Scientific paper

# Immobilization of Glucose Oxidase in GLYMO/MTEOS Sol-Gel Film for Glucose Biosensor Application

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## Abstract

An electrochemical biosensor was developed by using the sol-gel coating solution. The modified platinum electrode used in the study was constructed by immobilization of glucose oxidase under a layer of sol-gel film. The coating solution was prepared by using GLYMO, and MTEOS. Electrochemical measurements were carried out amperometrically by determining hydrogen peroxide produced by the enzymatic reaction between glucose and glucose oxidase. It was observed that the amperometric response of the enzyme electrode was linear for glucose concentrations in the range from 2 to10 mM. The response time of the biosensor to reach a steady-state current value was approximately 80 s. The glucose selectivity of the biosensor was determined in the presence of some interfering substances, such as lactose, sucrose, urea, uric acid, oxalic acid, and ascorbic acid.It was observed that the interfering molecules did not cause any observable signal. The stability of the sensor was investigated, and it was found hat the initial sensor response decreased approximately 44% over a period of 30 days of storage in dry state at 4 °C.

**Keywords:** Biosensor, Glucose, Glucose oxidase, GLYMO, MTEOS, Sol-gel

## 1. 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. Depending upon the electrochemical property to be measured by a detection system, electrochemical biosensors may be divided into amperometric, conductometric, and potentiometric biosensors. An amperometric biosensor may be the best approach to achieve accurate, specific, economic, and rapid monitoring of glucose. 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. It measures the resulting current changes on the modified working electrode due to direct oxidation of the products of the biochemical reaction.[1–9]

The key factor in the development of an enzyme-based amperometric glucose biosensor is the immobilization of glucose oxidase on the electrode surface. 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 immobilize the relevant enzyme in the construction of the amperometric biosensors.[8–12] Among the various modification procedures, 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 preparation, chemical inertness, negligible swelling, optical transparency, low-temperature encapsulation, tunable porosity, thermal stability, and biocompability.[13–15]

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.[16–18] This method is based on the hydrolysis and condensation reactions of liquid precursors to create a stable gel.[19] The sol-gel method is also used to obtain the composite materials. Organic-inorganic composite materials prepared by this method, which is termed ormosils, have many appli-

cation fields, like surface coating, corrosion protection and electrode modification.[20] Hybrid films prepared with organic-inorganic silanes via the sol-gel process are a type of composite material in which the inorganic and organic components are combined at the molecular level. The organic part of the hybrid material improves the adhesion between the coating and substrate while the inorganic part maintains the hardness and chemical durability of the coating. These coatings have been successfully used as enzyme immobilization matrix due to their biocompatibility. They can be readily prepared by hydrolysis and condensation of alkoxysilanes and organoalkoxysilanes.[21–24]

MTEOS and GLYMO are organoalkoxysilane and organofunctional alkoxysilane monomers, respectively, and they can be used to prepare the organic-inorganic hybrid films. MTEOS contains methyl, which is a non-reactive organic group. The entry of methyl group into the hybrid network leads to minor cross-linking. This group can act as filler in the silica network and result in small and narrow pore size distribution. In addition, MTEOS can bring hydrophobicity to the film by replacing the surface hydroxyl groups with methyl groups, which have a lower affinity towards water. GLYMO is essentially used as a binding agent in the silica coatings. It has a reactive functional (epoxy) organic group. The epoxy ring of GLYMO can be opened in acidic or basic conditions and highly adhesive films can be obtained by increasing the degree of cross-linking of the hybrid network. The organo functional group of GLYMO can react with amino group of biomolecules, and hence it can be used for immobilization of enzymes. The coating solutions containing GLYMO and MTEOS can form mechanically stable sol-gel thin films by ensuring a good chemical bonding to the electrode surface. A porous film can be obtained with a mixture of GLYMO/MTEOS, and the porosity of the film can be easily adjusted by changing the composition of the coating solution. The prepared films by using these silane compounds for biosensor applications can improve the long-term performance of the sensor.[25–29]

Several papers on the immobilization of glucose oxidase within the sol-gel matrix for the development of glucose biosensors have been reported in the literature. In these studies, various silane compounds have been used in the immobilization matrix to improve the stability, selectivity, reproducibility and other analytical parameters of the biosensor.[2, 21, 30–37]

This work reports a glucose biosensor prepared by immobilization of glucose oxidase with the silica sol-gel film on the platinum electrode surface. The sol-gel layer was prepared by mixing of (3-glycidoxylpropyl)trimethoxysilane and methyltriethoxysilane precursors. Electrochemical measurements were carried out amperometrically. The optimal values of the working potential and pH of buffer solution were determined. The electrochemical characteristics of the biosensor were investigated.

# 2. Experimental

## 2. 1. Chemicals and Solutions

(3-Glycidoxylpropyl)trimethoxysilane (GLYMO, 98%), methyltriethoxysilane (MTEOS, 99%), and 2-butoxyethanol were supplied from Aldrich. α-D-(+) glucose and glucose oxidase (GOx) from *Aspergillus Niger* were purchased from Sigma. HCl (37%) was obtained from Riedel-de-Haën. Double distilled water was used throughout the preparation and dilution of all solutions.

Phosphate buffer solution was prepared by using disodium hydrogen phosphate and potassium dihydrogen phosphate. The glucose 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 the β-D-glucose form.

## 2. 2. Preparation of Silica Sol-gel Solution

The sol-gel coating solution was prepared by mixing 1 mL of GLYMO, 0.4 mL of MTEOS, and 0.505 mL $H_2O$ in a glass vial. A 0.044 mL aliquot of concentrated HCl solution was added to the obtained mixture to accelerate hydrolysis of the silanes. The mixture in the 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 a stock solution. Then, a coating solution was prepared by mixing 1 mL of the stock sol solution and 3 mL of 2-butoxyethanol 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 the enzyme.

## 2. 3. Preparation of Enzyme Electrode

The enzyme solution was prepared by dissolving 5.1 mg of enzyme in 50 μL of 0.1 M PBS solution (pH = 7). A volume of 2 μL of this enzyme solution was dropped on the platinum electrode surface (2 mm diameter) and allowed to dry at room temperature for 30 min. After that, aliquots of 7 μL of the solution diluted with alcohol were carefully dropped on the enzyme adsorbed onto the surface of the platinum electrode and allowed to dry at room temperature for 48 h. The resulting enzyme biosensor was stored at 4 °C in a refrigerator when not in use.

## 2. 4. Methods and Instruments

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, a Ag/AgCl electrode saturated with KCl as the reference electrode, and a Pt wire coil as the auxiliary electrode.

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

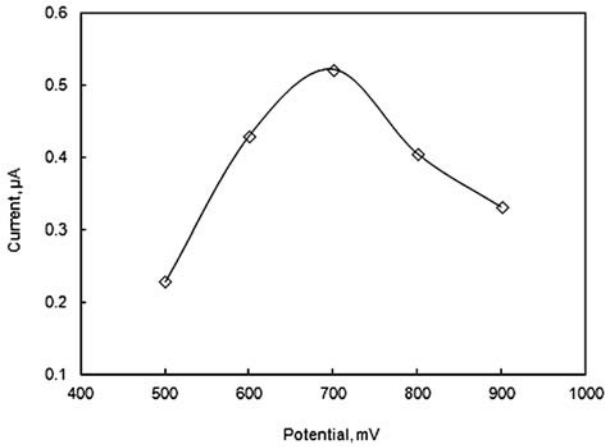


**Figure 2.** Effect of working potential on the sensor response.

# 3. Results and Discussion

## 3. 1. Effect of pH of Buffer Solution

The pH of the buffer solution has a very important effect on the sensitivity of the biosensor because the pH affects the bioactivity of glucose oxidase. Therefore, to determine the effect of buffer solution pH on the response of the biosensor, experiments were carried out by measuring the current response of the sensor to 10 mM glucose at different pH values at 700 mV. Figure 1 shows the effect of pH on the biosensor responses.As can be seen, the enzyme electrode exhibited a large response to glucose injections at pH 7. To obtain the maximum sensitivity of the biosensor, the optimal pH value of PBS was selected to be 7.

## 3. 2. Effect of Working Potential

The effect of the applied potential on the biosensor response was examined in the potential range from 500 to 900 mV versus the Ag/AgCl reference electrode. These experiments were performed by monitoring the response of the enzyme electrode to 10 mM glucose at pH 7. Figure 2 shows the effect of the working potential on the amperometric response of the biosensor. It can be seen that a maximum current response to glucose injections was obtained at a potential of 700 mV. To reach the highest current response in the amperometric measurement, the working potential was selected to be 700 mV.

## 3. 3. Electrochemical Characteristics of Biosensor

After determining the optimal values for the working potential and pH of the buffer solution, the electrochemical characteristics of the prepared biosensor, such as response time, linearity, selectivity, and stability, were investigated under the optimized experimental conditions.

Figure 3 shows a plot of the typical amperometric response of the silica/GOx electrode to the addition of aliquots of stock glucose solution. The glucose concentration for each injection was 2 mM. It can be seen that the

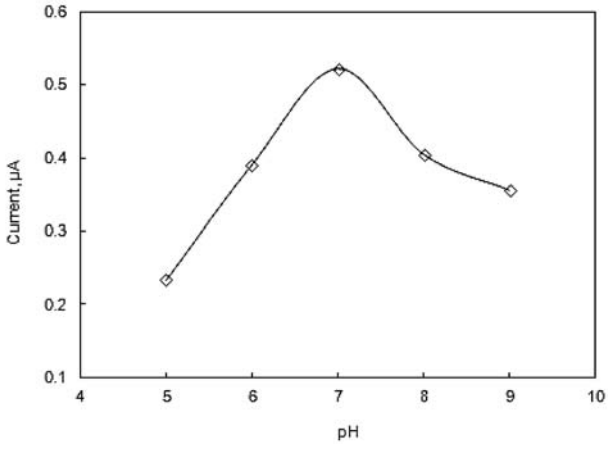


**Figure 1.** Effect of buffer solution pH on the sensor response.

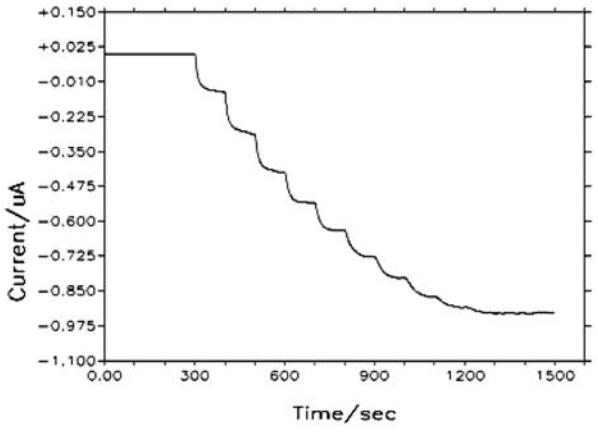


**Figure 3.** The amperometric responses of enzyme electrode to successive glucose injections.

enzyme electrode gave a rapid and sensitive response to changes in the glucose concentration. It was observed that the biosensor responded rapidly to the glucose and achieved a steady-state current value approximately within 80 s.

Figure 4 shows the calibration graph constructed for the enzyme electrode by using the steady-state amperometric responses given in Figure 3. It is clear from Figure 4 that the biosensor proves a linear response up to a concentration of 10 mM glucose. The sensitivity was determined to be 0.062 μA/mM.

The selectivity is one of the major characteristics of an amperometric glucose biosensor. The glucose selectivity of the designed biosensor in this study was examined in the presence of some electroactive (e.g., ascorbic acid, uric acid, and oxalic acid) and non-electroactive (e.g., lactose, sucrose, and urea) interfering molecules coexisting with glucose in real samples. Figure 5 shows the effect of the interfering species on the steady-state amperometric response of the biosensor. Each injection shown in Figure 5 corresponds to 2 mM of relevant substance. It can be seen that the enzyme electrode did not give a detectable signal for the electroactive and non-electroactive species while it responded successfully to glucose injections. However, it was observed that the glucose response decreased somewhat due to fouling. This decrease in the response towards glucose may be related to the blockage of the film pores due to contaminants. The biosensor showed good linearity to glucose injections in the range from 2 to 10 mM in the presence of the interfering substances ($R^2$ = 0.9887). Consequently, it can be said that the sol-gel layer effectivelyprotects the electrode surface from interfering molecules.

The stability of the glucose sensor was investigated by means of amperometric measurements over aperiod of one month using 10 mM glucose. The results of these experiments are shown in Figure 6.The enzyme electrode was stored in dry conditions at 4 °C to prevent the enzyme leaching through the sol-gel matrix when not in use. It was observed that the initial glucose sensor response decreased by 44% over a period of one month. This indicates that the activity of the enzyme under the sol-gel layer protects for a long time.

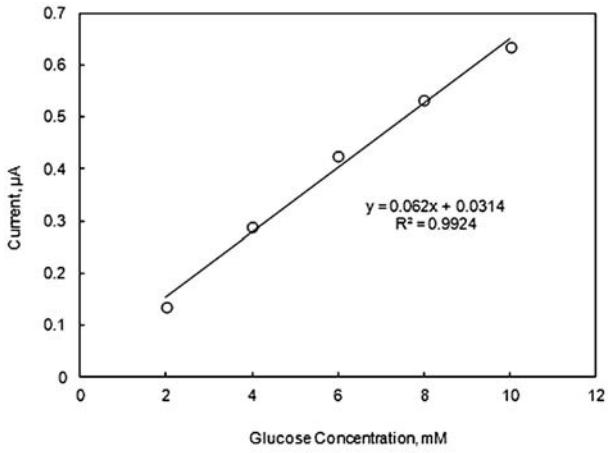


**Figure 4.** The calibration graph of the enzyme electrode.

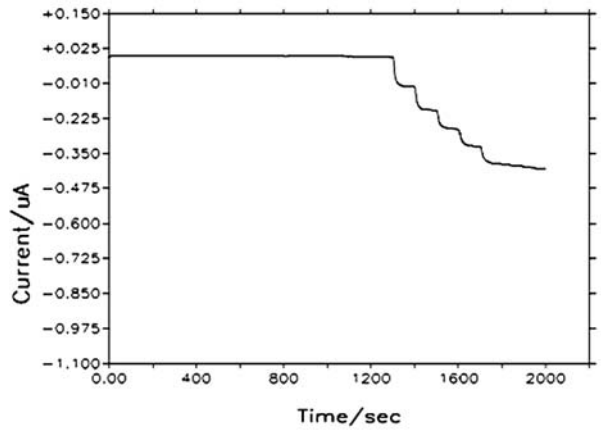


**Figure 5.** The selectivity of enzyme electrode to glucose in presence of interfering species.

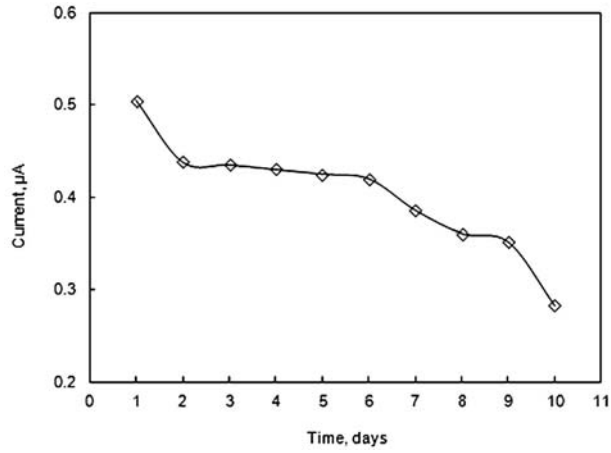


**Figure 6.** Stability of biosensor sensor.

## 4. Conclusions

In this work, a glucose biosensor with excellent biocompatibility was developed based on a sol-gel composite material. Glucose oxidase connected by physical adsorption on the platinum electrode surface was protected by a silica sol-gel film, which has been prepared using GLYMO and MTEOS. The resulting biosensor was used to detect glucose in a PBS buffer solution by means of amperometry. It was found that the enzymatic electrode showed high sensitivity, selectivity, a good stability and a reasonable linear range. It was observed that the glucose biosensor did not create any response from interfering substances.The lifetime of the glucose sensor indicates that a silica sol-gel matrix is a good immobilization medium for GOx. The results obtained demonstrate that the

sol-gel organic-inorganic hybrid material is an excellent matrix for development of an enzyme biosensor.

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## Povzetek


Razvili smo elektrokemijski biosenzor na osnovi sol-gel prekrivne raztopine. V študiji uporabljena modificirana platinska elektroda je bila narejena z imobilizacijo glukozne oksidaze pod plastjo sol-gel filma. Prekrivno raztopino smo pripravili z GLYMO in MTEOS. Pri elektrokemijskih meritvah smo amperometrijsko določili vodikov peroksid, ki je nastal z encimsko reakcijo med glukozo in glukozno oksidazo. Opazili smo, da je amperometrijski odgovor encimske elektrode linearen pri koncentraciji glukoze v območju 2 do10 mM. Odzivni čas biosenzorja do stabilnega toka je bil približno 80 s. Selektivnost biosenzorja za glukozo smo določili v prisotnosti nekaterih interferirajočih spojin, kot so laktoza, saharoza, sečnina, sečninska kislina, oksalna kislina in askorbinska kislina. Ugotovili smo, da interferirajoče spojine ne dajejo nobenega opaznega signala. Raziskali smo tudi stabilnost senzorja in ugotovili, da se začetni odziv senzorja zmanjša za približno 44 % po 30 dnevih shranjevanja v suhem stanju pri 4 °C.