

Glucose biosensor based on entrapment of glucose oxidase and myoglobin in silica gel by the sol-gel method

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Abstract. A spectrophotometric method is presented to determine glucose employing the sol-gel technique. Myoglobin (Mb) and glucose oxidase are encapsulated in a transparent and porous silica glass. The produced gel (xerogel) is then immersed in water where increments of glucose are added to the solution with stirring; glucose diffuses into the sol-gel glass pores and a series of reactions take place. Glucose is first oxidized by glucose oxidase and oxygen to gluconate and hydrogen peroxide is generated. The liberated hydrogen peroxide oxidizes the Mb heme (Fe^{2+} into Fe^{3+}). The higher is the glucose concentration added, the more is the H_2O_2 generated, and the more is the Mb oxidation (Fe^{2+} to Fe^{3+}) and as a result the higher is the absorbance at 400 nm (negative peak, lower absorbance value). All measurements are performed at this wavelength (400 nm), the negative peak obtained by subtracting the absorption spectra of Mb before and after oxidation. Measuring the slope of the absorbance decay versus time at 400 nm monitors increments of added glucose. Each glucose concentration has an accompanying unique decay curve with a unique slope. The higher is the glucose concentration; the steeper is the decay curve (higher slope value). The calibration curve was linear up to 40 mM.

1. Introduction

For many years, the glucose content of blood and urine was determined in the diagnosis of diabetes, a disease in which the blood glucose level is abnormally high and there is excessive urinary excretion of glucose. Now more sensitive methods for measuring blood glucose employ the enzyme, glucose oxidase in the presence of oxygen; gluconate and hydrogen peroxide are generated in this reaction [1,2].

Clark and Lyons first introduced the concept of glucose sensor in 1962. In their article dealing with continuous monitoring of blood chemistry, they suggested that a thin layer of soluble enzyme might be retained at the surface of an oxygen electrode using a dialysis membrane [3]. Glucose and oxygen would diffuse into the enzyme layer from the sample site and the consequent depletion of oxygen would provide a measurement of the glucose concentration. The first article describing an immobilized enzyme electrode was due to Updike and Hick in 1967 [4]. They immobilize the enzyme glucose oxidase in a polyacrylamide gel at an oxygen electrode. Since this pioneer work in the 1960s, reasonable research effort has been devoted to the development of glucose sensors by a number of research groups worldwide [5–8]. Recently, glucose sensor research is a relative mature and well-worked research field [9–11].

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The majority of sensors is based on electrochemical principles and employs enzymes as biological components for molecular recognition. Several new techniques for glucose sensing have been developed in clinical practice [12] as well as in biotechnology [13] and food industry [14]. This has inspired the development of an analytical method that can determine trace amounts of glucose with high precision when analytical separation techniques, such as chromatography and extraction, cannot be applied. Some methods with high sensitivity and accuracy, such as the biosensor [14], chemiluminometric peroxidase sensor [15] and label glucose oxidase method [16], have been reported, they require some specific apparatus and lengthy period of analysis. Analytical techniques for the determination of glucose utilizing a glucose biosensor based on an enzymatic membrane electrode were also discussed [15,17]. However, it is difficult to evaluate and maintain the sensitivity of an enzymatic electrode. Again, these methods require a specific apparatus (reactor or host). Immobilized enzyme methods have shown flexibility and applicability to the construction of analytical systems, since an enzyme only provides a selective catalytic effort for each individual reaction [18–26].

The sol-gel involves low temperature hydrolysis of a suitable metal alkoxide precursor followed by condensation to produce colloidal particles (sol), then gelation to form a wet network of porous metal oxide, and finally drying and shrinkage to form the xerogel (air dried gel) [27,28]. The sol-gel method is a convenient way to synthesize a host matrix for inorganic, organic and biomolecules. The substance to be encapsulated (the dopant) is added to the sol after partial hydrolysis of the precursor. As the degree of cross-linking from polycondensation increases, the gel becomes viscous and solidifies. The process continues during aging and the porous matrix is formed around the dopant molecules. The addition of the dopant molecule prior to the gelation process physically traps the dopant in the cross-linked network and ensures the homogeneous distribution of the dopant [29]. The principal advantages for this process are the room temperature (or lower) processing conditions, chemical inertness, negligible swelling effects, tunable porosity, the ease with which the microstructure of the material can be modified by varying the process parameters, and high purity of sol-gel derived glasses make them ideal for many types of sensor application [30,31]. For optimum biostability and reaction efficiency, the sol-gel matrix isolates the encapsulated biomolecule, protects it from self aggregation and microbial attack, and provide essentially the same local aqueous microenvironment as in biological media [32,33].

The sol-gel process has been frequently used for enzyme immobilization in biosensors, both bulk monolith gels and thin films were reported. Braun et al. [34] reported on the preparation of a 8×2 mm disk of tetramethyl orthosilicate (TMOS) derived xerogel doped with glucose oxidase, peroxidase, and a chromogenic dye for detection of glucose. Yamanaka et al. [35] investigated the activity of the activity of the encapsulated glucose oxidase with a photometric detection scheme. Tatsu et al. [36] prepared tetramethyl orthosilicate (TEOS) derived sol-gel monolith doped with glucose oxidase and used it as a glucose recognition element in a flow injection analytical system. Dave et al. [37] reviewed the sol-gel encapsulation methods for biosensors in 1994. Narang et al. [7] reported the characterization of thin sol-gel films derived from TEOS that are doped with glucose oxidase as a prototype for sol-gel based biosensor development. Chung et al. [38] encapsulated myoglobin in a TMOS monolithic glass matrix and were examined as a sensing element for measurement of dissolved oxygen. Lan et al. [39] examined a series of heme proteins including myoglobin encapsulated in monolithic TEOS gels, the trapped proteins retained their spectroscopic properties and chemical function. Jin et al. [4] reviewed the properties and applications of proteins encapsulated in sol-gel materials.

In the present work, Mb absorption spectra before and after oxidation by hydrogen peroxide are first generated. The difference produces a peak at 400 nm in the negative direction, all our measurements are recorded at this wavelength. The more Mb is oxidized the higher is the intensity of this peak.

To determine glucose in a solution: Mb and glucose oxidase are encapsulated in an optically transparent glass via the sol-gel method; the produced xerogel is then immersed in water in a vial. The glucose concentration to be determined is added into the vial where changes in their visible absorption spectra are monitored.

Measuring the slope of the absorbance decay versus time at 400 nm monitors increments of added glucose. The higher is the glucose concentration added, the more is the hydrogen peroxide generated and as a result more Mb is oxidized and the absorbance at 400 nm is increased. Each glucose concentration has an accompanying unique decay curve with a unique slope.

2. Experimental

2.1. Apparatus

Electronic absorption spectra were recorded on a spectrophotometer type Specord M 500 (Zeiss, Germany). Myoglobin spectra before and after oxidation were recorded over the range 200–700 nm. A 523 WTW (Williams Talhar and Wong International) pH meter with a WTW pH glass electrode was used for all pH measurements. Calibration of the pH meter was performed at regular intervals.

2.2. Reagents

Analytical grade reagents and deionized, ultra filtered water was used throughout. Mb, Glucose oxidase, and D(+)glucose were purchased from Sigma and were used without further purification. Tetramethyl orthosilicate (TMOS, 98%) was purchased from Aldrich.

A solution of about 200 μM of Mb was prepared by dissolving 8–10 mg in 2.0 ml phosphate buffer (pH = 6). Glucose oxidase solution was prepared by dissolving about 14 mg in 2.0 ml phosphate buffer (pH = 6). A 100-ppm stock solution of glucose was prepared; the remaining solutions were prepared by appropriate dilutions of the standard. Finally, a stock 4.93 mM of hydrogen peroxide was prepared.

2.3. Procedure

The base line for all the absorption spectra was generated using 2.0 ml of phosphate buffer (pH 6). The absorption spectrum of Mb over the range 200–700 nm was constructed by mixing 2.0 ml buffer and 0.2 ml of 200 μM Mb. The ferric Mb absorption spectra over the range 200–700 nm was performed by mixing 2.0 ml phosphate buffer (pH 6), 0.2 ml of 200 μM Mb, and 0.1 ml of 4.96 mM hydrogen peroxide.

The encapsulation of Mb and glucose oxidase in sol-gel glass was performed according to the following procedure: 1 ml of Mb and glucose oxidase was mixed with TMOS (1 ml) containing 0.5% polydimethylsiloxane (molecular weight: 162, Aldrich). The reaction mixture was gently shaken until mixture became homogeneous, and allowed to gel at room temperature. The gels were air-dried for two week at ambient temperature. The produced xerogels were 2 mm thick and 8 mm in diameter.

Glucose was determined according to the following procedure: the transparent glassy xerogel containing Mb and glucose oxidase was placed in the optical path of a spectrophotometer in a vial containing 6 ml water. At time 0, a concentrated solution of glucose was added, bringing glucose concentration to 10 mM. Enzymatic oxidation of glucose produced H_2O_2 , which oxidizes the Mb heme (Fe^{2+} into Fe^{3+}) and thus the absorbance decay at 400 nm was recorded. New glucose concentrations of 20, 40,

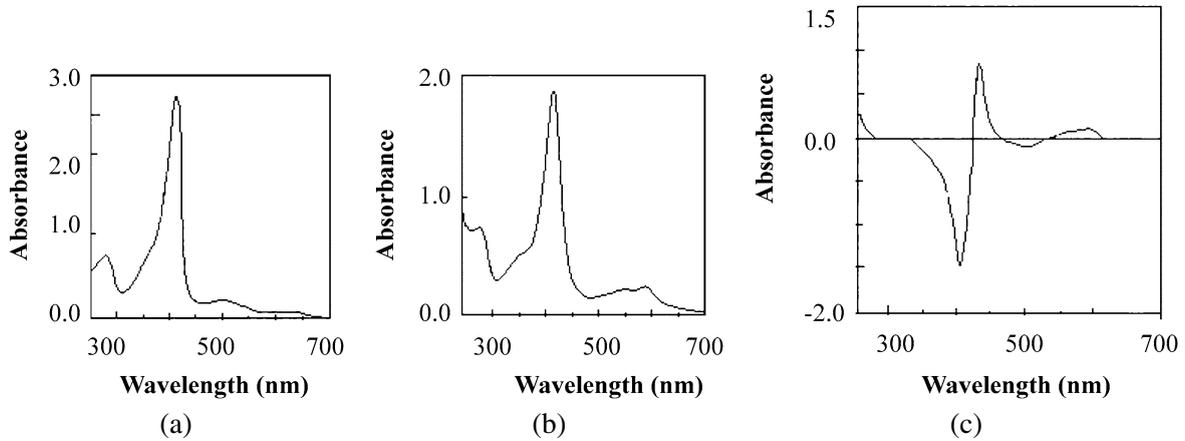


Fig. 1. Spectral changes of Mb before and after oxidation. (a) 2.0 ml buffer pH 6 and 0.2 ml of 200 μ M Mb; (b) 2.0 ml phosphate buffer pH 6, 0.2 ml of 200 μ M Mb, and 0.1 ml of 4.96 mM hydrogen peroxide; (c) The difference between Figs 1a and 1b.

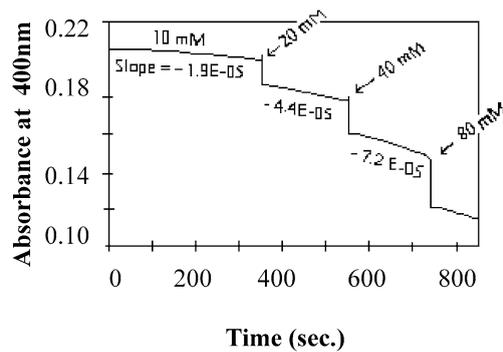


Fig. 2. Sol-gel optical glucose sensor. Xerogel (8×2 mm) containing Mb and glucose oxidase was placed in the optical pathway of a spectrophotometer in a vial containing 6 ml water. At time 0, a concentrated solution of glucose was added, bringing glucose concentration to 10 mM. Enzymatic oxidation of glucose produced H_2O_2 , which oxidizes Mb (Fe^{2+}) into Fe^{3+} . Absorbance decay curve versus time measured at 400 nm is recorded; slope for the curve is given underneath the decay line. New glucose concentrations of 20, 40, and 80 mM were added at 350, 550, and 750 seconds.

The calibration curve was constructed by plotting (a) decay slope (rate of absorbance change) at 400 nm versus concentration, a correlation coefficient of 0.975412 was obtained (Fig. 3); and (b) absorbance values at 400 nm measured 200 seconds after each addition of glucose versus concentration, the correlation coefficient was 0.992708 (Fig. 4). Each data point in Figs 3 and 4 represent the average value of five determinations, moreover, both figures show that the response is linear up to 40 mM.

These correlation data reveal that the rate of absorbance change is directly related to the glucose concentration. The correlation is apparently unique to the glass encapsulated Mb/glucose oxidase. The rate of absorbance change depends on the transport of glucose through the porous network of the gel matrix. The absorbance change rate represent the transport rate of glucose, the rate at which H_2O_2 is generated by oxidation of glucose by glucose oxidase, and the rate at which Mb iron is oxidized. The transport rate of glucose is expected to be rate limiting since glucose and Mb oxidize rapidly by glucose oxidase and H_2O_2 , respectively [18–26].

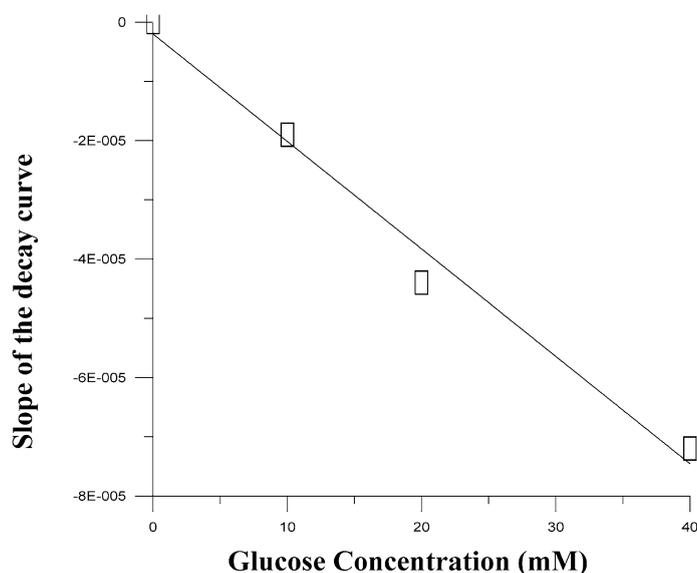


Fig. 3. Plot of slopes of the decay curves (rate of absorbance change) versus glucose concentration.

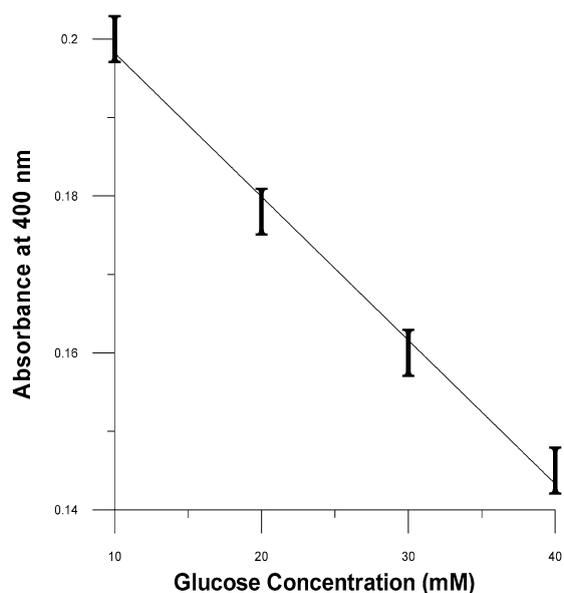


Fig. 4. Absorbance at 400 nm recorded 200 seconds after each addition of glucose versus glucose concentration.

The good correlation obtained in Figs 3 and 4 reveals not only that Mb and glucose oxidase are homogeneously distributed, but also that the pore structure is uniform throughout the glass gel. The experimental data demonstrate that the rate of absorbance change in sol-gel is directly proportional to the concentration of glucose.

We did not notice any denaturation or aggregation of Mb/glucose oxidase in sol-gel glass even for three months old gels [43]. We have found that Mb/glucose oxidase gels prepared and stored under identical conditions give the same kinetic behavior when exposed to glucose.

After Mb/glucose oxidase gels are exposed to glucose, the iron is converted into Fe^{3+} , but could be reconverted to the Fe^{2+} gel if it is to be reused. The regeneration of used gels could be accomplished by treatment with dithionite solution, which in this case reduces Mb (Fe^{3+}) into Mb (Fe^{2+}) gels. It is likely that other chemical reduction methods can be found as well. Thus, the used gels may be chemically reduced and reused for subsequent determinations.

4. Conclusion

Sol-gel glass encapsulated Mb and glucose oxidase in a monolithic gel derived from TMOS/polydimethyl siloxane has characteristics that make it an excellent sensor for glucose is reported here for the first time. These properties arise from a combination of the ability of heme-Mb to oxidize from the Fe^{2+} to the Fe^{3+} state by the hydrogen peroxide generated in the gel by the oxidation of glucose by glucose oxidase; and the optical transparency of the sol-gel matrix. The Mb/glucose oxidase encapsulated gels have been shown to exhibit three characteristics for a practical sensing element for glucose detection: (1) an optical response that is sensitive to glucose concentration; (2) an optical response that is established in few minutes; (3) relatively simple analytical equipment and procedures. The kinetics of Mb oxidation demonstrates that the device was glucose sensitive in the range between 0 and 40 mM, thus the encapsulated Mb/glucose oxidase sol-gel method has high potential as a new method for accurate and reproducible determination of glucose.

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