



# Hydrogen peroxide detection under physiological conditions by Prussian blue stabilized using a polyelectrolyte–surfactant complex matrix

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## ABSTRACT

Poly(allylamine)–dodecylsulfate complex (PA–DS) is able to form a stable and layered structure onto graphite electrodes and can efficiently retain negatively charged ions like ferrocyanide. In this context, Prussian blue (PB) is electrochemically synthesized from a solution containing K<sub>3</sub>[Fe(CN)<sub>6</sub>] and FeCl<sub>3</sub> at pH 1.7. The electrochemical behavior of the new system is investigated in different supporting electrolytes (K<sup>+</sup> and Na<sup>+</sup>) and in acid and neutral media. Remarkable, well-defined and reversible voltammetric responses are obtained, especially under physiological conditions (neutral pH and 150 mM NaCl). In these conditions, the modified electrode is able to electrocatalyze the reduction of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) at micromolar levels, with a low detection limit and good sensitivity. These results represent an important improvement regarding further uses of PB in bioanalytical applications.

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## 1. Introduction

In living organisms hydrogen peroxide is a signaling molecule in regulating various biological processes, for example vascular remodeling, immune cell activation, stomatal closure and root growth, therefore H<sub>2</sub>O<sub>2</sub> detection is relevant *per se*, as it was claimed by Chen et al. [1] in a recent review.

The electrocatalytic reduction or oxidation of hydrogen peroxide has been matter of intensive studies; besides heme-proteins, several materials have been explored for such a purpose as carbon nanotubes dispersed in matrixes as nafion, ionic liquids and polyethylenimine [2–4], perovskites-type oxides [5], graphene [6], graphene combined with gold nanoparticules [7], and Prussian blue [8–11], among others. Even though all these systems have shown comparable results, Prussian blue represents an interesting option regarding the simplicity of its synthesis and the cost effective production of sensors based on this material.

Prussian blue (PB) or iron(III) hexacyanoferrate is one of the most studied coordination compounds with numerous applications in several fields like electrochromism, magnetic materials, ion selective electrodes, charge storage devices, electrocatalysis and biosensors. In PB, the main structure comprises arrays of high-spin

Fe<sup>3+</sup> centers bridged by [Fe(CN)<sub>6</sub>]<sup>4-</sup> units, where a metal-to-metal charge transfer (MMCT) transition from [Fe(CN)<sub>6</sub>]<sup>4-</sup> to Fe<sup>3+</sup> gives rise to its intense blue color. In sensing application, PB is known as an artificial peroxidase due its ability to mediate hydrogen peroxide reduction.

PB sensing application of physiological samples is restricted due to two major drawbacks, the lack of operational stability in neutral solutions due to the reduced form of PB, Prussian White (PW), that can be decomposed by hydroxide ions [8], and the fact that electrochemical processes in PB films present a specific K<sup>+</sup> ion transport characteristic due to its ordered zeolite cagelike structure. In particular, Itaya et al. [9] reported the irreversible damage of a PB film caused by Na<sup>+</sup> ions in solution. Therefore, it is necessary to improve the stability of PB at relatively high pH and in presence of physiological concentrations of Na<sup>+</sup> ions to further develop *in vitro* diagnostics applications.

In the last years different studies demonstrated that positive charged ions from species as cetyltrimethylammonium bromide (CTAB), poly(vinylpyrrolidone), poly(allylamine) hydrochloride, poly(diallyldimethyldiammonium chloride) and tetrabutylammonium toluene-4-sulfonate can effectively enhance the electrochemical stability of metal hexacyanoferrate [8,10,11].

We have recently presented a complex formed by poly(allylamine)–dodecylsulfate (PA–DS) as the basis for a coating to modify electrode surfaces [12,13]. The structure of this complex can be explained considering that the positively charged polyelectrolyte acts as a scaffold, while the surfactant retains its

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tendency to assemble in layered structures; another important feature of this complex is the fact that can incorporate anions, as an example we have shown the incorporation of ferrocyanide; finally, the complex can be applied practically on any surface (graphite, gold, silicon, polycarbonate, alumina) maintaining a great stability and expanding further its application to other areas [13,14]. Considering this result and other previous reports using either polyelectrolytes [10,15] or surfactants [11,16,17] we decided to explore the synthesis and stabilization of PB using this polyelectrolyte–surfactant matrix.

In this work we present the synthesis of PB in presence of the PA–DS matrix, exploring its ability to obtain a stable modified electrode able to efficiently catalyze the reduction of hydrogen peroxide at neutral pH and in presence of  $\text{Na}^+$  ions at physiological concentration.

## 2. Materials and methods

### 2.1. Reagents and materials

Sodium dodecyl sulfate (SDS) and poly(allylamine) (PA, MW: 65,000) were from Sigma Aldrich, potassium ferricyanide ( $\text{K}_3\text{Fe}(\text{CN})_6$ ) and ferric chloride ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) were from Merck. All other reagents were analytical grade and were used as received. Graphite screen printing electrodes were constructed as previously described [18].

### 2.2. PB–PA–DS electrodes

Graphite electrodes are modified by applying a PA–DS dispersion (C|PA–DS) as already reported [13]. PB was synthesized by immersion of the C|PA–DS electrode in a solution containing 0.5 mM  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  and 0.5 mM  $\text{K}_3[\text{Fe}(\text{CN})_6]$  at pH 1.7 (0.02 M HCl–0.15 M KCl) and cycling the potential between  $-155$  and  $1045$  mV at a scan rate of  $0.1 \text{ V s}^{-1}$ . Modified ITO electrodes were prepared in the same way.

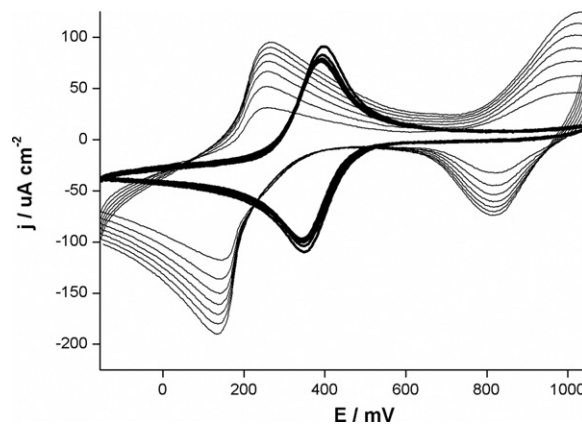
### 2.3. Measurements

Electrochemical experiments were carried out using a purpose-built potentiostat (TEQ-02). The system consisted of a working electrode, a platinum mesh counter electrode, and an Ag|AgCl reference electrode. Potentials are expressed against 3 M KCl|AgCl|Ag electrode. UV–vis absorption spectra were recorded on a Hewlett–Packard HP8453 diode array spectrophotometer; solid state UV–vis measurements were carried out with samples prepared on ITO glass electrodes. FTIR–ATR spectra were obtained using a Nicolet Magna-IR 560 Spectrometer.

## 3. Results and discussion

### 3.1. Electrochemical film formation and characterization

Polyelectrolyte–surfactant polymers are complexes of charged polymeric chains (polyelectrolytes) and oppositely charged small amphiphilic molecules (surfactants). The polyelectrolyte components can provide, for instance, mechanical strength and thermal stability, while the surfactants retain their tendency to assemble in layered structures [19]. They present the characteristic to form a stable colloidal suspension in water [20]. As it has been already reported [13], PA is a weak cationic polyelectrolyte of basic nature, with a reported pKa of 9.7. It is able to readily solubilize at neutral pH given a fully positively charged polymer. When this solution is mixed with an excess of SDS, a stable particle dispersion is produced. An interesting characteristic of this

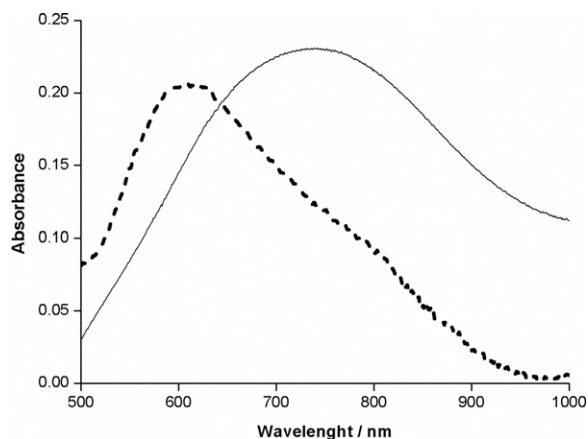


**Fig. 1.** Successive cyclic voltammograms showing PB film formation on a PA–DS modified graphite electrode (bold line), and PB film formation on a bare graphite electrode under the same conditions (thin line).

colloidal system is the simplicity of the particle preparation as far as the pH of the PA is below 10; considering the pKa of PA, at least half of the amino groups have to be protonated to induce the formation of a colloidal suspension. The white suspension containing the PA–DS complex can be applied on a graphite electrode, forming a stable composite on the surface. Here, an electrode modified in this way was immersed in a solution containing 0.5 mM  $\text{FeCl}_3$  and 0.5 mM  $\text{K}_3[\text{Fe}(\text{CN})_6]$  at pH 1.7. In this way PB was electrochemically synthesized by cycling the potential between  $-155$  and  $1045$  mV at a scan rate of  $0.1 \text{ V s}^{-1}$ . Fig. 1 shows the growth of PB in presence of the PA–DS complex (bold line) compared to PB formed on a bare electrode (thin line). Important differences can be observed between both films, the PB grown in presence of the PA–DS complex present an improvement in the reversibility of the couple with a peak difference of 48 mV against 133 mV for the PB grown on a bare graphite; also, the redox process is observed at a more positive potential ( $E_{1/2} = 0.37 \text{ V}$ ). A similar behavior is reported by Fiorito et al. [10], where multilayer films composed of PB and poly(allylamine hydrochloride) exhibit a redox couple at ca. 0.45 V. The second redox process observed on the bare electrode is also affected by the polyelectrolyte–surfactant structure appearing on a more positive potential where solvent decomposition simultaneously occurs.

This redox behavior is consistent with observations done by UV–vis absorptions experiments. The UV–vis absorption spectrum of PB–PA–DS in the solid state revealed a broad peak centered at 610 nm due to the  $\text{Fe}^{\text{II}}$  to  $\text{Fe}^{\text{III}}$  CT of PB in the PA–DS complex (Fig. 2, black dashed line). This absorption maximum is Blue-shifted from that of PB film formed on a bare ITO glass electrode. This suggests a strong interaction of the polyelectrolyte, positively charged, toward the cyanide complex, affecting the electron transference from  $\text{Fe}^{\text{II}}$  to  $\text{Fe}^{\text{III}}$  in PB. Uemura et al. [21] reported also larger absorption shifts to shorter wavelength (ca. 630 nm) in the solid state for PB nanoparticles protected by organic polymers such as poly(vinylpyrrolidone) (PVP) and poly(diallyldimethylammonium chloride) (PDDA), showing that the surface environments of the PB affect its inherent properties. Einaga et al. [15] also published that PB intercalated in bilayer films present analogous absorption shifts depending on the electronic nature of the bilayers.

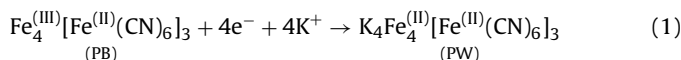
The FTIR–ATR spectrum of the PB film formed on a PA–DS modified graphite electrode exhibits a strong peak located at  $2073 \text{ cm}^{-1}$  associated with the cyano stretching mode of the CN groups in  $\text{Fe}^{\text{II}}\text{—CN—Fe}^{\text{III}}$  of PB (Fig. 3, bold line), presenting a similar shift as already reported for analogous PB systems [22].



**Fig. 2.** UV-vis absorption spectra for PB-PA-DS modified ITO glass electrode (black dashed line), and PB film formation on a bare ITO glass electrode under the same conditions (gray line).

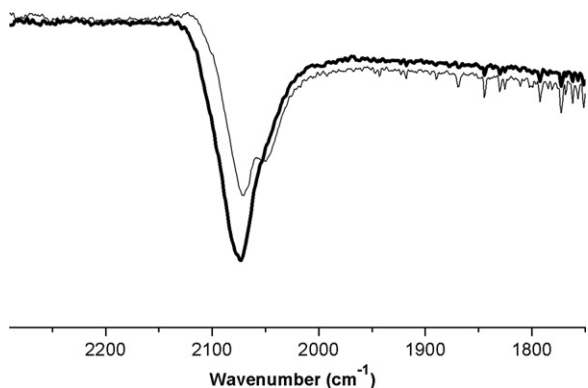
### 3.2. pH effect

The electrochemical process observed in Fig. 1 involves the following redox process:

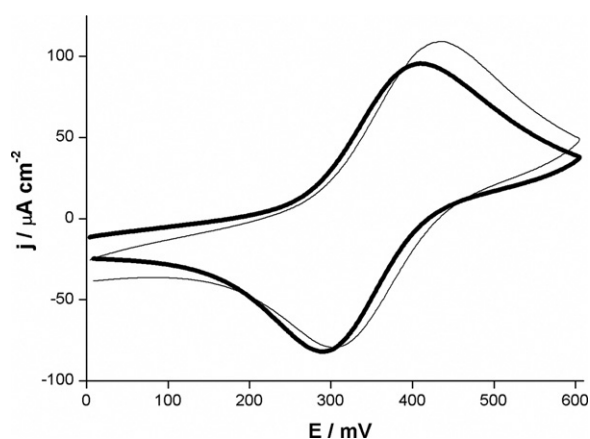


The reduced form of PB, Prussian White (PW) requires, for electroneutrality, the entry of  $\text{K}^+$  ions (or another appropriately sized electrolyte cation) into the lattice, and at high pH values it can be decomposed with the subsequent formation of iron hydroxo species; these facts hamper the use of PB in samples of clinical interest where the physiological pH is close to 7 and the presence of  $\text{Na}^+$ , different in size respect to  $\text{K}^+$ , is relatively high, for instance 140 mM in blood. Therefore, with the aim of stabilizing the response of PB at neutral pH and in presence of 150 mM  $\text{Na}^+$ , the PA-DS matrix was analyzed.

A PB-PA-DS modified graphite electrode was thoroughly rinsed with water and then immersed in acid medium, presenting the CV shown in Fig. 4 (gray line). Further, the same electrode was exposed to 150 mM KCl solution regulated at pH 7.0 with 10 mM Hepes (using  $\text{K}^+$  as counterion). Fig. 4 shows the CV obtained at pH 7.0 (black line) after 20 cycles. Compared to the response obtained in acid medium, the peak current observed at pH 7.0 is practically the same, while the peak potential is slightly shifted to lower potentials. Similar results were obtained by other authors at neutral pH using amino derivatives due to its ability to neutralize the effect of  $\text{OH}^-$  [8].



**Fig. 3.** FTIR-ATR spectra of the composite material PB-PA-DS (bold line) and of PB film formed on a bare graphite electrode (thin line).



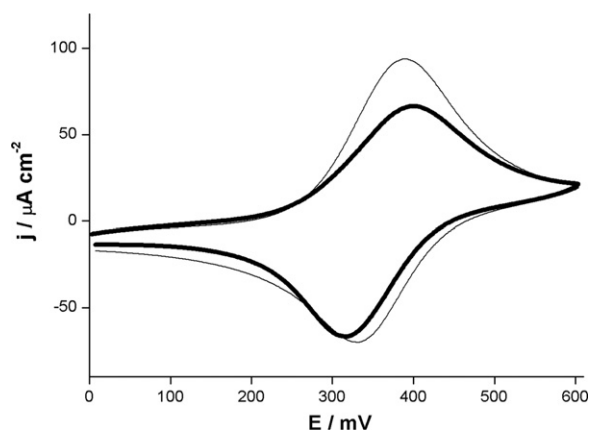
**Fig. 4.** Effect of pH on the CV of a PB-PA-DS modified graphite electrode in presence of 150 mM  $\text{K}^+$ ,  $\nu = 25 \text{ mV s}^{-1}$ ; pH = 1.7 (0.02 M HCl) (gray line) and pH = 7.0 (10 mM Hepes) (black line).

### 3.3. Ion effect

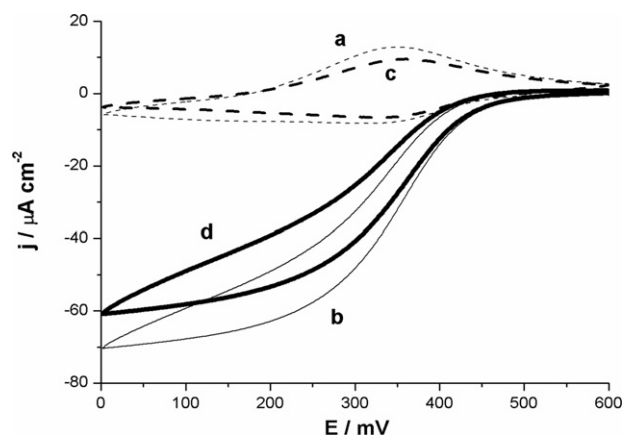
As PB redox process involves the exchange of cations (Eq. (1)), PB films exhibit electrolyte cation dependence on formal potentials and voltammetric shapes. Several authors have shown a charge reduction for PB films on graphite in presence of NaCl electrolyte compared to KCl [9,23]. This effect is attributed to the restrictions for the entry of hydrated  $\text{Na}^+$  ions (hydrated  $\text{Na}^+$  radius = 1.83 Å) into the PB lattice (channel radius = 1.6 Å), with respect to the entry of hydrated  $\text{K}^+$  ions (hydrated  $\text{K}^+$  radius = 1.25 Å). Even though it is possible to stabilize PB modified electrodes in the presence of a cationic surfactant, the lost of signal in solutions containing alkali ions other than  $\text{K}^+$  still remains [11].

As the PA-DS complex is formed by ion interactions, we decided to test the stability of the PB in this environment by immersion of the electrode in a 150 mM NaCl solution. Fig. 5 shows the response at two different pHs (1.7 and 7.0); the voltammograms are quite similar with a peak separation of 57 and 83 mV in acid and neutral pH, respectively. In comparison to the previous experiments carried out in presence of  $\text{K}^+$  ions, the peak separation is smaller in both pH conditions.

Remarkable, under these conditions (neutral pH and 150 mM  $\text{Na}^+$ ) reversible and stable signals are observed, an important result considering its application in sensing samples of biological origin.



**Fig. 5.** CV of a PB-PA-DS modified graphite electrode in presence of 150 mM  $\text{Na}^+$ ,  $\nu = 25 \text{ mV s}^{-1}$ ; pH = 1.7 (0.02 M HCl) (gray line) and pH = 7.0 (10 mM Hepes) (black line).



**Fig. 6.** Catalytic behavior at pH 7.0 (10 mM Hepes) for the same C|PB–PA–DS electrode,  $\nu = 10 \text{ mV s}^{-1}$ . CV in 150 mM KCl solution (a) and in presence of 1 mM  $\text{H}_2\text{O}_2$  (b). CV in 150 mM NaCl solution (c) and in presence of 1 mM  $\text{H}_2\text{O}_2$  (d).

### 3.4. Hydrogen peroxide catalysis

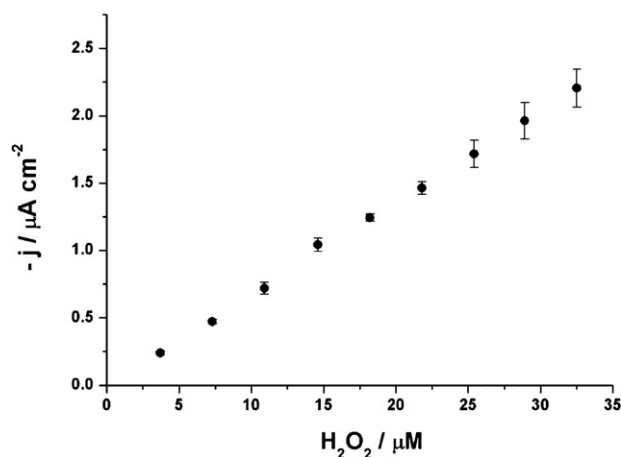
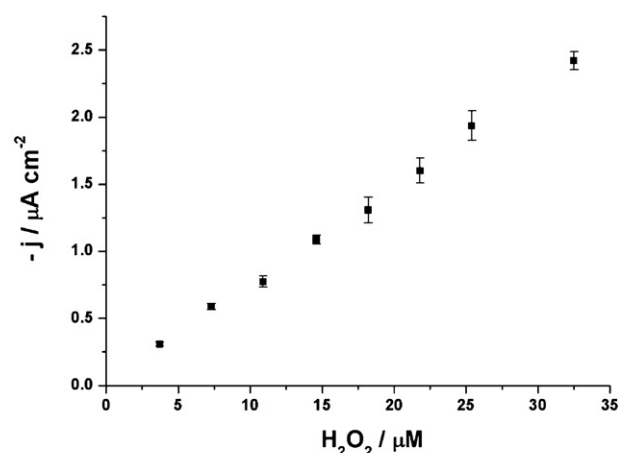
The PB–PA–DS modified graphite electrode is expected to find application in electrochemical detection of hydrogen peroxide. Moreover, the electrocatalytic properties of the modified electrode were tested in a solution regulated at pH 7.0, first in presence of  $\text{K}^+$  ions, then rinsed and tested in a solution containing  $\text{Na}^+$  ions at a physiological level. A close behavior can be observed in both cases (Fig. 6). It is evident that under physiological conditions this modified electrode exhibits a satisfactory catalytic response, since a current plateau is achieved and no oxidation peak is observed in the returning scans, typical features of an EC mechanism, a behavior not generally observed in others PB modified electrodes [24].

### 3.5. Chronoamperometric measurements

Chronoamperometric experiments were performed in order to study the performance of the PB–PA–DS modified electrodes during  $\text{H}_2\text{O}_2$  detection at physiological conditions. The experiments were carried out applying a potential of 0.0 V in a buffer solution at pH 7.0 (10 mM Hepes); first in presence of  $\text{K}^+$  ions, and later on tested in a solution containing  $\text{Na}^+$  ions at a physiological level. As mentioned before, a close behavior can be observed in both cases (Fig. 7).

The  $\text{H}_2\text{O}_2$  calibration curves were constructed carrying out amperometry at  $E = 0.0 \text{ V}$  under static conditions in the range of 3–35  $\mu\text{M}$  yielding a sensitivity of  $74.2 \mu\text{A mM}^{-1} \text{ cm}^{-2}$  in presence of  $\text{K}^+$  ions and a sensitivity of  $67.7 \mu\text{A mM}^{-1} \text{ cm}^{-2}$  under physiological conditions, with a detection limit of 1.3  $\mu\text{M}$  and 0.8  $\mu\text{M}$ , respectively, calculated on the basis of  $3\sigma$  ( $\sigma$  being the residual standard deviation of the  $y$ -intercept in the calibration curve). Chronoamperometric response curves (Figs. S1 and S2) are shown in the supporting information, showing a fast response to changes in concentrations.

Prussian blue based electrochemical detection of hydrogen peroxide has been matter of intense research due their immediate clinical and biological applications. Several works have been presented in the literature partially solving the issues presented in this work. Varma et al. [25] modified with Prussian blue a glassy carbon electrode in presence of tetrabutylammonium toluene-4-sulfonate and polyethyleneimine; the modified electrode was applied in a FIA system using a carrier solution at neutral pH and containing 0.1 M KCl. Liu et al. [24] prepared a thin film of PB deposited on a platinum electrode by self-assembly process including multiple sequential adsorption of ferric ions and hexacyanoferrate ions, in this case an impressive sensitivity was obtained at neutral pH; however, no studies regarding the effect of  $\text{Na}^+$  ions were presented. On



**Fig. 7.** Calibration curves obtained for  $\text{H}_2\text{O}_2$  detection at pH 7.0 (10 mM Hepes) from a chronoamperometry at  $E_{\text{app}} = 0.0 \text{ V}$  for the same C|PB–PA–DS electrode under static conditions, in 150 mM KCl solution (upper) and in 150 mM NaCl solution (lower).

the other hand, Vittal et al. [11] used CTAB modified electrodes to attenuate the effect of  $\text{Na}^+$  ions, without reporting results on the catalytic properties toward hydrogen peroxide detection of this system. More recently other published works presented the stabilization of Prussian blue based on the insertion of Nickel ions [26], by combination of PB with  $\text{Fe}_2\text{O}_3$  nanoparticles [27], or by the development of hybrid films containing Prussian blue, CTAB and gold nanoparticles [16]. Even though the measurements in these works are carried out close to neutral pH values, between 6 and 9, only one of them [16] presents hydrogen peroxide catalytic currents obtained in presence of 20 mM  $\text{Na}^+$ , far below from the values present in blood (140 mM), with a detection limit of  $1 \times 10^{-7} \text{ M}$  under stirring conditions. Finally, Salazar et al. [17] reported the electrochemical synthesis of PB using different cationic surfactants obtaining a sensitivity of  $630 \mu\text{A mM}^{-1} \text{ cm}^{-2}$  but under stirring and in the presence of 0.1 M KCl and 0.02 M HCl. Taking into account these previous works, the results presented here represent a successful trade off among different features required for the use of Prussian blue as an artificial peroxidase, being stable at neutral pH and with negligible effect of  $\text{Na}^+$  ions at physiological concentrations in order to achieve a low detection limit and good sensitivity. Finally, the results presented here were obtained in static conditions allowing its application in POCT devices and *in vivo* studies.

## 4. Conclusions

In this study, a new modified electrode composed of PB, PA and SDS is easily prepared from aqueous solution. PB grown in

the polyelectrolyte–surfactant matrix PA–DS presents several differences to either PB grown on a bare electrode or in presence of the polyelectrolyte in multilayer assemblies [10]. A more positive redox potential and a more reversible redox couple together with a Blue-shifted absorption maximum are observed in this system, suggesting that the surface protecting polymers significantly affect the Coulombic energy expended in transferring the electron from Fe<sup>II</sup> to Fe<sup>III</sup> in PB. This modified electrode can be easily prepared compared to other systems involving layer by layer deposition or the introduction of nanoparticles. The electrochemical behavior of this PB modified electrode shows remarkable features as reversible and stable signals under neutral pH and 150 mM Na<sup>+</sup>. In these conditions, the system exposed to hydrogen peroxide presents a catalytic response at micromolar levels, opening a new route to explore its application in biosensor development to be applied directly on biological samples.

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### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.snb.2013.02.081>.

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### Biographies

**Carina Gaviglio** received her Ph.D. degree from the University of Buenos Aires in 2009. She is currently completing her postdoctoral training on the development of new structures on electrodes surfaces for improving the performance of sensor devices, with application on samples of biological interest, under the supervision of Fernando Battaglini.

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