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Electrochemical comparison of two methods for polythionineelectropolymerization and its relevance as amperometric detector

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ABSTRACT

The electrochemical behavior of polythionine on different electrodes has been investigated by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Polymeric films were obtained by two electropolymerization methods: M1 using cyclic voltammetry or M2 in two steps, a constant potential oxidation and cycle potential sweeps. Those methodologies were compared by analyzing the electrochemical behavior of the films using two redox probes. The modified surfaces were also analyzed as electroanalytical sensors so phenol response was tested and a simple and reliable method for phenol quantification was proposed and validated, corresponding to an M2 glassy carbon modified electrode, using a 0.7 V working potential with a 4×10^{-6} to 2.7×10^{-5} M linear range, with those conditions a 3.6×10^{-6} mol L^{-1} LOD, 1.09×10^{-5} mol L^{-1} LOQ and a $(2.9 \pm 0.2) \times 10^{7}$ C_{Phenol} (mol L^{-1}) sensitivity are obtained.

1. Introduction

Dye surface modification can be achieved by different methods: covalent linkage to chemically modified surfaces [1], incorporation into polymeric films or macromolecular deposits [2] and deposition on the electrode by constant or cycled potential oxidation of a dye-containing solution (electropolymerization) [3–24]. Electropolymerization is one of the most common and useful techniques to obtain oligomer or polymer dye thin films. Thionine (Scheme 1) is a derivative of phenothiazine and is a water-soluble cationic dye. Different phenothiazine derivatives polymer thin films, e.g., Azure A [5–8], Azure B [9], Methylene blue [6,7,10,12–16,11], Methylene green [7,12] and Toluidine blue [17,18] have been prepared by electropolymerization.

Polythionine (PTH) has received considerable attention for electrodes modification and the electropolymerization process has also been studied [3,4,25]. PTH-modified electrodes were prepared basically by two methods (1) a potential-sweep in a relatively wide potential range (approximately 1.5 V) and (2) by two steps consisting of a constant potential oxidation followed by a cycling potential sweep in a relatively narrow potential window (approximately 0.6 V). Furthermore, both preparation methods were used under different experimental conditions such as media (acidic, neutral and slightly alkaline), substrate (gold, polished and activated glassy carbon, screen printed carbon and carbon paste electrodes), and potential windows [4,20–23,26,24]. Polythionine modified electrodes prepared using any of both methods have been used for different applications that can be mainly divided in two groups, as redox mediators (G1) or as electrochemical detectors (G2). For example, PTH-modified electrodes prepared by method (1) were used as redox mediators for H_2O_2 [26] and choline [20] determination and by method (2) as redox mediators for H_2O_2, [4] phenols [21] and ethanol [22] quantification. By the other side, electrochemical detectors using PTH modified electrode by method (1) for NADH [23] and by method (2) for hydroquinone and catechol [24] have also been proposed.

In this paper, we present a comparative analysis of both methodologies used to prepare PTH modified electrodes at different pH values by using two redox probes. In addition, we compare the behavior of two substrates glassy carbon and gold. Moreover, phenol was used to test modified electrodes analytical performance and an amperometric method for phenol detection is proposed.

2. Experimental

2.1. Chemicals

All reagents were obtained as analytical grade and used without further purification. Purified water obtained from a Milli-Q water

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M1	method 1, one step cycle potential sweep
M2	method 2, two steps, constant oxidation potential (preanodization potential) and a cycle potential sweep
m-T	first process observed in polythionine films (I) corresponding to the less crosslinked polymer composition
poly-T	second process observed in polythionine films (II) corresponding to the more compact polymer composition
GCE	glassy carbon electrode
GE	gold electrode
PTH	polythionine

purifying system (18 MΩ cm) was used for all experiments. Thionine, Phenol, $Ru(NH_3)_6Cl_3$, $K_3Fe(CN)_6$, Na_2HPO_4 and NaH_2PO_4 were purchased from Sigma–Aldrich (St. Louis, MS, USA). 0.05 M Phosphate buffer solution (PBS) was prepared by adjusting a mixture of 0.05 mol L^{-1} Na_2HPO_4/NaH_2PO_4 to the required pH value by adding either 1.0 mol L^{-1} hydrochloric acid (HCl) or sodium hydroxide (NaOH) solution.

2.2. Apparatus

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were performed with a CHI 660C electrochemical workstation (CHInstruments, USA). A conventional three-electrode system was used where either glassy carbon (GCE, 3 mm diameter) or gold (GE, 1 mm diameter), a platinum wire, and a Ag/AgCl (3 M KCl) electrode were used as working, counter, and reference electrodes, respectively. All potentials are referred to this reference electrode. The impedance spectra were analyzed by using the Z-view program. All electrochemical experiments were performed at room temperature. Amperometric experiments were carried out in a stirred 0.05 mol L^{-1} pH 7.0 PBS by applying 0.7 V.

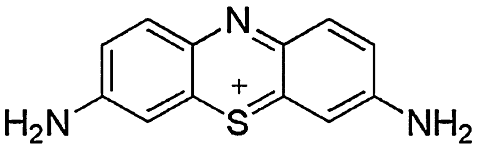
2.3. Preparation of modified electrode

Electropolymerization of thionine was performed by two methods that meet the requirements stated in Section 1: M1 using cyclic voltammetry or M2 in two steps, constant oxidation potential (preanodization potential) and a cycle potential sweep. GCE and GE were polished with 0.05 μm alumina/water slurry (Buehler, USA) on a polishing cloth to a mirror-like finish, followed by sonication and then rinsed with distilled water. The polished and cleaned electrodes were then modified by both methods (M1 and M2) at different conditions (pH values, electrode material) of electropolymerization in a solution containing 1×10^{-4} mol L^{-1} thionine.

3. Results and discussion

3.1. Thionine in solution: cyclic voltammetry

The electrochemical behavior of thionine was analyzed at two pH values in the −0.4 V to 0.4 V potential range. Fig. 1 shows the potentiodynamic *i*/*E* profiles with a glassy carbon electrode for 1×10^{-4} mol L^{-1} thionine in 0.10 mol L^{-1} phosphate buffers at pH 2.2 and 6.9. As it can be observed, a quasi reversible redox process (I, I^a and I^c) that depends on the solution pH is obtained, as spected for a diamine derivative. At neutral pH (6.9) the anodic potential peak (I^a) appears at −0.226 V and the cathodic one (I^c) at −0.258 V while for acid pH values (2.2) the whole process is defined at more positive potential values, with ca. a 0.3 V shift. Current density remains practically constant at both pH values, this behavior is in good agreement with those previously reported in the literature [4].



Scheme 1. Structure of thionine.

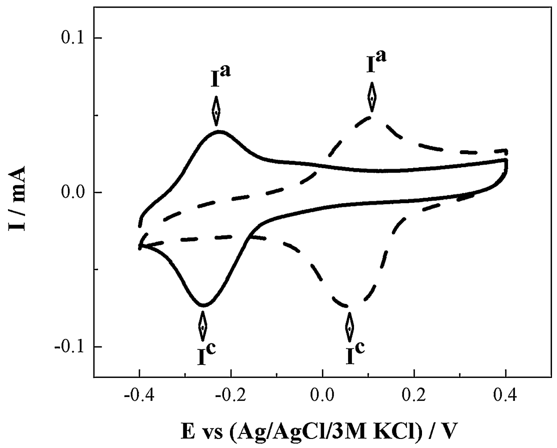


Fig. 1. Cyclic voltammograms of 1×10^{-4} mol L^{-1} thionine solution, in 0.10 mol L^{-1} phosphate buffer solution pH 6.9 (solid line) and 2.2 (dash line). Scan rate: 100 mV s^{-1}.

3.2. Comparative analysis of PTH-modified GCE and GE

As already mentioned in Section 2, methods for polymer growth are proposed. Fig. 2 shows cyclic voltammograms with a glassy carbon electrode using both procedures: M1 (A) and M2 (B) at pH 6.9. Films grown by both methods present two well defined process one at potential values corresponding to the thionine in solution (Fig. 1), with a potential peak value close to 0.1 V for pH 2.2 and to −0.2 V for pH 6.9; this process will be named from now on as m-T (I). However, when the switching potential becomes more positive, a new and different process at higher potential values than m-T is obtained, with a peak potential value close to 0.240 V at pH 2.2 and 0.007 V for pH 6.9 that will be named as poly-T (II, II^a and II^c) (Fig. 2). Method M1 (Fig. 2A) shows that the signal corresponding to process I shifts to higher potential values and remains practically constant with successive cycles while a current increase of the II process is observed. This fact can be assigned to polythionine growth. By the other side, with M2 (Fig. 2B) a preferential growth of m-T (I) compared to poly-T (II) is observed with an anodic potential peak at −0.224 V (close to thionine in solution). So according to the methodology used different films can be formed with more or less m-T or poly-T proportions.

The mechanism of thionine electropolymerization has been proposed [27–29]. It was commonly considered that initial adsorption of thionine monomer occurred on the electrode surface and the electropolymerization reaction started at fairly high potential values where reactive cation-radical species are formed. The monomer units in PTH are linked through —NH— bridges in the aromatic position [27]. It was reported that cation-radical species are formed at about +0.8 V for the electropolymerization of phenoxazines or phenothiazines if the parent monomer has primary amino group as ring substituents [28]. So, dye materials electropolymerization is achieved by dye monomer oxidation to its radical cation, these

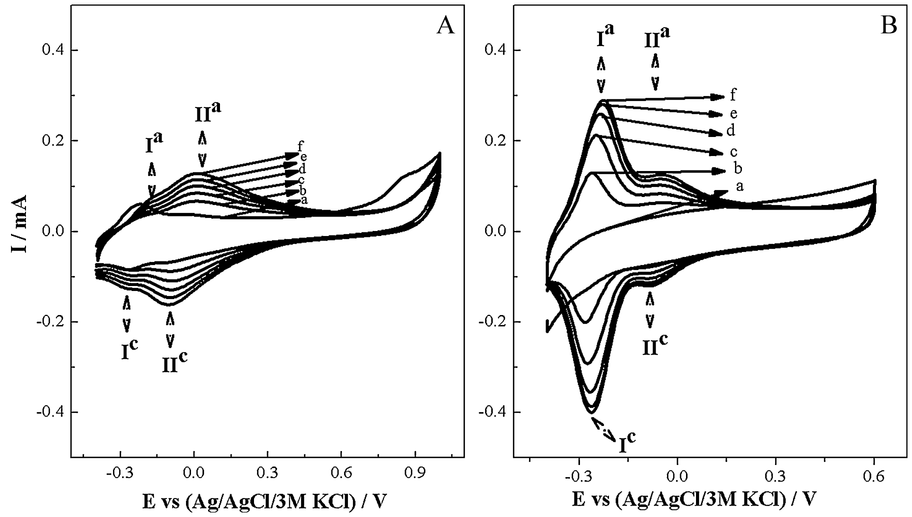


Fig. 2. Cyclic voltammetric curves of thionine electropolymerization on GC electrode in 0.05 mol L^{-1} phosphate buffer solution (pH: 6.9) containing 1.0×10^{-4} mol L^{-1} thionine. Scan rate: 50 mV s^{-1}. (A) M1 and (B) M2. (a) 1st scan, (b) 10th scan, (c) 20th scan, (d) 30th scan, (e) 40th scan and (f) 50th scan.

reactive cation radicals linked through the —NH— bridge to form polymerized thionine [27] with cycling potential sweep, as the commonly accepted manner. For that reason, in method M2, during the preanodization step, large amounts of radicals will form which in turn will produce many nucleus and short polymer formation, so a smaller poly-T than m-T signal is observed. It is worthy to note, that during preanodization step, a glassy carbon surface activation is obtained [4]. By the other side, with M1, radicals' generation is not so high allowing polymer chains to grow in length. Taking into account these mechanisms, all the parameters influencing films growth, such as electrode material, method of preparation, potential range (M1), preanodization potential (M2) and solution pH, were modified in order to analyze the electropolymerization procedure.

Fig. 3 presents peak current density (j_p) at gold electrodes corresponding to the oxidation process for m-T and poly-T at two pH values for films grown by both methods. Fig. 3A corresponds to the current density dependence with the cycling final potential values (M1), while Fig. 3B corresponds to the behavior for different preanodization potential values (M2). For both methods there is a potential value or potential range where a maximum peak current density is obtained, and these values are always lower at pH 6.9 than 2.2, in agreement with the electrochemical behavior observed with thionine in solution (Fig. 1). Furthermore, poly-T presents higher current densities than m-T for both methods at all potential values.

The same analysis was performed with glassy carbon, Fig. 4A and B. Films grown using M1 show a similar behavior as GE for all the different parameters. However, when films are grown using M2 a larger amount of m-T is obtained, being this effect more important for pH 6.9 at 1.5 V (Fig. 4B). So when GCE is used for surface modification, films with different m-T/poly-T proportions are obtained, this result allows to perform a comparative study. In summary with M1 at 1.0 V switching potential (the signal for poly-T is higher than m-T) while for M2 using a preanodization potential of 1.5 V (m-T signal is higher than poly-T). The greatest difference in m-T/poly-T proportion is obtained by M2 at pH = 6.9, while all the other conditions show less proportion differences. Moreover, films stability depends on the potential range used. Fig. 5 shows successive cycling voltammograms for different final potential values on GCE for films grown at both pH values and methods. It can be observed that at pH = 2.2 the film is stable up to potential value of 1.2 V (Fig. 5A and B). While for pH = 6.9 this stability is observed at lower potential value (1.0 V) (Fig. 5C and D). The peak corresponding to m-T process disappears for switching potential values higher than 0.80 V and the poly-T signal at 1.0 V (Fig. 5C and D). The same behavior is obtained at pH = 2.2, but at higher potential values (1.00 and 1.2 V respectively) (Fig. 5A and B). It should be noted that the

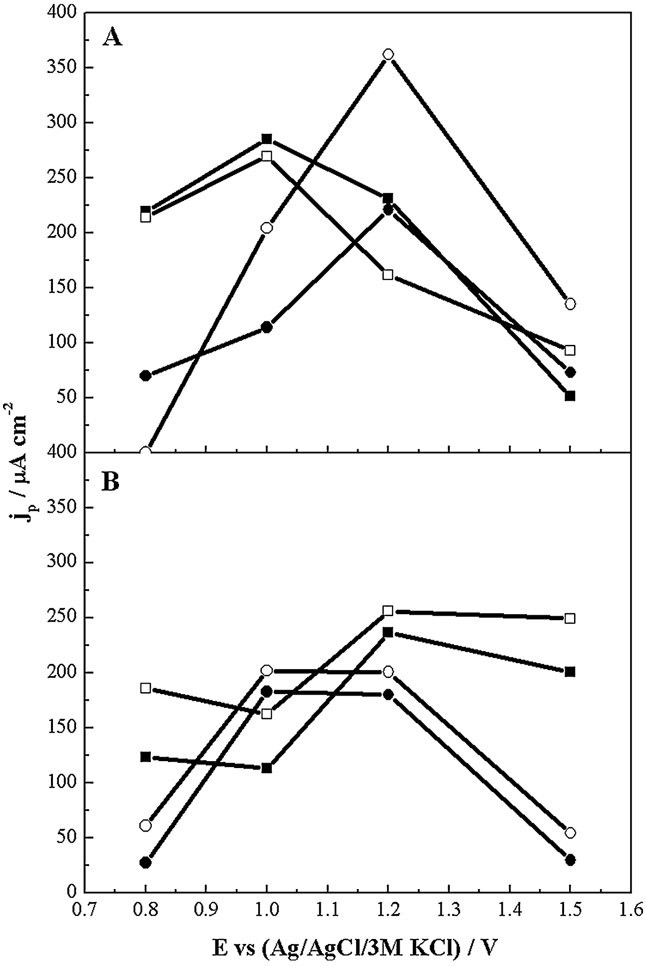


Fig. 3. Dependence of the polythionine anodic peak currents of poly-T (empty symbol) and m-T (filled symbol) for the final potential in M1 (A) and the preanodization potential in M2 (B) with GE at pH 2.2 (circle) and pH 6.9 (square).

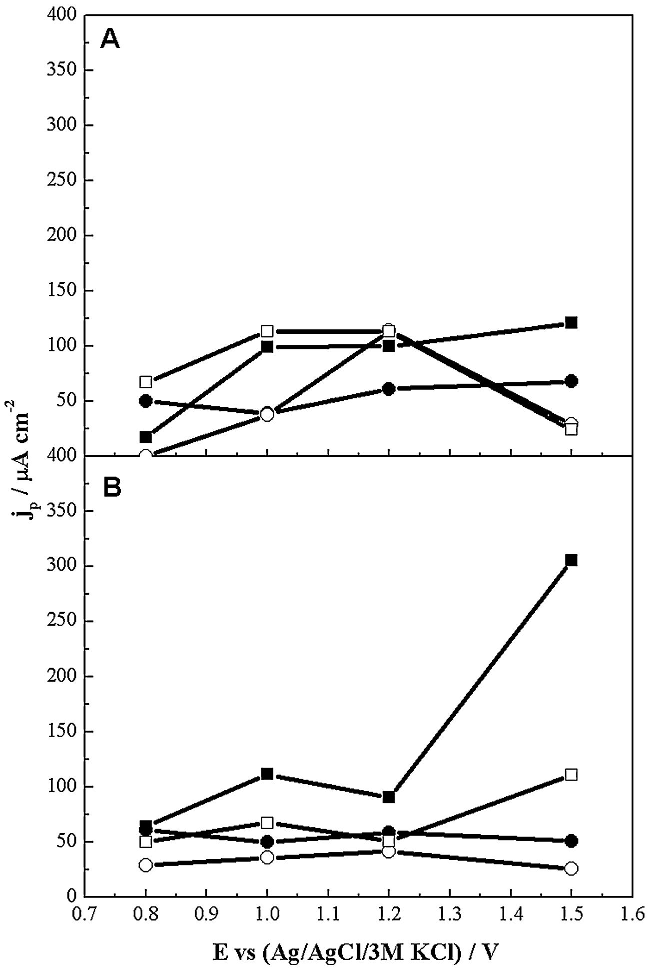


Fig. 4. Dependence of the polythionine anodic peak currents of poly-T (empty symbol) and m-T (filled symbol) for the final potential in M1 (A) and the preanodization potential in M2 (B) on GCE at pH 2.2 (circle) and pH 6.9 (square).

potential difference (0.20 V) between m-T and poly-T stability potential does not depend on the solution pH. This effect indicates that both films m-T and poly-T have the same behavior with the solution pH.

3.3. PTH modified electrodes characterization

CV and EIS with redox probes were employed to analyze the differences in the electrochemical behavior between GCE bare electrode and M1 and M2 thionine modified electrodes. Experiments were carried out at two different pH values and with two redox probes one with positive charge, $Ru(NH_3)_6^{3+/2+}$ and another with negative charge, $Fe(CN)_6^{3-/4-}$.

Fig. 6 shows cyclic voltammograms with modified GCE by both methods (M1 and M2) at pH 6.9 for $Ru(NH_3)_6^{3+/2+}$ (Fig. 6A and B) and $Fe(CN)_6^{3-/4-}$ (Fig. 6C and D). As it can be observed, when electrodes are modified with M1 (Fig. 6A and C) there is always a charge transfer reaction inhibition, indicated by both potential peaks (E_{pa} and E_{pc}) shift to higher values, a potential peak difference ($E_{pa} - E_{pc}$) increase and a current peak decrease, regardless the probe or the solution pH. A possible explanation could be that the film formed with M1 (majority poly-T) blocks the surface permeability and makes the charge transfer reaction more difficult. By the other side, M2 modified electrodes (Fig. 6B and D) show a different behavior as with both probes well defined current peaks are obtained, presenting a quasi reversible response. When $Fe(CN)_6^{3-/4-}$ is used as a probe two well defined current peaks are obtained at different potential values. However, with $Ru(NH_3)_6^{3+/2+}$ probe both current signals overlap and a single current peak with a higher value than that of polished electrode, is observed.

In order to get a deeper insight on the electrochemical process, EIS spectra were obtained. An ac signal of 10 mV in amplitude with a frequency range of 10^{-2} to 10^5 Hz was superimposed at the peak potential value of the oxidation redox couple. The impedance of the surface confined species is analyzed using a Randles circuit. The simplest model for surface-confined electroactive species includes contributions to the electrochemical response from four sources: uncompensated solution resistance (R_{el}), double layer film capacitance (C_{DL}), faradaic activity of surface-confined electroactive species, which is represented by a charge-transfer resistance (R_{CT}) and a Warburg element (W) that considers diffusion processes (see inset Fig. 7). Therefore, R_{CT} values can be determined from the spectra fitting using the mentioned equivalent electrical circuit. Fig. 7 shows the typical Nyquist plots obtained on bare GCE (•), and M2 thionine modified electrode (○) at pH 6.9 with $Ru(NH_3)_6^{3+/2+}$ (A) and $Fe(CN)_6^{3-/4-}$ (B) probes solution. For bare GCE, using equivalent circuit show in Fig. 7 inset, the solution resistance (R_{el}), is ca. $(1.0 \pm 0.2) \times 10^2\ \Omega\,cm^{-2}$, double layer film capacitance (C_{DL}) is around $3 \times 10^{-6}\ F\,cm^{-2}$, while the electron transfer resistance (R_{CT}) was found to be $(4.0 \pm 0.9) \times 10^3\ \Omega\,cm^{-2}$. Spectra with the $Ru(NH_3)_6^{3+/2+}$ probe (Fig. 7A) show that the R_{CT} did not a significant change with respect to the bare electrode. By the other side, with $Fe(CN)_6^{3-/4-}$ solutions a noticeably charge transfer resistance decrease is obtained $(4.0 \pm 0.8) \times 10^2\ \Omega\,cm^{-2}$ (Fig. 7B). Also, an almost straight line is observed, which can be assigned to a diffusion limiting step, probably inside the film, indicating that the modified electrode presents a different surface that favors ions permeation in good agreement with the lower R_{CT} value obtained (Fig. 7B). Probably, the electrostatic interaction between the negatively charged $Fe(CN)_6^{3-/4-}$ and the positively charged thionine film is also favoring the charge transfer reaction.

3.4. Analytical performance evaluation of the two polymerization methods M1 and M2 for phenol detection

Electrodes modified by both methods were used to detect phenol as a model analyte. For comparison also a M2 modified gold electrode was analyzed. Cyclic voltammetry experiments with $1 \times 10^{-3}\ mol\,L^{-1}$ phenol in phosphate buffer solution pH = 6.9 were performed with modified and bare electrodes at $50\ mV\,s^{-1}$ (Fig. 8). As it can be observed, either with M2 modified gold electrode and M1 modified glassy carbon electrode a current decrease or even an inhibition for phenol oxidation is obtained when compared to bare electrode (Fig. 8A and C). However, when the glassy carbon electrode is modified by M2 a current increase and a less current decrease during the second cycle, due to the phenol passivating film formation, is observed (Fig. 8B). These results agree with those obtained with redox probes.

3.4.1. Amperometric phenol detection with glassy carbon M2 modified electrode

As already mentioned M2 modification produces a better electrochemical response for phenol quantification (Fig. 8B), so this modified electrode was tested by chronoamperometry for phenol determination. Fig. 9 shows the hydrodynamic voltammograms ($1 \times 10^{-3}\ mol\,L^{-1}$ phenol) for bare and M2 modified glassy carbon electrodes; the applied potential was analyzed in the 0.0–0.9 V potential range, in the insert the corresponding calibration curves are also shown. As can be observed, there is a constant current increase for both electrodes for potential values higher than 0.6 V up to reach a maximum current value at 0.7–0.8 V; for that reason, a detection potential of 0.7 V was selected for all the experiments as

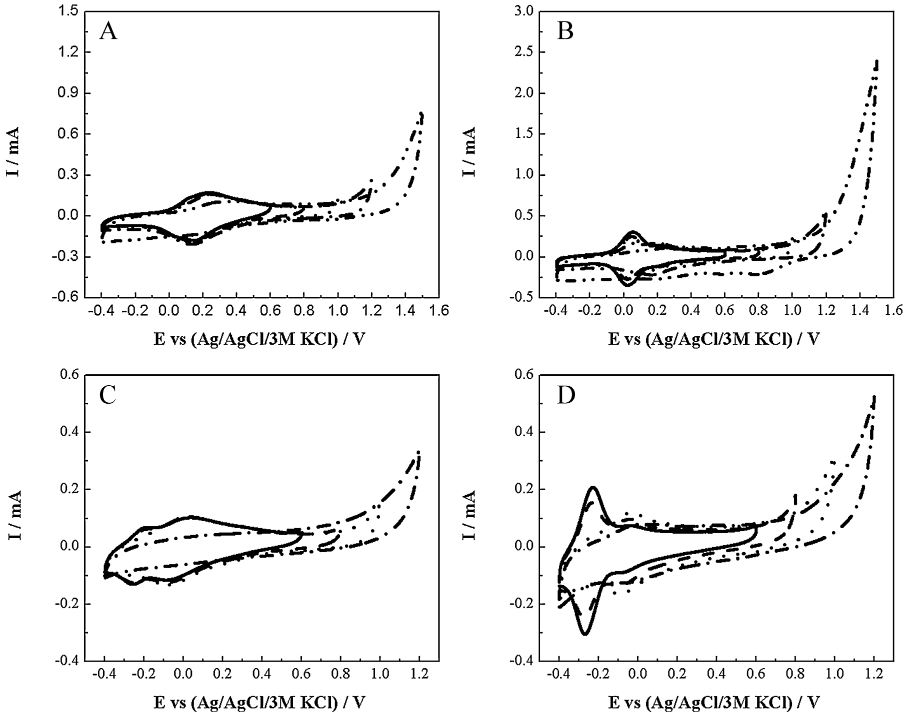


Fig. 5. CVs in phosphate buffer solution of PTH-modified GCE electrode at different final potential: (solid) 0.6 V, (dash) 0.8 V, (dot) 1.0 V, (dash dot) 1.2 V and (dash dot dot) 1.5 V. (A) M1 pH 2.2, (B) M2 pH 2.2, (C) M1 pH 6.9 and (D) M2 pH 6.9. Scan rate: 100 mV s^{-1}.

at higher potential values m-T film deterioration is observed (principal component for M2). The modified electrode shows a better sensibility for phenol determination than bare GCEs.

The linearity for M2 modified glassy carbon electrode was evaluated by linear regression analysis, calculated by the least-squares regression method. Calibration curves for phenol were linear over the 4×10^{-6}–2.7×10^{-5} mol L^{-1} concentration range. The detection limit (LOD) was measured as the lowest amount of the analyte that may be detected to produce a response which is significantly different from that of a blank. The limit of detection and quantification were calculated on the basis of the standard deviation of the response (δ) and the slope (S) of the calibration curves

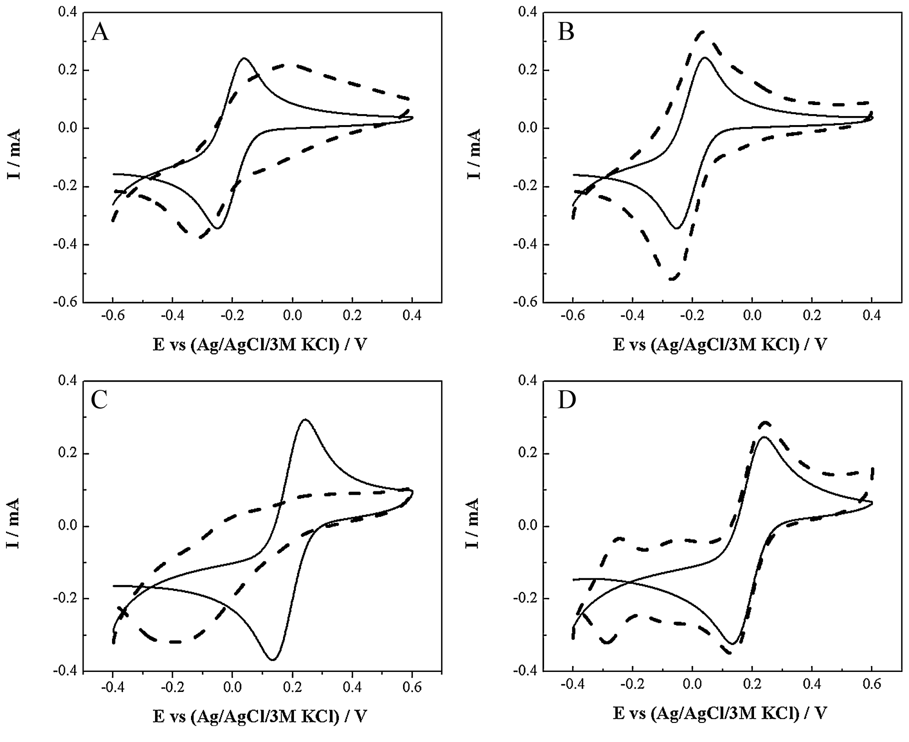


Fig. 6. CVs of 2×10^{-3} mol L^{-1} redox probe in phosphate buffer solution at PTH-modified electrode (dash line) and bare electrode (solid line). $Ru(NH_3)_6{}^{3+/2+}$ (A) M1, (B) M2 and $Fe(CN)_6{}^{3-/4-}$ (C) M1 and (D) M2. Scan rate: 100 mV s^{-1}.

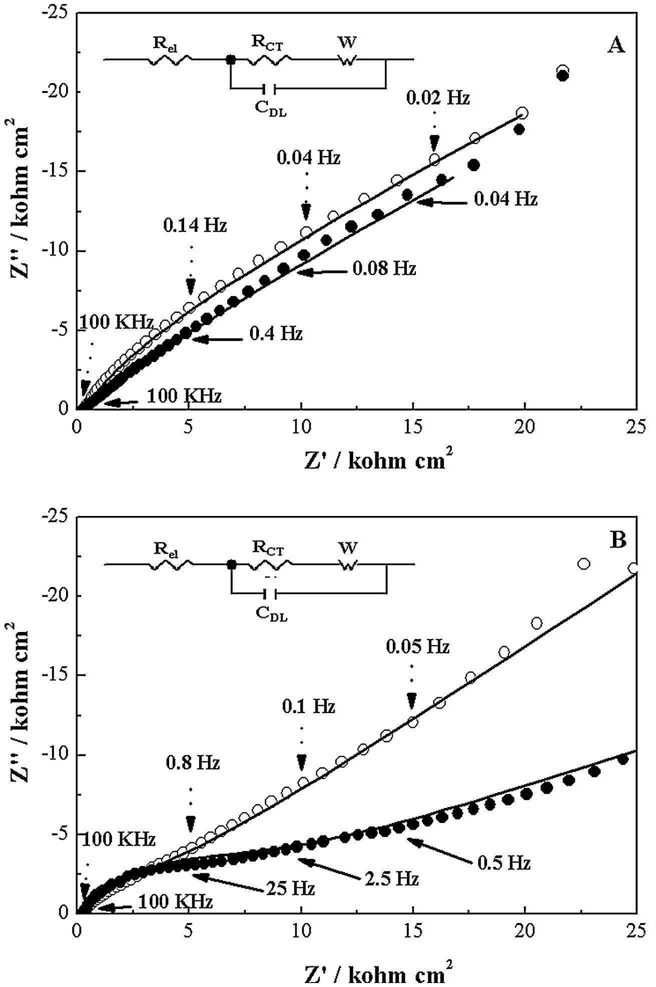


Fig. 7. Nyquist plots at anodic redox couple peak on GCE: experimental data (symbol) and optimum fit (line) of bare electrode (filled circle) and M2 (empty circle) at pH 6.9. (A) 1×10^{-3} mol L^{-1} $Ru(NH_3)_6{}^{3+/2+}$ solution and (B) 1×10^{-3} mol L^{-1} $Fe(CN)_6{}^{3-/4-}$ solution. Reference electrode: Ag/AgCl/3 M KCl. The insert: the EEC used to fit the EIS data.

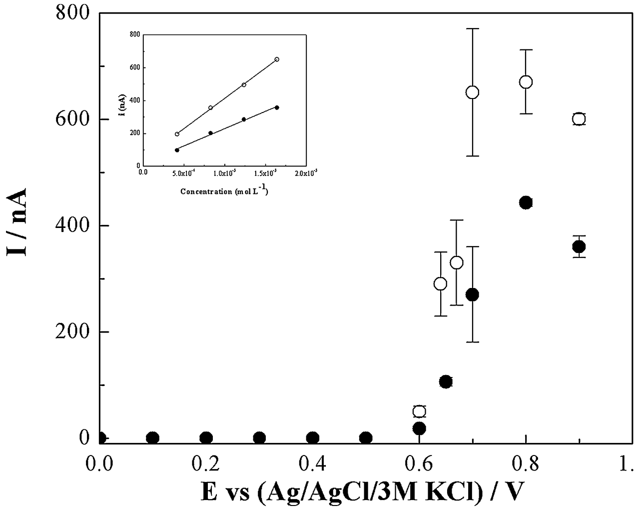


Fig. 9. Hydrodynamic voltammograms for 1×10^{-3} mol L^{-1} phenol in phosphate buffer, 0.05 mol L^{-1} (pH 6.9) with M2 PTH-modified GCE (empty circle) and bare electrode (filled circle). Inset: their respective calibration curves.

at the levels approaching the limits according to the equation LOD = 3.3(δ/S) and LOQ = 10(δ/S), being the LOD 3.6×10^{-6} mol L^{-1} and LOQ 1.09×10^{-5} mol L^{-1}. Typically, the regression equation for the calibration curve obtained as the average of 9 independent analyses and using five standard solutions was found to be

$$I(\text{nA}) = (2.9 \pm 0.2) \times 10^7\, C_{\text{Phenol}}\ (\text{mol L}^{-1}) + (10 \pm 3) \times 10^1$$

The assay precision was investigated with respect to both repeatability and reproducibility. Repeatability was determined by analyzing a 2.03×10^{-5} mol L^{-1} standard solution (five replicates), and is expressed as %CV, a value of less than 7% was obtained. Accuracy was determined by analyzing a known sample, comparing the measured value with the true value. Applying the *t* test, the experimental mean was not significantly different from the true value with 95% confidence (p = 0.7794), indicating that the proposed method is acceptable.

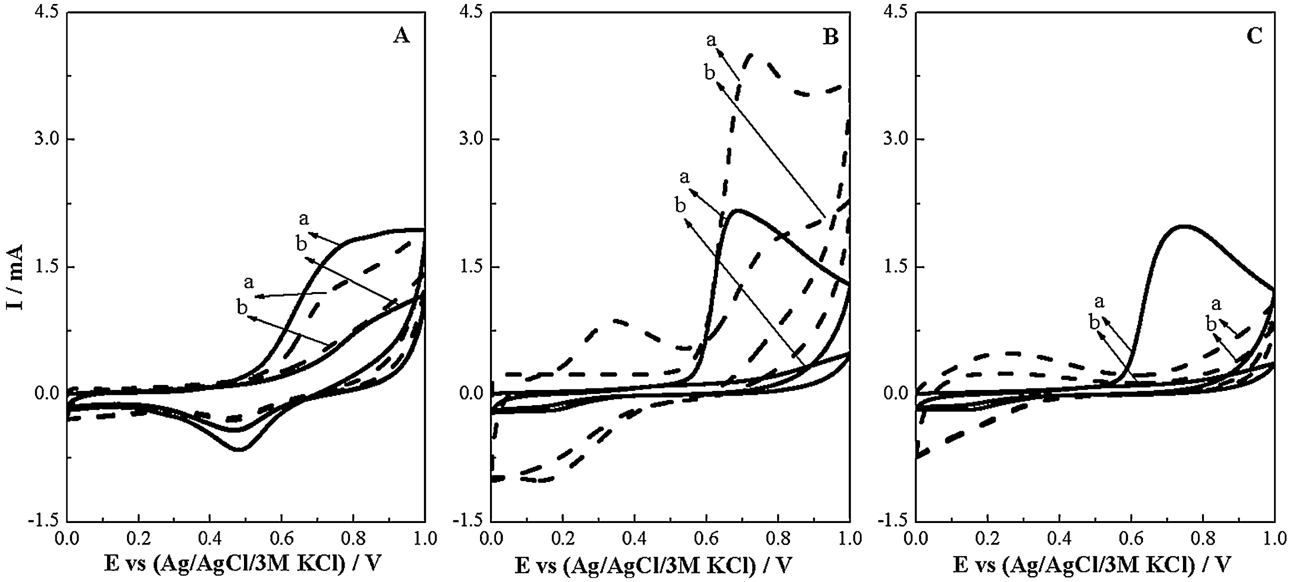


Fig. 8. CVs of 1×10^{-3} mol L^{-1} phenol in phosphate buffer solution at PTH-modified electrode (dash line) and bare electrode (solid line). (A) M2 GE, (B) M2 GCE and (C) M1 GCE. Scan rate: 50 mV s^{-1}. (a) 1st scan and (b) 2nd scan.

4. Conclusions

Two methods for polythionine film growth have been comparatively evaluated M1 and M2 that present different electrochemical characteristics. The polymeric film presents two compositions that are defined at different potential values named as m-T and poly-T, being their proportion dependent on the method and the pH used.

Modified glassy carbon electrodes were analyzed by CV and EIS using two redox probes. Electrodes modified by M2 at pH 6.9 present a lower R_{CT} value for negatively charged species than in all the other conditions. Furthermore, this modification increases the oxidation current signal and decreases the passivation process during phenol determination.

A simple and reliable method for phenol quantification was proposed and validated, corresponding to a M2 glassy carbon modified electrode. Using a 0.7 V working potential a 4×10^{-6}–$2.7 \times 10^{-5}\,\mathrm{mol\,L^{-1}}$ linear range is obtained with $3.6 \times 10^{-6}\,\mathrm{mol\,L^{-1}}$ LOD, $1.09 \times 10^{-5}\,\mathrm{mol\,L^{-1}}$ LOQ and a $(2.9 \pm 0.2) \times 10^{7}\,\mathrm{C_{Phenol}}\,(\mathrm{mol\,L^{-1}})$ sensitivity.

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