Pure & Appl. Chem., Vol. 70, No. 6, pp. 1301–1318, 1998.
Printed in Great Britain.

INTERNATIONAL UNION OF PURE
AND APPLIED CHEMISTRY

ANALYTICAL CHEMISTRY DIVISION
COMMISSION ON ELECTROANALYTICAL CHEMISTRY*

ANALYTICAL ASPECTS OF CHEMICALLY MODIFIED ELECTRODES: CLASSIFICATION, CRITICAL EVALUATION AND RECOMMENDATIONS

(IUPAC Recommendations 1998)

Prepared for publication by

WŁODZIMIERZ KUTNER[1], JOSEPH WANG[2], MAURICE L'HER[3] AND RICHARD P. BUCK[4]

[1]Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44, 01-224 Warsaw, Poland
[2]Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces, NM 88003, USA
[3]Faculté des Sciences et Techniques, URA CNRS 322, 6 avenue V. Le Gorgeu, B.P. 809-29285, Brest Cedex, France
[4]Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

*Members of the Commission during the period 1993–97 when the present report was prepared, were as follows:

Chairman: 1991–1997, R. P. Buck (USA); *Secretary:* 1991–1995, K. Tóth (Hungary); 1995–1997, S. Rondinini-Cavallari (Italy); *Titular Members:* M. F. Camoes (Portugal, 1995–1997); M. L'Her (France, 1991–1995); W. Kutner (Poland, 1995–1997); S. Rondinini-Cavallari (Italy, 1991–1995); K. Štulik (Czech Republic, 1989–1997); Y. Umezawa (Japan, 1991–1997); *Associate Members:* A. M. Bond (Australia, 1989–1997); K. Cammann (Germany, 1989–1995); M. F. Camoes (Portugal, 1987–1995); A. G. Fogg (UK, 1987–1997); L. Gorton (Sweden, 1994–1997); W. R. Heineman (USA, 1991–1995); S. Kihara (Japan, 1991–1997); W. F. Koch (USA, 1991–1995); W. Kutner (Poland; 1989–1995); E. Lindner (Hungary, 1995–1997); R. Naumann (Germany, 1995–1997); K. W. Pratt (USA, 1995–1997); E. Wang (China, 1987–1995); J. Wang (USA, 1991–1997); *National Representatives:* D. Bustin (Slovakia, 1994–1997); A. Covington (UK, 1987–1997); D. R. Groot (Republic of South Africa, 1994–1997); I. R. Gutz (Brazil, 1994–1997); S. S. M. Hassan (Egypt, 1994–1997); J.-M. Kauffmann (Belgium, 1992–1997); F. Kadirgan (Turkey, 1994–1997); H. Kim (Republic of Korea, 1994–1997); H. B. Kristensen (Denmark, 1988–1997); T. Mussini (Italy, 1989–1997); B. Pihlar (Slovenia, 1994–1997); H. P. van Leeuwen (Netherlands, 1993–1997); Y. Vlasov (Russia, 1995–1997). Names of countries given after Members' names are in accordance with the *IUPAC Handbook 1994–95 (Blackwell Science Ltd).*

Analytical aspects of chemically modified electrodes: Classification, critical evaluation and recommendations (IUPAC Recommendations 1998)

Abstract: Analytical aspects of chemically modified electrodes (CMEs) are critically reviewed. Effects of analyte and/or reagent accumulation, chemical transformation, electrocatalysis, permeability, ionic equilibria, controlled release, and change of mass, as well as combinations of these effects are evaluated and classified. Also, relevant definitions are provided and recommendations formulated for the most effective CME operation.

CONTENTS

1 INTRODUCTION

Chemically modified electrodes (CMEs) have attracted considerable interest over the past two decades as researchers have attempted to exert more direct control over the chemical nature of an electrode. The terminology, definitions and preparation methods of CMEs have been described and classified as well as recommendations provided in a recent IUPAC report (*1*). CMEs have found numerous important applications in, e.g., solar energy conversion and storage, selective electro-organic synthesis, molecular electronics, electrochromic display devices, corrosion protection, and electroanalysis. A vast review literature is available on the subject (*2-25*). The ability to manipulate the molecular architecture of the bulk matrix of an electrode and its surface in particular has led to a wide range of analytical applications of CMEs and created powerful opportunities for electroanalysis.

The objective of the present report is to classify and critically evaluate CMEs for analytical applications as well as to provide the relevant definitions and to formulate suitable recommendations for the most effective operation. For electroanalytical purposes, a CME can be designed (*3, 5, 6, 9, 13, 14, 16, 17, 20*) as a powerful, (predominantly voltammetric, amperometric, potentiometric, and also impedimetric and microgravimetric) sensing device, by deliberate modification of the surface or bulk matrix material of the electrode with a selected reagent (monomeric or polymeric) that governs its electrochemical properties. Such manipulation of the molecular composition of the electrode aims at improving sensitivity, selectivity and/or stability allowing for tailoring its response in order to meet analytical needs.

2 DEFINITIONS, CLASSIFICATION AND OPERATIONAL RECOMMENDATIONS

Several phenomena occurring at CMEs are exploited for electroanalytical purposes. These, assessed, classified and illustrated with selected examples below, include *analyte and/or reagent accumulation, chemical transformation, electrocatalysis, permeability, ionic equilibria, controlled release, and change of mass*. They are employed separately or in mutual combinations, simultaneously or, most commonly, in sequence.

2.1 Accumulation. From dilute solutions, accumulation (preconcentration, collection, ingress, or preferential uptake) of a substance in solution can be performed at the electrode modified with a suitable receptor (*3, 13-15, 20, 26*). For analytical purpose, either reagent or a target trace analyte is accumulated. By analogy to the protein-type biological cell-surface receptors, the CME receptors are the electrode-confined compounds which can interact selectively with target analytes or reagents.

Comment. *The use of the 'doping' and 'electrodoping' terms to describe accumulation of an ion or molecule in a CME film should be used with circumspection because these terms apply to the trace addition of the electron donating or accepting substances, i.e., dopants, to certain insulators in order to generate their semiconducting, p/n, properties, e.g., As or B to Si. Therefore, neither 'doping' nor 'electrodoping' is appropriate to describe ion ingress into polymer films in order to generate their conducting properties, e.g., ingress of anions to poly(pyrrole) or poly(aniline). This ion ingress is merely a charge balance effect irrelevant to the p/n properties of the polymers. These polymers are ion exchangers whose compositions are controlled by the interfacial potential difference (ipd) at the polymer film and the electrolyte solution. Also, the 'intercalation' term may be not applicable with this respect, since it applies to the foreign atom or compound insertion, reversible or irreversible, into a host crystal lattice where it occupies a discrete position. Exceptions include intercalation of spacially organized molecules, such as a DNA, immobilized on an electrode.*

The analyte accumulation in a small volume at the CME modifying layer usually precedes detection and its main purpose is to improve detectability. If this accumulation is preferential, because of selective interactions between the analyte and the immobilized reagent then, it can serve additionally as a separation step, thus improving the electrode selectivity (see Section 2.4). For instance, molecular recognition is displayed towards an analyte in solution by immobilized self-assembled monolayer films with terminal host or guest groups (*27*).

The accumulation at CMEs is chemical, i.e., based on both covalent and non-covalent bond formation. The accumulation by electrolysis, e.g., metals at analytical mercury electrodes, such as a hanging mercury drop electrode (HMDE) or a mercury film electrode (MFE), is excluded here. Moreover, these mercury electrodes are not chemically modified. The analyte or reagent can be accumulated at a CME in a monolayer or multilayer film; typical of the latter is the formation of a polymer film on the electrode to produce a polymer film electrode (PFE). The most typical accumulation mechanisms involve:

- Chemisorption (monolayer), for instance, by means of extended π-electron systems, at the Pt or carbon electrodes (*28-32*).

- Covalent bonding (monolayer or multilayer) to a compound (*33, 34*) which is immobilized at the electrode, e.g., by organosilanization, amidization, esterification or etherification. The analyte accumulation is governed by the reactivity of the modifying reagent.

- Electrostatic interactions of an ionic analyte or reagent with immobilized ion-exchange groups. The groups of both permanent and induced ion-exchange properties are used. Acid-base electro-inactive (e.g., sulfonic, or tetraalkylammonium) groups fall to the former category while redox (e.g., hexacyanoferrate) groups and charged conducting polymers (e.g., poly(pyrrole), poly(acetylene), or poly(aniline)) to the latter. Ion-exchange properties of the acid-base groups are controlled by appropriate pH adjustment of bathing solution while those of the redox groups as well as conducting polymers by tuning redox equilibria either chemically or electrochemically. The solution ions can be exchanged in a chemical modifier (*i*) monolayer, by means of, e.g., surface-confined ion-exchanging groups, or (*ii*) multilayer, by means of

immobilized films of zeolites (*35*) or clays (*36-38*), ion-exchange, conducting or redox polymers (*9, 23, 39, 40*), or bilayer lipid membranes (BLM) (*5, 16, 41*).

Comments. *(i) In addition to electrostatic interactions, hydrogen bonding (e.g., in case of lipids) and hydrophobic interactions (e.g., in case of antibodies) play a role in accumulation at BLMs (see Section 2.4). (ii) The exchange to solution, i.e., release (see Section 2.6), of the accumulated analyte ions may result in negative determination errors. A way to alleviate this problem is to transfer the PFE after accumulating analyte cations, such as Tl^{+} and Pb^{2+}, into a cell with a solid electrolyte that minimizes release of the cations (42, 43).*

Dynamic separation of ionic and neutral analytes is possible by abrupt charging and discharging of both redox polymer and conducting polymer film electrodes. For the former polymer, ions are accumulated rapidly, for instance, cations in charged poly(vinylferrocene), while salts and solvent are accumulated slowly (*44*). Therefore, certain selectivity can be achieved under dynamic charging conditions. For the latter polymer, differentiation of protons and anion is observed under dynamic charging and discharging of poly(aniline) (*45-48*).

Induced ion-exchange properties of an immobilized conducting polymer can be exploited for determination of electro-inactive ions in solution whose ingress to the film is necessary for charge compensation. For instance, poly(pyrrole) coating an electrode can be oxidized only if counter anions are available for compensation of a generated positive charge in the film. Therefore, the electro-oxidation current of poly(pyrrole) can be measured at sufficiently positive potential applied, if anions appear in a bathing solvent. This current can be used for amperometric determination of the anions (*49*).

- Complexation, including formation of a chelate or supramolecular complex, e.g., of metal cations or small organic molecules by immobilized ligands. Conversely, a ligand can be complexed by immobilized metal cations. Complexation can take place in a monolayer (*50*), or multilayer (e.g., by ligand complexing sites in polymer films (*51-53*)). For instance, selective sensing was demonstrated for a two-component monolayer membrane co-self-assembled on a gold electrode. The membrane was composed of diethyl sulfanediyldiacetate (DSEA) and octadecane-1-thiol (ODT). DSEA is an active (or sensing) and ODT inactive (or impermeable) film component. The ion-selective coordination of Cu^{2+} from solution by DSEA resulted in sensing even in the presence of Fe^{3+} (*54, 55*). Cu(I) was preconcentrated at the Nafion®-2,2'-biquinoline modified carbon electrode (*56*).

Comment. *Multiple coordination to a determined metal ion may create matrix effects, i.e., complexation can be severely hindered because some ligand sites may not be available for complexation due to steric or ligand motion factors (9).*

- Precipitation (multilayer), e.g., by electropolymerization or electrocoagulation (*40*).

- Partitioning, or extractive accumulation (multilayer), governed by size, charge and/or hydrophobicity of an analyte. For instance, analytes can be partitioned from aqueous solutions by synthetic polymers or BLMs (*16, 17*), or by natural polymers (bio-accumulation), such as cell walls of immobilized microorganisms (*57*).

Comment. *When considering the overall rate of accumulation by partitioning, the rate of transport through the membrane or inside pores should be taken into account.*

For assessment of selectivity and solution-film equilibria of the analyte or reagent, direct evidence of accumulation and/or release (see Section 2.6) can be obtained by using a radiotracer technique in combination with electrochemistry (*58-61*).

The accumulated analyte is subsequently determined by applying an appropriate voltammetric waveform (*9, 62, 63*), in a manner analogous to that developed earlier for stripping voltammetry at a HMDE or MFE (*3, 64-68*). This detection is usually combined with analyte release to solution (see Section 2.6). The resulting electrode mass change, followed with the electrochemical quartz crystal

microbalance (EQCM), can be exploited as a useful microgravimetric detection signal (see Section 2.7). Preconcentration by covalent bond formation of the electro-inactive analyte can be coupled with its chemical transformation into an electroactive form (see Section 2.2).

The CMEs with accumulated ions can be exploited for potentiometric determination of ions in solution (see Section 2.5).

Recommendations for successful amperometric or voltammetric determination of the analyte accumulated at the CME are, as follows.

- For non-specific or non-selective accumulation, the voltammetric peak potential of the analyte should differ greatly from that of the interfering substances.

- In the presence of non-accumulated electroactive interfering substances in the test solution, the analyte loaded CME needs to be transferred to the blank supporting electrolyte solution; that is, the medium needs to be changed before making the determination. In other words, a transfer or medium-exchange step of the determination procedure should be completed.

- Quantification of the accumulated analyte is possible for loads much smaller than those corresponding to the CME saturation (*9*).

- In the case of an analyte which cannot be stripped electrochemically off the electrode during or after voltammetric determination, chemical regeneration leading to an analyte-free CME is required for its reuse. For instance, in case of accumulation by ion exchange of a redox analyte ion characterized by not too large selectivity coefficient, a CME can be regenerated by exposing to the electro-inactive salt solution. Alternatively, disposable electrodes, for instance film-coated screen-printed electrodes (*69-71*), are recommended, if memory effects are permanent.

- In the case of ion-exchange accumulation, there is a competition for ion-exchange sites between ions of analyte, interfering substances and supporting electrolyte. Therefore, respective selectivity coefficients have to be taken into account. (*63*). Under semi-infinite diffusion conditions, the nature of charge transport through the film, i.e., electron hopping between redox sites and physical diffusion of redox analyte, affects the ion determination in solution in two ways. That is, the calibration plot of the voltammetric peak current against the analyte concentration in solution deviates either positively or negatively from the linear dependence. Positive deviations are predicted by a simple Dahms-Ruff model for the electron hopping being the charge transfer rate determining step. However, very few real systems exhibit this behavior (*10, 72*). Negative deviation may appear if physical diffusion of redox species in the film is the rate determining step. Therefore, the use of sufficiently thin films and/or sufficiently low potential scan rates are recommended in the case of extended linear concentration range of the calibration plot in order that the finite diffusion conditions are fulfilled. Then, the peak current is independent of the analyte diffusion coefficient in the film. However, the signal is less amplified due to ion-exchange accumulation under finite rather than semi-infinite diffusion conditions.

In the case of an electroactive (redox or conducting) polymer, potentials of the electrode redox processes of the polymer itself limit the potential range available for detection of accumulated ionic analytes on one hand and on the other, these processes can serve as internal standards for quantitative evaluation of detection signals. For instance, the ferrocene redox signal of the quaternized poly(vinylpyridine-vinylferrocene) copolymer film electrode is compared to that of the copper ions which can be accumulated in the film by complex formation with diethyl thiocarbamate which is immobilized by ion exchange in the film (*9*).

Comment. *The current signal of detection is proportional to the diffusion coefficient and concentration gradient of the analyte at the electrode. Apparently, this gradient is the largest for monolayer film accumulation at a maximum available coverage density of the analyte. For multilayer accumulation in the polymer film matrix, the apparent diffusion coefficient of analyte in the film is usually smaller by several orders of magnitude than that in solution. Nevertheless, the detection signal is enhanced because accumulation results in large local analyte concentration.*

2.2 Chemical transformation. An electro-inactive analyte can be reacted with the appropriate reagent immobilized at the CME to yield an electroactive product suitable for electrochemical determination. Electrode selectivity and sensitivity towards certain functional groups can be improved in this way. For instance, Pt electrodes were modified by adsorption of an allylamine layer for determination of ferrocenecarboxaldehyde (*28*). Electro-oxidation of the imine product of condensation of the carbonyl analyte and the immobilized amine gave rise to the detection signal. Alternatively, primary amines were determined by using a carbon paste electrode (CPE) modified with quaternized poly(vinylpyridine) having an anion functionalized aromatic aldehyde as a counter ion. The amines were reacted at the modified CPE and the electro-oxidation current of the resulting imine was used as the detection signal (*29*).

Also, the modifying effect of an analyte on the electrode-immobilized reagent can be exploited for analytical purposes. Accordingly, in a rather unusual application, Cs^+ was determined indirectly, in the presence of the Na^+ excess. This determination was based on the electrochemical response of iron sites within the selective cation-exchange $[NiFe(CN)_6]^-/[NiFe(CN)_6]^{2-}$ matrix modifying an Ni electrode (*73*). The mechanism of this selective detection consists in microstructure changes in the modifier lattice, induced by minute amounts of the analyte.

The recommended features of successful analyte transformation, suitable for detection at CME, involve:

- The cyclic voltammetry peak potential of the resulting electro-active couple should, favorably, be sufficiently separated from the peak potentials of immobilized reactants.

- For detectors modified with complex compounds, the analyte ion should be structurally compatible for triggering of the redox response of the central metal ion of the complex compound.

2.3 Electrocatalysis. For electroanalytical purposes, electrocatalysis at CMEs is used to amplify the detection signal. It consists in acceleration of heterogeneous electron transfer of the target analyte, which is slow at the same potential at a bare electrode, induced by an immobilized charge mediator, i.e., catalyst (*1, 5, 22, 74-77*). Electrocatalysis at a CME needs to be distinguished from mediation. Mediation implies that an immobilized redox couple generates heterogeneous electron transfer of a target redox analyte in solution that would occur just as readily at the same potential at a bare electrode if it were available. That is, electrocatalysis is accomplished by charge mediation but not all mediation results in electrocatalysis. The formal potential difference of the analyte and mediator redox couples should be favorable thermodynamically. Immobilized cobalt phthalocyanine (*51, 78*) and mixed-valent ruthenium cyanide complexes (*5*) are examples of useful charge mediators of appreciable electrocatalytic activity towards a wide range of analytes. Alternately, an analyte can be specifically accumulated at a CME and a catalyst can be present in the test solution. For instance, coupling of the reduction of the determined metal ion in an adsorbed metal complex with its chemical oxidation by oxidant present in solution gives rise to large (electrocatalytic) currents and thus improves detectability (*79*).

Slow electrode reactions of many important analytes require a potential greatly exceeding their formal redox potentials in order that these reactions proceed at desirably high rates. The acceleration of such kinetically-hindered electrode reactions by electrode-confined charge mediators permits the quantification of these analytes at less extreme potentials, because catalyzed electrode reactions usually occur near the formal potential of the mediator. By applying less extreme potentials, both detectability and selectivity can be improved significantly, as compared with those obtained at non-modified electrodes. Also, the electrode fouling is decreased which may occur in case of direct electrochemical conversion of the analyte at more extreme potentials at a bare electrode. Variations of the formal redox potential of the immobilized catalyst can be used effectively to discriminate between analytes. Since the rate of electrocatalysis depends primarily on the formal redox potential difference of the catalyst and analyte, selectivity can be tuned by selecting the proper catalyst. For instance, Fe(II) and Fe(III) are simultaneously detected at a dual electrode coated with electrocatalytic polymers (*80*).

Several different CMEs are fabricated for electrocatalysis, including (*i*) mediators immobilized in a monolayer film, (*ii*) multilayer films, or (*iii*) metal or semiconducting microparticles dispersed in the

host matrix ionomer or conducting polymer films. Electroanalysis is benefited by these CMEs in different ways (*22, 81*), as discussed below.

Redox reactions between the electrode-confined monolayer of a mediator and an analyte in solution, i.e., catalyzed redox reactions of analyte, result in catalytic currents, indicating a substantial chemical amplification of the detection signal. Under pseudo-first order conditions with respect to the immobilized catalyst reversibly exchanging charge at an electrode, the detection signal of the electrocatalytic current is, advantageously, proportional to the analyte concentration (for small kinetic parameter) if a transient technique, such as linear scan voltammetry, is used (*75, 82*). If a steady-state technique, such as voltammetry at the rotating disk electrode is used, then the catalyst does not need to exchange charge reversibly at the electrode and the catalytic limiting current is proportional to the analyte concentration in solution for all kinetic parameters. This behaviour is in contrast to the pseudo-first order electrocatalysis for both catalyst and analyte present in the bulk solution where the detection signal is proportional to the square root of the analyte concentration (*82*). In many cases, a mediator chemisorbed on the electrode surface is much more active than that being either present in solution (*31*) or immobilized in a polymer film (*83*). Optimum conditions of electrocatalytic determination at a CME are met when analyte diffusion in solution is the rate-determining step and charge propagation in the film is fast. This is most often the case if the electrocatalytic film is made of either an electronic or a very fast ionic conductor (*84, 85*).

Under a stationary regime (e.g., rotating disk or microelectrode voltammetry), catalyzed redox reactions between a polymer-embedded redox mediator (multilayer) and an analyte in solution result in limiting currents. The theory of electrocatalysis at PFE has been developed (*86-96*) for continuous and homogeneous polymer films and chemically reversible mediator-analyte cross-exchange redox reactions. The electrocatalytic signal dependence on the analyte and/or mediator concentration is governed by the nature of four conceivable rate-determining steps (or their combinations):

- Convective diffusion of analyte from the bulk solution to the film-solution interface.

- Diffusion of analyte through the film.

- Diffusion-like propagation of charge within the film by self-exchange redox reaction.

- Mediator-analyte cross-exchange redox reaction in the film.

Provided that migration and activity effects are negligible, the analyte determination is relatively simple (*86*) if kinetic limitations are absent at the film-solution interface, i.e., the overall reaction rate is determined by (*i*) the mediator-analyte cross-exchange redox reaction while diffusion-like charge propagation within the film and the substrate penetration through the film-solution interface are facile (case R), or (*ii*) diffusion of substrate through the film while diffusion-like charge propagation and cross-exchange redox reaction in the film are rapid (case S).

Electrocatalytic advantages of CMEs comprising the system of a metal or semiconductor microparticles embedded in an ionomer or conducting polymer film are as follows (*97, 98*).

- The systems are relatively easy to prepare.

- The catalysis and charge transport between the surface of an electrode support and the embedded catalytic microparticles are distinct.

- Just as for redox sites in a redox polymer, there is a three-dimensional dispersion of the catalytic microparticles throughout the polymer host matrix. Local microparticle loading may be made large, even though the total amount of the electrocatalytically active material is small. Recommendations for the optimum operation of the system are as follows (*98*).

- The electrocatalysis rate should be controlled by spherical diffusion of analyte to each catalytic microparticle rather than the mediator solubility.

- The electrocatalysis rate should be large, diameter of microparticles should be small, microparticle loading should be large and a polymer film should be thick.

Heterogeneous biocatalysis is a special type of electrocatalysis occurring at a biosensor which comprises an electrode modified with a bioreagent film (*1, 99, 100*). For heterogeneous biocatalysis, a biocatalyst (e.g., redox enzyme) is used as a bioreagent. Usually, catalytic biosensors operate amperometrically, however, chronopotentiometric detection was also proposed (*101*). Typically, biosensor lifetime, stability, reproducibility and calibration requirements are limited by the nature of the bioreagent and the method of its immobilization. Recommended features of successful biocatalyst immobilization and operation are as follows (*102*).

- Immobilization should be permanent.

- Biocatalyst activity should be large and free from deterioration as the result of immobilization.

- In case of fouling of an electrode, its surface should be protected by an electro-inactive film. Since a protective film of an adsorbed non-polymer substance can be desorbed, polymer protective films are more commonly used.

- Access of interfering redox substances to the electrode surface should be prevented by applying, for instance, a polymer film coating.

- Linear concentration range should be as large as possible.

- For reproducible calibration, diffusion rate in the film of the analyte (and mediator, if it is not permanently immobilized, see below) should remain constant.

- For rapid biosensor response and short recovery time, this diffusion should be fast and thickness of the film should be small.

- Internal calibration should be possible.

- Solution pH should be carefully controlled.

The recommendations presented above apply equally well for other catalysts except that a biosensor should reveal biocompatibility if it is to be used for *in vivo* applications.

A biocatalyst can be immobilized at an electrode in different ways, including irreversible adsorption, covalent linkage, complex formation (*103*), incorporation into an electrode matrix (e.g., carbon paste (*104*)), encapsulation within liposomes (*105*) or micelles (*106*) immobilized at electrodes, or by crosslinking in the matrix of either an insulating or electronically or redox-conducting polymer (*107-110*). More recently, a biocatalyst is covalently linked onto self-assembled monolayers (SAMs) of long alkane-1-thiols in order to promote its orientation on the electrode surface for facile charge transfer (*111-113*) and to decrease background currents (*111-114*). Rapid response and restricted linear concentration range are the respective advantage and disadvantage of this type of immobilization.

The charge exchange between the electrode and the immobilized biocatalyst involved in a catalytic redox sequence with a biological analyte in solution is either direct or it is aided by an auxiliary charge mediator.

Direct (or non-mediated) biocatalysis involves (*i*) electrode immobilized enzymes or, most often, antibodies which directly exchange charge with the electrode (*115*); most commonly, the consumed co-substrate or generated product is detected electrochemically, or (*ii*) it relies on amperometric detection of enzymatic labels attached to the antibodies (*116-118*).

In the case of mediated biocatalysis (*119-123*), the charge is shuttled by a redox mediator between an active redox site of the immobilized biocatalyst and the electrode surface. If the co-substrate is involved

in the enzymatic reaction then, the rate of reaction between enzyme and mediator should be much larger than that between enzyme and co-substrate in order that the detection signal be independent of the co-substrate concentration. The mediator, physiological or non-physiological, can be either present in the test solution or immobilized at the electrode. It is crucial that the mediator immobilization is accomplished in such a way that its undesired loss (leaking) to solution (which can be a physiological fluid) is prevented while its mediating activity is retained. Accordingly, an insoluble mediator is used or, otherwise, it is accumulated (see Section 2.1) by irreversible adsorption, ion exchange, covalent linkage to enzyme or a polymer, conducting polymer crosslinking in order to "wire" the biocatalyst redox sites to the electrode surface (*124*), or by supramolecular complex formation in the film of a polymer having inclusion sites (*71, 124, 125*). Immobilization of a mediator at a CME results in its large concentration at the biocatalyst detection sites which leads to large biocatalytic detection currents. A biosensor can be designed to detect enzyme activators or inhibitors. For example, the Mn^{2+} analyte accelerating the H_2O_2/O_2 cleavage by peroxidase was detected. The peroxidase was incorporated, together with the 1,2-naphthoquinone mediator, into a carbon paste (*126*). In another example, the enhancement of hydroxylation of phenol to 1,2-quinone by tyrosinase, immobilized in a poly(estersulfonic acid) polymer-modified glassy carbon electrode in presence of small water concentrations, was exploited for trace water determination in non-aqueous solvents (*127*). Mediated biocatalysis can be switched on and off with a suitably designed enzyme "switch". For instance, a glucose biosensor switch was constructed based on an insulating poly(benzene-1,2-diamine) film, containing glucose oxidase, which was polymerized onto a poly(aniline) modified electrode; tetrathiafulvalene in solution was a mediator (*128*).

For repeatable electrocatalytic use, it is required that chemical or electrochemical regeneration of the CME be possible. Otherwise, a CME can be used as a disposable electrode.

Comment. *Electrocatalysis is one of several very efficient methods of amplification of the detection signal. Other amplification methods involve, for instance, interaction between an analyte and a receptor embedded in a permeable membrane used for electrode modification. This interaction results in permeation of different ions or molecules from solution, due to opening of the membrane channels. Changes of conductivity of these ions or currents corresponding to redox processes of these ions or molecules at the electrode give rise to the enhanced detection signal (see Section 2.4).*

2.4 Permeability. Permeability is a general term describing discriminative transport through a membrane coating (*129*) that controls the access of analyte and interfering substances to the electrode surface. An electrode coated with the permeable membrane is accessible to the target analyte while interfering substances are rejected or prevented from reaching the electrode surface. Thus, the electrode selectivity is improved (*130, 131*). Permeability can be controlled either by thermodynamics (in terms of partition) or by the transport rate (governed by diffusion within the film). Under dynamic conditions, electrodes coated with a permeable film can reveal selectivity.

Comment. *Permeability must be distinguished from permselectivity. Permselectivity is a special case of permeability. A membrane reveals permselectivity if ions of one sign are selectively permeable.*

Mechanisms of the permeability transport are based on differences in properties, such as charge, size, shape, polarity or chirality of the analyte and interfering substances. For example, a cation-exchange polymer film coating an electrode is a membrane barrier to anions from solution while cations can freely partition into it. Obviously, the charge selectivity of an anion-exchange polymer film is opposite. A polyanionic perfluorosulfonated ionomer, i.e., Nafion®, (*132, 133*), and poly(estersulfonic acid) of the Kodak AQ series (*134, 135*) are examples of widely used cation-exchange permselective membranes while Tosflex® (*136*), an anion-exchanger analogue of Nafion®, is an example of an anion-exchange membrane. Cellulose acetate (*137*) and poly(carbonate) are typical examples of size-exclusion membranes. The electrode selectivity can be additionally enhanced by designing multilayer or mixed membranes that combine the transport properties of the individual membrane. A polycrystalline Pt electrode modified both with Nafion® and cellulose acetate, and used for direct oxidative determination of nitric oxide, illustrates combined anion- and size-exclusion discriminative properties of the modifying film (*137, 138*). The sieving properties of size-exclusion films can also impart higher electrode stability due to prevention of surface fouling by undesired precipitation or adsorption, for instance at MFEs (*139*).

An electrode modified with a SAM of long alkyl chain molecules having terminal acid/base or ionic groups can enhance or suppress selected redox reactions depending on the charge of the terminal group. For instance, SAMs of $HS(CH_2)_nCOOH$ on the Au electrode discriminated against ascorbates in the voltammetric determination of dopamine (*140*).

The permeability of the membranes can be combined with their other properties, such as electrocatalysis or biocatalysis. For instance, a charge mediator and/or redox enzyme can be attached or entrapped in the membrane (see Section 2.3).

Induced charge-exclusion properties of a CME are exploited for potential-controlled gating of ions. For instance, anion transport can be switched on and off selectively by electro-oxidation and electroreduction, respectively, of a poly(pyrrole) film electrodeposited on a porous Au electrode separating two electrolyte solutions (*141*).

Indirect ion-exchange immunoassay at a Nafion® modified electrode consists of accumulation, by ion exchange (see Section 2.1), of a cationic product of enzymatic redox transformation in solution of an anionic substrate, followed by voltammetric determination of the accumulated product (*142-144*).

Selective interactions of an analyte dissolved in an aqueous solution with a reagent, e.g., peptide, embedded in an artificial BLM on an electrode (see Section 2.1), alter both the electrostatic field and phase structure of the BLM giving rise to the detection signal (*16, 17, 41*). Often, this signal is amplified. Typically, the membrane charge or the rate of ion transport across the BLM is altered. Charging of the electrical double layer at the BLM surface by an analyte can result in ionization of surface functional groups, e.g., of the immobilized hydrolytic enzymes. At least three mechanisms of charge transport across the BLM can be distinguished:

- An ion can be carried by an ionophore, e.g., the K^+ cation can be transported by valinomycin.

- Charge can be shuttled through an impermeable membrane between the electrode surface and an analyte in solution. For instance, the NADH coenzyme, a bulky analyte, can be catalytically oxidized at a co-self-assembled monolayer composed of a π-conducting quinone derivative and insulating alkane-1-thiol (*145*).

- A channel available for permeation of a solution species through the impermeable membrane to the electrode surface can be opened. The operation principles of channel sensors, which belong to a larger group of chemiresistors, are based on this mechanism. There are at least two mechanisms of channel operation: channels are permanently open or they can be switched on and off.

In the case of permanently open channels, a redox analyte can be detected in solution due to the presence of receptor molecules in an impermeable monolayer membrane. At low concentrations, receptor sites behave as an array of molecular-size microelectrodes. For example, $Ru(NH_3)_6^+$ present in solution can be detected at an electrode modified with an impermeable monolayer membrane composed of a octanediol and octadecyl alcohol mixture and containing ubiquinone as the receptor molecule (*146*).

The operation principle of channels which can be switched on and off is based on analyte-triggered switching permeation through the membrane of the redox marker (indicator) ion or molecule. This permeation allows for amplification of the detection signal. Two different types of channels which can be switched on and off, intermolecular and intramolecular, can be distinguished.

Intermolecular channeling, operative for ion-channel sensors, involves the effect of the analyte species on the surface charge density of the receptor membrane (*147-150*). That is, a positively charged analyte can be selectively bound to a negatively charged receptor. As a result, the negative surface charge of the membrane is compensated and intermolecular voids are formed between receptor molecules. A negatively charged redox marker, which cannot penetrate the membrane in the absence of the analyte, can therefore traverse through the membrane intermolecular voids. For example, in a synthetic lipid membrane deposited on the electrode by means of the Langmuir-Blodgett technique, the channels are opened by the Ca^{2+} analyte channel switches (*147*). Specific interactions of the analyte with the membrane components

result in channel opening. The detection signal of the $Fe(CN)_6^{4-}$ redox marker anions, permeating through the open channels in the membrane, serves as an amplified measure of the Ca^{2+} analyte concentration. The channels can be reversibly closed by means of an analyte quencher, e.g., EDTA. This Ca^{2+} determination can serve as an illustration of indirect voltammetric determination of an electro-inactive species. There are also similar sensing systems using receptors of different charge signs or neutral receptors (*148, 149*).

Intramolecular channeling involves a redox marker flow through the channel-shaped receptors present in the membrane, in the absence of intermolecular voids (*150*). Intensity of the marker flow through the intramolecular channels is controlled by formation of supramolecular complexes of the receptor and analyte leading to channel blocking. For instance, a condensed membrane constructed of β-cyclodextrin (β-CD) derivatized with long alkyl chains, and transferred onto the surface of the highly oriented pyrolytic graphite electrode (PGE), is sterically permeable to 1,4-quinone. This permeation can be affected by several different analytes consisting of neutral organic guest molecules which form supramolecular complexes with β-CD (*150*). Intramolecular channeling is technically more demanding than intermolecular because of the inevitable occurrence of intermolecular voids which give rise to noise. But, advantageously, it is also operative for neutral analytes. While only monolayer membranes have been used for intramolecular channeling, monolayer, multilayer and polymer membranes have been used for the intermolecular channeling.

It is recommended that the following requirements be met for efficient sensing (*145*):

- The structure and orientation of a receptor molecule in the membrane should be such that an analyte in solution is allowed to approach the electrode surface sufficiently close so, that electron transfer is fast.

- The background faradaic current corresponding to interactions of analyte in solution with the defect sites of the membrane, in the absence of the gate molecule, is smaller than that which is due to the smallest concentration of the receptor molecules intentionally introduced into the membrane.

- In the case of channels which can be switched on and off, channel opening should be fast and reversible.

- For rapid response, the fewer the number of layers in the membrane the better.

2.5 Ionic equilibria. CMEs with selective ion-exchange (ionophore) films (membranes) are used as asymmetric ion selective electrodes (ISEs). That is, an electrolyte solution containing an analyte ion is on one side of the membrane and a solid electrode on the other. These CMEs are used predominantly as "zero current" potentiometric sensors. Interfacial potential difference (*ipd*), e.g., Donnan potential, is measured for such CMEs between the electrolyte solution and the film in an electrolytic cell (*151*) comprising a reference electrode, electrolyte solution containing an electro-active or electro-inactive analyte ion, and the CME with the ionomeric film. Seven different asymmetric cell configurations are distinguished (*151*) where *ipd* is measured either under equilibrium or non-equilibrium conditions.

A review of potentiometrically operating biosensors is given in (*152*). Selected examples of other potentiometrically operating PFEs are: determination of heavy metal cations at a Pt electrode coated with the quinoid polymer film which is modified with mercaptides of heavy metals of interest (*153*); determination of different electro-inactive anions at an electrode modified with poly(pyrrole) containing accumulated anions (*154-159*).

For PFEs modified with conducting polymers (*160-162*), or ion-exchangers (where the ion-exchange sites are redox couples incorporated either by covalent linkage (*163*) or ion exchange (*164*)), *ipd* contributes in a different way to formal potentials of the redox couples (*163-166*). Hence, the formal potentials are sensitive to the activity of ions in solution. If, additionally, redox equilibria are combined with acid-base equilibria then, *ipd* is affected by solution pH (*163*). Due to partitioning of buffer ions, the pH inside an ion-exchange film may differ from that of the solution (*167*).

For ion-exchange PFEs, *ipd* depends solely on the concentration of electro-inactive ions in solution only if the concentration of the hydrophobic redox couple incorporated in the membrane is small and a minor fraction of ion-exchange sites in the membrane is populated by this couple (*164*).

For conducting-polymer based CMEs, careful attention should be paid to side redox reactions between the polymer and solution components because these reactions may affect the measured *ipd* values (*160*). Because the total number of redox sites in the film is very small, even currents of a picoampere range, corresponding to redox processes of impurities, e.g., trace oxygen, can change the redox state of the polymers making determinations unreliable.

2.6 Controlled release. When accumulated at a CME, and at a PFE in particular, an analyte or reagent (see Section 2.1) can undergo quantitative release (egress, stripping, expulsion, or microdosing) to the test solution, under electrochemical or chemical control. Different mechanisms are involved in controlled release from the CME. These depend on the nature of the analyte or reagent and the method of accumulation.

- In the case of accumulation by covalent bonding, the anchoring bond is cleaved (ruptured) electrochemically (*168*).

- In the case of ion-exchange accumulation, an ionic (redox or acid-base) analyte or reagent is released by neutralizing of its charge or the charge of the ion-exchanging redox polymer film or conducting polymer film. Neutralizing of the accumulated redox species, or redox or conducting ion-exchanging polymers, can be accomplished either by a homogeneous redox reaction (with the use of a suitably selected redox reagent) or by heterogeneous (i.e., electrode) charge transfer (triggered by a suitably altered electrode potential). For the acid-base ionic analytes, a suitable pH change of the solution leads to their neutralization. Examples of electrochemically controlled release from conducting or redox PFEs are microdosing to solution of drugs (*169*), neurotransmitters (*170*) or some other biologically important molecules (*171*). Furthermore, electrochemically modulated liquid chromatography (EMLC) was developed (*172*), where retention of separated analytes by a conducting polymer is electrochemically controlled. The EMLC principle was illustrated with potential control of charge of a poly(pyrrole) film covering a glassy carbon bead stationary phase resulting in selective elution of amino acid derivatives (*172*).

- In the case of accumulation by supramolecular complex formation at a PFE modified with a polymer containing supramolecular sites, a guest analyte or reagent can be released by changing its charge and hence hydrophobicity (*170, 173-175*). For that purpose, charge transfer equilibria for the acid-base or redox guest couple are altered in the same manner as described above for release in the case of ion-exchange accumulation.

Comment. *For fast release, diffusion coefficients in the film of accumulated species should be large. If this release depends on charge switching of the film, then charge propagation through the film should be fast.*

2.7 Change of mass. Both accumulation and release of an analyte or reagent as well as several other electroanalytical processes can result in the inherent changes in the mass of the CME. These mass changes can be exploited as useful detection signals. The CME mass balance is described in the present document separately in order to distinguish the relevant microgravimetric (non-electrochemical) detection signal. Simultaneous electrochemistry and microgravimetry is accomplished by means of an electrochemical quartz crystal microbalance (*176, 177*), which is a piezoelectric mass sensing device using a thickness-shear-mode acoustic wave (*178*). In this device, the sign of the measured frequency change is opposite to the sign of the change in mass (*179*). For use with an EQCM, a CME is fabricated by covering one face of a metallized quartz resonator with a modifying film. The film-covered face contacts the solution. For controlled release applications, using the EQCM, the CME with a analyte-loaded conducting polymer or ion-exchange film is electrochemically switched. Examples are the release of adenosine 5'-triphosphate from a poly(pyrrole adenosine 5'-triphosphate) film (*171*) and the release of small cations from a Prussian blue film by potential switching (*180*). Alternately, the analyte or reagent

can be either electrochemically switched, or acid-base switched by changing of solution pH, for controlled release from inclusion-type polymer film electrodes (*173, 174, 181*).

The combination of CME and EQCM has several analytically attractive features (*182*) as follows.

- Mass detectability is very low and mass sensitivity is very high, on a pmol level, and can be conveniently tuned by a suitably adjusted switching potential.

- Mass determination is absolute; neither calibration nor additional assumptions are required within the frame of applicability of piezoelectric microgravimetry (*176, 177*). However, visco-elastic properties of the film material must be considered (*183, 184*).

- Analytes can be determined *in situ*, allowing for selection of optimum electrochemical conditions.

- Determination can be performed under dynamic conditions, e.g., under solution flow, due to a very short response time, usually of the order of a fraction of millisecond. Owing to this high resolution, a strategy was proposed to extract selectively the mass contribution of a single species transferred through the solution-film interface (*44*).

For the sake of selectivity mentioned above, it is recommended that the CME mass changes due to the solution-film transfer of the analyte or reagent be separated from other mass change components corresponding to the transfer of interfering substances, such as counter ions, salts and solvent molecules (*182*). This separation is possible provided that the extent of solvent transfer is independent of the electrolyte concentration in solution. Galvanostatically controlled switching can facilitate kinetic separation of the mass components (*44*).

3 CONCLUDING REMARKS

Despite the great potential of CMEs for analytical applications revealed by voluminous fundamental research, only a few examples of the commercial use of CMEs have been described. These include, for instance, metal/metal oxide reusable amperometric electrodes for determination of, e.g., alcohols, amines or carbohydrates (*185*). For successful practical applications, such as detection in liquid chromatography (*186*), flow injection analysis or batch determinations, the long-term stability and reproducibility of CMEs need to be improved while sensitivity and detectability of analytical CMEs should be made comparable or superior to that of non-modified electrodes. For such a rapidly growing area as analytical CMEs, the definitions, classifications and recommendations presented herein cannot address every construction detail and operation principle. It is apparent that periodical updating is needed with the further development of CMEs for analytical applications.

4 LIST OF ABBREVIATIONS

BLM - bilayer lipid membrane
β-CD - β-cyclodextrin
CME - chemically modified electrode
CPE - carbon paste electrode
DNA - deoxyribonucleic acid
DSEA - diethyl sulfanediyldiacetate
EMCL - electrochemically modulated liquid chromatography
EQCM - electrochemical quartz crystal microbalance
ipd - interfacial potential difference
ISE - ion selective electrode
HMDE - hanging mercury drop electrode
MFE - mercury film electrode
ODT - octadecane-1-thiol
PFE - polymer film electrode

PGE - pyrolytic graphite electrode
SAM - self-assembled monolayer

ACKNOWLEDGMENT

The authors thank Dr. C. Andrieux, R. Bilewicz, P. Kulesza, Royce W. Murray, and D. P. Nikolelis for helpful comments. Financial support of the Polish State Committee for Scientific Research to WK, through Grant No. KBN 2P 303 10 507, is gratefully acknowledged.

5 REFERENCES

1. R. A. Durst, A. J. Bäumer, R. W. Murray, R. P. Buck, and C. P. Andrieux, *Pure Appl. Chem.* **69**, 1317 (1997).
2. S. Dong and Y. Wang, *Electroanalysis* **1**, 99 (1989).
3. D. W. Arrigan, *Analyst* **119**, 1953 (1994).
4. A. J. Bard, *J. Chem. Educ.* **60**, 302 (1983).
5. J. A. Cox, R. Jaworski, and P. J. Kulesza, *Electroanalysis* **3**, 869 (1991).
6. M. W. Espenscheid, A. R. Ghatak-Roy, R. B. Moore III, Penner, R. M., M. N. Szentirmay, and C. R. Martin, *J. Chem. Soc., Faraday Trans. 1,* **82**, 1051 (1986).
7. L. R. Faulkner, *Electrochim. Acta* **34**, 1699 (1989).
8. R. J. Foster and J. G. Vos, *in* "*Comprehensive Analytical Chemistry*" (M. R. Smyth, J. G. Vos, and G. Svehla, eds.), Vol. 27, p. 465. Elsevier, Amsterdam, 1992.
9. A. R. Guadelupe and H. D. Abruña, *Anal. Chem.* **57**, 142 (1985).
10. G. Inzelt, *in* "*Electroanalytical Chemistry. A Series of Advances*" (A. J. Bard, ed.), Vol. 18, p. 191. Marcel Dekker Inc., New York, 1994.
11. A. Merz, *in* "*Topics in Current Chemistry, Electrochemistry IV*" (E. Steckhan, ed.), Vol. 152, p. 50. Springer-Verlag, Berlin, 1990.
12. R. W. Murray, *Acc. Chem. Res.* **13**, 135 (1980).
13. R. W. Murray, (ed.), "*Molecular Design of Electrode Surfaces.*" J. Wiley and Sons., Inc., New York. 1992.
14. R. W. Murray, A. G. Ewing, and R. A. Durst, *Anal. Chem.* **59**, 379A (1987).
15. Ŕ. W. Murray, *in* "*Electroanalytical Chemistry. A Series of Advances*" (A. J. Bard, ed.), Vol. **13**, p. 191. Marcel Dekker, Inc., New York, 1984.
16. D. P. Nikolelis and U. J. Krull, *Electroanalysis* **5**, 539 (1993).
17. K. Odashima, M. Sugawara, and Y. Umezawa, *Trends Anal. Chem.* **10**, 207 (1991).
18. J. Schreurs and E. Barendrecht, *Recl. J. Royal Nether. Chem. Soc.* **103**, 205 (1984).
19. G. G. Wallace, *in* "*Chemical Sensors*" (T. E. Edmonds, ed.), p. 132. Blackie, London and Glasgow, 1988.
20. J. Wang, *in* "*Electroanalytical Chemistry. A Series of Advances*" (A. J. Bard, ed.), Vol. 16, p. 1. Marcel Dekker, Inc., New York, 1989.
21. H. O. Finklea, *in* "*Electroanalytical Chemistry. A Series of Advances*" (A. J. Bard and I. Rubinstein, eds.), Vol. 19, p. 110. Marcel Dekker Inc., New York, 1996.
22. M. E. G. Lyons, *Analyst* **119**, 805 (1994).
23. K. Doblhofer, *in* "*Electrochemistry of Novel Materials*" (J. Lipkowski and P. N. Ross, eds.), p. 141. VCH, New York, 1994.
24. A. J. Bard, *in* "*Integrated Chemical Systems. A Chemical Approach to Nanotechnology*", Chap. 4. J. Wiley, Inc., New York, 1994.
25. H. C. Budnikov, V. N. Maystrenko, and Y. I. Murinov, "*The Voltammetry at Modified Electrodes and Ultramicroelectrodes*". Nauka, Moscow, 1994.
26. R. M. Kannuck, J. M. Bellama, and R. A. Durst, *Anal. Chem.* **60**, 142 (1988).
27. L. T. Zhang, L. A. Godinez, T. B. Lu, G. W. Gokel, and A. E. Kaifer, *Angew. Chem., Int. Ed. Engl.* **34**, 235 (1995).
28. J. F. Price and R. P. Baldwin, *Anal. Chem.* **52**, 1940 (1980).
29. A. R. Guadelupe, S. S. Jhaveri, K. E. Liu, and H. D. Abruña, *Anal. Chem.* **59**, 2436 (1987).
30. A. P. Brown and F. C. Anson, *Anal. Chem.* **49**, 1589 (1977).
31. Y. B. Lei and F. C. Anson, *Inorg. Chem.* **33**, 5003 (1994).

32. A. P. Brown, C. Koval, and F. C. Anson, *J. Electroanal. Chem.* **72**, 379 (1976).
33. A. Ruhe, L. Walder, and R. Scheffold, *Macromol. Chem. Macromol. Symp.* **8**, 225 (1987).
34. A. F. Diaz and K. K. Kanazawa, *IBM J. Res. Develop.* **23**, 239 (1979).
35. R. W. Murray, *Ann. Rev. Mater. Sci.* **14**, 145 (1984).
36. P. K. Ghosh and A. J. Bard, *J. Am. Chem. Soc.* **105**, 5691 (1983).
37. P. Labbe, B. Brahimi, G. Reverdy, C. Mousty, R. Blankespoor, A. Gautier, and C. Degrand, *J. Electroanal. Chem.* **379**, 103 (1994).
38. T. Wielgos and A. Fitch, *Electroanalysis* **2**, 449 (1990).
39. J. Wang and T. Martinez, *Anal. Chim. Acta* **207**, 95 (1988).
40. F. Beck, *Electrochim. Acta* **33**, 839 (1988).
41. D. P. Nikolelis, U. J. Krull, and A. L. Ottova, *in "Handbook of Chemical and Biological Sensors"* (R. F. Taylor and J. S. Schulz, eds.), p. 221. Institute of Physics Publishing, Bristol and Philadelphia, 1996.
42. G. Schiavon, R. Zotti, and G. Bontenpelli, *Anal. Chim. Acta* **264**, 221 (1992).
43. R. Toniolo, G. Bontenpelli, G. Schiavon, and G. Zotti, *J. Electroanal. Chem.* **356**, 67 (1993).
44. A. R. Hillman, N. A. Hughes, and S. Bruckenstein, *Analyst* **119**, 167 (1994).
45. D. Orata and D. A. Buttry, *J. Am. Chem. Soc.* **109**, 3574 (1987).
46. G. Horanyi and G. Inzelt, *Electrochim. Acta* **33**, 947 (1988).
47. M. Kalaji, L. Nyholm, and L. M. Peter, *J. Electroanal. Chem.* **313**, 271 (1991).
48. C. Barbero, M. C. Miras, O. Hass, and R. Kotz, *J. Electrochem. Soc.* **138**, 669 (1991).
49. Y. Ikariyama and W. R. Heineman, *Anal. Chem.* **58**, 1803 (1986).
50. G. T. Cheek and R. F. Nelson, *Anal. Lett.* **11**, 393 (1978).
51. J. A. Cox and P. J. Kulesza, *J. Electroanal. Chem.* **159**, 337 (1983).
52. S. V. Prabhu, R. P. Baldwin, and L. Kryger, *Anal. Chem.* **59**, 1074 (1987).
53. K. K. Kasem and H. D. Abruña, *J. Electroanal. Chem.* **242**, 87 (1988).
54. S. Steinberg and I. Rubinstein, *Langmuir* **8**, 1183 (1992).
55. I. Rubinstein, S. Steinberg, Y. Tor, A. Shanzer, and J. Sagiv, *Nature* **332**, 426 (1988).
56. Z. Gao, A. Ivaska, and P. Li, *Anal. Sci.* **8**, 3377 (1992).
57. J. Gardea-Torresday, D. Darnall, and J. Wang, *Anal. Chem.* **60**, 72 (1988).
58. G. Inzelt, G. Horanyi, and J. Q. Chambers, *Electrochim. Acta 32*, 757 (1987).
59. G. Inzelt and G. Horanyi, *J. Electroanal. Chem.* **230**, 257 (1987).
60. G. Inzelt and G. Horanyi, *J. Electrochem. Soc.* **136**, 1747 (1989).
61. G. Inzelt, G. Horanyi, and P. J. Kulesza, *Electrochim. Acta* **35**, 811 (1990).
62. K. Kalcher, *Electroanalysis* **2**, 419 (1990).
63. P. Ugo and L. M. Moretto, *Electroanalysis* **7**, 1105 (1995).
64. J. Wang and A. G. Fogg, *Classification and Definitions of Stripping Analysis*. IUPAC Report, Project 550/57/93, Commission on Electroanalytical Chemistry V. 5, *Pure Appl. Chem.*, to be submitted.
65. F. Vydra, K. Štulík, and E. Juláková, *"Electrochemical Stripping Analysis"*. Ellis Horwood, Chichester, 1976.
66. J. Wang, *"Stripping Analysis, Principles, Instrumentation and Applications"*. VCH, Deerfield Beach, FL, 1985.
67. A. G. Fogg, *Anal. Proc.* **31**, 313 (1994).
68. A. G. Fogg, *Anal. Proc.* **32**, 433 (1995).
69. J. P. Hart and S. A. Wring, *Electroanalysis* **6**, 617 (1994).
70. S. A. Wring and J. P. Hart, *Analyst* **117**, 1281 (1992).
71. Q. Chen, P. V. A. Pamidi, J. Wang, and W. Kutner, *Anal. Chim. Acta* **206**, 201 (1995).
72. M. Majda, *in "Molecular Design of Electrode Surfaces"* (R. W. Murray, ed.), Vol. 22, *Techniques of Chemistry,* Founding Ed., Weissberger, A., Series Ed., Saunders, W. H., Jr., p. 159. J. Wiley and Sons., Inc., New York, 1992.
73. L. J. Amos, A. Duggal, E. J. Mirsky, P. Ragonesi, A. B. Bocarsly, and P. A. Fitzgerald-Bocarsly, *Anal. Chem.* **60**, 245 (1988).
74. R. W. Murray, *Phil. Trans. R. Soc. Lond. A* **302**, 253 (1981).
75. C. P. Andrieux and J. M. Savéant, *J. Electroanal. Chem.* **93**, 163 (1978).
76. K. Aoki, K. Tokuda, and H. Matsuda, *J. Electroanal. Chem.* **199**, 69 (1986).
77. J. Zak and T. Kuwana, *J. Electroanal. Chem.* **150**, 645 (1983).
78. A. D. Jannakoudakis, N. Missaelidis, and E. Theodoridou, *Synth. Metals* **11**, 108 (1985).
79. C. M. G. van den Berg, *Anal. Chim. Acta* **250**, 265 (1990).

80. A. P. Doherty, R. J. Forster, M. R. Smyth, and J. G. Vos, *Anal. Chem.* **64**, 572 (1992).
81. S. A. Wring and J. P. Hart, *Analyst* **117**, 1215 (1992).
82. Z. Galus, "*Fundamentals of Electrochemical Analysis*". Ellis Horwood, New York, 1994, Chap. 11.
83. L. Ma and H. L. Li, *Electroanalysis* **7**, 756 (1995).
84. J. A. Cox and P. J. Kulesza, *J. Electroanal. Chem.* **175**, 105 (1984).
85. J. A. Cox and P. J. Kulesza, *Anal. Chem.* **56**, 1021 (1984).
86. C. P. Andrieux, J. M. Dumas-Bouchiat, and J. M. Savéant, *J. Electroanal. Chem.* **131**, 1 (1982).
87. F. C. Anson, *J. Phys. Chem.* **84**, 3336 (1980).
88. C. P. Andrieux and J. M. Savéant, *J. Electroanal. Chem.* **134**, 163 (1982).
89. C. P. Andrieux and J. M. Savéant, *J. Electroanal. Chem.* **142**, 1 (1982).
90. C. P. Andrieux, J. M. Dumas-Bouchiat, and J. M. Savéant, *J. Electroanal. Chem.* **169**, 9 (1984).
91. C. P. Andrieux and J. M. Savéant, *J. Electroanal. Chem.* **171**, 65 (1984).
92. C. P. Andrieux, P. Hapiot, and J. M. Savéant, *J. Electroanal. Chem.* **172**, 49 (1984).
93. C. P. Andrieux and J. M. Savéant, *Ann. Phys. (Paris)* **11**, 3 (1986).
94. J. A. Leddy, A. J. Bard, J. T. Maloy, and J. M. Savéant, *J. Electroanal. Chem.* **187**, 205 (1985).
95. X. Chen, He, P. and L. R. Faulkner, *J. Electroanal. Chem.* **222**, 223 (1987).
96. E. T. T. Jones and L. R. Faulkner, *J. Electroanal. Chem.* **222**, 210 (1987).
97. M. E. G. Lyons, D. E. McCormack, and P. N. Bartlett, *J. Electroanal. Chem.* **261**, 51 (1989).
98. M. E. G. Lyons and P. N. Bartlett, *J. Electroanal. Chem.* **316**, 1 (1991).
99. A. Hulanicki, S. Glab, and F. Ingman, *Pure Appl. Chem.* **63**, 1247 (1991).
100. D. R. Thevenot, K. Toth & G. Wilson *"Electrochemical Biosensors: Recommendations and Classification"* IUPAC Report, Project 550/31/87, Physical Chemistry Division, Steering Committee on Biophysical Chemistry and Analytical Chemistry Division, Commission on Electroanalytical Chemistry, *Pure Appl. Chem.*, to be submitted.
101. B. Uhe, W. Schuhmann, G. Janker, H.-L. Schmidt, and A. Janata, *Electroanalysis* **6**, 543 (1994).
102. S. A. Emr and A. M. Yacynych, *Electroanalysis* **7**, 913 (1995).
103. J.-i. Anzai, T. Hoshi, and T. Osa, *Chem. Lett.* , 1231 (1993).
104. L. Gorton, *Electroanalysis* **7**, 23 (1995).
105. M. F. Rosenberg, M. N. Jones, and P. M. Vadgama, *Biochim. Biophys. Acta* **1115**, 157 (1991).
106. D. M. Fraser, S. M. Zakeeruddin, and M. Graetzel, *Biochim. Biophys. Acta* **1099**, 91 (1992).
107. T. Ikeda, *Bull. Electrochem.* **8**, 145 (1992).
108. H. A. O. Hill and N. I. Hunt, *in* "*Methods in Enzymology*" (J. F. Riordan and B. L. Vallee, eds.), Vol. 227, p. 501. Academic Press, San Diego, 1993.
109. L.-H. Guo and H. A. O. Hill, *Adv. Inorg. Chem.* **36**, 341 (1991).
110. T. Ruzgas, E. Csöregi, J. Emnéus, L. Gorton, and G. Marko-Varga, *Anal. Chim. Acta* **330**, 123 (1996).
111. I. Willner, A. Riklin, B. Shoham, D. Rivenzon, and E. Katz, *Adv. Mater.* **5**, 912 (1993).
112. I. Willner, E. Katz, A. Riklin, and R. Kasher, *J. Am. Chem. Soc.* **114**, 10965 (1992).
113. E. Katz, A. Riklin, and I. Willner, *J. Electroanal. Chem.* **354**, 129 (1993).
114. S. Creager and K. G. Olsen, *Anal. Chim. Acta* **307**, 277 (1995).
115. L. Gorton, G. Jonsson-Pettersson, E. Csoregi, K. Johansson, E. Domingues, and G. Markovarga, *Analyst* **117**, 1235 (1992).
116. R. John, M. Spencer, G. G. Wallace, and M. R. Smyth, *Anal. Chim. Acta* **249**, 381 (1991).
117. J. L. Boitieux, G. Desmet, G. Wilson, and D. Thomas, *Ann. N.Y. Acad. Sci.* **613 (*Enzyme Eng.*10)**, 390 (1990).
118. I. Tsuji, H. Eguchi, K. Yasukouchi, M. Unoki, and I. Taniguchi, *Biosen. Bioelectron.* **5**, 87 (1990).
119. C. Bourdillon, C. Demaille, J. Moiroux, and J.-M. Savéant, *J. Am. Chem. Soc.* **115**, 2 (1993).
120. J. Jordan and M. K. Ciolkosz, *Solution Chem.* **20**, 995 (1992).
121. W. J. Albery, P. N. Bartlett, B. J. Driscoll, R. Lennox, and R. Bruce, *J. Electroanal. Chem.* **323**, 77 (1992).
122. T. Tatsuma, T. Watanabe, and Y. Okawa, *Anal. Chem.* **64**, 630 (1992).
123. T. Tatsuma and T. Watanabe, *Anal. Chem.* **64**, 630 (1992).
124. A. Heller, *J. Phys. Chem.* **96**, 3579 (1992).
125. W. Kutner, H. Wu, and K. M. Kadish, *Electroanalysis* **6**, 934 (1994).
126. M. H. Smit and G. A. Rechnitz, *Anal. Chem.* **64**, 245 (1992).
127. J. Wang and A. J. Reviejo, *Anal. Chem.* **65**, 845 (1993).
128. P. N. Bartlett and P. R. Birkin, *Anal. Chem.* **65**, 118 (1993).
129. R. P. Buck and E. Lindner, *Pure Appl. Chem.* **66**, 2527 (1994).

130. J. Wang, *Electroanalysis* **3**, 255 (1991).
131. P. Treloar, I. Christie, and P. Vadgama, *Biosensor, Bioelectronics* **10**, 195 (1995).
131. P. Treloar, I. Christie, and P. Vadgama, *Biosensor, Bioelectronics* **10**, 195 (1995).
132. I. Rubinstein and A. J. Bard, *J. Am. Chem. Soc.* **102**, 6641 (1980).
133. D. A. Buttry and F. C. Anson, *J. Electroanal. Chem.* **130**, 333 (1981).
134. T. Gennett and W. C. Purdy, *Anal. Chem.* **62**, 2155 (1990).
135. J. Wang and Z. Lu, *J. Electroanal. Chem.* **266**, 287 (1989).
136. L. Dunsch, J. Kavan, and J. Weber, *J. Electroanal. Chem.* **280**, 313 (1990).
137. J. Wang and L. D. Hutchins, *Anal. Chem.* **57**, 1536 (1985).
138. F. Pariente, J. L. Alonso, and H. D. Abruña, *J. Electroanal. Chem.* **379**, 191 (1994).
139. E. E. Stewart and R. B. Smart, *Anal. Chem.* **56**, 1131 (1984).
140. F. Malem and D. Mandler, *Anal. Chem.* **65**, 37 (1993).
141. P. Burgmayer and R. W. Murray, *J. Am. Chem. Soc.* **104**, 6139 (1982).
142. A. Le Gal La Salle, B. Limoges, J. Y. Anizon, C. Degrand, and P. Brossier, *J. Electroanal. Chem.* **350**, 329 (1993).
143. A. Le Gal La Salle, B. Limoges, and C. Degrand, *J. Electroanal. Chem.* **379**, 281 (1994).
144. B. Limoges, C. Degrand, P. Brossier, and R. L. Blankespoor, *Anal. Chem.* **65**, 1054 (1993).
145. M. Kunikate, K. Akiyoshi, K. Kawatana, N. Nakashima, and O. Manabe, *J. Electroanal. Chem.* **292**, 277 (1990).
146. R. Bilewicz, T. Sawaguchi, R. V. Chamberlain II, and M. Majda, *Langmuir* **11**, 2256 (1995).
147. M. Sugawara, K. Kojima, H. Sazawa, and Y. Umezawa, *Anal. Chem.* **59**, 2842 (1987).
148. M. Maeda, K. Tsuzaki, and M. Nakano, *J. Chem. Soc., Chem. Commun.* 1529 (1990).
149. S. Nagase, M. Kataoka, R. Naganawa, R. Komatsu, K. Odashima, and Y. Umezawa, *Anal. Chem.* **62**, 1252 (1990).
150. K. Odashima, M. Kotato, M. Sugawara, and Y. Umezawa, *Anal. Chem.* **65**, 927 (1993).
151. R. P. Buck and P. Vanýsek, *J. Electroanal. Chem.* **292**, 73 (1990).
152. E. A. H. Hall, "*Biosensors*," p. 268. Prentice-Hall, Englewood Cliffs, 1991, p. 268.
153. G. Arai, A. Fujii, and Y. I., *Chem. Lett.* , 1091 (1985).
154. A. Hulanicki, A. Michalska, and A. Lewenstam, *Talanta* **41**, 323 (1994).
155. S. Dong and G. Che, *Talanta* **38**, 11 (1991).
156. S. Dong and Z. Sun, *Analyst* **113**, 1525 (1988).
157. C. S. C. Bose, S. Basak, and K. Rajeshwar, *J. Phys. Chem.* **96**, 9899 (1992).
158. J. F. Pearson, J. M. Slater, and V. Jovanovic, *Analyst (Cambridge, U.K.)* **117**, 1885 (1992).
159. T. Okada, K. Hiratani, H. Sugihara, and N. Koshizaki, *Anal. Chim. Acta* **266**, 89 (1992).
160. A. Michalska, K. Maksymiuk, and A. Hulanicki, *J. Electroanal. Chem.* **392**, 63 (1995).
161. A. Lewenstam, J. Bobacka, and A. Ivaska, *J. Electroanal. Chem.* **368**, 23 (1994).
162. J. Bobacka, Z. Gao, A. Ivaska, and A. Lewenstam, *J. Electroanal. Chem.* **368**, 33 (1994).
163. J. Redeppenning, B. R. Miller, and S. Burnham, *Anal. Chem.* **66**, 1560 (1994).
164. R. Naegeli, J. Redeppenning, and F. C. Anson, *J. Phys. Chem.* **90**, 6227 (1986).
165. K. Doblhofer and R. D. Armstrong, *Electrochim. Acta* **33**, 453 (1988).
166. A. Fitch, *J. Electroanal. Chem.* **284**, 237 (1990).
167. G. Inzelt and J. Q. Chambers, *J. Electroanal. Chem.* **266**, 265 (1989).
168. A. N. K. Lau and L. L. Miller, *J. Am. Chem. Soc.* **105**, 5271 (1983).
169. M. Hepel and Z. Fijalek, *in* "*Polymeric Drug and Drug Administration*" (R. M. Ottenbritte, ed.), p. 79. ACS Symp. Ser. No. 545, Am. Chem. Soc., Washington DC, 1994.
170. B. Zinger and L. L. Miller, *J. Electroanal. Chem.* **181**, 153 (1984).
171. M. Pyo, G. Maeder, R. T. Kennedy, and J. R. Reynolds, *J. Electroanal. Chem.* **368**, 329 (1994).
172. R. S. Deinhammer, M. D. Porter, and K. Shimazu, *J. Electroanal. Chem.* **387**, 35 (1995).
173. W. Kutner, *Electrochim. Acta* **37**, 1109 (1992).
174. W. Kutner and K. Doblhofer, *J. Electroanal. Chem.* **326**, 139 (1992).
175. G. Bidan, C. Lopez, F. Mendes-Viegas, and E. Vieil, *Biosensors Bioelectronics* **9**, 219 (1995).
176. D. A. Buttry, *in* "*Electroanalytical Chemistry. A Series of Advances*" (A. J. Bard, ed.), Vol. 17, p. 1. Marcel Dekker, Inc., New York, 1991.
177. M. D. Ward and D. A. Buttry, *Science* **249**, 1000 (1990).
178. R. P. Buck, T. Ricco, E. Lindner, and W. Kutner, *"Electroanalysis with Piezoelectric Devices,"* IUPAC Report, Project 550/62/97, Commission on Electroanalytical Chemistry, V.5, *Pure Appl. Chem.*, to be submitted.

179. G. Sauerbrey, *Z. Phys.* **155**, 206 (1959).
180. M. R. Deakin and H. Byrd, *Anal. Chem.* **61**, 290 (1989).
181. W. Kutner, W. Storck, and K. Doblhofer, *J. Incl. Phenom.* **13**, 257 (1992).
182. A. R. Hillman, D. C. Loveday, S. M. J., S. Bruckenstein, and C. P. Wilde, *Analyst* **117**, 1251 (1992).
183. D. M. Soares, W. Kautek, C. Fruböse, and K. Doblhofer, *Ber. Bunsenges. Phys. Chem.* **98**, 219 (1994).
184. S. J. Martin, V. E. Granstaff, and G. C. Frye, *Anal. Chem.* **63**, 2272 (1991).
185. D. C. Johnson and W. R. LaCourse, *Electroanalysis* **4**, 367 (1992).
186. R. P. Baldwin and K. N. Thomsen, *Talanta* **38**, 1 (1991).