

A Disposable Glucose Biosensor Based on Diffusional Mediator Dispersed in Nanoparticulate Membrane on Screen-Printed Carbon Electrode

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ABSTRACT

A disposable glucose biosensor based on co-dispersion of a diffusional polymeric mediator and glucose oxidase (GOX) in nanoparticulate membrane on screen-printed carbon electrode was described. The biosensor exhibited classical features of a kinetically fast redox couple. At scan rates up to 200 mV/s, the peak-to-peak potential separation of the mediator was 59 mV at 25°C. In the presence of glucose, a typical catalytic oxidation current was observed, which reached a plateau at 0.30 V (*vs.* Ag/AgCl). Amperometric peak current at 5 s and the subsequent currents at longer test times were linear to the glucose concentration in the range of 5 to 600 mg/dl. By constructing a miniature channel on the screen-printed electrode and partially filling the channel with the nanoparticulate sensing membrane, glucose can be accurately determined in as little as 0.20 μ l of sample. Successful attempts were made in blood sugar analysis.

Keywords: glucose, biosensors, nanoparticles, ferrocene, glucose oxidase

INTRODUCTION

Since Clark and Lyons developed the first oxygen electrode-based enzyme electrode, enzyme-based biosensors have been used in an increasing number of clinical, environmental, agricultural, and biotechnological applications.^{1,2,3} To address the needs for frequent or continuous monitoring of glucose in diabetics, particularly in brittle diabetics, electrochemical glucose biosensors are, by far, the most widely employed. Since the two redox centers (FAD/FADH₂) of glucose oxidase (GOX) are prevented from transferring electrons to an electrode by an insulating glycoprotein shell,⁴ the presence of a mediator is necessary to achieve direct electron exchange between the electrode and the redox centers of GOX. Electrochemical glucose biosensors normally employ two major groups of mediators, hydrogen peroxide oxidation mediating reagents and glucose oxidation mediating reagents. The former usually require high operating potentials where unwanted reactions of coexisting electroactive constituents in blood complicate the interpretation of the signal. Moreover, being limited by the least concentrated substrate, oxygen, in the enzymatic reaction, these biosensors suffer from low sensitivities. On the other hand, glucose oxidation

mediating reagents offer a preferential catalytic oxidation of glucose at much lower potentials, and hence greatly enhance the selectivity of the corresponding blood sugar measurements. The sensitivity of the measurements is also greatly improved due to the replacement of oxygen by synthetic mediators that are usually in large excess in the systems. In these cases, the natural route of glucose oxidation catalyzed by GOX becomes a side-reaction, competing with the mediated one. Special attention must be paid to the effect of this reaction, and extensive effort must be made to minimize the degree of glucose consumption by dissolved oxygen in blood.

Unlike enzyme electrodes based on immobilized mediators, biosensors employing diffusional mediators are particularly attractive for developing disposable biosensors for diabetes home-care. Different forms of diffusional mediator-based systems have been employed by commercially available glucose biosensors. In most of the disposable glucose biosensors, the signal is a balance between the mediated reaction and the natural glucose oxidase catalyzed oxidation of glucose. Since each of the reactions has its own characteristics, the response of the biosensors is profoundly influenced by temperature, partial pressure of oxygen, pH, etc. A more challenging task in glucose biosensors development is to identify appropriate means of eliminating the interferences from blood cells and coexisting electroactive constituents. For example, high levels of red blood cells result in readings that are lower than the true values because of fouling of the sensing membrane and the increased magnitude of the side-reaction between oxygen and glucose. On the other hand, coexisting electroactive constituents such as ascorbic acid, uric acid, dopamine and acetaminophen all contribute to readings higher than the true values. Because of the importance of accurate blood sugar monitoring to the well-being of diabetic patients, it is highly desirable to have a sensing device that does not suffer from these drawbacks.

EXPERIMENTAL SECTION

Fig. 1 illustrates the fabrication of the disposable glucose biosensor. The nanocomposite membrane was screen-printed onto carbon strip using an aqueous slurry "ink" of poly(vinylferrocene-co-acrylamide) (PVFcAA), GOX, a poly(vinylpyridine-co-acrylic acid) (PVPAC) binder and alumina nanoparticles. A uniformed nanoparticulate membrane was printed on top of the

working area and dried at 37°C in a controlled environment. The thickness of the nanoparticle membrane was controlled by adjusting the total content in the ink while keeping a constant volume applied on the working area. After removing the protecting film of the double-sided adhesive tape, a Ag/AgCl coated polyester film was applied, forming a microchannel between the two films. Finally, individual sensors were cut off from the substrate.

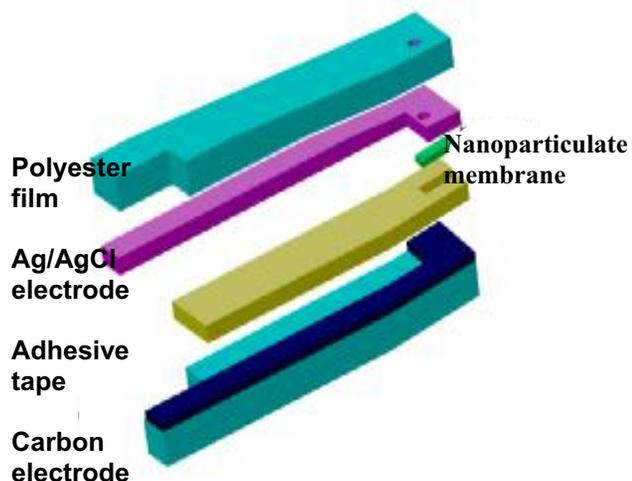


Fig. 1. Exploded view of the disposable glucose biosensor used in this work.

All electrochemical measurements were carried out with a model CHI 660A electrochemical workstation (CH Instruments, Austin, USA) at room temperature. Cyclic voltammetric measurements were performed using a conventional three-electrode system, consisting of a screen-printed carbon working electrode, a Ag/AgCl reference electrode (Cypress Systems, Lawrence, KS, USA) and a platinum wire counter electrode. To avoid the spreading of the printing ink beyond the 2-mm diameter working area, a patterned hydrophobic film was applied to the carbon electrode. To avoid electrode fouling and possible concentration changes in the ink, fresh electrode and ink were used for each voltammetric test. All glucose measurements were performed in a phosphate buffered saline (PBS) solution. In experiments where the pH was varied, 1.0 M HCl and 1.0 M NaOH solutions were used to adjust the pH of the PBS. In amperometric experiments, the working electrode was poised at 0.30 V (vs. Ag/AgCl).

RESULTS AND DISCUSSION

A typical cyclic voltammogram of the PVFcAA mediator in a plain nanocomposite ink is shown in Fig. 2. The electrode exhibited classical features of a diffusion-controlled kinetically fast redox couple. The peak current increased linearly with the square root of potential scan

rate, and the difference between the reduction and oxidation peak potential remained unchanged at 59 mV for scan rates up to 200 mV/s, showing that charge transfer from the mediator to the electrode is rapid.⁵ Spiking this ink with glucose did not change the voltammogram at all, which suggests that there is no catalytic oxidation of glucose by the mediator alone. Practically identical voltammograms, as that shown in Fig. 2 trace a, were obtained in the presence of different amounts of GOX, ranging from 0.10 to 20 mg/ml, indicating that the enzyme does not appreciably affect the electrochemistry of the Fc⁺/Fc redox couple in the ink. However, an addition of a very small amount of glucose to this ink resulted in an enhanced anodic current and a diminished cathodic current (Fig. 2 trace b). In addition, as can be seen in Fig. 2, the voltammogram was lifted up towards the anodic side around the redox potential of the mediator. Such changes are indicative of a typical chemically coupled electrode process (electrocatalysis).⁵

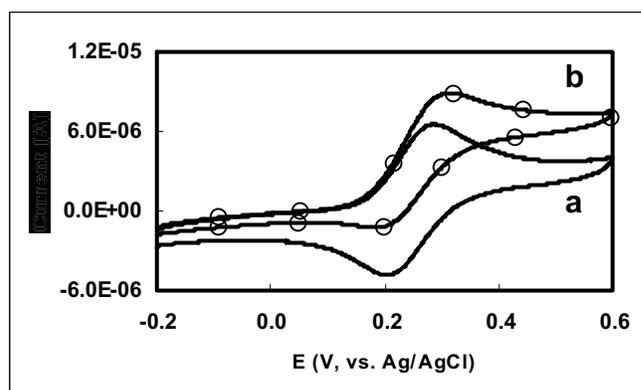


Fig. 2. Voltammograms of the nanoparticle printing ink (a) and (b) with the addition of 100 mg/dl glucose. Potential scan rate = 100 mV/s.

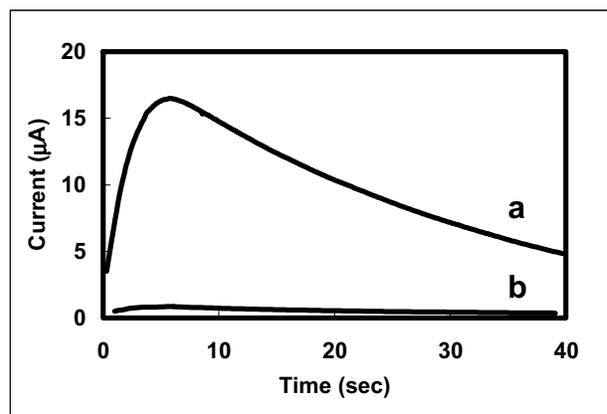


Fig. 3. Amperometric responses of the biosensor in PBS containing (a) 200 and (b) 0.0 mg/dl glucose. Poised potential = 0.30 V.

A typical amperometric response of glucose in an air-saturated PBS at the biosensor is shown in Fig. 3 trace a. Amperometric tests demonstrated that the biosensor has a rapid response time and high sensitivity to glucose. At 0.30 V, after spiking the glucose concentration, the oxidation current increased and reached the maximum very rapidly, within 5 s, followed by a gradual transient which maintains more than 60% of the peak current for a period of 20 s. No catalytic oxidation current was observed in a blank PBS buffer under identical experimental conditions (Fig. 3 trace b), but the presence of the nanoparticulate membrane did increase the background current and it took a considerably long time to drop to a minute level.

In order to obtain a satisfactory performance of the biosensor, the formulation of the nanocomposite ink was optimized. Since the catalytic reaction occurs between the mediator and GOX, the concentration of the mediator must be high enough to have a high sensitivity and linear relationship between the catalytic oxidation current and glucose concentration. Otherwise, the fraction of mediated glucose oxidation will be small and dependent on the amount of mediator in the membrane, instead of the glucose concentration. It was found that a mediator concentration of 15 mg/ml is best for our purpose and the optimal concentration of GOX was found to be 0.20 mg/ml, taking into consideration of both sensitivity and biosensor economy. The poise potential is expected to affect the amperometric response of the biosensor; it was therefore examined in the range of 0.0 to 0.70 V. The current sensitivity increased with increasing poise potential and reached a plateau at 0.30 V. A slight decrease in sensitivity was observed when the poise potential became more positive than 0.50 V, presumably due to an increased background current. Moreover, too high a poise potential compromises the accuracy of glucose measurements owing to complications from both the much increased background current and possible direct oxidations of a number of electroactive species at the underlying electrode. For amperometric measurements of glucose, the potential of the biosensor was therefore poised at 0.30 V. The dependence of the catalytic oxidation current of glucose on the thickness of the nanoparticulate membrane was also investigated. The catalytic oxidation current reached maximum for nanoparticulate membranes with thickness of 250-500 μm . Insufficient materials in thinner membranes resulted in lower sensitivity and the disappearance of the current peak, On the other hand, further increase in membrane thickness beyond 500 μm could inversely affect the membrane permeability for glucose and the oxidation products of the GOX-catalyzed reaction. In addition, longer response times were noted for thicker membranes.

As mentioned earlier, oxygen affects the sensitivity of the glucose biosensor because glucose oxidation by dissolved oxygen occurs simultaneously as a side-reaction. Initial amperometric tests on thin nanoparticulate membranes employing a hydrophobic polyvinylpyridine (PVP) binder showed that the response was higher in the

absence of oxygen than that with dissolved oxygen. At low glucose concentrations, e.g. 50 mg/dl, the competition with oxygen caused a significant decrease ($\sim 20\%$) in peak current. Hence, there was a need to suppress the oxygen interference in the system to achieve a highly selective and accurate biosensor. Introduction of acrylic acid units into the hydrophobic PVP resulted in a marked improvement of the biosensor performance. The resulting nanoparticulate membrane was highly hydrophilic, which improved the glucose/oxygen permeability ratio and optimized the accuracy and linearity of the biosensor response. The two amperometric graphs for 200 mg/dl glucose solutions bubbled with nitrogen and oxygen overlaid nicely with a difference of less than 5% in peak current. This illustrated that the biosensor was rather insensitive to the oxygen content in the samples

Unlike those utilizing surface-immobilized sensing membrane, the utilization of the non-conductive nanoparticulate sensing membrane offers great advantages over known disposable glucose biosensors in terms of selectivity. In the former systems, the sensing membrane is part of the electrode and is in direct contact with blood samples. Some constituents in blood, such blood cells, both red and white, proteins and ascorbic acid may interact with the sensing membrane and compromise the accuracy of blood sugar measurements. In this work, the nanoparticulate membrane is non-conductive, and therefore structurally and functionally is not part of the electrode. Catalytic oxidation of glucose only takes place at the electrode/nanoparticulate membrane interface. In other words, no electroactive species exchanges electrons with the electrode unless it passes through the nanoparticulate membrane to reach that interface. Thus, the nanoparticulate membrane provides a barrier to the passage of possible interferences of bulky species in blood such as cells and proteins. When this formulation was used to print the nanoparticulate membrane, the PVPAC binder serves a dual function in the sensing membrane: binding and analyte regulating. On rehydration, the membrane does not break up, but swells to form a gelled layer on the screen-printed carbon surface. Reactants, such as glucose and mediators move freely within this layer, whereas interfering species, such as red blood cells containing oxygenated hemoglobin are excluded. Anionic ascorbic acid and uric acid are expelled by the anionic PVAC polymer, and the partition of dissolved oxygen into the nanoparticulate membrane is largely minimized owing to the highly hydrophilic nature of this layer. This resulted in a sensing membrane whereby the amount of current generated in response to a given glucose concentration varied by less than 5.0% over a hematocrit range of 40-60% and in the presence of 0.20 mM ascorbic and 0.10 mM uric acid. Such desirable insensitivity towards the interfering constituents in blood was also observed in whole blood samples. Furthermore, the nanoparticulate membrane presented an analyte regulating layer for glucose too, significantly slowing down the transport of glucose so that the system was not kinetically controlled, thereby

extending the linear domain through the entire physiologically relevant glucose concentration range of 40 to 540 mg/dl.

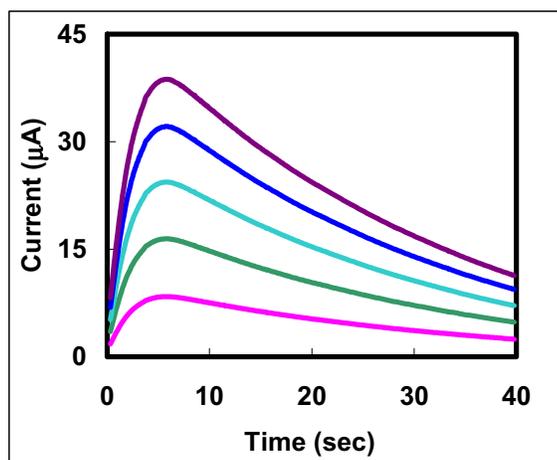


Fig. 4. Current responses to sequential increases of 100 mg/dl glucose from 100 to 500 mg/dl.

The sensitivity was ~ 76 nA/mg/dl for the peak current for glucose concentrations of ≤ 600 mg/dl. As seen in Fig. 4, the catalytic oxidation current was directly proportional to the glucose concentration up to 600 mg/dl, covering the entire physiologically relevant blood sugar levels. Interestingly, currents obtained at any point of time after the current peak was also linearly dependent on the glucose concentration, providing alternative sampling possibilities within the first 20 seconds of the amperometric tests. The precision was estimated from two series of 20 repetitive measurements of 40 and 300 mg/dl glucose solutions. The relative standard deviations were 4.0% and 8.6%, respectively. The detection limit, estimated from 3 times the standard deviation of repetitive measurements of 5.0 mg/dl glucose under optimal conditions, was found to be 1.8 mg/dl, which is limited by the charging current of the biosensor. More importantly, the blood sample volume needed for a single test was about 0.20 to 0.30 μl , the smallest sample volume amongst all the disposable glucose biosensors available on market. The stability tests were carried out at different temperatures. It was shown that the biosensor maintained 100% of its initial sensitivity for the first 180 days of storage at room temperature, lost 10% of its initial sensitivity after 60 min exposure at 50°C and about 50% of its initial sensitivity after 60 min at 60°C. This may be due to the loss of enzyme activity in the biosensor. The proposed method was successfully applied to the determination of glucose in whole blood (Table 1). The results were in good agreement with the reference values obtained with a yellow springs blood sugar analyzer (YSI Model 2300). The recoveries obtained were also good enough for practical use.

Table 1. Results of blood sugar analysis (average of 10 tests)

Sample	Glucose (mg/dl)	Reference Value (mg/dl)*	Recovery (%) (+ 50 mg/dl)
Blood 1	80	84	96.3
Blood 2	110	115	99.2
Blood 3	105	105	104
Blood 4	185	179	98.5
Blood 5	155	158	97.1

* Obtained with the YSI blood sugar analyzer.

In conclusion, we show here that glucose oxidase and PVFcAA can be readily and homogeneously dispersed into the nanoparticulate alumina together with the hydrophilic PVPAA binder, and the resulting membrane produced a typical catalytic oxidation current for glucose. The experimental results showed that the mediator retained its fast electron transfer properties and the GOX retained its catalytic activity after they were screen-printed onto the carbon electrode. They also demonstrated that this biosensor has good sensitivity and stability for blood sugar monitoring with a blood sample volume of as little as 0.20 μl . The use of screen-printing technique in the fabrication of the biosensor enables easy and low-cost mass production. These biosensor characteristics are promising for development of miniature glucose biosensors of high commercial values.

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