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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].

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

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

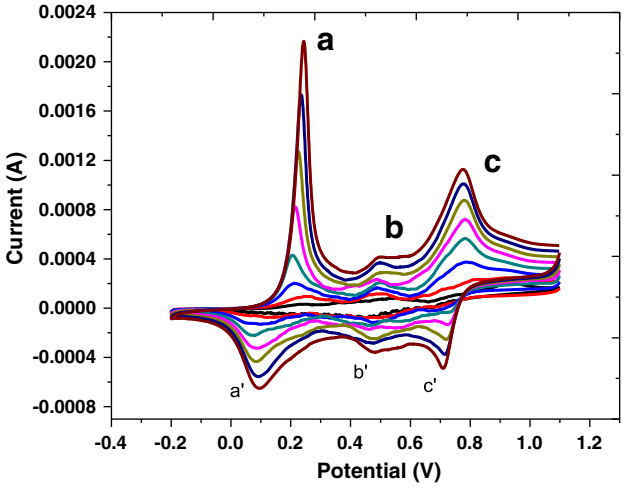


**Fig. 1.** Electrochemical polymerization of the PANI film onto the Pt electrode. Successive eight polymerization cycles are shown which corresponds to increase in the thickness of the film during deposition.

However, adsorptive nature of the electrode process was also observed after immobilization of the HRP enzyme onto the Pt/PANI electrode. As a substantial decrease in the peak current value in subsequent scans was observed after a steady state has reached, indicating that tamoxifen shows adsorptive characteristics at an enzyme immobilized electrode. A plot of log $I_p$ (peak current) versus log $\upsilon$ (scan rate) of slope 0.617–0.806 as shown in Fig. 2 demonstrated that the enzyme immobilized electrode is well agreed with the theory of diffusion-adsorption controlled process [53]. Thus it can clearly be attributed that the polymerization is electro-active in nature and mechanism of partial diffusion-adsorption took place through polymer chain. This has further demonstrated a rapid reversible electron transfer undergoing on the thin film surface of the conducting electro active polymer.

### 3.2. FT-IR spectroscopic studies

FT-IR spectra of electrochemically polymerized PANI films were obtained and characterized (Fig. 3 curve a). The absorption bands at 653 $cm^{-1}$ and 732 $cm^{-1}$ are attributed to the C-H vibrations in the benzene ring. The in-plane vibration of C-H bending mode in N=Q=N, Q–$N^+$H–B or B–$N^+$H–B (where Q = quinoid and B = benzenoid) is observed at 1156 $cm^{-1}$ in the FT-IR spectra. The presence of this absorption band is due to the polymerization of PANI i.e., polar structure of the conducting protonated form. In the spectra, bands between 1241 and 1351 $cm^{-1}$ are associated with C–N stretching in aromatic amines and 1625 $cm^{-1}$ due to C=C stretching of benzenoid rings and quinoid rings. Strong peak at 2345 $cm^{-1}$ is associated with –N≡N in diazonium salts. IR band at 2910–3450 $cm^{-1}$ corresponds to N–H stretching with hydrogen bonded amino groups and free O–H stretching vibration.

The FT-IR spectra (Fig. 3 curve b) is attributed to the successful immobilization of HRP enzymes on to the Pt/PANI film. The absorption peak at 1647 $cm^{-1}$ is observed due to presence of amide group on the surface of the HRP which strongly supported our contention on binding of HRP with PANI thin film surfaces.

### 3.3. Electrochemical Impedance Spectroscopic studies

EIS is an effective tool for studying electrochemical interfacial properties of surface modified electrodes [54–56]. Fig. 4 shows the Nyquist plots of Pt/PANI (curve a) and Pt/PANI/HRP bioelectrode

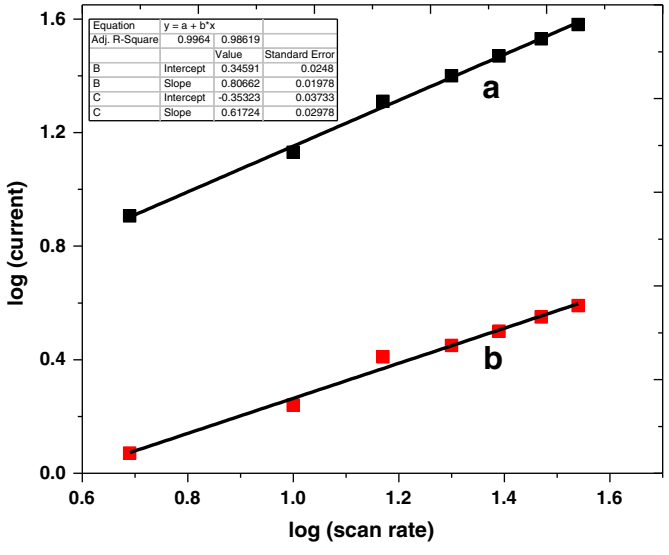


**Fig. 2.** The slope of peak current log (current). (a) Reduction and (b) oxidation versus log (scan rates) of the Pt/PANI/HRP in 0.1 M phosphate buffer solution of pH 6.8 containing 0.9% NaCl at different scan rates of 5 to 35 mV $s^{-1}$.

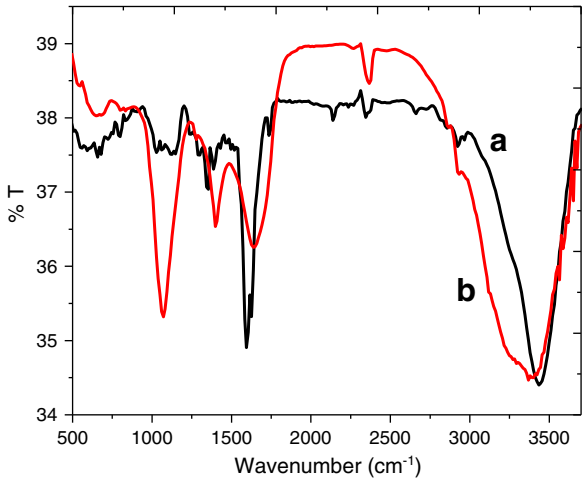


**Fig. 3.** FT-IR spectra of electrochemically polymerized thin film of (a) Pt/PANI and (b) Pt/PANI/HRP bioelectrode.

(curve b) carried out in 0.05 M phosphate buffer solution at pH 6.8 containing 0.9% NaCl. The semicircle diameters correspond to the electron transfer resistance ($R_{CT}$) at the electrode. The semicircle portion at higher frequencies corresponds to electron transfer process and linear part at lower frequencies corresponds to the diffusion process. (This is in agreement with the CV studies). It is observed that $R_{CT}$ value of Pt/PANI/HRP bioelectrode increases three times ($3.58\times10^3$ Ω) as compared to the Pt/PANI electrode ($1.15\times10^3$ Ω). This is in consistence with the facts that, immobilization of HRP on the electrode surface has offered electron transfer resistance in redox probe resulting in threefold increase of $R_{CT}$ value. This is further supported by the CV studies (Fig. 5) in which observation relating to decrease in peak current at Pt/PANI/HRP electrode (b) compared to Pt/PANI electrode (a) suggests successful immobilization on Pt/PANI electrode. These results of EIS, FT-IR and CV measurements indicate immobilization of HRP onto the surface of Pt/PANI film.

### 3.4. Reproducibility and storage stability of the biosensor

The lack of necessary operational and storage stability needed for commercial exploitation of biosensor is considered as one of the major hurdles. In order to check the performance of the HRP based biosensor for determination of tamoxifen, reproducibility and storage stability of the biosensor were carried out by keeping the biosensor

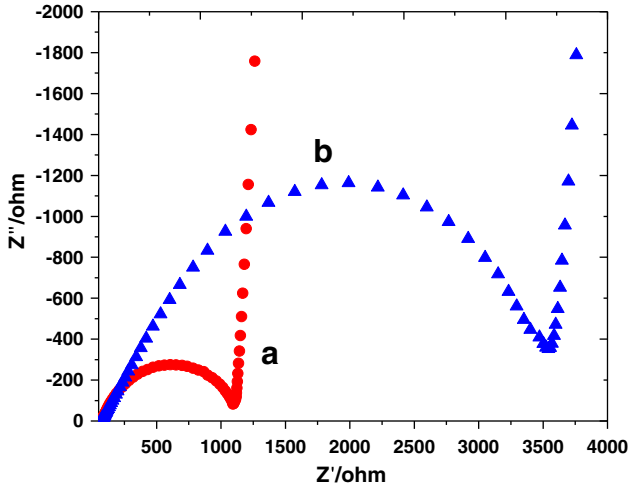


**Fig. 4.** EIS spectra of (a) Pt/PANI electrode and (b) Pt/PANI/HRP electrode in 0.1 M phosphate buffer solution containing 0.9% NaCl solution.

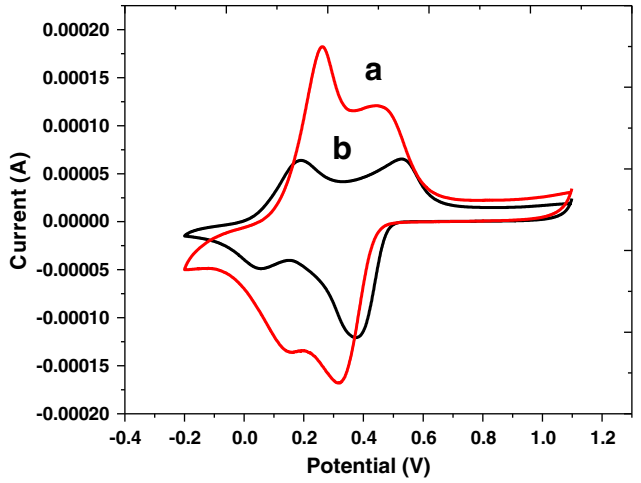


**Fig. 5.** Cyclic voltammogram (a) Pt/PANI and (b) Pt/PANI/HRP electrodes of constant scan rate 5 mV $s^{-1}$ in 0.1 M phosphate buffer solution of pH 6.8 containing 0.9% NaCl.

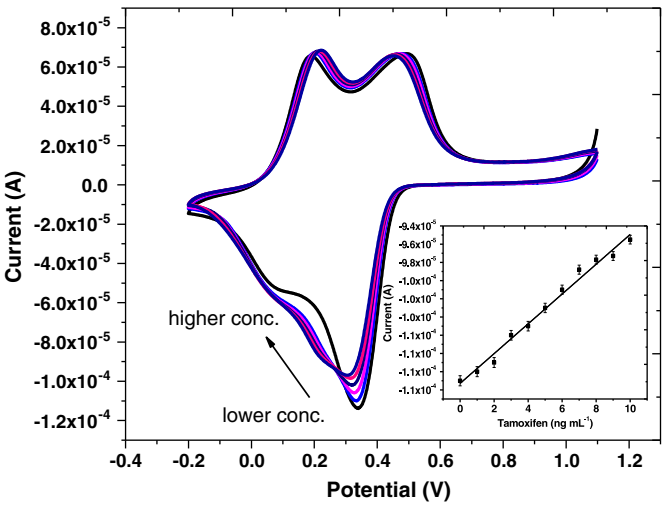


**Fig. 6.** Cyclic voltammograms showing different concentration of tamoxifen in 0.1 M phosphate buffer solution of pH 6.8 containing 0.9% NaCl at a scan rate of 5 mV $s^{-1}$ at Pt/PANI/HRP electrode. Inset figure shows different concentrations (1 ng $mL^{-1}$ to 11 ng $mL^{-1}$) of tamoxifen in increasing order.

electrode in the dark at 4 °C for 15 days and were analyzed at different times (every day). It has been seen that repeatable peak currents of tamoxifen (1 ng $mL^{-1}$) occurred up to 10 days and after that the peak current decreased significantly. Reproducibility and storage stability were calculated in terms of residual standard deviation yielding a value of 1.01% (n = 5). So, the present approach on Pt/PANI/HRP electrode as biosensor has demonstrated better reproducibility for a comparatively longer period and storage stability at 4 °C.

### 3.5. Application of method to the pharmaceutical dosage forms

The developed amperometric biosensor was successfully applied for determination of tamoxifen in tablets (*tamoxifen*). Filtration of tablet extract from undissolved excipients is not required. It is sufficient to dilute the aliquot from the supernatant layer with the supporting electrolyte (0.1 M phosphate buffer solution of pH 6.8 containing 0.9% NaCl). Voltammograms of tamoxifen in 0.1 M phosphate buffer solution of pH 6.8 containing 0.9% NaCl exhibit well defined cathodic peak at the potential range 0.30 ± 0.05 V. The current is due to partial diffusion–adsorption controlled and proportional to the concentration over a convenient range. The precision was estimated for 1–11 ng $mL^{-1}$ of the drug using the calibration graph and standard addition method. Representative voltammograms and calibration curve are shown in (Fig. 6). Inset figure of Fig. 6 shows that under the optimized condition the linear relationship between the tamoxifen concentration versus peak current is due to the generation of peak product hydrogen peroxide [57,58]. The proposed reaction mechanism of tamoxifen with HRP is illustrated below:

$$Tamoxifen_{oxd} + O_2 \overset{HRP}{\rightleftharpoons} Tamoxifen_{red} + H_2O_2, H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-.$$

The obtained mean percentage recoveries (%R) based on the average of five replicate measurements were found to be 89.5–95.5. The calculated detection limit is 0.073 ng $mL^{-1}$ with 1.0% relative standard deviation and the lower limit of quantification is 0.29 ng $mL^{-1}$ with 0.9% relative standard deviation. The analytical performance data of the proposed method are compiled in Table 1. The specificity of the method was investigated by observing any interference encountered from the excipients of the tablets mass and observed non-interference with the co-administered drug. To examine the ruggedness of the procedure, the within-day with 0.62% relative standard deviation and between-day with 0.72% relative standard deviation were evaluated by analyzing 1.0 ng $mL^{-1}$ sample of *tamoxifen* tablet six times a day for five consecutive days. The obtained result demonstrated good precision of our experimental task.

## 4. Conclusion

The electro-activity of tamoxifen on enzyme-based biosensor electrode was developed and studied for the first time. The electrochemical behavior of tamoxifen under the conditions described in this work is an irreversible process controlled by diffusion–adsorption. The advantage of this biosensor method of tamoxifen determination over the electrochemical method proposed in our previous article [32] is that this enzyme based technology gains practical usefulness from a combination of selective biochemical recognition with the high sensitivity of electrochemical detection. The proposed biosensor promise simple, low cost and rapid analytical tools which will represent a broad area of emerging technology ideally suited for point-of-care analysis. The proposed method has distinct advantages over other existing methods regarding sensitivity, selectivity, time saving and minimum detectability. Furthermore, in an earlier HPLC methods [59] and [60] for the determination of tamoxifen, sensitivity and lower limit of quantification were found to be 2.0 ng $mL^{-1}$ and 0.5 ng $mL^{-1}$ but in the present developed method it could be estimated up to a level of 0.29 ng $mL^{-1}$ with sensitivity of 1.6 μA ng $mL^{-1}$. In addition no sophisticated instrumentation is required. Consequently, the proposed method has a potential of a good analytical alternative in comparison to chromatographic methods for determining tamoxifen in pharmaceutical formulation. This developed method can also be adopted for pharmacokinetic studies as well as for quality control laboratory studies.

**Table 1**
Regression data of the calibration lines for quantitative determination of tamoxifen in dosage form (*tamoxifen*) by amperometric biosensor using Cyclic Voltammetry.

| Parameter | Dosage form |
|---|---|
| Measured potential (V) | 0.30 ± 0.05 |
| Linear range (ng $mL^{-1}$) | 1–11 |
| Slope (μA/μg $mL^{-1}$) | 1.53 |
| Intercept | 1.12 |
| Correlation coefficient ($r^2$) | 0.990 |
| %RSD of the slope ($S_b$) | 0.62 |
| %RSD of the intercept($S_a$) | 0.40 |
| Repeatability (%RSD) | 0.45 |
| % Recovery | 89.5–95.5 |
| LOD ng $mL^{-1}$ | 0.073 ± 0.1 |
| LOQ ng $mL^{-1}$ | 0.29 ± 0.9 |

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## References

[1] K. Kummerer, A. Al-Ahamed, B. Bertram, M. Wiessler, Chemosphere 40 (2000) 767.
[2] M.S. El-Shahawi, S.O. Bahaffi, T. El-Mogy, Anal. Bioanal. Chem. 387 (2007) 719.
[3] R. Jain, K. Radhapyari, N. Jadon, J. Electrochem. Soc. 154 (2007) F199.
[4] V.K. Gupta, R. Jain, K. Radhapyari, N. Jadon, S. Agarwal, Anal. Biochem. 408 (2011) 179.
[5] B.J. Furr, V.C. Jordan, Pharmacol. Ther. 25 (1984) 127.
[6] I.A. Jaiyesimi, A.U. Buzdar, D.A. Decker, G.N. Hortobagyi, J. Clin. Oncol. 13 (1995) 513.
[7] E. Robinson, G.G. Kimmick, H.B. Muss, Drugs Aging 8 (1996) 329.
[8] M.H. Gail, J.P. Constantino, J. Bryant, R. Croyle, L. Freedman, K. Helzlsouer, V. Vogel, J. Natl. Cancer Inst. 91 (1999) 1829.
[9] W.C. Park, V.C. Jordan, Trends Mol. Med. 8 (2002) 82.
[10] J. Cuzick, T. Powles, U. Veronesi, J. Forbes, R. Edwards, S. Ashley, P. Boyle, Lancet 361 (2003) 296.
[11] LHRH-Agonists in Early Breast Cancer Overview Group, Lancet 369 (2007) 1711.
[12] J. Cuzick, J. Forbes, R. Edwards, Lancet 360 (2002) 817.
[13] M. Baum, A. Buzdar, J. Cuzick, J. Forbes, J. Houghton, A. Howell, T. Sahmoud, Cancer 98 (2003) 1802.
[14] H.K. Crewe, L.M. Notley, R.M. Wunsch, M.S. Lennard, E.M. Gillam, Drug Metab. Dispos. 30 (2002) 869.
[15] Z. Desta, B.A. Ward, N.V. Soukhova, D.A. Flockhart, J. Pharmacol. Exp. Ther. 310 (2004) 1062.
[16] K.M. Fried, I.W. Wainer, J. Chromatogr. B 655 (1994) 261.
[17] R.R. Brown, R. Bain, V.C. Jordan, J. Chromatogr. 272 (1983) 351.
[18] J.E. Manns, S. Hanks, J.E. Brown, J. Pharm. Biomed. Anal. 16 (1998) 847.
[19] E.R. Kisanga, J. Gjerde, A. Guerrieri-Gonzaga, F. Pigatto, A. Pesci-Feltri, C. Robertson, D. Serrano, G. Pelosi, A. Decensi, E.A. Lien, Clin. Cancer Res. 10 (2004) 2336.
[20] A. Decensi, C. Robertson, G. Viale, F. Pigatto, H. Johansson, E.R. Kisanga, P. Veronesi, R. Torrisi, M. Cazzaniga, S. Mora, M.T. Sandri, G. Pelosi, A. Luini, A. Goldhirsch, E.A. Lien, U. Veronesi, J. Natl. Cancer Inst. 95 (2003) 779.
[21] J.M. Sanders, L.T. Burka, M.D. Shelby, R.R. Newbold, M.L. Cunningham, J. Chromatogr. B. Biomed. Sci. Appl. 695 (1997) 181.
[22] W. Lu, G.K. Poon, P.L. Carmichael, R.B. Cole, Anal. Chem. 68 (1996) 674.
[23] H.K. Adam, E.J. Douglas, J.V. Kemp, Biochem. Pharmacol. 27 (1979) 145.
[24] J.V. Kemp, H.K. Adam, A.E. Wakeling, R. Slater, Biochem. Pharmacol. 32 (1983) 2045.
[25] H.K. Lim, S. Stellingweif, S. Sisenwine, K.W. Chan, J. Chromatogr. A 831 (1999) 227.
[26] C. Murphy, T. Fotsis, P. Pantzar, H. Adlercreutz, F. Martin, J. Steroid Biochem. 28 (1987) 609.
[27] E.A. Lien, E. Solheim, S. Kvinnsland, P.M. Ueland, Cancer Res. 48 (1988) 2304.
[28] J. Wang, X. Cai, J.R. Fernandes, M. Ozsoz, D.H. Grant, Talanta 45 (1997) 273.
[29] Z. Fijalek, J. Chodkowski, M. Warowna, J. Electroanal. Chem. 226 (1987) 129.
[30] S.P. Chilukuri Sastry, J.S.V.M. Lingeswara Rao, K.R. Rao, Talanta 42 (1995) 1479.
[31] G. Xiao-xio, S. Zhang-Jug, T. Xin-juan, S. Jun-Feng, Anal. Lett. 41 (2008) 1225.
[32] R. Jain, Vikas, K. Radhapyari, Drug Test. Anal. 3 (2011) 743.
[33] A.K. Hemanth Kumar, V. Sudha, S. Swaminathan, G. Ramachandran, Indian J. Med. Res. 132 (2010) 390.
[34] M. Gerard, A. Chaubey, B.D. Malhotra, Biosens. Bioelectron. 17 (2002) 345.
[35] M.D. Imisides, R. John, G.G. Wallace, Chemtech 9 (1996) 261.
[36] X. Zeng, T. Ko, Polymer 39 (1998) 1187.
[37] N. Gospodinova, L. Telermezyan, Prog. Polym. Sci. 23 (1998) 1443.
[38] A. Bhattacharya, A. De, Prog. Solid State Chem. 24 (1996) 141.
[39] A.H. Parente, E.T.A. Marques Jr., W.M. Azevedo, F.B. Diniz, E.H. Melo, J.L. Lima Filho, Appl. Biochem. Biotechnol. 37 (1969) 267.
[40] W. Nadruz Jr., E.T.A. Marques, W.M. Azevedo, J.L. Lima Filho, L.B. Carvalho Jr., Braz. J. Med. Biol. Res. 2 (9) (1996) 347.
[41] M. Thangarathinavelu, A.K. Tripathi, T.C. Goel, I.K. Varma, J. Appl. Polymer Sci. 51 (1994) 1347.
[42] V. Leite, V.L. da Silva, W.M. Azevedo, E.H.M. Melo, J.L. Lima Filho, Biotechnol. Tech. 8 (1994) 133.
[43] K.F. Fernandes, C.S. Lima, H. Pinho, C.H. Collins, Process Biochem. 38 (2003) 1379–1384.
[44] G.D. Christan, W.C. Purdy, J. Electroanal. Chem. 3 (1962) 363.
[45] B. Olsson, L. Ögren, Anal. Chim. Acta 145 (1983) 87.
[46] P.R. Solanki, A. Kaushik, Anees A. Ansari, G. Sumana, B.D. Malhotra, Polym. Adv. Technol. 22 (2011) 903.
[47] R. Khan, W. Gorski, C.D. Garcia, Electroanalysis 23 (2011) 2357.
[48] R. Khan, N.C. Dey, A.K. Hazarika, K.K. Saini, M. Dhayal, Anal. Biochem. 410 (2011) 185.
[49] Anees A. Ansari, G. Sumana, R. Khan, B.D. Malhotra, J. Nanosci. Nanotechnol. 9 (2009) 1.
[50] D. Yu, B. Blankert, J.M. Kauffmann, Biosens. Bioelectron. 22 (2007) 2707.
[51] N.G.R. Mathebe, A. Morrin, E.I. Iwuoha, Talanta 64 (2004) 115.
[52] P. Monk, in: Fundamentals of Electroanalytical Chemistry, Willey, New York, 2001, p. 162.
[53] D.K. Josser, in: Cyclic Voltammetry: Simulation and Analysis of Reaction Mechanisms, VCH Publisher, 1993, pp. 27–70.
[54] R. Khan, M. Dhayal, Biosens. Bioelectron. 24 (2009) 1700.
[55] R. Khan, A. Kaushik, P.R. Solanki, A.A. Ansari, M.K. Pandey, B.D. Malhotra, Anal. Chim. Acta 616 (2008) 207.
[56] R. Khan, M. Dhayal, Electrochem. Commun. 10 (2008) 492.
[57] M.A. Alonso-Lomillo, O. Dominguez-Renedo, P. Matos, M.J. Arcos-Martínez, Bioelectrochemistry 74 (2009) 306.
[58] M.A. Alonso-Lomillo, J.M. Kauffmann, M.J. Arcos Martinez, Biosens. Bioelectron. 18 (2003) 1165.
[59] J. Maccallum, J. Cummings, J.M. Dixon, W.R. Miller, J. Chromatogr. B Biomed. Appl. 678 (1996) 317.
[60] Z. Yu-Bing, Z. Qian, Z. Jian-Jun, Y. Cui-Xia, X. Da-Wei, J. Pharm. Biomed. Anal. 46 (2008) 349.

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