ELECTROANALYTICAL METHODS BASED ON MODIFIED ELECTRODES: A REVIEW OF RECENT ADVANCES

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A. SUMMARY

Modified electrodes have advanced from the initial studies that were aimed at understanding electron transfer in films to applications in such areas as energy production and analytical chemistry. This review emphasizes the major classes of modified electrodes that are being explored for improving analytical methodology. In this regard, applications to detection in flow injection analysis, high performance liquid chromatography, and most recently, capillary electrophoresis are receiving the most attention. The primary focus is using modified electrodes to mediate oxidation in order to permit measurements in the presence of dissolved oxygen. The most significant advances will be shown to be on electrochemical determination of trace levels of amino acids, peptides, proteins, alcohols, and sugars; however, many examples of studies on inorganic ions also are reviewed. The problems related to long term stability and to restoration of activity after surface contamination are limitations of modified electrodes; several examples of how these problems have been addressed will be illustrated.

B. INTRODUCTION

Electrochemical methods can be divided into those in which a redox reaction occurs at a surface, such as voltammetry and amperometry, and those in which an interfacial potential comprises the signal, i.e., potentiometry. The present review is restricted to the former. A general limitation of these techniques is electrochemical inactivity at bare electrodes for the majority of species which undergo redox in solution or which are thermodynamically predicted to undergo heterogeneous redox. This limitation is especially apparent when studies are restricted to aqueous medium and to potential ranges in which the medium is not electrolyzed.

In recent years, the scope of applicability of voltammetry and related techniques to analytical measurements has been extended by modifying electrode surfaces. These modifiers often are thin films on platinum, gold and various forms of carbon. In other cases they are components of composite electrodes, complexes that are hosted by otherwise-passive films, or oxides on active metals such as nickel and copper. The modifiers facilitate redox either by mediation or by catalysis.

A general model for mediation at modified electrodes is promotion of the oxidation of insulin, I, by a mixed-valent ruthenium oxide film on glassy carbon /1,2/. Insulin is not electroactive at bare glassy carbon; indeed, except for the system described here, insulin has not been reported to be electroactive in aqueous solution. At about 1.1 V vs Ag/AgCl, ruthenium in the film is oxidized to Ru(VI). Subsequently, Reaction 1 occurs at the modified-electrode surface.

$$\mathrm{Ru(VI)} + n\,\mathrm{I} \rightarrow \mathrm{Ru(IV)} + \text{products} \qquad (1)$$

Reaction 1 does not produce current directly; however, at 1.1 V, Ru(IV) is immediately re-oxidized by the underlying glassy carbon electrode (Reaction 2), which produces anodic current.

$$\mathrm{Ru(IV)} \rightarrow \mathrm{Ru(VI)} + 2e^- \qquad (2)$$

The anodic current is directly proportional to insulin concentration in solution /2/, which in turn requires one or more of the following characteristics of the ruthenium-containing film: it is an electronic conductor, it undergoes facile electron self-exchange (electron hopping among the Ru centers), or it is sufficiently thin to permit electron tunneling from the glassy carbon to the modifier-solution interface.

The efficacy of this particular modifier for promoting insulin oxidation probably is due to a combination of mediation by Reaction 1 and catalysis by facilitating electron transfer to disulfide sites of insulin. In the latter regard, the oxidation product of sulfur-containing amino acids at this modified electrode has been shown to be sulfoxide and sulfone /3/. At bare electrodes passivating layers are formed because the fundamental electron transfer step in these cases produces thiyl radicals, which either react with the electrode surface or undergo coupling reactions to form insulating films /4/.

There are many examples where the modified electrodes promote reactions by catalysis rather than mediation. A recent example is the study by McCreery and co-workers /5/ on the role of surface sites on carbon on electron transfer kinetics. The presence of oxygen on the surface was demonstrated to accelerate the electron transfer rate during cyclic voltammetry of aquo-complexes such as the Fe(III/II) and Eu(III/II) systems. Selective blocking of carbonyl and hydroxyl sites demonstrated that the catalysis was related to the carbonyl sites acting as inner sphere probes.

Voltammetry of systems that undergo outer sphere electron transfer was not sensitive to the surface state of carbon and, hence, was quite independent of electrode pretreatment. It is well-known that generation of oxides on carbon surface enhances the rates of voltammetric oxidation for many species of interest in bioanalytical chemistry (dopamine is a notable example).

The present review considers modified electrodes that promote electron transfer by catalysis as well as by mediation. In many analytical applications, the mechanisms have not been studied sufficiently to ascribe them to general catalysis or mediation with certainty. In these cases, the general term, catalysis, will be used. The review is organized using the types of modifier as the major headings. Therefore, it should be recognized that readers interested in selected analytes often will find relevant information in more than one section of the review. Finally, the term "modified electrode" is not restricted to a common definition that the surface has been altered intentionally by a chemical procedure. Included in the present study are, for example, alloys, composites that contain dispersions of catalysts, and surfaces to which catalysts are adsorbed or deposited by a physical means.

A summary of the species investigated, the electrode modifier used, and the literature citation or citations is given in most major sections for references cited in that section. The same species may appear in more than one table, if more than one type of modifier has been used to investigate the species or the species class.

C. METAL OXIDE ELECTRODES

1. Transition Metal Oxides

Nickel and copper electrodes are used for the determination of carbohydrates, alcohols, and certain other analytes in basic solution. A general characteristic of these systems is that the presence of the metal oxide is required to promote the redox reaction. In their studies on the determinations of glucose and of alcohols by anodic amperometry at constant potential, Huber and coworkers /6,7/ were perhaps the first to use these electrodes for electroanalytical measurements. Catalysis was achieved by anodic formation of species such as $NiO(OH)_x$ on the surface in basic solution /8,9/. At this surface ethanol was oxidized to acetate /8/. Arikawa and Huber /9/ demonstrated that ethanethiol also was oxidized in this system. To prevent air-oxidation, the ethanethiol solutions were stored at pH

3. The determination was by flow-injection amperometry, a procedure which involved injection of 25 μL of the sample into a 0.1 M NaOH / 50 μM $NiSO_4$ carrier stream. The Ni(II) was necessary to provide electrode stability. The detection limits for a series of thiols were only in the range 30–200 μM, which is not as low as with other modified electrodes for sulfhydryl compounds (see the sections on platinum group metal oxides and on phthalocyanines), and the need to include the Ni(II) salt in the carrier is another limitation. The mechanism of the oxidation was reported to involve a reaction between a higher (than 2) oxide of nickel $NiO(OH)_x$ with a thiolate RS^- to yield a radical RS, which subsequently dimerized to RSSR. Such dimeric species often are responsible for electrode passivation. This mechanism was previously reported at a nickel oxide electrode /10/. When a mixed valent ruthenium oxide with cyano crosslinks (mvRuCN) film was the modifier, the detection limits for sulfhydryl compounds were lower, and the products were oxides of sulfur, which did not yield passivating films /3,11/, which are seen at platinum metal oxides (cf. section on platinum metal oxides).

Nickel-coated glassy carbon electrodes were used to determine aliphatic alcohols in basic solution in conjunction with HPLC separation or flow injection amperometric analysis /12/. The mechanism was reported to involve as key components a Langmuir-type adsorption on NiO and NiOOH and hydrogen abstraction from the carbon that is a to the OH site as the rate-determining step. The limits of detection (LOD) were determined on the basis of the slopes of linear regions of the calibration curves and signal-to-noise ratios of 3. With 0.2 M NaOH as the carrier stream in flow injection amperometry, the LODs for ethanol, ethylene glycol, glycerol and mannitol were 0.01, 0.02, 0.02 and 0.5 ppm, respectively. The film, which was prepared by drying an aliquot of $Ni(NO_3)_2$ on the glassy carbon surface and then conditioning at 500 mV vs Ag/AgCl, lost 2.3% of its activity after 50 repetitive injections of 10 ppm 2,3-butanediol.

The presence of hydroxyl groups on tetracycline and related compounds permitted their determination by flow injection amperometry at a nickel-modified glassy carbon electrode /13/. The surface modification was accomplished by oven-drying (at 40 °C) an aliquot of nickel sulfate on the glassy carbon surface. Mediation by the Ni(III(II) couple was suggested as the means of promoting the oxidations in 0.1 M KOH. A study of the cyclic voltammetric peak current as a function of scan rate demonstrated that the current was limited by diffusion of the analyte in the bulk solution, i.e., the mediation process and propagation of charge in the film were rapid relative to mass transport of analyte in solution. A series of these compounds was quantified at 550 mV vs Ag/AgCl at the mg/L level. Detection limits in the flow experiment were 0.03, 0.92, 0.34 and 1.81 mg/L for tetracycline, doxycycline, oxytetracycline and chlor-tetracycline, respectively, using the S(N ratio of 3 criterion for detection. The relative standard deviation of peak current was below 3% for about 200 injections; but the electrode lost reliability at that point. Based on the half-width of the peaks, for tetracycline the sampling rate was 75 injections per hour.

An alternative method for immobilizing Ni(II) on a glassy carbon surface was reported by E. Wang and Liu /14/. The surface was coated with a film of Eastman-AQ, a poly(ester sulfonic acid); subsequently, an aliquot of nickel sulfate was added. After each step the surface was dried. Among the analytes studied were various monosaccharides, mannose, serine, and lysine. Activity toward propylamine was stated, but data were not presented. This modified electrode allowed flow injection determinations at constant potential without the need to electrochemically reactivate the surface with a pulsed waveform. But it should be noted that application of such waveforms, as in the Pulsed Amperometric Detector (PAD) developed by Johnson and co-workers /15/, for example, is not characteristic of nickel oxide (or related transition metal oxide) electrodes. The PAD uses a sequence of three potentials applied to Pt or Au because the catalytic activity of these metals toward various analytes depends on whether the surface is a "low" or "high" oxide of the metal (or in some cases the oxide-free surface). The pulse train also allows removal of adsorbed products.

Copper oxide, either in the form of a film on a Cu electrode or as a deposit on glassy carbon, has been used for the amperometric determination of carbohydrates and underivatized amino acids in strongly basic solution. Generally, copper oxide has been used under potentiostatic conditions in conjunction with flow systems such as HPLC, flow-injection analysis, and capillary electrophoresis. The emphasis has been on the determination of carbohydrates /16–18/. Preparation methods have been summarized by Luo et al. /18/. Three procedures were reported, namely air-drying droplets of $Cu(NO_3)_2$ and NaOH successively on a glassy carbon surface to yield a copper oxide surface, electrolytically plating copper metal on glassy carbon, and electrolytically plating CuCl on glassy carbon. All three systems resulted in the oxidation of glucose in 0.15 M NaOH in the range of 480–500 mV vs Ag/AgCl. In addition, a copper wire could be used as the indicator electrode; a galvanic plating procedure in which $CuCl_2$ was the electrolyte, glassy carbon was the cathode, and a copper wire was the anode, yielded an active system. Indeed, the latter provided the superior analytical response. It is likely that regardless of the procedure used, the active surface was copper oxide /18/. Flow-injection experiments had detection limits for glucose at the pmol level, and 100 injections over a 1.5-h period gave a relative standard deviation of less than 1%.

In a very significant study, Stitz and Buchberger /19/ compared the performances of nickel, copper, and cobalt electrodes with glassy carbon that was modified with the oxides of these metals for the amperometric detection of alcohols and carbohydrates after HPLC separation. They also used spectroscopic methods to characterize the surfaces of such electrodes. Controlled potential electrolysis was used to generate the electrolysis products, which were subsequently identified. The authors confirmed that Ni(III) was the mediator responsible for the efficacy of this metal as a modifier. Cobalt was the least useful of these metals for studies in basic solution. They also established that the copper and nickel metal electrodes yielded a stability that was superior to their modified glassy carbon counterparts; but nevertheless, the detection limits in 0.2 M NaOH were comparable when glucose was the test analyte. Because it required less pretreatment and yielded greater long term stability, copper was recommended over nickel for analytical applications. An extensive list of analytes and electrolysis products at copper and nickel electrodes is provided in this report.

Sunohara *et al.* /20/ investigated the reduction and oxidation of nitrite where graphite-supported oxides of Mn, Fe, Co, Ni and Cu constituted the electrodes. Cobalt appeared to provide the lowest overpotential of this group of metals for both the oxidation and the reduction. The analytical utility relative to other catalysts for nitrite oxidation (mvRuCN, for example) is limited; however, the catalysis of the reduction may provide the basis for new analytical methodology.

An important application of the copper oxide system is amperometric detection after capillary electrophoretic (CE) separation. Detection limits for glucose, galactose and related compounds were in the range of 0.5–8 finols /17/. Similar results were reported for alditols. Replicate injections yielded relative standard deviations in the 5%–7% range. The copper electrode surfaces were stable. Single placements were used for a month, and it was not necessary to reactivate the surface during that period. The sensitivity decreased by about 10% over a two-weeks period of use. The specific electrode employed in this study was a 100-μm diameter copper wire placed in the wall-jet configuration. Zare and co-workers /21/ also reported the determination of carbohydrates by amperometry at a copper surface after CE separation; in this case, a copper fiber electrode coated with copper was the indicator electrode. The process was reported as mediated by Cu(III). It is noteworthy that adding borate to the 100 mM NaOH electrolyte decreased the sensitivity and degraded the separation efficiency.

The CE separation and amperometric determination of amino acids and peptides was reported /22/ using 50–100 mM NaOH and the copper electrode, as mentioned previously /17/. With 50 mM NaOH as the electrolyte, the following detection limits (fnol) were reported for a set of amino acids: arginine (1.6), tryptophan (0.8), lysine (6.4), phenylalanine (640), histidine (1.6), glutamine (240), leucine (160), isoleucine (160), methionine (6.4), valine (240), threonine (3.2), asparagine (3.2), proline (16), serine (1.6), alanine (64), glycine (6.4), cystine (16) and tyrosine (0.8). Cysteine, glutamic acid and aspartic acid did not give a response under these conditions; given that they are oxidized at the copper electrode, the lack of response undoubtedly was related to excessive migration times. Even at unmodified electrodes, tyrosine, tryptophan and cysteine are electroactive, but for the others, the anodic response was promoted by copper.

The mechanism of glucose oxidation at copper electrodes was studied by Marioli and Kuwana /23/. From voltammetric diagnosis of the mechanism, they surmised that C–C bond cleavage was involved rather than simply the formation of gluconic acid. Luo and Baldwin /16/ used large-scale electrolysis along with product identification to test the mechanism. The oxidation of glucose went by a 12-electron mechanism; formate was the only product identified. Thus, the hypothesis of Marioli and Kuwana /23/ was verified. Intermediates such as gluconic acid, glucaric acid and glucuronic acid were not identified, which further suggests that the electrolysis occurs with cleavage of C–C bonds as a primary step. This result is in marked contrast to studies performed at Pt and Au electrodes where formate as well as partial oxidation products are observed /15/.

Although it has been used primarily for fundamental studies, PbO_2 has shown promise as a catalyst for electroanalytical measurements. Brevett and Johnson /24/ investigated the oxidation of sulfite, thiosulfate, and dithionite at pure and doped PbO_2 films that were electroplated from 1 M $HClO_4$ containing 10 mM Pb(II) in the presence and absence of other metal ions.

The modified electrodes were used in basic media, in which they were stable for at least several hours. The oxidations did not yield only sulfate; the formation of some $S_2O_6^{2-}$, perhaps along with other polythionates, was suggested. Such dopants as silver and bismuth did not have an influence on the oxidation potentials but did influence the product distribution. Oxygen transfer by adsorbed hydroxyl radicals was suggested to occur. An earlier study had demonstrated that the heterogeneous rate constants for a variety of oxidations at PbO_2 were increased by doping with bismuth /25/. Indeed, the increase in rate for the oxidation of phenol was a factor of 500.

The aforementioned is only a partial listing of the electroanalytical studies that have been performed at transition metal oxide electrodes. Other metals, such as cobalt, manganese, and iron have been used /19,20,26,27/. A variety of applications to real samples has been presented, such as the use of the copper electrode in conjunction with HPLC to determine sugars in beer, soft drinks, and milk /28/.

2. Alumina

A conventional electrode surface that contains alumina generally is not considered a modified electrode; the electrochemical response at the bare surface, however, is altered in a manner very similar to that observed with other metal oxide films. The alumina can be present by intentional incorporation or as residual from a polishing step. In the latter regard, sonication of polished surfaces is the usual method of removing residual particles after polishing, but even ultrasonic cleaning may not remove all particles from surfaces such as glassy carbon. For example, a microparticle carbon layer that presumably was held by electrostatic attraction was observed on polished and sonicated glassy carbon /29/. It is unlikely that modification with alumina will lead to practical applications. This topic is reviewed herein primarily to exemplify the need to be cautious in polishing steps where alumina and related abrasives are employed.

When alumina is present on a glassy carbon electrode, certain electrode processes are catalyzed. For example, Zak and Kuwana /30/ observed that the potentials for the oxidations of catechol and 1,4-hydroquinone were lowered by about 150–200 mV in the presence of alumina. With such a surface, the determination of various catechols in the presence of ascorbic acid was possible.

Recently, E. Wang and co-workers /31/ used a glassy carbon electrode that was modified with a–alumina as a detector for liquid chromatography.

Local anesthetics were determined under potentiostatic conditions with 1.2 V vs Ag/AgCl as the applied potential. Although this represents a lowering of the overpotential, other modifiers undoubtedly would be preferred for analytical applications.

3. Platinum Group Metal Oxides

That the surface states of Pt and Au affect their activities toward different classes of analytes is well known. Johnson and co-workers /15/ devised an approach to controlling these surfaces states to be optimal for a targeted group of compounds; they recently reviewed the theory and application of this method. For example, the oxidation of aliphatic compounds is favored by the formation of AuOH or PtOH rather than AuO or PtO. Cleaning and restoring the surface is promoted by oxidation of the surface to the higher oxides followed by reduction to the metallic state. A pulse program that sequences the indicator electrode among three potentials is required to optimize measurement when the lower oxide is the active surface. Simple alcohols, glycols, and mono- and oligo-saccharides are among the compounds that are detected at oxide-free surfaces of Pt and Au; a wide variety of compounds, however, are oxidized under conditions in which the lower oxides form at the Pt and Au surfaces /15/. For example, sugar amines can be detected by a combination of their adsorption onto the bare electrode and their oxidation in conjunction with forming the lower oxide of Pt or Au. Amino acids and various organo-sulfur compounds are among the analytes that have been studied /15/. Because the determination of sulfur compounds involves pre-adsorption, the sensitivity depends on the time of exposure to the oxide-free surface /32/.

A typical example of an application of the pulsed amperometric detector (PAD) is the determination of aliphatic alcohols after separation by liquid chromatography /33/. Voltammetric studies of these alcohols at Pt in 50 mM $HClO_4$ demonstrated that oxidative desorption of the alcohol occurred as the lower oxide of Pt was formed, thereby providing the basis for the analytical measurement. In this medium, detection limits (ppm) obtained with the PAD for representative alcohols after chromatographic separation were the following: sorbitol (0.3), glycerol (0.2), ethanol (0.2), 2-propanol (1.1), propanol (0.3), and pentanol (2.3). The detection limits were the concentrations that yielded a signal of three-times the noise observed at the lowest concentration injected; the average noise was 13 nA. Many other applications have been reported, particularly to the determination of carbohydrates (34–36).

4. Iridium Oxides

Iridium oxide has been studied extensively as both a potentiometric pH electrode and a catalyst, especially for the oxygen evolution reaction, and also has been used in several electroanalytical studies. The analytical utility of this material was enhanced by a reported procedure for coating the oxide on glassy carbon and related materials /37/. Iridium oxide was electroplated on glassy carbon from a mixture of Pd(II) chloride and sodium hexacyanoiridate(IV) in 0.1 M HCl. Plating was effected by cycling the electrode over a potential range that was sufficiently negative to cause deposition of Pd at one limit and development of peaks for the Ir(IV/III) couple at the positive limit. Film growth was observed on glassy carbon only when the solution contained Pd(II); however, little palladium was found by electron spectroscopy in the film. A Pt electrode was coated in the absence of Pd(II) by an otherwise-identical procedure; this suggested that Pd(II) initiated the process by deposition of a metallic layer over the glassy carbon.

Determination of trace levels of thallium was reported at the iridium oxide electrode /38/. Because oxidation of Tl(I) was reversible at the electrode, differential pulse polarography was well-suited to Tl(I) determination. Linear calibration curves were obtained over the range 5–100 µM Tl(I) with a detection limit of 0.5 µM. The determination of H_2O_2 at a glassy carbon electrode modified with iridium oxide also has been reported. Both the oxidation /39/ and the reduction /40/ served as bases of determinations. The former is of interest because dissolved oxygen does not interfere. Flow-injection amperometry using 0.1 M KOH as the carrier and 0.0 V vs SCE as the applied potential yielded plots of anodic current vs concentration that were linear over the range 20 µM–36 nM /39/. A potentially important application of this electrode is the determination of hydroxide. At about 0.6 V vs SCE, OH^- is oxidized to O_2 at the iridium oxide electrode, with a current that is proportional to the OH^- concentration /41/. Further study of this process and the application of the iridium oxide electrode to the determination of biopolymers containing carbohydrate functionalities are topics that merit further investigation.

Table 1

Investigations with Metal Oxide Modifiers

Species Investigated	Electrode Modifier	Ref.
alcohols	copper oxide nickel oxide oxide of platinum or silver	16,17,19 7,8,12,19 33
amino acids	cobalt oxide copper oxide nickel oxide - Eastman - AQ polymer	27, 22 14
anesthetics (local)	α-alumina	31
arsenic(III)	iridium oxide	37
ascorbic acid	α-alumina	30
biological compounds	cobalt oxide	27
carbohydrates	copper(I) chloride copper (III) oxide nickel oxide-Eastman-AQ polymer	18 21 14
carbohydrates and derivatives	cobalt oxide copper oxide nickel oxide	27 16,17,19,23,28 6,19
catechol	α-alumina	30
cyclones	nickel oxide	13
hydrazines	manganese oxide	26
hydrogen peroxide	oxide of cobalt, iridium or manganese	26,27,39,40
1,4-hydroquinone	α-alumina	30
hydroxide ion	iridium oxide	41
nitrite ion	oxide of cobalt, copper, iron, manganese or nickel	20
peptides	copper oxide	22
propylamine	NiO-Eastman-AQ polymer	14
sulfur compounds	pure, Fe(III)-doped- or Ag-Bi-doped-PbO_2 platinum oxide	24, 32
thallium(I)	iridium oxide	38
thiols	nickel oxide	9, 10

D. POLYMERIC MIXED-VALENT METAL SYSTEMS

This group of electrode modifiers includes some of the most useful mediators in terms of the scope of application and long term stability. In general, such electrode modifiers are Prussian Blue analogues; it is important to recognize, however, that during the electrode modification procedure, certain of them are converted to mixed-valent metal oxides.

1. Polymeric Mixed-Valent Ruthenium Oxide Stabilized with Cyano Crosslinks (mvRuCN)

This modifier, which first was described in 1984 as a Prussian Blue analogue /42/, is deposited on surfaces such as glassy carbon and platinum by cyclic voltammetry of a mixture of $RuCl_3$ and $K_4Ru(CN)_6$ at pH 2. Subsequently, it was found that during the positive potential excursions, which are on the order of 1100 mV vs Ag/AgCl, the complex that forms initially is converted to an oxo-polymer (2). The film is stable on glassy carbon for a period of about 3 months, during which it is used for voltammetry of inorganic analytes /42/. For sulfur-containing amino acid analytes at concentrations of 1–100 μM in such flow systems as HPLC, the sensitivity under potentiostatic conditions is constant for about two weeks /43/. Even at concentrations below 1 μM, the stability is limited to about 3 weeks when the analytes are proteins and peptides. During that period, however, the sensitivity is constant (44), which is in contrast to that of other modified electrodes, where the normal behavior is to see a gradual loss in response during use. The following examples are representative of the reported applications of electrodes modified with mvRuCN.

The initial report dealt with the oxidation and determination of As(III) by linear scan voltammetry /42/. This analyte was selected because it is not electroactive at bare glassy carbon, which precludes imperfections in the film coverage as the cause of the observed current. Linear scan voltammetric currents were proportional to the concentration over the range 5 μM–2.0 mM. A linear least-squares fit of the data yielded a sensitivity of 22.0 ± 0.1 mA/M and a correlation coefficient of 0.9999. The results were not perturbed by the presence of 60 mg gelatin/L. Among the other inorganic analytes that are electroactive at mvRuCN are nitrite /45/, thiocyanate /46/, and hydrazine /47/.

J. Wang and Lu /47/ determined a series of hydrazines at mvRuCN-coated electrodes and at an electrode modified with cobalt phthalocyanine

(CoPC). Flow-injection amperometry at constant applied potential was employed. The mvRuCN modifier possessed advantages. The mvRuCN produced a more stable response; 30 repetitive injections of 5 mM hydrazine yielded a systematic decrease in response at CoPC in contrast to the constant response at mvRuCN. CoPC lost 63% of its sensitivity over the period whereas mvRuCN showed constant peak currents (1.4% relative standard deviation). Continuous cyclic voltammetry of 1 mM hydrazine yielded constant peaks with mvRuCN rather than the constant decrease found with CoPC. The operable pH range was greater for the mvRuCN system than for CoPC. With a 20 µL sampling loop, calibration data were reported for hydrazine, methylhydrazine and dimethylhydrazine. With hydrazine, the detection limit (S/N = 3) was 0.2 µM, where the signal and noise were taken from 5 µM hydrazine injections.

The most important applications of mvRuCN and related modified electrodes are to determinations of organic compounds, especially those of biochemical importance. The previously-mentioned determination of insulin at a mvRuCN-coated glassy carbon electrode was performed by flow injection amperometry at 960 mV vs Ag/AgCl with 0.2 M K_2SO_4 at pH 2 as the carrier solution /1/. A linear calibration curve was obtained for injections of 8.2–204 ng insulin with a 7.5 µL sample loop. The detection limit, which was taken as the concentration yielding a signal of 3 times the standard deviation of a measurement at the lowest concentration in the range, was 4.1 ng insulin/7.5 µL. Similar behavior was obtained with cystine, cysteine, methionine, glutathione, and glutathione disulfide under conditions that were compatible with chromatographic separation on a strong cation-exchange column /43/. Detection limits were in the range of 0.15–0.63 µM.

Kennedy *et al.* /48/ modified a carbon fiber microelectrode (9 µm dia) with mvRuCN for use in monitoring insulin secretion from individual pancreatic b–cells. When the modified microelectrode was used at pH 2, flow injection amperometry experiments were linear over 3 orders-of-magnitude and yielded a detection limit of 0.1 µM insulin, which was comparable to previously-reported analytical characteristics with an electrode of conventional size /1/. In a single cell, a response was seen upon application of glucose. The spiked currents observed with time were attributed to insulin secretion by exocytosis. Other species that are known to co-excrete with insulin were systematically eliminated as the source of the current; in this regard, the difference between the response at mvRuCN-coated carbon and bare carbon was an essential characteristic. An important limitation of mvRuCN was observed. When the electrode was operated under potentiostatic conditions at 850 mV vs SSCE in a pH 7.4 buffer, the sensitivity deteriorated rapidly. Stability was attained by resting the electrode at 400 mV between measurements. This is consistent with our studies of the stability of mvRuCN; stability under potentiostatic conditions at potentials positive of about 0.7 V vs Ag/AgCl is achieved only in the range $0 \leq pH \leq 5$.

Cataldi *et al.* /49/ determined a series of aliphatic alcohols by flow injection amperometry and HPLC at a mvRuCN-coated glassy carbon electrode. Here, 1050 mV vs Ag/AgCl was the applied potential, and elution in the HPLC experiments was performed with 25 mM H_2SO_4. With 10 µL injections, detection limits were 15, 4, 0.8, 1, and 2 ng injected for methanol, ethanol, 1-propanol, 1-butanol and 1-pentanol, respectively; detection limits were defined for S/N = 3 using background noise rather than standard deviation. It was noted that as the number of methylene groups increased in an homologous series, the response increased, which is in contrast to the behavior at a pulsed amperometric detector. Abstraction of hydrogen from the a-carbon was considered an important mechanistic step. The final products were considered as aldehydes and carboxyl compounds. The electrode was stable for several days under their conditions, in agreement with earlier reports.

A variety of modified electrodes that are closely related to mvRuCN have been reported. Generally these are ruthenium oxides, either in composites, including pastes or on titanium supports. J. Wang and Taha /50/ investigated electrodes prepared by mixing RuO_2 with graphite powder and mineral oil as indicators for flow injection amperometry. With 1.0 M NaOH as the carrier solution, the electrodes promoted the oxidation of carbohydrates at about 0.4 V vs Ag/AgCl. Among the analytes were glucose, fructose, ribose, galactose and gluconic acid; the responses were more sensitive than observed with disaccharides and glycerol. The catalytic stability was greater than observed with comparable modified electrodes, such as those prepared from phthalocyanines or copper oxide; however, the detection limits were not as low as those reported for the pulsed amperometric detector or phthalocyanine-containing carbon pastes. The best detection limits found for the RuO_2-containing pastes were 2–3 ng in a 20 µL injection. When alcohols were the analytes, this electrode system was stable for two weeks in a flow injection amperometry system /51/. A loss of sensitivity of only 6% was reported. The relative standard deviation of 60 injections of methanol was 4.3%. Although the extension to amperometric detection in HPLC was projected, it is important to note that carbon paste systems generally are not amenable to mobile phases with an organic solvent as a component.

The development of polishable composites represents an improvement over carbon pastes for preparing bulk-modified electrodes. These systems are described in detail later. One example is the use of RuO_2 as the modifier in a graphite-epoxy composite /52/. Flow injection amperometry with a 0.5 M NaOH carrier was employed to determine the saccharide antibiotics, streptomycin and novobiocin, at the µM-level and neomycin down to 10 µM-level. Of importance is that these systems had catalytic activity that was restored by polishing without introducing error above 10% difference among data sets. The determinations of streptomycin and neomycin were made at 350 mV vs Ag/AgCl with Ru(VI) as the mediator, and novobiocin was determined at 200 mV.

In summary, ruthenium oxide provides the basis for a variety of modified electrodes that possess activity toward sulfur-containing organic (including bio-organic) compounds, carbohydrates and a variety of inorganic species. The range of analytes is undoubtedly greater than those reported to date. Of particular interest is that poisoning of the surface by analytes such as biopolymers and by electrolysis products is not observed. This fact, along with the stability during storage in electrolyte solutions and air /42/, makes these electrodes very promising for routine application in applied laboratories. Through use in polishable composites, the need for electroplating a new film is avoided, which can enhance routine use in laboratories lacking the facility to modify electrode surfaces.

2. Prussian Blue Analogues

Metal hexacyanoferrates have been investigated as electrode modifiers. It usually is not apparent whether these modified electrodes have a film of the true Prussian Blue analogue,or whether the initial complex is converted to the oxo-form, as in the case of the mvRuCN system described previously. If the preparation and/or application procedure involves application of potentials positive of 500 mV vs Ag/AgCl, it can be suspected that this conversion occurs. It is known that continuous cycling voltammetry of complexes as simple as hexachloroferrate at a platinum electrode can result in partial conversion to an oxo-form under some conditions.

The possibility of using Prussian Blue as an electrode modifier for analytical studies is apparent in a report by Itaya *et al.* /53/. They found that the electrochemical reductions of O_2 and H_2O_2 were catalyzed by this material. Kulesza *et al.* /54/ demonstrated that Ni(II)-hexacyanoferrate catalyzed the reduction of Fe(III). The electrode was modified by first plating Ni onto glassy carbon and then oxidizing the metal in the presence of hexacyanoferrate. As the indicator electrode in an amperometric HPLC detector, the Fe(III) reduction was promoted at 200 mV vs SCE. Linear calibration curves were obtained over the range 0.2 µM–0.9 mM Fe(III). The detection limit was 40 nM Fe(III) using the criterion of the concentration that yielded a signal of two-times the uncertainty of the background current. By using an Fe(III)-selective chromatographic column, interference from other metal ions was eliminated, and common anions did not interfere.

A film on glassy carbon, prepared by cyclic voltammetry between 900 and -500 mV vs SCE in a mixture of 1 mM OsO_4 and 2 mM $K_4Ru(CN)_6$ in 0.5 M NaCl at pH 2, was demonstrated to catalyze oxidations and mediate reductions /55/. A result of the study, which used As(III) as the analyte, was a demonstration that oxygen-transfer was important in catalyzing the anodic process. In that this modifier and mvRuCN are similar, the study suggested the importance of oxygen transfer when mvRuCN was the modifier. In addition, X-ray data indicated that these modifiers were indeed inorganic polymers with cyano crosslinks.

An important characteristic of certain members of this family of modified electrode is that they are electroactive in the presence of only selected cations. Any electrochemical redox step involving a film immobilized on an electrode requires mobility of ions therein; otherwise, charge balance is not retained. Cox and Das /56/ presented preliminary data demonstrating that the combination of electroneutrality and size selectivity of these films allowed determination of selectively-mobile ions via the current developed by electrolysis of the film. This showed that the amount of electrolysis of certain films is limited by the concentration of the mobile ion(s) within the film. In that study a film was prepared on glassy carbon from Ag(I) and octacyanomolybdate(IV). Potassium ion was selectively mobile therein. The cyclic voltammetric current observed at this film was a function of the concentration of K(I) over the range 1 M to 0.1 mM. The calibration curve was nonlinear, and ammonium ion, which has the same hydrated ionic size as K(I), interfered. This concept subsequently was reported by Heineman and co-workers /57,58/ and by Ye and Baldwin /59/

for the determination of anions at polypyrrole and polyaniline, respectively, and by Bocarsly and co-workers /60/ for the determination of Cs(I) at a Prussian Blue analogue.

The most thorough study of the aforementioned approach for the determination of nonelectroactive ions was by Thomsen and Baldwin /61/. Electrodes were coated with either Ni(II) or Cu(II) hexacyanoferrate by electrolysis of the metal in the presence of the iron complex. A previous observation that there is a relationship between potential at which electroactivity of the film is achieved and the radius of the ion being tested /56,60/ was verified /61/. The Ni(II)-containing film provided the broader range of analytes, whereas the Cu(II)-containing film provided the greater selectivity. An important finding was that only the Cu(II)-hexacyanoferrate (CuHCF) film was sufficiently stable to employ in a flow system detector, so virtually all of their analytical experiments were performed with this system. With the CuHCF film, as long as Cs(I) was absent, the currents obtained for either K(I) or NH_4^+ remained within a 5% range during several hours of operation as a flow injection analysis detector. A single preparation of CuHCF was used for a few days of routine operation, during which it was stored either in air or in slowly-flowing electrolyte when not in use. Linear calibration curves for Rb(I) and Cs(I) were obtained over the range 20–0.2 μM; correlation coefficients were 0.998. As previously suggested, sensitivity was a function of the applied potential. For Rb(I) and Cs(I) the optimum potentials in terms of sensitivity were 550 and 650 mV vs Ag/AgCl, respectively.

The use of films that are electroactive in a manner related to the size and concentration of the ion in the contacting liquid phase remains a promising but under-explored approach to selective determination of nonelectroactive ions. Two limitations are that the stabilities are not sufficient for routine use and that a background in electrochemical methodology is required for both the preparation and application of these electrodes. The instability of these systems probably is related to dissolution of the film in one of its oxidation states, which certainly is the case for the silver-octacyanomolybdate system /56/. Coating the active film with a thin, microporous polymer that is not electroactive may enhance the stability without significantly compromising the sensitivity and response time. An optimized system would advance the state-of-the-art in detection of nonelectroactive ions, given the difficulty in using conductivity detection for HPLC in concentrated electrolytes.

Table 2
Investigations with Polymeric Mixed-Valent Metal System Modifiers

Species Investigated	Electrode Modifier	Ref.
alcohols	Ru(IV) oxide	49,51
amino acids, peptides, and proteins with thiol and/or disulfide bonds	mvRuCN	3,11,43,44
ammonium and Group 1 ions	hexacyanoferrate	61
As(III)	OsO_4 and hexacyanoruthenate mvRuCN	55 42,45[a]
carbohydrates, glycerol and sugar acids	Ru(IV) oxide	50
Fe(III)	Ni(II)-hexacyanoferrate	54
hydrazines	mvRuCN	47
hydrogen peroxide and oxygen	Prussian Blue	53
insulin	mvRuCN	1,48
neomycin, novobiocin and streptomycin	Ru(IV) oxide	52
thiocyanate	mvRuCN or iodine (ads)/cellulose acetate film	46

[a] Discussed in Section C.

E. COMPLEXES WITH SINGLE-METAL CENTERS AS MODIFIERS

This class of modified electrode includes systems that were among the first reported. Many studies in the early 1980s were performed on immobilizing charged, redox-active complexes in organic ionomer films on electrodes. Poly(4-vinylpyridine), PVP, and quaternized PVP were used extensively as anion-exchange films, and Nafion® was commonly used as a cation-exchange film. During that period the emphasis of the investigations was on the nature of charge transport in these films when loaded with complexes such as hexachloroiridate and bipyridyl ruthenium. These fundamental studies are not included in this review.

The first analytical applications of electrodes modified by chemical or physical attachment of an overlayer fall in this general class. Cheek and Nelson /62/ amidized a carbon paste electrode and, in an analogue of stripping voltammetry, accumulated Ag(I) complex at the surface. After a prescribed time the potential was scanned cathodically to develop a current peak that was related to the concentration of Ag(I) in solution. Cox and Majda /63/ accumulated Fe(III) at an electrode that was coated by an adsorbed film of adenosine-5'-monophosphate (AMP) and measured the cathodic stripping current after a prescribed preconcentration time. Unlike the Cheek and Nelson experiment, only the electrode surface was modified, and the stripping step restored the electrode to its initial condition. A 60-s preconcentration allowed the determination of Fe(III) over the range 10 nM–1 μM. Because AMP generally passivates Pt electrodes, the response was limited to those metals that not only complex with phosphate but also coordinate with the purine ring nitrogen /64/; hence, Cu(II) was the only cationic interferent that was found.

An advantage of voltammetric stripping methods that are based on ion-exchange or complex-formation as the preconcentration step is that they do not require the analyte to be electrochemically reversible. This feature was first illustrated by an investigation of Cox and Kulesza /65/ of Cr(VI) at a PVP-coated electrode. Rather than preconcentrating at open circuit, 0.9 V was applied to eject material preconcentrated from the prior experiment. The product was a non-electroactive complex of Cr(III) and fluoride. An 8-point working curve over the range 10 nM–μM with a 60-s preconcentration time followed by cathodic stripping voltammetry was linear. The correlation coefficient was 0.999, and the slope was 2.34 µA/µM. The standard deviation of the slope was 3.2%. The sensitivity was increased by using a longer preconcentration time and with differential pulse stripping. An important point was that the Cr(VI) results were not influenced by the presence of a 1000-fold excess of Cr(III), so that the approach permitted a degree of speciation for chromium in sample solutions.

Abruña and co-workers /66,67/ have advanced significantly the concept of chelation of metals at an electrode surface as a preconcentration method for voltammetric determination. Guadalupe and Abruña /66/ modified electrodes with films that had both electroactive and coordination centers. The electroactivity allowed quantifying the amount of the deposit, and the coordination sites permitted the preconcentration of metal ions. The use of the charge observed during cyclic voltammetry to quantify the deposition of a film was verified for very thin layers (on the order of a few monolayers) by the independence of faradaic charge on scan rate. An electrode that was modified with poly(bipyridine)vinylferrocene showed cyclic voltammetric peaks in the presence of Fe(III) in solution for both the ferrocene incorporated in the film and Fe(III) that was coordinated thereon. The analytical importance of the film's electroactivity is related to observation of saturation of the coordination sites by the test metal. In this regard, it is important to note that a major difficulty with stripping voltammetry after preconcentration on a modified electrode is the limited number of available coordination or ion-exchange sites; hence, in an unknown solution care must be taken to assure that the preconcentration time is in a range for which the resulting signal is indeed a function of concentration. This decision is simplified when a single potential scan provides data on the quantity of the film and on the amount of metal that has been incorporated during the preconcentration. Normalization of the response to the surface coverage also is a viable strategy for quantifying samples /67/. Among the applications of this approach reported by Abruña and co-workers /67/ is the determination of Hg(II) down to 1 x 10^{-8} M.

Cha and Abruña /68/ have presented a strategy for the preconcentration of metal ions at electrode surfaces that may lead to a speciation scheme. First, ligands with varying coordination strength were immobilized at the electrode surface; copper was the targeted metal. The modification step involved electro-initiated polymerization of a Ru(III,II)-containing film of about 2–3 monolayers thickness. Ligands such as Alizarine Red-S, Eriochrome Red-B and Bathocuproine sulfonate were introduced by immersion of the modified electrode in 5–10 mM solutions of these species. Copper was subsequently preconcentrated on the treated surface by ion exchange. The detection of copper down to the 50 nM level was achieved by voltammetric stripping after this preconcentration; this value represents a limit established by copper impurities in the reagents rather than the detection limit determined by the signal-to-noise ratio. Of particular interest was that the preconcentration varied with the presence of competing ligands in solution, thereby suggesting the opportunity to expand this methodology into a speciation scheme.

Martin and co-workers /69,70/ termed these approaches to analysis as ion-exchange voltammetry. They demonstrated that organic cations had a much higher affinity to Nafion® than did inorganic cations /69/. Because of this affinity factor, removing the organic substance after an experiment was problematic. Subsequently, they demonstrated that the favored preconcentration of organic cations was not limited to Nafion® and that use of an electroactive polymer film rather than Nafion® allowed electrochemical ejection of products. The determination of methyl viologen (MV^{2+}) was performed by ion-exchange voltammetry. Calibration curves were reported over the range of about 1–9 μM MV^{2+} with a correlation coefficient of 0.997. Ejection of the product was accomplished with a 15 min electrolysis at 800 mV vs SCE. In this regard, it should be noted that the reduction of MV^{2+} is reversible, so when the polymer film is oxidized to a cationic form, the MV^{2+} is electrostatically repelled.

Impregnation of organic ionomers with metal complexes is performed most commonly to introduce a redox mediator at the electrode surface. Such systems have been used for electroanalysis. For example, an electrode modified with a film of quaternized PVP into which hexacyanoiridate(IV) was incorporated mediated the oxidation of nitrite /45,71/. Calibration curves were obtained by cyclic voltammetry over the range 0.1–1.0 mM nitrite /71/ and down to 0.01 mM by flow injection amperometry /45/, but the plots were nonlinear, the detection limits were not impressive, and the electrode stability was poor. A direct comparison between these electrodes and systems using mvRuCN demonstrated that with both As(III) and nitrite as analytes, the mvRuCN-coated electrode was far superior in terms of analytical performance.

Liu and Abruña /72/ immobilized primary amines in carbon paste to determine aldehydes. For example, the complex $[Fe(CN)_5L]^{3-}$, where L was 4-aminopyridine, was mixed with graphite powder and ceresin wax in the preparation of the electrode. The basis of the method is the mediated oxidation of the aldehyde to the imine, although the reverse process (the reduction of the imine that is formed) also can be used. Reliable quantitative results were obtained at concentrations of 10 μM and above. The general concept employed in this study was very similar to that in an earlier study by Price and Baldwin,/73/, who immobilized allylamine on a Pt electrode and determined ferrocene-carboxylaldehyde by a variation of stripping analysis. They reported a detection limit of 0.1 μM after a 10 min preconcentration when differential pulse stripping was employed.

There is continued interest in metal complex systems as mediators because they can promote certain unusual electrochemical reactions. For example, the reduction of carbon dioxide has been reported by Abruña and co-workers /74,75/ at electrodes coated with films of iron, nickel, and cobalt complexes. O'Toole *et al.* /76/ also reported this electrode process using a film containing a rhenium complex on an electrode surface. To overcome the low electrocatalytic activity of an electroactive polymeric film on an electrode surface, a series of complexes of first-row transition metals with bis(terdentate) coordination around the metal center were prepared and used in a modified electrode /75/. A Co(II) complex resulted in three sets of peaks during a cyclic voltammetric scan between 0.0 and -2.0 V vs SSCE in dimethylformamide; one set was due to Co(II,I), and the others were attributed to the ligand. The reduction peak at -1.33 V was enhanced about six-fold in the presence of CO_2. The detection of formic acid after electrolysis provided evidence that the voltammetric behavior involved the reduction of CO_2. This and related studies may provide the basis for a CO_2 sensor. Indeed, given the problems of stability of modified electrodes based on immobilizing metal complexes at an electrodes surface, the most promising analytical applications may be amperometric sensors for gas-phase species.

Table 3
Investigations with Single-Metal Center Complex Modifiers

Species Investigated	Electrode Modifier	Ref.
benzaldehyde	aniline, 4-aminobenzoic acid or $[\mathrm{Fe(CN)_5(4\text{-}aminopyridine)}]^{3-}$	72
carbon dioxide	Co(II)-, Fe(II)- or Ni(II)-terdentates rhenium complexes [a]	75 76
Carbon dioxide and oxygen	Co(II)-, Fe(II)- or Ni(II)-terpyridine or vinylterpyridine	74
copper	polymeric film of Ru(II)[4-vinyl-4'-methyl-2,2'-bipyridyl] coupled to various organic complexing agents	68
Cr(VI)	PVP	65
Fe(III)	AMP	63,64
ferrocenecarboxylaldehde	allylamine	73
nitrite ion	hexachloroiridate(III,II)	45
organic cations	Nafion®	69
silver ion	amidization	62

[a] poly-*fac*-$[\mathrm{(4\text{-}methyl\text{-}4'\text{-}vinyl\text{-}2,2'\text{-}bipyridine)Re(I)(CO)_3L}]^{n+}$, where L = $\mathrm{Cl^-}$ or $\mathrm{CH_3CN}$

F. ELECTRODES MODIFIED WITH METAL PHTHALOCYANINES

Because they have been investigated so extensively and have such exceptional promise for analytical chemistry, the metal phthalocyanines are discussed separately from the modifiers in Section C. The general structure of metal phthalocyanines is shown in Figure 1. Their extreme insolubility in polar solvents makes them attractive for incorporation into carbon paste electrodes, which is the most commonly used configuration. Related modification procedures use polishable composites that contain this modifier /77/ or use screen-printing techniques /78/. In addition surface-coating has been used, most commonly by electropolymerizing substituted phthalocyanines /79/.

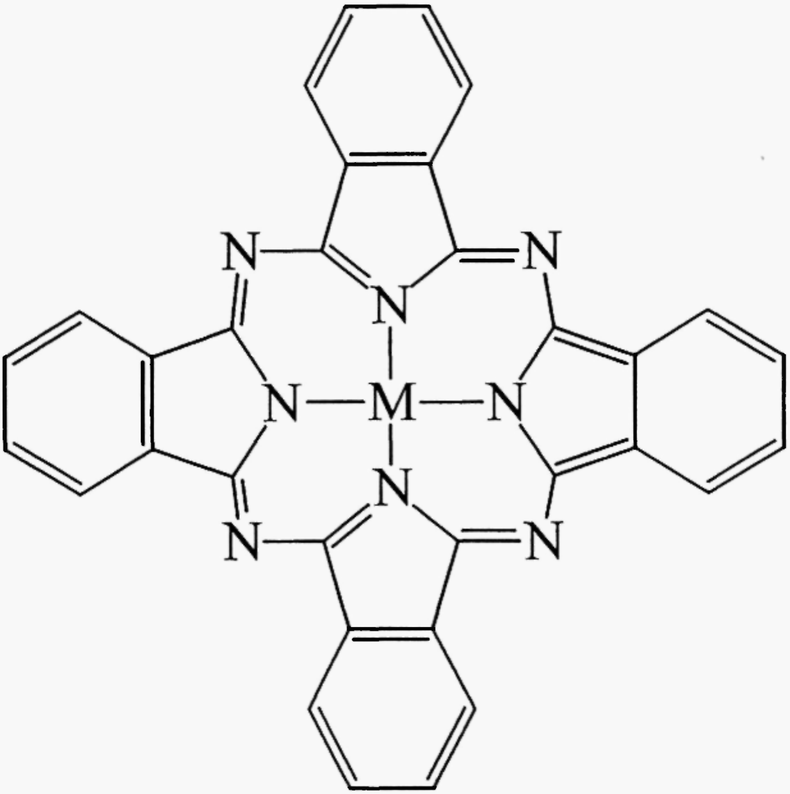

Fig. 1: The structure of a metalophthalocyanine. The symbol "M" represents the central metal atom

Although metal phthalocyanines previously were reported as electro-oxidation catalysts, the first systematic reports of using these materials in electroanalytical applications were by Baldwin and co-workers /80–83/. Cobalt phthalocyanine (CoPC) was mixed with graphite powder and Nujol oil for use as a bulk-modified electrode. The CoPC content was about 2% by weight. The electrode was employed in an amperometric flow cell serving as an HPLC detector. The targeted analytes were carbohydrates, which were separated by anion-exchange using 0.15 M NaOH as the mobile phase. Cyclic voltammetry of glucose, a typical analyte, at carbon paste in the presence and absence of CoPC provided evidence of glucose oxidation at about 0.4 V vs Ag/AgCl. The optimum potential for performing the amperometric measurements was determined by hydrodynamic voltammetry (HDV).

Flow injection or HPLC experiments with HDV are performed with the amperometric detector operated at several potentials. The amplitude of the current peaks obtained are plotted vs potential. If performed at a bare electrode with an electroactive analyte, the plot has the sigmoid shape characteristic of a dc polarogram. If the analyte is non-electroactive in the absence of a modifier, the plot that results with a modified electrode can have two general shapes. For simple catalysis the shape again resembles a dc polarogram; however, if the modifier mediates the electrode process (see Reactions 1 and 2), the hydrodynamic voltammogram has the shape of a peak. The onset of the process is related to the formal potential of the immobilized mediator. The mediated redox process yields a peak shape because for an oxidation at potentials well-positive of the formal potential the immobilized mediator is "anodically protected" from undergoing reaction with the solution-phase analyte. With CoPC as the mediator and glucose in 0.15 M NaOH as the analyte, the peak appears at 0.4 V vs Ag/AgCl /80/.

Using a 50 μL injection loop, Santos and Baldwin /80/ reported the following analytical results (detection limit; linear range) for the aforementioned system: glucose (18 ng; 2 μM–0.1 mM), galactose (18 ng; 2 μM–0.1 mM), maltose (34 ng; 2 μM–0.1 mM), lactose (34 ng; 2 μM–0.1 mM), ribose (75 ng; 10 μM–1.0 mM), fructose (90 ng; 10 μM–1.0 mM) and sucrose (171 ng; 10 μM–1.0 mM). A limitation of the method was that the CoPC mediator "fatigued" during potentiostatic operation. After four consecutive injections of glucose, the response at 0.4 V dropped to 30% of its initial value. Maintaining analytical performance required periodic application of about –0.3 V. This maintenance could not be delayed to the end of a multi-component chromatogram because the response for a given peak was influenced by the development of signals for previously-eluted components. Instead, a pulse sequence was applied; after 50 ms at the analytical potential, the electrode was switched to the restoration potential for a like time. It should be noted that such "fatigue" is atypical of a modified electrode containing a redox mediator. Systems such as mvRuCN are operated under true potentiostatic conditions. Indeed, it is not certain that fatiguing is inherent with CoPC operated potentiostatically. The cathodic pulse may have restored the surface of the paste in some indirect manner. In this regard, the reports on CoPC as a mediator are contradictory.

Tolbert *et al.* /84/ used a CoPC-containing carbon paste electrode for the amperometric detection of ribonucleotides. As in the previous study, the signal was generated at 0.4 V and pulses of equal duration at that potential and a negative, activity-restoration potential were applied. A systematic study was performed on cytidine. Cyclic voltammetry of cytidine, its pyrimidine base and its sugar ribose at conventional carbon paste and CoPC-containing carbon paste yielded the following information. At conventional carbon paste none of these compounds showed electrochemical activity. When CoPC was present, the ribose and the cytidine were electroactive, but a signal was not observed for the pyrimidine base. Hence, the electrochemical activity of cytidine at the CoPC-modified carbon paste electrode was due to oxidation of the sugar functionality. Introducing phosphate functionalities onto the ribose ring resulted in decreased sensitivity; the tri-phosphate resulted in less sensitivity than the mono-phosphate. As expected for a mediated system, the potentials at which the oxidations occurred were not influenced by this substitution. Cytidine, uridine, adenosine and guanosine behaved in similar manners in both cyclic voltammetric and hydrodynamic voltammetric experiments, thereby allowing amperometric detection of these compounds after separation of mixtures by HPLC. The detection limits were comparable to those obtained with spectrophotometric detection at 280 nm.

J. Wang *et al.* /85/ oxidized organic peroxides at a carbon paste electrode modified with CoPC and used the system for the flow injection determination of butanone peroxide, cumene hydroperoxide and t-butyl hydroperoxide down to the 2–8 ng level in 20 μL injections. Unlike the previously-described experiments, a basic carrier solution was not necessary. Cumene hydroperoxide responded over the pH range 3.5–9.0 with the same sensitivity; however, the experiment failed in more acidic media. The applied potential during the measurements was 0.7 V vs Ag/AgCl. As under the aforementioned conditions, application of a negative potential to restore the catalytic activity was required. The electrode did not respond to all peroxides that were tested, which can be an advantage in some cases where selectivity is desired.

The CoPC-modified pastes also can be used for inorganic determinations. Thiocyanate and selenocyanate were determined by flow injection amperometry using a pH 7 carrier solution /86/. A potential of 0.6 V vs SCE was used during the measurement half-cycle, and -0.3 V was used to reactivate the system. Higher sensitivity was observed at more positive potentials; however, the electrode stability was compromised. An important point was that the detection half-cycle had a period of 200 ms in order to allow the capacitive current to decay. In this regard potentiostatic measurements are advantageous over scanning experiments because the capacitive current is so low that faradaic currents from sub-micromolar concentrations can be measured above the background. The potential cycling that is necessary with metal phthalocyanine systems can compromise this feature; however, only 50 ms was sufficient to reactivate the CoPC. Linear calibration curves were reported for thiocyanate over the range 1–50 μM; the limit of detection was reported as 0.8 μM. This value was calculated from the concentration that gave a signal of $b + 3s_b$, where b was the (positive) intercept of the calibration curve's response axis, and s_b was the standard deviation of that intercept. The comparable analytical figures of merit for selenocyanate were 0.2–20 μM and 0.1 μM, respectively. During the study they noted that bromide and iodide were electroactive at this electrode and that cyanide was not electroactive.

Zagal *et al.* /87,88/ investigated the relationship between the identity of the metal that is coordinated to phthalocyanine and the catalytic activity toward various functionalities. These catalysts were adsorbed on carbon electrode surfaces. Generally, CoPC was the most active system, but the activity of FePC was only slightly less. Qi and Baldwin /89/ used FePC as the catalyst in an amperometric detector for organic peroxides. In contrast to an earlier-described study (85), the electrode process was a reduction. The pH and applied potential were controlled to allow reduction of these analytes at the FePC-modified electrode; dissolved oxygen interfered. Flow injection amperometry experiments with a 5-μL injection loop yield the following detection limits: peracetic acid (0.13 pmol), hydrogen peroxide (5.0 pmol), cumene hydroperoxide (0.50 pmol) and 2-butanone peroxide (1.0 pmol). These values represent an improvement over those reported with CoPC as an oxidation catalyst /85/. The linear dynamic ranges generally were 1–2 orders-of-magnitude. Other metal phthalocyanines were tested as reduction catalysts for these analytes. With NiPC, CuPC, VOPC, MnPC and H_2PC, no reduction was observed. With CoPC the reduction occurred at a potential more negative than that with FePC (0.35 V vs Ag/AgCl). Although it was neither discussed nor explicitly stated, apparently the reductions at FePC were performed under true potentiostatic conditions, i.e., not requiring the reactivation step that often is characteristic of applications of CoPC as oxidation catalysts.

An important extension of using modified electrodes as amperometric detectors for HPLC and flow injection analysis is to detection after separation by capillary electrophoresis (CE). Problems related to coupling electrochemical detectors and CE are not addressed herein, but they are described in papers cited by O'Shea and Lunte /90/. A CoPC-modified carbon paste electrode was designed for determination of thiols based upon their oxidation after separation by CE (90). The influence of CoPC loading on the sensitivity was examined. A 5% (wt)-CoPC level provided the optimum sensitivity. In contrast to the use of these pastes as detectors in HPLC, long term stability was achieved, apparently without the use of a reactivation potential. The stability relative to that seen in HPLC was attributed to the absence of organic solvent and the milder hydrodynamic conditions in CE. The detection limits, which were obtained by extrapolation of data obtained at 0.1 μM cysteine (CYS) and 1.0 μM glutathione (GSH), were 30 nM CYS and 0.3 μM GSH, using the criterion of a signal-to-noise ratio of 3. These corresponded to mass detection limits of 62 amol CYS and 600 amol GSH.

The scope of application of modified electrodes can be extended by coupling them to enzymatic reactors. In one example, a carbon paste electrode that contained CoPC was coated with a cholinesterase membrane in the design of an amperometric biosensor for organophosphate and carbamate pesticides /91/. The detection scheme was indirect. The sample contained butyrylthiocholine; with the electrode held at 400 mV vs Ag/AgCl in a stirred solution, a steady-state current is achieved, which corresponds to oxidation of thiocholine released in the enzymatic reaction. Addition of the analyte inhibits the enzymatic reaction, thereby decreasing the current observed.

The use of metal phthalocyanines in carbon pastes can be problematic for investigations requiring long term stability, especially when the electrodes are immersed in solvents that contain an organic component. One approach to alleviating problems associated with pastes is to incorporate the modifier in a hard, polishable composite. This method is reviewed in a later section.

Perhaps the most attractive means of immobilizing phthalocyanines on electrodes is to use electropolymerization of films. Guarr and coworkers /79,92/ prepared 4,4',4",4'"-tetra-aminophthalocyanines of nickel and cobalt by a published procedure and by repetitive cyclic voltammetrically deposited polyphthalocyanine films on glassy carbon. Tetrabutylammonium perchlorate in dimethyl sulfoxide was the supporting electrolyte, and the voltammetry was performed between 0.9 and –0.2 V vs Ag/AgCl. Sets of peaks at 0.34 and 0.21 V that grew with scan cycle provided evidence of film growth. Interestingly, in the absence of the monomer, the cyclic voltammograms of the modified electrode were featureless, which suggests that the films were extremely thin. A series of thiols were determined by HPLC with amperometric detection at a polyCoPC-modified glassy carbon electrode. If the positive potential was limited to values of 550 mV or less, the loss of sensitivity that was found in earlier studies /80–84,86/ was not observed. The authors speculated that the previously-observed loss in sensitivity of CoPC with time at the positive operating-potential was related to an irreversible oxidation of the phthalocyanine ring, but this was not related to the reported restoration of activity by periodic application of a negative potential. The analytical performances of polyCoPC films on glassy carbon and of CoPC-containing carbon pastes as electrodes for amperometric detection in HPLC were comparable when thiols were the analytes; however, the former electrode was more stable and allowed the use of organic solvents (up to 50% methanol) for at least a few hours. After 40 h, the sensitivity of measurements with polyCoPC dropped to about 80% of the initial value regardless of whether the mobile phase was 100% H_2O, 5% acetonitrile, 50% acetonitrile, or 50% methanol.

Ruiz *et al.* /93/ used polyNiPC-coated glassy carbon in a flow injection amperometric cell for the determination of the antioxidant, *t*-butylhydroxyanisole (BHA). An applied potential of 0.7 V vs SCE was used. Although the electrode was not reported to "fatigue" at this potential, it should be noted that the polymeric film was removed and the electrode apparently was re-plated frequently. Calibration curves were linear over the range 0.01 - 10 mg BHA/L, and the detection limit was 2.7 µg BHA/L (15 nM). Among the interferences in the method were propyl gallate, *t*-butylhydroxytoluene (BHT) and *t*-butyl- hydroxyquinone; the peak that was due to BHT was quite small, however, when compared with that for BHA at a comparable concentration.

In summary, metal phthalocyanines, especially CoPC, are among the most useful modifiers for electroanalysis. Because it can be employed in neutral and basic solutions, CoPC tends to complement mvRuCN. That it can be employed in basic solution makes it clearly superior to mvRuCN for studies of carbohydrates. The major limitation of metal phthalocyanines remains stability. Care must be taken in using them under potentiostatic conditions because of the "fatiguing" that is sometimes reported and is of still-unsolved origin. In the form of a polymer film on glassy carbon, the problems related to carbon pastes are diminished, i.e., it can be used with organic solvent; however, even in this preferred form, the long term stability doesn't match that of mvRuCN.

Table 4
Investigations with Metal Phthalocyanine Modifiers

Species Investigated	Electrode Modifier [a]	Ref.
arsenite, nitrite and S- or Se-containing anions	CoPC	86
ascorbic acid, cysteine, glutathione, hydrazine and thioguanine	CoPC	90
carbamate pesticides and organophosphate	CoPC/cholinesterase	91
carbohydrates	CoPC	80-83
cholate, hydrazine and penicillamine	CoPC	104
glutathione	CoPC	77
peroxides	CoPC or FePC	85,89
ribonucleotides	CoPC	84
t-butylhydroxyanisole	NiPC	93
thiols	CoPC	79, 92
uric acid	CoPC/cellulose acetate-uricase bilayer	78

[a] "PC" is an abbreviation for "phthalocyanine."

G. PORPHYRINS AS ELECTRODE MODIFIERS FOR ANALYTICAL STUDIES

The electrochemistry of porphyrins has been studied extensively. In many cases, the objective has been to investigate the redox chemistry of these materials. They frequently have been used as catalysts, but in these cases, the objective generally was not analytical measurement.

Catalysis of oxygen reduction has been studied extensively with porphyrins. One example is the report by Hayon *et al.* /94/, which includes several references to other catalytic studies with these materials. Polymeric films on glassy carbon were deposited by reacting the aniline groups of tetrakis(*o*-aminophenyl) porphyrins with nitrite in acidic solution. These films were metalated with cobalt, iron or nickel. The reduction of O_2 was investigated at the cobalt-substituted film. The data suggested that two Co(II) species were present and that these states had different reactivities toward O_2. The manganese-substituted film was used in the preparation of a glucose sensor based upon the film's ability to mediate the oxidation of glucose in the presence of glucose oxidase. An interesting point was that glucose oxidase could be incorporated into this chemically-polymerized deposit, whereas the incorporation of this enzyme into analogous electrochemically-polymerized films was not achieved. Amperometric measurement at 0.6 V vs Ag/AgCl yielded a calibration curve with a linear range that extended through the physiologically-relevant glucose concentration range of about 5 mM.

Malinski *et al.* /95/ used carbon fiber microelectrodes that were modified with conducting polymeric porphyrin films to determine unbound nickel in physiological cells. The electrode consisted of a sharpened carbon fiber with a diameter in the range 0.5 to 1 μm that was coated with 10–90 monolayers of polymeric tetrakis(3-methoxy-4-hydroxyphenyl)nickel porphyrin formed by oxidation of the monomer. The polymer was demetalated and inserted into a cell. The degree of remetalation was related to the unbound nickel concentration in the cell. The accumulation times were in the range 2 - 15 min. The probe was removed from the cell, placed in 0.1 M NaOH, and employed as the indicator electrode in linear scan voltammetry. Preliminary experiments with an electrode of conventional size showed that a linear relationship for the current, which is due to oxidation of Ni(II) to Ni(III) in the immobilized state, was obtained over the range 10 μM–10 mM with a detection limit of 8 μM. Solution experiments also were performed in the presence of albumin at the level of 50 mg in 5 mL of water. Currents due to Ni(II) oxidation and also the catalytic oxidation of water were obtained. On this basis they suggested that modified fiber electrodes indeed respond to unbound nickel in cells. The difference in nickel concentrations determined by the electrochemical method and by ^{63}Ni scintillation were attributed to bound nickel. It was assumed in this study, as in many other studies involving speciation of unbound metal, that there is no abstraction of the metal ion by the immobilized chelating agent from metal complex in the sample. No studies were performed to verify this fact; however, the ease with which the polymeric coating was demetalated may make the assumption reasonable, since this indicates that the polymeric nickel porphyrin is not a strong complex.

Perhaps the most exciting application is the determination of nitric oxide in biological systems, a field in which Malinski and coworkers are the pioneers. Nitric oxide is an important bioregulatory molecule, and abnormal physiological quantities of the compound can lead to several ill effects. Malinski *et al.* /96/ were able to measure the NO content in single cells to levels as low as 0.01 amol/cell. The base electrode was a carbon fiber which had been thermally sharpened. The electrode was modified with poly-tetrakis(3-methoxy-4-hydroxyphenyl)nickel porphyrin deposited in a manner analogous to that described in the previous paragraph, except that the polymer was not demetalated. To avoid interference from nitrite, the porphyrinic surface was covered with Nafion®, which is negatively charged and repels the nitrite anion, but which is highly permeable to NO. The integrity of the Nafion® film was verified by the absence of a Ni(II)/Ni(III) voltammetric peak, since this redox couple requires hydroxide, which also is repelled by Nafion©. Either differential pulse voltammetry (DPV) or amperometry was used to monitor NO. The reference electrode was not specified; however, in the work on intracellular nickel determination, Malinski *et al.* /95/ used a saturated calomel electrode, and they reported the nickel peak at 0.54 V compared to 0.52 V in this work.

Without the Nafion® coating, NO alone showed a peak at 0.63 V in pH 7.4 buffer at the modified electrode, and a solution of 1 μM NO and 20 μM nitrite showed a single peak at 0.80 V with a peak current three times that for NO alone. With the Nafion® coating, the NO peak was at 0.64 V, and a solution which was 1 μM NO and 20 μM nitrite showed a peak only 1% larger than for NO alone, with no change in peak potential. The oxidation was determined to involve loss of one electron, when the electrode was coated with Nafion®, and loss of 2.75 ± 0.22 electrons without the Nafion® coating. This was interpreted to mean that the negatively-charged Nafion® stabilized the positively charged NO^+; in the absence of the Nafion® coating, oxidation of NO leads to nitrite ion and its further oxidation to nitrate ion. The current was linear with NO concentration to 300 μM ($r = 0.994$; slope 2.05 nA/μM; DPV or amperometric method not specified). Detections limits were 20 nM for DPV and 10 nM for the amperometric method. This resulted in a detection limit of 0.01 amol for the volume equivalent of an average single cell, which is 2–4 orders of magnitude lower than the estimated amount of NO released per single cell. The response time (signal increase from 10 to 75%) for the amperometric mode was 10 ms.

A computer-controlled micropositioner was used to implant the electrode into a single cell, to place the electrode on the cell membrane's surface or to position the electrode at a controlled distance from the cell membrane. Studies were performed with both porcine aorta endothelium cells and porcine aorta cells. When 2 nmol of bradykinin was injected into the culture medium near endothelium cells, NO release was detected after 5 ± 0.5 s.

Flow-injection amperometry with cobalt tetraphenylporphyrin (CoTP) film on glassy carbon as the indicator electrode was used for the determination of hydrazine by Hou *et al.* /97/. The modification procedure involved placing small amounts of CoTP on a glassy carbon surface, grinding it against another glassy carbon surface and heating the surface at a ramped temperature, terminating at 750 °C, at which the surface was held for one hour. The heating was performed in a nitrogen atmosphere. After cooling the electrode was used as a modified electrode. Modification also was performed by contacting a polished glassy carbon electrode to a solution of CoTP. Cyclic voltammetry of hydrazine at the heat-treated electrode as a function of scan rate showed that the electrode process was controlled by the kinetics of the catalytic reaction. Electrocatalytic activity was not seen with ascorbic acid, cysteine, glucose, nitrite, thiocyanate and hexacyanoferrate; hence, the surface was considered to be selective toward hydrazine. Greater stability was claimed for the heat-treated CoTP than for cobalt phthalocyanine in a carbon paste, but this was on the basis of the cyclic voltammetry experiments. When the modification was performed by absorption without heating, the cyclic voltammetric experiments did not exhibit stability.

Flow-injection experiments were performed with 50 ng–2.4 µg hydrazine injected and 0.5 V vs SCE applied. A 10 µL injection loop was used. A correlation coefficient of 0.999 and a slope of 2.2 nA/ng were reported. With 10 ng injections used to establish a signal-to-noise ratio, a detection limit of 0.1 ng hydrazine was found; the criterion used was the concentration that yielded an S/N of 3.

In summary, the electrodes modified with porphyrins have interesting catalytic activities, but analytical applications have not been investigated to the degree that allows projection of unique characteristics except in one case. The most important application reported to date is that by Malinski *et al.* /96/, where nitric oxide in biological systems is the analyte.

Table 5

Investigations with Porphyrin Modifiers

Species Investigated	Electrode Modifier	Ref.
glucose	Mn(III) tetrakis(*o*-aminophenyl) porphyrin/glucose oxidase	94
hydrazine	Co tetraphenylporphyrin	97
Ni(II)	tetrakis(3-methoxy-4-hydroxyphenyl) porphyrin	95
nitric oxide	Ni(II) tetrakis(3-methoxy-4-hydroxyphenyl) porphyrin	96
nitrite ion	Fe(III) tetrakis(*o*-aminophenyl) porphyrin	94
oxygen (molecular)	Co(III) tetrakis(*o*-aminophenyl) porphyrin	94

H. GLASSY CARBON ELECTRODES DOPED WITH METALS

This class of modified electrodes refers to surfaces that contain small quantities of a modifier, such as platinum clusters, in contrast to surfaces coated with metal oxides. The nature of these surfaces has been described in a recent paper, which contains an extensive description of the various forms of carbon that are used as electrodes as well as a history of the use of glassy carbon as an electrode material /98/. One approach to doping was initiated by the development of low temperature (600 °C) methods for preparing glassy carbon from a variety of precursors /98/. The usual temperatures employed are above 2000 °C, which yields virtually pure carbon. The low temperature procedure provided routes to homogenous introduction of heteroatoms into the substrate. For example, mixtures containing poly(buta-1,3-diynyl-1,3-phenylene) and 0.25 or 1 atom percent of Pt(IV) oxide were pressed and heated at 600 °C. Electrodes prepared in this manner did not duplicate the catalysis of Pt electrodes toward hydrogen evolution, but the onset of this process was only about 100 mV more negative than at Pt when the formulation contained 1 atom percent of Pt(IV) oxide. Platinum in the form of a complex with poly(buta-1,3-diynyl-1,3-phenylene) yielded Pt clusters with dimensions of about 100 atoms, which were dispersed in the glassy carbon made with the low-temperature firing; the overpotential for hydrogen evolution at this electrode matched that at a polycrystalline-Pt electrode. Although the targeted applications of this technology have been in the energy field, important analytical applications can be envisioned.

J. Wang *et al.* /99/ prepared modified electrodes in which Pt- and Pd-particle dispersions were hosted by films on glassy carbon electrodes and reviewed the literature for accomplishing such modifications. In their study, the metals were distributed as electrodeposited microclusters in base-hydrolyzed cellulose acetate. The three-dimensional geometry of the clusters that are hosted by the film generally is acknowledged as causing the catalytic efficacy. Flow injection amperometry experiments illustrated the combined merits of catalysis by the Pt and ultrafiltration of the base-hydrolyzed cellulose acetate. Regarding the latter, peaks for the oxidation of NADH and hexacyanoferrate were markedly attenuated by the film. Catalytic oxidation of hydrogen peroxide and dopamine was illustrated.

Amperometric detection of bromide, iodide, thiosulfate, sulfite, and thiocyanate after ion chromatographic separation was achieved using a dispersion of Pt on glassy carbon /100/. The detection limits reported ranged from 1 ng I^-/mL to 20 ng Br^-/mL.

An interesting modified electrode for the amperometric detection of ceruloplasmin in HPLC was reported by Baldwin and coworkers /101/. A polyaniline film was electro-polymerized on glassy carbon, and 1 mM Fe(II) was included as a component of the mobile phase. This employed the known oxidative-promotion of 1,4-phenylenediamine and its derivatives by ceruloplasmin and Fe(II). Because the iron centers were not hosted by the film, this electrode is not technically in the category of doped glassy carbon; nonetheless, developing such a system, which would be more convenient than adding Fe(II) to the mobile phase, can be envisioned. Linear calibration curves were obtained over the range 3–100 μM, and the detection limit (the concentration that yields a signal of 3 times the noise) was 3 μM, i.e., 60 pmol ceruloplasmin per 20 μL injection.

Glassy carbon electrodes that used a polyaniline film as a host for assemblies of platinum/tin clusters were shown to catalyze the oxidation of methanol /102/. Polyaniline was selected because of its high conductivity and stability in acidic media. The polymerization was performed by cyclic voltammetry in 0.1– 0.5 M aniline solution in either sulfuric or perchloric acid. The catalyst also was deposited by cyclic voltammetry; here, the solution contained Pt(IV) and Sn(IV). Although the electrodes were studied in terms of applications to fuel cells, the low overpotential achieved for oxidation of methanol suggests utility in analytical chemistry.

Overall, glassy carbon doped with metals (either homogeneously or hosted by films on the surface) has not been explored sufficiently for analytical applications. It can be argued that with electrodes of small dimension, platinum group metals can be employed alone; however, there is a body of literature that demonstrates the advantage of small-dimension active sites in a conducting matrix for flow-system detectors. Greater sensitivity for a given geometric area can be accrued because of attenuation of the "electrochemical shadow" downstream from the active site and because of the hemispherical diffusion pattern (rather than planar diffusion) in the stagnant layer of solution adjacent to the active sites. It also has been suggested that adsorptive passivation by macromolecules is diminished by the use of very small active sites on electrodes.

Table 6
Analytical Investigations with Metal-Doped Glassy Carbon

Species Investigated	Electrode Modifier	Ref.
bromide, iodide, sulfite, thiocyanate and thiosulfate	Pt	100
Ceruloplasmin	polyaniline film	101
dopamine, ferrocyanide, NADH and peroxides	Pd or Pt/cellulose acetate film	99

I. POLISHABLE CONDUCTING CARBON COMPOSITE ELECTRODES

Bulk-modified electrodes made with conducting carbon composites have proven to be useful for work with analytes that directly or indirectly poison electrodes. A fresh surface can be obtained simply by polishing the composite. Electrodes of this design are reported to be robust and stable. These electrode designs do not have the problems of modifiers being leached, as is the case with carbon paste bulk-modified electrodes. The electrodes typically are made by mixing graphite powder and the modifier with epoxy or some other solidifying matrix.

Cataldi and Centonze /103/ studied nickel oxide distributed in a graphite powder and poly(vinyl chloride) (PVC) matrix. Electrodes were prepared by mixing known amounts of catalyst, 7% (w/w) PVC, and graphite powder with a minimal amount of tetrahydrofuran to form a slurry which hardened. Although alditols were not efficiently oxidized on unmodified graphite/PVC electrodes, the nickel oxide modified electrodes showed well-defined peaks for mediation of alditol oxidation by the Ni(III/II) redox couple. With a 0.1 M NaOH carrier, the response was linear up to 50 mM in flow injection experiments, and reported detection limits ranged from 0.02–0.10 mM. Detection limits were based upon a signal-to-noise ratio of 3, with the noise measured from the lowest concentration injected. The electrode showed little change in sensitivity (5%) for 10 mM sorbitol over 72 hours. Reproducibility of 10–20% was obtained by polishing the electrode with alumina (cf. Section

C.2 regarding possible effects of polishing with alumina), followed by conditioning the electrode in 0.1 M NaOH by cycling between 0.0 and 0.8 V vs Ag/AgCl.

Huang and coworkers /104/ showed that polishable composite electrodes bulk-modified with Cu_2O reduced glucose, whereas corresponding unmodified electrodes show sluggish kinetics. The electrodes were prepared by mixing carbon particles with a polyacrylic resin. At glucose concentrations higher than 1 mM, electrochemical regeneration of the catalyst became rate-determining at a rotating disk electrode. The electrodes demonstrated long term stability using flow injection amperometry. Replicate injections over 6 hours yielded a relative standard deviation of 1.8%. Cyclic voltammograms of glucose in 0.1 M NaOH showed one anodic peak at 0.55 V, as compared to two anodic peaks (-0.53 and 0.15 V) observed at gold electrodes, corresponding to the oxidation of aldehyde and alcohol groups, respectively.

Cox and Lewinski /105/ prepared composite electrodes by depositing mvRuCN on graphite powder. Because mvRuCN was not available in an isolatable form, electrodeposition onto graphite powder was required. Electrodeposition used a working electrode consisting of 52-mesh Pt gauze, which supported the graphite powder. The plating solution, which consisted of 2 mM K_4RuCN_6, 2mM $RuCl_3$ and 1 M KCl, adjusted to pH 2.0 with HCl, was pulled through the graphite powder with an aspirator. The potential was cycled 30 to 100 times between 0.5 and 1.1 V (vs Ag/AgCl, 3 M KCl) as the plating solution passed through the graphite bed. Voltammograms were in agreement with voltammograms produced by electrodeposition of mvRuCN on glassy carbon electrodes /42/. The mvRuCN-coated graphite powder was stable for at least 2 months when stored in acidified KCl or as air-dried powder, based upon comparison between freshly prepared composite and composite stored for 2 months; in fact, the composites showed no loss of catalytic activity during the one-year project /105/.

Catalytic activity was demonstrated with As(III), which is not oxidized at bare carbon. The most significant finding was that the sensitivity of voltammetric determinations did not change upon polishing the composite surface. When the surface was polished using 400- and 1200-grit carbide paper between each scan, 19 replicates of As(III) oxidation yielded a relative standard deviation of 3% in peak current. The catalytic activity of these electrodes also was tested on N-nitrosamines. A reproducibility study by flow injection analysis at 1.75 V on 50-μM N-nitroso-di(n-propyl)amine (NNDPA) samples yielded a peak current of 2.5 ± 0.1 μA. An important point in this study was that the mvRuCN composites performed well in micellar media. Flow-injection amperometry was performed in a carrier of 0.01 M hexadecyltrimethylammonium chloride buffered at pH 1.5 by phosphate. A test solution of 0.11 mM N-nitroso-di(n-phenyl)amine and 0.11 mM NNDPA yielded sensitivities of 0.16 and 0.14 A M^{-1} cm^{-2}, respectively. The sensitivity for NNDPA was considerably lower than the 0.28 A M^{-1} cm^{-2} observed in non-micellar media; other performance factors were the same, however, and the sensitivity is sufficient for electrochemical detection coupled to micellar HPLC separation.

J. MISCELLANEOUS MODIFIED ELECTRODES

Many important systems, which include some of the more novel types of modified electrodes, do not fit conveniently into one of the categories discussed in the previous sections. Conventional electrodes that have been activated in some manner are considered in this category. Some of these are actually the antithesis of a modified electrode, e.g., special treatments are used to remove chemisorbed oxygen on the surface of conventional Pt or carbon electrodes.

McCreery and co-workers /5,106/) have studied this topic extensively with carbon surfaces. In a recent paper /5/, the authors reported the influence of surface sites on electron transfer kinetics at carbon electrodes. Systems that undergo outer sphere electron transfer are not sensitive to the surface states, whereas those undergoing inner sphere electrochemical redox are catalyzed by the presence of surface oxides. Related studies are cited extensively therein.

Rice and McCreery /106/ showed that the electron transfer kinetics of laser-modified, highly-ordered, pyrolytic graphite correlates with edge plane defects. The authors found that cleaving the basal plane tends to cause edge plane defects, as does high laser power in the laser pre-treatment step. Their studies indicated that the heterogeneous rate constant of oxidation of hexacyanoferrate at edge-plane graphite was several orders of magnitude higher than at basal plane graphite. Laser pre-treatment at powers below 45 MW had nearly no effect on the heterogeneous rate constant. The rate constant rose between 45 and 90 MW of laser power for pre-treatment from 10^{-7} cm s^{-1} without pretreatment to 0.1 cm s^{-1}, and then reached a plateau or declined. This was interpreted as generation of edge-plane defects or randomness in carbon structure. The authors suggested an approximate formula for the effective heterogeneous rate constant, which is a weighted average of the electrode area's edge plane and basal plane fractions, and presented quantitative evidence to support the model.

Strein and Ewing /107/ investigated effects of laser pre-treating carbon-fiber microdisk electrodes on the heterogeneous kinetics of oxidizing dopamine, 4-methylcatechol and 3,4-dihydroxy-phenylacetate (DOPAC); digital simulation was employed to estimate changes in heterogeneous rate constants and oxidative transfer coefficient, b. The enhancement of peak currents and apparent b appeared to be a result of an increased active electrode area due to laser pre-treatment of the electrode. Linear dependences of half-wave potentials vs pH were found for dopamine between pH 2–7, for 4-methylcatechol between pH 2–8, and for DOPAC between pH 2–8 in both citrate/phosphate and phosphate buffers.

Zeolites have been used as modifiers that may enhance electroanalytical performance by a combination of serving as supports for catalysts, providing a degree of size-selectivity and allowing preconcentration /108/. Practical application to analytical chemistry will depend upon discovering convenient means of preparing stable forms of these electrodes /108/. Most investigations on zeolitic electrodes have focused on the development of electrochemical power sources. The general topic of zeolite-modified electrodes has been recently reviewed /109/.

Electrocatalytic oxidation of ascorbic acid has been reported by immobilizing 7, 7, 8, 8-tetracyanoquinodimethane (TCNQ) on edge plane pyrolytic graphite /110/. The modified electrode showed a linear response from 1–10 mM with a rate constant for the reaction between ascorbic acid and TCNQ of 1.8×10^6 M^{-1} s^{-1}.

Bonakdar and Mottola /111/ reported that carbon paste and glassy carbon electrodes with anchored Fe(II) and Fe(III) centers enhanced the signals from gaseous samples of NO_2, SO_2, H_2S and Cl_2. Carbon paste electrodes were prepared by mixing with tris[4,7-diphenyl-1,10-phenanthroline]iron perchlorate. Glassy carbon electrodes were modified by oxidative electrodeposition of tris[5-amino-1,10-phenanthroline]iron perchlorate. The authors reported that both electrodes were protected from poisoning by the electrode modification.

Carbon pastes have been modified with fumed silica to enhance their activities. Wang and Naser /112/ demonstrated that the oxidation of glucose is 13 times more sensitive with the addition of fumed silica to a glucose-oxidase/carbon paste electrode. The increase was attributed to greater enzyme activity and electrocatalytic action. Electrodes were prepared by mixing an enzyme/mineral oil slurry with graphite powder and rehydrated fumed silica. The improved sensitivity was attributed to the adsorptive and electrocatalytic properties of the silica nanoparticles. Although the electrode showed a 10% decrease in sensitivity over the first 8 hours, the electrode then was stable over a 14-day period with a 4% relative standard deviation over a 6-hour period. Addition of fumed silica also lowered the overpotential by 0.4 V.

Preconcentration techniques have been employed by incorporating complexing species into carbon pastes. Baldwin and coworkers /113/ used crown-ether- and cryptand-modified electrodes to preconcentrate Pb(II) and other metal ions. Freshly-produced modified surfaces showed little ability to preconcentrate; however, after seven or eight preconcentration cycles, the electrodes were stable for days or weeks. Possible explanations for this behavior are that repetitive preconcentration cycles either enhance the relative amount of complexing species at the surface or alter the orientation of the complexing species. For a 20-second preconcentration using a 7.5% dibenzo-18-crown-6 chemically modified electrode, the uptake of Pb(II) from a 1.0×10^{-5} M Pb^{2+} solution was investigated. The cations Hg(II), Cd(II), Tl(III), Cu(II), Zn(II), Ag(I), Na(I) and K(I) were examined as interferences at equimolar concentrations with Pb(II), except that Cd(II) was studied at a ten-fold higher concentration. None of these species interfered with the Pb(II) oxidation peak at -0.63 V, although Cd(II) produced an oxidation peak at -0.85 V. The dibenzo-18-crown-6 yielded a linear range 1.0 - 100 µM Pb(II) for a 20-s preconcentration. The linear range extended down to 0.5 µM for 2.2.2-cryptand electrodes. Detection limits (S/N = 3) were 1.0 and 0.5 µM, respectively. The authors did not specify the definition of noise; however, they used the term "limit of detection," which presumably means a signal three times the standard deviation of the intercept above the calibration curve's intercept on the response axis.

Lead has been preconcentrated using its affinity for alginic acids /114/ The composite electrode contained alginic acid in a carbon paste. The same study found that pectic acid could be used to preconcentrate copper.

Preliminary results indicated that a heparin-modified electrode could be used for preconcentration of copper. Cyclic voltammetry was used in all cases to generate a response.

Carbon paste electrodes doped with different metals show different catalytic activity. J. Wang and coworkers /115/ illustrated that they could catalyze the slow electrode reactions of hydrogen or organic peroxides, hydrazine compounds, ascorbic acid and dihydronicotinamide adenine dinucleotide with such electrodes. Modified electrodes were prepared by mixing Pd powder with carbon paste or electrodepositing Pd on carbon paste by cycling a Pd(II)-containing solution between 0.4 and -0.7 V vs SCE. The electrodes reduced aldehydes to alcohols and oxidized the alcohols back to the corresponding aldehydes by d.c. voltammetry /116/. Hydroxylamine oxidation also was catalyzed by mixing Pd in carbon pastes /117/. A linear relationship between 0.1 to 10 ng was found by flow injection amperometry with a detection limit (S/N = 3) of 20 pg hydroxylamine. The relative standard deviation of 5-ng injections was 4.0%.

Kulys *et al.* /118/ bulk-modified carbon paste electrodes by the addition of diaphorase and either Meldola blue (MaB) (7-dimethylamino-1,2-benzophenoxazinium chloride) or methylene green (MG) (4-nitro-3,7-bis[dimethylamino]-phenothiazinium chloride) and used these electrodes to determine NADH. Calibration data were fitted using the Michaelis-Menten equation. The apparent Michaelis constant was 3.6 mM for MG and 0.9 mM for MaB. Catalytic current was proportional to NADH concentration up to 350 µM and 600 µM, respectively, for MG- and MaB-modified electrodes; the respective sensitivities were 0.24 and 0.20 mA mM^{-1} cm^{-2}.

The use of modified electrodes in conjunction with solid-state electrolytes is a promising new approach to sensing gas-phase analytes. An interdigitated microelectrode array (IME) with the indicator array modified by mvRuCN (cf. Section D.1) was coated with Nafion® to design an amperometric gas-phase sensor /119/. For a 15-mL gas cell filled with Ar, linear response was reported for methanol vapors in the µmol range and N-nitrosamine vapors in the nmol range. The same modified IME, except with an overlayer of polymeric vanadium oxide deposited by a sol-gel process, allowed oxidation of NH_3 vapors by cyclic voltammetry /120/. The process was extended to the development of a solid-state, gas-phase amperometric sensor for µmol-levels of NH_3 /121/. In these examples, the Nafion® and the polymeric vanadium oxide, which are ionic conductors, served as the supporting electrolytes. A gas-phase sensor for methanol was described by Kulesza and Faulkner /122/, in which an ion-exchange membrane film impregnated with the ruthenium analogue of Prussian blue served as the mediator for oxidation.

An exciting use of modified electrodes is to use immobilized redox couples as mediators in enzymatic reactions. An excellent example along with a review of the related literature is presented in a report by Person *et al.* /123/. In this paper an immobilized redox polymer was used to regenerate NAD^+, which couples with NADH to serve as a co-factor for a variety of dehydrogenase-based reactions. The phenoxazine/phenothiazine system was the basis of the redox polymer; however, to improve its stability in alkaline solution, an amide was substituted on position 3 of the 7-dialkylamino form of these compounds. This topic also is included in a recent review on enzyme-modified microelectrodes for in vivo measurements /124/.

The NAD^+/NADH system was the subject of a recent study by Nowall and Kuhr /125/. The authors produced modified electrodes by cycling freshly-cleaved carbon fiber electrodes between 0.1 and 1.0 V at 50 Hz while adding 100 μM NADH by flow injection. Electrodes were then exposed to 1 mM ascorbate. Electrodes prepared in this method were useful only when the temperature was above 38 °C during the modification. The modified electrode showed low overpotentials for oxidation of dopamine and metabolites such as NADH, DOPAC, uric acid, and ascorbate. The modified electrode eliminated the irreproducibility of the NADH oxidation wave at carbon fiber microelectrodes. With the modified electrode the wave changed "only marginally" over 10,600 scans. The electrodes were stable for 4 days when stored in aqueous solutions and over 30 days when stored in air. The response was linear in the range 1 μM to 1 mM, with a limit of detection of 1 μM (S/N = 5). This response is an order-of-magnitude lower than identical electrodes that were not modified /126/. It should be emphasized that the detection limit definition of S/N = 5 corresponds to a confidence level in excess of 99.99%. Had the authors computed a detection limit for the more commonly used definition of S/N = 3, which corresponds to 99% confidence, the detection limit reported would have been considerably lower.

Finally, it should be noted that this review does not mention all modifiers that show promise in electroanalytical chemistry. Among the particularly interesting examples for extension from electrocatalysis research to electroanalytical applications are the polyoxometalates /127/ and over-oxidized polypyrrole /128/.

Table 7
Investigations with Miscellaneous Modified Electrodes

Species Investigated	Electrode Modifier	Ref.
Ag(I), Cd(II), Cu(II), Hg(II), Pb(II), Tl(III) and Zn(II)	crown ethers or 2.2.2-cryptand	112
aldehydes	Pd	115
amines and amino acids	mvRuCN	118
ammonia	mvRuCN	119,120
ascorbate, DOPAC, dopamine and uric acid	NADH	124
ascorbic acid	Pd, Pt or Ru 7, 7, 8, 8-tetracyanoquinodimethane diaphorase and either methylene green or meldola blue	114 109 117
carbohydrates [a]	glucose oxidase/1,1'-dimethylferrocene	90
Cl_2, H_2S, NO_2 and SO_2	perchlorate of tris[4,7-diphenyl-1,10-phenanthroline]iron or tris[5-amino-1,10-phenanthroline]iron	110
Cu and Pb	pectic or alginic acid	113
glucose	glucose oxidase/fumed silica	111
hydrazines and peroxides	Pd, Pt or Ru	114
hydroxylamine	Pd	116
NADH	diaphorase and either methylene green or meldola blue Pd, Pt or Ru NADH	117 114 124

[a] discussed in Section D.

K. CONCLUSIONS

It is apparent that modified electrodes have been developed that improve electroanalytical methodology for a wide range of important analytes. Such electrodes are especially useful for incorporation into detectors for HPLC, CE, and FIA. Yet, they are not used extensively in application laboratories where personnel who are untrained in electrochemistry are responsible for the measurements. Rectifying this lack of use will require commercially available systems that are (a) stable during use, (b) easily restored when poisoned, and (c) suited to storage in the dry state for long periods. Disposable versions of electrodes modified with mixed-valent ruthenium oxide crosslinked by cyano groups can meet these criteria because they have a long shelf-life when stored in the dry state; for commercial development and routine use, however, polishable composites may be more attractive. Otherwise, analytical applications of modified electrodes will continue to be restricted to research environments where they now are advancing science, especially through improved analytical methodology for biochemical investigations.

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