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Preparation and Characterization of Simple Amperometric Biosensor for Glucose with Alkali Nickel Hexacyanoferrate(II) Electrocrystallized on Nickel Electrode and Glucose Oxidase Immobilized in Bovine Serum Albumin Cross-linked with Glutaraldehyde

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Summary

Preparation and characterization of a simple amperometric enzyme-based glucose sensor is reported. The enzyme glucose oxidase is immobilized in bovine serum albumin by cross-linking with glutaraldehyde on the layer of alkali nickel hexacyanoferrate(II) electrocrystallized on the surface of nickel electrode. The selective biocatalytic effect of the enzyme glucose oxidase on the oxidation of glucose and catalytic effect of nickel electrode modified with alkali nickel hexacyanoferrate(II) on the reduction of hydrogen peroxide, produced by the ezymatic reaction, enables the amperometric glucose biosensor to operate using working potentials between –300 and –100 mV vs. SCE, where the oxidation of most important interferences present in human blood (ascorbic or uric acid) is not possible. Other characteristics of the reported biosensor are: practically independent current response of pH in the range between 6.7 and 7.7, response time for 0.3 mM glucose addition in sodium phosphate buffer solution (0.01 M phosphate ion, pH = 7.4) and 0.1 M of sodium perchlorate shorter than 20 s, relatively long time stability (during three months of operation the sensitivity decreased only 30% of the initial sensitivity) and linear range of glucose concentration up to 1.2 mM.

Keywords: biosensor for glucose, alkali nickel hexacyanoferrate, nickel electrode, glucose oxidase

Introduction

In the past six years there is an increased interest in development of different biosensors suitable for monitoring glucose concentration in different samples and at different concentration levels. In Science Citation Index from 1990 to 1993, there were reported 14, 16 and 16 papers per year, respectively, dealing with this topic. However, from 1993 to 1995 an increase by factor of ten in the papers published per year on glucose biosensors (160, 175 and 176, respectively) show an increased interest in developing a reliable, accurate and interference-free biosensor for this important analyte. Several most recent reviews are covering different aspects of preparation and applications of biosensor for glucose *(1–5)*. Despite so impressive number of papers on biosensors for glucose, Cristiansen and Jakobsen *(6)* discussed the reasons for slow penetration of enzyme-based biosensors into clinical chemical analysis, although many of the reported biosensors have been prepared for measuring the glucose directly in blood or in diluted blood samples *(7–10)*. Similar situation exists in the glucose biosensor application in food analysis and in bioreactor process monitoring and control. The main requirements in developing glucose biosensor suitable for food analysis, bioprocess monitoring and control and clinical applica-

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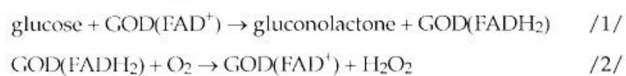
tion are: high accuracy and reliability in glucose determination directly in untreated samples or only diluted samples, interferences-free glucose determination, wide linear range, acceptable life-time and shelf-life and simplicity for mass production. To meet all these requirements it is not a simple task and it is not surprising that approximately fifty per cent of patents cover the glucose biosensors, in the same time when the fraction of sensor for this analyte is only about ten per cent of published papers *(6)*.

Many different techniques have been used to develop glucose sensor, most of them based on immobilized glucose oxidase (GOD) as an enzyme which selectively catalyzes the oxidation of glucose into gluconolactone with subsequent regeneration of its oxidized form, according to the following reactions *(11–16)*:



$$\text{glucose} + \text{GOD(FAD}^+) \rightarrow \text{gluconolactone} + \text{GOD(FADH}_2) \quad /1/$$

$$\text{GOD(FADH}_2) + O_2 \rightarrow \text{GOD(FAD}^+) + H_2O_2 \quad /2/$$

where GOD($FADH_2$) and GOD(FAD^+) represent reduced and oxidized enzyme forms, respectively. In reaction /2/ it can be seen that oxygen is necessary as an electron acceptor enabling oxidation of GOD($FADH_2$) into GOD(FAD^+) and producing hydrogen peroxide in the enzymatic reaction. Majority of investigated amperometric glucose biosensors measure the current of reduction or oxidation of H_2O_2, or reduction of oxygen, which is proportional to glucose concentration. However, electrochemical oxidation of hydrogen peroxide at relatively high positive potentials leads to the interference of many electroactive compounds present in the sample, which can be oxidized at positive potentials. To reduce high positive operating potentials, many different chemical substances (redox mediators) have been used as electron acceptors instead of oxygen *(17–21)*. Some authors have catalyzed oxidation of hydrogen peroxide by means of the noble group metals or their complex compounds *(22–24)*. Hexacyanoferrate ions in solutions have also been used as electron mediators *(25)* or for catalytic oxidation of reduced nicotine adenine dinucleotide (NADH) *(26)*.

The immobilization of GOD in most cases is performed by enzyme entrapment in an inert matrix or alternatively GOD is immobilized in different forms of thin film covering the base electrode surface. Graphite paste *(27)* or carbon fibers *(28)* have been reported as surfaces suitable for enzyme layer support. Conducting biocomposite materials made by mixing of graphite powder with epoxy resins, Teflon, poly(esters), poly(urethanes), etc. are also used for glucose biosensor matrices *(29–33)*.

This paper describes the preparation and characterization of a simple electrochemical glucose biosensor based on glucose oxidase, immobilized by cross-linking the enzyme using bovine serum albumin and glutaraldehyde on the surface of the alkali nickel hexacyanoferrate(II) modified nickel electrode. Nickel electrode modified with electrocrystallized alkali nickel hexacyanoferrate catalyzes the reduction of hydrogen peroxide, formed by enzymatic reaction /2/ at negative potentials. Depending on the interferences, the working potential can be selected between –300 and –100 mV *vs.* SCE, preventing the oxidation of most interferences present in different samples. The reported biosensor shows the current response practically independent of pH in the range between 6.7 and 7.7, response time for 0.3 mM glucose addition in solution of 0.1 M sodium perchlorate and sodium phosphate buffer solution (0.01 M phosphate ion, pH = 7.4) shorter than 20 s, relatively long time stability and linear range of glucose concentration up to 1.2 mM.

Materials and Methods

Chemicals and Reagents

All chemicals used were of analytical reagent grade. Glucose oxidase (GOD, from *Asperigillus niger*, 200 U/mg), bovine serum albumin (BSA), glutaraldehyde (GLA, w = 25%) and D(+)-glucose were obtained from »Sigma«. Potassium hexacyanoferrate(II) trihydrate, hydrogen peroxide (w = 30%), L(+)-ascorbic acid, and uric acid were from »Kemika« chemical company. The supporting electrolyte used for preparing the hexacyanoferrate film was 0.1 M sodium perchlorate containing 5 mM of potassium ferrocyanide in sodium phosphate buffer (0.01 M phosphate ion, pH = 7.4). All other solutions were prepared or diluted with sodium phosphate buffer solution (0.01 M phosphate ion, pH = 7.4). Deionized water was purified using a Milipore-MilliQ system. Samples of heparized human blood were supplied by Clinical Hospital Center »Rebro« and were free from human immunodeficiency virus and hepatitis B surface antigen.

Electrode Preparation

Nickel wire (2 mm in diameter and 5 mm long) was soldered with a copper wire, inserted into a glass tube (4–5 mm in diameter and 10 cm long) and sealed with epoxy resin to make the inner part of the tube water-tight. The formed nickel disc with a working area of 1 to 3 mm^2 (unless otherwise stated) was thoroughly rinsed in deionized water, acetone and finally air dried. A thin layer of black porous nickel was made on the surface of the disk using the known procedure *(34)*. After cleaning and drying, the layer of alkali nickel hexacyanoferrate(II) film was electrocrystallized on the top of the porous black nickel *(26)*.

Glucose oxidase (GOD) was immobilized by the glutaraldehyde-bovine serum albumin cross-linking procedure on the top of alkali nickel hexacyanoferrate layer. Into a volume of 1 mL of the 10% solution of BSA prepared in sodium phosphate buffer (pH = 7.4) the mass of 15 mg of GOD was added. This albumin solution was mixed well with equal volume of 2.5% glutaraldehyde solution and 5 μL of this mixture was deposited on the top of alkali nickel hexacyanoferrate(II) modified nickel electrode and left to dry for 20 minutes at room temperature in order to allow the formation of gel. The prepared enzyme electrode was conditioned overnight in phosphate buffer solution (pH = 7.4) at 4 °C.

Apparatus

All electrochemical experiments were performed using a EG&G PAR (USA) Model 264A Polarographic Analyzer, home-interfaced to an IBM compatible personal computer for data acquisition, manipulation and printout.

Three-electrode electrochemical cell was used having the electrochemical biosensor as a working electrode, saturated calomel electrode (SCE) as a reference electrode and graphite rod as an auxiliary electrode.

Results and Discussion

Characterization of the Glucose Biosensor

Cyclic voltammograms (18 scans) obtained, with a potential scan rate of 50 mV/s during the formation of alkali nickel hexacyanoferrate layer in a solution of sodium phosphate buffer (0.01 M phosphate ion, pH = 7.4), 0.05 M potassium ferrocyanide and 0.1 M sodium perchlorate, on the nickel electrode in potential range between –100 and 1000 mV, are shown in Fig. 1. The growth of alkali nickel hexacyanoferrate layer is clearly seen. The same patterns were obtained using scan rates of 10, 20 and 100 mV/s.

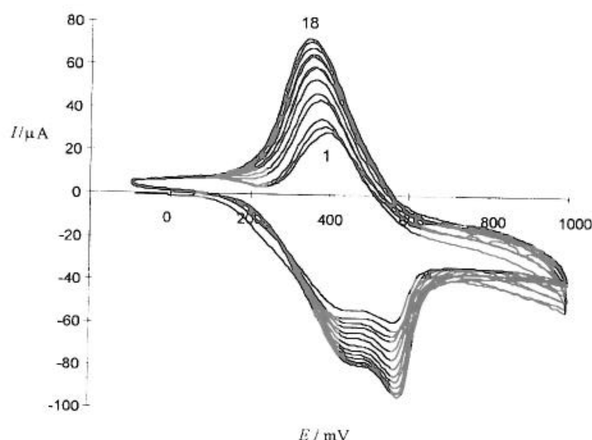


Fig. 1. Cyclic voltammograms (18 scans) recorded with potential scan rate of 50 mV/s using unmodified nickel electrode during the formation of alkali nickel hexacyanoferrate layer in solution of sodium phosphate buffer (0.01 M phosphate ion, pH = 7.4), 0.05 M potassium ferrocyanide and 0.1 M sodium perchlorate.

The cyclic voltammograms obtained with potential scan rate of 10, 20 50 and 100 mV/s (voltammograms 1, 2, 3 and 4, respectively) at alkali nickel heksacyanoferrate modified nickel electrode in sodium phosphate buffer solution are shown in Fig. 2. The cyclic voltammograms are broad suggesting that two steps of oxidation and reduction of the Fe(II)/Fe(III) couple in the crystallized alkali nickel hexacyanoferrate lattice on the electrode surface are taking place without changing the lattice. The compactness of the formed electrocrystallized layer can be estimated recording such cyclic voltammograms for different alkali nickel hexacyanoferrate modified nickel electrodes. It was found that when the current at negative potential is lower, the quality of the formed electrocrystallized alkali nickel hexacyanoferrate layer is better.

The unmodified nickel electrode cyclic voltammograms, recorded with potential scan rate of 50 mV/s, in deaerated solution of phosphate buffer (pH = 7.4) and

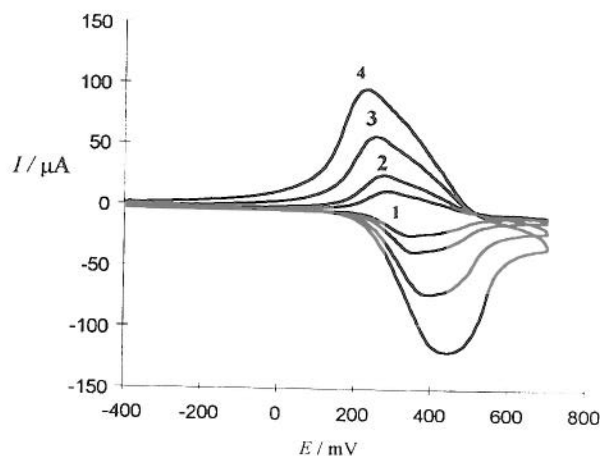


Fig. 2. Cyclic voltammograms recorded with different potential scan rates using alkali nickel hexacyanoferrate modified nickel electrode in solution of 0.1 M sodium perchlorate and sodium phosphate buffer (0.01 M phosphate ion, pH = 7.4). $\Delta E/\Delta t$ = 10, 20, 50 and 100 mV/s for voltammograms 1, 2, 3 and 4, respectively.

0.1 M sodium perchlorate and in the same phosphate buffer solution with added 1 mM solution of H_2O_2 are shown in Fig. 3 (voltammograms 1 and 2, respectively). The same experiment was made using alkali nickel hexacyanoferrate modified nickel electrode (Fig. 4). The increase of H_2O_2 reduction current in the later case and the increase of this current at more negative potentials show the catalytic effect of alkali nickel hexacyanoferrate modified nickel electrode, suggesting in the same time that it will be more favourable to select working potential as negative as possible.

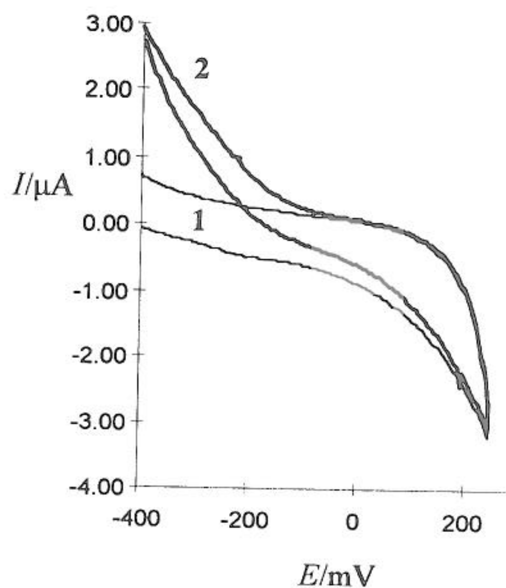


Fig. 3. Cyclic voltammograms recorded with scan rate of 50 mV/s using unmodified black nickel electrode in: (1) deaerated sodium phosphate buffer solution (pH = 7.4) and 0.1 M sodium perchlorate and (2) same solutions with 1 mM H_2O_2.

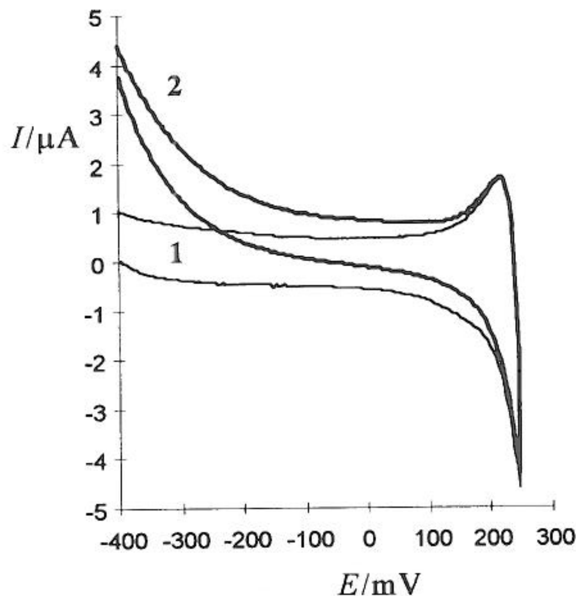


Fig. 4. Cyclic voltammograms recorded with scan rate of 50 mV/s using alkali nickel hexacyanoferrate modified nickel electrode in: (1) deaerated sodium phosphate buffer solution (0.01 M phosphate ion, pH = 7.4) and 0.1 M sodium perchlorate and (2) same solutions with 1 mM H_2O_2.

However, limitation at negative potentials are set by the oxygen, which should be present in the solution as electron mediator, and whose reduction at potentials more negative than –300 mV interfere with the electroreduction of hydrogen peroxide (Fig. 5, voltammogram 2).

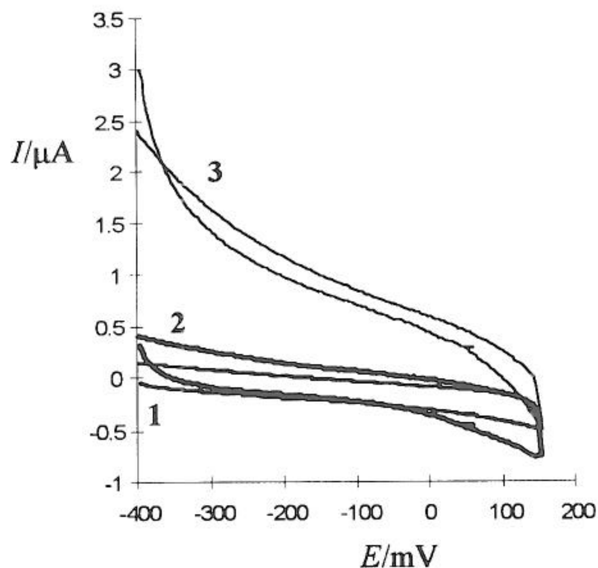


Fig. 5. Cyclic voltammograms recorded with scan rate of 50 mV/s using alkali nickel hexacyanoferrate modified nickel electrode in: (1) deaerated sodium phosphate buffer solution (0.01 M phosphate ion, pH = 7.4) and 0.1 M sodium perchlorate, (2) same non-deaerated solution with present oxygen and (3) same solution with 1 mM H_2O_2.

The influence of other possible interferences present in samples of human blood are shown in Fig. 6. The cyclic voltammograms were recorded using glucose biosensor in deaerated buffer solution (voltammogram 1 in Fig. 6) and in non-deaerated buffer solution with addition of 1 mM solution of H_2O_2, uric and ascorbic acids (voltammograms 2, 3 and 4, in Fig. 6, respectively). From these voltammograms it can be seen that in the potential range between –400 and 100 mV *vs.* SCE, there is no significant current response to neither uric nor ascorbic acid. The ascorbic acid produced significant oxidation current response only at potential of approximately 150 mV, which is 250 to 450 mV more positive than the possible glucose biosensor working potential set in the range between –100 and –300 mV. The strong catalytic effect of immobilized alkali nickel hexacyanoferrate on nickel electrode is seen from the higher current of H_2O_2 reduction at negative potentials. The increase of cathodic current at lower negative potentials resulted in higher sensor sensitivity whereas oxygen had a strong influence on the current response at potentials lower than –300 mV *vs.* SCE. As a consequence of these characterization a working potential of –200 mV *vs.* SCE is selected for further glucose determination.

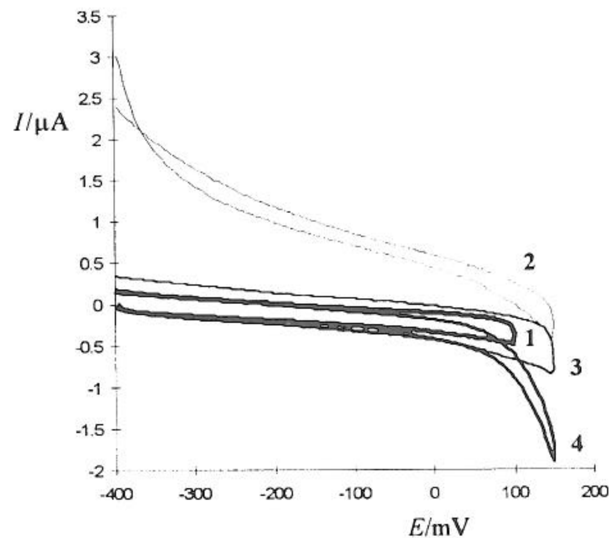


Fig. 6. Cyclic voltammograms recorded with scan rate of 50 mV/s using alkali nickel hexacyanoferrate modified nickel electrode in: (1) deaerated sodium phosphate buffer solution (0.01 M phosphate ion, pH = 7.4) and 0.1 M sodium perchlorate, (2) non-deaerated solution (1) with 1 mM H_2O_2, (3) non-deaerated solution (1) with 1 mM of uric acid and (4) non-deaerated solution (1) with 1 mM of ascorbic acid.

Chronoamperometric response at –200 mV *vs.* SCE of the GOD/alkali nickel hexacyanoferrate modified nickel electrode having larger area than the rest of the used electrodes (approximately 6.2 mm^2) for an addition of 0.5 mM of glucose in non-deaerated phosphate buffer solution (pH = 7.4) is shown in Fig. 7. The glucose was added in time »1«. The biosensor responded with current increase of approximately 0.07 μA and in relatively short time ($t < 60$ s) reached the constant value. In time

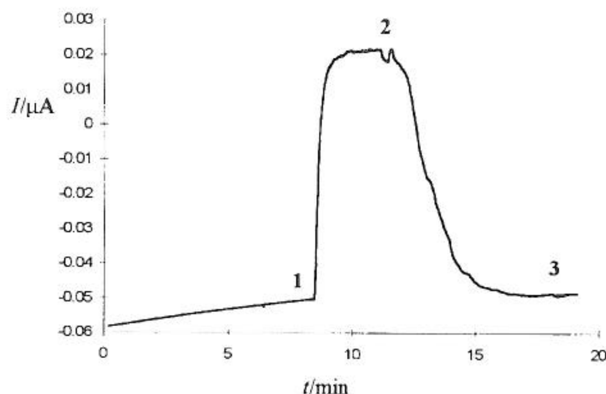


Fig. 7. Chronoamperogram of the glucose biosensor response on the addition of 0.5 mM of glucose in non-deaerated solution of sodium phosphate buffer (0.01 M phosphate ion, pH = 7.4) and 0.1 M sodium perchlorate. E_w = –200 mV *vs.* SCE; *A*(electrode) ≈ 6.3 mm^2.

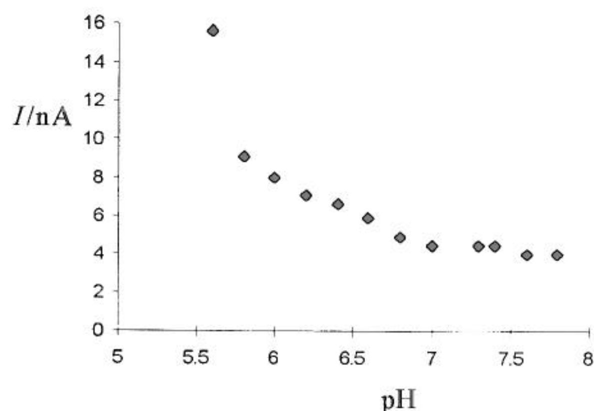


Fig. 8. Effect of pH on the current response of amperometric glucose biosensor. E_w = –200 mV *vs.* SCE, *c*(glucose) = 0.5 mM.

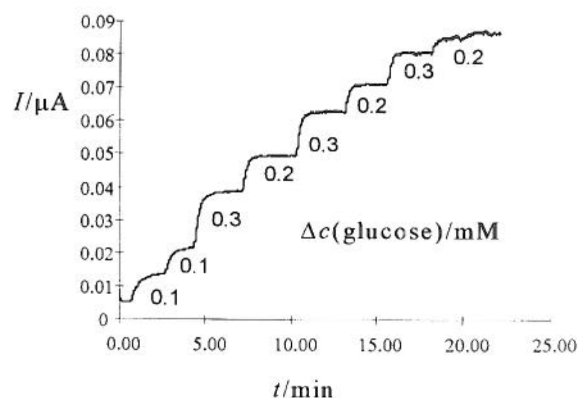


Fig. 9. Typical biosensor response to successive glucose addition in non-deaerated solution of sodium phosphate buffer (0.01 M phosphate ion, pH = 7.4) and 0.1 M sodium perchlorate. E_w = =–200 mV *vs.* SCE.

»2« the solution was purged with nitrogen. Nitrogen purging decreases the concentration of O_2 and consequently the concentration of H_2O_2, formed in enzymatic reaction /2/ in the solution decreases shown by current decrease. This current decrease reaches the constant background current value after 5 min. In time »3« another addition of 0.5 mM of glucose was made and the current response did not change, which means that oxygen molecule is the only responsible electron-acceptor or redox-mediator.

The influence of pH on biosensor sensitivity was also investigated. In Fig. 8 the current response of amperometric glucose biosensor is plotted *vs.* pH at working potentials of –200 mV *vs.* SCE. The biosensor sensitivity decreases with increasing of pH. In the pH range between 6.7 and 7.7 the biosensor response is practically independent of pH. Taking into account that the pH of the blood of healthy person is between 7.35 and 7.45, and in cases of some diseases it may eventually extend to 6.5 and 7.5, the proposed glucose biosensors might be very suitable for routine glucose determination in blood.

A typical example of amperometric biosensor response on successive addition of glucose obtained at working potential of –200 mV *vs.* SCE in sodium phosphate buffer solution (0.01 M phosphate ion, pH = 7.4) is shown in Fig 9. The saturation effect, typical for enzyme electrodes, can be seen at higher glucose concentrations. Calibration diagrams recorded in sodium phosphate buffer solution (0.01 M phosphate ion, pH = 7.4) at different working potentials –100, –200 and –300 mV (Table 1) show linear dependence up to 1.2 mM of glucose. At higher glucose concentration the biosensor response show non-linear response due to the saturation effect. At more negative potentials the biosensors sensitivity and background current are higher, while the correlation coefficients and lack of fits are lower (Table 1), most likely due to the oxygen interference at more negative potentials shown in Fig. 5.

The response time of the glucose biosensor is relatively fast. In Fig. 10 it is shown a chronoamperometric

Table 1. Parameters obtained from least squares linear regression of biosensor current response *vs.* glucose concentration, $I = (a \pm \text{std. err.}) + (b \pm \text{std. err.})\, c(\text{glucose})/\text{mM}$, obtained in 0.01 M phosphate buffer solution (pH = 7.4) at different working potentials (E_w)

E_w / mV	(a ± std. err.)/nA	(b ± std. err.)/(nA/mM)	r	L.O.F./%
–100	1.1 ± 2.2	38.8 ± 1.8	0.9958	4.8
–200	9.9 ± 3.4	55.0 ± 2.6	0.9946	5.4
–300	28.7 ± 7.0	92.0 ± 5.3	0.9918	5.8

r = correlation coefficient;

L.O.F. (lack of fit) = $\Sigma(I_{calc.}-I_{exp.})^2/\Sigma(I_{exp.})^2$

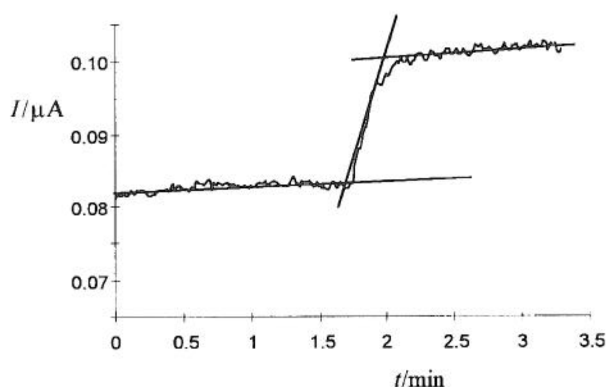


Fig. 10. Response time on a single 0.3 mM glucose concentration addition in non-deaerated solution of sodium phosphate buffer (0.01 M phosphate ion, pH = 7.4) and 0.1 M sodium perchlorate. E_w = –200 mV *vs.* SCE.

response profile of the biosensor in sodium phosphate buffer solution (0.01 M on phosphate ion, pH = 7.4) for an addition of 0.3 mM glucose. From this chronoamperogram the response time is estimated to be approximately 15 s, calculated as the time difference between a cross-sections of tangents on background current and the current jump after addition of glucose, and a cross-section of tangents on a current jump and on limiting current.

Glucose Determination in Samples of Human Blood

The glucose concentration in blood samples collected from diabetic patients varied from 1.2 to 20 mM. As the calibration graph obtained was linear up to 1.2 mM of glucose, the blood samples were diluted (in volume ratio 1 : 20) with solution of sodium phosphate buffer (0.01 M phosphate ion, pH = 7.4) and 0.1 M of sodium perchlorate. Results of glucose concentration obtained amperometrically were compared with those obtained spectrophotometrically using commercial glucose analyzer Ectachem 500 (Kodak, Rochester, USA). In Fig. 11 is shown a plot of glucose concentration difference, expressed in percentage of the value obtained spectrophotometrically, *vs.* the glucose concentration in blood samples. Results obtained using amperometric biosensor were an average of three separate measurements of the same sample. Good agreement (better than ±3%) was obtained for glucose concentrations in blood samples between 3 and 20 mM. For glucose concentration in blood samples below 3 mM the result obtained with amperometric biosensor were lower than those obtained using Ectachem 500 Analyzer for up to 20%. No adsorption of blood protein on the electrode surface was observed.

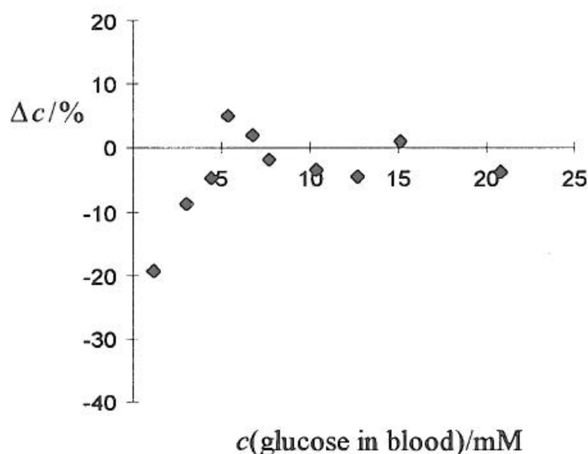


Fig. 11. Comparison between glucose concentration determined in human blood samples using amperometric glucose biosensor and commercial spectrophotometric Ectachem 500 Analyzer. $\Delta c/\% = 100 \cdot [c(\text{glucose, biosensor}) - c(\text{glucose, Ectachem})]/c(\text{glucose, Ectachem})$.

Conclusion

The described amperometric glucose biosensor prepared using glucose oxidase immobilized on the alkali nickel hexacyanoferrate modified nickel electrode *via* the BSA glutaraldehyde cross-linking procedure operates at very negative working potentials (–300 to –100 mV *vs.* SCE) preventing the oxidations of main interferences present in samples of human blood (ascorbic and uric acids). The biosensor has a fast response time and linear-concentration range up to 1.2 mM of glucose. Good agreement with spectrophotometric method using commercial glucose analyzer was obtained. Taking into account all the characteristics and long-term stability (only 30% of sensitivity decrease was observed after 3 months of operation) it can be concluded that this sensor might be suitable for routine work. Some possible improvements of the biosensor characteristics and its use as a detector in flow injection analysis are under investigation.

Acknowledgement

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References

1. S. P. J. Higson, P. M. Vadgama, *Med. Biol. Eng. & Comput. 32* (1994) 601.
2. A. Mulchandani, A. S. Bassi, *Crit. Rev. Biotechnol. 15* (1995) 105.
3. L. Gorton, *Electroanalysis, 7* (1995) 23.
4. P. Pantano, W. G. Kuhr, *Electroanalysis, 7* (1995) 405.
5. S. A. Jaffari, A. P. F. Turner, *Physiol. Measurement, 16* (1995) 1.
6. T. F. Christiansen, K. M. Jakobsen, *Acta Anaesthesiol. Scand. 39* (1995) 31.
7. L. Risinger, T. Buch-Rasmussen, G. Johansson, *Anal. Chim. Acta, 231* (1990) 165.
8. H. Gunasingham, C. Huattan, *Anal. Chim. Acta, 229* (1990) 83.
9. B. H. Ginsberg, *Clin. Chem. 38* (1992) 1596.
10. T. J. Ohara, R. Rajagopalan, A. Heller, *Anal. Chem. 66* (1994) 2451.
11. P. N. Bartlett, D. J. Caruana, *Analyst, 117* (1992) 1287.
12. P. N. Bartlett, R. G. Whitaker, *J. Electroanal. Chem. 224* (1987) 37.

13. D. Bélanger, J. Nadreau, G. Fortier, *J. Electroanal. Chem. 274* (1989) 143.
14. P. Janda, J. Weber, *J. Electroanal. Chem. 300* (1991) 119.
15. P. N. Bartlett, P. Tebbutt, C. H. Tyrrell, *Anal. Chem. 64* (1992) 138.
16. C. Malitesta, F. Palmisano, L. Torsi, P. G. Zambonin, *Anal. Chem. 62* (1990) 2735.
17. B. A. Gregg, A. Heller, *Anal. Chem. 64* (1992) 258.
18. C. E. Uhegbu, K. B. Lim, H. L. Pardue, *Anal. Chem. 65* (1993) 2443.
19. S. K. Beh, G. J. Moody, J. D. R. Thomas, *Analyst, 116* (1991) 459.
20. P. D. Hale, L. I. Boguslavsky, T. Inagaki, I. H. Karan, H. S. Lee, A. T. Skotheim, *Anal. Chem. 63* (1991) 677.
21. J. Wang, L. Fang, D. Lopez, H. Tobias, *Anal. Lett. 26* (1993) 1819.
22. J. Wang, J. Liu, L. Chen, F. Lu, *Anal. Chem. 66* (1994) 3600.
23. J. Wang, Q. Chen, *Analyst, 119* (1994) 1849.
24. J. Wang, L. H. Wu, Z. Lu, R. Li, J. Sanchez, *Anal. Chim. Acta, 228* (1990) 251.
25. P. N. Bartlett, Z. Ali, V. Eastwick-Field, *J. Chem. Soc. Faraday Trans. 88* (1992) 2677.
26. B. F. Y. Hin, C. R. Lowe, *Anal. Chem. 59* (1987) 2111.
27. W. Matuszewski, M. Trojanowicz, *Analyst, 113* (1988) 735.
28. E. R. Reynolds, A. M. Yacynych, *Electroanalysis, 5* (1993) 405.
29. J. Wang, K. Varughese, *Anal. Chem. 62* (1990) 318.
30. J. Wang, A. J. Reviejo, L. Angnues, *Electroanalysis, 5* (1993) 575.
31. F. Céspedes, E. Martinez-Fàbregas, S. Alegret, *Anal. Chim. Acta, 284* (1993) 21.
32. F. Céspedes, E. Martinez-Fàbregas, S. Alegret, *Electroanalysis, 6* (1994) 759.
33. S. Alegret, F. Céspedes, E. Martinez-Fàbregas, D. Martorell, A. Morales,E. Centelles, J. Muñoz, *Biosensors Bioelectronics, 11* (1996) 35.
34. S. Field, A. D. Weill: *Electro-Plating*, Pitman & Sons, London (1964) p. 296.

Priprema i karakterizacija jednostavnog amperometrijskog biosenzora za određivanje glukoze s alkalijskim niklenim heksacijanoferatom(II) elektrokristaliziranim na niklenoj elektrodi i glukoza-oksidazom imobiliziranom u albuminu govеđeg seruma s pomoću glutaraldehida

Sažetak

Opisana je priprava jednostavnog amperometrijskog enzimskog senzora za određivanje glukoze. Enzim glukoza-oksidaza imobiliziran je s pomoću albumina iz goveđeg seruma i glutaraldehida na sloj alkalijskog niklenog heksacijanoferata(II) elektrokristaliziranog na površinu niklene elektrode. Biosenzor se temelji na selektivnom biokatalitičkom učinku enzima glukoza-oksidaze pri oksidaciji glukoze i katalitičkom učinku niklene elektrode modificirane alkalijskim niklenim heksacijanoferatom(II) pri redukciji H_2O_2, *nastalog enzimskom reakcijom. To omogućuje da radni potencijal biosenzora za glukozu bude između –300 i –100 mV prema zasićenoj kalomelovoj elektrodi. U tom području potencijala ne dolazi do oksidacije askorbinske i mokraćne kiseline kao ni većine ostalih elektroaktivnih spojeva prisutnih u uzorcima ljudske krvi. Strujni odziv biosenzora u pH-području između 6,7 i 7,7 praktički je neovisan o pH, vrijeme odziva je manje od 20 s za dodatak od 0,3 mM otopine glukoze u otopinu pufera natrijeva fosfata (0,01 M fosfatnog iona, pH = 7,4) i 0,1 M natrijeva perklorata. Vrijeme uporabe je relativno dugo (tijekom tri mjeseca osjetljivost biosenzora opala je za 30% od početne osjetljivosti) i strujni je odziv linearan do 1,2 mM koncentracije glukoze.*