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Short communication

Clinical determination of glucose in human serum by a tomato skin biosensor

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ABSTRACT

Background: Glucose biosensors based on enzyme reaction of glucose oxidase were studied because the symptomatic therapy of diabetes mellitus requires reliable assessment of blood glucose level at frequent intervals. Tomato skin membranes have been successfully employed to entrap glucose oxidase for fabrication of glucose biosensor.
Methods: Glucose oxidase was immobilized onto the tomato skin and the enzyme membrane was then positioned on the surface of an oxygen electrode. The glucose concentration was quantified by the change of dissolved oxygen. All the serum samples were also simultaneously determined by a Hitachi 7060 chemistry analyzer.
Results: The response of the biosensor showed a linear relationship with a concentration range of 1.0–30.0 mmol/l glucose. The limit of detection was 0.20 mmol/l. Error Grid analysis demonstrated that 100% of the results fell within clinically acceptable zones A and B. The *F*- and *t*-tests showed no significant differences between the 2 methods. The recovery was 95.0–110.0% for 30 serum samples analysis.
Conclusions: The tomato skin biosensor possesses the advantages of simple fabrication, fast response time, low cost and high sensitivity. The results of our method are more accurate than and match well with the current clinical instrument method.

1. Introduction

Exact and rapid determinations of glucose in human blood are essential in the diagnosis and management of diabetes. Therefore, a large number of sensors have been introduced for monitoring glucose [1–3]. People who have diabetes or increased fasting levels of glucose have elevated blood glucose levels because of an inability to use insulin properly [4]. Worldwide numerous patients suffer from diabetes mellitus, a disease resulting from insulin deficiency which must be controlled by monitoring blood glucose and injecting accordingly daily doses of insulin as a medical treatment [5]. It is essential that a simpler, more-stable and cost-effective method should be developed for monitoring blood glucose in clinics and laboratories. In recent years, various membranes have been tested to entrap enzyme in order to retain good enzyme activity [6–9]. The symptomatic therapy of diabetes mellitus requires reliable assessment of blood glucose levels (glucose contents) at sufficiently frequent intervals [10]. And the glucose meters need to have the clinical evaluation [11,12].

In our preliminary work, tomato skin was found to possess good biocompatibility, gas-permeability and water-impermeability properties. It can be employed as an ideal enzyme immobilization bioplatform for fabricating fast-response enzyme-based biosensors and the biosensor possesses very good selectivity. Since many research groups have already used Clark-type electrodes to detect the change of oxygen concentration during enzymatic conversion of glucose [13,14], here we describe a simple amperometric analysis system for fast and accurate determination of glucose concentrations in human serum using a biosensor based on a glucose oxidase-immobilized tomato skin and a dissolved oxygen electrode.

2. Materials and methods

Glucose oxidase was initially immobilized onto the inner surface of a fresh tomato skin. The enzyme-immobilized membrane was subsequently positioned on the surface of a dissolved oxygen sensor. The detection scheme was based on the enzymatic reactions of glucose inducing the depletion of the dissolved oxygen in the solution. Then the decrease in the dissolved oxygen level was monitored and related to the glucose concentration. The proposed glucose biosensor was successfully applied to determine the glucose concentration in human serum and the results were satisfactory.

A tomato skin was carefully peeled from a fresh tomato and the flesh had been removed. It was cleaned with a copious amount of deionized water. An aliquot of 100 μl glucose oxidase solution (5.00 mg/ml) was added onto the inner surface of a tomato skin and left to dry. This procedure was repeated 3 more times. Then a 50-μl glutaraldehyde solution (5% w/w) as the cross linking reagent was dropped on the

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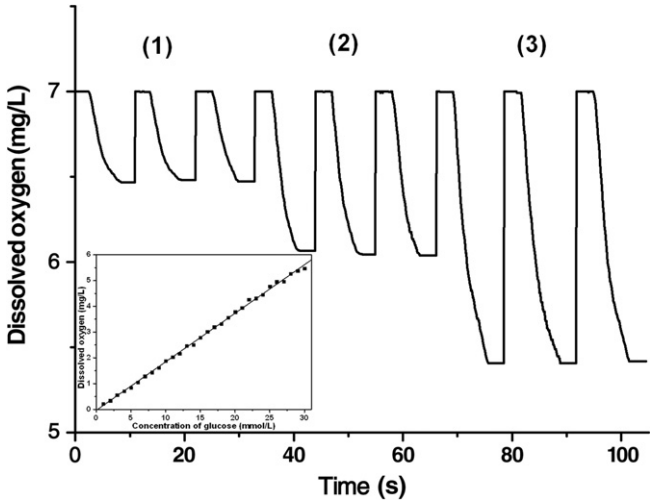

Fig. 1. The typical response of the biosensor on exposure to various blood serum samples. The concentrations of glucose were (1) 3.07, (2) 4.61, and (3) 7.63 mmol/l. The inset displays the linear regression curve for the biosensor from 1.0 to 30.0 mmol/l glucose.

membrane. After 2 h, the membrane was rinsed in a phosphate buffer (0.20 mol/l, pH 6.5) to remove excess glutaraldehyde and unimmobilized enzyme. After washing, the glucose oxidase-immobilized tomato skin was protected from light and stored at 4 °C until further use.

The glucose oxidase-immobilized tomato skin was positioned on the surface of a Pasco CI-6542 oxygen electrode (Pasco Scientific) and kept in a steady position by an *O*-ring. The enzyme electrode was immersed in a stirred 5.00-ml of 0.20 mol/l phosphate buffer at pH 6.5. Various standard or sample glucose solutions were injected into the phosphate buffer with the use of a syringe. The concentrations of glucose were calculated according to the change of oxygen in the buffer. The dissolved oxygen signal was captured at a sampling rate of 10/s and processed by a data logging system consisting of a Science Workshop 500 interface, and control software (Pasco Scientific) [15]. This study comprised 300 volunteers who, in morning fasting state, visited the outpatient clinic of the Chengdu First People's Hospital (Chengdu, Sichuan, P.R. China). Venous blood samples were drawn from each patient. The blood samples were centrifuged at 1252 ×*g* for 3–4 min at room temperature to separate the serum. 1.00 ml of the supernatant (blood serum) was diluted with 4.00 ml of 0.20 mol/l buffer (pH 6.5), and 1.00 ml of the resulting mixture was injected into the 5.00 ml buffer at which the enzyme electrode was used to monitor the oxygen content. The decrease in the oxygen level was found to be proportional to the glucose concentration. All the samples were submitted to the above processes and each test was performed in triplicate. And all the samples were also simultaneously determined by a Hitachi 7060 blood glucose analyzer in a hospital. Roche Diagnostics Precinorm® Universal and Precipath® Universal Control Sera were utilized as the quality control materials for monitoring accuracy and precision of the blood glucose analyzer. The glucose hexokinase method was used to determine the blood glucose concentration. The maximum concentration that can be determined is 33.3 mmol/l. The CV and batch-to-batch error were <2 and <3%, respectively.

3. Results

The injection of sample solution brought about a decrease in the signal to reach the minimum within 6–8 s (see Supplemental Fig. 1 in supplementary material). To evaluate the linearity of the biosensor, different concentrations of glucose were prepared for calibration. The response of the biosensor to glucose levels (1.0–30.0 mmol/l) shows a linear relationship with a linear regression equation of DO = 0.188 * [Glucose] − 0.0331 ([Glucose] = glucose concentration in mmol/l; DO = decrease in O_2 level in mg/l) and $r^2 = 0.9986$ as depicted in the inset of Figure. 1. The limit of detection was calculated to be 0.20 mmol/l from the calibration plot which produced an analytical signal equal to three times the standard deviation of the signal at zero value [16].

The storage stability was periodically evaluated for 6 months at 25 °C by exposing the biosensor to a 0.50-mmol/l glucose standard solution. The biosensor retained 90% of its initial response after 6-month storage at 25 °C.

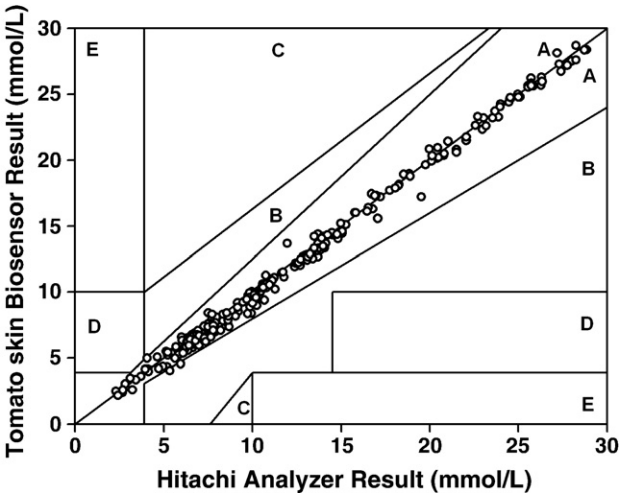

Fig. 2. The Error Grid analysis of two methods on exposure to various blood serum samples. Zone definitions: A, clinically accurate; B, deviating from the reference method by 20% but would lead to benign or no treatment error; C, deviating from the reference method by 20% and would lead to unnecessary corrective treatment errors; D, potentially dangerous failure to detect and treat blood glucose concentrations outside of desired target range; E, would result in erroneous treatment.

Moreover, the stability of the biosensors stored at 4 and 25 °C did not show any significant difference.

The effect of potential interferents in human serum on the response of the glucose biosensor was evaluated by exposing it to interferents. It was found that ascorbic acid, citric acid, uric acid, sucrose aminophenol, lactose and sodium chloride produced only slight interference.

The reversibility and repeatability of the biosensor were also evaluated by measuring the blood serum samples of various glucose concentrations (Fig. 1). The CVs were 1.80, 1.04 and 0.96% for mean (SD) glucose concentrations of 3.07 (0.071), 4.61 (0.068), and 7.63 (0.074) mmol/l (n=3), respectively. The serum samples were also divided into three concentration ranges, *i.e.*, <3.90, 3.90−6.10 and >6.10 mmol/l, according to the physiological and the hypoglycemic and hyperglycemic concentration. Ten measurements were taken for the three individual serum samples, one from each range. The CVs were 1.12, 0.88 and 1.07%, respectively. Reproducibility was evaluated by determining the glucose concentration of three serum samples on consecutive days. Between analyses, the samples were stored and protected from light at 4 °C. The between-day variations were 0.56, 0.49, and 0.63% for samples in the glucose ranges of <3.90, 3.90–6.10 and > 6.10 mmol/l, respectively. For the sensor-to-sensor reproducibility, 10 biosensors with different glucose oxidase-immobilized tomato skin membranes were constructed. These biosensors were applied to determine three serum samples, one from each range, and the CV was found to be 0.95, 0.84 and 1.07%, respectively.

The precision of our method was tested over 30 consecutive days. Ten samples were assessed daily. The maximum concentration was 29.31 (0.095) mmol/l and the CV was 1.04%. The minimum concentration was 3.07 (0.071) mmol/l and the CV was 1.21%. By linear regression analysis, the results of our biosensor (y) correlated well with those of the blood glucose analyzer (x) (y=1.005x−0.233 mmol/l, n=300, $S_{y/x}$=0.356 mmol/l, and r^2=0.9972). Error Grid analysis (Fig. 2) was used to show the clinical accuracy of this biosensor. Over the range of glucose tested, 99.3% of the results fell within zone A as "clinically accurate", and 0.7% fell within zone B as "deviating from the reference method by >20% but would lead to benign or no treatment error". The clinical performance of the two methods was observed to be identical based on the Error Grid analysis. The F-test and t-test also show no significant difference at the 90% confidence level between our proposed biosensor method and the automatic glucose analyzer.

Recoveries were evaluated using serum specimens (1.00 ml each). Specimens were supplemented with 10 μl of 0.20 and 0.40 mol/l glucose (final concentrations, 6.55 and 8.56 mmol/l). The recoveries were 102 and 101%. Thirty different serum samples (numbers 1 to 30) were used to examine recovery in the same way(see Supplemental Table 1 in supplementary material). The recoveries of our biosensor were 95.0%–108% (adding 2.0 mmol) and 95.5%–104% (adding 4.0 mmol). The recoveries of the automatic glucose analyzer were 101%–110% (adding 2.0 mmol) and 99.5%–111%.

4. Discussion

Based on the tomato skin immobilized glucose oxidase, a new blood glucose monitoring system was constructed. This biosensor was designed for rapid determination (6–8 s) of blood glucose concentrations over a wide range of glucose values (1.0–30.0 mmol/l). In order to determine if the biosensor is an acceptable system for measuring blood glucose concentrations, our study was aimed to its stability, accuracy and clinical performance. The blood samples covered a wide range of glucose concentrations (3.07–29.31 mmol/l). It shows that this study was sufficient to represent a larger population of diabetic patients [12].

The stability of the biosensor was assessed by adding 8.0 mmol/l glucose to the buffer solution (see Supplemental Fig. 2 in supplementary material). The signal was very stable before and after the injection of glucose for 83 min. And the biosensor could keep activity at least 6 months. The interference experiment indicated that the biosensor possesses very good selectivity.

The CVs of the biosensor at different glucose levels and experimental conditions were within the satisfactory interval (<2%). It suggests that the biosensor monitoring system provided precise measurements. In fact, the slight difference in tomato membrane performance brings the difference of response, but it can be eliminated by calibrating the biosensors prior to blood glucose determination. A currently marketed glucose monitoring system, Hitachi 7060 blood glucose analyzer, was selected for comparison. The Error Grid analysis showed that the biosensor glucose monitoring system had 100% of measurements in zones A and B. It indicates that the biosensor is a clinically acceptable device for measuring blood glucose concentrations. The linear regression analysis showed that the biosensor results correlated well with the Hitachi 7060 blood glucose analyzer results over the range of glucose concentrations measured. Recoveries were evaluated and it is obvious that our results are convincing. In addition, the results from the Hitachi 7060 blood glucose analyzer were more scattered than that of our biosensor, indicating that our method has better precision in measurement.

In conclusion, our study shows that the proposed glucose biosensor can be successfully applied to determine the concentration of glucose in human serum. The main advantages of this biosensor are simplicity of preparation, fast response time, low cost and high sensitivity. Besides, our results match well with the clinical automatic analyzer (Hitachi 7060) and are more precise. The promising features of our device are simple sensor design and ease of operation in comparison to other analyzer.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cca.2008.05.015.

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