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Palladium hexacyanoferrate hydrogel as a novel and simple enzyme immobilization matrix for amperometric biosensors

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Abstract

An amperometric glucose biosensor with glucose oxidase (GOx) immobilized into palladium hexacyanoferrate (PdHCF) hydrogel has been prepared and evaluated. The sensor was based on a two-layer configuration with biocatalytic and electrocatalytic layers separately deposited onto the electrode. To reduce the overpotential for reduction of hydrogen peroxide liberated in the enzyme catalyzed oxidation of glucose, an inner thin layer of nickel hexacyanoferrate (NiHCF) electrodeposited onto the surface of graphite electrode was used as an electrocatalyst. As an outer layer, the hydrogel of palladium hexacyanoferrate with entrapped glucose oxidase was used. Under optimal operating conditions (pH 5.0 and $E = -0.075$ V versus calomel (3.0 M KCl) reference electrode), sensor showed high sensitivity to glucose (0.3–1.0 μA/mM) and a response time of less than 30 s. The linear response to glucose was obtained in the concentration range between 0.05 and 1.0 mM in batch analysis mode and 0–7.0 mM in FIA. During the 32 days testing period, no significant decrease in the sensor sensitivity was observed. The sensor was applied for the determination of glucose concentration in fruit juice and yoghurt drink, and the results obtained showed good correlation with results obtained by reference spectrophotometric enzyme method.

Keywords: Glucose biosensor; Glucose oxidase; Hydrogel; Nickel hexacyanoferrate; Amperometric sensor

1. Introduction

In recent years, the use of hydrogels in the fabrication of biosensors received much attention. As matrices for enzyme immobilization, hydrogels posses some very attractive features: high water content providing biocompatible environment for immobilized enzyme and high mobility of analyte and reaction product(s), non-toxicity, and high chemical and hydrolytic stability. A range of organic hydrogels has been used to immobilize enzymes onto electrode surfaces. Enzyme immobilization into the hydrogel matrix is usually performed by mixing aqueous solutions of enzyme, water-soluble prepolymer, and suitable cross-linking agent or in the case of photopolymerization, a photoinitiator. By proper choice of water/prepolymer/cross-linking agent ratio, hydrogels with desired water content and mechanical properties can be obtained.

Some examples of organic hydrogels employed in biosensors fabrication are polycarbamyl sulphonate hydrogel used for immobilization of L-glutamate oxidase (Kwong et al., 2000) and different alcohol oxidases (Patel et al., 2001); carrageenan hydrogel for entrapment of cholesterol esterase and cholesterol oxidase (Crubliss et al., 1993); polyacrylamide hydrogel for immobilization of glucose oxidase (GOx) and urease (Jimenez et al., 1997); poly(2-hydroxyethyl methacrylate)–polypyrrole hydrogel composite used for entrapment of GOx, cholesterol oxidase and galactose oxidase (Brahim et al., 2002); and poly(ethylene glycol) hydrogel used for encapsulation of organophosphorus hydrolase enzyme (Russell et al., 1999). Organic hydrogels containing electron transfer mediators (so-called redox hydrogels) are also frequently used. A redox hydrogel formed by copolymerization of acrylamide and vinylferrocene (Bu et al., 1995) or that based on cross-linked poly(allylamine) with

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covalently attached ferrocene (Koide and Yokoyama, 1999) were used for immobilization of GOx. Several researchers have also reported biosensor applications of hydrogels containing redox active osmium complexes (usually with bipyridine ligands) acting as 'electron wires' connecting an enzyme redox center and an electrode. Examples are osmium-based redox hydrogels of poly(1-vinylimidazole) used for immobilization of GOx (Ohara et al., 1993), laccase, and tyrosinase (Daigle et al., 1998) or peroxidase and amine oxidase (Niculescu et al., 2000), redox hydrogels of poly(vinyl pyridine) used for encapsulation of GOx (Kenausis et al., 1996; Linke et al., 1994) and pyruvate oxidase (Gajovic et al., 2000) or osmium-based hydrogel of poly(allylamine) used for immobilization of lactate oxidase (Danilowicz et al., 1998).

In contrast to a wide variety of organic hydrogels employed in the fabrication of biosensors, inorganic hydrogels are not commonly used as matrices for enzyme immobilization. Silica, alumina, titanium dioxide, and vanadium pentoxide hydrogels readily prepared from organic precursors by sol–gel method (Avnir et al., 1994; Wang, 1999) have highly swollen structure that enables mobility and leaching of enzyme from a hydrogel matrix. Therefore, they are suitable for enzyme encapsulation only in the more condensed state in the form of xerogels.

In this work, we report the use of polymeric inorganic hydrogel of palladium hexacyanoferrate (PdHCF) as a novel matrix for enzyme immobilization in the fabrication of amperometric biosensors. The PdHCF hydrogel, described first by Bocarsly's group 10 years ago (Pfennig et al., 1993), can be readily synthesized from the ligand substitution reaction of K_2PdCl_6 by $K_3Fe(CN)_6$ in aqueous solution. In this reaction, two chloride ligands are displaced per palladium center and the formation of polymeric chains with iron and palladium centers bridged by cyanide ligands occurs. Interchain cross-linking leads to the formation of gel with high water content and a Pd:Fe stoichiometry of 2:1. Resulting hydrogel contains in excess of 95% water by weight; and once the gel is formed, it does not swell or dissolve when immersed in water (Pfennig et al., 1993). Since the PdHCF hydrogel is formed from coordination polymer in which the metal complex exists as an integral part of the polymer backbone through ligand bridge, the polymer network generated is geometrically constrained and hence, rigid. This, together with large water content and macro-porous structure providing facile diffusion of reactive species in and out of the polymer network, makes the PdHCF hydrogel an interesting candidate for enzyme immobilization matrix.

We chose glucose oxidase, the well known and the most often used enzyme for glucose determination in different matrices, as a model enzyme to test the suitability of the PdHCF hydrogel as a matrix for enzyme encapsulation. Glucose oxidase catalyses the oxidation of β-D-glucose to D-glucono-1,5-lactone and the enzyme is returned to its active state by transferring electrons to molecular oxygen, resulting in the production of hydrogen peroxide. Glucose concentration can be determined electrochemically by measuring the current resulting in oxidation or reduction of H_2O_2 formed. The reduction of H_2O_2 is the preferred choice in glucose determination because oxidation of possible interferences (viz. ascorbic acid) present in different sample matrices can be avoided. However, at potentials where H_2O_2 is reduced, reduction of dissolved oxygen also occurs producing high baseline current that reduces the sensitivity of the biosensor. In the literature, different metal hexacyanoferrates (Mattos et al., 2000; Milardović et al., 1997; Lin and Shih, 1999; Wang et al., 1999) were investigated as electrocatalysts in glucose biosensors in order to enable the reduction of H_2O_2 at potentials where electroreduction of dissolved oxygen does not occur. In this work, we chose nickel hexacyanoferrate (NiHCF) as an electrocatalyst for H_2O_2 reduction due to its high catalytic efficiency and good long-term and pH stability (Milardović et al., 1997). Graphite electrode modified with thin layer of electrodeposited NiHCF was used as a base on which the layer of the PdHCF hydrogel with entrapped GOx was deposited. The influence of different parameters such as working potential, electrolyte pH, storage conditions, and presence of easily reducible interferents on the sensor response was investigated. Under optimized conditions, biosensors were used for the determination of glucose in fruit juices and yoghurt drinks in batch analysis and flow injection analysis (FIA) mode.

2. Experimental

2.1. Materials and methods

Electrochemical experiments were performed in 0.1 M phosphate buffer using a Radiometer Voltalab PST050 potentiostat connected to the PC. All experiments were carried out at 25 ± 1 °C in three-electrode cell with the biosensor as working electrode and a Pt wire as the counter electrode. All potentials are referred to the calomel (3 M KCl) reference electrode except in FIA where Ag/AgCl (3 M KCl) reference electrode was used. Between the uses, biosensors were stored in refrigerator at 4 °C. Before the use, biosensors were kept at room temperature for 30 min.

Glucose oxidase (from *Aspergilus niger*, 117 U/mg) was purchased from Sigma (St. Louis, MO, USA). All other chemicals were reagent grade and were used without further purification. Spectrophotometric glucose determination in food samples was performed using a Boehringer enzyme kit (cat. no. 719620). Absorbance at 340 nm was measured with BioSpecord 200 spectrophotometer (Analytik-Jena, Jena, Germany).

2.2. Biosensor preparation

2.2.1. Electrode preparation and pretreatment

Graphite disc electrodes were used for preparation of biosensors. Rods of spectroscopically pure graphite (3 mm

in diameter) were cut to cylinders of 5 mm length and cylinders were sealed into glass tubes with epoxy. The piece of copper wire wound to spiral served as electrical contact to graphite inside the tube. Prior to the use, electrodes were polished on wet fine emery paper (P 400) and thoroughly rinsed with deionized water. Electrochemical pretreatment of electrodes was performed in 0.1 M phosphate buffer (pH 6.0) by cyclization between −1.5 and 1.5 V at 50 mV/s for 15 min.

2.2.2. Electrodeposition of NiHCF layer

Electrodeposition of thin electrocatalytic layer of nickel hexacyanoferrate, $K_{(2-x)}Ni_{(1+0.5x)}[Fe(CN)_6]$, ($x = 0$, 1), onto the surface of electrochemically pretreated electrodes was performed from solution containing 0.1 M KCl, 10 mM HCl, 0.5 mM $NiSO_4$, and 0.5 mM $K_3[Fe(CN)_6]$ by cycling the potential 50 times from 0.05 to 0.85 V at scan rate of 50 mV/s. After the layer of NiHCF was formed, electrodes were rinsed with deionized water, transferred to solution containing 0.1 M KCl and 10 mM HCl, and again the potential was cycled from 0.05 to 0.85 V at scan rate of 50 mV/s for 10 min. After that, electrodes were thoroughly rinsed with deionized water and allowed to dry in the air.

2.2.3. Immobilization of enzyme

The mass of 2 mg of GOx was suspended in 25 μL of 44 mM $K_3[Fe(CN)_6]$ in 0.1 M phosphate buffer (pH 6.0), 50 μL of deionized water was added, and the solution was allowed to stay at room temperature for 45 min. Subsequently, into this solution, 25 μL of 88 mM $PdCl_2$ in 0.15 M KCl was added. A volume of 5 μL of resulting mixture was pipetted quickly on the surface of the NiHCF modified graphite electrode and the electrode was allowed to stay in the air for 15 min. Five biosensors were prepared from one batch of enzyme and hydrogel solution. Three of them were stored immersed in 0.1 M phosphate buffer (pH 6.0), and remaining two biosensors were stored in air saturated with water vapor (i.e., in test tubes containing water with electrodes placed few centimeters above the water surface).

3. Results and discussion

3.1. GOx immobilization

Immediately after the addition of $PdCl_2$ solution into the solution containing GOx and $K_3[Fe(CN)_6]$ dissolved in phosphate buffer, the color of the mixture turns dark red indicating that the formation of polymeric palladium hexacyanoferrate species begins. We observed that the gelation time of immobilization mixture strongly depends on the amount of GOx added. For the mixture containing 2.5 mg of GOx per 100 μL of final solution, the gelation time was shorter than 60 s; and if the concentration of enzyme was higher than 4 mg of GOx per 100 μL of final solution, solution coagulated immediately after the addition of $PdCl_2$. For the mixture containing 2 mg of GOx per 100 μL of final solution (the composition chosen for GOx immobilization), the gelation time was about 3 min, sufficiently long to allow easy manipulation with immobilization mixture and its deposition on the electrode surface.

After the sol–gel transition was completed, thin red transparent layer of PdHCF hydrogel with entrapped GOx was formed on the electrode surface. Several combinations of electrode and electrode shield materials were tested as a base for the deposition of PdHCF hydrogel with entrapped GOx. It was found that the adhesion of the hydrogel to smooth surfaces (disc of smooth platinum with or without electrodeposited NiHCF layer, polyethylene, poly(vinylchloride), Teflon) was poor, and the peeling of hydrogel film was observed, especially under hydrodynamic conditions. The adhesion of the hydrogel to rough surface of spectroscopically pure graphite surrounded with epoxy seal was much better, and no peeling of hydrogel film from electrode surface was observed during 32 days testing period.

Cyclic voltammograms of PdHCF hydrogel containing GOx and deposited on bare graphite electrode, recorded in 0.1 M phosphate buffer (pH 6.0) over the potential window from −0.2 to 1.2 V, showed only one, small and ill-defined, irreversible reduction wave at about 0.1 V which disappeared in subsequent cycles. This wave can be attributed to the reduction of Pd(II) species from hydrogel to Pd metal (Pfennig et al., 1993). No additional waves were visible in the potential range studied indicating that the iron redox centers in hydrogel are not electroactive in accordance with previously published data (Pfennig et al., 1993).

3.2. NiHCF layer electrodeposition and its electrocatalytic properties

The formation of NiHCF layer on graphite electrode is shown in Fig. 1. Two pairs of overlapping peaks are visible

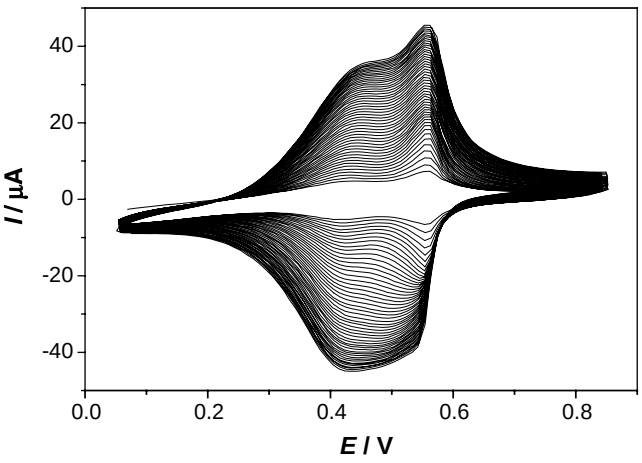

Fig. 1. Cyclic voltammograms for the formation of NiHCF film on graphite electrode recorded in 0.1 M KCl containing 10 mM HCl, 0.5 mM $NiSO_4$, and 0.5 mM $K_3[Fe(CN)_6]$; scan rate 50 mV/s.

on the voltammogram. Pair of peaks occurring at the most positive potentials can be attributed to the form of NiHCF with the approximate formula $K_2Ni[Fe(CN)_6]$, while the more negative pair of peaks is related to the oxidation/reduction of potassium depleted form of NiHCF with approximate formula $KNi_{1.5}[Fe(CN)_6]$ (Zamponi et al., 2003). Oxidation of both forms of NiHCF is accompanied by the simultaneous displacement of potassium ions and can be described by the following redox reactions (Zamponi et al., 2003):

$$K_2Ni^{II}[Fe^{II}(CN)_6] \leftrightarrow KNi^{II}[Fe^{III}(CN)_6] + e^- + K^+$$

$$KNi_{1.5}{}^{II}[Fe^{II}(CN)_6] \leftrightarrow Ni_{1.5}{}^{II}[Fe^{III}(CN)_6] + e^- + K^+$$

The behavior of the NiHCF modified graphite electrode in the presence of 20 mM H_2O_2 in phosphate buffer (pH 6.0) is shown in Fig. 2d. When compared to the voltammogram of the same electrode recorded in the supporting electrolyte alone (Fig. 2c), increase of reduction current at potentials smaller than 0.2 V as well as an increase of oxidation current at potentials greater than 0.65 V are visible in the presence of H_2O_2. In comparison to the reduction of H_2O_2 at a bare graphite electrode (Fig. 2b), the potential at which reduction of H_2O_2 begins is shifted in the anodic direction for approximately 0.2 V in the case of the NiHCF modified electrode. Moreover, the cathodic current due to H_2O_2 reduction is larger at the NiHCF modified electrode than at the bare graphite electrode (e.g., 55 μA for NiHCF modified electrode versus 2 μA for bare graphite electrode at −0.075 V) indicating that the electroreduction of H_2O_2 is greatly enhanced at the NiHCF modified electrode. In the potential range above 0.6 V where oxidation of H_2O_2 occurs, anodic currents recorded in the presence of H_2O_2 at the bare graphite electrode and the NiHCF modified electrode are almost equal indicating that oxidation of H_2O_2 is not catalyzed at the NiHCF modified electrode.

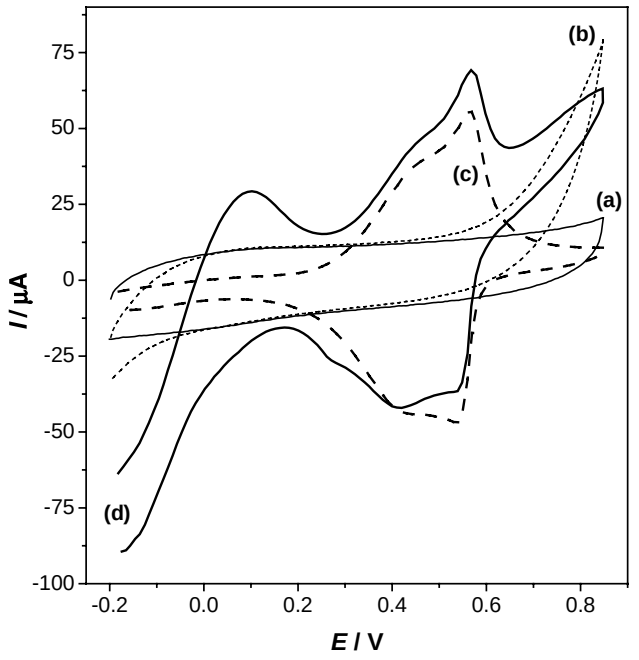

Fig. 2. Cyclic voltammograms recorded in 0.1 M phosphate buffer (pH 6.0) at bare graphite electrode (a and b) and NiHCF modified graphite electrode (c and d), without added H_2O_2 (a and c) and in the presence of 20 mM H_2O_2 (b and d); scan rate 50 mV/s.

3.3. Optimization of biosensor response

In Fig. 3, the sensitivity of the glucose biosensor is shown as a function of pH. Every point of the curve represents the averaged amperometric response for three successive additions of 10 μL aliquot of 0.1 M glucose standard solution into 10 mL of 0.1 M phosphate buffer. Maximum sensitivity of the biosensor is obtained in the pH range between 4.5 and 5.5. At pH values greater than 6, the sensor sensitivity slowly decreases with a rate of about 0.08 μA/mM per pH unit. In acidic region, at pH values below 4.5, the sensor sensitivity falls more rapidly; and at pH 3.5, it is reduced to ∼25% of maximum value. The pH value of 5.0 was selected as optimum for biosensor operation in all subsequent experiments.

As stated earlier, the potential window in which the biosensor can be operated is reduced at both the anodic and cathodic side by electrochemical oxidation of possible interferents and reduction of dissolved oxygen, respectively. In order to determine that potential window, a set of steady state hydrodynamic voltammograms were recorded in air-saturated and oxygen-free supporting electrolyte as well as in the presence of ascorbic acid as a model compound for easy-oxidable interferents (Fig. 4). As can be seen from the figure, in the potential range between −0.125 and −0.075 V, neither reduction of dissolved oxygen nor oxidation of ascorbic acid occurs at the NiHCF modified graphite electrode, and therefore, the working potential of biosensor must be set in that range. In order to minimize the background current due to oxygen reduction, the potential of −0.075 V was selected as optimum for biosensor operation in all subsequent experiments.

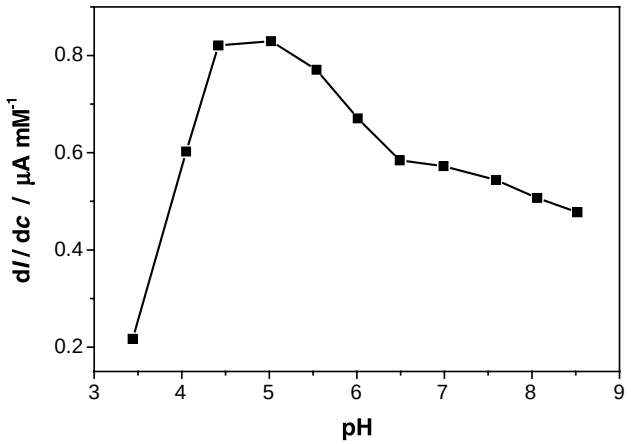

Fig. 3. Variation of the sensitivity of the biosensor with pH.

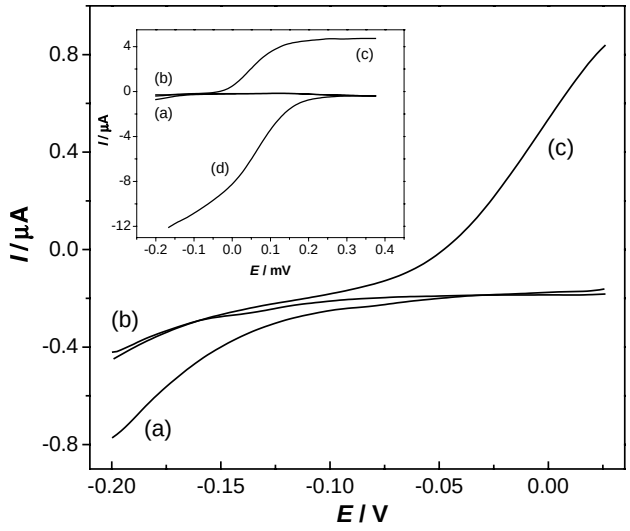

Fig. 4. Hydrodynamic voltammograms recorded in 0.1 M phosphate buffer (pH 5.0) at NiHCF modified graphite electrode: (a) supporting electrolyte saturated with air, (b) deaerated supporting electrolyte, (c) deaerated supporting electrolyte containing 1 mM ascorbic acid, and (d) deaerated supporting electrolyte containing 5 mM H_2O_2; scan rate: 2 mV/s.

3.4. Response characteristics of the biosensor

To investigate response characteristics of the biosensor, a current–time response after successive additions of aliquots of glucose standard to the phosphate buffer solution under stirring was recorded. Well-defined reduction current steps proportional to glucose concentration were obtained. Under the optimal conditions (pH 5.0 and working potential equal to −0.075 V) for all five biosensors linear calibration plots were obtained in the range from 0.05 to 1 mM ($r^2 > 0.995$). The slope of calibration plots (i.e., the sensitivity of biosensors) ranged from 0.3 to 1.0 μA/mM. The lowest detection limit (LOD) calculated as LOD = S.D./sensitivity (where S.D. is the standard deviation for the points used to construct the calibration plot and sensitivity is its slope), was found to be in the range from 3 to 14 μM. Response time of the biosensor (determined as the time needed for signal to obtain 95% of its steady state value) was typically under 30 s.

The ability of biosensor to operate under FIA conditions was also investigated. In Fig. 5, typical FIA response on subsequent injections of glucose standards is shown. Linear calibration plot is obtained in the range 0–7 mM glucose ($r^2 = 0.9996$), with the slope of 0.535 ± 0.005 μA/mM. The lowest limit of detection of 50 μM was calculated based on the slope of calibration plot and the standard deviation of calibration points.

When compared with previously published data on sensitivity of biosensors with GOx immobilized into various hydrogel matrices (calculated for the same electrode geometric area as in this work), better results were obtained for biosensor described here. For example, sensitivity of 0.028–0.043 μA/mM was reported for biosensors with GOx immobilized into redox hydrogel containing cross-linked osmium complex of poly(vinylpyridine) (Linke et al., 1994). Sensors with GOx cross-linked with albumin and poly(allylamine) based redox hydrogel showed sensitivity of 0.15 μA/mM (Koide and Yokoyama, 1999), while for biosensors based on GOx entrapped into polypyrrole-poly(2-hydroxyethyl methacrylate) hydrogel, composite sensitivity of 0.135 μA/mM was reported (Brahim et al., 2002). In comparison with previously reported biosensor based on the NiHCF modified nickel electrode with GOx immobilized by a glutaraldehyde/bovine serum albumin (BSA) cross-linking procedure (Milardović et al., 1997), both the response time and the sensitivity in FIA mode are enhanced with the biosensor design presented here. Since the same detection principle (i.e., electroreduction of H_2O_2) and electrocatalyst (NiHCF) were used in both biosensors, the 50-fold increase of sensitivity from 0.01 μA/mM for biosensor with GOx cross-linked with BSA to 0.5 μA/mM for biosensor with GOx entrapped into the PdHCF hydrogel can be attributed to better compatibility between enzyme and immobilization matrix and hence, better activity of enzyme.

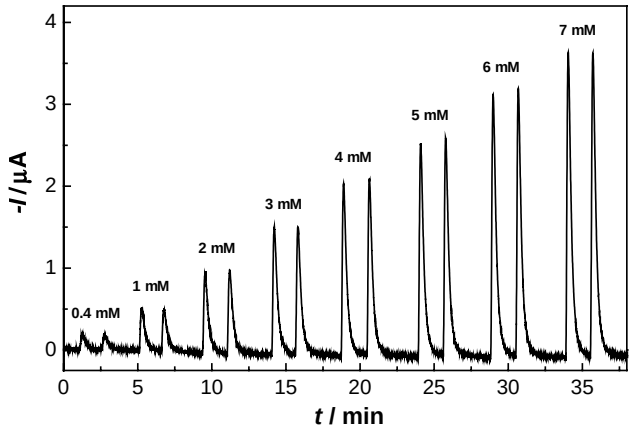

Fig. 5. Amperometric response of biosensor recorded for successive injections of 10 μL of glucose standard solutions in FIA mode (two injections of each standard solution are shown); supporting electrolyte: 0.1 M phosphate buffer, pH 5.0; applied potential: −0.035 V vs. Ag/AgCl (3 M KCl) reference electrode; flow rate: 1.0 mL/min.

3.5. Analysis of real samples

The biosensor was applied for glucose determination in two commercial food samples: fruit juice (referred as sample 1) and yoghurt drink (referred as sample 2). In Fig. 6a, amperometric response of the biosensor on successive additions of glucose standard and real sample into the cell containing phosphate buffer (batch analysis mode) is shown. Both samples were only diluted with phosphate buffer prior analysis, sample 1 in volume ratio ψ (sample 1, buffer) = 1:1, and sample 2 in ψ (sample 2, buffer) = 1:10. The glucose content in each sample was calculated from the ratio of the heights of current steps obtained upon the addition of standard and sample.

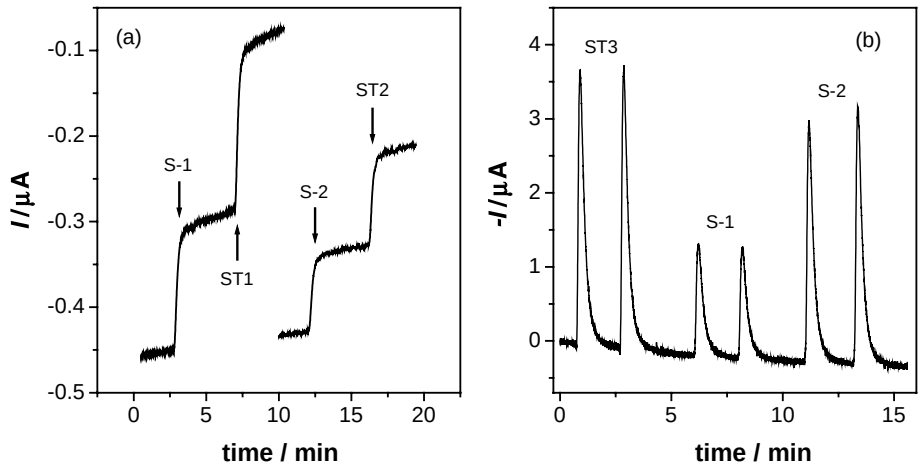

Fig. 6. Amperometric response of biosensor recorded for determination of glucose in (a) batch analysis mode and (b) FIA mode. (a) Additions of sample or glucose standard solution into the 10 mL of phosphate buffer are marked as follows: S-1, 20 μL of sample 1; ST1, 20 μL of 0.1 M glucose standard solution; S-2, 50 μL of sample 2; and ST2, 10 μL of 0.1 M glucose standard solution. (b) ST3, S-1, and S-2 indicate two successive injections of 10 μL of 4 mM glucose standard solution, samples 1 and 2, respectively. For dilutions of samples, see text. Supporting electrolyte: 0.1 M phosphate buffer, pH 5.0; applied potential: −0.075 V vs. calomel (3 M KCl) reference electrode (batch analysis mode) or −0.035 V vs. Ag/AgCl (3 M KCl) reference electrode (FIA mode); flow rate: 1.0 mL/min (FIA mode).

The glucose determination with the biosensor operating under FIA conditions was also performed (Fig. 6b). Prior analysis samples are diluted with phosphate buffer in volume ratios ψ (sample 1, buffer) = 1:100 and ψ (sample 2, buffer) = 1:50. Small volumes (10 μL) of glucose standard and diluted samples were injected into the phosphate buffer carrier stream and changes in reduction current caused by passage of sample or standard plug over the biosensor placed in wall-jet flow cell were recorded. From the ratio of the heights of peaks obtained, the concentration of glucose in each sample was determined.

In Table 1, the results obtained by batch analysis and FIA are compared with the glucose contents determined by reference spectrophotometric method for each sample. As can be seen, good agreement between results obtained by biosensor and reference method exists. Relative errors of 1.5 and 1.3% for batch analysis mode, and 5.8 and 2.9% for FIA mode were obtained for samples 1 and 2, respectively.

3.6. Repeatability and long-term stability of biosensor

Repeatability of the biosensor was tested in FIA mode by consecutive injections of 4 mM glucose standard within a period of 20 min, and the relative standard deviation of 3.8% (n = 10) was obtained. The long-term storage stability of biosensor was studied over the 32 days period. For this purpose, five biosensors were prepared and stored at 4 °C under different conditions: three sensors (labeled as E1–E3) were stored immersed in phosphate buffer (pH 6.0), while remaining two (labeled as E4 and E5) were stored in air saturated with water vapor. The sensitivity of biosensors was determined every 2 days as averaged amperometric response upon three additions of glucose standard measured in batch analysis mode. Immediately after the preparation, all five sensors showed sensitivity in the range between 0.4 and 0.55 μA/mM. For the sensors E1 and E3, decrease of sensitivity of 30–40% was observed during the first 6 days of storage, and then it remained constant for next period of 26 days. Sensors E2, E4, and E5 showed some increase of the sensitivity during the first 48 h of storage, and again, after that time they maintained their sensitivity until the end of the testing period. Such long-term behavior indicates that the compatibility of the PdHCF hydrogel with GOx enzyme is very good, and PdHCF hydrogel is promising matrix for GOx immobilization.

Table 1
Determination of glucose in real samples in batch analysis and FIA mode compared to the reference spectrometric enzyme method (sample 1, commercial fruit juice; and sample 2, yoghurt drink)

Sample	Amperometric biosensor		Reference spectrophotometric method
	Batch analysis	FIA	
	γ(glucose)/(g/L)		
1	27.9	29.1	27.5
2	31.0	32.3	31.4

4. Conclusion

Palladium hexacyanoferrate hydrogel has successfully been employed as a matrix for glucose oxidase immobilization onto the surface of graphite electrode modified with thin layer of nickel hexacyanoferrate. Due to the catalytic effect of nickel hexacyanoferrate for the electrochemical reduction of H_2O_2 liberated in GOx catalyzed oxidation of glucose, the prepared biosensor can be operated in the potential range where interferences neither of dissolved oxygen

nor of ascorbic acid occur. The sensor was applied for the determination of glucose concentration in fruit juice and yoghurt drink, and the results obtained showed good correlation with results obtained by reference spectrophotometric enzyme method. The biosensor showed good long-term storage stability indicating the good compatibility between the PdHCF immobilization matrix and the entrapped enzyme.

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