

Development in Voltammetric Analysis with Chemically Modified Electrodes and Biosensors

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A research done in the field of chemically modified electrodes (CMEs) within the last years is reviewed (208 references). Results obtained in our laboratory are related to those reported by other authors. Main areas are the theory and application of CMEs to the trace metal determination and speciation, electrocatalysis and mediated biocatalysis, utilization of polymer films as well as molecular recognition by DNA.

In the last two decades a big activity has been centred on the development of chemically modified electrodes (CMEs). In chemical sensing the emphasis of this relatively modern approach has been on improving the selectivity of electroanalytical measurement. Compared with other electrode concepts, the distinguishing feature of a CME is that generally quite a thin film of a selected chemical is bound to or coated on the electrode surface to endow the electrode with the chemical, electrochemical, optical, electrical, transport, and other desirable properties of the film in a rational, chemically designed manner. Recommended terminology and definitions concerning CMEs were introduced recently [1, 2]. CMEs can operate both amperometrically (or voltammetrically) and potentiometrically, however, they are generally used as amperometric sensors. A response reflects two separate components: a usual electrochemical one controlled by the potential and a special chemical one given by the reactivity of a confined chemical modifier. Hence, a chemically altered bare electrode exhibits new qualities concerning selectivity and sensitivity as well as against fouling and interferences.

Traditionally, conventional bulky electrodes (solid, carbon paste) are used as a support for the preparation of CMEs and testing new reagents and detection schemes [3–5]. The work on modified microelectrodes was stimulated by their potential application to flowing solution analysis (liquid chromatography, capillary electrophoresis, *etc.*) [6, 7]. A revolution step to the mass production of disposable electrochemical

sensors is represented by the development of screen-printing thick film technology [8–11]. Previously, we have reviewed the state-of-art for various types of CMEs [12–17]. It is the aim of this paper to review new investigations regarding analytical applications of CMEs and to demonstrate the progress we have made on this topic. The results of several concepts of CMEs are reported, such as selective binding and speciation of heavy metals, electrocatalysis and mediated biocatalysis, barrier, conducting and accumulation effects of polymeric materials, and biomolecular recognition.

Determination and Speciation of Heavy Metals

Electroanalytical techniques are well known as a simple and effective alternative to the atomic spectroscopic determination of heavy metals. As at adsorptive stripping voltammetry, the CMEs employ a nonelectrolytic preconcentration of trace analyte M due to its selective interaction with the chemical discriminator A,

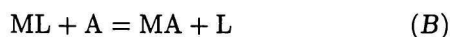


prior to the amperometric detection and have expanded the number of elements which can be determined voltammetrically with low detection limit, *e.g.* iron [18, 19]. The sensors were applied to the determination of copper [20–24], mercury and silver [25, 26], *etc.* Disposable thick film modified electrodes were introduced into stripping voltammetry to substitute

metallic mercury [27]. In principle, the electrode surface can be also modified *in situ*. The modification by surfactants was suggested for the accumulation of titanium, vanadium, and molybdenum [28–30] as well as silver [31] and iodine [32].

In this field, the diphenylcarbazone/graphite composite electrode for the mercury determination [33] and graphite electrodes with the surface modified by thia ligands for the silver [34] and copper [35] determination were introduced in our laboratory. The competitive complexation matrix effect was also evaluated [36]. A mathematical expression of selectivity derived for the CMEs with ion-exchange resin modifiers [37] allows to choose experimental conditions similarly as in the case of the preseparation of interferents [38].

It seems generally that electrochemical methods play an unsubstitutable role at special kinds of analysis. The determination of heavy metals speciation belongs surely to such analytical tasks. The possibility of preparing a family of sensors with different detection characteristics makes (bio)chemically modified electrodes suitable tools for the metal speciation especially at samples with various metal species and metal-complexing ligands of different strength or concentration. Based on the binding competition between a natural ligand in sample L and the electrode modifier A,



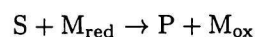
the CME reflects free metal ions and those gradually released from kinetically labile complexes by the dissociation during the time scale of metal accumulation. Hence, the analytical detection window can be shifted by the selection of the discriminator A and the sample is not contaminated with A.

Thermodynamics and kinetics of the metal accumulation at CMEs has been investigated. Relationships between the electrochemical signal and the concentration of both metal as well as ligand in solution according to the corresponding conditional stability constants or the selectivity coefficients of the electrode modifiers and time of measurement were verified [34, 35, 39–41]. Our results correspond with and represent additions to those obtained by other authors [42, 43]. A control of the analytical time-scale of measurement by hydrodynamic conditions at the metal accumulation step has been applied to the anodic stripping voltammetry (ASV) with CMEs. For copper species the validity of voltammetric criteria for the discrimination between the complex dissociation and diffusional rate control at CMEs was confirmed [39, 40]. A general approach to the determination of metal speciation based on the initial reaction rate of the (bio)chemical electrode modifier with metal species was developed [41]. The labile fraction of metal is operationally defined as it was shown by the comparison of results obtained with DPP, DPASV at the

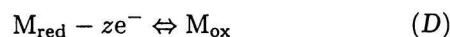
solid CME and the hanging mercury drop electrode as well as the ion-exchange column procedure [44]. The CMEs were also used for the determination of a sample complexation capacity and metal complexation stability constants [35, 40] as well as for the simple estimation of chelating properties of humic acids [45, 46] and solids [47].

Electrocatalytic and Biocatalytic Determinations

A need in the detection of compounds with poor spectrophotometric and electrochemical behaviour as well as in new enzyme electrodes has greatly stimulated the research of catalysis at CMEs. A chemical redox transformation of the substrate (biomolecule) S to the product P on the CME with the attached mediator couple M_{ox}/M_{red} can be described as follows



or



Nitric oxide belongs today to the most intensively studied small molecules. While the nickel central atom within the porphyrin environment is not essential for the detection of NO as demetallated porphyrin films give the same detection limits [48, 49], iron porphyrin films take part at the oxidative NO conversion [50]. Nickel-containing polymeric films [51], nickel and cobalt phthalocyanines [52], and composite modifiers on carbon microelectrodes [53] were described as mediators at the electrochemical detection of NO. A cobalt tetraaminophthalocyanine complex [54], palladium-substituted heteropolytungstate and heptadecatungstodiphosphate [55] as well as ytterbium and uranyl ions within Nafion coating [56] were applied to the electrodes for nitrite.

Electrocatalysis of the molecular oxygen reduction is of interest [57, 58]. We have shown the catalytic properties of a copper complex with the synthetic tetraazamacrocyclic ligand at the oxygen reduction in both aqueous and nonaqueous media [59, 60]. Metalloporphyrins were employed at the determination of hemoglobin [61] and toxic organohalides in aqueous solution [62], the nickel phthalocyanine at the HPLC detection of phenolic antioxidants [63]. Ruthenium dioxide deposited *in situ* at carbon fibre microelectrode was utilized at the flow injection amperometric determination of insulin [64]. Hydrazine was determined on a Nafion/Ru(III) film [65] and caffeine and acetaminophen in drug formula-

tions on a Nafion/ruthenium oxide pyrochlore [66]. An electrocatalytic reduction of DNA at the C₆₀: γ -cyclodextrin/Nafion modified glassy carbon electrode was investigated [67]. The tetrachlorophthalate(III) was bound as a catalyst and the perchlorate as an analyte at a carbon paste electrode (CPE) with liquid anion-exchanger [68]. Hydrogen peroxide was determined using manganese dioxide [69] and platinum/cobalt modifiers [70].

Among electrocatalysts and biosensor mediators, hexacyanoferrate in both ionic and nonionic forms is often considered even at nanosensors [71, 72]. Stable immobilization and electron-transfer performance of the mediator are sometimes rather limited. We have found an efficient attachment of the hexacyanoferrate anion to synthetic hydrotalcite as well as solid (Wofatit SBW) and liquid (tris-alkylamines) anion-exchangers [73]. The CMEs were utilized for the determination of ascorbic acid in real samples. The results are comparable with those obtained at the CME with mechanically immobilized copper hexacyanoferrate [74]. A simple *in situ* preparation and renewal of the surface-modified sensor may be of interest for the analysis in flowing stream. Carbon paste electrodes and microelectrodes bulk-modified with various metal oxides have been investigated for the electrooxidation of amino acids and used for their detection in capillary electrophoresis [75]. By this study, the copper, nickel, and cobalt oxides were confirmed to be the active modifiers in the case of application of metal wire indicating electrodes in strongly alkaline medium.

New materials and technologies for biosensors were reported and discussed [76, 77]. In spite of the wide use of electrochemical glucose monitors, over 80 % of the original literature deal with the glucose determination employing glucose oxidase, particularly its immobilization [78, 79] including the preparation of an enzyme ink for screen printing [80], new redox mediators [81], and interactions with conducting polymers [82]. Some working and cost advantages of microorganisms and tissue materials as biological elements in biosensors are generally known. A microbe-based CME with dry *Aspergillus niger* mycelium and ferrocene as the redox mediator has been investigated [17]. It was shown that some specific problems with the time response and signal repeatability are due to a nonhomogeneity of the biocomponent, a need of its rehydration as well as mediator leaching.

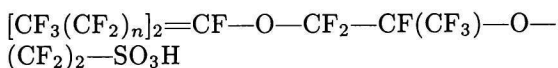
The detection of hydrogen peroxide is of interest in biosensing and redox mediators working at mild potentiostatic conditions are still searched [79, 83–89]. Derived from the reduction of peroxides mediated by a mixed ligand copper complex [90], a CPE was prepared with the bis(bathophenanthroline) copper complex bound electrostatically to the protonized amino groups of tris-octylamine used as a pasting liquid and it was utilized for the catalytic detection of H₂O₂ at –0.2 V *vs.* SCE in solution of pH 5 [91]. The glucose

oxidase-doped sensor exhibits a dynamic response to glucose within mmol dm^{–3} concentration range. This approach can be employed generally at oxidase-type biosensors with a simple *in situ* renewal of mediator loading.

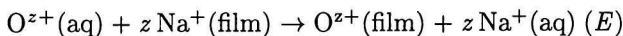
Electrocatalytic oxidation of NADH on the electrodes modified by cobalt hexacyanoferrate [92], redox dyes [93], and 2,6-dichlorophenolindophenol [94] was reported. Zirconium phosphate [95] and titanium phosphate [96] exhibit a strong stabilizing effect on redox properties of the NADH oxidation mediators such as phenothiazines and phenoxazines.

Applications of Polymer Materials

Nafion is the most commonly employed ion-exchange polymer. This perfluorinated negatively charged material of general formula



is able to preconcentrate onium ions



The polymer exhibits high stability, cation transport rate, and superselectivity against anions. Recently, a model for the internal structure of Nafion coatings was developed [97]. Nafion was used to anchor a mercury film electrode to the carbon substrate to yield an electrode stable under ultrasound conditions [98]. The Nafion coated mercury film electrode was also applied to chemical speciation of lead and cadmium in fresh water [99]. A protective role of the Nafion film was employed at the detection of dopamine [100, 101] as well as at ion-selective electrodes [102]. A utility of the Nafion membrane for enzyme immobilization was also described [103]. In studies with a Nafion-coated mercury film electrode (NCMFE), we have demonstrated some important advantages [104–106]. The Nafion membrane enhances significantly the signal stability at the ASV determination of trace metals, in particular in the case of solution exchange. The coating restricts fouling effects of the dodecyl sulfate anion up to the concentration 2 g dm^{–3}, the hyamine cation and uncharged surfactants within mg dm^{–3} concentration range.

Other commercially available ion-exchange polymers include the poly(estersulfonate) Kodak AQ 55, poly(styrene sulfonate), poly(acrylic acid), and poly(vinylpyridinium). The last mentioned one has been used, for instance, with mercury film for the selective determination of lead [107]. The effect of electrode coating on the detection limits was investigated [108]. Ion-exchange polymer-modified electrodes were also utilized as sensors in nonconducting media [109].

A development continues in the field of high-

molecular-mass organic films exhibiting electronic conductivity (poly(acetylene), poly(pyrrole), poly(aniline), poly(thiophene), and others). These films behave like metal phases (so-called synthetic metals), semiconductors or insulators depending on the nature and density of dopants. Conducting polymers were utilized for the immobilization of metal complexation agents [110] and enzymes [79, 111–114]. We have reported a catalytic oxidation of substituted hydroquinones on the poly(pyrrole)-modified electrode with the negative potential shift by 150 to 300 mV compared to the bare platinum electrode together with an increase in reversibility and current response [115]. Poly(pyrrole) containing metal complexes was applied in electroanalysis and electrocatalysis [116]. Overoxidized poly(pyrrole) exhibits ion-exchange properties with similar efficiency as Nafion [117]. It was used at an ultramicroelectrode for the measurement of dopamine in extremely small sample volumes [118]. Cation and anion selective poly(pyrrole) membranes were generated by the application of various dopants [119–121].

Electrocatalytic properties of other polymeric materials were also utilized. For instance, organic polymer dyes were used for the amperometric detection of the NAD/NADH couple and other biosubstrates [122–127], electropolymerized 3,4-dihydroxybenzaldehyde for hydrazine [128], and poly(methylene blue) modified electrode for hemoglobin [129]. Sensitivity of potentiometric pH transducers was addressed with poly(aniline) film [130] and glassy carbon as well as screen printed electrodes modified by processible poly(aniline) were employed for designing an urea biosensor [131]. Metal-tetraaminophthalocyanines electropolymerized on glassy carbon electrodes act also as potentiometric pH sensors over the pH range of 1–13 [132]. Poly(amino acids) such as poly(L-lysine) were used for an accumulation of inorganic and organic analytes as well as an improvement of the performance of screen-printed carbon and polished platinum and glassy carbon electrodes [133]. Nonconducting electropolymerized films such as poly(phenol) rep-

resent permselective, biocompatible coatings for amperometric enzyme electrodes which can also improve their stability to the action of whole blood [134–136].

Zeolites have been reviewed as materials suitable for a recognition at molecular level [137, 138]. Clay and zeolite-based sensors were developed for ascorbic acid [139], cytochrome c [140], dopamine [141, 142], paraquat [143], trace copper [19, 21], and mercury [144] as well as nonelectroactive cations [145]. Zeolite [146] and composite [147] modifiers were used to avoid leaching of redox mediators and enzymes.

Graphite-teflon composite based biosensors were prepared for the screening of phenolic compounds in aqueous medium [148]. Modified composite electrodes made of sol-gel materials offer better stability than CPEs and other composites [149]. At this approach a modifier is mixed with tetramethoxyorthosilicate followed by gelation and drying. Inorganic sol-gel polymers were reviewed [150]. They were used not only as the electrode substrate for metals determination [18], however, exhibit also important electrocatalytic effects, *e.g.* at the detection of insulin [63], ammonia [151], ethanol [152], heparin [153], *etc.*

Molecular Recognition by DNA Biosensors

With respect to potentialities of deoxyribonucleic acid (DNA) to recognize some ranges of analytes, the DNA-based electrochemical probes were reviewed [154–156] and compared with enzymatic and immunochemical biosensors [157]. Practical utilization of the DNA biosensors for environmental analysis in laboratory or field as well as clinical analysis and basic medical research is, however, still only at its beginning.

Considerable efforts have been made at the electrochemical detection of a sequence-specific DNA hybridization process. Formation of the DNA duplex (Fig. 1) from an immobilized single stranded (ss) DNA probe and a complementary target single strand can be detected using electroactive DNA intercalators and minor-groove binding indicators [158]. Short DNA se-

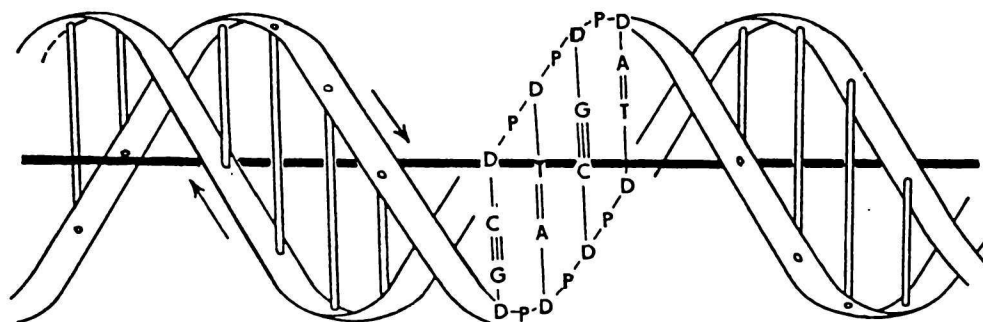


Fig. 1. Model of double stranded DNA.

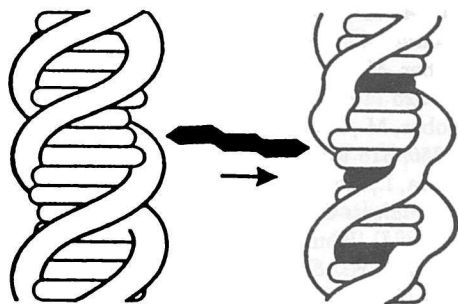


Fig. 2. Scheme of the intercalation process of Watson—Crick type bDNA [190].

quences related to human HIV [159], *Mycobacterium tuberculosis* DNA [160], *E. coli* DNA [161], and *Hepatitis B* virus [162] were determined. Nevertheless, known redox indicators do not satisfactorily fulfil requirements for high sensitivity and specificity [156]. Various electrode materials, DNA duplex indicators, intrinsic polynucleotide redox signals, and electrochemical detection modes were tested recently [163—170]. Polishable [171] and disposable [172] DNA hybridization biosensors were suggested. The ssDNA was incorporated into bilayer lipid membranes [173]. Peptide nucleic acids have been found to possess advantages such as strong adsorption on electrodes, highly specific and fast hybridization at room temperature, and possibility of using shorter probes [174, 175]. Procedures for the recognition and small-volume detection of DNA [176, 177] as well as the simultaneous determination of guanine and adenine contents in DNA, ribonucleic acid (RNA), and synthetic oligonucleotides using CME were described [178].

The double stranded (ds) DNA layer attached to the surface of a voltammetric transducer was used for the accumulation/detection of traces of drugs [179], hydrazines [180] and aromatic amines [181], the elucidation of a drug—DNA reactivity [182—184], the investigation of interactions of DNA with the anti-tumour agents daunomycin [185] and mitomycin C [186, 187] as well as other redox active molecules [188, 189]. Intercalation of analytes is a typical binding mode at dsDNA biosensors. It denotes an insertion of planar molecules of a minimum surface area of 0.28 nm^2 (optimum at three to four rings) between two stacked base pairs (Fig. 2) [190]. A partial deformation of the DNA tertiary structure occurs which can be considered as subtle damage to DNA [156].

We have applied DNA surface and bulk-phase modified carbon paste electrodes to the accumulation/voltammetric determination of electrochemically active phenothiazine and azepine type drugs [191] as well as catechin and acridine derivatives [192]. To validate the assay, a fibre fluorimetric measurement based on an effect of the tested compounds on the long wavelength dye ToPro-3:nucleic acid complex was

used [192]. The DNA modified screen-printed carbon electrodes have been investigated for the determination of nonelectroactive quinazoline derivatives using a voltammetric response of the competitive tris(phenanthroline) cobalt(III) redox marker and a chronopotentiometric response of the guanine residue [193]. The disposable sensors promise wide medical and environmental applications including home diagnostic systems [194].

Direct electroactivity of nucleotides [195], a.c. voltammetric response [196—198], and chronopotentiometry [199] were used in a study of DNA damage represented by a deep DNA degradation (strand breaks, damage or release of DNA bases). While the carbon electrode is only suitable transducer for the hybridization biosensors, the mercury electrode is more sensitive to the detection of DNA damage [156]. Association interactions of two chemical nuclease mimetic copper complex compounds of 1,10-phenanthroline (phen) and the tetraazamacrocyclic ligand TAAB with calf thymus dsDNA have been investigated using solution and surface-based methods [200]. A general trend in transition of the interaction mode between the charged metal species and the DNA duplex from dominantly electrostatic to dominantly intercalative with the increase in ionic strength was confirmed. A simple and sensitive bioassay procedure based on a change in the voltammetric signals of both the Co(phen)_3^{3+} redox marker and the DNA guanine moiety was proposed for the evaluation of damage to DNA in solution as well as GCE-attached DNA.

CONCLUSION

The application of CMEs in research and chemical analysis is very large today. Modified electrodes were successfully utilized for the preconcentration and detection of trace organic compounds. Chemical modifiers, redox mediators, and protective films are widely used at biosensors for various analytes. CMEs can be found in new areas. For instance, nonelectroactive cations have been detected amperometrically in capillary electrophoresis using a graphite electrode modified with mixed valent ruthenium iron cyanide [201]. Problem of protein adsorption at glassy carbon electrode was solved by a covalent bond of tetraethylene glycol diamine [202].

We can await further investigations of the probes with biomolecules such as DNA and cyclodextrins [203—208] as simple warning analytical systems for the detection of drugs as well as risk compounds and pollutants. With respect to this, the manufacture of thick film strips as low-cost sensing and diagnostic devices for a single use will also continue. The development of portable and on-line monitoring systems in future will be based on the electrochemical detection with CMEs.

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