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Effect of GLYMO/TEOS/MTEOS Sol-Gel Film Thickness on the Responses of Glucose Biosensor

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Abstract: In the present study, the effect of the film thickness on the responses of a glucose biosensor was investigated. The modified platinum electrodes used in the study were constructed by immobilization of glucose oxidase under a film layer of the sol-gel coating solution. The sol-gel coating solutions were prepared by using (3-Glycidoxylpropyl) trimethoxysilane (GLYMO), tetraethoxysilane (TEOS), and methyltriethoxysilane (MTEOS). Electrochemical measurements were carried out amperometrically by determining hydrogen peroxide released by the enzymatic reaction between glucose and glucose oxidase. It was found that the amperometric responses of the sensor decreased with increasing the volume of coating solution dropped on the adsorbed enzyme. It was determined that the film thickness has a significantly influence on the sensor responses. It was observed that the amperometric responses decreased by 85% when the volume of the coating solution dropped on the electrode increased from 4 µL to 12 µL.

Keywords: Biosensor; glucose; glucose oxidase; sol-gel.

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INTRODUCTION

The routine analysis of glucose in various physiological fluids is one of the most frequent operations in a clinical chemical laboratory. The convenient, rapid, safe and precise determination of blood sugar in diabetes patients is important for the treatment and control of diabetes. Glucose can be detected by various methods, such as electrochemical, colorimetric, and optical methods. Among these methods, enzyme-based electrochemical biosensors are widely used for the determination of glucose. They may be divided into conductometric, potentiometric, and amperometric biosensors depending upon the electrochemical property to be measured by detector system. The amperometric biosensors are more attractive than the others due to their high sensitivity and wide linear range. Enzymatic detection of glucose by an amperometric biosensor is based on the monitoring of hydrogen peroxide formed by the enzymatic reaction between glucose and glucose oxidase. The resulting current changes due to the oxidation of hydrogen peroxide on the modified working electrode were measured as a function of time (1-4).

The most important subject in the development of an enzyme-based amperometric glucose biosensor is the immobilization of glucose oxidase on the surface of the working electrode. A number of immobilization techniques, such as physical entrapment, chemical immobilization in an inert matrix, and covalent attachment to electrode surfaces have been used to attach the relevant enzyme in the construction of the amperometric biosensors. Among the various modification procedures, the sol-gel technology has attracted wide spread interest to immobilize the biomolecules in the design of the biosensor due to its distinct advantages, such as low temperature requirement, chemical inertness, negligible swelling, optical transparency, low-temperature encapsulation, tunable porosity, thermal stability, and biocompability (5-7).

The sol-gel process is a chemical synthesis method used in the preparation of glass and ceramics, thin films and coatings, fine powders, fibers and some others. This method is based on the hydrolysis and condensation reactions of liquid precursors to produce a stable gel. The sol-gel method can be also practiced to prepare the hybrid coating by using organic-inorganic silane compounds. Organic-inorganic composite materials prepared by this method have many application fields, like surface coating, corrosion protection and electrode modification. These coatings have been successfully performed as enzyme immobilization matrix due to their biocompatibility (5, 8). Several papers on the immobilization of glucose oxidase within the sol-gel matrix for the preparation of glucose biosensors have been found in the literature. In these studies, different silane compounds have been practiced as the immobilization matrix to improve the stability, selectivity, reproducibility and other analytical parameters of the biosensor (9-11).

In this study, the effect of the film thickness on the responses of the glucose biosensor prepared by immobilization of glucose oxidase on the platinum electrode surface using the sol-gel films has been reported. The sol-gel film was prepared by mixing of (3-glycidoxylpropyl)trimethoxysilane, tetraethoxysilane and methyltriethoxysilane precursors.

MATERIAL AND METHODS

The sol-gel coating solution was prepared by mixing 1 mL of (3-Glycidoxylpropyl)trimethoxysilane (GLYMO, 98%, Aldrich), 0.4 mL of tetraethoxysilane (TEOS, 98%, Aldrich), 0.4 mL of methyltriethoxysilane (MTEOS, 99%, Aldrich), and the calculated amount of distilled water in a glass vial. A calculated aliquot of concentrated HCl (37%, Riedel-de-Haën) solution was added to the mixture to accelerate hydrolysis of the silanes. The mixture in glass vial was stirred until a clear and homogeneous solution was obtained and stored at room temperature for 24 h. This solution was used as the stock sol solution. Then, a coating solution was prepared by mixing 1 mL of the stock sol solution and 3 mL of 2-butoxyethanol (Aldrich) in a separate glass vial. This final solution was stirred for 2-3 h and stored at room temperature for 24 h. The solution diluted with alcohol was used for the immobilization of glucose oxidase.

The enzyme solution was prepared by dissolving 5.1 mg of glucose oxidase (Aspergillus Niger, Sigma) in 50 µL of 0.1 M phosphate buffer solution (pH=7) at room temperature.

Phosphate buffer solution (PBS) was prepared by using disodium hydrogen phosphate and potassium dihydrogen phosphate. The glucose (α-D-(+) glucose, Sigma) stock solution (0.2 M) was prepared in distilled water and left at room temperature for 24 h prior to use to ensure the presence of β-D-glucose form.

The platinum electrode was chosen as working electrode for the preparation of the sol-gel modified glucose biosensor. A volume of 2 µL of the enzyme solution was dropped on the cleaned platinum electrode surface (2 mm diameter) and allowed to dry at room temperature. After that, the coating solutions at different volumes to obtain the varied film thickness were carefully dropped on the enzyme adsorbed onto the surface of the platinum electrode and allowed to dry at room temperature.

Electroanalytical measurements were carried out with a BAS 100 W (Bionalytical Systems, Inc.) electrochemical analyzer. All experiments were performed by using a conventional electrochemical cell with a three-electrode system, comprising a modified platinum electrode as the working electrode, an Ag/AgCl electrode saturated with KCl as the reference electrode, and a Pt wire coil as the auxiliary electrode.

Phosphate buffer solutions (PBS) used in the amperometric studies were aerated by bubbling air for about 20 min prior to use. Then, three-electrode system was immersed into 10 mL of PBS solution. The solution was stirred by using a magnetic bar to provide the convective mass transport during the experiments. A predetermined constant working potential versus Ag/AgCl was applied to the cell, and the background current was allowed to reach the steady state before glucose injections. The resulting amperometric response due to the oxidation of hydrogen peroxide formed by the enzymatic reaction was measured as a function of time, and the graphs of the current versus time were continuously recorded.

RESULTS AND DISCUSSIONS

The responses of a biosensor are affected from various parameters, such as film thickness, amount of enzyme, compound of coating solution, pH of solution, applied potential, and presence of interfering species. The film thickness is probably the most important one among these parameters because the species to be measured reaches the electrode surface by diffusing through the film. Therefore, the amount of substance diffused through the film varies depending upon whether the film is thick or thin. At the same time, the film porosity affects the response of sensor.

In a biosensor, enzyme is generally contact with electrochemical transducer, which converts an observed change into a measurable signal. A coating layer is located onto the enzyme layer, which is the recognition element of biosensor, to immobilize the enzyme. The substrate is diffused from the sample solution to the recognition element passing through the coating layer, and where an enzymatic reaction occurs between the substrate and enzyme. The product formed as a result of the enzymatic reaction is transformed by transducer the evaluable signal associated with the amount of the product. Thus, the analyte concentration in the sample solution is indirectly detected by biosensor. Consequently, it can be said that the determination of a substrate concentration by a biosensor is based mainly on a sequential two-step process including diffusion and chemical reactions. In here, the diffusion step is directly related with the thickness of the coating layer or film on the enzyme.

The overall reaction between glucose and glucose oxidase in a glucose biosensor can be written as follows:

$$Glucose + O_2 \xrightarrow{glucose\ oxidase} Gluconic\ acid + H_2O_2 \quad (1)$$

Because the glucose does not directly measure electrochemically, the amount of glucose in the sample solution can be indirectly determined by monitoring of the consumed oxygen, the produced gluconic acid or the released hydrogen peroxide. In the amperometric determination of glucose, the monitoring of hydrogen peroxide has the advantage of being simpler, especially

when miniaturized devices are concerned. Hydrogen peroxide measurements are commonly carried out on a platinum electrode at an oxidation potential of around 0.6-0.7 V versus Ag/AgCl reference electrode, and the alterations in the anodic current are recorded (in Eq. 2).

$$H_2O_2 \xrightarrow{Pt} O_2 + 2H^+ + 2e^- \qquad (2)H_2O_2 \xrightarrow{Pt} O_2 + 2H^+ + 2e^- \qquad (2)$$

It is related to the glucose concentration whichever the chemical species mentioned above is measured. Thereby, the glucose concentration determined is proportional with the glucose amount that can pass through the coating layer. Accordingly, the film or coating layer thickness has a very important effect on the response of biosensor.

In this work, the enzyme electrodes have been prepared by dropping the sol-gel coating solution at volumes of 4, 5, 6, 7, 8, 9, 10, 11, and 12 µL on the enzyme layer adsorbed onto the platinum electrode. The results of the electrochemical tests performed using these electrodes are given in Fig. 1. In this figure, only the experimental results of the electrodes prepared by dropping aliquots of 4, 5, 6, 7, 8, and 9 µL of the sol-gel solution have been illustrated. Each injection indicated in Fig. 1 corresponds to 2 mM concentration of the glucose. For all the enzymatic electrodes obtained by dropping in the range of 4-12 µL of the sol-gel solution, the amperometric current responses for a total concentration of 10 mM glucose at the end of five injections have been shown in Table 1. A graph of the values of the amperometric responses versus each volume of the coating solution given in Table 1 are plotted in Fig. 2. As can be seen from Figs. 1-2, and Table 1, the amperometric responses of the enzymatic electrodes were decreased with increasing the amount of the sol-gel coating solution dropped on the electrodes. It was determined that the amperometric response decreased by 85% when the volume of the coating solution dropped on the electrode increased from 4 µL to 12 µL. As can be seen from Fig. 1, the responses of the enzymatic electrodes are high up to 7 µL of the sol-gel coating solution, however they are irregular responses. The similar responses to each other have been observed for 5, 6, and 7 µL of the films. The responses taken from the enzymatic electrode prepared with 8 µL of the coating solution are more uniform, and the similar results have been obtained for the successive measurements performed using this electrode. The recorded responses from the electrodes prepared beginning from 9 µL of the coating solution have been increasingly diminished. The amount of glucose passed through the pores of the resulting sol-gel film is high when the thin film is in question. As a consequence, the biosensor responses become large due to the high amount of hydrogen peroxide formed after enzymatic reaction. As the film becomes thicker, the amperometric responses of sensor decrease significantly because of a decrease in glucose diffusion through the thick sol-gel film. The thinner films may appear advantageous in terms of the sensor responses. But, the selectivity of an amperometric glucose biosensor can affect adversely from the application of

thin films because some electroactive species (e.g., ascorbic acid, uric acid, and oxalic acid) together with glucose in the real biological samples is present.

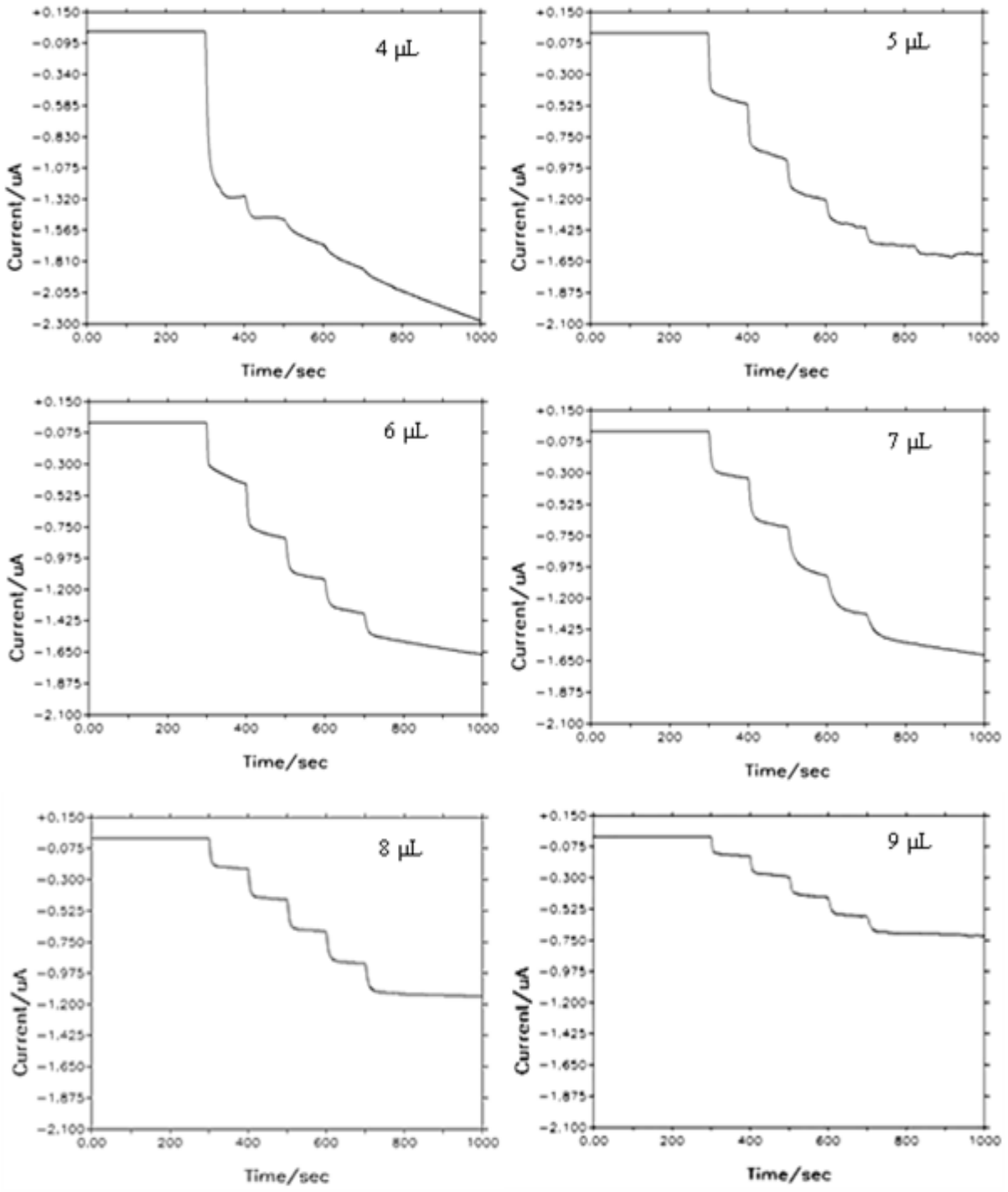

Figure 1: The amperometric responses of enzyme electrodes prepared by dropping the coating solution at different volumes.

Table 1: The values of amperometric current responses of enzyme electrodes for a total concentration of 10 mM glucose.

Volume of Coating Solution, µL	Response, µA
4	2,28
5	1,60
6	1,62
7	1,61
8	1,09
9	0,72
10	0,58
11	0,49
12	0,34

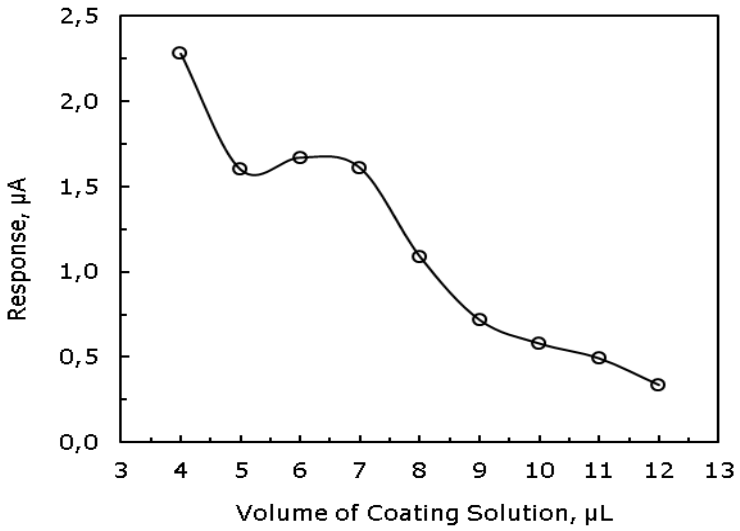

Figure 2: The graph of amperometric current responses of enzyme electrodes for a total concentration of 10 mM glucose at various volumes of coating solution.

These species can oxidize on the electrode surface by passing through the sol-gel film and can interfere to the current responses of the released hydrogen peroxide by enzymatic reaction. In addition to this, the leaching of enzyme through the pores of the thin sol-gel films can take place from the electrode surface into the sample solution. These negativities may overcome by using the thicker sol-gel films. But in this case, the mass transfer from the sample solution to the enzyme under the film layer becomes difficult and the sensor responses decrease. When the thicker sol-gel films are used, the diffusion pathway can lengthen and the pores of the film can block. Hence, the whole glucose injected may not pass from the thick sol-gel film, and ultimately the amperometric responses of glucose sensor decrease significantly.

CONCLUSIONS

In this study, the effect of the amount of the sol-gel coating solution on the responses of the glucose biosensor has been examined. The enzymatic electrodes were produced by entrapping under a silica sol-gel film of the glucose oxidase adsorbed on the platinum electrode surface. The silica sol-gel coating solutions have been prepared by using GLYMO, TEOS, and MTEOS. It was observed that the amperometric responses decreased with increasing the volume of coating solution dropped on the adsorbed enzyme. A decrease in the amperometric responses can be attributed that the diffusion of glucose through the resulting thick sol-gel films becomes difficult. According to the experimental findings determined, it can be said that the film thickness or the amount of the coating solution dropped on the electrode has a significantly influence on the sensor responses. The experimental findings showed that the current responses were decreased by 85% when the volume of the coating solution dropped on the electrode increased from 4 µL to 12 µL.

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