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Electrochemical evaluation of non-electroactive drug erythromycin in trace amount at biological samples by continuous cyclic voltammetry

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ABSTRACT

A fast continuous cyclic voltammetry was used as a detection method for the monitoring of erythromycin in a flow-injection system. A special computer-based numerical calculation method (using Fast Fourier Transformation) is introduced here for enhancing the analyte signal and noise reduction. During the measurements, the potential waveform (consists of potential steps for cleaning, stripping and potential ramp) was continuously applied on an Au disk microelectrode with a 25 μm radius. The objective of the performed experiments was the inspection of the effects of different parameters on the method sensitivity. After the experiments' completion, it was concluded that the method was linear for the concentration range of 7–733,000 pg/ml ($r = 0.998$) with a limit of detection and quantitation 2.4 and 7 pg/ml, respectively. The stripping time was less than 200 ms. Effects of rest potential, sweep rate, and delay time on the sensitivity and selectivity of the method were investigated.

1. Introduction

Erythromycins are broad-spectrum antibiotics that exhibit high activity against nearly all Gram-positive and Gram-negative bacteria. It is a macrolide antibiotic that has an antimicrobial spectrum similar to or slightly wider than that of penicillin, and is often used for people that have an allergy to penicillins. Most of erythromycin is metabolised by demethylation in the liver. Its main elimination route is in the bile, and a small portion in the urine. Erythromycin is available in enteric-coated tablets, slow-release capsules, oral suspensions, ophthalmic solutions, ointments, gels, and injections.

Owing to their extensive use in infectious disease therapy, several procedures have been reported for their determination, such as, spectrophotometry [1–3], and electrochemistry [4–8], HPLC techniques with UV, fluorescence, or electrochemical detection (ECD) which were previously used for the detection of erythromycin concentration in human plasma [9–17]. Wang et al. have reported the oxidation behavior and determination of erythromycin at a glassy carbon electrode. In that work, the voltammetric behavior of the antibiotic erythromycin at the pre treated glassy carbon electrode has been described [18]. Voltammetric techniques are generally rapid and economical in the determination of some organic and inorganic compounds in aqueous systems with a sensitivity range of parts-per-billion. Additionally, due to the movement of the analyte zone in an electrochemical flow cell, the application of these techniques requires fast analyte accumulation and fast potential scanning, which need very small electrodes [19,20].

The use of voltammetric techniques has been further stimulated by the advent of ultramicro electrodes (UMEs), due to their steady state currents, higher sensitivity, increased mass transport and their ability to be used in electroanalysis in solutions with high resistance [21]. UMEs, for example, have been applied as sensors in various techniques such as flow injection analysis [22,23], cardiovascular monitoring and organic compounds analysis [24,25]. Some investigations were also done, to find the effects of various parameters on the sensitivity of the proposed method. Some of the advantages of the proposed method are as following the removal of oxygen from the test solution is not required any more, the detection limit of the method is sub-nanomolar and finally, the method is fast enough for determination of such compounds, in a wide variety of chromatographic methods. Furthermore, in this paper, a special computer based numerical method is introduced, for calculation of the analyte signal and noise reduction.

1.1. Theory

In Fig. 1, the diagram of applied waveform potential during cyclic voltammetric measurements is shown. The potential waveform consists of three parts; a) cleaning part (part EC), potential steps, E_{c1} and E_{c2} (which are used for oxidizing and reduction of the electrode surface, respectively), by which electrochemical cleaning of the electrode surface takes place, b) E_c, where accumulation of analyte takes place, and c) the final part potential ramp, in which current measurements take place.

The waveform potential was continuously applied on an Au disk microelectrode (12.5 μm radius). It must be noted that in this case, the

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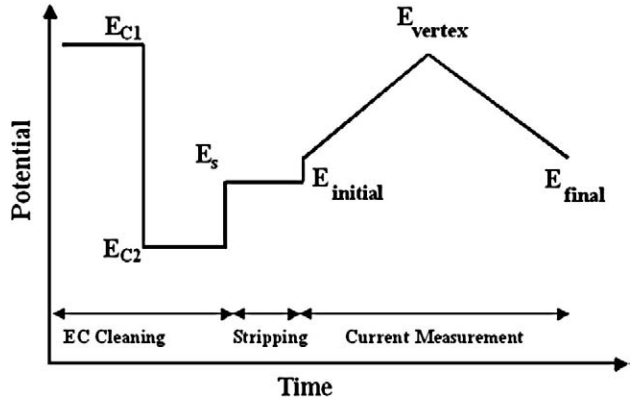

Fig. 1. The schematic of applied potential waveform.

current changes (result of injected analyte) at the voltammograms can be caused by various processes, which take place at the electrode surface. Those processes include; a) oxidation and reduction of adsorbed analyte, and b) inhibition of oxidation and reduction of the electrode surface by the adsorbed analyte. Indeed, in order to see the influence of the adsorbed analyte on the oxidation and reductions peaks of the gold surface, the scan rate must be set at very high rates (e.g. >20 V/s).

However, during the scan, some of the adsorbed analyte molecules are desorbed. Depending on the rate of those processes and scan rate, the amount of the desorption analyte molecule (during the scan) can be changed. The important point which has to be mentioned is some parts of the adsorbed analyte molecule still remain on the electrode surface that make inhibition of red/ox process of the electrode surface.

It is well understood that the crystal structure of a polycrystalline gold electrode, strongly depends on the condition of applied potential waveform [21]. Therefore various potential waveforms were examined in order to obtain a reproducible electrode surface (or a stable background signal). In fact, application of cyclic voltammetry for determination of electro active compound mainly faces to low stability of the background signal, due to changes occurring in the surface crystal structure during oxidation, and reduction of the electrode in each potential cycle. In this work, after examination of various potential wave forms, the best potential to obtain a stable background during the measurement was the waveform shown in Fig. 1.

The electrochemical oxidation process of gold surface started with electrosorption of hydroxyl ion, which at more positive potentials was going to form a gold oxide and undergoes structural rearrangement [24]. The surface oxidation can be initiated by adsorption of water molecule and then at more positive potential AuOH forms leading to the formation of a two-dimensional phase of gold oxide;

$$2Au + 3H_2O \rightarrow Au_2O_3 + 6e + 6H^+. \quad (1)$$

An example of recorded CVs is shown in Fig. 2 (a, b). Fig. 2a shows a sequence of CVs recorded during the flow analysis for the determination of the drug. The volume of the injection was of 50 µl of 8.0×10^{-7} M erythromycin (in 0.05 M H_3PO_4) into the eluent solution containing 0.05 M H_3PO_4. The time axis of the graph represents the time of the flow injection experiment. In the absence of erythromycin, the shape of the CV curves is typical for a polycrystalline gold electrode in acidic media [27]. Fig. 2b shows the absolute current changes in the CVs curves after subtracting the average background 4 CVs (in the absence of the analyte). To obtain the current change at first 4–5 voltammograms without drug injection should be recorded. Then the new voltammograms recorded after injection was subtracted of the mentioned voltammograms. As can be seen, this way of presentation of the electrode response gives more details about the effect of adsorbed ion on currents of the CV. The curves show that current changes mainly take place at the potential regions of the oxidation and reduction of gold. When the electrode–solution interface is exposed to erythromycin, which can be adsorbed on the electrode, the oxide formation process becomes strongly inhibited. In fact, the inhibition of the surface process causes significant change in the currents at the potential region, and as a consequence the profound changes in the shape of CVs take place. Universality of the detector in this mode is very advantageous for chromatographic analysis, where a mixture of compounds is present in sample.

It must be noted that, theoretically, in this method, the analyte response can be affected by the thermodynamic and kinetic parameters of adsorption, the rate of mass transport and electrochemical behavior of the adsorbed species. The free energy and the rate of adsorption depend on the electrode potential, the electrode material, and to some extent, on the choice of the concentration and type of supporting electrolyte. By taking points into consideration, in order to achieve maximum performance of the detector, the effect of

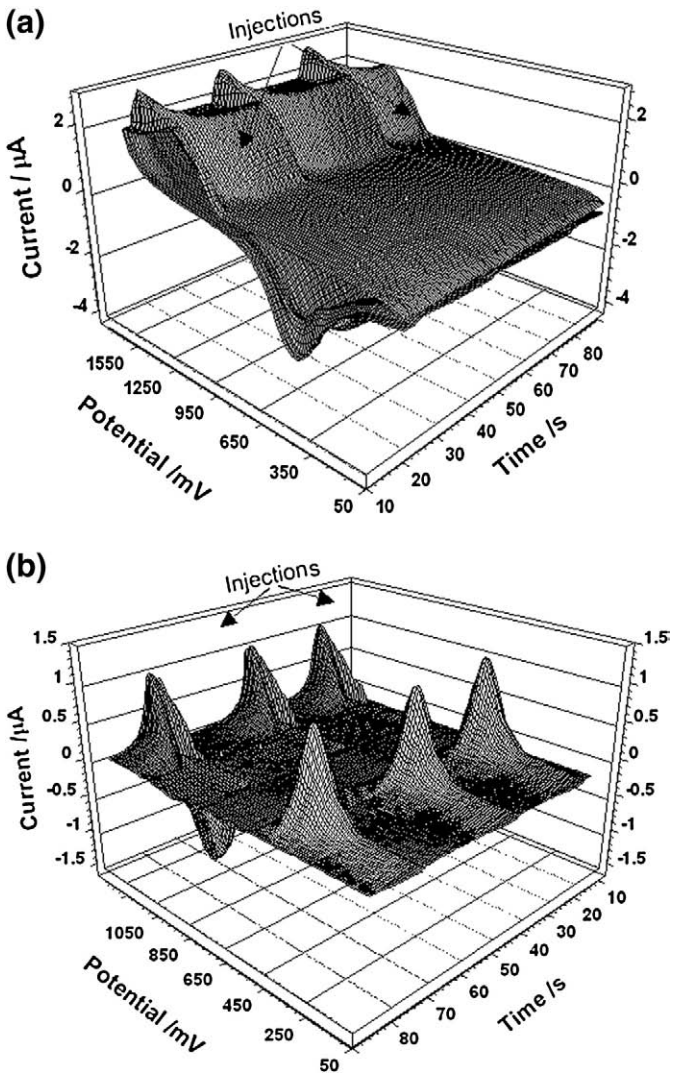

Fig. 2. a) Cyclic voltammogram at a 12.5 µm Au ultramicroelectrode recorded during a flow-injection experiment. The eluent was 0.05 M H_3PO_4, the flow rate, and the sweep rate were 3 ml/min and 80 V/s, respectively. For the electrode conditioning, each scan was preceded by 100 ms (at 1600 mV) and 100 ms (at 300 mV), respectively. The accumulation time was 300 ms at 400 mV. The injected solution (50 µL) contained 8.0×10^{-7} M erythromycin in 0.05 M H_3PO_4. b) Curves result of subtraction of an average CVs (in the absence of the analyte) from of the CVs in (a).