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Kinetic Analysis of Glucose with Wireless Magnetoelastic Biosensor

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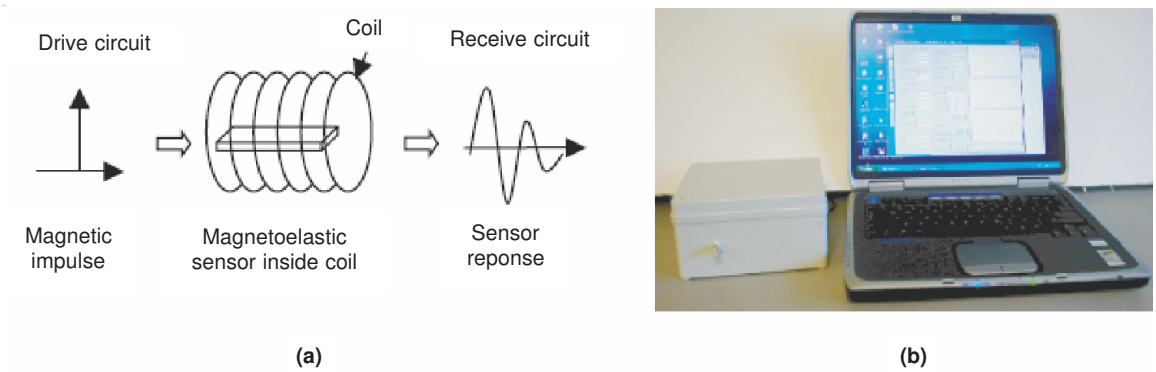
A wireless remote-query disposable magnetoelastic biosensor was developed for the kinetic study of glucose. The sensor was fabricated by applying a magnetoelastic ribbon with a layer of pH-sensitive polymer and upon it a sensing film containing bovine serum albumin (BSA) and glucose oxidase (GOX). The GOX-catalyzed hydrolysis of glucose decreases the solution pH, resulting in the polymer shrinking and consequently the resonance frequency of the magnetoelastic sensor increasing. The kinetic parameters were measured to be 8.46 mM (Michaelis constant) and 769 Hz/min (maximum initial rate). A linear fitting was obtained between 2 and 15 mM of glucose with a regression coefficient of 0.99 (n = 5). The proposed sensor can determine 1.45 mM of glucose.

Key Words: Glucose oxidase, Wireless, Magnetoelastic, Biosensor, Glucose.

INTRODUCTION

Our research is concerned with the development of wireless, inexpensive biosensors that can be used for kinetic study on glucose oxidase activity. Diabetes mellitus is an important chronic metabolic disorder that results from the body's total or partial deficiency of insulin, resulting in hyperglycemic glucose values. Numerous methods such as spectrophotometry[1-3], amperometry[4,5], HPLC[6], polarimetry[7] and capillary electrophoresis[8] for glucose analysis have been reported.

In this work, a wireless magnetoelastic biosensor was developed for glucose measurement by applying a magnetoelastic thick-film ribbon with a bio-sensing layer containing GOx and catalase with BSA as the support medium. **Scheme-I** (a) illustrates the operating principle of a magnetoelastic sensor. In response to time-varying magnetic field a magnetoelastic sensor longitudinally vibrates at a fundamental characteristic resonance frequency that inversely depends upon the sensor length. Since the magnetoelastic ribbon is also magnetostrictive, the mechanical vibration in turn generates a magnetic flux that can be remotely detected using a pick-up coil. No physical connections between the sensor and the detection system are required, nor does the sensor require an internal power supply such as a battery. In recent years, various magnetoelastic biosensors have been developed for the analysis and quantification of enzymes[9,10], bacteria[11], proteins[12,13].



Scheme-I: Schematic showing operation of the wireless magnetoelastic sensor (a) and the magnetoelastic sensor-reader box (b, on left) connected to a portable computer *via* a RS 232 port. The computer interface allows for user-control of measurement parameters, data display and storage

EXPERIMENTAL

Glucose oxidase (EC 1.1.3.4, 217 units/mg) and catalase from bovine liver (EC 1.11.1.6, 2860 units/mg) was purchased from Sigma Chemical Co. Bovine serum albumin (BSA) was purchased from Sigma Co. One unit of glucose oxidase is defined as the amount of enzyme needed to oxidize 1 μmol β-D-glucose to D-gluconolactone and H_2O_2 per min at pH 5.1, 35 ºC. One unit of catalase is defined as the amount of enzyme needed to decompose 1.0 μmol H_2O_2 per min at pH 7.0, 25 ºC. Acrylic acid, poly(ethylene glycol) diacrylate (PEGD) and isooctyl acrylate were purchased from Aldrich (Milwaukee, USA) and purified using an inhibitor remover disposable column (Aldrich) prior to use. 2,2-Azo*bis*(isobutyronitrile) (AIBN), dimethylaminopropyl-3-ethylcarbodiimide (EDC) and N-hydroxysuccinimide (NHS) were purchased from Aldrich and used as received. Bayhydrol 110, an anionic dispersion of an aliphatic polyester urethane resin in water/N-methyl-2-pyrrolidone solution (50 % w/v) was purchased from Bayer Corp (Pittsburgh, PA). All chemicals were of analytical grade. Double distilled water was used throughout the experiment. Sensors in rectangles in size of 18 mm × 6 mm × 28 μm were cut from a 28 μm-thick ribbon of Metglas alloy 2826MB1 donated by Honeywell Corporation. The resonant frequency of the uncoated sensor in air is approximately 110 kHz.

Sensor fabrication: The pH-sensitive polymer[14] was synthesized by a free radical copolymerization of acrylic acid and isooctylacrylate in N,N-dimethyl formamide. Briefly, in 50 mL solution containing acrylic acid and isooctylacrylate with an initial mole ratio of 4:1 and an overall monomer concentration of 4.5 mol/L were added 0.02 mol % PEGD as cross-linker and 0.4 mol % AIBN as initiator. After 1 h bubbling of nitrogen to deoxygenate the solution, the temperature was raised to 65 ºC to start the polymerization and maintained for 2 h to complete the polymerization under nitrogen atmosphere. The polymer was rinsed with toluene to remove any unreacted components and then dried in a vacuum oven at 80 ºC under reduced pressure (10 Torr) overnight.

The magnetoelastic sensor was ultrasonically cleaned in a Micro-Cleaning solution, followed by water and acetone rinse and then dried in a stream of nitrogen. To both sides of the cleaned sensor 8 μL of bayhydrol 110 was applied. The polyurethane-coated sensor was dried in air and then heated at 150 ºC for 2 h to form a protective membrane, which offers a NH group for binding the pH-sensitive polymer and protects the iron-rich magnetoelastic substrate from corrosion. The polyurethane-protected sensors were then coated with 12 μL of 23 g/L pH-sensitive polymer solution in ethanol containing 0.46 g/L EDC and 0.38 g/L NHS. The polymer coated sensor was dried in air and then heated in an oven at 120 ºC for 2 h to form amide bonds between the pH-sensitive polymer and polyurethane film. The sensor achieved at this point in the fabrication process can be used as a reference sensor to calibrate for the effect of pH in the glucose concentration measurements. To fabricate the working glucose sensor, the sensor was first dipped in a solution containing 0.02 % EDC and 0.02 % NHS in water and rinsed with water. A solution containing 2 g/L GOx, 0.2 g/L catalase, 3 % BSA and 0.02 % glutaric dialdehyde in water was prepared and set at 4 ºC for between 5 and 30 min to enable the cross-linking reaction. Then 20 μL of the solution was applied on the sensor. The resulting sensor was dried in air at room temperature and stored at 4 ºC for 2 days to ensure strong cross-linking of the sensing film. The unreacted GOx, catalase, chitosan and glutaric dialdehyde were removed by rinsing the sensor with 10 mM phosphate buffer solution (PBS) containing 10 mM NaCl. The resulting biosensor was stored at 4 ºC while not in use. Fig. 1 shows the fabrication procedure.

Measurement: Prior to use, the magnetoelastic biosensor coated with pH polymer and GOx was activated in 2 mM pH 6.5 PBS for 20 min to wet the film. It was then steeped in 2.5 mM PBS (containing 5 mM KCl) and incubated at 37 ºC for *ca.* 10 min to let the response stabilize. After that, 40 μL 0.1 M glucose solution was added into the detection cell. The resonant frequency was recorded with a magnetoelastic sensor reader.

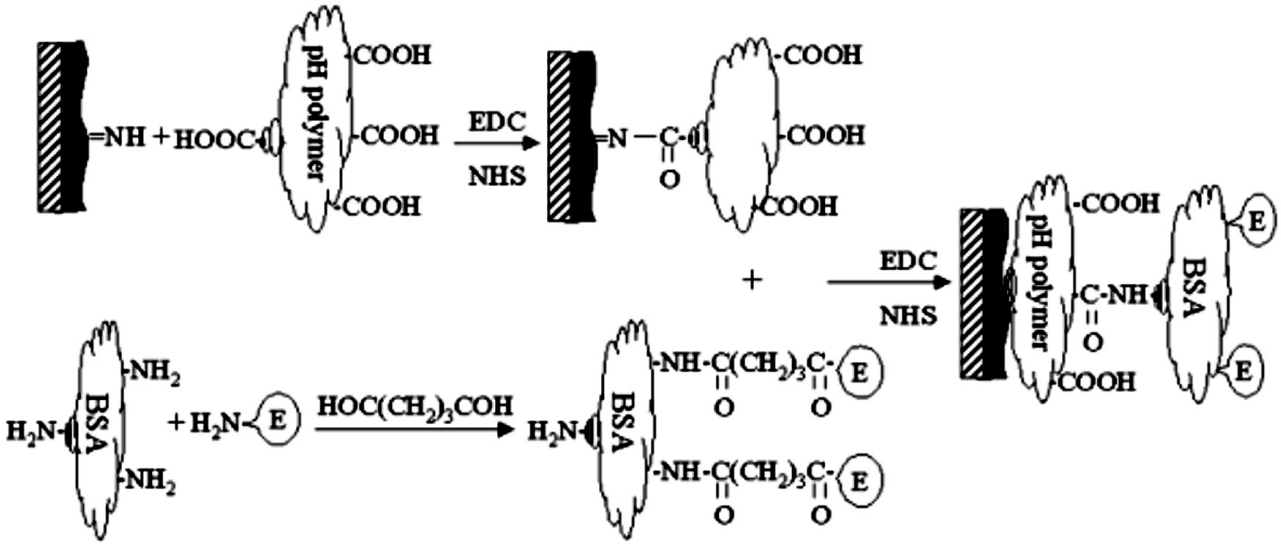


Fig. 1. Schematic diagram showing the procedures for the immobilization of GOx onto the pH-sensitive polymer on magnetoelastic sensor. (E: GOx)

RESULTS AND DISCUSSION

Effect of temperature and buffer concentration: Fig. 2 shows that the maximum activity occurs between 35 and 38 ºC. While the temperature was higher than 40 ºC, GOx was deactivated rapidly. Considering the measurement condition and the enzyme stability, experiments were carried out at 37 ºC.

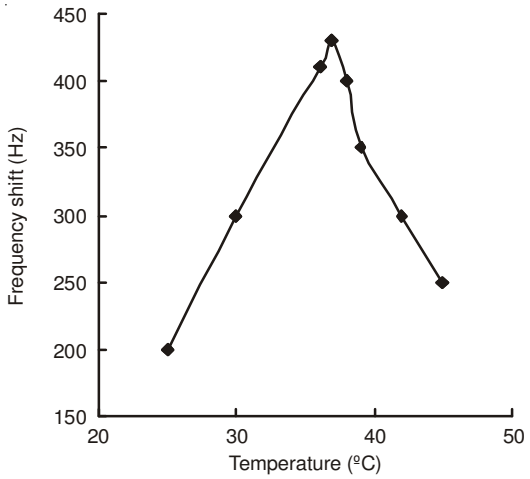


Fig. 2. Influence of temperature on the GOx activity at pH 6.5, [GOx] = 0.4 U/mL and [glucose] = 5 mM

As the proposed sensor is a pH-sensitive sensor, high concentration buffer would offset the sensor response. The buffer would neutralize a part of the H^+ produced by the enzyme-catalyzed reaction, resulting in a decrease in the response. The response, however, did not increase with decreasing the buffer concentration (Fig. 3). The maximum response was observed in 2 mM buffer solution. When the buffer concentration is less than 2 mM, a drop in the response was observed, suggesting that PBS also affected the GOx activity. It was surmised that the -OH of PBS could form hydrogen bonds with an enzyme, resulting in a breaking of the hydrogen bonds inside the enzyme, which slightly disturbs the enzyme conformation and makes the active sites of the enzyme more flexible, consequently enhancing the enzyme activity. With increasing the PBS concentration, the GOx activity increases but the buffer capacity also increases, which gives a maximum response at 2 mM PBS.

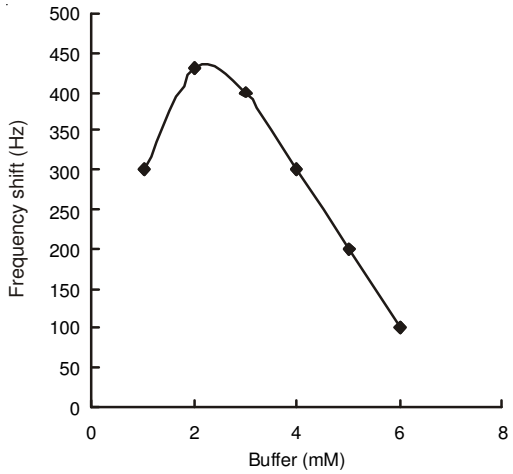


Fig. 3. Influence of the buffer concentration on the GOx activity at 37 ºC, pH 6.5, [GOx] = 0.4 U/mL and [glucose] = 5 mM

Measurements of the kinetic parameters: The reaction of the GOx-catalyzed hydrolysis of glucose is:

$$\beta\text{-D-glucose} + O_2 + H_2O \xrightarrow{GOx} \beta\text{-D-gluconic acid} + H_2O_2 \quad (1)$$

$$H_2O_2 \xrightarrow{Catalase} 0.5O_2 + H_2O \quad (2)$$

The overall reaction becomes:

$$\beta\text{-D-glucose} + 0.5O_2 \xrightarrow{GOx+catalase} \beta\text{-D-gluconic acid} \quad (3)$$

The dissociation of gluconic acid produces H^+, resulting in polymer shrinking and consequently the resonance frequency of the sensor increasing due to the decreased mass load on the sensor.

The production of gluconic acid results in the pH-sensitive polymer shrinking and consequently the resonance frequency increasing. Fig. 4 shows the dual-reciprocal curves of the 52-gluocse concentration and the initial rate where the substrate glucose was directly injected in solution. The frequency shift within the first 5 min was used to calculate the initial hydrolysis rate (v) since a linear response was observed within the first 5 min. The enzyme-catalyzed reaction is basically coincidental to the Lineweaver-Burk plot, from which the kinetic parameters can be estimated:

$$\frac{1}{V} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}} \quad (4)$$

where V and V_{max} are the initial hydrolysis rate and the maximum hydrolysis rate in Hz/min, respectively. [S] is the glucose concentration in mM. A linear fitting was obtained between 2 and 15 mM of glucose with a regression coefficient of 0.99 (n = 5). The kinetic parameters were calculated to be V_{max} = 769 Hz/min and K_m = 8.46 mM. The Michaelis constant (8.46 mM) is equivalent to 7.01 mM according to the method proposed by Mala Ekanayake *et al.*[14], the GOx hydrolysis of glucose (at pH 6.5) using a nano-porous electrode.

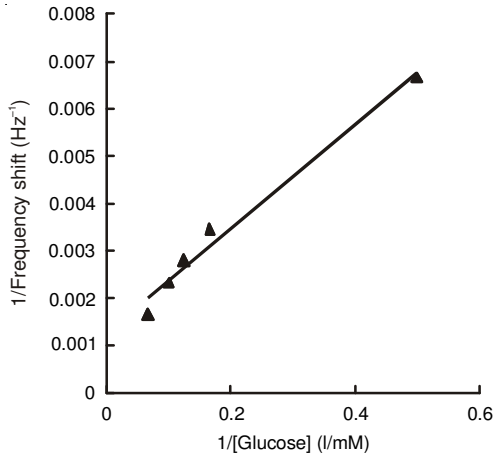


Fig. 4. Dependence of the reciprocal of frequency shift on the reciprocal of 5'-glucose concentration in 0.4 U/mL GOx solution at 37 ºC and pH 6.5

Glucose assay: GOx at an activity of 217 units/mg purchased from Sigma Co. was used to prepare the calibration solutions. Glucose was detected using the sensor immobilized with GOx. Fig. 5 shows the calibration curves obtained with

the fixed GOx. It shows linear responses between 2 and 15 mM of glucose with regression coefficients of all > 0.99 (n = 5). Defining the limit of detection (LOD) as three times the signal, the LODs were calculated to be 1.45 mM. The sensor-to-sensor reproducibility was investigated by measuring the sensor response to 0.4 U/mL GOx at pH 6.5, 37 ºC with a RSD of 3.4 % in six parallel measurements. The sensor proposed in this paper can be used to determine 2-15 mM of glucose.

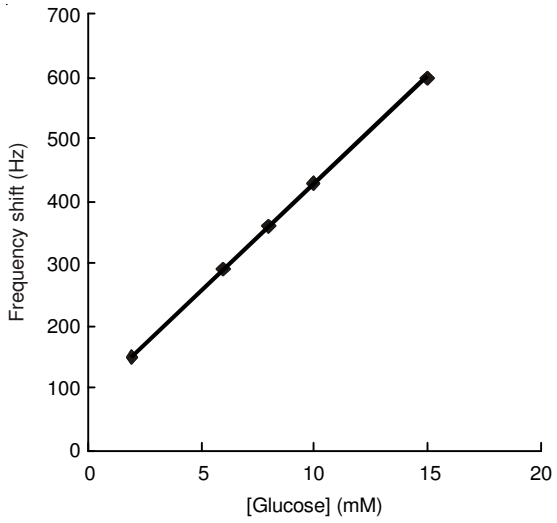


Fig. 5. Dependence of the reciprocal of frequency shift on the reciprocal of glucose concentration in 0.4 U/mL GOx solution at 37 ºC and pH 6.5

Conclusion

A magnetoelastic glucose biosensor is based on the pH change resulting from the co-enzyme couple reaction, was successfully fabricated and tested. The sensor platform has the following advantages: easy construction, high sensitivity with a detection limit of 1.45 mM and wide dynamic range from 2-15 mM. The kinetic parameters were calculated to be V_{max} = 769 Hz/min and K_m = 8.46 mM.

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