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Preparation and application of a new glucose sensor based on iodide ion selective electrode

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Abstract

An electrode for glucose has been prepared by using an iodide selective electrode with the glucose oxidase enzyme. The iodide selective electrode used was prepared from 10% TDMAI and PVC according our previous study. The enzyme was immobilized on the iodide electrode by holding it at pH 7 phosphate buffer for 10 min at room temperature. The H_2O_2 formed from the reaction of glucose was determined from the decrease of iodide concentration that was present in the reaction cell. The iodide concentration was followed from the change of potential of iodide selective electrode. The potential change was linear in the 4×10^{-4} to 4×10^{-3} M glucose concentration (75–650 mg glucose/100ml blood) range. The slope of the linear portion was about 79 mV per decade change in glucose concentration. Glucose contents of some blood samples were determined with the new electrode and consistency was obtained with a colorimetric method. The effects of pH, iodide concentration, the amount of enzyme immobilized and the operating temperature were studied. No interference of ascorbic acid, uric acid, iron(III) and Cu(II) was observed. Since the iodide electrode used was not an AgI–Ag_2S electrode, there was no interference of common ions such as chloride present in biological fluids. The slope of the electrode did not change for about 65 days when used 3 times a day.

Keywords: Glucose electrode; Membrane electrode; Determination; Glucose in blood; Iodide electrode

1. Introduction

Enzyme electrodes are being used for the measurement of different organic substrates. In most of them a potentiometric sensor, eg.oxygen [1] carbon dioxide [2], or ammonium ion electrodes [3] have been used, but some of them use different voltammetric measuring techniques.

Determination of glucose in human fluids is very important. The development of an electrochemical glucose biosensor with an immobilized enzyme on an electrode surface has been the aim of several recent studies [4–7]. In some of these procedures the enzyme glucose oxidase is used. This enzyme catalyzes the oxidation of glucose to gluconic acid in the presence of oxygen and hydrogen peroxide is formed. Hydrogen peroxide formed during this reaction can be determined with amperometric [4,5,8] or potentiometric [9–11] sensors. In one work [8] a glucose amperometric sensor was prepared where glucose oxidase was immobilized on platinized platinum. Here, glucose oxidase has been covalently bound to poly-2-aminoaniline film. The linear response range was 0.2–20 mM but significant interference of ascorbic acid was observed. Electrodes stored at 4 °C under wet conditions lost 40–50% of their initial glucose response in three days. An amperometric enzyme electrode [4] prepared by glucose oxidase immobilized in poly (vinylferrocenium) showed peak current values which was linear up to 75 mM glucose concentration. The electrode response decreased during the first 20 days. The interference of 2.5 mM ascorbic acid and uric acid has been studied and no noticeable effect was observed. Glucose oxidase membrane systems based on poly(vinyl chloride) matrices have also been prepared [10] for glucose determination. Here, glucose oxidase was entrapped in plasticized PVC and an iodide ion selective electrode was used for iodide monitoring. This electrode had a mean slope of 74 mV/decade of glucose and a lifetime of 7 days. The present work describes the preparation and application of a new potentiometric glucose sensor based

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on glucose oxidase immobilized on an iodide ion sensitive electrode.

2. Experimental

2.1. Apparatus and reagents

Potential measurements were made with JENWAY 3030 Ion Analyser. A double junction Ag/AgCl electrode 9240368, filled with saturated KCl, was used as the outer reference electrode. The enzyme was immobilized on our previously prepared iodide electrode. For the pH measurements, the ion analyzer with 924005 combined pH electrode is used. All measurements were made with a 30 ml glass cell prepared for this purpose. A magnetic stirrer was used throughout the experiments. All reagents used were analytical reagent grade (Merck). Triply distilled water was used for the preparation of solutions.

2.2. Preparation of the electrode

The iodide electrode on which the enzyme was immobilized was prepared according to the procedure developed by us [12]. For this purpose about 180 mg PVC dissolved in 5 ml tetrahydrofuran (THF), 60 mg of ion exchanger, tridodesyl methyl ammonium iodide, dissolved in 1 ml THF and 0.2 ml of dibutylphtalate (DBF) are mixed and stirred. After evaporation of the solvent the film membrane is cemented to a PVC tube with inner diameter of 10mm, the tube is filled with 0.1 M KI and 0.1 M NaCl solution. A home-made Ag/AgCl electrode is immersed as the inner reference.

For the immobilization procedure, first 25 mg of enzyme is dissolved in 5 ml phosphate buffer (pH = 7). The iodide electrode prepared as the above given procedure is kept in it for 2 h at room temperature. This electrode was stored in pH 7 buffer at +2 °C when not in use.

The measurements are made in 19 ml pH 7 phosphate buffer, 1 ml 4×10^{-3} M Mo(VI) and in the presence of 0.01 M iodide solution.

3. Results and discussion

Glucose is oxidized by air oxygen in the presence of glucose oxidase enzyme and hydrogen peroxide is formed, which reacts with iodide ion quantitatively. Thus, this reaction can be used for the determination of glucose when known concentration of iodide is present. The decrease of iodide concentration will be proportional to glucose concentration according to the following reactions.

$$\text{glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

$$H_2O_2 + 2I^- \xrightarrow{\text{Mo(VI)}} I_2 + H_2O$$

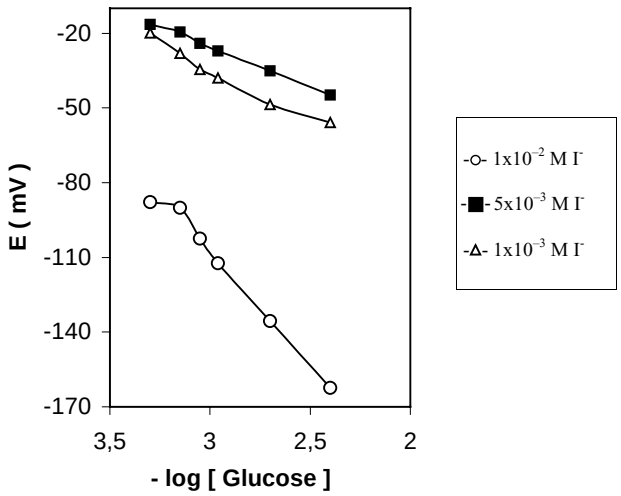

Fig. 1. The effect of iodide concentration on the slope of the electrode.

Molybdenum(VI) or peroxidase enzyme can be employed as the catalyst, [9–11], the last has the advantage of higher efficiency. As can be seen two moles of iodide is used for one mole of glucose. Thus the change of concentration of iodide after reaction with glucose can be used for the determination of glucose.

In this work we used iodide electrode for the determination of iodide concentration before and after the reaction with glucose.

3.1. Effect of iodide concentration

Since one mole of glucose uses two moles of iodide its concentration has to be higher than glucose concentration. But it cannot be too high since according to the Nernst equation the log term will not be large enough to measure the change in ΔE value. Thus the optimum concentration of iodide has to be determined for a glucose concentration that is in the range of glucose present in blood. For this purpose solutions with 19 ml pH 7 phosphate buffer and 1 ml 4×10^{-3} M (Mo(VI)) have been prepared containing various iodide concentrations of 1×10^{-3}, 5×10^{-3} and 1×10^{-2} M. Their potentials were measured and then after each glucose addition once more the potentials were measured. The glucose concentrations in the cell were in the range of blood serum, changing from 4.5×10^{-4} M (80 mg/100 ml) to 4×10^{-3} M (660 mg/100 ml blood). As can be seen from Fig. 1 the slope was the highest for 10^{-2} M iodide concentration.

3.2. Effect of pH and buffer concentration

Adjustment of pH is important both for the immobilization of enzyme and for the reaction between hydrogen peroxide and iodide. At different pH values changing from 8 to 5.5 the response of electrode has been measured against glucose concentration when 0.01 M iodide was present. Whereas there was nearly no response at pH values of 5.5, 6, 6.5,

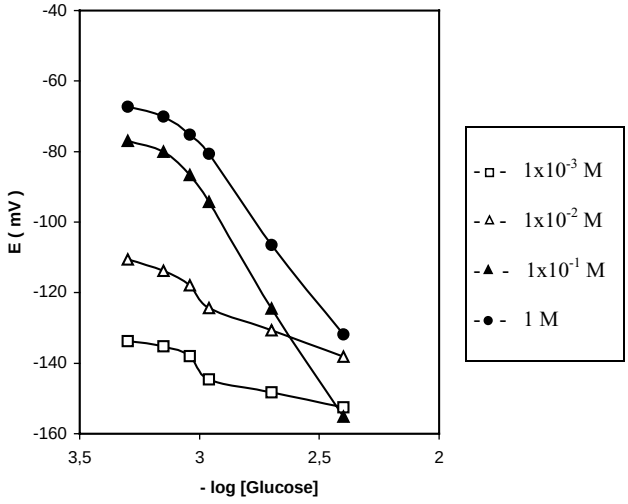

Fig. 2. The change of potential with buffer concentration (10^{-2} M I^-).

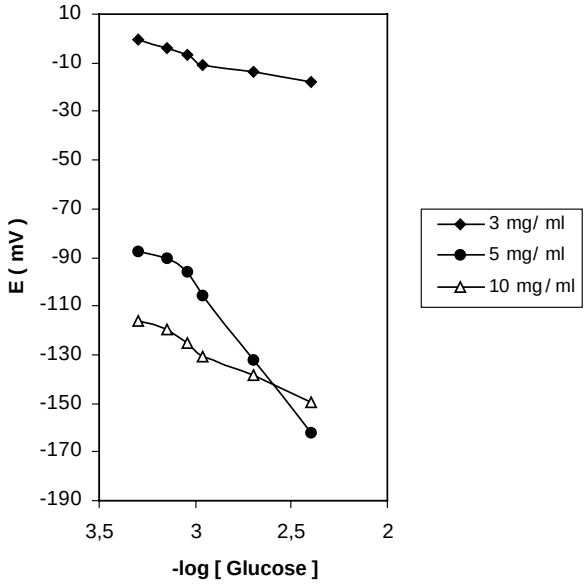

Fig. 3. The effect of immobilized enzyme quantity on the slope of the electrode (10^{-2} M I^-).

7 and 8, the slope was the largest at pH 7. At this pH the ion exchanger (mentioned in Section 3.3) is positive and enzyme is negative, thus it is the most convenient pH for immobilization and for the reaction.

Phosphate buffer has been chosen because of its pH working area. Its response for glucose has been investigated at buffer concentrations changing from 1.0 to 10^{-3} M. The change of potential against glucose concentration is given in Fig. 2. As can be seen 0.1 M buffer concentration had the largest slope.

3.3. Immobilization of enzyme

The ion exchanger TDMAI on the iodide electrode becomes a positive charge at pH = 7, at this pH the enzyme becomes minus charge and thus the enzyme will be immobilized on the electrode surface. The quantity of enzyme will be important since only one part of it can be immobilized on the electrode surface. For this purpose iodide electrode was dipped into pH 7 phosphate buffer solutions each containing 3, 5 and 10 mg/ml enzyme for 2 h. It was washed with distilled water and its response has been measured for glucose concentrations in the presence of 0.01 M iodide. As can be observed from Fig. 3, the slope was the largest for 5 mg/ml enzyme.

3.4. Effect of temperature

Optimum temperature is very important since the enzyme activity will increase with temperature, but on the other hand at high temperatures there may be thermal deactivation of the enzyme and also decrease of O_2 concentration. In a solution containing 0.01 M iodide, the potential of a solution of 2×10^{-3} M glucose has been followed between temperatures of 25–55 °C within 5 °C intervals. The maximum activity of the enzyme was obtained at 30 °C, Fig. 4.

3.5. Reproducibility, response and lifetime

This electrode did not loose its activity for 65 days when used 3 times a day. The response time was measured at different glucose concentrations. As can be seen from Fig. 5 the response was almost immediate. The lifetime of the electrode is also very good compared with other electrodes [5,8,10,11]. The slope of the linear portion was about 79 ± 2 mV per decade change in glucose concentration.

3.6. Interference studies

The product of enzymatic reaction is hydrogen peroxide, thus reducing agents such as uric acid and ascorbic acid, two compounds commonly found in biological fluids, may interfere [8,9]. The strong interference of uric acid and ascorbic

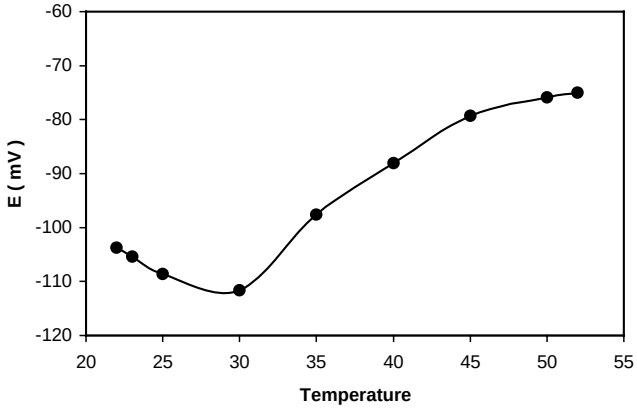

Fig. 4. The effect of temperature on the activity of the enzyme (2×10^{-3} M glucose, 10^{-2} M I^-).

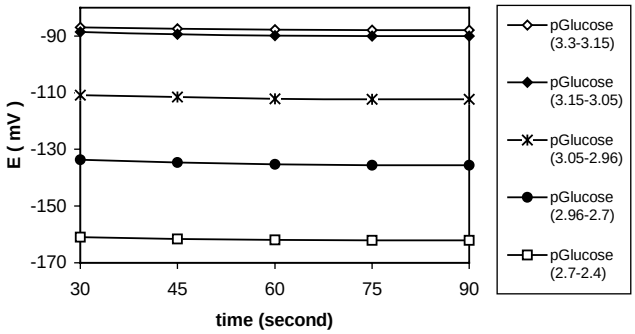

Fig. 5. The dependence of response time of the electrode on the change of concentration.

Table 1
Selectivity coefficients ($k^{pot}_{A,B}$) for the glucose electrode in mixed solutions (in the presence of 4.5×10^{-4} M glucose)[a]

	B			
	Uric acid	Ascorbic acid	Fe^{3+}	Cu^{2+}
$k^{pot}_{A,B}$	5.7×10^{-4}	4.6×10^{-4}	2.3×10^{-4}	4.8×10^{-4}

[a] A: glucose; B: interfering ion.

acid in a former study [9] was eliminated after pre treatment with hydrogen peroxide. With our new electrode in the presence of 0.01 M iodide and 4.5×10^{-4} M glucose there was no interference from the above mentioned substances in the concentration ranges that are commonly encountered in biological fluids (0–2.5 mM). The selectivity constants determined by using the mixed solution method [13] are given in Table 1. Since the iodide sensitive electrode used here was not a Ag_2S–AgI, but it was an ion exchanger membrane [12], no interference was observed from the ions (Cl^-, SO_4^{--}) commonly found in biological fluids.

3.7. Measurement of glucose in blood serum

It was found that, with this new electrode the glucose could be determined with high accuracy and precision, Table 2. For a solution containing 65 mg glucose/100 ml the result obtained for 4 measurements was 64 ± 2 mg/100 ml.

The blood samples that were analyzed for their glucose quantity were obtained from the University Health Center. They were first centrifuged with a speed of 9000 round/min

Table 2
Determination of glucose in known samples[a]

Glucose (mg/100 ml)	Glucose determined with the new electrode (mg/100 ml)		
x_i	$\bar{x}$	S	CI*
65	64	1.4	64 ± 2

[a] Number of samples, $N = 4$; 95% confidence interval.

Table 3
Glucose levels in three different blood samples

Quantity given from Health Center (mg/100 ml)	Glucose levels, with the new electrode (mg/100 ml), deviation from mean ($n = 4$)
127	123 ± 1
123	119 ± 1
113	110 ± 2

and these were used for glucose determination. First the potential of a solution containing 19 ml buffer (pH = 7), 1 ml 4×10^{-3} M Mo(VI) and 0.01 M iodide was measured. A 0.1 ml of serum sample was added and the potential was once more measured. Then two standard additions of 0.1 M glucose (each 0.1 ml) were made and potentials were measured. From the change of potentials the amount of glucose in blood was determined. Blood samples shall not wait long time, otherwise glucose will be lost because of destruction. If it has to wait additions of fluoride or ascorbic acid is needed.

Glucose quantities for three different blood samples are given in Table 3 with the results of University Health Center (colorimetric) for comparison.

4. Conclusions

A new enzyme based electrode is prepared by using an iodide selective electrode. Here one electrode works as enzyme holder and at the same time it monitors the iodide concentration. Since the iodide electrode is constructed with an ion exchanger and not with AgI, it does not show any interference of most common ions such as chloride and sulfate. The prepared sensor displayed very good performance in regard to reproducibility, sensitivity and long lifetime. It shows linear response in the 75–650 mg/100 ml concentration range with a slope of about 79 mV per decade change of glucose.

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References

[1] S. Updike, G.P. Hicks, Science 158 (1967) 270.
[2] G.G. Guilbault, F. Shu, Anal. Chem. 44 (1972) 1261.
[3] G.G. Guilbault, G. Nagy, Anal. Chem. 45 (1973) 417.
[4] H. Gülce, H. Özyörük, S.S. Çelebi, A. Yýldýz, J. Electroanal. Chem. 394 (1995) 63.
[5] Li-Xian Sun, F. Xu, T. Okada, Talanta 47 (1998) 1165.
[6] L. Campanella, M. Tomassetti, Selective Electrode Rev. 11 (1989) 69.

[7] M. Macsini, G.G. Guilbault, Biosensors 2 (1986) 147.
[8] J. Losada, M. Pilar, Electroanalysis 9 (18) (1997) 1416.
[9] G. Nagy, L.H. Von Storp, G.G. Guilbault, Anal. Chim. Acta 66 (1973) 443.
[10] I.K.Al. Hitti, G.J. Moody, J.D.R. Thomas, Analyst 109 (1984) 1205.
[11] A. Merkoçi, E. Fabregas, S. Alegret, Sens. Actuators B 60 (1999) 97.
[12] G. Somer, Ş Kalaycı, G. Ekmekçi, Sens. Actuators B 81 (2001) 122.
[13] K. Srinivasan, G.A. Rechnitz, Anal. Chem. 41 (1969) 1203.