

Amperometric responses of some biosensor-related analytes at electrodes coated with semipermeable polymer layers: a comparative study

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Amperometric responses of hydrogen peroxide, ascorbate and paracetamol have been studied at platinum electrodes coated with Nafion or electropolymerized polypyrrole and poly(o-phenylenediamine) layers. Among the three layers studied, the Nafion layer shows superior properties. It possesses high permeability to hydrogen peroxide (95% as compared to naked platinum) and drastically diminishes responses from common interferences like ascorbate (up to 1–4%) and paracetamol (up to 46%). The electropolymerized poly(o-phenylenediamine) layer also shows good permeability to peroxide (90%), whereas its ability to repel ascorbate (up to 11–46%) does not appear to be as efficient as that of Nafion. The electropolymerized polypyrrole layer retards the permeability of peroxide and paracetamol up to 60% and increases the response to ascorbate by the factor of 1.9–2.6, probably due to its electrocatalytic action.

Key words: amperometric analysis, hydrogen peroxide, ascorbate, nafion, polypyrrole, poly(o-phenylenediamine)

INTRODUCTION

Semipermeable and selective polymer coatings are widely used for electrochemical sensors and biosensors. There are plenty of polymers known that, when coated as thin films at an electrode surface, discriminate the transport of solution species to an electrode either by their mass or electric charge. Basically, two different procedures for electrode modification by selective polymer coatings are applied in the development of sensors. First, a solution of a suitable polymer in an organic solvent can be distributed uniformly over the electrode surface, yielding a uniform polymer film upon drying. This procedure appears to be simple and reliable, however, a non-uniformity of the resulting film by thickness and even the appearance of uncoated electrode areas are the major drawbacks of this procedure, especially when the background electrodes of a complex configuration are used. Following an alternate procedure, the electrode can be coated by electrochemical polymerization, resulting in a more uniform distribution of a polymer layer over the electrode surface.

Presumably, the most popular selective coating consists of perfluorinated ion-exchange resin Nafion®. A thin film of this material allows the diffusion of low-molecular-weight species like hydrogen peroxide, and repels negatively charged species like ascorbate. Below is a short overview of some recent applications of this versatile material. Nafion and poly(o-phenylenediamine) coatings over a Prussian blue layer at the carbon fiber electrode have been reported to improve the selectivity of the electrode to hydrogen peroxide against common interferences like ascorbic acid (AA) and uric acid (UA) [1]. The Nafion coating was applied to reduce AA or UA interferences over a GOD containing colloidal gold-modified carbon ionic liquid electrode [2]. Placing the Nafion membrane between a platinum electrode and mucin-albumin hydrogel matrix containing lactate oxidase resulted in minimization of AA interferences to practically undetectable levels in lactate assay [3]. An increase of selectivity over AA and UA with the use of the Nafion coating has been reported for a dopamine biosensor based on monoamine oxidase immobilized on glutaraldehyde activated eggshell membrane [4]. A combined Nafion and poly(o-phenylenediamine) layer was adopted for a biosensor based on D-aminoacid oxidase

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suitable for D-serine monitoring in brain, whereas AA interference has been found to be negligible [5]. A glassy carbon electrode, modified with a hybrid Nafion and poly(3-methylthiophene) film, permitted a superior separation of voltammetric responses from dopamine and AA by shifting the oxidation peak of the latter toward less positive potential [6]. Positively charged epinephrine showed an enhanced electrochemical response at a CNT-Nafion electrode compared to a simple CNT electrode, enabling one to detect this analyte in the presence of a large excess of AA [7]. The glassy carbon electrode was modified consecutively with MWCNT, quercetin and Nafion, and used for detection of dopamine, virtually eliminating the interference of AA [8]. In this electrode configuration, CWNT increases the current output by five times, quercetin diminishes the oxidation potential of dopamine by 60 mV, and Nafion repels AA. A nickel hexacyanoferrate coated graphite wax composite electrode was additionally coated with Nafion and successfully evaluated for dopamine detection in urine in the presence of AA interference [9]. A cobalt hexacyanoferrate and Nafion coated glassy carbon electrode showed high selectivity to dopamine over AA [10]. Selective detection of dopamine in the presence of AA has been reported by covering the hexacyanoferrate-based composite electrode with the Nafion layer [11]. A needle type electrode, free from AA and UA interferences, based on Nafion and GOD layer coated Pt-Ir wire, was proposed for real-time monitoring of blood glucose level in fish [12]. A carbon fiber microelectrode, modified by Nafion and SWNTs, exhibited an enhanced electroanalytical voltammetric sensitivity and selectivity to dopamine in the presence of AA [13]. A layer containing carbon coated iron nanoparticles in chitosan at the glassy carbon electrode was overcoated with the Nafion layer, and the resulting structure was used in dopamine assay [14].

For oxidase type enzyme-based biosensors, the selective coating should allow the diffusion of a reaction product, hydrogen peroxide, and suppress the penetration of interfering substances usually present in an analyte solution, like AA, UA, or paracetamol. In the preceding work, we studied amperometric responses of these species at an electrode, covered with a polyaniline layer, and surprisingly found no discrimination of hydrogen peroxide and paracetamol, and acceleration of ascorbate electrooxidation by the polyaniline layer [15]. The present work is aimed at a comparative study of amperometric responses of hydrogen peroxide and two most important interferences, ascorbic acid and paracetamol, at a platinum electrode covered with a layer of Nafion and polypyrrole.

EXPERIMENTAL

Pyrrole, poly(o-phenylenediamine), hydrogen peroxide, ascorbic acid and other chemicals of analytical or reagent grade were used. Nafion® perfluorinated resin solution (5 wt.%) in a mixture of lower aliphatic alcohols and water

(Aldrich) was used as received. Paracetamol (acetaminophen) was obtained from a local drug store. Throughout the work, a BAS-Epsilon model potentiostat along with a C3 cell stand (Bioanalytical Systems Inc., West Lafayette, USA) were used. The experiments were performed in a 15 ml one-compartment electrochemical cell, arranged with a flat circular platinum wire working electrode, press-fitted into a plastic holder, 1.6 mm in diameter (2 mm² surface area) (Bioanalytical Systems Inc., West Lafayette, USA), glassy carbon counter electrode, and Ag/AgCl electrode, filled with 3N NaCl solution, as a reference. All potentials given below relate to this reference electrode. During the experiments, the electrolyte was continuously stirred with a magnetic stirrer.

The electrode was covered with the Nafion layer by placing a portion (ca. 100 µl) of Nafion solution onto the electrode surface and allowing to dry at an ambient temperature. In some experiments, two droplets were consecutively applied. Electropolymerization of pyrrole or o-phenylenediamine at the electrode was performed at a controlled potential of 0.8 V for 5, 10 or 15 min from a solution containing 0.1 M of potassium chloride and 0.1 M of pyrrole or o-phenylenediamine, respectively. After preparation, the modified electrode was rinsed with water and immersed into 0.1 M phosphate buffer solution pH 7.0, containing additionally 0.1 M of KCl, where all current-concentration dependencies were recorded. Small portions of stock analyte solutions were injected into the operating cell with a microsyringe. The electrode was operated at a controlled potential of 0.6 V. 0.1 M phosphate buffer solution pH 7.3, containing additionally 0.1 M of KCl, was used as a background electrolyte.

RESULTS AND DISCUSSION

Hydrogen peroxide is the most important analyte in biosensors. Oxidase-based biosensors produce hydrogen peroxide in an enzyme-catalyzed reaction, which is then detected amperometrically at a controlled electrode potential of ca. 0.6 V vs. Ag/AgCl, applied to the platinum or gold background electrode. Fig. 1 shows the dependence of the electrode current on the hydrogen peroxide concentration, both for the naked platinum electrode and the same electrode, coated with the Nafion layer.

A linear interdependence is observed within the concentration range tested up to 0.6 mM. Essentially, the slope of linear interdependencies does not differ markedly for both types of electrodes. For the platinum and Nafion coated platinum electrodes, the slope and thus the sensitivity of the electrode are 6.65 µA/mM (or 332 µA/mM cm²) and 6.37 µA/mM (or 318 µA/mM cm²), respectively. It follows that the layer of Nafion polymer does not retard markedly the diffusion of hydrogen peroxide from the bulk solution to the electrode surface and diminishes the sensitivity only by 4–5%. The rise of the electrode current output up to 95% of its maximum value upon addition of hydrogen peroxide occurs in less than 10 s, both for the naked and Nafion coated electrodes. A small cur-

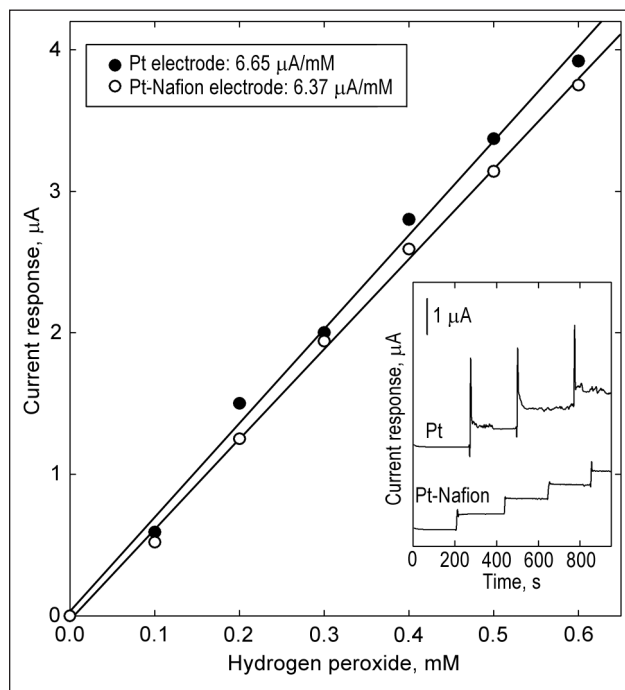


Fig. 1. Dependence of electrode current response on hydrogen peroxide concentration for the unmodified platinum electrode and the same electrode modified by 1 portion of Nafion (as indicated). Inset: current-time profiles for the same electrodes, as obtained upon addition of hydrogen peroxide (each step represents an increment of 0.1 mM of peroxide)

rent noise was also observed for the Nafion coated electrode, as compared to the naked platinum electrode (shown in the inset of Fig. 1).

For ascorbate, a linear dependence of the current response on its concentration is also observed at the platinum electrode up to 0.6 mM at the electrode operation potential of 0.6 V (Fig. 2).

The sensitivity for ascorbate at the naked platinum electrode appears to be about 3.3 times lower than that for hydrogen peroxide, being 1.88 $\mu\text{A}/\text{mM}$ (or 94 $\mu\text{A}/\text{mM cm}^2$). As distinct from hydrogen peroxide, the Nafion layer placed at the electrode surface drastically reduces the current response from ascorbate. For two Nafion coatings studied, a drop of sensitivity up to 0.075 $\mu\text{A}/\text{mM}$ (or 3.75 $\mu\text{A}/\text{mM cm}^2$) for a thinner coating and 0.021 $\mu\text{A}/\text{mM}$ (or 1.05 $\mu\text{A}/\text{mM cm}^2$) for a thicker coating was obtained. As compared to the naked platinum, a drop of the current response to ascorbate up to 4.0 and 1.1% is observed for two Nafion coatings studied, respectively. As for hydrogen peroxide, the rise of the current output to its maximum value in case of ascorbate proceeds fast, and a small noise is also observed for the Nafion coated electrodes, as depicted in the inset of Fig. 2. The results obtained show a high efficiency of a thin Nafion coating, containing negatively charged sulfonate groups in its structure, in the electrostatic repel of negatively charged ascorbate anions.

Next to ascorbate, paracetamol (acetaminophen) presents the well known interference for peroxide-detecting biosensors.

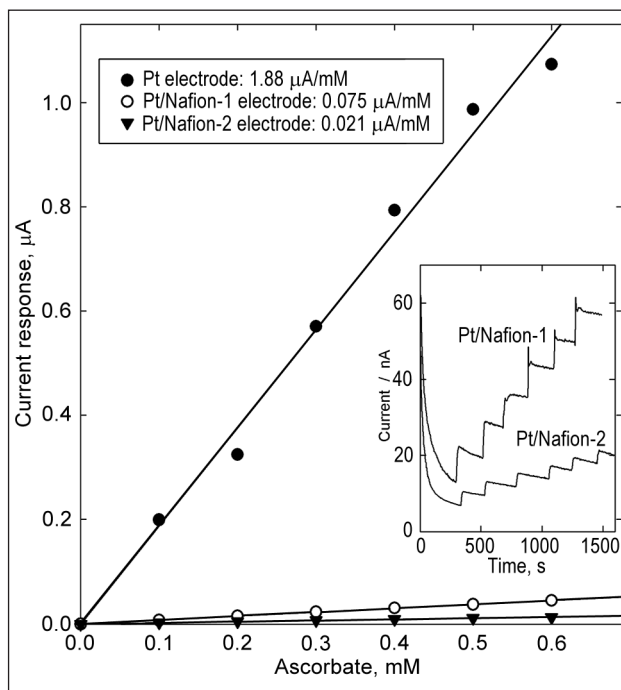


Fig. 2. Dependence of electrode current response on ascorbate concentration for the unmodified platinum electrode and the same electrode modified by 1 or 2 portions of Nafion (as indicated). Inset: current-time profiles for the same electrodes, as obtained upon addition of ascorbate (each step represents an increment of 0.1 mM of ascorbate)

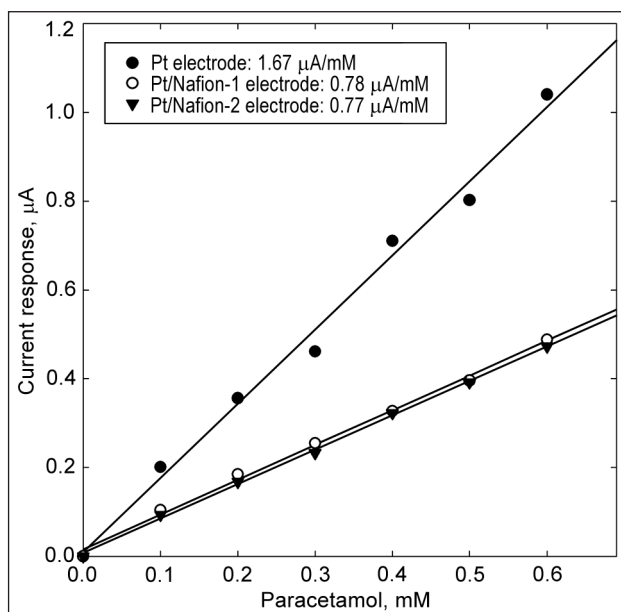


Fig. 3. Dependence of electrode current response on paracetamol concentration for the unmodified platinum electrode and the same electrode modified by 1 or 2 portions of Nafion (as indicated)

A linear dependence of the current output for the platinum electrode on the paracetamol concentration, as it was tested under the same conditions as for peroxide and ascorbate (Fig. 3), results in a sensitivity of 1.67 $\mu\text{A}/\text{mM}$ (83.5 $\mu\text{A}/\text{mM cm}^2$).

Coating of the electrode with the Nafion layer diminishes the electrode sensitivity to paracetamol up to 0.77–0.78 $\mu\text{A}/\text{mM}$ (38–39 $\mu\text{A}/\text{mM cm}^2$), i. e. up to 46% of its initial value. Paracetamol does not possess negatively charged ionizable groups in its structure, thus, the discrimination by the molecular size is the most probable reason for the decrease of the current response at the Nafion coated electrode.

As a reasonable alternative, semipermeable layers of polypyrrole and related materials are often used for the same purpose, particularly to reject ascorbate interference [16]. Fig. 4 displays the response of the polypyrrole coated electrode to the same three analytes.

For hydrogen peroxide, the electrode sensitivity drops up to 0.602 of its initial level by coating the electrode with polypyrrole at electropolymerization time of 5 min under the conditions specified. Similarly, the electrode response to paracetamol diminishes up to 0.620 upon its coating with polypyrrole under the same conditions, as compared to the naked platinum electrode. It follows that the polypyrrole layer retards the diffusion of paracetamol roughly twice, thus

resembling the action of the Nafion coating. In contrast to the Nafion layer, however, polypyrrole significantly reduces the response of the coated electrode to hydrogen peroxide. This combination, however, offers no advantage of the polypyrrole coating over Nafion. For ascorbate, conversely, a strong enhancement of the electrode response has been observed at the polypyrrole coated electrode (Fig. 3). In this case, the response depends on the thickness of the modifier layer. For polypyrrole layers, prepared by electropolymerization for 5 or 10 min, an enhancement of response by the factor of 1.903 and 2.628 has been observed, respectively. Obviously, electrocatalytic oxidation of ascorbate proceeds at polypyrrole coated electrodes. A related effect has been observed and discussed earlier for polyaniline coated electrodes [15].

For the electrode modified with the poly(*o*-phenylenediamine) (POPD) layer, prepared by the electropolymerization procedure, a small drop of sensitivity to hydrogen peroxide is observed, not exceeding 10%. In this respect, the behaviour of the POPD layer resembles that of Nafion. POPD also showed a well expressed repelling behaviour to ascorbate (Fig. 5).

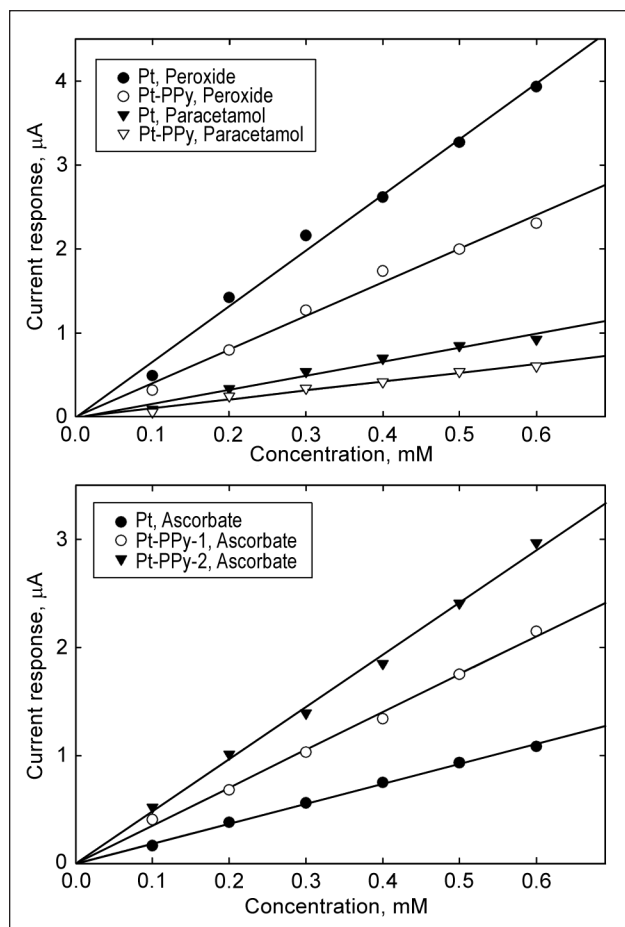


Fig. 4. Top: dependence of the current response of platinum or polypyrrole modified (electropolymerization time: 5 min) platinum electrodes on the concentration of hydrogen peroxide or paracetamol (as indicated). Bottom: same for ascorbate, as obtained for platinum electrodes modified with polypyrrole at electropolymerization time of 5 min (1) or 10 min (2)

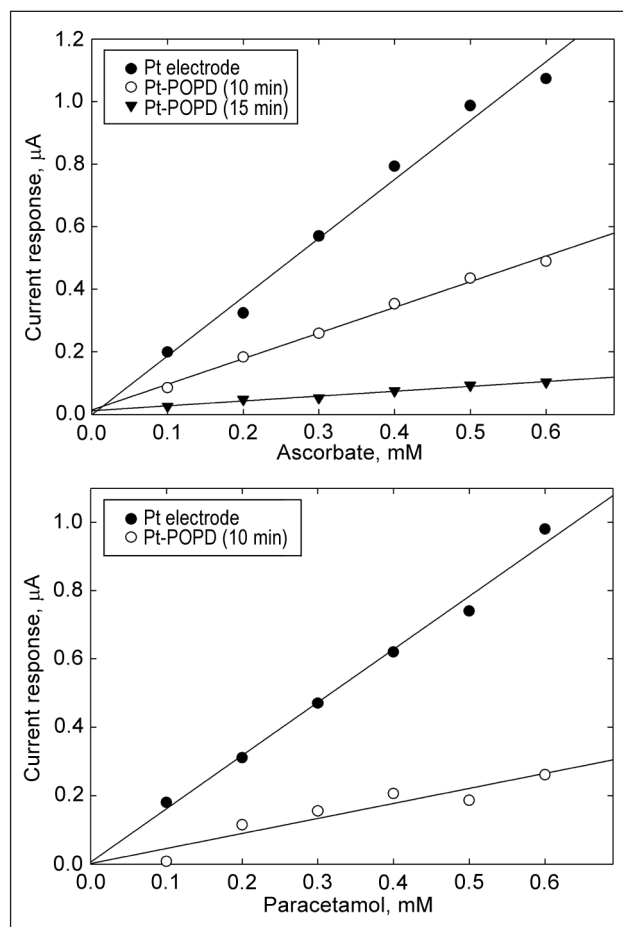


Fig. 5. Top: dependence of the current response of platinum or poly(*o*-phenylenediamine) modified (electropolymerization time: 10 or 15 min, as indicated) platinum electrodes on the concentration of ascorbate. Bottom: same for paracetamol, as obtained for the platinum electrode modified with poly(*o*-phenylenediamine) at electropolymerization time of 10 min.

For POPD layers, prepared by electropolymerization for 10 or 15 min, the decrease of the current output to ascorbate up to 46% or 11% (from 94 to 43 or 9.5 $\mu\text{A}/\text{mM cm}^2$) was obtained, respectively. For paracetamol, the decrease of the current output up to 28% (from 77.5 to 22 $\mu\text{A}/\text{mM cm}^2$) was obtained for POPD films, prepared by electropolymerization for 10 min. It follows that the layer of POPD shows well expressed, although some lower repelling properties towards ascorbate, and similar or slightly better repelling properties towards paracetamol, as compared to the Nafion layer.

CONCLUSIONS

Three different polymer layers coated over a platinum electrode show different behavior to electroanalytically important solution species – hydrogen peroxide, ascorbate and paracetamol. The drop-coated Nafion layer has a very high permeability for peroxide and nearly full impermeability for ascorbate. The electropolymerized poly(o-phenylenediamine) layer has a good permeability for peroxide and somewhat lower discrimination for ascorbate, whereas the electropolymerized layer of polypyrrole shows an enhanced electrochemical response to ascorbate, as compared to uncovered platinum, probably due to its electrocatalytic action. For peroxide-detecting biosensor application, the Nafion coating suits best among the three polymer coatings studied.

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References

1. P. Salazar, M. Martin, R. Roche, R. D. O'Neill, J. L. Gonzalez-Mora, *Electrochim. Acta*, **55**, 6476 (2010).
2. X. Y. Liu, X. D. Zeng, N. N. Mai, et al, *Biosens. Bioelectron.*, **25**, 2675 (2010).
3. M. R. Romero, F. Ahumada, F. Garay, A. M. Baruzzi, *Anal. Chem.*, **82**, 5568 (2010).
4. P. Joshi, H. C. Joshi, S. K. Sanghi, S. Kundu, *Microchim. Acta*, **169**, 383 (2010).
5. Z. M. Zain, R. D. O'Neill, J. P. Lowry, et al, *Biosens. Bioelectron.*, **25**, 1454 (2010).
6. V. T. Huong, T. Shimanouchi, D. P. Quan, H. Umakoshi, P. H. Viet, R. Kuboi, *J. Appl. Electrochem.*, **39**, 2035 (2009).
7. L. B. Ou, Y. N. Liu, J. X. Wang, L. Zhang, *J. Nanosci. Nanotechnol.*, **9**, 6614 (2009).
8. P. Y. Chen, R. Vittal, P. C. Nien, K. C. Ho, *Biosens. Bioelectron.*, **24**, 3504 (2009).
9. S. J. R. Prabakar, S. S. Narayanan, *Electroanalysis*, **21**, 1481 (2009).
10. S. S. L. Castro, R. J. Mortimer, M. F. De Oliveira, N. R. Stradiotto, *Sensors*, **8**, 1950 (2008).
11. R. Thangamuthu, Y. C. Wu, S. M. Chen, *Electroanalysis*, **21**, 994 (2009).
12. H. Endo, Y. Yonemori, K. Hibi, et al, *Biosens. Bioelectron.*, **24**, 1417 (2009).
13. H. Jeong, S. Jeon, *Sensors*, **8**, 6924 (2008).
14. G. S. Lai, H. L. Zhang, D. Y. Han, *Microchim. Acta*, **160**, 233 (2008).
15. S. Kozlovskaja, G. Baltrūnas, A. Malinauskas, *Microchim. Acta*, **166**, 229 (2009).
16. A. Malinauskas, R. Garjonytė, R. Mažeikienė, I. Jurevičiūtė, *Talanta*, **64**, 121 (2004).

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KAI KURIŲ ANALIČIŲ AMPEROMETRINIO ATSAKO ANT ELEKTRODŲ, PADENGŲ PUSIAU LAIDŽIAIS POLIMERŲ SLUOKSNAIS, PALYGINAMASIS TYRIMAS

Santrauka

Vandenilio peroksido, askorbato ir paracetamolio amperometrinis atsakas buvo tiriamas ant platinos elektrodų, padengtų nafiono ar elektrochemiškai polimerintų polipirolo bei poli(o-fenilendiamino) sluoksniais. Iš trijų tirtųjų sluoksnių geriausiomis savybėmis pasižymi nafiono sluoksnis, kuris gerai praleidžia vandenilio peroksidą (amperometrinis atsakas siekia 95 % nepadengtos platinos elektrodo atsako) ir efektyviai sulaiko askorbatą (atsakas tesiekia 1–4 %) bei paracetamolį (46 %). Geru pralaidumu vandenilio peroksidui (90 %) pasižymi poli(o-fenilendiaminas), tačiau savo gebėjimu sulaikyti askorbatą (11–46 %) jis gerokai nusileidžia nafionui. Elektrocheminės polimerizacijos būdu gautas polipirolo sluoksnis sumažina peroksido ir paracetamolio pralaidumą iki 60 %, tačiau dėl elektrokatalizinio aktyvumo 1,9–2,6 karto padidina askorbato atsaką.