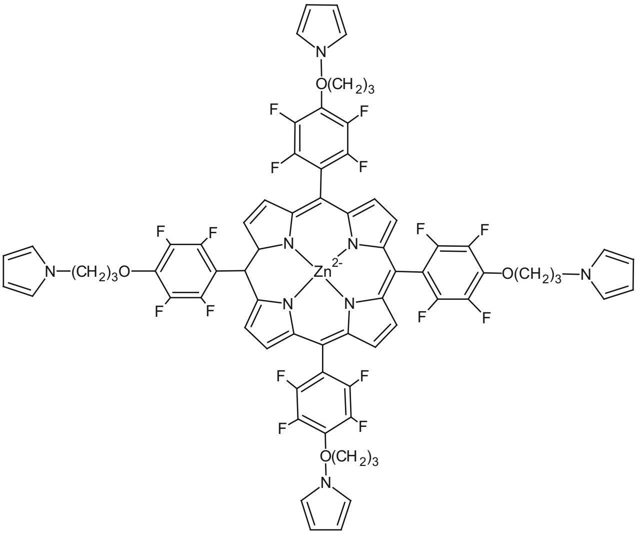


Fig. 3.31 Monomer of metallotetraphenylporphyrine bearing four Py residues at which polymerization occurs, leading to a network [131]

energy of the porphyrin complex. However, statistical data about the actual possibility of using the finding for analytical purposes are not given.

In Ref. [131], Zn(II) and Mn(III) tetraphenylporphyrin complexes are covalently linked to (1) one or (2) four PPy chains (see Fig. 3.31) through long alkyl residues, forming a network in the latter case. The electropolymerized coating exhibits electrocatalytic properties and seems promising in respect of H_2O_2 detection and quantification, by cathodic reduction in aqueous solution, even in the presence of O_2, envisaging applications in biosensing. Moreover, catalytic oxidation of dissolved NO has been realized at an electrode coated by polymerization of Py residues at each of the four phenyls of an Mn tetraphenylporphyrin redox center.

The length of the alkyl chain from the PPy to the porphyrin ring leads the redox centers to assume well defined oxidation states, being inside a fairly conductive matrix at the same time [132]. Detection of NO released in vivo by endothelial cells, under stimulation of endothelial growth factor, was successfully performed. A layer of Nafion® coating the electrode modification prevents interference from anionic species, such as NO_2^- [133].

An electrode modified by a mono or submonolayer of poly[Ni(protoporphyrin IX)] has demonstrated to be effective in quantitative determination of dissolved SO_2 by electrocatalysed oxidation, also acting as a barrier with respect to interfering anions [134].

3.8.6 Phthalocyanine Ring

Fewer articles deal with electrochemical generation of metallopolymers bearing phthalocyanine residues or differently including phtalocyanine within the conductive π-electron system. However, proposals to use amperometric sensors based on similar modifiers are particularly interesting. Coatings similar to those formed by poly(metallophenylporphyrine) are reasonably assumed to form (see Fig. 3.30).

Electropolymerized Ni(II)-tetraaminophthalocyanine-coated electrodes have been used [135] for electrocatalytic oxidation of dopamine. The results of the determination are exhaustively treated and the interference of ascorbic and uric acids, as well as of amino acids and inorganic ions, has been studied. The selectivity toward given potentially interfering species has been strongly enhanced by the electrostatic action of a Nafion® coating in a bilayer electrode modification. Of note, tests in biological matrices, such as plasma and urine, are reported. In Ref. [136], Mn(II)-tetraaminophtalocyanine is electropolymerized onto a Pt microelectrode and has been shown to be effective in the determination of NO_3^-, i.e., a powerful oxidant and cytotoxic agent. In Ref. [137], electropolymerized PPy functionalized by Mn(II)-phtalocyanine constitutes the electrode coating. The polymer presents marked electronic interaction between the PPy chain and the metal phthalocyanine complex; it exhibits electrocatalytic properties in the reduction of molecular oxygen. In Ref. [138], though no analytical application is proposed, an appealing metallopolymer has been electrogenerated, starting from the synthesis of the relevant monomer: a Ru complex with a substituted phtalocyanine ring bridges the N atoms of pyridine rings, in turn bridged to bithiophene moieties in 3 and 5 positions.

3.8.7 Cyclam Cycle

Cyclam, i.e., 1,4,8,11-tetraazacyclotetradecane, linked to PTh chain [139], has been used to complex Ni(II), obtaining effective redox matching (see Fig. 3.32). The

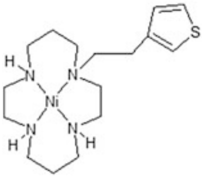


Fig. 3.32 Th monomer β-functionalized by Ni-cyclam complex [139]

complex is separated from the organic chain by an alkyl bridge that overcomes the steric hindrance of the bulky cyclam residue. Of note, a co-polymerization of Th and 3-methylthiophene was necessary in order to achieve a stable deposit on the electrode substrate. PEDOT functionalized by Ni-cyclam complex [140] has also been reported to grow, by electropolymerization, on an electrode surface. The Ni (II)/(III) reversible process is clearly observed. Despite the interest in obtaining a metallopolymer containing an Ni(II) center suitable to activate electrocatalytic processes, to the best of our knowledge these materials have not found an application in amperometric sensing.

3.8.8 Sulphur-Based Ligands

Metallopolymers in which Ni(II) [141–143], Pd(II) [142, 144], and Au(III) [142, 144] are complexed by sulphur-containing ligands are reported in the literature, under the form of dithiols or dithioethers. Side Th groups are suitable to lead to the corresponding metallopolymers. These have been synthesized and characterized, but are not used in electroanalysis. On the other hand, they are often poorly conductive, and p-doping is only obtained at relatively high potentials.

3.8.9 Phosphine Ligands

Th derivatives bearing phosphine functionalities complexing metals such as Pd (II) [145, 146], Rh(I) [147], and Ru(II) have been polymerized; in this case, applications in electroanalysis are also still missing, although easy conformational change, potentially interesting with respect to specificity in metal coordination, have been evidenced. A further unexplored interesting aspect of phosphines, as well as of other ligands such as CO, lies in the well-known capability to stabilize the metal center in low oxidation states.

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Chapter 4
Ion Exchange Polymers

This chapter deals with a number of non-conducting polymers possessing net positive or negative charge. They impart the electrode a specific reactivity toward charged species in solution, exploitable in many electroanalytical contexts, as detailed in the following. Table 4.1 reports the most common anionic and cationic polymers used in electroanalysis; they are, for the most part, commercially available.

4.1 Polymers for Ion Exchange Voltammetry

The positive or negative charges brought by polymers of this class allow the pre-concentration of oppositely charged analytes in the close proximity of the electrode transducer, i.e., the conducting substrate on which the polymer is deposited. The process is driven by ion exchange equilibrium between electroactive counter-ions present in solution, i.e., the analyte, and non-electroactive counter-ions originally incorporated into the polymer coating. As an example, in the case of metal ion detection with a Nafion® coating, the pre-concentration equilibrium can be written as:

$$\mathrm{An}^{n+} + n(\mathrm{R}-\mathrm{SO}_3^-\mathrm{X}^+) \leftrightarrows n(\mathrm{R}-\mathrm{SO}_3^-)\mathrm{An}^{n+} + n\mathrm{X}^+$$

where $-\mathrm{SO}_3^-$ residues indicate the ion-exchanging sites of the polymer, X^+ is an electrochemically inert counter-ion, chosen with $z = 1$ for the sake of simplicity, and An^{n+} is the multiply charged electroactive analyte. Regeneration of the coating after the voltammetric detection can be achieved by exposing the film to a suitably high concentration of non-electroactive salt solution, to expel the analyte from the film.

The ability to pre-concentrate the analyte in the close proximity of the electrode surface leads the sensor to reach high sensitivity and low detection limits, generally resulting two or three orders of magnitude lower than those achieved when using

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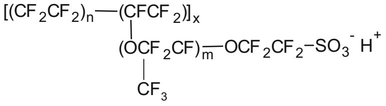
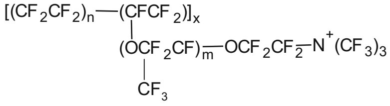
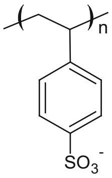
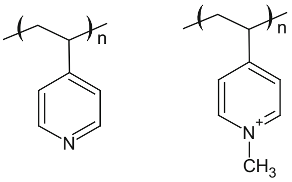
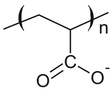
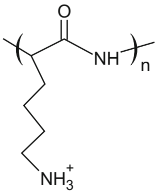
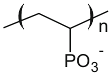
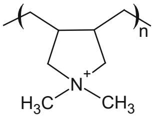
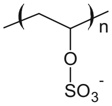
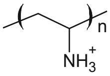
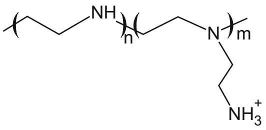
Table 4.1 Examples of ion exchange polymers applied in electroanalysis

Anionic polymers	Cationic polymers
 $[(CF_2CF_2)_n-(CFCF_2)]_x$ $(OCF_2CF)_m-OCF_2CF_2-SO_3^-\ H^+$ CF_3 Nafion®	 $[(CF_2CF_2)_n-(CFCF_2)]_x$ $(OCF_2CF)_m-OCF_2CF_2-N^+(CF_3)_3$ CF_3 Tosflex®
 Poly(sodium-4-styrenesulphonate)	 Poly(4-vinylpiridine) – Poly(N-methyl-4-vinylpiridine)
 Poly(acrylic acid)	 Poly(L-lysine)
 Poly(vinyl phosphonic acid)	 Poly(dimethyldiallylammonium)
 Poly(vinyl sulphate)	 Poly(allylamine)
	 Poly(ethyleneimine)

the relevant unmodified electrode [1–3]. The concentration inside the polymer is usually so high, compared with that in the solution, that higher currents are registered with respect to bare electrodes despite the lower diffusion coefficient of the species in the polymer phase.

The selective interaction of the coating with the analyte can be exploited for speciation, allowing the quantification of one specific chemical species under which the analyte is present [4]. On the other hand, coatings containing an ion-exchange polymer can drastically reduce the effects of interferences, thanks either to size or to electrostatic exclusion effects [5–8].

Owing to the great interest in the development of sensors for heavy metal detection made possible by the use of a cation-exchange polymer, Nafion® still represents the polymer most frequently used in ion-exchange voltammetry [9, 10]. As depicted in Table 4.1, it consists of a fluoropolymer bearing sulphonate-exchange residues. The main reason for the wide diffusion of this polymer lies in its chemical structure, characterized by hydrophilic domains, constituting the ion exchange sites, linked to long and flexible organic chains [11]. This hydrophobic skeleton makes the coating stably adhere at electrode surfaces.

Tosflex® represents the cationic homologous of Nafion®, bearing quaternary ammonium residues [2, 4, 9, 10, 12, 13]; for instance, Tosflex® is suitable for the detection of heavy metals in the form of anionic complexes.

Besides heavy metal determination, many different organic and inorganic ionic species, such as organic acids and catecholamines, have been quantified by taking advantage of the ability to pre-adsorb them at the electrode surface [8–10].

In addition to the two polymers listed above, several electroanalytical applications have been reported for poly(sodium-4-styrenesulphonate) [1], poly(L-lysine) [14], poly(allylamine hydrochloride) [15] and poly(diallyldimethylammonium) chloride [3]. However, due to the higher density of ion-exchange sites in these polymers, they are all characterized by a higher solubility in water, negatively affecting the stability of the anchoring. More stable systems can be obtained by cross-linking stabilization procedures, once the polyelectrolyte is deposited on the electrode surface [1, 14, 16].

In many cases, ion-exchange electrode coatings are simply deposited by slow evaporation of solvent from fairly diluted solutions, drop casted onto metal electrode surfaces. Due to the insulating nature of ion-exchange polymers, care must be taken to limit the thickness of the coating. On the other hand, it should also be taken into account that the coating finally results permeable enough to aqueous solutions to allow the entrance of the analyte, so that the polymer does not constitute a barrier to the analyte in approaching the electrode. Alternatively, ion-exchange polymers can be included in the bulk of the electrode, within either carbon paste or ink in a screen-printed electrode [17].

4.2 Polymers for Inclusion of Redox Mediators

The ability of ion-exchange polymers to incorporate different ions can be exploited in a different context, i.e., to fix redox active species capable of activating electrocatalytic processes, i.e., to act as redox mediator, for the determination of a target analyte. The most spontaneous choice consists of the inclusion of

$[Fe(CN)_6]^{3-}$ in anion-exchange polymers, typically in Tosflex® [12, 18]. To the same purpose, several examples are also reported involving Nafion® for the anchoring of positively charged metal complexes [19] or of organic species [20].

The redox mediator can be fixed either to a polymer film pre-formed on the electrode surface or to a polymer dissolved in solution; in the last case, the deposition occurs in a subsequent step. One of the main problems arising from the use of these electrode coatings lies in the progressive leaching of the redox mediator when exposed to the solution under analysis. Some strategies have been proposed to overcome this drawback, e.g., covering the polymer film with some fixing agent. As an example, the addition of sol-gel makes the coating highly rigid, i.e., more suitable to trap the redox mediator [18].

Following a different approach, the ability to fix metal complexes by an ion-exchange polymer coating can be exploited for the synthesis of metal nanoparticles onto electrode surfaces: charged metal precursors are adsorbed on ion-exchange polymer films, and metal nanoparticles are subsequently formed inside the polymer by chemical or cathodic reduction [21].

4.3 Polymers for Gas Sensors

Ion-exchange polymers have also been used in a very different frames, i.e., for the formation of membranes at the basis of amperometric sensors working in the gas phase [22, 23] or in low-conductive liquid media [22, 24]. The working electrode consists of a porous metal layer, usually made of Pt or Au, grown on the one side of an ion-exchange membrane, generally of Nafion®. This arrangement is typically obtained by in situ chemical reduction of an appropriate metal salt to form the porous metal electrode. On the opposite side, the membrane is in contact with an electrolyte solution in which the counter and the reference electrodes are located. The membrane acts as an insoluble solid polymer electrolyte (SPEl), in close contact with the porous electrode surface; this configuration makes the system suitable to detect analytes in low conductive media, even including gas phases. When the working electrode is polarized at a suitable potential, reduction or oxidation of the analyte occurs at the electrode surface. In order to maintain the electroneutrality within the membrane, ions are exchanged with the internal electrolyte solution; on the opposite side of this solution, anodic or cathodic reactions, respectively, occur at the auxiliary electrode. A general scheme reporting the peculiar amperometric sensor described is reported in Fig. 4.1.

Many different volatile analytes can be analysed with similar systems [23], including SO_x, NO_x, H_2, O_2, ozone, petroleum components, and aldehydes [25]. When considering the application in gas detection, SPEl are characterized by shorter response times and higher sensitivity with respect to conventional gas membranes that require the diffusion of the gaseous analyte throughout, in order to dissolve it in the internal electrolyte solution. However, the problem of irreversible drying out of the membrane constitutes a main drawback for actual use of

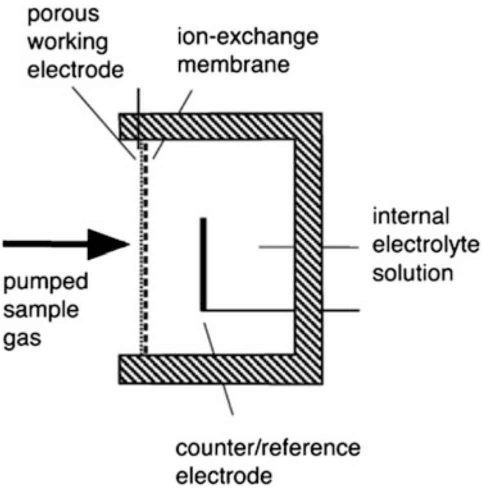


Fig. 4.1 Scheme of a general electrochemical cell based on an SPEl membrane (Reproduced from Ref. [23] with the permission of Elsevier)

SPEl-based devices. This probably constitutes the main reason that induced the development of a new class of amperometric gas sensors; these are based on a thin layer of a room temperature ionic liquid deposited on the surface of either a three electrode cell or a microelectrode array [26–28].

4.4 Other Applications of Ion-Exchange Polymers

Finally, note that Nafion® is most frequently involved in electrode coatings for reasons that differ from those previously discussed. Although it is difficult to compile an exhaustive list of all the possible applications of similar electrode deposits, two specific applications deserve to be cited.

The former consists of a significant enhancement of sensor selectivity when Nafion® is added as the outermost layer of an electrode coating: the electrocatalytic properties of many electrode coatings that are the object of different chapters of this monograph are combined with the selectivity conferred by this ion-exchange polymer. In this respect, a most frequently reported application is addressed to lower the ascorbic acid interference in amperometric sensors for glucose or for dopamine determination.

As a further application, Nafion® is generally used to stably fix enzymes on electrode surface; the very low solubility of this polymer allows stable deposition of the biomolecule on the transducer, and its marked hydrophilic character allows the enzyme to be effectively in contact with species in solution.

We emphasize here that, for both these applications, the use of charged polymers different from Nafion® is also possible. However, Nafion® is commercially available, possesses well known properties and is usually preferred.

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Chapter 5
Monolayers

One of the most common methods employed to tune the properties of a surface consists of the formation of a monomolecular layer of organic species, possessing a thickness of up to a few nanometers [1–13]. Molecules that form monolayers are normally more or less strongly adsorbed or even covalently bound. The adsorption of species is a spontaneous phenomenon, since clean electrode surfaces are frequently thermodynamically unstable in a real environment, due to the presence of dangling bonds. Adsorption of molecules leading to mono- and multilayers reduces the overall energy of the system consisting of substrate and adsorbed species.

The applications of monolayers range from the development of electronic components and biocompatible devices to lubricating thin layers, corrosion prevention, and so on. The aim of this chapter is to sketch the structure, properties, and preparation methods of monolayers and to discuss the relevant most important electroanalytical applications: it is focused on the most common monolayers used in the frame of amperometric sensors.

The properties of monolayers can be exploited in anchoring nanostructures, such as metal or metal oxide nanoparticles at a substrate, constituting a bridge between the substrate and the nanostructure. In the case of both planar substrates and nanostructures, monolayers may be used to modify the surface for inducing or favoring the growth of other materials, such as conducting polymers. Hence, the description of phenomena strictly related to monolayer formation and relevant physico-chemical properties is essential for full comprehension of nanostructured materials on electrode surfaces, as described in Chap. 6. Moreover, thick layers of organic materials, typically based on polymers, have been already treated in Chaps. 2, 3, and 4.

Self-assembled monolayers (SAMs) actually constitute peculiar cases of monolayers [1–8], consisting of adsorbed or covalently anchored molecules on a substrate surface that undergo a self-organization process: after adsorbing on the substrate, the final structure of the SAM consists of a packed well-ordered thin film. One of the first examples of such a system was reported in 1946 [14], but only in the last 30 years the relevant structures and properties have been deeply

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in Electrochemistry, DOI 10.1007/978-3-662-45103-8_5

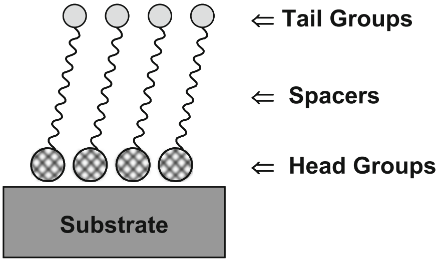


Fig. 5.1 Basic structure of a monolayer

investigated. We use the term ‘monolayers’ with reference to both disordered systems and SAMs. Calling every monolayer a SAM, independent of the actual structure, is quite a common choice in the literature; however, the meaning of the acronym, though appealing, may be a source of misunderstanding when improperly used.

A typical molecule suitable for the formation of monolayers (see Fig. 5.1 for the particular case of a SAM structure) possesses a *head group*, which is chemisorbed or covalently anchored on the surface, and a *tail group* at the opposite side of the molecule; the latter is at the interface between the thin film and the external environment, typically consisting of a liquid phase when considering electroanalytical applications. A *spacer* is located between these two groups; the interactions among the spacers play a basic role in conditioning the final structure and stability of the monolayer. It is possible to tune the chemical and physical properties of such a thin film by changing the chemical composition of head and tail groups as well as that of the spacer.

In the following sections, the most important aspects regarding substrates, head groups, and spacers is discussed. An enormous number of different tail groups have been reported so far; they are discussed at the end of this chapter, together with the relevant analytical applications, since the different strategies for amperometric sensing through monolayers mainly take advantage of the chemical properties of the tail groups.

5.1 Anchoring Monolayers to Substrate

A large number of different molecules are used for the preparation of monolayers on a substrate. The most widely studied species belong to one of the following classes [1–8, 15]:

1. Organosulphur compounds
2. Organosilicon compounds
3. Amines
4. Carboxylic acids
5. Phosphor-based species

Organosulphur compounds represent the most common class of molecules exploited for formation of monolayers [1–8]. Many different kinds of similar molecules, e.g., alkanethiols, dialkyl disulphides, dialkyl sulphides, thiophenes (Ths), thiophenols, and cysteines, have been reported to form monolayers on different substrates mainly consisting of (1) metals (e.g., Au, Ag, Cu, Pt, or Hg) or (2) semiconductors, such as indium tin oxide (ITO) and GaAs. Among the possible systems, the most extensively studied are those based on thiols adsorbed on Au. Although fundamental studies have also been carried out on the analogous Se and Te molecules, these have led to a limited number of applications in electroanalysis.

As for organosilicon compounds, alkylchlorosilanes, alkylalkoxysilanes, and alkylaminosilanes can be linked through hydroxyl groups protruding from a surface: surface polymerization is coupled to contemporary formation of bonds between coating and surface, using Si and O atoms, respectively (Fig. 5.2) [1–8]. When considering surfaces suitable for electroanalytical applications, these monolayers have been successfully prepared on conducting oxides, such as ITO. Only a few studies have reported on the surface polymerization of organosilicon compounds on metals such as Au [16].

The preparation is not straightforward, due to the strong influence of the ubiquitous presence of water: most of the starting molecules are, in fact, sensitive to moisture, which leads to polymerization of the Si-based groups; consequently, they are difficult to handle. The reproducibility and degree of order of the monolayers are strongly affected by this variable, which constitutes a possible heavy drawback.

Amine residues, particularly from primary amines, have been extensively investigated as head groups [17]. Their behavior is similar to that of thiols, although the adsorption energy is reported to be significantly lower [18]. Furthermore, carboxylic acids can be adsorbed on metal oxides (Ag_2O, CuO, Al_2O_3) by interaction between the carboxyl groups and the positively charged metal centers [1–8].

Finally, monolayers based on phosphorus can be divided into two quite different classes, namely those based on phosphines and those on phosphonic acids [1–8]. The species belonging to the former class stably adsorb on metal surfaces, e.g., on Au, thanks to the presence of a lone electron pair interacting with the metal orbitals; those of the latter class prefer oxide surfaces.

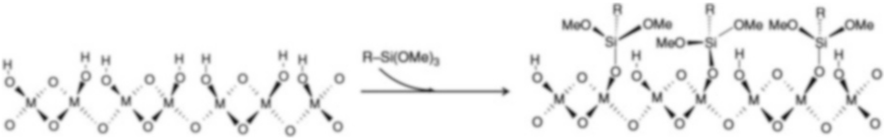


Fig. 5.2 An example of reaction involving the formation of monolayers based on organosilicon compounds: the anchoring of trimethoxyorganosilane molecules to a metal oxide surface is depicted (Reproduced from Ref. [11] with the permission of Wiley-VCH)

Independent of the tail group chosen, monolayers are normally prepared in a liquid phase by simply dipping the substrate into a dilute solution of the organic molecules, in the millimolar concentration range. The adsorption kinetics of the head groups is so fast that high coverage is normally reached in a few seconds at room temperature, only being slow in the case of very dilute solutions [6]. Ordering due to the rearrangement of spacer and tail groups starts simultaneously to the adsorption process, but its completion may need many hours. Ordering allows the increase of the stability and of the degree of coverage.

Monolayers on the electrode surfaces can also be obtained from the vapor phase, typically by exposing the substrate to vapors of the molecules constituting the monolayer. The deposition can be carried out in (ultra) high-vacuum conditions or by positioning the substrate over a dilute solution of the species to anchor, at ambient pressure. In most cases, the molecules to deposit are pretty small, so the relevant vapor pressure is quite high; alternatively, a heated crucible can be employed to enhance the volatility of the molecules. Although the final structure of the monolayer is similar to that obtained from the solution phase, the solvation of the molecules plays a key role in the rearrangement step, as in the case of 1,4-benzenedimethanethiol on Au [19–22]. Hence, different deposition techniques allow the tuning of the final structure of the monolayers, in terms of molecular orientation.

As to grafting with marked covalent character, the most widely investigated systems are based on diazonium salts that can be anchored at a number of quite different electrode surfaces, e.g., C or Pd (Fig. 5.3) [23–26]. The grafting occurs through chemical or electrochemical reduction of the diazonium moiety, leading to the release of nitrogen from the precursor molecules and concurrent formation of C-C or C-metal bonds. It is worth noticing that the reaction is not self-limited, so that formation of multilayers is also possible.

The exploitation of oxidation or reduction reactions occurring on the surface of a polarized electrode [24, 27] can lead to the formation of a variety of monolayers depending on the nature of the organic species. As an example, amines, alcohols, and carboxylic acids can be anchored to metal and C-based surfaces through anodic oxidations; similarly, cathodic reductions can induce grafting of vinyl moieties.

Finally, literature reports some cases of adsorption of relatively large disk-shaped molecules on electrode surfaces. Porphyrins, phthalocyanines, and the relevant metal complexes [28] constitute prototypical examples of similar systems, in which a metal cation is coordinatively inserted inside a macrocycle. Different anchoring strategies can be envisioned (Fig. 5.4) [29], both on metal [30–35] and on C-based substrates [36]. Similar macrocyclic complexes possess large π-conjugated systems that can be favorably adsorbed flat on the substrate (Fig. 5.4a). Alternatively, the macrocycle can be functionalized with a single spacer and a head group, leading to monolayers in which the aromatic systems stand upon the surface thanks to the thermodynamically favored configuration (Fig. 5.4b). A third possibility consists of multiple spacers and head groups bound to the macrocycles, leading to a sort of ‘octopus’ configuration (Fig. 5.4c). Metalloporphyrins or metallophthalocyanines can be bound at a surface through a monolayer in which the tail group

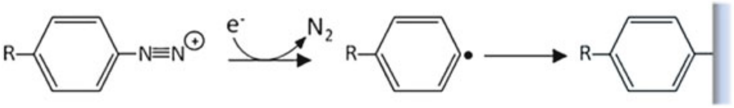


Fig. 5.3 Reduction of a diazonium salt leading to covalent grafting on a surface (Reproduced from Ref. [1] with the permission of Springer)

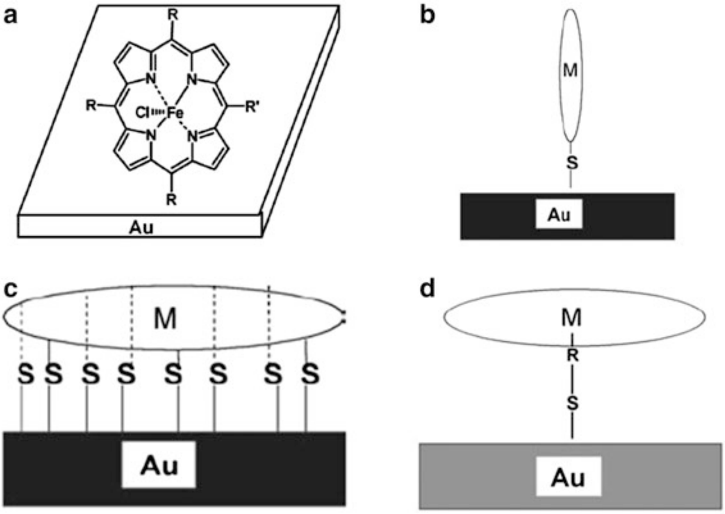


Fig. 5.4 Different adsorption geometries for disk-shaped molecules on electrode surfaces: (**a**) flat, (**b**) vertical, (**c**) octopus, (**d**) umbrella. For the sake of simplicity, the molecule in vertical configuration is orthogonal to the substrate, different tilt angles being actually possible (Reproduced from Refs. [29] and [30] with the permission of World Scientific and Elsevier, respectively)

acts as an axial ligand of the metal; this arrangement is often called ‘umbrella’ configuration (Fig. 5.4d).

In addition, surface complexes are obtained through the formation of coordination complex of adatoms, i.e., atoms protruding from the terraces of the metal substrate and exhibiting the lowest possible coordination number with other surface atoms, with suitable ligands dissolved in solution (Fig. 5.5) [37, 38]. However, this fascinating method of preparing metal complexes on a metal surface has not been exploited in the frame of electroanalysis.

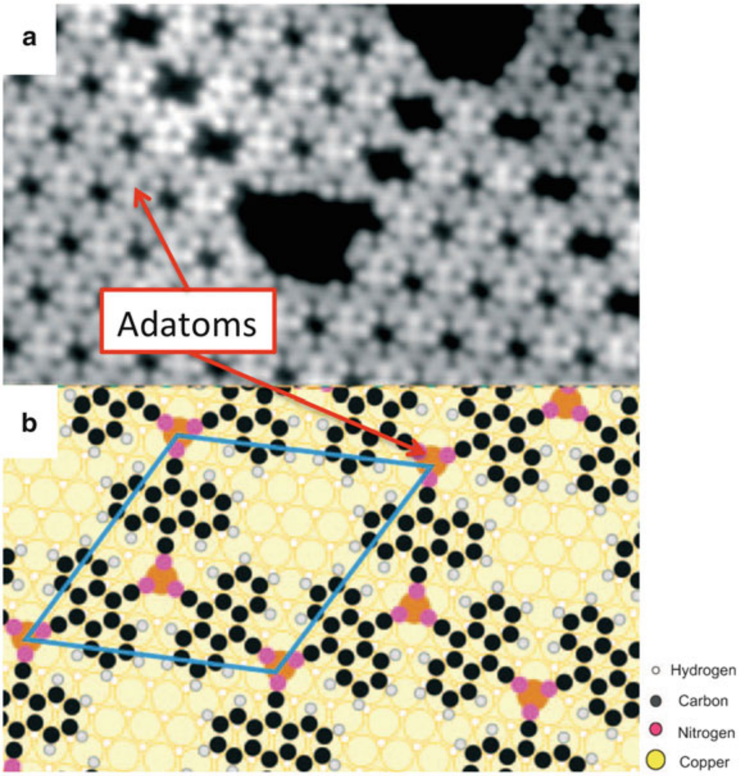


Fig. 5.5 An example of surface complex involving Cu adatoms: 9,10-anthracenedicarbonitrile on Cu(111). (**a**) scanning tunneling microscopy image (17 × 10 nm); (**b**) relevant model (Reproduced from Ref. [37] with the permission of Wiley)

5.2 Common Substrates for Preparation of Monolayers

Au constitutes the most common substrate for the preparation of monolayers suitable for amperometric sensing. Other noble metals, such as Pt and Pd, have been employed, together with some more electropositive metals such as Cu and Ag. Noble metals possess a number of advantages: (1) the procedures for modification with monolayers are well established; (2) the substrates are relatively pure and chemically inert, so that the surface composition and structure are well defined; (3) a number of different head groups can be stably anchored; (4) a number of patterning methods of the substrates have been developed. However, the raw materials are quite expensive and the cleaning procedures of the substrates are not always straightforward, as described in the following.

Metal alloys have been only occasionally investigated to such a purpose, due to the complexity of the system. The surface composition of metal alloys, in fact, is not simple to determine, since compositional gradients are possible, and the surface may be composed by a patchwork of different phases. In addition, investigations on metal glasses, such as Vitreloy, are still very limited in number, also because these materials are, in turn, commonly based on alloys.

As for C-based materials, graphite, glassy carbon, and boron-doped diamond represent the most commonly used substrates. The materials are relatively chemically inert, although different head groups can be adsorbed on the surface. In the case of graphite and glassy carbon, at variance with noble metals, the nature of the surface is still poorly defined and strongly dependent on the preparation procedure and polarization potential. In particular, the presence of different oxygenated species, e.g., alcohol, carboxylic, or ketone moieties, has been reported [39, 40]. Some materials, such as the doped diamond, are also quite expensive and not easily available in the bulk form.

The limited conductivity of most metal oxides and of semiconductors prevents the use of most of them in amperometric sensors. On the other hand, some conducting metal oxides, such as ITO and fluorine-doped tin oxide (FTO), are widely employed. These substrates possess good electrical conductivity and optical transparency, creating the possibility of coupling electrochemical and optical characterizations; in addition, the signal arising from the presence of a target analyte can take advantage either of two different transductions, i.e., the electrochemical or the optical, or even couple them in spectroelectrochemical sensing. However, the chemical stability and the accessible potential window are less appealing with respect to noble metals. In addition, the amorphous nature of the surface restricts the exhaustive characterization of the monolayer; as an example, high-resolution imaging through scanning tunneling microscopy is challenging.

The degree of order and of coverage for a coating monolayer is strongly dependent on the nature of the substrate surface. In the case of metal substrates, a high degree of order and of coverage can be obtained only when the number of surface defects, such as vacancies, adatoms, kinks, etc., is limited, and the sizes of the terraces are as large as possible. This condition is dramatically fulfilled in the case of clean single crystal surfaces, which exhibit large terraces, even a few micron wide. These surfaces can be cleaned in vacuum, after a proper sputtering and annealing procedure, or in air, after a proper electrochemical cleaning. However, single crystal surfaces are of poor significance in the frame of electroanalysis, due to their cost and the complexity of the preparation: their use is relegated to fundamental investigations. In most cases, polycrystalline surfaces are employed that show only small terraces, i.e., few tens of nanometers wide. The structure of monolayers prepared on polycrystalline surfaces is often assumed to be similar to that observed in the case of a single crystal. Obviously, this assumption most often constitutes a crude approximation, because the degree of order is often much lower for polycrystalline materials. On the other hand, some strategies can be adopted to increase the order degree even when using polycrystalline substrates: thin metal films can be thermally treated, e.g., by a flame [41, 42] in order to increase the size of the terraces. In the case of thiol molecules, the final substrate resembles a single crystal as to degree of order of the monolayer.

5.2.1 Substrate Preparation

The substrates should be properly treated before grafting the monolayer. In particular, excellent cleanness is key in order to obtain monolayers possessing a good quality in terms of degree of order and coverage, stability, and reproducibility. The cleaning procedures are based on a precise knowledge of the surface chemistry of the substrate; a number of different methods have been developed so far, including electrochemical oxidation-reduction, flame annealing, dipping in strongly oxidizing media, e.g., the piranha solution, and plasma cleaning [39–44]. Many variants are reported in the literature, differing from one another for some experimental details, such as the concentration of the reagents or the temperature. However, only few systematic studies emphasizing the differences in the monolayer structure deriving from the cleaning treatment have been published so far [41–43].

It is strongly advisable to use the substrates immediately after the cleaning process, since they spontaneously adsorb adventitious contaminants from the atmosphere or the liquid phase.

5.2.2 Arrangement of the Head Groups on the Substrate Surface

Despite the large number of papers on the topic, a thorough understanding of the nature of the chemical interaction between the molecules constituting the monolayer and the substrate has not been reached yet. This is because a precise characterization of the head group, once anchored on the substrate, is quite challenging. It is commonly accepted that charge transfer occurs between the adsorbed head group and the atoms of the substrate surface. For instance, in the case of thiols, hydrogen atoms are supposed to be lost as a consequence of the adsorption. However, the fate of the hydrogen atoms still remains debated: the atoms could be adsorbed on the surface or even diffuse into the crystal structure of the substrate [1, 6].

A further question concerns the deposition of organic species containing unsaturated functionalities: although aromatic rings are considered essentially untouched by the deposition process, the rupture of the aromatic rings adsorbed on the surface has been verified in several cases, even on noble metal surfaces. It is clear that the occurrence of similar reactions by adsorption is sometimes underestimated, although it affects the chemical nature of the electrode coating and sheds new light on the actual reactivity of supposedly inert noble metals. As an example, it has been reported [45, 46] that adsorption on Au and Pt of Th derivatives may induce dramatic decomposition to species such as atomic sulphur and thiols. In this respect, as a support to the great meaning of the definition of the actual nature of the monolayers, it is worth evidencing that such a layer also constitutes the very first in a multilayer deposit: the substrate|deposit interface may be quite different to that thought or even modeled.

When monolayers are prepared from a liquid phase, the nature and concentration of the species dissolved in solution are critical. In particular, the presence of ions (e.g., due to the use of a buffered medium) can strongly influence the adsorption of molecules constituting the monolayer. The adsorption energy of many anions can be significant: the organic molecules have to displace both solvent molecules and ions at the surface, in order to anchor the substrate; the effect of this phenomenon is often underestimated in the literature.

5.2.3 Adsorption Sites for the Head Groups

In general, head groups are supposed to form an ordered arrangement on crystalline metal and metal oxide surfaces. The exact location of the adsorption sites and the two-dimensional arrangement of the head groups still constitute a debated issue, even in the simplest cases. The preparation of the substrate and the experimental details of the formation of the monolayer exert profound influence on the final structure. In many cases, the two-dimensional arrangements exhibit vacancies called pinholes. In addition, the mobility on the surface of some head groups, particularly of thiols, is significant. Conversely, the mobility of organosilicon head groups is negligible, which has been identified as one of the main causes of the lower degree of order of the resulting monolayer with respect to thiols.

In the case of metal surfaces, different adsorption sites for organic molecules forming monolayers have been identified [47]. The exact nature of the adsorption sites depends, obviously, on that of the substrate and, in particular, on the surface crystallographic structure. Literature reports a number of investigations on metals possessing face-centered cubic crystal structure, such as Au and Pt; in particular (111) and (100) crystallographic faces have been investigated [47]. In these cases, the head group can be adsorbed, to a first approximation, on (1) a hollow site (i.e., among three adjacent metal atoms); (2) on a bridge site (i.e., between two metal atoms); (3) on a top site (i.e., only connected to a single metal atom), as shown in Fig. 5.6a. A prototypical example given by the adsorption of alkanethiol on Au (111) [47, 48] (Fig. 5.6b). When the head group is particularly bulky, such as in the case of oligomers or large aromatic molecules, precise identification of the adsorption site is definitely speculative.

Due to the steric hindrance of the spacers and of the tail groups, the head groups do not saturate all the possible adsorption sites on the surface. For this reason, in many cases the extent of the interaction between two adjacent head groups is scarce. As an example, in the case of thiol molecules, the distance between two adjacent S atoms is approximately 5 Å [48]. The formation of S-S bond on the surface is hard, and very few papers report formation of S-S bonds on an Au surface [49]. Organosulphur compounds, and particularly thiol molecules, possess an additional peculiarity: the adsorption of the head group may on its own induce

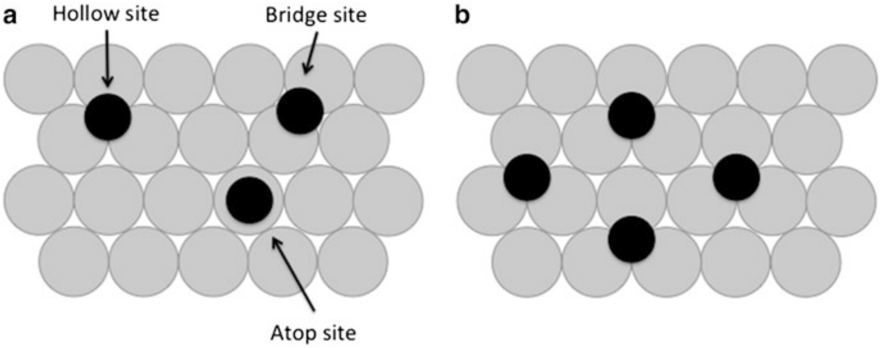


Fig. 5.6 (**a**) Different adsorption sites in the case of a face-centered cubic (111) crystal face. (**b**) adlayer adsorption geometry on (111) surface

surface defects, e.g., one atom deep valley or pit, on the Au surface, as testified by scanning tunneling microscopy investigations [6].

As for crystalline metal oxides, their surface exposes both oxygen atoms and metal cations [47, 50]; furthermore, different hydroxylated species are almost always present on the surface, forming residues with different acidity degrees [51]. Head groups, particularly carboxylate moieties, can assume a well-ordered arrangement on these surfaces [52]. However, a significant fraction of the oxide substrates used for the preparation of monolayers does not show a crystalline surface. The amorphous nature leads to a poorly defined substrate, in terms of adsorption sites for head groups and of morphology, on which the monolayer grows. In addition, single crystals, which are very important in fundamental studies, are difficult to prepare; this is the case of ITO. Similarly, easily oxidisable metals such as Ag exhibit a very thin layer of oxide on the surface, from one monolayer to many nanometers thick; the formation of these oxide layers is often underestimated, and the substrate is erroneously considered to be pure metallic in nature.

It has been cited above that adsorption processes may happen, altering the surface nature and arrangement of the substrate atoms. In most cases, the electronic perturbation due to the adsorption affects the outermost two or three layers of substrate atoms [53]. In addition, relaxation or reconstruction of the crystal surfaces have been reported [47]. The term relaxation refers to a change of the distance between the atoms in correspondence to a surface or an interface, with respect to the bulk; the change does not affect the regular distribution of the atoms (i.e., the so-called periodicity) along the plane of the substrate|deposit interface. On the contrary, reconstruction does refer to change of the periodicity of the atoms along the substrate|deposit interface plane.

Specific molecules may adsorb on the surface via two head groups. In many cases, the two moieties are located at the two ends of the molecule, separated from each other by a spacer. A competition occurs between these two head groups for access to the biding sites on the substrate. The molecule can finally assume a stand-up configuration similar to the conventional monolayer (Fig. 5.7): one moiety is

Fig. 5.7 The two possible adsorption geometries of dithiol molecules

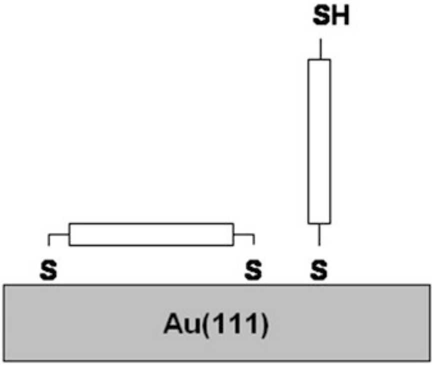
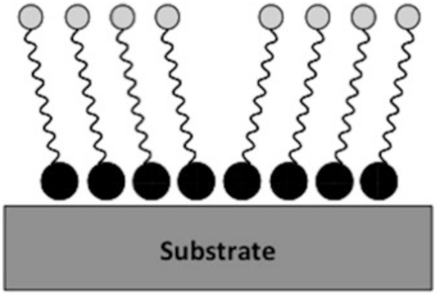


Fig. 5.8 Ideal self-assembled monolayer exhibiting two different azimuthal domains



anchored to the substrate and the other end is left dangling. Alternatively, both moieties can adsorb on the surface, forming a sort of bridge. Dithiol molecules constitute a prototypical example: depending on the preparation conditions and the nature of the molecules, either of the adsorption geometries is preferred [19–22].

The crystallographic surface structure of the substrate and the arrangement of the head groups exert an important effect on the distance between adjacent tail groups and on the tilt angle of the spacers with respect to the substrate [54]. As an example, in the case of thiol-based SAMs, the tilt angle with respect to the normal to the surface for alkylthiols deposited on Au(111) is ca. 30°, since such a chain configuration maximizes the packing density of the alkyl chains, favoring the forces ascribing compactness to the SAM as a whole [55, 65]. The angle decreases in the case of Ag(111), since the spacing between the head groups is lower, which leads to a maximum packing density in correspondence to a lower tilting. It is worth noticing that adsorbed molecules in a single crystalline terrace normally form different domains, each one characterized by the same tilt angle but different azimuthal angle, as shown in Fig. 5.8.

5.3 Spacers

The most common spacer groups are based on alkyl chains. The number of C atoms varies from 1 to more than 16. The length of the alkyl chains is very important for the formation of a stable and highly ordered monolayer: once the nature of the substrate is suitable and the rearrangement time is prolonged enough, a proper SAM may form. If the alkyl chain consists of six or more C atoms it is possible to obtain ordered structures in ambient conditions (i.e., -CH_2- and -CH_3 groups are in *all-trans* configuration). On the other hand, in the case of shorter or very long chains, the presence of conformational defects (*gauche* defects) is likely (Fig. 5.9) [57].

In the case of SAMs, an even or odd number of C atoms in the alkyl spacer leads to a slightly different orientation of the tail group, which is known as the 'odd–even' effect (Fig. 5.10) [58, 59]. This phenomenon may have important consequences in electroanalysis [60], affecting the degree of interaction between the receptor in correspondence of the tail group and the analyte in solution. However, the odd–even effect in electroanalytical contexts is often masked by the change of degree of order and of conductivity due to the different length of the spacers employed.

In the case of thiols, the Au-S-C angle can vary according to S hybridization: it holds around 180° or 104° in the case of sp and sp^3 hybridization, respectively. The sp^3 configuration seems to be energetically favored, though such a conclusion is still dubious [48].

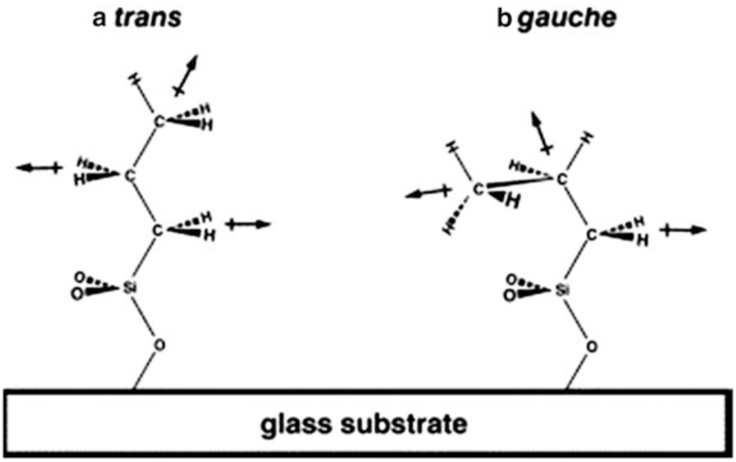


Fig. 5.9 An example of gauche defects is spacers: two conformational isomers of n-propyltrichlorosilane monolayer on glass, namely (**a**) trans and (**b**) gauche configurations (Reproduced from Ref. [57] with the permission of Elsevier)

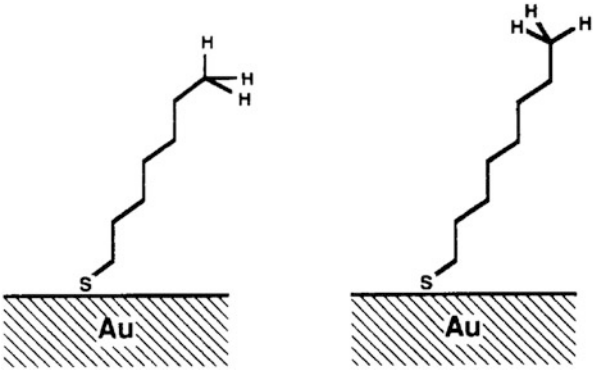


Fig. 5.10 An example of the odd–even effect. The orientation of the bonds of tail group (-CH_3) depends on the number of C atoms in the spacer (Reproduced from Ref. [54] with the permission of American Chemical Society)

In most cases, the packing of the spacers is mainly controlled by van der Waals [1–8] and aromatic, e.g., π-π, [61–63] interactions. There are few examples of hydrogen bonds, (e.g., due to the presence of amide moieties [64]) or of dipole–dipole interactions (e.g., due to sulphone groups) between adjacent spacers [65]. Covalent bonds between atoms of adjacent spacers are only occasionally reported, as in the case of the formation of C-C bonds between alkyl chains, induced by electron irradiation [66]. Disorder in the spacer ensemble may be due to the head and tail groups: in the case of bulky groups, the density of the molecules on the substrate is low. As a result, the forces between adjacent spacers are weak, disorder arises in the spacer moieties, and consequently in the SAM as a whole.

5.4 Stability of the Monolayers

In the case of monolayers prepared by simple adsorption, the stability of the substrate–deposit system represents a critical aspect. The adsorption energy is difficult to estimate: the overall value is related to the strength of the anchoring of the head group and of the interaction energy between the spacers and between the tail groups belonging to adjacent adsorbed molecules. The adsorption energy is estimated to be close to ca. 40–50 kcal/mol in the case of highly ordered thiol-based monolayers possessing a relatively long alkyl chain [6, 8]. A particular case consists of monolayers bearing bulky tail groups: the resulting density of the molecules on the substrate is low, reducing the overall stability of the system. In order to increase the stability of the monolayer, a usual approach consists of the use of molecules possessing multiple head groups, as in the case of tripodal thiol adsorbed on Au [67]. Caution must be paid to the possibility that not all the head groups are anchored to the substrates [68].

Particular attention has been devoted to the influence of the temperature on the structure and degree of order of monolayers, an increase of temperature leading to a lower order. The thermal stability of alkanethiols is, in fact, quite low: thiol molecules start desorbing above 70 °C [55, 69]. Some authors report the healing of defects in monolayers by a thermal treatment; however, the closeness of the desorption and healing temperatures discourages the use of this method when aiming to reduce the density of defects in the monolayers [70]. Moreover, the thermal treatment may induce structural changes such as the change of S hybridization from sp^3 to sp and the formation of disulphide on Au(111) surface [48]. Monolayers prepared using solutions of thiol molecules at different temperatures showed that the overall substrate area corresponding to pinholes is constant, but the number of pinholes decreases at increasing temperatures [8, 70].

The stability of thiol monolayers in atmosphere is fair for short time lengths; they are sensitive to the presence of traces of ozone at the level present in the common environment, which causes oxidation of S head groups, with consequent desorption. Hence, it is strongly advised to store the system in solution [71–73].

As for the stability of the molecules to use, the product of the -SH group oxidation depends on the experimental conditions; different species containing oxygen have been reported, all of them weakly adsorbed on metal surfaces. In addition, thiols are easily oxidatively destroyed by $OH^{\bullet}$ radicals [74].

5.5 Reactivity of the Monolayers

The chemistry of organic monolayers deposited on a substrate has been deeply investigated [8, 75–79]; a few examples are reported in Fig. 5.11. The reactivity is normally ascribed to the tail group: the chemical modification may induce the anchoring of a second molecule which represents the actual functionality for the target analyte. Moreover, examples of chemical reactions between spacers have also been reported [78]. It is worth noticing that electromagnetic radiations have been employed to trigger chemical reactions. In particular, the orientation of the adsorbed molecules can be modulated. As an example, cis-trans conversion of double bonds present in the spacer occurs upon absorption of photons in the UV-visible range (Fig. 5.12) [80].

The preparation of multilayers based on the same or on different structural units may take advantage of the reactivity of the monolayers and particularly of the tail groups. Different strategies have been adopted; as an example, dithiol molecules lead to multilayers thanks to the formation of an S-S bond between molecules on the surface [20–22]. The defects present in a single monolayer are inherited by the monomolecular layer anchored to its tail group. Hence, the degree of order of a multilayer is usually much lower with respect to the first monolayer on the substrate. In the majority of cases, bi- or tri-layers are prepared, although few hundreds of micron thick multilayers can be also obtained.

It is worth noting that molecules anchored at a substrate exhibit peculiar behaviors with respect to the analogous molecules in solution. One of the most

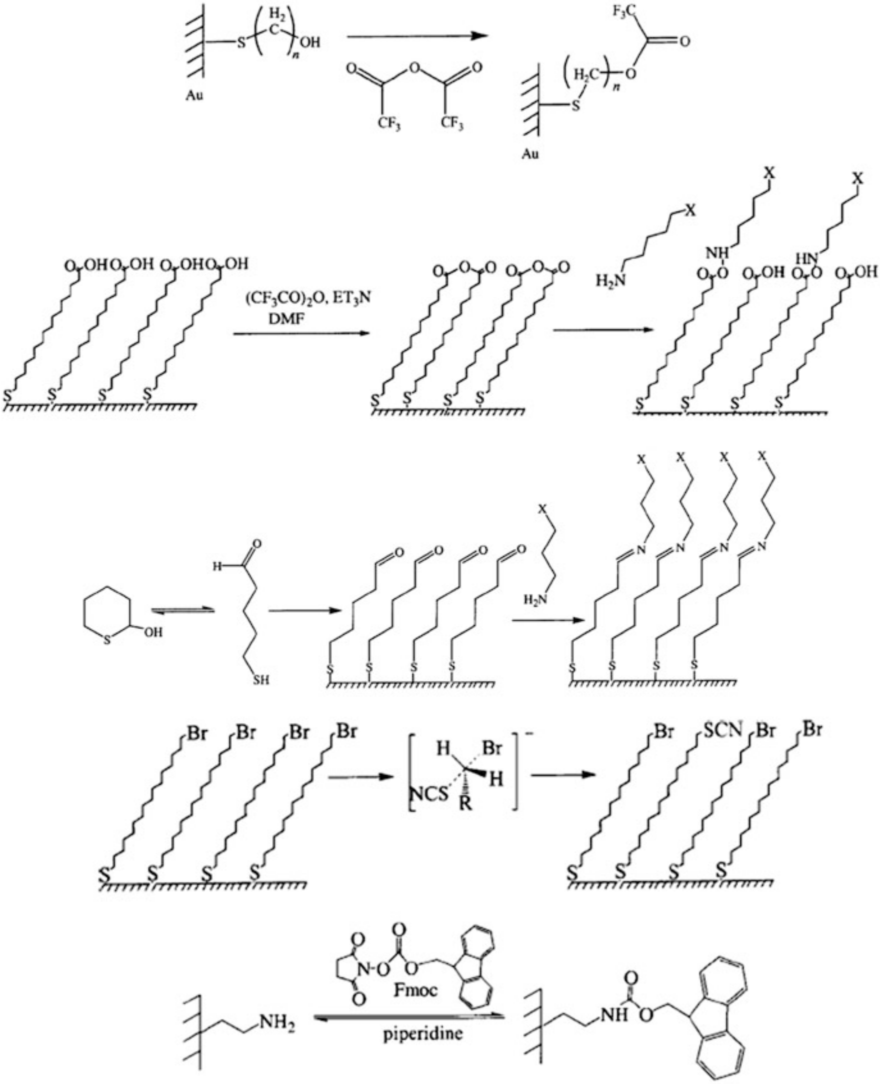


Fig. 5.11 Some representative examples of chemical reactions involving monolayers (Reproduced from Ref. [77] with the permission of Wiley)

notable examples consists of the change of the acidity or basicity character. The determination of pK_a values is quite challenging in the case of monolayers. Many authors report a significant decrease of the acidity of the molecules after the adsorption process [78].

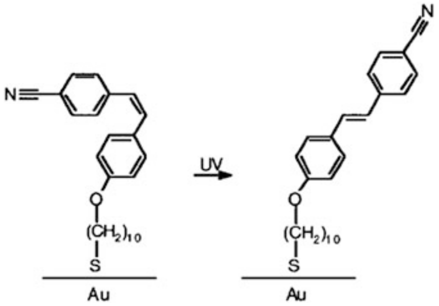


Fig. 5.12 An example of *cis-trans* conversion triggered by UV radiation (Reproduced from Ref. [80] with the permission of American Chemical Society)

5.6 Mixed Monolayers

Mixed monolayers (i.e., monolayers consisting of different adsorbed molecules) constitute an interesting evolution of the surface modifications discussed so far [6]. Different strategies can be adopted to realize similar systems. The use of a solution or vapor phase containing more than one single species constitutes one of the simplest strategies. Different molecules compete with each other for adsorbing on the substrate, and different surface compositions are possible according to the intrinsic affinity of the species to the substrate, the composition of the starting solution/gas phase, and other experimental conditions. A second possibility consists of the use of molecules possessing more than one spacer and tail moieties, e.g., dialkyl disulphides and dialkyl sulphides [6]. One of the main limitations of such a method lies in the restricted composition variability that can be achieved.

One of the most common surface processes involving monolayers lies in the so-called place-exchange reaction [81–83] (Fig. 5.13): the molecules absorbed on the surface can be partially replaced by other molecules, so that the final monolayer contains a mixture of two or more species. As an example, thiol molecules deposited on Au surfaces can be partially replaced by amine residues directly bound to oligonucleotide moieties [84].

The reaction usually takes place in a solution containing a suitable concentration of entering molecule. The substitution efficiency depends on (1) the adsorption energy of the head groups; (2) the length of R' and R spacer chains; (3) the presence of bulky groups in the precursor molecules, which can induce steric hindrance; (4) the concentration of the entering molecules in solution. The reaction exhibits a 1:1 stoichiometry and usually does not involve side reactions, such as the formation of disulphides or of oxidized sulphur species in the case of thiol-based monolayers. The presence of defects on the substrate and in the monolayer gives the most spontaneous explanation to this exchange reaction. The molecules in the correspondence of defects are less tightly and uniformly surrounded by analogous entities, viz. they are less constrained by the other molecules and are more easily accessible by species in solution or in a vapor phase: the kinetics of the exchange process in correspondence to the defects is very fast [81]. However, a slow

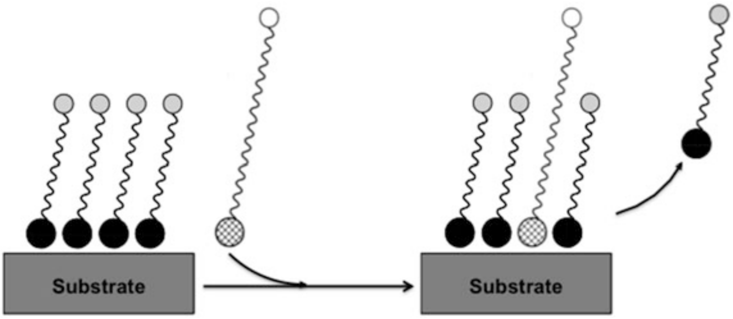


Fig. 5.13 Scheme of place exchange reaction

exchange reaction has also been found to occur in the correspondence of the surface covered by a compact monolayer [81].

All the strategies discussed so far suffer from a potential drawback, i.e., phase segregation. It has been reported that, in a mixed monolayer, the single components can be localized within domains, leading to a patchwork surface [85, 86]. This arrangement of the molecules often minimizes the overall energy of the system, thanks to the maximization of the attractive interaction between similar molecules within each domain.

5.7 Monolayers Based on Oligomers and Polymers

Oligomeric and polymeric species adsorbed on a substrate play a crucial role in many systems devoted to amperometric sensing. When considering multilayers possessing a thickness not exceeding a few nanometers, their nature is conditioned, in one way or another, by the closeness to the substrate. As a consequence, the properties of the resulting thin films resemble those of monolayers.

Although a number of systems and reaction pathways have been proposed for anchoring polymeric species on electrode surfaces [78], intrinsically conducting polymers (ICPs) and relevant building blocks represent the most important cases in the frame of amperometric sensing. As discussed in Chap. 2, polymers or oligomers of aniline (An), pyrrole (Py), and Th derivatives [87, 88] are the most widely diffused ICPs. Although these materials have been thoroughly investigated, only a minor fraction of the relevant studies deals with their behavior at the electrode|film interface. Additional ICPs have been proposed in recent years, such as polyphenylenes and carbazoles [87, 88]; however, their properties at the interface are, by far, even less thoroughly investigated.

Three major classes of monolayers based on An, Py, and Th derivatives can be identified: (1) monolayers possessing one An, Py, or Th moiety as the head group (Fig. 5.14a); (2) monolayers possessing one An, Py, or Th moiety as the tail group

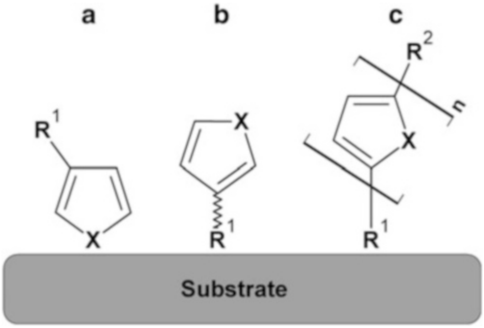


Fig. 5.14 Examples of molecules representative of monolayers possessing an aromatic derivative as the (**a**) head group, (**b**) tail group or (**c**) spacer. One of the simplest cases, i.e., the case of one aromatic heterocyclic ring, is sketched for **a**

(Fig. 5.14b); (3) monolayers possessing one or more similar heterocyclic rings in the spacer chain (Fig. 5.14c).

It is worth noting that the aromatic heterocyclic ring could compete with a different head group present in the molecule in the adsorption process on the substrate. As an example, an interesting case is represented by monolayers consisting of molecules bearing both thiol and thiophene groups, which are both suitable to act as the head group, so much so that, in the case of 2-mercapto-3-n-octylthiophene (Fig. 5.15), it is not clear which one of the S atoms is actually anchored at the surface [89].

The interaction of An, Py, and Th derivatives with metal or metal oxide surfaces may also lead to chemical reactions that differ from the simple anchoring of the single ring to the electrode surface [76]. As an example, the formation of oligomeric and polymeric species from the relevant monomers, as a consequence of the adsorption on the metal surface, has been reported (Fig. 5.16a) [45, 90]. Surface polymerization may involve the reduction of the metal substrate with concomitant oxidation of the monomer and consequent oligo- or polymerization reaction. The addition of a proper oxidizing species has also been experienced [91]. Unfortunately, the relevant mechanism is still unknown.

Surface polymerization can also take place on metal oxides. The reaction may occur thanks to the oxidation of the heterocyclic monomeric head, by possible concomitant reduction of metal cations in the oxides. Zeolites are the most widely studied systems suitable for this purpose: monomers diffuse from gas or solution phase into the pores, where polymerization takes place [92]. Similar phenomena also occur in the case of layered inorganic oxides, such as clays. An example is given by the polymerization of Th confined within adjacent montmorillonite layers [93].

The systems resulting from adsorption of oligomers and polymers of An, Py, or Th derivatives have often been considered in literature. The adsorption geometry of polymeric monolayers consisting of macromolecules is quite complex, since it is strongly variable in dependence of the nature of the polymer and the deposition conditions. Many papers have been published with this respect [95, 96]. One of the most common configurations consists of the anchoring of short segments of the chain (trains) to the substrate, the rest of the backbone forming loops and tails (Fig. 5.17).

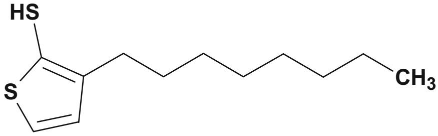


Fig. 5.15 2-mercapto-3-n-octylthiophene

On the other hand, when using the denomination 'oligomer' we refer to a species possessing a number of structural units lower than around 10–12. In the case of unsubstituted Th, a number of structural units higher than six to eight leads to oligomers possessing electronic properties similar to those of the polymer. Sexithiophene, in fact, is one of the most widely studied oligomers, representing the shortest prototype of polythiophene with respect to the energy gain in delocalization of polarons and biporarons within [97, 98]. This molecule has also been proposed for a number of applications in the frame of organic electronics.

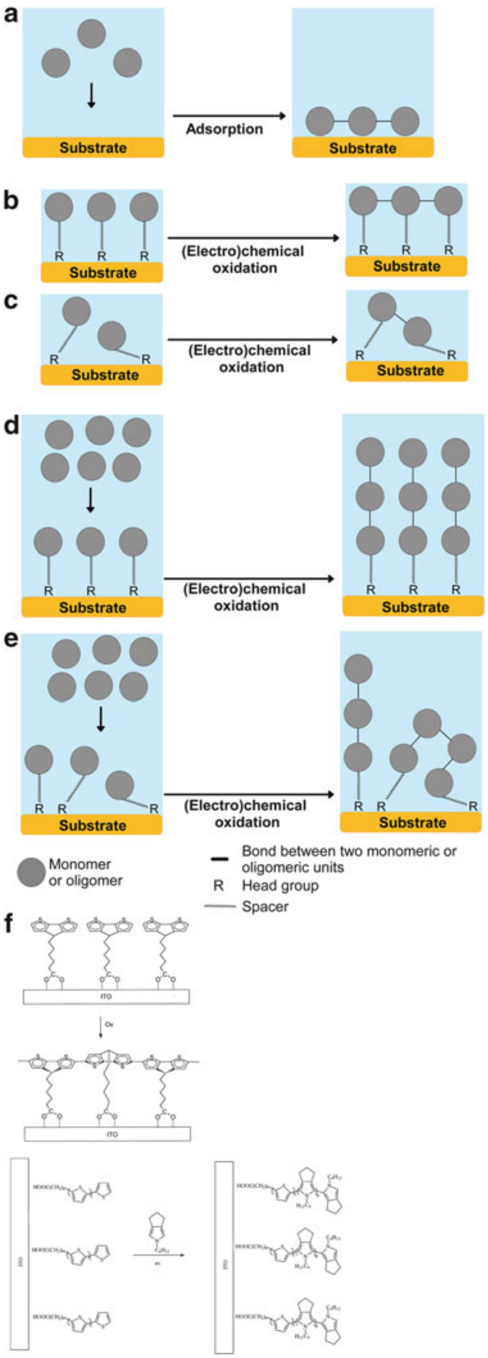
Not surprisingly, similar to the case of monomeric derivatives, the adsorption of oligomers can lead to the formation of polymeric species (Fig. 5.16a). As an example, the polymerization of terthiophene on Ag surface has been reported [99].

Anchoring of monolayers bearing an oligomeric or polymeric derivative as the tail group constitutes an interesting case (Fig. 5.16b–d). Similar monolayers are prepared by starting from shorter adsorbed molecules possessing a monomeric or oligomeric residue in the tail position. Longer oligomeric or even polymeric chains have been found to form through different processes, e.g., by exposure to oxidizing species, by UV radiation, or by polarization of the underlying conductive substrate at a suitable potential [100–102]. If the reaction involves adjacent adsorbed molecules, the thickness of the polymeric layer is self-limited. It is evident, in fact, that the adsorption geometry of the polymeric or oligomeric backbones is primarily influenced by the configuration of the starting monolayer, preceding the occurrence of the polymerization reaction. As an example, short oligothiophenes bearing carboxylic groups have been anchored to ITO surface; the superimposition of an electrical potential has led to the formation on an ultra thin film [103].

5.8 Electrochemistry of Monolayers

Electrical conduction between head and tail is most often essential for charge transfer to the tail groups, and finally to the analyte, either attached to the tail group or in solution, in the frame of amperometric sensors. Actually, most of the relevant investigations have been carried out in the frame of organic electronics, since the transport phenomena through a monolayer is of key importance for devices such as the thin film transistors. Most of these studies have been based on the use of scanning probe techniques, particularly of scanning tunneling microscopy and spectroscopy, and narrowly spaced couple of electrodes in front of each other.

Fig. 5.16 (**a**) Spontaneous polymerization of low-molecular-weight derivatives or oligomers on a substrate; (**b**) polymerization of monomeric or oligomeric residues located at the outermost part of a monolayer: ideal, highly ordered system; (**c**) more realistic case that takes into account the presence of molecules possessing different configurations; (**d**) growth through chain propagation starting from monomeric and oligomeric residues bound to the surface: ideal, highly ordered system; (**e**) more realistic case that takes into account the molecular disorder and possible coupling of adjacent chains; (**f**) examples taken from the literature (Reproduced from Ref. [94] with the permission of Springer)



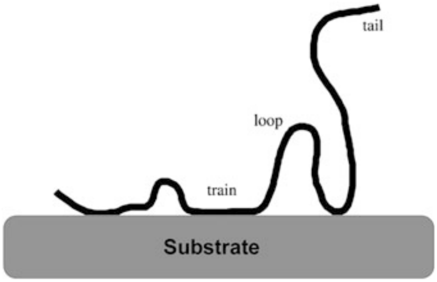


Fig. 5.17 Typical adsorption geometry of a polymeric chain

In addition, electrochemical investigations on the electron transfer processes through monolayers have been carried out by changing the nature of the tail groups; in particular, ferrocene and Ru complexes have been directly linked as terminal groups of alkyl chains of different lengths, in order to clarify the mechanism of conduction through the spacer. Similar investigations have also been carried out using redox species dissolved in solution as probes. At variance with experiments dealing with scanning probe microscopy, the current signal recorded in electrochemical experiments also includes the charge exchanged in correspondence to defects present in the monolayer, i.e., to pinholes.

Different mechanisms have been invoked to give a rationale to the charge transport, mainly ascribable to hopping and tunneling phenomena [3, 60, 104–106]. To a first approximation, the Arrhenius equation holds for hopping conduction mechanism:

$$\sigma = \sigma_0 \, e^{\frac{E_a}{kT}}$$

where σ is the conductivity, σ_0 is the pre-exponential term, E_a the activation energy, k the Boltzmann constant, and T the temperature.

Tunneling conduction mechanism is regulated by the Simmons equation:

$$j = \frac{1}{d} e^{-\beta d}$$

where

$$\beta = \frac{8\pi m F}{h} A$$

where j is the current density, d the thickness of the monolayer, m the electron mass, F the tunneling barrier height, h the Plank’s constant, and A a parameter accounting for the asymmetry of the potential profile. β is often called the tunneling coefficient and assumes values typically below 0.6 $Å^{-1}$ and between 0.6 and 1 $Å^{-1}$ for conjugated and alkyl-based molecules, respectively.

Monolayers in which π-electron conjugated systems are present exhibit good electrical conduction. Literature dealing with molecular electronics often refers to these systems as 'molecular wires'. They are normally based on (oligo)phenylenes, (oligo)thiophenes, hydrocarbon chains containing double and triple C-C bonds, and relevant mixtures [107]. It is evident that an increasing length of the chain, despite the increase of the number of aromatic rings and double bonds in the spacer, leads to an increment of the resistivity of the system [108]. On the other hand, the increase of the number of methylene groups in the spacer on its own drastically reduces the electrical conductivity [104].

The modification of surfaces with monolayers strongly modifies the electrochemical responses with respect to those recorded on a bare electrode [56, 109]. Once more, the most widely investigated systems in electrochemistry are based on alkylthiols adsorbed on Au. After anchoring a compact monolayer at a conductive substrate, cyclic voltammetric tests relative to $[Fe(CN)_6]^{3-/4-}$ and $[Ru(NH_3)_6]^{3+/2+}$ in solution show anodic-cathodic peak systems that are lower than those recorded on the same species on bare electrode. The coverage degree by similar monolayers is essentially complete, as demonstrated by a.c. impedance spectroscopy and by cyclic voltammetry; as a consequence, the electroactive species can only reach the Au surface through pinholes, which makes the electrochemically active area much smaller. As expected, the capacitor, to which the system substrate|monolayer|solution can be assimilated, is influenced by the nature of the alkyl thiol: the longer the alkyl chain, the lower the capacitance. On the other hand, in the case of monolayers possessing a low degree of order and coverage, the diffusion of the species in solution through the monolayer is not completely hindered. Hence, electroactive species can reach the substrate, and electrode charge transfer may occur.

Monolayers based on thiols are stable within quite a wide range of potential values (between ca. −0.8 V and +0.6 V vs. Saturated Calomel Electrode [SCE]) [110]. At more negative potentials, a reductive desorption takes place, according to the following reaction:

$$\mathrm{R-S-Au+e^{-}\rightarrow R-S^{-}+Au}$$

The charge spent for thiol electrochemical desorption can be exploited to estimate the degree of coverage and the reproducibility of the monolayer formation process. The desorption potential depends on the length of the alkyl thiol molecule: the longer the chain, the lower the desorption potentials. In general, the reaction starts occurring below ca. −0.8 V vs. SCE. Short thiol molecules exhibit only a single redox process; conversely, for longer thiol molecules, the charge transfers may be spread out over a wide potential range, due to different possible energy content of the different single molecules. In general, the longer the chain, the lower the desorption potentials.

At potentials more positive than ca. +0.6 V vs. SCE, oxidative desorption takes place. The relevant electrode mechanism is much less clear, and different pathways have been proposed. Many authors suggest that the break of not only the Au-S bond but also of other bonds, such as C-C bonds, occurs [110].

5.9 Analytical Applications

A number of electroanalytical applications of electrodes modified by monolayers have been reported so far and reviewed by many authors [1, 8, 29]. The use of similar electrodes in amperometric sensing is particularly appealing, thanks to the possibility to prepare a large number of different systems in a straightforward manner. The precursor molecules, suitable for the preparation of monolayers by simple dipping in a diluted precursor solution, are often commercially available. In the majority of cases, a receptor devoted to the detection of a specific analyte constitutes the tail group of the monolayer. Examples include organic ligands for metal ions, antibodies for the relevant antigens, and oligonucleotide chains for the complementary nucleobase sequence. The presence of the receptor in the outermost part of the electrode coating allows it to be effectively in contact with the solution containing the analyte. At the same time, the receptors are normally quite close to the underlying conductive substrate, favoring the charge transfer processes, as described in Sect. 5.8. Full coverage of the electrode surface by the monolayer and a high degree of order is normally sought in order to increase the density of receptors on the monolayer surface and to isolate the underlying substrate from the solution. The estimate of these features is not always straightforward, especially in the case of bulky receptors. As a result, in many cases the degree of order is largely overestimated: the actual structure of the monolayers is more disordered and inhomogeneous than that proposed in schemes often reported. On the other hand, in some situations, the formation of disordered monolayers is deliberately pursued. As an example, the determination of Cu by anodic stripping voltammetry in the presence of fouling species, namely surfactants, has been carried out on Au surfaces coated by a disordered monolayer of 2-mercaptoethane sulphonate [111]. The monolayer protects the underlying Au surface by the fouling due to the surfactants, though allowing the diffusion of the analyte towards the electrode. A similar strategy is also followed for the development of genosensors, where the deposition of thiols possessing short alkyl chains protects the underlying substrate from not specific interactions with oligonucleotide chains in solution [84].

Although long-term stability (from a few weeks to a few months) has been reported [112], the most frequent drawback of monolayers lies in the poor chemical and thermal stability, leading to sensing elements with a limited shelf-life and lifetime. Special precautions, such as refrigerated storage, are often required in order to extend the shelf-life.

Representative examples of amperometric determinations through electrodes modified by monolayers are reported in Table 5.1. We choose not to report an

Table 5.1 Selected analytical applications of monolayers

Receptor	Substrate	Head group	Analyte	Refs.
Alkyl chains	Au	SH	Drugs	[121]
Amines	Au	SH	Ascorbic acid and dopamine	[124]
Alcohols	Au	SH	2,4,6-Trinitrotoluene and 2,4-dinitrotoluene	[129]
Carboxylic acids	Au	SH	Dopamine	[125]
Ethylenediaminetetraacetic acid	Au	SH	Cu^{2+}, Pb^{2+}	[122]
Sulphonic acids	Au	SH	Cu^{2+}	[111]
Thiols	Au	SH	Cd^{2+}	[123]
Cavitands				
Calix[4]pyrroles	Au	SH	Cl^{-}	[120]
Cyclodextrins	Au	Multiple SH moieties	Glucose	[133]
Boronic acids	HOPG	Alkyl chain	Catechols	[126]
	Au	SH	Dopamine	[127]
Metal complexes				
Fe porphyrin	Au	SH	L-histidine	[30]
Fe porphyrin	Au	None (molecule adsorbed flat on Au)	L-histidine	[30]
Fe phthalocyanine	Au	SH	SCN^{-}	[34]

exhaustive list, since the number of literature reports has skyrocketed in the last 15 years. The reviews cited in the first lines of this section may be of help in this respect. Our choice was rather that of drawing some general considerations from the literature. As discussed previously, for obvious reasons of convenience and cost, polycrystalline substrates strongly predominate in the electroanalytical applications of monolayers. Conventional-size disk electrodes are routinely employed. They are flanked by thin films prepared by sputtering and by lithographic techniques and by screen-printed electrodes based on composite inks containing tiny Au, Pt, or carbon particles [113–116]. One of the main advantages offered by screen-printed electrodes consists of the ability to employ mass-produced, cheap, disposable electrochemical cells; however, the reproducibility of the electrode surface is lower than with other fabrication techniques. Lithographic techniques and microfluidics allow also the miniaturization of the whole electrochemical cell [117–119].

The vast majority of the articles deal with studies carried out in buffered aqueous solutions, even if some examples of application in organic media, e.g., CH_2Cl_2, are also present in the literature [120]. As for many other electrode coatings, only relatively few investigations in simulated [30] or real matrices [121] have been carried out.

When not considering bio-sensors, heavy metals [111, 122, 123] and easily oxidisable species such ascorbic acid and catecholamines [124–127] are among the most widely studied analytes. These classes of chemical species have also been so often chosen because they are of key importance in environmental and biochemical contexts [128]. In addition, they have been widely investigated using different kinds of modified electrodes, thus representing a sort of benchmark of the effectiveness of the strategy followed in the realization of the electrode modification. The determination of metal ions is usually carried out by anodic stripping technique, involving the application of a suitable cathodic potential for metal pre-concentration in the first phase of the analysis. The exact condition in which the metal deposited in the first step is present is still poorly investigated; in particular, it is not clear whether the deposit is on the top of the monolayer, forming pillars in correspondence of defects, or is located between the substrate and the monolayer.

Besides the already cited metal ions, a large number of different chemical species have been investigated, such as amino acids [30], drugs [121], explosives [129], inorganic ions (Cl^- [120], SCN^- [34], etc.), and oxidizing species [130]. The amperometric detection of these species takes advantage of different mechanisms of interaction of the analyte with the tail group of the SAM, such as complexation, electrostatic [131] attraction or repulsion, hydrophobic interaction [121], formation of covalent bonds, or size exclusion effects [111].

Charged monolayers have been employed to selectively attract or repel charged species dissolved in solution, possessing opposite or similar electrical charges, respectively. The most deeply investigated system consists of ascorbic acid and dopamine: at pH ca. 7, the former molecule is negatively charged while the latter is positively charged; electrostatic filtering allows the acquisition of signals coming from only one of the two species [131].

Electrocatalytic phenomena ascribed to the interaction between the analyte and the receptor are often reported [125]. It is worth noting that electrocatalysis on monolayer-coated surfaces have also been investigated in the frame of applications different form sensors, e.g., organic syntheses and fuel cells [132]. It is evident that the information gained in these studies can be fruitfully exploited in the near future in order to develop sensing systems. With this respect, an interesting new frontier consists of the use of persistent organic free radicals [132]; although their use in amperometric sensing is still missing, the results gained in organic synthesis are also promising for applications in electroanalysis.

It is worth noting that, in some cases, modified electrodes may be suitable to determine analytes that are not electroactive. In these cases, the generation of the current signal may be induced by activation of different mechanisms. As an example, the determination of chloride ions, which are not electroactive in usual experimental conditions, has been carried out using a thiol-based monolayer bearing tetrathiafulvalene-calix[4]pyrroles as the tail group [120]. The inclusion of chloride ions in the cavitand leads to a significant shift of the voltammetric peak ascribed to the redox processes of the tail groups, which has been employed as the species generating the analytical signal: the potential is linearly related to the logarithm of the chloride concentration. Another peculiar example is given by the use of a monolayer coating based on α-cyclodextrin as the tail group bounding an electroactive species, namely ferrocene [133]. The amperometric signal due to the anodic oxidation of ferrocene decreases when the electrode system is in contact with glucose: glucose molecules, which are a non-electroactive species under these experimental conditions, substitute for ferrocene molecules.

Despite the advantages outlined above, some possible drawbacks have to be evidenced. Complete reversibility of the interaction between the analyte and the receptor is not always exhibited by the receptors present in the monolayers. This not only occurs due to the formation of covalent bonds, but also because of some adduct formation reactions and has led to the widespread use of disposable systems. In addition, it is well known that amperometric sensing constitutes one of the most important detection systems for flow injection, chromatographic, and electrophoretic devices. The lack of reversibility of the interaction and the poor stability of the coating monolayer gives a rationale to the limits of meaningful applications in this field [121]. However, the availability of low-cost, disposable working electrodes based on Au, Pt, and C [134] allows for the envisioning of new perspectives for similar electrode systems modified by monolayers.

Conversely, the development of affinity biosensors constitutes a field of application where monolayer-modified electrodes are of great importance, as testified by the abundant literature [135–142]. Antibodies, aptamers, and strands of nucleic acids are the most common biological recognition elements used for the selective detection of the target analyte. These biomolecules require to be stably grafted on the electrode surface through a monolayer without losing their activity [143].

The analyte, dissolved in solution, normally interacts with receptors located on the tail of the monolayer. This step generally requires quite a long immersion step, followed by vigorous washing of the system, devoted to removing interfering species giving rise to non-specific interactions. Hence, it is evident that the formation of a very stable monolayer of the bio-receptors on the electrode surface is of primary importance to obtain high-performance amperometric sensors. Furthermore, affinity biosensors consist mainly of disposable systems, so that the accuracy of the sensor is strongly conditioned by the reproducibility of the monolayer formation.

The presence of a more or less ordered plane of receptor biomolecules on the electrode surface favors the analyte–receptor interaction, since the approach of the analyte molecules to the receptor is not hindered by the presence of a more or less thick coating. Thiol-based molecules anchored on Au substrate represent the most common family of electrode modifications in amperometric biosensing based on monolayers. Two different strategies can be adopted. The receptor can be functionalized with a moiety suitable to be anchored at the substrate, typically bearing a thiol end group. This strategy requires a careful choice of the synthesis, since bio-activity could be lost if harsh experimental conditions are employed. However, the subsequent adsorption process on the electrode surface requires only one step. Despite the complexity of some synthetic procedures, a number of commercial biological receptors bearing a thiol moiety are available, since a similar detection strategy is well consolidated. Alternatively, it is possible to take advantage of the reactivity of the monolayer tail groups discussed in Sect. 5.5. The

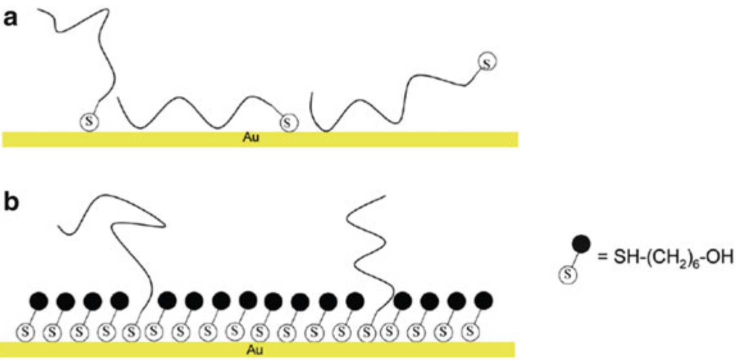


Fig. 5.18 An example of the importance of mixed monolayer in the case of single-stranded DNA molecules bearing a –SH group adsorbed on Au substrate: (**a**) the degree of order is poor when DNA strands are the only adsorbed molecules; (**b**) the use of a mixed monolayer based on DNA strands and mercaptohexanol leads to more ordered adsorbed nucleic acid molecules (Reprinted from Ref. [136] with permission of Royal Society of Chemistry)

creation of amide bonds with –COOH tail groups represents the most widely adopted strategy. On the other hand, in this case the anchoring of the biomolecule requires two steps. In addition to formation of covalent bonds, the anchoring of the bio-molecules on the electrode surface can also take place via electrostatic interactions, chelation, and formation of hydrogen bonds [140, 144]. However, it is evident that, for the operating conditions previously described, the formation of covalent bonds is generally preferred.

Mixed monolayers have been widely employed in affinity biosensors, in order to prevent non-specific adsorption of dissolved species on the electrode surface, to reduce the steric hindrance between biological receptors, and to increase the degree of order of the bio-receptors on the electrode surface (Fig. 5.18).

A widely diffused strategy, followed to increase the sensitivity of affinity biosensors, consists of the formation of bio-receptor monolayers on nanostructured surfaces, replacing the most common smooth ones. The effect of the use of a rough surface is not merely to increase the number of bio-receptors on the electrode surface, but, in particular, to induce a lower steric hindrance between bio-receptors (Fig. 5.19A) [145]. Consequently, a higher amount of target analyte can form an adduct with bio-receptors on the electrode surface and the final resulting analytical signal is more intense (Fig. 5.19B). It is evident that the choice of the most proper nanostructure morphology is of primary importance to impart the sensor the sensitivity sought [146].

Similarly, electrode surfaces can be modified by monolayers of proteins in enzymatic biosensors. Amine terminal groups from lysine residues of the protein are typically used for the formation of very stable imine bonds; to such a scope, electrode surfaces are modified with monolayers containing –COOH tail groups. Different functional groups present in the polypeptide chain can also be exploited, as in the example reported in Fig. 5.20.

On the other hand, when considering enzymatic biosensors, the deposition of monolayers on flat surfaces is generally avoided; in this case a low number of

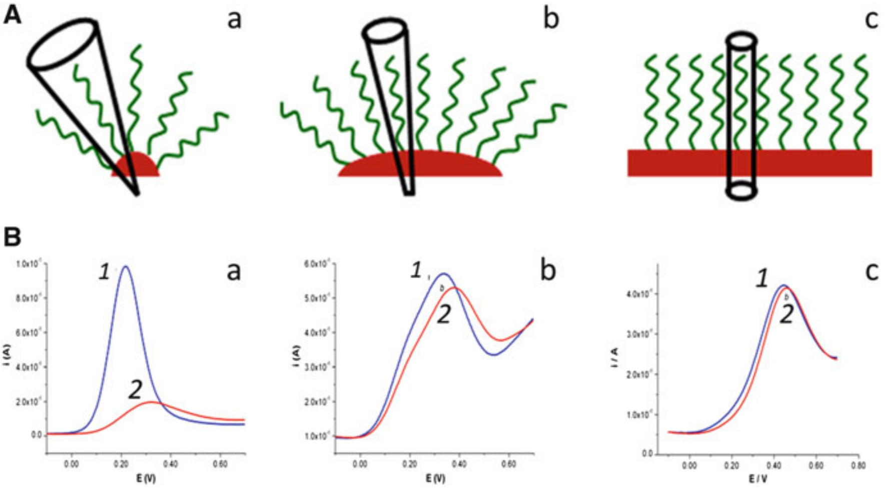


Fig. 5.19 (**A**) The disposition of single-strand oligonucleotide chains in monolayers deposited on Au surfaces characterized by a different mean roughness: (**a**) ca. 10 nm, (**b**) ca. 100 nm, (**c**) flat (Reproduced from Ref. [145] with the permission of American Chemical Society). (**B**) Relevant voltammetric signal obtained before (*1*) and after (*2*) hybridization with the complementary oligonucleotide chain in a solution of $[Fe(CN)_6]^{3-}$ electroactive probe (Reproduced from Ref. [146] with the permission of Springer)

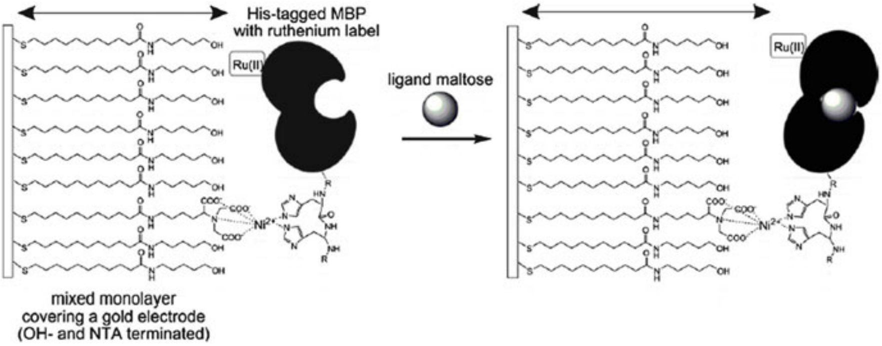


Fig. 5.20 An example of an amperometric biosensor based on a bilayer of organic molecules. Maltose-binding protein (MBP) bearing a Ru(II/III) redox label is anchored to the electrode surface through a histidine (His) tag. The tag allows the formation of a metal complex with an Ni(II) ion and a nitrilotriacetic acid (NTA) residue located on the tail group of a thiol molecule adsorbed on Au electrode surface. The formation of an adduct between the analyte, namely a maltose molecule, and MBP, leads to a rearrangement of the protein on the surface, changing the effectiveness of the charge transfer between Ru(II/III) redox couple and the electrode surface (Reproduced from Ref. [147] with the permission of American Association for the Advancement of Science)

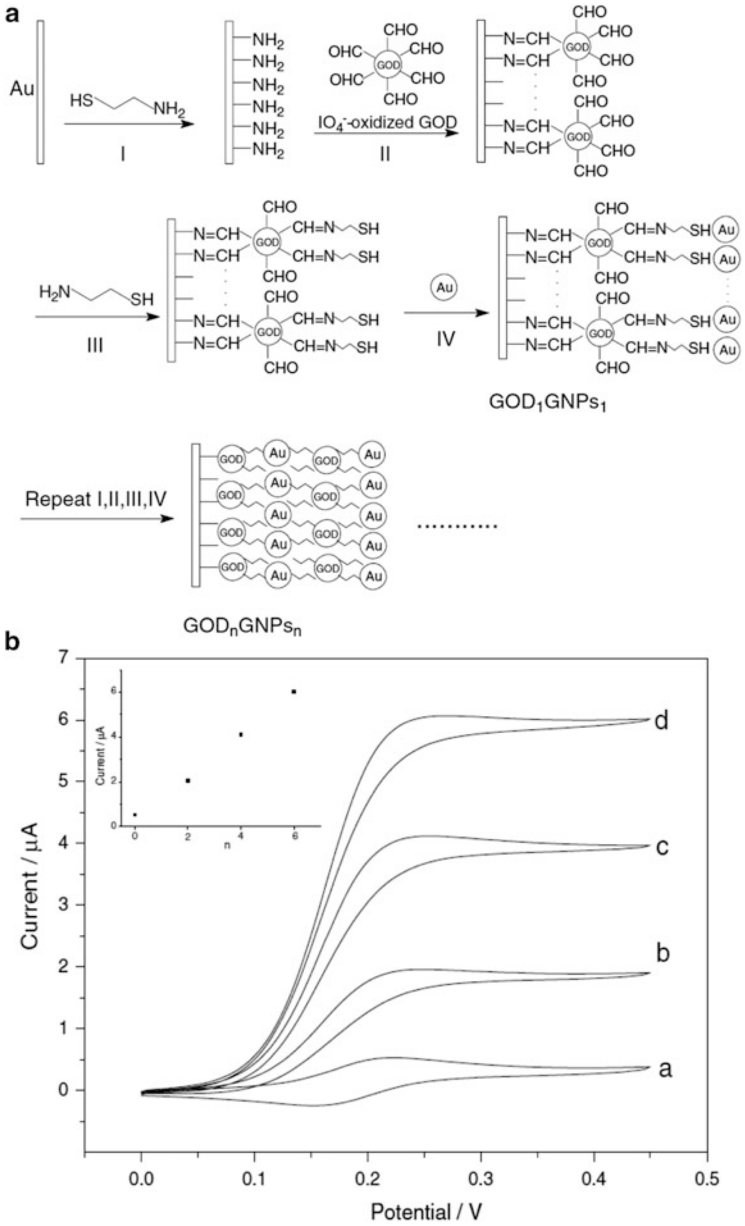


Fig. 5.21 (**a**) Layer-by-layer construction of the glucose oxidase (GOD) – Au nanoparticle (Au) multilayer films on an Au electrode surface. (**b**) Cyclic voltammograms, in the presence of 20 mM glucose, of the multilayer-modified electrodes containing a different number of GOD/Au bilayers: (*a*) zero; (*b*) two; (*c*) four and (*d*) six; *inset*: plots of anodic plateau currents vs. numbers of GOD/Au bilayers. All curves were registered in the 0.1 M, pH 6.8 phosphate buffer solution containing 0.25 mM ferrocenemethanol as the redox mediator (Reproduced from Ref. [148] with the permission of Elsevier)

bio-catalytic centers are finally present on the electrode surface, resulting in sensors characterized by a too low sensitivity. In this respect, the formation of a multilayer of enzyme molecule is definitely preferable, since the resulting density of recognition elements on the electrode surface is higher. To better show this effect, Fig. 5.21 reports the amperometric signal obtained at multilayer-modified electrodes in the same solution of glucose varying the number of layers deposited: the higher the number of layers deposited containing glucose oxidase, the higher the signal.

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Chapter 6
Nanosized Materials

Novel nanosized materials have been proposed at an increasing rate over the past two decades, and have influenced many different scientific and technological fields as soon as the community revealed and developed their particular characteristics [1–5]. Their use in electroanalysis has become so widespread that electrode modifications based on these materials are often the preferred solution to the realization of effective amperometric sensors.

The main advantages of using nanosized materials in electroanalysis derive directly from their dimensions:

1. the atoms constituting defects such as vertexes and corners reveal the particular reactivity of this material in electrocatalytic processes;
2. the electroactive area expands when nano-objects are deposited onto electrode surfaces, because of the high surface-to-volume ratio of the nano-objects;
3. by passing from a smooth to a nanostructured surface, the diffusional regime changes from being linear to exhibiting a significant radial component;
4. the small spatial dimensions of nano-objects may allow for a direct intimate electrical connection with biological recognition elements;
5. the very small dimensions make it possible to fix a number of molecules stably on the nanostructured electrode surface.

The outstanding properties of these materials have fostered the publication of so many reviews and books to date that it is difficult even to list them all [5–32]. On the other hand, it should be observed that the prefix ‘nano’, which should indicate objects of nanometer dimensions, is often adopted to indicate objects whose dimensions could be more spontaneously expressed by micrometer units. Actually, the functional distinction between nano- and micro-objects is not univocal and depends on the nature of the material. More precisely, it depends on the characteristics exhibited by the material, as is explained in the following.

First, we must highlight that this chapter focuses on electroanalytical applications of nanosized materials and, for this reason, only electrical conductors are considered. These consist mainly of metal nanoparticles (NPs), carbon nanotubes

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(CNTs), graphene, and, to a lesser extent, metal oxides, quantum dots (QDs), metal clusters, and fullerenes.

6.1 Classification of Nanosized Materials

The variety of compositions and shapes reported to date makes it quite difficult to outline a univocal classification system for nanosized materials. One possible distinction is based on the dimensionality of the structures, as illustrated in Table 6.1 [33].

The classification scheme shown is not definite. For example, the distinction between NPs and clusters cannot be established on the basis of dimensional criteria. Although the term 'cluster' is used for small Au NPs [34], in principle, they are characterized by a well-defined structure [35], while the mobility of the surface atoms in the NPs does not allow one to ascribe them an exact geometrical shape. Similarly, although transmission electron microscopy (TEM) images depict carbon black particles as spherical and they are thus classified as 0D nano-objects, they actually consist of disordered graphene sheets.

Other authors [36] suggest a different classification, suitable to categorize a larger number of mono- and multi-component nanomaterials. In particular, the authors identify 36 classes of nanostructures containing up to three different components.

In most cases, nanostructures possess an inorganic core of metal, carbon, or semiconductor material. A few examples of nanostructured coatings based on pure conductive polymers [37] have also been reported in the literature, as better described in Sect. 6.5. Most metal nanomaterials consist of noble metals, although Ag and Cu have also been reported [8, 11, 13–15, 17–20, 23, 24, 26–28]. For electroanalytical applications, NPs constitute the most common nanostructures, followed by nanowires [38, 39]. Although the former appear spherical, they are actually a polyhedral shape (Fig. 6.1).

Carbon nanostructures (Fig. 6.2) comprise a variety of materials [42] based mainly on carbon atoms with sp^2 hybridization. The most widespread in the field of electroanalysis are CNTs [1, 25, 29, 30, 43, 44] and graphene [1, 7, 9, 12, 21, 22, 45–49], although applications involving fullerene [50] and carbon black [51] have also been reported. Both CNTs and graphene are graphite derivatives, consisting of rolled or planar sheets, respectively. Both single- and multi-wall CNTs have been

Table 6.1 Dimensionality of nanosized materials

Denomination	Examples
Zero-dimensional (0D)	Nanoparticles, clusters, carbon black, diamondoids, fullerenes
One-dimensional (1D)	Nanowires, nanotubes
Two-dimensional (2D)	Graphene
Three dimensional (3D)	Tetrapodal structures, nanoporous systems, fractal objects

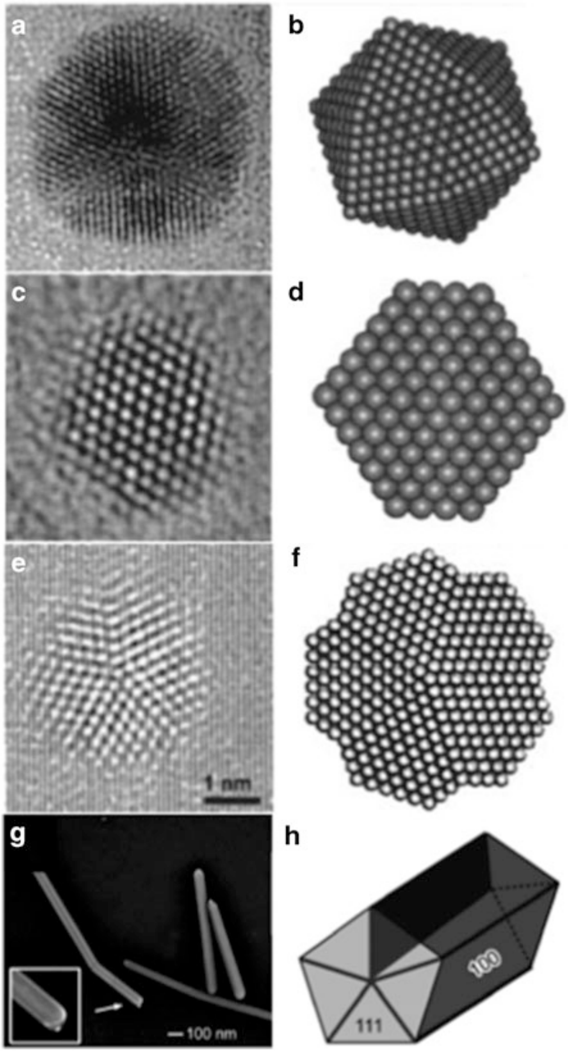

Fig. 6.1 Transmission electron microscopy images of Au nanoparticles (NPs) at atomic resolution level. (**a**) Icosahedral, (**c**) cubo-octahedral, and (**e**) truncated decahedral NP shapes are shown, together with their hardball models (**b**, **d**, **f**, respectively). (**g**) and (**h**) are the scanning electron microscopy image of Ag nanowires and the relevant geometrical model (Reproduced from Refs. [40] and [41] with the permission of American Institute of Physics and American Chemical Society, respectively)

employed, although a sound comparative assessment of their performance has not been published. Although CNTs are often considered to consist of uniform sp^2 structures, they present a number of structural defects, such as vacancies at the level of atoms or even rings, interstitial inclusions [52, 53], and oxygenated species [1, 25, 29, 30, 43, 44]. The effect of similar defects on the electrocatalytic properties of the material is still being debated.

Semiconductors, generally consisting of metal oxide NPs such as TiO_2, MnO_2, and CuO_x, or QDs such as CdSe and CdS are used less frequently in electroanalysis, although more reports have appeared in the literature over recent years [14, 15, 57, 58].

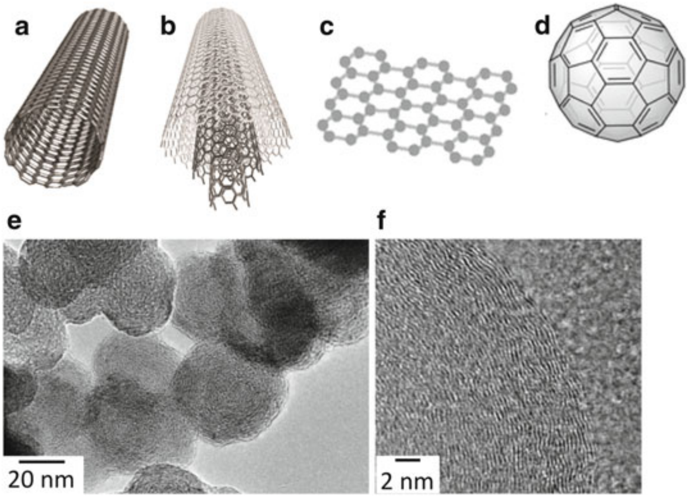

Fig. 6.2 Carbon-based materials used in electroanalysis: schemes for (**a**) single- and (**b**) multi-wall carbon nanotubes, (**c**) graphene, (**d**) C_{60} fullerene; (**e**) transmission electron microscopy images of carbon black particles (Reproduced from Refs. [45, 54, 55], and [56] with the permission of Springer, American Chemical Society and InTech, respectively)

6.2 Synthesis Techniques

The performance of electroanalytical sensors is strictly related to size and shape of the nanosized material employed. This means that, in order to define reliable property–structure relationships, the nano-objects must possess a similar shape and narrow size distribution, i.e., characterized by a relative standard deviation of less than 20 %. Many parameters that depend on the synthetic procedure adopted affect the size, size distribution, and shape of the resulting nano-objects.

There are two basic approaches to the synthesis of nanosized materials: top-down or bottom-up methodologies (Table 6.2). Top-down refers to pulverization of bulk material into fine particles that can be collected as solid powder, suspended in a liquid, or deposited directly on the electrode surface. The particles are usually obtained by physical methods, such as thermal evaporation, sputtering, or laser ablation. In addition, metallic nanosized particles can also be obtained via electrochemical synthesis, exploiting the dissolution of a sacrificial anode [59].

Table 6.2 Most important synthetic methods for some classes of nanomaterials

Nanomaterial	Bottom-up approaches	Refs.	Top-down approaches	Refs.
Metal nanostructures	Chemical and electrochemical reduction of metal salts in solution and at liquid|liquid interface	[67–74, 84]	Electrochemical synthesis from sacrificial anode	[59, 95, 96]
	Electrochemical deposition onto a substrate	[75, 76]	Thermal evaporation	[33, 81, 95, 96]
	Decomposition of organometallic compounds	[77]	Sputtering	[70, 81, 95, 96]
	Biosynthesis through living organisms	[60, 78–80]	Laser ablation	[33, 81, 95, 96]
Metal oxide nanostructures	Precipitation	[67, 82–83]	Sputtering	[33, 81, 95, 96]
	Sol-gel	[67, 82–84		
	Electrodeposition	[83]		
	Synthesis in microemulsion	[3, 71]		
	Hydrothermal synthesis	[67, 82, 85]		
	Sonochemical synthesis	[69, 82]		
CNTs	Chemical vapor deposition	[86, 87]	Arc discharge	[86, 95, 96]
			Laser ablation	[86]
Graphene	Chemical vapor deposition	[88–90]	Graphite exfoliation	[88–90, 95, 96]
QDs	Wet chemical synthesis	[4, 67, 91	Thermal evaporation	[4, 95, 96]
	Sol-gel	[4, 67, 91]	Sputtering	[4, 95, 96]
	Synthesis in microemulsion	[4, 67, 91]		

(continued)

Table 6.2 (continued)

Nanomaterial	Bottom-up approaches	Refs.	Top-down approaches	Refs.
	Decomposition of organometallic compounds	[4, 67, 91]		
Polyoxometallates	Wet chemical synthesis	[92–94]		
Fullerenes (buckyball clusters)			Arc vaporization	[94–98]
			Laser ablation	[94–98]
Metal clusters	Wet chemical synthesis	[71]		
Polymeric nanostructures	Electrochemical deposition	[37]		

The bottom-up strategy relies on wet chemical or gas phase methodologies. In particular, there are at least four families of solution-based syntheses [59, 60]:

1. chemical reduction of metal salts;
2. electrochemical reduction of a metal salt and concomitant deposition onto a substrate;
3. decomposition of organometallic compounds;
4. biosynthesis by living organisms.

Obviously, for specific application in the frame of amperometric sensing, chemical reduction and electrochemical synthesis constitute the solutions of choice, but the actual possibility to effectively modulate and control the dimension of the nano-objects still constitutes a challenging issue.

With regard to a gas phase approach, chemical vapor deposition and laser pyrolysis have been proposed, although these methods have been only occasionally employed in the development of amperometric sensors [61, 62].

Many synthetic procedures, including most of the wet chemical ones, are relatively simple, so researchers often prefer synthesizing nanosized materials on their own. This allows them to adjust the properties of the materials by properly adapting the preparation methodology. On the other hand, procedures based on complex and expensive instrumentation, such as required when preparing CNTs, may leave no choice but to purchase the material on the market. Over the past 20 years, the number of firms commercializing a variety of nanomaterials have increased enormously, in parallel to the diffusion of these materials in many applications.

Sometimes, further treatments of the nano-objects are necessary to obtain best performance from the resulting modified electrode. For example, highly conductive graphene is generally obtained through chemical or cathodic reduction of poorly conductive graphene oxide [64, 65]. Graphene oxide, prepared by simple exfoliation of graphite flakes, presents the advantage of being stable in aqueous suspension, with the result to be easily stored and processed.

Impurities can play a major role in conditioning the properties of the nanostructures. The presence of impurities is detrimental in terms of relating the electroanalytical performances of the nano-objects with their own actual nature, due the unpredictable influence of a foreign material. Some of the most well-known examples of extraneous species in the case of nanomaterials are traces of metal catalysts affecting some of the commercial products. CNTs suffer from such a drawback due to the synthetic procedures followed [44]: the actual electrocatalytic properties of CNTs are often ascribable to the presence of similar impurities. For this reason, a variety of purification protocols has been developed to minimize the presence of extraneous species. In particular, the dispersion of CNTs in strong acidic media is one of the most popular procedures to remove metal impurities [63], although this chemical treatment presents several problems and seems to significantly shorten the length of the CNTs. A further example of undesired species is given by graphite impurities in fullerenes, CNTs, and graphene [66]. Foreign species are deliberately added in only a few cases, to tune the electrochemical behavior of the nanostructures (see Sect. 6.8).

6.3 Stability in Solution

Many nanosized materials produced by bottom-up syntheses need to be stably dispersed in solution, so as not to undergo aggregation or coalescence. Stabilization is achieved by surrounding the core with a shell (Fig. 6.3) consisting of a more or less ordered and dense organic monolayer. This cloud constitutes the interface between the nano-object and the solution. In the case of bottom-up synthetic techniques, it also plays a key role in limiting the growth of the inorganic core.

The Derjaguin and Landau, Verwey and Overbeek (DLVO) theory and its extensions [99] predict the actual achievement of stability by nanosized materials in solution. The same theory has been employed to describe the stability of colloids; interestingly, the term ‘colloid’ is sometimes used to refer to nano-objects, particularly NPs, dissolved in solution. One of the most important characteristics threatening the stability of nano-objects in solution lies in the spontaneous tendency of neighboring cores to join with one another. Two main processes may actually occur: aggregation or coalescence [100, 101]. Aggregation refers to the formation of groups of nano-objects due to interaction among the cores or among the encapsulating layers of adjacent nano-objects. After aggregation, the cores retain their individuality, so the properties typical of nanosized materials are at least partially preserved. On the other hand, coalescence refers to the formation of nanostructures with different shapes and larger core dimensions than the original ones, so they may lose the characteristics of true nanosized materials. In particular, coalescence may lower the surface-to-volume ratio of the fine particles, leading to an increase of core dimension and, ultimately, to the loss of the characteristic properties of nanomaterial.

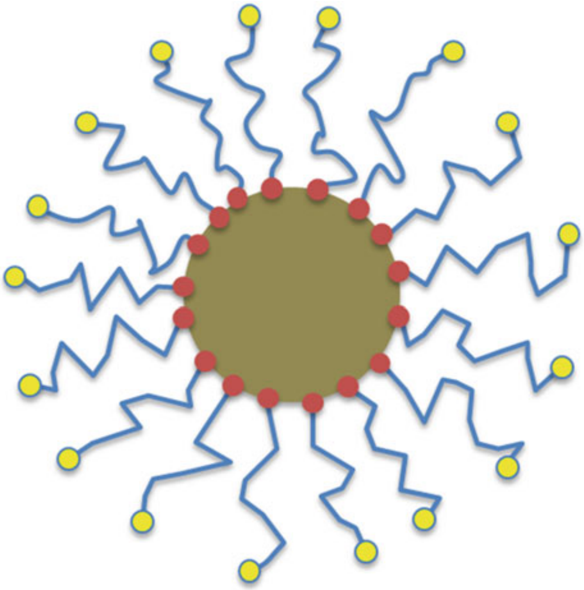

Fig. 6.3 Simplified scheme of an inorganic core surrounded by an encapsulating shell. The main chemical functionalities of the organic molecules are evidenced. *Red balls*: anchoring groups; *yellow balls*: functionalities. The case of a nanoparticles is represented

To prevent aggregation and coalescence, stable dispersion of nano-objects in solution is generally achieved by exploiting repulsive forces between encapsulating clouds. The origin of the stability of the dispersion may be of various natures [70, 102]:

1. electrostatic stabilization (Fig. 6.4a) relies on electrostatic repulsion among nano-objects, attributable to the net positive or negative charge of the encapsulating cloud, e.g., citrate or chloride ions surrounding Au NPs, or carboxylic groups on the surface of CNTs, formed by acid treatment;
2. steric stabilization (Fig. 6.4b) is based on steric repulsion of the organic layers of neighboring nano-objects, e.g., alkyl thiols or polymers surrounding Au NPs.

As for electrostatic stabilization, the role of ionic strength is crucial [70, 99, 102]: the increase in total concentration of ions leads to weakening of the repulsive forces due to ‘screening’ of the net charge on the surface of the nano-objects. In this condition, aggregation of nano-objects may occur.

The solvent itself may act as the capping agent [59], and even the impurities present in the solvent media may play a role in stabilization. In addition, during the synthesis, the nano-object precursors may release species suitable to play such a role. For example, Au NPs can be prepared in solution phase without the addition of a dedicated molecule to stabilize the growing NPs, if $AuCl_4^-$ is used as the precursor: Cl^- anions are released during the reduction process [103]. Cl^- anions act as labile, though effective, encapsulating agents. Some examples of species suitable to act as encapsulating agents of the indicated nano-objects are reported in Table 6.3.

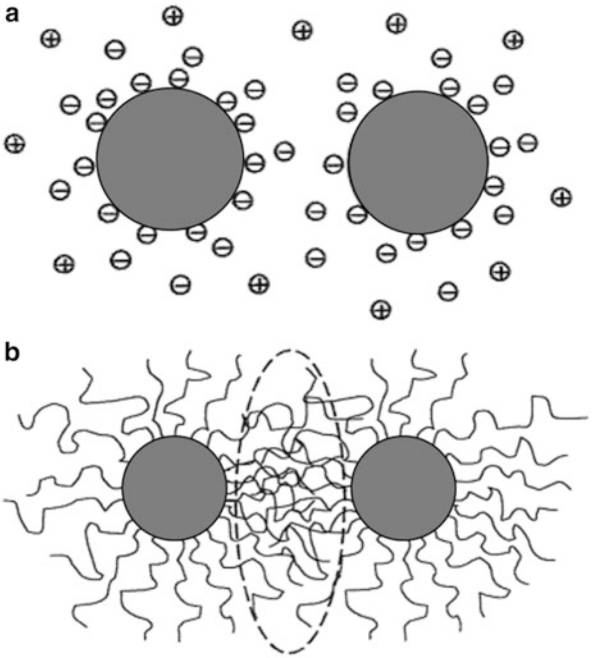

Fig. 6.4 (**a**) Electrostatic and (**b**) steric forces induce stable dispersion of nano-objects in solution. Nanoparticles (NPs) are represented (Reproduced from Ref. [70] with the permission of American Chemical Society)

Table 6.3 Examples of encapsulating agents for selected nano-objects

Encapsulating agent	Nanomaterials	References
Thiols	Au NPs encapsulated by 1-dodecanethiol	[104]
Amines	Pt NPs encapsulated by 1-dodecylamine	[105]
Tetraalkylammonium salts	Ni, Cu, and Au NPs encapsulated by tetraoctylammonium bromide	[102]
Inorganic anions	Au NPs encapsulated by chloride anions	[103]
Carboxylic acids	Au NPs encapsulated by citrate anions	[63, 106]
	CNTs treated with strong acidic solutions	

6.4 Synthesis of Nanostructures within Templates

Alternatively, more or less rigid enclosures acting as templates can be used in order to control the growth of the nano-objects. A few representative examples are reported hereafter. It is worth noticing that most nanostructures can constitute a template for the growth of a second nanostructured component on their surfaces or inside their pores or cavities.

Microemulsion formation represents one of the most popular template syntheses: suitable surfactant molecules are dissolved in a hydrophobic solvent to form small vesicles containing an aqueous medium (Fig. 6.5) [63, 102–107]. Such a methodology is adopted in the synthesis of metal NPs: the chemical reduction of metal ion precursors to zero-valence metal occurs in the aqueous phase. After a

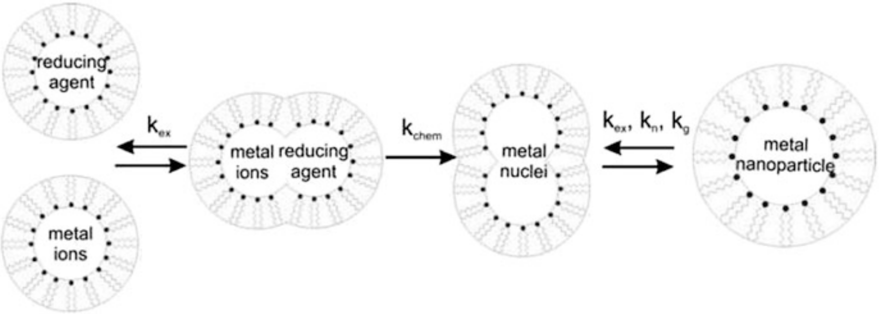

Fig. 6.5 Example of microemulsion synthesis: chemical reduction of metal precursors aiming at preparing metal nanoparticles (Reproduced from Ref. [71] with the permission of InTech)

suitable purification procedure, encapsulated nano-objects are obtained. Pseudo-spherical NPs represent the most common product of this synthetic approach; the shape of the nano-objects can be tuned by carefully choosing the surfactant mixture, taking advantage of the large number of different shapes the droplets can assume in solution.

A further typical example of template-based synthesis widely employed in developing systems for electroanalysis is given by the electrogeneration or electroless deposition of noble metal nanostructures inside the pores of membranes, typically based on polycarbonate or alumina (Fig. 6.6) [108]. By electrodeposition, the electrode surface is obtained through the coating of one of the membrane surfaces with a thin layer of a conductive material, typically Au, by sputtering or thermal evaporation. Nanowires or nanotubes anchored on the electrode surface are the nano-objects that result from removal of the membrane. The template can also be retained on the electrode surface to keep the nano-objects well separated from each other, e.g., when the aim is to obtain a nano-electrode array. The size of the nano-objects, in terms of length and diameter, can be controlled by tuning the dimensions of the pores and the deposition conditions; unfortunately, the nano-objects are often randomly arranged on the membrane. Alternatively, the membrane can be removed and the nano-objects separated from the surface, e.g., in order to disperse them in solution. A similar approach can exploit inorganic membranes consisting of alumina, obtained by anodization of Al foils [109].

A further popular example of the use of a template lies in the formation of the so-called inverse opal on planar surfaces (Fig. 6.7) [16, 111, 112]. Opal is a well-known inorganic material based, to a first approximation, on an ordered arrangement of packed silica spherical (nano)particles. An inverse opal is synthesized starting from an opal-like structure based, for example, on latex spheres. The voids among the spheres are filled with a foreign material, e.g., a metal deposited through cathodic reduction. The original template material is dissolved, leading to

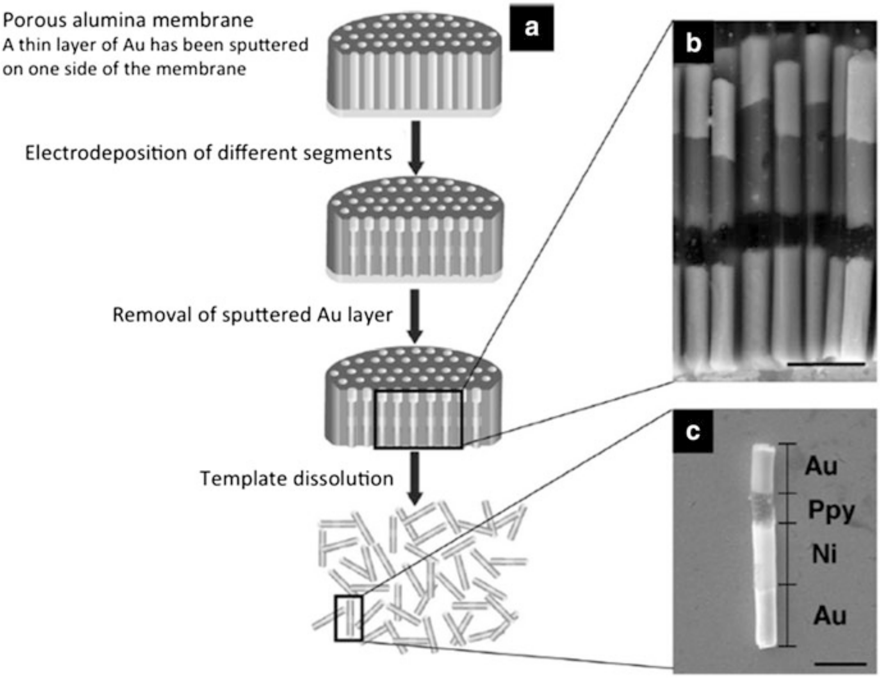

Fig. 6.6 (**a**) Synthetic procedure for Au-polypyrrole (Ppy)-Ni-Au multi-segmented nanowires; (**b**) cross-section image of the nanowires inside the alumina template obtained through scanning electron microscopy; (**c**) scanning electron microscopy image of a single nanowire showing the different components. *Scale bar*: 1 μm (Reproduced from Ref. [110] with the permission of Wiley)

an ordered structure containing only the foreign material. To reach a high degree of order, latex spheres possessing a monodisperse size distribution are mandatory.

Finally, disordered metallic foam can be obtained starting from a bimetallic material containing two distinct phases. A proper (electrochemical) etching procedure, more or less correctly called de-alloying, leads to the dissolution of one of the metallic phases (Fig. 6.8) [113, 114].

A characteristic synthesis through a template exploits the surface defects of suitable substrates to achieve a material deposition under the form of a nanostructure. As an example, the step edges of the terraces of highly oriented pyrolitic graphite (HOPG) constitute preferential nucleation sites for electrodeposited noble metal NPs (Fig. 6.9) [115].

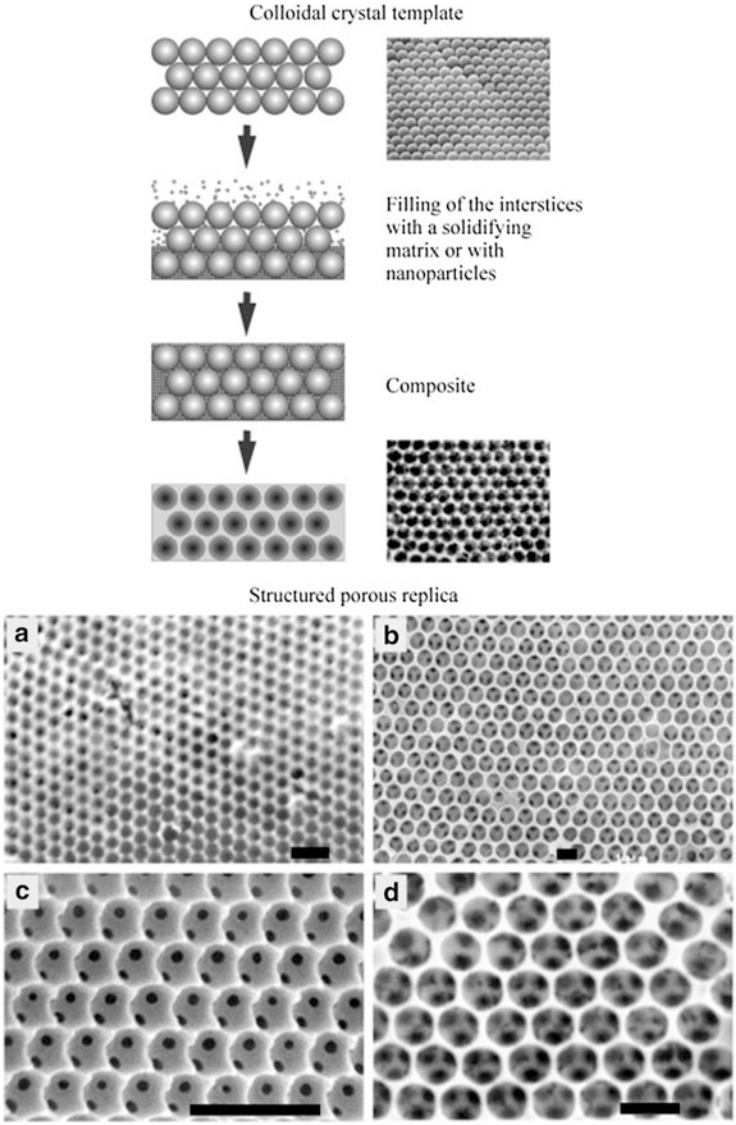

Fig. 6.7 Synthetic procedure of inverse opal and a few representative scanning electron microscopy images of the resulting material (**a–d**). *Scale bars*: 1 μm (Reproduced from Ref. [112] with the permission of Elsevier)

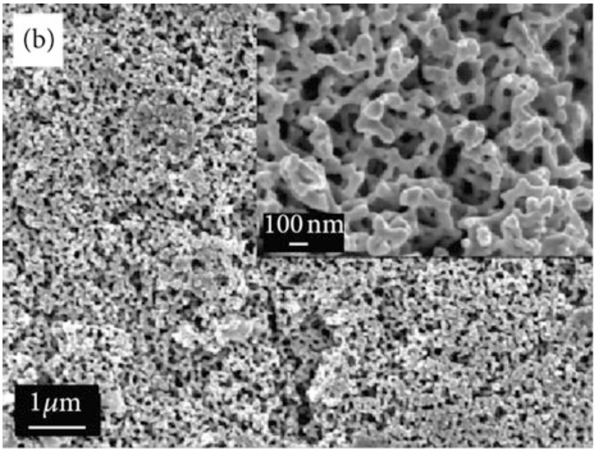

Fig. 6.8 Au nanoporous structure obtained from an Au–Zn alloy. A representative image at higher magnification level is shown in the inset (Reproduced from Ref. [114] with the permission of Hindawi)

6.5 Synthesis of Polymeric Nanostructures

The most important organic nanostructures of use in electroanalysis are based on intrinsically conductive polymers (ICPs). A large number of polymeric nanostructures, both supported on the electrode surface and dispersed in solution, have been prepared through the use of a template, taking advantage of the strategies described in Sect. 6.4. A few examples are reported in Table 6.4 and Fig. 6.10.

Literature reports a number of alternative synthetic approaches [37, 116, 117]. The most widespread procedures for the synthesis of the polymeric backbones are based on chemical or electrochemical polymerization from a diluted solution of the monomers. The mechanisms that are active during formation of polymer chains are similar to those reported in Chap. 2. Different shapes have been reported for the resulting nano-objects; however, NPs, nanowires, and nanotubes represent the most popular. Note that the surface of polymeric nanostructures is not rigid as in the previously described metal- and carbon-based cores. On the contrary, the surface of these nanostructures often consists of a network of flexible polymeric chains and exhibits a significant porosity. Amorphous phases are proper of the most part of similar nanostructures: the polymeric chains possess a high degree of disorder. Hence, the encapsulation process of dispersed polymeric nano-objects differs with respect to metal- and carbon-based materials: the conventional adsorption on well-defined specific sites does not take place, although electrostatic interactions between the polymeric nano-object surface and the capping molecules may also be operative. With respect to bulk polymeric films described in Chap. 2, polymeric nanostructures possess a higher surface-to-volume ratio and a lower resistance to the diffusion of species into them. In addition, their size may allow direct charge transfer between redox centers of enzymes and the electrode surface in enzymatic biosensors (see Sect. 6.10.3), which is prevented when using bulk ICP-modified electrodes.

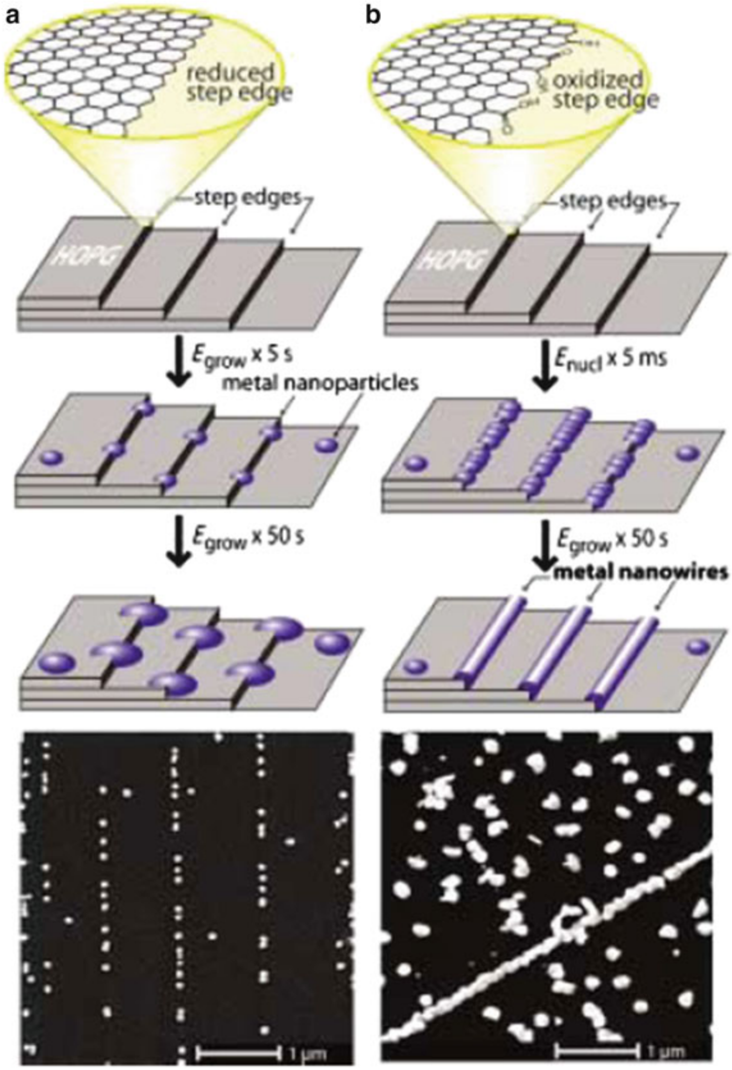

Fig. 6.9 Synthesis of Au nanoparticles and nanowires exploiting highly oriented pyrolitic graphite (HOPG) step edges (Reprinted from Ref. [115] with the permission of Wiley)

Independent of the actual intention to prepare nanostructured surfaces, the preparation of ICP films (see Chap. 2) on a planar substrate in most cases leads to the formation of a granular material based on nano- and micro-particles [118–121].

Table 6.4 Representative examples of polymeric nanostructures

Nanostructure	Electrode preparation	Analyte	Refs.
PPy nanowires	Electropolymerization in Al_2O_3 template; deposition on Pt electrode trough electrophoresis	NO_3^-	[122]
Dodecylbenzene sulphonate-doped PANI NPs	Ink-jet printing of chemically polymerized NPs through a soft template; substrate: carbon-based electrode	NH_3	[123]
(a) Polystyrene/PANI core/shell particles	Electrodeposition of PANI on PS particles deposited on a glassy carbon electrode	Reduced nicotinamide adenine dinucleotide	[124]
(b) PANI hollow spheres			
(c) Nanoporous PANI			
(a) Polystyrene/PANI core/shell particles	Electrodeposition of PANI on PS particles deposited on glassy carbon	NO_2^-	[125]
(b) Nanoporous PANI			
PANI dendrites	Electrogeneration in the presence of p-toluene sulphonate ions	Glucose	[126]

PANI polyaniline, *PPy* polypyrrole

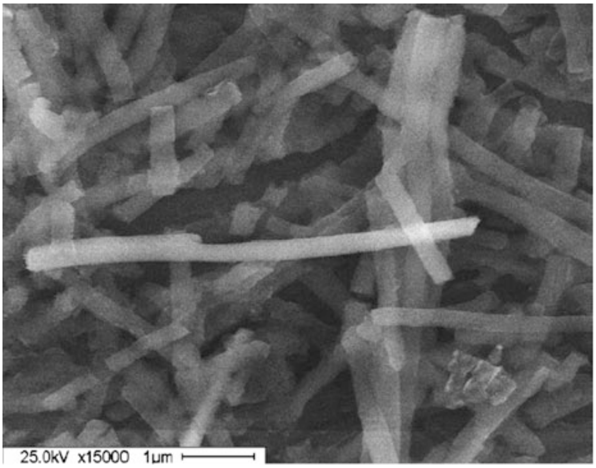

Fig. 6.10 Polypyrrole nanowires synthesized through alumina membrane template (Reproduced from Ref. [122] with the permission of Elsevier)

6.6 Synthesis of Metal Nano-Objects through Chemical Reduction of Metal Salts

Chemical reduction of metal salts represents the most popular method for the synthesis of metallic nanostructures, in particular consisting of Au, Pt, and Ag [122–126]. The synthesis of nanostructures can be described, to a first approximation, by three subsequent steps: (1) reduction of the suitable metal salt, (2) nucleation, and (3) growth of the nuclei. The second step leads to the formation of stable nuclei ('seeds') that subsequently grow to the final NP size. The second and third steps can follow two different pathways: (1) autocatalytic pathway, where the reduction of the metal ions occurs at the surface of the NPs, or (2) collision pathway, where the formation of the nuclei occurs by collision between metal atoms from the previous reduction step. However, the exact reaction mechanism is poorly defined even in the most extensively investigated systems, owing to the high number of different chemical species involved and to the high reaction rate.

It is very difficult to classify the reducing agents commonly used in the first step because a large number of different species have been successfully employed in recent years. The most important are as follows [59, 127]:

1. borohydride salts such as $NaBH_4$;
2. carboxylic acids such as citrate ions;
3. alcohols such as glycols.

6.6.1 *Brust's Method*

Among the different synthetic procedures, Brust's method [128–133] has been and still is one of the most widely employed for the synthesis of Au and Pt NPs. The reaction takes place in a two-phase system consisting of water and toluene at room temperature (Fig. 6.11). Metal salts, typically $NaAuCl_4$ or K_2PtCl_6, are dissolved in water, and a phase-transferring agent, usually a tetraalkylammonium salt, is employed for the solubilisation of the metal salt in the toluene phase [133, 134]. Afterwards, the encapsulating agent (thiol molecules) is added to the organic solution. In the case of Au, the change in color of the solution from yellow to white after addition of thiol molecules is diagnostic of the occurrence of their oxidation, with the contemporary partial reduction of Au(III) to Au(I). Under vigorous stirring, the reducing agent, i.e., an aqueous solution of $NaBH_4$, is added, causing the toluene to become dark red in color. The reactions taking place are schematized as follows:

Transfer of metal precursor dissolved in water into organic phase

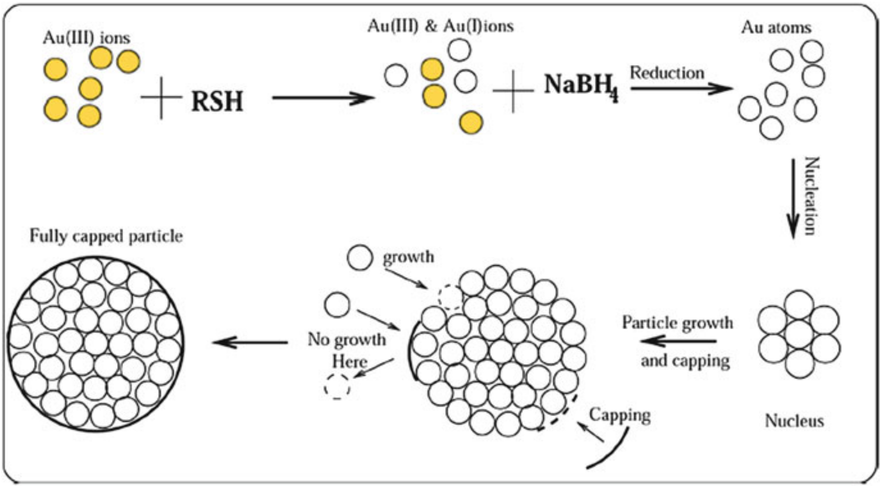

Fig. 6.11 Simplified scheme of Brust's method (Reproduced from Ref. [132] with the permission of American Chemical Society)

$$AuCl^-_{4(aq)} + R_4N^+Br^-_{(org)} \rightarrow R_4N^+AuCl^-_{4(org)} + Br^-$$

Reactions in organic phase

$$R_4N^+AuCl^-_{4(org)} + nRSH \rightarrow -Au(SR)_{n-2} - + RSSR + R_4N^+ + 4Cl^- + nH^+$$

$$-Au(SR)_{n-2} - + BH^-_4 + RSH + RSSH \rightarrow AuNP \text{ encapsulated by RS moieties}$$

$$R_4N^+AuCl^-_4 + BH^-_4 + RSH + RSSH \rightarrow AuNP \text{ encapsulated by RS moieties}$$

where R, aq, org refer to an alkyl chain and the aqueous and organic phases, respectively.

The final product of the synthesis is a core/shell system: the resulting metallic nano-objects are encapsulated by an organic monolayer consisting of thiol molecules. The organic molecules control the growth of the nano-objects and prevent aggregation. The thiol monolayers, often indicated as three-dimensional (3D) self-assembled monolayers (SAMs; see Chap. 5), are similar in structure and properties to the SAMs on planar (111) metal surfaces (two-dimensional [2D] SAMs). The resulting nano-objects can easily be purified and are stable both in dried form and in solution. The size distribution is usually narrow and it is possible to graft the nano-objects onto a planar substrate, obtaining a mono- or multi-layer film, as reported in Sect. 6.9. The medium size of the nano-objects is strongly affected by the thiol-to-metal molar ratio and by the temperature of the synthesis environment: the dimensions decrease and the distribution width increases by using a high thiol-to-metal molar ratio and a low temperature.

Brust's method is extremely flexible: the composition of the metal core can be tuned by changing the nature and the concentration of the metal salts; in particular, the formation of different multimetallic NPs has been reported, e.g., Au-Ag, Au-Cu, Au-Pd, Au-Ag-Cu [135]. The most widely used class of species used to stabilize the metal core dispersion, in the case of Brust's method, are alkylthiols, although many other sulphur-based molecules have been reported, such as disulphides and thioethers [69, 130]. In particular, the solubility can be changed by adsorption of ω-substituted alkyl thiols. A polar group, such as −OH or −COOH, in ω position with respect to the sulphur-based group, leads to the formation of a polar surface layer in correspondence to the outer part of the organic shell surrounding the metal core: the nano-objects become soluble in polar solvents. Finally, it is worth noting that a synthetic procedure similar to the Brust's one has been adopted to synthesize nano-objects encapsulated by a different class of molecules, such as amines [136].

6.6.2 Turkevich's Method

Another important synthesis method for metal nano-objects is Turkevich's method [106, 137, 138]; the reactions take place in hot water. Similar to Brust's method, metal salts are employed, e.g., $NaAuCl_4$ or K_2PtCl_6. Citrate ions act as reducing and encapsulating agents at the same time. However, the reaction mechanism is not known in detail. In particular, many authors suggest that citrate ions are adsorbed on the metal surface, leading to an electrostatic stabilization of the nano-objects. Other ions dissolved in solution are candidates for stabilization by adsorption on the metal surface. The overall reaction suggested [106] is as follows:

$$\begin{aligned} 2AuCl_3 + 3({}^{-}OOCCH_2)_2C(OH)COO^{-} \rightarrow {} & 2Au + 3({}^{-}OOCCH_2)_2CO + 6Cl^{-} \\ & + 3H^{+} + 3CO_2 \end{aligned}$$

Furthermore, when following this procedure, the medium size of the NPs and the relevant size distribution can be tuned by varying the metal-to-citrate ratio and the temperature of the reaction medium. The nano-objects are stable for weeks or even months in the same solution as used for the synthesis. On the other hand, the increase of the ionic strength of the solution or the removal of the solvent leads to partial coalescence of the metal nuclei; hence, nano-objects cannot easily be purified. These phenomena can be explained by considering that the encapsulating shell is not so strongly adsorbed on the metal surface as thiol molecules are. Conversely, the adsorption energy seems to be relatively small. This seemingly negative attribute can be exploited to easily substitute the original encapsulating shell with more strongly adsorbed molecules, as in the case of the formation of hybrid materials (see Sect. 6.7 and 6.8).

6.7 Reactivity of the Encapsulating Agent

The chemical nature of the capping agent [139–146] exerts a decisive influence on the properties of a nanosized material, because the encapsulating shell constitutes the interface between the inorganic core and the surrounding environment. The organic cloud plays a role in conditioning the behavior of the NPs as a whole, depending on the following characteristics:

1. the strength of the interaction between the organic cloud and the inorganic core;
2. the permeability of the organic shell to species in solution;
3. the reactivity of the outermost surface of the organic layer.

In particular, the strength of the bond between encapsulating agent and core can vary significantly. Strong chemical bonds are generally preferred when specific functionalities brought by the stabilizing molecules are planned to constitute the site of interaction with the analyte. This is the case of DNA strands stably anchored to Au NPs by means of thiol groups: in genosensing systems, effective coupling with the complementary DNA is specifically sought. On the other hand, if organic layers are well packed, the inorganic core is insulated from the species in solution. This is why labile encapsulating agents or, in general, flexible residues that do not form a compact cloud around the metal core, are preferred whenever the interaction between the analyte and the inorganic core is essential for the sensor to properly exploit the characteristics that are most often required, e.g., to activate electrocatalytic processes. Furthermore, when the synthesis of the nano-objects is part of a more complex process, the use of labile capping molecules may be crucial to allow the exchange with other agents that possess specific reactivity. For example, in the formation of composite materials, the interaction of the core with the second component (e.g., with polymeric chains) is essential to achieve the formation of a stable bicomponent material [147, 148]. Moreover, to obtain Au NPs surrounded by nucleic acid strands, substitution by DNA [149] or peptide nucleic acid (PNA) [150] molecules may follow the synthesis of Au cores capped by labile citrate ions.

As mentioned above, the outermost surface of the organic layer most strongly conditions the interaction of the nano-objects with the external environment. Organic moieties can induce selective interactions with the analyte in solution; for instance, carboxylic groups on the surface of CNTs form complexes with heavy metals [151]. Another possibility, as illustrated in Fig. 6.12, is that specific functional groups activate chemical reactions by anchoring a second layer bearing the actual receptors for the target analyte. Finally, the outermost surface of the organic shell can induce stable anchoring of the nano-object to an electrode surface. This last aspect is discussed in detail in Sect. 6.9.1.

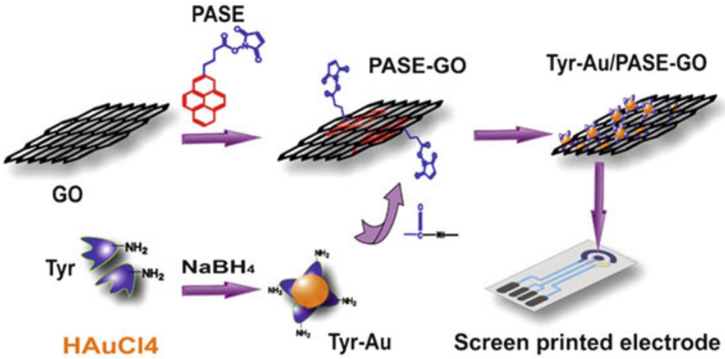

Fig. 6.12 Assembly process of tyrosinase (Tyr) modified Au nanoparticles (NPs) on chemically functionalized graphene oxide (GO) sheets by 1-pyrenebutanoic acid, succinimidyl ester (PASE) anchoring, and subsequent deposit on screen-printed electrodes. The formation of Tyr-Au/PASE-GO occurs via the formation of an amide bond between the amine residue on Tyr-Au and the PASE carboxylate functionality (Reprinted with permission of the authors. Reproduced from Ref. [152] with the permission of Elsevier)

6.8 Multicomponent Materials

To take advantage of the characteristics of nanosized materials, many multicomponent materials, conventionally called ‘hybrids’, have been proposed to date. Although the scientific community has not yet agreed upon a univocal definition of ‘hybrid’, Gomez-Romero and Sanchez have proposed a general classification for bicomponent systems, i.e., the most frequently analysed materials of this class [153]. Figure 6.13 illustrates a similar classification scheme, suitably adapted to the nanosized materials most commonly reported in electroanalytical applications. Composites constitute a family of hybrid materials that play major roles in electroanalysis. The International Union of Pure and Applied Chemistry (IUPAC) defines them as “multicomponent materials comprising multiple, different (non-gaseous) phase domains in which at least one type of phase domain is a continuous phase” [92]. Alternative classifications have been suggested by other authors [154], but the chosen approaches are less comprehensive, i.e., are suitable to classify only a fraction of the hybrid materials reported so far in the frame of amperometric sensors.

Hybrids are very appealing from a scientific and technological point of view, particularly with respect to catalysis and electrocatalysis, because the properties of these systems often do not derive from the mere sum of those of the single components: in most cases, synergic action notably improves the individual performance [155]. Mechanisms at the basis of synergic effects are often poorly investigated, also due to the complexity of the reaction pathways. However, spillover effects and co-catalysis have been identified as the main causes for the performance enhancement of multicomponent materials compared with the individual one-component materials [155].

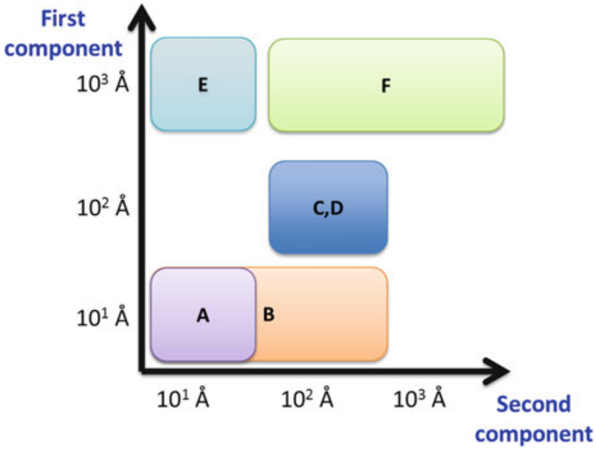

Fig. 6.13 Tentative classification of bi-component hybrids most commonly reported in electroanalytical applications. *A* alloy and core shell metal structures, *B* nanoparticles (NPs) and carbon nanotubes (CNTs) encapsulated by a thin polymeric layer, *C* NPs grafted on the surface of CNTs and graphene, *D* mixture of NPs, *E* fullerenes included in polymeric matrices, *F* NPs and CNTs in polymeric matrices (Reproduced from Ref. [169] with the permission of Springer)

Many materials reported in Fig. 6.14 have been widely applied in electroanalysis. Typical examples include metal NPs deposited on the surface of CNTs, and ICPs including NPs, graphene, or CNTs [52, 93–101]. Several quite interesting multicomponent materials (Fig. 6.14a–c) [34, 102, 103, 156–158] have not been studied deeply as monocomponent materials in terms of their potential in electroanalysis. Three main classes of bi-component materials at a nanoscale level may be identified:

1. substitutional and interstitial alloy structures;
2. core-shell structures;
3. one component partially segregated on the surface of a nano-object made of a second component.

Similar structures usually consist of metals or semiconductors, as in the case of QDs. Although the synthesis of these multicomponent nanosized materials is based on techniques similar to those employed for monocomponent materials, important experimental changes should be adopted [34]. For example, in the case of bimetallic nano-objects, if the synthesis starts from metal salts, the order in which the components are reduced constitutes one of the most important synthetic variables. The simplest method consists of the contemporary reduction of two different metal salts. Alternatively, by performing the reduction of the two metals in two subsequent steps, the second reduction generates a coating on the surface of the first metal. Some authors [156, 159] suggest that intimate contact between nano-objects of different composition, as in the bicomponent nanosized material system reported in Fig. 6.14d, may induce properties very similar to those of true alloys of the

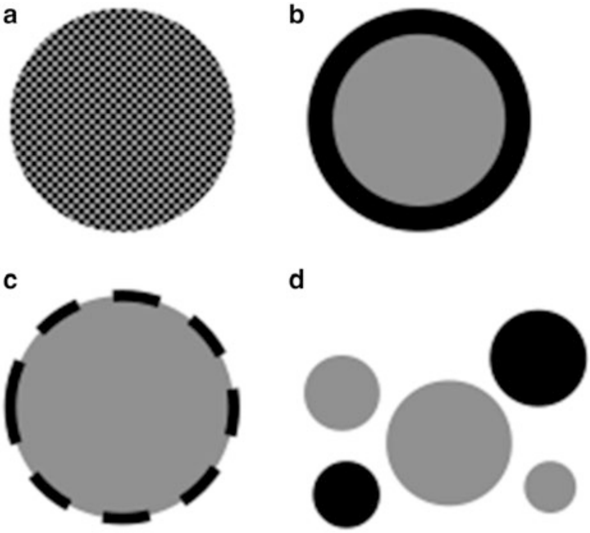

Fig. 6.14 Main bicomponent nanosized materials: (**a**) substitutional and interstitial alloy and (**b**) core-shell structures; (**c**) partial surface segregation; (**d**) a mixture of nano-objects with different compositions. Nanoparticles are represented

same two metals, which is plausible due to the 'nano' dimension of the objects in bicomponent systems.

Further examples of bicomponent materials based on carbon consist of doped fullerenes, CNTs, and graphene. The nature of these carbon-based nanostructures is significantly modified by the substitution of some carbon atoms with foreign metallic and non-metallic atoms (Fig. 6.15). In addition, fullerenes and CNTs can host similar species inside their own structures. However, the effectiveness of these systems in electroanalysis is still poorly investigated [160, 161], despite their popularity in other scientific fields.

On the other hand, different forms of doped diamond-like materials, particularly boron-doped diamond (BDD), have been widely employed as electrode materials in the form of (nanostructured) thin films [162]. The use of these materials in electroanalysis exploits the wide potential window (Fig. 6.16), chemical inertness, mechanical robustness, and small background current typical of this class of materials.

6.9 Grafting Nanosized Materials onto a Substrate

Grafting nanosized materials onto a substrate is a very important topic for practical applications [164], including electroanalysis. The choice of the suitable procedure is crucial in order to obtain the desired characteristics of the surface. In particular, the morphology of the resulting nanostructure, the accessibility to the

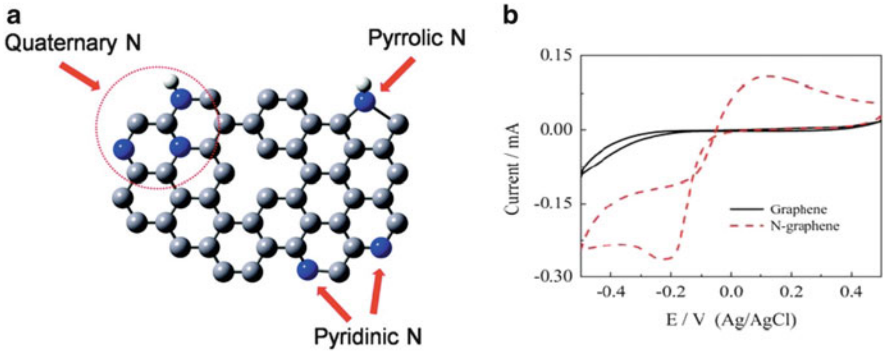

Fig. 6.15 An example of doped carbon-based nanostructures: graphene sheet doped with nitrogen atoms. (**a**) Possible chemical state of N atoms and (**b**) electrochemical response of doped and undoped graphene-modified electrodes in the presence of 5 mM H_2O_2 (Reproduced from Refs. [160] and [161] with the permission of American Chemical Society and Royal Society of Chemistry, respectively)

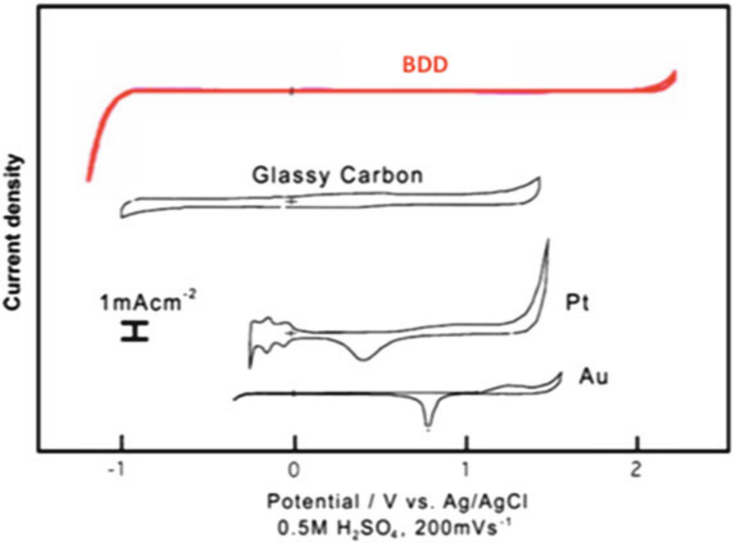

Fig. 6.16 Voltammetric responses of boron-doped diamond (BDD) and Pt electrodes in 0.5 M H_2SO_4 (Reproduced from Ref. [163] with the permission of Springer)

inorganic cores by the species in solution and the spatial arrangement of the nano-objects on the underlying surface represent the most important aspects to consider.

The procedures most often reported in the literature consist of grafting pre-synthesized nano-objects through a monolayer or a thicker film, e.g., including nano-objects inside an ICP film. These techniques allow precise control of the nature of the nano-objects in terms of size, shape, encapsulating agent, etc.

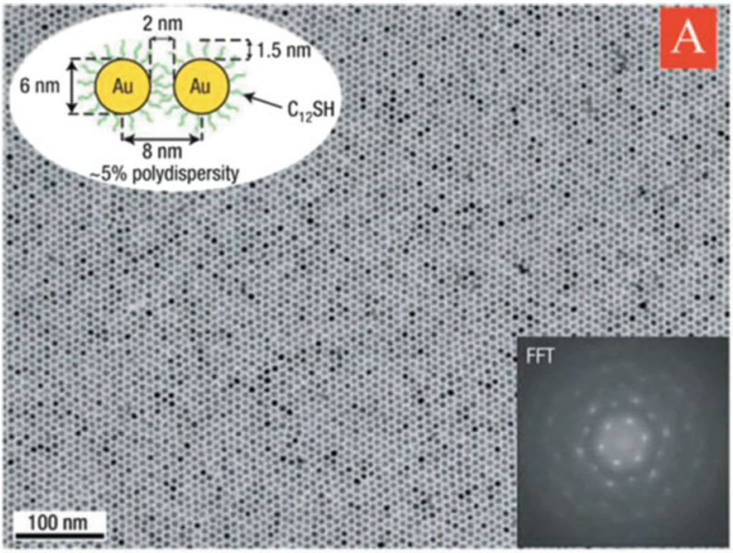

Fig. 6.17 Transmission electron microscopy image of a monolayer of Au nanoparticles capped by dodecanethiol molecules deposited on a planar substrate by drop casting. The *upper-left inset* is a sketch of the arrangement of two adjacent NPs, i.e., the interdigitation of the capping layers. The Fourier transform of the image shown in the *lower-right inset* evidences the high degree of order of the NPs (Reproduced from Ref. [165] with the permission of Royal Society of Chemistry)

Alternatively, synthesis and deposition can be carried out simultaneously: metal and metal oxide nanostructures can be obtained by cathodic reduction of the relevant precursors, with concomitant deposition onto the substrate. This approach is, in principle, more simple than the previous one, also thanks to the absence of an encapsulating agent; however, control of the size distribution and shape of the nano-objects could be more difficult.

It is worth noting that nano-objects, especially when monodisperse, possess the spontaneous tendency to form well packed structures on a surface [165, 166]. The most notable example is the packing of NPs on a flat substrate: 1D, 2D, and 3D arrangements of NPs have been reported. An example of 2D arrangement is shown in Fig. 6.17. Packing of the nano-objects can be obtained by simple solvent evaporation of a solution on planar substrates; however, in most cases, this process leads only to a short-range order. The interaction between the encapsulating layers of adjacent NPs plays a major role; as an example, well-packed monolayers of alkylthiol molecules surrounding noble metal NPs exhibit interdigitation of alkyl chain, leading to the formation of ordered structures. The formation of ordered 3D structures leads to the creation of the so-called colloidal crystals, based on a nano-object disposition similar to the opals previously described (see Sect. 6.4).

6.9.1 Grafting of Pre-formed Nano-Objects through Monolayers

Pre-synthesized nano-objects, of either inorganic or organic nature, can be deposited onto conductive substrates under the form of a more or less ordered monomolecular organic layer (Chap.5), with thickness up to a few nanometers [139, 140, 146]. Schemes of the main methods reported in the literature are shown in Fig. 6.18 [69, 167].

Organic species bearing two suitable functional groups may be used for bonding monolayers of nano-objects to electrode surfaces. The group at the outermost surface of the monolayer should form strong chemical bonds with either the inorganic core (Fig. 6.18a) or the capping organic layer (Fig. 6.18b). Typical examples are thiol or amine tail groups employed to graft on noble metal cores of NPs, or carboxylic acids anchored to amine-functionalized CNTs or NPs. The portion of molecules between the two functional groups conditions the final structure and stability of the monolayer. Changing the chemical composition of the organic molecule forming the monolayer [1–8, 168] deeply modifies the chemical and physical properties of such a thin film.

In terms of electroanalytical applications, organosulphur compounds are the most common class of molecules used to form monolayers [1–8]. The most extensively studied systems are based on thiols adsorbed on Au. Monolayer depositions are commonly performed in liquid phase, simply by dipping the substrate into a diluted solution of organic molecules in the millimolar concentration range.

Deposition of nano-objects by exploiting the organic shell surrounding the inorganic core represents a special case in which a functional group suitable for bonding the nano-object directly to the bare substrate is present in the outermost portion of the encapsulating cloud (Fig. 6.18c).

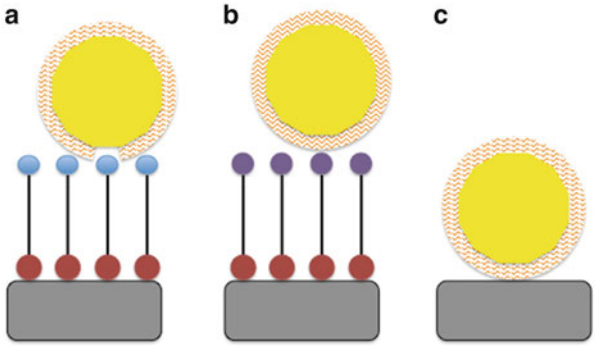

Fig. 6.18 Three methods for deposition of nano-objects onto electrode surfaces through monolayers. The case of an ordered monolayer is shown: anchoring of a nanostructure through interaction between the tail group of the monolayer and (**a**) the nanostructure core or (**b**) the nanostructure encapsulating cloud; (**c**) grafting through direct interaction between the bare substrate and the nanostructure encapsulating cloud

6.9.2 Grafting Pre-formed Nano-Objects in the Form of Hybrid Material

Hybrid materials can be synthesized in solution and subsequently deposited onto electrode surfaces. In many cases, the deposition is performed simply by drop casting of a solution of a hybrid material. Unlike grafting through monolayers, thicker films are generally obtained in this case.

No precise classification of different preparation methods is available, so that only a selection of the most important deposition methods exploited in electroanalysis is reported in Table 6.5 [169, 170].

In most cases, nano-objects are stably anchored to electrode surfaces by inclusion in an organic matrix. The matrix can simply act as a support for the nano-objects or can also improve the performance of the sensing system, activating synergy. If the organic matrix is an insulator, the ratio of the quantities of the two materials must be suitably chosen to allow charge percolation through. On the other hand, if the matrix is conductive, the fraction of nano-objects can vary within a wider range. In the frame of hybrid materials for electroanalysis, ICPs constitute the most important class of organics, possessing significant electrical conductivity [169], although a few notable examples of redox polymers have been reported [103, 183–188].

6.9.3 Simultaneous Synthesis and Deposition of Nano-Objects

There are a number of procedures for simultaneous formation of metal and metal oxide nano-objects and relevant deposition onto electrode surfaces [189–192]. In the case of metal nanostructures, the processes involve chemical or cathodic reduction of the corresponding metal salts (Fig. 6.19) and may generate an electrode surface modified by pristine nano-objects (Fig. 6.19a) or by hybrid coatings (Fig. 6.19b, c). The synthesis of nano-objects at the same time as deposition onto the electrode surface provides quick and easy formation of nanostructured surfaces. However, although many authors claim that the size of the resulting nanostructures can be controlled by suitable choice of the deposition parameters, the resulting size distribution is generally quite broad.

The term ‘electroless deposition’ is often adopted to describe the process of chemical deposition of nanostructures of a foreign metal onto a substrate in the absence of an applied electrical potential [193]. Two distinct deposition methods should be distinguished. In the first, often called autocatalytic deposition, a suitable reducing agent is added to the solution to activate a reduction reaction of the metal ions on the substrate surface. In the so-called galvanic displacement process, the reducing agent is absent: the deposition of a foreign metal onto the surface of a metal substrate occurs by redox reaction between a dissolved salt of the metal and

Table 6.5 Most important methods for grafting nano-objects onto electrode surfaces with formation of hybrid materials

Method	Example	Ref.
Dispersion of nano-objects and polymers in solution and subsequent deposition by drop casting or spin coating	Ag NPs encapsulated by hexadecyl ammine mixed with poly (3-hexylthiophene) in chloroform	[171]
	Single-wall CNTs mixed with polyaniline in N-methyl-2-pyrrolidinone	[172]
Inclusion in sol-gel	Embedding of Au NPs encapsulated by citrate ions in a (3-mercatopropyl)-3-methoxysilane sol	[173]
Electrogeneration of polymers on a substrate in the presence of nanostructures	Inclusion of Au NPs encapsulated by *N*-dodecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulphonate during the electrogeneration of poly (3,4-ethylenedioxythiophene) films	[174]
Chemical synthesis of a soluble polymer in the presence of nano-objects and subsequent deposition by drop casting or spin coating	Chemical polymerization of pyrrole in the presence of CNTs in acidic aqueous solution containing an oxidizing species, namely persulphate ions	[175]
Adsorption of nano-objects on a preformed polymeric film	Au NPs encapsulated by tetraoctylammonium bromide adsorbed on poly (3,4-ethylenedioxythiophene) film surface	[176]
Layer-by-layer deposition	Alternate deposition of anionic Au NPs encapsulated by 11-mercaptoundecanonate and a cationic polythiophene	[177, 178]
Langmuir-Blodgett and Langmuir-Schaeffer deposition	Co-deposition of poly(3-hexylthiophene) and Au NPs encapsulated by dodecanethiol	[179, 180]
Electrophoresis	Co-deposition of metal oxide NP includes and different soluble polymers	[181, 182]

the surface atoms of the substrates. The formation of metal nano-objects occurs concurrently to partial dissolution of the substrate surface.

Electrodeposition of nanostructures by cathodic reduction of a metal salt at a suitable substrate represents the most important alternative synthetic procedure. Different methods have been employed for the deposition of metals, particularly of noble metals [194]. The electrodeposition may occur from different media; although most of the studies deal with aqueous solutions, organic solvents and ionic liquids have also been employed [195]. An extensive literature dealing with electrodeposition of metals has been published due to the importance of such processes in industrial applications, e.g., in corrosion protection and in enhancement of mechanical and aesthetic properties [75, 194, 196–198].

As already cited, nanostructures can be anchored to bare substrates (Fig. 6.19a) or to pre-synthesized polymeric films on electrode surfaces (Fig. 6.19b). Alternatively, the nano-objects and the polymer matrix can be formed simultaneously

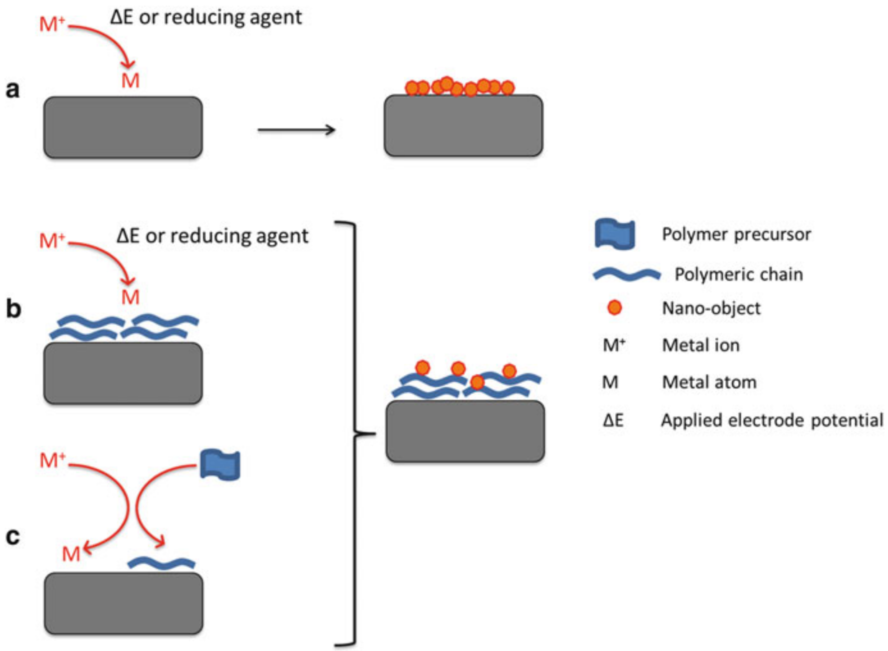

Fig. 6.19 Formation of metal nanostructures on surfaces, through different reduction processes of the relevant metal salts. The cases are shown of (**a**) pristine nano-objects and (**b**, **c**) hybrids with a polymer

(Fig. 6.19c). In this procedure, the reducing agent consists of the relevant monomer molecules. The presence of the synthesized polymeric component or of the monomeric precursor during formation of the nano-objects may lower the extent of aggregation, because the organic chains may act as seeds for the formation of nanostructures or even as encapsulating agents.

As for metal oxides, several different procedures have been proposed for the deposition of the relevant nanostructures, e.g., CoO_x, MnO_x, CeO_x, and TiO_x NPs [199]. The most common procedure consists of inducing variation of the solution pH in close proximity to the substrate surface, by the reduction of water, dissolved oxygen, or nitrate ions. The local increment of the pH value leads oxide/hydroxide species to precipitate onto the electrode surface [197].

Table 6.6 A few selected examples of analytical applications of nanomaterials

Analytes	Nanomaterials	Refs.
Ascorbic acid and catecholamines	Metal oxide particles, CNTs	[200, 201]
	Metal NPs	[18, 202, 203]
	Enzymatic biosensors on nanostructures	[204]
Carbohydrates	CNTs, nanoporous metal structures	[205]
	Different biosensors	[206]
	Nanoporous materials, metal NPs, CNTs	[203, 207]
Amino acids	Enzymatic biosensors on nanostructures	[204]
Strong oxidizing species	Metal and metal oxide nanoparticles, CNTs	[203, 208]
Phenols	Enzymatic biosensors based on metal and metal oxide nanoparticles	[209]
Alkaloids	Differently modified CNTs	[210]
Explosives	CNTs	[211]
Heavy metals	Metal NPs	[212, 213]
	Enzymatic biosensors based on nanostructures	[214]
Inorganic anions	Nitrite: different materials	[215]
Pesticides	Metal oxide NPs, CNTs	[14, 216]
Nucleic acids	Composite materials based on ICPs, CNTs and metal NPs	[203, 217, 218]

6.10 Electroanalytical Applications

As mentioned above, many amperometric sensors rely on one or more of the nanosized materials described above [1–32]. In the following sections, we highlight how the performance of amperometric sensors, in terms of sensitivity, detection limits, and selectivity, can be significantly improved by the use of materials under the form of nano-objects. Table 6.6 lists the most significant classes of analytes determined through amperometric sensors, together with a few representative references.

6.10.1 Nanosized Materials in Electrocatalytic Reactions

Nanostructured surfaces induce specific interactions with the analyte that, in the case of non-reversible charge transfers occurring via inner sphere mechanism, may induce lowering of the required overvoltage for the process to occur and, consequently, even lead to higher and sharper current peaks at less extreme potential values (Fig. 6.20).

Electrocatalysis is often invoked to account for specific behavior of nanostructured surfaces compared with bare surfaces. It is evident that electrocatalysis refers, in this case, to lowering of the activation energy of the charge transfer step, rather

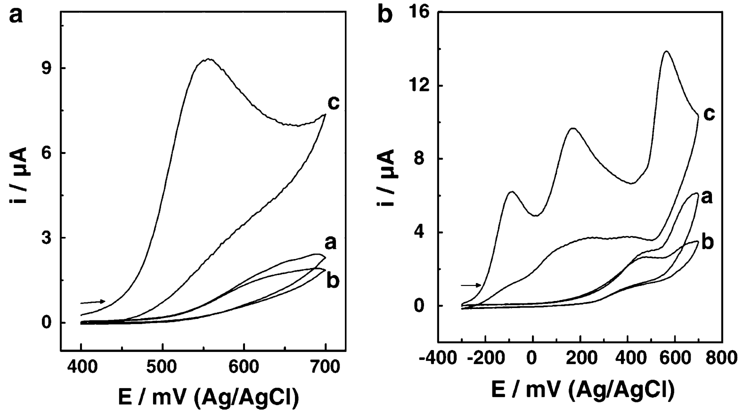

Fig. 6.20 Cyclic voltammograms recorded on 0.25 mM NO_2^-, 0.1 M phosphate buffer solution (pH 7.2) (**a**) in the absence and (**b**) in the presence of 0.25 mM N_2H_4 and SO_3^-. The responses collected at (*a*) bare, (*b*) sol-gel, and (*c*) sol-gel/Au nanoparticles modified glassy carbon electrodes are reported; 0.02 Vs^{-1} potential scan rate (Adapted with permission of the authors. Reproduced from Ref. [219] with the permission of Elsevier)

than to the redox action of a mediator (see Chap. 3); the two cases may be similar from a thermodynamic point of view, but different from a mechanistic viewpoint. However, it is difficult, to say the least, to identify the mechanisms involved in analyte–surface interaction on the basis of the results of electrochemical measurements. The need to couple electrochemical investigations with microscopic and spectroscopic studies is the main constraint to achieve a reliable definition of such complex electrode mechanisms that are operative when the electroactive surface of the electrode system is involved, not simply as electron exchanger, but also in terms of the chemical and physical specific characteristics. Some clues can be extrapolated from electrocatalytic studies carried out in relation to fuel cells. In particular, it has been demonstrated that the electro-oxidation of different species, such as alcohols and carbohydrates, on the surface of noble metals exploits the formation of hydrated oxides on the surface [220].

Understanding the mechanisms involved in surface–analyte interaction is even more challenging when the performance of the sensor takes advantage of co-catalytic effects due to a hybrid material. In these cases, the electrode coatings may provide electrocatalytic performance superior to that of the two single components (Fig. 6.21).

Other factors, in addition to electrocatalysis, need to be accounted for to explain the improvement of sensor sensitivity when using nanosized materials, e.g., the increase of the electroactive surface area and reduced fouling. Although not always adequately considered, electrode fouling, in fact, broadens and lowers the voltammetric peaks because of progressive modification of the electroactive sites during the voltammetric scan. In this respect, the antifouling effect of the

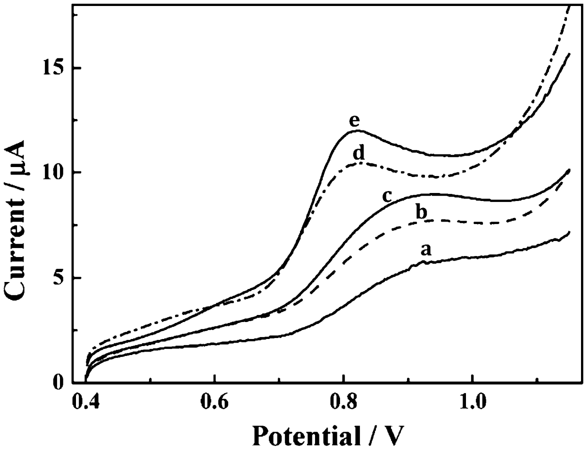

Fig. 6.21 Linear sweep voltammograms recorded at modified glassy carbon electrodes in 5.0 μM NO_2^-, 0.1 M phosphate buffer solution; 0.1 Vs^{-1} potential scan rate. Different electrode coatings have been considered: (*a*) carbon nanotubes (CNTs), (*b*) CNTs-Chit, (*c*) $OMIMPF_6$-CNT-Chit (*d*) ($OMIMPF_6$-CNT) gel, (*e*) ($OMIMPF_6$-CNT) gel-Chit (Chit: chitosan; $OMIMPF_6$: 1-octyl-3-methylimidazolium hexafluorophosphate) (Adapted with permission of the authors. Reproduced from Ref. [221] with the permission of Wiley)

nanostructured surface results not only in more reliable signals, but also in more repeatable and sharper peaks.

6.10.2 Nanosized Materials in Stripping Voltammetric Techniques

Nanostructured surfaces cause an increase of the electroactive area, which leads to a higher level of pre-concentrated analyte on the electrode surface that can be exploited in the case of stripping voltammetry (Fig. 6.22). Of course, this leads to an increase in sensitivity and, most often, to lowering of the detection limit.

Moreover, sharper peaks and better resolution also characterize the voltammetric signal in the stripping potential scan, so that the signals of different metals in solution tend to be more resolved from each other and present increased height. In this respect, the differences between bare and nanostructured surfaces are thought to be due to a different interaction of the resulting zero-valence metals with the electrode surface: the presence of atoms with low coordination numbers in the nanostructured surface may induce weaker chemical interactions, kinetically and even thermodynamically favoring the anodic stripping step. A significant shift of the re-oxidation potential has consequently been observed [222, 223].

Among the proposed systems for the determination of metal ions [11], nanostructured surfaces based on Bi and Sb represent valid alternatives to Hg. On the

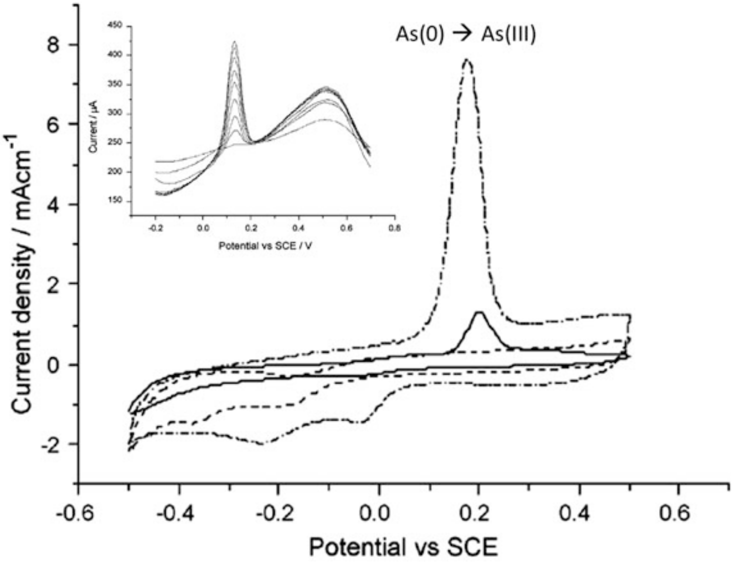

Fig. 6.22 Cyclic voltammetric responses of 0.5 mM As(III) in 0.1 M HCl at bare Au electrode (*solid line*), at carbon nanotubes (CNTs) (*dashed line*), and at Au nanoparticles/CNTs (*dash dotted line*) modified glassy carbon electrodes; 0.1 Vs^{-1} potential scan rate. Inset reports square wave anodic stripping voltammetric responses in 10–70 nM As(III) solutions (pre-deposition: −0.4 V vs. saturated calomel electrode for 120 s; stripping: $f = 50$ Hz, $E_{sw} = 20$ mV, $\Delta E_s = 2$ mV) (Adapted with permission of the authors. Reprinted from Ref. [222] with the permission of Elsevier)

other hand, NPs of noble metals are also widely used, especially for determining As and Hg, thanks to their affinity to zero-valence metals.

Many amperometric sensors developed to quantify heavy metals present carbon nanostructured surfaces, mainly consisting of CNTs and graphene. The interaction of these nanosized materials with analytes is due to carboxyl groups present in correspondence with defects of the nanostructure. However, the possible involvement of metal impurities normally present in CNTs, as well as the capability of sp^2 hybridized carbon sites to adsorb analyte molecules from the solution, should also be assessed.

On the other hand, the possible contemporary adsorption of many organic and inorganic species present in the solution may affect the selectivity of the resulting sensors and limit the effective application of carbon-based electrode materials in real matrices. Some expedients have been proposed to reduce this negative effect, e.g., the deposition of CNTs on a Nafion® coating, which acts as an electrostatic filter.

Improved sensor response to several organic species can be achieved by exploiting the high adsorption capability of carbon surfaces, in adsorptive stripping voltammetry (AdSV). This procedure, based on very similar principles of solid phase microextraction (SPME), implies that analytes are pre-concentrated at carbon nanostructured surfaces prior to voltammetric detection. It has been successfully exploited, for example, to detect electroactive agents such as herbicides [224] and nitro-derivatives [225]. Electrocatalytic mechanisms similar to those previously described once more give rise to voltammetric responses characterized by very sharp peaks positioned at less extreme potentials.

In the case of stripping analyses, the sensor performance can be enhanced by functionalization of the nanosized material with organic moieties, inducing selective interaction (i.e., formation of ‘adducts’ of different nature) due to adsorption as well as formation of complexes with the target analyte. For example, this strategy has been widely exploited for detecting heavy metals [11] by anchoring carboxylic, amino, or thiol groups to the outermost surface of the inorganic core.

As is clear from the mechanisms described, the choice of the proper morphology for the nanostructured surface plays a key role in defining the properties of the sensor. Pre-synthesized nano-objects have been anchored to the underlying electrode surface by means of organic [148] or inorganic [226] polymeric films; the increase of the sensor sensitivity has been ascribed to an increase in the electroactive area. In addition, compared with monolayers, the porous polymer binder anchors a higher number of nano-objects at the electrode|solution interface.

6.10.3 Nanosized Materials in Bio-Catalytic Sensors

A specific class of amperometric sensors acquires particularly high selectivity thanks to the use of biological species as the recognition elements for well-defined analytes [204, 206, 209, 214, 215]. Several chemical species are detected with bio-catalytic sensors, i.e., sensors that exploit the mediation of a specific enzyme. Some examples include the detection of glucose by means of glucose oxidase [206] and of phenol derivatives by means of tyrosinase [209]. Enzymes consisting of redox active proteins can also be used, as in the case of hemoglobin in the catalytic reduction of NO_2^- [215]. In addition to high selectivity and sensitivity, another benefit of using biological elements lies in the possible extension of the lower detection limit.

The role of nanosized materials in electrode coatings promises improvements of the performance of biosensors compared with conventional ones. In addition to a significant increase of the number of biological receptors at the solution|electrode interface, the nano-dimension itself and the electrocatalytic properties of nano-objects can positively affect the performance of these biosensors.

As to the nano-dimension of the material, the enzyme adapts its 3D configuration to the morphology of the underlying nanostructure without inducing protein denaturation, resulting in intimate contact between the nano-objects fixed to the electrode surface and the redox active sites of the enzyme, which are often located deep inside the protein. In other words, nano-objects act as electrical bridges between the conductive, polarized substrate and the bio-receptor, providing effective charge transfer typical of ‘third-generation’ biosensors.

In addition, the advantage taken from the electrocatalytic properties of nanosized materials is particularly meaningful for enzymes requiring the presence of NAD^+ as the cofactor, or leading to H_2O_2 production, which is the final analyte to detect at the electrode. As stated above, the use of nanostructured surfaces leads to detection of the analyte at less extreme potential values, in correspondence with voltammetric peaks characterized by higher repeatability and resolution.

Among different nanosized materials, Au NPs and CNTs are the most commonly used to obtain stable anchoring of proteins to electrode surfaces. The most frequently adopted procedure consists of forming amide bonds between amino groups of the peptide chains and carboxylic groups on the outermost surface of the nanosized material. Alternatively, deposition procedures can rely on electrostatic attraction between positively or negatively charged encapsulating shells and enzymes with opposite charge, as a function of the relevant isoelectric point and pH value of the solution. This last deposition process can also be reiterated in a so-called layer-by-layer deposition (see Chap. 4 for a more detailed description) to increase the number of biological elements on the electrode surface. Due to the hydrophilic nature of many deposits, even proteins in the internal part of the coating may be in close contact with the solution, so interactions between the biological element and the analyte are favored.

6.10.4 Nanosized Materials in Affinity Biosensors

Affinity biosensors, namely genosensors, immunosensors, and aptasensors, take advantage of the characteristics of nanostructured surfaces. The literature dealing with similar biosensors is particularly abundant, and a number of sensing systems based on different nanostructures have been proposed for a variety of analytes. In this respect, the list of detectable species ranges from ‘simple’ ions (e.g., heavy metal ions [227, 228]) to more complex pathogenic microorganisms [229]. It seems reasonable to draw out here general considerations that, in one way or another, are common to most of the proposed nanostructures.

Many papers simply attribute improved sensitivity to the higher density of biological elements anchored on such a surface. On the other hand, only a few authors take into consideration the correlation between sensor performance and morphology of the nanostructure [230–232]. They conclude that the marked curvature of NPs affects the spatial disposition of bio-receptor molecules on the surface, resulting in a poorly packed structure that facilitates the access of the complementary bio-molecule to form the receptor–analyte adduct. This result indicates that the careful control of the substrate morphology constitutes the basis for obtaining particularly sensitive sensors.

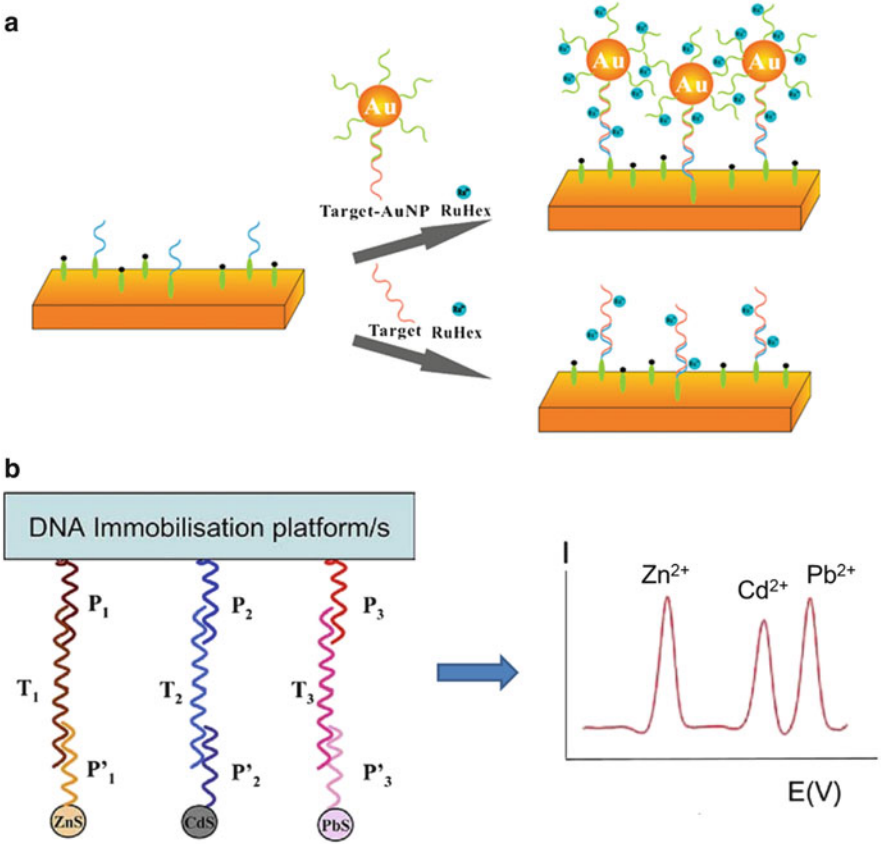

Fig. 6.23 Schematic representation of two analytical methods involving nanosized materials in genosensors. (**a**) Amplified detection of DNA by means of Au nanoparticles (NPs) functionalized with the complementary oligonucleotide sequence. The anchoring of DNA on the electrode surface is detected by exploiting a positively charged redox probe, namely $[Ru(NH_3)_6]^{3+}$, interacting with negatively charged phosphoric groups of DNA. A higher amount of electroactive species is present in close proximity to the electrode surface when using NP functionalized strands (Reproduced from Ref. [233] with the permission of American Chemical Society). (**b**) Multiple detection of three DNA sequences (T_1, T_2, T_3, respectively) by means of hybridization with DNA strands (P_1, P_2, P_3, respectively) anchored to a surface and labeling with different quantum dots (QDs), namely ZnS, CdS, and PbS, bound to P'_1, P'_2, P'_3 DNA strands, respectively; after hybridization, QDs are dissolved and the relevant metal ions are quantified by anodic stripping voltammetry (Reproduced from Ref. [31] with the permission of Elsevier)

Nanosized materials, particularly Au NPs and QDs, may improve the performance of affinity sensors also acting as labels for the formation of the receptor-analyte adduct. The amperometric signal of many affinity sensors, in fact, does not come from an electrochemical process directly involving the analyte. Redox active species often need to be added to solution, and many different strategies can be adopted to collect the amperometric signal indirectly: nano-objects can be used as a label certifying the occurrence of the hybridization reaction. Figure 6.23 reports two examples among the many different approaches proposed in the literature that involve nanosized materials; they aim at illustrating the main advantages of using nanosized tags in the specific case of genosensors. Similar strategies can also be exploited for other affinity sensors.

6.11 Final Remarks

As specified at the beginning of the chapter, the number of papers focusing on the use of nanostructures in amperometric sensing is increasing rapidly. In many cases, the nanosized materials involved and the techniques proposed for the deposition onto electrode surfaces are actually variants of well-known systems and procedures. On the other hand, some of the most innovative experimental approaches are so complex to realize, or require so much expensive instrumentation, that they are very rarely adopted. Moreover, novel systems, such as multimetallic nano-objects and some multicomponent composite materials, still need to be adequately studied with respect to fundamental properties. In addition, some nano-objects developed in different contexts, e.g., organic electronics and catalysis, might be of great interest in the frame of amperometric sensors.

Some of the most popular nano-objects, e.g., Au NPs encapsulated by thiol molecules or citrate ions, can be synthesized in the laboratory, even in large quantities. The possibility of making use of commercial products is often one of the most important factors determining the popularity of some nano-objects. For example, it is undoubtedly true that the number of applications involving CNTs is significantly higher than those involving non-commercial carbon nanostructures. When nano-objects that have been suitably functionalized with organic moieties are required, the uncertain availability on the market constitutes significant obstacle. Custom syntheses are usually time-consuming and not trivial at all. Noteworthy, the number of different commercial precursors for the preparation and grafting of nano-objects is expanding all the time.

Analyses of responses aiming to define the characteristics of the electrode deposit and the details of the mechanism operative in anodic oxidations or cathodic reductions are also very challenging and rare in the case of amperometric sensing on modified electrodes, due to the complexity of most systems with respect to the bare ones. On the other hand, the superior performance of new computers may lead to unprecedented sophisticated simulations. In the case of composites based on nanosized materials, studies of the diffusion to micro- and nano-electrode systems may be exploited [234]. Similarly, calculations regarding the reaction mechanisms based on density functional theory have been exploited to give a rationale to the performance of a number of electrocatalysts [235].

The combination of different analytical techniques will open new approaches to sensing. In particular, the combination of optics and electrochemistry seems very promising. In this respect, only a few examples of light addressable electrodes and photoelectrochemical sensors have been reported [236, 237]. Finally, in the frame of an unusual hyphenation, it deserves to be cited that irradiation of electrochemical cells with microwaves or ultrasounds [237–239] has been proven to increase the sensitivity of the sensing system.

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Chapter 7
Silica-Based Materials and Derivatives

This chapter describes a variety of silica-based inorganic materials, mainly consisting of clays, zeolites, and pure SiO_2. The driving force for the use of these materials as an electrode coating was their abundance on the earth crust, although the outlook has been enriched by the development of a wide number of synthetic derivatives. Pure silica-based materials only consist of Si-O groups; different inorganic units are also present in clays and zeolites, particularly based on Al. Furthermore, the class of clays also includes a variety of different derivatives, e.g., Mg/Al anionic clays, conferring the electrode quite interesting and specific physico-chemical properties of interest in electroanalytical applications. Common features of all these materials are the good stability at high temperatures and in extremely oxidative conditions, which increases the possibility of reaching extremely positive electrode potentials.

Synthetic derivatives are more effective in an electroanalytical context than the corresponding natural materials, because their structure and composition are more defined and, thus, reproducible. Impurities are generally present in natural products, even after complex purification procedures [1].

An interesting feature of the use of these inorganic materials as electrode coatings lies in the presence of charged functionalities possibly giving rise to selective interactions with species in solution. In the case of zeolites, selectivity is enhanced by a well-defined three-dimensional structure acting as a size filter for species exceeding the dimension of the pores.

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7.1 Clays

7.1.1 *General Aspects*

When considering the natural mineral form, clays mainly consist of aluminosilicates: alternated silicate and gibbsite sheets constitute the basic components of clays. In the silicate layer, three oxygen atoms of the tetrahedral SiO_4^{4-} units are shared by different units to form the layered structure reported in Fig. 7.1a. Free oxygen atoms, shown just above each Si atom in the figure, do not belong to one sheet, but may serve to link two adjacent sheets with each other.

Gibbsite sheets (Fig. 7.1b) contain octahedral $[Al(OH)_6]^{3-}$ units, where each hydroxide ion is co-ordinated to two Al^{3+} cations. Thus, only two-thirds of the hydroxide sites are occupied in bridging two Al ions. Analogously, brucite sheets, typical of some natural cationic and anionic clays, contain $Mg(OH)_2$ units where each hydroxide ion is bonded to three cations.

The cationic layers of mineral clays consists of silica sheets linked to gibbsite-like sheets: oxygen atoms form a bridge between two adjacent sheets. As an example, the 1:1 structure formed by the link of one silica sheet with one gibbsite sheet leads to kaolinite. Many natural clays contain 2:1 structures, where a gibbsite-like sheet is interposed between two silica layers, oxygen atoms being located on those apices of the tetrahedra that are shared with the octaedra. This structure is typical of clays belonging to the smectite family (see Fig. 7.2), namely montmorillonite, nontronite, and hectorite; one of the general formulas proposed for each one of these clays is reported in Table 7.1 [2]. Other clay minerals exploited as electrode modifiers mainly belong to the vermiculite, laponite, and sapiolite families.

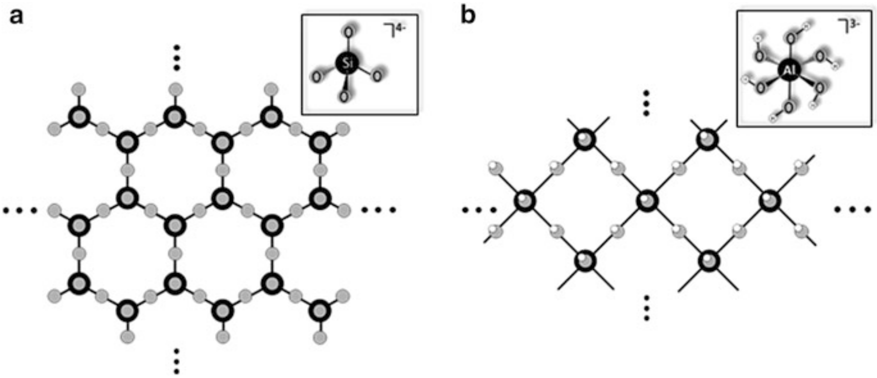


Fig. 7.1 (**a**) Tetrahedral unit of SiO_4^{4-} (*inset*) in the layer of silicates and (**b**) octahedral unit of $[Al(OH)_6]^{3-}$ (*inset*) in the layer of gibbsite

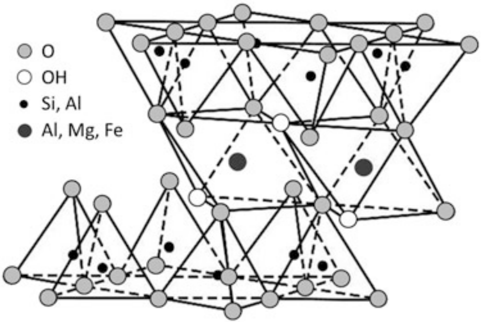


Fig. 7.2 Schematic representation of a 2:1 silica and gibbsite sheet typical of smectite clays

Table 7.1 Denomination and chemical formula of main mineral clays used as electrode coatings [2]

Clay type	Name	Formula
Cationic 1:1	Kaolinite	$Al_2Si_2O_5(OH)_4$
Cationic 2:1 dioctahedral	Montmorillonite	$(Al_{2-x}Mg_x)Si_4O_{10}(OH)_2(Na_x^+, nH_20)$
	Nontronite	$Fe_2^{3+}(Si_{4-x}Al_xO_{10})(OH)_2(Na_x^+, nH_20)$
Cationic 2:1 trioctahedral	Hectorite	$(Mg_{3-x}Li_x)Si_4O_{10}(OH)_2(Na_x^+, nH_20)$
	Laponite (synthetic hectorite)	$(Mg_{5.5}Li_{0.5})Si_4O_{10}(OH)_2(Na_x^+, nH_20)$
	Vermiculite	$Mg_3(Si_{4-x}Al_xO_{10})(OH)_2(Na_x^+, Ca_{x/2}^{2+}nH_20)$
Cationic	Sepiolite	$(Mg_{8-y-z}R_{y-3}^{3+})(Si_{12-x}R_x^{3+})$ $(OH)_4(OH_2)_4R_{x-y+2}^{2+}(H_2O)_8$
Anionic	Hydrotalcite	$[Mg_6Al_2(OH)_{16}]CO_3(4H_20)$
	Layered double hydroxide (LDH)	$[M_{1-x}^{II}M_x^{III}(OH)_2]X \cdot nH_2O$

The dimension of clay particles is often less than 1 μm in diameter, which locates them in the colloidal size range. As a consequence, they possess a high surface-to-volume ratio of which many catalytic and electrocatalytic applications can profitably take advantage. Moreover, due to the layered structure, they can swell by incorporation of water, leading to a significant volume increase. This capability is improved by the presence of divalent or monovalent cations in the sheets, replacing Al^{3+} or Mg^{2+}, respectively; this results in an overall negative charge on the layers. The electrical neutrality is maintained by cations localized in the interlayer regions. These cations, mainly consisting of Na^+ and Ca^{2+} in natural clay minerals, possess a high mobility and are thus exchangeable when the clay is suspended in an electrolytic solution. The cation exchange capacity (CEC) is a measure of the capability of a clay to exchange the original cations in the interlayer region with those present in a solution. Typical values of CEC are in the range 0.80–1.50 mmol/g for montmorillonite. However, the extent to which the layer expansion occurs is not only directly related to the type of clay, but also to the nature and concentration of the electrolyte. It is worth noticing that 1:1 kaolinite clays are characterized by very poor swelling capacity, resulting in a very low CEC value, which limits its use in an electroanalytical frame.

The distance between the anionic layers is generally evaluated by X-ray diffraction. Although it is conditioned by the amount of water and cationic species intercalated, the usual inter-lamellar distance for a natural Na-montmorillonite is ca. 12 Å; it can be significantly reduced when the clay is heated, or increased by suspension in an electrolytic solution. The interlayer distance of the natural material can be highly increased by introducing polyhydroxy metallic precursors [3–7]; the subsequent thermal treatment induces the formation of metal oxides in the form of pillars, which act as cross-linkers between the silicate layers. Due to this structure, the final material is named pillared clay. It exhibits a microporous structure, the pores possessing well defined size and shape, suitable to activate selective interaction with the target analyte, allowing pre-concentration inside the coating, close to the electrode [8].

A different approach to the modification of the physico-chemical properties of natural cationic clays involves the chemical functionalization of the aluminosilicate layer with alkylammonium groups. First attempts along this direction were based on simple adsorption of these positively charged organic derivatives exceeding the CEC of the clay [9, 10]. Recently, a more stable functionalization of the native material was realized: strong covalent bonds were obtained by letting silanol groups react at the surface of the clays with different alkylammonium silane derivatives [11, 12]. Both these approaches led to the formation of an external organic layer characterized by a net positive charge [13–15]. Due to their chemical structure, these materials are generally called organoclays.

Similar to the natural cationic clays, anionic clays, namely hydrotalcites (Fig. 7.3), are also present in nature. They are based on positively charged brucite-like layers containing Mg and Al hydroxides, described by the general formula $[Mg_6Al_2(OH)_6]^{2+}$; the electroneutrality of the material as a whole is guaranteed by included anions, typically CO_3^{2-} in the natural rocks, localized in the interlayer region.

The number of anionic clays was increased by the synthesis of a series of similar solids, namely layered double hydroxides (LDHs), possessing the general formula $[M^{II}_{1-x}M^{III}_{x}(OH)_2]X \cdot (H_2O)_n$, where both Mg(II) and Al(III) are replaced by different metal ions, conferring the material the characteristics sought. A similar chemical structure is generally indicated as M^{II}/M^{III} - X; for short, this denomination will be also used throughout the chapter. The structure and properties of these materials can be modulated by varying the nature of M^{II} and M^{III}, their molar ratio, i.e., the x parameter in the formula, as well as the nature of X (i.e., the anion in the interlayer region).

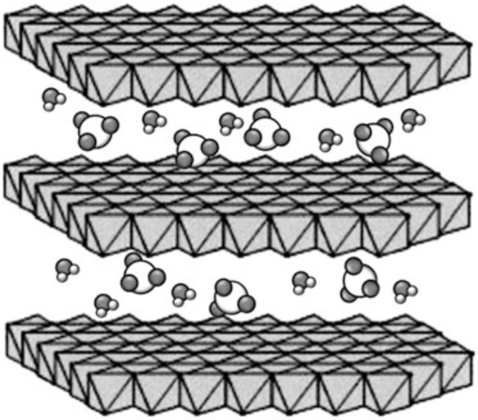


Fig. 7.3 Schematic representation of hydrotalcite characterized by CO_3^{2-} anions and H_2O molecules intercalated between positively charged $[Mg_6Al_2(OH)_6]^{2+}$ layers

7.1.2 Electrochemical Behavior of Clay-Modified Electrodes

Clays represent one of the most widely explored classes of inorganic nanostructures in mineralogy. Although the application of clay minerals in heterogeneous catalysis can often be directly related to the reactivity of the material as such, the possibility of stably including a suitable catalyst inside the three-dimensional structure constitutes a remarkable added value, and has often been exploited. The properties of clays were more recently widened further by the synthesis of a large number of derivatives, modulated as a function of the properties sought. All these aspects make clays a very interesting material for electroanalytical applications.

Due to its easy availability in nature, the early electrochemical works concerning the use of clay-modified electrodes (ClMEs) were carried out on Na^+-montmorillonite [16, 17]. The advantage of using such a modifier lies in the ability to strongly adsorb redox active cations inside the aluminosilicate layers by simply soaking the electrode in a solution containing them. Different metal complexes, particularly those consisting of 2,2′-bipyridine derivatives and $[Ru(NH_3)_6]^{3+}$ have been incorporated into the clay lattice, showing voltammetric behavior typical of species that undergo reversible redox processes [17]. As observed in Fig. 7.4a, b, the half-wave potentials for all these species are near those obtained at a bare electrode. However, in many cases, only 10–30 % of the amount of ions incorporated were found electroactive [16], and the current relative to the relevant charge transfer rapidly decreases upon immersion of the film in a pure electrolyte solution [20]. In the same reference, a detailed investigation shows that species stably included into the galleries and at the external surfaces through an ion-exchange mechanism are electroinactive, while only the fraction exceeding the CEC of the clay actually is. In order to undergo electrode oxidation or reduction, the intercalated species

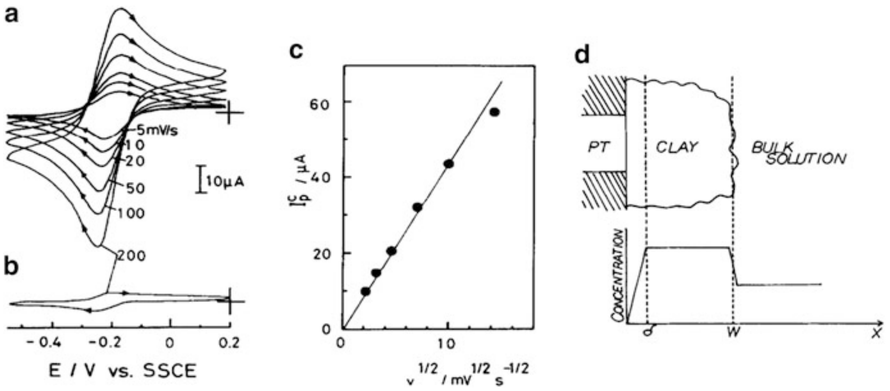


Fig. 7.4 Cyclic voltammograms of 6×10^{-5} M $[Ru(NH_3)_6]^{3+/2+}$, 0.1 M Na_2SO_4, aqueous solution (pH 7.0) containing 20 mM phosphate buffer obtained at (**a**) montmorillonite clay modified (0.73 μm thickness) and (**b**) bare electrode. (**c**) Dependence of the cathodic peak current on the square root of the potential scan rate for the modified electrode. (**d**) Diagram of the concentration of species adsorbed inside the clay film (Pictures **a**–**c** are reproduced from Ref. [18] with the permission of Elsevier and **d** from Ref. [19] with the permission of Clay Minerals Society)

must first diffuse out of the interlayer spaces, so the opportunity to exploit the geometry of the gallery spaces is lost.

The fraction of intercalated species emerging as electroactive is less stably trapped inside the coating, being mainly localized in microchannels formed inside the film, filled with the electrolytic solution. The presence of these channels is also invoked to explain why neutral and anionic species, e.g., $[Mo(CN)_8]^{4-}$ and $[Fe(CN)_6]^{4-}$, can also diffuse into the cationic clay film [6, 21].

Similar to cationic clays, redox active anions can be incorporated between adjacent layers of anionic clays, as evidenced by the relevant voltammetric traces. Several examples include metal complexes, such as $[Fe(CN)_6]^{4-}$, $[Mo(CN)_8]^{4-}$, $[IrCl_6]^{2-}$ [22–24], polyoxometalate [25], and redox active organic anions [24, 26].

The charge conduction inside native clay films consists of quite a complex mechanism discussed in many scientific articles. It involves heterogeneous electron-transfer reactions at the clay|electrode interface and homogeneous charge-transport reaction inside the clay film. Electron hopping between neighboring electroactive sites, diffusion of redox active species inside the clay, and concurrent migration of counter-ions of the supporting electrolyte are operative. A linear dependence of the current on the square root of the potential scan rate is observed (see Fig. 7.4c), demonstrating the diffusion-controlled nature of the charge transfer process.

The most interesting aspect emerging from these first studies carried out in the 1980s lies in the fact that, despite the lower diffusion coefficient of these species inside the clay with respect to the solution phase, the current values recorded at ClMEs are significantly higher than those at the bare electrode (see Fig. 7.4a, b). This aspect indicates the high local concentration of electroactive cations inside the film, especially when considering the very low fraction of species that are actually

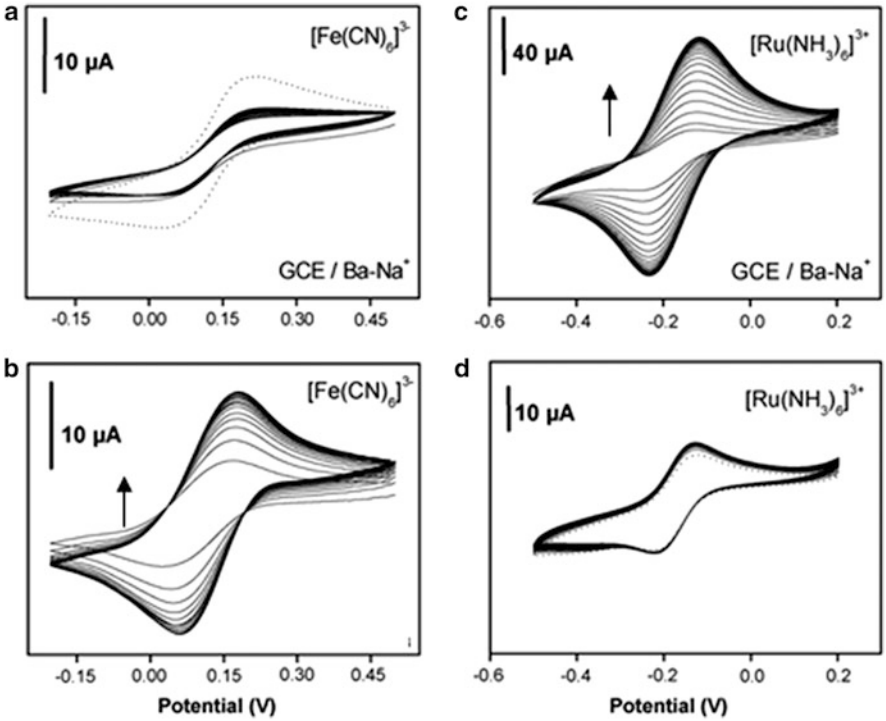


Fig. 7.5 Multisweep cyclic voltammograms recorded at 0.05 Vs^{-1} in 0.1 M NaCl solution containing 1.5 mM $[Fe(CN)_6]^{3-}$ or 1.5 mM $[Ru(NH_3)_6]^{3+}$. Signal recorded on (**a**, **c**) native smectite clay and (**b**, **d**) the relevant organoclay. The *dotted line* on (**a**) and (**d**) represents the signal of $[Fe(CN)_6]^{3-}$ and $[Ru(NH_3)_6]^{3+}$, respectively, on the bare glassy carbon electrode (Reproduced from Ref. [15] with the permission of Elsevier)

electroactive. As described in the following, this property has been exploited in the development of sensors for the analysis of positively charged species.

After the first studies, the number of applications of cationic clays in electroanalysis increased, realizing that neutral and anionic species can also be massively intercalated inside the coating after functionalization of the native material to form organoclays: charge neutrality at the positively charged organic external zone of the coating requires the inclusion of counter-anions inside the clay structure. They can participate to anion-exchange equilibrium with other electroactive anions in the solution. In this way, redox active anions, such as $[Fe(CN)_6]^{4-}$, $[Mo(CN)_8]^{4-}$, and $[Fe(C_2O_4)_3]^{3-}$, have been incorporated [10, 15], giving rise to electrochemical responses similar to those reported in Fig. 7.5a. Obviously, at variance with the native clay, the synthesized material finally acts as an electrostatic filter toward cationic species (see Fig. 7.5b).

The major drawback with the use of natural clays as electrode coatings lies in their intrinsic electrochemical inertness, i.e., in the insulating character of the layered backbone. This poses serious obstacles to the charge percolation throughout the film. In order to improve electrical conductivity, Villemure et al. proposed the synthesis of a series of clay derivatives containing redox active metal ions within the layered structure, namely Fe, Co, and Ni for cationic clays, and Co, Ni, and Mn for anionic clays [2, 27–29]. The voltammetric traces recorded with these modified electrodes show a reversible peak system ascribed to the transition metal inside the clay structure (see Fig. 7.6a). The effectiveness of these redox centers in charge percolation inside the clay layer through inner redox reaction was verified by insertion of electroactive species in the clay inter-layers. As an example, Fig. 7.6b reports the voltammetric traces obtained for $[Fe(CN)_6]^{3-}$ at a conductive Mg/Mn-CO_3 modified electrode, with respect to the signal obtained, under the same experimental conditions, at a non-conductive Zn/Al-Cl clay. The higher currents registered at the conductive ClME are ascribed to the contribution of electroactive species belonging to the CEC fraction, which are involved in the electrochemical process as a consequence of the more conductive character of the inorganic skeleton.

To better clarify the role played by the electroactive metal ions in the lattice, electrodes modified with the Ni/Al-Cl films were tested by electrochemical impedance spectroscopy, and the results obtained were compared with those of electrodes modified with the nonconductive Zn/Al-Cl clay [30]. It was observed that the resistance to charge transfer depends on the applied potential, decreasing from more than 10^6 Ω at the open circuit potential, to a minimum of about 60 Ω for the partially oxidized film. Conversely, the impedance spectra of Zn/Al-Cl-modified electrodes are much less notably affected by the value of the applied potential. As expected, in the case of conductive LDH, the mechanism of charge percolation throughout the film is quite complicated, involving electron hopping between neighboring redox active metal centers and necessary entrance of hydroxides from the solution to ensure electroneutrality inside the film [31, 32]. The final result is that the overall electrochemical process is neither diffusive nor surface controlled, as inferred from the trend of the peak current at varying the potential scan rate.

It is also important to mention here that the presence of electroactive species in the lattice structure can induce electrocatalytic properties exploitable for electroanalytical applications. With respect to all properties described, the introduction of conductive clays is a definite step forward in the application of ClMEs for amperometric sensing.

A more recent approach followed to improve the conductivity of clay films consists of the introduction of metal nanoparticles (NPs) [33] or graphene [34]. Obviously, the efforts to develop these composite materials originate with the well-known electrocatalytic properties of the nanosized filler, inducing the search for supports able to meet with many different experimental requirements.

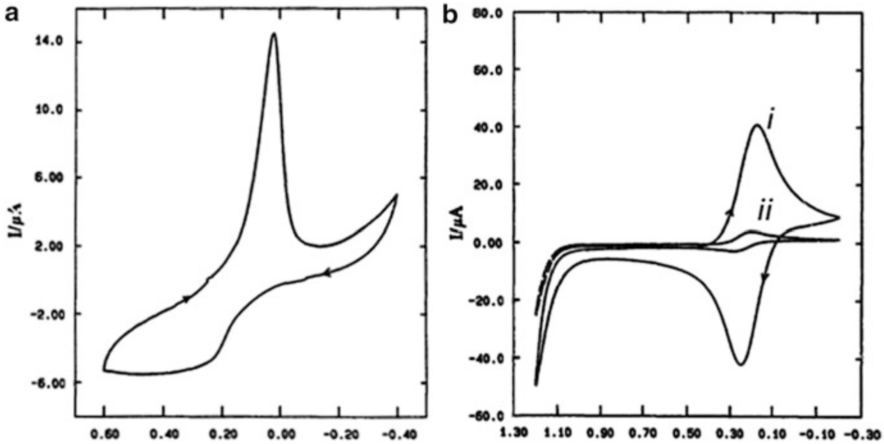


Fig. 7.6 Cyclic voltammograms of Mg/Mn-CO_3 modified electrode in 0.1 M KCl, (**a**) in the absence and (**b**) in the presence of 0.1 mM $[Fe(CN)_6]^{3-}$ (*curve i*); *curve ii* in **b** reports the voltammetric trace recorded at a non-conductive Zn/Al-Cl electrode coating. 0.020 Vs^{-1} potential scan rate (Reproduced from Ref. [29] with the permission of Elsevier)

7.1.3 Deposition of Clays on the Electrode Surfaces

Similar to the case of many different electrode materials, the formation of a carbon paste electrode [35–38] constitutes the most spontaneous method to obtain an electrode consisting of a clay as the electroactive solid. Graphite is generally mixed with the clay powder after reducing the clay particle size below 1–5 μm; the clay is wetted before the formation of the mixture, to remove impurities present in the native rock and to introduce water molecules in the interlayer space in order to expand its dimension.

However, the most frequent way to obtain a ClME consists in the formation of a film coating. Different deposition procedures can be followed depending on the thickness required. Among others, evaporation of the solvent from a clay aqueous suspension is the simplest and most widely used method for film thickness ranging from a few hundreds of nanometers to several micrometers. In order to obtain a more homogeneous, reproducible, and thinner film, a spin coating deposition technique is also adopted. Due to the poor stability of clay films obtained by these methods, alternative approaches imply the entrapment of clay particles into polymeric membranes [39–41], epoxy resin [40], or sol-gel structure [42].

Langmuir-Blodgett and electrophoretic techniques are two less frequently used methods for obtaining ClMEs starting from clay suspensions. In the former procedure, the clay suspension also contains a tetraalkylammonium salt, in order to obtain an adduct suitable to form a Langmuir film at the water|air interface; the subsequent immersions of the substrate in the monolayer obtained after hydrophobic–hydrophilic orientation allows the formation of thin films consisting of 6–30 layers [43–45]. The electrophoretic technique simply requires the application of a fairly high potential difference (from 2 to 12 V) between a working and a counter electrode; this process results in the formation of a very uniform film, characterized by a thickness ranging from few to few tens of nanometers, depending on the deposition time [46].

LDH-modified electrodes have been also obtained by a layer-by-layer (LbL) deposition technique, by exploiting the net positive charges on the brucite-like layers [47]: after delamination of suitably synthesized LDHs [48, 49], the positively charged component of the film is thus obtained. The anionic counterpart consists of an organic polymer, such as poly(sodium 4-styrenesulphonate) [48, 49], of negatively charged electroactive species, such as metal porphyrins [50], phthalocyanines [51], Au NPs surrounded by an anionic cloud [52], or a specific enzyme deposited from buffered solutions at pH values above the isoelectric point [53].

All the previously described methods involve a two-step procedure comprising the purification of the native clay or its chemical synthesis, e.g., by a co-precipitation method, followed by deposition onto the electrode surface. Conductive LDH-modified electrodes can also be obtained in a single step with an electrochemical approach. In this case, the electrode is dipped in a solution containing the nitrate salts of both the bivalent (Co^{2+}, Ni^{2+}, Mg^{2+}, Zn^{2+}) and the trivalent (Al^{3+}) metals necessary to the LDH structure: the application of a negative potential able to induce NO_3^- and hydrogen reduction leads to an increase of the pH value in the close proximity of the electrode surface [54], with consequent precipitation of the metal hydroxides under the form of the layered structure typical of hydrotalcites [55–58]. From a thermodynamic point of view, the electrochemical reduction of NO_3^- is favored with respect to the reduction of the cations to the relevant zero-valence metal. By choosing the suitable composition of the electrolytic solution and the time length of polarization, it is possible to modulate the composition and thickness of the coating, as well as to obtain porous structures [59]. The higher stability of the film coatings and the ability to modify any conducting supports to obtain even very complex geometries constitute further advantages with respect to the two-step approaches previously described.

7.1.4 Application of Clays in Electroanalysis

The applications of ClMEs to electroanalytical issues can be roughly divided according to three main goals, corresponding to three relevant methodologies followed: (1) determination of chemical species pre-concentrated by adsorption at the outermost surface and inside the bulk of the layered structure; (2) determination of species whose oxidation or reduction is electrocatalysed by the clay; (3) determination of specific species by biocatalytic reactions, thanks to the stable immobilization of a suitable enzyme in the clay film.

The aim of this section is to describe the main strategies that encouraged researchers to use ClMEs in electroanalysis. Due to the high number of applications reported in the literature, only a few of them are discussed here, chosen in order to give accounts of different situations.

Pre-concentration

Due to the ion-exchange capability that is a specific property of each clay, ClMEs were spontaneously first applied to the determination of either positively or negatively charged species, employing cationic or anionic ClMEs, respectively. Some representative examples are reported in Table 7.2 [1]. The advantage of the use of similar modified electrodes compared with bare electrodes lies in the ability to pre-concentrate ionic species in the interlayer region of the clay structure, with consequent improvement of both sensitivity and selectivity exerted by the filtering effect induced by the material toward charged species.

The analysis is carried out in two steps: in the first one, the analyte is adsorbed on the electrode coating, generally at open circuit potential and under controlled stirring of the solution. After careful washing of the surface, the electrode is transferred into a solution containing a suitable supporting electrolyte, where the analyte is quantified through a voltammetric technique. To optimize the experimental parameters, it must be taken into account that the ion-exchange capability decreases as the ionic strength of the dipping solution is increased, due to the replacement by the electrolyte of exchangeable ions in the clay-layered structure; conversely, such a competitive ion exchange is exploited to regenerate the electrode coating after a measurement [60, 61].

Many applications of cationic clays are directed toward the detection of heavy metals [1]. In this case, the analytical method followed is very similar to an anodic stripping voltammetric technique: a step reducing pre-concentrated metal ions to the zero valence state is performed and the analytical signal is collected in the subsequent anodic re-oxidation step [61]. The selectivity of the clay toward a target metal ion can be improved by a functionalization of the material with suitable alkylsilane derivatives [64, 65, 80] or by intercalation of a specific organic ligand inside the clay interlayers [66, 67, 81].

As previously highlighted, despite the negative charge of the smectite layers, anionic species can also partially diffuse into the clay, thanks to the presence of microchannels. Although the anion exchange capacity of the clay minerals is about four to five times lower than CEC, examples are found in which montmorillonite-modified electrodes are effective in the detection of heavy metals, under the form of a relevant anionic metal complex [68, 69]. This aspect renders ClMEs suitable for the speciation of heavy metals in many applicative contexts.

At the same time, neutral species can also be, to a certain extent, adsorbed and quantified via a similar analytical approach [70–72]. Organoclays are suitable to improve the sensitivity of the sensor: the high affinity toward non-ionic species allows these materials to be profitably exploited to pre-concentrate several organic species. Thanks to the development of organoclays synthesized on purpose, several important analytes, mainly belonging to the classes of herbicides [73, 75] and

Table 7.2 Examples of the determination of chemical species pre-concentrated inside the clay film

Analyte	Electrode coating	Clay assembling	Ref.
Fe^{3+}	Montmorillonites	Carbon paste	[60]
Cu^{2+}	Vermiculite	Carbon paste	[61]
Cu^{2+}	Laponite/polythiophene	Langmuir-Blodgett	[62]
Cd^{2+}	Kaolinite	Carbon paste	[63]
Pb^{2+}	Thiol functionalized kaolinite	Carbon paste	[64]
Hg^{2+}	Amine or thiol functionalized organoclay	Carbon paste	[65]
Pb^{2+}	1,10-Ortophenantroline functionalized montmorillonite	Carbon paste	[66]
Pb^{2+}	Tripolyphosphate functionalized kaolinite	Carbon paste	[67]
$(HgCl_4)^{2-}$	Montmorillonites	Carbon paste	[68]
$(AuCl_4)^-$	Montmorillonites	Carbon paste	[69]
Methylviologen	Laponite	Dialysis membrane	[39]
Linuron	Sepiolite	Carbon paste	[70]
Methocarbamol	Montmorillonites	Carbon paste	[71]
Silymarin	Montmorillonites	Carbon paste	[72]
2,4-Dichlorophenol	Hectorite	Clay/sol-gel film	[73]
Methylparathion	Smectite organoclay	Drop casting	[15]
4-Chlorophenol	Montmorillonite organoclay	Carbon paste	[74]
Mesotrione	Smectite organoclay	Drop casting	[75]
I^-	Friedel's salt	Carbon paste	[76]
IO_3^-	Zn/Al-CO_3 LDH	Drop casting	[77]
Bisphenol A	Mg/Al-CO_3 LDH	Drop casting	[78]
Methylparation	Ni/Al-NO_3	Electrogenerated	[79]

pesticides [15, 74, 82, 83], have been detected with improved sensitivity compared with natural cationic clays. As an example, Fig. 7.7 reports the voltammetric traces relative to reduction of mesotrione, an herbicide whose chemical structure is reported in the inset of the same figure. As observed, the current peaks recorded at organoclay-modified electrodes (curves *c* and *d*) are significantly higher than those both at bare (curve *a*) and at Na-smectite-modified (curve *b*) electrodes.

At variance with the high number of applications of cationic clays as pre-concentrating materials, only a few examples are found about the use of anionic clays [77–79]. They involve both LDHs as such, for the adsorption of electroactive anions [76], and the modification of conventional anionic clays for the analysis of organic species; in this last case, anionic surfactants are adsorbed in the interlayer region of the clay, to increase the distance between adjacent brucite-like layers and to induce a higher hydrophobic character that is more suitable for the adsorption of organic species [84, 85].

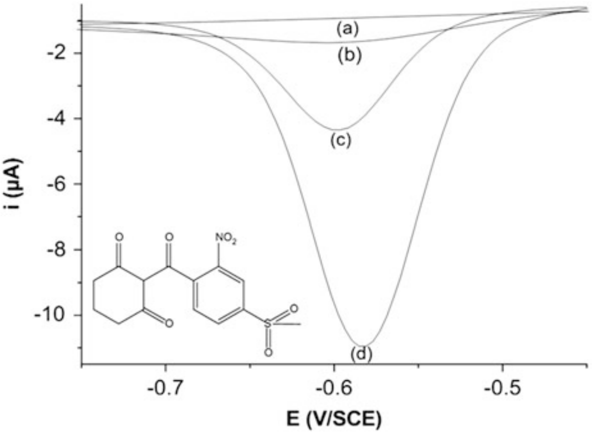


Fig. 7.7 Square wave voltammetric traces recorded on 2×10^{-5} M mesotrione, 0.1 M acetate buffer solution (pH = 6) at a (*a*) bare glassy carbon electrode, (*b*) natural Cameroonian smectite-type clay-modified electrode, (*c*) cetyltrimethylammonium functionalized smectite-type clay-modified electrode, and (*d*) didodecyldimethyl ammonium functionalized smectite-type clay-modified electrode. *Inset* reports the chemical structure of mesotrione (Reproduced from Ref. [75] with the permission of Elsevier)

Electrocatalysis

Due to the variety of chemical compositions, several methodologies have been proposed to make the clay film suitable to induce electrocatalytic processes. Table 7.3 reports some examples representative of the strategies described in the following.

The first approach consisted of exploiting the ion-exchange capability of both anionic and cationic clays to intercalate ionic redox active species, capable to act as redox mediators, in the interlayer region. In this case, the clay simply acts as the support to the actual catalyst [94–97]. However, the small fraction of iron present in the montmorillonite structure (ca. 2.7 %) was also found to be sufficient for activating electrocatalysis; for this reason, the use of the clay possessing the highest amount of iron in the main structure, namely nontronite, appears more suitable to achieve higher sensitivity [87, 88, 98].

An effective way to include a catalyst inside brucite-like layers is an LbL deposition technique [51, 52, 89, 90]: exfoliated anionic clays constitute one of the components of the multilayers, while the actual electrocatalytic element possesses an opposite charge and acts as the second component of the film. This technique allows the deposition of very thin films with a homogenous and controlled disposition of the catalytic sites inside the coating. Moreover, it allows a higher number of electroactive sites to be effective in the catalytic reaction.

Despite the importance of the systems described, when electrocatalytic properties are sought, the conductive LDHs are the clay coatings of choice; to such a purpose, it is possible to exploit the presence of redox active metal sites in the

Table 7.3 Examples of the determination of chemical species thanks to electrocatalytic processes

Analyte	Electrode coating	Clay assembling	Catalyst	Ref.
H_2O_2	Mg/Al-$[Fe(CN)_6]^{3-}$	Drop casting	$[Fe(CN)_6]^{3-}$	[86]
Arbutin	Nontronite	Drop casting	Fe sites in nontronite	[87]
Aminotriazole	Nontronite	Drop casting	Fe sites in nontronite	[88]
Dopamine	Mg/Al-Co (II) phthalocyanine	LbL	Co(III) phthalocyanine	[51]
Glucose	Zn/Al-AuNPs	LbL	Composite material	[52]
H_2O_2	Co/Al-naphtol green B	LbL		[89]
Ascorbic acid	Ni/Al-naphtol green B	LbL		[90]
Methanol	Ni/Al-Cl LDH	Drop casting	Ni(III) in the brucite-like layers	[32]
Glucose	Ni/Al-NO_3 LDH	Electrogeneration	Ni(III) in the brucite-like layers	[91]
Glyphosate	Ni/Al-NO_3 LDH	Electrogeneration	Ni(III) in the brucite-like layers	[92]
Salicylic acid	Co/Al-NO_3 LDH	Electrogeneration	Co(IV) in the brucite-like layers	[93]
Dopamine	Zn/Al-graphene	Drop casting	Composite material	[32]

brucite-like layers. Ballarin et al. [31, 32] reported that Ni(II) sites inside Ni/Al-Cl clay coatings catalyze the oxidation of methanol and higher alcohols in strong alkaline solutions. The oxidation of the analyte occurs at potentials at which Ni (II) oxidation takes place, indicating that catalysis does occur. Starting from these first works, the study was extended to different conductive LDHs and to the analysis of several carbohydrates, which are typical polyhydric compounds [40, 41, 91], as well as to aliphatic and aromatic amines [99]. It was observed that Ni centers act as redox mediators for both alcohol and sugar oxidation, whereas only polyhydric compounds can be oxidized at Co/Al-based LDH modified electrodes; the different behavior between the two materials was ascribed to the lower oxidation potential of the Co(II) sites of the clay [100]. Co/Al-NO_3 modified electrodes have been proposed for the selective determination of alcohols in real matrices, such as commercial untreated beers.

We should remember that the oxidation processes at LDH modified electrodes is rather complex: the analyte is oxidized both at the solution/coating interface and inside the LDH coating. The actual occurrence of the reaction between the Ni active sites and the substrate is conditioned by the diffusion rate of the analyte, both in solution and inside the film, as well as by the kinetics of the reaction between the analyte and the electroactive sites of the electrode coating.

Bio-Catalysis

Clay coatings have been widely applied to the development of catalytic biosensors, showing to be suitable to stably anchor proteins. Biosensors were developed bearing glucose oxidase (GOD), horseradish peroxidase (HRP), polyphenol oxidase (PPO), and hemoglobin (Hb). Table 7.4 reports a few examples from the literature, aiming to show the main strategies followed to exploit both cationic and anionic clays for developing effective biosensors [116]. The advantage of using these inorganic materials mainly consists of the swelling they undergo in water, which allows protein inclusion and immobilization, by a simple adsorption process, inside a host matrix characterized by a high hydrophilic character. As convincingly claimed by several authors [116, 117], these advantages finally lead to catalytic biosensors with superior performance in terms of sensitivity and detection limits, with respect to biosensors based on sol-gel or on polymers as the support component.

The protein–clay interaction is generally based on the negative charges of the enzymes, when deposited at pH values higher than the relevant isoelectric point. The enzyme can electrostatically interact with the positive charges at brucite-like layers in LDHs [103, 118] or with the surfactants added to positive clays to form organoclays [13, 119].

In the majority of cases reported, the preparation of these biosensors simply consists of the deposition of a fixed aliquot of an aqueous suspension of a protein/clay mixture onto the electrode surface, and subsequent evaporation of the liquid phase [13, 101, 118]. Alternatively, to make the bioactive centers available to the catalytic reaction with the relevant analyte and to reduce the amount of enzyme required, the enzyme fixation can be carried out at a pre-deposited clay film [104, 119, 120]. For the same reason, LbL deposition has also recently been exploited: the negatively charged protein acts as the anionic polyelectrolyte to interpose between exfoliated brucite-like layers [53] or, vice versa, positively charged myoglobin or HRP are intercalated between layers of negatively charged montmorillonite NPs [121]. Finally, when considering conductive LDHs, the enzyme can be adsorbed at the brucite-like layers during the electrogeneration of the clay film [122, 123].

Due to the rather weak electrostatic interactions involved, cross-linking agents, e.g., glutaraldeide (GA) [13, 103, 104, 112, 118, 122] and chitosan [101, 102] are often added to prevent the release of the adsorbed enzymes from the electrode coating.

Suitable redox centers can also be inserted in the electrode coating with the aim to catalyze the oxidation or reduction of the product coming from the enzymatic reaction, such as H_2O_2 or nicotinamide adenine dinucleotide (NADH). This approach may allow the detection of the analyte at potential values where interfering species are not electroactive [107] and, in some cases, has also been proved to improve the electrode sensitivity [119]. Redox mediators can be adsorbed in the interlayer region of the clay [103, 105, 106, 109, 118] or constitute the brucite-like layers of conductive LDHs [104, 122]. Alternatively, bi-enzymatic electrode coatings have also been proposed [53, 108]. Organic polymers, namely poly(pyrrole–

Table 7.4 Examples of the determination of chemical species by enzymes fixed on clay films

Analyte	Electrode coating	Clay assembling	Catalyst	Ref.
Glucose	Smectite organoclay/GOD+GA	Drop casting	–	[13]
Catechol	Smectite organoclay/PPO+GA	Drop casting	–	[13]
Phenol	Laponite/chitosan/PPO	Drop casting	–	[101]
Glucose	Laponite/chitosan/GOD	Drop casting	–	[102]
Glucose	[Mg/Al-ferrocene-carboxylate]/GOD+GA	Drop casting	Ferrocene-carboxylate	[103]
Glucose	[Ni/Al-NO_3]/GOD+GA	Electrogeneration	Ni(III) in brucite-like layers	[104]
Catechol	Laponite/PPO/Azure B/GA	Drop casting	Azure B	[105]
Glucose	[Zn_3/Al-Cl]/GOD	Drop casting	–	[106]
Glucose	[Zn_3/Al-Cl]/GOD/ferrocenemethanol	Drop casting	Ferrocenemethanol	[107]
Glucose	Laponite/GOD/HRP/graphite	Drop casting	HRP	[108]
Rutin	Laponite/PPO/Azure B/GA	Drop casting	Azure B	[109]
NO	Montmorillonite/mioglobine	Drop casting	Heme sites[a]	[110]
Ethanol	Laponite/alcohol dehydrogenase/polymethylene blue	Drop casting	Polymethylene blue	[111]
Lactate	Laponite/lactate dehidrogenase/polymethylene blue	Drop casting	Polymethylene blue	[111]
Phenol	Laponite/PPO/GA/poly(azure B)	Drop casting	Poly(azure B)	[112]
NO	Montmorillonite/polyvinyl alcohol/Hb	Drop casting	Heme sites	[113]
Xanthine	[Zn_3–Al–Cl]/xanthine oxidase/GA	Drop casting	Inhibition of allopurinol	[114]
NO_3^-	Laponite/nitrate reductase/methylviologen/poly (pyrrole-viologen)/GA	Drop casting	Methylviologen	[115]

[a] In this case, the analytical signal consists of the linear shift of the formal potential for Mb(Fe^{II})/Mb(Fe^{III}) redox couple as a function of the logarithm of NO concentration

viologen), poly(azure B), and polymethylene blue, were also added to the clay coatings in order to combine the mechanical stabilization of enzymes on the clay coating and to induce a charge transfer mediation, with activation of electrocatalytic processes [108, 111, 112].

A further advantage observed in the use of LDH-modified electrodes in the development of glucose biosensors lies in the possibility of avoiding the interference of negatively charged species, mainly consisting of ascorbate anions, without the addition of external membranes, which can reduce the enzyme efficiency [104].

7.2 Zeolites

7.2.1 General Aspects

Zeolites constitute a class of well ordered aluminosilicates, exhibiting a microporous structure with pore and channels possessing dimensions typically within the range 4–15 Å. This aspect ascribes zeolites a large surface area and a well defined three-dimensional structure: different species in solution are discriminated on the basis of the possibility of entering the pores, as conditioned by the relevant steric hindrance.

Similar to clays, zeolites are widely present in nature, and a number of synthetic derivatives have enriched the panorama of physico-chemical properties exploitable in electroanalysis. Naturally occurring zeolites consist of Si and Al atoms exhibiting tetrahedral coordination with O atoms. Each O atom is, in turn, bounded to two Si or Al atoms, resulting in SiO_2 and AlO_2 sub-structures (see Fig. 7.8). Due to the +3 oxidation state of Al, the AlO_2 subunit possesses -1 formal charge that is counterbalanced by positive counter-ions in the micropores, mainly consisting of alkaline (Na^+, K^+) or alkaline-earth (Mg^{2+}, Ca^{2+}) cations. These extra-framework cations are part of the zeolite structure, but possess a considerable freedom of movement, e.g., exit from the solid is possible, which confers zeolites ion-exchange properties exploitable to include cations. These may consist in a redox mediator or in the target analyte.

Ions isoelectronic with Al^{3+} and Si^{4+} have been used to prepare synthetic zeolites possessing peculiar catalytic properties. B^{3+}, Ga^{3+}, Fe^{3+}, and Cr^{3+} are reported to be suitable for the formation of metallosilicates, and Ge^{4+} and Ti^{4+} for the preparation of metalloaluminates. They all form three-dimensional structures with pores and channels possessing specific size. These can be modulated thanks to the relatively high flexibility of the metal–oxygen–metal and oxygen–metal–oxygen bonds, leading to the formation of rings containing a different, though fixed, number of atoms. As a direct consequence, the pore size distribution is not continuous, but rather discrete, and is related to the number of O atoms in the rings: for conventional zeolites, the number is generally between 6 and 12, leading to pore sizes ranging from 3 to 8 Å, depending on the structure. However, pore sizes of 15 Å can also be found.

The most common zeolite frameworks in electroanalytical applications are reported in Fig. 7.8. Apart from the three-dimensional arrangement, the classification of aluminosilicate zeolites is based on the Si/Al ratios, as reported in Table 7.5. In turn, it is related to the ion-exchange capability of the material. Zeolite A presents the highest density of Al^{3+} sites, leading to a 1:1 ratio between Si^{4+} and Al^{3+}; this structure is not present in nature, but can be synthesized. A higher number of Si^{4+} sites is present in naturally occurring faujasite and in the relevant synthetic products, namely zeolites X and Y. Mordenite possesses the highest Si/Al ratio among the naturally occurring zeolites, assuming a value of 5. Highly siliceous zeolites can also be produced in the laboratory: they contain a very low amount of

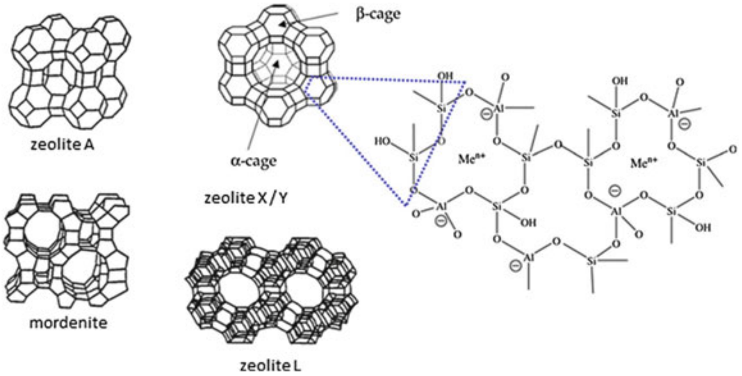


Fig. 7.8 Three- and two-dimensional representation of the framework structure of different zeolites (Adapted from Ref. [124] with the permission of Elsevier)

Al^{3+}, with an Si/Al ratio tending to very high values. The result is, obviously, a very low ion-exchange capability.

7.2.2 Electrochemical Behavior of Zeolite-Modified Electrodes

The main interest with zeolites in electroanalysis is related to the increase of the electrode surface due to a material characterized by high ion-exchange capability; this allows pre-concentration of cationic analytes possessing a dimension lower than that of the pores. In fact, the very first studies on zeolite-modified electrodes (ZMEs) demonstrated that higher currents can be registered in responses to species in solution when using similarly coated electrodes compared with bare ones [126, 127]: cationic species can easily be inserted into the porous structure, thanks to the ion-exchange capability of the aluminosilicate framework [128, 129]. By exploiting this capability, the electrochemical responses are higher the longer the soaking time of pure ZMEs in the solution containing the species of interest [130–133]. In spite of the insulating nature of this silica-based material and of the lower diffusion coefficients of the species in the coating compared with the solution, similar to what has already been discussed in the case of ClMEs, the higher currents registered indicate that a high amount of electroactive species can actually be incorporated very close to the electrode surface. Obviously, with the aim of improving the sensitivity of the electrochemical response at best, zeolite powder is generally mixed with a conductive binder, as is described in more detail hereafter.

The electrochemical behavior of ZMEs is described in exhaustive reviews [17, 134], and the mechanisms involved in charge percolation processes inside ZMEs are also at the basis of the applications of these materials in electroanalysis and of the strategies followed to exploit their properties. The charge percolation process is

Table 7.5 Physical and chemical properties of zeolites used in electrochemical studies [125]

Type	Unit-cell compound (typical, fully hydrated)	Pore dimension (Å)	Typical Si/Al	Void fraction (cm^3/cm^3)
A	$Na_{12}[(AlO_2)_{12}(SiO_2)_{12}] \cdot (H_2O)_{27}$	4.1 Å	1.0	0.47
X	$Na_{86}[(AlO_2)_{86}(SiO_2)_{106}] \cdot (H_2O)_{264}$	7.4 Å	1–1.5	0.50
Y	$Na_{56}[(AlO_2)_{86}(SiO_2)_{136}] \cdot (H_2O)_{250}$	7.4 Å	>1.5–3	0.48
L	$K_9[(AlO_2)_9(SiO_2)_{27}] \cdot (H_2O)_{22}$	7.1 Å	2.6–3.5	0.32
Mordenite	$Na_8[(AlO_2)_8(SiO_2)_{40}] \cdot (H_2O)_{24}$	6.5−7.0 Å	4.7–5	0.28

influenced by intrinsic characteristics of the zeolite and by experimental parameters that are reflected in the ion-exchange capability of the material: the size of the pores, the nature of the electroactive species, and the dipping time. Moreover, the nature of the supporting electrolyte and, in particular, the size and charge of the cation, also play a key role in conditioning the electrochemical response, as is evident from the possible conductivity mechanisms first proposed by Shaw and co-workers [130], and subsequently revised by Dutta and Ledney [125]. The three proposed mechanisms can briefly be schematized as follows:

Mechanism 1: intra-zeolite charge percolation

$$\mathrm{E}^{m+}_{(z)} + n\mathrm{e}^- + n\mathrm{C}^+_{(s)} \leftrightarrows \mathrm{E}^{(m-n)+}_{(\mathrm{z})} + n\mathrm{C}^+_{(z)}$$

Mechanism 2: extra-zeolite charge percolation

$$\mathrm{E}^{m+}_{(\mathrm{z})} + n\mathrm{C}^+_{(\mathrm{s})} \leftrightarrows \mathrm{E}^{m+}_{(\mathrm{s})} + n\mathrm{C}^+_{(\mathrm{z})}$$

$$\mathrm{E}^{m+}_{(\mathrm{s})} + ne^- \leftrightarrows \mathrm{E}^{(m-n)+}_{(\mathrm{s})}$$

Mechanism 3: surface-mediated charge percolation

$$\mathrm{E}^{m+}_{(\mathrm{z,\ surf})} + n\mathrm{e}^- + n\mathrm{C}^+_{(\mathrm{s})} \leftrightarrows \mathrm{E}^{(m-n)+}_{(\mathrm{z,surf})} + n\mathrm{C}^+_{(\mathrm{z,surf})}$$

$$\mathrm{E}^{(m-n)+}_{(\mathrm{z,\ surf})} + n\mathrm{C}^+_{(\mathrm{z,surf})} + E^{m+}_{(\mathrm{z,bulk})} \leftrightarrows \mathrm{E}^{m+}_{(\mathrm{z,surf})} + n\mathrm{C}^+_{(\mathrm{z,surf})} + \mathrm{E}^{(m-n)+}_{(\mathrm{z,\ bulk})}$$

where E is the electroactive species with charge m^+, C^+ is the electrolyte cation, chosen as a monovalent one for simplicity, the suffixes '*z*' and '*s*' indicate zeolite and solution phases, respectively, and the subscripts '*surf*' and '*bulk*' indicate the zeolite surface and bulk ion-exchange sites, respectively. According to mechanism 1, electroactive species are reduced or oxidized directly in the bulk of zeolite particles, the charge percolation mechanism involving adjacent redox species; counter-ions from the solution are requested to migrate into the electrode coating to compensate for the arising excess of charge. According to mechanism 2, the electroactive species are first exchanged by the electrolyte cations, thus diffusing out from the zeolite to the underlying polarized substrate. A third, more recently proposed, mechanism invokes a surface-mediated charge transfer: the electrode process first involves chemical species located in the outermost boundaries of the zeolite particles closest to the conductive substrate, where they are more available to charge transfer with the polarized substrate. In the second step, these species act as mediators in the redox transformation of species located in the bulk of the solid. Also in this case, each step requires charge compensation by the electrolyte cations.

Although the way in which charges are transferred through the film, involving electroactive species located in the microporous structure of the zeolite, has induced enormous research efforts [125, 135], it constitutes a still existing problem. A general assumption is that all the reported mechanisms may be operative, depending on the nature of the electroactive species. Mechanism 1 was verified for bulky species irreversibly trapped inside the coating, e.g., metal complexes directly formed inside the zeolite [136–139]. The inclusion of species larger than the zeolite pores into the particles of the host material requires specific synthetic strategies [140], so that the term ‘ship-in-a-bottle complexes’, generally used in this case, appears quite appropriate. When included inside this inorganic material, they can potentially exert new reactivity, since they are physically trapped within the channels, also forming strong boundary-associated complexes.

Obviously, mechanism 2 has been proposed for species of small size, such as those fixed within the zeolite framework by ion exchange, which can move freely thorough the channel system and can easily be extracted from the zeolite by exchange with the electrolyte cations [129–131, 141–145]. Although, on the one hand, this mechanism renders a high number of species enclosed inside zeolite electroactive, it results in a progressive decrease of the voltammetric response when experiments with long running times are performed.

7.2.3 Deposition of Zeolite onto Electrode Surface

Apart from the selectivity imparted by the porous and negatively charged framework, one of the main advantages offered by the use of zeolites as chemical modifiers of the electrode surfaces lies in the high stability under extreme conditions: they are thermally stable up to 700–1100 °C, depending on the aluminum content, and the high-silica zeolite structures persist in acidic media. Furthermore, they can be dehydrated and rehydrated in a reversible way without any damage to the framework structure. Conversely, they also present two main drawbacks: (1) the poor cohesion of individual crystals of micrometric size requires the addition of a second component to act as a binder; (2) the insulating nature of the inorganic framework requires the second component to possess a conductive nature. To solve both these problems, zeolite particles are most often mixed with carbon grains to form carbon paste electrodes [132–134, 146–155]. Despite the simplicity and rapidity of this approach, the main drawback lies in the hydrophilic character of the inorganic grains, inducing strong impregnation of the external layer of the film for prolonged use in aqueous media. This process causes progressive modifications of the amperometric response, i.e., strong memory effects in successive experiments with the same electrode. This aspect forces the removal of the external layer of the coating between two subsequent measurements, to eliminate the hydrated layer. An alternative way to overcome this problem consists of increasing the hydrophobic character of the surface, e.g., by using solid paraffin [156, 157] or resins [158] as the binder, replacing paraffin oil. A different strategy lies in decreasing the thickness of the composite by realizing screen-printed electrodes, where the more usual carbon ink is substituted by the zeolite-based composite [159, 160]. The resulting zeolite-carbon strips possess a typical thickness of 200 μm and contain zeolite particles embedded inside a carbon-polymer matrix.

Zeolite particles may also constitute one of the components of the electrode coating. First approaches to formation of two-component zeolite-based films were carried out by depositing zeolite particles onto the electrode surface in the form of a layer [161, 162], coated by an external film capable of imparting the necessary mechanical stability in the frame of a typical bilayer system. However, a simple mixture of all the components, followed by deposition through drop casting or spin coating, is generally preferred in electroanalytical applications [163, 164]. For reasons similar to those previously described, graphite grains are generally added to a non-conductive polymer matrix that acts as the binder [163, 165–168]. The formation of the coating can be very simply carried out by following these approaches, without the need for particular instrumentation; on the other hand, the resulting film can be poorly homogeneous. Several different strategies have been proposed more recently to overcome these drawbacks. They mainly consist of electrophoretic and self-assembly depositions. In the former, a continuous [169, 170] or pulsed [171] electrode polarization at highly positive potential is applied, inducing the precipitation of negatively charged zeolite particles onto the electrode surface. Good control of the film thickness and homogeneity is achieved with this deposition method. An external polymeric layer is generally applied to improve the mechanical stability of the film. Conversely, self-assembly can be performed by inducing either (1) formation of covalent bonds between the zeolite particles and a suitably functionalized electrode surface [172, 173], e.g., by silanization of zeolite particles with trimethoxysilane functionalities present on the electrode surface [173], or (2) electrostatic interactions in an LbL deposition, by alternating negatively charged zeolite particles with a cationic polyelectrolyte [174–177].

7.2.4 Analytical Applications

From an electroanalytical point of view, zeolites potentially offer a variety of properties required to an electrode coating: (1) shape, size, and charge selectivity, capable of discriminating the target analyte among different species in solution;

(2) physical and chemical stability typical of inorganic materials; (3) ion-exchange capability, exploitable for analyte pre-concentration in the close proximity of the electrode surface; (4) hydrophilic character, suitable to interact with biological elements for the development of catalytic biosensors. We present here an overview of the main strategies applicable to zeolites, and the approaches followed in the realization of the relevant ZMEs. Only a few representative examples, among all the possible analytes and sensors proposed in the literature, are reported. For a more exhaustive analysis of the papers published in this field, we direct the reader to several reviews concerning these topics [134, 178, 179].

Pre-Concentration of Analytes

As already evidenced, the most meaningful characteristic of zeolites lies in the cation exchange capability. The advantages of using ZMEs, exploited whenever the pre-concentration of the analyte is necessary to improve the sensitivity and the detection limit of the analysis, lies in the size, shape, and charge selectivity imparted by the three-dimensional network: under equilibrium conditions, only positively charged and neutral species, with a dimension suitable to penetrate into the channels of the zeolite coating, can diffuse into the film to undergo charge transfer at the underlying substrate electrode (see Fig. 7.9).

Apart from determination of heavy metal ions [132, 151, 164, 177, 180], which for obvious reasons represents the widest area of applications, some positively charged organic molecules, e.g., dopamine [133, 152], epinephrine [133], and paraquat [160], have also been the object of several studies. Two important aspects have been taken into account in trying to improve the performance of ZMEs in the pre-concentration step. The way in which zeolite is anchored on the electrode surface should be chosen in order to make the pores effectively available to include the target analyte; the immersion time of the electrode in the sample solution must be long enough to induce a marked pre-concentration of the analyte, but not too long to make the chemical regeneration of the electrode surface difficult, due to the supporting electrolyte entrance into the zeolite particles [129].

Indirect Determination

The determination of several non-electroactive species is also possible at ZMEs, by means of an indirect approach briefly schematized in Fig. 7.10a. Zeolite films are ‘doped’ with redox active species, mainly consisting of Cu^{2+}. According to mechanism 2 previously outlined, these species undergo an extra-zeolite charge transfer: the presence in solution of cations possessing dimensions suitable to enter the zeolite pores and presenting competitive affinity for the zeolite causes the expulsion of initially included electroactive species and their reduction at the substrate. The extent of the current is consequently proportional to the concentration of the inert cationic analyte [181].

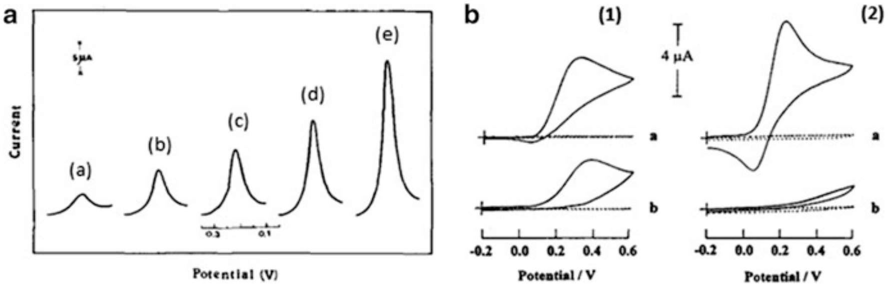


Fig. 7.9 (**a**) Differential pulse voltammograms for a zeolite-modified electrode (ZME) after deposition of 1 ppm Ag^+ solution for different pre-concentration times (*a*) 1; (*b*) 2; (*c*) 3; (*d*) 5; (*e*) 10 min; (Reproduced from Ref. [132] with permission of Elsevier). (**b**) Cyclic voltammograms of 2×10^{-4} M (*a*) dopamine and (*b*) ascorbic acid at (*1*) pure and (*2*) 10 wt.% zeolte-modified carbon paste electrodes (Reproduced from Ref. [133] with the permission of Elsevier)

Apart from the size, charge, and shape, the selectivity of zeolites with respect to a given cationic analyte may also be related to the peculiar affinity of the aluminosilicate toward some species. For this reason, the affinity of the aluminosilicate for each chemical species may differ markedly from one zeolite to another, and the most suitable choice of the electrode material must be carefully evaluated. As an example, the affinity of alkali metal cations for zeolite A decreases in the order $Na^+ > K^+ > Li^+ > Cs^+$, whereas in zeolite Y the corresponding order is $Cs^+ > K^+ > Na^+ > Li^+$. This effect has obvious consequences on the sensitivity and selectivity of the amperometric sensor.

Due to the very fast ion-exchange equilibrium, such an analysis can be performed either in batch or even in flow injection mode [147, 182, 183], as in the example reported in Fig. 7.10. This property allows the application of ZMEs in the frame of detectors for ion chromatography. In this case, the renewal of the electrode that includes more or less high quantities of the analyte may consist of a step in which a short stream of the pristine electroactive species is made flow on the electrode surface to restore the initial capacity of the zeolite. The chromatographic carrier reaching the detection zone either contains a size-excluded cation, as happens in Fig. 7.10, or consists of pure water, as in the case of ion chromatography with a suppression device [156, 183].

Catalytic Reactions

The presence of zeolite at the electrode surface may be, on its own, suitable to catalyze the electron transfer in charge of the analyte, as it happens in the case of 4-nitrophenol oxidation [146]. However, as is easily seen from the examples in Table 7.6, the most frequently followed approach consists of exploiting the

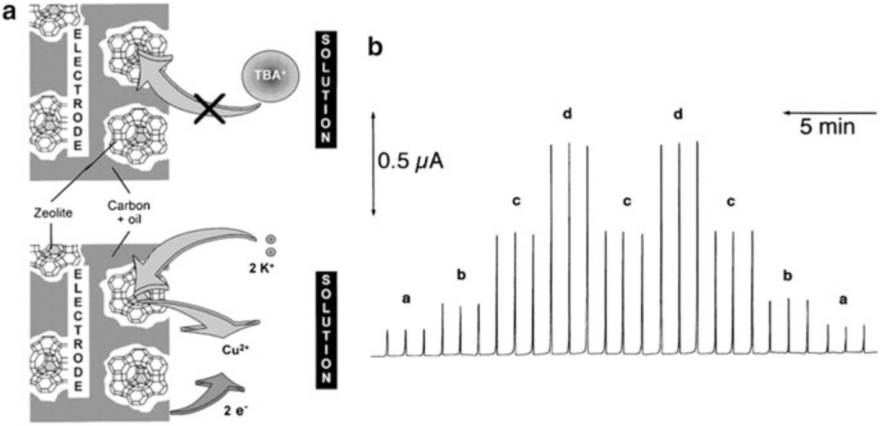


Fig. 7.10 (**a**) Schematic representation of the detection principle for non-electroactive species by means of indirect amperometry at the Cu-doped zeolite-modified electrode (ZME). (**b**) Amperometric responses in flow injection analysis of a Cu^{2+}-doped zeolite Y carbon paste-modified electrode to successive injections of K^+ samples: (*a*) 5.0×10^{-5} M, (*b*) 1.0×10^{-4} M, (*c*) 2.5×10^{-5} M, and (*d*) 5.0×10^{-4} M. Flow rate: 5 ml/min; applied potential: −0.25 V. The dimension of the tetrabutylammonium cation (TBA^+) carrier does not allow it to enter the zeolite and, hence, to interfere with K^+ determination (Reproduced from Ref. [147] with the permission of Elsevier)

ion-exchange capability of zeolites in the incorporation of redox mediators suitable to activate electrocatalytic processes. When small redox active species are inserted within the zeolite, the electrochemical signal takes advantage of the high mobility of these species to reach the underlying electrode surface according to mechanism 2; however, as a consequence of this charge percolation mechanism, these sensors suffer from poor stability, due to partial leaching of the redox mediator. Alternatively, bigger redox active species can also be inserted in the zeolite cages by following the previously mentioned ‘ship in a bottle’ strategy to obtain a higher long-term stability. On the other hand, as already evidenced in the frame of the discussion of mechanism 1, in this case less intense electrochemical responses, strongly affected by ohmic drop, are obtained, due to the insulating character of this inorganic material.

More recently, the upsurge of nanosized materials has encouraged researchers to devote efforts to the formation of several composites in which carbon nanostructures [184, 185] and metal NPs [173, 186] are mixed with zeolite. Similar composites combine the electrocatalytic properties of the nanomaterials with the selectivity imparted by the inorganic structure or, more simply, with the enormous enlargement of the surface due to zeolite material, also suitable to fix nano-objects. In this case, the high conductivity of the nanostructured component overcomes the problem of the insulating nature of the zeolite component.

Table 7.6 Examples of the determination of chemical species with zeolite-modified electrodes (ZMEs)

Analyte	Zeolite	Zeolite assembling	Ref.
4-Nitrophenol	Zeolite mixture	Carbon paste	[146]
Nitrite/nitrate	Ag(I) doped zeolite (clinoptilolite)	Expanded graphite-epoxy composite film	[15]
Ascorbic acid	Methylene blue in mordenite	Carbon paste	[148]
NADH	Ferrocene in zeolite Y	Carbon paste	[149]
L-dopamine	Trinuclear Ru amine complex in zeolite Y	Carbon paste	[150]
H_2O_2	Methylene green – zeolite X	Carbon paste	[153]
Ascorbic acid	Fe(III) doped zeolite Y	Mixed with graphite powder in a polystyrene film	[165]
Ascorbic acid	Cu(II) doped zeolite A	Mixed with graphite powder in a PVC film	[168]
Uric acid	Fe(III) doped zeolite Y	Mixed with graphite powder in a polystyrene film	[167]
Dopamine	2,4,6-Triphenylpyrylium in zeolite Y	Paraloid B72 film	[166]
Cysteine	Co(II) doped zeolite Y	Carbon paste	[155]
Ascorbic acid/dopamine/uric acid/tryptophan	Fe ion-doped natrolite zeolite-multi wall carbon nanotube	Film dispersion in chitosan	[184]
Ascorbic acid/dopamine	Cu(II) doped zeolite A-graphene	Drop casting	[185]
Ethanol	Au NPs – zeolite Y	Zeolite film + Au NP electrogeneration	[186]

Biocatalytic Reactions

Zeolites allow the contemporary immobilization of enzymes and of a suitable redox mediator, in the frame of catalytic biosensors. Several examples of the possible application of ZMEs to biosensing are reported in Table 7.7. Due to the large size of biomolecules compared with the dimension of the pores of zeolites, the biological receptors cannot be included into the inorganic material. Maybe this is why the first example of biosensors based on ZMEs is relatively recent (1995) [159]. However, two main advantages can be identified in the use of ZMEs for the development of biosensors: (1) the hydrophilic character of zeolites, which enables massive substrate penetration into the internal zone of the electrode coating, leading to the exposition of a high level of enzymes to the solution, for active interaction with the analyte [187, 189, 190]; (2) the ability to fix a suitable mediator inside the channels, through an ion-exchange process [159]. Unfortunately, as already highlighted, a serious drawback lies in the progressive leaching of the redox mediator caused by ion exchange with electrolyte cations at the basis of the electrochemical signal. By considering this last aspect, biosensors that do not require the presence of a redox mediator, e.g., cytochrome C or tyrosinase, are more effective. In this case, the ability to use nanosized zeolites [176], possessing particularly a large surface,

Table 7.7 Examples of the determination of chemical species through zeolite-based biosensors

Analyte	Electrode coating	Zeolite assembling	Ref.
Phenol	Methylphenazonium-zeolite Y/tyrosinase	Screen printed	[159]
H_2O_2	Methylene green-zeolite Y/HRP	Polystyrene film	[187]
Glucose	Ferrocene-zeolite/GOD	Carbon paste	[154]
H_2O_2	Zeolite Y/cytochrome C	Polyvinyl alcohol film	[188]
H_2O_2	Zeolite L – PDDA[a] multilayer/cytochrome C	LbL	[175]
Phenol	β-nanozeolite – PDDA[a] multilayer/tyrosinase	LbL	[176]
H_2O_2	Zeolite/myoglobin or hemoglobine	LbL	[177]
NO_2^-	Zeolite/myoglobin or hemoglobine	LbL	[177]

[a] Poly(diallyldimethylammonium chloride)

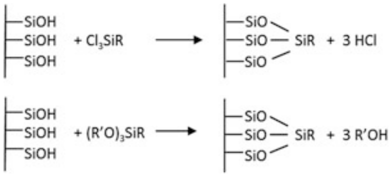
capable of stably fixing a very high number of biological elements on the electrode surface, is quite interesting.

7.3 Additional Silica-Based Materials

Silica-modified electrodes (SMEs) attracted the interest of scientists much later than the previously described materials. Although silica has long been widely used in chromatographic columns, acting as the support material to so many stationary phases, the use in electroanalysis only goes back to the middle of the 1990s. Actually, silica presents very interesting properties for electroanalytical applications: (1) the adsorption capacity due to acid/base character, suitable to pre-concentrate chemical species at the electrode surface; (2) the possibility to covalently link an enormous variety of functional groups acting, for example, as the actual redox mediators; (3) the thermal stability; (4) the ability to obtain the modified electrodes through very easy and cheap synthetic procedures. The frequent use of SMEs in the late 1990s was due to the development of *sol-gel* materials [191, 192], which allow the stable deposition of many redox mediators on the electrode surface, and the encapsulation of enzymes without alteration of the biological activity [193]. In recent years, the interest for silica-based materials has renewed thanks to the development of ordered mesoporous structures [179, 194], which represent a new class of materials possessing a well defined three-dimensional structure, consisting of channels with various configurations, e.g., lamellar or hexagonal, possessing dimensions ranging from 2 to 50 nm. Similar to what has already been discussed in the case of ZMEs, such a structure leads to a very large electrode surface, with the ability to adsorb a high number of species, either the analyte in the sample solution or the most suitable recognition element. It is clear that, for many aspects, these materials strictly recall what has already been discussed for zeolites; for this reason, many common aspects are discarded here. One of these similarities consists of the total absence of any electrical conductivity, so that the strategies for making these materials suitable to constitute electrodes should provide a way to improve charge transfer between the solution coating interface and the underlying conductive substrate. In the following, we discuss the different classes of materials and the relevant properties and applications, together with the main strategies followed to realize the electrode system.

7.3.1 Silica Gel

Pure SiO_2 gel particles, generally simply dispersed inside a carbon paste matrix, can be used for the development of amperometric sensors; examples are listed in Table 7.8. Similar modifications primarily take advantage of the chemical reactivity of silanol groups, capable of adsorbing different organic and inorganic species. Such interactions can be exploited for the accumulation of the target analyte at the electrode surface, prior to the voltammetric quantification [194–198]. At the same time, the reactivity of silica materials can be modulated in a way very similar to that used for the construction of a wide series of stationary phases in chromatography, i.e., by covalently fixing different molecules possessing functionalities suitable for the specific application sought [199, 201]. The reaction is generally carried out with chloro- and alkoxy-organosilanes:



Functionalization of the silica surface can be also obtained with inorganic derivatives [200, 202, 204, 205], acting as redox mediators in electrocatalytic reactions involving the analyte; chloride derivatives are also used in this case, thanks to the ability to react with the silanol groups on the particle surface:

$$n \equiv \mathrm{SiOH} + \mathrm{MCl}_x \rightarrow (\equiv \mathrm{SiO})_x\mathrm{MCl}_{(x-n)} + n\mathrm{HCl}$$
$$(\equiv \mathrm{SiO})_x\mathrm{MCl}_{(x-n)} + (x-n)\mathrm{H_2O} \rightarrow (\equiv \mathrm{SiO})_n\mathrm{M(OH)}_{(x-n)} + (x-n)\mathrm{HCl}$$

where M is a generic metal ion.

Concerning amperometric biosensors, SiO_2 coatings have been generally used as such, i.e., without the need for a proper redox mediator [203, 204]. The advantage of the use of such an electrode material lies in the enlargement of the surface area suitable for enzyme deposition, making a larger level of biological receptor easily available for biocatalytic reaction. In this respect, titanium functionalized silica gel, obtained by the sequence of reactions written above, where M = Ti, can be advantageously exploited. In fact, it is suitable to achieve higher stability of the enzyme on the electrode surface, thanks to the good affinity of TiO_2 for the carboxylic groups of the terminal amino acid residues of the enzymes [204]. A similar strategy has also been adopted for the development of biosensors based on different siliceous structures [206].

Table 7.8 Examples of amperometric sensors consisting of silica particles differently fixed at the electrode surface

Analyte	Electrode coating	Analytical strategy	Ref.
Metamitron (herbicide)	SiO_2 in carbon paste	Pre-concentration	[195]
Cu(II)	SiO_2 in carbon paste	Pre-concentration	[196]
Hg(II)	SiO_2 in carbon paste	Pre-concentration	[197]
Insulin	SiO_2 in carbon paste	Pre-concentration	[198]
$H_2C_2O_4$	Co(II) tetrasulfophthalocyanine adsorbed on 3-N-propylpyridinium chloride functionalized silica gel in carbon paste	Electrocatalysis	[199]
Hydrazine	Ni(II) tetrasulfophthalocyanine on titanised silica gel in carbon paste	Electrocatalysis	[200]
O_2	Cu(II) complexed with 3-aminopropyltriethoxy silane immobilized on silica gel incorporated in carbon paste	Electrocatalysis	[201]
O_2	Co(II) porphyrin immobilized on Nb_2O_5-functionalized silica gel in carbon paste	Electrocatalysis	[202]
Glucose	GOD on a SiO_2 film	Bio-catalysis	[203]
Phenol	HRP on titanised silica gel in carbon paste	Bio-catalysis	[204]
L-lactate	Lactate oxidase and meldola's blue adsorbed on SiO_2 particles coated with niobium oxide and dispersed in a carbon paste electrode	Bio- and electro-catalysis	[205]

7.3.2 *Sol-Gel*

Sol-gel materials combine the high versatility of silicates previously described with the high stability and durability typical of solid electrodes or solid electrode coatings [207, 208]. Sol-gel synthesis is a very simple process, taking place through condensation reactions generally starting from alkoxysilane derivatives. The usual whole procedure for the formation of the suitable material constituting the electrode for amperometric sensing can be schematized in four main steps:

1. *Hydrolysis*

$$Si(OR)_4 + 4H_2O \leftrightharpoons Si(OH)_4 + 4ROH$$

Both tetraethoxysilane (TEOS) and tetramethoxysilane (TMOS) are used as precursors for sol-gel formation. The hydrolysis reaction generally takes place in a water–alcohol mixture and is usually catalyzed either by acid or by basic aqueous solutions.

2. *Condensation*

$$\mathrm{Si(OH)_4 + Si(OH)_4 \leftrightarrows (OH)_3Si\text{-}O\text{-}Si(OH)_3 + H_2O}$$
$$\mathrm{Si(OH)_4 + Si(OR)_4 \leftrightarrows (OH)_3Si\text{-}O\text{-}Si(OR)_3 + ROH}$$

   These reactions give rise to the formation of molecular clusters evolving to a sol, due to progressive formation of a cross-linked structure characterized by siloxane bonds involving different structural units.

3. *Gelification*

   In this step, colloidal particles link to one another to form a three-dimensional network. During this step, different kinds of redox active species [209] and/or biomolecules [210–212] can be stably inserted into the inorganic network to find stable inclusion inside the electrode coating; alternatively, particularly stable inorganic species can also be added to the initial reactant mixture [213]. The physical contact between these active (bio)molecules and species in solution occurs thanks to the hydrophilic nature of the silica material when using suitable alkoxysilane derivatives. It is obvious that, thanks to such a characteristic, many bio(catalytic) sensors have been developed based on the sol-gel deposition technique. During the gelification process, graphite grains can also be added to increase the conductivity of the resulting material. Alternatively, metal NPs can play such a role [214, 215], also thanks to their effective electrocatalytic properties toward different species [214–216].

   After the addition of all the components necessary to obtain an electrode surface with the characteristics sought, sol-gel can be differently deposited onto a solid electrode surface for the formation of a film [211, 216, 217] or modeled in various configurations including bulky cylinders, rods, or fibers [218]. Alternatively, sol-gel-modified electrodes are produced by the screen-printing fabrication technique [210, 214].

4. *Solvent evaporation*

   This process results in the formation of a xerogel and, subsequently, of a solid electrode coating or electrode body, depending on the procedure followed in the previous step.

As is evident from the description, bulk sol-gel electrodes resemble carbon paste electrodes in many aspects [219, 220]:

1. They allow easy and stable inclusion of effective redox mediators or of specific functionalities, imparting the electrode selective interactions with the target analyte.
2. They may possess a certain degree of permeability toward species from the solution.
3. They can exploit the peculiar reactivity of silica functionalities described above.
4. Their surface can be rapidly renewed by simple mechanical polishing.
5. They are particularly robust so they do not require any organic binder, which could affect the electrochemical response.

It is worth mentioning that beside the ‘classic’ synthetic approach followed for sol-gel synthesis, a different method has also been proposed [221]; in this case, the silanization is activated by an ultrasonic cavitation, so that hydrolysis is promoted in the absence of any additional solvent and the time needed for the reaction to occur is reduced drastically. For the synthetic conditions adopted, this material is called sono-gel, and the mixture of sonogel with graphite grains leads to highly conductive electrodes. Furthermore the properties of the electrode can be suitably addressed by the addition of specific chemical species during the gelification step [222].

Organic functionalities can also be included in the sol-gel by formation of true covalent bonds [206]; in this case, effective organic–inorganic hybrid materials are obtained. These materials are generally referred to as ORMOSILs (ORganically MOdified SILicates). They can easily be synthesized using suitably functionalized alkoxysilanes, $R'_n Si(OR)_{4-n}$ (where R is an alkyl chain, R′ a suitable organic or metallorganic functionalization, and n ranges from 1 to 3), as the precursor or one of the precursors for sol-gel reactions. The use of ORMOSILs can be exploited in different situations: (1) to tune the hydrophilic/hydrophobic character of the material, i.e., the wettability of the electrode coating and the consequent diffusion of species from the solution [222]; (2) to stably fix suitable electrocatalytic centers able to impart the material specific properties [216–218]; (3) to introduce specific functionalities able to selectively adsorb the target analyte from a mixture of species in the solution [223, 224]; (4) to induce stable deposition of enzymes acting as the selective recognition elements at the electrode/solution interface [225, 226].

In any case, it is clear that, for its own nature, sol-gel material is suitable for the formation of many different composite materials [207]: inorganic and organic polymers, as well as many different kinds of nano-objects possessing effective electrocatalytic properties, can find stable inclusion into a host matrix possessing all the characteristics previously listed. The most simple approach consists of adding the filler to the siliceous matrix after sol-gel formation. However, composite materials can also be obtained by synthesizing the sol-gel film in the presence of the precursor of the filler, e.g., a monomer [227] or a metal complex [228]. The synthesis of the composite material is finalized in a subsequent step, e.g. by a chemical oxidation or reduction, leading to polymer chains or metal nanoparticles, respectively, included inside the sol-gel matrix.

A few examples of the huge number of applications of sol-gel-modified electrodes in electroanalysis are listed in Table 7.9, which also tries to pick up the advantages of such a material in different analytical contexts.

Mesoporous Silica

As already mentioned, the evolution of silica to a mesoporous three-dimensional structure has more recently attracted the interest of the scientific community [179, 207, 232]. These materials are obtained by carrying out the sol-gel synthesis in the

Table 7.9 Examples of sol-gel-modified electrodes in electroanalysis

Analyte	Electrode coating	Analytical strategy	Ref.
Cr(VI)	Pyridine functionalized sol-gel	Pre-concentration	[224]
Fe(II)	4,7-dimethyl-1,10-phenanthroline and graphite powder in sol-gel	Pre-concentration	[229]
Ni(II)	Dimethylglyoxime and graphite powder in sol-gel	Pre-concentration	[230]
NO_2^-	Mo_8O_{26} and graphite powder in sol-gel	Electrocatalysis	[213]
L-cysteine/ glutathione	$Ru[(tpy)(bpy)Cl]PF_6$ and carbon grains in sol-gel	Electrocatalysis	[231]
Glucose	Au NPs fixed in a 3-mercaptopropyl functionalized sol-gel	Electrocatalysis	[216]
Ascorbic acid/ uric acid	Methylene blue in sol-gel film	Electrocatalysis	[217]
Dopamine	Ag^+ fixed in 3-mercaptopropyl functionalized sol-gel	Catalysis	[218]
Glucose	GOD, ferrocene, and graphite powder in sol-gel	Bio-catalysis	[214]
Cholesterol	Cholesterol oxidase and HRP in sol-gel	Bio-catalysis	[211]
H_2O_2	HRP on AuNP grafted on a thiol functionalized sol-gel	Bio-catalysis	[225]
Anti-immuno-globulin G	Immunoglobulin G and graphite powder in sol-gel	Antigen-anti-body affinity	[210]
NO_2^-	Cytochrome C nitrite reductase in sol-gel on pyrolytic graphite electrode	Bio-catalysis	[212]
L-lactate	Lactate dehydrogenase covalently linked to carboxylic functionalized sol-gel	Bio-catalysis	[226]

presence of surfactant micelles, acting as templates for the attainment of pores and channels with well defined and uniform structure; they are subsequently removed from the material through extraction procedures. Due to the exclusively synthetic nature of this class of materials, their names are acronyms, originating from the laboratory in which they were first synthesized (for instance, MCM is for Mobil Composition of Matter). As an example of the synthetic procedure adopted for the formation of this kind of material, Fig. 7.11 reports the synthesis of MCM-41, consisting of regular hexagonal channels.

Depending on the nature of the surfactant and on the synthetic conditions chosen, it is possible to synthesize a number of mesoporous materials possessing sieving properties and enormous enlargement of the specific surface area typical of zeolites, together with a marked reactivity typical of silica-based materials, which allows stable anchoring of suitable organic groups. Contrary to zeolites and to sol-gel, the inorganic framework is characterized by a more uniform three-dimensional structure, with pores and channels possessing a mean diameter from 2 to 50 nm; this structure allows a higher number of binding sites to be reached by species from the solution [233]. A direct consequence of this feature is the ability to

Fig. 7.11 Schematic representation of the synthesis of MCM-41, involving the condensation of tetraethoxysilane (TEOS) in the presence of surfactant molecules (●⁓)(Reproduced from Ref. [207] with the permission of Royal Chemical Society)

obtain higher sensitivity for sensors made by this material, compared with those of amorphous character [179, 207].

Mesoporous silica can be functionalized according to approaches very similar to those already discussed for sol-gel [207]. Apart from the exploitation of the weak interactions with the acid Si-OH terminal groups, the formation of more stable covalent Si-C bonds appears more promising. This process can be carried out either through a post-synthesis grafting of organo-silane reagents or by performing the sol-gel synthesis with an organoalkoxysilane and tetraalkoxysilane mixture. This second approach allows for a better control of the composition of the material and of the distribution of the organic functionalities; on the other hand, it may limit the order of the three-dimensional structure at high concentrations of organosilane derivative.

Similar to that discussed previously for other Si-based materials, the formation of modified electrodes based on mesoporous silica requires specific strategies to improve the conductivity of the coating [179]. In many electroanalytical applications, mesoporous (organo)silica particles are dispersed inside a carbon paste or, alternatively, inside a carbon ink for the deposition of screen-printed electrodes. At the same time, deposition onto a solid electrode surface under the form of a thin film is also a frequent practice. The direct deposition of silica particles from a suspension containing the particulate material is probably the simplest, and more used, approach. The stability of the film is generally enhanced by dispersing Si particles into a polymer coating or covering a layer of Si particles with a polymer coating. Obviously, in this case, carbon particles may also be added to improve the conductivity. The formation of similar films allows the attainment of coatings possessing the stability typical of polymer coatings; however, the organic binder can negatively affect the performance gained.

Alternatively, thin layers of mesoporous silica can be prepared in a single step by a so-called evaporation-induced self-assembly (EISA) procedure [234]: the sol solution is deposited onto the electrode surface, and water evaporation occurs concomitantly with micelle formation and condensation of the material; the template surfactant is extracted after that complete dryness is reached. A well defined voltammetric response is obtained at the resulting electrode system, due to the species that can diffuse throughout the coating after surfactant removal.

More recently, Walcarius et al. [235] developed a new approach to obtain mesostructured silica films: electro-assisted self-assembly (EASA). This was developed by exploiting a very simple electrochemical method: the application of a suitable negative potential to a hydrolyzed sol solution also containing the surfactant generated OH^- species in the close proximity of the electrode surface. Condensation of the silane precursors is thus induced, progressively leading to the formation of the inorganic coating with a well packed mesoporous structure growing perpendicularly to the electrode surface. This particular structure facilitates mass transport throughout the film.

Since the electroanalytical strategies involving mesoporous silica materials are very similar to those already described for SiO_2 and sol-gel based electrodes, for specific examples of applications we redirect the reader to exhaustive reviews [179, 207, 232].

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