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CHARACTERIZATION OF SURFACTANT-MODIFIED PRUSSIAN BLUE SCREEN-PRINTED CARBON ELECTRODES FOR H_2O_2 DETECTION

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Abstract: *An improved H_2O_2 sensor based on the electrocatalytic reduction of H_2O_2 on Prussian Blue modified screen-printed electrode was developed. A comparative study regarding different procedures for modifying the carbon screen-printed electrodes (SPE) with Prussian Blue (PB) was carried out in this work. Several procedures for PB deposition on the SPE electrodes were tested: electrochemical deposition (galvanostatic, cyclic voltammetry) and chemical deposition by the reaction of $K_3[Fe(CN)_6]$ with $FeCl_3$. The electro-deposition method was optimized in the presence of an anionic surfactant.*

Key words: *Prussian Blue, Screen-printed carbon electrodes, H_2O_2 determination.*

1. Introduction

Prussian Blue (PB), or ferric hexacyanoferrate, is one of the most ancient coordination materials. In 1978 it was reported that PB forms electroactive layers after chemical deposition onto a Pt electrode surface, a fact which has opened a new area in fundamental investigation of this unique inorganic polycrystal [11]. Soon after, in 1981, Itaya et al. proposed a simple method for PB deposition based on a electrochemical reduction of a ferric-ferricyanide solution [8].

The reduced form of Prussian Blue (also called Prussian White) has a catalytic effect for the reduction of O_2 and hydrogen peroxide. Also, the oxidised form of Prussian Blue showed a catalytic activity for the oxidation of hydrogen peroxide. For many years, the structure of PB has been a subject of investigation in order to explain the electrochemical behaviour and its catalytic activity. An exhaustive literature survey on this subject can be found in the review published by Ricci and Paleschi [16].

The first sensors for hydrogen peroxide based on PB modified glassy carbon electrode were reported by Karyakin et al. [9], [10]. The PB deposited on these sensors has an electrocatalytic effect on the reduction

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of H_2O_2, allowing its detection at potentials close to 0 V, thus making possible the coupling with oxidase enzymes while avoiding or reducing the electrochemical interference.

The direct hydrogen peroxide amperometric detection 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 V using PB modified electrodes has enormous advantages and applications in many fields [7], [14].

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 electrodes (SPEs) 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 [13].

In the last years, a number of applications based on screen-printed electrodes modified with PB for glucose, thiocholine, pesticides determination were reported [15], [18].

2. Objectives

Recently it was reported that the surface modification of electrodes with PB in presence of added surfactant offers enhanced film growth, improved stability and excellent electrochemical reversibility [17]. Although, studies to date have focused mainly on the use of cationic surfactants like cetyltrimethyl ammonium bromide (CTAB), in this work we have studied the influence of the anionic surfactant dioctyl sulfo-succinate sodium salt (AOT) on the electrochemical properties of the PB film deposited on a screen printed carbon electrode.

3. Materials and Methods

3.1. Apparatus

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

3.2. Reagents

All chemicals from commercial sources were of analytical grade. 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. Double-distilled water was used throughout.

3.3. Electrodes

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

3.4. 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 minutes [15]. 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 on 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 [15]. The PB modified electrodes were stored dry at room temperature in the darkness. 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.

3.5. 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 [14]. 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 °C 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) [12].

The PB-modified electrodes were stored at room temperature in the dark.

4. Results and Discussion

4.1. Prussian Blue deposition

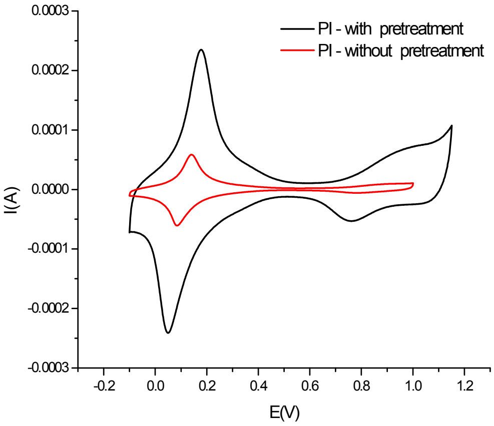
The influence of the pre-treatment and thermal stabilization steps in the chemical and electrochemical procedures for PB deposition on the SPE electrodes were tested.

Figure 1 shows the voltammograms of the PB modified SPCE, with and without the pretreatment, respectively. 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.

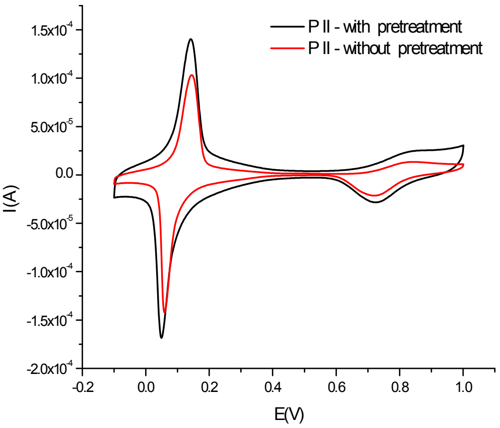
The oxidation and reduction peaks centered at 0.10 V are much narrower and well shaped for the PB sensors obtained via electrochemical deposition compared with those obtained via chemical deposition.

One can observe that in the case of PB chemical deposition (Procedure I), the electrochemical pretreatment has a major influence on the formation of PB. In the electrochemical deposition (Procedure II and II), the pretreatment also influence in a positive way, but not so dramatically.

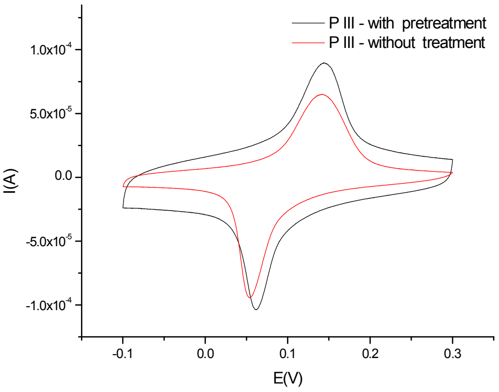
In Figure 2 the cyclic voltammograms obtained for the SPCE modified PB using the electrochemical procedure in presence and absence of the anionic surfactant AOT are shown. The PB sensor was prepared in presence of AOT showed for the first redox couple higher I_{ox} and I_{red}.



A (procedure I)



B (procedure II)



C (procedure III)

Fig. 1. *Influence of the pretreatment step on the cyclic voltammograms recorded for the PB sensors (electrolyte 0.1 M KC in 0.1 M HCl; 50 mV/s)*

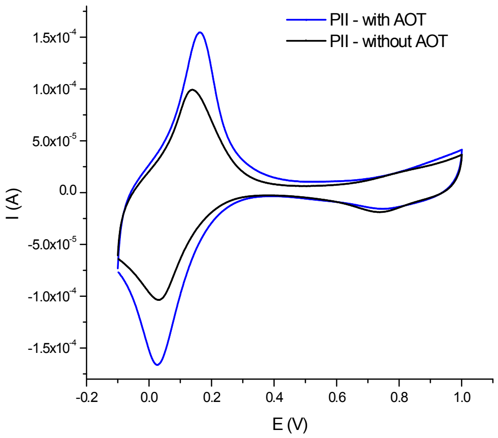


Fig. 2. *Influence of the AOT on the cyclic voltammograms of PB sensor (electrolyte 0.1 M KCl in 0.1 M HCl; 50 mV/s)*

The influence of the thermal stabilization by keeping the electrodes at 100 °C 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 H_2O_2, but the operational stability was greatly 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 couple centered

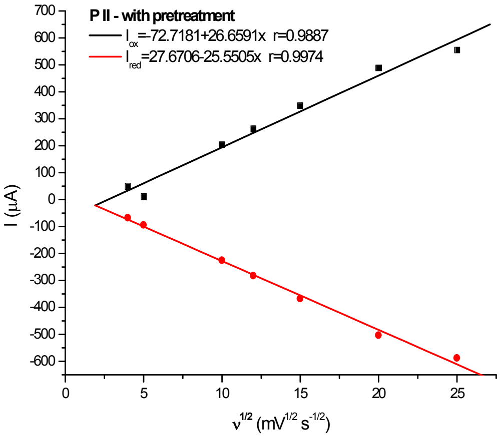


Fig. 3. *Variation of I_{ox} and I_{red} vs. square root of scan rate in electrolyte solution (0.1 M KCl, 0.1 M HCl)*

at 0.10 V. Plotting the I_{ox} and I_{red} vs. square root of scan rate showed a linear relationship (Figure 3), the result indicating that diffusion of electrolyte across the PB layer controls the electrode process.

This behaviour was observed for all the three tested procedures used for PB deposition.

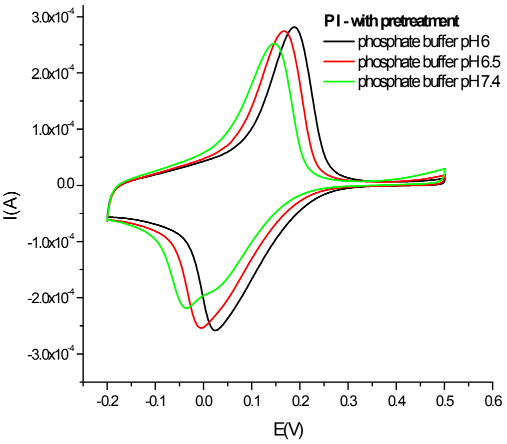
4.2. Electrocatalytic reduction of H_2O_2

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 [3], [4].

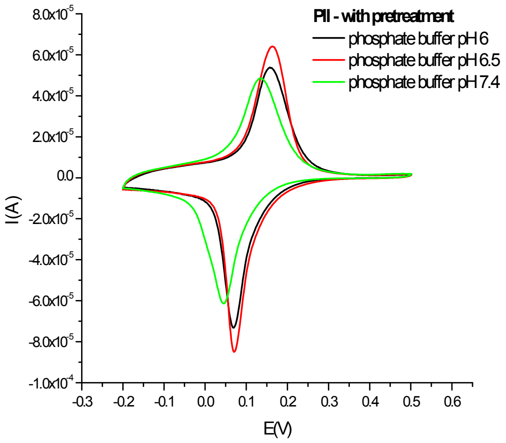
Recently has been demonstrated that electrochemical pretreatments induce some cracking on the surface carbon electrodes with the increase of its effective working area [6]. The sensors developed in this work use oxidase, which catalyse the hydrogen peroxide then being detected electrochemically, using aPB redox mediator [2]. This behaviour confirms the results reported in the literature [1].

The pH influence on the electrochemical determination of H_2O_2 using the PB modified SPCE was studied at pH ranging from 6-7.4 (Figure 4). For all the tested electrodes the highest reduction peaks were obtained for pH 6.5. For all the following studies an optimum electrolyte solution was used; the 50 mM phosphate buffer, pH 6.5.

The sensors developed in this work use oxidase, which catalyse the hydrogen peroxide then being detected electrochemically, using PB redox mediator [2].



Procedure I



Procedure II

Fig. 4. *Influence of electrolyte solution pH on the response of PB modified SPCE to 1 mM* H_2O_2

In order to select the working potential to be applied when measuring H_2O_2 the amperometry technique was used. Potentials ranging from –100 mV to +200 mV were applied. For a selected concentration of hydrogen peroxide (100 μM) we have plotted the current intensity *vs.* applied potential (Figure 5). The lowest background noise and the highest signal for H_2O_2 measurement was recorded for the PB sensor prepared via the electrochemical procedure (PII).

Therefore, we selected –50 mV as working potential, considering both the response intensity and operational stability.

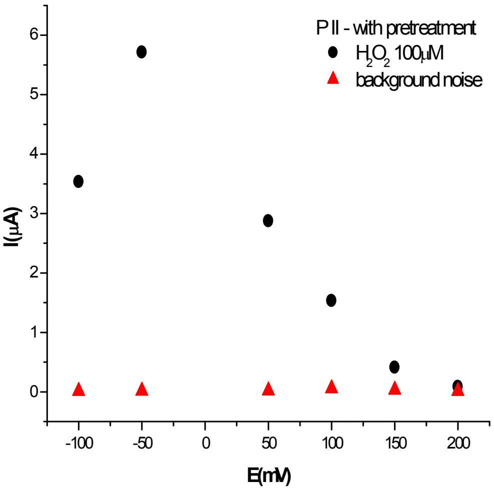


Fig. 5. *Amperometric response of 100 µM H_2O_2 vs. applied potential* (*electrolyte: 50 mM phosphate buffer, pH 6.5*)

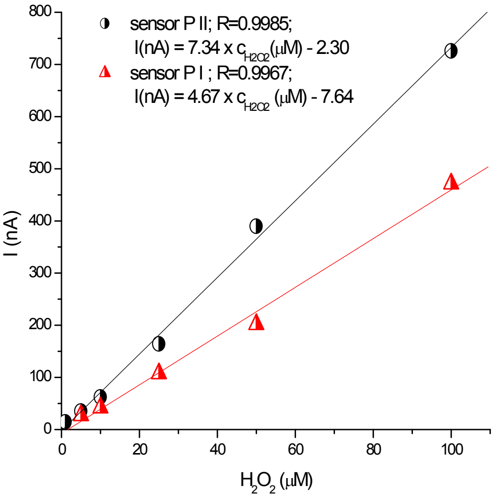


Fig. 6. *Calibration plots for detection H_2O_2, using chronoamperometry* (*E = –50 mV/s*)

4.3. Performances of the PB sensor

The developed PB sensors may be used for H_2O_2 determination using as electrochemical techniques: amperometry (under stirring); flow injection amperometry and chronoamperometry.

The amperometry under stirring technique gave the best results for the PII sensors in rapport with the background noise and response time. Even though the sensitivity recorded with sensors PI is higher than that obtained for PII and PIII, the linear range of concentration for PI is shorter (data not shown).

Also this electrochemical technique may be applied for on-field measurements using a portable potentiost/galvanostat.

According to the measurement results, the linear range of the PB sensors was from 1 µM to 100 µM H_2O_2 with the linear correlation of 0.9967 for the P I (chemical deposition) and, respectively, of 0.9985 for the P II (galvanostatic deposition).

The sensitivity of the PII sensors was 50% higher than of the PI (Figure 6). For both types of sensors the detection limit was 0.5 µM H_2O_2.

The reproducibility of the single electrodes was examined for 10 successive assays at a H_2O_2 concentration of 50 µM. The relative standard deviation (RSD) was 3.6%, 2.5% and 4.8% for PB sensors prepared according to the PI, PII, and respectively PIII.

The stability of the PII sensor under storage conditions (dried, at room temperature, darkness) was assessed for a period of 45 days using a solution of 50 µM hydrogen peroxide by making a determination in triplicate every working day. After 45 days the response of PII sensor was 94% of the initial response.

The operational stability of the PII sensor was also checked by using the sensor 6 hours/day for different H_2O_2 concentrations. The PII sensor proved to be very robust in operational conditions. Only, after 18 days was a 35% reduction in the response observed.

5. Conclusions

Three methods for preparation of Prussian Blue modified screen printed carbon electrodes were compared in order to design a sensitive and very robust sensor for hydrogen peroxide as a platform for oxidase based biosensors, resulting in the selection of the galvanostatic procedure (PII) as the most efficient.

For the first time it is reported the use of the anionic surfactant AOT in the electrochemical procedure which resulted in a more sensitive and reproducible PB sensor.

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