Synthesis, characterization and immobilization of Prussian blue nanoparticles. A potential tool for biosensing devices†

Pablo A. Fiorito, Vinicius R. Gonçales, Eduardo A. Ponzio and Susana I. Córdoba de Torresi*

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Prussian blue (PB) particles with the size of *ca.* 5 nm were synthesized and immobilized in a multilayer structure, as a strategy for the potential development of an amperometric transducer for oxidase-enzyme-based biosensors. Multilayer films composed of PB and poly(allylamine hydrochloride) (PAH) were prepared *via* layer-by-layer (LbL) sequential deposition. The process was carefully monitored by UV–vis spectroscopy and cyclic voltammetry. The increase of the redox current peaks during the layer-by-layer deposition demonstrated that charge propagation within the film occurs. Linear increase of UV–vis absorbance with the number of deposited bilayers indicates that well-organized systems have been elaborated. ITO electrodes coated with PB/PAH films were used successfully for detecting H_2O_2, sensitivity being dependent on the number of PB/PAH layers.

The monitoring of hydrogen peroxide is of great importance in medicine, environmental control and industry.[1] Prussian blue (PB), a typical hexacyanoferrate, is a well-known electrocatalyst used in the analytical determination of this compound. The peculiar characteristics of the reduced form of PB, Prussian white (PW), is able to catalyze the electrochemical reduction of hydrogen peroxide at low potentials.[2] Karyakin *et al.* showed that the catalytic rate constant for H_2O_2 reduction at PB films was 3×10^{-3} mol^{-1} L s^{-1},[3] similar to that obtained with peroxidase. Prussian blue has been intensively investigated in the form of thin polycrystalline electrodeposited films. The use of nanoparticles can improve the analytical performance for hydrogen peroxide detection; nano-sized particles have unique physical and chemical properties, often showing very interesting peculiarities unmatched by their bulk counterpart.[4] The large surface-to-volume ratio and the increased surface activity of nanoparticles, when compared to those of bulk materials, enable their use in catalysis and sensing.

Prussian blue nanoparticles were synthesized by a method previously reported by Liu *et al.*[5] and modified by using sonochemistry. In order to build-up a hydrogen peroxide sensor, Prussian blue nanoparticles were immobilized by assembly onto a flat electrode by using the layer-by-layer (LbL) method.[6] In the conventional LbL process, substrates are alternately dipped in solutions containing anionic and cationic species, which adsorb onto the solid support mainly *via* electrostatic interactions (for highly charged polyelectrolytes). One advantage of the LbL method is that it allows remarkable nanometre-level control over the thickness of the resulting layers and, within the limit of interpenetration of the polyelectrolyte layers, over vertical structuring.[7] Since the LbL technique is based on dipping flat substrates in aqueous solutions, there would be basically no limitation in size and shape of these substrates with a water-based environmental friendly process. There are numerous reports describing the inclusion of nanoparticles into LbL assembled polyelectrolyte-based multilayer films. Various inorganic (*e.g.* silica, magnetite, gold and semiconductor) nanoparticles have been LbL assembled with oppositely charged polyelectrolytes.[8] In this paper, we report the multilayer build-up of thin films of Prussian blue nanoparticles, prepared by the alternate adsorption of the oppositely charged linear polyelectrolyte poly(allylamine hydrochloride) (PAH) onto ITO (indium–tin oxide, sheet resistance $< 20\ \Omega$ $square^{-1}$).

Prussian blue nanoparticles were synthesized by the reaction of $FeCl_3$ and $K_3Fe(CN)_6$ in the presence of a slight excess of H_2O_2 in an ultrasonic bath ($\sim$40 kHz). They were dispersed in water and a dialysis procedure was carried out over 5 days in order to obtain a very stable, blue colored dispersion that was used to prepared LBL multilayer films.

Fig. 1 shows the HRTEM micrographs of PB nanoparticles obtained by this procedure. It can be observed spherical nanostructures of *ca.* 5 nm diameter (Fig. 1a) with well-defined atomic planes (Fig. 1b), these data being corroborated by X-ray diffraction experiments (see supplementary information provided). As mentioned before, sonochemical stirring has been used as a modification of the synthetic approach proposed by Liu *et al.*[5] It must be pointed out that, while these authors obtained nanoparticles measuring *ca.* 50 nm in diameter, in this work the use of sonochemistry allows us to obtain spherical nanostructures ten times smaller with a very narrow size distribution.

The growth of the films prepared by LbL, was followed by UV–vis absorption spectroscopy by recording successive spectra after the deposition of each PAH/PB bilayer. Fig. 2 shows that the absorbance recorded at 710.7 nm, increases linearly with the number of bilayers indicating that PB nanoparticles are effectively immobilized by controlling the thickness of the film at the nanoscale level. On the other hand, it can be observed that the amount of PB immobilized in each bilayer is almost the same. Cyclic voltammetry experiments were carried out with films of different amounts of PAH/PB bilayers (see electronic supplementary information (ESI)†). Typical *j–E* electrochemical responses were obtained showing a well-defined redox couple at *ca.* 0.45 V (*vs.* Ag/AgCl/$Cl^-_{(sat)}$), corresponding to Prussian white (PW) to Prussian blue conversion. The current intensity of the voltammetric peaks increases linearly with the amount of bilayers, putting in evidence that PB nanoparticles are electrically connected to each other due to the well-known interpenetration phenomenon among layers that allows charge transfer through an electron-hopping

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† Electronic supplementary information (ESI) available: Prussian blue XRDP pattern, dependence of voltammetric response on number of bilayers. See http://www.rsc.org/suppdata/cc/b4/b412583e/
*storresi@iq.usp.br

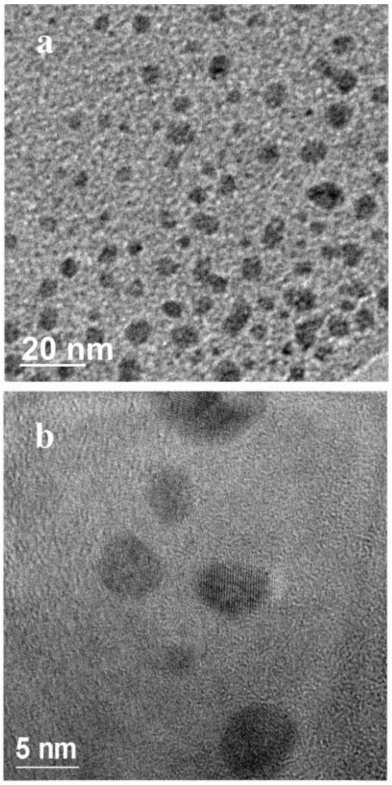

Fig. 1 HRTEM micrographs of Prussian blue nanoparticles synthesized by sonochemical method.

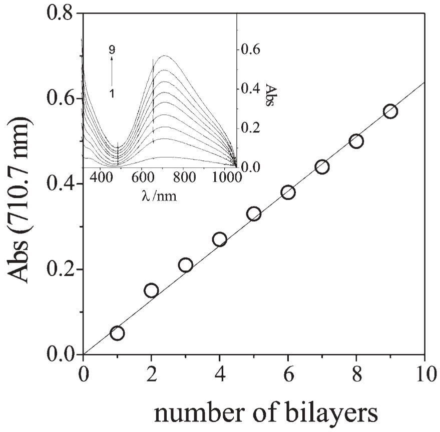

Fig. 2 Absorbance *vs.* number of bilayers recorded at 710.1 nm for PAH/PB nanoparticles films. The inset shows the absorbance spectra recorded after each bilayer deposition.

process. As mentioned before, Prussian blue can be electrochemically reduced to Prussian white (PW), which is capable of catalysing the reduction of hydrogen peroxide at low potentials, according to eqn. (2).

$$\underset{\text{PB}}{Fe_4[Fe(CN)_6]_3} + 4e^- + 4K^+ \rightarrow \underset{\text{PW}}{Fe_4K_4[Fe(CN)_6]_3} \qquad (1)$$

$$\underset{\text{PW}}{Fe_4K_4[Fe(CN)_6]_3} + 2H_2O_2 \rightarrow$$

$$\underset{\text{PB}}{Fe_4[Fe(CN)_6]_3} + 4OH^- + 4K^+ \qquad (2)$$

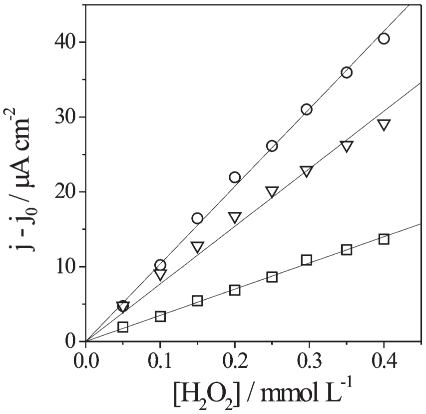

Fig. 3 Linear range analytical curves obtained for H_2O_2 detection for LBL films of (□) 5, (∇) 10 and (○) 15 PAH/PB nanoparticles bilayers. $E_{app} = 0.0$ V in a KCl 0.1 mol L^{-1} + HCl 0.1 mol L^{-1} electrolytic solution.

Recently, an oxalate biosensor based on the immobilization of oxalate oxidase onto a Prussian blue modified electrode was reported to show high performance and stability.[9] Further, an electrodeposited Prussian blue film was used as the substrate for the effective immobilization of glucose oxidase by the LbL technique for the preparation of a reliable glucose biosensor.[10] In this way, the synthesis and immobilization of PB nanoparticles present a very elegant strategy for preparing sensing devices based on oxidase-enzymes. The LbL technique opens enormous possibilities of preparing different molecular architectures with the use of PB nanoparticles.

Fig. 3 shows the experimental curves obtained for the amperometric detection of H_2O_2 performed with electrodes of different amounts of PAH/PB nanoparticle bilayers. It can be seen that, for all electrodes, a good linear range is obtained up to 0.4 mmol L^{-1} of H_2O_2. On the other hand, attention must be paid to the fact that the analytical response depends strongly on the amount of bilayers. The calculated sensitivities increases linearly with the amount of immobilized PB being 34.5 μA $mmol^{-1}$ L cm^{-2} for the electrode containing 5 bilayers, 76.8 for 10 bilayers and 103.5 for 15 bilayers. These results clearly indicate electrical connections among nanoparticles in different bilayers in the sense of obtaining an amplification of the analytical response with the amount of catalyst controlled at the nanoscale level.

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Pablo A. Fiorito, Vinicius R. Gonçales, Eduardo A. Ponzio and Susana I. Córdoba de Torresi*
Instituto de Química, Universidade de São Paulo, C.P. 26077, 05513-970, São Paulo (SP), Brazil. E-mail: storresi@iq.usp.br

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