Preparation and Properties of Polybenzidine Film-Coated Electrode as an H_2O_2 Selective Polymeric Material

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ABSTRACT: By means of electrochemical polymerization, polybenzidine-modified electrodes were prepared in an aqueous monomer solution at a potential of 0.7 V versus Ag/AgCl. The permselective character of the polybenzidine electrode prepared in a one-step procedure was examined for electroactive (ascorbic acid, oxalic acid, and hydrogen peroxide) and nonelectroactive (lactose, sucrose, and urea) species. Influence of the various parameters on the permselective properties of the polybenzidine membrane was systematically investigated and the optimal values for these parameters were determined. It has been found that polybenzidine membrane showed selective permeation for hydrogen peroxide while blocking the permeation of electroactive and nonelectroactive interferents through film. In brief, it is claimed that this polybenzidine film can be used as a coating material to prevent interferences in electrochemical biosensor applications. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci 70: 2227–2234, 1998

Key words: polybenzidine film; permselective membrane; selective permeation

INTRODUCTION

In recent years, a number of articles concerning the preparation of polymer-modified electrodes by electrochemical polymerization of a variety of compounds have been reported. These polymeric electrodes have been successfully used in various application areas, such as gas sensors,[1–5] batteries,[6,7] corrosion protection,[8] biosensors,[9–15] and permselectivity.[16–19] The polymeric film used as a coating material in the biosensor and/or modified electrode construction must be selective to the related substrate while preventing the permeation through the film of the electroactive or non electroactive species.

In oxidase or peroxidase based biosensors, the measured hydrogen peroxide concentration is proportional to the substrate concentration. In biological liquids, where the substrate is being measured, some electroactive (oxalic acid, ascorbic acid, etc.) and nonelectroactive (lactose, sucrose, and urea) substances are also present. The former interferes with the measured signal, while the latter fouls the electrode surface.

Chemical and morphological characteristics and film thickness, especially in the case of nonconducting polymers, are the most important factors affecting the permselectivity of the polymeric film obtained. Electropolymerization is a suitable method to control film thickness by altering the amount of charge consumed during polymerization.

D'Eramo et al.[20] showed that polymerization of benzidine (4,4′-diaminobiphenyl) involves N—N coupling or C—N coupling in the *ortho* position of the aromatic ring. However, the exact mechanism of the polymerization has not yet been fully elucidated.

In the study presented here we report on the electrochemical synthesis, optimization, and investigation of the permselectivity of the polybenzidine film for some electroactive and nonelectro-

active substances such as ascorbic acid, oxalic acid, hydrogen peroxide, lactose, sucrose, and urea.

EXPERIMENTAL

Reagents

Benzidine as the monomer was supplied from Fluka and used as received. All the other chemicals, such as ascorbic acid, oxalic acid, lactose, sucrose, urea, and KCl were of analytical grade and purchased from Sigma Chemical Company (St. Louis, MO) or E. Merck (Darmstadt, Germany). All the experiments, except for electrochemical polymerization, were run in a PBS (phosphate buffer salts, pH = 7.0) solution. Ascorbic acid solutions used in the permselectivity measurements were prepared immediately before use. In polymerization, nitrogen-saturated aqueous KCl solution was used as supporting electrolyte. Deionized and doubly distilled water was used in the preparation of all the electrolyte solutions.

Instrumentation

For the electrochemical polymerization, cyclic voltammetry, linear sweep voltammetry, and potentiostatic measurements, a BAS 100 W (Bioanalytical Systems, Inc.) electrochemical analyzer was used.

All electrochemical operations were carried out with a standard three-electrode electrochemical cell consisting of a bare or polybenzidine-coated Pt electrode (geometric area, 2.01 mm^2) as the working electrode (BAS, MF-2013), together with a Ag/AgCl reference electrode (BAS, MF-2063) and a Pt wire coil auxiliary electrode. A Jenway 3010 pH-meter was used to perform the pH measurements.

Electropolymerization

Prior to electropolymerization, platinum disc electrodes, to be used as the working electrode, were cleaned according to the standard procedure[21] and polished with successively finer grades of diamond polishing compounds and aqueous alumina slurry (Johnson Matthey Catalog Comp., USA) down to 1.5 μm.

The aqueous electropolymerization solution consisting of 2 mM benzidine and 0.1 M KCl was degassed with high purity nitrogen for 10 min, and the electrolysis solution was blanketed with

Figure 1   Cyclic voltammograms of the bare Pt electrode in 0.1 M KCl (A) and 0.1 M KCl + 2 mM benzidine (B). Scan rate: 50 mV/s.

the same gas during polymerization. Polybenzidine films were electrochemically synthesized at a potential of 0.7 V versus Ag/AgCl.

Polybenzidine films with various thicknesses were prepared by monitoring the charge consumed during polymerization. Polymer coated electrodes were removed from the electrolysis solution and rinsed with pure water. Visual examination revealed the formation of thin and homogenous films of dark-blue color on the electrode surface.

Voltammetric Measurements

Linear sweep voltammetry was used for the determination of electrooxidation potential of the hydrogen peroxide on the polymer coated electrode. For the permselectivity experiments, the responses to the electroactive and nonelectroactive test substances on the polybenzidine-coated electrodes were measured using a cell of three electrodes, all immersed in a PBS solution.

During the amperometric measurements, the PBS solution was kept under gentle stirring at room temperature. Before the injection of the test substances into the PBS solution, the background current was allowed to decay to a steady state, which took 5 min at most. The change in anodic current by the injection of the test substances was then recorded as a function of time.

RESULTS AND DISCUSSION

In the preliminary experiments, seeking for a suitable electropolymerization potential of the

Figure 2 Linear sweep voltammograms of the polybenzidine electrode in PBS (A) and PBS + 25 mM H_2O_2 (B). Scan rate: 50 mV/s.

benzidine in aqueous KCl solution, cyclic voltammograms on the bare Pt electrode in the absence and presence of the monomer were recorded. As shown in Figure 1, there was an anodic peak around 0.7 V, which seemed to decrease in magnitude and shift to higher po-

Figure 3 The effect of film thickness on the H_2O_2 response of the polybenzidine electrode.

Figure 4 The behavior of ascorbic acid on the polybenzidine (A) and bare electrode (B). Each step corresponds to 1 m*M* ascorbic acid.

tential values on the subsequent cycle. This behavior is typical for electrochemically grown nonconducting polymeric films. Electropolymerization potential for benzidine was chosen as 0.7 V, so as to secure thin polymeric films at a slow electrolysis rate. Thus, a period of ca. 25 ± 2 min were required for a passage of typical charge of 2.0 mC.

Figure 5 The behavior of oxalic acid on the polybenzidine (A) and bare electrode (B). Each step corresponds to 1 m*M* oxalic acid.

Figure 6 The effect of monomer concentration on H_2O_2 response of the polybenzidine electrode.

To determine the oxidation potential of electro-active hydrogen peroxide in the PBS solution, linear sweep voltammograms, in the absence and presence of hydrogen peroxide on the polybenzidine-modified electrode, are depicted in Figure 2.

In the region of hydrogen peroxide oxidation, 0.7 V was chosen as the working potential mainly for two reasons: (1) with the polymer electrode a working potential higher than the electropolymerization potential is customarily avoided be-

Figure 7 Effect of pH on H_2O_2 response of the polybenzidine-coated electrode.

Figure 8 The permselectivity of the optimized polybenzidine electrode. (Each injection corresponds to 1 mM.) At 600th second ascorbic acid, at 700th second oxalic acid, starting 800th second hydrogen peroxide injections were made at every 100th second.

cause of possible overoxidation of the polymeric film, and (2) the use of higher potentials widens the spectrum of interferents that can be encountered in the biological liquids.

Permselectivity and the Effect of Film Thickness of Polybenzidine

The effect of the film thickness on the hydrogen peroxide response was investigated. As indicated in Figure 3, the amperometric response obtained for the hydrogen peroxide increased with increasing film thickness and reached a maximum value when about 2 mC charge was passed; thereafter, response progressively decreased with the increasing film thickness. Therefore, it was accepted that films that were prepared with a charge of 2 mC had an optimal thickness and were used for the later interference studies.

To examine permselectivity characteristics of the polybenzidine-modified electrode to ascorbic acid and oxalic acid, steady-state amperometric responses to the mentioned interferents on the bare Pt and polybenzidine electrode of optimal thickness are illustrated in Figures 4 and 5. From these figures, it can be seen that the polybenzidine-coated electrode was selective for hydrogen peroxide, meanwhile preventing unwanted re-

sponses from the electroactive ascorbic acid and oxalic acid.

Effect of Monomer Concentration

Monomer concentration determines the rate of polymerization, which in turn affects the morphology and permeability character of the polymeric film. Figure 6 indicates the effect of monomer (benzidine) concentration on the hydrogen peroxide response at the polybenzidine electrodes prepared with the same charge. As shown in this figure, amperometric response increased with increasing benzidine concentration up to 1.5 mM, after which a trend of decrease was observed. Thus, optimal benzidine concentration was chosen as 1.5 mM.

pH Effect

The effect of the pH of PBS solution on the hydrogen peroxide response at the polybenzidine-coated electrode was examined over the range of pH 5.0–9.0. From Figure 7 it is seen that polybenzidine electrode has a relatively higher response in the neutral region.

After the optimization of parameters (charge, monomer concentration, and the pH of measure-

Figure 9 The permselectivity of the optimized polybenzidine electrode. (Each injection corresponds to 1 m*M*.) At 500th second lactose, at 600th second sucrose, at 700th second urea, starting 800th second hydrogen peroxide injections were made at every 100th second.

ment solution) to provide the highest response for hydrogen peroxide, permselectivity of the optimized polybenzidine-modified electrode was tested once again. Steady-state amperometric responses to successive injections of the test substances are shown for the electroactive species in Figure 8, and for the nonelectroactive species in Figure 9.

As expected, it was found that polybenzidine-coated electrode responded to only hydrogen peroxide injections among the electroactive substances examined. Moreover, it was seen that these hydrogen peroxide responses were not affected in the presence of the fouling substances.

CONCLUSION

It has been demonstrated that a polybenzidine film electrode can be prepared by electrochemical polymerization of the benzidine in aqueous solution. After optimization of the experimental parameters affecting film properties, it was found that the optimized polymeric membrane prevented common interferents while allowing hydrogen peroxide permeation through the film.

As a result, it can be claimed that this permselective polymeric electrode can be used as an inner and/or protective membrane to deal with the interference problems in the hydrogen peroxide based biosensor construction.

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