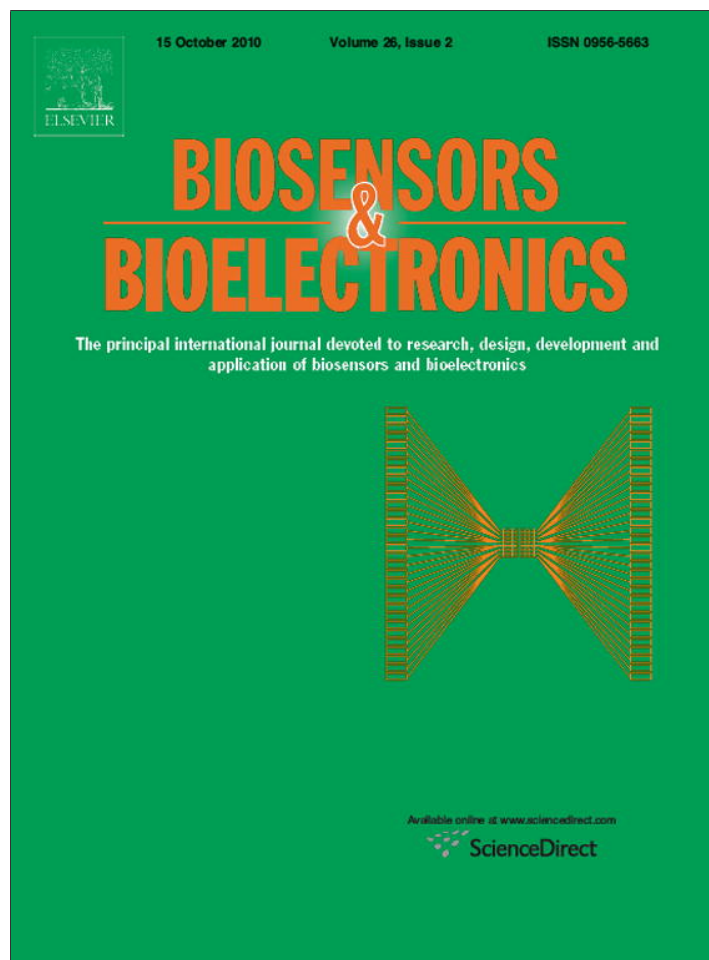


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Short communication

Structure effects of self-assembled Prussian blue confined in highly organized mesoporous TiO₂ on the electrocatalytic properties towards H₂O₂ detectionMartín Gaitán^a, Vinicius R. Gonçalves^c, Galo J.A.A. Soler-Illia^b,
Luis M. Baraldo^{a,*}, Susana I. Córdoba de Torresi^c^a INQUIMAE-Facultad de Ciencias Exactas y Naturales - UBA, Pabellón 2, Ciudad Universitaria, C1428EHA, Ciudad de Buenos Aires, Argentina^b Unidad de Actividad Química, Comisión Nacional de Energía Atómica, Centro Atómico Constituyentes, Av. Gral Paz 1499, San Martín, B1650KNA Buenos Aires, Argentina^c Instituto de Química, Universidade de São Paulo, C.P. 26077, 05513-970 São Paulo, SP, Brazil

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ABSTRACT

Here we report the derivatization of mesoporous TiO₂ thin films for the preparation of H₂O₂ amperometric sensors. The coordination of the bifunctional ligand 1,10 phenantroline, 5,6 dione on the surface Ti(IV) ions provides open coordination sites for Fe(II) cations which are the starting point for the growth of a layer of Prussian blue polymer. The porous structure of the mesoporous TiO₂ allows the growth, ion by ion of the coordination polymer. Up to four layer of Prussian blue can be deposit without losing the porous structure of the film, which results in an enhanced response of these materials as H₂O₂ sensors. These porous confined PB modified electrodes are robust sensors that exhibit good reproducibility, environmental stability and high sensitivity towards H₂O₂ detection.

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1. Introduction

Prussian blue (PB) modified electrodes are excellent catalysts for H₂O₂ reduction at low potentials, minimizing the interference caused by reductant species (Cheng et al., 2007; Karyakin et al., 1994). Consequently, they have been widely studied as mediators in oxidase enzymes based biosensors (Karyakin et al., 1995; Ricci and Palleschi, 2005). Different techniques have been used for the incorporation of Prussian blue into the electrode including electrochemical deposition (Karyakin et al., 1994, 1995), sequential adsorption of ions (Guo et al., 1999) mechanical attachment to the electrode (Dostal et al., 1995) and layer by layer deposition of nanoparticles (Fiorito et al., 2005; Liu et al., 2009; Wang et al., 2007). The sensitivity of the resulting electrodes depends strongly on the method of fabrication and it has been observed that nanostructured electrodes present an enhanced current response (Karyakin et al., 2004). The increased area of the nanostructured electrodes is also a desired feature because it

increases the contact with the other components of the sensor. This property makes them attractive as mediators for enzyme based biosensors. Several configurations of Prussian blue films and enzymes have been explored and these efforts have produced suitable sensors for a variety of substrates (Heimer et al., 1996).

The development of a nanostructured Prussian blue film processed as a robust, sensitive and highly reproducible supported system with a large surface area would constitute a step forward in this area. These properties could be achieved using the well characterized family of mesoporous oxide thin films (MOTF) as a platform. The MOTF, prepared using the surfactant template technique (Crepaldi et al., 2003) exhibit very large surface areas, tailored pore size, surface and connectivity, which make the entire pore system readily accessible to species in solution (Sanchez et al., 2008). These characteristics make their functionalization, by tailored organic groups or inorganic complexes, an open field of diverse possibilities in the development of new catalysts, sensors, selective membranes, etc. (Soler-Illia and Innocenzi, 2006). In this communication we report the functionalization of thin TiO₂ mesoporous films by coordination assisted layer by layer deposit of Prussian blue (PB) polymer and its performance as H₂O₂ sensors.

* Corresponding author. Tel.: +54 11 31793115; fax: +54 11 45763341.

E-mail addresses: baraldo@qi.fcen.uba.ar, luis.baraldo@gmail.com (L.M. Baraldo).

2. Materials and methods

2.1. Mesoporous TiO₂ thin films

Mesoporous TiO₂ thin films were deposited by dip-coating an ITO conductive substrate in a Ti(IV) ethanol–water sol, containing a non-ionic block-copolymer template (Pluronic F127), followed by thermal treatment at 350 °C, as previously reported (Crepaldi et al., 2003). This procedure generates a highly organized mesoporous film with 8 nm diameter pore arrays, derived from a uniaxially distorted cubic *Im3m* mesostructure ($a = 16$ nm). Films with 30% pore volume and 130 nm thickness were used in this work.

In previous works, we have demonstrated by using electrochemical techniques that these pores are easily accessible to small and medium sized molecules. In addition, we have shown that pore surfaces can be modified with organic functional groups, which can tune the properties, like philicity and, accessibility, of the whole pore system (Otal et al., 2006). This concept can be extended to achieve the homogeneous assembly of PB within the pores. In order to incorporate the coordination polymer on the TiO₂ surface we have taken advantage of the bifunctional ligand 1,10 phenantroline 5,6 dione (Calderazzo et al., 1999; Smith and Cagle, 1947). As observed for similar ligands (Jankovic et al., 2009; McNamara et al., 2008), the dione functionality binds irreversibly to the Ti(IV) surface sites, generating a very stable functional monolayer. The nitrogen atoms on the ligand remain available for coordination. Thus, when the modified titania film is exposed to a Fe(II) solution, cation coordination readily takes place. Subsequent exposure of this modified electrode to a K₃Fe(CN)₆ solution leads to the observation of a blue color, characteristic of the metal to metal charge transfer transition of Prussian blue. The immersion sequence in Fe(II) and [Fe(CN)₆]³⁻ solutions can be then repeated several times. This cycle allows building up the material layer by layer. As the two reactants have different coordination functionalities, they can only react with the modified surface, preventing the formation of aggregates and preserving the mesoporous structure.

2.2. Electrode preparations

The ITO electrodes were immersed in a 0.5 M 1,10 phenantroline 5,6 dione solution in ethanol, for 10 h and rinsed with ethanol. To remove the excess ligand from the substrate surface, the samples were dried. Incorporation of the Prussian blue polymer was accomplished by immersion of the ITO into a Fe(SO₄)₂ 0.5 M solution for 30 min, followed by immersion into a K₃Fe(CN)₆ 0.5 M solution for 30 min. After each treatment the modified electrode was removed from the solution, rinsed in deionized water and dried. This procedure was repeated for every double layer until the desired number of layers was deposited. The active area was 0.25 cm².

3. Results and discussion

3.1. UV–vis spectra

Development of the PB films onto the titania walls can be followed through the evolution of UV–vis spectra. Even for the first layer, the metal to metal charge transition is already noticeable due to the large surface of MOTF film. The energy of this transition is substantially shifted to the blue compared with the observed transition for the bulk PB. Upon the addition of further layers of Prussian blue this intensity of the transition grows, indicating an increase in the amount of polymer deposited, and the band maxima shifts to the red approaching the value observed for the bulk Prussian blue (S3). This shift can be related to a change in the structure of the polymer as more ions become available to establish a three-dimensional

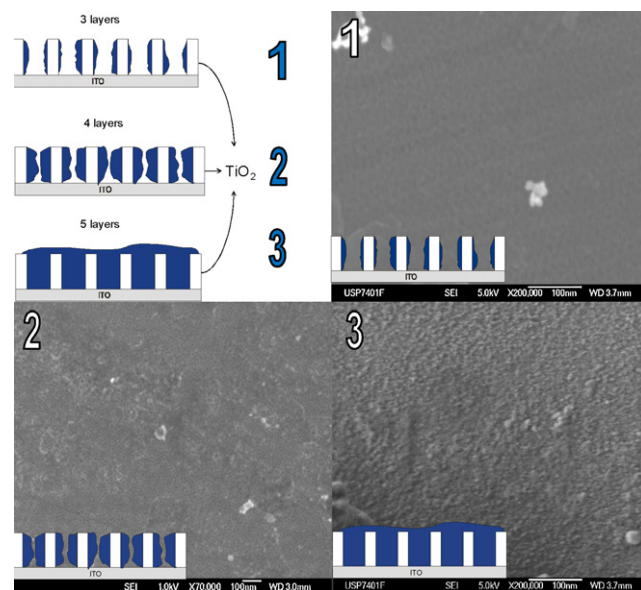


Fig. 1. FEG-SEM images of electrodes with 3, 4 and 5 layers of Prussian blue.

network, decreasing the number of defects. The evolution of FTIR spectra of the films confirms the above observation. For the first layer, a strong peak at 2080 cm⁻¹ is clearly observed, which can be assigned to the cyanide stretch for the [Fe(CN)₆]⁴⁻ moiety, similar to the observed in bulk PB. The presence of [Fe(CN)₆]⁴⁻ indicates that, for the first layer, the film already presents the electronic configuration observed in the bulk Prussian blue, due to the redox reaction between the added [Fe(CN)₆]³⁻ and the Fe(II) ions already incorporated into the film.

3.2. Change of the film structure with the number of layers

FEG-SEM images of the films indicate that the mesoporous structure is preserved for the initial Prussian blue layers. The mesopore structure is clearly visible for a modified electrode built up with three and four layers (Fig. 1 and S5). On the other hand, for electrodes modified with five layers the porous structure is lost and it is replaced by a granulostructure typical of the Prussian blue films deposited by sequential coordination of ions (Itaya et al., 1986).

The change in the structure of the films is also reflected in the ability of the electrode to incorporate more Prussian blue layers. The incorporation of the PB layers can be followed by FTIR (cyanide stretch at 2080 cm⁻¹, S3), UV–vis (metal to metal charge transfer band, S3) and its electrochemical response (reduction of the Fe(III), S4). The evolution of the electrodes followed by these three techniques clearly shows that after the fourth layer the amount of PB polymer incorporated in each layer, is greatly diminished. This behavior is consistent with the proposed filling of the pores. Upon the lost of the porous structure the coordination takes place only at the geometrical surface of the electrode which is two orders of magnitude smaller than the surface of the porous electrodes and hence very little polymer is incorporated after the fifth step.

3.3. Chronoamperometric measurements

Chronoamperometric experiments in a three-electrode electrochemical cell were performed in order to study the performance of Prussian blue modified electrodes during H₂O₂ detection. The experiments were carried out in systems containing 5 mL of KCl 0.1 mol L⁻¹ + HCl 0.1 mol L⁻¹ or 5 mL of KCl 0.1 mol L⁻¹ + HCl

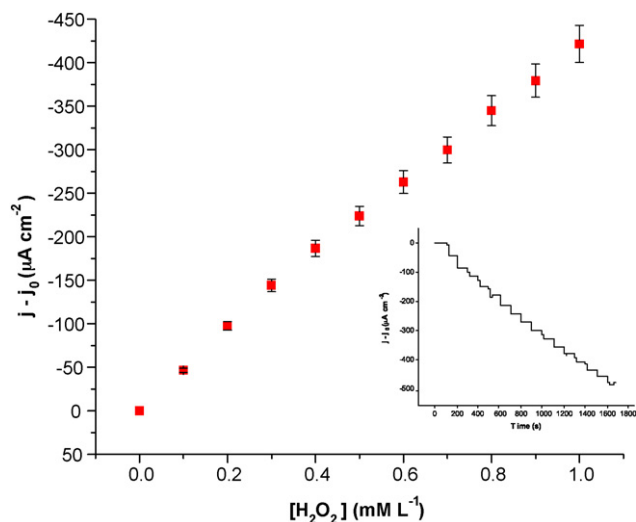


Fig. 2. Analytical curve obtained for H_2O_2 detection for mesoporous electrode modified with four layers of PB. The detection limit is $0.03 \pm 0.01 \mu\text{M}$. Time response = 10 s (until completely stabilized the current). $E_{\text{app}} 0.0\text{V}$ in a $\text{KCl } 0.1\text{ mol L}^{-1} + \text{HCl } 0.1\text{ mol L}^{-1}$ electrolytic solution.

0.1 mol L^{-1} with moderate stirring during the measurements. Initially, the work electrode was held at the operational potential of 0.0V , just below the observed reduction wave (S4), until background current stabilization. After this time, $10\text{ }\mu\text{L}$ of a freshly 50 mmol L^{-1} H_2O_2 solution were successively added to construct the amperometric curves. Each H_2O_2 addition corresponded to a 0.1 mmol L^{-1} increment in the analyte concentration. Fig. 2 shows the amperometric response of a electrode modified with four layers of Prussian blue towards H_2O_2 detection. The modified electrodes are remarkably robust and stable. We have tested the reutilization of these electrodes for seven times and we have not seen any significant loss of activity. The reported sensitivities are also very reproducible, as electrodes with the same amount of layers give consistently the same value of sensitivity with a variation of only a five percent. The high reproducibility must be related to the extremely controlled size (diameter/depth) of the pores achieved by the sol–gel method for preparing the mesoporous platforms. In this way, the amount of TiO_2 sites available for coordination of the bifunctional ligand is very similar for different electrodes and the amount of Prussian blue that can be coordinated in each deposited layer is nearly always the same. The observed sensitivities depend strongly on the number of layers (S4). A noticeable increase in the sensitivity is observed after the deposition of the second layer. Addition of further layers continue to increase the sensitivity of the electrodes until reaching a value of $415 \pm 20\text{ }\mu\text{A cm}^{-2}\text{ mmol}^{-1}\text{ L}$ for the electrodes with four layers of Prussian blue. In this case, the sensor exhibited a linear range up to 1.4 mM , with a detection limit of $0.03 \pm 0.01\text{ }\mu\text{M}$ (calculated as three times the background noise of each step of the chronoamperogram, see S4). This value is similar to the detection limit achieved with Prussian blue based nanoelectrode arrays and it is lower than the conventional electrodeposited Prussian blue (Karyakin et al., 2004).

3.4. Effect of the structure change on the sensitivity of the electrodes

The mesoporous structure of the film makes most of the Prussian blue active sites accessible to H_2O_2 , which can easily diffuse through the pore network and across the few layers of deposited material. Hence the assembly of more layers of Prussian blue increases the amount of polymer available to react with H_2O_2 and a

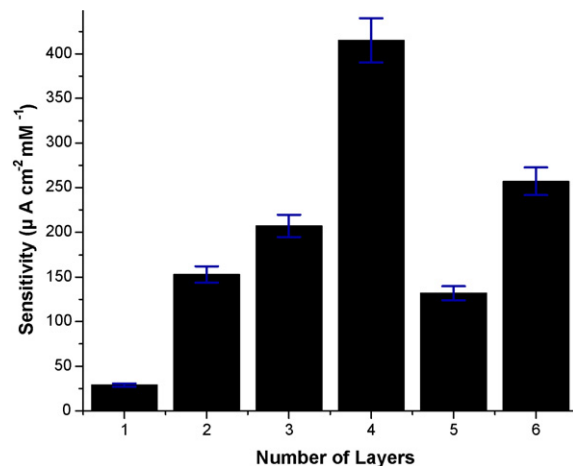


Fig. 3. Relation of the sensitivity with number of PB layers. Error bars correspond to the mean of seven different electrodes.

higher response is observed. The sensitivity of these modified electrodes is among the highest reported for Prussian blue films (Ricci and Palleschi, 2005). In a previous paper (Fiorito et al., 2005) it was reported $305\text{ }\mu\text{A cm}^{-2}\text{ mmol}^{-1}\text{ L}$ for electrodeposited PB films or up to $103.5\text{ }\mu\text{A cm}^{-2}\text{ mmol}^{-1}\text{ L}$ for electrodes prepared by the immobilization of PB nanoparticles. However, after the deposition of the fourth layer, further deposit of Prussian blue results in a drastic decrease of the sensitivity to $132 \pm 6\text{ }\mu\text{A cm}^{-2}\text{ mmol}^{-1}\text{ L}$. This result is very interesting because it is a direct indication of the confinement behavior of Prussian blue inside the mesoporous structure. The addition of a fifth bilayer leads to the filling of the pore volume, as depicted in Fig. 1. In this case, the superficial active sites available to the H_2O_2 decrease significantly as the surface/volume ratio is not so advantageous anymore. Building up the sixth bilayer, the sensitivity increases again to $257 \pm 13\text{ }\mu\text{A cm}^{-2}\text{ mmol}^{-1}\text{ L}$ because there is more PB present, but the surface/volume ratio continues similar to the observed for the bulk PB (Fig. 3).

4. Conclusions

The high response and the high surface area of these electrodes make them particularly suitable for the development of biosensors using new types of enzymes. For example, the enzymatic activity of some oxidases is low and the small amounts of H_2O_2 produced by these enzymes have precluded their efficient use in a biosensor. The high response of the electrodes described here could be enough for the detection of the activity of this kind of enzymes, expanding the range of substrates that could be detected using this type of electrodes.

In summary, we have shown that functionalized mesoporous TiO_2 thin films can be excellent amperometric sensors. In this case, we have modified the surface of the TiO_2 film with a bifunctional ligand that allow the introduction of Fe(II) ion with open coordination sites on the surface. Then, the PB coordination polymer is assembled layer by layer. This procedure results in a thin layer of PB that does not disrupt the mesoporous structure of the film and allows control on the amount of PB confined in the pores. The thin TiO_2 films modified with Prussian blue are robust amperometric sensors that exhibit high reproducibility, environmental stability and high sensitivity for the H_2O_2 detection. Moreover it is possible to control several variables of their structure as the number of PB layers, the thickness and, pore size of the film. Correlation of sensor response with these variables could result in modified electrodes with even better sensing properties.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.07.026.

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