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REVIEW ARTICLE

The Use of Chemically Modified Electrodes for Liquid Chromatography and Flow-Injection Analysis

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ABSTRACT

The use of chemically modified electrodes (CMEs) for liquid chromatography and flow-injection analysis is reviewed. Electrochemical detection with CMEs based on electrocatalysis, permselectivity, ion flow in redox films, and ion transfer across the water–solidified nitrobenzene interface is discussed in terms of improving the stability, selectivity, and scope of electrochemical detectors, and the detection of electroinactive substances. More than 90 references are included.

INTRODUCTION

Electrochemical (EC) detection in flowing streams has become a powerful tool for chemical, clinical, pharmaceutical, and environmental sciences in recent years [1], largely because it offers excellent sensitivity and moderate selectivity toward electroactive compounds. Operation with constant potential (amperometric detection) for solid electrodes, especially carbon electrodes, is the most common mode due to the ease of operation and simplicity of instrumentation. However, further improvements are needed to extend the power and scope of this technique, which includes liquid chromatography (LC) and flow-injection analysis (FIA).

First, many compounds that are important biologically and environmentally overpotential, or show no response within a potential window at solid electrodes. Direct EC detection usually requires high potential for such compounds. This can produce large background current, resulting in inferior detection limits. Therefore, this detection mode is applicable only for small amounts of such compounds.

Second, passivation and/or deactivation of the electrode surface, due to the adsorption of macromolecules (e.g., proteins and surfactants) or of reaction products, greatly affects the stability of electrode response.

Third, coexisting components, which may be present in concentrations much larger than the analytes, may severely interfere with the determination of trace analytes. Complicated sample pretreatments are often employed to eliminate or separate interfering components. It is highly desirable to couple sample pretreatment procedures in situ with the detection scheme.

Fourth, amperometric detection usually does not offer identification information for effluents. Preliminary quantitation may be achieved by plotting hydrodynamic voltammograms with replicate experiments varying the applied potentials.

Fifth, the majority of substances are electroinactive and cannot be detected directly by amperometric detection. EC makes it possible to extend the range of compounds that can be detected, which is a desirable universal goal.

To obviate the foregoing problems, several detection schemes such as multiple-electrode detection [1a], pulsed amperometric detection [2], rapid scanning potential detection [1e], indirect EC detection [3], and post- and precolumn derivatization [4] have been proposed. Here, we shall discuss another promising route based on tailoring of the electrode surface—the application of chemically modified electrodes (CMEs)— for improving quantitative measurements and extending the scope and power of EC detection in flow systems.

CMEs have attracted a great deal attention over the past decade in a wide range of potential applications in electrochemical technology, particularly in chemical analysis and energy conversion, with possibilities in information storage and display, as well [5]. From the view of analytical chemistry, the deliberately tailored electrode surfaces (CMEs) offer a specific chemical and physical environment for catalytic reaction, ion exchange, complexation, permselectivity, and the incorporation of biocomponents for the analytes. These properties have been reviewed for the applications in analytical chemistry [6]. This review discusses applications of CMEs in flow analysis (LC and FIA). Different routes of CMEs in flow systems reported in the literature are summarized in Table 1.

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TABLE 1 EC detection Schemes Using CMEs for Flow Analysis

Based on electrocatalysis
Adsorption or coating CME
Incorporation into carbon paste
Electrodeposition
Conducting polymer
Inorganic film
Electrochemical pretreatment
Laser activation
Permselectivity
Size exclusion
Charge exclusion
Multifunction: combined electrocatalysis and permselectivity
Ion flow in redox polymer for electroinactive ions
Ion transfer across solidified nitrobenzene–water interface

BASED ON ELECTROCATALYSIS

Mediator Catalytic Reaction

For many important compounds, the actual redox potentials are much higher than the formal potential at solid electrodes [7], leading to inaccessibility by EC detection. CMEs employing immobilized redox mediators can facilitate the electron transfer of such analytes. The overall reaction scheme can be represented as follows:

$$\text{At the electrode} \qquad R - ne \rightarrow O \qquad (1)$$

$$\text{At the electrode–mediator/solution} \qquad O + S \xrightarrow{K_{ch}} P + R \qquad (2)$$

where R and O are the reduced and oxidized forms of the mediator, and S is the analyte to be detected.

When the potential is poised at the limiting current range of reaction 1, the mediator (or catalyst) exists as O at the electrode surface. As the analyte (S) is eluted from an LC column or FIA valve and reaches the CME surface by the means of the carrier solution (mobile phase), it rapidly reacts with O to produce R, which then oxidizes reversibly at the electrode, whereupon the current (peak response) is observed. The peak current is proportional to the concentration of analyte(s).

To achieve EC detection, the reactions above should have the following characteristics.

1. The overall catalytic reaction should take place at the potential of the formal potential of the catalyst, which must be reasonably low (e.g., < +1.0 V vs. SCE for oxidation) to maintain the desirable detection limit.
2. The formal potential of the catalyst should be close to that of analyte.
3. The catalytic reaction of S at the CME should occur at lower potentials than that at the naked electrode, to ensure reduction or oxidation at a favorable reaction rate.
4. The electrode surface should have sufficient coverage of the catalyst to ensure the catalytic efficiency for the entire analytical linear range.
5. For long analysis times, the CME must be stable and must be insoluble in the LC or FIA carrier solution containing organic solvents.

The application of CMEs in flow systems can be traced to the work by Johnson et al. (see, e.g., ref. 8), who used iodide treatment (resulting in iodine adsorption) of a platinum electrode for selective reduction of chromium (VI) in ion-exchange chromatography. Significant advances were made by Baldwin's group via the use of cobalt phthalocyanine (CoPC) incorporated into a carbon paste electrode (CPE). These and other CMEs based on electrocatalysis for flow analysis are summarized in Table 2.

Since CoPC is insoluble in many solvents and the central metal exhibits good catalytic behavior toward many organic compounds, a renewable CME can be obtained by incorporating the electrocatalytic center into the CPE. Baldwin *et al.* [9] first reported the CoPC–CPE for FIA oxidation detection of hydrazine. A detection limit of 6.4 pg had been obtained at the very low potential of −0.1 V. Thiol compounds, which proved to be kinetically hindered for oxidation (> 1.0 V) at carbon electrodes, can be easily oxidized at 0.75 V with the electrocatalysis of Co(II) → Co(III) at the CoPC-CPE [10]. This reaction succeeds for cysteine, homocysteine, *N*-acetylcysterine, glutathione, and 6- mercapurine, but not for cystine methionine, and malathion.

Carbohydrates, including the reducing sugars, exhibit large overpotential of electrooxidation at glassy carbon (GC) electrodes. Nevertheless, these compounds can be easily oxidized by many chemical oxidizing agents. Using CoPC-CPE, glucose can be oxidized irreversibly at 0.42 V [11]. The oxidation product degrades the electrode severely; therefore, pulse potential detection has been proposed for "cleaning" the electrode. With 0.39 V of detection potential and −0.30 V of activation potential, nanogram levels of glucose can be detected [11a,b]. This detection scheme has been extended for alditols and acidic sugars [11c]. The oxidation of ribose-containing compounds such as the ribonucleosides cytidine, uridine, adenosine, and guanine also was found to be catalyzed at 0.4–0.5 V in 0.15 M NaOH solution, but deoxyribonucleosides such as thymidine and the purine and pyrimidine bases did not exhibit this behavior [12].

Another type of CME also reported by Baldwin's group [13] used a GC electrode immersed in Cu(II) solution to yield a film of Cu(III) species. Carbohydrates are easily oxidized at the Cu(III)-GCE. a nanogram-level detection limit has been obtained for monosaccharides, disaccharides, and related compounds with simple amperometric detection.

The α-keto acids are another group of compounds that can be oxidized catalytically at CoPC-CME. Electrooxidation of these compounds is also sluggish at carbon electrodes. At CoPC-CME, the oxidation potentials are lowered to 0.75 V [14]. With liquid chromatography with

TABLE 2 CME Detection Based on Electrocatalysis

CME	*Mode of Use*	*Detected Compound*	*Ref.*
I_2/Pt	Adsorption	Cr(VI)	8
CoPC/CPE	Incorporation	Thiol compounds, hydrazine, oxalic acid, α-keto acids, carbohydrates, alditols, acidic sugars, ribonucleosides	9–12, 14
Cu(III)/GC	Oxidation	Carbohydrate, amino sugars, alditols, acidic sugars	13
btdpy-PVF/Au	Adsorption	Cytochrome c	16
MB/graphite	Adsorption	Myoglobin, hemoglobin	17
TB/GC	Deposition	Myoglobin, hemoglobin	18
PAN/GC	Deposition	Ceruloplasmin	19
PCz,PTh-RVC	Deposition	Tricyclic drugs	22
PB/GC	Deposition	Hydrazine	25
I^--CA/Pt	Adsorption	SCN^-	26
Ru-Ru$(CN)_6$/GC	Deposition	As(III), SCN^-, insulin, hydrazine	20,26,27,29
Ni-Fe$(CN)_6$/GC	Deposition	Fe(III)	28
I^--PVP/Pt	Adsorption	NO_2^-	26b
$IrCl_6^{4-}$-PVP/GC, Pt	Incorporation	NO_2^-	26c
MnTPP/GC	Adsorption	AA, OA, cysteine, acetaminophen, penicillamine	30
CoTPP/GC	Heat treatment	Hydrazine	31
Os$(bpy)_3^{2+/3+}$-Nafion/GC	Incorporation	Thiol compounds	32
Phenoxazine/graphite	Adsorption	NADH	33
$AuCl_4^-$/Au	Oxidation	AA, catecholamines	34
[Ru$(bpy)_2(PVP)_5Cl]Cl$/GC	Coating	NO_2^-, Ni $(HDTC)_2$	35
α-Alumina/GC	Adsorption	AA, oxalic acid, catecholamines	41

Abbreviations: bpy, 4,4′-bipyridine; HDTC, bis(2-hydroxyethyl)dithiocarbamate.

electrochemical analysis (LCEC), the detection limit for oxalic acid is 0.3 pmol; other α-keta acids (pyruvic, phenylpyruvic, α-ketoglutaric, and α-ketoisocaproic) are in the range of 150–1000 pmol. This has been applied for the detection in serum and urine [14b].

Electrochemical detection of biological macromolecules (e.g., cytochrome c, myoglobin, hemoglobin), in flow system is very important in biological studies. The extended three-dimensional structure of proteins makes the electroactive center inaccessible. In addition, protein adsorption onto the electrode surface causes subsequent passivation. Most biological macromolecules thus exhibit such slow rates of electron transfer that no useful current can be observed at conventional electrodes, even with the application of relatively large overpotentials. The use of a low molecular weight "mediator" or "accelerator" has been successfully applied to improve the rate of electron transfer between biological macromolecules and electrodes [15]. Schlager and Baldwin [16] adapted this approach for LCEC. The poly(vinylferrocene) (PVF) coated gold electrode, with a subsequently absorbed layer of 4,4′-bithiodipyridine (btdpy) as accelerator, was used for the detection of reduced and oxidized cytochrome c at +0.15 and −0.15 V, respectively, after size exclusion chromatographic separation. The detection limit is about 10–20 pmol.

A similar approach by adsorbing the phenothiazine mediator titrants methylene blue (MB) and thionine onto a graphite electrode was used for the electroreductive detection of myoglobin and hemoglobin [17]. Recently, we found that another phenothiazine mediator, toluidine blue O (TB), (electrodeposited at −0.4 V onto a GCE) can accelerate the electroreduction of myoglobin and hemoglobin [18]. As shown in Figure 1A, the TB mediates the reduction of myoglobin and hemoglobin at about −0.1 V. A stable FIA detection of the two redox proteins was obtained at −0.3 V (Figure 1B). Another redox protein, the copper protein ceruloplasmin, has been successfully detected at a polyaniline-coated GC electrode [19], as was demonstrated for the analysis of human serum. Insulin is a small protein molecule, and its direct electrooxidation is difficult. Cox and Cray [20] successfully used a Ru-Ru$(CN)_6$ inorganic film CME for the FIA oxidative detection of insulin based on the electrocatalysis toward cysteine in the protein. Insulin can be detected with FIA techniques down to 4.1 ng.

Conducting polymers represent newly developing electrode materials and are actively studied now because of their potential applications [21]. Depositing such films onto electrode surfaces can provide a unique chemical analytical environment. Polymer film has good conductivity and is rich in heteroatoms (e.g., N, S, etc.) as well as

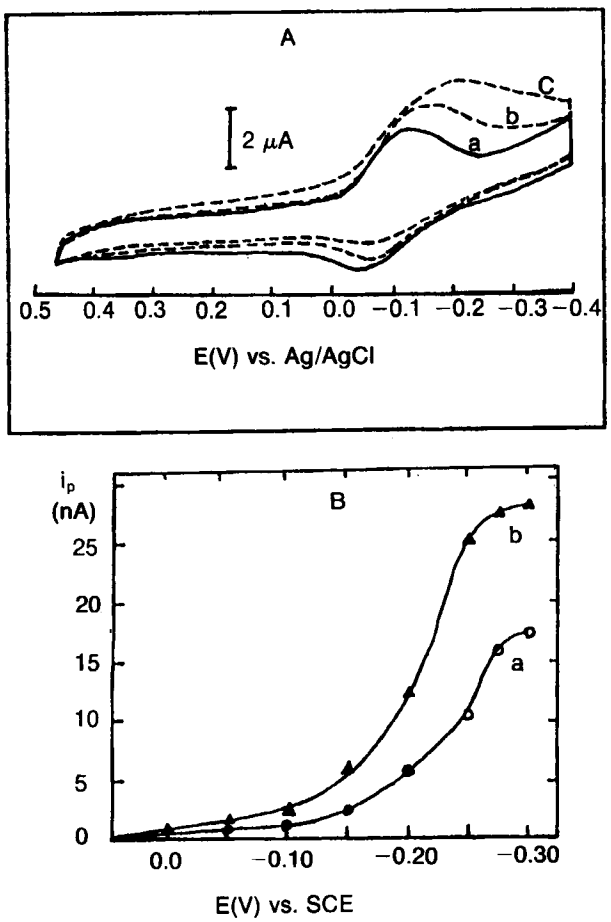


FIGURE 1. (**A**) Cyclic voltammograms of TB CME. Curve a, pH 4.6 acetate buffer; curve b, (a) + 5.0 × 10^{-6} M myoglobin; curve c, (a) + 1.5 × 10^{-5} M myoglobin; scan rate: 50 mV/s. (**B**) Hydrodynamic voltammograms at TB CME: 0.2 mg/mL each of myoglobin (a) and hemoglobin (b).

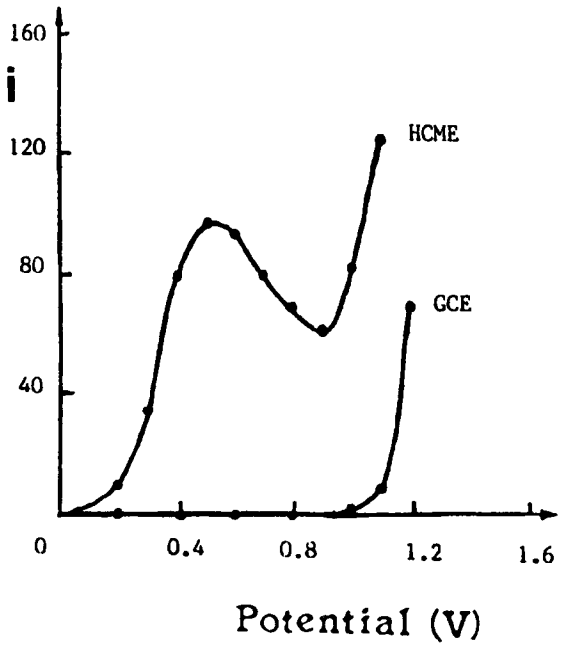
easy to prepare. Purdy et al. [22] deposited polycarbazole and polythiophene film onto a reticulated vitreous carbon surface for the electrocatalytic oxidation of tricyclic drugs. An electrochemical response was seen for amitriptyline, nortriptyline, and protriptyline, all of which previously had been reported to be electrochemically inactive, while iminostilbene, imipramine, and carbamazepine demonstrated similar electrochemical behavior at both polymer and conventional carbon electrodes. Another example is the use of polyaniline for facilitating the electron transfer of copper proteins [19]. As discussed below, poly(3-methylthiophene) films can provide anti fouling properties toward the electrooxidation of phenols [23].

Mixed-valent inorganic films, including Prussian blue (PB) and cupric hexaferrocyanide (CuHFC), were found to have catalytic properties [24]. We have recently found that PB can electrocatalytically oxidize hydrazine, at a potential of about 0.90 V, to give a very stable response [25]. A PB analogue, mixed-valent ruthenium cyanide, displayed excellent catalytic stability toward the oxidation of inorganic species, such as As(III) and SCN^- [26, 27] and Fe(III) [28]. Recent papers also illustrate that such films can catalyze the oxidation of insulin [20] and hydrazine [29]. Wang and Lu [29] reported an additional size-selective advantage by using codeposition of polyaniline and mixed-valent ruthenium cyanide.

Acceleration of the sluggish redox processes of many biologically important compounds, including ascorbic acid (AA), penicillamine, acetaminophen, cysteine, and oxalic acid (OA), at a GC electrode coated with an adsorbed layer of manganese(III) *meso*-tetraphenylporphyrin (MnTPP), was reported by Wang and Golden [30]. This greatly enhanced electrochemical quantitation by differential pulse and cyclic voltammetry in addition to amperometric detection for LC and FIA. We have recently found that a CoTPP/GC electrode with heat treatment (HCME) at 750°C exhibits catalytic reduction of oxygen [6b], and displays a very stable catalytic oxidation of hydrazine in acidic media [31]. As indicated from the hydrodynamic voltammograms, (Figure 2), the overpotential was reduced remarkably at the HCME. The heat treatment electrode could be used for several weeks in a flow system. Nevertheless, this electrode showed no catalytic effect toward ascorbic acid, cysteine, oxalic acid, hydraquinone, and other inorganic species such as SCN^- and NO_2^-, indicating a different mechanism compared to the MnTPP-GC electrode [30].

It is obvious from the discussion above that the incorporation of electrocatalytic centers into electrode surfaces is an attractive route to the improvement of EC

FIGURE 2. Hydrodynamic voltammograms of 20 ppm hydrazine sulfate at GCE and 5 ppm hydrazine sulfate at HCME. Mobile phase, 0.1 M KH_2PO_4 (1 mM EDTA), pH 4.5; flow rate, 0.5 mL/min.



detection. The problem of optimizing the immobolizing methods remains to be solved. A drawback of carbon paste electrodes is their poor mechanical and chemical stability when used with high contents of organic solvents. Other film modification procedures, such as adsorption or coating methods [26, 30–35], do not provide polishable and durable electrodes in many cases. The composite electrodes reported recently by Wang's group [36] (mixing epoxy and catalyst), and Shaw's group [37] using carbon particles or carbon fiber containing copolymers of vinylferrocene or vinylpyridine in a cross-linked polystyrene matrix, may have potential advantages for flow systems in which these electrodes are robust and polishable. Another avenue given by Heineman *et al.* [38], who employed the γ-irradiation cross-linking for the polymer film electrodes, resulting in a polymeric network film, could also be used or improving stability in flow EC detection.

Electrode Surface Pretreatments

Glassy carbon is an ideal electrode material for LCEC detection [1a]. The surface property of the electrode influences remarkably the reversibility of an electrochemical reaction, showing a large overpotential for many biologically important molecules. In recent years, many surface pretreatment procedures have been proposed to enhance the electrochemical reaction [39]. Zak and Kuwana [40] reported that metal oxide particles dispersed on the GC surface showed catalytic oxidation toward many organic compounds (e.g., ascorbic acid, hydroquinone, catechols). Wang and Frerha [41] employed this modification scheme for LCEC detections. The overpotential was largely reduced for ascorbic acid, oxalic acid, and catecholamines at the α-alumina dispersed GC detector. Other surface pretreatment procedures, including chemical, plasma, vacuum, heat treatments, laser irradiation, and electrochemical treatments, have been reported for studying surface properties and electrocatalysis [39, 42]. Among these procedures, electrochemical pretreatment is the most reliable for flow system applications due to its simplicity and effectiveness. It is generally accepted that the treatment introduces functional groups (e.g., phenolic, quinonic) and enhances the adsorption of electroactive species at the electrode [42].

Table 3 shows the compounds detected by electrochemically pretreated electrodes [43–54]. It is clear that the operation potential is lowered and the detection limit is improved after such pretreatment. Some of these compounds had been detected by CME based on electrocatalysis (Table 2); however, the electrochemical pretreatment is simpler and offers stable response. No theoretical prediction has been proposed for the compounds to be detected by electrochemical treatment procedures, which must be determined experimentally.

Recently, Wang and Peng [55] reported the use of electrochemical pretreatment to improve the stability of an EC detector. The presence of surfactant (hemoglobin) does not interfere with the response of the EC detector, and the adsorption of analyte (chlorpromazine) or the reaction products of dinitrobenzene is reduced. The use of electrochemical pretreatment for the GC electrode detector can be used as a routine operation to achieve better stability in EC detection [55]. We have found that EC pretreatment is an effective method for activating the carbon fiber based detector and for maintaining stability

TABLE 3 Applications of Electrochemical Pretreatment of Glassy Carbon Electrodes

Method	*Detected Compound*	*Potential (V)*	*Detection Limit*	*Ref.*
+1.4 V, 40 min	Catecholamines	0.21	10 pmol	43
+1.4 V, 40 min	6-Mercapurine, xanthine	0.8	0.01–0.1 ppm	44
+1.75 V, 5 min; −1.2 V, 5–10 s	Hydrazine	0.5	10 pg	45
	AA, NADH, DA, etc.	Decreased	Not improved	46
+1.75 V, 5 min; −1.2 V 1 min	S^{2-}	1.0	0.1 ppm	47
Open 2 min	Timolol, oxprenolol	1.32	1–2 ng	48
6.0 V, 2 min	Oprenolol	1.32	2 ng	49
+1.9 V, 2 min	Glutathione	1.1	100 pg	50
	Methione, tyrosine, oxipurinol, lactic acid, pyruvic acid	1.7	1 ng	51
		1.2	0.8–30 ng	52
+1.5 V, 10 min; −1.0 V, 1 min	CN^-	1.0	0.75 nmol	53
+1.8 V, 5 min; −1.2 V, 10 s	Tetracyclines	1.2	Improved 2–5-fold	54

[56]. Most compounds did not respond at the fresh fiber electrode. The peak current increased remarkably after electrochemical pretreatment. In addition, the electrochemical activity of a passivated electrode can be restored by simple pretreatment for 30–60 seconds in the mobile phase.

In situ laser activation was recently proposed by McCreery *et al.* for a GC electrochemical detector [57]. Improved reversibility and detection limits were found for many compounds, such as glutathione, resorcinol, and 5-hydroxytryptamine. The GCE degraded by the fouling of phenol oxidation products could be activated by this pretreatment, showing improved stability.

PERMSELECTIVITY

If the electrode surface is tailored with a permselective layer of chemicals, significant selectivity improvements can be obtained. In this way, only desirable compounds are able to penetrate through the "sieve" to exchange electrons at the electrode. Various chemical and physical properties can be employed for such purposes—for example, molecular size, charge, conformation, and hydrophobicity. Permselectivity based on molecular size and charge has been realized by coating an appropriate polymer layer on the electrode. Several factors should be considered when using such a film-coated electrode for flow system detection.

1. Is the film adhering roughly to the electrode surface under a vigorously flowing solution that may have some organic solvent content?
2. Does the film act as an "off–on" switch? The exclusion or selectivity property is obviously important. It is highly desirable that the switch level (i.e., the magnitude of the selectivity) of the film be controllable to meet specific needs.
3. Can the analytes/products diffuse rapidly into and out of the film, to minimate peak tailing?

Size Selectivity

Film porosity controls molecular size selectivity. Cellulose acetate (CA) film coated platinum was first reported by Sittampalam and Wilson [58] for the selective FIA detection of H_2O_2. Large molecules such as proteins, which cannot diffuse into the film, do not adsorb onto and passivate the electrode. The analytical stability of biological samples was greatly improved. Besides, other electroactive species (such as ascorbic acid) do not interfere with the determination of H_2O_2 because of the small porosity of the CA film, which allows only selective penetration of H_2O_2. An improved procedure was proposed by Wang and Hutchins [59], who hydrolyzed CA films in basic solution (0.07 M KOH) to enhance film porosity. Molecular weight selectivity can be realized by controlling the hydrolysis time. For example, compounds with molecular weight larger than 400 do not respond at the CA film electrode for 30 minutes of hydrolysis time. Besides the size selectivity of CA film, electrode contamination was largely reduced; for example, electrode fouling by the oxidation products of phenols was reduced and surfactant species such as proteins were excluded by the CA film. However, the current response at CA-coated electrodes was decreased compared with that at bare electrode (i_c/i_b). The i_c can be represented as follows [60].

$$i_c = i_b \frac{1}{1 + P_s/P_m}$$

where the P_s and P_m are the permeability coefficients of analytes in the solution and film, respectively. So i_c/i_b may be used as a qualitative parameter for the effluents. This scheme had been achieved by parallel dual electrodes with a coated and a bare electrode for simultaneous measurements of i_c, i_b, and i_c/i_b to obtain the identification information (peak purity, integrity, etc.).

The base-hydrolyzed CA film does not offer size exclusivity if the hydrolysis is allowed to proceed until molecules of about 450 daltons can pass through the film, and if there is instability after long hydrolysis periods due to the loss of the acetyl group [61]. The method of the phase inversion process, for making reverse osmosis membranes, was adopted to make modified electrodes with the goal of decreasing thickness, improving response time, and understanding the influence on CA membrane porosity of both casting solution composition and casting conditions. The best size selectivity had been obtained in the few thousand daltons range by controlling the casting composition of acetone, CA, and $MG(ClO_4)_2$ [62].

Recently, a poly(3-methylthiophene) (PMTh) film electrodeposited onto a GC electrode was reported to improve response stability toward phenol oxidation (which severely degrades the solid electrode by the radical polymerization process). Using a PMTh electrode, the FIA peak current remained unchanged for 15 repetitive injections of 3×10^{-4} M phenol, while the peak current was decreased by 37% at a bare GC electrode [23], indicating the effective block of oxidation products "clinging" to the electrode. The antifouling property of this film electrode is still unclear, probably due to a different oxidation pathway.

Charge Selectivity

If an ionic film is coated onto the electrode, the film will exclude species of the same charge and exchange with species of the opposite charge. Several polymeric films had been used for this purpose, including Nafion (perfluorosulfonate ionomer) [63, 64], Eastman-AQ (polyestersulfonic acid cation exchanger) [65], and polyelectrolyte [66]. Their main properties for EC detection are listed in Table 4.

Nafion, a cation exchanger made by Du Pont, is highly stable in aqueous solution. A Nafion film has been proved to be suitable for the preparation of modified electrodes, and fundamental studies have been made for both the dynamics of charge transport and the thermodynamics of ion-exchange reactions [5a, 67]. Adams and coworkers [68] deposited Nafion on a miniature graphite

TABLE 4 Properties of Detection Based on Permselectivity

	Modified Film		
Specificity	*Cellulose Acetate*	*Nafion*	*Polyvinyl-pyrrolidone*
Selectivity	Low molecular weight	Cation	Anion
Sensitivity (i_c/i_b)	≈0.1	>0.75	>0.44
Antifouling	Protein, oxidation of phenol	Protein	/
Flow-rate dependency	0.064–0.078	0	−0.27
Organic solvent	Unstable in AN Stable in MeOH	10% Acetonitrile, 25% methanol	10% MeOH
Stability	Good	Good	Bad

electrode (used to detect neurotransmitter-related species in rat brain) to eliminate the interference of anionic species (ascorbic acid, uric acid) and studied the ion exchange and transport of neurotransmitters in Nafion film on conventional and microelectrode surfaces. Wang et al. had successfully incorporated the Nafion film onto a GCE for EC detection [63], showing the improved selectivity toward catecholamines that are cationic in the LC pH range, while excluding the interfering species (ascorbic acid and uric acid). The in situ separation procedure had been combined with the detection mode. As shown in Figure 3, the anionic species did not respond, the response of neutral molecules was decreased by two-thirds, and the cations responded on the same scale. The Nafion film also showed antifouling properties toward the adsorption of proteins [63b].

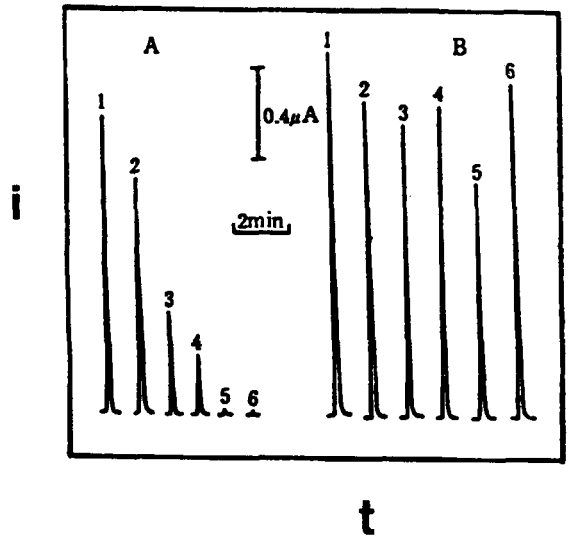
A new commercially available polymer, Eastman-AQ55D, was reported recently by Wang *et al.* [65]. Ion-exchange properties and permeability characteristics similar to those for the Nafion film were found, at lower costs.

Detailed studies of catechols transporting through Nafion on GCEs were recently given by Kok *et al.* [69]. The film diffusion coefficients of catecholamines were found to be in the range of 0.5–3.0 × 10^{-7} cm^2/s. In the flow system, proper choice of solution pH results in improved shielding of the electrode from interfering compounds, which are converted into anions. Methanol may be added to the solution up to 25% (v/v) without reducing the stability of the film (for EW 1100 Nafion), and the methanol content of the solution has little influence on the selectivity. Film thickness must be kept as low as possible, to reduce response time.

Multiple-layer modified electrodes can further enhance the selectivity of an EC detector. By coating CA film at the Nafion film GCE, size selectivity can be added to the charge selectivity of Nafion-coated electrodes, and the antifouling property is greatly increased [70]. A similar scheme was used by Bindra and Wilson [64] for pulsed amperometric detection of glucose at the Nafion-coated gold electrode with a precast layer of collagen (100 μm thick) or CA (30 μm thick) membrane as the outer protective layer.

Another form of charge selectivity, anionic selectivity, has been realized by coating the electrodes with cationic polymer. The cationic polyelectrolyte poly(4-vinylpyridine) (PVP) was one of the first polymers used for modifying electrode surface [71]. Much research effort has been directed to the characterization of the transport and electrostatic binding of multiple-charged anions at the electrode coated with PVP. PVP-coated GC flow detectors were described by Wang *et al.* [71], offering significant selectivity improvements in a manner (charge exclusion) analogous to that of Nafion film. Protection from organic surfactants can be coupled with the charge exclusion effect by using a bilayer coating, with a CA film atop the PVP layer [72].

FIGURE 3. FIA response of Nafion-coated detector. **(A)** Electrode coated with 5 μL 1% Nafion **(B)** Bare electrode. Mobile phase, 0.1 M KH_2PO_4 + 1 mM EDTA, pH 7.0; flow rate, 0.5 mL/min; potential, 0.60 V (vs. SCE); injection volume, 20 μL. Peaks: (1) 5 ppm dopamine, (2) 5 ppm epinephrine, (3) 2 ppm catechol, (4) 2 ppm hydroquinone, (5) 5 ppm 3,4-dihydroxylbenzoic acid, and (6) 5 ppm ascorbic acid.



MULTIFUNCTIONAL CHEMICALLY MODIFIED ELECTRODES

Electrocatalysis and permselectivity can be combined to enhance the quantitative and qualitative aspects of flow detection. As discussed above, a CoPC-modified electrode was shown to decrease by several hundred millivolts the potential required for electrooxidation of several irreversibly oxidizable species, and it played an important role in electrocatalytic detection. The size and charge exclusion characteristics of polymeric coatings such as CA, Nafion, and PVP have offered substantial improvements in the selectivity and stability of amperometric measurements. The incorporation of electrocatalytic centers into the foregoing polymeric coatings can provide additional advantages of chemical analysis. At these new microstructures, CA [73] or Nafion [66] serves as a template that establishes the structure and transport characteristics, while CoPC serves to enhance the electron transfer kinetics. The mixed CoPC/CA or CoPC/Nafion coating was found to present properties superior to those of either component alone. Improvements in stability, scope, and selectivity of amperometric detection were obtained for such multifunctional operations. Another electrocatalytic center, $Os(bpy)^{2+/3+}$, immobilizing in the Nafion film, was used by He *et al.* [32] for the electrocatalytic oxidation of thiol compounds in LCEC detection.

Platinum microparticles dispersed in polymer film have been reported using polyvinyl acetate, PVP, polypyrrole, and polyaniline polymer films for the reduction of hydrogen and the oxidation of methanol [74]. Recently, the combination of the electrocatalytic activity of platinum and the charge selectivity of Nafion was reported [75] as a unique electrode system that is useful for the EC detection of catecholamines.

DETECTION BASED ON ION FLOW IN THE REDOX POLYMER FILM CME FOR ELECTROINACTIVE IONS

The doping and undoping of anions can be coupled with the redox process of conducting polymers [21]. Ikariyama and Heineman [76] first developed this property for the determination of electroinactive anions. In the process of electrooxidation of polypyrrole, the polymer changes from a reduced (nonconductive, undoping) state to a conductive state. Meanwhile the anion is doped into the polymer film to keep charge neturality. When the film is reduced, the anion is released. When a zwitterion electrolyte, glycine, is used as the mobile phase, the background current is very small at 0.90 V because of the exclusion of the polymer film to glycine. As the anion (e.g., CO_3^{2-}, PO_4^{3-}, CH_3COO^-) is injected, a peak current is observed because of the doping of anions into the film, enhancing the oxidation of polypyrrole film. Then the potential is switched back to −0.3 V, where the anion is released back to the mobile phase. The injection can be repeated. The above presence of electroinactive anions in the concentration range of 10–100 μM can be detected using this doping–undoping scheme. Another conducting polymer, polyaniline, has been recently used by Baldwin and Ye [77] for the determination of anions according to the same mechanism as polypyrrole. However, polyaniline film is more stable and dopes larger amount of anion than polypyrrole film. Also the potential does not need to switch back for each injection [77]. Polythiophene offers similar properties [78].

Flow of compensated ions in the redox process of CME is a popular characteristic of redox film electrodes [24]. Recently, Deakin and Byrd [79] described the use of Prussian blue film, deposited on a quartz microbalance (QCM), for monitoring the change of current and frequency during redox process. K^+ was used as compensated cation, which diffuses into and out of the PB film in the course of the electrochemical reaction, leading to the mass change (frequency change) at QCM. Indeed, 0.1 mM K^+ can be detected at the potential of 0.10 V with FIA by recording current and frequency. This detection scheme was refined by Thomsen and Baldwin [80] using cupric hexaferrocyanide deposited on GCE. The following reaction occurred as the anion was injected:

$$Cu_3^{II}[Fe^{III}(CN)_6]_2 + 2e^- + 2K^+ \rightleftharpoons K_2Cu_3^{II}[Fe^{II}(CN)_6]_2$$

K^+ and NH_4^+ can be detected by using FIA and ion chromatography with the detection limits of 10^{-6} M and 5×10^{-7} M, respectively. Electrode stability was improved by coating Nafion film onto the CuHFC film. However, this detection mode cannot provide linear pressure with the concentration of cations [80].

ELECTROCHEMICAL DETECTION BASED ON ION TRANSFER ACROSS THE SOLIFIFIED NITROBENZENE–WATER INTERFACE

Electrochemistry at the liquid–liquid interface has received great interest in recent years [81]. This has opened a new area of electrochemical research into another class of substances that may not undergo electron transfer at the electrodes, and makes possible the determination, by means of direct electroanalysis, of a variety of electroinactive ions that can transfer across the liquid–liquid interface. Here, we consider the application of ion transfer across the solidified nitrobenzene–water interface to be another type of chemically modified electrode when the liquid–liquid interface is operated in three-electrode mode, although the current response mechanisms are inherently different.

A nitrobenzene phase solidified by polyvinyl chloride was reported first by Senda and coworkers [82], based on results of ion-selective electrode experiments. This makes the liquid–liquid interface a portable electrode. They even constructed this interface as a microvoltammetric electrode, for clinical applications such as the determination of potassium ion [83]. A preliminary report about ion transfer across a polymer gel–liquid interface as a voltammetric detector for a flow system was given by Marecek et al. [84].

Recently, we have constructed a thin-layer amperometric detector based on ion transfer across the water–solidified nitrobenzene (W/SNb) interface (Figure 4A)

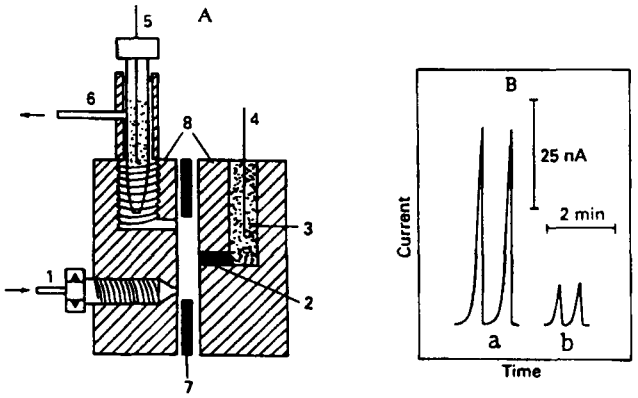


FIGURE 4. (**A**) Schematic diagram of the thin-layer flow-through cell. (1) solution inlet. (2) 0.05 M TBATPB (nitrobenzene) + PVC + agar, (3) 0.05 M TBACl (H_2O) + agar, (4) Ag/AgCl, working electrode, (5) Ag/AgCl + 0.05 M TBACl, reference electrode, (6) solution outlet, auxiliary electrode, (7) PTFE gasket (100 μm), and (8) PTFE cell bodies. (**B**) FIA peaks for (a) 0.2 mM tetracycline and (b) terramycin. Mobile phase, 0.1 M KH_2PO_4 + 1 mM EDTA, pH 2.0; potential, 0.40 V.

[85]. The nitrobenzene phase was solidified by polyvinyl chloride (PVC) and agar with tetrabutylammonium tetraphenylborate (TBATPB) as the supporting electrolyte. The solidified nitrobenzene phase with Ag/AgCl-TBACl (agar) was used as working electrode, as in a conventional amperometric detector. The response characteristics of the detector were studied for several ions, such as Cs^+, tetrabutylammonium (TBA^+), tetramethylammonium (TMA^+), acetylcholine (ACh^+), choline (Ch^+), ClO_4^-, IO_4^-, and ReO_4^-. The detection limit of FIA is in the range of 4–40 ng, which 2 orders of magnitude lower than that at conventional EC detectors because of the lower diffusion coefficients in the solidified nitrobenzene phase [86]. Also demonstrated was the possible application of such detectors for liquid chromatography, if the column is properly selected.

Ion transfer across the liquid–liquid interface may be processed by simple ion transfer, facilitated by ionophore, or coupled with a chemical reaction [81]. Potential application of these mechanisms incorporated into the amperometric detection of flow systems is straightforward. Recent developments in this laboratory show that many biologically important substances (cholines and related compounds, antibiotics, etc.) can transfer across the liquid–liquid interface. An application of such detectors is the FIA determination of tetracycline and terramycin in drugs [87]. Figure 4B shows the FIA peaks of tetracycline and terramycin at the detector. When a constant potential of 0.40 V is applied, both antibiotics transfer across the interface, the process being facilitated by protons, with a linear current response for 2–200 μM solutions of tetracycline and 5–300 μM solutions of terramycin. The results obtained were in good agreement with those given by biological assay. This indicates that such a detection mode is an accurate, convenient, and fast method for the determination of tetracycline and terramycin in drugs.

CONCLUDING REMARKS

The application of CMPs in flow detection has enjoyed considerable success and has recently stimulated extensive activity. Lack of stability and simplified preparation of CMEs is still the main factor limiting the widespread application of this approach. New types of catalyst and new immobilizing polymer materials should be developed to meet the challenge of trace analysis in complex biological fluids. Recent publications on inorganic membranes [88], heat treatment of perfluorosulfonate ionomer [89], composite electrodes [90], and hydrophobic barriers [91] should be useful for future investigations using EC detection. It is expected that many chromatographic procedures, such as sample pretreatment, chemical derivatization, and enzymatic reactions, could be simplified by coupling/integrating into the detecting electrodes.

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REFERENCES

1. (a) P. T. Kissinger, In *Laboratory Techniques in Electroanalytical Chemistry,* P. T. Kissinger, and W. R. Heineman, Eds., Dekker, New York, 1984, pp. 611–635. (b) K. Stulik, V. Pacakova, and E. Wang, *Pure Appl. Chem.* submitted. (c) D. M. Radzik and S. M. Lunte, *CRC Crit. Rev. Anal. Chem.* **20** (1989) 317. (d) H. Gunasingham and B. Fleet, In *Electroanalytical Chemistry,* Vol. 16, A. J. Bard, Ed., Dekker, New York, 1989, pp. 80–180. (e) S. G. Weber, J. T. Long, *Anal. Chem. 60* (1988) 903A.
2. D. S. Austin-Harrison, and D. C. Johnson, *Electroanalysis, 1* (1989) 189.
3. (a) E. S. Yeung, *Acc. Chem. Res. 22* (1989) 125. (b) J. Ye, R. P. Baldwin, K. Ravichandran, *Anal. Chem. 58* (1986) 2337.
4. I. S. Krull, C. M. Selavka, C. Duda, and W. Jacobs, *J. Liquid Chromatogr. 8* (1985) 2845.
5. R. W. Murray, In *Electroanalytical Chemistry,* Vol. 13, A. J. Bard, Ed., Dekker, New York, 1984, pp. 191–368.
6. (a) R. W. Murray, A. G. Ewing, and R. A. Durst, *Anal. Chem.* 59 (1987) 379A. (b) S. Dong, and Y. Wang, *Electroanalysis, 1* (1989) 99.
7. R. N. Adams, *Electrochemistry at Solid Electrodes,* Dekker, New York, 1969.
8. J. H. Larochelle and D. C. Johnson, *Anal. Chem. 50* (1978) 240.
9. K. M. Korfhage, K. Ravichandran, and R. P. Baldwin, *Anal. Chem. 56* (1984) 1154.
10. M. K. Halbert and R. P. Baldwin: (a) *Anal. Chem. 57* (1985) 591; (b) *J. Chromatogr. 345* (1985) 43; (c) *Anal. Chim. Acta, 187* (1986) 89.
11. (a) L. M. Santos and R. P. Baldwin, *Anal Chem. 59* (1987) 1766; (b) L. M. Santos and R. P. Baldwin, *Anal. Chim. Acta, 206 (1988)* 85; (c) A. T. Tolbert, and R. P. Baldwin, *Electroanalysis, 1* (1989) 389.
12. A. M. Tolbert, R. P. Baldwin, and L. M. Santos, *Anal. Lett., 22* (1989) 683.

13. S. V. Prabhu and R. P. Baldwin: (a) *Anal. Chem. 61* (1989) 852; (b) *Anal. Chem. 61* (1989) 2258.
14. L. M. Santos and R. P. Baldwin: (a) *Anal. Chem. 58* (1986) 848; (b) *J. Chromatogr. 414* (1987) 161.
15. F. A. Armstrong, H. A. O. Hill, and N. J. Walton, *Acc. Chem. Res. 21* (1988) 407.
16. J. W. Schlager and R. P. Baldwin, *J. Chromatogr. 390* (1987) 379.
17. J. Ye and R. P. Baldwin, *Anal. Chem. 60* (1988) 2263.
18. J. Zhou and E. Wang, *Electroanalysis* in press.
19. J. Ye, R. P. Baldwin, and J. W. Schlager, *Electroanalysis, 1* (1989) 133.
20. J. A. Cox and T. J. Cray, *Anal. Chem. 61* (1989) 2462.
21. *Handbook of Conducting Polymers,* T. A. Skothein, Ed. 5 Dekker, New York, 1986
22. D. J. Turk, S. A. McClintock, and W. C. Purdy, *Anal. Lett. 18* (1985) 2605.
23. J. Wang and R. Li, *Anal. Chem. 61* (1989) 2809.
24. (a) K. Itaya, I. Uchida, and V. D. Neff, *Acc. Chem. Res. 19* (1986) 162. (b) P. J. Kulesza, T. Jedral, and Z. Galus, *Electrochim. Acta, 34* (1989) 851. (c) F. Li and S. Dong, *Electrochim. Acta, 32* (1987) 1511.
25. W. Hou, and E. Wang, in preparation.
26. (a) J. A. Cox, T. Gray, and K. R. Kulkarni, *Anal. Chem. 60* (1988) 1710. (b) J. A. Cox and P. J. Kulesza, *Anal. Chim. Acta, 158* (1984) 335. (c) J. A. Cox and K. R. Kulkarni, *Analyst, 111* (1986) 1219; *Talanta, 33* (1986) 911.
27. J. A. Cox and P. J. Kulesza, *Anal. Chem. 56* (1984) 1021.
28. P. J. Kulesza, K. Brajter, and E. Dabek-Zlotorzynska, *Anal Chem. 59* (1987) 2776.
29. J. Wang and Z. Lu, *Electroanalysis, 1* (1989) 517.
30. J. Wang and T. Golden, *Anal. Chim. Acta, 217* (1989) 343.
31. W. Hou, H. Ji, and E. Wang, in preparation.
32. P. He, X. Chen, and P. T. Kissinger, *Proceedings of the Second Beijing Conference and Exhibition on Instrumental Analysis,* p. 1323
33. G. A. Marko-Varga, *Anal. Chem. 61* (1989) 831.
34. M. S. Tunuli, *Talanta, 35* (1988) 697.
35. (a) J. N. Barisci, G. G. Wallace, E. A. Wilke, M. Meaney, M. R. Smyth, and J. G. Vos, *Electroanalysis, 1* (1989) 245. (b) G. G. Wallace, M. Meaney, M. R. Smyth, and J. G. Vos, *Electroanalysis, 1* (1989) 357.
36. J. Wang, T. Golden, K. Varughese, and I. El-Rayes, *Anal. Chem. 61* (1989) 508.
37. (a) J. Park and B. R. Shaw, *Anal. Chem. 61* (1989) 1460.
38. L. A. Coury, Jr., E. W. Huber, E. M. Birch, and W. R. Heineman, *J. Electrochem. Soc. 136* (1989) 1044.
39. G. N. Kamau, *Anal. Chim. Acta, 207* (1989) 1.
40. J. Zak and T. Kuwana, *J. Electroanal. Chem. 150* (1983) 645.
41. J. Wang and B. Freiha, *Anal. Chem. 56* (1984) 2266.
42. H. Ji and E. Wang, *Acta Chim. Sinica, 47* (1989) 867.
43. H. Imai, H. Yoshida, T. Masujima, and M. Owa, *Bunseki Kagaku, 30* (1981) 561.
44. H. Imai, H. Yoshida, T. Masujima, and M. Owa, *Anal. Lett. 16* (1983) 1109.
45. K. Ravichandran and R. P. Baldwin, *Anal. Chem. 55* (1983) 1782.
46. K. Ravichandran and R. P. Baldwin, *J. Liquid Chromatogr. 7* (1984) 2031.
47. A. G. Fogg, M. A. Fernández-Arciniega, and R. M. Alonso, *Analyst, 110* (1985) 851.
48. M. R. Gregg, *Chromatographia, 20* (1985) 129.
49. M. R. Gregg, *Chromatographia, 21* (1986) 705.
50. K. Iriyama, T. Iwamato, and M. Yoshiura, *J. Liquid Chromatogr. 9* (1986) 955.
51. K. Iriyama, T. Iwamato, and M. Yoshiura, *J. Liquid Chromatogr. 9* (1986) 2955.
52. K. Iriyama, T. Iwamato, and M. Yoshiura, *J. Chromatogr. 400* (1987) 263.
53. A. G. Gogg and R. M. Alonso, *Analyst, 112* (1987) 1071.
54. W. Hou and E. Wang, *Analyst, 114* (1989) 699.
55. J. Wang and T. Peng, *Anal. Chem. 58* (1986) 1787.
56. W. Hou, H. Ji, and E. Wang, *Anal. Chim. Acta, 230* (1989) 207.
57. K. Sternitzke, R. L. McCreery, C. S. Bruntlett, and P. T. Kissinger, *Anal. Chem. 61* (1989) 1989.
58. G. Sittampalam and G. S. Wilson, *Anal. Chem. 55* (1983) 1608.
59. J. Wang and L. D. Hutchins, *Anal. Chem. 57* (1985) 1536.
60. D. A. Gough and J. K. Leypoidt, *Anal. Chem. 51* (1979) 439.
61. L. D. Hutchins-Kumar, J. Wang and T. Peng, *Anal. Chem. 58* (1986) 1019.
62. L. S. Kuhn, S. G. Weber, and K. Z. Imail, *Anal. Chem. 61* (1989) 303.
63. (a) J. Wang, T. Peng, and T. Golden, *Anal. Chim. Acta. 194* (1987) 129. (b) H. Ji and E. Wang, *J. Chromatogr. 410* (1987) 111.
64. D. S. Bindra and G. S. Wilson, *Anal. Chem. 22* (1989) 2566.
65. (a) J. Wang and T. Golden, *Anal. Chem. 61* (1989) 1397. (b) J. Wang and Z. Lu, *J. Electronanal. Chem. 266* (1989) 287.
66. J. Wang and R. Li, *Talanta, 36* (1989) 279.
67. L. D. Whiteley and C. R. Martin, *J. Phys. Chem. 93* (1989) 4650.
68. (a) G. Nagy, G. A. Gerhardt, A. F. Oke, M. E. Rice, R. N. Adams, R. B. Moore, III, M. N. Szentirmay, and C. R. Martin, *J. Electroanal. Chem. 188* (1985) 85. (b) G. A. Gerhardt, A. F. Oke, G. Nagy, B. Moghaddam, and R. N. Adams, *Brain Res. 290* (1983) 390.
69. A. J. Todos, W. J. J. Ozinga, H. Poppe, and T. W. Kok, *Anal. Chem. 62* (1990) 367.
70. J. Wang, and T. Peng, *Anal. Chem. 58* (1986) 3257.
71. L. R. Faulkner, *Electrochim. Acta. 34* (1989) 1699.
72. J. Wang, T. Golden, and T. Peng, *Anal. Chem. 59* (1987) 740.
73. J. Wang, T. Golden, and R. Li, *Anal. Chem. 60* (1988) 1642.
74. K. M. Kost, D. E. Bartak, B. Kazee, and T. Kuwana, *Anal. Chem. 60* (1988) 2379.
75. H. Gunasingham, C. Tan, and T. Aw, *J. Chromatogr. Sci. 27* (1989) 672.
76. Y. Ikariyama, and W. R. Heineman, *Anal. Chem. 58* (1986) 1803.
77. J. Ye and R. P. Baldwin, *Anal. Chem. 60* (1988) 1979.
78. R. C. Russell, I. C. Lee, A. Galal, O. Y. Ataman, H. Zimmer, and H. B., Mark, Jr. *Pittsburgh Conference Abstract,* No. 670, Atlanta, March 6–10, 1989.
79. M. D. Deakin and H. Byrd, *Anal. Chem. 61* (1989) 290.
80. K. N. Thomsen, and R. P. Baldwin, *Anal. Chem. 61* (1989) 2594.
81. (a) E. Wang and Z. Sun, *Trends Anal. Chem. 7* (1988) 39. (b) J. Koryta, *Electrochim. Acta, 24* (1979) 293; *29* (1984) 445; *33* (1988) 189. (c) H. H. Giraut and D. J. Schiffrin, in *Electroanalytical Chemistry,* Vol. 15, A. J. Bard, Ed., Dekker, New York, 1989, pp. 1–141.
82. T. Osakai, T. Kakutani, and M. Senda, *Bunseki Kagaku, 33* (1984) E371.
83. M. Senda, *Proceedings of the Third Beijing Conference on Instrumental Analysis,* Beijing, 1989, F5.

84. V. Marecek, H. Janchenova, M. P. Colombini, and P. Papoff, *J. Electroanal. Chem. 217* (1987) 213.
85. E. Wang and H. Ji, *Electroanalysis, 1* (1989) 75.
86. V. Marecek, and M. P. Colombini, *J. Electroanal. Chem. 241* (1988) 133.
87. H. Ji and E. Wang, *Analyst, 113* (1988) 1541.
88. C. J. Miller, and Majda, *Anal. Chem. 60* (1988) 1168.
89. R. B. Moore, III and C. R. Martin, *Macromolecules, 21* (1988) 1334.
90. Q. Zhou, L. L. Miller, and J. R. Valentine, *J. Electroanal. Chem. 261* (1989) 147.
91. J. Wang and Z. Lu, *Anal. Chem. 62* (1990) 826.