Applied Biochemistry and Biotechnology
https://doi.org/10.1007/s12010-019-02979-2

Development of Voltammetric Glucose-6-phosphate Biosensors Based on the Immobilization of Glucose-6-phosphate Dehydrogenase on Polypyrrole- and Chitosan-Coated Fe_3O_4 Nanoparticles/Polypyrrole Nanocomposite Films

Selmihan Sahin[1] · Ismail Ozmen[1] · Gizem Yildirim Bastemur[2] · Sabriye Percin Ozkorucuklu[3]

Received: 20 November 2018 / Accepted: 19 February 2019/Published online: 28 February 2019

Abstract

Polypyrrole (PPy) and PPy-containing chitosan-coated Fe_3O_4 have been electrochemically polymerized on pencil graphite electrodes (PGEs). After the resulting electrodes were characterized by SEM-EDS analysis, glucose-6-phosphate dehydrogenase (G6PD) was immobilized onto these electrodes via glutaraldehyde. The biosensors prepared for the chronopotentiometric detection of glucose-6-phosphate (G6P) at 0.25 $mAcm^{-2}$ were studied and optimized at different parameters such as the pH of supporting electrolyte, the temperature, and $NADP^+$ and G6P concentrations related with the analytical performance of the biosensors. PPy/G6PD (BS-1) and CS/Fe_3O_4-PPy/G6PD (BS-2) biosensors showed a broad linear response in the concentration range 0.025–0.25 mM and 0.0025–0.05 mM, and their detection limits for G6P and the RSD values were determined as 0.008 mM and 0.002 mM and 3.80% and 4.60% after 15 times usage, respectively. The interference study with various major blood components such as urea, glucose, and cysteine was performed to evaluate the selectivity of the biosensors. The proposed BS-2 biosensor showed almost free response from available interferences in blood serum with a recovery of 91 to 110%. The developed biosensors could be used in the G6P level measurement of medical samples.

Keywords Glucose-6-phosphate dehydrogenase · Glucose-6-phosphate · Polypyrrole · Chitosan-coated Fe_3O_4 nanoparticle · Biosensor

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12010-019-02979-2) contains supplementary material, which is available to authorized users.

✉ Ismail Ozmen
ismailozmen@sdu.edu.tr

Extended author information available on the last page of the article

Introduction

G6PD is a NAD(P)-dependent oxidoreductase which catalyzes the conversion of G6P to 6-phosphoglucono-δ-lactone (6PG) with the concomitant reduction of $NADP^+$ [1]. G6P has an important role for pentose phosphate pathway (PPP) of all organisms such as bacteria, plants, and animals [2]. Its relative concentration gives information about activities of enzymes such as glucose-6-phosphate dehydrogenase (G6PD), phosphoglucose isomerase, hexokinase, and phosphoglucomutase, due to being their substrate or product, and also it has a relation with the regulations of some enzymes, such as protein kinase and glycogen synthase [3].

Various methods have been used for determination of G6P such as spectroscopic, chromatographic or radioactive techniques. However, there is a great attention to develop an enzyme-based biosensor for G6P determination because all of these methods have disadvantages (time-consuming, labor-intensive, expensive) [2, 3]. Several kinds of biosensors have been studied for the measurements of G6P, so far. These are based on a combination of G6PD with different enzymes (salicylate hydroxylase, glutathione reductase, *p*-hydroxybenzoate hydroxylase), polymers (polyethyleneimine), or/and mediators (ferrocene, tetracyanoquinodimethane, Prussian blue nanoparticles) [2–7].

The electron transfers between biological macromolecules and the electrode can be mediated by conducting polymer. The most common conducting polymers are poly(acetylene), poly(3,4-ethylenedioxythiophene) (PEDOT), poly(thiophene), poly(phenylene vinylene) (PPV), poly(pyrrole), and poly(aniline). These can be synthesized chemically and electrochemically [8]. Among these conducting polymers, PPy is important for molecular recognition system because it can be used in a neutral pH region, and its stable films can properly be polymerized on different substrate materials. Therefore, it has been widely studied for the immobilization of enzymes, nucleic acids, and antibodies [9, 10]. However, bulky form of PPy transfers electrons relatively slow. To overcome this problem, PPy can be combined with nanostructured materials, and thus, the sensitivity is increased due to faster diffusion in the sensing area [11, 12]. Use of nanoparticles such as Fe_3O_4, zinc oxide (ZnO), and cerium oxide (CeO_2) for biosensor construction has gained much interest in recent years due to their high surface reaction activity, large surface-to-volume ratio, strong adsorption ability, and high catalytic efficiency. Such properties of nanoparticles make them extremely attractive for immobilization of desired molecules. Furthermore, nanoparticles provide fast electron transfer between the electrode and the enzyme's active site. Among metal oxide nanoparticles, Fe_3O_4 nanoparticles are biocompatible and show superparamagnetic behavior, so it can be considered as the most interesting materials [13, 14]. Previously, Fe_3O_4 nanoparticles in composite films have been used in different biosensor applications to improve the electron transfer and increase the surface area of the sensors for immobilization of enzyme and thus possess the good electrocatalytic activity [11, 13–19].

The aim of this study is to enhance the sensing capabilities of G6P biosensor, such as LOD, stability, and biocompatibility by using PPy film and PPy composite film with CS/Fe_3O_4 nanoparticles. For this aim, we prepared BS-1 and BS-2 biosensors for detection of G6P (PGE) and compared their performances. Surface properties and content of the prepared electrodes were evaluated with SEM-EDS analysis. The proposed biosensors exhibit fast and sensitive responses to G6P without any electron mediators. Parameters, such as temperature, pH of supporting electrolyte, and $NADP^+$ and G6P concentration, that related with the analytical performance of the biosensor were studied and optimized. The stability of the prepared electrodes and the influence of possible interferents were also examined. The resulting biosensors exhibited a good analytical performance for the determination of G6P.

Materials and Methods

Chemical and Reagents

Pyrrole (98%), hydrochloric acid (HCl, 35%), and glutaraldehyde were purchased from Sigma–Aldrich, Germany. All the other chemicals used in the study were of analytical grade and used as received. Purification of G6PD from bovine erythrocytes and preparation of chitosan-coated magnetic nanoparticles (CS/Fe_3O_4) were reported in the previous study [20].

Apparatus

Electropolymerization and all electrochemical measurements were performed using Autolab PGSTAT302N Potentiostat/Galvanostat and controlled by a Nova 1.11 software version (Ecochemie, The Netherlands). Conventional three-electrode system equipped with bare pencil graphite electrodes (PGE) or the prepared BS-1 and BS-2 as the working electrode, Ag/AgCl electrode as the reference electrode, and a platinum electrode as the auxiliary electrode were used for all electrochemical measurements. The pH values of solutions were determined by using an MA 235 model (Mettler Toledo, Switzerland) pH-Ion meter with InLab 416 Ag/AgCl glass electrode. Elmasonic S 15 ultrasonic cleaner (Germany) was used for cleaning the electrodes. Scanning electron microscopy (SEM) images were obtained from PEIQUANTA 250 FEG.

Preparation of the BS-1 and BS-2 Electrodes

A Noki pencil model 2000 (Japan) was used as a holder for graphite leads (Tombo, HB, 0.5 mm diameter, Japan). The PGE was obtained by cutting the leads into 3 cm-long sticks. PGEs were washed with distilled water to remove the impurity and dried at room temperature before use. Then, PGE was immersed in the polymerization solution.

The PPy electrodes were prepared by electrochemical polymerization of PPy on the PGE at a constant current density of 0.35 mA cm^{-2} during 900 s from an aqueous solution of 0.5 M HCl containing 0.2 M pyrrole.

In order to obtain CS/Fe_3O_4-PPy electrodes, the electropolymerization was carried out applying constant current density of 0.35 mA cm^{-2} for 900 s in aqueous solution of 0.5 M HCl containing 0.2 M pyrrole and CS-Fe_3O_4 with 0.75 ratio (CS-Fe_3O_4/pyrrole).

The G6PD was immobilized on these electrodes by covalent immobilization via glutaraldehyde (GA). First, PPy and CS/Fe_3O_4-PPy electrodes were immersed in 1.2 *w*/*w* % GA for 1 h. The electrodes were washed with distilled water and then immersed in G6PD solution and incubated at 4 °C for 3 h. After that, the G6PD immobilized electrodes were washed with 100 mM Tris HCl (pH 8.0) buffer to remove any unbound enzyme from the electrode surfaces and were stored at 4 °C when not in use.

Optimization Study

The experimental parameters such as pH of the electrolyte and temperature and the concentration of cofactor ($NADP^+$) were investigated in order to optimize the analytical performance of the developed biosensors. Voltammetric responses of the prepared biosensors were examined in the presence of different major blood constituents, such as glucose, urea, and cysteine.

Electrochemical Measurements

Chronopotentiometric measurements were performed by placing the electrode in a magnetically stirred reaction mixture (8 mL), which contained 100 mM Tris–HCl buffer (pH 8.0), 0.1 M $MgCl_2$, 1.25 mM $NADP^+$, and G6P. Working current was 0.25 mA for all G6P measurements. The resulting equilibrium potential vs. Ag/AgCl electrode was then measured after each addition of standard G6P solution in a three-electrode cell.

To demonstrate the usefulness of the proposed biosensors, G6P-spiked human serum samples were used as analytes for the chronopotentiometric measurements by using prepared BS-1 and BS-2 biosensors.

Operational Stability

The reusability of the proposed biosensors was studied by chronopotentiometric G6P measuring 15 repeated times in a single day. The total mean value was calculated, and the relative standard deviation (RSD) provided the analytical precision.

Results and Discussion

Preparation of the BS-1 and BS-2 Electrode

Electrochemical polymerization of PPy and PPy-containing CS/Fe_3O_4 on the PGE electrode at constant current density of 0.35 $mAcm^{-2}$ during 900 s are given in Fig. 1.

As it can be seen from Fig. 1, during first ~50 s, the electrode potential increases rapidly as a result of electrode surface being coated by PPy film, and then the potential is constant at about 0.6 V corresponding to the polymerization and deposition of the PPy on the initially formed film. The stabilization potential obtained for the formation of the CS/Fe_3O_4-PPy nanocomposite film was apparently lower than the pure PPy film. It is indicated that the resulting nanocomposite film had better conductivity. We deduced that the decreased potential of electropolymerization was due to the presence of Fe_3O_4 in the polymer, which could prevent the overoxidation of the film during the polymerization process, leading to the decrease of required energy for polymer film formation [21].

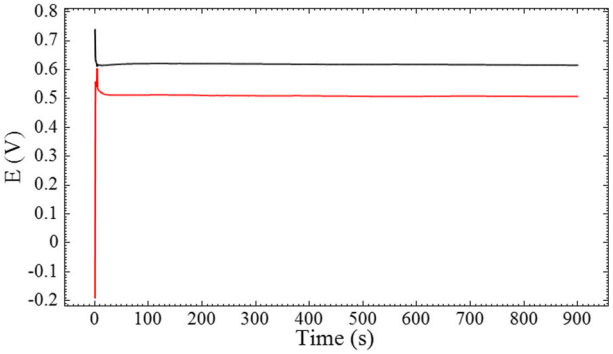

Fig. 1 Chronopotentiometric curves registered during the galvanostatic synthesis (0.35 mA cm^{-2}) of the PPy film (black line) and the CS/Fe_3O_4-PPy nanocomposite film (red line) on the PGE

Surface morphological studies of the prepared PPy and CS/Fe_3O_4-PPy electrodes were investigated using SEM. PPy film has typically globular and cauliflower-like structure (Fig. 2a), while CS/Fe_3O_4-PPy nanocomposite film has smoother surface (Fig. 2b) [22]. Figure S1 shows bare PGE electrode surface and PGE electrodes coated with PPy film and CS/Fe_3O_4-PPy nanocomposite film with 500 μm magnificence.

Further evidence for successful preparation of the electrodes could be obtained from EDS data (Table S1). When compared, the EDS results of PPy film-coated PGE and bare PGE and the appeared N peak indicated that PPy film was successfully coated on PGE. The presence of 0.14% of iron in the CS/Fe_3O_4-PPy nanocomposite film coated on the PGE was also verified.

Immobilization efficiency of G6PD on BS-1 and BS-2 electrode was 44% and 68%, respectively. Using of CS/Fe_3O_4 nanoparticles increased immobilization of G6PD on the modified electrode surface [22].

Effect of $NADP^+$ Concentration on Biosensor Responses

The $NADP^+$ concentration effect on the BS-1 and BS-2 biosensor response was investigated. The responses of the biosensors to 1.0 mM G6P were monitored by adding various concentration of $NADP^+$ to the reaction mixture (Fig. 3). The response of both biosensors increased with the increasing $NADP^+$ concentration and became saturated at 1.25 mM $NADP^+$. Therefore, 1.25 mM of $NADP^+$ was used for further experiments. Also, it has been shown that the reduction of $NADP^+$ was performed at a lower concentration with the BS-2 biosensor by comparison with BS-1 biosensor. So, we could say that $NAD(P)^+$-dependent G6PD reactions can be performed more effectively by the BS-2 biosensor without any mediator. The similar results were obtained by Kim and Yoo, [23] with tin oxide metal nanoparticle, Kim et al. [24] with Fe_2O_3/carbon black and Liu et al. [25] with silica nanoparticle.

Optimization of the Experimental Conditions

The experimental conditions—the optimum pH and temperature—affecting the sensitivity of the biosensor were optimized. The pH of the buffer is important to determine the sensitivity of the biosensors because the pH of the system affects the activity of enzymes and the stability of nanoparticles. The effect of the pH of supporting electrolyte on the biosensor responses was studied in the pH range of 6.5 to 9.0. The BS-1 biosensor response increased with the increase

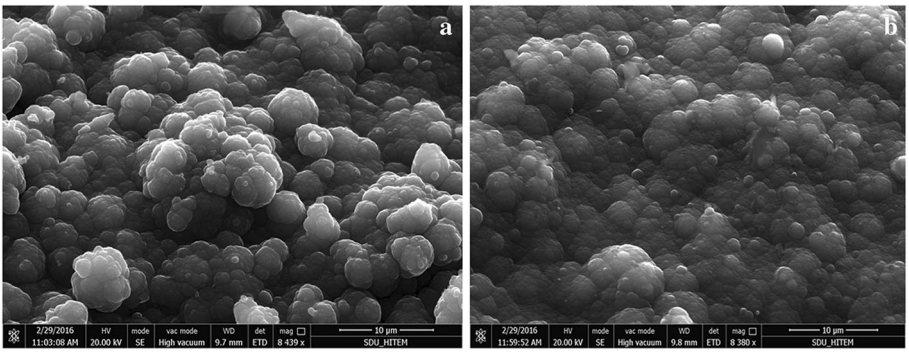

Fig. 2 SEM images of **a** PPy film and **b** CS/Fe_3O_4-PPy nanocomposite film on the PGE

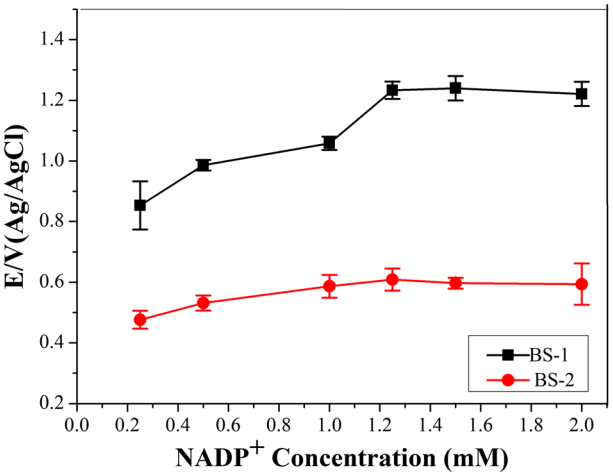

Fig. 3 Chronopotentiometric response of the BS-1 and BS-2 biosensors to 1 mM G6P for various loadings of cofactor NADP+ ranging from 0.25 to 2 mM

of pH and became maximum at pH 8.5 (Fig. 4a). However, the response of the BS-2 biosensor did not significantly change with pH. As shown in Fig. 4b, the maximum response was observed at pH 8.0. The optimum pH of the enzyme depends on the basic behavior of support materials as well as ionizable groups of the enzyme. It can be said that the use of the CS/Fe_3O_4 nanoparticles might cause the change of the enzyme and support materials interactions, which could influence by the pH.

The effect of the temperature on the biosensors response was studied ranging from 30 to 60 °C. The relative responses to G6P of the BS-1 (Fig. 5a) and the BS-2 (Fig. 5b) biosensors gradually increased until 40 °C and then began to decrease as the temperature increased above 40 °C. The relative responses (%) decreased above 40 °C, because of the thermal inactivation of enzymes on the electrode surfaces.

Response Characteristics of G6P Biosensors

Chronopotentiometric responses of the both biosensors increased with the addition of varying amounts of G6P under the optimized conditions (Fig. 6) and were linear in a concentration range of 0.025 to 0.250 mM (BS-1, Fig. 7a) and 0.0025 to 0.05 mM (BS-2, Fig. 7b) G6P.

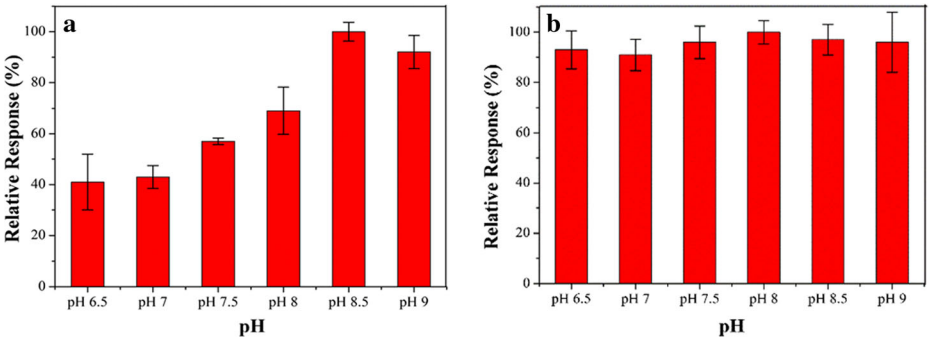

Fig. 4 The effect of change in pH values ranging from 6.5 to 9.0 on BS-1 (**a**) and BS-2 (**b**) biosensors

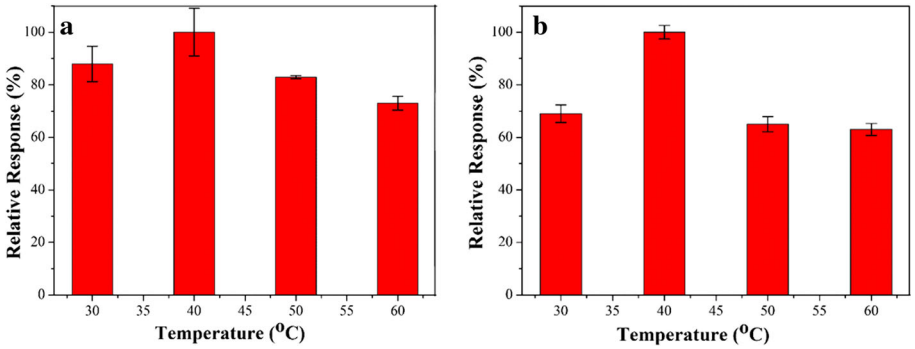

Fig. 5 Relative response of the BS-1 (**a**) and BS-2 (**b**) biosensors to 1 mM G6P at various temperature (30, 40, 50, 60 °C)

Linear range and LOD value of the proposed biosensors for G6P determination is compatible with numerous biological samples such as blood, saliva, and cellular cytoplasm (Table 1). The enzyme electrodes had a rapid response time (4 s). The limit of detection (LOD) values of G6P for BS-1 and the BS-2 biosensors were determined as 0.008 mM and 0.002 mM, respectively ($S/N = 3$), which are much lower than the concentration of G6P available in human blood (Table 1). The BS-2 biosensor had lower LOD value and linear range than the BS-1 biosensor. This could be related with that the association of magnetic nanoparticles and PPy provide better electron transfer due to properties of Fe_3O_4 nanoparticles. Previously, Haddaoui et al. [26] have obtained similar results. The performance of the proposed biosensors was compared with that of other G6P biosensors reported in the literature, LOD, detection range, and response time (Table 2). It was seen from Table 2 that all reported G6P sensors were amperometric. The most of them have small detection ranges, less stability, and long response time; whereas, the proposed biosensors showed a lower detection limit and a broad detection range and shorter response time compared with several reported G6P sensors. Thus, it can be said that many disadvantages of the reported methods could be overcome by using the methods for the preparation of the proposed biosensors.

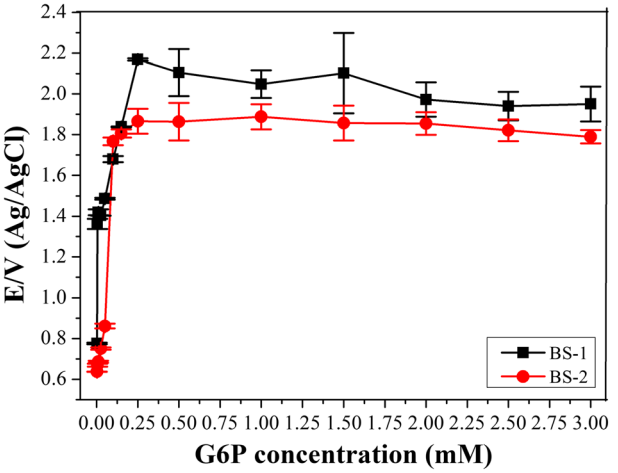

Fig. 6 Response of the BS-1 and BS-2 biosensors to various loadings of G6P ranging from 0.0025 to 3 mM

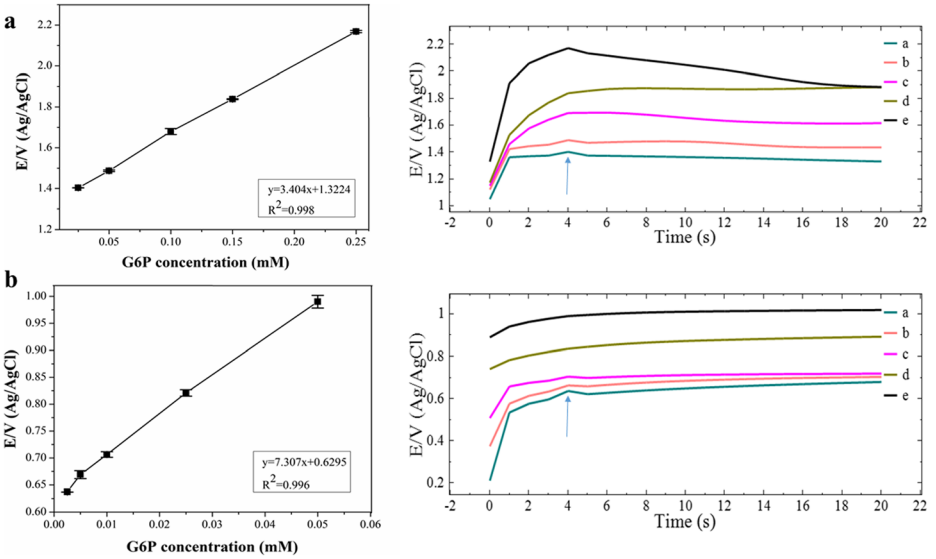

Fig. 7 Calibration curves (left) and chronopotentiometric responses (right) of BS-1 (**a**) and BS-2 (**b**) biosensors for G6P estimation. G6P concentrations (mM): **a** 0.025 (a), 0.05 (b), 0.1 (c), 0.15 (d), 0.25 (e); **b** 0.0025 (a), 0.005 (b), 0.01 (c), 0.025 (d), 0.05 (e)

Previously, different methods have been used for G6P determination such as spectrophotometric, fluorimetric, LC-MS, and specific colorimetric methods (Table 2). Each method's linear range and LOD value generally vary considerably. BS-1 has broad linear range, and both of the proposed biosensors can show good performance for detecting G6P level at low concentrations in biological samples (Table 2). Finally, we can conclude that the CS/Fe_3O_4 nanoparticles could provide determination of G6P at a lower concentration. It can be considered that proposed biosensors can be as a valid alternative to the other G6P determination methods due to several advantages such as ease of use, rapidity, cost-effectiveness, and portability.

Operational Stability

Operational stability of the prepared biosensors was evaluated in terms of relative standard deviation (RSD) by measuring the responses of biosensors with 0.25 mM G6P for 15 repeated times (Fig. 8). The RSD values of the BS-1 and the BS-2 biosensor response were 3.80% and 4.60%, respectively, which were in < 10%, allowing reliable and sensitive responses to be obtained with both biosensors.

Table 1 Normal concentration of G6P in biological systems. (http://www.hmdb.ca/metabolites/HMDB0001401)

Biospecimen	Value	Age
Blood	29.1 ± 6.8 μM	Adult (> 18 years old)
Cellular cytoplasm	26–50 μM	Adult (> 18 years old)
Saliva	2.42 ± 1.55 μM	Adult (> 18 years old)

Table 2 Comparison of performances of the proposed biosensors with various G6P biosensors and different methods

Methods/transducer	Detector elements	LOD (μM)	Detection range (μM)	Response time (sec)	Reference
Clark-type electrode	G6PDH, salicylate hydroxylase, and NAD^+	15	20–470	20	[6]
Graphite carbon electrode	G6PDH, GR, $NADP^+$, and polyethyleneimine ferrocene	–	50–20	–	[7]
Carbon paste electrode	G6PDH, $NADP^+$, tetracyanoquinodimethane, polyethyleneimine, and Mg^{2+}	50	Up to 80	30–50	[4]
Screen printed electrode	G6PDH, $NADP^+$, and p-hydroxybenzoate hydroxylase	1.2	2–1000	20	[3]
Porous platinum black electrode	G6PDH from *B. stearothermophilus*	–	–	–	[27]
Screen printed electrode	G6PDH, GR, $NADP^+$, and Prussian blue nanoparticles	2.3	10–1250	15	[2]
Spectrophotometry	–	3	3.4–180	–	[28]
Fluorimetry	–	0.1	0.3–10	–	[29]
LC-MS	–	1.5	5.0–100	–	[30]
Specific colorimetry	–	0.15	0.5–200	–	[28]
PGE	G6PDH, $NADP^+$, and PPy	8	25–250	4	Current study
PGE	G6PDH, $NADP^+$, PPy, and CS-Fe_3O_4 nanoparticles	2	2.5–50	4	Current study

Interference Study

In the real sample, some materials may involve the electrochemical reaction and affect the biosensor responses such as glucose, urea, and cysteine. The effect of these interfering substances on the BS-1 and BS-2 biosensors was investigated at constant density of

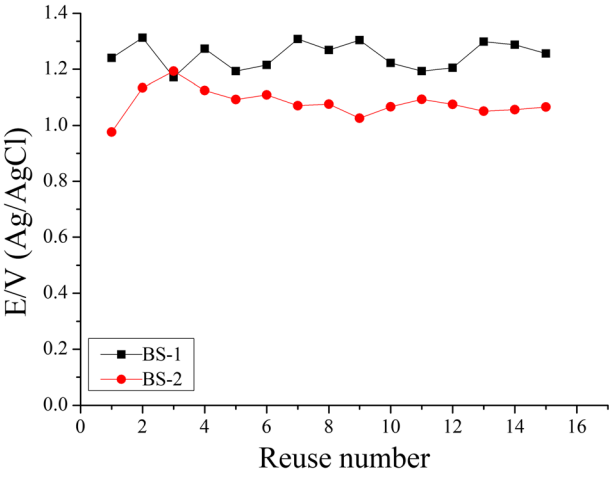

Fig. 8 Operational stability of the BS-1 and BS-2 biosensors

Table 3 Determination of G6P in blood serum samples

BS-1 biosensor		BS-2 biosensor	
G6P concentration mM	Recovery (%)	G6P concentration mM	Recovery (%)
0.025	74.0	0.005	110
0.03	79.0	0.01	103
0.05	80.0	0.025	100
0.1	72.0	0.03	93
0.15	70.0	0.05	91

0.25 mA/cm^2 in 100 mM Tris–HCl buffer (pH 8.0). The responses for a fixed concentration of G6P were compared with the potential value obtained in the presence of the interfering species. The biosensors were evaluated with 0.25 mM interferents in the detection of 0.25 mM of G6P. The influence of the interferents to response of biosensors was summarized in Table 2 by calculating the deviation of response (%) indicated as below:

$$\text{Deviation of response } (\%) = [(I\text{p}-I\text{a})/I\text{a}] \times 100$$

*I*p Response of the biosensor to X M G6P in presence of interferent at certain concentration
*I*a Response of the biosensor to X M G6P

It was shown that the responses of the BS-1 and BS-2 biosensor increased between 1 and 8% at glucose and urea, while the response of the BS-1 biosensor increased by 4.3% at cysteine, had no effect on the response of BS-2 biosensor.

Analysis of G6P in Human Serum

In order to investigate the possible application of these biosensors in clinical analysis, the modified electrodes were also tested in human serum. For this aim, human serum samples were spiked with known concentrations of G6P, and chronopotentiometric measurements were done using the BS-1 and BS-2 biosensors. The results of G6P content to be determined by using those biosensors in blood serum were listed in Table 3. It was found that BS-2 biosensor gave better results, which are consistent with the added amount of G6P in serum samples. While the recovery values of the spiked serum sample ranged from 91 to 110% for BS-2 biosensor, those values were 70% and 73% for PPy/G6P biosensor. Previously, Banerjee et al. [2] obtained the recovery values of the spiked serum sample ranged from 94 to 105.2% with amperometric G6P biosensor based on Prussian blue nanoparticle-modified screen-printed electrode.

Based on this study, it can be said that the proposed BS-2 biosensor is almost free from interferences present in serum sample and can be effectively used for analysis of available G6P in blood serum.

Conclusions

In this study, the PPy film was used as a suitable matrix for enzyme immobilization. BS-1 and BS-2 biosensors were prepared and compared for the measurement of G6P. BS-1 electrode was formed by immobilization of G6PD on PPy electrochemically polymerized on the PGE. Also, BS-2 electrode was obtained by the same procedure in the presence of CS/Fe_3O_4

nanoparticles embedded in PPy matrix. The experimental results clearly showed that both of the biosensors exhibited response to the G6P. The immobilized G6PD displayed excellent catalytic property to G6P. But, the BS-2 nanocomposite film offered better results by decreasing potential. CS/Fe_3O_4 nanoparticles in the biosensing interface provided not only immobilization of G6PD on the surface conveniently but also improved the electron transfer between electrode surface and analyte. The proposed biosensors showed improved performance in terms of low response time (4 s), low detection limit, broad detection range, and good stability. The developed BS-2 biosensor is almost free from interferences present in blood serum with recovery between 91 and 110% and can be effectively used for analysis of G6P content in blood serum. It can be said the PPy film and CS/Fe_3O_4-PPy nanocomposite film could be a good candidate for the highly sensitive and selective G6P biosensors by providing electrochemical and biocompatible microenvironment in the immobilization studies of the G6PD.

Funding Information The authors thank the Suleyman Demirel University Research Funds (Project number: 4795-OYP-D2-17).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Srivastava, P. K., & Singh, S. (2013). Immobilization and applications of glucose-6-phosphate dehydrogenase: a review. *Preparative Biochemistry & Biotechnology, 43*(4), 376–384.
2. Banerjee, S., Sarkar, P., & Turner, A. P. (2013). Amperometric biosensor based on Prussian blue nanoparticle-modified screen-printed electrode for estimation of glucose-6-phosphate. *Analytical Biochemistry, 439*(2), 194–200.
3. Cui, Y., Barford, J. P., & Renneberg, R. (2007). Development of a glucose-6-phosphate biosensor based on coimmobilized p-hydroxybenzoate hydroxylase and glucose-6-phosphate dehydrogenase. *Biosensors & Bioelectronics, 22*(11), 2754–2758.
4. Bassi, A. S., Tang, D., & Bergougnou, M. A. (1999). Mediated, amperometric biosensor for glucose-6phosphate monitoring based on entrapped glucose-6phosphate dehydrogenase, Mg^{2+} ions, tetracyanoquinodimethane, and nicotinamide adenine dinucleotide phosphate in carbon paste. *Analytical Biochemistry, 268*(2), 223–228.
5. Tzang, C. H., Yuan, R., & Yang, M. (2001). Voltammetric biosensors for the determination of formate and glucose-6-phosphate based on the measurement of dehydrogenase-generated NADH and NADPH. *Biosensors & Bioelectronics, 16*(3), 211–219.
6. Cui, Y., Barford, J. P., & Renneberg, R. (2007). Development of an interference-free biosensor for glucose-6-phosphate using a bienzyme-based Clark-type electrode. *Sensors and Actuators B: Chemical, 123*(2), 696–700.
7. Suye, S.-i., Zheng, H., Okada, H., & Hori, T. (2005). Assembly of alternating polymerized mediator, polymerized coenzyme, and enzyme modified electrode by layer-by-layer adsorption technique. *Sensors and Actuators B: Chemical, 108*(1-2), 671–675.
8. Aydemir, N., Malmstrom, J., & Travas-Sejdic, J. (2016). Conducting polymer based electrochemical biosensors. *Physical Chemistry Chemical Physics, 18*(12), 8264–8277.
9. Geetha, S., Rao, C. R., Vijayan, M., & Trivedi, D. C. (2006). Biosensing and drug delivery by polypyrrole. *Analytica Chimica Acta, 568*(1-2), 119–125.
10. Berkkan, A., Seçkin, A. I., Pekmez, K., & Tamer, U. (2009). Amperometric enzyme electrode for glucose determination based on poly(pyrrole-2-aminobenzoic acid). *Journal of Solid State Electrochemistry, 14*, 975–980.

11. Abdul Amir Al-Mokaram, A. M. A., Yahya, R., Abdi, M. M., & Muhammad Ekramul Mahmud, H. N. (2016). One-step electrochemical deposition of polypyrrole–chitosan–iron oxide nanocomposite films for non-enzymatic glucose biosensor. *Materials Letters, 183*, 90–93.
12. Montoya, P., Mejía, S., Gonçales, V. R., Torresi, S. I. C. d., & Calderón, J. A. (2015). Performance improvement of macroporous polypyrrole sensor for detection of ammonia by incorporation of magnetite nanoparticles. *Sensors and Actuators B: Chemical, 213*, 444–451.
13. Tran, L. D., Nguyen, B. H., Van Hieu, N., Tran, H. V., Nguyen, H. L., & Nguyen, P. X. (2011). Electrochemical detection of short HIV sequences on chitosan/Fe3O4 nanoparticle based screen printed electrodes. *Materials Science and Engineering: C, 31*(2), 477–485.
14. Kaushik, A., Khan, R., Solanki, P. R., Pandey, P., Alam, J., Ahmad, S., & Malhotra, B. D. (2008). Iron oxide nanoparticles-chitosan composite based glucose biosensor. *Biosensors & Bioelectronics, 24*(4), 676–683.
15. Cheng, Y., Liu, Y., Huang, J., Li, K., Xian, Y., Zhang, W., & Jin, L. (2009). Amperometric tyrosinase biosensor based on Fe_3O_4 nanoparticles-coated carbon nanotubes nanocomposite for rapid detection of coliforms. *Electrochimica Acta, 54*(9), 2588–2594.
16. Wang, S., Tan, Y., Zhao, D., & Liu, G. (2008). Amperometric tyrosinase biosensor based on Fe3O4 nanoparticles-chitosan nanocomposite. *Biosensors & Bioelectronics, 23*(12), 1781–1787.
17. Yang, L., Ren, X., Tang, F., & Zhang, L. (2009). A practical glucose biosensor based on Fe(3)O(4) nanoparticles and chitosan/nafion composite film. *Biosensors & Bioelectronics, 25*(4), 889–895.
18. Zhang, W., Li, X., Zou, R., Wu, H., Shi, H., Yu, S., & Liu, Y. (2015). Multifunctional glucose biosensors from Fe_3O_4 nanoparticles modified chitosan/graphene nanocomposites. *Scientific Reports, 5*(1), 11129.
19. Tan, X., Zhang, J., Tan, S., Zhao, D., Huang, Z., Mi, Y., & Huang, Z. (2009). Amperometric hydrogen peroxide biosensor based on horseradish peroxidase immobilized on Fe3O4/chitosan modified glassy carbon electrode. *Electroanalysis, 21*(13), 1514–1520.
20. Sahin, S., & Ozmen, I. (2016). Determination of optimum conditions for glucose-6-phosphate dehydrogenase immobilization on chitosan-coated magnetic nanoparticles and its characterization. *Journal of Molecular Catalysis B: Enzymatic, 133*, S25–S33.
21. Montoya, P., Jaramillo, F., Calderón, J., Córdoba de Torresi, S. I., & Torresi, R. M. (2010). Evidence of redox interactions between polypyrrole and Fe3O4 in polypyrrole–Fe3O4 composite films. *Electrochimica Acta, 55*(21), 6116–6122.
22. Patel, S. K., Choi, S. H., Kang, Y. C., & Lee, J. K. (2017). Eco-friendly composite of Fe3O4-reduced graphene oxide particles for efficient enzyme immobilization. *ACS Applied Materials & Interfaces, 9*(3), 2213–2222.
23. Kim, Y. H., & Yoo, Y. J. (2009). Regeneration of the nicotinamide cofactor using a mediator-free electrochemical method with a tin oxide electrode. *Enzyme and Microbial Technology, 44*(3), 129–134.
24. Kim, Y. H., Kim, T., Ryu, J. H., & Yoo, Y. J. (2010). Iron oxide/carbon black (Fe2O3/CB) composite electrode for the detection of reduced nicotinamide cofactors using an amperometric method under a low overpotential. *Biosensors & Bioelectronics, 25*(5), 1160–1165.
25. Liu, W., Zhang, S., & Wang, P. (2009). Nanoparticle-supported multi-enzyme biocatalysis with in situ cofactor regeneration. *Journal of Biotechnology, 139*(1), 102–107.
26. Haddaoui, M., Sola, C., Raouafi, N., & Korri-Youssoufi, H. (2018). E-DNA detection of rpoB gene resistance in mycobacterium tuberculosis in real samples using Fe3O4/polypyrrole nanocomposite. *Biosensors & Bioelectronics, 128*, 76–82.
27. Kazuhito Aoki, Hiroko Suzuki, Yoshihiro Ishimaru, Shigeru Toyama, Yoshihito Ikariyama, Takeaki Iida, (2005) Thermophilic glucokinase-based sensors for the detection of various saccharides and glycosides. *Sensors and Actuators B: Chemical 108*(1–2), 727–732
28. Aiping Zhu, Roberto Romero, Howard R. Petty, (2011) An enzymatic colorimetric assay for glucose-6-phosphate. *Analytical Biochemistry 419*(2), 266–270
29. Aiping Zhu, Roberto Romero, Howard R. Petty, (2009) An enzymatic fluorimetric assay for glucose-6-phosphate: Application in an in vitro Warburg-like effect. *Analytical Biochemistry 388*(1), 97–101
30. Carla Antonio, Tony Larson, Alison Gilday, Ian Graham, Ed Bergström, Jane Thomas-Oates, (2007) Quantification of sugars and sugar phosphates in Arabidopsis thaliana tissues using porous graphitic carbon liquid chromatography-electrospray ionization mass spectrometry. *Journal of Chromatography A 1172(2)*, 170–178

Affiliations

Selmihan Sahin[1] · Ismail Ozmen[1] · Gizem Yildirim Bastemur[2] · Sabriye Percin Ozkorucuklu[3]

[1] Arts and Science Faculty, Department of Chemistry, Suleyman Demirel University, Cunur, 32260 Isparta, Turkey

[2] Institute of Science, Department of Molecular Biology and Genetics, Istanbul University, Fatih, 34134 Istanbul, Turkey

[3] Science Faculty, Department of Molecular Biology and Genetics, Istanbul University, Fatih, 34134 Istanbul, Turkey