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Comparison of Lead Dioxide and Cerium Dioxide as Mediators for Carbon Paste Electrodes in Flow Injection-Amperometric Detection of Hydrogen Peroxide

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Abstract: Carbon paste electrodes (graphite / paraffin oil), bulk-modified with lead dioxide and cerium dioxide, were used as sensors for the amperometric determination of hydrogen peroxide in flow injection analysis. Experimental parameters, such as applied working potential, flow rate of the carrier and injection volume were optimized with a thin-layer flow-through cell. The method was validated with respect to calibration curve, linear dynamic range, detection limit, repeatability and reproducibility.

Keywords: cerium dioxide, lead dioxide, mediators, FIA, hydrogen peroxide

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Introduction

Hydrogen peroxide is a side product of many oxidative biological reactions and is therefore an important parameter for monitoring of biological processes. Due to the increasing number of patients suffering from diabetes, considerable attention has been focused on the development of glucose sensors. The electrochemical detection of the analyte often proceeds via hydrogen peroxide which is formed during the enzymatic oxidation of glucose by glucose oxidase with the aid of oxygen. The ultimate goal is an automated continuous and noninvasive *in vivo* monitoring system for glucose levels in the blood which necessitates miniaturization of the respective biosensors that would allow the possibility of implantation or the use of microdialysis probes [1-3].

Due to their wide exploitable potential range, low electrical resistance, low residual current, chemical inertness, and low cost, carbon electrodes are considered to be extremely suitable candidates for this purpose. They favor easy surface modifications with mediators and enzymes. Many different forms of carbon, such as glassy carbon, carbon fibers, carbon pastes, carbon nanotubes and graphite films have been used as substrate transducers in biosensor fabrication [4-6].

Despite the high specifity of the enzyme, the response of the corresponding sensors may still be influenced by electroactive substances present in the biological sample matrix, such as ascorbic acid and uric acid. Therefore, the main aim is to reduce the high overpotential of hydrogen peroxide (usually around +0.7 V vs. ref.el. for its oxidation, and -0.8 V for its reduction at bare electrodes) by means of a mediator introduced as a modifier to the electrode surface or into the electrode bulk.

Quite a few mediators have been used so far for lowering the overpotential for the electrochemical conversion of H_2O_2. The use of Prussian Blue (PB) and its analogues, which exhibit strong electrocatalytic activity, well-defined redox transitions and electrochromicity [7], has been particularly successful. Several researchers have reported highly selective and sensitive first generation amperometric oxidase biosensors based on PB modified electrodes [8, 9]. Such devices lower the overpotential for hydrogen peroxide reduction and enable the low-potential monitoring of glucose. Due to the good performance of PB, much attention has been paid to the development of PB analogues such as cuprous, cobalt, nickel, chromium and mixed hexacyanoferrates, with a view to improve the electrode stability, especially under more alkaline conditions, and increasing its sensitivity to H_2O_2 [10 – 12].

As regards mediators, it was demonstrated that various metal oxides are suitable modifiers for both, carbon pastes and carbon inks, in order to prepare carbon paste electrodes (CPEs) and screen printed carbon electrodes (SPCEs). In principle, there are two ways which may be followed. The first is the use of more frequent oxides such as MnO_2 [13 – 17], Fe_3O_4 [18], FeO [19], SnO_2, CuO, Fe_2O_3 [20], etc. The second one is the use of oxides of platinum group metals; they are more expensive (anyway, their consumption is not too high), but the resulting sensors are less affected by various side reactions (dissolution in acidic media, etc.). Thus, the electrocatalytic properties of RuO_2 [21, 22] and RhO_2 [23] were exploited in biosensors for the determination of hydrogen peroxide, glucose and other biologically important compounds. Oxides of the remaining group of platinum metals (PdO, OsO_2, IrO_2 and PtO_2) were subjected to similar studies more recently [24].

The paper presented here investigates the possibilities to use lead dioxide and cerium dioxide as mediators in bulk-modified carbon paste electrodes for the amperometric determination of hydrogen peroxide.

Experimental

Chemicals

All chemicals used were of analytical reagent grade, and solutions were prepared with highly pure water obtained with a purification system (Milli-Q Integral System, Millipore, Massachusetts). The concentration of hydrogen peroxide stock solution (p.a., Roth, Karlsruhe, Germany) was 30%, and it was diluted daily as required. Phosphate buffer solution, pH 7.5, was prepared from the appropriate volumes of stock solutions containing 18.0 mL 0.2 M sodium dihydrogenphosphate ($NaH_2PO_4 \cdot H_2O$, p.a., Sigma-Aldrich) and 84.0 mL 0.2 M disodium hydrogenphosphate ($Na_2HPO_4 \cdot 7H_2O$, p.a., Sigma-Aldrich) with the addition of 100.0 mL water.

Apparatus

Voltammetric experiments were performed on a PalmSens electrochemical analyzer (Palm Instruments BV, Houten, The Netherlands) operated via "PalmSensPC" 9 software. In the case of cyclic or hydrodynamic voltammetric measurements (batch system), the external electrode stand included a three-electrode cell with a corresponding carbon paste electrode as the working electrode, a platinum wire (99.99%) as the auxiliary, and an Ag/AgCl/3 mol L^{-1} KCl as the reference electrode electrode; all potentials are quoted vs. this reference electrode. The electrode body of the working electrode consisted of a Teflon rod with an opening diameter of 7 mm, 3 mm deep, and filled with unmodified or modified carbon paste; contact was made with a copper wire. Stirring of the solution was carried out with a magnetic bar rotated at approx. 300 rpm.

The equipment for flow injection analysis (FIA) contained a high performance liquid chromatographic pump (Waters 600E, Waters, Millford, USA), injection vale (model 5041, 0.8 mm i.d. PTFE tubing, 100 µL loop; Pharmacia, Karlsruhe, Germany) and a thin-layer flow cell (model CC5, Bioanalytical Systems Inc. BASi, West Lafayette, USA); it was equipped with a modified screen-printed carbon working electrode and a Ag/AgCl (3 M KCl)

reference electrode (model RE6, BASi); the back plate of the cell (steel) served as counter electrode. Currents were recorded with a potentiostat (model 100W, BASi) operated with the correspondedn software. The pH was measured using a pH-meter (pH Meter Thermo Orion, Model 210 A+) in conjunction with a combined glass electrode.

Procedures

Preparation of the CPEs. Unmodified carbon paste ("CPE") was made by intimate hand-mixing of 1.0 g graphite powder (RW-C, Ringsdorff-Werke GMBH Bonn-Bad Godesberg, Germany) with the 320.0 µL of paraffin oil (Uvasol, Merck) as liquid binder.

The modified pastes ("PbO_2-CPE", "CeO_2-CPE") were prepared by substituting 5 % (m:m) of the carbon powder moiety by the corresponding oxide (PbO_2, p.a., or CeO_2, p.a., Sigma-Aldrich).

For FIA-amperometric measurements lead dioxide-modified screen-printed electrodes ("PbO_2-SPE") were used. Carbon ink (type C2030519P4, Gwent Group, Torfaen, UK) was mixed thoroughly with 5 % (m:m) of lead dioxide and screen printed on Laser-pre-etched (40x10 mm) sintered alumina plates (115x165 mm, CoorsTek, Golden, USA) using a metal screen of 100 µm thickness. The printed electrode area was 35x3 mm. The electrode plates were dried at room temperature overnight and then broken into the individual sensors.

Preliminary amperometric measurements were performed with CPEs, PbO_2-CPEs and CeO_2-CPEs in the presence of phosphate buffer (0.1 M, pH 7.5) as supporting electrolyte. The concentration of H_2O_2 in the test solution was 100.0 µg mL^{-1}. The investigated working potentials ranged from -0.5 V to +0.5 V with all electrode types. The solutions were deaerated by passing an argon stream through them for 10 min, and the amperometric curves were registered at ambient temperature. The analytical signals obtained for the oxidation of H_2O_2 with different CPEs at different potentials were evaluated on the basis of the current intensities. All experiments were carried out in triplicate.

All FIA-amperometric measurements were performed with PbO_2-SPE in the presence of phosphate buffer (0.1 M, pH 7.5) as supporting electrolyte/carrier. The tested potentials were +0.3, +0.4 and +0.5 V, the flow rate was 0.4 mL min^{-1}, and the injected volume was 100.0 µL. For quantitative determinations the oxidation signals of H_2O_2 at all applied potentials were measured for the following concentrations: 5.0, 10.0, 20.0, 50.0 and 100.0 µg mL^{-1}. All experiments were carried out in triplicate.

Results and Discussion

Cyclic Voltammetry

Cyclic voltammetric studies (not shown) uncovered that with unmodified carbon paste the oxidation of hydrogen peroxide to oxygen sets in at potentials above +0.7 V. When using lead dioxide as a modifier it can be found that PbO_2 catalyzes the oxidation of hydrogen peroxide at potentials more positive than +0.3 V, similar to manganese dioxide (eqn 1).

$$H_2O_2 + PbO_2 \rightarrow PbO + H_2O + O_2 \qquad (1)$$

As a result an oxidation current can be observed at sufficiently positive potentials due to the electrochemical reconstitution of lead dioxide (eqn 2).

$$PbO + H_2O \rightarrow PbO_2 + 2\,H^+ + 2\,e^- \qquad (2)$$

The overall reaction of eqns (1) and (2) is the „catalyzed“ oxidation of hydrogen peroxide according to eqn (3) which now proceeds at significantly lower potentials than in the absence of the mediator.

$$H_2O_2 \rightarrow O_2 + 2\,H^+ + 2\,e^- \qquad (3)$$

The electrocatalytic effect was pronounced with lead dioxide, but could not be detected with cerium dioxide. As cyclic voltammetry is not very sensitive, cerium dioxide was also included in the ensuing amperometric studies to investigate in more details its ability to catalyze the oxidation of the analyte.

At negative potentials no catalytic effect could be detected neither with lead dioxide nor with cerium dioxide. In case of CeO_2 it is not surprising because the substance is a very strong oxidant itself. Basically it could be possible with PbO_2 that the reduced form of the oxide (PbO), present at negative potentials due to the reduction of the modifier, is oxidized by hydrogen peroxide and will induce an additional reduction current of Pb(IV). But such behavior could not be verified in any case. Strong onset of apreciable reduction currents started at -0.7 V and below due to direct reduction of hydrogen peroxide.

Hydrodynamic Amperometry

The behavior of CPE, PbO_2-CPE and CeO_2-CPE was compared with hydrodynamic amperometry in phosphate buffer (pH 7.5) by adding consecutively 100.0 μg mL^{-1} of H_2O_2 to the solution. The investigated potential range was between -0.5 V and +0.5 V.

In case of the unmodified CPE the obtained signals, especially in the positive range of the applied potentials (where the oxidation signal of the investigated analyte was expected), were on the level of the background current and its respective noise. No significant signals could be detected (Fig. 1), even with higher concentrations of H_2O_2 (350 μg mL^{-1}). A typical example is shown in Fig. 1 with an operating potential of +0.2 V; very similar curves were obtained at more positive potentials (investigated up to +0.5 V).

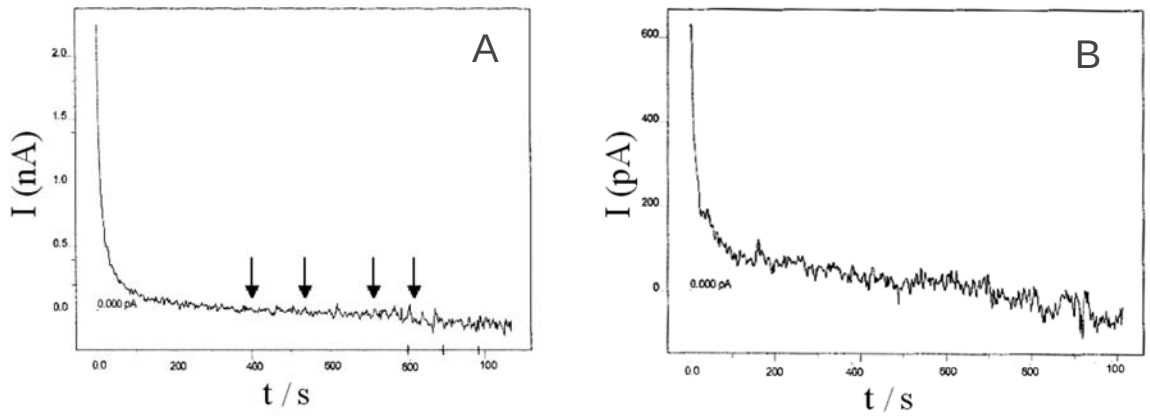

Figure 1. *Batch hydrodynamic amperograms obtained with an unmodified CPE at nano* (A) *and pico* (B) *amperometric current scales*; supporting electrolyte of 0.1 M phosphate buffer (pH 7.5), stirred solution; operating potential +0.2 V. The arrows show the addition of 100.0 μg mL^{-1} H_2O_2.

In case of the CeO_2-modified CPE very similar behavior could be observed (Fig. 2). No significant oxidation currents were obtained in the presence of H_2O_2 at an operating potential of +0.2 V. If the potential of the CeO_2-CPE was set at +0.3 V, a small oxidation current of about 2 nA was obtained for the same concentration of H_2O_2 (2B). Almost equal values were also obtained at the potentials of +0.4 and +0.5 V.

Lead dioxide as a modifier provokes at a working potential of +0.3 V significant oxidation signals already after addition of portions of 100.0 μg mL^{-1} of H_2O_2. Namely, the test measurements showed that the signal at +0.3 V is by about 20x more intensive than the background, and exhibits an increasing tendency up to a working potential of +0.5 V (Fig. 3).

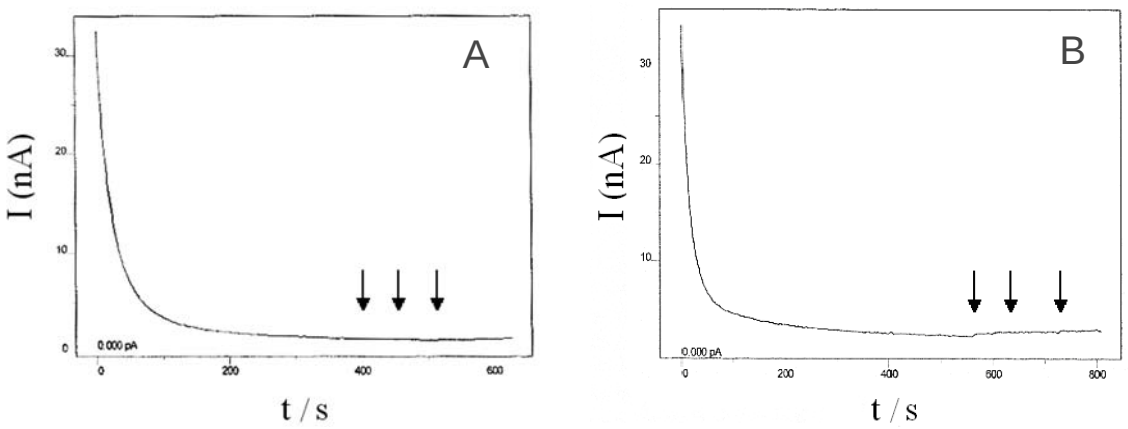

Figure 2. *Batch hydrodynamic amperograms of the CeO_2-CPE at the working potential of +0.2 V* (A) *and +0.3 V* (B); supporting electrolyte: 0.1 M phosphate buffer (pH 7.5); stirred sol. The arrows show the injection of 100.0 μg mL^{-1} H_2O_2.

The amperograms show that the working potential suitable for the detection of hydrogen peroxide may be set between +0.3 and +0.5 V. Higher potentials will probably give even higher signals but have not investigated in this work because such potentials are not attractive for practical applications due to the risk of oxidation of other substances and to the possibility of direct detection of the analyte without mediator. The hydrodynamic amperograms also show that passivation of the electrode occurs resulting in a decrease of the current after some time. Fortunately this effect was not observed with flow injection analysis, where, due to the transient character of the technique, the electrode quickly regenerates after contact with the analyte. Nevertheless, some preliminary calibration plots were established for the batch amperograms (Fig. 3) to estimate the effect.

A graphical comparison of the current responses of the CeO_2-CPE and CPE (Fig. 4A), shows that the modified electrode did not exhibit significant electrocatalytic activity. On the other hand, the compartive diagram presented in Figure 4B shows that the PbO_2-CPE gives a manifold increase in the current response. At the potential of +0.3 V the intensity of the reduction signal is increased by 8 times, at +0.4 V by 55 times, and at +0.5 V by 60 times compared to the CPE response.

Flow Injection Analysis

The batch amperometric studies showed that lead dioxide is a convenient modifier for the detection of hydrogen peroxide at positive potentials ≥ +0.3 V. Therefore, amperometric

studies were made using lead dioxide-modified electrodes in FIA which seems an appropriate tool to circumvent the quickly decreasing response curves in batch amperometry.

Screen printed electrodes behave very similarly as carbon paste electrodes from point of view of electrochemical properties, but they are mechanically more robust and easier to handle than the latter ones. SPEs seem also a good support for the attachment of membranes containing biological entities for eventual designs of biosensors. For these reasons PbO_2-modified screen printed carbon electrodes were used for all investigations with FIA.

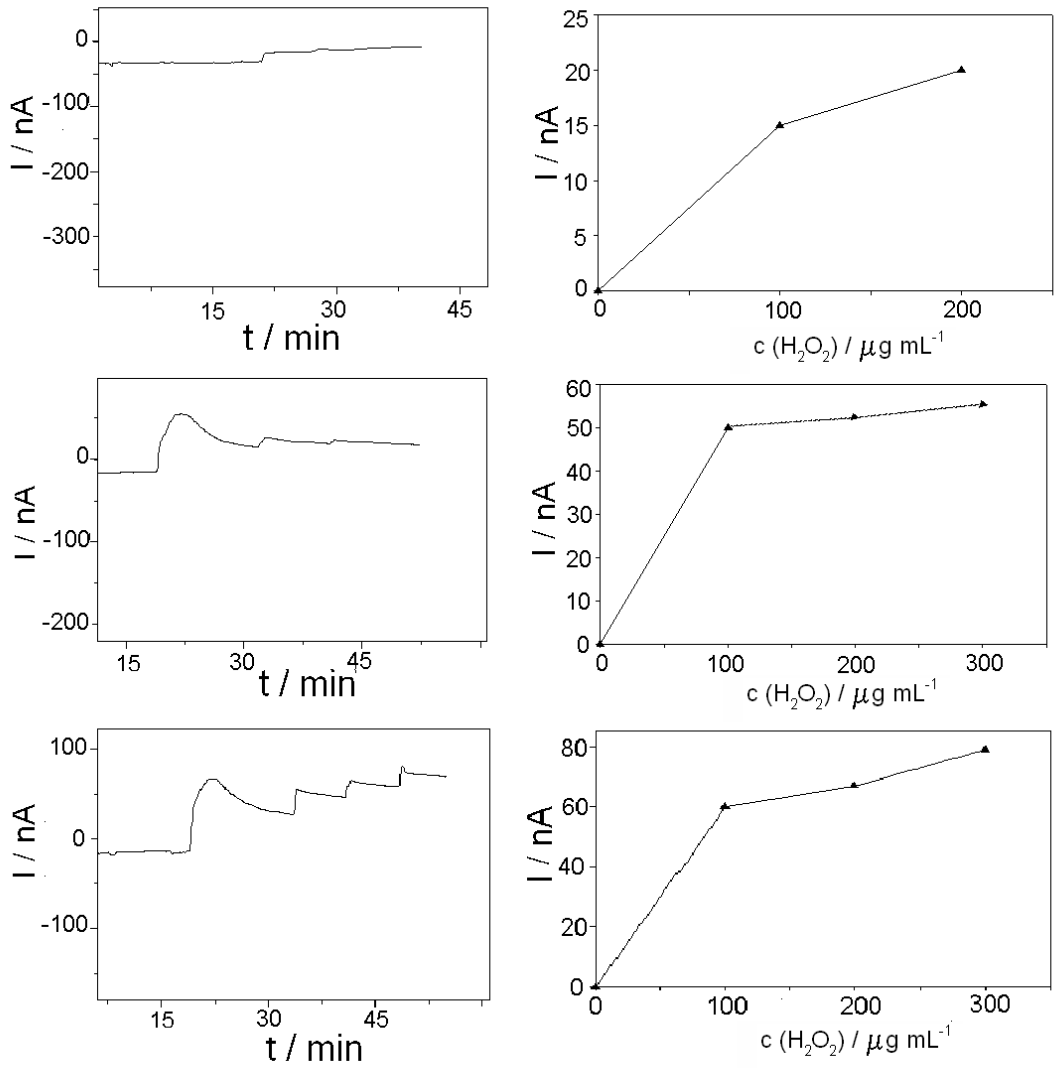

Figure 3. Typical hydrodynamic amperograms for additions of portions of 100 µg mL^{-1} H_2O_2 recorded using a PbO_2-CPE at different working potentials +0.3 V (A), +0.4 V (C) and +0.5 V (E), and the corresponding current responses as a function of hydrogen peroxide concentration in the range of 0.0-300.0 µg mL^{-1} (B,D,F); supporting electrolyte: 0.1 M phosphate buffer (pH 7.5); stirred solution.

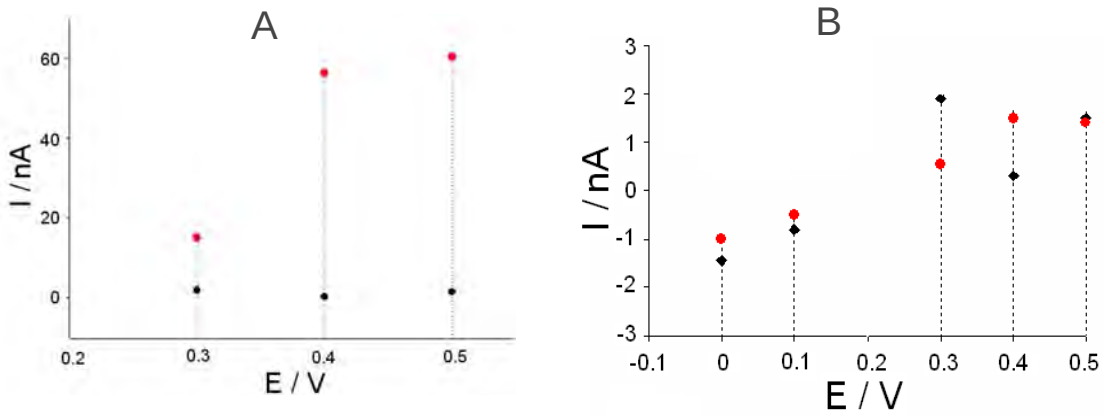

Figure 4. *Comparison of batch amperometric responses of an unmodified CPE with a CeO_2-CPE* (A) *and with a PbO_2-CPE* (B); supporting electrolyte: 0.1 M phosphate buffer (pH 7.5); stirred solution.

Fig. 5 shows the amperometric signals with PbO_2-SPEs polarized at +0.3, +0.4 and +0.5 V in the flow injection analysis for the different concentrations of hydrogen peroxide (5.0, 10.0, 20.0, 50.0 and 100.0 μg mL^{-1}). As can be seen, the signals obtained under all experimental conditions may be used for the determination of hydrogen peroxide. Like in the case of hydrodynamic amperometry, the most intensive signals are obtained at the working potential of +0.5 V.

For the above concentration range, the following equations were derived for different applied working potentials (eqns 4-6):

$$|I_p| = 2.64\,c + 1.876,\ r = 0.992;\quad E_p = +0.3\ V \qquad (4)$$

$$|I_p| = 11.99\,c - 37.88,\ r = 0.989;\quad E_p = +0.4\ V \qquad (5)$$

$$|I_p| = 17.14\,c - 52.875,\ r = 0.991;\quad E_p = +0.5\ V \qquad (6)$$

In the equations I_p is the peak current in nA and c is the concentration in μg g^{-1}.

On the basis of the slopes of the obtained calibration graphs it can be concluded that the response obtained at a potential of +0.5 V is approximately by 6.5 times more sensitive compared to that at +0.3 V. Nevertheless, the latter operation potential can also be used as shown in Fig. 6. At the working value of +0.5, V the limit of detection (3 σ) is 0.3 μg mL^{-1}, and the limit of quantification is 1.0 μg mL^{-1}, when the RSD does not exceed 2 %.

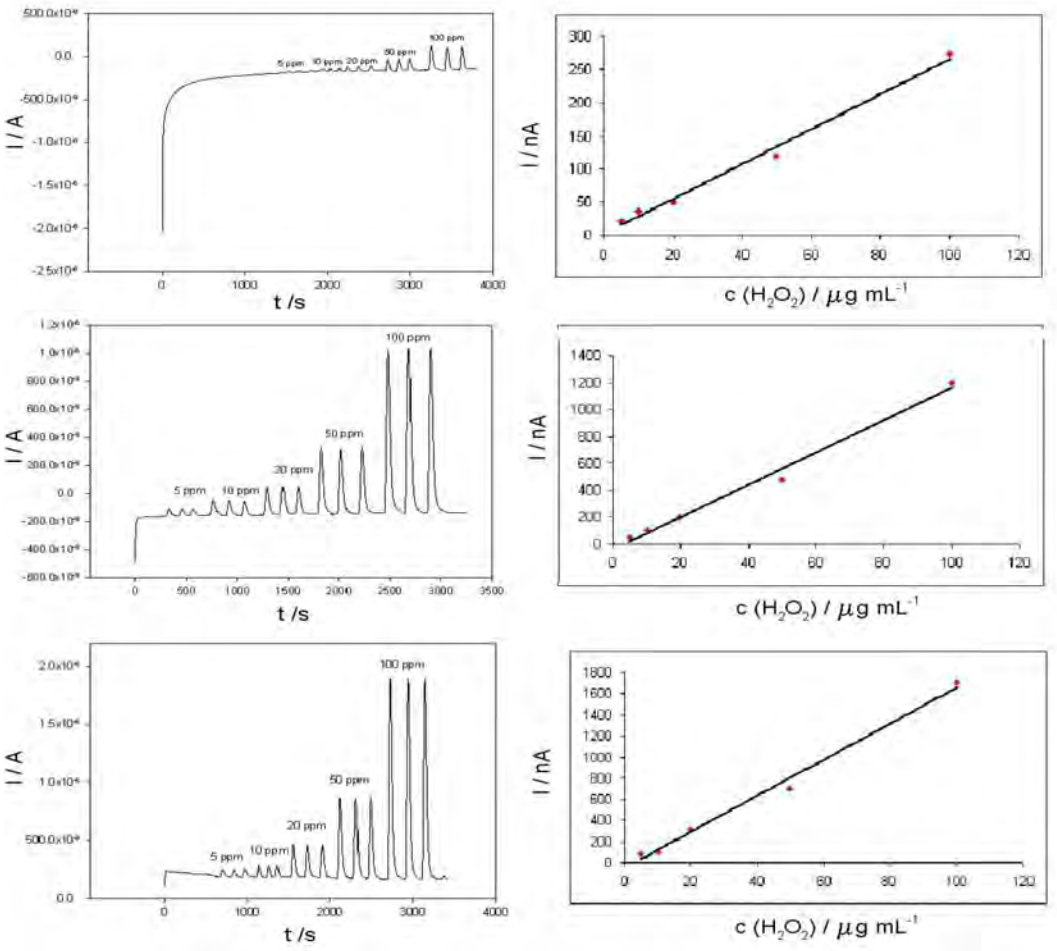

Figure 5. FIA amperograms of a PbO_2-modified SPE recorded at a working potential of +0.3 V (A), +0.4 V (C) and +0.5 V (E), and the corresponding calibration graphs (B,D, F); carrier: 0.1 M phosphate buffer (pH 7.5); flow rate: 0.4 mL min^{-1}, injection volume: 100 µL. *Note:* Images (A-F) from top to bottom and from left to right.

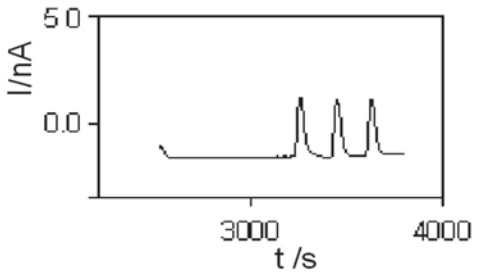

Figure 6. FIA-amperometric signals of the PbO_2-CPE at the working potential of +0.3 V. H_2O_2 concentration 100.0 µg mL^{-1}. Other parameters as in Fig.5.

Based on the data obtained, it can be concluded that PbO_2 is a potentially good mediator in the detection of the H_2O_2 evolved by the action of an oxidase (e.g., glucose oxidase) incorporated in the amperometric biosensor. Future investigations will be focused on the development of biosensors, especially those for glucose, and employing CPE and SPE modified with lead dioxide, PbO_2 .

Conclusions

This study was concerned with potential electrocatalytic properties of lead dioxide and cerium dioxide incorporated as mediators in CPEs for the amperometric determination of hydrogen peroxide. It was found that the nature of the CPE determines to a great extent its electrochemical characteristics and analytical application and that lead dioxide is a promising mediator for amperometric analysis.

The choice of the most suitable electrode for the determination of hydrogen peroxide was carried out on the basis of measuring the intensity, shape, and reproducibity of the corresponding hydrodynamic amperometric signals obtained using the CPE modified with lead dioxide and cerium dioxide. It was found that the intensity of the oxidation signal obtained with the PbO_2-CPE at the working potential of +0.3 V is higher by 8, at +0.4 V by 55, and +0.5 V by 60 times compared to that of the unmodified CPE. On the basis of the obtained results it can be concluded that cerium dioxide did not exhibit significant electrocatalytic activity.

The FIA results indicate that SPEs modified with lead dioxide are applicable at working potentials at and above +0.3 V, reasonably up to +0.5 V, with increasing sensitivity at higher potentials. The linear range of measurement is between 5.0 and 100.0 μg mL^{-1} at all the tested values of working potential.

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