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Amperometric Uric Acid Biosensor with Improved Analytical Performances Based on Alkaline-Stable H_2O_2 Transducer

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Electrodes modified with Prussian blue containing structurally incorporated Ni^{2+} ions were employed as the alkaline-stable H_2O_2 transducers in amperometric uric acid biosensors based on urate oxidase. The employed H_2O_2 transducers enabled operation of the biosensor at low cathodic potentials and in mild alkaline media, near the optimal pH of urate oxidase. At the pH value of 9.0 and working potential of –0.05 V, the biosensor exhibited a linear response towards uric acid in the concentration range of 2.5 to 200 μM, with the sensitivity of 26.9 ± 0.4 mA M^{-1} cm^{-2} and detection limit of 0.65 μM. The biosensor was successfully applied for determination of uric acid in samples of commercial dairy products.

Keywords: Uric acid; biosensor; Prussian blue; hydrogen peroxide; alkaline medium

1. INTRODUCTION

Uric acid (2,6,8-trihydroxypurine) is the main final product of purine metabolism in humans. It is an important marker in clinical diagnosis because the elevated uric acid concentration in blood (hyperuricemia) is associated with a number of diseases, such as gout, insulin resistance, hypertension, and renal insufficiency [1,2]. High level of serum uric acid is also considered as a risk factor for myocardial infarction and stroke [3]. Therefore, the methods for rapid, sensitive and selective determination of uric acids in body fluids are of great interest in clinical chemistry. The analysis of uric acid in food is also of importance because a diet low in purine and its derivates is usually prescribed for the patients suffering from hyperuricemia. In this context, it is especially interesting to monitor the levels of uric acid in milk and dairy products, which are the major protein sources in low-purine diet [4]. Raw animal milk contains between 10 and 25 mg/L of uric acid [5], and therefore, the

increased consuming of milk and dairy products in a daily basis may influence the serum uric acid level [6].

Numerous analytical methods have been developed for the determination of uric acid, ranging from the simple colorimetric methods based on commercially available enzymatic kits or immobilized enzymes [7], fluorometric and chemiluminescence methods [8,9], or flow-injection methods [10], to the high-resolution separation methods, such as capillary electrophoresis [11] and high-performance liquid chromatography [12,13]. Electroanalytical methods based on direct electrochemical oxidation of uric acid have also been developed [14]. Although very simple and highly sensitive, these methods usually lack the selectivity necessary for the determination of uric acid in complex samples of biological origin due to the interferences caused by the presence of other easily oxidizable substances (e.g. ascorbic acid and dopamine) in the sample matrix. The selectivity of direct electrochemical uric acid determination can be improved by the use of catalytically modified electrodes, on which the interferences of ascorbic acid and dopamine can be greatly reduced or even totally eliminated [15-18].

As an alternative to the analytical methods mentioned above, various types of uric acid biosensors were developed and tested in the analysis of uric acid in complex real samples [19-28]. In these biosensors, selective and sensitive recognition of uric acid is achieved by using enzyme urate oxidase (uricase), which catalyzes oxidation of uric acid ($C_5H_4N_4O_3$) to allantoin ($C_4H_6N_4O_3$) in the presence of molecular oxygen:

$$C_5H_4N_4O_3 + O_2 + 2\,H_2O \rightarrow C_4H_6N_4O_3 + CO_2 + H_2O_2 \quad (1)$$

Typically, the operation of uric acid biosensors is based either on the measurement of consumption of O_2 [20,26,28] or production of H_2O_2 [19,21-25,27] in enzymatically catalyzed reaction (1). Uric acid biosensors of the latter type are prevailing due to their simpler construction and shorter response time in comparison to the biosensors based on dissolved oxygen probes. Since H_2O_2 is electroactive, these biosensors typically employ an amperometric transduction [21-25,27].

Two problems are encountered in development of amperometric uric acid biosensors based on electrochemical detection of enzymatically produced H_2O_2. The first is connected with the low specific activity of commercially available urate oxidase, which rarely exceeds 20–25 units per mg and typically is lower than 10 units per mg, while the second is related to the relatively high optimal pH of urate oxidase, which lies near the pH value of 9.0 [29]. Due to the low catalytic activity of enzyme, the rate of H_2O_2 production in enzymatically catalyzed reaction (1) is low, resulting in the low current signal of the biosensor. This puts the special requirements on the H_2O_2 transducer employed in the construction of the biosensor. In order to obtain a low detection limit and consequently, a wide dynamic range of the biosensor, the transducer must exhibit a high sensitivity and very low noise. The latter can be achieved if the biosensor is operated at mild cathodic potentials (around 0.0 V vs. Ag/AgCl), at which the background current is minimized. However, due to the slow kinetics of H_2O_2 reduction on most of the commonly employed electrode materials, the appreciable H_2O_2 reduction currents can be achieved at these potentials only on catalytically modified electrodes. In terms of sensitivity and selectivity, one of the best inorganic catalysts for electrochemical reduction of H_2O_2 is Prussian blue (iron(III) hexacyanoferrate(II), $KFe[Fe(CN)_6]$) [30-34]. Unfortunately, due to the instability of Prussian blue in neutral and alkaline media [35,36], Prussian blue-based transducers are not suited for use in uric acid biosensors operating at optimal pH of urate oxidase. Due to this

limitation, majority of the amperometric uric acid biosensors developed so far operated either at (i) the pH value which is lower than the optimal pH of urate oxidase, or (ii) anodic potentials at which the transduction is based on the oxidation of enzymatically produced H_2O_2. Both of these approaches can result in degradation of biosensor performances, either due to the lowering of sensitivity and linearity of the biosensor response (caused by the lower enzyme activity, i.e. lower K_M and v_{max}, at pH values differing from the optimal pH of enzyme [37]), or due to the lower signal-to-noise ratio and increased background current of the biosensor (caused by the operation at anodic potentials).

Recently, we found that the partial substitution of nitrogen-coordinated iron ions in the crystal lattice of Prussian blue by Ni^{2+} ions can greatly enhance the alkaline stability of Prussian blue, without appreciably lowering its electrocatalytic activity towards the reduction of H_2O_2 [38]. In this work, amperometric H_2O_2 transducer based on the Ni^{2+}-modified Prussian blue was employed in the fabrication of uric acid biosensor, with the aim to enable the operation of the biosensor at mild cathodic potentials and in alkaline media, near the optimal pH of urate oxidase, and thus, to improve the performances of the biosensor. Analytical performances and stability of the developed biosensor were evaluated and its applicability in the analysis of complex real samples was tested by using the biosensor in determination of uric acid in commercial dairy products.

2. EXPERIMENTAL PART

2.1. Chemicals and instrumentation

Urate oxidase (EC 1.7.3.3, from *Bacillus fastidiosus*, 9 units per mg) and bovine serum albumin (BSA, ≥98 %) were purchased from Sigma Aldrich. All other chemicals were of reagent grade and were used without further purification. Reagent solutions were prepared with deionized water additionally purified in Millipore Synergy ultrapure water system (Millipore, USA).

Electrochemical measurements were carried out using a Voltalab 50 potentiostat (Radiometer, France). Measurements were performed at room temperature in a conventional three-electrode cell, with a Pt wire serving as the counter electrode and a Hg/Hg_2Cl_2/3.5M KCl reference electrode (E = 0.250 V *vs.* SHE). Disc electrodes (A = 0.071 cm^2) fabricated from spectroscopic graphite rods were employed as working electrodes in all experiments.

Flow injection analysis (FIA) experiments were performed with a home-made FIA system consisting of a two-channel peristaltic pump (Minipuls II, Gilson, USA), an electrically actuated 6-port Cheminert injection valve (Valco, USA) equipped with a 50 μL sample loop, and an electrochemical flow-through detector cell. HPLC analyses were carried out on a HP 1090 (Hewlett-Packard/Agilent, USA) liquid chromatography system equipped with a diode array detector.

2.2. Biosensor preparation

2.2.1. Preparation of amperometric H_2O_2 transducers

Amperometric H_2O_2 transducers used for the fabrication of uric acid biosensors consisted of a thin electrocatalytic film of Ni^{2+}-modified Prussian blue deposited on the surface of spectroscopic

graphite disc electrodes. Prior to the deposition of electrocatalytic film, the electrodes were abrasively cleaned on a fine (1200 grit) emery paper, polished on a filter paper, and finally electrochemically pretreated by potential cycling between –0.75 and 1.0 V in 0.1 M KCl/0.01 M HCl solution for 10 cycles. Electrochemical deposition of Prussian blue on pretreated graphite electrodes was performed by cyclic polarization of the electrodes between 0.55 and –0.15 V in solution containing 0.1 M KCl, 0.01 M HCl, 5 mM $FeCl_3$, and 5 mM $K_3[Fe(CN)_6]$ for 12 cycles. After the deposition of Prussian blue, the electrodes were equilibrated in 0.1 M KCl/0.01 M HCl solution by potential cycling between –0.15 and 1.05 V for 10 cycles. Finally, Ni^{2+} ions were incorporated into the structure of Prussian blue by cyclic polarization of the electrodes between 0.60 and –0.15 V in solution containing 0.1 M KCl, 0.01 M HCl, and 0.01 M $NiCl_2$ for 15 cycles, followed by equilibration in 0.1 M KCl/0.01 M HCl solution by potential cycling in the same potential range for 5 cycles. A scan rate of 50 mV/s was employed in all voltammetric procedures described above.

2.2.2. Enzyme immobilization

Immobilization of urate oxidase on the surface of amperometric H_2O_2 transducers was performed by cross-linking of enzyme with BSA. For this purpose, the amounts of 5.0 mg of urate oxidase and 5.0 mg of BSA were dissolved in 100 μL of 0.1 M Britton-Robinson buffer (pH = 9.0) and the mixture was left to stand at room temperature for 30 min. A volume of 25 μL of glutaraldehyde solution (prepared by mixing 35 μL of 50 % (w/w) aqueous glutaraldehyde solution with 500 μL of deionized water) was subsequently added to the enzyme/BSA mixture. The aliquots of 5 μL of the resulting mixture were pipetted on the surface of amperometric H_2O_2 transducers and left to stand in air for 15 minutes, after which the obtained biosensors were rinsed with deionized water and stored in buffer at 4 °C. Ten biosensors were prepared from the same enzyme immobilization batch.

2.3. Determination of uric acid in real samples

Water extracts of twelve samples of commercial dairy products (yoghurts, kefirs, acidophilus and sour milks, probiotic milk products) were prepared by mixing 15.0 g of the sample with 10 mL of deionized water on rotating mixer for 30 min, followed by centrifugation at 4500 rpm for 15 min. The supernatants were filtered through an ordinary filter-paper and stored at –18 °C until analyzed.

For the determination of uric acid with the developed amperometric biosensor, the volume of 1.0 mL of 0.6 M Britton-Robinson buffer containing 0.6 M KCl was added to 5.0 mL of the sample water extract. The aliquot of the resulting mixture was added into an electrochemical cell containing 8.0 mL of 0.1 M Britton-Robinson buffer/0.1 M KCl solution (pH 9.0) and the cathodic current of the biosensor polarized at –0.05 V was recorded. Quantification of uric acid was performed by standard addition method.

HPLC determination of uric acid was performed on an analytical Aminex HPX-87H column (300 x 7.8 mm, Biorad, USA) at 45 °C, by using 0.2 % (w/w) H_3PO_4 as a mobile phase at a flow rate of 0.6 mL/min. Detection was performed at 210 and 280 nm. Before the analysis, the water extracts of samples were filtered through a 0.45 μm filter.

3. RESULTS AND DISCUSSIONS

3.1. Electrochemical properties and operational stability of the H_2O_2 transducer

Hydrogen peroxide transducers were prepared by a simple two-step electrochemical procedure, as described in *Sect. 2.2.1*.

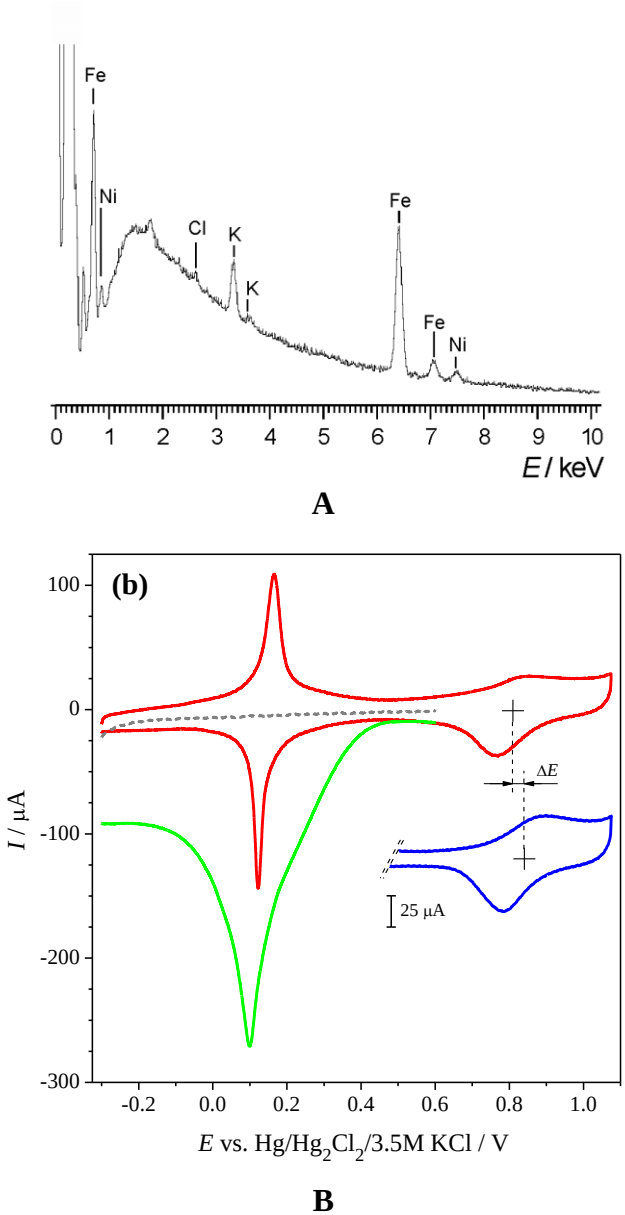

Figure 1. EDX spectrum (a) and cyclic voltammogram (b, red line) of Ni^{2+}-modified Prussian blue obtained after the cyclic polarization of Prussian blue film between 0.60 and –0.15 V in 0.1 M KCl / 0.01 M HCl / 0.01 M $NiCl_2$ solution for 15 cycles. Inset in Fig. (b): cyclic voltammetry response of carbon-coordinated iron ions in unmodified Prussian blue. The difference between the mid-peak potentials of pair of peaks shown in the inset and the corresponding pair of peaks in Ni^{2+}-modified Prussian blue is indicated by ΔE. Green and gray traces in Fig. (b) represent the linear sweep voltammograms recorded in the presence of 5 mM H_2O_2 on Ni^{2+}-modified Prussian blue and unmodified graphite electrode, respectively. Supporting electrolyte: 0.1 M KCl / 1 mM HCl; scan rate: 50 mV/s.

First, a thin film of Prussian blue was electrochemically deposited on the surface of spectroscopic graphite electrodes. In the second step, Ni^{2+} ions were incorporated into the crystal lattice of Prussian blue through an electrochemically driven insertion-substitution mechanism [39,40], by repetitive cyclic polarization of Prussian blue-modified electrodes in 0.1 M KCl solution containing 0.01 M $NiCl_2$. The incorporation of Ni^{2+} ions was confirmed by energy dispersive X-ray (EDX) spectroscopy, which showed the presence of Ni L_α and Ni K_α lines at 0.85 and 7.46 keV, respectively, in spectra of Prussian blue films obtained after the potential cycling in $KCl/NiCl_2$ solution (Fig.1.a). A typical cyclic voltammogram of the Ni^{2+}-modified Prussian blue film is shown in Fig. 1.b. The voltammogram is characterized by the appearance of two pairs of peaks with the mid-peak potentials of $E_1 = 0.140$ V and $E_2 = 0.805$ V (in 0.1 M KCl/0.01 M HCl), which can be, by analogy with the cyclic voltammetry response of the unmodified Prussian blue [41], attributed to the reversible redox transitions of nitrogen-coordinated and carbon-coordinated iron ions, respectively, in the crystal lattice of Prussian blue. In comparison to the pair of peaks associated with the carbon-coordinated iron ions in unmodified Prussian blue, the corresponding peaks in Ni^{2+}-modified Prussian blue showed a slight (≈35 mV) shift in the cathodic direction (inset in Fig. 1.b) due to the partial substitution of nitrogen-coordinated iron ions with Ni^{2+} ions [39] and the formation of mixed nickel-iron hexacyanoferrate, $K_{1+x}\{Ni_xFe_{1-x}[Fe(CN)_6]\}$ [42]. From the Ni/Fe molar ratio of 0.10 ± 0.01, as determined by the EDXS analysis of Ni^{2+}-modified Prussian blue films, the value of x equal to 0.18 can be calculated, corresponding to the film composition given by the formula $K_{1.18}\{Ni_{0.18}Fe_{0.82}[Fe(CN)_6]\}$.

Electrochemical reduction of hydrogen peroxide on Ni^{2+}-modified Prussian blue commences at ca. 0.45 V in 0.1 M KCl solution (Fig. 1.b, green line), which is approximately 0.6 V more positive than the potential at which the reduction of H_2O_2 starts on the unmodified graphite electrode under identical conditions (Fig. 1.b, gray line), clearly demonstrating the electrocatalytic activity of Ni^{2+}-modified Prussian blue towards the reduction of H_2O_2.

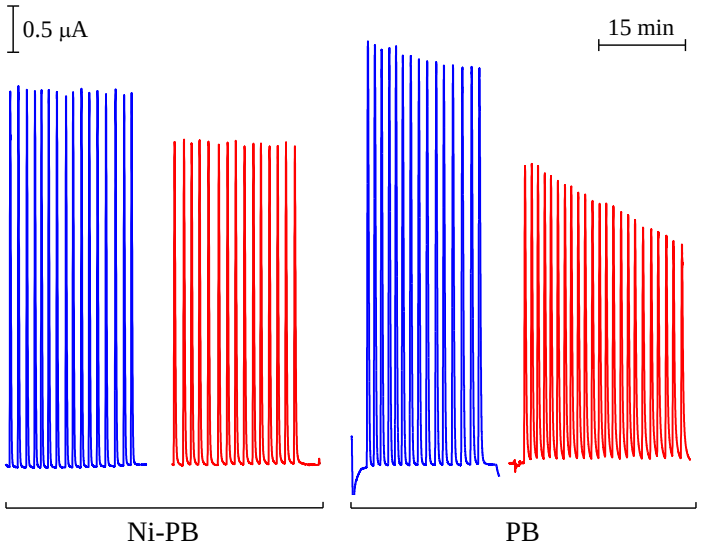

Figure 2. Fiagrams recorded on Ni^{2+}-modified Prussian blue (Ni-PB) and unmodified Prussian blue (PB) upon the injections of 50 μL of 250 μM H_2O_2 into the carrier stream buffered to pH 7.0 (blue line) and 9.0 (red line). Carrier: 0.1 M Britton-Robinson buffer / 0.1 M KCl; flow rate: 0.8 mL/min; operating potential: –0.05 V.

Since the primary goal of the present work was to develop the uric acid biosensor capable of operating at pH near the optimal pH of urate oxidase, the operational stability of the H_2O_2 transducer in mild alkaline media is an important condition that must be meet in order to ensure the functionality of the biosensor. The stability of Ni^{2+}-modified Prussian blue in neutral and mild alkaline media was examined under the flow-injection (FIA) conditions, by recording the changes in reduction current due to the injections of 250 μM H_2O_2 into the carrier stream buffered to pH 7.0 and 9.0 (Fig. 2.). As evident from the obtained fiagrams, the H_2O_2 transducer based on Ni^{2+}-modified Prussian blue exhibited a stable response on H_2O_2 at both pH values. The degradation of the transducer response was observed only at pH values greater than 9.5. The repeatability of the FIA signal, expressed as a relative standard deviation of the current peaks obtained upon the consecutive injections of 250 μM H_2O_2, was found to be 0.6 % ($n = 16$) and 0.7 % ($n = 15$) at pH 7.0 and 9.0, respectively. Under the identical conditions, the transducer based on unmodified Prussian blue exhibited a significantly lower stability, especially in alkaline medium, in which the FIA signals due to the injections of 250 μM H_2O_2 decreased in the intensity for almost 30 % during the 30 min exposure of the transducer to the carrier stream of pH 9.0 (Fig. 2.). These results clearly demonstrate the increase of the alkaline stability of Prussian blue films achieved by the incorporation of Ni^{2+} ions into the structure of Prussian blue.

Under the hydrodynamic conditions, the catalytic current due to the reduction of H_2O_2 on Ni^{2+}-modified Prussian blue exhibited a diffusion limited plateau at potentials lower than 0.0 V (not shown). Since the background current of the biosensor increased at potentials more negative than –0.1 V due to the reduction of dissolved oxygen, the potential of –0.05 V was chosen as an optimal working potential of the H_2O_2 transducer.

3.2. Analytical performances of the biosensor

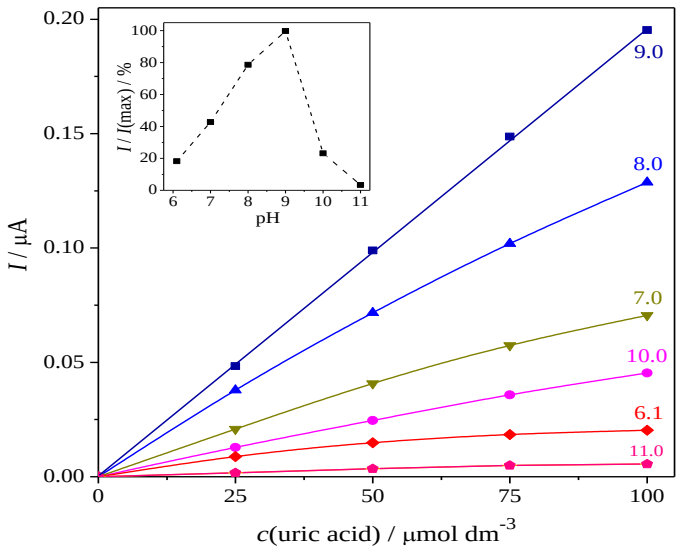

Figure 3. Dependence of the biosensor current on the concentration of uric acid at pH values of 6.1, 7.0, 8.0, 9.0, and 10.0. Inset: dependence of the biosensor sensitivity towards uric acid on pH (the sensitivities were normalized with respect to the maximum sensitivity observed at pH 9.0). Supporting electrolyte: 0.1 M Britton-Robinson buffer / 0.1 M KCl; operating potential: –0.05 V.

The influence of pH on the biosensor response was evaluated in the pH range between 6.0 and 11.0. The measurements were performed under the batch conditions (in magnetically stirred cell), with the biosensor polarized at –0.05 V, by recording the changes of the cathodic current induced by four successive additions of 25 μM of uric acid into the measuring cell. The obtained results (Fig. 3.) show that both the sensitivity and the linearity of the biosensor response are strongly influenced by pH. At pH equal or lower than 7, the biosensor exhibited a low sensitivity and a markedly non-linear response at concentrations of uric acid greater than 50 μM. Both of these parameters improved by increasing the pH of the medium. At pH 9.0, the highest sensitivity and a fully linear response over the examined range of uric acid concentrations were observed. Further increase of pH resulted in a sharp decrease of the biosensor sensitivity (inset in Fig. 3.), most probably caused by the combined effect of decreased enzyme activity [37] and hydrolytic degradation of the Ni^{2+}-modified Prussian blue film employed as an electrocatalyst in the H_2O_2 transducer at pH values greater than 9.5. Therefore, the pH value of 9.0 was selected as an optimal working pH of the biosensor.

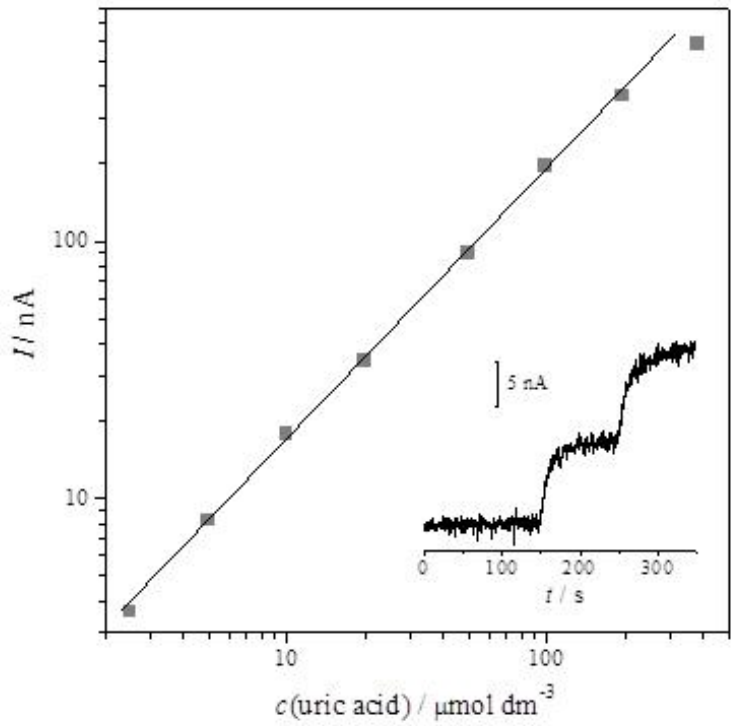

Figure 4. Calibration plot for uric acid measured with the uric acid biosensor. Inset: amperometric response of the biosensor upon two successive additions of 5 μM of uric acid. Supporting electrolyte: 0.1 M Britton-Robinson buffer / 0.1 M KCl, pH = 9.0; operating potential: –0.05 V.

The calibration plot for uric acid measured at pH 9.0 under the batch conditions and at working potential of the biosensor of –0.05 V is shown in Fig. 4. A linear ($r^2 = 0.9987$) response of the biosensor was observed in the concentration range of uric acid between 2.5 and 200 μM, with the sensitivity of 26.9 ± 0.4 mA M^{-1} cm^{-2} and the limit of detection (LOD, S/N = 3) of 0.65 μM. Under the conditions specified above, the response time of the biosensor was between 25 and 30 s (inset in Fig.

4.). Sensor also exhibited a low noise, which was found to be typically 0.4 nA (rms). Repeatability of the current response, expressed as a relative standard deviation of ten consecutive measurements of 100 μM of uric acid performed with the same biosensor, was found to be 2.8 %. In order to evaluate the reproducibility of the biosensors, five biosensors were randomly chosen from the group of biosensors prepared from the same enzyme immobilization batch and the sensitivity of each biosensor was determined from the slope of calibration plot measured in the concentration range of uric acid between 10 and 100 μM. The sensitivities thus obtained exhibited a relative standard deviation of 6.3 %, indicating a good reproducibility of the current response within the group of biosensors prepared under identical conditions.

Ascorbic acid and dopamine were chosen as the model compounds of electrochemical interferents present in real samples, and their influence on the biosensor response was examined. It was found that the addition of 200 μM of dopamine or ascorbic acid caused no changes in the current response of the biosensor in the presence of 50 μM of uric acid. It was also found that the presence of glucose, lactose, citric, pyruvic and lactic acid in the concentration of 5 mM have no influence on the biosensor response towards uric acid.

To assess the long-term stability of the biosensors, the sensitivity of five biosensors was monitored periodically over the period of 42 days (Fig. 5). During the first week of storage, the biosensors lost ca. 15 % of their initial sensitivity. After this period, the loss of sensitivity became more gradual and at the end of the six-weeks monitoring period, the biosensors still exhibited approximately 65 % of their initial sensitivity.

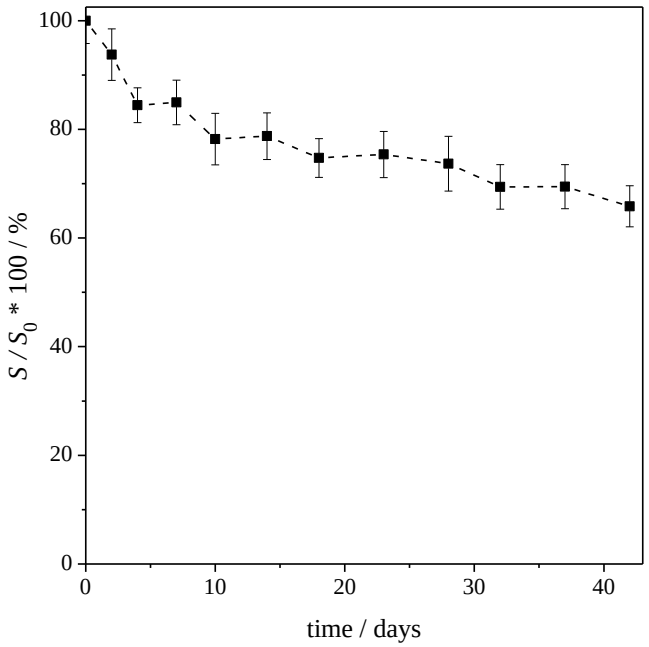

Figure 5. Long-term stability of the uric acid biosensor. The points represent the mean sensitivity towards uric acid of five biosensors, normalized with respect to the mean sensitivity of the biosensors measured on the first day of use. Error bars indicate the standard deviation of the measured sensitivities. Supporting electrolyte: 0.1 M Britton-Robinson buffer / 0.1 M KCl, pH = 9.0; operating potential: –0.05 V.

The comparison of analytical performances of the biosensor described in this work with the performances of other amperometric uric acid biosensors operating on similar principle (i.e. electrochemical detection of enzymatically produced H_2O_2) is presented in Table 1. Although the developed biosensor exhibited only moderate sensitivity, its linearity, covering two orders of magnitude of uric acid concentrations, is significantly better than the linearity of the majority of biosensors listed in Table 1. The developed biosensor is also characterized by a very low LOD, which can be attributed to the favorable working potential of the employed H_2O_2 transducer (–0.05 V). This potential falls into the electrochemically "quiet" region where neither the electrochemical reduction of dissolved oxygen, nor the oxidation of easily oxidizable compounds present in the sample matrix occur, resulting in the low noise and low background current, and therefore, the high signal-to-noise ratio of the biosensor.

Table 1. Analytical performances of some amperometric uric acid biosensors based on electrochemical detection of enzymatically produced hydrogen peroxide.

H_2O_2 transduction principle	Linearity (μM)	Sensitivity ($mA\,M^{-1}\,cm^{-2}$)	LOD (μM)	pH	*E* (V)	Ref.
oxidation on Co-phthalocyanine modified carbon electrode	$13 – 6 \cdot 10^3$	1.52	13	9.2	0.4 [b]	[27]
oxidation on Nafion-covered microelectrode array	15 – 175	176	–	7.0	0.8 [c]	[28]
reduction on Ir-modified carbon electrode	100 – 800	110	10	7.0	0.25 [a]	[21]
reduction on Prussian blue modified ITO electrode	0.1 – 0.6	150	–	7.4	0.0 [b]	[22]
reduction on microperoxidase-11 modified Au electrode	5 – 150	3.4	2.0	8.5	–0.1 [c]	[23]
oxidation on polyaniline/poly-pyrrole modified Pt electrode	2.5 – 85	2.2	1.0	9.0	0.4 [a]	[24]
reduction on hemoglobin/chito-san modified carbon electrode	2 – 30	420	0.85	7.0	–0.325 [a]	[25]
reduction on Ni^{2+}-modified Prussian blue	2.5 – 200	26.9	0.65	9.0	–0.05 [d]	this work

[a] vs. Ag/AgCl electrode [c] vs. Ag/AgCl/3M KCl electrode – not reported
[b] vs. Hg/Hg_2Cl_2/KCl(sat.) electrode [d] vs. Hg/Hg_2Cl_2/3.5M KCl electrode

In order to evaluate the applicability of the developed biosensor in analysis of real samples, the biosensor was employed for the determination of uric acid in twelve samples of commercial dairy products. The obtained results are shown in Table 2. along with the results of uric acid analysis obtained by the ion-exclusion HPLC method [43]. The uric acid content determined by the biosensor showed a high correlation with the corresponding value obtained by HPLC (Fig. 6.; slope of the correlation line, $a = 0.978$; correlation coefficient, $r^2 = 0.9978$), indicating that the developed biosensor can be successfully employed for determination of uric acid in complex real samples.

Table 2. Uric acid content in commercial dairy products determined by the developed amperometric biosensor and the ion-exclusion HPLC method. Samples of the same product type, but originating from different manufacturers are indicated by numbers 1, 2, etc.

Sample	Uric acid content (mg / 100 g)	
	Biosensor	HPLC
yoghurt 1	2.02	1.94
yoghurt 2	0.52	0.49
yoghurt 3	1.31	1.41
kefir 1	1.00	1.08
kefir 2	0.79	0.74
acidophilus milk 1	1.12	1.02
acidophilus milk 2	2.14	2.20
sour milk 1	2.63	2.80
sour milk 2	1.73	1.90
probiotic 1	2.90	2.93
probiotic 2	2.28	2.20
probiotic 3	1.95	2.03

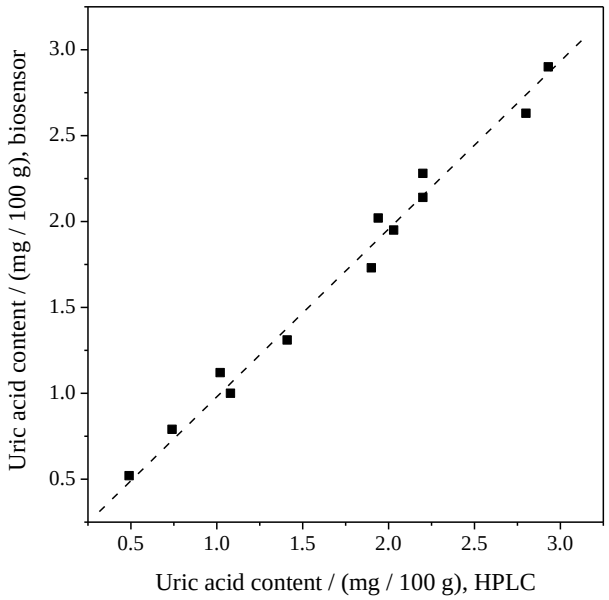

Figure 6. Correlation between the uric acid contents in commercial dairy products determined by the developed uric acid biosensor and the ion-exclusion HPLC method. The regression line is indicated by dashed trace.

4. CONCLUSIONS

Investigations performed in this work showed that Ni^{2+}-modified Prussian blue can be successfully employed as an electrocatalyst for H_2O_2 reduction in urate oxidase-based amperometric

uric acid biosensor. Due to the high electrocatalytic activity of Ni^{2+}-modified Prussian blue towards the reduction of H_2O_2, which was retained even in mild alkaline media, the developed biosensor was capable of operating at low cathodic potentials and near the optimal pH of urate oxidase of 9.0. This resulted in favorable analytical performances of the biosensor in terms of linearity (which covered two orders of magnitude of uric acid concentrations), selectivity, signal-to-noise ratio, and limit of detection. The high stability of Ni^{2+}-modified Prussian blue makes this form of Prussian blue an attractive H_2O_2 transducer for applications in amperometric biosensors based on other oxidase enzymes, which optimal pH falls into the neutral and mild alkaline range (e.g. xanthine oxidase and choline oxidase).

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