

ELECTROCHEMICAL SENSORS FOR ENVIRONMENTAL MONITORING: A REVIEW OF RECENT TECHNOLOGY

by

JOSEPH WANG
Department of Chemistry and Biochemistry
New Mexico State University
Las Cruces, New Mexico 88003

Solicitation No.
LV-94-012

Project Officer
Kim Rogers
EMSL - U.S. EPA
P.O. Box 93478
Las Vegas, NV 89139-3478

National Exposure Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Notice

The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development (ORD), funded and managed in the compilation of this research review. It has been subjected to the Agency's peer review and has been approved as an EPA publication. The U.S. Government has the right to retain a non-exclusive, royalty-free license in and to any copyright covering this article.

Abstract

Electrochemical sensors are expected to play an increasing role in environmental monitoring. Significant technological advances during the 1980's and early 1990's are certain to facilitate the environmental applications of electrochemical devices. This report surveys important advances in electrochemical sensor technology, including amperometric or potentiometric biosensors, chemically modified electrodes, stripping-based metal sensors, and other tools for on-site field testing. Such devices should allow one to move the measurement of numerous inorganic and organic pollutants from the central laboratory to the field, and to perform them rapidly, inexpensively, and reliably. Representative environmental applications and future prospects are discussed.

Contents

Notice	ii
Abstract	iii
Introduction	1
Principles	2
Electrochemical Biosensors	3
Chemically Modified Electrodes for Environmental Monitoring	6
Stripping-based Metal Sensors	8
Ion and Gas Selective Electrodes	11
Conclusions	12
References	13

Tables

Table 1. Typical Environmental Applications of Stripping Analysis	9
Table 2. Examples of Electrochemical Sensors and Biosensors for Environmental Analysis	12

Figures

1. Electrochemical biosensors: biorecognition and signal transduction	3
2. Enzyme (tyrosinase) electrode for monitoring phenolic compounds	4
3. Amperometric immunosensor based electroactive-(A) and enzyme (B) tagged antigen	5
4. Electrocatalysis at modified electrodes; electron transfer mediated reaction between the target analyte and surface-bound catalyst	7
5. Steps in anodic (A) and adsorptive (B) stripping voltammetry of trace metals, based on electrolytic and adsorptive accumulation, respectively, of target metal analytes	10

Introduction

Electroanalytical chemistry can play a very important role in the protection of our environment. In particular, electrochemical sensors and detectors are very attractive for on-site monitoring of priority pollutants, as well as for addressing other environmental needs. Such devices satisfy many of the requirements for on-site environmental analysis. They are inherently sensitive and selective towards electroactive species, fast and accurate, compact, portable and inexpensive. Such capabilities have already made a significant impact on decentralized clinical analysis. Yet, despite their great potential for environmental monitoring, broad applications of electrochemical sensors for pollution control are still in their infancy.

Several electrochemical devices, such as pH- or oxygen electrodes, have been used routinely for years in environmental analysis. Recent advances in electrochemical sensor technology will certainly expand the scope of these devices towards a wide range of organic and inorganic contaminants and will facilitate

their role in field analysis. These advances include the introduction of modified- or ultramicroelectrodes, the design of highly selective chemical or biological recognition layers, of molecular devices or sensor arrays, and developments in the areas of microfabrication, computerized instrumentation and flow detectors.

The EPA's Office of Research and Development is currently pursuing the development of environmental monitoring technologies which can expedite the characterization of hazardous waste sites in the U.S. Relevant to this objective, is the review and evaluation of currently reported field analytical technologies. The objective of this report is to describe the principles, major requirements, prospects, limitations, and recent applications of electrochemical sensors for monitoring ground or surface waters. It is not a comprehensive review of these topics, but rather focuses on the most important advances and recently reported devices which hold great promise for on-site water analysis.

Principles

The purpose of a chemical sensor is to provide real-time reliable information about the chemical composition of its surrounding environment. Ideally, such a device is capable of responding continuously and reversibly and does not perturb the sample. Such devices consist of a transduction element covered with a biological or chemical recognition layer. In the case of electrochemical sensors, the analytical information is obtained from the electrical signal that results from the interaction of the target analyte and the recognition layer. Different electrochemical devices can be used for the task of environmental monitoring (depending on the nature of the analyte, the character of the sample matrix, and sensitivity or selectivity requirements). Most of these devices fall into two major categories (in accordance to the nature of the electrical signal): amperometric and potentiometric.

Amperometric sensors are based on the detection of electroactive species involved in the chemical or biological recognition process. The signal transduction process is accomplished by controlling the potential of the working electrode at a fixed value (relative to a reference electrode) and monitoring the current as a function of time. The applied potential serves as the driving force for the electron transfer reaction of the electroactive species. The resulting current is a direct

measure of the rate of the electron transfer reaction. It is thus reflecting the rate of the recognition event, and is proportional to the concentration of the target analyte.

In potentiometric sensors, the analytical information is obtained by converting the recognition process into a potential signal, which is proportional (in a logarithmic fashion) to the concentration (activity) of species generated or consumed in the recognition event. Such devices rely on the use of ion selective electrodes for obtaining the potential signal. A permselective ion-conductive membrane (placed at the tip of the electrode) is designed to yield a potential signal that is primarily due to the target ion. Such response is measured under conditions of essentially zero current. Potentiometric sensors are very attractive for field operations because of their high selectivity, simplicity and low cost. They are, however, less sensitive and often slower than their amperometric counterparts. In the past, potentiometric devices have been more widely used, but the increasing amount of research on amperometric probes should gradually shift this balance. Detailed theoretical discussion on amperometric and potentiometric measurements are available in many textbooks and reference works.¹⁻⁵

Electrochemical Biosensors

The remarkable specificity of biological recognition processes has led to the development of highly selective biosensing devices. Electrochemical biosensors hold a leading position among the bioprobes currently available and hold great promise for the task of environmental monitoring. Such devices consist of two components: a biological entity that recognizes the target analyte and the electrode transducer that translates the biorecognition event into a useful electrical signal. A general schematic diagram for the operation of electrochemical biosensors is shown in Figure 1. A great variety of schemes for implementing the electrochemical biosensing approach, based on different combinations of biocomponents and electrode transducers have been suggested. These rely on the immobilization of enzymes, antibodies, receptors or whole cells onto amperometric or potentiometric electrodes. Fundamental aspects of these devices have been reviewed in the literature.⁶⁻⁸

Enzyme electrodes have the longest tradition in the field of biosensors. Such devices are usually prepared by attaching an enzyme layer to the electrode surface, which monitors changes occurring as a result of the biocatalytic reaction amperometrically or potentiometrically. Amperometric enzyme electrodes rely on the biocatalytic generation or consumption of electroactive species. A large number of hydrogen-peroxide generating oxidases and NAD^+ -dependent dehydrogenases have been particularly useful for the measurement of a wide range

of substrates. The liberated peroxide or NADH species can be readily detected at relatively modest potentials (0.5-0.8V vs. Ag/AgCl), depending upon the working electrode material. Lowering of these detection potentials is desired for minimizing interferences from coexisting electroactive species. Potentiometric enzyme electrodes rely on the use of ion- or gas-selective electrode transducers, and thus allow the determination of substrates whose biocatalytic reaction results in local pH changes or the formation or consumption of ions or gas (e.g. NH_4^+ or CO_2). The resulting potential signal thus depends on the logarithm of the substrate concentration. Proper functioning of enzyme electrodes is greatly dependent on the immobilization procedure.

The design of enzyme electrodes is such that the current or potential measured is proportional to the rate limiting step in the overall reaction. For reactions limited by the Michaelis-Menten kinetics, a leveling off of calibration curves is expected at high substrate concentrations. Mass-transport limiting membranes can be used to greatly extend the linear range. This will also lead to a slower response. The signal may be dependent also upon the pH of the water sample or its heavy-metal content that affect the enzymatic activity. Attention should be given also to the long-term stability of these devices, due to the limited thermostability of the biocatalytic layer. Improved immobilization and use of thermophilic or 'synthetic' enzymes should be useful for

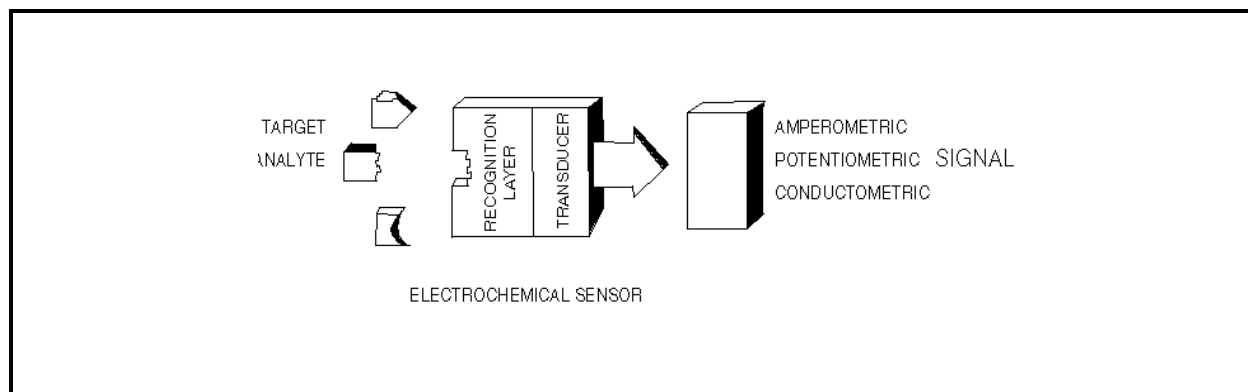


Figure 1. Electrochemical biosensors: biorecognition and signal transduction.

extending the lifetime of enzyme electrodes (particularly in connection with field applications). Mass producible, disposable enzyme electrodes can be readily fabricated (as common for clinical self-testing of blood glucose), and used as 'one-shot' throwaway devices.

Several enzyme electrodes have already proven useful for the task of environmental monitoring. For example, several groups reported on highly sensitive amperometric biosensors for phenolic compounds.⁹⁻¹⁵ Such devices rely on the immobilization of tyrosinase (polyphenol oxidase) onto carbon- or platinum transducers, and the low potential detection of the liberated quinone product (Figure 2). Assays of industrial wastes or natural water have been documented,¹²⁻¹⁴ including possible remote phenol sensing¹³ and single-use on-site sensing.^{14,15} Similarly, low potential biosensing of organic peroxides or hydrogen peroxides can be accomplished at peroxidase-modified electrodes.^{16,17} "Class-selective" enzyme electrodes, based on tyrosinase or peroxidase, can be used for semi-quantitative field screening. They can also be used as detectors for liquid chromatography, hence providing quantitation of the individual substrates.¹⁸ The organic-phase activity of these enzymes should be useful not only for chromatographic separations, but also in connection with rapid solvent extraction procedures. Other enzymes, such as sulfite oxidase, nitrate reductase, nitrilase, alcohol dehydrogenase, or formaldehyde dehydrogenase have been employed for electrochemical biosensing of environmentally-relevant species such as sulfite,¹⁹ nitrate,²⁰ organonitriles,²¹ alcohols,²² or formaldehyde,²³ respectively. Most of the above devices

offer low (micromolar) detection limit, good precision (RSD = 1-3%) and fast (30-60 sec.) response.

In addition to substrate monitoring, it is possible to employ enzyme electrodes for measuring various toxins (via the perturbation/modulation of the enzyme activity). For example, the inhibition of enzymes, such as cholinesterase, tyrosinase, or peroxidase, has led to useful biosensors for organophosphates and carbamates pesticides,²⁴ cyanide,²⁵ or toxic metals.^{26,27} The resulting (inhibition) plots thus reflect the enzyme inhibition kinetics. Such enzyme inhibition devices may thus be useful as early warning poison detectors. Improved specificity may be achieved by designing multi-enzyme arrays that offer a "fingerprint" pattern of the individual inhibitors. Analogous detection of benzene or herbicide contaminations and of anionic surfactants can be accomplished by immobilizing whole cells onto electrodes and monitoring the modulation in the microbial activity.^{28,29,30} Another environmentally important microbial sensor offers rapid estimate of BOD (biochemical oxygen demand), hence replacing the long (5 day) conventional BOD test.³¹ The use of whole cells (instead of isolated enzymes) can increase the sensor stability and allows regeneration of the bioactivity (via immersion in a nutrient media). Other whole cell electrodes, relying on plant tissues (such as mushroom or horseradish) have been used for detecting phenolic and peroxide substrates (of their tyrosinase and peroxidase enzymes). While offering prolonged lifetimes, such tissue electrodes may suffer from side reactions due to the coexistence of several enzymes.

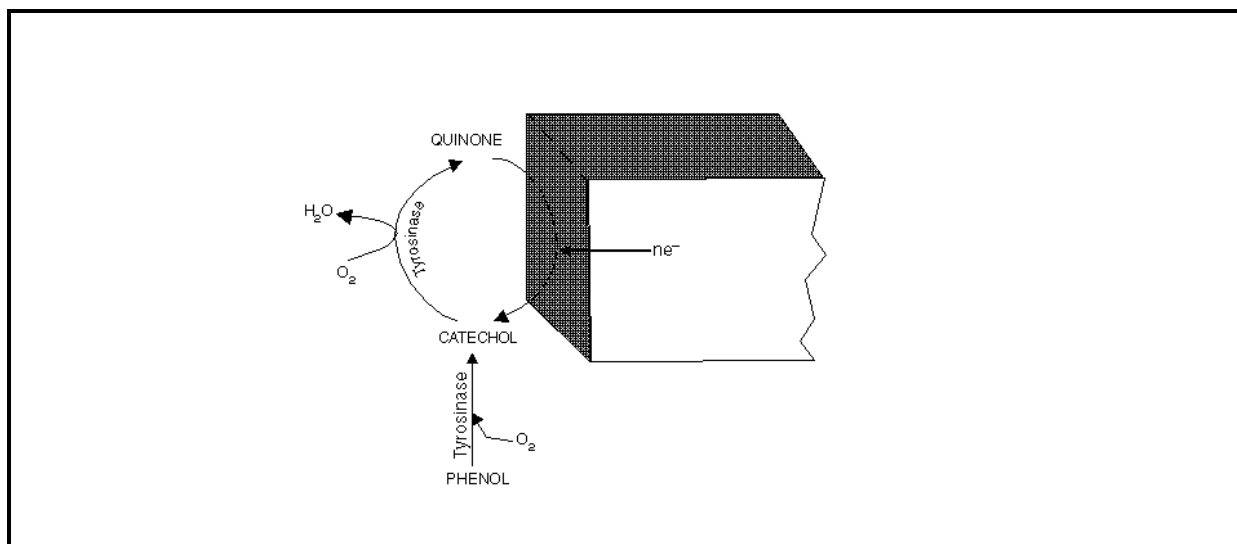


Figure 2. Enzyme (tyrosinase) electrode for monitoring phenolic compounds.

Affinity electrochemical biosensors, employing natural binding molecules as the recognition element should also play a growing role in future environmental monitoring. In this case the recognition process is governed primarily by the shape and size of the receptor pocket and the analyte of interest. Particularly promising are electrochemical immunosensors due to the inherent specificity of antibody-antigen reactions.³² Disposable immunoprobes based on mediated electrochemistry have been developed.³³ In addition to immunosensors, the environmental arena may benefit from the production of electrochemical immunoassay test kits. Such assays commonly rely on labelling the antigen with an electroactive tag (Figure 3A), or with an enzyme that acts on a substrate and liberates an electroactive product (Figure

3B). A wide variety of enzymes are suitable (peroxidase, alkaline phosphatase, etc.), and there is also a wide choice of substrates for these enzymes. New test kits, developed for the clinical market, may be readily adapted for environmental monitoring. Other promising concepts are based on specific binding between membrane-embedded receptors and target analytes³⁴ or the hybridization of electroactive markers by surface-bound DNA.³⁵ Amperometric or potentiometric transducers are useful to follow these binding events. Genetic engineering technology is currently being explored for designing binding molecules for target analytes.

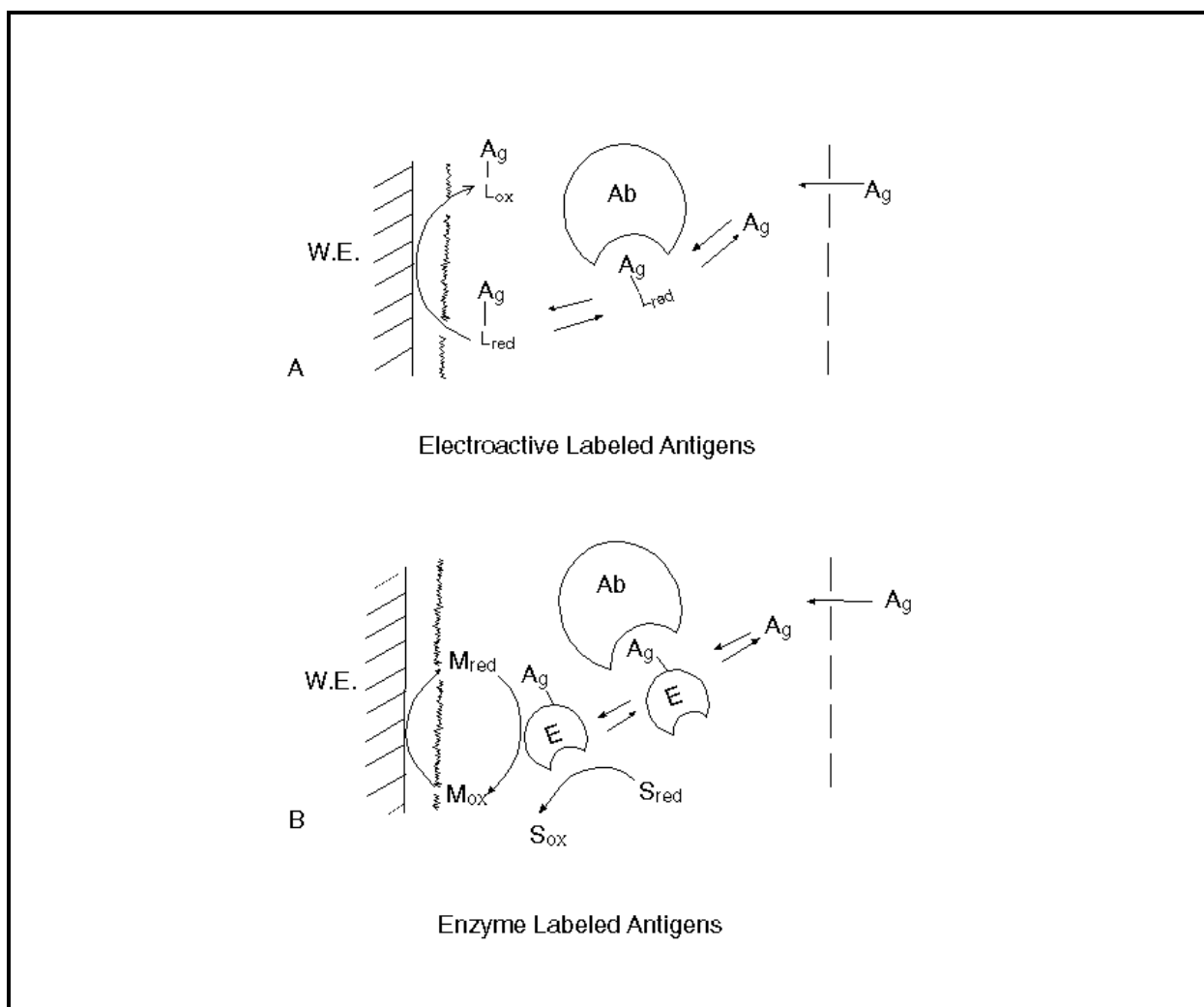


Figure 3. Amperometric immunosensor based electroactive-(A) and enzyme (B) tagged antigen.

Chemically Modified Electrodes for Environmental Monitoring

Chemical layers can also be used for imparting a high degree of selectivity to electrochemical transducers. While conventional amperometric electrodes serve mainly for carrying the electrical current, powerful sensing devices can be designed by a deliberate modification of their surfaces. Basically, the modification of an electrode involves immobilization (on its surface) of reagents that change the electrochemical characteristics of the bare surface. Inclusion of reagents within the electrode matrix (e.g. carbon paste) is another attractive approach for modifying electrodes. Such manipulation of the molecular composition of the electrode thus allows one to tailor the response to meet specific sensing needs. The new “mercury-free” surfaces address also growing concerns associated with field applications of the classical mercury drop electrode. Theoretical details on modified electrodes can be found in several reviews.³⁶⁻³⁸

While sensors based on modified electrodes are still in the early stages of their lifetime, such preparation of structured interfaces holds great promise for the task of environmental monitoring. There are different directions by which the resulting modified electrodes can benefit environmental analysis, including acceleration of electron-transfer reactions, preferential accumulation or permselective transport.

Electrocatalysis involves an electron transfer mediation between the target analyte and the surface by an immobilized catalyst (Figure 4). Such catalytic action results in faster electrode reactions at lower operating potentials. Various catalytic surfaces have thus been successfully employed for facilitating the detection of environmentally-relevant analytes (with otherwise slow electron-transfer kinetics). These include the electrocatalytic determination of hydrazines³⁹ or nitrosamines⁴⁰ at electrodes coated with mixed-valent ruthenium films, monitoring of aliphatic aldehydes at palladium-modified carbon paste,⁴¹ sensing of nitrite at a glassy carbon electrode coated with an osmium-based redox polymer,⁴² of nitrate at a copper modified screen printed carbon electrode,⁴³ monitoring of organic peroxides at cobalt-phthalocyanine containing carbon pastes,⁴⁴ and of

hydrogen peroxide at a copper heptacyano-nitrosylferrate-coated electrode.⁴⁵

Preconcentrating modified electrodes can also be useful for environmental sensing. In this case an immobilized reagent (e.g. ligand, ion-exchanger) offers preferential uptake of target analytes. This approach enjoys high sensitivity because it is a preconcentration procedure. A second major advantage lies in the added dimension of selectivity, which is provided by the chemical requirement of the modifier-analyte interactions. Such improvements have been documented for the measurement of nickel, mercury, or aluminum ions at dimethylglyoxime,⁴⁶ crown-ether,⁴⁷ or alizarin⁴⁸ containing carbon pastes, respectively, monitoring of nitrite, chromium, or uranyl ions at ion-exchanger modified electrodes,⁴⁹⁻⁵¹ and of copper at an algaemodified electrode.⁵² Covalent reactions can be used for analogous collection/determination of organic analytes, e.g. monitoring of aromatic aldehydes at amine-containing carbon pastes.⁵³ Routine environmental applications of these preconcentrating electrodes would require attention to competition for the surface site and the regeneration of an ‘analyte-free’ surface.

Another promising avenue is to cover the sensing surface with an appropriate permselective film. Discriminative coatings based on different transport mechanisms (based on analyte size, charge, or polarity) can thus be used for addressing the limited selectivity of controlled-potential probes in complex environmental matrices. The size-exclusion sieving properties of various polymer-coated electrodes offer highly selective detection of small hydrogen peroxide or hydrazine molecules.^{54,55} In addition, surface passivation (due to adsorption of macromolecules present in natural waters) can be prevented via the protective action of these films.

More powerful sensing devices may result from the coupling of several functions (permselectivity, preconcentration or catalysis) onto the same surface. Additional advantages can be achieved by designing arrays of independent modified electrodes, each coated

with a different modifier and hence tuned toward a particular group of analytes. The resulting array response offers a unique fingerprint pattern of the individual analytes, as well as multicomponent analysis (in connection with statistical, pattern-recognition procedures). Use of different permselective coatings or catalytic surfaces thus hold great promise for multiparameter pollution monitoring. The development of electrochemical sensor arrays has been reviewed

recently.⁵⁶ Related to this are new molecular devices based on the coverage of interdigitated microarrays with conducting polymers.^{57,58} Eventually we expect to see molecular devices in which the individual components are formed by discrete molecules. Modification of miniaturized screen-printed sensor strips can also be accomplished via the inclusion of the desired reagent (e.g. ligand, catalyst) in the ink used for the micro-fabrication process.

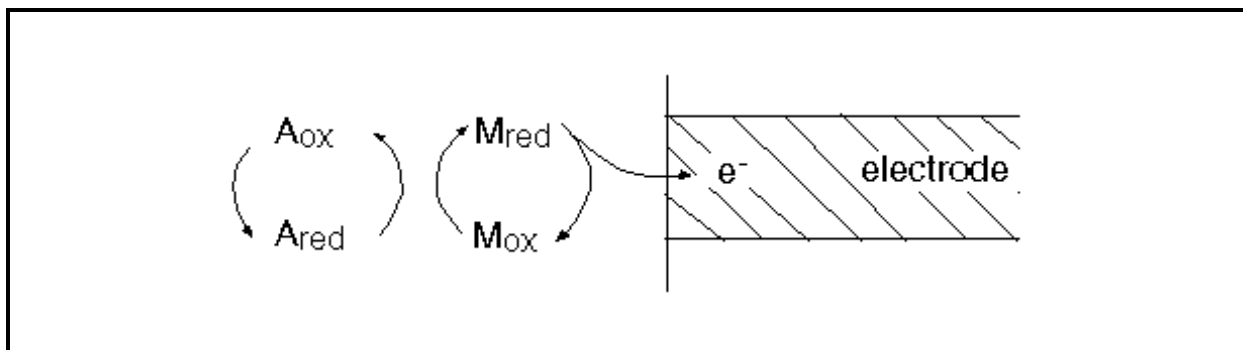


Figure 4. Electrocatalysis at modified electrodes; electron transfer mediated reaction between the target analyte and surface-bound catalyst.

Stripping-based Metal Sensors

The most sensitive electroanalytical technique, stripping analysis, is highly suitable for the task of field monitoring of toxic metals. The remarkable sensitivity of stripping analysis is attributed to its preconcentration step, in which trace metals are accumulated onto the working electrode. This step is followed by the stripping (measurement) step, in which the metals are “stripped” away from the electrode during an appropriate potential scan. About 30 metals can thus be determined by using electrolytic (reductive) deposition or adsorptive accumulation of a suitable complex onto the electrode surface (Figure 5). Stripping electrodes thus represent a unique type of chemical sensors, where the recognition (accumulation) and transduction (stripping) processes are temporally resolved. Short accumulation times (of 3-5 min) are thus sufficient for convenient quantitation down to the sub-ppb level, with shorter periods (1-2 min) allowing measurements of ppb and sub-ppb concentrations. The timeconsuming deaeration step has been eliminated by using modern stripping modes (e.g. potentiometric or square-wave stripping), that are not prone to oxygen interferences. Stripping analysis can provide useful information on the total metal content, as well as characterization of its chemical form (e.g. oxidation state, labile fraction, etc.).⁵⁹ Field measurements of chromium(VI) represent one such example.^{60,61} Overlapping peaks, formation of intermetallic compounds and surfactant adsorption represent the most common problems in stripping analysis.

Various advances in stripping analysis should accelerate the realization of on-site environmental testing of toxic metals. New sensor technology has thus replaced the traditional laboratory-based mercury electrodes and

associated cumbersome operation (oxygen removal, solution stirring, cell cleaning, etc.). Of particular significance are new stripping-based tools, such as automated flow systems for continuous on-line monitoring,⁶²⁻⁶⁴ disposable screen-printed stripping electrodes for single-use field applications,⁶⁵ or remote/submersible devices for down-hole well monitoring or unattended operation.^{66,67} Portable and compact (hand-held), battery-operated stripping analyzers are currently being commercialized for controlling these field-deployable devices. In addition to providing on-site realtime information, such in-situ devices should minimize errors (due to contamination or loss) inherent to trace metal measurement in a centralized laboratory. Stripping analysis has been extensively used by marine chemists on board ships for numerous oceanographic surveys.⁶² Relevant examples of environmental applications of stripping analysis are given in Table 1.

In addition to trace metal pollutants, it is possible to employ new adsorptive stripping procedures for measuring low levels of organic contaminants that display surface-active properties (e.g. detergents, oil components). However, due to competitive adsorption such schemes usually require a prior separation step. Another version of stripping analysis, cathodic stripping voltammetry, can be used for measuring environmentally-relevant anions (e.g. S^{2-} , I, Br⁻) or sulfur- or chlorine-containing pollutants (e.g. pesticides) following their oxidative deposition onto the working electrode. Additional background information on stripping analysis and its environmental opportunities can be found in various books or reviews.⁷⁷⁻⁸⁰

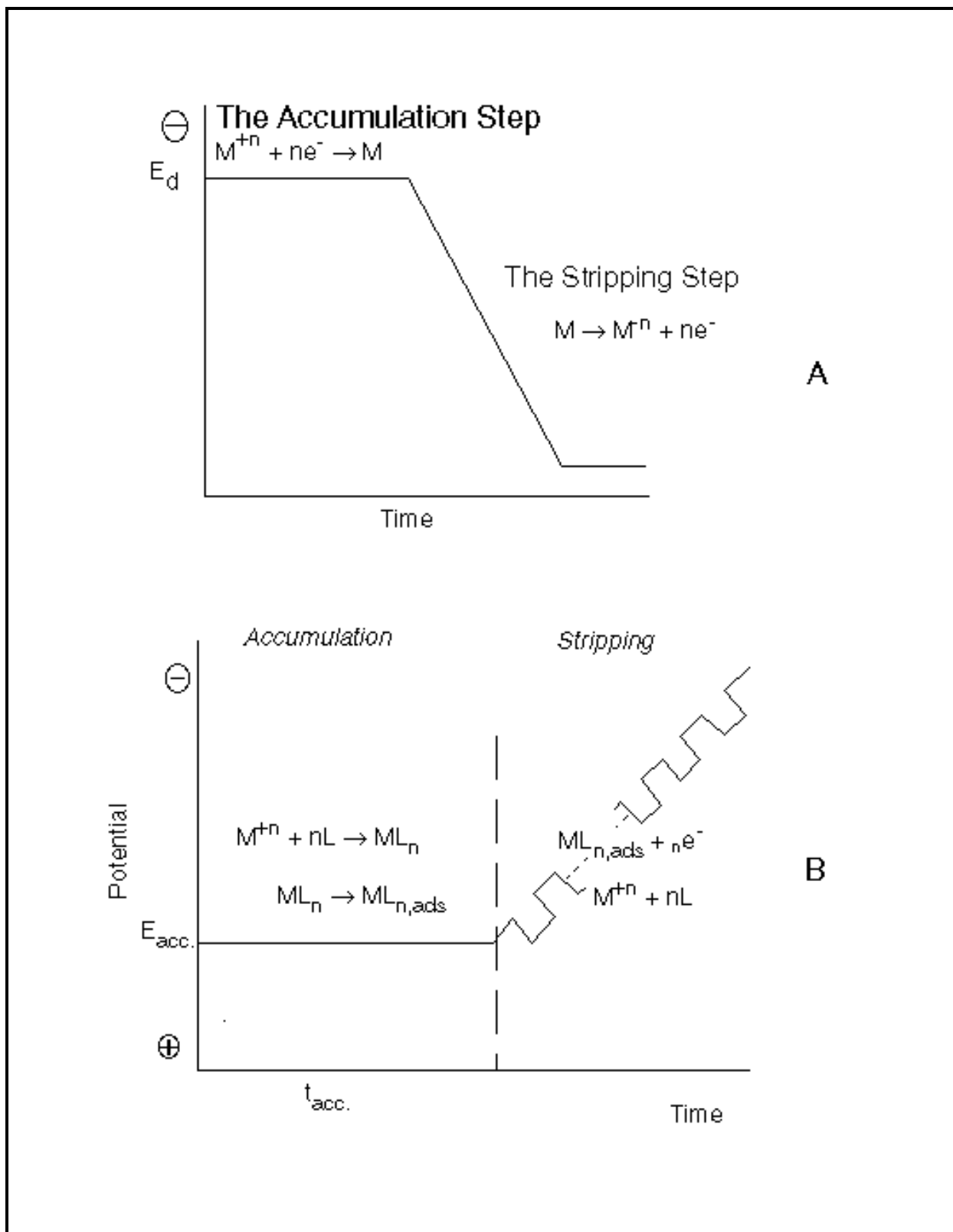


Figure 5. Steps in anodic (A) and adsorptive (B) stripping voltammetry of trace metals, based on electrolytic and adsorptive accumulation, respectively, of target metal analytes.

Table 1. Typical Environmental Applications of Stripping Analysis

Trace Metal	Matrix	Electrode	Stripping Mode	Ref.
As	Natural waters	Gold	Differential pulse	68
Cd	Lakes and Oceans	Mercury film	Differential pulse	65,69
Cr	Seawater Sediments	Mercury drop	Adsorptive	61 60
Cu	Tap water	Mercury film	Potentiometric	70
Hg	Seawater	Gold	Differential pulse	71
Mn	Natural waters	Mercury drop	Potentiometric	72
Ni	Seawater	Mercury drop	Adsorptive	73
Pb	Lakes and Oceans, Sediments	Mercury film Mercury film	Differential pulse Potentiometric	65,69 60
Se	River water	Gold	Potentiometric	74
Tl	Natural waters	Mercury film	Differential pulse	75
U	Groundwater Sediments	Mercury drop	Adsorptive	76

Ion and Gas Selective Electrodes

Ion selective electrodes offer direct and selective detection of ionic activities in water samples. Such potentiometric devices are simple, rapid, inexpensive and compatible with on-line analysis. The inherent selectivity of these devices is attributed to highly selective interactions between the membrane material and the target ion. Depending on the nature of the membrane material used to impart the desired selectivity, ion selective electrodes can be divided into three groups: glass, solid, or liquid electrodes. Many ion selective electrodes are commercially available and routinely used in various fields.

By far the most widely used ion selective electrode is the pH electrode. This glass-membrane sensor has been used for environmental pH measurements for several decades. Its remarkable success is attributed to its outstanding analytical performance, and in particular to its extremely high selectivity for hydrogen ions, broad dynamic range, and fast and stable response. Various solid-state crystalline membrane electrodes have been shown useful for monitoring environmentally-important ions, such as F^- , Br^- , CN^- , S^{2-} or Cu^{+2} .⁸¹ The calcium and nitrate ion-exchanger sensors represent environmentally useful liquid membrane electrodes. The synthetic design of macrocyclic polyether ionophores has led to liquid membrane electrodes for heavy metals, such as lead or zinc.⁸² Anion selective liquid membrane electrodes have

been developed in recent years for sensing of phosphate or thiocyanate. New technologies of thin film (dry-reagent) slides or semiconductor chips will certainly facilitate field monitoring of ionic analytes.⁸³ The principles and applications of ion selective electrodes have been reviewed.⁸⁴⁻⁸⁶

The rapid detection of ammonia or oxygen plays a vital role in pollution control. Gas sensing electrodes are highly selective devices for monitoring these (and other) gases. Such sensors commonly incorporate a conventional ion selective electrode, surrounded by an electrolyte solution and enclosed by a gas permeable membrane. The target gas diffuses through the membrane and reacts with the internal electrolyte, thus forming or consuming a detectable ionic species. The ammonia selective probe uses an internal pH glass electrode in connection with an ammonium chloride electrolyte. The glass electrode detects the decreased activity of protons. While most gas sensors rely on potentiometric detection, the important oxygen probe is based on covering an amperometric platinum cathode with a Teflon or silicon rubber membrane. Handheld and remote oxygen probes are available commercially.⁸⁷ Potentiometric sensors for other gases (SO_2 , NO_2 , HF , etc.) have been designed by using different membranes and equilibrium processes.

Conclusions

Electrochemical sensor technology is still limited in scope, and hence cannot solve all environmental monitoring needs. Yet, a vast array of electrochemical sensors have been applied in recent years for monitoring a wide range of inorganic and organic pollutants (Table 2). We are continuously witnessing the introduction of new electrochemical sensing devices, based on a wide range of chemical or biological recognition materials. In addition, mass production techniques (adapted from the microelectronic industry) enable the fabrication of extremely small and reproducible, and yet inexpensive (disposable), sensing devices. Such devices are being coupled with light and user-friendly microprocessor-based instrumentation.

Fast-responding electrochemical sensors are also being adapted for detection in on-line monitoring or flow-injection systems (as needed for continuous monitoring or field screening applications). Other advances of selective and stable recognition elements,

“smart” sensors and molecular devices, remote electrodes, multiparameter sensor arrays or micromachining and nanotechnology, are certain to have a major impact on pollution control. Additional efforts should be given to the development of new immobilization procedures (that increase the stability of the biocomponent), to the design of new electrocatalysts (that facilitate the detection of additional priority pollutants), to the replacement of classical mercury electrodes with well-defined solid surfaces, to address the fouling and degradation of electrochemical sensors during use, to the development of immunoassay-based electrochemical sensors and of remote electrodes for unattended operations, and introduction of multi-sensor systems for simultaneous monitoring of several priority contaminants. On-going commercialization efforts, coupled with regulatory acceptance, should lead to the translation of these and future research efforts into large scale environmental applications.

Table 2. Examples of Electrochemical Sensors and Biosensors for Environmental Analysis

Analyte	Recognition	Recognition Process	Transduction Element	Ref. mode
Benzene	Modulated microbial activity	Whole cell	Amperometry	28
Cyanide	Enzyme inhibition	Tyrosinase	Amperometry	25
Hydrazines	Electrocatalysis	Ruthenium catalyst	Amperometry	39
Lead	Ion recognition	Macrocyclic ionophore	Potentiometry	82
Mercury	Preconcentration	Crown ether	Voltammetry	47
Nickel	Preconcentration	Dimethylglyoxine	Voltammetry	46
Nitrite	Preconcentration	Aliquat 336 ion exchanger	Voltammetry	49
Nitrosamines	Electrocatalysis	Ruthenium catalyst	Amperometry	40
Peroxides	Biocatalysis	Peroxidase	Amperometry	16,17
Pesticides	Enzyme inhibition	Acetylcholinesterase choline oxidase	Amperometry	16,17
Phenol	Biocatalysis	Tyrosinase	Amperometry	9-15
Sulfite	Biocatalysis	Sulfite oxidase	Amperometry	19
Uranium	Preconcentration	Nafion	Voltammetry	51

References

1. Janata, J., Principles of Chemical Sensors. Plenum Press, New York, 1989, 749pp.
2. Kissinger, P. Heineman, W., Laboratory Techniques in Electroanalytical Chemistry. Dekker, New York, 1984, 749pp.
3. Wang, J., Analytical Electrochemistry. VCH Publishers, New York, 1994, 198pp.
4. Brett, C., Brett, A.M.O., Electrochemistry: Principles, Methods and Applications. Oxford University Press, Oxford, 1993, 427pp.
5. Covington, A.K. (ed.), Ion Selective Electrode Methodology. CRC Press, Boca Raton, 1978, 150pp.
6. Turner, A.P., Karube, I., Wilson, G., Biosensors: Fundamentals and Applications. Oxford Science Publications, Oxford, 1987, 770pp.
7. Frew, J., Hill, H., Electrochemical Biosensors. Anal. Chem. 59:933A, 1987.
8. Kobos, R., Enzyme-Based Electrochemical Biosensors. Trends Anal. Chem. 6:6, 1987.
9. Wang, J. Lu, F., Lopez, D., Amperometric Biosensor for Phenol Based on a Tyrosinase/Graphite Epoxy Biocomposite. Analyst 119:455, 1994.
10. Wang, J., Lu, F., Lopez, D., Tyrosinase-Based Ru-Dispersed Carbon Paste Biosensor for Phenols. Biosens. Bioelectronics 9:9, 1994.
11. Bonakdar, M., Vilchez, J., Mottola, H.A., Bioamperometric Sensors for Phenol Based on Carbon Paste Electrodes. J. Electroanal. Chem. 266:47, 1989.
12. Camponella, L., Beone, T., Sammartino, M., Tomassetti, M., Determination of Phenol in Wastes and Water Using Enzyme Sensor. Analyst 118:979, 1993.
13. Wang, J., Chen, Q., Remote Electrochemical Biosensor for Field Monitoring of Phenolic Compounds. Anal. Chem., submitted.
14. Kotte, H., Grundig, B., Vorlop, K., Strehlitz, B., Stottmeister, U., Methylphenazonium-Modified Enzyme Sensor based on Polymer Thick Films for Subnanomolar Detection of Phenols. Anal. Chem. 67:65, 1995.
15. Wang, J., Chen, Q., Microfabricated Phenol Biosensors based on Screen-Printing of Tyrosinase-Containing Carbon Ink. Anal. Letters, in press (28 (7), 1995).
16. Wang, J., Freiha, B., Naser, N., Romero, E. Wollenberger, U., Ozsoz, M., Evans, O., Amperometric Biosensing of Organic Peroxides with Peroxidase Modified Electrodes. Anal. Chim. Acta 254:81, 1991.

17. Csoregi, E., Gorton, L., Marko-Varga, G., Tudos, A., Kok, T., Peroxidase-Modified Carbon Fiber Microelectrode in Flow-Through Detection of Hydrogen Peroxide and Organic Peroxides. *Anal. Chem.* 66:3604, 1994.
18. Ortega, F., Dominguez, E., Burestedt, E., Emneus, J., Gorton, L., Marko-Varga, G., Phenol Oxidase-based Biosensor as Selective Detection Units in Column Liquid Chromatography for the Determination of Phenolic Compounds. *J. Chromatogr.* 675:65 1994.
19. Abu-Nader, P., Vives, S.S., Mottola, H., Studies with a Sulfite-Oxidase Modified Carbon Paste Electrode for Detection/Determination of SO₂ in Continuous Flow Systems. *J. Electroanal. Chem.* 284:323, 1990.
20. Cosnier, S., Innocent, C., Jouanneau, Y., Amperometric Detection of Nitrate via a Nitrate Reductase Immobilized and Electrically Wired at the Electrode Surface. *Anal. Chem.* 66:3198, 1994.
21. Liu, Z., Wang, Y., Kounaves, S., Brush, E., Determination of Organonitriles Using Enzyme-Based Selectivity Mechanism. *Anal. Chem.* 65:3134, 1993.
22. Wang, J., Gonzalez-Romero, E., Amperometric Biosensing of Alcohols at Electrochemically Pretreated Glassy Carbon Enzyme Electrodes. *Electroanalysis* 5:427, 1993.
23. Weng, L., Ho, M., Nonidez, W., Amperometric Determination of Formaldehyde via Hexacyanoferrate Coupled Dehydrogenase Reaction. *Anal. Chim. Acta* 233:59, 1990.
24. Marty, J., Sode, K., Karube, I., Biosensor for Detection of Organophosphate and Carbamate Insecticides. *Electroanalysis* 4:249, 1992.
25. Smit, M., Rechnitz, G.A., Toxin Detection Using a Tyrosinase-Coupled Oxygen Electrode. *Anal. Chem.* 65:380, 1993.
26. Dolmanova, I., Shekhovtsova, T., Kutcheryaeva, V., Assay of Enzyme Effectors. *Talanta* 34:201, 1987.
27. Shekhovtsova, T., Chernetskaya, S., Determination of Mercury at the pg/ml Level Using Immobilized Horseradish Peroxidase. *Anal. Letters*, 27:2883, 1994.
28. Tan, H., Cheong, S., Tan, T., An Amperometric Benzene Sensor Using Whole Cell *Pseudomonas putida* ML2. *Biosens. Bioelectronics* 9:1, 1994.
29. Rawson, D.M., Willmer, A., Turner, A.P., Whole Cell Biosensor for Environmental Monitoring. *Biosensors* 4:299, 1989.
30. Nomura, Y., Ikebukuro, K., Yokoyama, K., Takeuchi, T., Arikaza, Y., Ohno, S., Karube, I. A Novel Microbial Sensor for Anionic Surfactant Determination. *Anal. Letters* 27:3095, 1994.
31. Riedel, K., Biochemical Fundamentals and Improvement of the Selectivity of Microbial Sensors - A Minireview, *Bioelectrochem. Bioenerg.* 25:19, 1991.
32. Rosen, I., Rishpon, J., Alkaline Phosphatase as a Label for a Heterogeneous Immunochemical Sensor. *J. Electroanal. Chem.* 258:27, 1988.
33. Weetall, H., Hotaling, T., A Simple, Disposable Electrochemical Sensor for Clinical and Immuno-Assay. *Biosensors* 3:57, 1987.

34. Leech, D. Rechnitz, G., Neuronal Biosensors: A Progress Report. *Electroanalysis* 5:103, 1993.
35. Millan, K., Mikkelsen, S., Sequence Selective Biosensor for DNA Based on Electroactive Hybridization Indicators. *Anal. Chem.* 65:2317, 1993.
36. Murray, R.W., Ewing, A.G., Durst, R., Chemically Modified Electrodes: Molecular Design for Electroanalysis. *Anal. Chem.*, 59:379, 1987.
37. Wang, J., Modified Electrodes for Electrochemical Sensors. *Electroanalysis* 3:255, 1991.
38. Arrigan, O., Voltammetric Determination of Trace Metals and Organics After Accumulation at Modified Electrodes. *Analyst* 119:1953, 1994.
39. Wang, J., Lu, Z., Electrocatalysis and Detremination of Hydrazine Compounds at Glassy Carbon Electrodes Coated with Mixed-Valent Ru(III,II) Cyanide Film. *Electroanalysis* 1:517, 1989.
40. Gorski, W., Cox, J., Amperometric Determination of N-Nitrosamines in Aqueous Solution at a Electrode Coated with a Ru-Based Inorganic Polymer. *Anal. Chem.* 66:2771, 1994.
41. Cai, X., Kalcher, K., Studies on the Electrocatalytic Reduction of Aliphatic Aldehydes on Pd-Modified Carbon Paste Electrodes. *Electroanalysis* 6:397, 1994.
42. Doherty, A., Forster, R., Smyth, M., Vos, J., Development of a Sensor for the Detection of Nitrite Using a Glassy Carbon Electrode Modified with the Electrocatalyst [(Os)(bipy)₂(PVP)₁₀Cl]Cl. *Anal. Chim. Acta* 255:45, 1991.
43. Fogg, A., Scullion, S., Edmond, T., Birch, B., Direct Reductive Amperometric Determination of Nitrate at a Copper Electrode Formed In-Situ in a Capillary Fill Sensor Device. *Analyst* 116:573, 1991.
44. Wang, J., Angnes, L., Chen, L., Evans, O., Electrocatalysis and Amperometric Detection of Organic Peroxides at Modified Carbon Paste Electrodes. *Talanta* 38:1077, 1991.
45. Gao, Z., Ivaska, A., Pin, L., Kuaizhi, L., Jianjun, Y. Electrocatalysis and Flow Injection Analysis for Hydrogen Peroxide at a Chemically Modified Electrode. *Anal. Chim. Acta*, 259:211, 1992.
46. Baldwin, R., Christensen, J., Kryger, L., Voltammetric Determination of Ni(II) at a Chemically Modified Electrode Based on DMG Containing Carbon Paste. *Anal. Chem.*, 58:1790, 1986.
47. Wang, J., Bonakdar, M., Preconcentration and Voltammetric Measurement of Mercury with Crown-Ether Modified Carbon Paste Electrode. *Talanta* 35, 277, 1988.
48. Downard, A., Kipton, H., Powell, J., Xu, S., Voltammetric Determination of Aluminum using a Chemically Modified Electrode. *Anal. Chim. Acta* 251:157, 1991.
49. Kalcher, K., A New Method for the Voltammetric Determination of Nitrite. *Talanta* 33:489, 1986.
50. Cox, J., Kulesza, P., Stripping Voltammetry of Cr(VI) at a Poly(4-vinyl pyridine)-Coated Pt Electrode. *Anal. Chim. Acta* 154:71, 1983.
51. Ugo, P., Ballarin, B., Daniele, S., Mazzocchin, A., Electrochemical Behavior and Preconcentration of Uranyl(VI) at Nafion-Coated Glassy Carbon Electrodes. *J. Electroanal. Chem.* 324:145, 1992.

52. Gardea, J., Darnall, D. Wang, J., Bioaccumulation and Measurement of Copper at an Alga-Modified Carbon Paste Electrode. *Anal. Chem.* 60:72, 1988.
53. Liu, K., Abruna, H., Electroanalysis of Aromatic Aldehydes with Modified Carbon Paste Electrodes. *Anal. Chem.* 61:2599, 1989.
54. Sasso, S., Pierce, R., Walla, R., Yacynych, A., Electropolymerized 1,2-Diaminobenzene as a Means to Prevent Interferences and Fouling and to Stabilize Immobilized Enzyme in Electrochemical Biosensor. *Anal. Chem.* 62:1111, 1990.
55. Wang, J., Chen, S., Lin, M., Use of Different Electropolymerization Conditions for Controlling the Size-Exclusion Selectivity at Polyaniline, Polypyrrole and Polyphenol Films. *J. Electroanal. Chem.* 273:231, 1989.
56. Diamond, D., Progress in Sensor Array Research. *Electroanalysis* 5:795, 1993.
57. Matsue, T., Electrochemical Sensors Using Microarray Electrodes. *Trends Anal. Chem.* 12:100, 1993.
58. Bidan, G., Electroconducting Polymers: New Sensitive Matrices to Build Up Chemical or Electrochemical Sensors, A Review. *Sensors Actuators B*6:45, 1992.
59. Florence, T.M., Electrochemical Approaches to Trace Element Speciation in Waters. *Analyst* 111:489, 1986.
60. Olsen, K., Wang, J., Setiadji, R., Lu, J., Field Screening of Chromium, Zinc, Copper, and Lead in Sediments by Stripping Analysis. *Environ. Sci. Technol.* 28:2074, 1994.
61. Boussemart, M., van den Berg, C.M.C., Ghaddaf, M., The Determination of Chromium Speciation in Seawater Using Catalytic Cathodic Stripping Voltammetry. *Anal. Chim. Acta* 262:103, 1992.
62. Zirino, A., Lieberman, S., Clavell, C., Measurement of Cu and Zn in San Diego Bay by Automated Anodic Stripping Voltammetry. *Environ. Sci. Technol.* 12:73, 1978.
63. Wang, J., Sediadji, R., Chen, L., Lu, J., Morton, S., Automated System for On-Line Adsorptive Stripping Voltammetric Monitoring System of Trace Levels of Uranium. *Electroanalysis* 4:161, 1992.
64. Clark, B., DePaoli, D., McTaggart, D., Patton, B., On-Line Voltammetric Analyzer for Trace Metals in Waste Water. *Anal. Chim. Acta* 215:13, 1988.
65. Wang, J., Tian, B., Screen-Printed Stripping Voltammetric/Potentiometric Electrodes for Decentralized Testing of Trace Lead. *Anal. Chem.* 64:1706, 1992.
66. Tercier, M., Buffle, J., Zirino, A., De Vitre, R., In-Situ Voltammetric Measurement of Trace Elements in Lakes and Oceans. *Anal. Chem. Acta* 237:429, 1990.
67. Wang, J., Larson, D., Foster, N., Armalis, S., Lu, J., Rongrong, X., Olsen, K., Zirino, A., Remote Electrochemical Sensor for Trace Metal Contaminants. *Anal. Chem.*, in press (1995).
68. Bodewig, F., Valenta, P., Nurnberg, H., The Determination of As(III) and As(V) in Natural Water by Differential Pulse Anodic Stripping Voltammetry. *Fres. Z. Anal. Chem.* 311:187, 1982.

69. Poldoski, J., Glass, G. ASV at Mercury Film Electrode: Baseline Concentrations of Cd, Pb, and Cu in Selected Natural Waters. *Anal. Chim. Acta* 101:79, 1978.
70. Jagner, D., Sahlin, E., Axelsson, B., Ratana-Ohpas, R., Rapid Method for the Determination of Copper and Lead in Tap Water Using a Portable Potentiometric Stripping Analyzer. *Anal. Chim. Acta* 278:237, 1993.
71. Gustavsson, I., Determination of Mercury in Seawater by Stripping Voltammetry. *J. Electroanal. Chem.* 214:310, 1986.
72. Eskilsson, H., Turner, D., Potentiometric Stripping Analysis for Manganese in Natural Waters. *Anal. Chim. Acta* 161:293, 1984.
73. Achterberg, E., van den Berg, C.M.C. In-Line U.V. Digestion of Natural Water Samples for Trace Metal Determination. *Anal. Chim. Acta* 291:213, 1994.
74. Gil, E., Ostapczuk, P., Potentiometric Stripping Determination of Mercury, Selenium, Copper and Lead at a Gold Film Electrode in Water Samples. *Anal. Chim. Acta* 293:55, 1994.
75. Bonelli, J., Taylor, H., Skogerbae, R., A Direct Differential Pulse ASV Method for the Determination of Thallium in Natural Water. *Anal. Chim. Acta* 118:243, 1980.
76. Wang, J., Wang, J., Lu, J., Olsen, K., Adsorptive Stripping Voltammetry of Trace Uranium. *Anal. Chim. Acta* 292:91, 1994.
77. Wang, J., Stripping Analysis, VCH Publishers. New York, 1985, 159pp.
78. Wang, J. Anode Stripping Voltammetry as an Analytical Tool, *Environ. Sci, Technol.* 16:104A, 1982.
79. Tercier, M., Buffle, J., In-Situ Voltammetric Measurements in Natural Waters: Future Prospects and Challenges. *Electroanalysis* 5:187, 1993.
80. Wang, J., Decentralized Electrochemical Monitoring of Trace Metals: From Disposable Strips to Remote Electrodes. *Analyst* 119:763, 1994.
81. Frant, M. History of the Early Commercialization of Ion-Selective Electrodes. *Analyst* 119:2293, 1994.
82. Simon, W., Pretsch, E., Morf, W., Ammann, D., Oesch, U., Dinten, O., Design and Application of Neutral Carrier-based Ion Selective Electrodes. *Analyst* 109:207, 1984.
83. Jacob, E., Vadasdi, E., Sarkozi, L., Colman, N., Analytical Evaluation of i-STAT Portable Clinical Analyzer and Use by Nonlaboratory Health-Care Professionals. *Clin. Chem.* 39:1069, 1993.
84. Rechnitz, G., Ion and Bio-selective Membrane Electrodes. *J. Chem. Ed.*, 60:282, 1983.
85. Lewenstam, A., Maj-Zurawska, M., Hulanicki, A., Application of Ion-selective Electrodes. *Electroanalysis* 3:727, 1991.
86. Oesch, U., Ammann, D., Simon, W., Ion Selective Membrane Electrodes for Clinical Use. *Clin. Chem.* 32:1448, 1986.
87. "YSI Water Quality Products, 1994," YSI Inc., Yellow Springs, OH.