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Immobilization of purine bases on a poly-4-aminophenol matrix

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Abstract Conducting polymers or semi-conductors have various features that make them excellent materials for the immobilization of biomolecules and the rapid transfer of electrons necessary for the production of biosensors. Conducting electroactive polymers of poly-4-aminophenol have been developed as sensors to detect the purine bases (adenosine triphosphate, ATP and guanosine triphosphate, GTP) of DNA. The electrooxidation of 4-aminophenol onto a graphite electrode in the presence of perchloric acid yielded thin polymer films. The conductivity was studied by cyclic voltammetry and surface morphology by optical microscopy and interferometry. The immobilization and detection of ATP and GTP on a graphite electrode or modified electrode coated with poly-4-aminophenol was studied by cyclic voltammetry. Systematic variation of the experimental conditions that influenced the electrode reaction, particularly the pH of the electrolytic solution, showed that the oxidation potentials of the immobilized ATP or GTP in the modified electrodes decreased with increasing pH of the electrolyte. When these conditions were optimized based on voltammetric measurements, modified electrodes coated with poly-4-aminophenol were found to be efficient in immobilizing purine bases, and increased the amplitude of the ATP and GTP signals by ~1.5 and ~24 times, respectively, when compared with non-coated graphite surfaces.

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Introduction

The chemical structures of conducting polymers are amenable to modification, and chemical modeling and synthesis allow the alteration of their electrical and mechanical properties [1]. Conducting polymers are generally compatible with biological molecules in neutral aqueous solutions [1] and can efficiently transfer the electrical charges produced by biochemical reactions to electronic circuits [2]. The conductivity of conducting polymers is influenced by various factors, including polaron length, conjugation length, overall length and the charge transfer to adjacent molecules [1, 3].

Coating electrodes with conducting electroactive polymers (CEPs) under mild conditions provides numerous possibilities for immobilizing biomolecules and for producing bioaffinity or biodetectable reagents, as well as for improving their electrocatalytic properties, rapid electron transfer and direct communication in the development of analytical signals and new analytical applications [4]. CEPs contain conjugated pi-electron backbones that account for their unusual

electrochemical (high electrical affinities and conductivities and low ionization potentials) and optical (low energy optical transitions) properties that have previously been found only in inorganic materials [1].

Electropolymerized films have received considerable attention in the development of biosensors and biochips, and their use is rapidly increasing. Most of the work on biomolecules entrapment has been done using redox or electron-conducting polymers such as polyaniline, polypyrrole and polythiophene [5]. The discovery of polyaniline in 1862 [6] was followed by that of its four oxidation states at the beginning of the 20th century [7]. Polyaniline is the most successfully used polymer in gas sensors (see [8] for a recent review) and is one of the most important conducting polymers [9]. Polyaniline was the first conducting polymer to be commercialized and currently has applications ranging from batteries [10] to biosensors [11]. Numerous studies have investigated the usefulness of polyaniline films in organic and aqueous electrolytes, but the molecular structure and properties of its derivatives have been few studied [12, 13].

Some polymeric films containing amine groups, for example polytyramine, used as materials for biosensing devices contain one amine per moiety, i.e. a very high surface concentration of reactive sites for the immobilization of biomolecules. These sites can act as reactive groups for the covalent binding of oligodeoxynucleotides through a carboxamide bond or a phosphoramidate linkage [14].

Purine derivatives have important functions in nature, with adenine and guanine nucleotides, together with those of thymine and cytosine, being important monomer units of nucleic acids. Investigation of the electrochemical behavior of these molecules can improve our understanding of electron transfer reactions in DNA [15, 16]. In addition to their role in various biological processes [17], these nitrogenated bases are useful precursors in the synthesis of a wide variety of antiasthma and anti-AIDS drugs [18, 19] because of their participation in energy transduction and in cell signaling [20].

Electrochemical methods provide a promising approach for studying the immobilization of purines on the surface of electrodes coated with polymeric films [5]. The electrochemical processes involved in the oxidation of purine bases are similar to those involved in enzymatic oxidation reactions and are of crucial importance in interpreting DNA drug/metal interactions that result in oxidative damage of this nucleic acid [15].

The detection of DNA sequences through the hybridization of oligonucleotides to their complementary strands is possible [21–23]. Such methods have been used to detect polymorphisms and simple base mismatches [24].

The electrochemical behavior of single- and double-stranded DNA and oligonucleotides has been extensively characterized [25–29], including the redox processes of DNA bases and important aspects of the adsorption behavior on different electrodes such as mercury or carbon [30, 31]. Adsorption is the most convenient way to immobilize oligodeoxynucleotides on solids, but these macromolecules may be released from the surface during washing or simply through hybridization with targets, resulting in probe loss from the electrode surface. Hence, it is preferable to immobilize oligodeoxynucleotides on surfaces through covalent binding.

Adenine and guanine are monomeric units of nucleic acids, and guanine residues are frequently used as biomarkers in biosensors. In its oxidized form, the mediator which electrogenerated cations useful for achieving indirect reductions or oxidations of substrates can remove one electron from guanine residues in the hybridized nucleic acid target [32]. The electrochemical mechanisms involved in the oxidation of guanine and adenine in solution have been investigated [33], but no study has focused on their oxidation on the surface of electrodes coated with polymeric films derivated of poli-4-aminophenol. Polymers frequently show adsorption phenomena, particularly conducting polymers, which often have a charged structure, even in a reduced state.

The main objective of this work was to study the immobilization of adenosine triphosphate (ATP) and guanosine triphosphate (GTP) on the surface of graphite electrodes with or without electropolymerized films derived from 4-aminophenol. The immobilization of the purine bases on poly-4-aminophenol films was studied as a function of the pH of the solution and polymer formation. Electrodes coated with poly-4-aminophenol showed improved analytical characteristics and considerably enhanced the electrochemical signal associated with the detection of purine derivatives.

Experimental

Chemicals and solutions

All of the chemicals used were of analytical grade. The solutions were prepared with deionized water from a Millipore Milli-Q system (resistivity = 18.2 MΩ cm). 4-Aminophenol solution was prepared immediately before use. The purines used were obtained from Sigma. Stock solutions of ATP and GTP were prepared

in water, pH 5.5. Graphite (99.9995%) was used as the working electrode. All of the experiments were done at room temperature (25 ± 1 °C).

Apparatus

Voltammetric measurements, electropolymerization and cyclic voltammetry were done in a three-electrode cell using a PAR 273A potentiostat. Nitrogenated bases were detected using a 6 mm graphite disk electrode (GDE) as the working electrode. A platinum plate electrode was used as an auxiliary electrode. All of the potentials were measured with reference to a saturated calomel electrode (SCE).

The surface morphology of poly-4-aminophenol films were assessed by optical microscopy using a Zeiss Amplival microscope. Film thickness was measured with an interferometer Laser – UBM Measurement and Analysis System.

Mechanical preconditioning of the graphite electrodes

The graphite electrodes were polished using alumina oxide (0.3 μm slurry) before each electrochemical assay. After polishing, the electrodes were rinsed with deionized water. The electrode was then sonicated for 5 min in an ultrasound bath and rinsed again with water.

Electropolymerization and immobilization of nitrogenated bases

The monomer solutions were deoxygenated by bubbling with N_2 prior to electropolymerization. Polymeric films derived from 4-aminophenol were deposited on the surface of graphite electrodes by cyclic voltammetry. 4-Aminophenol (2.5×10^{-3} mol L^{-1}) was electropolymerized on the working electrode in a solution of $HClO_4$ (0.1 mol L^{-1}) by cycling the potential between +0.4 V and +1.87 V versus SCE at a variety of scan rates. After electropolymerization, the modified electrode was rinsed in deionized water to remove unreacted monomer.

This electrodes without film and modified electrodes with polymeric films were preconditioned by applying a +1.10 V oxidation potential for 300 s. After this treatment, ATP and GTP were immobilized on the surface of these preconditioned electrodes by adsorption at room temperature (25 ± 1 °C). Fifteen microliters of a stock solution of ATP or GTP (20 mmol L^{-1}) was placed on the surface of these electrodes, followed by incubation in a desiccator for 4 h. After immobilization of the nitrogenated bases, cyclic voltammetry was done using acetate (0.1 mol L^{-1}, pH 4.5) and phosphate (0.1 mol L^{-1}, pH 7.4) buffers as the electrolytic solutions.

pH studies

The buffers used to study the influence of pH were 0.2 mol L^{-1} Na_2HPO_4/0.1 mol L^{-1} citric acid pH 2.2–8.0, prepared according to McIlvaine [34] and 0.1 mol L^{-1} borate/sodium hydroxide solutions for pH 9–12. The purine bases were immobilized in the surface of electrodes coated with poli-4-aminofenol (25 potential scans).

Results and discussion

Polyaminophenol film synthesis and characterization

In this study, the polymerization was done in acidic aqueous medium since this yields films with improved conductivity [14]. Strongly adhering poly-4-aminophenol films were applied to the surface of a graphite disk working electrode by cycling between 0 and +1.87 V versus SCE, with 100 potential scans. The voltammogram of a graphite electrode for 100 cycles in solution of 4-aminophenol is shown in Fig. 1.

Figure 2a shows the cyclic voltammogram obtained with a graphite electrode coated with poly-4-aminophenol. in $HClO_4$ at pH 2.0. Waves of oxidation and reduction were observed at +475 mV and +420 mV, respectively.

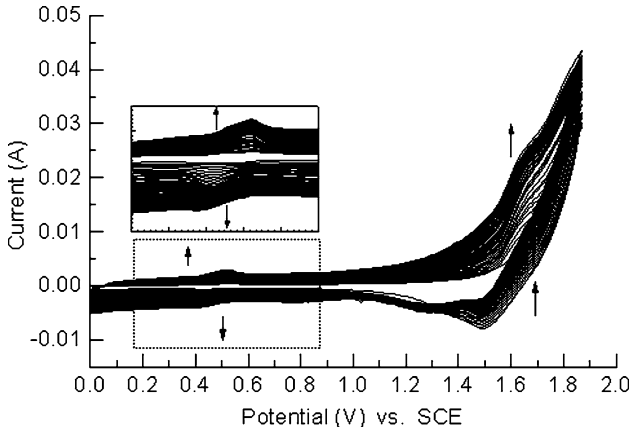

Fig. 1 Cyclic voltammogram of a graphite electrode in solution containing 2.5×10^{-3} mol L^{-1} 4-aminophenol in 0.1 mol L^{-1} $HClO_4$, 0.05 V s^{-1} (100 potential scans). The arrows indicate increase of current values. Inset: polymeric film formation

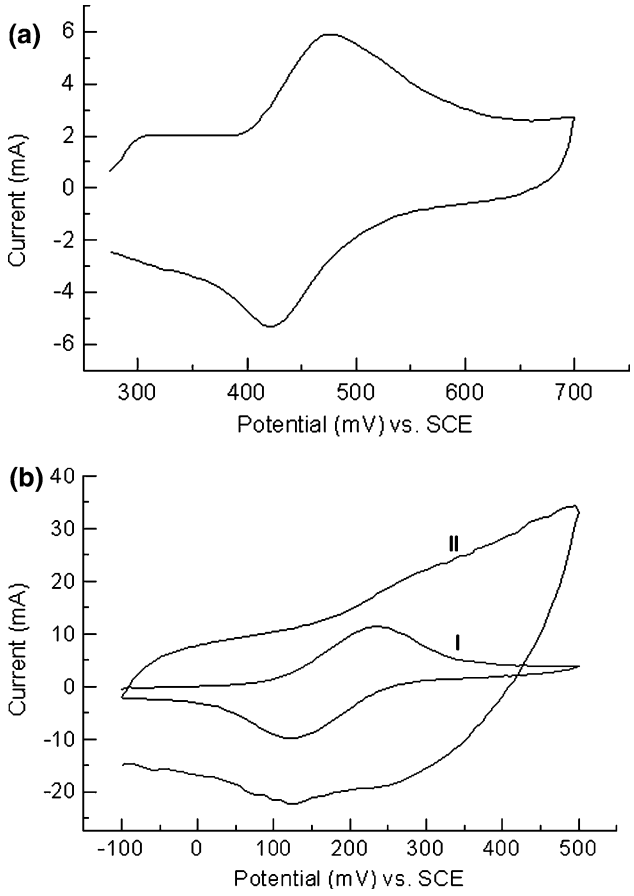

Fig. 2 (**a**) Cyclic voltammogram of poly-4-aminophenol on a graphite electrode. Medium: $HClO_4$ (0.5 mol L^{-1}); 50 mV s^{-1}. (**b**) Cyclic voltammogram of a graphite electrode without (I) and with (II) a poly-4-aminophenol film. Medium: aqueous solution containing $K_3Fe(CN)_6$ (5 mmol L^{-1}), $K_4Fe(CN)_6$ (5 mmol L^{-1}) and KNO_3 (0.1 mol L^{-1}), 100 mV s^{-1}

The increase in the electrochemical response of the redox pair suggests the covering of the electrode surface by the conducting polymer with characteristics that facilitated electron transfer as confirmed in an aqueous solution containing $K_3Fe(CN)_6/K_4Fe(CN)_6$ (Fig. 2b).

Reproducible cyclic voltammograms (up to 100 scans) were obtained without loss of the electro-activity of the polymer. When the polymeric films were compared as a function of the number of potential scans at a constant anodic potential limit, there was a reduction in the current of the oxido-reduction waves with the increase of the scans.

Analysis of the rugosity profile (Fig. 3), extracted profile (Fig. 4) and surface topography (Fig. 5) showed that electrodeposition resulted in complete coverage of the surface with poly-4-aminophenol and produced a film thickness of about 2.5 μm.

Purine immobilization

ATP and GTP undergo oxidation when immobilizated by adsorption in graphite electrodes. Figure 6 shows the voltammetric behavior of ATP in phosphate buffer (pH 7.4) and GTP in acetate buffer (pH 4.5) in which all of the experimental curves were corrected for the background measurements. This correction of the original voltammograms allowed clearer identification of the peaks.

In electrodes coated with a polymeric film containing immobilized purines, the magnitude of the current increased ~1.5 times for ATP (Fig. 6a) and ~24 times for GTP (Fig. 6b) compared to non-coated electrodes. In addition, the oxidation peaks for ATP and GTP shifted towards more positive potentials by 45 and 55 mV, respectively.

The most probable explanation for the wave of ~0.80 V, in electrodes modified with polymeric films, indicated by the arrows in Fig. 6b is that the guanosine used was slightly contaminated by guanine, a similar finding has been reported [16, 35]. The adsorption of guanine can attenuate the guanosine signal by reducing the electrode surface area available for the reaction.

None peak of guanine was found to studies with electrode without film, suggesting that the amount of immobilized guanine in the surface of the graphite electrode, in the GTP sample, is not enough to be detected, confirming the increase in the immobilization

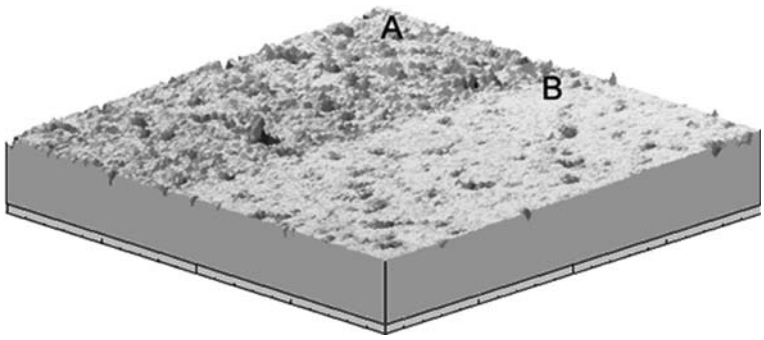

Fig. 3 Rugosity profile by interferometry of a graphite electrode (**a**) and an electropolymerized film of 4-aminophenol (50 mV s^{-1}) (**b**), 200 scans

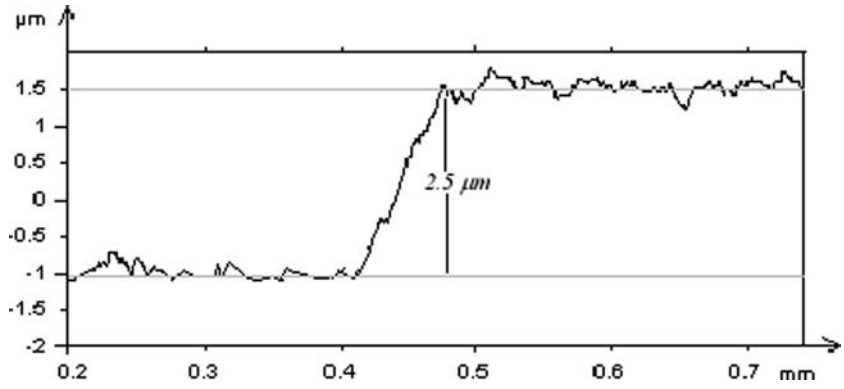

Fig. 4 Extracted profile by interferometry of a graphite electrode and a graphite electrode with electropolymerized film of 4-aminophenol (50 mV s^{-1}), 200 scans

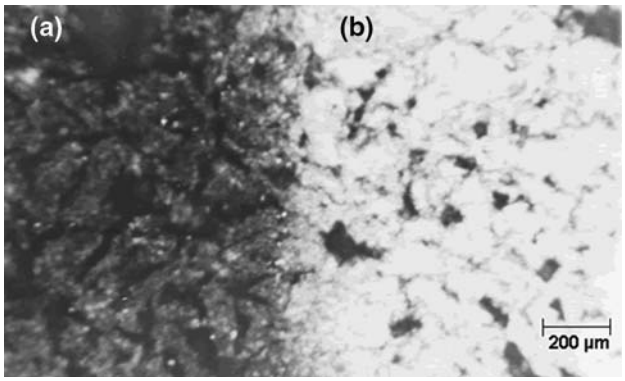

Fig. 5 Optical microscopy image of a graphite electrode without (**a**) and with (**b**) a conducting film of poly-4-aminophenol. Medium: 2.5×10^{-3} mol L^{-1} 4-aminophenol in $HClO_4$ (0.5 mol L^{-1})

efficiency in the surface of modified electrode with polymeric film.

Figure 7 shows the pH-dependence of the cyclic voltammetric potentials of ATP (Fig. 7A) and GTP (Fig. 7B) over the pH range of 2 to 12.

The E_{ox} versus pH plots were linear for the graphite electrodes and were expressed by the equations E_{ox} (ATP) = 1.5223 – 0.02901 × pH [mV vs. SCE] and E_{ox} (GTP) = 1.24 – 0.0448 × pH [mV vs. SCE], with correlation coefficients of approximately 0.99 for both.

The peak potential for the peak oxidation of ATP and GTP decreased with increasing pH, which suggested that the oxidation of these two molecules in acidic solution involved mechanisms similar to those that occur at neutral and alkaline pH. The slope of the E_{ox}–pH plot for ATP and GTP over the pH range studied was 29 and 45 mV per pH unit, respectively.

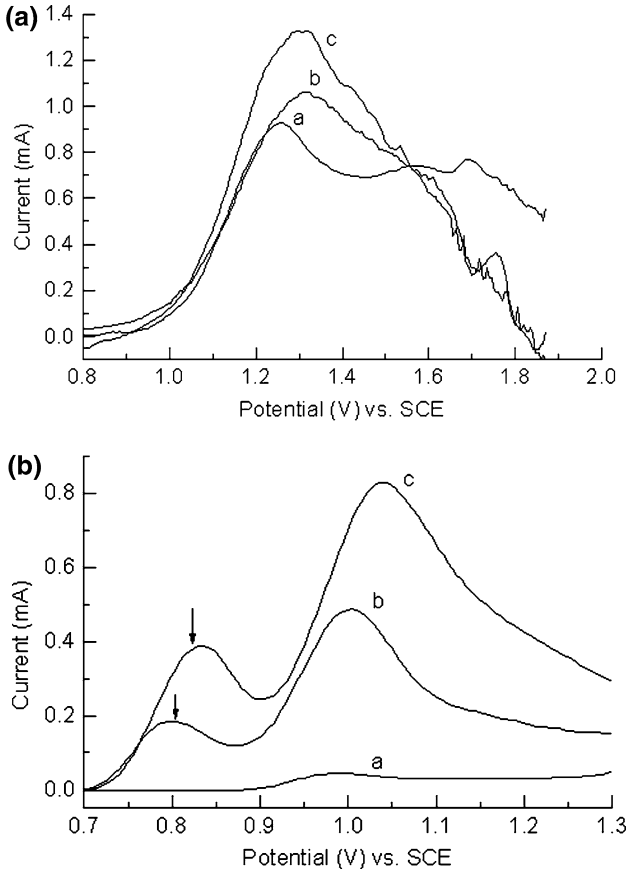

Fig. 6 (**a**) Cyclic voltammograms for ATP immobilized on electrodes using 0.1 mol L^{-1} phosphate buffer, pH 7.4. (**b**) Cyclic voltammograms for GTP immobilized on electrodes using 0.1 mol L^{-1} acetate buffer, pH 4.5. Scan rate: 5 mV s^{-1}. (a) no film; (b) poly-4-aminophenol, 25 scans; (c) poly-4-aminophenol, 100 scans. The arrows indicate guanine oxidation

Conclusions

It was possible the formation of electroactive polymeric films derived of 4-aminophenol in graphite electrodes, by cyclic voltammetry.

The electropolymerization resulted in complete coverage of the graphite surface with a film thickness of about 2.5 μm.

These modified electrodes coated with poly-4-aminophenol were found to be efficient in immobilized purine bases. The oxidation potentials of the immobilized ATP

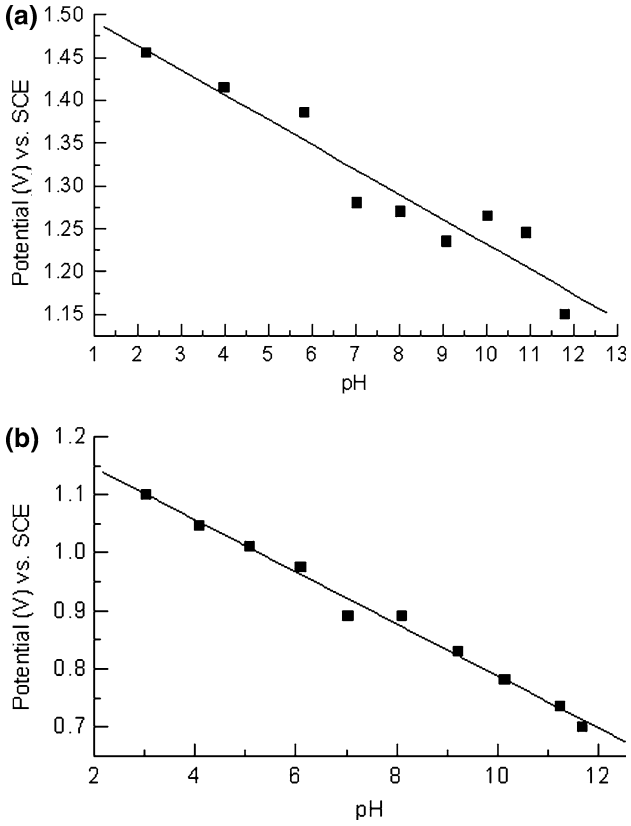

Fig. 7 Influence of the pH of the supporting electrolytic solution on the cyclic voltammetric peak potential of ATP (**a**) and GTP (**b**)

or GTP in the modified electrodes decreased with increasing pH of the electrolyte.

The modified electrodes increased the amplitude of the ATP and GTP signals by ~1.5 and ~24 times, respectively, when compared with non-coated graphite surfaces.

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