

SCREEN-PRINTED CARBON ELECTRODES MODIFIED WITH PRUSSIAN BLUE AND A NON-CONDUCTING ELECTROPOLYMERIZED FILM FOR SELECTIVE DETERMINATION OF H₂O₂ IN BEVERAGES

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Abstract

The development of a highly selective and sensitive sensor for H₂O₂ in beverages such as natural juices, is described in this work. The sensor is based on the deposition of Prussian Blue (PB) onto screen-printed carbon electrodes (SPCEs) followed by the electropolymerization of a non-conducting film. Several procedures for PB deposition on the SPE electrodes were tested: electrochemical deposition (potentiostatic, cyclic voltammetry) and chemical deposition. The electrochemical and analytical properties of the SPCE/PB sensors had been evaluated and the potentiostatic method for PB deposition was selected for the further development of the H₂O₂ sensor. In order to develop a robust sensor for H₂O₂ determination in samples with complex matrix, we covered the PB layer with an electropolymerized non-conducting film with a high permselectivity for H₂O₂. This film is a copolymer based on 2,6-DHN (2,6-dihydroxynaphthalene) and APEA (2-(4-aminophenyl)-ethylamine). The SPCE/PB/copolymer sensor demonstrated improved stability in operational conditions and excellent interference rejection properties. This sensor may be successfully applied on-field, using a portable potentiostat-galvanostat and the chronoamperometry technique, as well as in a laboratory bench flow injection analysis system with amperometric detection. The developed sensor was able to measure H₂O₂ in the linear range 1 μM – 500 μM (R=0.9989), with a detection limit of 0.5 μM. The SPCE/PB/copolymer sensor maintained for a long period its response for H₂O₂ (94% response was retained after 60 days).

Keywords: screen printed carbon electrode, Prussian Blue, nonconductive copolymer, H₂O₂, beverages

INTRODUCTION

The direct amperometric detection of hydrogen peroxide at conventional electrodes is possible only at 0.6 V vs. Ag/AgCl. At this potential, the presence of easily oxidizable compounds present in real samples (ascorbic acid, bilirubin, uric acid, etc.) can easily interfere in the measurement, being oxidized at the electrode together with hydrogen peroxide. For this reason the detection of H₂O₂ at potentials around 0 using electrodes modified with electrochemical modifiers, such as Prussian Blue (PB), has enormous advantages and applications in many fields [1-3].

The first sensors for hydrogen peroxide based on PB modified glassy carbon electrode were reported by Karyakin et al [4]. The PB deposited on these sensors has an electrocatalytic effect on the reduction of H₂O₂, allowing its detection at potentials close to 0 V, thus making possible the coupling with oxidase enzymes while avoiding or reducing the electrochemical interference.

Screen-printed electrodes are frequently used in analytical applications because of their unique properties such as small size, low detection limit, fast response, high reproducibility, etc. [5].

Screen-printed carbon electrodes (SPCEs) are devices that are produced by printing different inks on various types of plastic or ceramic

substrates. The composition of the inks used for printing on the electrodes determines the selectivity and sensitivity required for each sensor development. Screen-printed electrodes are inexpensive, simple to prepare, versatile and suitable for the mass-production of disposable electrodes [6].

The aim of this work was to develop a simple, robust and portable sensor based on PB and a non-conducting copolymer which are deposited on a SPCE for hydrogen peroxide determination in beverages (commercial juices).

MATERIALS AND METHODS

Apparatus

Electrochemical measurements were carried out using a μ Autolab type III potentiostat/galvanostat computer controlled by the GPES software, as well as a portable PalmSens potentiostat/ galvanostat controlled via the PalmSensPC software.

The flow injection analysis system consisted from a four-channel Minipuls 3 Gilson peristaltic pump fitted with tygon tubing (1.52 mm id) used for the propulsion of fluids, an injection valve (Rheodyne, 7725i model) and a flow cell special for SPCE from Dropsens, Spain. The valve loop volume was 100 μ L. Fittings and connectors were used to connect the different components of the manifold. The optimum flow rate was 0.36 mL/minute. The detector was the same potentiostat/galvanostat used for voltammetric measurements.

Electrodes

Screen-printed carbon electrodes (SPCEs) model DRP-110 purchased from DropSens (Spain) were used for electrochemical measurements. In this case the electrochemical cell is composed by a graphite working electrode ($d = 4$ mm), a graphite auxiliary electrode and a silver pseudoreference electrode, with silver electric contacts deposited on a ceramic substrate.

Reagents

All chemicals from commercial sources were of analytical grade. Iron chloride (FeCl_3), potassium ferricyanide $\text{K}_3[\text{Fe}(\text{CN})_6]$, HCl 37%, sodium chloride, hydrogen peroxide (30%), were purchased from Sigma-Aldrich. AOT (Dioctyl sulfo-succinate sodium salt) was from Carlo Erba. The monomers 2,6-dihydroxynaphthalene (2,6-DHN) and 4-(2-

aminoethyl)aniline (APEA) were from Aldrich and respectively Fluka. Double-distilled water was used throughout.

2,6-DHN and APEA were dissolved in 0.1M phosphate buffer pH 7.4. Hydrogen peroxide (Fluka) was prepared daily in phosphate buffer, pH 6.5.

Modification of SPCE with Prussian Blue

Three procedures were investigated for PB film formation on the working electrode of screen printed carbon electrodes in order to prepare sensitive and robust PB sensors for H_2O_2 determination. The tested procedure were based on: chemical, galvanostatic and cyclic voltammetry based deposition.

Prior to Prussian Blue modification, the SPCEs were pretreated in the presence of 50 mM phosphate buffer in 0.1 M KCl, pH 7.4, by applying the potential of + 1.7 V versus Ag/AgCl for 3 minute. For the *chemical deposition* of PB films, two solutions were prepared. Solution 1: 100 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$ in 10 mM HCl. Solution 2: 100 mM FeCl_3 in 10 mM HCl. Prussian Blue modification of SPCE was then accomplished by placing 5 μ l of precursor solution 1 and 5 μ l of precursor solution 2 onto the working electrode area [7]. The solution was left onto the electrode for 10 min and then rinsed with a few millilitres of 10 mM HCl. The electrodes were then left 90 min in the oven at 100° C to obtain a more stable and active layer of PB.

For electrochemical deposition of PB the galvanostatic and cyclic voltammetry techniques were tested. The *galvanostatic deposition* was made in a mixture (solution 3) of 2.5 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$, 2.5 mM FeCl_3 and 1 mM AOT prepared in 100 mM KCl and 100 mM HCl solution by applying the potential of 0.4V for 40 sec [1]. After a gentle rinsing with water, the sensor was placed in a solution of 100 mM KCl in 100 mM HCl and a number of 20 cycles, between - 0.2 and 0.4 V, at a scan rate of 50 mV/s, was run. The *cyclic voltammetrydeposition* was carried out in the solution 3 (20 cycles, between - 0.2 and +0.4 V, at a scan rate of 50 mV/s) [14].

The deposition of the PB film was confirmed by performing cyclic voltammetry in 50 mM phosphate buffer, pH 7.4.

The PB modified electrodes were stored dry at room temperature in the darkness.

Electrodeposition of non-conducting films on SPCE/PB

The SPCE/PB sensors were covered with a non-conducting copolymer electrodeposited using the cyclic voltammetry technique. The copolymer was synthesized from a solution of 0.9 mM 2,6-DHN and 10 mM APEA by cycling for 10 – 20 times the potential from + 0.2 V to +1.1 V with a scan rate of 5 – 10 mV/sec.

RESULTS AND DISCUSSIONS

SPCE / PB sensors characterization

After the preparation, the SPCEs modified with PB (SPCE / PB) where characterized by cyclic voltammetry, in 0.1 M KCl prepared 0.1M HCl, in order to prove the PB film formation.

Figure 1 shows the specific voltammograms of the SPCE / PB sensors prepared according to the procedures described in ‘Materials and methods’ section. Two characteristic peak couples corresponding to conversion of high and low spin ions appear with formal potentials of 0.10 V and 0.80 V. The oxidation and reduction peaks centred at 0.10 V are much narrower and well shaped for the PB sensors obtained via electrochemical deposition compared with those obtained via chemical deposition. However, the SPCE / PB obtained by cyclic voltammetry showed the lowest oxidation and reduction peaks, and also some other experiments led to the conclusion that the PB film was too thin and fragile. For this reason, the most important further studies were done on the SPCE / PB sensors obtained by chemical and galvanostatic deposition.

The influence of the thermal stabilization by keeping the electrodes at 100° for 90 min was also studied. No evident differences between the treated and nontreated PB electrodes were observed regarding the response of the electrodes in KCl, phosphate buffer or for H₂O₂, but the operational stability was greater improved for the electrodes stabilized via the thermal treatment.

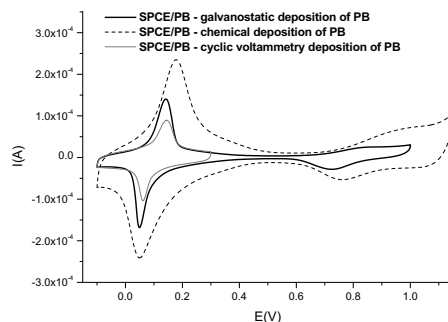


Figure 1. Influence of the deposition method of PB on the cyclic voltammograms recorded for the SPCE / PB sensors (Electrolyte 0.1 M KCl in 0.1M HCl; 50 mV/s)

The effect of potential scan rate on the oxidation (I_{ox}) and reduction peak (I_{red}) currents was studied for the redox couple present around 0.10 V. Plotting the I_{ox} and I_{red} vs. square root of scan rate showed a linear relationship (figure 2), the result indicating a diffusion limited process. This behaviour was observed for all the three tested methods for PB deposition.

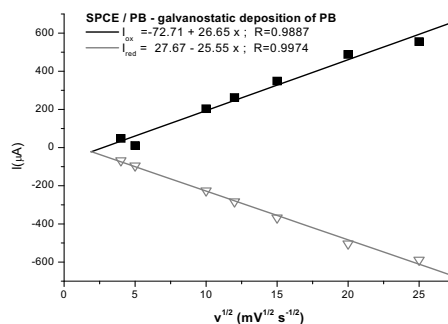


Figure 2. Variation of I_{ox} and I_{red} vs. square root of scan rate in electrolyte solution (0.1M KCl, 0.1M HCl)

The pH value of the electrolyte solution is an important parameter for H₂O₂ determination using the PB modified SPCEs. The stability and sensitivity of the PB sensor may be affected by the hydroxide ions which can break the Fe-(CN)-Fe bonds, but also by the protons which may block the electrochemical reactivity of PB [8].

The pH influence on the electrochemical determination of 1 mM H₂O₂ using the SPCE / PB was studied at pH ranging from 6-7.4 (figure 3). For all the tested electrodes the highest reduction peaks were obtained for the

pH 6.5. For all the following studies, as optimum electrolyte solution, was used the 50 mM phosphate buffer, pH 6.5.

The selection of the optimum working potential to be applied when measuring H_2O_2 was studied for the next electrochemical techniques: chronoamperometry, amperometry in stirred solution and amperometry in flow injection system (FIA). Potentials ranging from -100 mV to +200 mV were applied in presence of a selected concentration of hydrogen peroxide (100 μM). The highest signal was obtained for the potentials of -50 mV when working in chronoamperometry and of -100 mV in amperometry (for both, stirred solutions and FIA). The lowest background noise and the highest signal for H_2O_2 measurement was recorded for the PB sensor prepared via the galvanostatic procedure.

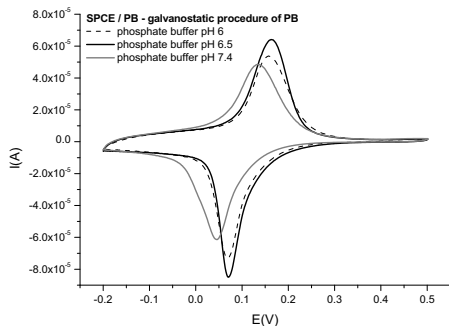


Figure 3. Influence of electrolyte solution pH on the response of SPCE / PB to 1mM H_2O_2

Regarding the hydrogen peroxide determination, the amperometry under stirring technique gave the best results for the SPCE / PB sensor prepared via galvanostatic method in rapport with the background noise and with the response time. Even that the sensitivity recorded with sensor prepared by chemical deposition of PB is higher than that obtained for that obtained via electrochemical procedures, the linear range of concentration for PI shorter (data not shown).

Cronoamperometry technique is very advantageous due to the fact that the screen printed electrodes allow working with a very low volume of sample (100 μL), the determination is fast and reproducible, and does not require a time for working electrode polarization. Also this electrochemical

technique may be applied for on-field measurements using a portable detector.

According to the measurement results, the linear range was from 1 μM to 100 μM H_2O_2 with the linear correlation of 0.9967 for the sensor obtained by chemical deposition of PB and, respectively, of 0.9985 for the sensor prepared by galvanostatic deposition.

The sensitivity of the galvanostatic prepared sensors was with 50 % higher than obtained via the chemical deposition (figure 4). For both type of sensors the detection limit was 0.5 μM H_2O_2 .

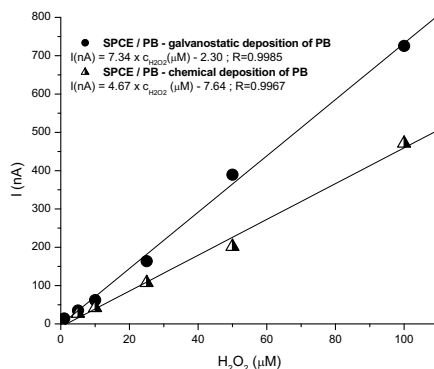


Figure 4. Calibration plots for detection of H_2O_2 , using chronoamperometry technique ($E = -50$ mV/s)

Characterization of SPCE / PB / copolymer sensors

The PB exhibits excellent catalytic activity for the electroreduction of hydrogen peroxide, but the operational stability of PB is still a matter of concern in real samples with complex matrix, such as the food samples. In order to protect the PB layer, the coverage with non-conducting films as the poly(*o*-aminophenol) [1] or with ionomers as Nafion [9] was reported.

In this work we report the use, for the first time, of the non-conducting copolymer electrosynthesized from a mixture of 2,6-DHN and APEA for protection of the PB layer. The copolymerization was performed via the cyclic voltammetry technique by cycling the potential from + 0.2 V to +1.1 V. In figure 5 one can observe that the irreversible oxidation peak at the +0.65 V present in the first three cycles disappears in the following cycles. The oxidation peak current decrease proves the

formation of a non-conducting film on the surface of the SPCE / PB sensor.

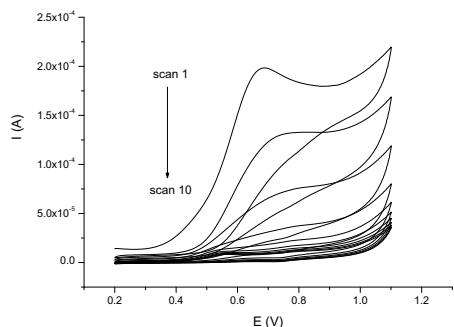


Figure 5. Cyclic voltammograms recorded during the formation of the copolymer poly(DHN – APEA) on the SPCE / PB prepared via the galvanostatic method (10 mM APEA; 0.9 mM 2,6-DHN; 10 mV/s; 10 cycles)

The optimization of the copolymer formation related to the hydrogen peroxide determination was performed studying the influence of the scan rate and number of cycles.

In figure 6, one can observe the influence of the number of cycles used for the copolymer electrodeposition on the calibration graphs obtained by chronoamperometry. A higher number of cycles led to a higher thickness of the copolymer layer and consequently to a more difficult diffusion of H_2O_2 across it.

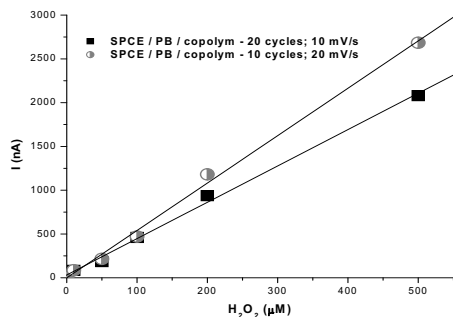


Figure 6. Influence of the number of cycles used for copolymer electrodeposition on chronoamperometric calibration graphs ($E = -0.1$ V)

In Figure 7 the influence of the scan rate used during the copolymer electrodeposition process on the H_2O_2 determination in chronoamperometry is presented. The scan rate has a major influence on the film porosity. A low scan rate, as 5 mV/s, lead to a copolymer film less

porous, which acts as a barrier especially for higher concentration of H_2O_2 .

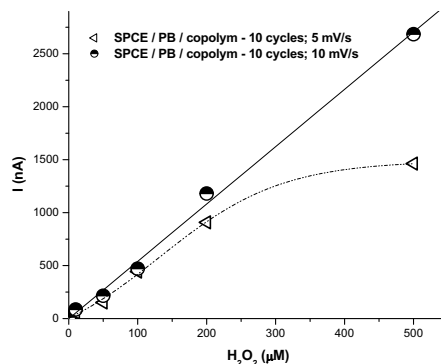


Figure 7. Influence of the scan rate used for copolymer electrodeposition on chronoamperometric calibration graphs ($E = -0.1$ V)

By comparing figures 4 and 6, it can be concluded that an important feature achieved by the copolymer electrodeposition on the PB layer was the extending of the linear concentration range up to 500 μ M in chronoamperometry.

The optimum conditions for copolymer electrodeposition were: scan rate = 10 mV/s; number of cycles = 10; potential range = 0.2 V - 1.1 V. Another important characteristic of the SPCE / PB / copolymer sensors is represented by a higher operational stability even in flow injection analysis conditions. In figures 8 is presented the FIA amperogram recorded for the PB sensor covered with the poly(DHN – APEA) copolymer.

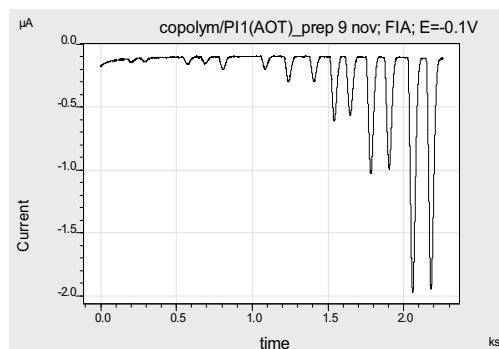


Figure 8. FIA amperogram recorded for the SPCE/PB/copolymer sensor ($v_{inj}=100$ μ L; flow rate=0.36mL/min; H_2O_2 concentration injected in duplicate = 2; 5; 10; 20; 50; 100; 200 μ M)

Also, in FIA conditions the sensitivity for H₂O₂ detection was much higher comparing with those obtained in chronoamperometric conditions.

The possible interference of compounds present in beverages, such as ascorbic acid and glucose, was tested. Glucose showed no interference in H₂O₂ determination with SPCE/PB/copolymer sensor. Ascorbic acid may interfere, giving a false negative signal, only if the ascorbic acid concentration is much higher than of H₂O₂.

The SPCE/PB/copolymer sensor maintained for a long period its response for H₂O₂ (94% response was retained after 60 days)

Real sample analysis

Sometimes, for the aseptic packaging of natural fruit juices, hydrogen peroxide is used as a chemical agent for sterilization. However, the hydrogen peroxide residues in higher concentration are irritative for the skin and may affect the human health.

The developed SPCE/PB/copolymer sensor was applied for the fast and simple determination of H₂O₂ in several commercial fruit juices. The sample treatment consisted only in dilution with 50 mM phosphate buffer, pH=6.5.

In table 1 is presented the H₂O₂ concentration determined in the tested samples with the SPCE/PB/copolymer sensor by chronoamperometry.

Table 1. H₂O₂ concentration in several commercial fruit juices

sample	H ₂ O ₂ (μM)
Orange juice (Granini)	3.12
Peach juice (Prigat)	n.d.*
Apple Juice (Granini)	n.d.
Orange pulpy juice (Cappy)	n.d.
Orange juice (Fanta)	2.48

*n.d. = not detectable

The results demonstrated that the level of hydrogen peroxide concentration used in tested juices preservation is very low.

CONCLUSIONS

In this work, it was developed a robust and cost-effective sensor based on SPCE modified with PB and an electropolymerized non-conducting film for H₂O₂ determination. The experimental results showed that the copolymer film remarkable improves the operational

stability of the PB sensor. The SPCE/PB/ copolymer sensor has an excellent electrocatalytic activity for the reduction of H₂O₂, with a broad linear range from 1 μM to 500 μM, and a detection limit of 0.5 μM. Furthermore, introduced into a FIA system, the sensor proved a great operational stability. The developed sensor was successfully applied to the determination of H₂O₂ in commercial juices.

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REFERENCES

1. Ping, J., Wu, J., Fan, K., Ying, Y., 2011. An amperometric sensor based on Prussian blue and poly(o-phenylenediamine) modified glassy carbon electrode for the determination of hydrogen peroxide in beverages. *Food Chemistry* 126, p. 2005–2009
2. de Mattos, I. L., da Cunha Areias, M. C., 2010. Automated determination of glucose in soluble coffee using Prussian blue–glucose oxidase–Nafion modified electrode. *Talanta* 66, p. 1281–1286.
3. Haghighi, B., Hamidi, H., Gorton, L., 2010. Electrochemical behavior and application of Prussian blue nanoparticle modified graphite electrode. *Sensors and Actuators B* 147, p. 270–276
4. Karyakin, A.A., Gitelmacher, O., Karyakina, E.E., 1994. A high-sensitive glucose amperometric biosensor based on Prussian Blue modified electrodes. *Anal. Lett.* 27 (15), p. 2861–2869.
5. Dominguez Renedo, O., Alonso-Lomillo, M.A., Arcos Martiinez, M.J., 2007. Recent developments in the field of screen-printed electrodes and their related applications. *Talanta* 73, p. 202–219
6. Pchelintsev, N.A., Millner, P.A., 2007. Development of surface activated screen-printed carbon transducers for biosensors application. *Anal. Let.* 40 (7), p. 1317 – 1332.
7. Ricci, F., Palleschi, G., 2007. Sensor and biosensor preparation, optimisation and applications of Prussian Blue modified electrodes. *Biosens. Bioelectron.* 21, p. 389–407.
8. de Mattos, I.L., Gorton, L., Ruzgas, T., Karyakin, A.A., 2000. Sensor for hydrogen peroxide based on Prussian Blue modified electrode: improvement of the operational stability. *Anal. Sci.* 16, p. 795–798.
9. Ping, J., Mao, X., Fan, K., Li, D., Ru, S., Wu, J., Ying, Y., 2010. A Prussian blue-based amperometric sensor for the determination of hydrogen peroxide residues in milk. *Ionics* 16, p. 523–529.