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# Chemically modified electrodes as amperometric sensors in electroanalysis

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ABSTRACT. The state and prospects in the development of new fields of electroanalytical chemistry, namely amperometric sensors based on chemically modified electrodes, are examined. The methods for the construction of these electrodes, the types of modifying agents, and the mechanisms of their response to substrates are discussed. The analytical possibilities of chemically modified electrodes, including amperometric biosensors based on them, in the solution of problems associated with ecology, medicine, and pharmacology are demonstrated.

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## I. Introduction

The conviction that success in research and analysis is associated with the degree of purity of the surfaces of electrodes has held sway in electroanalytical chemistry for a long time. Specialists in electroanalysis endeavoured to achieve an ideal insert surface reproducible from measurement to measurement. Such a surface was provided by a mercury drop flowing from a capillary (mercury dropping electrode). The creation of the mercury dropping electrode gave rise to a renaissance in voltammetry and related methods. However, there exist a number of factors which hinder the extension of voltammetry in various practical applications:

the laborious nature of the electroanalytical methods associated with the use of the mercury dropping electrode;

the toxicity of the material of this electrode;

the difficulty of interpreting the results of measurements in cases where the adsorption of the depolariser and intermediate species, the effect of impurities, and the effect of the structure of the electrical double layer, etc. complicate the electrochemical process.

Apart from mercury, other materials and compositions have been used for electrodes.[2] Among them, the so called chemically modified electrodes (CME) have gained the greatest recognition. The study of the American investigators Miller and Van de Mark[3] on the application of an electrode coated with an electrically conducting polymer film was published in 1978. During modification, a chemical compound or polymer film is deposited in a special way on the electrode surface. This alters its capacity for the voltammetric response as a result of the appearance of new electrocatalytic properties. Electrocatalytically active compounds, for example metal, complexes with organic ligands or certain organic compounds capable of functioning as ligands, or enzymes are "bound" to the electrode surface either chemically (covalently), or as a result of adsorption, or by incorporation into the polymer film. The "contamination" of the electrode surface, with which electroanalytical chemists struggled in earlier days, came to be used beneficially and purposefully under control. The quality of the modification is monitored as carefully as the purity of the electrode surface has been estimated previously.

In principle, the concept of the "chemically modified electrode" can be treated in a broad sense. For example, any electrode the molecular structure of the surface of which has been acted upon appropriately (chemically or physically), altering thereby the electrochemical and in particular the amperometric response, can be included among chemically modified electrodes. In a recently published monograph,[4] certain problems of the modification of electrodes by polymer films, their electrocatalytic properties, the mechanisms of charge transfer through these films, and also their use in organic electrochemistry were discussed in detail.

Chemically modified electrodes can be used for various purposes. Thus, stereospecificity in electrosynthesis can be achieved with the aid of such electrodes. This was observed, for example, when cyclodextrins were used as modifying agents.[5] Chemically modified electrodes have been used[6–8] for electroanalytical purposes. Since the voltammetric cell with two electrodes can be regarded as the prototype of the amperometric sensor, the interest in chemically modified electrodes can be explained by the endeavour to construct on the basis of such a cell a kind of sensor with a specific amperometric response to a particular substance—the substrate. Thus the search for a new type of chemically modified electrodes led to a new chemical sensor. The growing stream of information about this field has now led to the accumulation of such a large amount of data that the discussion of all the characteristic features of the operation of chemically modified electrodes by modification methods is impossible in a single review. We shall therefore consider only the principal aspects of studies in the field of chemically modified electrodes and their practical applications as specific amperometric sensors.

## II. Amperometric sensors based on chemically modified electrodes

### 1. Methods for the modification of electrode surfaces

As noted above, the modification of an electrode can be achieved by acting in different ways on its current conducting surface. The chemical compound (the modifying agent) is usually deposited in a special way on the electrode surface, imparting to the electrode new electrocatalytic properties. The following procedures are used for the immobilisation of the catalyst:

irreversible adsorption on the electrode surface;

chemical binding with fixation of the catalyst via different groups with formation of covalent bonds;

incorporation in a polymer film;

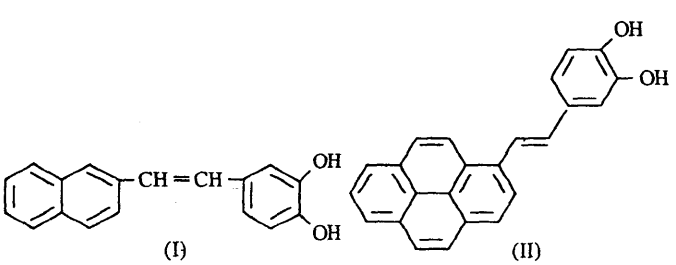
inclusion of various substances, including inorganic materials, in a paste made up of graphite materials.

Modified surfaces containing the so called sensor layer are usually subdivided into three groups depending on the nature of this layer. These can be (1) thin layers of a low-molecular-weight compound, (2) polymer films, and (3) inorganic films. It is not always possible to draw a clear-cut boundary between the groups listed above.[7]

Thus, immobilisation on the electrode surface or in its bulk phase results in the fixation of electrochemically active groups, species, or definite microstructures. The molecules of the modifying agent or its species retain their original nature, i.e. their chemical and physicochemical properties, which they possessed whilst in solution. A number of reviews have been devoted to methods for the immobilisation of modifying agents.[9,11]

The simplest modification procedure is physical or chemical adsorption. Its advantage consists in the fact that it does not require special reagents for the grafting of the catalyst; it is sufficient to activate and purify the electrode surface before depositing the modifying agent. However, its lifetime on the surface is relatively short and it gradually escapes into the solution. Various procedures increasing the retention time of the catalyst on the electrode have been described; they were developed mainly for the immobilisation of various biological materials in the creation of biosensors for a particular substrate.[11] The adsorption of polycyclic aromatic compounds on carbonaceous materials can serve as an example.[10,12] It is based on the principle of the structural similarity of the benzene rings and the hexagons on the carbon surface. Furthermore, adsorption is promoted by the low solubility of these compounds in aqueous solutions.

Adsorption usually results in the immobilisation of several layers of the organic modifying agent, for example, in the case of catalytic oxidation of NADH in the catechol [compounds (I) and (II)]–quinone [the oxidised forms of compounds (I) and (II)] system. The quinones serve as a mediating oxidising agent adsorbed on the graphite electrode. With increase in the number of aromatic rings in the catechols, the degree of their adsorption increases.[10,13]




(I) (II)

The modifying agent was retained on the electrode surface as a result of adsorption forces at a particular potential or its solution was deposited on the electrode surface with the aid of a micropipette with subsequent evaporation of the solvent.[10] This method is satisfactorily reproducible.

In order to increase the surface area of the platinum electrode, the latter is coated with platinum black. This procedure has been extended to retain enzymes as catalysts in biosensors, which will be discussed below.

A more stable immobilisation of the modifying agent can be ensured with the aid of covalent binding.[9,11] Before the chemical binding of the modifying agent, the electrode surface is activated. It is known that the surfaces of carbonaceous materials contain, after a special treatment, quinonoid, carboxy-, and phenolic groups,[2] which are capable of forming covalent bonds, for example, the amide bond, with suitable groups in the modifying agent. Amidation has been used to graft to the surface of a carbon electrode a series of aromatic amines serving as oxidants for ascorbic acid.[14,15] In this case, the carboxy-groups on the electrode surface initially react with thionyl chloride or acetyl chloride with formation of the C–Cl covalent bond which is then converted into an amide bond as a result of treatment with an amine:[6]

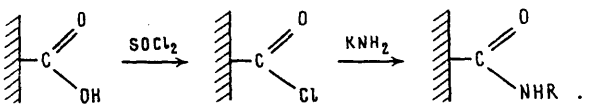



Fig. 1 illustrates schematically the modification of an electrode with the aid of covalent grafting.

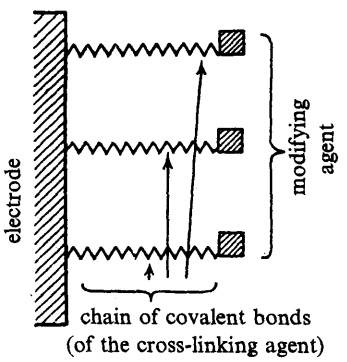
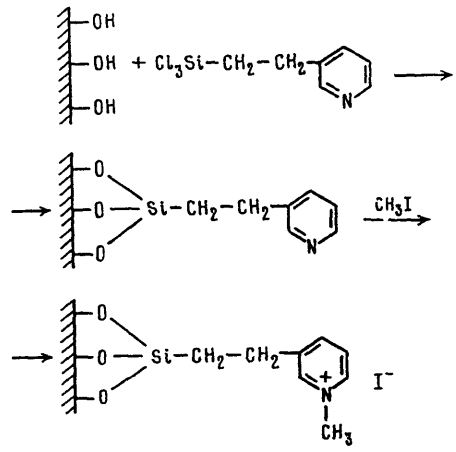



**Figure 1. Schematic illustration of an electrode modified by the covalent grafting of a reagent.**

Other examples of covalent binding are silanisation and the formation of ether and C–C bonds.[6,16] In this case treatment of a metallic electrode containing oxides with strong acids leads to the

formation of numerous centres with hydroxy-groups, which then interact with chloro- or alkoxy-silanes:



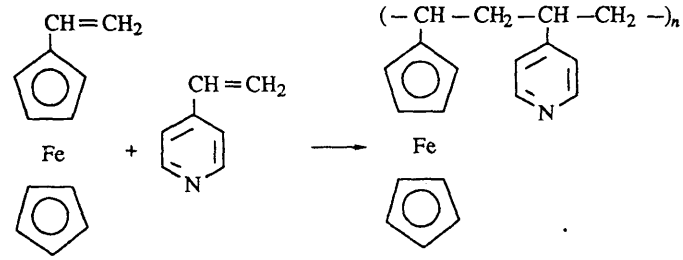
After quaternisation with the aid of methyl iodide, electrode surfaces with new properties are obtained. The coating of the electrode with such a film makes it possible to protect its surface against contamination by, for example, serum proteins, which is particularly important in immunoenzyme analysis.[17] The resulting film acquires ion-exchange properties, by virtue of which it is capable of incorporating in its structure the transferring agent anion $Fe(CN)_6^{4-}$ responsible for the analytical signal. The methods for the activation on the surface of the electrode itself (chemical, thermal, and electrochemical) have been examined in a review.[6]

Procedures for the activation of the surface with subsequent immobilisation of the reagent (catalyst) by covalent binding have been described.[11] Such an activation procedure is used mainly in the construction of biosensors,[11] but it can also be applied to other chemically modified electrodes.

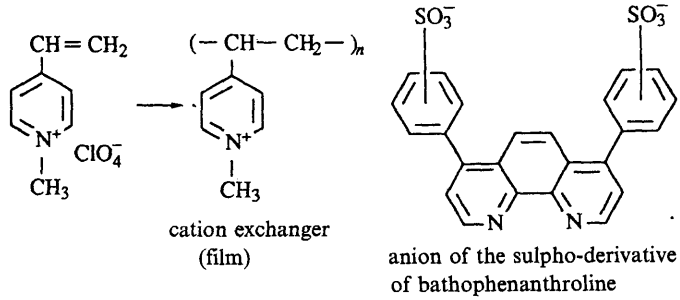
The commonest method for the immobilisation of the reagent is apparently its retention in a polymer film.[3,18–21] For example, an oxidation-reduction modifying agent can be bound to a polymer film on the electrode as a result of the electrostatic interaction of a polyanionic or polycationic electrolyte with a reagent carrying the opposite charge. This procedure is frequently employed to immobilise multicharged ions, for example, $IrCl_6^{2-}$,[22] $Ru(bipy)_3^{2+}$, $Co(bipy)_3^{2+}$, $Ru(NH_3)_6^{3+}$,[23] and $Fe(CN)_6^{4-}$,[24] where bipy represents bipyridyl.

The commonest polyelectrolytes of this kind are perfluorinated Nafion and its analogues.[25,26] In the first studies on chemically modified electrodes, electroactive polymers of the type of polyvinylferrocene were used for the modification. Subsequently, it was noted that polymer films make it possible to achieve reactions and processes such as ion exchange, membrane separation, deposition, coordination, and acid–base and oxidation-reduction equilibria on which analytical chemistry is based. The electroactive fragments of polyvinylferrocene responsible for the reversible electron transfer are the monomer units. In other films, the monomer units perform the function of ligands in reactions with metal ions. For example, the ruthenium(III) complex of EDTA is retained by coordinate bonds in a polyvinylpyridine film.[27]

The polymer film may be polyfunctional, for example, it may contain a complex-forming group in addition to the oxidation-reduction couple. The former binds selectively a particular metal ion, while the redox couple ensures contact with the electrode surface. The copolymer of vinylferrocene (ensures the presence of redox centres) and vinylpyridine (vinylbipyridyl, vinylphenanthroline) can serve as a film of this kind for chemically modified electrodes:[28]



In certain cases, the modifying agent is retained in the polymer film as a result of an ion-exchange interaction: for example, between the anionic form of the modifying agent and the cation-exchange resin:



cation exchanger (film)

anion of the sulpho-derivative of bathophenanthroline

The polymer film may contain several tens of thousands of electroactive centres.[29] The electrical conductivity of the films ensures the transfer of electrons and ions. Polymer films with bound electroactive centres (metal ions or complexes) conduct current as a result of electron exchange between the reduced and oxidised forms of these centres. The thicker the films, the greater their stability and the greater the catalytic effect and the corresponding amperometric signal. However, the coordination of a particular metal ion and the transport of charges may be hindered in films of this kind. The oxidation-reduction centres may be incompletely involved in electrocatalytic steps in thick films even at a low rate of change of the polarising potential.[30]

The electron transfer in chemically modified electrodes is influenced by the structure of the polymer and the disposition of the electroactive fragments in the polymer chain. The formal redox potential of the electroactive fragment depends on its local environment in the polymer structure. The morphology of the polymer film is influenced by the solution and the supporting electrolyte. Films characterised by a high ion-exchange capacity, insoluble in water, and swelling in aqueous solutions have been recognised as promising. Although strongly swelling films are most suitable for chemically modified electrodes because electron transfer in them is fastest, nevertheless they can partly dissolve, particularly if the polymer has a linear structure. In order to prevent this, cross-binding with the aid of bifunctional reagents (for example, glutaraldehyde) is used. The modifying agent molecules are then bound to the polymer, the electrode surface, and to one another (Fig. 2). This method is used in those cases where a long-lived

chemically modified electrode and the stability of its sensor component are required.

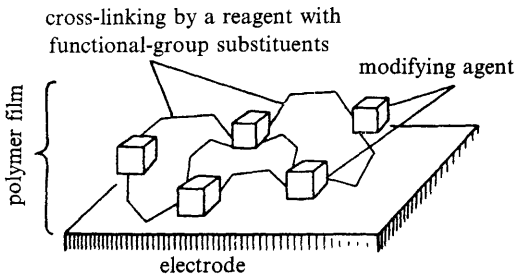



**Figure 2. Schematic illustration of an electrode whose surface has been coated by a film with a cross-linked modifying agent.**

Polyelectrolyte coatings are formed either by immersing the electrode in a solution (films of the Langmuir–Blodgett type) or by evaporating aliquot parts of the polyelectrolyte solution deposited on the electrode surface. The polyelectrolyte is then retained on the surface by adsorption forces. Electropolymerisation is also used to coat the electrode surfaces with films.

The immobilisation of modifying agents in the paste of a carbon-paste electrode (CPE) merits special attention because of the simplicity and ease of the method of preparation of the electrode and convenience in practical use. The modifying agent together with the carbon powder and the binding hydrophobic filler (oil) are carefully mixed in specified proportions until a homogeneous mixture is obtained and the latter is then placed in the cavity of the carbon paste electrode. The surface of such a modifying electrode is readily renewed mechanically and the amount of the modifying agent can also be readily monitored. The carbon paste electrode can be regarded as an alternative to the mercury dropping electrode (m.c.e.) in view of the ease of the renewal of its surface.

Towards the middle of the 1980s, inorganic materials as modifying agents for chemically modified electrodes attracted the attention of investigators. The layers on the electrode surface, based mainly on zeolites as well as various clays, metal oxides, silicates, etc., exhibit both chemical (in relation to the action of strong acids, oxidants, and other reagents) and thermal stability.[31]

Electrode films made up of zeolites contain three-dimensional rather than two-dimensional structures. The use of the pore dimensions in zeolites for the recognition of particular molecules or ions (the sieve effect), the possibility of discrimination among the latter as regards charge, and also combination of electrocatalysis and heterogeneous catalysis impart extremely attractive properties to such chemically modified electrodes. The cation-exchange capacity of the zeolites, which can be regulated, is also important in such cases. The combination of these factors ensure a fairly high selective response by the chemically modified electrodes. The use of commercial zeolite powders[32–34] has been described: alumino-silicates, aluminophosphates, silicatoaluminophosphates (M = Li, Mg, Be, or a transition metal). The zeolite matrix in the film on the chemically modified electrode plays the role of the "host" for the concentration or retention of a molecule or other species of suitable size ("guest"), which in the case of subsequent reactions and electrodes generate the analytical signal.

A suspension of zeolite powders in solution in a polymer is used to coat the electrode surface. After the deposition of a drop of such a mixture on the electrode, the solvent is evaporated. The retaining film-forming agent is usually polystyrene. The reproducibility of the surface of such a chemically modified electrode does not present special difficulties. Mixtures of zeolites and electrically conducting powders are also promising. The carbon paste composition (the carbon paste electrode) is convenient in this respect. Composite electrodes based on a mixture of a zeolite with a carbon powder and a polymer have also been described. The zeolite and carbon powder particles are then retained with the aid of a copolymer of styrene and a cross-linking reagent (divinylbenzene). The electrode chemically modified with the aid of such a composite mixture has a hard surface which can be readily polished and reproduced. The response time of such a chemically modified electrode is shorter than that of the carbon paste electrode.

It is appropriate to note that the guest–host concept in the mechanism of the response of chemically modified electrodes can be productive not only in the case of zeolites as modifying agents. A wide variety of macrocyclic compounds, forming stable complexes with metal ions, ensure a fairly high selectivity of the determination when present in the sensor component of the chemically modified electrode. This will be discussed below.

## 2. Electrocatalysis on chemically modified electrodes

It is known that by no means all organic substances are electrochemically active in the accessible range of potentials. Furthermore, the electrode reactions of some of them occur with a high overpotential and the analytical signal under these conditions can acquire a distorted form and can be poorly reproducible. One of the attractive approaches for the elimination of these undesirable phenomena consists in the use of mediators (M) which transport electrons between the electrode and a particular compound (substrate). The mediator species M and M* are components of a reversible oxidation-reduction system capable of subsequently reacting chemically with the substrate S under homogeneous conditions, i.e. in solution:

$$M \pm e \rightleftharpoons M^*, \quad (1)$$

$$M^* + S \rightleftharpoons M + S^* \quad (2)$$

$$S^* \longrightarrow P \text{ (products)}. \quad (3)$$

Thus it is possible to eliminate or greatly diminish the influence of heterogeneous factors (for example, the orientation of the substance at the electrode surface, the effect of the electrode field, the roughness and cleanliness of the surface, etc.) on the rate and degree of completion of the electrochemical reaction of the substrate S. The experimentally observed increase in the mediator current in the presence of the substrate compared with the current in its absence is a source of information about the homogeneous reaction occurring.[10] The substrate can be involved in this way in the electrochemical reaction at the normal potential of the M/M* couple and the overpotential characterising the electrode reaction of the substrate can be reduced. Since M* is regenerated, the homogeneous electron transfer can be included among catalytic processes.

Catalytic processes with subsequent homogeneous oxidation-reduction reaction are described by the familiar mechanism (Fig. 3).[35]

A necessary condition for the occurrence of the homogeneous electron transfer reaction is the stability of M* and the irreversibility of the conversion of the substrate S. Direct electron transfer to S is

characterised by an overpotential determined either by the slowness of the electron transfer stage or by the influence of the subsequent chemical reactions. The mechanism of the homogeneous reaction (2) can be various (Fig. 3). If the reversible M/M* couple functions merely as an electron transferring agent, then one is dealing with oxidation-reduction catalysis[35] or with a homomediator system.[36] On the other hand, if the adduct M*S is forming during the reaction and then decomposes with regeneration of M, then one is dealing with chemical catalysis[35] or a heteromediator system.[36] The pathways in the chemical reactions of the adduct M*S are determined by the experimental conditions (electrode, solvent) and therefore influence the process selectivity.

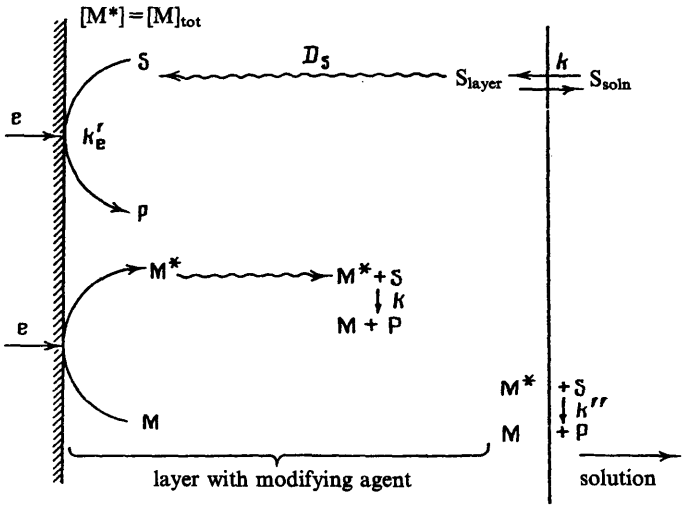



**Figure 3. Schematic representation of the electrochemical process on a chemically modified electrode with a polymer coating.**

The thermodynamic condition for the occurrence of homogeneous catalysis is that the standard potential of the catalyst couple $E^0_{M/M^*}$ should be more negative (positive) than the potential of the electrode substrate reduction (oxidation) reaction $E^0_{S/P}$, where $E^0_{S/P} - E^{1/2}_S$ is the overpotential. Under these conditions, the regeneration of M depends on the rate of the irreversible reaction (3) (Fig. 3), which shifts the equilibrium in reaction (2) to the right. The irreversible reaction (3) can represent a combination of several stages, which sometimes influence the catalytic current.

Saveant and co-workers[35] considered the difference between the potentials $E^0_{M/M^*}$ and $E^{1/2}_S$ to be the principal factor determining the catalytic effect. This conclusion is based on an experimental fact—the increase in the catalytic current with decrease in $\Delta E$ for a number of systems. It is assumed that the rate of the homogeneous transfer is higher the closer the energies of the lowest unoccupied molecular orbitals of the transferring agent and the substrate. The rate of homogeneous electron transfer is appreciable already at $E = 0.2-0.7$ V.

The use of the phenomenon of homogeneous electron transfer in electroanalysis stimulated the search for new ways of immobilising the mediators on a current-conducting surface in order to create amperometric sensors for a specific substrate. The methods for the modification of electrodes examined above ensure a high local concentration of the oxidation-reduction centres for a low overall amount of the catalyst and make it possible to consume the latter economically and to avoid special operations involving the separation of the catalyst from the reaction products.

In the study of the amperometric response of chemically modified electrodes with an immobilised mediator, it is possible to establish the influence of immobilisation on the electrochemical parameters of the mediator, the distribution of redox centres within the coating, and the location where the catalytic reaction occurs, to find the relation between the thickness of the coating, the amount of the reagent, and the catalytic current, and to determine the stability of the chemically modified electrode in time and the reproducibility of the analytical signal, i.e. to estimate a series of metrological and operational characteristics.

The study of a large group of mediators bound covalently to the electrode surface and comparison of their behaviour in solution and under heterogeneous conditions showed that the thermodynamic characteristics of the catalysts are relatively insensitive to immobilisation. Cases where the covalent bond involves an electroactive group in the molecule of the modifying agent may be an exception. In this case the maximum difference between the standard redox potentials $\Delta E^0$ is ~90 mV.[37]

The kinetic requirement for the rapid establishment of the electrochemical equilibrium $M \rightleftarrows M^*$ (stage 1, Fig. 3) is fulfilled best for compounds for which the oxidation-reduction process is associated with the small Gibbs free energy characterising the reorganisation of the structure of the reactant molecule and of its solvation shell as a result of electron transfer.[38] Therefore, one uses such modifying agents as transition metal complexes with organic ligands[39] or organic compounds whose structure contains conjugated π-bonds or metallocenes.

Among the main reasons inducing the difference in $\Delta E^0$ noted above, one may specify the following: (1) the increase in the energy of reorganisation of the solvent and the change in the stereochemistry of the modifying agent, which may accompany the electron transfer involving the immobilised centres (for example, as a consequence of Coulombic repulsion); (2) the influence of immobilisation on the retention of the planar structure of the mediator (for example, in the case of a metal complex); (3) the strong interaction of the mediator with the support (this affects the potential); (4) the interaction between the catalyst molecules in the oxidised and reduced forms.[10] All these factors act more or less appreciably for any immobilisation method.

The mediator performs its function of an electron transferring agent via an outer sphere or an inner sphere mechanism. In the outer sphere interaction between M* and S, an electron simply "jumps" between these species (redox catalysis). In the inner sphere mechanism (chemical catalysis), the mediator plays the role of an electron transferring agent and simultaneously binds the substrate into the intermediate. Thus the reduction of oxygen to hydrogen peroxide on a chemically modified electrode based on the porphyrin-cobalt complex (CoP) incorporated in a Nafion film, proceeds in fact via the chemical catalysis mechanism.[40] The electron exchange takes place within the adduct between the substrate $O_2$ and the modifying agent, i.e. within the complex $CoPO_2$. In the chemical catalysis, there is a possibility of the transfer of two electrons. For a sufficiently large number of catalytic cycles in the construction of the chemically modified electrode, one may deposit thin films (or even a monolayer). In practice, it is best to use under these conditions an

electrode having a large contact surface area, for example, one made from a porous or fibrous material.[41]

The principal requirements which must be not by mediators are listed below:

the mediator must be stable in relation to atmospheric oxygen (its reduced form should not, for example, be oxidised in air);

the oxidation-reduction potential of the mediator must be low and virtually independent of the pH, i.e. the potential of the M/M* redox couple is not a function of $H^+$;

the stage in which the mediator interacts with the substrate must be rapid, which is best achieved for an inner sphere mechanism;

the mediator must not be transferred from the surface of the chemically modified electrode to the test solution;

the mediator must be non-toxic.

The disadvantages which are encountered in studies with chemically modified electrodes include a decrease in the activity of the modifying agent on immobilisation or its change as a function of time, possible side reactions which deactivate irreversibly the modifying agent, as well as certain other side processes have been noted. However, these disadvantages of chemically modified electrodes are manifested to a greater extent when they are used for electrosynthesis than in electroanalysis.[41]

The theory of charge transfer in a layer of an immobilised modifying agent as well as the reactions of the substrate occurring on the chemically modified electrode have been described by different workers.[42–53] Depending on the immobilisation procedure, the modifier can be deposited, as noted above, on the surface both in the form of a monolayer and in the form of a film having a definite thickness. Multilayer coatings are as a rule discussed in the literature and the monolayer is regarded as a special case. The processes occurring on the surface of chemically modified electrodes are extremely varied; new transport stages and chemical reactions, any of which may limit the rate of the overall process, are manifested here. Fig. 3 presents a general scheme for the amperometric response on a chemically modified electrode with a polymer catalyst film.[52]

The rate of electron transfer in the direction of the redox centres through the electrode–modifying agent interface is fairly high and is determined by the potential. The M and M* centres participate in the electron transfer within the layer.[54] The charge transfer is partly associated either with the diffusion of counterions having oxidation-reduction properties if polycationic film includes an ion exchanger (see, for example, Anson et al.[55] and Pickup and Murray[56]) or with the movement of electrons along the conducting polymer chain.[57] The overall diffusion coefficient $D_c$ characterises this movement of charges.

It has been suggested that the substrate enters the layer of the modifying agents slowly via diffusion. This process is characterised by a partition coefficient $K$. In the film itself, the migration of the species S is affected both by the pore size[58] and by the density of the polymer film impregnated with the solvent.[59] The diffusion coefficient of the substrate $D_S$ in the layer of the modifying agent can be lower by 3–4 orders of magnitude than in solution.[60,61] It may happen that not all the immobilised modifying agent species participate in the reaction of the substrate, only some of them being involved, which depends on the kinetic and transport parameters $k$, $k'_e$, $k''$, $D_S$, and $D_e$. The reduction of the substrate S directly on the electrode surface (determined by the rate constant $k_e$) can occur only in the case of thin films[62] and is not typical for the usual layers. The difference in the behaviour of the modifying agent bound directly to the surface and present in the film (i.e. in its reaction layer) is explained by the difference in the solvation of the modifying agent.

The use of polymer coatings differing in the structure and composition of the films opens yet another pathway to the attainment of selectivity in the analytical determinations. For example, ascorbic acid and dopamine, which are oxidised on the carbon glass electrode at virtually identical potentials, can be separated on a chemically modified electrode coated by polyvinylpyridine in which the $IrCl_5^{3-}$ transferring agent has been incorporated.[63] Both the peak potentials on the voltammograms and the rates of the catalytic reaction of these substrates differ appreciably under these conditions.

The establishment of the thickness of the coating on the electrode (for the maximum concentration of the modifying agent in the latter), which affects the catalytic activity, is important. The extent $X$ of the reaction zone, determined by the modifying agent (in the film)/solution boundary, is estimated from the relation

$$X_L = (D_S/k[M]_{tot})^{1/2}. \quad (4)$$

For the electrode surface/modifying agent boundary layer, this distance from the electrode surface is determined from the formula

$$X_0 = (D_e/kK[S]_{soln})^{1/2}. \quad (5)$$

Here $[S]_{soln}$ is the substrate concentration in solution at the boundary near the polymer film and the subscripts $L$ and O indicate the distance from the surface of the electrode proper where the reaction zone begins.

Then types of reaction zones, depending on the site where the modifying agent is located, have been proposed in a detailed study of the mechanisms of the response of chemically modified electrodes[64] (Fig. 4). Eqns. (4) and (5) describe the extreme cases where the reaction zone is located directly in the solution and at the electrode proper. If the rate constant for the reaction

$$M^* + S \xrightarrow{k} M + P$$

in the layer of the modifying agent is zero, the substrate reaches the surface of the electrode proper ($Ek'_e$ type of location). If the electron transfer and the mass transfer of the substrate are reversible, then the reaction proceeds in the layer of the modifying agent and for a low value of $k$ the reaction zone is diffused throughout the thickness of the layer of the modifying agent (V$k$ type of location). When $k$ is large, the reaction zone narrows down to the size of the layer (RZV type of location).

For reaction zones located on the surface of the modifying agent (types P$k''$ and VP$k$), two cases are distinguished. In the reaction zone with P$k''$ type of location, a homogeneous reaction takes place at a high rate (high catalytic effect; $k_2 > 10^4$ litre$^2$ mol$^{-1}$ s$^{-1}$). The thickness of the sensor layer is then unimportant. Since the thickness of the film may be decisive for electron transfer, it is best to use thin coatings or even a monolayer of the modifying agent for chemically modified electrodes.

The VP$k$ zone occurs for thick coatings and electrodes. In the ideal case the extent of this zone is described by Eqn. (4). Since the concentration of the electrocatalytic centres in the layer is several times greater than that in the solution, for a sufficient mass transfer of substrate into the layer ($D_S \simeq 10^6$ cm$^2$ s$^{-1}$) the quantity $k_2$ in the layer may be appreciably smaller than in the solution ($k_2 > 10$ litre$^2$ mol$^{-1}$ s$^{-1}$). It has been suggested that porous film forming

agents be used fot the immobilisation of the modifying agents on such chemically modified electrodes and that the pore diameter in the layer should be approximately an order of magnitude greater than the diameter of the substrate molecule,[41] the optimum thickness of the layer being $\sim 1$ μm.[52]

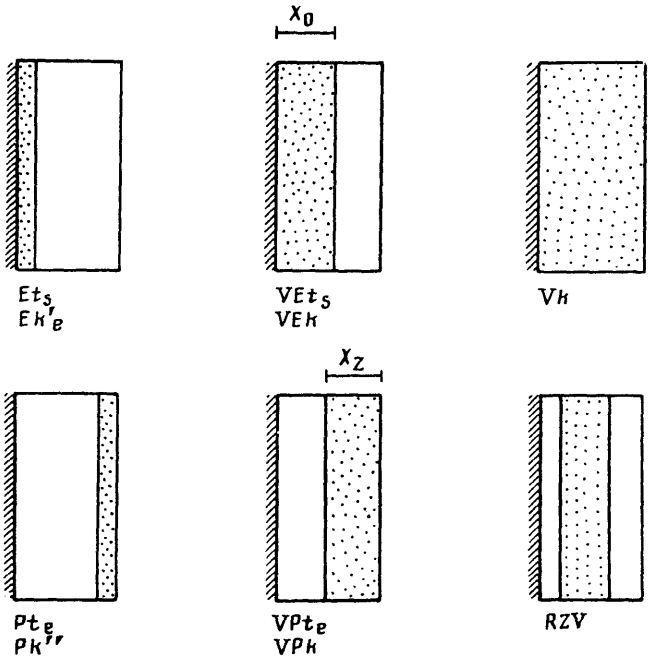



**Figure 4.** Types of reaction zones in the film of modifying agent on the surface of a chemically modified electrode. Location of the zone: E—electrode; VE—layer at the electrode; V—bulk-phase layer; P—surface of the chemically modified electrode facing the solution; VP—layer at the surface of the chemically modified electrode; RZV—reaction zone in the bulk phase layer. Rate-determining stage: substrate transfer ($t_S$) and electron transfer ($t_e$). Rates of reactions: electrochemical reaction ($k_e$) and catalytic reaction in the layer ($k$) or on the surface ($k''$) of the modifying agent (according to Bartlett[64]).

It follows from the data described above that effective catalytic action is observed when the entire bulk of the immobilised catalyst is involved in the process (case V$k$), which is difficult to achieve in practice, or when the reaction layer is involved (VP$k$), its reaction zone being at the maximum distance from the modifying agent/solution interface and extending in the direction towards the surface of the electrode proper. The ratio of the limiting catalytic current at a rotating disc electrode to the limiting current calculated from the Levich equation has been adopted as a measure of the catalytic efficiency of the chemically modified electrodes. For $X < X_{opt}$, the catalytic efficiency of the modifying agent increases with increase in $X$. For $X > X_{opt}$, a much greater consumption of the modifying agent (which is frequently expensive) is required for a slight increase in efficiency.

Apart from the rotating disc electrode, voltammetry, especially its cyclic version, which makes it possible to determine the rate constant for the reaction of the mediator with the substrate in solution ($k_2$), is used for the qualitative assessment of the observed kinetic effects. The nature of the catalytic reaction of the substrate can be established in this instance from the cyclic curves.[65] For a low rate of the subsequent chemical reaction (M* + S) and its low constant $k_f = k_2[S]$, the shape of the voltammogram is close to that of the cyclic curve characteristic of a reversible electrode process ($i_p \propto V^{1/2}$; $i_{pa}/i_{pc} = 1$; $\Delta E_p \simeq 0.057/n$ (volts); here $V$ is the rate of change of the polarising potential, $i_{pa}$ and $i_{pc}$ are the currents of the anodic and cathodic peaks, $\Delta E_p$ is the difference between the potentials of these peaks, and $n$ is the number of electrons participating in the oxidation-reduction reaction).

For higher values of $k_f$, the voltammogram contains one step (for example, the cathodic step), the limiting current of which is proportional to $k_f^{1/2}$ and is independent of the rate of change of the polarising potential. In the intermediate situations with average values of $k_f$, the height of the cathodic peak of the mediator M in the presence of the substrate S is greater, while the height of the anodic peak is lower than in the absence of S. These changes in the peak currents are clearer for low rates of change of the potential ($V = 1-10$ mV s$^{-1}$).

The diagnostic criteria described in the literature for cyclic voltammetry are used for the qualitative description of the electrochemical behaviour of chemically modified electrodes in the substrate solution.[65] We may recall that useful information can be extracted from the dependence of $i_p/V^{1/2}$ on $V$. The quantity $k_f$ is found from a series of cyclic voltammograms recorded for different rates of scanning of the potential.[65]

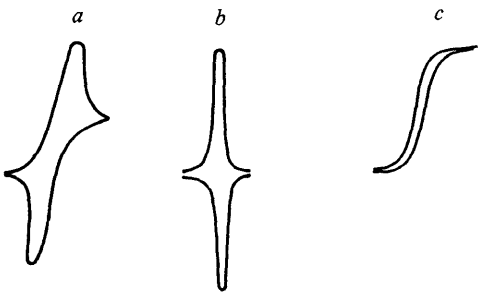



**Figure 5.** Typical cyclic voltammograms for different types of behaviour of the mediator in the chemically modified electrode: *a*, reversible behaviour of the mediator in solution; *b*, ditto but on the surface of the chemically modified electrode; *c*, catalysis at a high rate ($k_f$).

As was to be expected, cyclic voltammograms for an immobilised mediator in the absence of the subsequent chemical reactions involving the redox couple contain reversible peaks and we have $i_p \propto V^{1/2}$, $i_{pa}/i_{pc} = 1$, and $\Delta E_p = 0$. Fig. 5 presents for comparison the cyclic voltammograms corresponding to the cases described above.

### 3. Immobilisation of electron transferring agents in chemically modified electrodes

It follows from subsection 1 that the methods for the retention of modifying agents on the surfaces of electrodes are very varied and one can therefore put forward a large number of procedures for the fixation of the oxidation-reduction centres participating in the catalytic response of the chemically modified electrode. However, in practice, mainly polymer films are used to immobilise electron transferring agents[4,9] because of the simplicity of the operation, its reproducibility, the adequate stability of the chemically modified electrode obtained, and the possibility of varying the film thickness. Here account must be taken only of the fact that the material of the polymer is frequently non-indifferent to the transferring agent, which affects, for example, its formal redox potential.

The electron transferring agent is immobilised in different ways. One of them involves the electrochemical preparation of a conducting polymer with the simultaneous incorporation of the catalyst in the latter. For example, in the electropolymerisation of pyrrole in solution in the presence of hexacyanoferrate(II) ions, quinone, porphyrins, phthalocyanines, and certain other catalysts, films containing these transferring agents are obtained. The polypyrrole films are used more often than other coatings in chemically modified electrodes because polypyrrole is electrically conducting and its thick films do not induce an appreciable passivation of the electrode, are retained both on metal oxide surfaces and on carbonaceous materials, and are chemically stable during the period necessary for analysis.

The use of an *N*-substituted pyrrole as a monomer in electropolymerisation leads to still more stable films. Their polymerisation is accompanied by the immobilisation of bipyridyl and porphyrin metal complexes, ferrocene, anthraquinone, phenothiazine, dyes, and other compounds.[66] However, the polymer films based on *N*-substituted pyrroles exhibit a lower conductivity compared with the unsubstituted analogue, which can be avoided by copolymerising pyrrole and an *N*-substituted pyrrole.[41] Other compositions comprising two polymers are also used.[67,68] The substituted pyrrole as the monomer may contain in its structure groups involved in electron transfer. The substituents in the pyrrole molecule ultimately determine the property of the polymer coating on the chemically modified electrode which influences the mass transfer of the substrate.[69]

The commonest electron transferring agents are the bipyridyl complexes of ruthenium and osmium[57,70] as well as iron and cobalt.[71] They are immobilised in films based on polyvinyls. The porosity of the films, which determines their transport properties, is achieved in different ways, for example, by activating the polymerisation process by means of plasma.[72] The transport of species with electrocatalytic properties from the solution into the polymer film occurs sometimes during a comparatively long period.[73] The cyclic alteration of the potential of the chemically modified electrode over a short time interval in the region of the formal redox potential accelerates this process.[61,74]

As mentioned above, the retention of the species of the modifying and transferring agent can be achieved by means of electrostatic interaction forces. In such cases, an ion exchanger of the type of Nafion is widely used as the retention matrix. Its selectivity in the charge (ion) transfer process is a maximum in relation to hydrophobic species. Another example of the use of electrostatic interactions for the purpose of immobilisation is the fixation of ruthenium hexacyanoferrate(III) complexes on anionic or cationic surfactants immobilised in their turn with polystyrene film.[75] In order to ensure the selectivity of the electrocatalytic response, which is determined by the size and shape of the substrate particles, the modifying and transferring agent is immobilised in various clays or zeolites, which are retained on the electrode surface by polymers, including polymers formed by electropolymerisation.[76] The clay-modified electrode can adsorb certain surfactants, which then extract from the solution transferring agents of the type of hexacyanoferrate(III) ions, ferrocene, etc.[77]

Apart from the immobilisation of transferring agents with the aid of polymer films, their adsorption on the electrode surface is employed. Copper and cobalt complexes with organic ligands and aromatic compounds are immobilised in this way. This method is frequently used to immobilise enzymes or other biological materials in the construction of biosensors.[78] For example, an adsorbed film of microorganisms is capable of retaining combined transferring agents and electrocatalysts comprising hexacyanoferrate(III) ions or ruthenium(III) complexes.[79] The insoluble hexacyanoferrates(II), deposited on the electrode surface, can also function as transferring agents.[80–83] Ruthenium complexes, ferrocene, and benzoquinone are retained on the surface of carbon glass after its electrochemical treatment at an anodic potential.[84]

### 4. Chemically modified electrodes as biosensors

If a biological material, for example an enzyme or an enzyme-containing preparation, is incorporated in the signal-generating layer of the chemically modified electrode, then such an electrode is called a biosensor or a biochip. Other names are also used.[85] The modifying agent, in the given instance an enzyme, is immobilised by all the procedures which were described above for simpler transferring agents. Much attention has been devoted to the principles of the operation of biosensors and their applications. A series of special reviews have been published, including reviews in Russian journals.[85,86] Here is it appropriate to consider the problems concerning biosensors of the same kind as those arising in the development of chemically modified electrodes. It must be emphasised that studies on chemically modified electrodes, carried out independently and simultaneously with the development of biosensors, made a definite contribution to the expansion of the biosensor field. It was found that many results of studies on chemically modified electrodes have led to advances in the study of biosensors up to the level of their practical application.

In principle, any biosensor can be regarded as one of the versions of the chemically modified electrodes. Since the general principles of the functioning of amperometric sensors based on immobilised enzymes were examined previously,[85] there is no need to return to this problem.

The operation of amperometric biosensors is based either on the direct electron transfer between the active centre of the immobilised enzyme and the surface of the electrode proper or on the involvement of a mediator. In the latter case the biolayer giving rise to a specific response of the substrate is deposited on the modified surface containing an electron transferring agent. In this so called bulk phase technology, the individual components of the biochip constitute, as it were, a physical ensemble. Although such biosensors have now found practical applications, there are difficulties in their preparation on a mass scale. In addition, biochips with comparatively thick membranes have a long response time; complications arise also in their calibration.

The principal requirements which must be met by the mediators in chemically modified electrodes also apply to the biosensors based on them. It must be emphasised that the stage involving the

interaction of the mediator with the active centre of the enzyme must be fairly rapid, i.e. the mediator must behave in essence as if it were a substrate of the enzyme. Ultimately two catalytic cycles occur on such an electrode (Fig. 6).

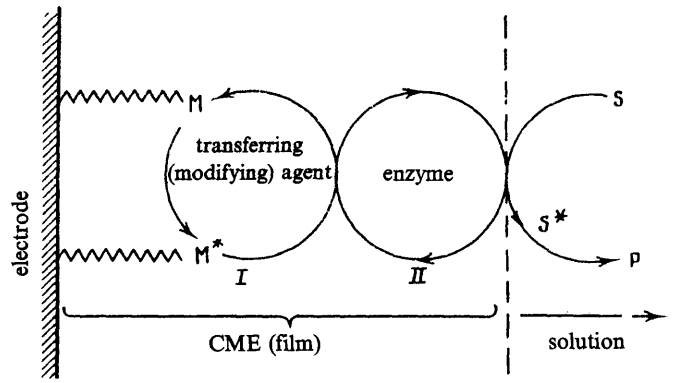



**Figure 6. Schematic illustration of the operation of a chemically modified electrode with two catalytic cycles.**

Amperometric biosensors are usually based on the so called oxyreductase enaymes in which the electron acceptors are, for example, atmospheric oxygen, nicotinamide-adenine dinucleotide ($NAD^+$) or nicotinamide-adenine dinucleotide phosphate ($NADP^+$). These components of the chemically modified electrode participate in the recyclisation of the enzyme after its reaction with the substrate. Enzymes employing $O_2$ as the electron acceptor are usually referred to as oxidases, whereas enzymes operating in combination with $NAD^+$ or $NADP^+$ are referred to as dehydrogenases or reductases. Among the enzymes in these two groups, oxidases are usually employed for the construction of biosensors.[9,11]

Without going into the details of the practical aspect of the problem of biosensors, the reader being referred to the reviews mentioned above,[9,11] we may note that their selectivity is determined by the specificity of the action of the enzyme and by the nature of the electrochemical reaction proper, in which the components of the enzymic process participate. Oxidases are as a rule highly specific in relation to particular components of the biological specimen. On the other hand, the system transforming the response of the biological reaction involved in the recognition of the substrate molecule into electric current (the system comprising the transferring agent or mediator, also referred to as the transducer) is not always quite as selective as one would wish. Indeed, many metabolites may be oxidised at a platinum microelectrode (transducer) at its operating potential. Thus ascorbic and uric acids, aminoacids, urea, and other components of the biological specimen analysed are oxidised at this electrode at a working potential of +0.6 V.

In order to increase the selectivity of such biosensors, use is made of special films operating on the basis of the principle of filters. For example, in order to suppress the influence of the above components and also of cysteine and proteins, which can be present in biological specimens, for example, in blood serum, use is made of a polymer film based on 1,2-diaminobenzene obtained by electrochemical polymerisation.[87] Such a biosensor with an immobilised glucose oxidase yields satisfactorily reproducible results in the course of 1–1.5 months. Glutaraldehyde may be used to increase the thermal stability of the biolayer.

The advantage of a biosensor with a film permeable only to substrate molecules of a particular size consists also in the fact that such devices have a low noise level in the recording of a useful signal and can be employed in flow injection analysis.

As mentioned above, dehydrogenases can serve as the enzymes in many biosensors.[11] These enzymes operate in combination with $NAD^+$ or $NADP^+$, the so called coenzymes. During the enzymic process, they are consumed in an amount equivalent to the amount of the substrate. The coenzymes must therefore be regenerated during the operation of dehydrogenase biosensors. This is achieved in a catalytic cycle on an electron transferring agent immobilised on the surface of the transducer proper. Quinone-hydroquinone derivatives are usually employed for this purpose (cycle I, Fig. 6). The coenzyme participates in the second catalytic cycle, which determines jointly with the enzyme the specificity of the response (cycle II, Fig. 6).

A low working potential is applied to such electrodes with an immobilised modifying agent, which imparts to them certain useful properties. Thus an advantage of the biosensors working on the principle of chemically modified electrodes with a double catalytic cycle is the possibility of increasing the selectivity of the response by limiting the number of specimen components, capable of reacting electrochemically when a potential is applied, by increasing the signal/noise ratio. These biosensors have a linear response over a wider range than the biosensors without a modifying agent.

Direct electron transfer between the active centre of the enzyme and the working electrode, i.e. the bioelectrocatalysis proper, is also a method for increasing the metrological and operational characteristics of biosensors. In this case, there is no mediator-transferring agent, in contrast to the previous scheme. A number of reviews and articles have also been devoted to the problem of direct electron transfer.[88–92]

The efficiency of biocatalysis based on an idealised model has been discussed.[88,89] It has been shown that the catalytic activity of enzymes on the surfaces of electrodes can be so great that the amperometric response is determined by the diffusion of the substrate, while the immobilisation of the enzymes ensures a high current density. A limited number of examples of the direct electron transfer from the active centre of the enzyme to the electrode have been described in the literature. Thus the possibility of using cytochrome *c* as the catalyst of the electrochemical reduction of molecular oxygen has been investigated. The rate of formation of hydrogen peroxide increases in the presence of this biocatalyst.[90] Evidence indicating the possibility of the direct electron transfer from the active centre of the enzyme to the electrode has been obtained for the oxygen–laccase–electrode system.[91,92]

Among the amperometric biosensors giving rise to a response in the absence of a mediator, the greatest interest attaches to those where the electrode (the carrier of the immobilised enzymes) is constructed from electrically conducting materials, for example, organic salts of the type tetracyanoquinonedimethane–tetrathiofulvalene ($TCQ^-TTF^+$).[93] Thus the oxidation of lactate is accelerated catalytically when cytochrome $B_2$ is immobilised on the complex of $TCQ^-$ with *N*-methylphenazinium. This biocatalytic system has been used as a basis for the functioning of the biosensor for lactate.[94] If instead of cytochrome one uses in this system glucose oxidase immobilised on the same organic salt by means of

irreversible adsorption, then the biosensor makes it possible to determine glucose with an activity which does not change in the course of 100 days.[94] Other enzymes are also adsorbed on this organic conductor. Biosensors for purine, hypoxanthine, and xanthine have been constructed on the basis of such an assembly.[95] Finally one should note that various microorganisms and cells immobilised in a biolayer are used in biosensors, including those with an amperometric response (see, for example, Ivnitskii et al.[86]).

## III. Applications of chemically modified electrodes in electroanalytical chemistry

Various practical applications of chemically modified electrodes have been described so far in the literature. Amperometric sensors and the analysers based on them usually give rise to a measured signal which is linear over a wide range as a function of concentration and has a lower detection limit compared with potentiometric sensors. It is noteworthy that each sensor, transducer, or detector, working in accordance with the principles of chemically modified electrodes, is constructed for the solution of a particular specific practical problem and functions under rigorously defined conditions.

After the modification of the electrode surface by one of the methods described above, the resulting chemically modified electrode is introduced into the solution to be analysed. If metal ions are to be determined, then in the course of a certain time (from several minutes to several tens of minutes) the ion is bound (absorbed) on the electrode surface or in its bulk phase as a result of the reaction with the modifying agent. If an immobilised ligand serves as the modifying agent, then the ion is bound as a result of complex formation. The selectivity of the determination is attained not only by selecting the appropriate ligand but also by regulating the pH and the concentration of masking agents.[7] Metal ions may accumulate on the chemically modified electrode due to the electrostatic interaction forces resulting from ion exchange.[7,9,10] Multicharged ions can then be concentrated from highly dilute solutions.

Measurement of the voltammetric response after the electroaccumulation stage gives rise to a peak on the curves whose height (or area bounded by the corresponding contour) is used to determine the concentration of the given ions in the solution. If the electrochemical reaction involving the metal ions to be determined is reversible, then virtually any version of voltammetry or differential-pulse technique can be used to measure the signal. In this case the chemically modified electrode permits single determinations, after which its surface has to be regenerated with repeated modification.[7] On the other hand, if the electrode reaction is irreversible and the metal–ligand bond is ruptured during it but the immobilised ligand is not degraded, then the chemically modified electrode can be used repeatedly.[7]

The lower limit of the determination using chemically modified electrodes depends on the number of binding centres on the electrode surface, the stability of the complexes formed, the parameters of the distribution of ions between the solution and the absorbing phase of the electrode with incorporated modifying agent, and also on the method of recording the voltammetric signal.

Among inorganic ions, those of heavy metals have attracted most attention by investigators, which is associated primarily with the problems of the ecological monitoring of natural and waste waters, air, soils, and agricultural products. The problem of the sensitivity and selectivity of the determination has always been and remains of current interest, particularly now when the importance of analytical monitoring in the community has increased.

Inversion voltammetry combined with chemically modified electrodes has been used recently to increase the sensitivity of the determination. Detection limits for metal ions are then attained at the level of the capacity of physical methods such as atomic absorption voltammetry, which is used most often for such purposes in environmental monitoring. Thus the principles of inversion voltammetry have been used to determine copper(II) on a carbon paste electrode modified with di-8-quinolinyl disulphide.[96] After reduction to quinoline-8-thiol, the latter binds copper(II) ions in a stable complex. The detection limit for copper(II) with the aid of such an electrode reaches $8 \times 10^{-10}$ M, which is close to the detection limit for copper with the aid of the mercury film electrode.

The carbon paste electrode modified with 2,2′-dithiobipyridyl has been proposed for adsorption accumulation and subsequent voltammetric determination of silver with a detection limit of $8 \times 10^{-9}$ M. The analysis is hindered by the presence of mercury and copper, but bismuth, antimony, cadmium, cobalt, nickel, and zinc ions present in a 100-fold excess do not interfere.[97]

The carbon glass electrode whose surface has been coated with poly-4-vinylpyridine containing mercury(II) ions makes it possible to determine heavy metal ions (lead, cadmium, and copper).[98] The preliminary accumulation is carried out at a potential of about $-1$ V for not more than 3 min, after which the differential-pulse voltammogram is recorded.[98]

The graphite electrode modified with diphenylcarbazone has been proposed for the determination of traces of mercury(II) in aqueous solutions.[99] It is made by pressing a mixture of the modifying agent with graphite powder. The mercury(II) ions are concentrated from solutions containing nitric acid. The solution is then changed and the differential-pulse voltammogram is recorded in the new supporting electrolyte. The detection limit is $5 \times 10^{-8}$ M (for accumulation in the course of 10 min). The determination is interfered with by silver ions, chromate ions, and strong complex-forming ligands.

The principles of inversion voltammetry have also been used to determine small amounts of cobalt with the aid of a carbon paste electrode.[100] The modifying agents were a cation exchanger and phenanthroline. The cobalt(II) detection limit was $8 \times 10^{-10}$ M. Conditions permitting the analytical determination of this ion in the presence of a number of heavy metal cations have been found.

As an illustration, Table 1 presents data obtained in the determination of certain toxic metal ions, which characterise the analytical possibilities of chemically modified electrodes in voltammetry and its modifications. Although Table 1 does not exhaust all the literature data on the problems involved in the use of chemically modified electrodes in analytical practice, the measured concentrations of metal ions demonstrate convincingly that voltammetry combined with chemically modified electrodes can compete with a method such as atomic-absorption spectrophotometry.

Not only metal ions but also organic compounds are concentrated on the surface of chemically modified electrodes. Ecological as well as medical and pharmacological problems dictate also in this case the choice of the objects for analysis. Substrates with complex structures, proteins, medicinal preparations, vitamins, biologically active compounds, including pesticides, etc. are determined with the aid of voltammetry combined with chemically modified electrodes. Table 2 presents examples illustrating the possibilities of chemically modified electrodes in the electroanalysis of organic substances.

**Table 1. Voltammetric determination of inorganic ions in solution with the aid of chemically modified electrodes.**

| Ion to be determined | Material of electrode | Modifying agent | Method of measurement of signal | Concentration determined, M | Refs. |
|---|---|---|---|---|---|
| Cu(II) | Carbon glass | 3-Aminopropyltriethoxysilane | Inversion voltammetry, differential-pulse technique | $1\cdot10^{-9}-3\cdot10^{-7}$ | [101] |
| | | Poly(pyrrole-*N*-carbodithionate) | Substance concentrated at −0.6 V; cyclic voltammetry | $10^{-5}$ | [102] |
| As(III) | | Ruthenium(II, III) cyanides | Catalysis of oxidation, voltammetry | $5\cdot10^{-6}-2\cdot10^{-3}$ | [103] |
| As(III), Te(II) | Graphite | Mercury complexes | Inversion voltammetry | $3\cdot10^{-9}-5\cdot10^{-10}$ | [104] |
| Sb(III) | | Triphenylmethane dyes | – | $3\cdot10^{-8}-4\cdot10^{-9}$ | [104] |
| Ag(I) | Carbon paste | Diethylenetriamine | – | $10^{-11}-10^{-7}$ | [106] |
| Bi(III) | | Mercaptans | – | $1\cdot10^{-7}-2\cdot10^{-5}$ | [106] |
| Hg(II) | Graphite | Diphenylsemicarbazone | – | $10^{-9}$ | [99] |
| $UO_2^{2+}$ | Carbon glass | Tri-n-octylphosphine oxide | Voltammetry | $4\cdot10^{-9}-2\cdot10^{-8}$ | [107] |
| $DO_2^{2+}$ | | | Inversion voltammetry | $10^{-8}$ | [108] |
| $NO_2^-$ | Platinum | Poly(4-vinylpyridine) and iodine | Voltammetry | $4\cdot10^{-6}-2\cdot10^{-3}$ | [109] |
| | Carbon glass | $IrCl_6^{3-}$ in a polyvinylpyridine film | Flow-injection analysis | $10^{-4}-10^{-3}$ | [110] |
| | | Macrocyclic nickel complex in a Nafion film | Cyclic voltammetry | $10^{-4}$ | [74] |
| $SCN^-$ | Platinum | Acetylcellulose film with iodide | Flow-injection analysis | $5\cdot10^{-6}-1\cdot10^{-4}$ | [111] |
| | Carbon glass | | | $2\cdot10^{-7}-4\cdot10^{-6}$ | [111] |

It does not mention the substrates of enzymes used in biosensors because their determination has already been discussed in detail.[10,11,85]

Table 2 shows that, among the methods for immobilisation of modifying agents on the electrode surface, the incorporation of such agents into a polymer film or into the bulk of the carbon paste electrode is usually employed. Non-traditional solutions are rarely encountered.

Various hydrophobic materials deposited directly on the surface of a solid electrode may be included among unusual modifying agents.[120–122] Thus many medicinal substances reducing blood pressure, of the type of reserpine and rescinnamine, can be determined on lipid-modified electrodes.[121] After the deposition of the lipid film on the carbon glass surface of the electrode, compounds are accumulated in the course of 10 min, whereupon they permeate actively into the hydrophobic layer. The voltammogram is then recorded. The detection limit is $5 \times 10^{-9}$ M.

Polar electroactive substances (up to a 100-fold excess) do not affect the results of the determination. Electrodes modified by lipid films respond selectively to the presence of hydrophobic organic substances containing the electroactive groups in their structure.[122] Hydrophilic compounds are incapable of permeating into the lipid layer during its accumulation, which prevents their reactions on such a chemically modified electrode. Studies on lipid films prepared on the basis of distearoylphosphatidylcholine and dioleolylphosphatidylcholine showed that the electrode reactions on such chemically modified electrodes are due to the presence of double bonds in the alkenyl chains of the lipids.

Graphite electrodes with stearic acid deposited on their surface have been used to determine dopamine and norepinephrine in rat brain tissues *in vivo*.[120] The carboxy-groups of the modifying agent influence the oxidation potentials of these biogenic amines shifting them towards more positive values. The effectiveness of this chemically modified electrode in the presence of metabolites—electroactive and neurochemical compounds belonging to the group of catecholamines and present in tissue liquids. It has been suggested that there is a relation between the form of the voltammetric signal and the activity of dopamine.

In many instances, chemical modification of the electrodes alters the parameters of the electrochemical reaction and makes the process more reversible, while the form of the analytical signal becomes more convenient. It has been established that the oxidation and reduction of one of the coenzymes in many biological catalysts and in PQQ {sodium salt of 2,7,9-tricarboxy-1*H*-pyrrolo[2,3-*f*]quinoline-4,5-dione—a growth stimulator in a number of microorganisms} on

**Table 2. Voltammetric determination of organic compounds in solution using chemically modified electrodes.**

| Compound determined | Material of electrode | Modifying agent | Method of measurement of signal | Concentration determined, M | Refs. |
|---|---|---|---|---|---|
| Ascorbic acid | Carbon glass, carbon paste | Aminophenols | Voltammetry | $10^{-5}-10^{-3}$ | [15] |
| | Graphite | Polypyrrole | | $10^{-4}$ | [112] |
| Catecholamines | Platinum | Iodide ion | Differential-pulse technique | $8\cdot10^{-7}$ | [113] |
| Amines | Carbon paste | Polyvinylpyridine, benzaldehyde-4-carboxylic acid | Square wave voltammetry | $3\cdot10^{-5}-10^{-2}$ | [114] |
| Glutathione | | Cobalt-phthalocyanine | HPLC | $10^{-10}$ | [115] |
| Thiols | Ditto | | Ditto | $2.7\cdot10^{-9}$ | [115] |
| Ferredoxin | Gold | Di(4-pyridyl) disulphide | Voltammetry | $4\cdot10^{-4}-9\cdot10^{-5}$ | [116] |
| Oxalic acid | Graphite | Ruthenium and iron complexes in a Nafion film | Voltammetry and chemiluminescence | $5\cdot10^{-2}$ | [117] |
| Hypoxanthine | Carbon paste | Hydroxymethylferrocene + xanthine oxidase in a cellulose triacetate film | Voltammetry | $6\cdot10^{-7}$ | [118] |
| | Graphite | Ferrocene, iron-phthalocyanine | Voltammetry | $1.5\cdot10^{-7}$ | [115, 119] |
| Dopamine, norepinephrine | | Stearic acid | | $1.5\cdot10^{-7}$ | [120] |
| Reserpine, rescinnamine | Carbon glass | Lipids | Inversion voltammetry | $5\cdot10^{-9}$ | [121] |
| Hydrophobic organic compounds with multiple bonds | Platinum | Phosphatidylcholine | Voltammetry | $5\cdot10^{-9}$ | [122] |
| Alcohols | Graphite | $Fe[(CN)_6]^{3-}$ in a polyvinylpyridine film | Flow-injection analysis | $2\cdot10^{-6}$ | [123] |
| Phenols | | Ditto | Cyclic voltammetry, flow system | $0.5\cdot10^{-6}$ | [124] |
| Dopamine, ascorbic acid | Carbon glass | $Fe_4[Fe(CN)_6]_3$ | Cyclic voltammetry | $10^{-2}$ | [125] |
| | Platinum | Ferrocene | | $10^{-4}-10^{-3}$ | [126] |
| | Carbon glass | $IrCl_3^{2-}$ in a polyvinylpyridine film | | $10^{-3}$ | [127] |
| Cysteine | | $Os(bipy)_3^{3+}$ in a Nafion film | HPLC | $2\cdot10^{-5}-10^{-3}$ | [128] |

platinum and gold electrodes are irreversible. However, if these electrodes are coated by a polypyrrole film, then cyclic voltammograms show reversible two-electron peaks.[129] If pyrrole is electropolymerised in the presence of PQQ, then the latter is incorporated in the polymer matrix, retaining its oxidation-reduction properties, i.e. it can play the role of a mediator. The latter factor leads to the possibility of preparing biosensors for the determination of sugars.

In the solution of the problems of monitoring (continuous analysis) on the real time scale of the on-line or in-line type, a notable place has been assigned to voltammetry and chemically modified electrodes. In many instances, the aim of the development of chemically modified electrodes was in fact to use them as amperometric transducers in a stream, for example, in flow-injection analysis (FLA) or in high performance liquid chromatography (HPLC). These problems have been partly discussed in other reviews.[11,85] A special review devoted to chemically modified electrodes for the detection of substances in a stream of liquid has been published.[130] In FIA and HPLC, modified electrodes make it possible to ensure high productivity of the determination and ultimately lead to the accumulation of a large amount of information.

Methods for the modification of electrodes designed to impart to them a specific response have begun to be used recently in the miniaturisation of systems for analytical monitoring. This aspect of research and development is regarded as promising because it is possible to obtain ultimately miniature electrodes which permit measurements *in vivo* in tissues or even in individual cells. For example, an ultramicroelectrode based on a carbon fibre modified with tetrakis(4-hydroxy-3-methoxyphenyl)porphyrin in a polymer film has been prepared.[131] It makes it possible to determine nickel in individual biological cells. The film itself, on the surface of the ultramicroelectrode, is formed on electrochemical oxidation of the modifying agent. The number of layers in this film at the tip of the carbon fibre (0.5–1 μm in diameter) reaches 10–90. The calibration plot is linear in the concentration range $5 \times 10^{-5}$–$5 \times 10^{-3}$ M. The detection limit is $\sim 10^{-6}$ M nickel(II).

The miniaturisation of chemically modified electrodes makes it possible to construct portable analysers working in an autonomous regime and ensuring the continuous monitoring of biologically active substances in many spheres of human activity.

However, one should note that the chemically modified electrode incorporated in the analyser system is a product of extremely laborious scientific research. Although this problem is in fact of current interest and the need for such sensors is continuously growing, nevertheless more time is required to obtain a device or the corresponding instrument with the required operating properties. Therefore the view that sensors represent the future of analytical chemistry is not without foundation.[85] Advances in related fields of science, particularly in microelectronics, have been attracting increasing attention by researchers in the field of chemically modified electrodes and sensors. The use of the so called planar technology, which is used to create integrated circuits, instead of the traditional three-dimensional technology, in the construction of sensors will probably make it possible to proceed to their mass scale production. The microstructure of the surfaces of electrodes chemically modified with the aid of a multilayer coating has to be monitored already at the molecular level.

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