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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 $K_3[Fe(CN)_6]$ and $FeCl_3$ at pH 1.7. The electrochemical behavior of the new system is investigated in different supporting electrolytes (K^+ and Na^+) 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_2O_2) 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.

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_2O_2 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^{3+} centers bridged by $[Fe(CN)_6]^{4-}$ units, where a metal-to-metal charge transfer (MMCT) transition from $[Fe(CN)_6]^{4-}$ to Fe^{3+} 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^+ 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^+ 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^+ 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 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 ($K_3Fe(CN)_6$) and ferric chloride ($FeCl_3{\cdot}6H_2O$) 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 $FeCl_3{\cdot}6H_2O$ and 0.5 mM $K_3[Fe(CN)_6]$ 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 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, an stable particle dispersion is produced. An interesting characteristic of this 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 $FeCl_3$ and 0.5 mM $K_3(FeCN_6)$ 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 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 ($E_{1/2} = 0.37$ 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.

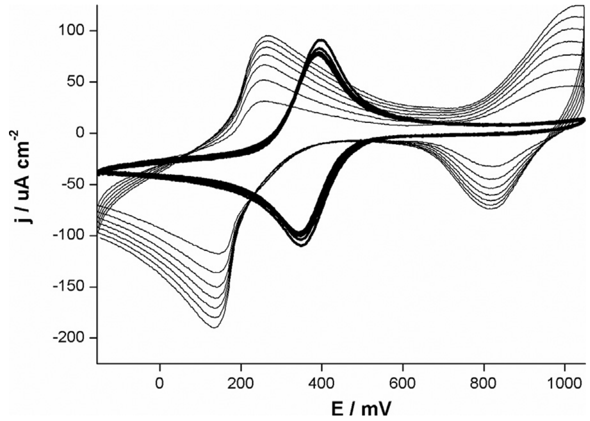

Fig. 1. Successive cyclic voltammograms showing PB film formation on a PA–DS modified graphite electrode (bold line), and PB film formation on a bare graphite electrode under the same conditions (thin line).

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 Fe^{II} to Fe^{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 Fe^{II} to Fe^{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 cm^{-1} associated with the cyano stretching mode of the CN groups in Fe^{II}—CN—Fe^{III} of PB (Fig. 3, bold line), presenting a similar shift as already reported for analogous PB systems [22].