



MODIFIED PRUSSIAN BLUE SCREEN PRINTED ELECTRODES FOR H_2O_2 DETECTION

Florentina HUTANU¹, Gheorghe GUTT^{1*}

¹Ștefan cel Mare University of Suceava, Faculty of Food Engineering, Universității Street, 720229, Suceava, Romania, g.gutt@usv.ro

*Corresponding author

Received 10 January 2013, accepted 21 February 2013

Abstract *In this work, presents recent developments in the electrochemical application of disposable screen-printed sensors, to the type of materials used to modify the working electrode. The sensor was based on the electrocatalytic reduction of H_2O_2 on Prussian Blue modified screen-printed electrode. A comparative study regarding different procedures for modifying the carbon screen-printed electrodes (SPE) with Prussian Blue (PB) was carried out in this work. Two 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 $FeCl_3$. Also, the influence of the pretreatment of SPE (+1.7V for 3 min in PBS, pH 7.4) and of the stabilization of PB deposited on SPE (by heating at 100°C) were evaluated. The developed sensors were optimized with respect to the lowest limit of detection achieved for amperometric detection of H_2O_2 . Analytical parameters, such as detection limit, linearity range and sensitivity have been evaluated, together with operational and storage stability. The improved electro-deposition methods, pH stability and permeability of the optimized PB film provide a further boost in its sensitivity for H_2O_2 detection, which is a critical parameter in biosensor design and application.*

Keywords: Screen printed electrodes, H_2O_2 determination, Prussian Blue.

1. Introduction

In 1978, Neff [1] reported a thin layer of Prussian Blue deposition on platinum foil by chemical method. Prussian Blue, is a prototype of mixed-valence transition metal hexacyanoferrates, has been widely used as an electron transfer mediator in amperometric biosensor due to its for electrocatalysis.[2-5] Only 4 years later, Itaya et al. [6] showed that the reduced form of Prussian blue (also called Prussian white, PW) had a catalytic effect on the reduction of both O_2 and H_2O_2 , many

publications have appeared exploring its electrocatalytic, electromagnetic and electrochemic properties [7]. Itaya et al. [8] demonstrated the most important feature of Prussian Blue (in terms of analytical application). It was in fact shown that the reduced form of Prussian Blue (also called Prussian White) had a catalytic effect for the reduction of O_2 and hydrogen peroxide. Many efforts have been made to improve the selectivity of carbon based electrochemical sensors, mainly through their modification with redox mediators [9]. However, direct hydrogen peroxide

amperometric detection at conventional electrodes is only possible 0.6 V vs. Ag/AgCl. At this potential, the presence of easily oxidisable compounds such as ascorbate, bilirubin, urate etc. can easily interfere in the measurements, being oxidised at the electrode together with hydrogen peroxide [10].

Prussian Blue modified electrodes represent very attractive detectors for hydrogen peroxide because they work at an applied potential around 0 V vs. Ag/AgCl with no other electrochemical interferences [11].

Objectives

The aim of this work was to compare three procedures for PB film formation on the working electrode of screen printed carbon electrodes in order to prepare sensitive and robust PB sensors for H₂O₂ determination. The tested procedure were based on: chemical, galvanostatic and cyclic voltammetry based deposition.

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 [13]. This approach also increases the PB sensor stability in neutral and basic media. 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 screen printed carbon electrode.

2. Materials and methods

2.1. 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.

2.2. Reagents

All chemicals from commercial sources were of analytical grade. Iron chloride (FeCl₃), potassium ferricyanide K₃[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.

2.3. 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 = 4mm), a graphite auxiliary electrode and a silver pseudoreference electrode, with silver electric contacts deposited on a ceramic substrate.

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 and ceramic substrate. These electrodes are for working with microvolumes, for decentralized assays or to develop specific sensors. The electrodes were produced in 75 units packs. Each sensor consists of three printed electrodes, a carbon working and two silver electrodes, acting as counter and pseudoreference, respectively.

Screen-printed electrodes [12] are frequently used in analytical applications because of their unique properties such as small size, low detection limit, fast response, high reproducibility, etc. [13]. Advantages of screen-printed electrodes is

that they are inexpensive, simple to prepare, versatile and suitable for the mass-production of disposable electrodes [14]

2.4 Chemical deposition of Prussian Blue

Prior to Prussian Blue modification, screen-printed electrodes were pretreated in presence of 50 mM phosphate buffer in 0.1 M KCl, pH 7.4, by applying the potential of + 1.7 V versus Ag/Ag Cl for 3 minute [15]. For the chemical deposition of PB films two solutions were prepared. Solution 1: 100 mM $K_3[Fe(CN)_6]$ in solution 10 mM HCl.

Solution 2: 100 mM $FeCl_3$ in solution 10 mM HCl Prussian Blue modification of screen printed was then accomplished by placing 10 μ l 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 obtained more stable and active layer of Prussian Blue. [14]

2.5. Electrochemical deposition of Prussian Blue

Prior to Prussian Blue modification, screen-printed electrodes were pretreated as in previous section. PB modification of SPE eas a accomplished by placing a drop (40 ml of total volume) of precursor solution onto the working electrode area.

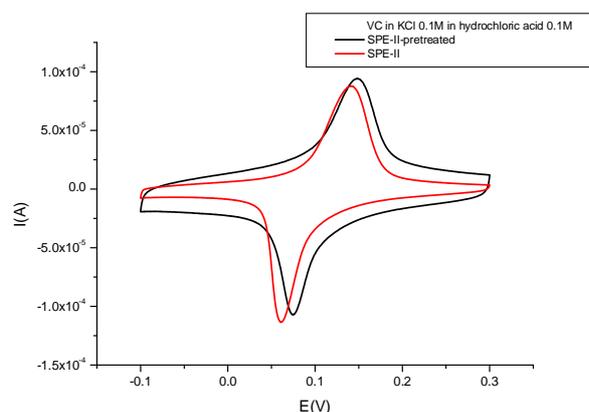
The deposition was made in a mixture KCl 100 mM of 0.1 M $K_3[Fe(CN)_6]$, 0.1M $FeCl_3$ prepared in 100 mM KCl and 100 mM HCl solution by applying the potential of 0.4V for 40 sec [15]. After a gentle rinsing with water, the sensor was placed

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. After some deposition cycles, the PB modified electrode was dried at 100° for 1 h to obtain a more stable and active PB layer [16]

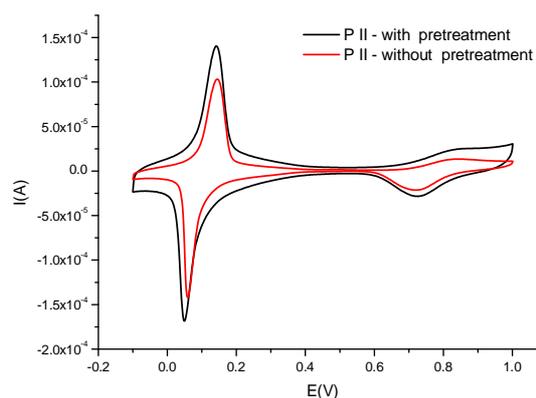
3. Results and discussion

3.1. 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.



A (procedure I)



B (procedure II)

Fig. 1. Cyclic voltammograms obtained for the SPCE modified PB using the chemical and electrochemical procedure 0.1 M KCl in 0.1M HCl; 50mV/s.

In Fig. 2 are presented the cyclic voltammograms obtained for the SPCE modified PB using the chemical procedure in presence and absence of the anionic surfactant AOT at concentration H_2O_2 3mM. The PB sensor prepared in presence of AOT showed for the first redox couple higher I_{ox} and I_{red} .

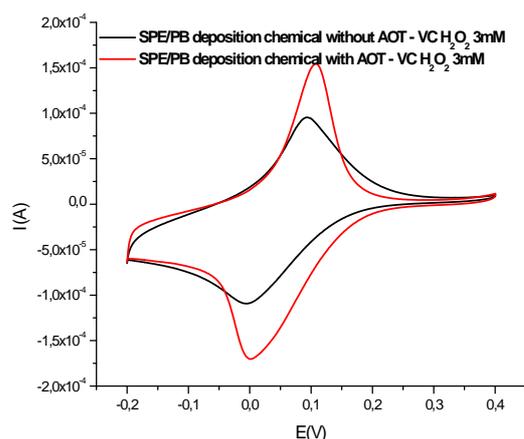


Figure 2. Cyclic voltammograms, in KCl 0.1 M in HCl 0.1 M; the scan rate 50 mV/s, modified PB/AOT using the chemical procedure.

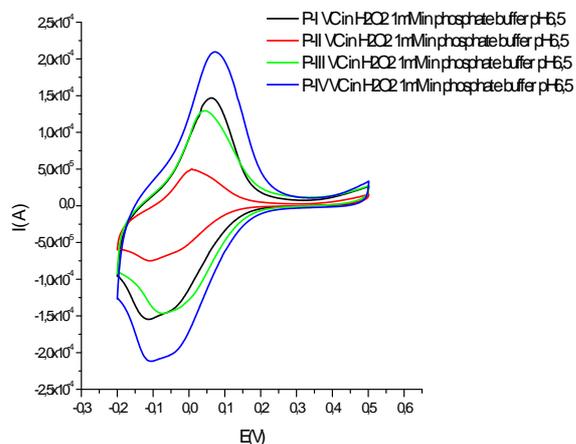


Figure 3. Cyclic voltammograms of PB sensor 0.1 M KCl in HCl; E = -50mV/s, phosphate buffer pH 6,5, H_2O_2 1mM.

The influence of the thermal stabilization by keeping the electrodes at 100° for 90 min was also studied [17]

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 greater improved for the electrodes stabilized via the thermal treatment.

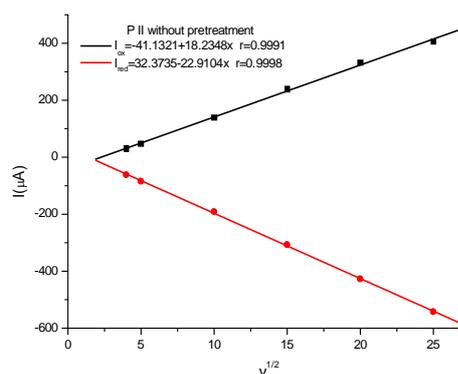


Figure 4. Variation of oxidation and reduction vs. square root of scan rate in electrolyte solution 0.1M KCl, 0.1M HCl

Plotting the oxidation and reduction vs. square root of scan rate showed a linear relationship (Fig. 4), the result indicating that diffusion of electrolyte across the PB layer controls the electrode process. This behaviour was observed for all the two tested procedures used for PB deposition.

3.2. Electrocatalytic reduction of H_2O_2

The pH influence on the electrochemical determination of H_2O_2 using the PB modified SPCE was studied at pH ranging from 6.5 (Fig. 3). For all the tested electrodes the highest reduction peaks were obtained for the pH 6.5. In order to select the working potential to be applied when measuring H_2O_2 it was used the amperometry technique.

Therefore, we selected the -50 mV as working potential, considering both the response intensity and operational stability. The applied potential used -50 mV was selected in our previous paper [18]

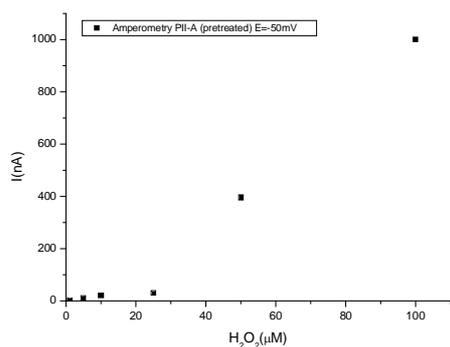


Figure 5. Amperometry response procedure II, from these sensors was evaluated by amperometric measurement in turbulent solution.

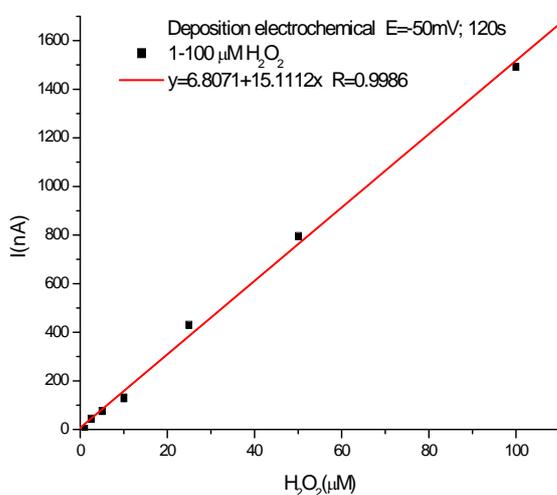


Figure 6. Calibration curves obtained by successive additions of solution H₂O₂ phosphate buffer pH 6.5, E= -50 mV/s.

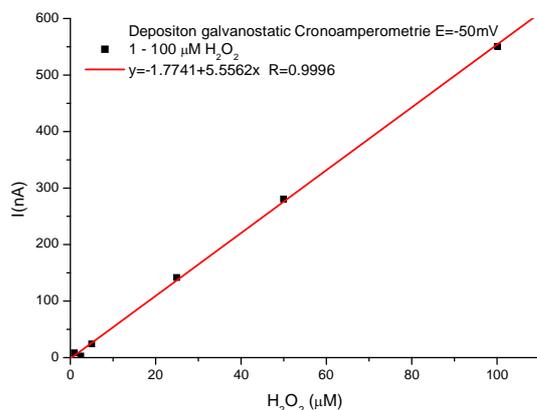


Figure 7. Calibration curves obtained for H₂O₂ detection performed at electrodes with PB layers in the 1-100 μM concentration range. Applied potential -50mV/s.

Equation obtained for the linearity is $y = 1.77 + 5.55x$, with a correlation coefficient $r^2 = 0.9996$ deposition galvanostatic for the deposition electrochemical $y = -6.8 + 15x$ $r^2 = 0.998$. The time required to achieve 90% of corresponding steady state current was 10 s.

3.3. Performances of the PB sensor

The developed PB sensors was used for H₂O₂ determination using as electrochemical techniques: amperometry and chronoamperometry. According to the measurement results, the linear range of the PB sensors was from 1-100 μM, H₂O₂ with the linear correlation of 0.9996 for the deposition galvanostatic and chemical deposition $r^2 = 0.998$. For both type of sensors the detection limit was 0.5 μM H₂O₂. The relative standard deviation (RSD) was 3.6 %, for PB sensors prepared according to the procedures mentioned.

4. Conclusions

Two methods for preparation of PB modified SPCEs were compared in order to design a sensitive and robust sensor for hydrogen peroxide as a platform for oxidase based biosensors, resulting in the selection of the galvanostatic procedure (SPE II-A) as the most efficient.

5. Acknowledgments

The authors acknowledge to the project POSDRU/CPP107/DMI1.5/S/78534 for financial support.

6. References

- [1].V. D. NEFF, Electrochem. Soc. 125 (1978) 886.
- [2].A.A. KARAYAKIN, O.V. GITELMACHER, E.E. KARAYAKINA, Anal. Chem. 67 (1995) 2419.
- [3].G. ZHAO, J.J. FENG, Q.L. ZHANG, S.P. LI, H.Y. Chen, Chem. Mater. 17 (2005) 3154

- [4]. F. RICCI, A. AMINE, D. MOSCONE, G. PALLESCHI, *Biosens. Bioelectron.* 22 (2007) 854
- [5]. L.V. LUKACHOVA, E.A. KOTEL'NIKOVA, D.D' OTTAVI, E.A. SHKERIN, E.E. KARYAKINA, D. MOSCONE, G.PALLESCHI, A. CURULLI, A.A. KARAYAKIN, *IEE Sens. J.* 3 (2003) 3.
- [6]. K. ITAYA, K. SHIBAYAMA, H. AKAHOSHI, S. TOSHIMA, *J. Appl. Phys.* 53 (1982) 804
- [7]. F. RICCI, , G.PALLESCHI, *Biosens. Bioelectron.* 21 (2005) 389-407.
- [8]. ITAYA, K., SHOJI, N., UCHIDA, I., 1984a. Catalysis of the reduction of molecular oxygen to water at Prussian Blue modified electrodes. *J. Am. Chem. Soc.* 106, 3423-3429
- [9]. CASS, A.E.G., DAVIS, G., FRANCIS, G.D., HILL, H.A.O., ASTON, W.J., HIGGINS, I.J., PLOTKIN, E.V., SCOTT, L.D., TURNER, A.P.F., 1984. Ferrocene mediated enzyme electrode for amperometric determination of glucose. *Anal. Chem.* 56, 667-671.
- [10]. SCHELLER, F.W., PFEIFFER, D., SCHUBERT, F., RENEBERG, R., KIRSTEIN, D., 1987. Application of enzyme amperometric biosensors to analysis of real object. In Turner, A.P.F., KARUBE, I., WILSON, G.S. (Eds.), *Biosensors: Fundamental and Applications*. Oxford University Press, Oxford, UK (Chapter 18).
- [11]. F. RICCI, , G.PALLESCHI, *Biosens. Bioelectron.* 21 (2005) 389-407.
- [12]. Q. DOMINGUEZ RENEDEO, M.A. ALONSO-LOMILLO, M.J. ARCOS MARTINEZ Recent developments in the field of screen-printed electrodes and their related applications *Talanta* 73 (2007) 202-219
- [13]. IVANILDO LUIZ de MATTOS, LO GORTON, *Tautgirdas Ruzgas Biosensors and Bioelectronics* 18 (2003) 193-200
- [14]. Ricci, A. Amine, G. Palleschi, D. Moscone *Biosens. & Bioelectronics* 18 2003 165-174
- [15]. F. RICCI, A. AMINE, D. MOSCONE, G.PALLESCHI, Prussian Blue based screen printed biosensors with improved characteristics of long-term lifetime and pH stability . In: *Biosensors Bioelectron.* (2003) Vol. 18, p. 165-174.
- [16]. F. RICCI, , G.PALLESCHI, *Biosens. Bioelectron.* 21 (2005) 389-407.