Molecular and Cell Biotechnologies

ISSN 1993-6842 (on-line); ISSN 0233-7657 (print)
Biopolymers and Cell. 2017. Vol. 33. N 2. P 124–134
doi: http://dx.doi.org/10.7124/bc.00094A

UDC 543.553+544.722+543.64+543.94

Highly selective amperometric biosensor for uric acid determination in real samples

O. A. Zinchenko[1], L. V. Shkotova[1], T. U. Kulynych[2], O. O. Zinkina[3], A. P. Soldatkin

[1] Institute of Molecular Biology and Genetics, NAS of Ukraine
150, Akademika Zabolotnoho Str., Kyiv, Ukraine, 03680

[2] Taras Shevchenko National University of Kyiv
64, Volodymyrska Str., Kyiv, Ukraine, 01601

[3] Institute of High Technologies, Taras Shevchenko National University of Kyiv
2, korp.5, Pr. Akademika Hlushkova, Kyiv, Ukraine, 03022
helen_nazarenko@yahoo.com

Aim. To develop an amperometric biosensor based on immobilized uricase (1.7.3.3) from *Arthrobacter Globiformis* and a platinum disk electrode for the detection of uric acid in biological fluids. **Methods.** To obtain a highly selective detection of the uric acid concentration, an additive semi-permeable polymer film was formed on the surface of a platinum disk electrode by electro-polymerisation of *m*-phenylene diamine. The enzymatic selective layer was formed on the poly-*m*-phenylene diamine membrane using uricase immobilized in BSA matrix by a non-toxic crosslinking agent – poly(ethylene glycol) diglycidyl ether (PEGDE). **Results.** An influence of possible interfering substances – ascorbic acid, cysteine, urea, glucose, glutamic acid and lactic acid – was studied. Almost no effect of these electrochemical compounds on the biosensor re-sponse was found, indicating that the selectivity of the developed biosensor is very high. The bio-sensor characteristics were determined: detection limit 0.001 mM (s/n = 3), linear working range 0.008–0.218 mM, sensitivity 165 μA•mM^{-1} cm^{-2}. The biosensor stabi-li-ty and reproducibility were studied and shown. **Conclusions.** The developed biosensor was validated by comparing the results of the urine samples analysis provided with the biosensor and the spectrophotometric method (correlation coefficient r = 0.99).This biosensor is found to be promising method for uric acid detection in the real samples.

Keywords: uricase; amperometric biosensor; uric acid; *m*-phenylene diamine; poly(ethylene glycol) diglycidyl ether.

Introduction

Uric acid (2,4,6 – trihydroxypurine) is an end product of the purine metabolism in humans. A number of diseases, sometimes very serious, are known to be caused by the violations of

purine metabolism, generally declared as a significant increase of uric acid in the blood. It can result for instance, in the development of hyperuricemia (Lesch–Nyhan syndrome) [1] and gout [2–3]. According to the epidemiological studies, on average 0.01–0.37 % of adults worldwide suffer from gout, caused by the kidney failure, as well as hematological diseases, myocardial infarction, and heart failure [2]. Several medical investigations demonstrated that an increased level of uric acid in human serum was a risk factor for cardiovascular disease [4]. The normal level of uric acid ranges from 240 to 520 μM in blood serum and 1.4 to 4.4 mM in urine [5], increasing by 3–4 times under at pathology.

Therefore, the determination of uric acid concentration in biological fluids is important in the laboratory practice. A number of methods are used for the analysis – chemical [6], enzymatic-colorimetric [7], chemiluminescent [8–9], fluorescent [10], voltammetric-colorimetric [11], enzymatic-spectrophotometric [12], mass spectrometric [13], high performance liquid chromatography [14], capillary electrophoresis, and amperometry [15].

However, despite the noticeable diagnostic importance of the quantity of uric acid concentration, its analysis is not widely used in clinical practice because of the low specificity and complex procedure of the existing methods.

The Biosensors can be considered as a good alternative method of the uric acid detection [16].

In this work an amperometric biosenor for the determination of uric acid has been developed using a promising method of the uricase immobilization with poly(ethylene glycol) diglycidyl ether (PEGDE) on the transducer surfaces. PEGDE, an important component of the redox hydrogels, is broadly used in the commercial devices. It is a non-toxic chemical, which is utilized for the enzyme fixation. It contains two epoxy groups able to react with the amino-, hydroxyl- and carboxyl groups of enzymes [17]. Under the influence of temperature PEGDE reacts with amino groups of the enzyme at higher rate and forms a matrix for uricase immobilization on the electrode surface, which is very similar to the matrix formed with glutaraldehyde.

Previously it was shown [18] that the immobilization with PEGDE has a slight effect on the enzyme catalytic parameters and at the same time the immobilized enzyme is stable and selective.

Materials and Methods

Materials

Uricase (1.7.3.3.), 15-30 U/mg of protein from *Artrobacter globiformis*, production of "Sigma" (Germany); uric acid (99% purity), production of "Sigma" (Hungary); 99% ethanol, production of "Fluka" (Germany); bovine serum albumin (BSA) (fraction V), production of "Sigma" (Germany); poly(ethylene glycol) diglycidyl ether (Mn 500), production of "Sigma" (Japan); 1,3 phenylenediamine, production of "Sigma-Aldrich Chemie GmbH; borate buffer ($Na_2B_4O_7x10H_2O$-HCl), pH 8.5, $Na_2HPO_4 \cdot 7H_2O$ and $KH_2PO_4 \cdot H_2O$, production of "Helicoη" (Russia); 3 % solution of hydrogen peroxide (Teteriv, Kiev region, Ukraine); $Na_2SO_4 \cdot 10H_2O$, production of Mikhailovsky chemical reagent factory (Russia). Polymix buffer solution (NaH_2PO_4, $Na_2B_4O_7$, Tris-HCl,

KCl, NaON and citric acid) production of "Helicoη" (Russia). All chemicals used were of analytical reagent grade.

A scheme of measuring setup

Amperometric measurements were carried out in the 2 ml electrochemical cell using a potentiostat/galvanostat PalmSens (Netherlands production) controlled by the PalmSens PC programme (Fig. 1). The three-electrode circuit consisted of platinum working, platinum auxiliary and Ag/AgCl reference electrodes [19].

The amperometric platinum transducers were studied with regards to the signal reproducibility and reliability by cyclic voltamperometry in the potential range of 0 to +1.0 V versus the Ag/AgCl reference electrode (a speed of potential involute was 0.05 V/s). The experiments were carried out in 5 mM borate buffer, pH 8.5.

Procedure of deposition of additional poly-m-phenylene diamine membranes on the surface of amperometric platinum electrodes

The unmodified amperometric platinum transducers are characterized by their sensitivity to a variety of electroactive substances. To improve the transducer selectivity two approaches were used: reduction of working potential and deposition of semi-permeable membranes on the transducer surface. Such membranes are permeable for small compounds, such as hydrogen peroxide, and are a barrier for diffusion of bigger compounds to the electrodes. For the membrane deposition, a bare transducer was immersed in 1 mM solution of *m*-phenylene diamine (*m*-PD), afterwards five cyclic voltammograms were obtained (if more than five cycles of polymerization are performed the membrane becomes too dense, which results in the significantly reduced sensitivity of the transducer to hydrogen peroxide). *m*-Phenylene diamine was dissolved in 10 mM potassium-phosphate buffer, pH 7.2. The initial potential was 0 V, the end potential +0.9 V, the rate of potential change was 0.2 V/s in the presence of 5 mM *m*-phenylene diamine. Before the subsequent deposition of the bioselective membrane, the surface of poly-*m* -PD-modified transducer was thoroughly washed with distilled water. The technique of deposition of the poly-*m*-phenylene diamine membrane was adapted from [20].

The cyclic voltammogram of poly-*m*-PD electropolymerisation demonstrates a decrease of the peak values in each next cycle caused by the formation of a polymer film on the working electrode surface.

Preparation of bioselective membrane

To prepare an uricase-based bioselective element the required amounts of the enzyme and BSA were dissolved in 20 mM phosphate buffer, pH 8.5, with addition of glycerol for the enzyme stabilization and prevention of early drying of the solution on the transducer surface. The obtained solution was mixed with PEGDE just before the membrane preparation. The final mixture contained 50 mg/ml uricase, 50 mg/ml BSA, 5 % of glycerol and 40 mg/ml PEGDE in 20 mM phosphate buffer, pH 8.5. The prepared mixture was immediately deposited on the sensitive surface of platinum disk electrode; 0.1 μl was needed to cover it completely. For immobilization the electrode was placed in the oven at 55 ^{o}C for 2h (see Fig. 1).

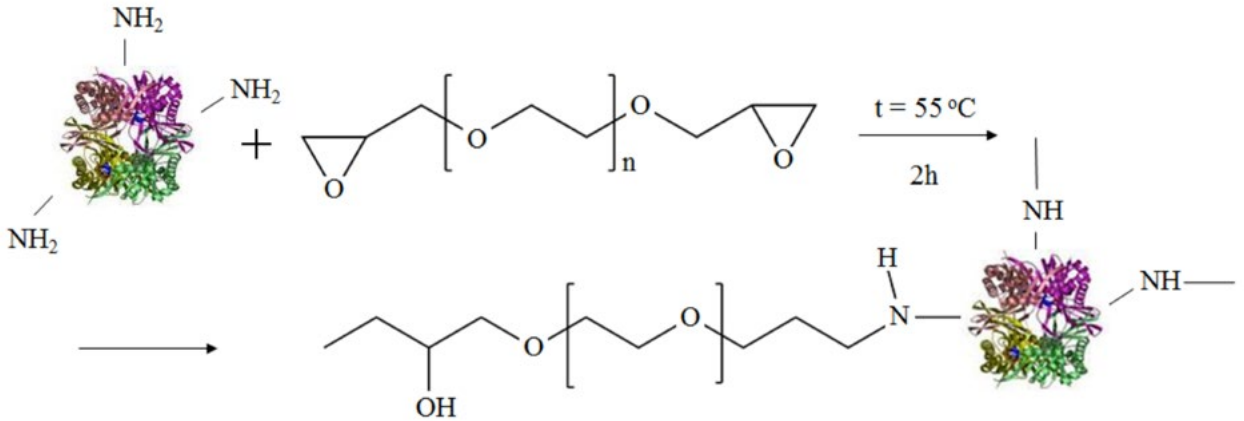


Fig. 1. Scheme of the enzyme immobilization by PEGDE [18].

Preparation of uric acid solution.

5 mM solution of uric acid in the 5 mM borate buffer, pH 8.5, was prepared just before the measurement. For complete dissolution of uric acid the solution was heated to 50 °C. pH of uric acid solution was adjusted to pH 8.5 with 1M NaOH.

Procedure of measuring substrates in model solutions

Biosensor measurements of the uric acid concentrations were carried out at room temperature in an open electrochemical cell filled with working buffer (mostly 5 mM borate buffer, pH 8.5) at vigorous stirring. Before addition of uric acid, the transducers were kept in the working buffer solution until the stable signal (base line) was obtained. The substrate concentration in the electrochemical cell was changed by adding aliquots of the substrate stock solutions. The experiments were carried out in at least three replications.

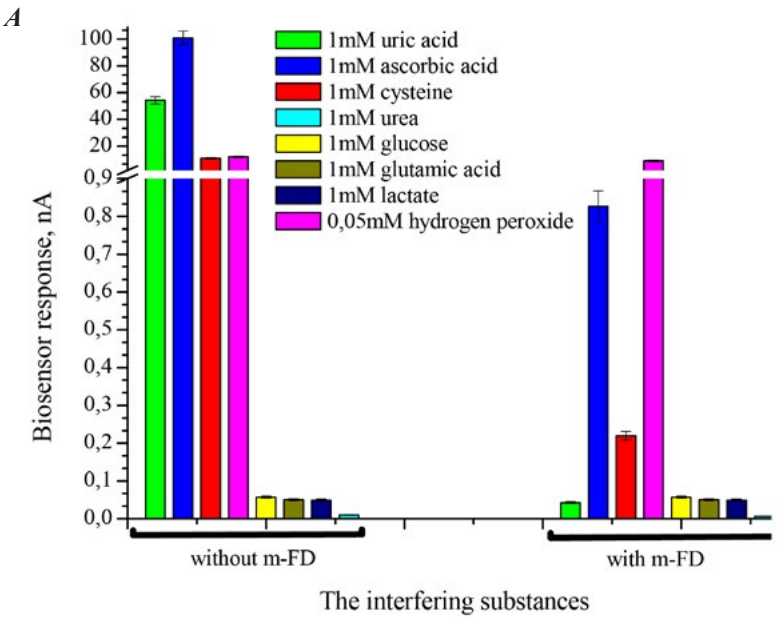
Statistics

Statistical package Microsoft Excel 10 was used for statistical analysis of the results, the average values and standard deviations were calculated; the results were considered as reliable at $p < 0.05$.

Results and Discussion

Transducer optimization

The biosensor selectivity depends on two major factors – selectivity of an enzyme (an enzyme-substrate reaction) and selectivity of a transducer (influence of electrochemical interferents). To minimize the effect of interfering substances, it was created a semipermeable selective membrane, which prevents an access of undesirable active compounds to the transducer surface. An additional polymeric membrane obtained by electrochemical oxidation of water-soluble monomer phenylene diamine was used for the purpose. The best sensitivity and selectivity were reported in case of *meta*-phenylene diamine, whereas *ortho*-phenylene diamine provided high sensitivity but low specificity, *para*-phenylene diamine - low sensitivity and selectivity [20].



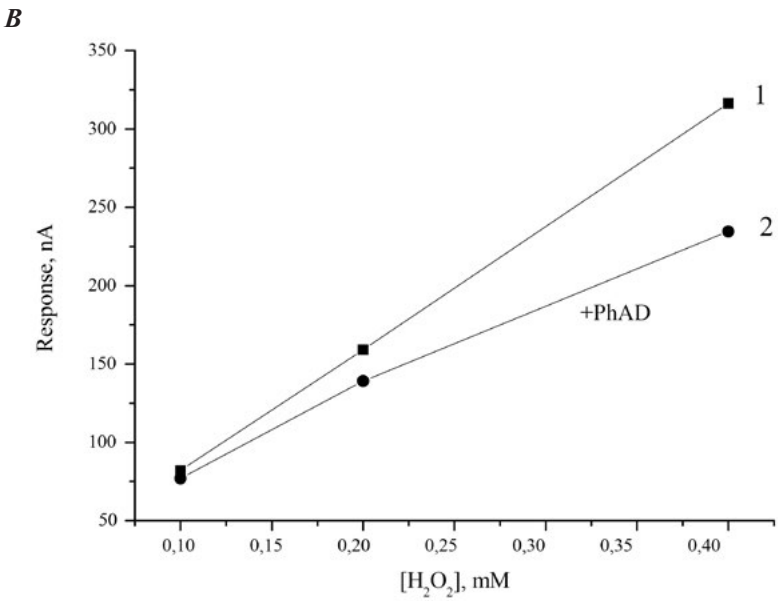


Fig. 2. Comparison of responses of platinum electrodes (A) (bare and covered with poly-m-phenylene diamine layer) to uric acid, hydrogen peroxide and potentially possible interfering substances. Sensitivity to hydrogen peroxide of platinum electrodes: bare (B(1)) and modified with electropolymerised layer of poly-m-phenylene diamine (B(2)). Measurement conditions: 5 mM borate buffer (pH 8.5), at potential of +0.4 V versus reference electrode.

The selectivity of bare electrodes and electrodes coated with poly-*m*-phenylene diamine to possible interferents was studied via assessing their sensitivity response to uric acid, ascorbic acid, cysteine, glutamic acid, urea, lactate, glucose. As shown in Fig.2 (A), bare electrodes responded to a wide range of interfering substances, whereas the polymer-coated electrodes almost did not react to them.

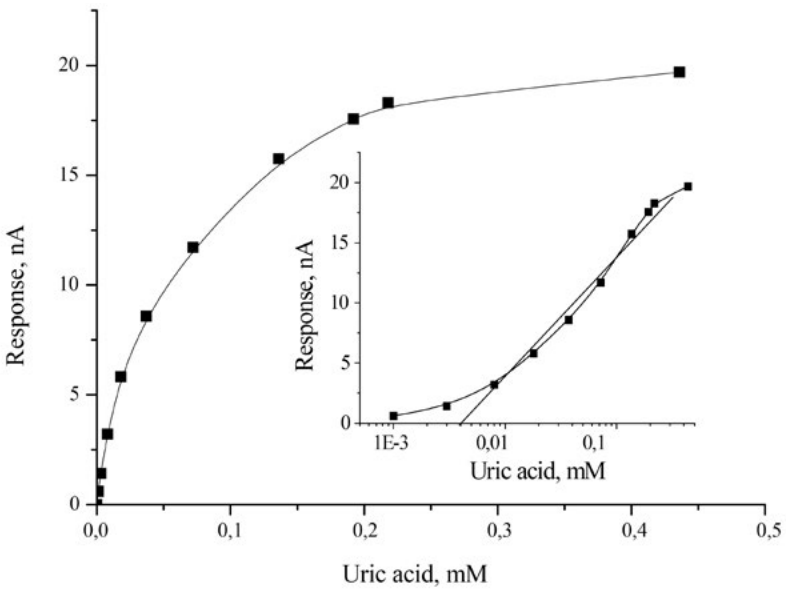


Fig. 3. Calibration curves for the uric acid sensitive biosensor. Measurement conditions: 5 mM borate buffer (pH 8.5), at potential of +0.4 V versus reference electrode.

Hence, in practical measurements, the minimal sensitivity of transducer to possible interfering substances can be neglected and this design of transducers can be successfully used for the further development of the biosensor for uric acid analysis.

A comparison of the sensitivity of bare and modified electrodes to hydrogen peroxide showed that after deposition of a poly-*m*-phenylenediamine layer onto the platinum electrode surface, the sensitivity to H_2O_2 decreased slightly (see Fig.2 (B)).

Biosensor

All known methods for the enzymatic determination of uric acid using uricase are based on the enzymatic oxidation of uric acid with production of allantoin, hydrogen peroxide and carbon dioxide:

Uricase

$$\text{Uric acid} + H_2O + O_2 \rightarrow$$
$$\rightarrow \text{Allantoin} + H_2O_2 + CO_2$$

Decomposition of electrically active substance hydrogen peroxide results in generation of electrons, which can be registered by amperometric transducer

$$H_2O_2 \rightarrow 2H^+ + 2e^-$$

In the work presented, we used uricase for creation of the biosensor and the enzyme was immobilized on the transducer surface by poly (ethylene glycol) diglycidyl ether crosslinking.

The measurements were generally carried out at room temperature in an intensively stirred electrochemical open cell system. The typical dependence of the biosensor response on the uric acid concentration (calibration curve) is presented in Fig. 3.

As can be seen from Fig 3 the apparent Michaelis-Menten constant *(Km)* is 0.06 mM and *Imax* = 22 nA for uricase immobilized in a bioselective membrane. A linear dependence of the biosensor response was observed in the range of uric acid concentration 0.008 – 0.218 mM (s/n =3), with the sensor sensitivity of 165 $\mu A \cdot mM^{-1} \cdot cm^{-2}$ (the calibration curve

presented in linear coordinates). Under the conditions described, the time of biosensor response was about 5–10 s. The presentation of the biosensor calibration curve in semi-logarithmic coordinates gives the possibility to extend the linear dynamic range and to shift the range of uric acid detection to higher concentration.

A low value of Michaelis–Menten constant (0.06 mM) indicates that immobilized uricase in the BSA-PEGDE layer has high affinity to uric acid. The detection limit of the uricase biosensor was estimated as 0.001 mM (s/n =3).

Since the biosensor working characteristics strongly depend on the experimental conditions, it was important to examine an effect of ionic strength and pH on the biosensor response. The dependence of amperometric biosensor response on the NaCl concentration is shown in Fig.4(A).

An analysis of the impact of NaCl concentrations revealed a little effect on the response of amperometric uricase-based biosensor. Notably, it is typical for most enzyme amperometric biosensors [21].

As known, each enzyme has a specific working optimum pH value. For some enzyme it can be changed after immobilization, shifting into alkaline or acid region. This phenomenon can be explained in the first place by the change in conformation of immobilized enzyme and redistribution of charges within the enzyme-BSA-PEGDE system. The pH-dependence of the sensor response was investigated for 3, 8 and 18 μM uric acid over the pH range of 6.0–11.0 in the universal buffer solution [22]. The experimental results show (see Fig.4 (B)) an atypical picture of dependence of the biosensor responses on pH; it means that the activity of immobilized uricase is weakly

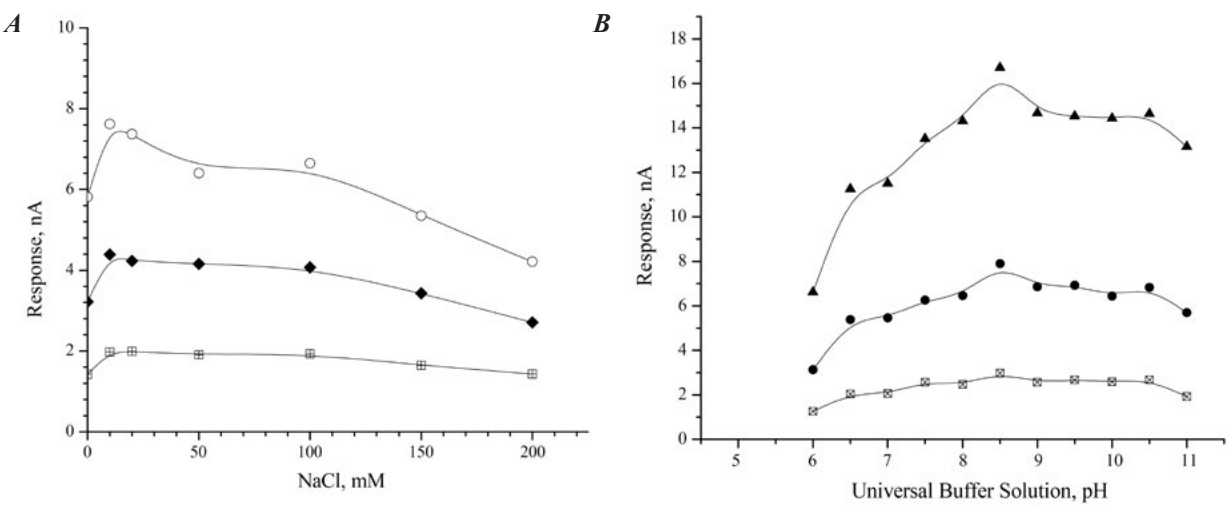


Fig. 4. Dependence of the response of amperometric uricase-based biosensor on the concentration of NaCl in a buffer solution (A). Measurement conditions: 5 mM borate buffer, pH 8.5. Dependence of the response of amperometric biosensors based on immobilized uricase on pH of polymix buffer solution (B) (2.5 mM NaH_2PO_4, 2,5 mM $Na_2B_4O_7$, 2,5 mM Tris-HCl, 2,5 mM KCl, 2,5 mM NaON and 2,5 mM citric acid). Measurement was carried out at potential of + 0.4 V versus reference electrode.

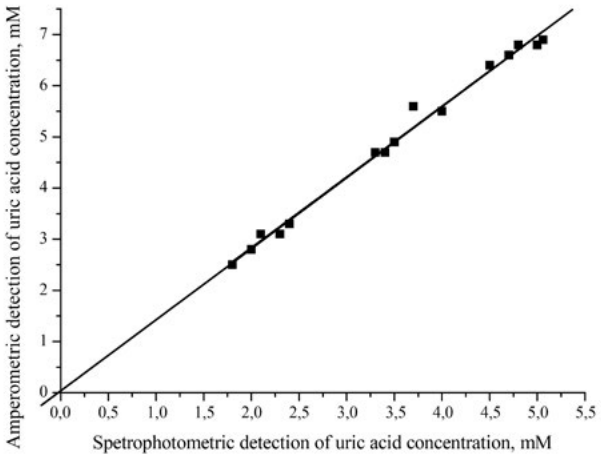


Fig. 5. Correlation between the values of uric acid concentrations in urine determined by the developed biosensor and the hospital standard spectrophotometric methods.

dependent on the pH changes in this range. The form of curves can be explained by an increase in the immobilized enzyme stability and/or by the limitation of substrate diffusion in a bioselective membrane [23]. The highest activity was observed at pH 8.5, which was chosen as optimal for further work.

The response reproducibility and operational stability, the most important biosensor characteristics, were studied. The biosensor responses to different concentrations of uric acid were measured over one working day with 30-min intervals, the biosensor with immobilized uricase being kept between measurements in buffer solution at room temperature. The biosensor was characterized by high reproducibility (relative standard deviation of the signals did not exceed 5 %) and high operational stability (the bioselective element did not lose its activity during 5–6 h).

To study long-term stability of the biosensor developed, the responses to the same concentration of substrate were evaluated with 100-hour intervals between subsequent measurements (data not presented). During the first 100 hours of storage, the biosensors lost 30 % of the initial sensitivity. In 400 hours, the biosensors still exhibited approximately 50 % of their initial sensitivity. The loss of activity is probably associated with the relatively low stability of uricase.

Analysis of real samples

An efficiency of the developed biosensor for in the analysis of real samples was evaluated by comparing the values of uric acid concentration in the urine samples of 15 volunteers measured by the presented biosensor method (axis Y) with the results obtained by spectrophotometric method [24] (axis X) (Fig. 5). A high correlation was demonstrated (correlation coefficient r = 0.99).

Conclusion

The electrochemical biosensor based on the poly(*m*-phenylenediamine) modified platinum disk electrode as a transducer and immobilized uricase from *Arthobacter globiformis* as a sensitive element has been developed for detection of uric acid in the presence of electrochemical compounds. It provides a sensitive, selective and rapid methods of uric cid analysis.

The amperometric biosensor for detection of uric acid in biological fluids was developed using uricase immobilization by a non-toxic crosslinking agent – poly(ethylene glycol)diglycidyl ether.

The main characteristics of the developed biosensor were estimated. The biosensor demonstrated fast response (5s), high sensitivity and selectivity, linear working range of 0.01 – 0.22 mM with detection limit of 0.001 mM (s/n =3).

The biosensor was tested when analyzing the urine samples of 15 volunteers. The results correlated with those obtained by the standard spectrophotometric method. Thus, the developed biosensor can be applied for monitoring the level of uric acid in urine at norm and pathology during the medical examination of population.

Acknowledgments

The authors gratefully acknowledge the financial support of this study by the National Academy of Sciences of Ukraine in the frame of Scientific and Technical Program "Sensor systems for medico-ecological and industrial-technological requirement: metrological support and experimental operation".

REFERENCES

1. *Lesch M, Nyhan WL.* A Familial disorder of uric acid metabolism and central nervous system function. *Am J Med.* 1964;**36**:561–70.
2. *Maksudova A, Salikhov I, Habirov R, Halfina T.* The gout. Moscow: Medpress-inform, 2012. 96 p.
3. *Heinig M, Johnson RJ.* Role of uric acid in hypertension, renal disease, and metabolic syndrome. *Cleve Clin J Med.* 2006;**73**(12):1059–64. PubMed
4. *Alderman M, Aiyer KJ.* Uric acid: role in cardiovascular disease and effects of losartan. *Curr Med Res Opin.* 2004;**20**(3):369–79.
5. *Raj C, Ohsaka T.* Voltammetric detection of uric acid in the presence of ascorbic acid at a gold electrode modi-fied with a self-assembled monolayer of heteroaromatic thiol. *J Electroanal Chem.* 2003; **540**: 69–77.
6. *Isdale IC, Buchanan MJ, Rose BS.* Serum uric acid estimation. *A modified chemical method.* Ann Rheum Dis. 1966;**25**(2):184–5.
7. *Lorentz K, Berndt W.* Enzymic determination of uric acid by a colorimetric method. *Anal Biochem.* 1967; **18**(1):58–63.
8. *Hong H-C, Huang H-J.* Flow injection analysis of uric acid with a uricase-and horseradish peroxidase-coupled Sepharose column based luminol chemiluminescence system. *Anal Chim Acta.* 2003, **499**(1–2):41–6.
9. *He D, Zhang Z, Huang Y, Hu Y, Zhou H, Chen D.* Chemiluminescence microflow injection analysis system on a chip for the determination of uric acid without enzyme. *Luminescence.* 2005;**20**(4–5):271–5.
10. *Galbán J, Andreu Y, Almenara MJ, de Marcos S, Castillo JR.* Direct determination of uric acid in serum by a fluorometric-enzymatic method based on uricase. *Talanta.* 2001;**54**(5):847–54.
11. *Abdullin IF, Bakanina YuN, Turova EN, Budnikov GK.* Determination of uric acid by voltammetry and coulometric titration. *J Anal Chem.* 2001; **56**(5): 45356.
12. *Rocha DL, Rocha FRP.* A flow-based procedure with solenoid micro-pumps for the spectrophotometric determina-tion of uric acid in urine. *Microchem J.* 2010; **94**(1):53–9
13. *Perelló J, Sanchis P, Grases F.* Determination of uric acid in urine, saliva and calcium oxalate renal calculi by high-performance liquid chromatography/mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2005;**824**(1–2):175–80.
14. *Dai X, Fang X, Zhang C, Xu R, Xu B.* Determination of serum uric acid using high-performance liquid chroma-tography (HPLC)/isotope dilution mass spectrometry (ID-MS) as a candidate reference method. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2007;**857**(2):287–95.
15. *Xu DK, Hua L, Li ZM, Chen HY.* Identification and quantitative determination of uric acid in human urine and plasma by capillary electrophoresis with amperometric detection. *J Chromatogr B Biomed Sci Appl.* 1997;**694**(2):461–6.
16. *Soldatkin AP, Dzyadevych SV, Korpan YI, Sergeyeva TA, Arkhypova VN, Biloivan OA, Soldatkin OA, Shkotova LV, Zinchenko OA, Peshkova VM, Saiapina OY, Marchenko SV, El'Skaya AV.* Biosensors. A quarter of a century of R&D experience. *Biopolym Cell.* 2013; **29**(3):188–206.
17. *Vasylieva N, Marinesco S, Enzyme Immobilization on Microelectrode Biosensors, in: Microelectrode*

Biosensors. Eds. *Marinesco S, Dale N, Neuromethods, 2013: 95–114.*
18. *Vasylieva N, Maucler C, Meiller A, Viscogliosi H, Lieutaud T, Barbier D, Marinesco S.* Immobilization method to preserve enzyme specificity in biosensors: consequences for brain glutamate detection. *Anal Chem.* 2013;**85**(4):2507–15.
19. *Shkotova LV, Slast'ia EA, Zhyliakova TA, Soldatkin OP, Schuhmann W, Dziadevych SV.* [Amperometric biosensor for ethanol analysis in wines and grape must during wine fermentation]. *Ukr Biokhim Zh (1999).* 2005;**77**(1):96–103.
20. *Schuvailo OM, Soldatkin OO, Lefebre A, Cespuglio R, Soldatkin AP.* Highly selective microbiosensors for in vivo measurement of glucose, lactate and glutamate. *Anal Chim Acta.* 2006; **573–574**:110–6.
21. *Goriushkina TB, Shkotova LV, Gayda GZ, Klepach HM, Gonchar MV, Soldatkin AP, Dzyadevych SV.* Amperometric biosensor based on glycerol oxidase for glycerol determination. *Sens Actuators B.* 2010, **144**(2):361–7.
22. *Lurie Yu.* Handbook of analytical chemistry. Moscow: Chemistry, 1967.
23. *Trevan M.* Immobilized enzymes: An introduction and applications in biotechnology. *John Wiley & Sons, Chichester.* 1980.
24. *Zhao Y, Yang X, Lu W, Liao H, Liao F.* Uricase based methods for determination of uric acid in serum. *Micro-chim Acta.* 2009; **164**(1–2):1–6.

Високоселективний амперометричний біосенсор для визначення сечової кислоти в реальних зразках

О. А. Зінченко, Л. В. Шкотова, Т. Ю. Кулинич, О. О. Зінкіна, О. П. Солдаткін

Мета. Розробити амперометричний біосенсор на основі іммобілізованої урікази (1.7.3.3) з *Arthrobacter Globiformis* і платинового дискового електрода для визначення сечової кислоти в біологічних рідинах. **Методи.** Для досягнення високоселективного визначення концентрації сечової кислоти на поверхні платинового дискового електрода сформована додаткова напівпроникна мембрана шляхом електрополімерізації м-фенілендіаміна. Ферментний селективний шар сформований на полі-м-фенілендіамінові мембрані з використанням урікази, що іммобілізована в матриці БСА, в якості зшиваючого агента використовували нетоксичний поліетиленгліколь дигліцидиловий естер (ПЕГДЕ). **Результати**. Досліджено вплив інтерферуючих речовин: аскорбінової кислоти, цистеїну, сечовини, глюкози, глутамінової кислоти, молочної кислоти на активність розробленого біосенсору і показана відсутність впливу цих електрохімічно активних речовин на відгук біосенсора, що свідчить про дуже високу селективність розробленого біосенсора. Визначені наступні характеристики біосенсора: мінімальна концентрація, що визначалась 0.001 мМ (s/n = 3), робочий лінійний діапазон 0.008–0.218 мМ, чутливість 165 мкА·мМ$^{-1}$ см$^{-2}$. Також досліджені і показані операційна стабільність біосенсора і його стабільність при зберіганні. **Висновки.** Апробація розробленого біосенсора при аналізі реальних зразків сечі показала хорошу кореляцію даних із класичним спектрофотометричним методом (коефіцієнт кореляції r = 0.99). Таким чином, даний біосенсор є перспективним методом і може бути застосований в медичній діагностиці для визначення сечової кислоти в реальних зразках.

Ключові слова: уріказа; амперометричний біосенсор; сечова кислота; м-фенілендіамін; поліетиленгліколь дигліцидиловий естер.

Высокоселективный амперометрический биосенсор для определения мочевой кислоты в реальных образцах

Е. А. Зинченко, Л. В. Шкотова, Т. Ю. Кулинич, О. А. Зинкина, А. П. Солдаткин

Цель. Разработать амперометрический биосенсор на основе иммобилизированной уриказы (1.7.3.3) из *Arthrobacter Globiformis* и платинового дискового электрода для определения мочевой кислоты в биологических жидкостях. **Методы**. Для достижения высокоселективного определения концентрации мочевой кислоты на поверхности платинового дискового электрода была сформирована дополнительная полупроницаемая мембрана путём электрополимеризации м-фенилендиамина. Ферментный селективный слой сформирован на поли-м-фенилендиаминовой мембра-

не с использованием уриказы, иммобилизированной в матрице БСА, в качестве сшивающего агента использовали нетоксичный диглицидиловый эфир полиэтиленгликоля (ПЭГДЭ). **Результаты.** Исследовано влияние интерферирующих веществ: аскорбиновой кислоты, цистеина, мочевины, глюкозы, глутаминовой кислоты, молочной кислоты на активность разработанного биосенсора и показано отсутствие влияния этих электрохимически активных веществ на отклик биосенсора, что свидетельствует об очень высокой селективности разработанного биосенсора. Определены следующие характеристики биосенсора: граничная определяемая концентрация 0.001 мМ (s/n = 3), рабочий линейный диапазон 0.008–0.218 мМ, чувствительность 165 мкА•мМ$^{-1}$ см$^{-2}$. Также исследованы и продемонстрированы операционная стабильность биосенсора и его стабильность при хранении. **Выводы.** Апробация разработанного биосенсора при анализе реальных образцов мочи показала хорошую корреляцию данных с классическим спектрофотометрическим методом (коэффициент корреляции r = 0.99). Таким образом, данный биосенсор является перспективным методом и может быть использован в медицинской диагностике для определения мочевой кислоты в реальных образцах.

Ключевые слова: уриказа; амперометрический биосенсор; мочевая кислота; м-фенилендиамин; полиэтиленгликоль диглицидиловый эфир.

Received 15.01.2017