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ORIGINAL PAPER

Response of hydrogen peroxide, ascorbic acid, and paracetamol at a platinum electrode coated with microfilms of polyaniline

Svetlana Kozlovskaja · Gintaras Baltrūnas · Albertas Malinauskas

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Abstract The amperometric response of hydrogen peroxide, ascorbic acid and paracetamol (which are known interferents in many enzymatic biosensors) have been studied at polyaniline (PANI) modified platinum electrodes in pH 7.0 buffer solution. Platinum electrodes covered with the films of electropolymerized PANI display reduced response both to hydrogen peroxide and paracetamol, depending on the thickness of the film. An almost 1.5 to 2.1-fold decrease in anodic current is observed both for peroxide and paracetamol in case of PANI films having a redox capacity ranging from 5 to 400 μC cm^{-2}. Ascorbate, in contrast, causes an increase in current due to the electrocatalytic nature of this process. It is concluded that PANI coatings do not warrant an adequate discrimination between the analytes studied.

Keywords Sensor · Hydrogen peroxide · Ascorbate · Paracetamol · Polyaniline · Modified electrode

Introduction

Electrochemical biosensors based on oxidase type enzymes are widely used in clinical analysis and process control. For the development of biosensing devices, immobilization of enzymes at electrode surface is of great importance. Next to widely used traditional techniques like crooslinking with inert proteins, new techniques are extensively developed in the past decade. Among them, immobilization into or onto electropolymerized layers of specific species at the electrode surface seem to be highly promising. For this aim, electropolymerized layers of polyaniline, polypyrrole and other related polymers have been used and well reviewed [1–7]. Electropolymerized layers have some advantages over other related techniques like easiest of preparation, inexpensive materials and methods, and specific properties like electric conductivity enabling a direct (mediatorless) electron exchange between enzyme and electrode.

One of the most useful properties of electropolymerized films is their permselectivity. This property is determined either by the presence of specific groups in the polymer structure, or by variations in polymer pore size. Overoxidized polypyrrole is probably most often used as a layer well suited for attenuation of ascorbate. This layer is usually prepared by pyrrole electropolymerization and subsequent electrochemical treatment to produce negatively charged groups on its surface. These groups repel negatively charged interference species like ascorbate, allowing a free diffusion of uncharged species of analytical interest like *e.g.* dopamine [8–11]. Following another way, electrodeposited polymers discriminate analytes and interference species by the size. In case if the polymer has very compact structure, it allows the diffusion of small species to electrode surface, and retards the permeability of bigger ones. The most useful application of these semipermeable electropolymerized layers relate to oxidase-type biosensors. As usual, these sensors detect hydrogen peroxide formed in the course of enzyme-catalyzed reaction. Since hydrogen peroxide presents small species differing in size from usual interferences present in solution like ascorbate, uric acid or

S. Kozlovskaja · G. Baltrūnas · A. Malinauskas
Department of Physical Chemistry, Vilnius University,
Naugarduko 24,
Vilnius LT-03225, Lithuania

A. Malinauskas (✉)
Institute of Chemistry,
Goštauto 9,
Vilnius LT-01108, Lithuania
e-mail: albertas.malinauskas@chi.lt

acetaminophen, semipermeable electropolymerized layers seem to be most suited for discrimination of these interferences from hydrogen peroxide. Among a few polymers known, poly(*o*-phenylenediamine), an electrogenerated polymer having a dense structure, is well suited for this purpose [12, 13].

Next to permselectivity, some elecropolymerized films show electrocatalytic effect to solution species. Among them, polyaniline is known to catalyze anodic oxidation of ascorbic acid. Based on this, different electrode configurations have been proposed for electrochemical assay of this important analyte [14]. The present work has been aimed to comparative study of electrochemical responses for three analytes, hydrogen peroxide, ascorbate, and paracetamol, at polyaniline modified electrode.

Experimental

Aniline and ascorbic acid (both of Fluka, www.sigmaaldrich.com) have been used as received. Paracetamol (acetaminophen) and hydrogen peroxide have been obtained from a local drug store. Throughout the work, μ-Autolab Type III potentiostat (Eco Chemie, The Netherlands, www.ecochemie.nl) has been used. The experiments have been performed in a custom built 20 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 mm^2 surface area) (Bioanalytical Systems Inc., West Lafayette, USA, www.bioanalytical.com), platinum wire counter electrode, and saturated Ag/AgCl electrode as a reference. All potentials given below relate to this reference electrode. Before experiments, platinum working electrode was polished with alumina slurry. The cell has been arranged with a magnetic stirrer.

Polyaniline modified electrode has been prepared by two different procedures. Following the procedure of electropolymerization at a controlled potential, the electrode has been immersed into 0.5 mol L^{-1} sulfuric acid solution containing 0.05 mol L^{-1} of aniline, and controlled potential of 0.8 V was applied for different time ranging up to 20 min. Following potential cycling procedure, the electrode has been subjected to potential cycling within the limits of -0.1 to 1.0 V at a potential scan rate of 50 mV s^{-1} for different time ranging up to 30 min. In some cases, the amount of electrodeposited polyaniline has been calculated from cyclic voltammograms recorded in 0.5 mol L^{-1} solution of sulfuric acid not containing aniline. After preparation, the modified electrode has been rinsed with water, and immersed into 0.1 mol L^{-1} phosphate buffer solution pH7.0, containing additionally 0.1 mol L^{-1} of KCl, where all current-concentration dependencies have been recorded. Small portions of stock analyte solutions have been injected into the operating cell with a microsyringe. The electrode has been operated at a controlled potential of 0.6 V.

Results and discussion

Two traditional techniques were used in preparing of polyaniline (PANI) modified platinum electrode. First, electropolymerization has been performed at a controlled potential of 0.8 V from an acidic solution containing aniline monomer. Following this way, the amount of PANI eletrodeposited, and thus the thickness of a modifier layer, depend greatly on the duration of electropolymerization. Electropolymerization of aniline is known to bear autocatalytic character, thus, the amount of PANI deposited does not depend linear on the duration of the process [15–18]. Figure 1 shows cyclic voltammograms for PANI modified electrodes prepared at different electropolymerization time. Within the scan limits of -0.1 to 0.5 V, electrodes show usual redox behaviour with a pair of anodic and cathodic peaks with a midpoint value ($E_{pa} + E_{pc}$)=0.16 V and a peak separation of 0.11 V. An increase of anodic and cathodic peaks for the resulting modified electrodes with an increase of electropolymerization time is well seen in Fig. 1. The fastest growth of PANI film proceeds between 5 and 15 min of polymerization, whereas slower growth is observed at prolonged procedure (up to 20 or 30 min), most probably

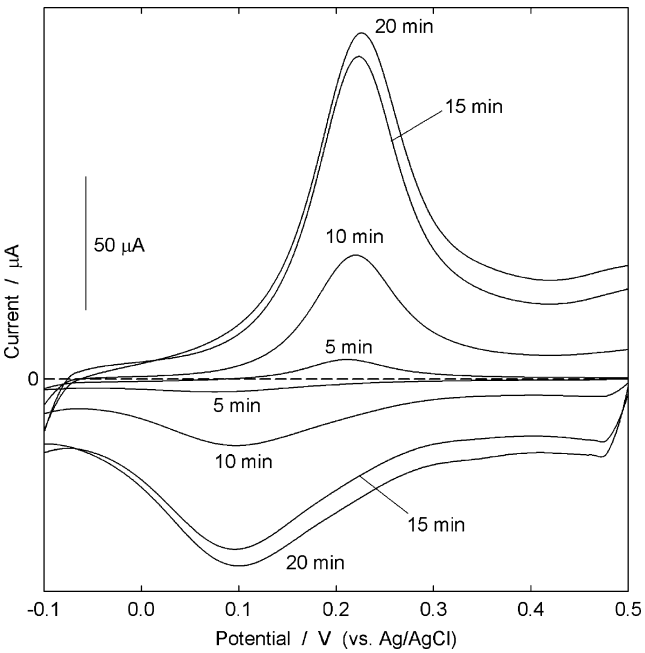

Fig. 1 Cyclic voltammograms of polyaniline modified electrodes, as obtained in 0.5 mol L^{-1} solution of sulfuric acid within potential scan limits of -0.1 to 0.5 V at a scan rate of 50 mV s^{-1}. Electrodes have been prepared by electropolymerization from 0.5 mol L^{-1} solution of sulfuric acid containing 0.05 mol L^{-1} of aniline at a controlled potential of 0.8 V for different time ranging from 5 to 20 min (*as indicated*)

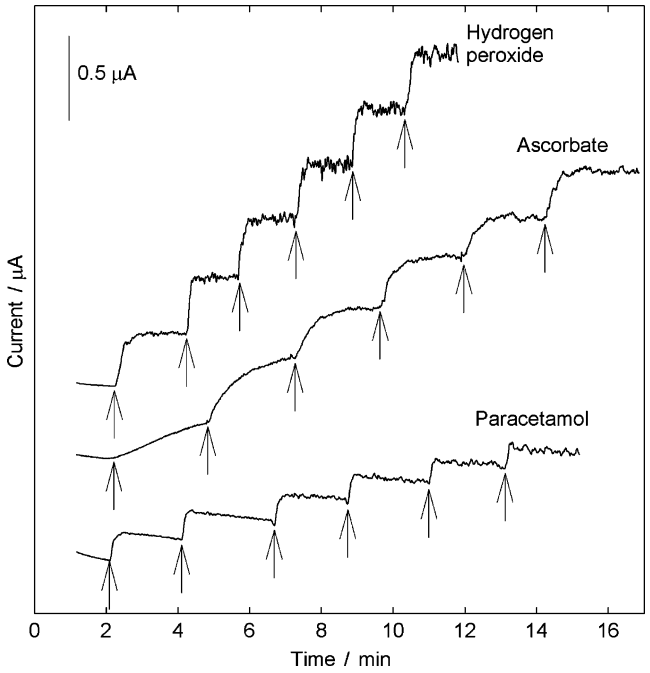

Fig. 2 Cathodic current response of polyaniline modified electrode to repeating additions of hydrogen peroxide, ascorbate, and paracetamol (*as indicated*). Electrode has been prepared by electropolymerization at a controlled potential of 0.8 V for 10 min in a solution of 0.5 mol L^{-1} sulfuric acid containing 0.05 mol L^{-1} of aniline, and has been operated in 0.1 mol L^{-1} phosphate buffer solution pH7.0 containing 0.1 mol L^{-1} of KCl at a controlled potential of 0.6 V. The corresponding substances have been added in portions of 0.1 mmol L^{-1} at the moments indicated by the arrows

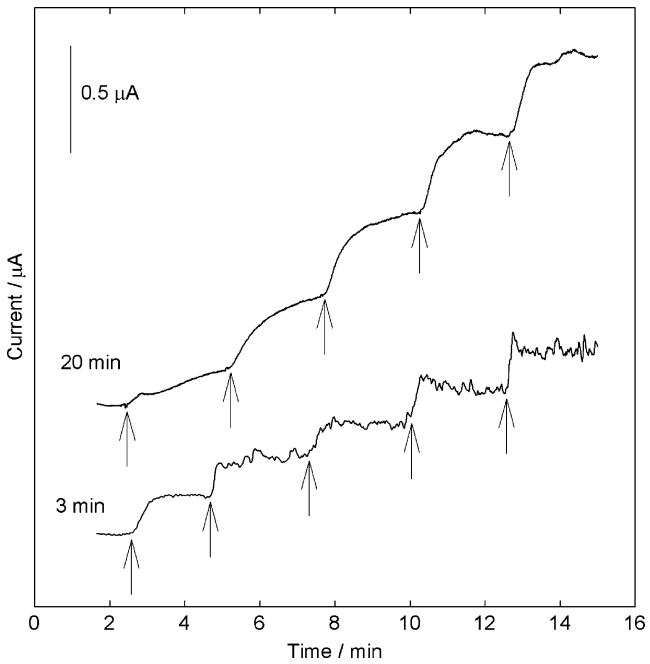

Fig. 3 Cathodic current response of polyaniline modified electrode to repeating additions of ascorbate, as obtained for polyaniline modified electrodes prepared by electropolymerization at a controlled potential of 0.8 V for 3 and 20 min (as indicated). Other conditions as in Fig. 2

because of a faster decomposition process for thick polymer films, as discussed earlier [15]. From the data obtained, an amount of PANI electrodeposited could be calculated by integration of the corresponding CVs. For electropolymerization time ranging from 3 to 20 min, an amount of PANI, expressed in terms of electric capacity, ranging from 5 to 400 μC cm^{-2} has been obtained.

When operated at a controlled potential of 0.6 V in phosphate buffer solution pH7.0, PANI coated electrodes prepared show an anodic current response to all analytes used, *viz.* hydrogen peroxide, ascorbate, and paracetamol. Some representative responses are presented in Fig. 2. By

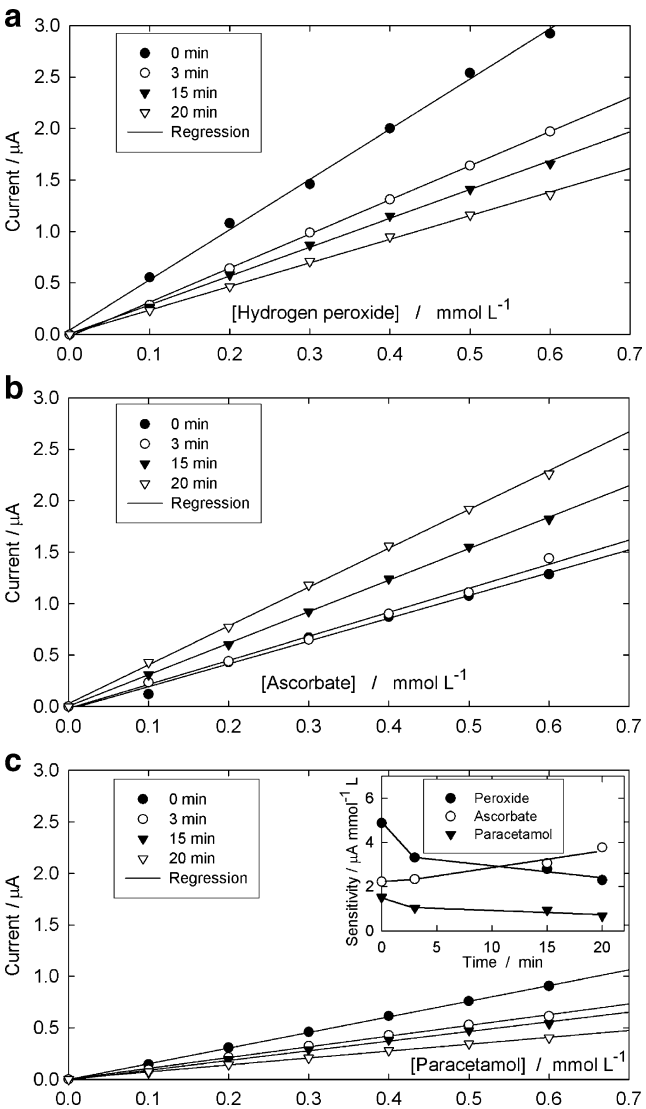

Fig. 4 Dependence of cathodic current on concentration of hydrogen peroxide (*A, top*), ascorbate (*B, middle*), and paracetamol (*C, bottom*), as obtained at polyaniline modified electrodes, prepared by electropolymerization in solution of 0.5 mol L^{-1} sulfuric acid containing 0.05 mol L^{-1} of aniline at a constant potential of 0.8 V for 0 min (bare platinum electrode), and 3, 15, or 20 min (*as indicated*). An inset in C shows dependence of electrode sensitivity to three different analytes used (*as indicated*) on electropolymerization time

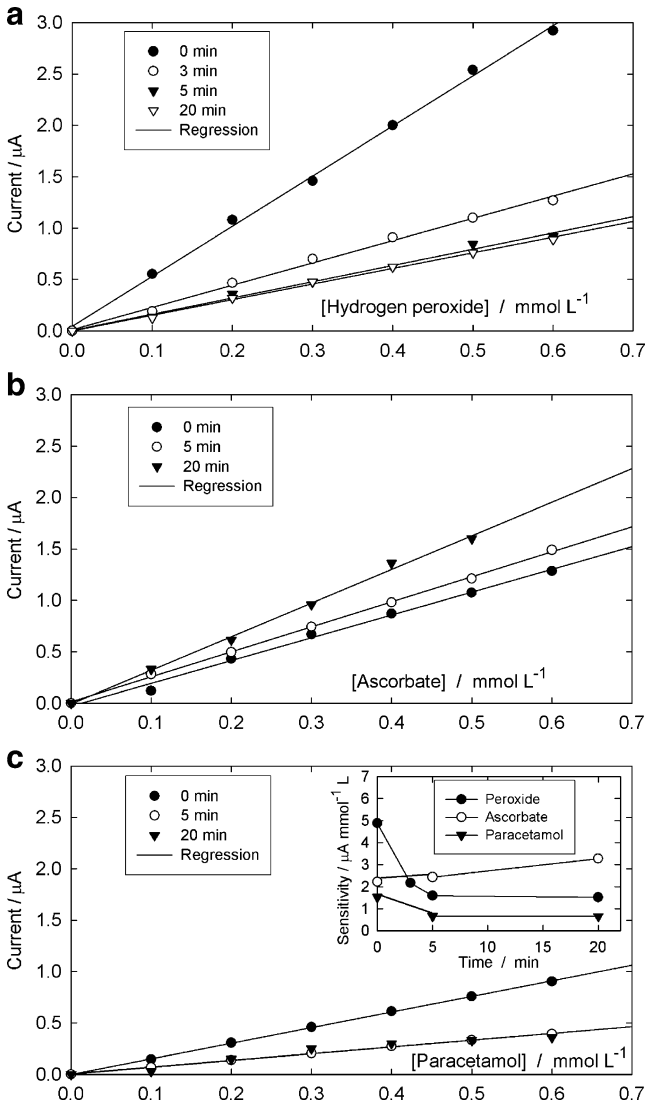

Fig. 5 Same as in Fig. 4, as obtained for polyaniline modified electrode prepared by electropolymerization in solution of 0.5 mol L^{-1} sulfuric acid containing 0.05 mol L^{-1} of aniline by potential cycling procedure at a scan rate of 50 mV s^{-1} within the potential window of -0.1 to 1.0 V for 0, 3, 5, and 20 min (*as indicated*)

adding of a small amount of hydrogen peroxide to a stirred solution, anodic current grows up and reaches its definite limiting value. At an unmodified electrode, the maximum (limiting) current is reached in approx. 10 s, whereas somewhat slower growth of current response is observed at modified electrodes. For hydrogen peroxide and paracetamol, typical current rise times are within the range of 10 to 20 s, depending on the thickness of PANI film.

Quite different behaviour is observed for ascorbate. Upon addition of the first aliquot of ascorbate, a slow increase of anodic current is observed, reaching its steady state in 3 or even more minutes. Addition of the second and further aliquots of ascorbate result in even faster growth of current reaching its maximum (diffusion controlled) value (Fig. 2). Earlier, we interpreted this specific behaviour by its autocatalytic nature [19, 20]. In pH-neutral solution, polyaniline presents in its non-conducting deprotonated form, thus, no electrocatalytic reactions could be expected to proceed at PANI modified electrode in pH-neutral solution. Since electrodeposited PANI layer is presumable of a porous nature, ascorbate diffuses through the pores to the background electrode surface, where its electrooxidation proceeds. In the course of this electrode reaction, protons are produced in an equimolar amount to oxidized ascorbate, causing a decrease of a local pH within PANI layer and thus turning PANI into its protonated and conducting state. Once turned, PANI catalyzes anodic oxidation of ascorbate. Therefore, autoacceleration of anodic oxidation of ascorbate is observed. The results obtained present additional indirect evidence for this mechanism. The data for hydrogen peroxide, ascorbate and paracetamol, presented in Fig. 2, relate to the same PANI film. Thus, porous PANI film prepared under the conditions used allows the diffusion not only of peroxide, but also of much larger paracetamol species. Since ascorbate and paracetamol present chemical species of comparable dimensions, the diffusion of ascorbate through these pores appears to be not restricted. In order to prove this assumption, we tested anodic current responses for ascorbate at electrodes, covered with PANI films of different thickness. Figure 3 shows the corresponding dependences, obtained at the thinnest and thickest PANI coatings. It is well seen that much faster response is obtained at thinner PANI film. For PANI film, obtained by electropolymerization for 3 min (with the redox capacity of 5 μC cm^{-2}), the rate constant for initial current rise of 6.38×10^{-1} min^{-1} has been obtained by the treatment of the data according to 2-parameter single

Table 1 Sensitivity of PANI-coated electrodes to analytes

Electrode modification mode	Duration, min	Sensitivity, μA $mmol^{-1}$ L		
		to hydrogen peroxide	to ascorbate	to paracetamol
No modification	0	4.88	2.22	1.52
Controlled potential	3	3.32	2.33	1.04
	20	2.29	3.77	0.67
Potential cycling	3	2.17	2.35	0.66
	20	1.52	3.27	0.65

exponential rise to maximum equation. In contrast, for the thickest PANI film, prepared by electropolymerization for 20 min (resulting in the redox capacity of 400 μC cm^{-2}), the rate constant for initial current rise of $4.35 \times 10^{-4} min^{-1}$ has been obtained. Comparing these data, it could be concluded that an increase of the thickness of PANI film by *ca.* two orders of magnitude causes a decrease of the rate constant by *ca.* three orders of magnitude. Also, electrocatalytic behaviour of PANI towards ascorbate electrooxidation could be deduced from the data presented in Fig. 3. For thin PANI films (5 μC cm^{-2}), anodic current steps obtained do not differ markedly in their height from those obtained at a bare platinum electrode. For thick PANI films (400 μC cm^{-2}), however, the steps appear approximately 1.5-fold higher. This shows a substantial increment from electrocatalytic oxidation mechanism to the net current response, next to a simple oxidation of ascorbate due to usual diffusion through the pores of electrodeposited PANI.

For all three analytes tested, a linear dependence of anodic current response on analyte concentration has been obtained in submillimolar concentration range, both for electrodes prepared by electropolymerization at a controlled electrode potential (Fig. 4), and under potential cycling conditions (Fig. 5). From these dependencies, important conclusions could be drawn. For hydrogen peroxide, the greatest slope of a calibration graph, and thus the highest electrode sensitivity has been obtained for unmodified (bare) platinum electrode (Table 1). Electropolymerization of aniline at a controlled potential for 3 or 20 min causes nearly 1.5-fold or 2.1-fold decrease of sensitivity, respectively. Even higher decrease of sensitivity has been obtained for electrodes, modified by potential cycling procedure: nearly 2.2-fold and 3.2-fold decrease has been observed for 3 or 20 min of potential cycling. It follows that, as compared to electropolymerization at a controlled potential, potential cycling yields more compact and less permeable to hydrogen peroxide PANI films. As it could be expected, an adverse order of electrode sensitivities has been obtained for ascorbate (Figs. 4 and 5). Even a thin PANI film (5 μC cm^{-2}) deposited over a platinum electrode increases the sensitivity by 5 or 6% depending on polymerization procedure used. For the thickest PANI films (400 μC cm^{-2}), a 1.7-fold increase of sensitivity is observed (Table 1). For paracetamol, a decrease of sensitivity is observed after the modification of electrode with PANI layer. For thinnest and thickest PANI films used the sensitivity to paracetamol drops by the factor of 1.46 and 2.27, respectively.

Conclusions

Perhaps the most important conclusion from the results obtained is that the anodic current response both to hydrogen peroxide and paracetamol is retarded nearly to the same degree at PANI modified platinum electrodes. Pursuing possible applications of PANI films for biosensors, it should be noted that, although PANI is often considered as a suitable matrix for enzyme immobilization, it shows almost no discrimination ability between hydrogen peroxide as a product of enzyme-catalyzed reaction, and paracetamol as possible interference. For ascorbate, adversely, an increase of current response is observed by covering of electrode by PANI layer. As a result, PANI modified electrode shows an enhanced sensitivity to ascorbate, as compared to bare platinum electrode. This makes it possible to use this modified electrode as an amperometric sensor for ascorbate.

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