

Sensor for Hydrogen Peroxide Based on Prussian Blue Modified Electrode: Improvement of the Operational Stability

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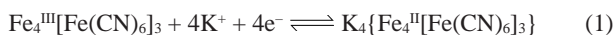
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Improvement of the operational stability of amperometric sensors based on Prussian Blue (PB) modified glassy carbon electrodes is presented. The long term performance of the sensors was evaluated by injection of hydrogen peroxide (5 μM in potassium buffer) solutions in a flow-injection system during a period of 5 – 10 h. The following parameters were investigated and correlated with the performance of the sensor: the times for electrodeposition and electrochemical activation, temperature, storage time, pH, composition of the buffer solution and of volume sample injected. These analytical characteristics of the modified electrode can be emphasized: initial sensitivity of $0.3 \text{ A cm}^{-2} \text{ M}^{-1}$, detection limit of *ca.* $0.5 \mu\text{M}$, precise results (r.s.d. < 1.5%) and possibility to carry out around 50 samples ($50 \mu\text{L}$) per hour.

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Prussian Blue, $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3 \cdot n\text{H}_2\text{O}$ is a classical mixed valence compound.¹ The overall structure of the cubic Fe(III)-N-C-Fe(II) framework corresponds to the face-centered space group^{1,2} and there is a substantial interaction between the two kinds of Fe-ions, as indicated by the appearance of a broad intense absorption band at 14100 cm^{-1} , assigned as the Fe(II) \rightarrow Fe(III) charge transfer transition.^{1,2} Further, there are two proposed formulae of Prussian Blue (PB), which have been called “insoluble PB” ($\text{Fe}_4^{\text{III}}[\text{Fe}^{\text{II}}(\text{CN})_6]_3$) and “soluble PB” ($\text{KFe}^{\text{III}}\text{Fe}^{\text{II}}(\text{CN})_6$).^{3,4} It is believed that the degree of hydration influences the conducting properties of these compounds.^{5,6}

The insoluble form of PB on the electrode can be oxidized to Prussian Green (PG) as well as reduced to Prussian White (PW, Everitt's salt) at approximately +0.7 and -0.1 V vs. SCE respectively.⁷



The reduced form of PB (Prussian White) has a catalytic activity for the reduction of molecular oxygen and of hydrogen peroxide in aqueous acidic electrolytes and can act as an electrocatalyst for hydrogen peroxide reduction present in solution or formed in the course of an enzyme-catalyzed reaction.^{8,9} Since this electrocatalytic process proceeds at a low electrode potential (*ca.* -0.1 V vs. SCE), it is possible to eliminate the effect of interfering species to develop sensors with a suitable catalytic surface for the amperometric determination of hydrogen peroxide at a low potential.^{9,10} In this sense, the electrodeposition of the PB film has been investigated in diverse materials such as carbon paste,⁸ Pt⁹ and glassy carbon.¹⁰ Under certain deposition conditions, the PB film can selectively catalyze the electroreduction of hydrogen peroxide in the presence of molecular oxygen.¹⁰

Hydrogen peroxide is present in countless biological reactions as the main product of several oxidases,^{11,12} and is an important parameter for the monitoring of these bio-processes.^{11–14} Many

analytical techniques are available to determine H_2O_2 , including fluorometry,¹⁵ chemiluminescence,¹⁶ photometry¹⁷ and voltammetry.¹⁸ Among applications, the determination of hydrogen peroxide has been realized in matrices such as foods^{19–21} and pharmaceuticals^{22–24} and in the environment.^{25–27} In recent years, one of the most widely used electroanalytical methods relies on the use of peroxidase.^{12,14,28} A number of PB-modified electrodes and also electrodes modified with similar metal-ion cyanide complexes have been reported and proposed for highly selective monitoring of hydrogen peroxide. However, until now, these electrodes still face a number of drawbacks with regards to long-term stability and sensitivity to pH-changes.

Thus the main purpose of the present work was to present a study directed to the improvement of the operational stability of Prussian Blue-modified electrodes as hydrogen peroxide sensors. For this task, a systematic investigation was undertaken of the electrochemical characteristics and chemical composition for the formation of the PB film.

Experimental

Materials, reagents and standards

Concentrated solution (30%) of H_2O_2 was purchased from Aldrich (Steinheim, Germany). All reagents were prepared with chemicals of analytical-reagent grade and double-distilled water (Millipore-Q). For the electrodeposition of PB onto glassy carbon electrodes, the following aqueous solutions were used: reagent R₁, 2.5 mM FeCl_3 + 2.5 mM $\text{K}_3\text{Fe}(\text{CN})_6$ + 0.1 M KCl + 0.1 M HCl; reagent R₂, 0.1 M KCl + 0.1 M HCl; reagent R₃, 0.05 M $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ + 0.1 M KCl with the pH adjusted to 5.5 and reagent R₄, 0.1 M $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ + 0.1 M KCl. For investigation of the effect of cations, chloride solutions 0.1 M of sodium, potassium and cesium, and 0.05 M of magnesium, calcium and barium were used. Hydrogen peroxide solution (0.1 M) was prepared by dissolving 100 μL of 30% hydrogen peroxide in 10 mL of double distilled water and stored at 4°C when not in use. Working standard solutions (5 – 50 μM) were freshly prepared by dilution of the stock solution with buffer (R₃).

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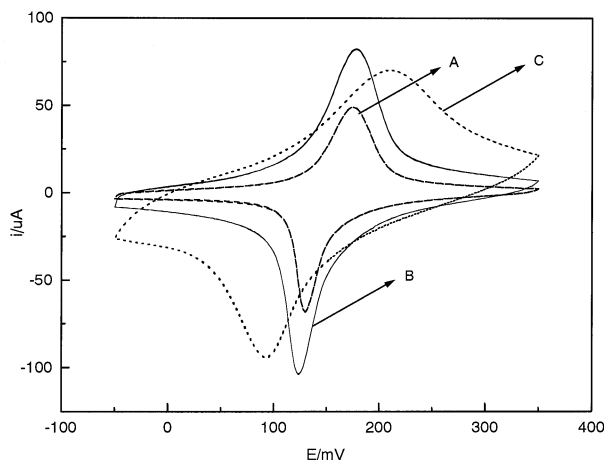


Fig. 1 Cyclic voltammograms for 20 (A), 40 (B) and 60 s (C) electrodeposition times; time of the activation = 600 s; scan rate = 50 mV s⁻¹ in R₂ solution. The scanning was started at 350 mV vs. SCE.

Apparatus and deposition of the Prussian Blue film

Electrochemical measurements were performed using an EG&G PAR 273 potentiostat-galvanostat system (Princeton, NJ). Hydrodynamic experiments were carried out with a LC4B electrochemical analyzer (Bioanalytical Systems Inc., West Lafayette, IN). Electrochemical deposition of PB onto the glassy carbon electrodes and cyclic voltammetric investigation of the PB modified electrodes were performed in a three-compartment electrochemical cell containing a platinum net auxiliary electrode and a saturated SCE reference electrode. Glassy carbon disk electrodes (diameter 3.0 mm, Sigradur-G, HTW Hochttemperatur/Werkstoffe GmbH, Thierhaupten, Germany) were used as working electrodes. Flow-injection experiments were carried out in a programmable Ismatec system (Zürich, Switzerland) with two peristaltic pumps (variable and invariable) furnished with Tygon pumping tubes and 0.7 mm i.d. Teflon tubing. A homemade flow-through amperometric cell was of the confined wall-jet type²⁹ and connected to the outlet of the flow-injection system. The inlet section of the cell contained the Ag/AgCl reference electrode in a circular chamber filled with 0.1 M KCl from an external syringe. This chamber contacted the working electrode space *via* four holes (0.3 mm) concentrically surrounding the inlet (0.5 mm). The auxiliary electrode was a platinum wire encircling the outlet chamber. The glassy carbon disk electrodes (diameter 3.0 mm) were used as working electrodes. The distance between the nozzle and working electrode was about 2 mm.

Prior to surface modification, the glassy carbon electrode was mechanically polished with alumina powder (Al₂O₃, 1 μm) until a mirror finish was obtained. Unless otherwise indicated, the potential scan rate in cyclic voltammetry experiments was 50 mV s⁻¹. Electrodeposition of the Prussian Blue film was accomplished with some modifications of earlier work.¹⁰ The actual deposition of Prussian Blue was accomplished in a R₁ solution by applying a constant potential to the glassy carbon electrode at +400 mV vs. SCE for 20, 40 and 60 s. The electrode was then carefully washed with water and transferred into a R₂ solution and electrochemically cycled for various times between 350 and -50 mV vs. SCE (25 cycles). After washing with water, it was dried for 1 h at 100°C. After that, the modified electrode was immersed into R₃, it was conditioned by keeping at -50 mV vs. SCE for 60, 300 and 600 s and by cycling between 350 to -50 mV vs. SCE (25 cycles).

The sample (H₂O₂) was injected into the flow system by means of a 50 μL sampling loop; it was pushed by its carrier stream flowing through the 20-cm coiled reactor (R₃ sol. at 0.8 mL min⁻¹) towards the detector with a fixed potential (-50 mV vs. Ag/AgCl) applied to the working electrode. The passage of the processed sample through the electrochemical cell produced an amperometric transient response signal with a peak current proportional to the hydrogen peroxide content. Precision is expressed as relative standard deviation estimated after successive measurements.

Results and Discussion

Increasing the time of electrodeposition of the Prussian Blue film onto the glassy carbon electrode resulted in an increase in the surface coverage (*Γ*, Fig. 1). For electrodeposition times between 20 and 60 s, *Γ* presented values between 2 and 6 nmol cm⁻² assuming a transfer of four electrons per unit cell Fe₄[Fe(CN)₆]₃·12H₂O.⁴ Independent of the deposition time (up to 60 s) the formal potential (*E*^{o'}, calculated as the mean value of the anodic and cathodic peak potentials³⁰) was found as *ca.* 150 mV in all cases and is in concordance with the value previously reported for PB-modified glassy carbon electrodes.¹⁰ However, for 60 s a significant increase of the capacitive current was noted compared with that of 20 s, suggesting an increase in the surface area or that the double layer was affected by the presence of the PB film. Also, for this deposition time, an impressive increase of the peak separation (*ΔE_p*) was observed from 50 (related with 20 and 40 s) to 117 mV (this change can be explained by the increase of the ohmic resistance of the film).

The figure shows cyclic voltammograms of the PB-modified electrode registered between -50 and +350 mV in solution R₂: one clear anodic and one clear cathodic wave are shown. These peaks were attributed to the reversible redox interconversion of Prussian Blue and Prussian White or the oxidized and reduced forms, respectively. The *ΔE_p* value found as 117 mV represents a deviation of the Nernstian behavior,^{30,31} and as a consequence the time equal to 60 s was disregarded. For further experiments, a deposition time of 40 s was selected, since under this condition, a higher surface coverage was observed compared with 20 s. In previous investigations, the electrocatalytic activity is also linearly dependent on the surface coverage, as long as the electron transfer remains reasonably fast.³²

Fixing the electrodeposition time as 40 s, the influence of the electrochemical activation time for the PB film was investigated. Increasing this time within 60, 300 and 600 s (with the working electrode polarized at -50 mV vs. SCE in solution R₃, see Experimental) a beneficial effect on the stability of the film was observed. In fact, when this time was raised from 60 to 600 s and the electrode was employed for successive hydrogen peroxide measurements in the FIA system (for 3 h), the observed response current (for 5 μM of H₂O₂ in R₃) decreased *ca.* 10%. For 300 s this decrease was found to be 18% and for 60 s a decrease of *ca.* 28% of the initial value was observed. In order to avoid false interpretations because of the instability of the hydrogen peroxide solution, this solution was exchanged for a freshly prepared one every 1.5 h. Therefore, an electrochemical activation time of 600 s was chosen as the time for the electrochemical activation of the Prussian Blue film for further pretreatment.

The polycrystal (PB precipitate contains an average of 14 water molecules per unit Fe₄[Fe(CN)₆]₃) affects the conducting properties and as a consequence the stability of the corresponding PB modified electrodes.^{5,6} Thus the influence of

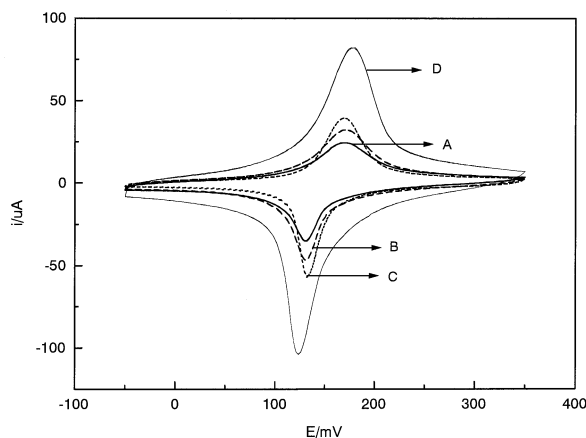


Fig. 2 Cyclic voltammograms obtained at different temperatures and storage after pretreatment: freshly (A), 20°C during 48 h (B), 20°C during 24 h (C) and 100°C during 1 h (D); electrodeposition and activation times = 40 and 600 s; scan rate = 50 mV s⁻¹ in R₂ solution.

drying of the films was examined by keeping the electrode in a thermostat (20 and 100°C) before the activation in R₃ reagent (see Experimental). The electrodes were maintained for 24 or 48 h at 20°C or 1 h at 100°C. Figure 2 shows that, as expected, the presence of water lowers the electroactivity of the Prussian Blue film evaluated as the detectable surface coverage. When the modified electrode was exposed at 100°C for 1 h, an enlargement of the amount of electroactive PB was found on the electrode surface compared with the increment observed to that found for 20°C (Fig. 2). Moreover, it is possible to note a slight decrease of the electroactivity of the film after a longer storage condition (48 h, in air with exclusion of light at ambient temperature). This confirms earlier work⁷ in which was observed a significant decrease of electroactivity of the PB-modified electrode storage time in a desiccator even under light protection. The decomposition of the modifier resulting in a decrease of electroactive molecules on the surface was found to be the main reason. It is important to note that, despite a significant decrease of the electroactivity, the presence of water (zeolitic) did not change the position of the redox peaks, which were maintained separated by the same magnitude (ΔE_p close to 50 mV). This observation confirms the initial belief that the film is permeable to water molecules.⁵⁻⁷ Then for further experiments, the PB film was treated under 100°C for 1 h before the activation in phosphate buffer solution (R₃ reagent).

The pH value was shown to be a very important parameter for the stability of the PB films. At pH >7 the peak current response for H₂O₂ decreased to about 20% of its initial value after 1 h of monitoring. The decrease in current of H₂O₂ reduction was also followed by a change in the color of the electrode surface from the initial dark blue to yellow, as earlier reported.¹⁰ This can be explained by the presence of hydroxide ions, which are known to be able to break the Fe-(CN)-Fe bonds.¹⁰ In contrast, when the acidity of the initial solution was increased, more stable electrodes were achieved. However, when the concentration of HCl exceeded 0.1 M, a decrease was observed in the growth rate of the inorganic polycrystal. This dependence may be expected because, on one hand, monovalent cations are involved in the reduction process of PB (H⁺ may replace K⁺ to a certain degree in the reaction: Fe₄(III)[Fe(CN)₆]₃ + 4K⁺ + 4e⁻ ↔ K₄Fe₄(II)[Fe(CN)₆]₃) and of H₂O₂ (H₂O₂ + 2H⁺ + 2e⁻ ↔ 2H₂O);⁷ on the other hand, protons may block the

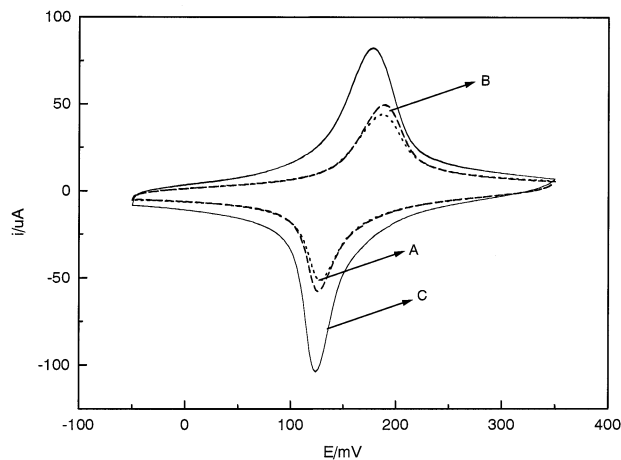


Fig. 3 Cyclic voltammograms of the PB films activated in 0.05 M K₂HPO₄/KH₂PO₄ + 0.1 M KCl (A); 0.1 M K₂HPO₄/KH₂PO₄ + 0.1 M KCl (B) and 0.1 M KCl (C); electrodeposition and activation times = 40 and 600 s; scan rate = 50 mV s⁻¹ in R₃ (A and B) and R₂ (C).

electrochemical reactivity of PB,³ yielding a maximum in the current-pH dependence and causing a significant drop below pH 1. Therefore, for the electrodeposition step the solution employed had a pH of *ca.* 1 (see reagent R₁ in Experimental).

We considered that cations such as K⁺, NH₄⁺, Rb⁺ and Cs⁺ promote the electroactivity (can be expected to transport through the crystal of PB during the reduction of the iron ions) and others such as Na⁺, Li⁺, H⁺, Mg²⁺ and Ca²⁺ can be related as blocking cations of the Prussian Blue films.^{3,10} Thus experiments were done to investigate some of these cations and select the better buffer solution. The idea was to put in the same buffer solution different cations to improve both the electroactivity and the stability of the films. Then, the electrochemistry of PB was examined in the presence of chloride solutions of K⁺, NH₄⁺, Rb⁺, Cs⁺, Na⁺, Li⁺, Mg²⁺, Ca²⁺ and Ba²⁺ (all pH values of the solutions were adjusted to pH 5.5). In all cases, except that for K⁺ ion, reaction (Eq. (1)) is blocked and no *i*-*V* maxima were observed after some cycles confirming earlier work.³ After that, the composition of the buffer solution was defined with K⁺ ion (see reagent R₃ in Experimental).

In Fig. 3, it is possible to note that when the CVs were run in the presence of 0.1 and 0.05 M K₂HPO₄/KH₂PO₄ + 0.1 M KCl with the pH adjusted to 5.5 (curves A and B, respectively) and were compared to that run in 0.1 M KCl (curve C), the voltammograms were altered. The half-widths of the wave became larger and the peak currents were significantly lower both for the anodic and cathodic scans. Additionally, the ΔE_p was slightly increased from *ca.* 50 to 60 mV *vs.* SCE. Despite these observations, when the PB film was cycled in this buffer medium (K₂HPO₄/KH₂PO₄), an increase in stability was observed. In fact, when the electrode was used to monitor hydrogen peroxide (5 μM) in potassium buffer (R₃, see Experimental) the current signal stayed constant after 5–6 h of continuous running. However, with a prepared electrode cycled in KCl solution the decrease in signal for H₂O₂ was *ca.* 15% after 2–3 h. This behavior (higher stability) was confirmed by using the electrode prepared (activated) in more concentrated buffer = 0.1 M K₂HPO₄/KH₂PO₄ + 0.1 M KCl, pH 5.5 (R₄ reagent). As can be observed in Fig. 3, the CVs were similar (curves A and B).

Then, for further experiments, a buffer was chosen consisting

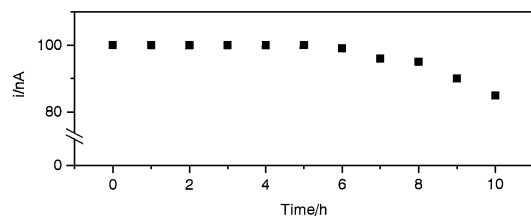


Fig. 4 Long time stability of the Prussian Blue modified electrode loading in the FIA system: recorder output of a $5 \mu\text{M}$ H_2O_2 ($50 \mu\text{L}$) in R_3 ; carrier stream at 0.8 mL min^{-1} in R_3 ; applied potential = -50 mV vs. Ag/AgCl .

of 0.05 M in $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ plus 0.1 M KCl with the pH adjusted to 5.5 (R_3 reagent, see Experimental). This solution was used both for the electrochemical activation of the PB film and as the carrier stream in the flow-injection system.

After setting the parameters of the flow-injection system, the long-term stability of the modified electrode was evaluated by repeated injections of standard-solution ($5 \mu\text{M}$ H_2O_2 in R_3 reagent) during 10 h . The system robustness was emphasized after these experiments, because no significant variation in the baseline was noted. Figure 4 shows the performance of the PB modified electrode for hydrogen peroxide determination. Until the 5th h the H_2O_2 current was maintained constant and equal to 100 nA (initial sensitivity of $0.3 \text{ A cm}^{-2} \text{ M}^{-1}$). At the end of the 10th h , slight variations in the recorded current were found usually $<10\%$. With the proposed system, about 50 samples can be run per hour; for a typical $50 \mu\text{M}$ hydrogen peroxide, the relative standard deviation of 50 injections was estimated to be $<1.5\%$.

The operational stability of the PB modified electrode was improved (from $1-2 \text{ h}^{10}$ to $5-7 \text{ h}$) by fixing the times of deposition (with solution at pH 1 , R_1 reagent) and electrochemical activation (with solution at pH 5.5 , R_3 reagent) of the films as 40 and 600 s , respectively. Also, the elimination of the water of the PB precipitate (zeolitic water) by heating it up to 100°C for 1 h contributed to increase the performance of the sensor.

The Prussian Blue-modified glassy carbon electrode demonstrated potentialities (with good initial sensitivity and detection limit) to be used for hydrogen peroxide monitoring. In this way and in view of its inherent selectivity, it can be employed for the determination of hydrogen peroxide in different matrices (after some preliminary experiments) such as pharmaceutical formulations and cosmetics, atmospheric samples and foods.

The performance of the PB electrode (constant signal to H_2O_2 after 5 h) means that it can be suitable for the development of biosensors based on *oxidases* immobilized onto the modified surface. Experiments are in progress and will be reported separately (ref. 33).

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