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Electrochemical reduction of nitroprusside on a glassy carbon electrode modified by poly-L-lysine films

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Abstract The present work describes the preparation and characterization of polyelectrolyte coatings of poly-L-lysine (PLL) to modify a glassy carbon electrode and its application to the pre-accumulation of nitroprusside (NP). The effects of the coating on the electrochemical reduction of NP were investigated. The performance of the modified electrode indicates that the drug can be immobilized by electrostatic interaction and the voltammetric signal monitored at all pH values in the range of 2–12. The strong interaction between NP and PLL stabilizes the complex on the electrode surface and minimizes the chemical reaction of lost CN^- ions as a subsequent reaction of electron transfer, which could improve the action mechanism of NP.

Keywords Sodium nitroprusside · Poly-L-lysine · Chemically modified electrode

Introduction

During the last decade, nitric oxide (NO) has become the subject of growing interest in the field of biology, medicine, and pharmacy. NO is a biologically active molecule present in the body, which can play an important role in vascular regulatory effects such as vasodilatation, neurotransmitter, and inhibition agent of platelet aggregation [1, 2]. The NO

molecule has a short half-life, limiting its diffusion, which can be affected by its local concentration, type of biological sample matrix, and source of production [3]. Considering its physiological importance and short lifetime, studies conducted to understand controller systems for the releasing of NO have significant importance.

Among parenteral drugs destined for use in hypertensive emergencies, the nitroprussiate has been in general use for a long time. Nitroprussiate ($Na_2[Fe(CN)_5NO]$) is a nitrosyl complex, which has been widely applied in medical clinical applications, whose potential use is related to the liberation of NO in blood vessels. Nevertheless, some studies [4, 5] have pointed out the fact that along with liberating NO, nitroprussiate can also liberate cyanide, which can interfere in the drug action mechanism. Therefore, particular emphasis has been given in literature to the reactive aspects of nitroprusside (NP) and the circumstances in which it can liberate NO, as it can provide a means to work either as a NO scavenger or deliver NO under biologically relevant conditions.

The electrochemical reduction of the NP ion in an aqueous solution on a mercury electrode has been the subject of continuing investigation with respect to its electrochemical properties [6–15]. These studies are focusing on both the physical–chemical properties of the coordinated nitrosyl group and also on the understanding of the biological role of NP as an in vivo source of NO. In an aqueous solution, the electrochemical reduction in NP occurs in three steps. The more recent results [13] indicate that the first reduction step is attributed to $[Fe(CN)_5NO]^{2-}/$ $[Fe(CN)_5NO]^{3-}$, in which the NO^+ ligand has been reduced to NO. The product generally loses the axial cyanide ligand, giving $[Fe(CN)_4NO]^{2-}$ in a pH-dependent chemical step, which in alkaline solution is the rate-limiting step. The ion $[Fe(CN)_4NO]^{2-}$ is the reactant of the second reduction process. The remaining $[Fe(CN)_5NO]^{3-}$ and the product of

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disproportionation reaction involving the second reduction step product induced by proton availability leads to a final four-electron reduction product of NP, [Fe(CN)$_4$NH$_2$OH] at a more negative potential. Therefore, investigations dealing with the electrochemical reduction in NP immobilized on an electrode surface coated by polymeric films should be interesting, as it could alter the control of the dissociation of the NO in the complex.

Polyaminoacids are a versatile class of synthetic materials tested for the development of several novel drug delivery systems, as they are biodegradable polymers, whose degraded product is a nontoxic monomer. Among these, the use of these kinds of polymers with low molecular weight (30,000–70,000) has been investigated as an ideal system able to concentrate, protect, and liberate the drug at a specific site of the body by manipulation of its bio-distribution profile [16–21]. In addition, they can present several functional groups having a controlled chain, and they are easily excreted in urine.

Poly-L-lysine (PLL) is a synthetic polyaminoacid that has been used as a model compound for the study of more complex proteins and as a new alternative polymer for drug delivery. In aqueous solutions below pH 10.5, the NH$_2$ groups in PLL are in the protonated state, and the PLL exists in a random coil conformation [22]. Above this pH, the PLL in solution acquires the α-helical conformation. However, at higher temperatures (≥80 °C), the β-sheet conformation is formed [22]. This can promote a stable film on the electrode surface, which can be successfully used as an electrochemical sensor for several anionic substrates [23–35].

Nevertheless, a film of PLL adheres better to the surface of a glassy carbon electrode (GCE) when the PLL is partially cross-linked by means of glutaraldehyde (GA). A film composition of 97.5% PLL/2.5% GA gives good adhesion and retains the anionic exchange capability of the PLL [35, 36] and can be used successfully as an electrochemical sensor for anionic specimens.

As poly-L-lysine can be a model of a polyaminoacid derivative able to concentrate $\left[\text{Fe(CN)}_6\right]^{3-}$ anions in a very effective way [35], it can also be utilized for the initial investigation into the effects of surface immobilization of NP on PLL films to understand its reductive mechanism. On the basis of this above-mentioned application, the present study describes the electrochemical behavior of NP anchored to a modified electrode with a PLL film coating.

Experimental

Apparatus and reagents

An EG&G (PARC) 362 potentiostat controlled by a compatible microcomputer with AVOLM software was used to carry out the cyclic voltammetric experiments. A three-electrode system consisting of an EG&G (PARC) Ag/AgCl (3 mol l^{-1}) reference electrode, a platinum wire auxiliary electrode, and a GCE as working electrode were used. Unless specified otherwise, all potentials reported here are referenced to this Ag/AgCl electrode. The pH measurements were carried out using a Micronal pH meter, model B222, with a Micronal combined pH/reference electrode.

Suprapur grade reagents supplied by Merck and demineralized water from a Milli-Q system (Millipore) were used in the preparation of all solutions. The studies were carried out in Britton–Robinson (B–R) buffer (0.4 mol l^{-1} in each of acetic, phosphoric, and boric acids) adjusted to the required pH using 0.2 mol l^{-1} sodium hydroxide solution. A stock solution of NP 1×10^{-2} mol l^{-1} (Allergan–Frumtost laboratory) was obtained by direct dissolution of the salt in deionized water (Milli-Q) and used immediately. PLL (MM= 77,300) hydrobromide and GA 25% solution in water were obtained from Sigma. Tests with the cyanide ion were carried out adding known volumes of 1×10^{-3} mol l^{-1} KCN in solution containing NP solution at pH 12. The addition of NO in aqueous solution previously deaerated was obtained by bubbling the gas deriving from a reaction between 8 HNO$_3$+3 Cu→3 Cu(NO$_3$)$_2$+2 NO+4 H$_2$O in a separated compartment under nitrogen atmosphere.

Working electrode preparation

The GCE (3-mm diameter) was polished with alumina (0.3 μm, Buehler) and then washed and dried at room temperature. Solutions of the product of the reaction between PLL and GA were prepared using different ratios of the two constituents: PLL (1% *w/v*) and GA (0.05% *w/v*). A 10-μl sample of the aqueous solution was placed on the polished electrode surface. The tip of the pipette was used to spread the solution evenly over the whole surface. The inverted electrode was then placed in a drying oven at 80 °C for 2 min. After this drying procedure, the electrode was rinsed thoroughly with water and was then immersed in a solution of B–R buffer at pH 5.6.

General procedure The performance of the modified electrode was tested by two procedures in which NP was accumulated. In the first method, the electrode had been immersed in a solution containing NP. The curves were recorded after sufficient time had elapsed to accumulate the drug over a controlled time period and continuous stirring. The electrode was transferred to a new buffer solution (previously purged with nitrogen), which did not contain the analyte after being previously washed with deionized water. In the second procedure, the modified electrode was immersed directly in a solution containing NP, which was then accumulated by continuous potential cycling between defined potential recording the corresponding cyclic voltammograms.

Results and discussion

Voltammetric characteristics

Figure 1 presents typical cyclic voltammograms obtained for reduction in 1.0×10^{-3} mol l^{-1} NP in 0.40 mol l^{-1} B–R buffer at pH 5 on the GCE. The NP is voltammetrically reduced on the GCE in a process of only two steps. The first one is seen at around −0.44 V (peak I), attributed to the reduction in NO^+/NO in the complex [7–13]. The absence of a voltammetric signal for the reverse scan indicates the loss of the axial cyanide ligand giving $[Fe(CN)_4NO]^{2-}$ after one-electron transfer [13]. In agreement with literature [7–13], the process is similar to that observed for the mercury electrode where the electrodic process is followed by a chemical reaction preponderant on the acidic condition involving the release of cyanide. The second reduction step (peak II) is marked by a well-defined pair of peaks at −0.64/−0.58V, where the anode-to-cathode peak height ratio ($I_{II}*/I_{II}$) is around 1 and $E_{pII*} - E_{pII} = 60$ mV, clearly indicating that the process is reversible and involves one-electron transfer [37]. This behavior also agrees with literature [7–13], where it is proposed that the $[Fe(CN)_4NO]^{2-}$ is reduced to $[Fe(CN)_4NO]^{3-}$ by one-electron transfer involving a complex reaction mechanism of disproportionation [9]. The peak current for both peaks were proportional to $\nu^{1/2}$ indicating that both electrodic processes are diffusion controlled [37]. Although, on the mercury electrode, the authors indicated a new reduction step at a more negative potential attributed to the remaining $[Fe(CN)_4NO]^{3-}$ and the product of disproportionation reaction involving the second reduction step, there was no evidence of similar process on the GCE.

The modified GCE coated with mixture of PLL/GA at a proportion of 97.5:2.5% was immersed in a solution of 1.0×10^{-3} mol l^{-1} NP in B–R at pH 5, and the obtained
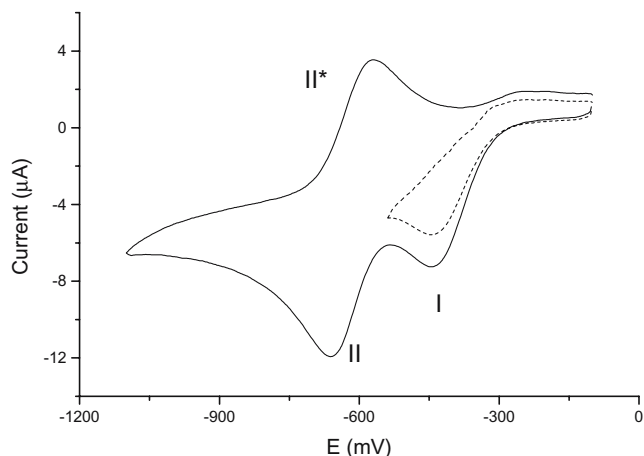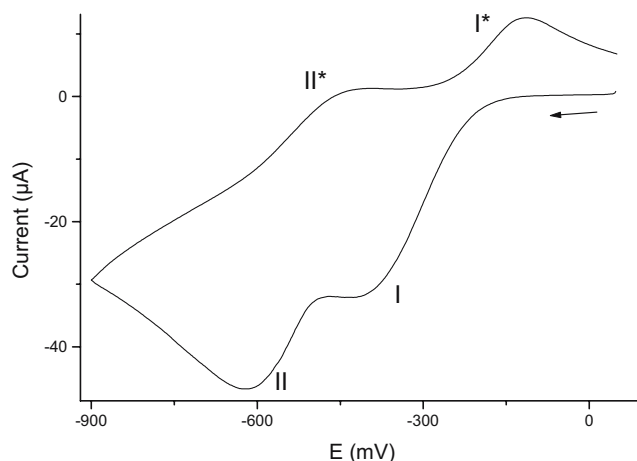

Fig. 2 Cyclic voltammogram obtained for the reduction of 1×10^{-3} mol l^{-1} NP in B–R buffer at pH 5 on GCE modified by PLL/GA film (97.5:2.5 *v/v*). Scan rate=100 mV s^{-1}

cyclic voltammogram is shown in Fig. 2. The cyclic voltammograms present both reduction peaks at −0.35 V (I) and −0.62 V (II) with higher intensities than those obtained for the bare electrode. In addition, both peaks exhibited the corresponding anodic peak in the reverse scan, including peak I* at −0.20 V, which was not verified on bare electrode. This behavior indicates that the product of the first reduction step is stabilized by the PLL film and presents the peak (I*) on the reverse scan. This phenomenon is better demonstrated in Fig. 3, where the multi-scan cyclic voltammograms were recorded limiting the potential scan to −0.45V for the PLL-coated electrode immersed in a solution containing 1×10^{-4} mol l^{-1} of NP in pH 5. The analysis of the first peak indicates that both cathodic and anodic peak currents increase with successive scans clearly indicating that the NP is gradually incorporated into the PLL film on the GCE surface along with its reductive product. Taking into consideration that the PLL presents
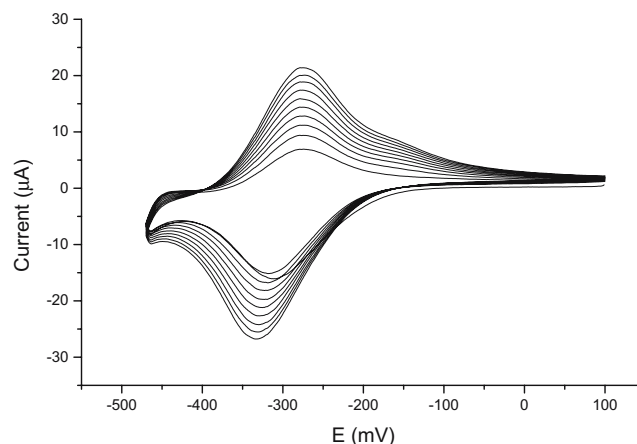

Fig. 1 Cyclic voltammograms obtained for the reduction of 1×10^{-3} mol l^{-1} NP in B–R buffer at pH 5 on a GCE. *Scan rate* 100 mV s^{-1}

Fig. 3 Successive cyclic voltammograms obtained for the reduction of 1×10^{-4} mol l^{-1} in B–R buffer at pH 5 on GCE coated with films of PLL/GA (97.5:2.5 *v/v*). *Scan rate* 100 mV s^{-1}
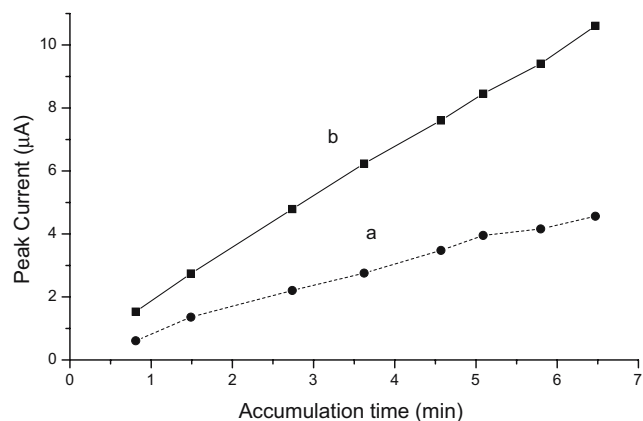
Fig. 4 Effect of accumulation time on GCE coated with PLL film and loaded in a solution of 2×10^{-5} mol l^{-1} NP and transferred to a B–R buffer at pH 5 (*a*) peak I and (*b*) peak II

positively charged amino groups in an alkaline medium (pKa$=10.44$) [9] and that the NP is a free anion at pH 5, the strong affinity that PLL coating exhibited to the NP seems to be due to electrostatic interaction or even interaction with amine groups available in the PLL film [38]. Similar results are obtained at all pH values higher than 3, and it is clearly evidenced that the incorporation of NP onto the film hinders the subsequent chemical reaction involving losses of CN$^-$, which was observed on bare electrode in solution.

Effect of accumulation time

The influence of accumulation time on the peak current (Fig. 4) was investigated from 0 to 6 min under open-circuit conditions using the measurements of the peak current obtained for 2×10^{-5} mol l^{-1} of NP in B–R buffer solution at pH 5. The NP loading incorporated into the PLL/GA film was monitored voltammetrically after transference to a new supporting electrolyte solution at pH 5. The peak current obtained for both cathodic peaks increased linearly, suggesting that the NP accumulates onto the available sites of the PLL film on the electrode surface, and this interaction is strong enough to be monitored later away from the environment of previous adsorption.

To investigate the adherence of NP onto the electrode surface film coating, cyclic voltammograms were carried out comparing the cathodic response of the GCE modified by PLL/GA loaded with NP. This accumulation was obtained under open-circuit conditions after immersion in 2×10^{-5} mol l^{-1} of NP in B–R buffer at pH 5 for 5 min. Then the loaded electrode was transferred to a new supporting electrolyte solution. The first voltammogram recorded was similar to that obtained in the original solution. On carrying out successive potential cycling applied to the electrode, the peak current decreased, until it was only a fifth the height of the first scan once the current had stabilized. This was shown later to be owing to

the loss of the NO ligand after the second reduction step of the complex and not the poisoning of the film.

Effect of PLL/GA

Cross-linking the PLL involves a reaction of some of the amino groups that are on the PLL film along with the aldehyde group of the GA, which, mixed together, form a Schiff base. This product increases the adherence of PLL onto the electrode surface, but it can also reduce the number of amino sites available on the PLL film to bind NP. The effect of the PLL concentration in the coating solution on the electrode response was investigated. To this end, coating the carbon disk with 10 μl samples of a mixture of PLL/GA (MM$_{PLL}$$=77,300$) giving the following ratios: 50:50; 75:25; 95:5; and 97.5:2.5 (PLL%/GA% m/m). The film was treated as described in "Experimental," and the cyclic voltammograms were recorded using the modified electrode after immersion in a 1×10^{-4} mol l^{-1} NP solution in B–R buffer at pH 4. The reduction peak currents obtained with these four electrodes were represented in Fig. 5. The peak current values clearly indicate that the lower the extent of cross-linking the greater the binding of NP and the greater the signal size [34, 36]. The 97.5:2.5 PLL/GA coating was selected for use in subsequent experiments. This coating gives acceptable anion exchange properties while giving the required improvement of adhesion to the GCE surface. This confirms the findings that the occurrence of free amine groups in the PLL film is important to pre-concentrate NP onto the electrode, and this condition is favorable at a low concentration of GA.

Therefore, the results indicate that the GCE modified incorporating PLL/GA films can be successfully used to pre-concentrate NP and also to promote a stable complex under a reductive condition.
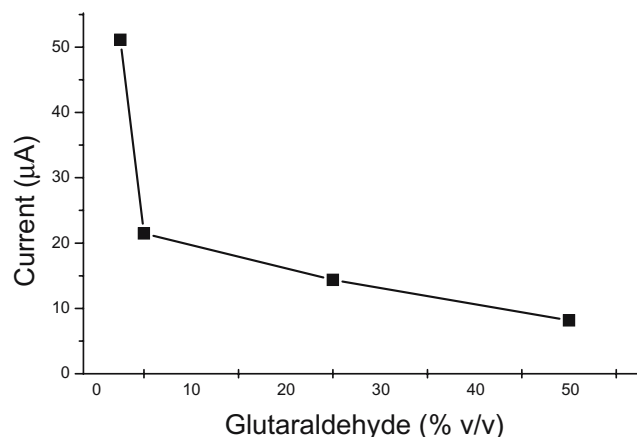

Fig. 5 Effect of film composition on the maximum peak current obtained from cyclic voltammograms recorded after 5 min of immersion of the GCE modified in 1×10^{-4} mol l^{-1} of NP in B–R buffer at pH 4. Film composition: PLL (50%)/GA (50%); PLL (75%)/GA (25%); PLL (95%)/GA (5.0%); PLL (97.5%)/GA (2.5%)
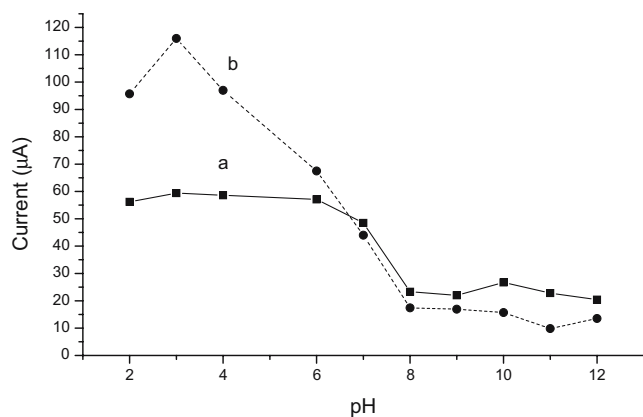
Fig. 6 Graphs of maximum peak current obtained for peak I (**a**) and peak II (**b**) obtained from cyclic voltammograms recorded for GCE coated with PLL/GA film and loaded with 1×10^{-3} mol l^{-1} NP

Effect of pH

The influence of the pH on the NP accumulation process onto the GCE/PLL/GA was examined over a range of pHs from 2 to 12. Once the PLL/GA-coated electrode was loaded in 1×10^{-3} mol l^{-1} NP at different pH values from 2 to 12, it was transferred to a new solution of supporting electrolyte. The analyte was pre-concentrated on the film at all pH values investigated, as shown in Fig. 6. The first peak is highest at pH<6 when the maximum signal is obtained, but there is a measurable current even at pH> 8 when the peak current is constant. On GCE without modification, it is seen that the reversibility of the first peak in alkaline solutions is improved, as the rate constant for reaction of release of cyanide is a relatively slow chemical step, but this reaction is faster in acidic media because, in this case, the reduction may be followed by the fast formation of HCN [9, 13]. The second peak is higher than the first one at all pH ranges lower than 6, but its intensity decreases and becomes smaller under an alkaline condition.

At pH 12, the second peak is almost neglected on the cyclic voltammogram at bare electrode, as shown in Fig. 7. However, when NP is accumulated onto the electrode surface, it presents a well-defined second peak even at pH 12, as shown in Fig. 8a (Curve A).

The cyclic voltammograms recorded for GCE modified with poly-L-lysine immersed in a solution containing 1.0×10^{-4} mol l^{-1} NP over all pH ranges from 8 to 12 indicated that both shape and peak intensity are also strongly influenced by pH variation. But at all pHs investigated, it was possible to see the two main peaks at around −0.35 and −0.60 V. On the modified electrode, the peak is at least 100 times higher than that of the bare electrode, indicating that even under alkaline conditions, PLL film is pre-concentrating the analyte. This means that at pH<10, the positive charge of the PLL film improves the interaction with the NP. But under alkaline conditions, the electrode charge is zero or slightly

negative (free amine groups). Therefore, PLL films are still pre-concentrating both NP and its derivative on the electrode surface. This behavior suggests that along with the electrostatic interaction, there will be a possible complexation with the PLL amine groups.

In agreement with literature [38], some cyanide complexes ($[Fe(CN)_6]^{4-}$ can act as catalysts in the Berthelot reaction, and this complex can stabilize chloroamine, forming a new possible complex denoted as $[Fe(CN)_5NH_2Cl]^{3-}$. The formation of a complex between NP and the amino acid histidine have also been described in literature [39]. Nevertheless, under neutral or alkaline conditions, this reaction can be substituted by the formation of an aquopentacyanoferrate complex generated after hydrolysis of the original product. Therefore, PLL-bearing amino groups could be acting in a similar way to pre-concentrate reduced NP.

To understand these results, PLL-modified electrodes were directly immersed in a solution of 1×10^{-4} mol l^{-1} of sodium NP at pH 12, and the cyclic voltammograms were recorded limiting the scan to −0.45 V, where the respective curve is shown in Fig. 8 (Curve A). A clear increase in both the cathodic and anodic peaks for successive scan can be observed. This behavior was not observed previously for the bare electrode, suggesting that the product generated after the first electron transfer is also immobilized onto the electrode surface.

Taking into account that, on the bare electrode, the NP reduction in acidic medium pH lower than 6 is always characterized by two reduction waves at −0.33 and −0.55 V and that there is no oxidation peak for the reverse scan in aqueous solution, which is attributed to a loss of CN^- after the first reductive step [12, 13], the use of PLL could be a good strategy to minimize the reaction responsible for the toxicological effects of NP. The second wave at more negative potential is seen in all the pH range of 2–12 on
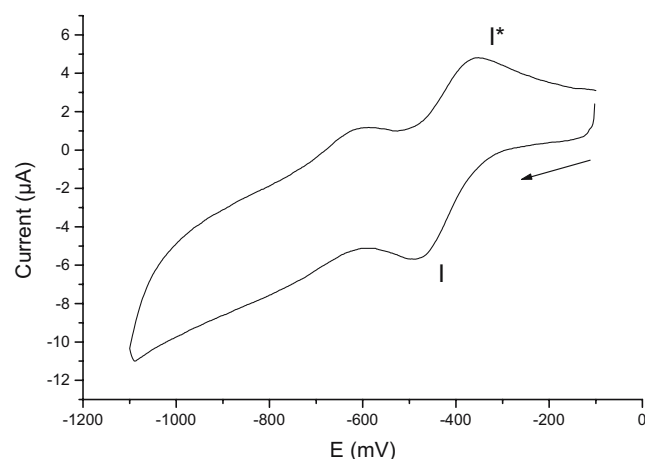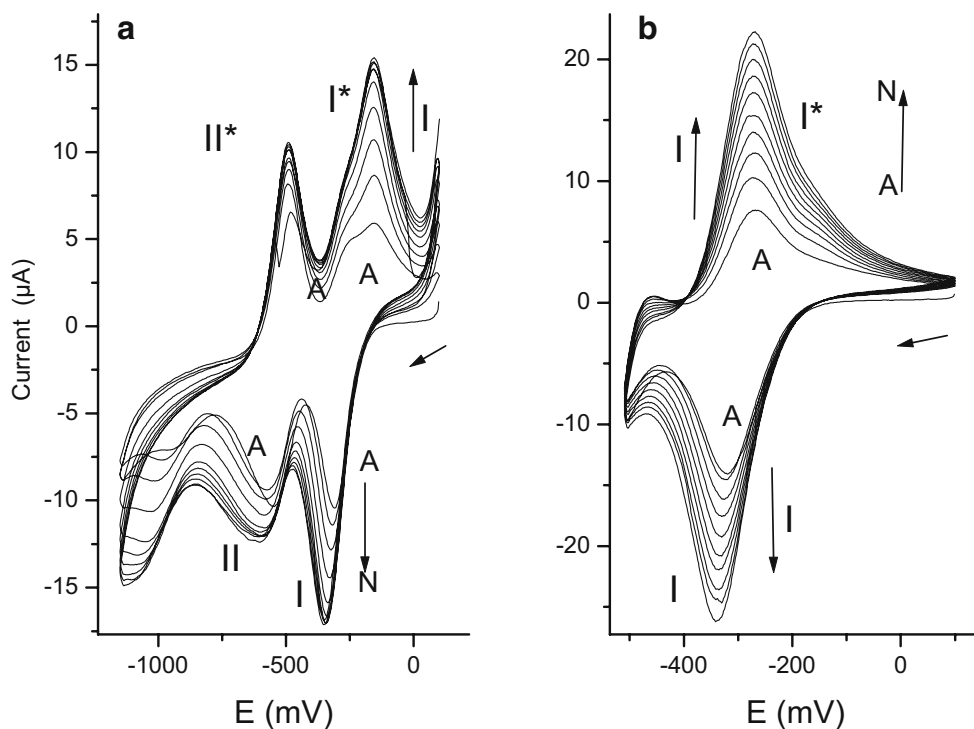

Fig. 7 Cyclic voltammogram obtained for the reduction of 1×10^{-3} mol l^{-1} NP in B–R buffer at pH 12 on GCE. *Scan rate* 100 mV s^{-1}

Fig. 8 Successive cyclic voltammograms obtained for the reduction of 1×10^{-4} mol l^{-1} in B–R buffer at pH 12 on GCE coated with PLL/GA (97.5:2.5% v/v) film. Scan from 0 to −1.2 V (**a**). Scan from 0 to −0.45 V (**b**). *Scan rate* 100 mV s^{-1}


GCE coated by PLL film and also presents an anodic peak for the reverse scan, which increases the reversibility of the electrodic process. This is attributed to a minimization of both the protonation and the disproportionation reaction after a possible second reduction step forming [Fe(CN)$_5$NO]$^{3-}$/[Fe(CN)$_5$NOH]$^{3-}$ as proposed by Masek et al. [7] or forming aquopentacyanoferrate [Fe(CN)$_5$H$_2$O]$^{3-}$ after the liberation of NO [39].

Voltammetric characterization of the reduction product

Successive cyclic voltammograms in a wider potential scale window gave an interesting aspect to understand the process on the PLL film. A repetitive scan (60 cycles) for the GCE modified by PLL/GA films loaded with NP in a new supporting electrolyte has shown voltammograms that present a clear diminishing of both reductive peaks (I and

Fig. 9 **a** Successive cyclic voltammograms obtained for oxidation of 1×10^{-4} mol l^{-1} [Fe(CN)$_6$]$^{4-}$ in B–R buffer at pH 4 on glassy-carbon electrode coated PLL:GA film. **b** Cyclic voltammograms obtained for glassy-carbon electrode coated with PLL:GA film loaded with NP (100 cycles) and transferred to a new solution of B–R buffer at pH 4.0 (*Curve A*). Standard addition of 1×10^{-4} mol l^{-1} (*B*); 2×10^{-4} mol l^{-1} (*C*); 3×10^{-4} mol l^{-1} (*D*); and 4×10^{-4} mol l^{-1} (*E*) of [Fe(CN)$_6$]$^{4-}$ solution
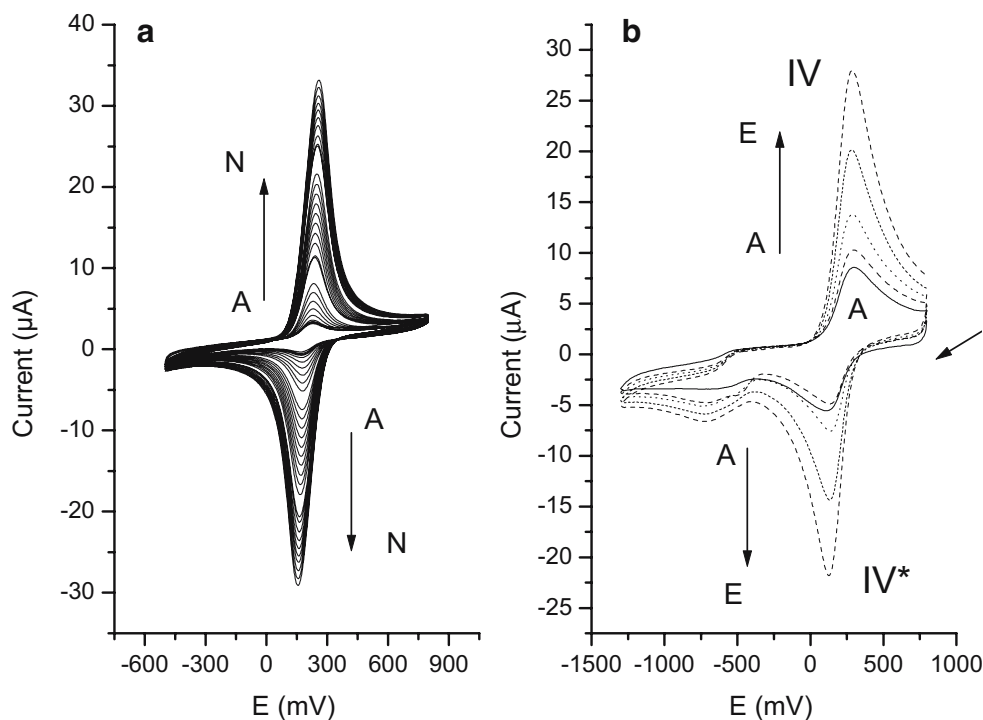
II) attributed to NP concomitant to the occurrence of a new peak at +0.2 V (peak IV) that increases with successive scan. The final products incorporated onto the film were washed and transferred to a new solution of B–R buffer at pH 4, whose cyclic voltammogram is shown in Fig. 9b (Curve A). This cyclic voltammogram produces a very stable signal with a reversible characteristic. The voltammetric signal increases when $[Fe(CN)_6]^{4-}$ is added to the solution, as shown in Fig. 9b (Curves A–E). These results indicate that probably the final product is retained on the electrode surface and is electro-active. This could be attributed to the $[Fe(CN)_5H_2O]^{3-}$ complex coated on the electrode due to a specific interaction with a PLL amino group, which could be reduced at a potential closer to the $[Fe(CN)_6]^{4-}$ complex. The electrochemical behavior is also confirmed by literature [39], which reports a polarographic wave at $E_{1/2}$ values at around +0.20 V for polarographic reductions of $[Fe(CN)_5H_2O]^{3-}$ complexes.

To confirm the possibility of forming a $[Fe(CN)_5 H_2O]^{3-}$ complex on the electrode surface, cyclic voltammograms recorded for oxidation of 1×10^{-4} mol l^{-1} of $[Fe(CN)_6]^{4-}$ on PLL film were carried out under the same experimental condition, and these cyclic voltammograms are shown in Fig. 9a. The cyclic voltammograms present a typical behavior expected for a film formation where $[Fe(CN)_6]^{3-}$ is incorporated on the PLL film, and the voltammogram shape and peak potential are identical to that obtained after NP reduction by successive cycles from −1 to +1 V.

To test this hypothesis, cyclic voltammograms were carried out for NP in B–R at pH 12 on the PLL films in the absence and presence of 1×10^{-5} to 1×10^{-3} mol l^{-1} of CN^- ion generated by a chemical process described in "Experimental." Cyclic voltammograms recorded for the reduction in CN^- ion on GCEs modified by PLL films do not show any electrochemical response. The addition of the CN ion to the NP film also does not promote any alteration up to 1×10^{-3} mol l^{-1}.

Conclusion

The cross-linking reaction between PLL and GA leads to an easy way of modifying a GCE and permits the preparation of an electrode with high retention capability of PLL. NP is efficiently immobilized on PLL films, and this modified electrode has a promising use for the study of NP/polyaminoacid interaction. The electrochemical reaction of the NP incorporated on PLL film has been improved, and the drug is reducible at all pH ranges, from 2 to 12. At all pH investigated, the film improves the stability of the reduced NP complex, and its first reduction involves the reduction in NO^+/NO in the complex, but the losses of CN^-

as a ligand is not preponderant in the process, which is the main concern regarding the use of NP. Further, this information will be of value in the development of electrochemical sensor devices based on the PLL-modified GCE and other materials for NP determination and the development of methods using PLL as drug delivery vehicles.

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