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PRUSSIAN BLUE BASED SCREEN PRINTED BIOSENSORS WITH IMPROVED CHARACTERISTICS OF LONG TERM AND pH STABILITY

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Abstract *In this article is reported the effect of anionic surfactants AOT (dioctyl sulfosuccinate) in the electrodeposition of Prussian Blue (PB) onto screen-printed carbon electrodes (SPCE). The SPCE/PB/AOT electrode displayed a significant improvement of its electrochemical properties and of its stability compared with PB modified SPCE formed in absence of surfactant. The new electrodeposed PB/AOT film proved excellent electroanalytic properties for glucose determination and is a promising material for assembling of biosensors. In addition, the effects of pH value, applied potential, electroactive interference and the stability of the biosensor were discussed. The applicability to food analysis was also evaluted.*

***Key words**: Prussian Blue, surfactant anionic, modified electrode.*

1. 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 H_2O_2 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].
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].

This work aims to evaluate the possibility of using Prussian Blue modified screen printed electrodes substrates for the development of biosensors assembled with glucose oxidase, for the determination of glucose, in food analysis.

2. Materials and Methods

Apparatus

Electrochemical measurements were carried out using a Autolab 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 100µL. Fittings and connectors were used to connect the different components of the manifold.

Electrodes

Screen-printed carbon electrodes (SPCEs) model DRP-110 purchased from DropSens (Spain) were used for electrochemical measurements.

Reagents

All chemicals from commercial sources were of analytical grade. Glucose oxidase from Aspergillus niger, 232 U mg, 28 mg/mL, and D-(+)- glucose (97%) were obtained from Sigma.7,7 mL of gluocose oxidase were dissolved in 50 mM potassium phosphate 100 mM sodium acetate, 250 mM KCl, pH = 5,5. Stock solutions of glucose were prepared in water distilited and stored at 4° when not use. The stock solution of glucose was allowed to mutarotate at room temperature overnight before use. Iron chloride ($FeCl_3$), potassium ferricyanide $K_3[Fe(CN)_6]$, HCl 37%, sodium chloride, hydrogen peroxide (30%), were purchased from Sigma-Aldrich. AOT (Dioctyl sulfo-succinate sodium salt) was from Carlo Erba. The 2,6-dihydroxynapthalene and 4-(2-aminoethyl) aniline were from Aldrich. Double-distilled water was used throughout 2,6-dihydroxynapthalene and 4-(2-aminoethyl)aniline were dissolved in 0.1M phosphate buffer pH 7.4.

2.1 Preparation of the modified screen-printed electrodes with Prussian Blue

Chemical deposition of Prussian Blue

Prior to Prussian Blue modification, screen-printed electrodes (SPE-C) 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 [7]. For the *chemical deposition* of PB films (*Procedure I*), two solutions were prepared. Solution 1: 100 mM $K_3[Fe(CN)_6$ 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 µl of precursor solution 1 and 5 µl of precursor solution 2 onto the working electrode area. The drop was carefully placed exclusively on the working electrode area, in order to avoid the formation of PB on the reference and counter electrodes which may increase the internal resistance of the system. The solution was left onto the electrode for 10 min and then rinsed with a few milliliters 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 [7]. The PB modified electrodes were stored dry at room temperature in the darkness. An activation was performed before of the first use by applying a potential of 0.0 V during 3 min in

50 mM phosphate buffer in 0.1 M KCl , pH 7.4.

2.2. Electrochemical deposition of Prussian Blue

Prior to Prussian Blue modification, screen-printed electrodes were pretreated as in previous section. The galvanostatic and cyclic voltammetry techniques were tested for the electrochemical deposition of PB.The *galvanostatic deposition* (*Procedure II*) was made in a mixture (solution 3) of 2.5 mM $K_3[Fe(CN)_6,]$, 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 [8]. 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 films were stabilized by keeping the electrodes at 100° for 90 min. The presence of the PB film was confirmed by performing cyclic voltammetry in 50 mM phosphate buffer, pH 7.4. The *cyclic voltammetry deposition* (*Procedure III*) was carried out in the solution 3 (20 cycles, between - 0.2 and +0.4 V, at a scan rate of 50 mV/s) [9].

3. Results and Discussions

Electrodeposition of non-conducting films on SCPE/PB

The SPCE/PB biosensors 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.

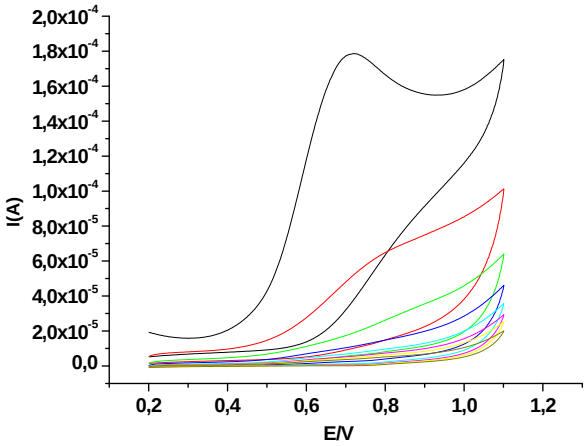

Figure 1. Influence of the deposition method of PB on the cyclic voltammograms recorded for the SPCE / PB poly(DHN – APEA).

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 glucose, but the operational stability was greater improved for the electrodes stabilized via the thermal treatment.

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.

Recently, we reported a new procedure for PB modification on graphite particles based on in situ chemical synthesis of PB, obtaining PB-modified electrodes with high stability even at basic pH values [10,11].

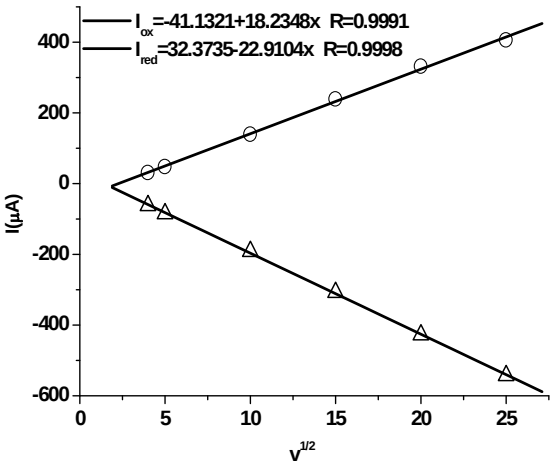

Figure 2. Variation of I_{ox} and I_{red} vs. square root of scan rate in electrolyte solution (0.1M KCl, 0.1M HCl)

The pH value of the electrolyte solution is an important parameter for H_2O_2 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].

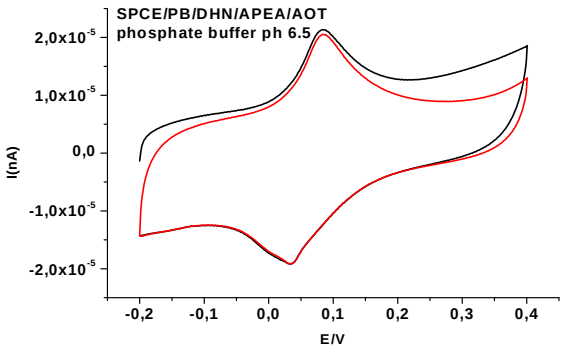

Figure 3. Influence of electrolyte solution pH on the response of PB modified SPCE to phosphate buffer pH 6.5

The pH influence on the electrochemical determination of glucose using the SPCE / PB was studied at pH ranging 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 (100µM). 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.

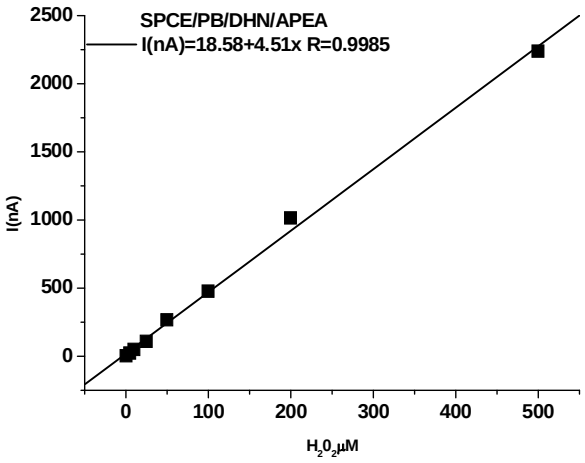

Figure 4. Influence of electrolyte solution pH on the response of SPCE / PB to 500 µM H_2O_2

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. 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.

According to the measurement results, the linear range was from 1 µM to 500 µM H_2O_2 with the linear correlation of 0.9967 for the sensor obtained by chemical deposition [12] 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 5). For both type of biosensors the detection limit was 2.5 mM - 4mM concentration glucose.

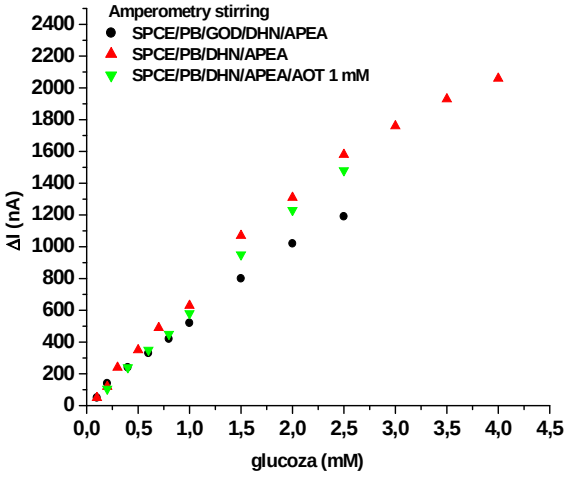

Figure 5. Calibration plots for amperometric determination of (E = -50 mV/s)

Characterization of SPCE/ PB/ DHN/APEA/AOT 1mM biosensors

The PB exhibits excellent catalytic activity for the electroreduction of glucose, 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) [11] or with ionomers as Nafion [9-10] was reported.

In this work we report the use, for the first time, of the non-conducting monomers electrosynthesized from a mixture of 2,6-DHN and APEA for protection of the PB layer. The electropolymerization was performed via the cyclic voltammetry technique by cycling the potential from + 0.2 V to +1.1 V. In figure 6 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 biosensor.

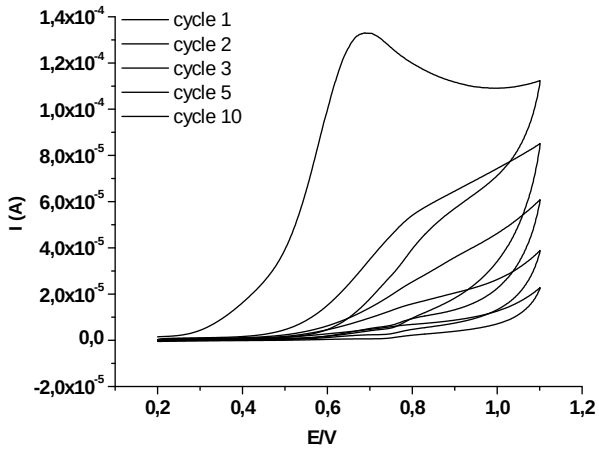

Figure 6. Cyclic voltammograms recorded during the formation of the monomer DHN – APEA on the SPCE / PB prepared via the galvanostatic method 10 mM APEA; 0.9 mM 2,6-DHN; 10 mV/s; 10 cycles

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 7, one can observe the influence of the number of cycles used for the glucose oxidase electrodeposition on the calibration graphs obtained by chronoamperometry.

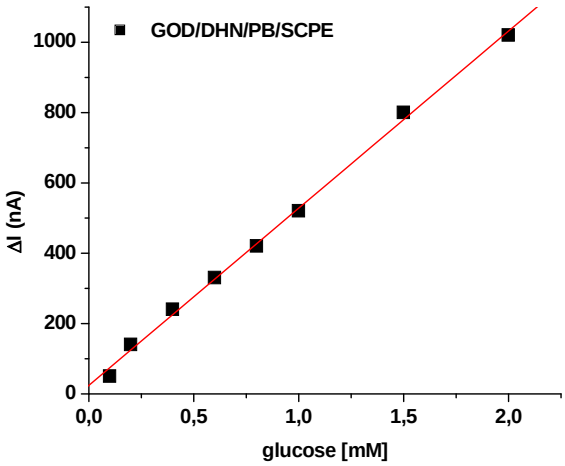

Figure 7. Influence of the number of cycles used for electrodeposition chemical on chronoamperometric calibration graphs (E = - 0.1 V)

In Figure 7 the influence of the scan rate used during the polimer electrodeposition process on the glucose 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 polimer film less porous, which acts as a barrier especially for higher concentration of glucose 4 mM.

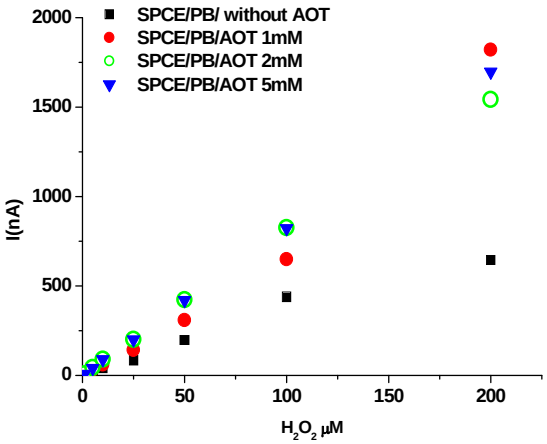

Figure 8. Influence of the scan rate used for polimer electrodeposition on chronoamperometric calibration graphs (E = -0.1 V)

The influence of several AOT concentration (1 mM, 2 mM and 5 mM) on the analytical performances of the PB sensor for H_2O_2 sensing were evaluated. The stability of the SPCE/PB/AOT 1 mM sensor under storage condition was assessed for a period of 90 days using a solution of 50 μM H_2O_2 by making a determination in triplicate at least twice on week.

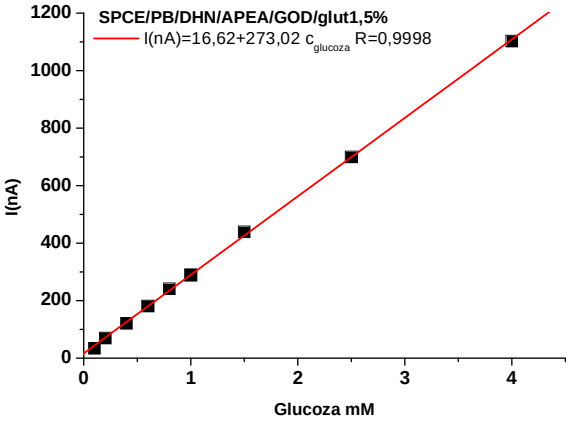

Figure 9. Influence of the scan rate used for monomer SPCE/PB/DHN/APEA/GOD/AOT 1 mM electrodeposition on chronoamperometric calibration graphs , 50mV/s, E = - 0.1 V

The optimum conditions for polimer 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 / monomers biosensors is represented by a higher operational stability even in flow injection analysis conditions. In figures 10 is presented the FIA amperogram recorded for the PB biosensor covered with the poly(DHN – APEA) monomers.

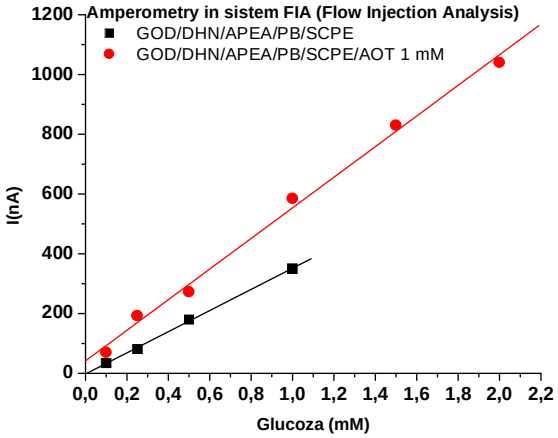

Figure 10. FIA amperogram recorded for the SPCE/PB/DHN/APEA/AOT 1mM biosensor (v_{inj}=100 µL; flow rate=0.36mL/min; glucose concentration injected in duplicate = 2; 5; 10; 20; 50; 100; 200 µM)

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

The possible interference of compounds present in beverages, such as ascorbic acid and glucose, was tested. Ascorbic acid may interfere, giving a false negative signal, only if the ascorbic acid concentration is much higher than of H_2O_2.

The SPCE/PB/DHN/APEA/GOD/AOT 1 mM biosensor maintained for a long period its response for glucose (94% response was retained after 60 days).

Real sample analysis

Sometimes, for the aseptic packaging of natural fruit juices, gluose is used as a chemical agent for sterilization. However, the glucose residues in higher concentration are irritative for the skin and may affect the human health. The developed SPCE/PB/DHN/APEA/AOT/GOD biosensor was applied for the fast and simple determination of glucose in several commercial fruit juices. The sample treatment consisted only in dilution with 50 mM phospahate buffer, pH=6.5. In figure 11. is presented the glucose concentration determined in the tested samples with the SPCE / PB / DHN / APEA / GOD / AOT biosensors by chronoamperometry.

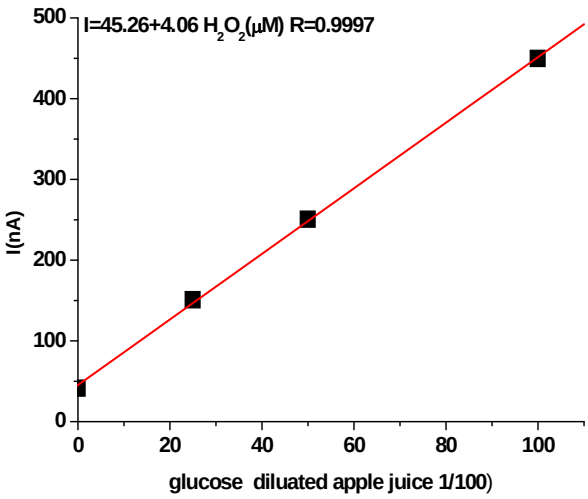

Figure 11. The glucose concentration determined in the tested samples

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

4. Conclusions

In this work, it was developed a robust and cost-effective biosensor based on SPCE modified with PB and an electropolymerized non-conducting film for glucose determination. The experimental results showed that the polimer film remarkable improves the operational stability of the PB biosensor. The SPCE/PB/ polimer biosensor has an excellent electrocatalytic activity for the reduction of glucose. Furthermore, introduced into a FIA system, the sensor proved a great operational stability. The developed biosensor was successfully applied to the determination of glucose in commercial juices.

5. Acknowledgements

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6. References

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