

Modification of electrodes with catalytic, size-exclusion films

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Abstract The application of electroanalytical methodology to the study of liquid-phase samples can be complicated by the adsorption of sample components on the electrode surface. Macromolecules are particularly problematic in this regard. An early means of addressing this problem was to use a membrane permeable to the analyte as a barrier between the sample phase and the electrochemical cell. Amperometric determination of oxygen in biological fluids is a historically important example. This approach was refined by modifying electrodes with semi-permeable, conducting films applied directly to the surface of the working electrode. Cellulose acetate is an example of a conductive material that blocked adsorption of compounds in biological samples but was permeable to analytes such as hydrogen peroxide. Modification of electrodes with ion-exchange films and, more recently, porous sol-gel films

was an expansion of this methodology. A complicating factor was that oxidation or reduction of most analytes requires a catalyst. The development of films that are size-exclusion barriers to interferents and incorporate an electron-transfer catalyst is described.

Keywords Passivation · Voltammetry · Modified electrodes · Selective films · Electrocatalysis · Cellulose acetate · Sol-gels

Foreword

In the 1960s, the cyclic voltammetry, also termed stationary-electrode polarography at that time, was emerging as an important method for diagnosis of electrode reactions, and the related technique, stripping analysis, was being applied for trace-level determinations. Irving Shain, with whom I was a postdoctoral associate, was a leader in both areas. Application of these methods required long exposure of working electrodes to samples relative to the use of dropping mercury electrodes. The exposure time exacerbated problems related to adsorption of matrix components. Early in my academic career I was funded to develop a method for the determination of nitrate in surface water. For an electroanalytical method, a major challenge was to protect the electrode from contamination by adsorption of matrix components, but the needs to include a catalyst in the supporting electrolyte and to preconcentrate the analyte were also important. From my time at the University of Wisconsin, I was familiar with the work of Walter Blaedel on the use of Donnan dialysis for the transport of ions from a low ionic-strength sample into a concentrated receiver electrolyte. Extension of his studies yielded a design in which a three-electrode voltammetry

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cell was separated from the sample by an ion-exchange membrane. For general applications, there were several limitations of this approach, including the quantity of catalyst required and the role of the difference in ionic strength at the membrane surfaces on the transport rate. The objectives of our early study can now be met by designing and fabricating nanometer-scale films with targeted characteristics on electrode surfaces. In this paper, the history of the development of modified electrodes that have size-exclusion and catalytic properties is described.

Introduction

The decrease of sensitivity with time of contact of a working electrode to samples that contained surface-active components became a problem of high importance when solid electrodes were required rather than the dropping mercury electrode. Monitoring oxygen in blood during surgical procedures is representative of this general problem. As detailed in a monograph [1], Clark et al. [2] pioneered a general approach to the mitigation of passivation of the working electrode for oxygen determinations where a current path through the sample is not required. The anode and cathode were separated from the sample by a membrane permeable to oxygen (e.g., polyethylene and Teflon), and an internal electrolyte bridged these electrodes. Other studies in that period used working electrodes that were coated with materials such as collodion [1], but the Clark electrode was significantly more robust and had an internal current path. The limited permeability of the membranes used in the original Clark oxygen electrode was a restriction on the scope of analyte to which this device could be used. A major advance was the use of cellulose acetate to separate aqueous samples from electrochemical cells; subsequently, it was immobilized on electrode surfaces to provide a size-exclusion barrier. The success of cellulose acetate in protecting electrodes from passivation by adsorption led to the use of electrodes modified with poly(4-vinylpyridine) (PVP) and related polymers that are permeable to anions; Nafion, a perfluor-sulfonated polymer that is permeable to cations; and sol-gel films, which can be prepared to provide a size-exclusion barrier as well as a degree of charge-selective permeable. These materials, alone and in combination with electron-transfer catalysts, are used to exemplify the development of a branch of electrochemistry at modified electrodes that is of important in applications where the contacting liquid phase contains species that are passivating when in direct contact with the working electrode. Also discussed is the transition from employing these materials as barriers between the sample and the electrochemical cell to using them directly on the electrode.

Modification of electrodes with cellulose acetate

The first systematic studies aimed at blocking interference of macromolecules on electroanalytical measurements used cellulose acetate (CA). Initial investigations employed CA barriers between aqueous samples and an electrochemical cell that contained a mercury working electrode, exploiting the facile diffusion of metal ions across this membrane [3]. Addition of a complexing agent to the electrolyte permitted preconcentration of metal ions in the electrolysis chamber, a process that was facilitated by the ability of the CA to size-exclude diffusion of the complexing agent from the electrolyte into the sample.

A major advance was the demonstration by Sittampalam and Wilson [4] that the size-exclusion property of a CA film was realized when this material was cast as a film on a Pt electrode. Here, the film was formed by dissolving CA (39.8% acetyl content) in a mixture of cyclohexanone and acetone, pipetting onto Pt, and drying. The resulting Pt|CA electrode was used in an amperometric detector (0.80 V vs Ag|AgCl) in a flowing electrolyte (0.1 M phosphate buffer at pH 7.4; flow rate, 0.5 mL min⁻¹) with H₂O₂ as the analyte (0.028–1.40 nmol H₂O₂ in 20- μ L injections) and bovine serum albumin (BSA) as the test interferent. When Pt was the electrode, the sensitivity decreased with consecutive injections of BSA-containing samples and was not restored by flow of BSA-free electrolyte. In a comparison of initial trials at freshly prepared electrodes on H₂O₂ samples that contained 0.2% BSA, the sensitivity of the bare Pt electrode was 50% of that recorded in the absence of BSA, whereas the analogous result with a Pt|CA electrode showed a decrease of only 5% due to the BSA. An additional characteristic of the CA film is that the sensitivity of the Pt|CA electrode toward ascorbate, a common component of biological samples, is only 0.4% of that toward H₂O₂; therefore, separating these species prior to amperometric detection of H₂O₂ can be avoided in many cases.

Two potential limitations of CA-coated electrodes are that they respond to only very small analytes and it does not include an electron-transfer catalyst, a need of particular importance in the electrochemical investigation of biological compounds. The former was addressed by hydrolysis of the CA film in 0.07 M KOH for controlled times up to 50 min prior to electrochemical applications [5]. As shown in Fig. 1, increasing the hydrolysis time increases the CA-film permeability, and the response for a given hydrolysis time increases with decreasing molecular size for the sequence phenol, acetaminophen, estriol, and NADH. The data for Fe(CN)₆⁴⁻ oxidation is anomalous in terms of size-dependent sensitivity; instead, the low current reflects the electrostatic repulsion effect that was reported previously [4]. A stable response with repetitive injections of 4 \times 10⁻⁴ M phenol was observed.

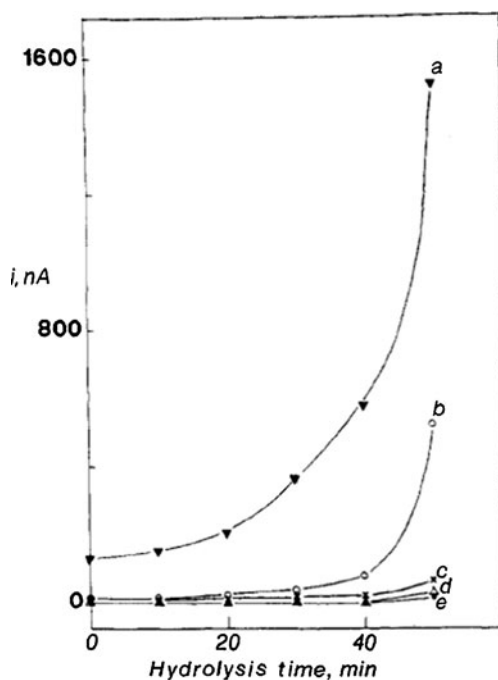


Fig. 1 Influence of the time of hydrolysis in 0.07 M KOH of CA films on Pt on the flow-injection amperometry (FIA) signal for the oxidation of species of varying molecular size. FIA conditions: electrolyte, 0.05 M phosphate buffer at pH 7.4; flow rate, 0.75 mL min⁻¹; applied potential, 0.8 V vs Ag|AgCl for *a*, *b*, *d*, *e* and 1.1 V for *c*; injection volume, 20 μ L. Solutes: *a* phenol, *b* acetaminophen, *c* estriol, *d* NADH, and *e* K₄Fe(CN)₆ at 6×10^{-4} M (*a*) or 4×10^{-4} M (*b*–*e*). Reprinted with permission from Wang and Hutchins [5] (Fig. 1)

The second general limitation involves incorporation of an electrochemical catalyst. Because CA is strongly basic, incorporating a catalyst by ion exchange is problematic, particularly in acid solution. This problem has been addressed primarily by casting composites of CA with either an ion-exchange component [6] or a catalyst [7, 8] onto an electrode surface. When the precursor solution contains CA and ZrO₂ and the cast film is reacted with phosphoric acid, thereby forming zirconium phosphate, the uptake of methylene blue (MB) by ion exchange occurs [6]. The MB mediates the oxidation of NADH. In this study, the CA served to prevent agglomeration of the ZrO₂ during film formation; whether it retains the ability to mitigate passivation by adsorption was not discussed; however, by comparison to an earlier report on the oxidation of NADH, it is likely that the high sensitivity of the electrode reaction mediated by MB resulted from a CA structure of a porosity too high to provide protection from irreversibly adsorbed species such as proteins. In contrast, a composite of CA and cobalt phthalocyanine (CoPC) was cast as a film on a glassy carbon electrode under conditions where the size-exclusion property of CA was retained [7]. The CoPC served to catalyze the oxidations of oxalic acid and hydrazine. The response toward the oxidation of oxalic

acid was influenced by the hydrolysis of the CA component of the film. Without hydrolysis, cyclic voltammetry of 1×10^{-4} M oxalic acid did not produce a current above the background, whereas after a 40-min treatment in 0.07 M KOH, a well-defined anodic peak was observed. In a similar manner, a composite of CA and 2,6-dichlorophenolindophenol (DCPI) was cast on a GC electrode and applied to the electrochemical oxidation of sulfide [8].

The efficacy of the DCPI mediator is illustrated by Fig. 2. The amplification of the anodic current and the attenuation of the cathodic current related to DCPI when sulfide is present (Fig. 2b) suggests that the oxidation of sulfide is via a mediated electron transfer. Of importance is that the peak current for the oxidation of sulfide increases with the time of hydrolysis of the CA in the composite. The anodic current corresponding to Fig. 2b, except with 1×10^{-4} M sulfide, increases with the time of hydrolysis in 0.07 M KOH at a rate of 20 nA min⁻¹ at 0.08 V. While the electrode was applicable to the determination of sulfide in urban wastewater, there was a 12% loss of sensitivity after a 10-h flow of buffer at 0.4 mL min⁻¹.

Alternatives to co-deposition of CA and a catalyst are to deposit the catalyst after the film is formed and to apply CA to an electrode that is modified with a catalyst. In a study that foreshadows recent methods of forming metal nanoparticles for use as catalysts, Wang et al. [9] deposited Pt in the 0.2–0.4- μ m range in the voids of a hydrolyzed CA film on GC (GC|CA- μ Pt). The resulting electrodes lowered the overvoltage for the oxidation of H₂O₂ by ca. 0.5 V relative to bare GC. The sensitivity of the FIA determination of H₂O₂, NADH, Fe(CN)₆²⁻, and dopamine was increased with GC|CA- μ Pt relative to that at a Pt-coated GC electrode. The retention of the size-exclusion property of CA was demonstrated by the amperometric determination

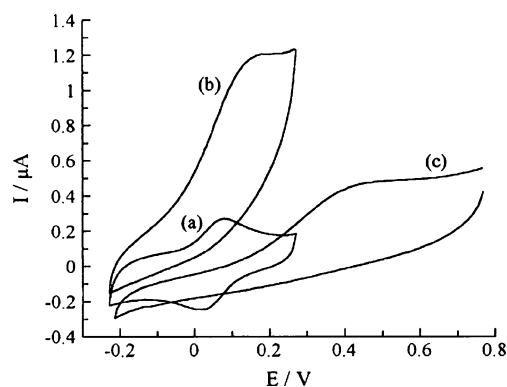


Fig. 2 Electrochemical oxidation of sulfide at GC modified with a composite of CA and DCPI after a 6-min hydrolysis in 0.07 M KOH. Voltammograms in *a* supporting electrolyte, 0.25 M phosphate buffer at pH 7.25 in 0.5 M KCl; *b* 1×10^{-3} M sulfide in the supporting electrolyte; *c* repeat *b* at bare GC. Reprinted with permission from Florou et al. [8] (Fig. 3)

of hydrazine in the presence of electroactive species of larger size. Although not systematically investigated, the ability of CA and similar porous structures to protect catalysts from poisoning by adsorption of solutes in electrolytes was predicted. An overlayer of CA to protect an electrode that is modified with an electron-transfer catalyst is the second alternative. Gilbert et al. [10] cast a CA film over an electrode modified with CoPC to detect H_2O_2 as part of an indirect determination of phosphate.

While CA is of historical importance as an electrode modifier that protects surfaces from fouling by adsorption, it is not unique in its ability. A recent study compared several membrane materials as protectors of electrode surfaces from bio-fouling [11]. The tests were performed as follows: A gold electrode was covered with various polymers, the $\text{Ru}(\text{NH}_3)_6^{3+,2+}$ redox couple and the reduction of dissolved oxygen were the electrochemical probes, albumin was the fouling agent, and cyclic voltammetry was the electrochemical method. The materials tested as coatings on the electrode were Nafion, chitosan, CA, fibronectin, and a composite of polystyrene sulfonate and polylysine (PSS/PL). The voltammetric data indicated that albumin adsorbed on the PSS/PL film and that chitosan was very ineffective in mitigating passivation. Nafion and CA were considered good protecting agents, although a degree of hindered diffusion was observed; it was proposed to result from partial blocking of the pores. Fibronectin provided the best protection against bio-fouling of this group, but the reported need to prepare it from bovine plasma may hinder its wide-scale utilization. The mechanism of its attenuation of fouling of electrode surfaces is like that of CA, namely size exclusion.

Electrodes modified with films that contain ion-exchange functionality

As CA was being investigated as an electrode modifier in the 1980s, parallel and more extensive investigations were occurring on the use of films with ion-exchange properties on electrode surfaces. The primary objective was not protection against fouling by size exclusion, but rather these films generally were investigated for their ability to immobilize electrochemical catalysts at electrode surfaces. In many cases, these studies did consider ion/charge transfer across the films. The resulting behavior in terms of application in hostile media often included protection against fouling as a result of the hydrophilic nature of ion-exchange materials. Nafion, which is weakly basic because of its sulfonate sites, along with poly(4-vinylpyridine) and its quaternized form, an anion-exchange polymer, is a representative of these materials.

Historically, the use of ion-exchange films as electrode modifiers followed promising results on mitigating sample

matrix effects when these materials were configured as membranes that separated complex samples from the supporting electrolyte of electrochemical cells or from ion-selective electrodes. Blaedel and coworkers described the theory, demonstrated the potential for analytical preconcentration of analyte ions by Donnan dialysis into relatively high ionic-strength receiver electrolytes, and employed this transport in the determination of sodium in large-volume samples at an ion-selective electrode in contact a thin film of electrolyte coupled to the sample by a cation-exchange membrane [12–14]. We extended Donnan dialysis to the voltammetric determination of nitrate [15, 16]. The anion-exchange membrane between the sample and the supporting electrolyte retained the catalyst (La^{III} or Zr^{IV}) in the electrolyte, provided preconcentration of nitrate, and blocked potential interferents such as Zn^{II} and 0.001% gelatin from entering the electrochemical cell. In the latter case, size-exclusion or limited adsorption of neutral species on the membrane provided the separation.

The intentional chemical modification of electrode surfaces initially involved anchoring ligands such as amines to the surface of tin oxide electrodes by silane chemistry [17] but was quickly expanded to include covalent immobilization of redox agents [18] and casting ion-exchange polymer films on electrodes. Because they did not involve the development of electroanalytical methodology as a primary purpose, investigating interference from concomitants in the supporting electrolyte, particularly via a size-exclusion process, generally was not an objective. A notable exception was the report by Witkowski and coworkers [19] of a means of obtaining a permselective layer on an electrode from a cast film of a conducting polymer. Here, polypyrrole was “over-oxidized” at 1.0 V in basic medium to yield a non-conducting film that was highly selective for the transport of cations, but overall the major focus was on the immobilization of redox-active species and the mechanism of charge transfer at the resulting modified electrodes [20–22]. We developed an analog of stripping voltammetry in which a charged analyte was preconcentrated at an electrode modified with a film of ion-exchange material and subsequently quantified by voltammetric oxidation or reduction [23]. In this manner, the determination of Cr^{VI} was accomplished without interference by 10 mg L^{-1} gelatin [23]. The method subsequently was termed ion-exchange voltammetry [24]. Whether the elimination of interference by this surfactant was from size exclusion or the hydrophilic nature or the protonated PVP film was not established; however, the reported affinity of Nafion for hydrophobic cations [25] suggests that with these films, size exclusion is likely to play a role in blocking surfactants from interfering with voltammetry of accumulated ions. Moreover, when a GC electrode was modified, first, with a layer of a mixed-valence ruthenium

oxide catalyst and, second, with a Nafion film, voltammetry of 4-nitroso-*N,N*-diethylaniline, 1-amino-2-phenylethane, cysteine-glycine, and *N*-nitrosodiphenylamine was observed; hence, the Nafion was permeable to these compounds given that the electrocatalytic oxidation at the Nafion|catalyst interphase was achieved [26]. More common is the incorporation of the proposed electrochemical catalyst in the film by an ion-exchange reaction even though the current-limiting step becomes dependent on a combination of charge-transfer kinetics and charge propagation within the ion-exchange layer [27–29].

The above studies document the ability of films of ion-exchange materials to serve as the foundation of electrocatalytic electrodes, but the ability to discriminate against species that have the potential to passivate electrodes by adsorption is not readily predicted because partitioning into materials such as Nafion is facile for charged hydrophobic species rather than predicted by the Hofmeister series of ion-exchange interactions. To address this problem, composites of size-exclusion materials (e.g., CA) and hosts for electrochemical catalysts (e.g., Nafion) have been investigated.

Bilayers of size-exclusion and ion-exchange materials as electrode modifiers

A “sandwich” comprising an electrode material coated with ion-exchange (inner) and size-exclusion (outer) layers can be predicted to have several merits for electrochemical measurements. Physically small species that are targeted for oxidation or reduction can transport across the outer layer while species larger than the pore size of the cladding material will be blocked. The layer of ion-exchanger can transport charge, small neutral species, and ions of the appropriate charge sign; hence, it provides selectivity of targeted ionic species over those of opposite charge sign. Providing they are sufficiently small and/or of appropriate charge sign, the products of the electrode reaction can be transported away from the electrode. Preconcentration of ionic species in the ion-exchange layer can be achieved, thereby accruing the favorable characteristics of stripping voltammetry. The ion-exchange sites allow the film to be modified with a catalyst of the appropriate charge sign.

Perhaps the first reports of the use of these bilayers were by Wang and Tuzhi [30, 31]. They developed a GC|Nafion|CA electrode for the determination of dopamine in the presence of other organic cations of larger size such as norepinephrine. The CA was hydrolyzed for various times to optimize the size-exclusion property [30]. The efficacy of this sandwich configuration for the determination of a small, neutral molecule, nitric oxide, was demonstrated by Pariente and coworkers [32]. At a Pt electrode in neutral solution, the oxidations of NO and NO₂[−] by cyclic

voltammetry both occur near 0.9 V vs SCE. At Pt|Nafion, the signal from NO₂[−] is not observed because of Donnan exclusion of that anion by the sulfonated membrane. When the pH 7 phosphate buffer contains NO, an anodic current that is proportional to NO concentration is developed over the range 60–480 μM ($r=0.994$). The data were obtained at constant potential, 0.9 V, under hydrodynamic conditions. A CA overlayer was used to prevent passivation by the adsorption of proteins.

Pt|Nafion and Pt|Nafion|CA electrodes were compared for the measurement of H₂O₂ released by enzymatic reactions of cholesterol (the analyte) [33]. The test interferent was 0.75% (v/v) Triton X-100; a pH 7.5 phosphate buffer served as the supporting electrolyte in the flow-injection amperometric monitoring of H₂O₂. The Pt|Nafion was unstable under these conditions; however, Pt|Nafion|CA was stable for 3 weeks of use in serum samples albeit at the cost of sensitivity. With the CA layer, the sensitivity was reported as only 0.25% of that at the corresponding Pt|Nafion electrode, but there is no indication that the pore size of the CA was optimized by hydrolysis.

An example of the combination of electrocatalysis with an electrode that also contains a bilayer comprising an ion-exchange and a size-exclusion material has been reported [34]. In this case, the working electrode was carbon paste that was modified with a mixture of peroxidase and ferrocene. A Nafion|CA overlayer was applied. The objective was to detect H₂O₂ in the presence of ascorbate by its reduction at 0.1 V vs Ag|AgCl in the flow-injection mode. A variety of membrane combinations was compared. Nafion was superior to polycarbonate in terms of rejection of ascorbate. However, the stability of the Nafion-coated electrode was only a few hours unless a CA overlayer was applied. With this bilayer film, the electrode was stable for more than 1 month, and the detection limit (signal-to-noise ratio of 6) in a 20-μL injection was 10 nM (200/fmol; Fig. 3). This system permitted the indirect determination of biologically important species such as glucose based on the

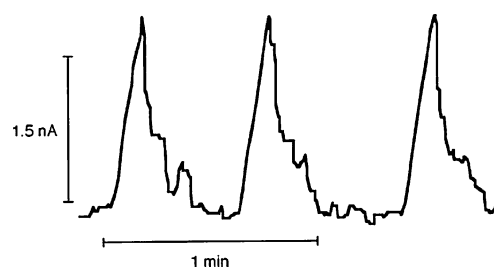


Fig. 3 Flow-injection amperometry of hydrogen peroxide at an electrocatalytic carbon paste electrode coated with a Nafion/CA bilayer. H₂O₂ concentration, 10 nM; injection volume, 20 μL; flow rate, 0.5 mL min^{−1}, electrolyte, pH 7.0 phosphate buffer containing 0.3 M KCl; applied potential, 0.100 V vs Ag|AgCl. Reprinted with permission from Yamamoto et al. [34] (Fig. 3)

electrochemical monitoring of H_2O_2 released by enzymatic reactions.

In summary, the bilayer systems are an approach to solving an important issue in electroanalytical chemistry, namely the need for electrodes to function in hostile media and to gain an element of selectivity without sacrifice of conductivity. These problems are important in certain fuel cell systems, particularly those related to biological systems. The bilayer design also has provided a route to long-term stability. Important questions that have not been addressed sufficiently are how to incorporate catalytic functionality and how to avoid perturbation of electrode behavior from the accumulation of electrolysis products. In the former case, the use of complexes bound to ion-exchange sites as electron-transfer mediators in a bilayer system is an approach that merits study and can be predicted to be effective in that such mediators in single layers on electrodes have been reported extensively. The latter case may represent a fundamental limitation of a bilayer approach.

Electrodes modified with sol–gel films

The most recent advance in the design of electrodes that combine size exclusion and the ability to incorporate catalysts is modification with films of sol–gels. By inclusion of organosilanes in the precursor sol, the structure that is obtained can include functional groups such as ion-exchange and metal complexation sites. That is, the merits of the above-described bilayers potentially can be accrued without their limitations. The resulting SG films are physically and chemically robust and potentially have long-term stability.

For electrochemical applications, the permeability of sol–gel films is a key issue. The target species must transport from the film–sample interface to the electrode surface unless the film contains a redox mediator that makes the outer surface of the film the site of electron transfer. Even in the latter case, the sol–gel must be permeable to the electrolyte to maintain charge balance. Permeability depends on pore size and film thickness. Regarding pore size, from studies on the formation of monolithic solids by ambient-temperature sol–gel processing, the general rule is that with hydrogen-ion catalysis, the product is a polymer chain (little branching), and with base-catalyzed hydrolysis, a highly branched product, a nanometer-scale particulate, is formed. In the former case, the subsequent condensation reaction forms a microporous solid, whereas in the latter case, a mesoporous solid is produced [35, 36]. A microporous material has pore widths less than 2 nm, and mesoporous solids have pore widths in the range 2–50 nm [37].

Historically, the influence of pore size on electrochemical measurements in sol–gel solid electrolytes was considered indirectly in studies focused on the role of residual

water. In the first report, Oliver et al. used the voltammetric current for the oxidation of a ferrocene derivative to estimate the gelation point of a sol [38]. Audebert et al. [39] employed the potentiostatic current–time behavior to investigate diffusion during the formation of silica and zirconia sol–gels. The primary decrease in diffusion was observed at a point about three times the gelation time. Here, they surmised that the redox probe was no longer influenced primarily by the interstitial liquid but rather included a major contribution from interaction of the probe with the oxo-polymers in the shrinking network. The measurement of the diffusion coefficient is complicated because the true concentration of the probe in the solid electrolyte is not known; however, we demonstrated that by varying the scan rate over a range that causes transition from hemispherical diffusion to linear diffusion, the apparent diffusion coefficient, D_{app} , as well as the effective concentration in a SG solid electrolyte can be determined in that two equations for the two unknowns are generated [40]. The general conclusion reached by Audebert et al. [39] that rigid sol–gels can yield liquid-like diffusion was supported by measurement of D_{app} of polyoxometalates [40] and hexacyanoferrate [41] in silica monoliths. We directly investigated the role of pore size by preparing silica monoliths in the presence of a surfactant at a level well above the critical micelle concentration [42], a procedure that yields mesoporous rather than microporous SGs [43]. This use of surfactants was well-known as a means of controlling pore size in zeolites [44]. With phosphotungstate as the redox probe in silica monoliths, D_{app} was about two orders of magnitude greater when mesopores were templated. It is important to note that D_{app} in sol–gels is also influenced by the relationship between the charge sign of the pore wall and that of the probe [45, 46]. Under a given set of conditions, D_{app} of $\text{Fe}(\text{CN})_6^{3-}$ is greater in a tetramethoxysilane (TMOS)-derived sol–gel relative to that in an organically modified silica (ormosil) where the precursor is a mixture of TMOS and a trimethoxy analog with a functionality that introduces a quaternary amine.

Electrochemical applications that employ size exclusion by SGs require the modification of electrodes with films of these materials. In the first report, Pt was coated from a sol formulated to yield a borosilicate sol–gel (BSG) composition of 84% SiO_2 , 12% B_2O_3 , and 4% Na_2O [47]. Spin-coated films were prepared with varying thickness by control of the amount of sol pipetted on the electrode surface. The sol–gels were thermally cured. Permeability was measured by voltammetry of a solute, [(tetramethylphenanthroline) $_3\text{Os}](\text{PF}_6)_2$, herein designated as Ph_3Os (Fig. 4). Two points are particularly interesting. The shapes of the voltammograms at Pt|BSG suggest that there is a hemispherical diffusion component of the current-limiting process, which is indicated by a transition from the time-

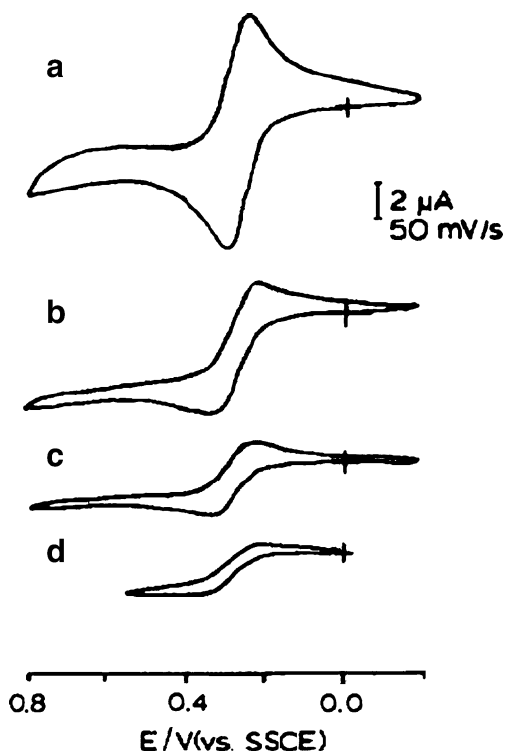


Fig. 4 Voltammetry of 0.8 mM Ph_3Os in acetonitrile with a 0.1 M Et_4NClO_4 supporting electrolyte at a Pt electrode coated with varying thickness (number of spin coats) of a borosilicate, BSG, sol-gel, and curing temperature. *a* bare Pt; *b* 15 spin coats, 4 h at 400 °C; *c* 30 spin coats, 17 h at 700 °C followed by an etch for 5 s in 0.5% HF; *d* same as *c* but without an etching step. Reprinted with permission from Lundgren and Murray [47] (Fig. 4)

dependent current decay after the peak in Fig. 4a to a time-independent plateau in Fig. 4d. Second, even after treatment at a high temperature (Fig. 4d), the BSG is permeable. Not only does the Ph_3Os penetrate the film but also the film retains conductivity. A strong influence of B_2O_3 on the SG properties is suggested by the absence of annealing.

The importance of pore size of SG films on electrodes has led to investigation of the influence of processing conditions other than pH on this parameter. They include the water-to-alcohol ratio in the precursor solution, the identity of the precursor and the resulting steric effects on structure, and the drying/aging conditions. Common precursors of films are TMOS, tetraethoxysilane (TEOS), and methyltrimethoxysilane (MeTMOS), the product of which is an organically modified silica sol-gel (ormosil). A variety of substituent other than methyl groups has been used. Of these parameters, factors that determine the relative rates of the condensation step and the loss of solvent are especially important [35, 48]. In turn, they are dependent on the method of forming the SG film.

Dip coating and spin coating are the most common method of fabricating sol-gel films [35, 49], of which the latter is utilized more in electrode modification. With these

methods, the evaporation of solvent is rapid, which results in less porous sol-gels than observed with monoliths that are prepared under analogous conditions [48]. Shapiro and coworkers have examined the interaction of sol-gel processing conditions on the thickness and permeability of spin-coated films used for electrochemical applications [50]. A mixture of TMOS, methanol, water, and HCl comprised the precursor solution. The film was formed by pipetting an aliquot onto a substrate followed by spinning for 1 min. Variables were the dilution factor (2, 10, and 50) and hydrolysis time (0 or 1 day) prior to pipetting onto the surface. The spin rate was varied over the range, 2,000–6,000 rpm. The results summarized in Fig. 5 demonstrate that films ranging from ca. 20 to 350 nm were obtained with these conditions. The influence of the preparation procedure on the permeability was determined by adding an electroactive species, ferrocene monocarboxylic acid (FMA), to the initially prepared solution. Films fabricated by dilution prior to hydrolysis did not show electrochemical activity of FMA, whereas those formed from solutions hydrolyzed for 1 day prior to dilution showed quasi-reversible behavior. The result was explained by dilution prior to hydrolysis leading to the formation of small oligomers that, in turn, result in dense sol-gels with small pore volumes.

The development of electrochemically assisted methods of deposition of SG films was a major advance in the design of electrodes modified with electrocatalytic, size-exclusion films on electrodes. As detailed in the initial report [51], electrolysis is used to control the pH of the interface between an electrode and a SG precursor solution. For example, the reduction of water or hydronium increases the pH of the interface which initiates and sustains the condensation and polycondensation of a SG precursor. In

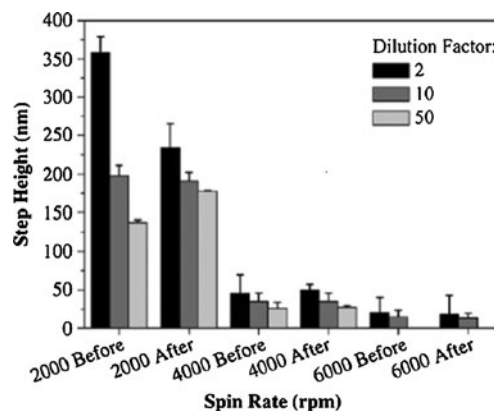


Fig. 5 Influence of precursor concentration, rotation rate, and hydrolysis time prior to film formation on the thickness of spin-coated sol-gel films derived from TMOS. Substrate, *n*-Si(1,1,1); precursor, methanol/TMOS/HCl mole ratio 5.1:1:13.6. Reprinted with permission from Shapiro et al. [50] (Fig. 1)

this study [51], MeTMOS was the precursor. Electrochemically assisted processing (EAP) was accomplished at potentials between -0.3 and -1.5 V vs Ag|AgCl for 1 h in stirred solution, after which the resulting film was dried for 1 h at 70 °C or for 1 week at room temperature. Cyclic voltammetry of $\text{Fe}(\text{CN})_6^{3-}$ at this ormosil-coated electrode is shown in Fig. 6. Comparison of trace A1 to B2 illustrates that the ITO is virtually passivated by the film after deposition of the ormosil for 30 min at -1.1 V vs Ag|AgCl. This method has been refined and extended to other precursors such as titanium tetra-*n*-propoxide [52], zirconium tetra-*n*-propoxide [53], and TMOS [48]. Details of this processing method are available in recent reviews [46, 49, 54].

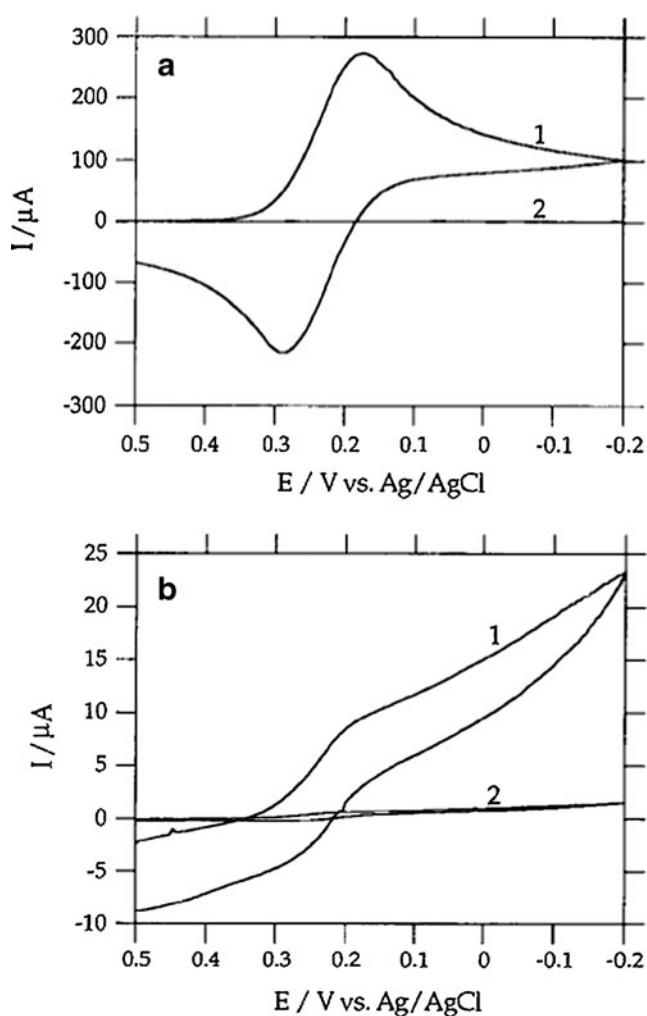


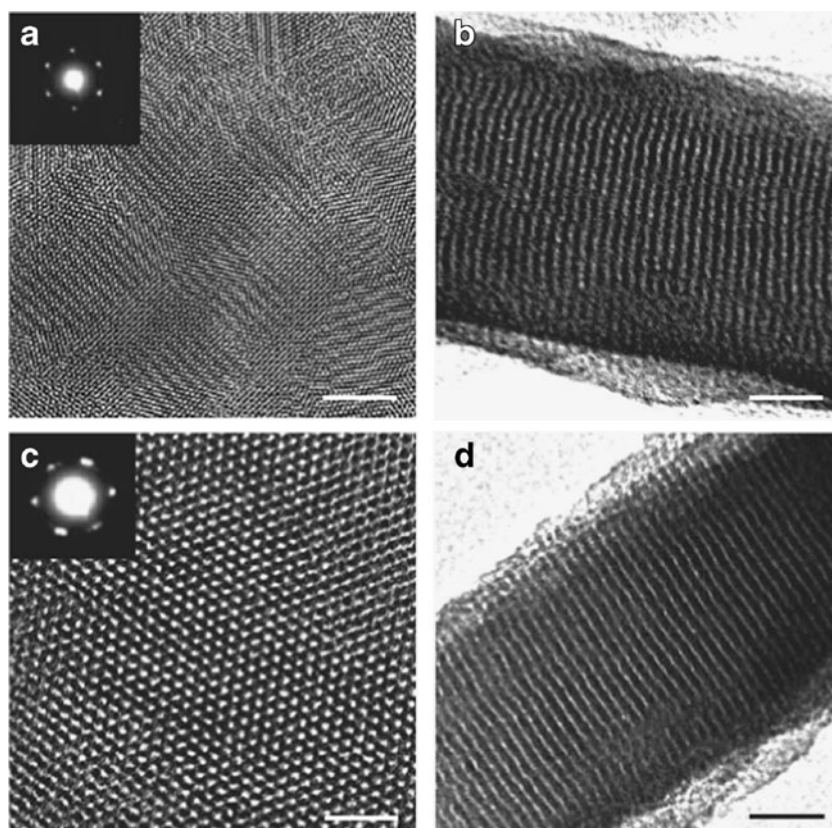
Fig. 6 Cyclic voltammetry of 2 mM $\text{Fe}(\text{CN})_6^{3-}$ in 0.1 M KCl at an ITO electrode modified with an ormosil film deposited by an electrochemically assisted process from a precursor comprising MeTMOS, ethanol, water, KNO_3 , and a 1 -mM phthalate buffer at pH 3.5 . **a** Before (1) and after (2) application of -1.1 V vs Ag|AgCl for 30 min in the precursor solution; **b** after applying the potential for (1) 5 min and (2) 30 min. Reprinted with permission from Shacham et al. [51] (Fig. 6)

Walcarius et al. [55] developed an approach to electrochemically assisted SG film formation that yielded a mesoporous layer. The SG processing is somewhat related to evaporation-induced self-assembly (EISA) [56] in that the film is formed in the presence of an organized surfactant. In EISA, dip coating a substrate with a silica precursor in the presence of a surfactant such as cetyltrimethylammonium bromide (CTAB) followed by evaporation of the solvent causes the CTAB to progress in concentration to levels that first yield micelles and eventually lamellar liquid crystals. By control of pH, the gelation is suppressed to permit silica–surfactant self-assembly. For electrochemical applications, EISA has a limitation in that the resulting mesopores are parallel to the substrate surface. Under potential control, the surfactant orientation is altered from that in the bulk solution, which permitted deposition of Pt with pores in the 4 -nm range that were perpendicular to the electrode surface [57]. Using this concept on a mixture of TEOS and CTAB along with electrochemical perturbation of the pH of the precursor solution from 3.0 (1 mM HCl) to a basic value by reduction of hydronium ion, highly organized mesopores of silica on the electrode were obtained (Fig. 7) [55]. Using sols aged for 2.5 h, the thickness varied linearly with time at the applied potential. For example, at -2.2 V with a glassy carbon electrode, the growth rate was about 8 nm s^{-1} for up to 30 s. Homogeneous structures in the range 40 – 150 nm were obtained.

A related procedure is to use a thiol-substituted silane such as mercaptopropyl trimethoxysilane (SH-PTMS) as a component of the precursor solution and an electrode (e.g., Au) at which a self-assembled monolayer of the thiol forms [58]. A mixture containing SH-PTMS, TEOS, and MeTMOS was prehydrolyzed for 2.5 h, after which a gold electrode was immersed therein. This immersion time was limited so that only a partial monolayer (via Au–S formation) was assembled. In this regard, to obtain a mesoporous film in the subsequent electrochemically assisted SG formation, a compact self-assembled monolayer needs to be avoided. The voltammograms displayed in Fig. 8b–d have the characteristic shape for a current limited by hemispherical diffusion. This behavior requires that the pore length in the film is short enough to prevent the time of diffusion through the pore from being the current-limiting step. In this case, the SG-coated electrode has the characteristics of a microelectrode array.

Although electrochemically assisted formation of mesoporous SG films on electrodes is a major step in the development of size-exclusion electrochemistry, for practical application to a wide range of electroanalytical measurements, the ability to extend the pore size to values beyond those that can be achieved by control of the sol–gel processing conditions (e.g., pH, length of side chains on

Fig. 7 Transmission electron microscopy of a silica film deposited from a TEOS, CTAB solution at pH 3 at a potential that (1) organizes the surfactant with pores parallel to the electrode surface and (2) provides a basic interface by the reduction of hydronium, water, and/or the supporting electrolyte anion. **a** Low magnification top view on gold, **b** high magnification cross section on gold, **c** high magnification top view on glassy carbon, and **d** high magnification cross section on glassy carbon. Scale bars correspond to **a** 50 nm and **b–d** 20 nm. Reprinted with permission from Walcarius et al. [55] (Fig. 1)



ormosils) and by organizing the structure with surfactants is required. Moreover, the incorporation of electron-transfer catalysts is necessary for many applications, particularly those involving biological compounds as analytes.

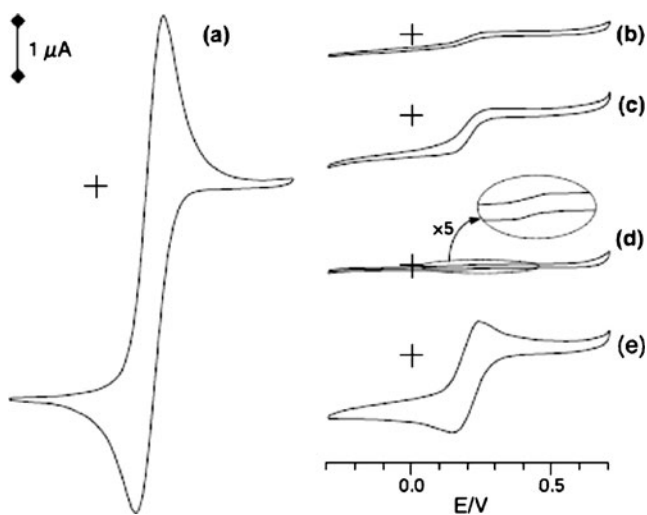


Fig. 8 Cyclic voltammetry of 1 mM $\text{Fe}(\text{CN})_6^{3-}$ at a gold electrode coated by a combination of an electrochemically assisted process with self-assembly of a mercapto-substituted silane. **a** Bare Au; **b–d** SH-PTMS/TEOS ratios of 5%, 10%, and 20%, respectively; **e** characteristic trace when the SG film is cracked. Electrolyte, 0.1 M NaNO_3 ; scan rate, 5 mV s^{-1} . Reprinted with permission from Sayen and Walcarius [58] (Fig. 3)

The inclusion of templating agents in the precursor solution is a convenient method for imparting the desired porosity in SG films. For example, sol-gel derived, mesoporous tungsten oxide was prepared by inclusion of a tri-block copolymer in the precursor; the copolymer was removed by calcination after a dip-coated film was made [59]. Cyclic voltammetry showed an anodic peak, presumably the oxidation of W^{V} , after application of -0.8 V. A significantly higher current density was observed than when the tungsten oxide film was prepared in the absence of the copolymer. Khrarov and coworkers spin-coated TMOS-derived SG films that were doped with 0.3–1.0 μm polystyrene (PS) spheres [60, 61]. After formation of these films on glassy carbon, the polystyrene was dissolved with chloroform. Imaging illustrated that the voids comprised pores that extended through the silica film. The resulting electrodes were tested by cyclic voltammetry of various solutes in an aqueous supporting electrolyte. Voltammetry of $\text{Ru}(\text{NH}_3)_6^{3+}$ yielded current–voltage curves that are characteristic of this complex, whereas when the films were made in the absence of PS under otherwise-identical conditions, the characteristic voltammetry was not seen. Moreover, the current increased with time of immersion of the electrode in 1 mM $\text{Ru}(\text{NH}_3)_6^{3+}$, which suggested accumulation of this complex on the negatively charged walls of the pores in the silica [61] (Fig. 9).

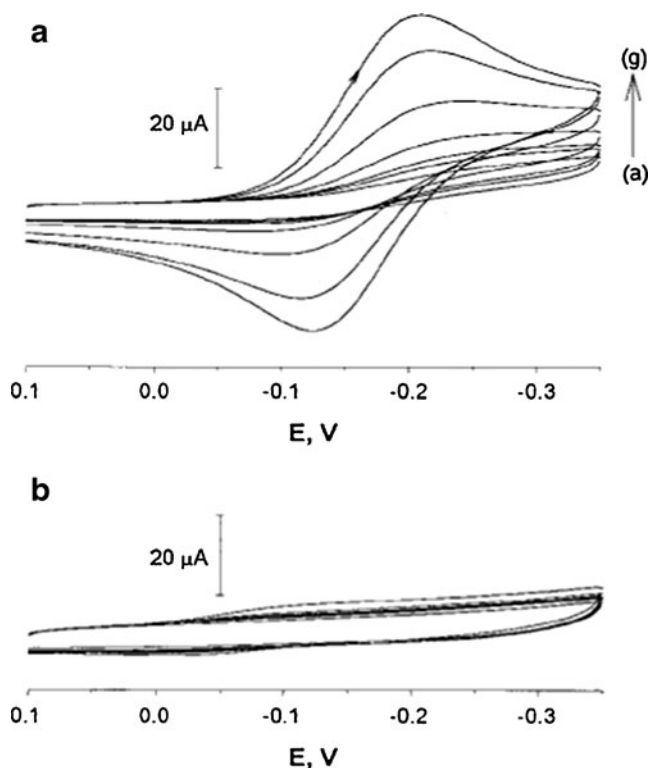


Fig. 9 Cyclic voltammetry of 1 mM $\text{Ru}(\text{NH}_3)_3^{3+}$ at a spin-coated, TMOS-derived SG film on a glassy carbon electrode. **a** Film templated with 0.5- μm polystyrene beads that were dissolved prior to the voltammetry; **b** TMOS-derived film prepared without a templating agent. Curves *a–e* represent immersion times in the $\text{Ru}(\text{NH}_3)_3^{3+}$ of 0, 20, 40, 60, 80, 100, and 120 min prior to voltammetry. Electrolyte, 0.1 M KNO_3 ; scan rate, 100 mV s^{-1} . Reprinted with permission from Khramov et al. [61] (Fig. 6)

In a report of an electroanalytical method for the determination of a phospholipid, we demonstrated both the efficacy of size exclusion of a templated SG film and a route to incorporation of an electrochemical catalyst in a SG formed by an electrochemically assisted process [62]. The film was formed from a TEOS precursor that contained β -cyclodextrin (CD) as the templating agent and a rhodium acetate dimer as the catalyst. CD is a toroid with a ring size of about 0.7 nm, and Rh^{II} mediates oxidations at about 0.9 V vs $\text{Ag}|\text{AgCl}$. Except for the inclusion of CD and the catalyst, the film was formed in accord with Shacham and coworkers [51–53]; in particular, the electrode was withdrawn from the solution at 50 $\mu\text{m s}^{-1}$ [52, 63] while under potential control (2.35 V vs a Pt quasi-reference electrode). Because CD was reported previously to form stacks on electrodes [64], it was hypothesized that pores perpendicular to the electrode surface in the SG will be formed. The pore size was smaller than that of the test phospholipid, phosphatidylcholine (PC), which is necessary because phospholipids are well-known to passivate electrodes by strong, irreversible adsorption. With this film design, PC was chemically oxidized by Rh^{III} at the SG|sample interface by a

process that involved electrochemical generation of Rh^{III} at the electrode–SG interface and electron self-exchange of $\text{Rh}^{\text{II,III}}$ through the 230-nm film to yield Rh^{III} at the SG|sample interface (Fig. 10). After five scans under the conditions in Fig. 10b, the current for the $\text{Rh}^{\text{II,III}}$ couple decreased by about 20% because of some adsorption of PC on the SG, but the current was readily restored to the original value after treatment in 0.1 M Bu_4NPF_6 in methanol (Fig. 10c). These data suggest that the ring size of CD was important in that it permitted facile charge transport through SG film while preventing passivation of the base electrode by the PC. A more direct illustration of size exclusion by a CD-templated SG film on an electrode is the influence of 10 μM PC on the cyclic voltammetry of 10 mM ferrocene. The addition of the PC to the sample lowered the anodic peak current by only 5%, whereas at a bare electrode, the PC passivated the electrode.

The continued development of sol–gel films for size-exclusion voltammetry will require versatility of control of the pore size. In this regard, poly(amidoamine) (PAMAM) dendrimers are promising as templating agents. Generations (G) 4–10 PAMAM adsorb in a homogeneous pattern to various substrates such as mica and gold [65, 66]. The mean diameter of the adsorbed particles is dependent on the generation number (Fig. 11) [66]. The same trend applies to G4-PAMAM [65]. The adsorbed PAMAM served as a templating agent in the electrochemically assisted deposition of a TEOS-derived SG film by the method described by Walcarius et al. [55] but with a precursor solution that contained 1.0 mM G4-PAMAM and a 0.32 ratio of CTAB/TEOS [67]. Prior to applying the modified electrode, the

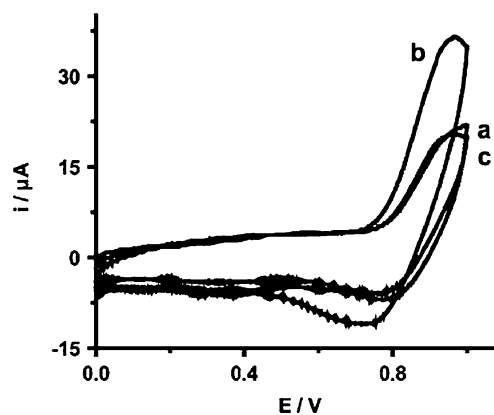


Fig. 10 Cyclic voltammetry of phosphatidylcholine (PC) at a gold electrode modified by electrochemically assisted processing of a TEOS-derived sol–gel film templated with cyclodextrin. The sol–gel phase was doped with a Rh^{II} catalyst. Sequence: *a* initial scan in the supporting electrolyte showing redox of the $\text{Rh}^{\text{II,III}}$, *b* 0.1 mM PC in the supporting electrolyte and *c* repeat of scan in supporting electrolyte after treating the electrode by ten scans in 0.1 M Bu_4NPF_6 in methanol. Supporting electrolyte, 0.1 M Bu_4NPF_6 (aq); scan rate, 50 mV s^{-1} . Reprinted with permission from Wandstrat et al. [62] (Fig. 5)

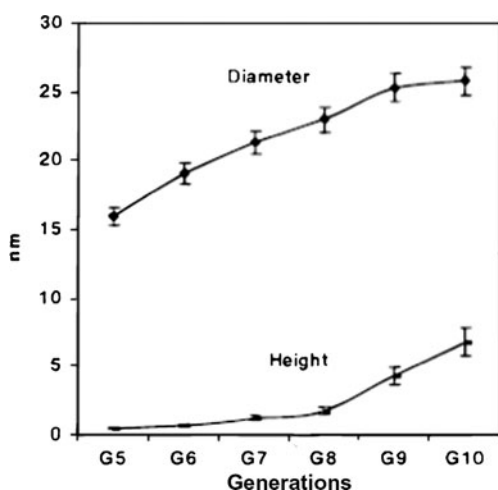


Fig. 11 Influence of generation number of poly (amidoamine) dendrimers on the size of particles deposited by spin coating on mica. Imaging was performed by 3D atomic force microscopy in the tapping mode. Reprinted with permission from Li et al. [66] (Fig. 5)

G4-PAMAM and CTAB were removed by 20-min treatment with a low-pressure oxygen plasma [68, 69] and in an equal-volume mixture of ethanol and 0.1 M HCl (aq). The pore diameter, 10 ± 5 nm, was determined by scanning electron microscopy, which also showed that the treatment did not damage the SG film. Cyclic voltammetry of 5-hydroxytryptophan (5-HTPP) at this electrode gave a reproducible current–voltage curve for at least 20 continuous cycles at 50 mV s^{-1} , whereas a bare electrode was passivated [67]. The prevention of passivation was attributed to blocking polymerization of the cation radical formed by the electron-transfer reaction because of spatial restrictions from the nanowell environment, an interpretation consistent with mass spectrometric evidence of the electrochemical polymerization of aniline being blocked in microporous silica [70]. The absence of passivation permitted the determination of 5-HTPP from its oxidation at constant potential in a flow-injection system with a linear dynamic range of 0.5–60 μM and a detection limit of 17 nM. In these electroanalytical measurements, the SG-coated electrode was further modified with cobalt hexacyanoferrate as a catalyst. The Co^{2+} was ion-exchanged onto the silica and then reacted with hexacyanoferrate. In future work, it will be necessary to identify more sophisticated methods to modify these electrodes with catalysts, especially when the desired electrochemical reaction is performed at the base of the templated pores.

Future directions

From its roots where cellulose acetate was the material of interest, the modification of electrodes with films that

combine size exclusion and electrocatalysis has grown toward fabrication of films that are more physically and chemically robust and that are amenable to interrogation by methods in addition to electrochemistry. Sol–gel films meet these requirements, especially the formulations that are optically transparent. They are readily doped, including incorporation of organic reagents, to obtain materials with, for example, ion-exchange, chromophore, and chelation properties [71]. The continued growth of interest in sol–gel processing by the chemistry community is likely to lead to further advances in the formulation of SG films on electrodes. Modification of SG precursors to entrap ionic liquids is an example [72]. In terms of the development of films on electrodes that provide size exclusion, it can be anticipated that tuning pore size in narrow distributions beyond the simple classifications of microporous, mesoporous, and macroporous will be an area of increasing activity.

The development of EAP methods was a major advance in fabrication of SG films. It can be anticipated that it will find increased utilization, particularly because it provides multiple methods of controlling the film thickness and porosity and the ability to grow films without rapid loss of solvent. The latter results in increased densification of the SG phase relative to that obtained by casting, dip coating, and spin coating. Achieving a dense SG phase generally is desired when control of the size and size distribution of pores by use of a template is employed. For applications requiring films that are mesoporous, especially near the 50-nm diameter value that is the boundary with macroporosity, but do not require a narrow size distribution, ambigel films [73], which are processed with a low surface-tension solvent exchanged for water prior to drying, are likely to experience increased attention.

Immobilization of catalysts in size-exclusion, SG films has not been extensively investigated. Well-established methods based on cellulose acetate and organic ion-exchange materials are generally amenable to SG films except for those fabricated by EAP. With EAP, adapting methods for inclusion of catalysts requires consideration of whether the catalyst will affect the deposition of the SG and whether it will be stable under the deposition conditions. A particularly promising means of forming such films by EAP is to co-deposit metal nanoparticles [74]. Other routes to doping SG films with metal nanoparticles have demonstrated the catalytic activity of such a composite [75]. Immobilization of catalysts within templated pores is an option meriting more investigation. In this regard, the size and distribution of pores in these films comprise a grid for deposition of arrays of nanoparticles. With films that are sufficiently thin and non-tortuous, so that the term $(2Dt)^{1/2}$ exceeds the thickness at a given experiment time, t , the pores will have the characteristic a nanoelectrode array at which currents limited by hemispherical diffusion can be

obtained. This mass transport limit enhances the ratio of Faradaic to capacitive current relative that of a linear diffusion system.

The exposed electrode surface at the base of these pores is amenable to modification with catalysts by methods used on conventional electrodes provided that the chemistry involved does not perturb the SG surface. In this manner, layer-by-layer electrostatic assembly may be a route to immobilizing a wide range of catalysts, including enzymes, but this application requires elimination of the negative surface charge on silica and related sol–gel materials. The use of ormosils, including the coating of a TEOS-derived sol–gel with a layer of organically modified silane [76], is one method to make the SG hydrophobic. An approach we are presently investigating is to methylate the negative sites of templated SG films derived from TMOS by EAP (JA Cox, unpublished results).

Thin films of other oxides such as alumina have been fabricated with controlled thickness and pore size (e.g., thickness, 500 nm; pore size, variable over the range 20–150 nm [77]). The resulting electrode arrays have been used for a variety of fundamental and applied studies, particularly where these honeycomb materials were used as supports for lipid bilayers. Applications include investigations such as studies of lateral mass and charge transport and of enzyme immobilization for electrochemical reactions [78–80]. Platforms made by sol–gel processing are a promising alternative. They have already been used extensively in conjunction with lipid bilayers, including the measurement of lateral diffusion [81]. The degree of control of porosity now attainable with SGs and the ability to interrogate these materials by electrochemistry in combination with optical techniques suggest that investigations employing lipid bilayers on templated SGs will increase in scope.

An important question is: will the incorporation of catalytic, conducting centers into SG films obviate the need for size-exclusion pores to mitigate passivation? In this regard, SGs, especially with hydrophobicity tuned by use of ormosils or by end-capping, are less subject to adsorption than are traditional electrode surfaces. In addition, catalysts for oxidation of organic compounds can change the course of electrode reactions involving coupled chemical reactions so that products that are not adsorbed are obtained [82, 83]; an example is conversion of a disulfide into a sulfenic acid at a bismuth-doped lead oxide electrode [83]. Future studies can be envisioned in which the outer surface of doped SG films without templated pores serves as the plane at which the electron-transfer reaction occurs and adsorption of neither the product of said reaction nor the targeted species is observed. Inclusion of nanoparticles in the SG is perhaps a route to the design of such films.

The primary applications of catalytic, size-exclusion SGs are expected to be in the area of electroanalytical measure-

ments; however, electrodes modified with these materials are potentially applicable to any electrolytic or galvanic reaction in hostile media. Moreover, the ability to change the pathway of chemical reactions coupled to electron transfer may find applications to electrosynthesis. Finally, other methods of fabricating grids with controlled dimensions are experiencing increased utilization. Deposition of block copolymers followed by dissolution of one of the units and printing various geometries by electrochemistry at the tips of imaging systems such as atomic force microscopes are examples of methods that are anticipated to become more important in the near future.

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