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Oxidation and flow-injection amperometric determination of 5-hydroxytryptophan at an electrode modified by electrochemically assisted deposition of a sol–gel film with templated nanoscale pores

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ABSTRACT

The oxidation of 5-hydroxytryptophan (5-HTPP) yielded a passivating polymeric film at an indium tin oxide (ITO) electrode. Coating ITO with a nanoscale sol–gel film with a mesoporous structure was shown to change the pathway of the chemical reaction coupled to the electron transfer. The sol–gel film was deposited by an electrochemically assisted process, and the mesoporosity was imparted by including generation-4 poly(amidoamine) dendrimer in the precursor solution. The dendrimer was removed subsequently with an atmospheric oxygen plasma. This electrode remained active during cyclic voltammetry and controlled potential electrolysis of 5-HTPP, which was attributed to dimer, rather than polymer, formation from the oxidation product. Mass spectrometry confirmed this hypothesis. The anodic current was limited by the electron-transfer kinetics. Modification of the sol–gel film by inclusion of cobalt hexacyanoferrate, which catalyzes the oxidation, resulted in a diffusion-limited current. Determination of 5-HTPP by flow-injection amperometry had a detection limit of 17 nM.

1. Introduction

Electroanalytical measurements in real-world samples are often hindered or precluded by adsorption of matrix components, of the analyte and/or of the products of the electron-transfer reaction. In the common case of passivation by matrix components, an approach to circumventing this limitation is to modify the electrode with a film that is permeable to the analyte but blocks transport of concomitants. Perhaps the first report of this electrode design was by Sittampalam and Wilson [1] who used hydrolyzed cellulose acetate (CA) as the barrier in an amperometric method for the determination of hydrogen peroxide in a complex matrix. Here, the pore size permitted facile diffusion of the analyte but was smaller than the size of the surface-active matrix components. The use of CA as the electrode modifier has several limitations including inability to dope the material with a catalyst, inexact control of pore size and film thickness, and limited physical stability. To overcome such limitations of CA and the explore other potential attributes of nanoporous domains for electrochemical reactions, we are investigating the modification of electrodes with nanometer-scale, mesoporous sol–gel films [2].

The method for preparing sol–gel films in the present study is based on recent reports of their electrochemically assisted formation. A common sol–gel process consists of the hydrolysis, condensation and polycondensation of a tetraalkylorthosilicate to form microporous or mesoporous silica. This process is initiated and sustained by changing the near-neutral pH of a stable precursor sol to an acidic or basic value at which sol–gel processing is catalyzed. Conventional sol–gel processing is used to produce a variety of geometries such as monoliths and micron-scale cast or spin-coated films. In electrochemically assisted methods [3–6], electrolysis of water in quiescent, unbuffered sol generates an acidic (at positive potentials) or basic (at negative potentials) environment exclusively at the electrode–liquid interface. Mandler and co-workers [4–6] initiated this process by applying a potential sufficient to electrolyze water to an electrode immersed in an electrolyte that contained a sol–gel precursor, zirconium-tetra-n-propoxide [5]; a typical electrolysis time was 15 min. The film thickness was varied intentionally over the range from tens of nanometers to ca. 1000 nm. The thickness achieved depended on such factors as the applied potential, the nature of the electrode material, and the water content of the supporting electrolyte. In concert, these factors varied the rate of change of interfacial pH that, in turn, controls the rates of condensation and of polycondensation. The sol–gel films prepared in this manner are highly resistive; therefore, they are well-suited to corrosion protection.

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A variation of the procedure, namely the inclusion of β-cyclodextrin (CD) in the precursor solution, yielded porous films that maintained the electrochemical activity of the substrate [2]. An indium tin oxide (ITO) electrode was coated with silica by applying 2.35 V versus a Pt quasi-reference electrode for 30 min in a solution comprising 2-propanol as the bulk liquid-phase, lithium perchlorate as the supporting electrolyte, water to serve as the proton source upon electrolysis, CD as the templating agent, and tetraethylorthosilicate (TEOS) as the sol–gel precursor. The resulting film-coated ITO electrode was withdrawn from the plating bath at 50 μm min^{-1} to provide controlled drainage of the liquid from the electrode surface. The vertical stacking of CD on the electrode [7] resulted in the formation of a sol–gel film with controlled porosity. For the voltammetric determination of phospholipids, the composite of CD and sol–gel was doped with bis(acetate)dirhodium-11-molybdophosphate, which is an oxidation catalyst [8], by inclusion of this complex in the precursor solution. At this modified electrode, the oxidation of phosphatidylcholine (PC) was observed by cyclic voltammetry, and the anodic current persisted during repeated potential scans. In contrast, at bare ITO the adsorption of PC passivated the electrode. Evidence was that the oxidation of a test species, 1.0 mM ferrocene, was blocked when 100 μM PC was present [2].

Walcarius et al. [3] described an approach to electrochemically assisted deposition of sol–gels that yielded nm-scale mesoporous films with pores perpendicular to the electrode surface. The procedure combined base-catalyzed sol–gel processing of tetraethylorthosilicate (the pH was locally increased at the electrode surface by electrochemical reduction of hydronium and water in unbuffered solution) with orientation of channels in cetyltrimethylammonium bromide (CTAB) assembled in hemimicelles at the electrode surface by the electric field. The films were characterized after dissolution of the CTAB. They exhibited high pore density (75,000 pores μm^{-2}) and pore volume. The diffusion coefficient of ferrocene ethanol, 3.5×10^{-6} cm^2 s^{-1}, in these films demonstrated its facile mobility therein.

Pore size of a sol–gel matrix has been shown to influence the pathway of electrochemical reactions performed therein. The electrochemical oxidation of an aniline as a dopant in a microporous silica sol–gel [9] and in a mesoporous organically modified sol–gel [10] was investigated. The primary product was a dimer in the former and a polymer in the latter, which is a result consistent with a restriction of the size of the product of a chemical reaction following electron transfer in a microporous domain. The sol–gel matrix can affect reaction pathways by means other than pore size. For example, UO_2^+ formed by the electrochemical reduction of UO_2^{2+} in a silica sol–gel monolith rapidly disproportionated whereas UO_2^+ was stable when the reduction UO_2^{2+} was at an electrode immersed in aqueous solution [11]. Catalysis of the disproportionation reaction was related to the ion-exchange reaction between the UO_2^+ and the negative sites of the silica. Interactions with sol–gel matrices influence other types of reactions as well. For example, hydrogen bonding of the starting compound with silanol sites catalyzed the photochemical conversion of *trans*-4-methoxy-4′-(2-hydroxyethoxy)-azobenzene to the *cis* configuration [12].

The present study is an extension of preliminary work on the analytical utility of sol–gel films with controlled porosity as electrode modifiers. The initial study employed films that were doped with a redox mediator (a Rh^{II} complex) and had pores templated with CD, which has a toroid structure with a 7-nm cavity. Because the pores were smaller than the size of the analyte, the mediated oxidation occurred at the interface of the Rh^{II}-doped sol–gel film and the electrolyte solution that contained the analyte [2]. This study focused on an analyte that was small enough to diffuse through the pores to the electrode surface. The test species that was selected, 5-hydroxytryptophan (5-HTPP), had been shown previously to undergo chemical oxidation by a pathway that was influenced by pore size of the matrix [13]. When the 5-HTPP was a component of a microporous silica sol–gel monolith, chemical oxidation resulted in dione formation whereas dimer was formed in mesoporous silica. The voltammetry of 5-HTPP in a silica sol–gel monolith was investigated at a boron-doped diamond electrode [14]; however, passivation of the electrode precluded generation of sufficient product for identification. The gradual passivation of the working electrode surface during the oxidation of 5-HTPP was mitigated by the use of a catalyst such as a Ru^{II}metallodendrimer incorporated into a carbon composite [15]. For the present study, cobalt hexacyanoferrate (CoHCF) was selected because it has been reported to catalyze the oxidation of substrates related to 5-HTPP, including tryptophan, and it is convenient to prepare at the surface of electrodes [16].

Because our previous study showed a difference between microporous and mesoporous silica matrices in terms of the pathway of 5-HTPP oxidation [13], amine-terminated generation 4-polyamidoamine dendrimer (PAMAM), which has a calculated sphere diameter of 4.5 nm [17], was used rather than CD to template the pore size. An additional reason for the choice of PAMAM was that these dendrimers have a diameter that depends on generation in a known manner [18], so successful fabrication of porous sol–gel films in the present study can be applied to future investigations on the role of pore size on the voltammetric behavior of a variety of analytes.

2. Material and methods

Potassium ferricyanide, potassium chloride, monobasic potassium phosphate, dibasic potassium phosphate, cobalt chloride, ethanol, acetonitrile, sodium nitrate, and hydrochloric acid were ACS Reagent Grade from Fisher Scientific (Fair Lawn, NJ). The 5-HTPP was from Sigma (St. Louis, MO). The following were obtained from Aldrich (Milwaukee, WI): tetraethylorthosilicate (TEOS), amine-terminated generation 4-polyamidoamine (PAMAM), 98% purity; α-cyano-4-hydroxycinnamic acid (CHCA), 97% purity; and CTAB. Water used in this study was house-distilled that was further purified with a Barnstead NANO pure II system.

Electrochemical experiments were performed on CH Instruments (Austin, TX) Models 400, 660B or 800 electrochemical workstations. The working electrode was indium tin oxide (ITO) from Delta Technologies (Stillwater, MN). The ITO was rinsed with ethanol, dried under nitrogen, and cut into squares with 1.5 cm edges prior to use as electrodes. Using a rubber o-ring, a 0.32 cm^2 portion of this ITO was isolated as the working electrode. All potentials were measured and reported against an Ag|AgCl, 3 M KCl reference electrode (Bioanalytical Systems, West Lafayette, IN). The counter electrode was platinum gauze. The matrix-assisted laser desorption/ionization mass spectrometry (MALDI) experiments were performed with a Bruker Ultraflex III instrument with a time-of-flight mass analyzer (Billerica, MA).

The flow-injection amperometry (FIA) experiments were performed with a system comprised of a 30-mL syringe pump (Pharmaseal Laboratories, Glendale, CA), Rhyodyne 7010loop injector, and a thin layer electrochemical cell (Bioanalytical Systems, West Lafayette, IN). The cell with a Pt auxiliary electrode and a Ag|AgCl reference electrode was modified to incorporate a sol–gel coated ITO (0.32 cm^2) working electrode.

Mesoporous silica sol–gel films were deposited on the ITO electrodes under potentiostatic conditions adapted from Walcarius et al. [3]. A typical precursor solution consisted of 13.6 mmol TEOS, 10 mL ethanol, 10 mL 0.1 M $NaNO_3$ (aqueous), 1.0 mM PAMAM, and 1.0 mM HCl (aqueous), to which 4.35 mmol CTAB was added while stirring. The pH was adjusted to 3.0 with dilute HCl. The