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Voltammetric analysis of acyclovir at glassy carbon/oppy/templated electrode

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Abstract. This study had the purpose to analyse the antiviral drug acyclovir employing a voltammetric method that permits its quantification sensitively and reliably in pharmaceutical preparations. This investigation consisted of optimizing the voltammetric parameters and involved the chemical modification of the electrode. Among Au, Pt, and GC electrodes, the latter showed the best behaviour, which was modified by means of electrochemical and chemical pretreatments, using 0.10 M $LiClO_4$ as supporting electrolyte, 20.0 mM pyrrole, and 1.0 mM acyclovir as templating species. Polypyrrole was then overoxidized in 0.10M phosphate buffer at 0.95 V vs Ag/AgCl. This approach enhanced the limit of quantification, the stability, and sensitivity. The glassy carbon electrode coated with molecularly imprinted, overoxidized polypyrrole (OPPy) behaves as a cation exchanger due to dedoping and loss of conjugation. Square wave voltammetry allowed determining acyclovir in 0.10 M phosphate buffer, pH 4.00. The pulse height and frequency were 40 mV and 50 Hz. The limit of detection was 0.20 µM. Its quantitation in a pharmaceutical preparation by multiple addition of standard, a content of (206.0 ± 4.2) mg was determined, R^2 was 0.9986; the amount of Acy was 200 mg. The voltammetric method is of easy application, less expensive, and as sensitive as HPLC.

1. Introduction

Acyclovir is a guanosine analog; (2-amino1,9-dihydro-9-(2-hydroxyethoxymethyl)-6H-purine-6 one), is the most commonly used antiviral drug used in the treatment of infections caused by the varicella-zoster virus and the herpes simplex virus [1]. An inadequate consumption causes adverse reactions, for example, neurotoxicity, urticaria, diarrhoea, and fainting. There exist different analytical approaches to ACy quantification. Lotfy et al [2] reported two sensitive and precise methods for the determination of ACy in presence of its metabolite (Guanine). Regression analysis showed excellent correlation in the concentration ranges 10-60 μgmL^{-1} and 20 -120 μgmL^{-1} with percentage recoveries of 100.06 ± 0.89 and 99.62 ± 0.66 for methods A and B, respectively. Other methods include spectrophotometry [3-4], colorimetry [5], spectrofluorometry [6], HPLC [7-8], and voltammetry [9 – 11].

Our concern consisted in modifying the working electrode surface. First, it was activated by potential cycling between -1.6 V and +1.6 V vs Ag/AgCl in 0.10 M PBS. Then pyrrole was electropolymerized. The charge injected was carefully controlled to prepare a thin film of the polymer. Polypyrrole has received the most attention due to its easy preparation, high stability and wide range of applications. PPy shows its electric conductivity and electrochemical redox activity [12].

Molecular imprinting techniques are suitable and accepted methods for the recognition and isolation of key biological or biochemical target molecules, due to the great potential advantages of using molecularly imprinted polymers (MIPs) instead of natural receptors and enzymes such as their superior stability, low cost, and easy preparation. The general principle of molecular imprinting is based on processes where monomers are polymerized in the presence of a target analyte (the imprinting molecule) which acts as a molecular template. This procedure can be accomplished via either reversible covalent bonding or non-covalent interactions between monomers and imprinting molecules. Toth´s group [13] reported the use and characterization of ultrathin overoxidized polypyrrole (OPPy) films templated with adenosine, inosine and ATP, grown on GC. High sensitivity at the ultrathin film electrodes indicated that the interactions with the film rather than in-film transport controlled the response of the film electrodes in slow scan voltammetry. The electropolymerization method provides a reliable, simple, and rapid technique of controlling the thickness of the conductive polymer film. The polymer PPy can undergo charge transfer reactions, while OPPy is an ion conductor with perm selective properties.

In this work, a thin OPPy film coated GCE was assayed with the aim of enhancing selectivity, stability, and sensitivity in the quantification of acyclovir. The film was imprinted with Acy to create interaction sites in the architecture of the polymer once the template molecule is released from it. After overoxidation, the positive charge of PPy is lost and the OPPy film became a cation perm selective membrane.

2. Experimental

2.1. Reagents and solutions

A Stock solution of acyclovir (1.00 mM) was prepared in 0.10 M phosphate buffer of pH 4.00 and used as supporting electrolyte. All other reagents were analytical grade and prepared in double distilled water. Acetonitrile was HPLC grade. Pyrrole was from Sigma. Potassium phosphate dibasic and potassium ferricyanide were from Mallinckrodt. Acetonitrile and methanol of HPLC grade, purchased from Fisher.

2.2. Instrumentation

A 660E CHI bipontentiostat was used for the electrochemical measurements. The cell consisted of a glassy carbon working, Ag/AgCl as the reference, and Pt as the counter electrodes. The electrodes were purchased from BAS. The cell has a capacity of 10 mL. Prior the experiments all solutions were purged with nitrogen for ten minutes. The potentiostat was connected to a PC for data acquisition. The HPLC measurements consisted of an 1100 Agilent system, an ODS column. The mobile phase was methanol/0.020 M acetic acid, 5:95 (v/v), DAD at 280 nm. The internal standard was guanine.

Before modification, the GCE was cleaned with a micro polishing powder of 0.025 µm alumina/water slurry on a microcloth for three minutes and sonicated in deionized water for a minute. Then, the electrode was washed in an ultrasonic bath with isopropanol and doubly distilled water for three minutes. The GCE area was determined by chronocoulometry using 0.10 M $Fe(CN)_6^{3-}$ in 0.10 M KCl ($D_o = 7.63x10^{-6}$ $cm^2.s^{-1}$).

2.3. General Procedure

PPy films were grown onto GC and electrosynthetized modifying the procedure recommended by Hsueh. [13] which consisted of preparing 10.0 mL of 0.10 M LiClO4, 0.020 M Pyrrole, 200 µL water and adding acetonitrile HPLC grade to the mark. The solution was degassed for ten minutes with N_2(g). Chronocoulometry (CC) was used from a potential step from 0.0 to 0.75 V, pulse width of 100 ms, in order to control the charge of ca 35µC/cm^2. The overoxidation of PPy was conducted by CC in 0.10 M PBS, pH 7.0 from 0.0 to 0.95 V vs Ag/AgCl, a pulse width of 800 ms. Each PPy formation is followed by overoxidation and this scheme was repeated until the signal coming from the reduction of 0.10 M ferricyanide in 0.10M KCl disappeared. In average, six coatings were applied. Figure 1, shows how ferricyanide response decreased with overoxidation. To synthetize OPPy template, to the initial matrix, we added 0.50 mM acyclovir and repeated the same procedure to form OPPy/Acy.

Overoxidized PPy and OPPy/Acy thin film GC electrodes gave reproducible results, for at least forty measurements, of 100.0 µM Acy by SWV. The relative standard deviation was about 4%. The OPPy/Acy templated electrodes were more sensitive and stable than the OPPy ones. From calibration graphs, we deduce the sensitivity and linear dynamic range of bare GC, PPy, OPPy, and OPPy/Acy electrodes. The imprinted molecule enhanced response and linear range.

The GC/OPPy/Acy electrode displayed a significant improvement of the anodic peak current of Acy. Under our conditions, the templated electrode showed a wide linear response range from 0.50 µM to 10.0 µM with a detection limit of 0.20 µM (based on 3 sb) and a quantitation limit of 0.50 µM. A 50.0 µM Acy solution was submitted to SWV and the peak current was measured every day for seven days and the average current was 0.1270 µA, the relative standard deviation was ca 6.5%. In the same day, in a period of 12 hours, five measurements of Acy were made and the relative standard deviation was 3.3%.

The OPPy templated electrode was also applied to the direct determination of Acy in a commercial formulation of 200 mg, using the method of multiple addition of standard. Square wave voltammetry permitted to determine the sample content of Acy. It resulted to be 206± 4 mg. The percent relative error was 3.0%. We compared this analysis with the HPLC method and the results did not show significant differences at 95% level of confidence. The HPLC system consisted of a Agilent 1100, with acetic acid 0.020 M/ methanol (95:5) as mobile phase, flow rate 1.0 mL.min^{-1}, wavelength 280 nm, DAD, a 15.0 cmx3mmx 3.7µm ODS column. The internal standard was guanine.

3. Results and Discussion

3.1. Optimal parameters for SWV

At the bare GCE, a 1.00 mM solution of Acy was analyzed by SWV to find out the best electrolyte and concentration, pH, frequency, and amplitude. The experimental outcomes were 0.10 M PBS, pH 4.00, 40 Hz, and 50 mV, respectively. To electropolymerize pyrrole, the optimal conditions were 0.10 M LiClO4, 20.0 mM Py, 200 µL H20, in 10.0 mL of acetonitrile solution. The potential step varied from 0.0 to 0.75 V vs Ag/AgCl and for overoxidation of PPy with 0.50 mM Acy as templating molecule, the potential step changed from 0.0 to 0.95 V in 0.10M PBS, pH 7.00. Both procedures were carried out using chronocoulometry. To suppress the cyclic voltammetric response of 0.10 M $Fe(CN)_6^{3-}$ in 0.10 M KCl, sweep rate 25 mV/s, electrode area 0.0036 cm^2, four to six coatings were needed. Figure 2 displays this behaviour, which indicated that the OPPy film is free of pinholes [13], and is now an ion exchange membrane.

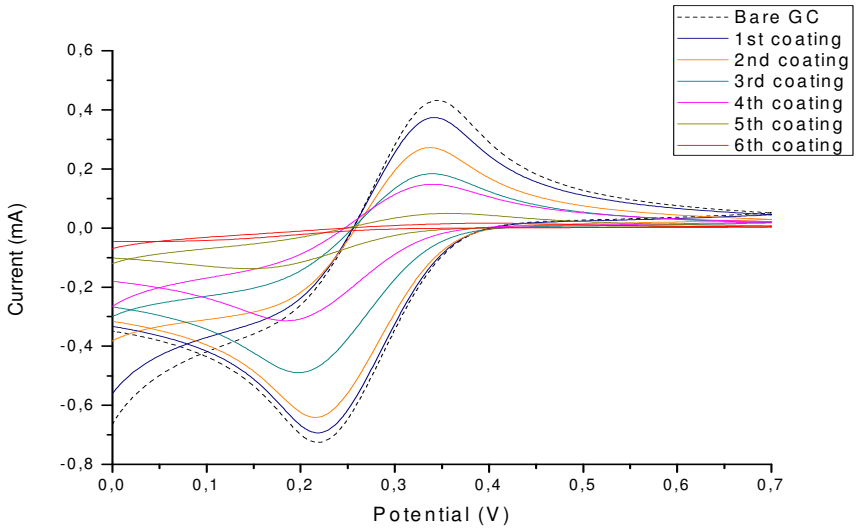
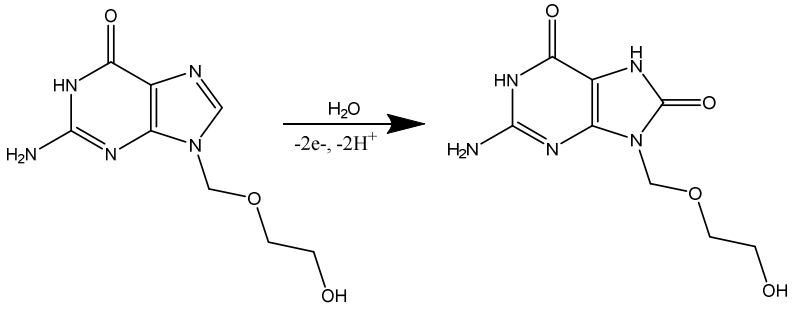


Figure 1. Response of 0.10 M $Fe(CN)_6^{3-}$ in 0.10 M KCl after repeated PPy coating/overoxidation. Scan rate25 mV/s, A = 0.0036 cm^2.

3.2. Diffusion controlled process

To know if the process was controlled by diffusion or by adsorption or if there was a contribution of both processes, cyclic voltammetry was performed. There is a linear relationship between the peak current and the sweep rate, this was evaluated between 5.0 and 125 mV/s (Plot 2A). Since the correlation coefficient of the linear graph obtained was very close to one (0.9977), the data indicated a process controlled by diffusion. In addition, a graph of the logarithm of the peak current versus the logarithm of the sweep rate should give a slope close to 0.5 (Plot 2B). The experimental results gave a slope of 0.483, indicative of a process controlled by diffusion. The irreversible oxidation of Acy is a 2e, $2H^+$ process, (scheme 1).



Scheme 1. Electrochemical oxidation of Acy according to Shahrokhian et al [14].

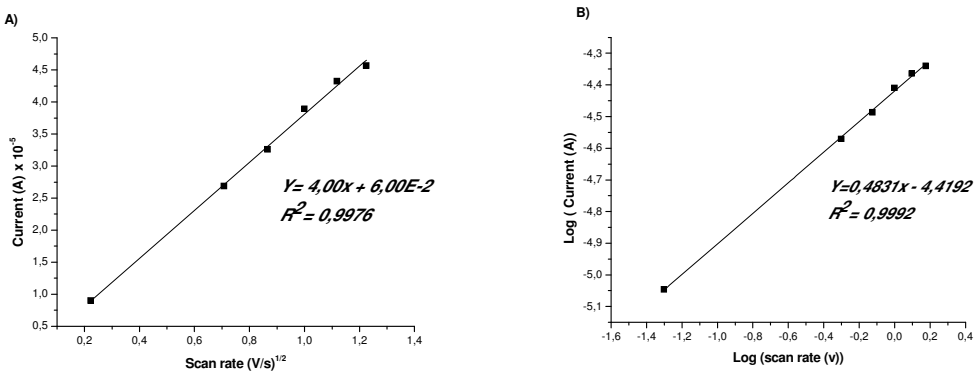


Figure 2. Peak current vs scan rate A) and log peak current vs log scan rate B), at a GCE 1.0 mM solution of Acy, buffer pH 4.00.

3.3. Influence of GC/OPPy/Acy on sensitivity and stability

Since nitrogen, carboxylic, carbonyls groups in OPPy films permit additional hydrophobic and electrostatic interactions with the templating molecule, Acy; the sensitivity is greatly enhanced as shown in figure 4. The slopes of the calibration curves of Acy at the bare and OPPy/ACy were 0.622 and 2.020. The correlation coefficient in both cases was 0.9995, as displayed in figure 3. The templated electrode increased its slope 3.25 with respect to the bare GCE. The limit of quantitation improved to 0.50 µM, measured through the calibration graph.

The use of molecularly imprinted polymers has received a lot of concern. There are many applications of MIPs in areas as sample preparation, food and environmental analysis, biosensors, chemical separation, selective extraction, catalysis, or molecular sensing [15].

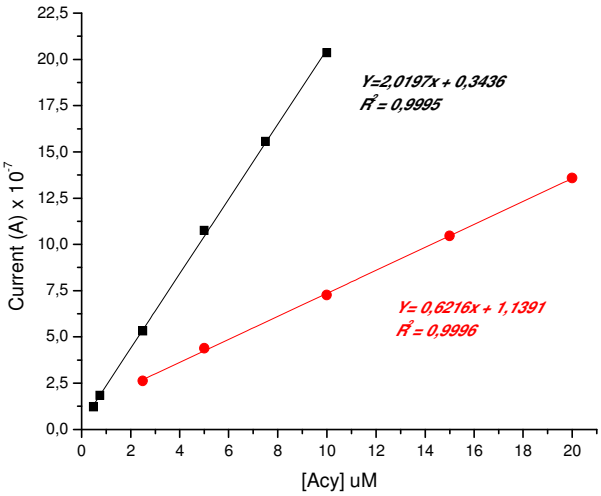


Figure 3. Calibration graphs of Acy at bare GC (black) and at GC/OPPy/Acy (red).

4. Conclusions

Our results allowed to determine Acy in a sensitive and reliable way. The GC/OPPy/Acy gave reproducible oxidation peak currents. For analytical purposes, the modified electrode displayed good electrochemical behaviour with significant increase of the peak current compared to the bare GC. A well-resolved diffusion controlled voltammetric peak was obtained in phosphate buffer solution, pH 4.0. The limit of quantification was 60 $ng.mL^{-1}$. A linear calibration graph from 0.50 µM to 10.0 µM had a correlation coefficient of 0.9995 and a slope of 2.020, which was 3.25 higher than the bare electrode. The method was successfully applied to direct assays of Acy tablets using the multiple addition of standard. The GC/OPPy/templated electrode can be used for at least forty measurements without losing reproducibility by the use of cyclic voltammetry. The imprinting molecule is released during overoxidation of PPyand diffuse towards the surface of the electrode, leaving vacancies in the polymer architecture with favorable interactions with the analyte (hydrogen bonding). The method of electropolymerization (by chronocoulometry), provided a simple way of controlling the thickness of the polymer film, by controlling the charge. Thin layers of the OPPy polymer favor the perm selectivity of the ion exchange membrane.

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