Comparative Study of Electrochemical Sensors Based on Enzyme Immobilized into Polyelectrolyte Microcapsules and into Chitosan Gel

Yulia V. Plekhanova,*1† Sergei A. Tikhonenko,*2 Alexey V. Dubrovsky,*2 Alexander L. Kim,*2,*3 Egor V. Musin,*2,*3 Gou-Jen Wang,*4 Iren E. Kuznetsova,*5 Vladimir V. Kolesov,*5 and Anatoly N. Reshetilov*1,*5†

*1 *PSCBR RAS G. K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Prospekt Nauki 5, Pushchino, Moscow Region 142290, Russia*
*2 *FSBIS Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Institutskaya St. 3, Pushchino, Moscow Region 142290, Russia*
*3 *Lomonosov Moscow State University, Leninskie Gory, Moscow 119991, Russia*
*4 *Department of Mechanical Engineering, National Chung-Hsing University, Taichung 402, Taiwan*
*5 *A. Kotelnikov Institute of Radio Engineering and Electronics, Russian Academy of Sciences, Mokhovaya St. 11-7, Moscow 125009, Russia*

The characteristics of an electrochemical biosensor based on a Prussian-blue screen-printed electrode containing glucose oxidase incorporated into polyelectrolyte microcapsules (PMC) are considered. PMC with the embedded enzyme were formed using sodium polystyrene sulfonate and poly(allylamine hydrochloride). The characteristics were compared with those of the enzyme immobilized in chitosan gel. We assessed the dependences of biosensor signals on the composition of the buffer solution, on the glucose concentration; the operational and long-term stabilities. The enzyme immobilized in PMC proved to be more sensitive to buffer molarity at a maximum within 35 – 40 mM. The apparent Michaelis constants were 1.5 and 4.1 mM at the immobilization in, respectively, chitosan and PMC. The developed biosensors were used to assay commercial juices. The biosensors' data on the glucose contents were shown to have a high correlation with the standard spectrophotometric assay (0.92 – 0.95%), which implies a possible application of the fabricated biosensors in foodstuff analysis.

Keywords Electrochemical biosensor, screen-printed electrode, glucose oxidase, polyelectrolyte microcapsules, chitosan, glucose

(Received April 14, 2019; Accepted May 23, 2019; Advance Publication Released Online by J-STAGE May 31, 2019)

Introduction

The development of biosensors and biofuel cells is associated with the search for and development of methods for immobilization of the biocatalyst on the electrode surface, so that the biocatalyst is active for as long as possible. For this reason, the search for novel biocompatible materials to create electrodes and the use of new polymers to bind the biocatalyst on the electrode surface are topical.

The activity of enzymes can be preserved at a high level by encapsulation. The enzymes are immobilized in capsules from various materials, such as liposomes,[1] polymers,[2] sol-gels,[3] hydrogels,[4] *etc.* The layer-by-layer coating of oppositely charged polyelectrolytes for immobilization of enzymes[5-7] enables their use as microcontainers and microreactors in various industries, for example, food,[8] cosmetic,[9] and pharmaceutical.[10] Earlier, for urease and lactate dehydrogenase, it has been shown that the immobilization of enzymes in polyelectrolyte microcapsules (PMC) makes it possible to maintain enzyme activity at a high level for several months and also protects encapsulated enzymes from proteinase K;[11] encapsulated catalase is protected by PMC from the action of microorganisms and protease.[12] Enzymes immobilized in polyelectrolyte capsules can be used to create biosensors, *e.g.*, for potentiometric assays of urea[13-15] or acetylcholine,[16] optical (fluorescent) determination of glucose.[17]

The catalytic properties of glucose oxidase (GOx) have been well investigated. At the same time, immobilization of the enzyme in polyelectrolyte capsules may change its structure and, as a consequence, its catalytic activity. Thus, the binding of a number of negatively charged polyelectrolytes to lactate dehydrogenase and glutamate dehydrogenase has been shown to break down the tertiary and partially secondary structure of the enzymes and, in this way, to totally inactivate their activity.[18] The use of polyelectrolytes of opposite chirality has also led to a change in the activity of immobilized horseradish peroxidase and GOx.[19]

One of the methods used to control the catalytic activity of the

† To whom correspondence should be addressed.
E-mail: plekhanova@ibpm.pushchino.ru (Y. V. P.); anatol@ibpm.pushchino.ru (A. N. R.)

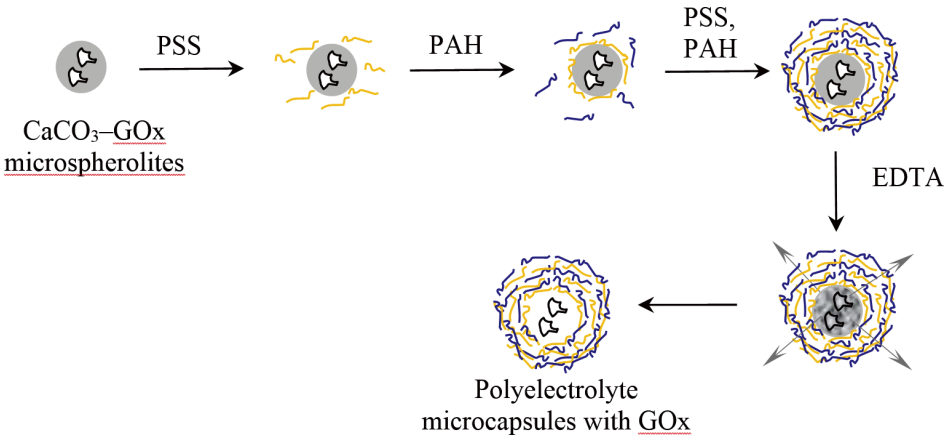

Fig. 1 Scheme of fabricating polyelectrolyte microcapsules with the enzyme.

enzyme in biosensors is the determination of hydrogen peroxide released during oxidation of the substrate. An electrocatalyst of hydrogen peroxide reduction is iron hexacyanoferrate (Prussian blue), which is used to modify the measurement electrodes.[20,21] The possibility of a selective determination of hydrogen peroxide by its reduction reaction in the presence of oxygen on electrodes with iron hexacyanoferrate at a zero potential and a pH close to neutral has been demonstrated.[22]

The aim of this work was to compare the characteristics of the glucose biosensor prepared using two techniques of immobilization of the enzyme glucose oxidase on the surface of a Prussian-blue screen-printed electrode–encapsulation of the enzyme in polyelectrolyte layers or in chitosan gel. Immobilization into chitosan for a comparison was chosen because, due to the attractive properties of chitosan (biocompatibility, biodegradability and ease of use), this technique is often used to develop biosensors.[23,24]

Experimental

Reagents

The following reagents were used: dibasic trihydrate potassium phosphate, sodium hydroxide, sodium chloride, glucose, acetic acid (Diakon, Russia); iron(III) chloride, potassium chloride, calcium chloride, sodium carbonate (Khimmed, Russia); hydrochloric acid, hydrogen peroxide (30% solution), sodium carbonate (Reakhim, Russia); chitosan (low molecular weight), potassium hexacyanoferrate(III), sodium polystyrene sulfonate (PSS, 70 kDa), poly(allylamine hydrochloride) (PAH, 70 kDa), ethylenediaminetetraacetic acid (EDTA), 4-aminoantipyrine, phenol, glucose oxidase (EC 1.1.3.4) from *Aspergillus niger* (activity, 185000 U/g), horseradish peroxidase (1.11.1.7) (activity, 1 kU/g) (Sigma-Aldrich, USA).

Formation of a Prussian blue-based chemical sensor

Screen-printed three-contact electrodes (Color Electronics, Moscow, Russia) were used as working electrodes. Prussian blue was precipitated on the electrode surface from a reaction mixture containing 0.1 M $FeCl_3$ and 0.1 M $K_3[Fe(CN)_6]$ in the background electrolyte (0.1 M KCl, 0.1 M HCl). The electrode was activated (a potentiodynamic treatment of the Prussian blue electrode in a cyclic mode in the background electrolyte within the range of potentials from – 0.05 up to 0.35 V) in accordance with the method described in a study.[22]

Fabrication of polyelectrolyte microcapsules with incorporated glucose oxidase

Formation of composite $CaCO_3$-protein microspherolites. An equal volume of a sodium carbonate solution (0.33 M) was added to a 0.33-M calcium chloride solution containing 3 mg/mL GOx and intensively stirred on a magnetic mixer. The mixing was continued for 30 s, after which the suspension was kept up to the complete sedimentation of formed particles.[25] The maturation of microspherolites was monitored by a light microscope. Then, the supernatant was decanted, and the sediment was washed with water and used to produce PMC. The particles formed were 5 ± 1 μm in size.

Preparation of PMC. Polyelectrolyte microcapsules were produced by the alternate adsorption of oppositely charged polyelectrolytes on disperse microparticles (nuclei), followed by the dissolution of the nuclei. Alternate adsorption of PAH and PSS on the surfaces of $CaCO_3$ microspherolites was carried out in polyelectrolyte solutions (2 mg/mL) containing 0.5-M NaCl. Each adsorption step was followed by a triple washing of fabricated capsules with a 0.5-M NaCl solution, which was required to discard non-adsorbed polymer molecules. Particles were separated from the supernatant by centrifugation. After a required number of layers was deposited, carbonate nuclei were dissolved in a 0.2-M solution of EDTA for 12 h. Produced capsules were washed three times with water to remove nuclei' decomposition products. Capsules contained 6 polyelectrolyte layers. The concentration of protein in a capsule was 0.66 ng/capsule. The suspension of capsules at a concentration of 3×10^9 particles/mL was stored in bidistilled water at a temperature of 4°C. The formation of polyelectrolyte capsules around the enzyme is shown schematically in Fig. 1.

Formation of glucose biosensors

1) Polyelectrolyte capsules in the amount of 5 μL with incorporated GOx were applied onto the surface of a Prussian blue working electrode and dried at room temperature for 30 min. The enzyme concentration on the electrode surface was 139 μg/cm^2.

2) A mixture of GOx (6 mg/mL) and chitosan (2% solution in 1% acetic acid)[26] at a volume ratio of 1:1 was prepared; 5 μL of the mixture was applied onto the surface of a Prussian blue working electrode and air-dried for 30 min at room temperature. The concentration of the enzyme on the electrode surface was 211 μg/cm^2.

Between measurements, the biosensors were stored at a

temperature of 4°C in the dark. The measurements were carried out at a temperature of 20°C in a 1-mL cuvette at constant stirring in a sodium–potassium–phosphate buffer solution. The molarity, pH, content of sodium chloride in the buffer were varied. The measurements were performed on an EmStat 3 potentiostat (PalmSens, Netherlands) at a constant zero potential fed to the working electrode relative to the reference electrode (Ag/AgCl). The measured parameter was the amplitude of the signal; it was assessed by the difference between the initial and final levels before and after the introduction of glucose.

As real samples to determine the concentration of glucose, we used beverages diluted tenfold with a buffer solution to a concentration required for the assay: 1) Global Village reconstituted clarified apple juice, Yuzhnaya Sokovaya Kompaniya, Russia; 2) Fruto-Nyanya reconstituted clarified apple juice, Progress Ltd., Russia; 3) Fruktovyi Sad orange pulp nectar, Lebedyansky Ltd. Russia; 4) Fruktovyi Sad multifruit pulp nectar, Lebedyansky Ltd., Russia; 5) Home-made wine, 2-year-aged, Izabella grape; 6) Aloe-Fresh non-alcoholic non-carbonated pasteurized beverage, Velta-Penza Ltd., Russia; 7) El Kurador Sangria sugary wine drink, Hauser Weinimport GmbH, Germany.

Spectrophotometric assay of glucose

The assay is based on the photometric determination of a compound colored during the oxidation of chromogenic substrates.[27] The oxidation of glucose by air oxygen in the presence of glucose oxidase produces hydrogen peroxide. Hydrogen peroxide oxidizes the chromogenic substrate (4-aminoantipyrine) in the presence of phenol and peroxidase (catalyst) to produce a colored compound, quinoneimine. The coloration intensity of this compound is directly proportional to the concentration of glucose in the sample. Measurements were carried out using a Specord m40 spectrophotometer at a wavelength of 500 nm.

Results and Discussion

Dependence of biosensor signals on buffer solution

The optimal pH for glucose oxidase to function is pH 5.6.[28] The maximal amplitudes of the biosensor responses during the immobilization of the enzyme in chitosan gel were observed within the range of pH 6.0 – 7.0; during immobilization in PMC, pH 5.5 – 6.0 (Fig. 2(A)). An insignificant shift of the optimum to the alkaline region and a broadening of the optimal pH range is, most probably, due to conditions created by immobilization of the enzyme.

The catalytic activity of the immobilized enzyme was also affected by the molarity of the buffer solution (Fig. 2(B)). Within the investigated range of its changes for the enzyme immobilized in chitosan, biosensor signals are seen to grow monotonically; the signal amplitudes reach the greatest values at 40 – 50 mM of the buffer solution. At the same time, for the encapsulated enzyme the amplitude depends on the value of the molarity in a bell-shaped way; the maximum in this case is observed at a buffer solution concentration of 30 – 40 mM.

A high ionic strength of a solution (greater than 100 mM) negatively affects the stability of polyelectrolyte complexes,[29] but increases its conductance by facilitating the transfer of protons onto the electrode. This is reflected on the generation of biosensor and biofuel cell signals;[30] the addition of sodium chloride leads to a decrease of the internal resistance of the system, and to an increase of the power.[31] Figure 2(C) plots the

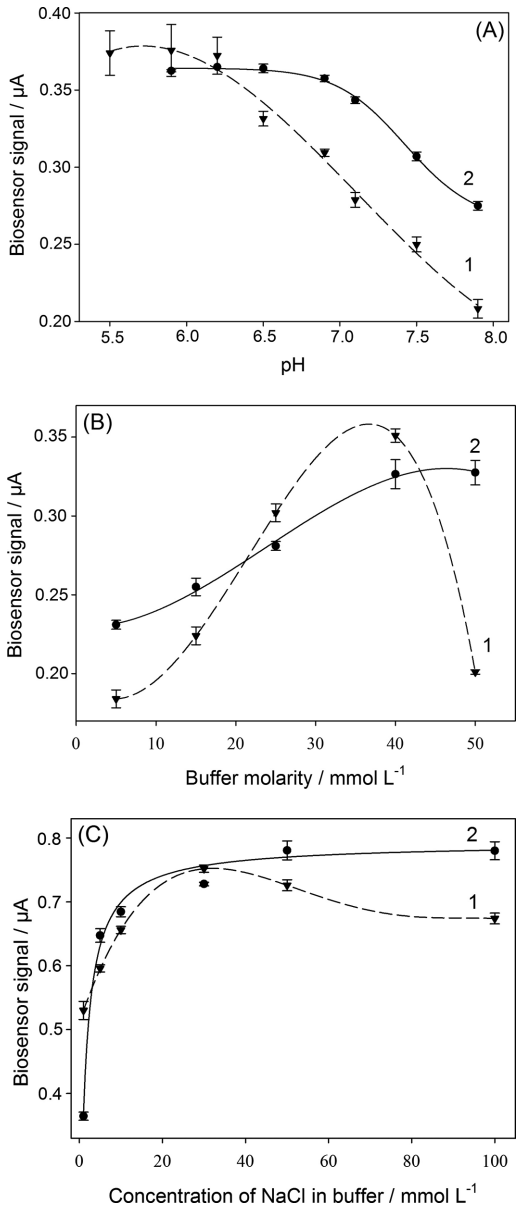

Fig. 2 Dependence of biosensor signals on the composition of the buffer solution for a biosensor based on glucose oxidase immobilized in polyelectrolyte capsules (1) and in chitosan gel (2): (A) on pH of the buffer solution; (B) on molarity of the buffer solution; (C) on the concentration of NaCl in the buffer solution. Concentration of glucose, 5 mM for a biosensor with polyelectrolyte capsules, and 1.24 mM for a biosensor with chitosan gel.

dependences of biosensor signals on the NaCl concentrations in the buffer. An increase of the NaCl concentration increases the signal, whereas for the encapsulated enzyme at a NaCl concentration of 25 mM and higher the signal decreases. The observed dependence can be partially explained by the data of Fig. 2(B): at a buffer solution molarity rise the dependence of the signal amplitude passes through a maximum; as the concentration of sodium chloride is increased, we observe in fact a non-monotonic dependence, which also passes through a maximum. Thus, it can be summed up that high concentrations of sodium chloride (greater than 80 mM) have a negative effect on the activity of the enzyme, which leads to decreased biosensor signals; an increase of the concentration beyond 30 mM has a negative effect on the activity of the enzyme in the capsule.

As can be seen from a comparison of Figs. 2(B) and 2(C), potassium ions render a more negative effect (the buffer molarity increases due to both potassium and sodium ions), whereas an increase in the concentration of sodium ions (Fig. 2(C)) beyond the level of 30 mM leads to a drop in the level of biosensor signals by ~10% (and in the case of a joint increase of the concentrations of potassium ions and sodium ions beyond the level of 30 mM the drop is ~44%).

Calibration dependences of biosensor signals on glucose concentration

Dependences of biosensor signals on the glucose concentration are plotted in Fig. 3. Major characteristics of the investigated biosensors are given in Table 1. The ranges of glucose determination using the encapsulated enzyme differ from those for the enzyme immobilized in chitosan gel. Thus, the lower and upper detection limits are higher, and the linear range is broader, in the case of the encapsulated enzyme. The sensitivity coefficient, however, is higher when using chitosan for immobilization (1.17 *vs.* 0.12 μA/mM). Works[6,32] have shown that the layer-by-layer application of oppositely charged polyelectrolytes does not prevent the penetration of low-molecular compounds into the interior of the capsule. Glucose is a low-molecular compound, so the change of enzyme activity can be due only to immobilization. As is known, the Michaelis constant shows the extent of affinity of the substrate and enzyme:[33] the lower is the value of the constant, the higher is the affinity of the enzyme to substrate. Thus, the immobilization of the enzyme in PMC leads to a decrease in the affinity of the enzyme to the substrate (Table 1).

The reproducibility of the results of measurements by biosensors was assessed based on a statistical treatment involving a sampling of 15 measurements for a glucose concentration of 1 mM. The variation coefficient for the biosensor with the encapsulated enzyme was 4.5%; the mean value of the signal and the standard deviation were 151.31 ± 6.88 nA. For a biosensor with GOx immobilized in a chitosan gel, the variation coefficient was 4.6%; the mean value of the signal and the standard deviation was 587.37 ± 27.08 nA.

Assessment of long-term stability

For GOx incorporated into polyelectrolyte capsules, the signal

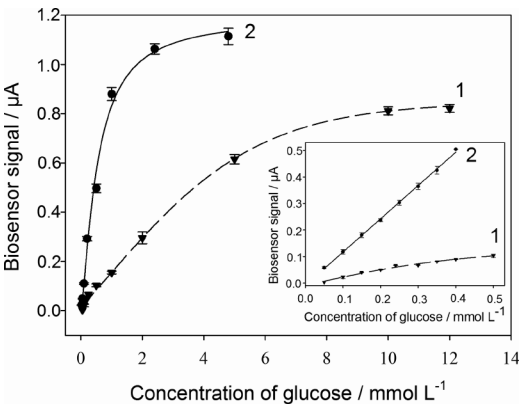

Fig. 3 Dependence of biosensor signals on the concentration of glucose for a biosensor based on glucose oxidase immobilized in polyelectrolyte capsules (1) and in chitosan gel (2). Inset, the initial linear-range segments of the concentration dependences.

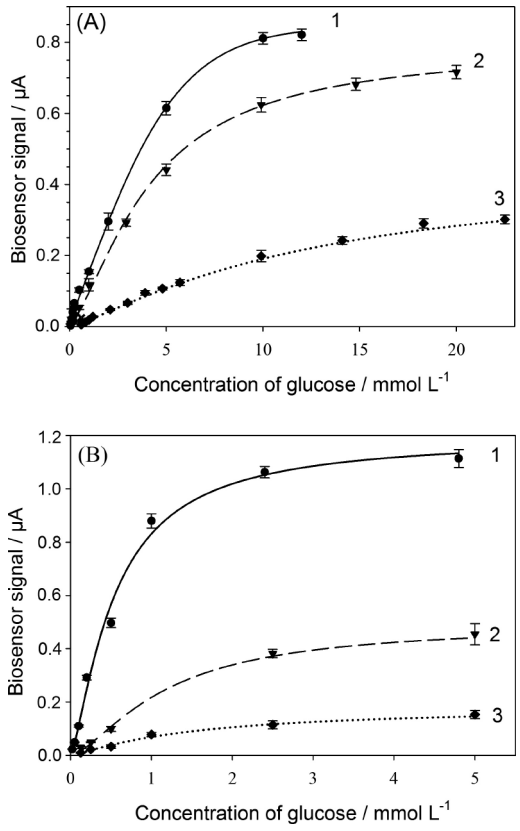

Fig. 4 Long-term stability of the glucose biosensors: (A) glucose oxidase immobilized in polyelectrolyte capsules (1, the first 24 h of biosensor formation; 2, in 5 months; 3, in 8 months); (B) glucose oxidase immobilized in chitosan gel (1, the first 24 h of biosensor formation; 2, in 2 months; 3, in 12 months).

Table 1 Characteristics of the fabricated glucose biosensors

Parameter	In polyelectrolyte capsules	In chitosan gel
Values of parameters of the calibration dependence	V_{max} = 1.069; h = 1.223; K_M = 4.071	V_{max} = 1.398; h = 1.176; K_M = 1.517
Linear detection range/mM	0.10 - 5.50	0.05 - 0.60
Regression equation for the linear segment	$y = 0.1184x + 0.0456$	$y = 1.1749x - 0.0032$
Sensitivity coefficient/μA mM^{-1}	0.12	1.17
Minimum detection limit/mM	0.10	0.05
Detection range/mM	0.10 - 10.00	0.05 - 5.00

An equation describing the calibration dependences: $V = \frac{V_{max}S^h}{K_M^h + S^h}$. Correlation coefficient for calibration dependences and for regression equation for the linear segment of R_2 is 0.99.

Table 2 Changes in major parameters of the calibration curve in storage of the enzyme biosensor

Parameter	Immobilization in polyelectrolyte capsules			Immobilization in chitosan gel	
	Day 1	After 5 months	After 8 months	Day 1	After 2 months
V_{max}/μA	1.069	0.775	0.445	1.398	0.491
K_M/mM	4.071	4.142	12.641	1.517	1.166
Enzyme activity, %	100	75	20	100	43
Minimum detection limit/mM	0.10	0.16	0.80	0.05	0.10
Detection range/mM	0.10 – 10.00	0.16 – 15.00	0.80 – 18.00	0.05 – 5.00	0.10 – 4.00

Table 3 Results of measurements of real samples

Technique	Immobilization in polyelectrolyte capsules		Immobilization in chitosan gel		Spectrophotometry, λ = 500 nm	
Sample	Biosensor readings, $a \pm$ SD/mM	Measurement error, %	Biosensor readings, $a \pm$ SD/mM	Measurement error, %	Standard method readings, $a \pm$ SD/mM	Measurement error, %
1	129.00 ± 1.87	1.5	142.18 ± 0.35	0.2	136.95 ± 2.56	1.9
2	136.24 ± 5.10	3.7	167.43 ± 6.67	4.0	147.51 ± 5.83	4.0
3	156.04 ± 6.10	3.9	192.41 ± 3.00	1.6	149.46 ± 6.43	4.3
4	180.01 ± 1.44	0.8	171.00 ± 4.52	2.6	159.88 ± 11.00	6.9
5	204.20 ± 11.33	5.5	198.67 ± 3.44	1.7	178.38 ± 1.03	0.6
6	37.20 ± 1.96	5.3	24.22 ± 0.94	3.9	28.82 ± 2.05	7.1
7	306.94 ± 15.62	5.1	216.16 ± 8.45	4.0	192.25 ± 12.4	6.5

a, The mean value of measurements performed in 3 – 5 repeats; SD, standard deviation. Samples are described in Experimental.

was ~75% of the initial level after 5 months of storage of the biosensor between measurements at 4°C; after 8 months, 20% (Fig. 4(A)). For GOx immobilized in chitosan gel, the biosensor signal was 43% of the initial level after 2 months; 13%, after 1 year (Fig. 4(B)). Table 2 shows changes in the major parameters of the biosensors in storage.

For capsules with the enzyme stored in bidistilled water at 4°C for 11 months followed by their immobilization on the electrode the biosensor signal was about 50% of the initial level (data not shown). Therefore, the activity of the enzyme decreases slower for capsules with the enzyme stored in solution than those immobilized on the electrode, the biosensor being stored dry at 4°C. Thus, encapsulation contributes to preserving the activity of the enzyme, including as a biosensor component, at a sufficiently high level for a long time, which is consistent with data for other enzymes, *e.g.*, for urease.[11,15]

Both methods of immobilizing GOx on the surface of a screen-printed electrode may find use in the development of biosensors. The simplicity of immobilizing the enzyme in chitosan gel and small time required to form a biosensor enable speeding up and simplifying the assay based on this immobilization technique; at the same time, immobilization in polyelectrolyte capsules makes it possible to increase the lifetime of the biosensor and to maintain the activity of the enzyme on the initial level.

Effects of real-sample components on biosensor signals

When assaying real samples, it is required to take into account the effects of their major components. Thus, biosensor signals in glucose assays of wine beverages can be affected by the ethanol contents. We investigated biosensor signals for the introduction of glucose in buffers with ethanol contents within the range of 0.1 – 2.0 M. Upon the introduction of glucose at a concentration of 1.0 and 5.0 mM, the signal of the biosensor did not change; the variation coefficient did not exceed 5%. Thus, the content of ethanol in a sample within the concentration range of 0.1 – 2.0 M (0.5 – 9.2%) shall not affect the biosensor signal or introduce additional measuring error. Therefore, the developed biosensor can be used to measure the glucose contents in products that contain alcohol up to 9.2%.

The content of acid in juices should not exceed 3 g/L; in nectars, 5 g/L. We investigated the effect of citric acid within the concentration range of 0.06 – 0.90 mM on biosensor signals during the introduction of glucose at a concentration of 5 mM. The occurrence of citric acid did not change the biosensor signal; therefore, its presence in samples up to a concentration of 31 mM (6 g/L) shall not affect the glucose assay.

Use of the biosensors for assays of glucose in beverages

Monitoring of the level of sugar in foodstuffs is a necessary procedure for assessing the quality of product. The sugar contained in juices consists mainly of glucose, fructose and sucrose. Glucose increases the sugar level in the blood and stimulates the secretion of insulin faster than other sugars.[34] Therefore, its content in foodstuffs is an important parameter, especially for diabetic patients. The developed biosensors were tested on real samples of juice and alcoholic beverages (Table 3). The results of measurements were compared with the data of the standard glucose assay of samples by spectrophotometry. The correlation coefficient of the data between measurements made by the biosensor with GOx immobilized in chitosan gel and by the standard technique is 0.95. The correlation coefficient of the data between measurements made by the biosensor with GOx immobilized in polyelectrolyte capsules and by the standard method is 0.92. The correlation coefficient of the data between measurements made by two types of biosensors is 0.88. The correlation data are shown in Fig. 5. Thus, the developed biosensor is capable of determining the glucose content in

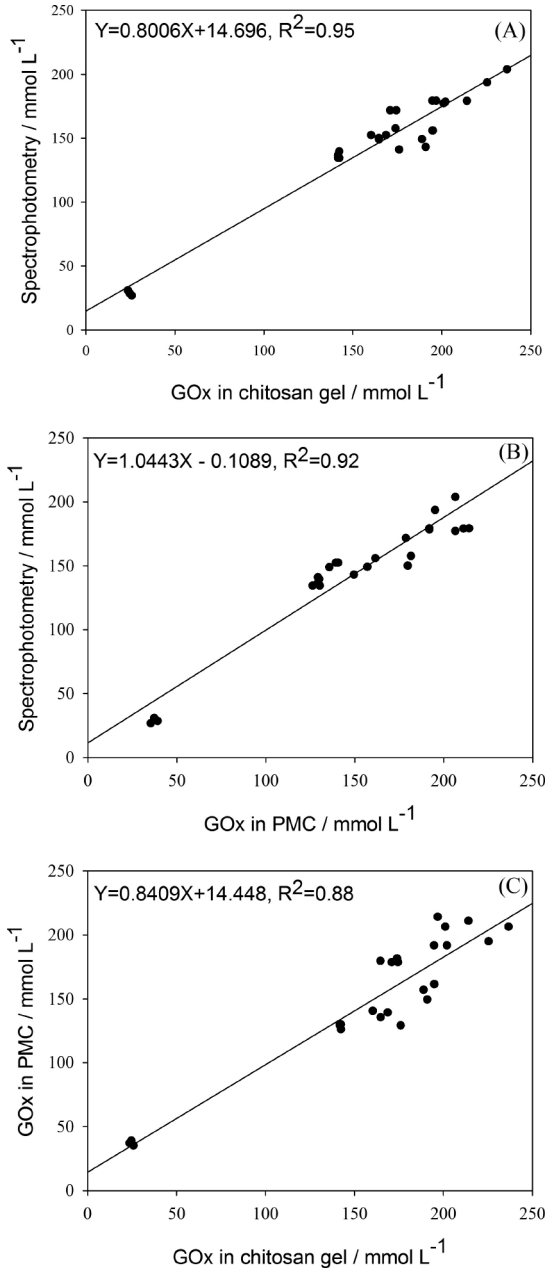

Fig. 5 Graphical correlation of data produced by various glucose assays of real samples: (A) spectrophotometric assay, glucose oxidase immobilized in chitosan gel; (B) spectrophotometric and biosensor assays, glucose oxidase immobilized in polyelectrolyte microcapsules; (C) two types of biosensors.

complex matrices comprising both other sugars and compounds of other classes, which is indicative of the specificity of the biosensor.

Conclusions

Thus, we showed that the activity of the enzyme in capsules depended on the molarity and sodium chloride concentration in the buffer solution; the presence of potassium ions had a greater effect on the decrease of the signal than sodium ions. The sensitivity coefficient of the biosensor based on GOx immobilized in chitosan gel was by an order of magnitude higher than that of the biosensor based on GOx immobilized in polyelectrolyte capsules (1.17 and 0.12 μA/mM, respectively). However, the linear range of glucose determination for the biosensor based on PMC is broader (0.10 – 5.50 and 0.05 – 0.60 mM, respectively). In one study,[35] its authors used quartz sensors to also study GOx-containing polyelectrolyte capsules. The application of natural polyelectrolytes, alginate and chitosan, led to the swelling of films and, correspondingly, to a change of the reaction to glucose, as it can be incorporated into produced polyelectrolyte complexes. They showed that, by varying the film crosslinking time, pH, extent of alginate acetylation, the sensitivity of capsules to glucose can be controlled. We showed that the use of PMC based on sodium polystyrene sulfonate and poly(allylamine hydrochloride) to immobilize the enzyme enables increasing the lifetime of the biosensor for at least up to half a year (the apparent Michaelis constant of 4.1 mM did not practically change for over 5 months of storage). We have shown earlier[36] that a modification of polyelectrolyte capsules with the enzyme by carbon nanotubes enables increasing the biosensor signal, sensitivity, glucose-detection lower limit. At the same time, many studies point to a toxicity of nanomaterials.[37,38] In the present work, we increased the amount of capsules on the biosensor surface from 17.5×10^4 (used in work[36]) up to 15×10^6 and, thus, succeeded in changing the biosensor's main parameters, sensitivity (which changed from 0.05 up to 0.12 μA/mM) and detection range (which changed from 1.0 – 25.0 to 0.1 – 10.0 mM), as well as in decreasing the glucose detection limit (from 1.0 mM down to 0.1 mM) without using nanotubes. Although the sensitivity of the biosensor with nanotubes is still higher (0.30 μA/mM) and the glucose detection lower limit is lower (0.05 mM), the biosensors presented in this work can find practical applications. The biosensors that we developed were used to assay glucose in real samples of juice; the obtained data were shown to have a high correlation with the data by the standard spectrophotometric glucose assay. A strong retention of polyelectrolyte capsules with the enzyme on the surface of a graphite electrode enables producing a multiple use biosensor, which makes it economically viable and competitive with other glucose assay techniques.

Acknowledgements

The authors acknowledge Victor Selivanov for providing language help. The work was supported by a Russian Science Foundation grant No. 18-49-08005.

References

1. J. M. Rodriguez-Nogales and A. Delgadillo, *J. Mol. Catal. B*, **2005**, *33(1–2)*, 15.
2. A. Ramanavicius, A. Kausaite, and A. Ramanavicien, *Sens. Actuators, B*, **2005**, *111–112*, 532.
3. A. C. Pierre, *J. Biocatal. Biotransform.*, **2004**, *22*, 145.
4. X. Zhu, Y. Ma, C. Zhao, Z. Lin, L. Zhang, R. Chen, and W. Yang, *Langmuir*, **2014**, *30*, 15229.
5. M. J. McShane, *Methods Mol. Biol.*, **2011**, *679*, 147.
6. K. Etsuo, *Bioseparation*, **1998**, *7*, 241.
7. S. Yabuki, *Anal. Sci.*, **2011**, *27*, 695.
8. M. A. Cerqueira, A. C. Pinheiro, O. L. Ramos, H. Silva, A. I. Bourbon, and A. A. Vicente, in "*Micro and Nano Technologies, Emerging Nanotechnologies in Food Science*", ed. R. Busquets, **2017**, Elsevier, 11.
9. A. Munin and F. Edwards-Lévy, *Pharmaceutics*, **2011**, *3*, 793.

10. A. Larrañaga, M. Lomora, J. R. Sarasua, C. G. Palivan, and A. Pandit, *Prog. Mater. Sci.*, **2017**, *90*, 325.
11. B. I. Sukhorukov, S. A. Tikhonenko, E. A. Saburova, A. V. Dubrovsky, Y. N. Dybovskaya, and L. I. Shabarchina, *Biophysics*, **2007**, *52*, 575.
12. F. Caruso, D. Trau, H. Mohwald, and R. Renneberg, *Langmuir*, **2000**, *16*, 1485.
13. V. I. Ternovsky, Y. V. Chernokhvostov, M. G. Fomkina, and M. M. Montrel, *Biofizika*, **2007**, *52*, 825.
14. M. M. Montrel, A. I. Petrov, V. I. Ternovsky, and M. G. Fomkina, RF Patent, **2008**, 2333231.
15. A. N. Reshetilov, Y. V. Plekhanova, S. A. Tikhonenko, and A. V. Dubrovskii, *J. Anal. Chem.*, **2015**, *70*, 1368.
16. L. O. Yagodina and Y. V. Chernokhvostov, *Vestn. Kazan Technological University*, **2013**, *16*, 117.
17. L. I. Kazakova, N. P. Sirota, T. V. Sirota, and L. I. Shabarchina, *Russ. J. Phys. Chem. A*, **2017**, *91*, 1828.
18. E. A. Saburova, B. I. Sukhorukov, M. E. Bobreshova, and L. I. Elphimova, *Biochemistry (Moscow)*, **2000**, *65*, 976.
19. C. Ding, H. Sun, J. Ren, and X. Qu, *Anal. Chim. Acta*, **2017**, *952*, 88.
20. P. A. Fiorito, C. M. A. Brett, and S. I. C. de Torresi, *Talanta*, **2006**, *69*, 403.
21. J. Li, J. D. Qiu, J. J. Xu, H. Y. Chen, and X. H. Xia, *Adv. Funct. Mater.*, **2007**, *17*, 1574.
22. A. A. Karyakin, O. V. Gitelmacher, and E. E. Karyakina, *Anal. Lett.*, **1994**, *27*, 2861.
23. A. Baranwal, A. Kumar, A. Priyadharshini, G. S. Oggu, I. Bhatnagar, A. Srivastava, and P. Chandra, *Int. J. Biol. Macromol.*, **2018**, *110*, 110.
24. M. Hasanzadeh, N. Shadjou, and M. de la Guardia, *TrAC*, **2018**, *102*, 210.
25. A. I. Petrov, D. V. Volodkin, and G. B. Sukhorukov, *Biotechnol. Prog.*, **2005**, *21*, 918.
26. X. Wang, H. Gu, F. Yin, and Y. Tu, *Biosens. Bioelectron.*, **2009**, *24*, 1527.
27. D. Barham and P. Trinder, *Analyst*, **1972**, *97*, 142.
28. A. P. Nechayev, S. E. Traubenberg, and A. A. Kochetkova, "*Food Chemistry*", 4th ed., **2007**, GIORD Publishers, St. Petersburg, RF, 640.
29. S. A. Tikhonenko, E. A. Saburova, E. N. Durdenko, and B. I. Sukhorukov, *Russ. J. Phys. Chem. A*, **2009**, *83*, 1781.
30. J.-Y. Nam, H.-W. Kim, K.-H. Lim, H.-S. Shin, and B. E. Logan, *Biosens. Bioelectron.*, **2010**, *25*, 1155.
31. O. Lefebvre, Z. Tan, S. Kharkwal, and H. Y. Ng, *Bioresour. Technol.*, **2012**, *112*, 336.
32. M. J. McShane, *Methods Mol. Biol.*, **2011**, *679*, 147.
33. A. Fersht, "*Enzyme Structure and Mechanism*", Reading, San Francisco, **1977**, 371.
34. B. M. Lee and T. M. S. Wolever, *Eur. J. Clin. Nutr.*, **1998**, *52*, 924.
35. S. Belbekhouche, S. Charaabi, L. Picton, D. Le Cerf, and B. Carbonnier, *Carbohydr. Polym.*, **2018**, *184*, 144.
36. A. Reshetilov, Y. Plekhanova, S. Tarasov, S. Tikhonenko, A. Dubrovsky, A. Kim, V. Kashin, A. Machulin, G.-J. Wang, V. Kolesov, and I. Kuznetsova, *Membranes*, **2019**, *9*, 53.
37. R. Madannejad, N. Shoaie, F. Jahanpeyma, M. H. Darvishi, M. Azimzadeh, and H. Javadi, *Chemico-Biological Interactions*, **2019**, doi: https://doi.org/10.1016/j.cbi.2019.04.036.
38. L. Yang, X.-B. Luo, and S.-L. Luo, "*Assessment on Toxicity of Nanomaterials*", ed. X. Luo and F. Deng, **2019**, Chap. 9, Elsevier Inc., 273.