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Investigation the electroanalytical behavior of the tamoxifen as breast anticancer drugs using differential pulse anodic adsorptive striping and it's extraction from tablets

Z. Deris Falahiye
Department of Chemistry, Faculty of Science, Islamic Azad University, Arak Branch, P.O.B: ۳۸۱۳۵-۵٦۷, Arak, Iran
Email: z_deris_f@yahoo.com

M. Jalali
Department of Chemistry, Faculty of Science, Islamic Azad University,Omidiyeh Branch, P.O.B: ٦۳۷۳۱-۹۳۷۱۹, Omidiyeh, Iran
Email: jalalimehdian@yahoo.com

M. Alimoradi
Department of Chemistry, Faculty of Science, Islamic Azad University, Arak Branch, P.O.B: ۳۸۱۳۵-۵٦۷, Arak, Iran
Email: m-Alimoradi@IAU-Arak.AC.IR

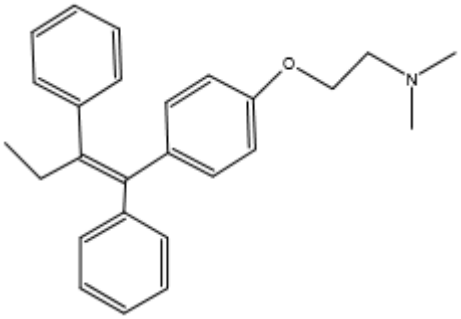
Abstract

In this work, the electrochemical behavior of tamoxifen as an anti cancer drug were addressed at a glassy carbon electrode (GCE). Cyclic voltametry (CV) and chrronoamperometry were used to understand the electrochemical characteristics of tamoxifen (Tam) In Britton-Rubinson (BR) buffer (pH= ٤,٢). Based on the results of the recorded CV, the electrodeposition and anodic striping behavior of the Tam were investigated at the surface of GCE. To find the best condition for taking a sharp analytical peak concerning the electro-oxidation of Tam, differential pulse anodic adsorptive striping voltammetry (DPAASV) was studied. The primary experiments demonstrated that the DPAASV presents a sufficient oxidation peak current at approximately ١,١ V vs Ag/AgCl. Therefore, the effects of different parameters such as; deposition potential, deposition time, pH and the electrocleaning condition has been studied and optimized. The obtained results shown that the -١,٤ v, ٣٠s, pH=٤,٢ and cleaning in H_2SO_4 ٠,٥ M are the optimal values, respectively. Then the calibration curve was plotted in the range of ١ to ١٠ μM and the limits of detection (LOD) and quantitation (LOQ) were calculated to be ٠,٦٢١ and ٢,٠٧ μM, respectively. The mean, standard error and relative standard deviation (RSD) for five replicates of ٤,٠ μM were found to be ٤,١ μM, ٢,٦٥ % and ٣,٦٢ %, respectively. To estimate the application potential of the proposed method, the extraction of Tam from tablets containing ٢٠ mg Tam were investigated and optimized. Finally, the proposed method was successfully employed for determination of Tam in spiked physiological samples.

Keywords: Tamoxifen, DPAASV, Striping voltametry, deposition

۱. Introduction

Tamoxifen, [Z]-۲-[٤-(۱،۲-diphenyl-۱-butenyl)-phenoxy]-N,N-dimethylethylamine (Tam), a nonsteroidal anti- estrogen, has been the most important hormonal agent for treatment of breast cancer for more than two decades, and recently has been approved as a long-term chemo preventive agent for breast cancer in healthy women at high risk for developing breast cancer.[۱-٤] Tamoxifen undergoes chemical transformation to its phase I metabolites in vivo, resulting in a series of modified species, predominately through methylation or hydroxylation of the benzene rings on the tamoxifen structure, to structures such as ٤-hydroxytamoxifen.[۱]



There are a variety of bioanalytical methods that have been developed to determine the concentrations of Tam in biological fluids and pharmaceutical preparations. Methods developed for Tamoxifen analysis include capillary electrophoresis,[٥] and chromatography used in conjunction with a range of detection techniques.[٦] Liquid chromatography coupled to detection by mass spectrometry is particularly gaining acceptance.[۷-۸] Electrochemical techniques have received significant attention in analysis of pharmaceuticals, due to their low detection limits and rapid time frame. They offer the analyst a technique for the analysis of drugs that is rapid, simple and low cost[۹]. Electrochemical studies of Tamoxifen has centred upon the properties of Tamoxifen by constant current potentiometric stripping at a glassy carbon electrode [۱۰] and voltammetric analysis at a carbon paste electrode [۱۱], however due to the importance of the drug there is a desire to have a validated method for rapid determination of Tamoxifen in pharmaceutical preparations, and also increase our understanding of the electrochemistry in biological fluids not only of Tamoxifen, but of its phase I metabolites[٦]. Many organic compounds exhibit surface-active properties that are manifested by their adsorption from solution onto the surface of a solid phase[۹]. This phenomenon forms the basis for adsorptive stripping voltammetry (AdSV), where the species to be determined are accumulated on the electrode by adsorption. Adsorptive stripping voltammetry has been demonstrated as a sensitive analytical method for a wide range of pharmaceutical compounds that can be adsorbed onto an electrode surface [۱۲-۲۲]. The present work is concerned with the determination of Tam by anodic adsorptive striping voltametry.

۲. Experimental

۲،۱. Chemicals

All chemicals used were of analytical reagent grade quality and were employed without further purification. The tamoxifen was purchased from Sigma- Aldrich. Tablets containing Tamoxifen citrate labelled ۲۰ mg Tam were purchased from commercial sources. Britton–Robinson (B-R) buffers of pH ۲–۱۲ (mixtures of ۰،۰٤ mol/L acetic, orthophosphoric, and boric acids; adjusted to the required pH with ۱ M sodium hydroxide solution) were prepared and used as supporting electrolytes with ۲۰ % methanol added on pure samples to ensure drug solubility.

۲,۲. Instruments

A potentiostate/galvanostate model PG state ۳۰۲ N (Metrohm-Autolab, Swithzerand) equuiped with a RDE system and a three-electrodes cell was employed to record the voltamograms. Ag/AgCl and a Pt rod were used as reference and counter electrod, respectively. The working electrod used in this work was GCE (۳ mm). A pH meter model ۸۲۷ Metrohm (Swithzerland) were used to adjust the pH of the solutions.

۲,۳. Analytical procedure

۱۰ mL Tam containing solution was transferred to the electrochemical cell. Then the deposition of the Tam was conducted at -۱,٤ V vs Ag/AgCl at GCE in MeOH-B-R (۲۰٪, pH=٤,۲) for ۳۰ second. Then after ۱۰ min equlibrium, the differential pulse anodic striping voltamogram (۰-۱,٤ V) was recorded in a condition as pulse amplitude ۲٥ mV, pulse interval ۰,٥ s and the scan rate of ۱۰ mV s^{-1}.

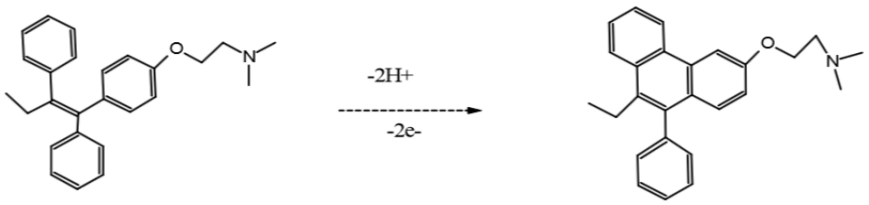
۲,۳,۱ Extraction of the Tam from ground tablets

In order to optimize the extraction of the Tam by proposed DPAASV, ten tablets were weighed and the average mass per tablet was determined and then ground to a homogeneous fine powder in a mortar. A portion of the finely ground material equivalent to ۲۰ mg of TAM was dissolved in ۳ mL methanol, and stirred in a batch process for ٥ min, then filterd by filter paper. Then ۰,۱ mL of this sample diluted up to ۱۰ mL methanol (۲۰٪ V V^{-1}) BR buffer (pH=٤,۲). Finally, ۰,٥ mL of new solution was diluted to ۱۰ mL by fresh methanol (۲۰٪ V V^{-1}) B-R buffer (pH=٤,۲) before DPAASV analysis. Then, the extraction recovery (ER%) of the Tam were calculated based on the results obtained by calibrated DPAASV.

۳.Results and discusion

۳,۱. Cyclic voltametry study

Cyclic voltamograms of Tam at a bare GCE in B-R buffer (pH=۷) were shown in Fig ۱. As can be seen, in the presence of Tam an oxidation peak was appeared at ۱,۱ V vs Ag/AgCl. This peak can be attributed to the cyclization oxidative reaction (Scheme. ۱) of Tam at the surface of GCE. It is obvious that no peak was observed in cathodic sweep. Therefore, the oxidation of Tam is not a reversible electrochemical process. However, this oxidation peak was followed in differential pulse striping voltametery.



(Scheme.۱)

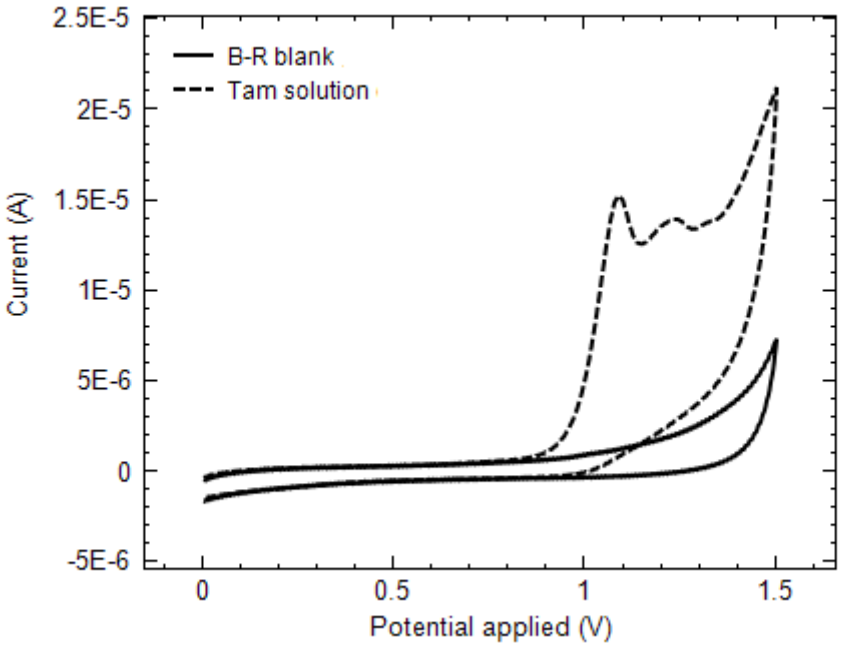


Fig ۱. The cyclic voltamograms of B-R bufer and Tam in B-R.
Conditions; scan rate=۱۰۰ mV s^{-1}, pH=٤,۲

۳.۲. Optimization the DPAASV at GCE

All following experiments were carried out under conditions of Tam concentration ۱۰۰ µM, B-R ۰,۰٤ M, deposition potential -۱,٤ V, mixing rate ٤۰۰ rpm and deposition time ۲٤۰; except when that parameter was under investigation.

۳. ۲. ۱ The effect of the pH of B-R

pH was first parameter that was investigated. For this poupose a set of experiments were designed in the pH range of ۲ to ۱۲. Then, under the mentioned conditions, the DPAASV were carried out and the obtained voltamograms shown in Fig ۲(a). To explain the effect of pH on the peak current, the peak current vs pH was also plotted in Fig ۲(b). As can be seen, the best peak current was achieved at pH=٤,۲ B-R. It is obvious that when pH diminishes below ٤,۲, the peak current decreases slightly. This is due to the high concentration of H^+ that can be prevented the oxidation of Tam (Schem ۱). But, for the pHs greater than ٤,۲ the peak current decreases with increase the pH. It is clear that the current produce on the surface of working electrode can be limited by counter electrode reaction. In pHs greater than ٤,۲ the H^+ concentration depleted and the hydrogen evolution reaction (HER) dimineshes and limites the Tam oxidation peak current. Therefore, the pH=٤,۲ was choosen as optimal value in the next experiments. It was observed that no peak appeared in pH ۸, ۱۰ and ۱۲ that can be supported this idea.

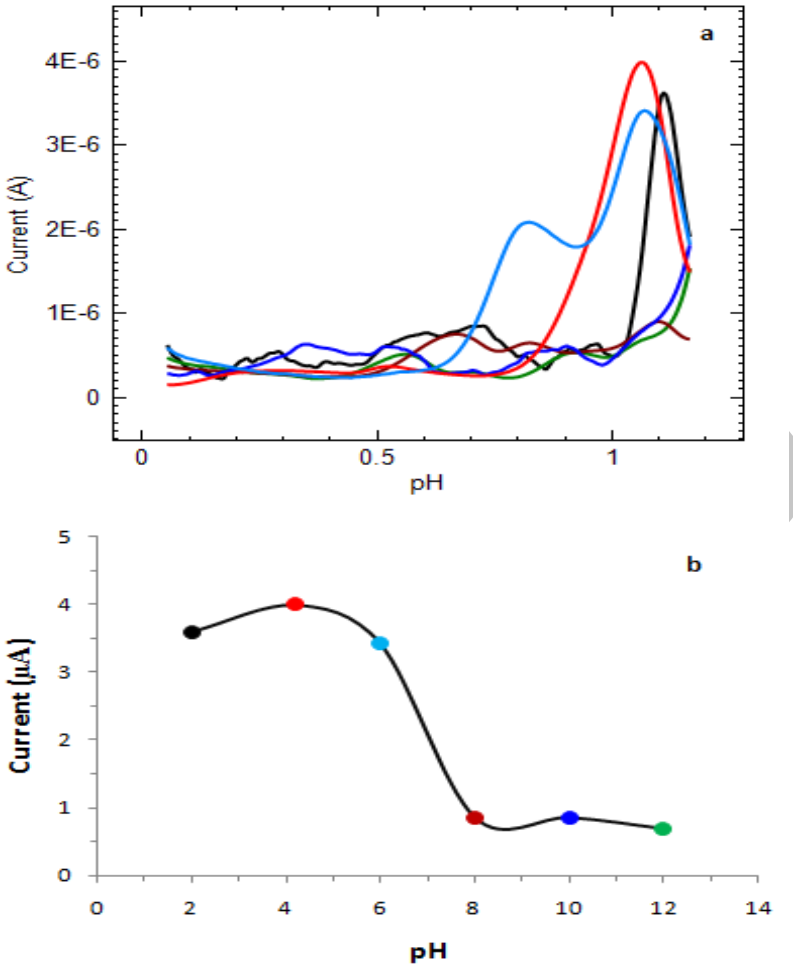


Fig ۲. The obtained voltamograms in different Ph (a) and peak current vs pH plot (b).

۳. ۲. ۲ *The effect of deposition potential*

In this case the deposition potential was varied srom -۱،۸ to ۱ V. The obtained voltamogrames were recorded in Fig ۳(a) and the peak current vs deposition potential were ploted in Fig ۳(b). As can be seen the best peak current resulted with deposition potential -۱،۸ V. When the deposition potential shifted to more positive values, the current peak decreases. Based on this results, it can be stated that a adsorptive process contributes in the deposition of Tam. Because the Tam can not be reduced on the surface of GCE. Therefire the deposition potential -۱،۸ V was selected as optimal value in next experiments.

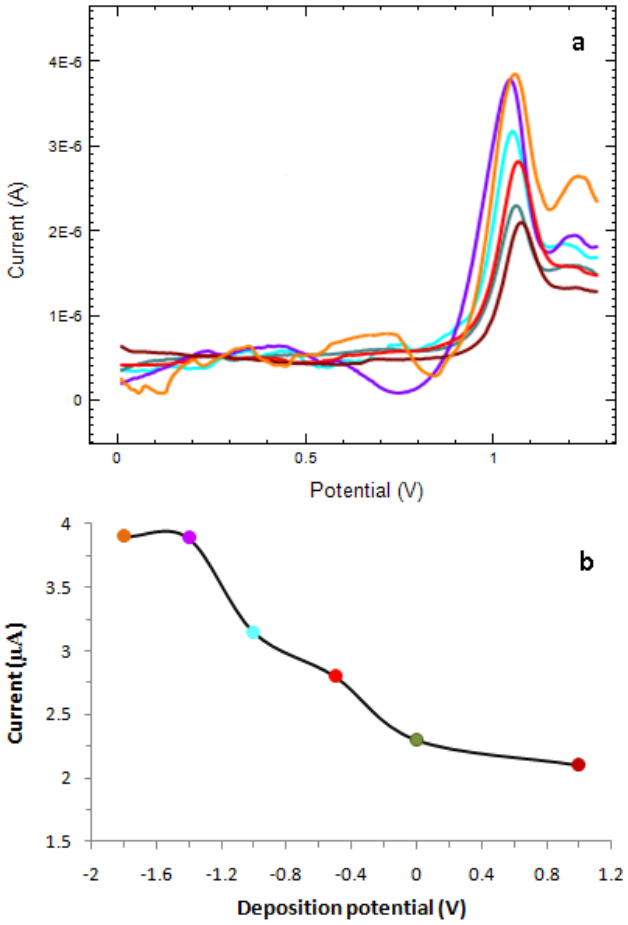


Fig ۳. The obtained voltamogram in different deposition potentials (a) and peak current vs deposition potential (b)

۳. ۲. ۳ *The effect of deposition time*

The effect of deposition time was investigated in the range of ۳۰ to ۳۰۰ s Fig ٤(a). As can be seen the greatest peak current was obtained after ۳۰ s deposition time. Some reasons may be affect the peak current in different deposition time. But, what observed in this case the maximum of the maximum of the variation of peak current is approximately o.٥ µM. however, to decrease the analysis time, ۳۰ s deposition time was selected in subsequent experiments.

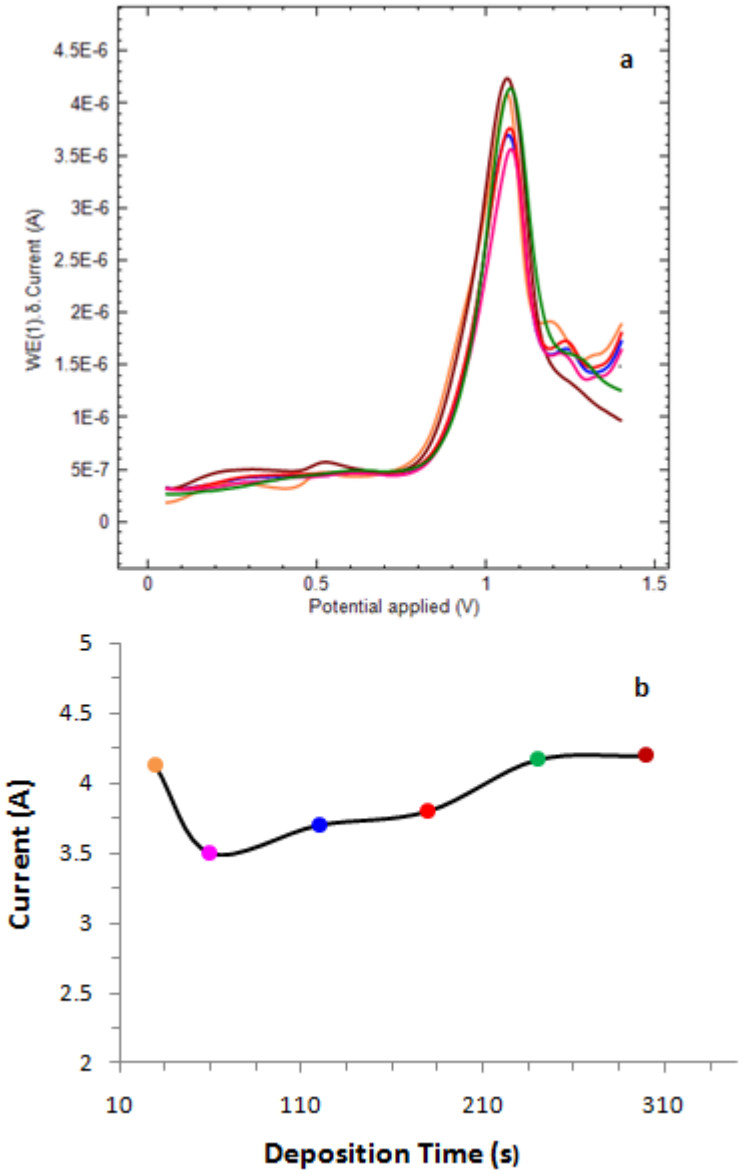


Fig ٤. The obtained voltamogram in different deposition time (a) and peak current Vs deposition time plot (b).

٣. ٢. ٤. *Calibration curve and figures of merit*

Under the optimum conditions include; deposition pH ٤،٢, deposition potential -١،٨ V, deposition time ٣٠ s, the calibration curve was plotted in the range of ١-١٠ µM. The obtained voltamogram were recorded in Fig ٥(a) and the calibration plot was shown in Fig ٥(b). As can be seens a good linearity was obtained in this range of Tam concentration. After the calibration of the proposed method, the blank DPAAS behavior was performed to obtain the blank standard deviation for LOD and LOQ calculations as follows:

LOD= ٣sb/m (١)

LOQ= ١٠ sb/m (٢)

Where sb is the standard deviation of the signal at ١،١ V and m is the slope of calibration curve. The LOD and LOQ were round to be ٠،٦٢١, ٢،٠٧ µM, respectively.

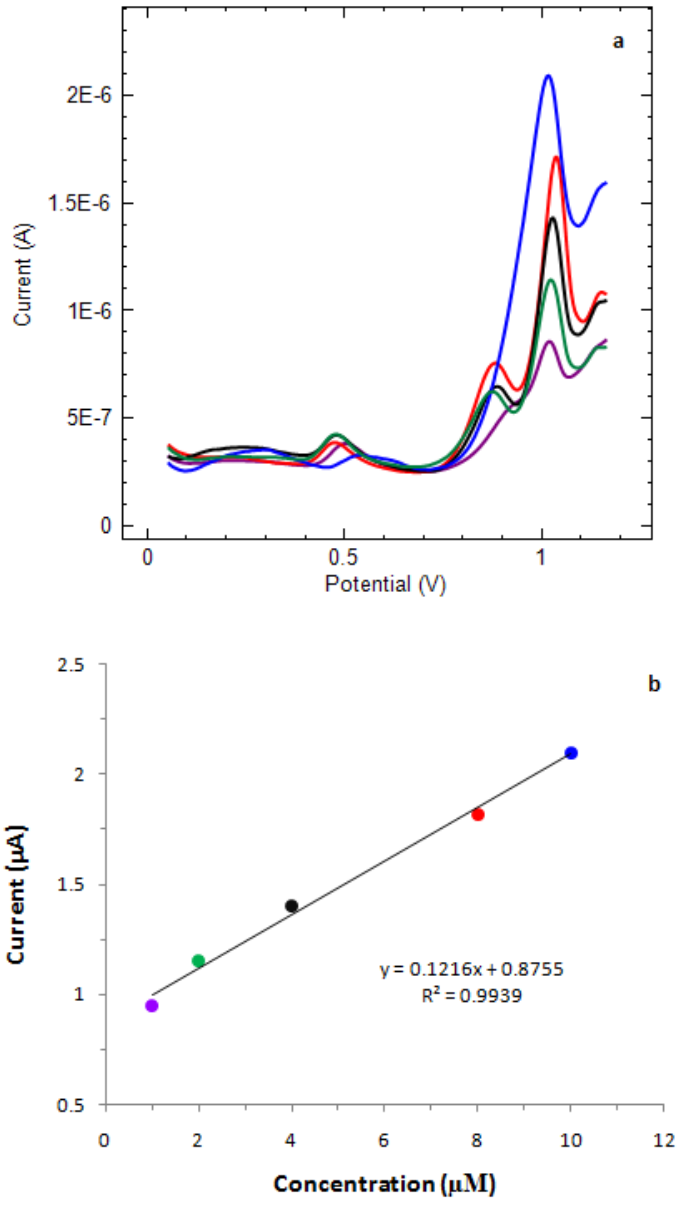


Fig ٥. The obtained voltamograms of the Tam standard solution (a) and the calibration curve (b).

٣,٢,٥ precision and accuracy of the proposed DPAASV.

To evaluate the precision (RSD) and accuracy (error%) of the proposed method, the ٥ replicates of ٤ µM Tam were the five voltamogram were shown in Fig ٦. The ٥ concentration were obtained by calibration equation. Finally, the concentration average, error and RSD were found to be ٤,١٠٦, ٢,٦٥%, ٣,٦٢%, respectively. The results are in well agreements with the accuracy and precision of a analytical method.

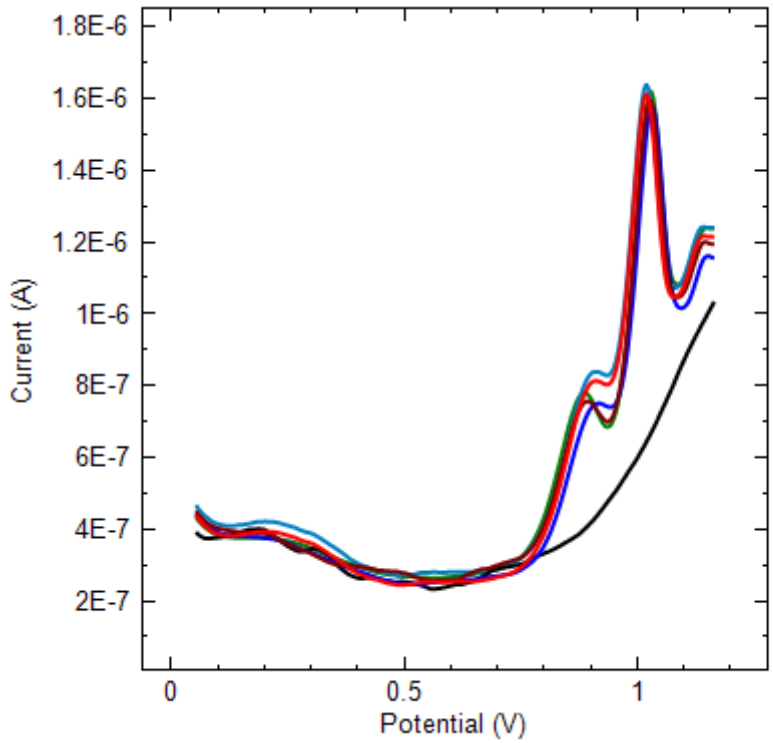


Fig ٦. The obtained voltamograms of the blank and ٥ replicates of ٤ µM Tam.

٣,٣. Extraction of Tam from tablets

As an applied result of this work, the extraction conditions of the Tam from tablets by MeOH was investigated. In this case, the effect of extraction time and MeOH volume were investigated.

٣.٣.١. The effect of extraction time

The effect of contact time between grounded tablet and MeOH was studied from ٥ to ٤٠ min. the peak cuttent of the Tam oxidation was followed as a signal of the extraction performance . The current peak vs time (min) was plotted in the range of ٥ to ٤٠ min (Fig ٧). As can be seen, the best performance is achieved after ٥ min extraction. When the extraction time increase the performance reduces, because the rest of the additives used in tablet formulation can be functioned as a adsorbent and subsequent reduces the extraction performance.

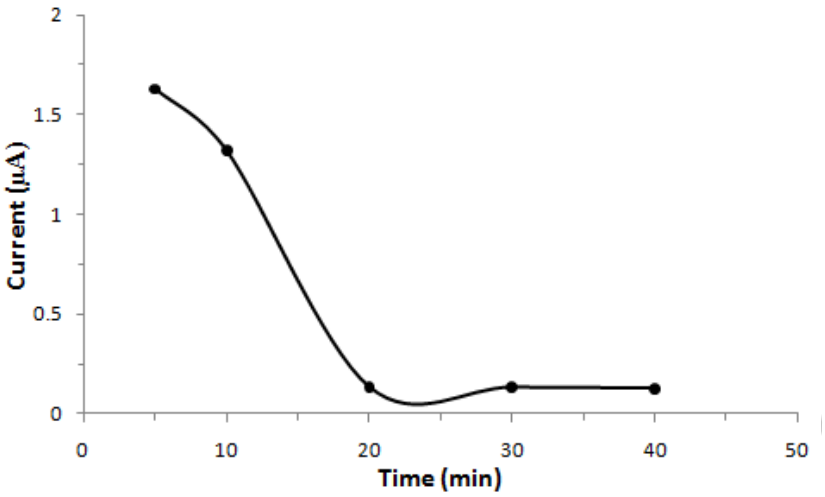


Fig ۷. The effect of contact time in the extraction of Tam from tablets by MeOH.

۳. ۳. ۲. The effect of MeOH volume

In order to investigate the effect of MeOH in the extraction of Tam, the MeOH volume was varied from ۱ to ۸ mL. The obtained voltamogram shown in Fig ۸. After the calculation of the tam cincentration by calibration curve, the extraction recovery percentage (ER%) were calculated based on the Eq. ۳ and Eq. ٤ as follow:

$ER\%=C_{exp}/C_{th}$ (۳)

$C_{th}=۲,۷\times۱۰^{-۵}/V_{MeOH}(mL)$ (٤)

Where the C_{exp} and C_{th} are the experimental and expected concentration of Tam. The $۲,۷\times۱۰^{-۵}$ is a constant that result from dilution calculation. As can be seen the best ER% was obtained by ۳ mL MeOH. However this optimized procedure can be used for extraction and monitoring of the Tam content in pharmaceutical formulations.

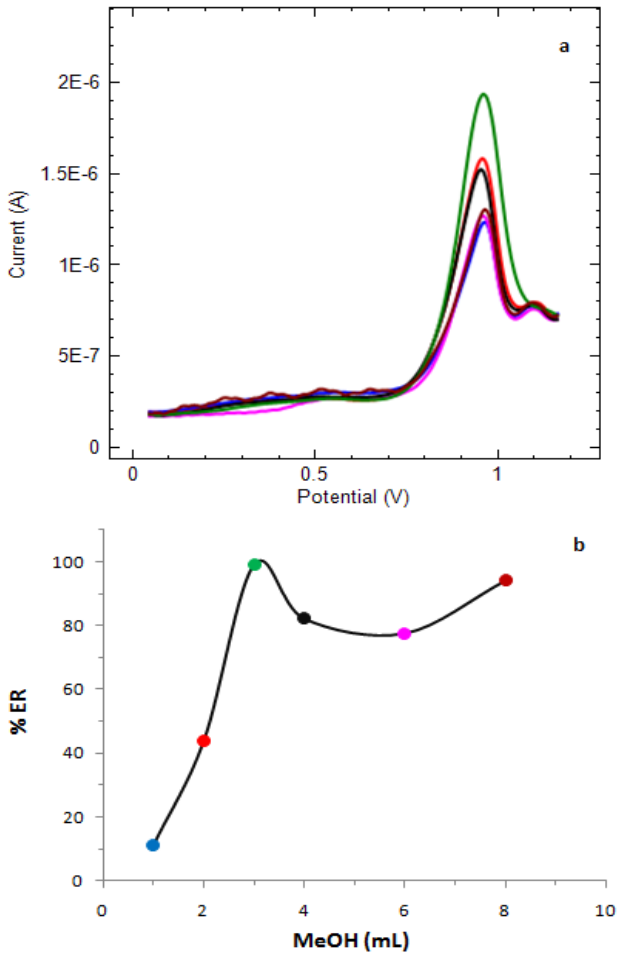


Fig ۸. The voltamograms result from the extraction of Tam by different volume of MeOH (a) and the extraction recovery vs MeOH curve (b).

٤. Conclusion

The electroanalytical behavior of Tam in B-R buffer at the surface of GCE was investigated. The effect of different parameters that affect the peak current of Tam in anodic striping have been stadied. At the optimized conditions, a calibration curve with a good linearity was achieved. Finally, the proposed DPAASV was succesfully employed for extraction and monitoring Tam drug in tablets.

٥. Acknowledgment

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