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Detection of anticancer drug tamoxifen using biosensor based on polyaniline probe modified with horseradish peroxidase

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ABSTRACT

Amperometric biosensor based on horseradish peroxidase immobilized via glutaraldehyde on the polyaniline modified platinum electrode shows evidenced promising characteristics in detecting anticancer drug tamoxifen. The sensor was fabricated simply by adsorbing horseradish peroxidase enzyme on the electrode surface for which Cyclic Voltammetry was used to monitor the electro-catalytic reduction of tamoxifen under diffusion-adsorption controlled conditions. Fourier Transform Infrared Spectroscopy, Cyclic Voltammetry and Electrochemical Impedance Spectroscopic techniques are used to characterize the electrochemical interfacial properties of surface modified electrodes. The first-hand effort on modified biosensor within Platinum/Polyaniline/Horseradish peroxidase biosensor system has demonstrated excellent electro-analytical properties with biosensor sensitivity of 1.6 μA ng mL^{-1}. The optimum limit of detection and limit of quantification are 0.07 ng mL^{-1} and 0.29 ng mL^{-1} respectively for the determination of anticancer drug tamoxifen. It is felt that the present study will help in improving our knowledge of cost-effective quantitative determination of tamoxifen in metabolized biological fluids and other pharmaceutical formulations.

1. Introduction

The concern for better public health and environmental implications has given rise to a significant interest in the analysis of drugs in bulk, dosage and biological fluids that has gathered tremendous momentum in the sphere of analytical chemistry [1–4]. To devise a simple, rapid, sensitive and accurate method for the determination of active ingredient in drugs is, therefore, welcomed and in fact necessary. Tamoxifen ([Z]-2-[4-(1, 2-diphenyl-l-butenyl)-phenoxy]-N, N-dimethylethylamine), an oral nonsteroidal antiestrogen drug used in the prevention and treatment of breast cancer [5–8], was first approved in the United Kingdom in 1973 and by the Food and Drug Administration in the United States in 1977 [9] for reduction of women's risk to Estrogen Receptor-positive breast cancer [10,11]. However, the long-term use of tamoxifen is subjected to controversy due to its estrogen agonistic properties which may lead to the development of endometrial cancer and thromboembolic diseases [12,13]. Tamoxifen is an extensively metabolized drug that produces *N*-desmethyltamoxifen, 4-hydroxy tamoxifen, tamoxifen-*N*-oxide, hydroxytamoxifen, and *N*-didesmethyltamoxifen [14,15].

In view of the importance of tamoxifen in the treatment of breast cancer, several methods have been developed and reported for quantitative analysis of tamoxifen and its metabolites in biological fluids and pharmaceutical formulations, based on high-performance liquid chromatography (HPLC) [16–19], nonaqueous capillary electrophoresis (CE) [20,21], thin layer chromatography (TLC) [22,23], potentiometry [24], liquid chromatography–mass spectrometry (LC–MS) [25], gas chromatography–mass spectrometry (GC–MS) [26,27], polarography [28], spectrophotometry [29] and voltammetry [30–32]. The most extensively used HPLC method not only consumes a considerable time but also involves a large volume of organic solvent [33]. The development of electrochemical biosensor with enhanced sensitivity and selectivity for direct determination of a tamoxifen is, therefore, of utmost importance. It is known that electrochemical biosensors based on conducting polymers offer many advantages and new possibilities to detect biologically significant compounds and they have been extensively used as support for enzyme immobilization [34]. Conducting polymers such as polyaniline (PANI) is one of the most intensively studied polymers due to its excellent stability in different solutions, good electronic properties, and strong biomolecular interactions [35]. Polymers of the PANI family including chemically synthesized PANIs are potentially effective components toward application in enzymatic immobilization of biochemical engineering [36–40]. The chemically synthesized PANIs can also provide suitable polymeric support over and above its chemical characteristics, such as ease of preparation, high yield, high stability to extreme temperature and pH, and resistance to attacks from micro-organisms [41,42]. The performance of some polymers of the PANI family for horseradish peroxidase (HRP) immobilization has been reported [43].

The present study deals with the development of an electrochemically polymerized PANI amperometric biosensor modified with HRP as an excellent, fast and cost-effective analytical method for the determination of anticancer drug tamoxifen in bulk form and in pharmaceutical

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formulation. In this first-hand approach, an attempt is also made to elucidate the electrochemical and enzymatic reactions based on modified Pt/PANI/HRP biosensor system involved in the determination of tamoxifen.

2. Materials and methods

2.1. Reagents and materials

Tamoxifen of 99% purity was obtained from the Biochem Pharmaceuticals Industries, Mumbai, India. The tablet containing tamoxifen citrate (*tamoxifen*) labeled 20 mg and 10 mg were obtained from commercial sources. A stock solution of tamoxifen 10 mg mL^{-1} was prepared by direct dissolution in methanol and thereof freshly diluted solutions were prepared by accurate dilution with 0.1 M phosphate buffer solution of pH 6.8 containing 0.9% NaCl for experimental investigations. Phosphate buffer solution of 200 mL capacity with ionic strength 0.1 [44] in the pH range 2–12.0 were prepared in deionized water by adding appropriately measured amounts of 85% H_3PO_4, KH_2PO_4, Na_2HPO_4, and Na_3PO_4 and used as supporting electrolyte. All the reagents used in the present study were of analytical and molecular biology grade and obtained from Sigma Aldrich. HRP was also procured from Sigma Aldrich.

2.2. Instrumentation

Electrochemical measurements were performed using a Potentiostat/Galvanostat/ZRA (Gamry Reference 3000, United States of America) with Gamry Echem Analyst Software. Platinum electrode, Ag/AgCl (3 M KCl) and a platinum wire were used as working, reference and auxiliary electrode, respectively. Film surface areas for sensor on platinum (Pt) electrode were 3 mm in diameter. Cyclic Voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS) were carried out in a 20 mL Dr. Bob's electrochemical cell stand. Alumina micropolish and polishing pads were used for electrode polishing. The FT-IR spectrum of solid complex was recorded using KBr pellets on an IR, spectrophotometer (Spectrum 100 with software version CPU32).

2.3. Polyaniline film formation

Prior to the electro-polymerization, the Pt electrode was polished on 1, 0.3 and 0.05 μm alumina slurries (make Buehler-Gamma Micropolish) and then thoroughly rinsed with de-ionized water after each polishing step. Polymerization was achieved in a potentiodynamic mode in 0.2 M aniline per 1 M HCl solution following standard methodology of Olsson and Ogren [45]. Based on the optimum increasing trend (8 cycles) of amperometric biosensor response the potential was cycled between −0.2 V to 1.1 V at a scan rate of 50 mV s^{-1}.

2.4. Biosensor preparation

HRP 1 mg mL^{-1} (173 U) was freshly prepared by direct dissolution in 0.1 M phosphate buffer solution of pH 6.8 and stored at 4 °C for experimental investigations [46]. In order to prepare the biosensors, the HRP was directly immobilized with PANI electrode by adsorption technique (overnight dipping in a special assembled cell to allow the uniform distribution of enzymes on the surface of Pt/PANI matrix) using 0.1% glutaraldehyde mediator as a cross linker and incubated at 4 °C overnight. The conditions for the immobilization of the enzyme were selected based on prior studies [47–50]. The biosensors were rinsed with a buffer solution to remove loosely-bound material and preserved at 4 °C in pH 6.8 phosphate buffer solution for further use.

2.5. Electrochemical measurements

The cell used for the electrocatalytic reduction of tamoxifen consisted of biosensor, platinum wire, and Ag/AgCl as the working, counter and reference electrode respectively. The 10 mL test solution containing 0.1 M phosphate buffer (pH 6.8) containing 0.9% NaCl was degassed with nitrogen after each addition of small amounts of 1 ng mL^{-1} tamoxifen. Cyclic voltammograms were performed at a scan rate of 5 mV s^{-1} at concentration range of studied tamoxifen ranging from 1 to 11 ng mL^{-1}.

3. Results and discussion

3.1. Cyclic voltammetric studies

Polymerization of PANI on to the Pt electrode in 0.2 M aniline with 1 M HCl solution for eight cycle process at potential window of −0.2 V to +1.1 V with a scan rate of 50 mV s^{-1} is presented in Fig. 1. Cyclic voltammogram of PANI in acidic medium exhibits three redox voltammetric peaks. Redox peaks (a, a′) and (b, b′) are due to the catalytic effect of PANI and redox peak (a, a′) is attributed to the transformation of PANI from the reduced leucoemeraldine state of partially oxidized emeraldine. Moreover, the peaks b, b′ are related to the redox couple reaction of p-benzoquinone and the peaks c, c′ can be attributed as the result of transitional reaction of PANI from leucoemeraldine to pernigraniline [51]. The voltammetric peak current and number of cycles as a measure of biosensor response have shown positive relationship up to eight electro-polymerization and beyond that it demonstrates negativity.

The reversibility in reduction process was investigated by using CV. The cyclic voltammetric behavior of the PANI film was studied at different scan rate (5 to 35 mV s^{-1}) in 1 M HCl and 0.1 M phosphate buffer solution containing 0.9% NaCl. With increasing scan rate (i) the peak potential increases, (ii) the peak current increases steadily and (iii) the peak current function, $i_p/AC\upsilon^{1/2}$ exhibits uniformity. Plot of i_p against $\upsilon^{1/2}$ have evidenced significant straight line relationship of Randles–Sevcik nature with positive correlation coefficient of 0.992 supporting mass transport as a means of diffusion [52].

$$i_p(\mu A) = 0.3 \times \upsilon^{1/2}\left(mVs^{-1}\right) + 0.6; r^2 = 0.992; n = 6(1\ MHCl)$$

$$i_p(\mu A) = 0.2 \times \upsilon^{1/2}\left(mVs^{-1}\right) + 0.9; r^2 = 0.991; n = 6(Phosphate\ buffer\ solution)$$

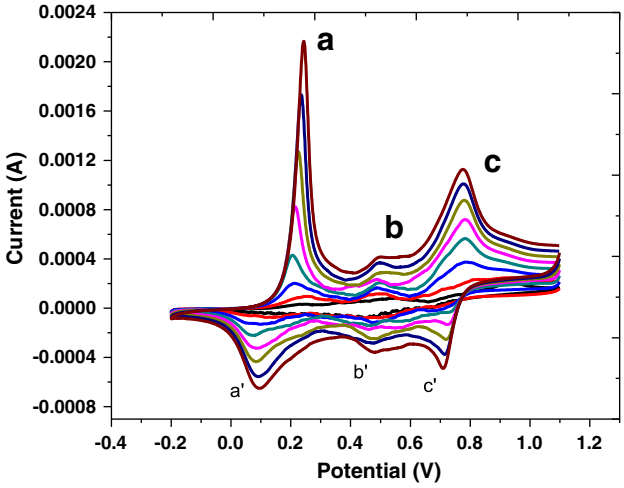

Fig. 1. Electrochemical polymerization of the PANI film onto the Pt electrode. Successive eight polymerization cycles are shown which corresponds to increase in the thickness of the film during deposition.