

Application of SiO₂–poly(dimethylsiloxane) hybrid material in the fabrication of amperometric biosensor

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Abstract

Silica xerogel was widely used in the development of biosensors by coupling the desired biological components to various transducers. Unfortunately, the application of xerogels is limited due to their poor mechanical properties and poor structural maintenance of entrapped biomaterials. In this study, the availability of poly(dimethylsiloxane) (PDMS)-modified silica sol–gel (TEOS/PDMS Ormosil) glass in the immobilization of enzymes and its application in the fabrication of amperometric biosensor was investigated. We demonstrated that most of activity of encapsulated horseradish peroxidase can be preserved in PDMS-modified SiO₂ glass compared with conventional silica xerogel. The synthesized monoliths exhibited high transparency and crack-free. The enzyme electrode based on glucose oxidase encapsulated TEOS/PDMS Ormosil glass on Pd electrode was fabricated and characterized electrochemically. The characteristics of the biosensor were studied by cyclic voltammetry and chronoamperometry. The biosensor exhibited a series of good properties: high sensitivity (1.63 μA mM⁻¹ analyte), short response time and high reproducibility. The results indicate that TEOS/PDMS Ormosil glass has great potential to the immobilization of biomaterials as well as the fabrication of biosensors. © 2006 Elsevier B.V. All rights reserved.

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1. Introduction

The entrapment of enzymes in a porous silica sol–gel has attracted a great deal of interest because of its potential for use in industry [1–3]. Silica sol–gel technology offers several advantages, such as processing under mild conditions, ability to form nanoparticles/thin-films/monoliths [4–6] and chemical inertness [7], to the immobilization of biomolecules. The formation of the silica mesoporous matrix enables entrapped enzymes to retain sufficient activity and stability and allows the reactant molecules to easily reach the entrapped enzymes. Moreover, the optically transparent nature of the silica sol–gel glass not only ensures that the biological characteristics of entrapped enzymes can be easily studied [8], but also makes it suited for the fabrication of optical biosensors.

Although silica sol–gel glass exhibits several advantages over other materials to the immobilization of biomolecules, the brit-

tleness is the major drawback that limits its applications in the industry [9]. To eliminate this disadvantage several methods, such as utilize surfactant to protect the sol–gel film [10], generate a sol–gel derived ceramic-carbon composite material [11] and generate a sol–gel derived organic-inorganic hybrid material [12], are proposed to make use of its advantages. The sol–gel derived organic-inorganic hybrid material is the organic modification of sol–gel matrix by using organoalkoxysilane modifiers. Take the advantage of sol–gel process the organoalkoxysilane can be readily incorporated in the silicate network to produce organically modified ceramic matrixes (Ormosils). The incorporation of organoalkoxysiloxanes in the silicate matrix reduces the degree of cross-linking, improve film adhesion to its supports and enhance the mechanical properties of sol–gel matrix. Moreover, additional functional groups can be introduced into silica matrix for anchoring of sensing elements or bio-components [12,13].

Poly(dimethylsiloxane) (PDMS) processes specific properties like transparency, good chemical and thermal stability and similarity of its backbone structure (–Si–O–) to that of tetraethylsiloxane (TEOS, an inorganic precursor for SiO₂) [14–19].

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The mechanical properties of Ormosil are suggested to be improved by the incorporation of PDMS into SiO₂ network [14–19]. Moreover, PDMS-modified silica sol–gel glass attracts widespread interests due to its applications as a gain media in laser materials, non-linear optical materials, chemical sensors, pH sensors and protein immobilization [20–24]. In this work, we further show that PDMS-modified silica sol–gel glass has a great potential in the protein immobilization and the fabrication of amperometric biosensors.

2. Experimental

2.1. Materials

2,2'-Azino-bis(3-ethylbenzthiazolin-6-sulfonate (ABTS), diaminobenzidine (DAB), tetraethylorthosilicate (TEOS), silanol-terminated polydimethylsiloxane (PDMS, MW approximately 1700 g mol⁻¹), horseradish peroxidase (HRP, 116 U mg⁻¹) and glucose oxidase (GOx, EC1.1.3.4, 240 U mg⁻¹) were purchased from Sigma. Pd-electrode was obtained from Roach. Hydrogen peroxide (30% H₂O₂), buffers and salts were obtained from Merck (Darmstadt, Germany). All other reagents and chemicals were analytical grade, and purchased from Sigma. Aqueous solutions were prepared in doubly distilled water.

2.2. Apparatus

Amperometric and cyclic voltammetric experiments were performed with an Electrochemical Analyzer (CHI-400). All experiments were carried out with a conventional three-electrode system with the enzyme electrode as the working electrode and a standard platinum electrode as the auxiliary electrode. All the potentials given here were relative to an Ag/AgCl (saturated KCl) reference electrode. A 10 mL electrochemical cell and a magnetic stirring bar were used. All experiments were conducted in ambient conditions at approximately 25 °C.

2.3. Preparation of organically modified sol–gel glass

The TEOS sol was prepared by mixing 6.6 mL TEOS, 4.9 mL H₂O and 0.75 mL 0.1 M hydrochloric acid at room temperature and stirring for at least 30 min. The TEOS/PDMS sol was prepared by mixing TEOS sol and PDMS in a ratio of 3:1 (v/v) [14]. The condensation was initiated by adding 0.32 mL 2 M Tris–HCl buffer (pH 9) and 0.3 mL HRP stock solution (1 mg mL⁻¹) into the resulting hydrolyzed TEOS or TEOS/PDMS sol and mixed gently at room temperature. The final concentration of doped HRP was 2 U mL⁻¹. The resulting HRP/sol mixture (30 μL each) was then added into each well of a microtiter plate to form aerogel disk or spot on the glass substrate. Following aging and drying for 24 h, aerogels were then stored at 4 °C or under the room temperature. For activity assay, the HRP-entrapped SiO₂ glass and PDMS-modified silica sol–gel glass (TEOS/PDMS Ormosil) were pre-warmed and re-hydrated under the room temperature for 5 min before each experiment. The microscopic images were obtained by monitoring the glass substrate contain-

ing HRP-doped SiO₂ and TEOS/PDMS Ormosil disks under a differential interference contrast (DIC) microscope (Olympus).

2.4. Preparation of enzyme electrode

The Pd electrode was rinsed with doubly distilled water before each experiment. To prepare GOx-immobilized composite electrode 0.3 mL GOx stock solution (1 mg mL⁻¹) and 0.32 mL 2 M Tris–HCl buffer (pH 9) were added into the TEOS/PDMS sol and mixed gently at room temperature. Total GOx encapsulated in TEOS/PDMS Ormosil was 120 U. The resulting GOx-TEOS/PDMS sol (5 μL) was then spread on the surface of the Pd-electrode and allowed to dry and age at 4 °C for at least 24 h.

2.5. Activity assay

The activity of entrapped HRP in silica sol–gel glass or TEOS/PDMS Ormosil was measured colorimetrically in the presence of ABTS or DAB as the substrate. A reaction solution that contained 2.9 mM H₂O₂ and 0.5 mM ABTS or 2 mg mL⁻¹ DAB in 100 mM sodium acetate (pH 5.0) was prepared. The activity assay of HRP in aerogel was conducted by adding reaction mixture (100 μL) into each well of the microtiter plate and incubating it at room temperature for 5 min. The reaction was determined by measuring absorbance at 425 nm. The activity assay of HRP-doped SiO₂ sol–gel glass or TEOS/PDMS Ormosil disks on the glass substrate was performed by laying 20 μL 2 mg mL⁻¹ DAB in pH 7.4 phosphate buffer on top of each disk at room temperature for 10 min to allow brownish dye to deposit on the spot.

3. Results and discussion

3.1. Effect of PDMS on the mechanical property of silica sol–gel glass

Although silica sol–gel has been extensively used in the protein encapsulation and immobilization, this material can be brittle, may undergo cracking owing to hydration stresses. The usage of organic-inorganic composite material may overcome this problem. PDMS, an organic modifier, has been used as a glassy matrix to dope organic dyes for the preparation of laser materials, non-linear optical materials, luminescence solar concentrator and protein immobilization [20–26]. Its application in the preparation of enzyme-encapsulated silica sol–gel, however, has not been studied. This work wants to address the effect of PDMS on the mechanical property of silica sol–gel and on the stability of entrapped enzymes. To prepare a TEOS/PDMS Ormosil without cracking and is suited for protein immobilization various ratios of TEOS and PDMS were tested. We found that more than 25% (v/v) PDMS might be required to prevent TEOS/PDMS Ormosil from cracking (data not shown). This result is similar to the observation of Mackenzie and co-workers [14–19], by which the Ormosil containing PDMS (over 20 wt%) may present a rubbery property, called “ceramic rubber”. Thus, the TEOS/PDMS sol was prepared by mixing TEOS sol and

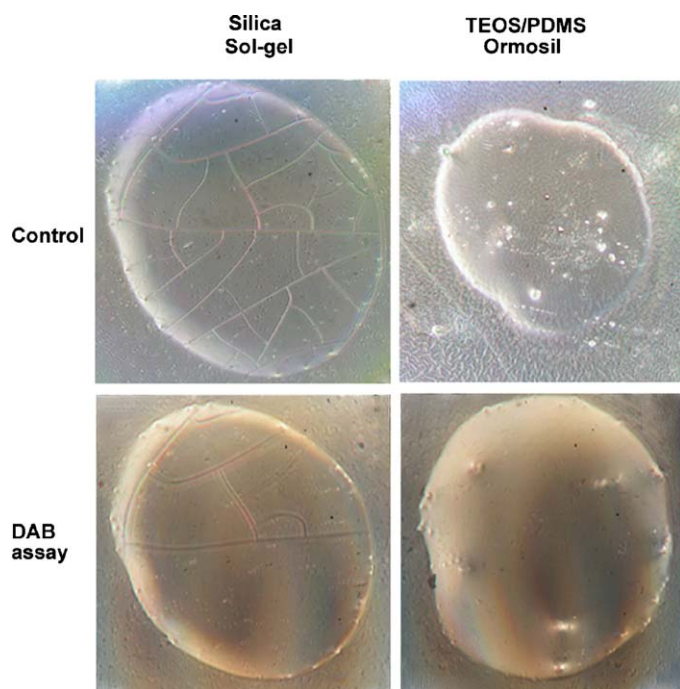


Fig. 1. The microscopic images of silica sol-gel and TEOS/PDMS Ormosil glasses on the surface of the glass plate. The activity of HRP encapsulated in silica matrix with or without the modification of PDMS was tested by laying reaction solution (20 μL) containing 2 mg mL⁻¹ DAB and 2.9 mM H₂O₂ in sodium acetate buffer, pH 5.0.

PDMS in a ratio of 3:1 (v/v) [14]. As shown in Fig. 1, the SiO₂ matrix cracked during aging even stored at 4 °C. In contrast, the surface of TEOS/PDMS Ormosil remained smooth without any cracking even after activity assay. This result suggests that the mechanical property of original SiO₂ matrix is enhanced by the incorporation of PDMS. Furthermore, both silica sol-gel glass and TEOS/PDMS Ormosil exhibited the ability to entrap HRP and retain its activity as suggested by the brownish dye deposit in both samples (Fig. 1).

3.2. Stability of enzyme entrapped in TEOS/PDMS Ormosil

The ability of TEOS/PDMS Ormosil to stabilize entrapped enzyme was tested in this study. The activity of HPR entrapped in silica matrix with or without organic modifiers after storage was determined by colorimetric method. Since glycerol was usually used to stabilize proteins in aqueous solution, we also want to know whether it can enhance the stability of entrapped protein in silica matrix. As shown in Fig. 2, following 24 h of drying and aging, the activity of the entrapped HRP declined rapidly in 9 days in silica matrix without organic modifiers no matter what temperature (4 °C or 25 °C) they were stored. A similar result was also observed in silica sol-gel encapsulated glucose oxidase (GOx) [12]. About 75% activity of SiO₂-encapsulated GOx was lost after storage at 4 °C for just 1 day. It has been shown that, following gelation, the initial gels are probably soft and contained lots of water (50–80%) and large pores (up to 200 nm in diameter) [27], in which the entrapped proteins might be unaltered and hence reserved their native structure and most

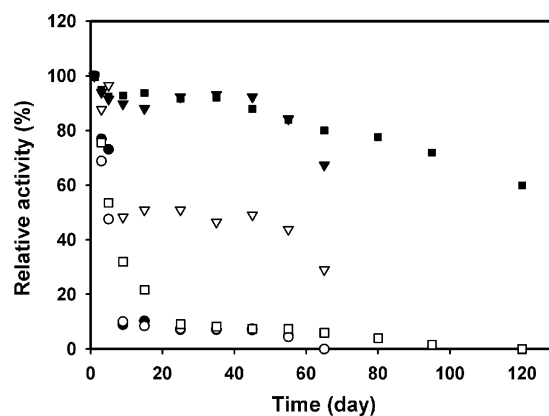


Fig. 2. Stability of HRP encapsulated in conventional silica sol-gel glass and in TEOS/PDMS Ormosil matrix. The HRP-silica sol-gel discs without (● and ○) or with 5% glycerol (▼ and □) and TEOS/PDMS Ormosil (■ and □) were stored at 4 °C (●, ▼ and ■) or 25 °C (○, ▽ and □) and assayed to determine their remnant activity at the specified times. The results were means of data obtained from three independent experiments.

of their activity. However, after aging over a period of days to weeks a further cross-linking of silica matrix takes place and strengthens the silica network [27,28]. Although silica matrix is a highly porous material, extensive cross-linking within matrix may constrain the conformation of the entrapped enzymes and reduce their activity.

Alternatively, following aging and drying, most of interstitial water will be removed, leading to protein denaturation presumably due to the reduction of hydrophobic effect [29] or simply due to protein-silica interactions [30]. The hydrophobic effect is known to be a major factor in stabilizing the three-dimensional structure of protein [29,31]. It is possible that the silica interface surrounding proteins may reorganize the remaining water molecules into a structure unfavorable the maintenance of the hydrophobic effect on protein folding [23,24,32]. The alteration of hydrophobic effect could probably be prevented by the presence of hydrophilic sugars or polyols [29]. This postulation was partially demonstrated by the result observed on the HPR-entrapped silica sol-gel containing 5% glycerol (Fig. 2). At least 50% activity of entrapped HRP could be retained in the silica matrix containing 5% glycerol at 25 °C in air; whereas, at 4 °C, as much as 90% activity of entrapped HRP could be retained for about 45 days before a rapid activity decrease (Fig. 2). This result indicates that glycerol is useful in stabilizing the activity of enzymes entrapped in silica matrix.

Interestingly, more than 80% activity of entrapped HRP in TEOS/PDMS Ormosil could be retained at 4 °C for at least 80 days in air (Fig. 2). Even about 60% activity of entrapped HRP could be preserved after 120 days of storage in air. However, when stored at 25 °C, the activity of entrapped HRP in TEOS/PDMS Ormosil also declined about 90% in 9 days. This result suggests that, as an organic modifier, PDMS can greatly enhance the stability of entrapped proteins at low temperature in silica matrix. One way for PDMS to prevent protein denaturation to occur is probably to relief constrain on the structure of entrapped proteins by reducing the level of cross-linking in silica matrix. On the other hand, PDMS contains hydrophobic

methyl groups on its backbone, which may elevate hydrophobicity within the hydrophilic silica matrix. A slightly increase in hydrophobicity within a hydrophilic silica matrix may support a high kinetic barrier that prevents the unfolding of proteins from their native conformation [33–36]. The results from this and other studies [22–24] suggest that TEOS/PDMS Ormosil can be a potential protein immobilizing material in many industrial applications, especially in the fabrication of biosensors.

3.3. Electrochemical response of enzyme electrode

Ormosils have been widely used in the fabrication of biosensors due to their high porosity, physical rigidity, chemical inertness, low biodegradation and high thermal stability [37–42]. In previous experiments, we have demonstrated that TEOS/PDMS Ormosil was suited to immobilize HRP. Its application in the fabrication of biosensors has not been studied. Therefore, an enzyme electrode with GOx-TEOS/PDMS Ormosil composite was prepared and its electrochemical responses were tested by cyclic voltammetry. Cyclic voltammograms of enzyme electrode at scan rate 25 mV s^{-1} were recorded in blank buffer solution (Fig. 3, curve b) and also in buffer solution containing various concentrations of glucose (Fig. 3, curves c–e). In the absence of glucose, only the background current was observed. A clear anodic current appeared, when glucose was added. This anodic current corresponds to the electrochemical oxidation of H_2O_2 generated by GOx on the enzyme electrode. With an increase in the concentration of glucose, the anodic current increased, indicating the maintenance of biocatalytic activity of GOx within the TEOS/PDMS Ormosil matrix. This result suggests that TEOS/PDMS Ormosil is suitable for the fabrication of biosensors.

3.4. Characterization of enzyme electrode

The pH dependence of the enzyme electrode was investigated over the pH range 5.0–8.5 in 50 mM phosphate buffer in

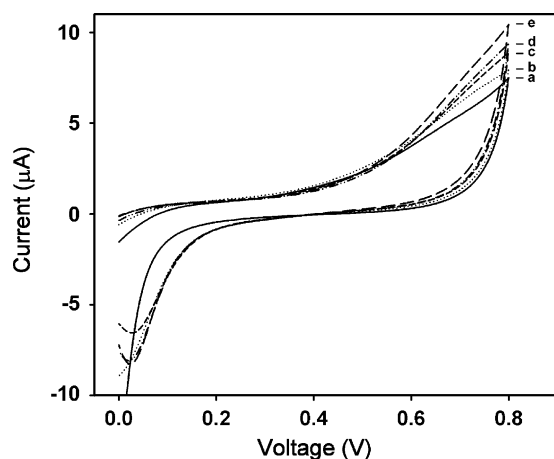


Fig. 3. Cyclic voltammetry of unmodified Pd-electrode (a) and GOx-TEOS/PDMS Ormosil composite electrode in the absence (b) and the presence of 1 mM (c), 5 mM (d) and 10 mM glucose (e) in 0.05 M phosphate buffer, pH 7.4 at the scan rate of 25 mV s^{-1} .

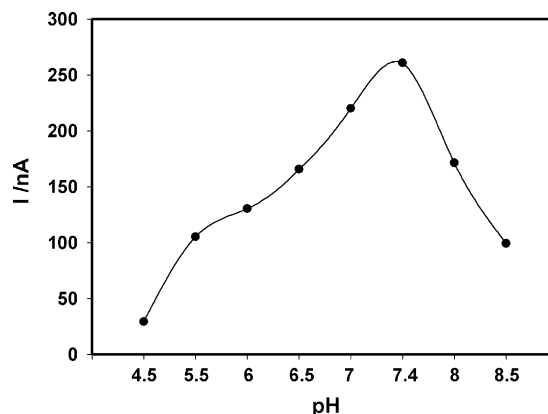


Fig. 4. Effect of pH on the electrochemical responses of TEOS/PDMS Ormosil-derived GOx electrode. Reactivity of GOx was studied amperometrically with $50 \mu\text{M}$ glucose in 0.05 M phosphate buffer, pH 7.4. Working potential was 0.5 V vs. Ag/AgCl.

the presence of $50 \mu\text{M}$ glucose with a working potential at 0.5 V versus Ag/AgCl (Fig. 4). The optimum biosensor response is achieved in the pH range 7.0–7.4. Lowering or increasing the pH resulted in a decrease of its biocatalytic activity. This result is in agreement with the optimum pH of 7.0–7.4 for SiO_2 immobilized GOx [43,44] and similar to that of 6.5–7.0 for free glucose oxidase [45]. Therefore, pH 7.4 was selected for the subsequent studies since the greatest response has been obtained at this pH.

A current–time plot of the enzyme electrode on successive step changes of glucose concentration is illustrated in Fig. 5. The enzyme electrode could reach a steady-state current within 25 s. This result is similar to the reported results for GOx electrodes based on pure silica sol–gel glass [46–48] and the hydrogel [49], but better than the reported results for GOx electrodes encapsulated in other Ormosils and pure silica sol–gel [36–38,50]. A typical calibration graph of the steady-state current versus glucose concentration was determined. The linear range spans the concentration of glucose from 0.5 to 50 mM with a correlation coefficient of 0.990, while the lowest glucose concentration can be detected is $50 \mu\text{M}$ (61.9 nA). With this dynamic range, the TESO/PDMS Ormosil-based GOx electrode exhibits a potential to be used clinically in determining glucose in serum with a physiological concentration of around 5.5 mM.

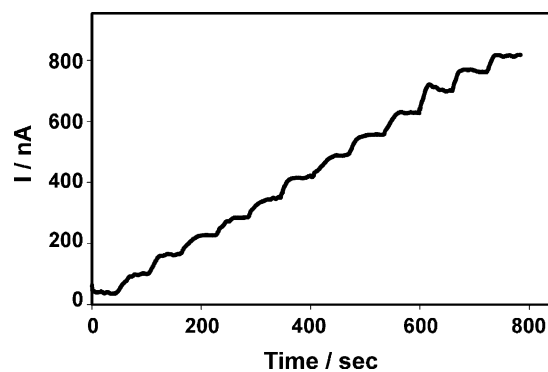


Fig. 5. Typical steady-state current response of the Ormosil-derived GOx electrode with successive addition of $50 \mu\text{M}$ glucose. The electrochemical responses were determined in 0.05 M phosphate buffer, pH 7.4 with a working potential of 0.5 V vs. Ag/AgCl.

The reproducibility of the biosensors was also studied using a repetitive operation mode under room temperature. The electrode reproducibility was estimated from the response to 100 μM glucose after at least thirty enzyme reactions. A mean current response of $163 \pm 5 \text{ nA} (100 \mu\text{M})^{-1}$ was determined with a relative standard deviation of 3%. Although the porosity of PDMS/TEOS Ormosil was not investigated directly in this study, it was represented indirectly through the study of the leakage of encapsulated HRP in Ormosil by a repetitive activity assay. The result shows that more than 95% activity of the encapsulated HRP remained even after 30 runs of activity assay. This study suggests that the prepared PDMS/TEOS Ormosil is a porous structure that allows small molecules to freely diffuse in and out of the matrix without losing the encapsulated HRP during the activity assay. Furthermore, the TEOS/PDMS Ormosil-derived GOx electrode exhibits a good operational reproducibility, suggesting the capability of being used in a flow injection analysis system. Further study showed that the TEOS/PDMS Ormosil-derived GOx electrode remained stable at 4 °C for at least 2 months (data not shown).

4. Conclusion

In this work, we demonstrated that TEOS/PDMS Ormosil could be a potential organic–inorganic hybrid material for protein immobilization as well as in the fabrication of biosensors. This hybrid material is proved to be useful for the protein immobilization, because it cannot only prevent the cracking of conventional silica sol–gel glass, but also maintain most of the activity of encapsulated enzymes for at least 80 days. Furthermore, the potential of TEOS/PDMS Ormosil in the fabrication of amperometric biosensor was also demonstrated in this work. The resulting amperometric biosensor exhibits high sensitivity, high reproducibility and long term stability, which compares favorably to that fabricated with conventional silica or other organic–inorganic hybrid materials. This TEOS/PDMS Ormosil preparation strategy can be readily expanded to the immobilization of other biomaterials as well as the fabrication of various types of biosensors.

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