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Ultrasensitive flow-injection electrochemical method for detection of anticancer drug tamoxifen

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ABSTRACT

This paper presents the optimization of instrumental and solution parameters for determination of tamoxifen in urine and plasma and formulation by fast Fourier transform square wave voltammetry (SWV) using a gold microelectrode in flow-injection system. The samples are subjected by the same buffer solution and are injected in the flow-injection apparatus. By applying a novel square wave voltammetry method to perform as a sensitive method the voltamograms are recorded. The method used for determination of tamoxifen by measuring the changes in admittance voltammogram of a gold ultramicroelectrode (in 0.05 mol L^{-1} H_3PO_4 solution) caused by adsorption of the tamoxifen on the electrode surface. The best sensitivity was achieved using a frequency of 600 Hz and a medium composed of 0.05 mol L^{-1} phosphate buffers at pH 2.0. The best performance was obtained with the pH value of 2, pulse amplitude 25 mV, frequency 600 Hz, accumulation potential of −100 mV and accumulation time of 0.5 s. Furthermore, signal-to-noise ratio has significantly increased by application of discrete fast Fourier transform (FFT) method, background subtraction and two-dimensional integration of the electrode response over a selected potential range and time window. Calibration plots are given for solutions containing 1.0×10^{-11} to 3.0×10^{-6} mol L^{-1} of tamoxifen. The detection limit is calculated to be 3.0×10^{-12} mol L^{-1} (~2 pg mL^{-1}). The relative standard deviation at concentration 2.0×10^{-8} M is 6.1% for five reported measurements.

1. Introduction

Trans isomer of (*Z*)-2-[*p*-(1,2-diphenyl-butenyl)phenoxy]-*N*,*N*-dimethyletylamine (tamoxifen) is a hormone treatment developed over 20 years ago. It lowers the risk of breast cancer coming back (recurring) or developing in the other breast. It belongs to a class of drugs called selective estrogen receptor modulators (SERMs), which have both estrogenic and antiestrogenic effects. Tamoxifen has the same nucleus as diethylstilbestrol but possesses an additional side chain (trans isomer) which accounts for its antiestrogenic activity [1]. Tamoxifen is extensively metabolized after oral administration. *N*-desmethyl tamoxifen is the major metabolite found in plasma. *N*-desmethyl tamoxifen activity is similar to tamoxifen. The prolonged binding of tamoxifen to the nuclear chromatin of these results in reduced DNA polymerase activity, impaired thymidine utilization, blockade of estradiol uptake, and decreased estrogen response [2,3]. There were some reports on determination of that by using Capillary gas chromatographic in the presence of a number of antidepressants in urine [4] and Capillary zone electrophoresis [5], but these methods are so expensive and time consuming. The method which introduced in this paper is too sensitive, inexpensive and fast for detection of tamoxifen.

The combination of ME with square wave voltammetry (SWV) has recently been shown to be advantageous for environmental detection of several compounds [6]. The adaptation of this technology to ASV of tamoxifen on gold ME could provide a substantial improvement for rapid analysis [7–9]. This paper describes a fundamentally different approach to SWV measurement, in which the detection limits are improved, while preserving the information content of the SW voltammogram. The approach is designed to separate the voltammetric signal and background signal in frequency domain by using discrete fast Fourier transformation (FFT) method. This separation allows, digitally filtrating some of the noises and decreasing the bandwidth of the measurement. Further improvement in the signal was gained by two-dimensional integration of the electrode response over a selected potential range and time window of the signal. Although at sufficiently high scan

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rates CV can approximate an ac voltammetric technique and can be used to investigate electrode surface phenomena such as physical adsorption, FFT–SWV may be a more appropriate technique for monitoring analyte adsorption, as the potential dependence of analyte adsorption may be more clearly characterized. SWV measures the current response while rapid alternating potentials are applied during a staircase scan, whereas CV, which uses only a forward and reverse linear dc scan, is not sensitive to the potential dependence of changes that occur in the double layer.

2. Experimental

2.1. Apparatus and reagents

All solutions were prepared in double-distilled water using analytical grade reagents. Reagents in use in preparation of the stock eluent solution for flow-injection analysis (0.05 mol L^{-1} H_3PO_4 and NaOH 1 mol L^{-1} used for adjusting pH of the eluent) were obtained from Merck Chemicals. In all experiments all solutions were made up in the background electrolyte solution, and were used without removal of dissolved oxygen.

The equipment for flow-injection analysis included a 10 roller peristaltic pump (Home made) and a four-ways injection valve (Supelco Rheodyne Model 5020) with a 50 μL sample loop. In all experiments, described in this paper, the flow rate of eluent solution was 0.5 mL min^{-1}.

The Au–ME was constructed from a 25 μm diameter gold wire (Goodfellow). It is the same as what we used in our previous papers [10–20]. In all measurements, an Ag (s) | AgCl (s) | KCl (aq, 1 mol L^{-1}) reference electrode was used. The auxiliary electrode was made of a Pt wire, 1 cm length and 0.5 mm in diameter.

2.2. Sample preparation assay

Twenty tablets were weighed, finely powdered and portions equivalent to 20 mg tamoxifen were transferred into 100 mL volumetric flask; 50 mL distilled water was added, shaken thoroughly to dissolve, made up to volume and mixed well. Suitable aliquots of solution were filtered through a Millipore filter (0.45 μm). 1 mL of the filtered solution was diluted with distilled water in a 100 mL volumetric flask. Then 50 μL of the resulting solution was added to a 100 mL volumetric flask and made up to volume with 0.05 mol L^{-1} phosphoric acid to yield starting concentration of 2.0 ng mL^{-1}.

2.3. Determination of tamoxifen in human urine and plasma

1 mL of untreated urine containing 50 ng mL^{-1} tamoxifen was placed into a 5 mL volumetric flask and diluted with water to the mark. A 1 mL of this solution was diluted with pH 2 buffer solution to 10 mL into a volumetric flask. Then 50 μL aliquot was injected into the FIA system.

For the determination of tamoxifen in plasma, 100 μL aqueous tamoxifen solutions (50 ng mL^{-1}) were added to 100 μL of untreated plasma. The mixture was vortexed for 30 s. In order to precipitate the plasma proteins, the plasma samples were treated with 20 μL perchloric acid $HClO_4$ 15%. After that, the mixture was vortexed for a further 30 s and then centrifuged at 6000 rpm for 5 min. Then 50 μL aliquot of the obtained supernatant was injected into the FIA system. The voltammograms were recorded according to the above recommended procedure. The voltammograms of samples without tamoxifen do not show any signal that can interfere with the direct determination, so external calibration can be used.

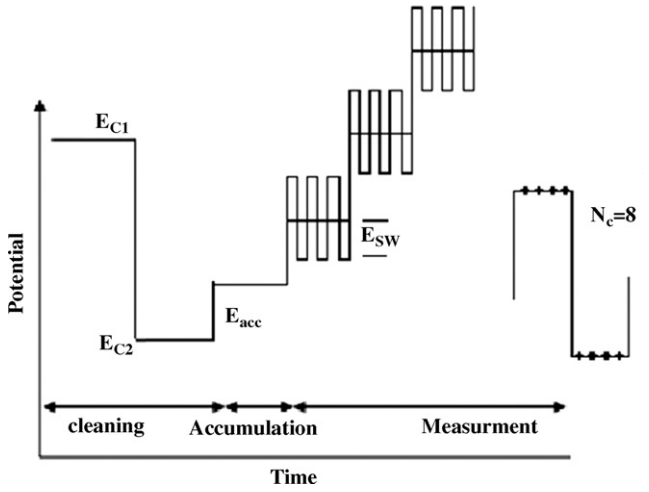

Fig. 1. The diagram of potential waveform used in measurements.

2.4. Electrochemical setup

All electrochemical experiments were done using a setup comprised of a PC PIV equipped with a data acquisition board (PCL-818H, Advantech Co.) was used to output an analog waveform to the working electrode and acquire current readings from the working electrode that connected to a custom made potentiostat. Most of the waveform parameters could be modified from within the software; including the pre- and post-scan potential/time, square wave frequency/amplitude, dc ramp initial/final potential, and ramp time.

In this new method, to improve the detector sensitivity, the FFT–SWV technique was modified in the potential excitation waveform and current sampling and data processing. Fig. 1 shows the potential waveform consisted of three sections; (a) electrode conditioning and (b) accumulation part (c) measurement the potential waveform contained three additional potential steps, E_{c1} to E_{c2} (for cleaning the electrode surface) and Es (for accumulation of tamoxifen). As is shown in Fig. 1, the measurement part of the waveform contains multiple SW pulses with amplitude of E_{SW} and frequency of f_o, were superimposed on a staircase potential function, which was changed by a small potential step of ΔE. The values of potential pulse of SW (E_{SW}) and ΔE were in a range of few mV (10–50 mV). In potential ramp, the currents sampled four times per each SW polarization cycle.

3. Results and discussion

Fig. 2 shows the changes in the electrode admittance, of the gold electrode in 0.05 mol L^{-1} H_3PO_4 into the eluent solution, caused by the injection of a solution of 50 μL of 5.0×10^{-8} mol L^{-1} tamoxifen. The FFT–SW modulation had amplitude of 10 mV and a frequency of 800 Hz. Before each scan, the electrode was held at E_{c1} potential (1400 mV) for 60 ms, the E_{c2} potential at −200 mV for 60 ms and accumulation potential; Es at −100 mV for 500 ms.

The single peak at potential 1000 mV at the voltammogram is due to the adsorption process of drug and inhibition of redox behavior of gold electrode as shown in Eq. (1). When the electrode potential passes the zero charge potential, changes in the double layer capacitance is caused by the reorientation of water molecule and ion exchange at the Helmholtz layer. Since such processes (e.g., water molecule reorientation) are too fast, the pseudo capacitance peak can be observed easily in various electrolytes even at frequencies above 1 MHz. The second peak, with a shoulder, is related to