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Surface renewable sol–gel composite electrode derived from 3-aminopropyl trimethoxy silane with covalently immobilized thionin

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Abstract

Sol–gel technique has been used for the covalent immobilization of the water-soluble mediator, thionin to construct a bulk modified, leak free composite electrode. This renewable composite electrode provides stable immobilization matrix for thionin via glutaraldehyde crosslinking. In the electrode composition the sol–gel precursor 3-aminopropyltrimethoxy silane serves as the host for immobilization of thionin, thereby preventing its leakage. An additional precursor methyl trimethoxy silane endows hydrophobicity and limits the wetting section of the modified electrode. Cyclic voltammetric characterization of the modified electrode in the potential range of 0.2 to −0.6 V exhibited stable redox peaks with a formal potential of −0.273 V, corresponding to immobilized thionin. This chemically modified electrode exhibits good electrocatalytic activity for the reduction of H_2O_2 at a lower potential of −0.35 V. The reduction current of the modified electrode increases linearly in the range of 3.44×10^{-6} M to 3.07×10^{-3} M H_2O_2 with a detection limit of 1.38×10^{-6} M. The stable and quick response (5 s) during chronoamperometry shows the potential application of the modified electrode for flow system analysis. The low potential operation (−0.35 V) favoured selective determination of H_2O_2. The composite electrode exhibits distinct advantages of polishing in the event of surface fouling as well as simple preparation, good chemical and mechanical stability, economical and remarkable long-term stability (more than 1 year). The applicability of the present sensor for H_2O_2 determination proposes a method for the detection of other biologically significant analytes.

Keywords: Thionin; Sol–gel composite; Covalent immobilization; Hydrogen peroxide; Cyclic voltammetry

1. Introduction

Determination of H_2O_2 using reliable methods is of great importance in the fields of food, clinical, pharmaceutical, biological, industrial and environmental analyses. Conventional methods for the determination of H_2O_2, including titrimetry (Hurdis and Romeyn, 1954), fluorimetry (Genfa et al., 1991), spectrophotometry (Lobnik and Cajlakovic, 2001), chemiluminescence (Rocha et al., 2005) often suffer from complicated methods and expensive equipments. Electrochemical methods surpass these defects with advantage of simplicity, selectivity and sensitivity (Lin et al., 2005; Tseng et al., 2005). The direct electrochemical determination of H_2O_2 is very difficult at most of the conventional electrode surfaces. Often detection of H_2O_2 by oxidation at higher potentials suffers from the interferences due to easily oxidisable species, resulting in inaccuracy in determination. Biosensors have also been used as a sensitive way of detecting H_2O_2, but these sensors are costly and lack long-term stability due to its inherent instability (Ruzgas et al., 1996; Miao and Tan, 2000; Chaubey and Malhotra, 2002). Chemically Modified Electrodes (CMEs) using mediators have emerged as an effective alternative for H_2O_2 sensor development (Garjonyte and Malinauskas, 1998; Wang et al., 2004). Direct electroanalysis of several species encounters numerous problems such as high overpotential, fouling of the electrode surface, irreversibility and interference of other electroactive species. Hence a great deal of work has been devoted to the design of CMEs since they display significant improvements compared to the unmodified electrodes by combining the intrinsic properties of the modifier to a selected redox process (Walcarius, 2001). CMEs have

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demonstrated improvements in the electron transfer reactions of the compounds at the electrode (Zen et al., 2003).

A wide range of electrode modification processes has been proposed aiming at the sensitivity, selectivity and stability during particular application (Nalini and Sriman Narayanan, 2000; Deepa and Sriman Narayanan, 2001; Ravi Shankaran and Sriman Narayanan, 2002). Among these sol–gel method of designing electrochemical sensors has become attractive due to the ease of preparation of solids displaying diversity in terms of chemical composition, structure and tailor made properties. Sol–gel process provides an alternative route for the analytical chemists to tailor supporting matrices with the required versatility and flexibility (Alber and Cox, 1997; Collinson, 1998; Walcarius, 1998; Tess and Cox, 1999; Wang, 1999). Immobilization of different mediators and reagents by sol–gel route, in the chemically inert inorganic host has proven to be a simple way to incorporate recognition species effectively (Lev et al., 1997).

Lev et al. launched a valuable route for the preparation of stable host matrix by the introduction of new class of sol–gel carbon-ceramic electrodes (Tsionsky et al., 1994). These ceramic composite electrodes (CCEs) possess the advantages of simplicity of fabrication, tunability of physical characteristics, surface renewability, mechanical rigidity, porosity, chemical and thermal stability which makes them particularly attractive for electrochemical sensor development. The potential advantage of using this type of electrodes is that, they allow bulk modification of the entire electrode material with additives such as mediators, activators or other components that would increase the stability, amplitude and selectivity of the response signal. In contrast to the conventional electrode counterparts such as solid platinum, gold or glassy carbon that allow only surface modification, a new active electrode surface is obtained by simple polishing or cutting. The active section of the electrode is not clogged upon repeated polishing due to the brittleness of the sol–gel silicate backbone and the active section of the electrode can be renewed by a simple mechanical polish after every use or contamination (Lev et al., 1995; Pankratov and Lev, 1995; Gun and Lev, 1996; Wang et al., 1996).

The simple doping of the modifiers during sol–gel processing would have a concomitant detrimental effect on the electrochemical response while developing sensors for in vivo or environmental analysis. The main problem associated with such sensors is the difficulty to maintain the long-term stability, since the electrochemical activity of the modified electrode gradually decreases due to the detachment and dissolution of the mediator from the electrode. This problem can be solved by covalent linking of the mediator with sol–gel precursors. Bifunctional sol–gel precursors such as (3-aminopropyl) trimethoxy silane and (3-mercaptopropyl) trimethoxy silane offers the additional advantage of surface modification due to the presence of free amino and thiol groups that could be used as the binding site for covalent attachment (Ravi Shankaran et al., 2002; Salimi et al., 2003). These functional sol–gel precursors facilitate the construction of sensing materials with possible control over the selective modification, orientation and distribution of catalytic sites (Jin and Brennan, 2002; Shen et al., 2005).

In the present work, chemically modified thionin ceramic composite electrode was constructed by sol–gel route. The modified electrode is comprised of percolating graphite powder for conduction and 3-aminopropyltrimethoxy silane for the covalent immobilization of the thionin mediator and methyl trimethoxy silane for controlling the hydrophilic/hydrophobic nature of the composite and both these silanes serves as binder to construct a surface renewable, rigid and porous composite. The covalently modified mediator acts as the electrocatalyst for the reduction of H_2O_2. The analytical applications of the modified electrode as an amperometric sensor for H_2O_2 under both static and dynamic conditions were verified.

2. Experimental

2.1. Chemicals and reagents

3-Aminopropyl trimethoxy silane (APTMOS), methyl trimethoxy silane (MTMOS), graphite powder (1–2 μm) were products of Aldrich and used for the preparation of composite electrode. Thionin (TH) was purchased from Himedia. Glutaraldehyde (25%) was received from s.d. fine chemicals. A 50% H_2O_2 solution obtained from Merck was diluted freshly everyday and used. The exact concentration of H_2O_2 was obtained by titration with standard potassium permanganate solution. All other chemicals were of analytical grade and used as received. Aqueous solutions were made up with doubly distilled water.

2.2. Electrochemical measurements

Electrochemical instrumentation was a CHI 400 A Electrochemical Analyzer (CH Instruments, USA). A three-electrode configuration consisting of thionin immobilized ceramic composite electrode (TH/CCE) as working electrode, saturated calomel electrode (SCE) as reference electrode and Pt wire as counter electrode was used. Cyclic voltammetric experiments were performed in a static supporting electrolyte solution. For amperometric measurements a magnetic stirrer and a stirring bar provided a convective environment. All potentials were measured and reported with respect to SCE. pH measurements were made with an Elico pH meter (model LI 120). All solutions were deoxygenated by bubbling high purity N_2 for 15 min prior to experiments.

2.3. Procedure

2.3.1. Construction of thionin immobilized ceramic composite electrode (TH/CCE)

The sol–gel mixture was prepared by mixing immediately 0.6 ml MTMOS, 0.4 ml APTMOS, 0.6 ml MeOH and 0.02 ml of HCl (10 mM) for 5 min. The ratio of MTMOS to APTMOS was optimized to this value to maintain the desirable hydrophobicity/hydrophilicity. To the resulting sol–gel mixture added 0.04 ml of glutaraldehyde (25%) and shaken thoroughly for 30 min to allow the terminal amino group of APTMOS to react with the bifunctional crosslinker. Then added 28 mg of thionin to the above mixture and continued shaking for another