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ELECTROCHEMICAL APPROACH FOR THE SENSITIVE DETERMINATION OF ANTICANCER DRUG EPIRUBICIN IN PHARMACEUTICALS IN THE PRESENCE OF ANIONIC SURFACTANT

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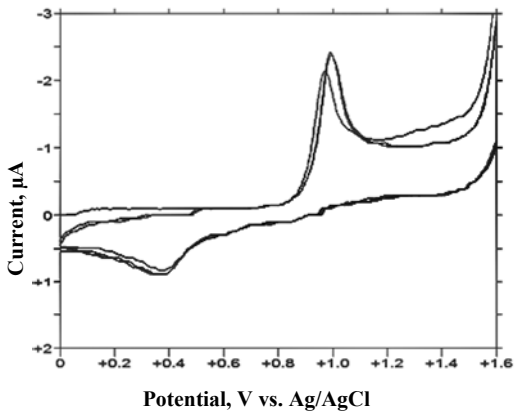
In this research, sensitive, rapid, different electrochemical methods were developed for the determination of anticancer drug epirubicin. The aim of the study was to fully validated determination of epirubicin in pharmaceuticals, by means of electroanalytical methods. The detailed electrooxidative behavior of epirubicin was investigated using cyclic, differential pulse and square wave voltammetry at boron-doped diamond electrode. The possible oxidation mechanism was discussed. Surfactant effect was also examined using 1×10^{-3} M sodium dodecyl sulphate. The oxidation process was found nearly irreversible over the pH range studied and exhibited diffusion controlled electrode process. All experimental parameters have been optimized and the following studies were realized under the optimum conditions. The sensor used in this research is suitable for the analysis of the trace amounts of epirubicin in pharmaceuticals. The proposed methods were applied to commercial preparations and average percentage recovery was in good agreement between each other (differential pulse and square wave voltammetry).



INTRODUCTION

Epirubicin (EPR), (10-(4-amino-5-hydroxy-6-methyl-oxan-2-yl) oxy-6,8,11-trihydroxy-8-(2-hydroxyacetyl)-1-methoxy-9, 10-dihydro-7H-tetracene-5, 12-dione) (Scheme 1a), which is an antineoplastic agent, used for breast, pancreatic, lung and ovarian cancers. It has similar activity with doxorubicin and has been approved for use worldwide since the 1990s.[1-4] Most of the anthracyclines appear to form a complex with DNA by intercalation between the DNA strands. Therefore they can inhibit replication and transcription attributed to interference with topoisomerase-DNA cleavable complex and helicase activity by anthracyclines. However, the mechanism of antitumour action for EPR has not been completely elucidated. Approximately 11 to 15 % of EPR is eliminated primarily via the hepatabiliary system, within the urine as unchanged drug and metabolites.[5]

The extensive development of pharmaceutical field requires sensitive analytical methods for the determination of anticancer agents because of their side effects. For this reasons there is need for simple, sensitive, accurate, time saving and economical methods for its determination in bulk

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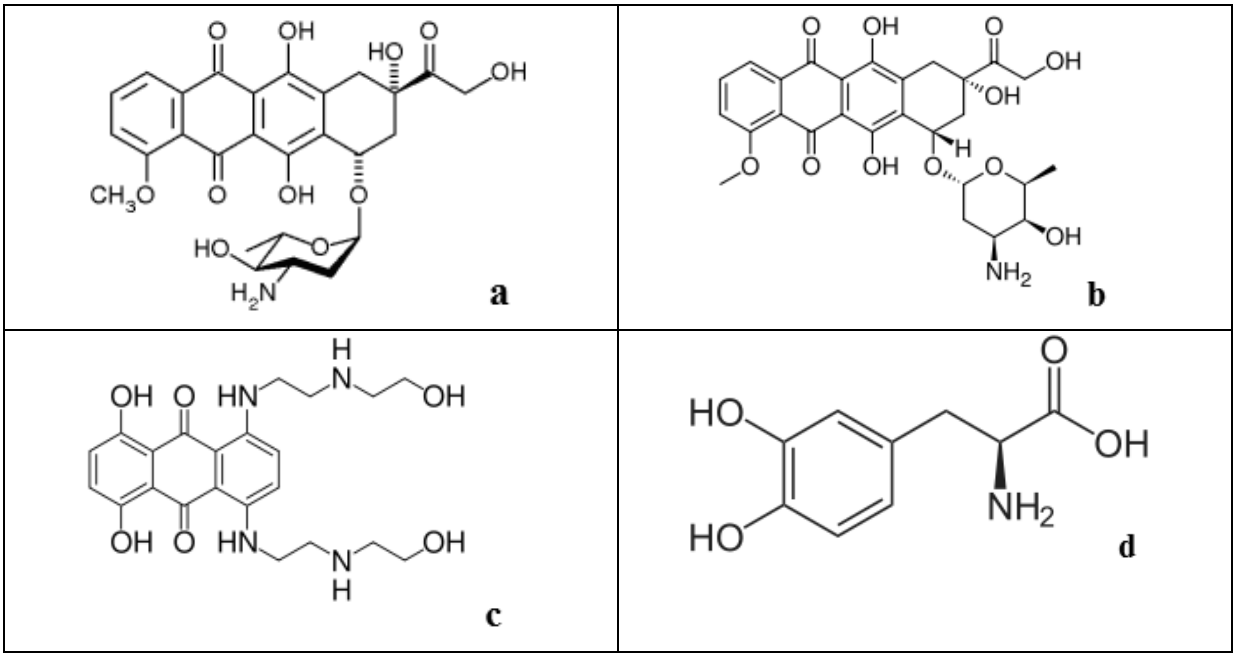
solutions, biological samples and pharmaceuticals. Several chromatographic methods such as LC-UV,[6-10] electrochemical,[11] MS/MS detection,[12-16] fluorescence detection[17] and capillary electrophoretic methods[18] have been used for the individual or multicomponent analysis of EPR in biological samples. Electrochemical methods have proved to be highly sensitive for the analysis of drugs in pharmaceuticals and biological samples owing to the straight forwardness, low cost and relatively short analysis time comparing to the other analytical techniques. However, only one voltammetric method is available for the determination of EPR in pharmaceutical formulations using carbon nanotubes modified electrode.[19] In addition there is only limited voltammetric study between EPR and DNA interaction.[20, 21]

Surfactants contains both polar (hydrophilic) and nonpolar (hydrophobic) groups. This amphiphilic structure property makes them surface-active agents, providing important applications such as active cleaning agents for all kinds of washing. In the presence of a suitable surfactant, most water insoluble materials can be readily solubilized.[22] Surface active agents play great role in various fields of pharmaceutical analysis. Surfactants are often used as selective masking agents to improve not only sensitivity but also selectivity of electrochemical methods.[23,24] The aggregates of surfactants, such as micelles, liquid crystalline, vesicles etc. could enhance the stabilized content and the control release behavior of drugs are widely studied as drug delivery systems. Adsorption of surfactants on electrodes and solubilization of electrochemically active compounds in micellar aggregates might significantly change the redox potential, charge transfer coefficients and diffusion coefficients of electrode processes.

Boron-doped diamond (BDD) electrodes have been proposed for applications in analysis due to their exceptional chemical inertness and mechanical strength. BDD film is electrically conducting and has found applications as electrode material. BDD electrodes can be used at very high potential values, either negative or positive, without promoting electrode decomposition.[25] A literature survey reveals that no electrochemical data were available concerning the voltammetric behavior of EPR either at BDD electrode or in the presence of surfactants.

The aim of this work is to carry out a detailed investigation on the voltammetric behavior and possible oxidation mechanism of EPR on BDD electrode using cyclic voltammetric (CV), differential pulse voltammetric (DPV), and square wave voltammetric (SWV) techniques, to develop simple, sensitive, rapid, low cost and reliable voltammetric methods for the determination of EPR using modern pulse voltammetric techniques in bulk materials and pharmaceutical dosage forms. These techniques did not require any time consuming sample pre-treatment or extraction step prior to drug assay. In addition, the other aim of this work is to realize surfactant effect on electrochemical response.



Scheme 1 – Structures of EPR (a), doxorubicin (b), mitoxantrone (c), L-dopa (d).

RESULTS AND DISCUSSION

Cyclic voltammetric behavior of EPR

The voltammetric behavior of EPR on BDD electrode was examined as details in different supporting electrolytes and pH values. Fig. 1 shows the repetitive CVs of 50 μg mL^{-1} EPR in 0.1 M H_2SO_4 using BDD electrode in the range between 0 and 1.8 V at a scan rate of 100 mV s^{-1}. EPR gave one sharp and well defined anodic peak at about +0.97 V. By reversing at +1.80 V, only one reduction signal at about +0.37 V corresponding to the anodic response was observed on the cathodic branch. Repetitive CVs revealed that the peaks increased upon the second and subsequent scans owing to weakly reactant adsorbed. The net effect is an increase in the height of the anodic peak because both adsorbed and diffusing reactants contribute the current.[26]

Influence of pH

Further work was dedicated towards studying the influence of nature and pH of the supporting electrolyte. All electroanalytical methods were carried out to characterize the effect of solution pH on the current and the potential of EPR, however only the results of DPV technique was reported for peak potential versus pH (Fig. 2a). The related parameters were evaluated over the pH range from 0.3 to 10.0 in different supporting electrolytes on 50 μg mL^{-1} EPR. It was found that the peak potential was shifted negatively with the increase of pH, indicating that the oxidation of EPR was a pH-dependent reaction. The peak potential shifted to less positive values, together with a decrease in peak currents with increasing the pH of the buffer solutions. 3D plots of peak potential versus solution pH were shown in Fig. 2b.

The plot of peak potential versus solution pH gave straight line which can be expressed by the following equation for the 1st (less positive) peak in all supporting electrolytes,

$$E_p\ (mV) = -\ 81.798\ pH + 1103.1,\ r = 0.993\ (n=10)$$

From the above equation the slope was found as -81.798 mV/pH, which is very close to the theoretical value of -59.0 mV, demonstrating that equal amounts of electrons and protons which are implicated in the rate-determining steps.

For the irreversible case, the equation given below can be used;

$$[E_p - E_{p/2}] = 48 / \alpha n\ mV\ at\ 25\ ^oC.^{27}$$

where E_p is the peak potential, $E_{p/2}$ is the half wave potential, α is the electron transfer coefficient and n is the electron transfer number. From this equation n was found 2.3 (~2).

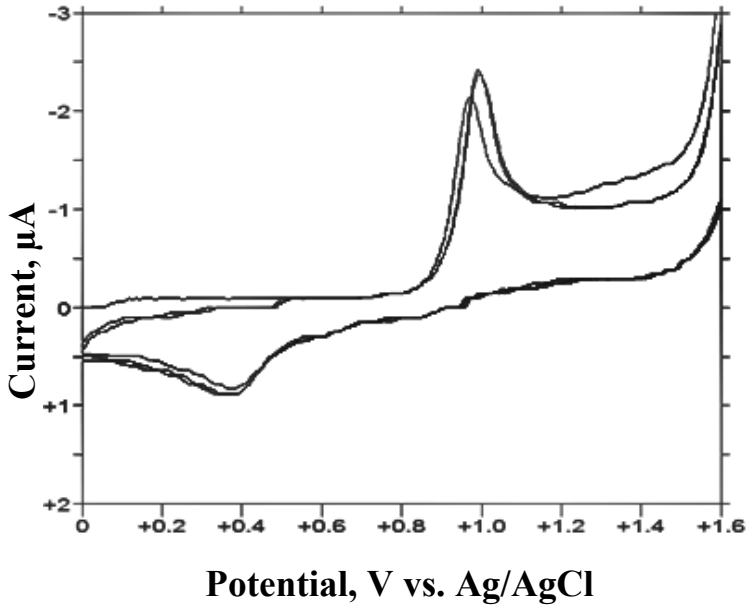


Fig. 1 – The repetitive CVs of 50 μg mL^{-1} EPR in the potential range of 0-1.80 V, with a scan rate of 100 mV s^{-1} in 0.1 M H_2SO_4 with BDD electrode.

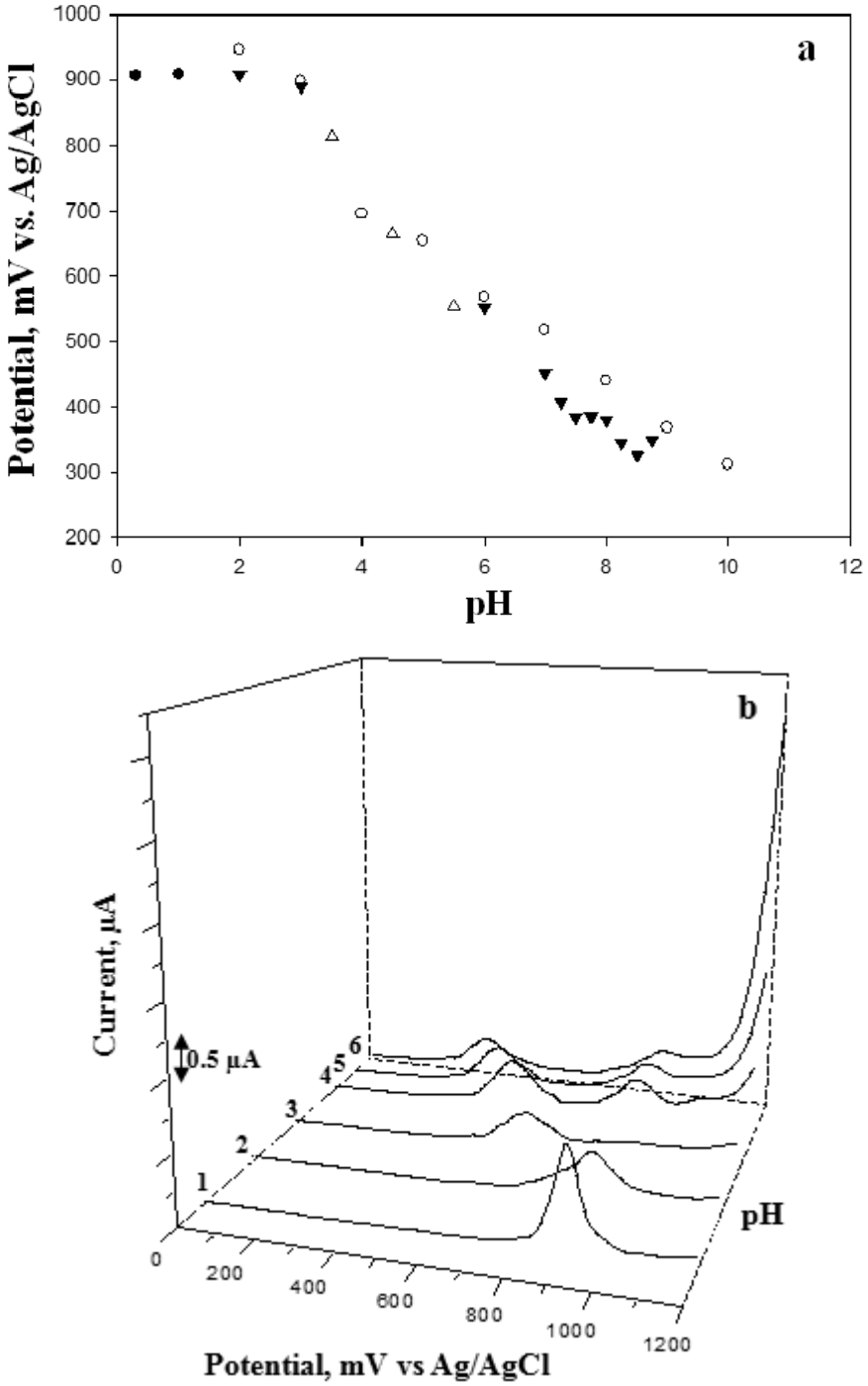


Fig. 2 – (a) Plot of E_p vs pH of 50 µg mL^{-1} EPR solution, (•) 0.1 M H_2SO_4 (○) 0.04 M BR buffer (▲) 0.2 M phosphate buffer (Δ) 1.0 M acetate buffer and (b) 3D plot of DP voltammograms; (1) 0.1 M H_2SO_4, (2) pH 3.0 BR buffer, (3) pH 5.0 BR buffer, (4) pH 7.0 BR buffer, (5) pH 8.0 BR buffer, (6) pH 9.0 BR buffer.

As can be seen in Fig. 2b, 0.1 M H_2SO_4 solution is not only resulted in EPR oxidation peak with good intensity but also resulted in good peak shape. Cyclic, DP and SW voltammograms of EPR exhibited one well-defined anodic peak in all buffer solutions, until pH 5.0 (peak1). After this pH value a second peak appeared which was at more positive potential (peak 2). The second peak appeared at about +0.90 V in all pH solutions (pH>5). From Ip-pH experiments, maximum current was obtained in 0.1 M H_2SO_4, phosphate buffer at pH 7.5 and BR buffer at pH 2.0. Therefore these mediums were used for further experiments.

Influence of scan rate

Useful information involving electrochemical mechanism can usually be acquired from the relationship between the peak current and the scan rate. Therefore, the electrochemical oxidation behavior of EPR at different scan rates from 5-750 mVs^{-1} was also studied in different buffer solutions. The oxidation peak shifted towards more positive potentials, as the scan rate increased a typical behavior of irreversible electrochemical reactions. It was noticed that the oxidation peak become broader and almost disappeared at higher scan rates. There is a good linear relationship between peak current and square root of scan rate in different buffer solutions. The anodic peak current of EPR increases linearly with the root of the scan rate ($\upsilon^{1/2}$) in the range of 5-750 mVs^{-1} and can be expressed as:

$$I_p\ (\mu A) = 0.168\ \upsilon^{1/2} - 0.098,\ r = 0.995$$
(n=9) for 0.1 M H_2SO_4

$$I_p\ (\mu A) = 0.158\ \upsilon^{1/2} - 0.193,\ r = 0.993$$
(n=9) for pH 2.0 BR buffer

$$I_p\ (\mu A) = 0.103\ \upsilon^{1/2} - 0.043,\ r = 0.997$$
(n=8) for pH 7.5 phosphate buffer

Furthermore, a plot of logarithm of peak current ($\log I_p$) versus logarithm of scan rate ($\log \upsilon$) gave a straight line within the same scan rate. There was a linear relation between $\log I_p$ and $\log \upsilon$ and the linear relationship was obtained as:

$$\log I_p = 0.482 \log \upsilon - 0.765,\ r = 0.995$$
(n=9) for 0.1 M H_2SO_4

$$\log I_p = 0.532 \log \upsilon - 0.950,\ r = 0.994$$
(n=9) for pH 2.0 BR buffer

$$\log I_p = 0.450 \log \upsilon - 0.859,\ r = 0.995$$
(n=8) for pH 7.5 phosphate buffer

The slopes of the equations are very close to the theoretically expected value of 0.5 which indicates the diffusion controlled process.[28] Both the correlation coefficient of I_p versus $\upsilon^{1/2}$ and the slope of $\log I_p$ versus $\log \upsilon$ confirm that the diffusion-controlled nature of the electrode processes.

The peak potential (E_p) (V) of the irreversible peak shifted anodically with increasing sweep rate. Indeed, the plot of E_p versus $\log \upsilon$ (V/s) gives a straight line with the equations of

$$E_p = 0.0465 \log \upsilon + 1.024,\ r = 0.990$$
(n=9) for 0.1 M H_2SO_4

$$E_p = 0.0626 \log \upsilon + 1.027,\ r = 0.994$$
(n=9) for pH 2.0 BR buffer

$$E_p = 0.0496 \log \upsilon + 0.500,\ r = 0.992$$
(n=8) for pH 7.5 phosphate buffer

which indicates that the rate control is a first order step following electron transfer.[29]

This behavior was constant with the electrochemical nature of the reaction in which the electrode reaction is coupled with an irreversible follow-up chemical step.[30]

Surfactant effect

To improve the sensitivity of the electroanalytical responses, the effect of SDS, which was added to the working solutions, on the peak current and peak potential were investigated (Fig. 3). The effect of SDS concentration on the voltammetric behavior of the EPR was evaluated for 20 μg mL^{-1} EPR in 0.1 M H_2SO_4 solution. In the presence of 1×10^{-3} M SDS, EPR showed sharper anodic peak, which is almost 3 times higher than those without SDS using BDD electrode (Fig. 3a).

As can be seen in Fig. 3a and 3b, 1×10^{-3} M SDS concentration is not only resulted in EPR oxidation peak with high intensity but also gave the good peak shape. Also background current level was lower in the presence of 1×10^{-3} M SDS than in its absence. The increase in SDS concentration induced a shift in EPR peak potential towards less positive potential values (from 972 mV to 944 mV).

Finally, SDS concentration optimized for further studies was 1×10^{-3} M, because of either providing the highest current peak or well-defined anodic peak at about +0.90 V.

Controlled potential coulometry

By using controlled coulometry, the number of electrons transferred, n values were calculated from the charge consumed by the very low desired concentration of EPR. The measurements were occurred in acidic media and total charge consumed for the electroanalysis was obtained and substituted into Faraday's equation;

$$Q = n\ F\ N$$

where Q is the total charge consumed for the electrolysis, n is the number of electrons transferred, F is the Faraday constant (96500 C) and N is the number of molar equivalents.

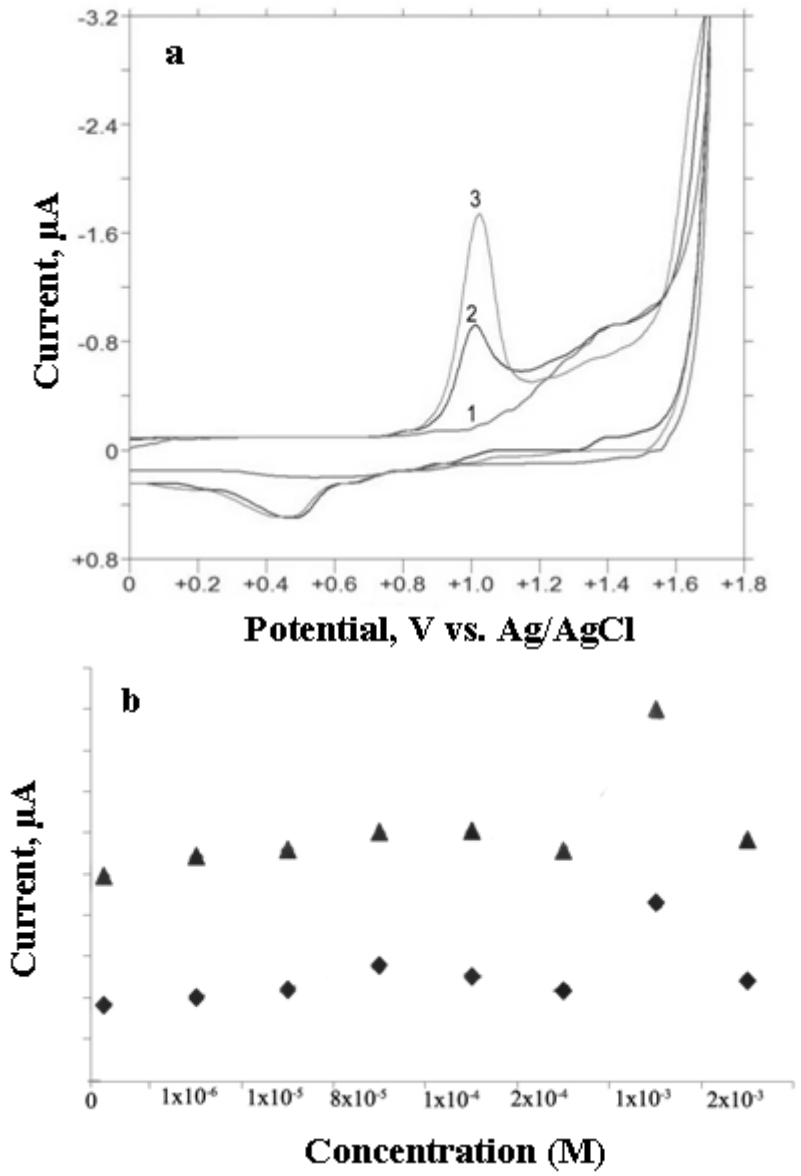


Fig. 3 – a) CVs of (1) 0.1 M H_2SO_4 (2) 20 µg mL^{-1} EPR in the absence of 1×10^{-3} M SDS in 0.1 M H_2SO_4 (3) 20 µg mL^{-1} EPR in the presence of 1×10^{-3} M SDS in 0.1 M H_2SO_4 (b) Plot of I_p vs. SDS concentration (▲) using DPV method (♦) using SWV method.

The number of electrons transferred was calculated from Faraday's equation. The exhaustive electroanalysis of 2 mL 2×10^{-10} M EPR was carried out at 0.1 M H_2SO_4 and 0.80 V. The total charge consumed was 32.3 µC and the number of electrons transferred was two for the anodic peak of EPR in acidic media.

Oxidation pathway mechanism

CV is the most suitable method for investigation of the redox behavior of the drug active compounds which can give insights into its metabolic fate.[31-34] CV curves might have profound effects on the understanding of the redox mechanism related to the activity of EPR. The EPR molecule is extensively metabolized in vivo.[1-4] In some cases, it has been suggested that the electrode mechanisms might mimic enzyme reactions by the researchers. Therefore, mechanism enlighten is important.

For the identification of the responsible oxidation group of EPR, the CV curves were compared with the curves of some selected model compounds which contain phenol moieties to exclude the possibility of the oxidation part of EPR. Even though the exact oxidation mechanism was not determined, some conclusion about the potential electroactive center under working conditions could be reached. The course of anodic oxidation of phenolic compounds is remarkably complex. In general, the oxidation of phenol in a

solution at high pH will generate the phenoxy radical giving an additional oxidation and reduction process. Many species involved in process are related to one another by a series of electron and proton transfers that may occur as the result of biomolecular interactions. Voltammetric studies show the expected irreversible electron process leading to formation of quionon structure of EPR. Some model compounds such as doxorubicin, mitoxantrone and L-dopa (Scheme 1 b, c, d) were studied for understanding redox mechanism related to the activity of EPR using CV measurements. L-dopa has hydroxyl groups on benzene ring. As can be seen in Fig. 4 these groups gave a sharp peak at about +0.90 V which is close to EPR oxidation peak. EPR, doxorubicin and mitoxantrone also have nearly same molecular structures. They have hydroxyl groups in benzene ring that is inside the quionon structure and these molecules gave similar oxidation behavior and close oxidation potentials (Fig. 4). So, it may be suggested that the oxidation of EPR is from the hydroxyl groups that are inside the quinon ring.

The electrochemical oxidation of EPR appears to be a complex process and different reaction pathways might be possible. EPR contains highly electroactive hydroxyl groups on the benzene rings which makes it suitable for electrochemical detection. According to the molecular structure of EPR, literature knowledge, and the obtained experimental results, the oxidation mechanism of EPR may be postulated by an initial oxidation with two electrons and the conversion of hydroxyl group to quinone, which was electroactive in both acidic and alkaline media[4,5,31] (Scheme 2). The electrooxidation of EPR appears to be a complex process. EPR oxidizes in all supporting electrolyte via initial two electron oxidation, including fast chemical reactions with water to give the quinon structure.[35,36]

Calibration curve

For BDD electrode, according to the obtained results, it was possible to apply DPV and SWV techniques to the quantitative analysis of EPR in the presence of 1×10^{-3} M SDS. 0.1 M sulphuric acid solution was selected as the supporting electrolyte for the quantification as EPR gave maximum peak current at about pH 1.0. DP and SW voltammograms obtained with increasing amounts of EPR showed that the peak current increased linearly with increasing concentration, as shown in Fig. 5a and 5b. It was found that the plots of I_p versus concentration showed linearity over the EPR concentration in the range of 0.5 to 40.0 μg mL^{-1} for both methods. The linear equations were:

I_p (μA) = 0.041C - 0.020 r=0.998 (n=6) for DPV
I_p (μA) = 0.047C - 0.039 r=0.998 (n=7) for SWV

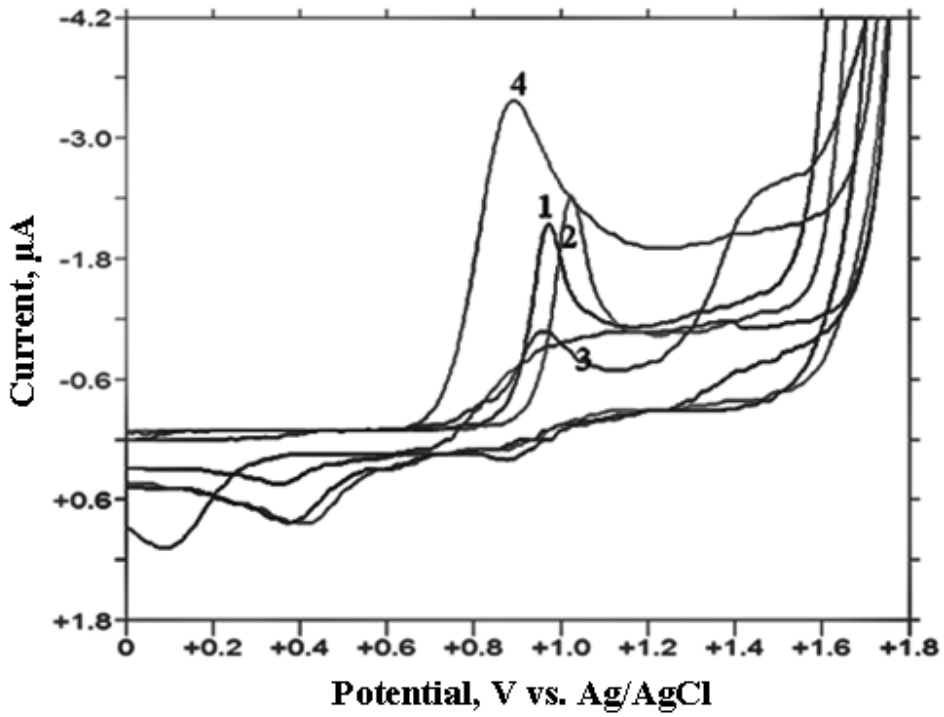
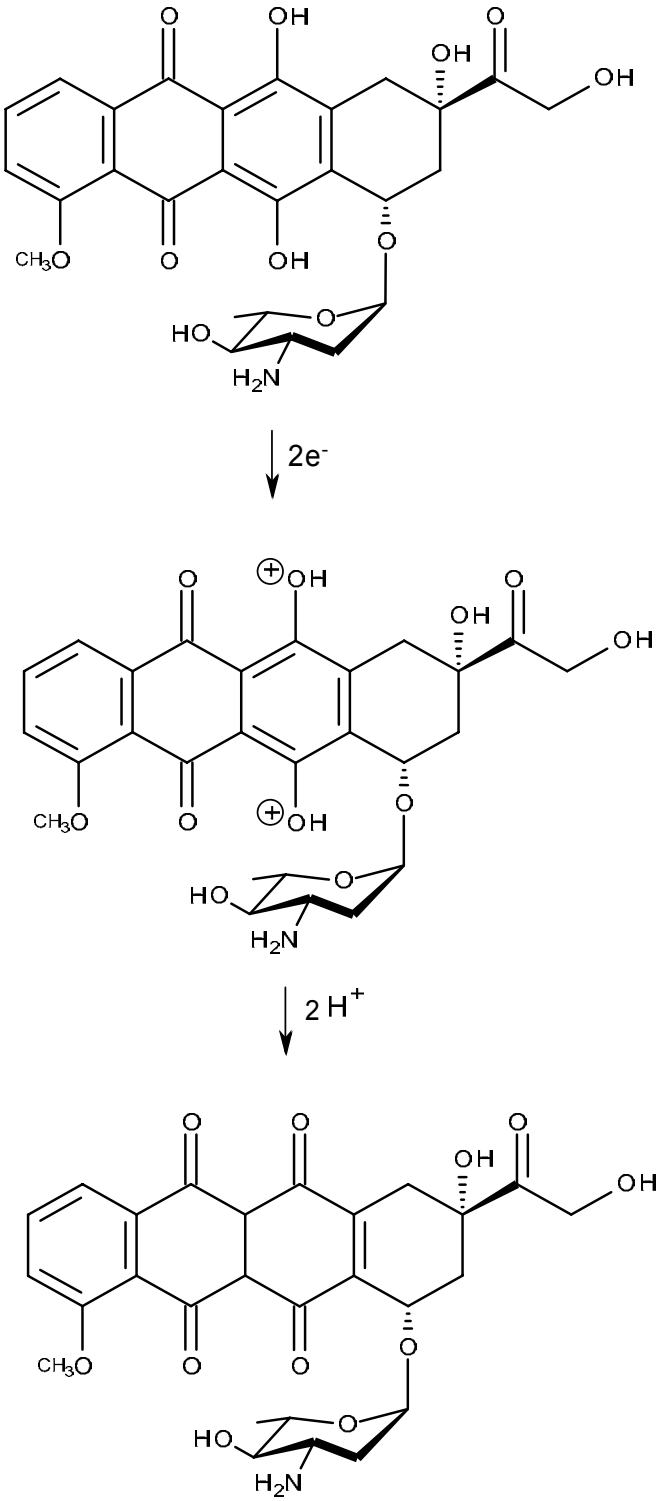


Fig. 4 – CVs of 50 μg mL^{-1} EPR and model compounds in 0.1 M H_2SO_4. (1) EPR, (2) doxorubicin, (3) mitoxantrone, (4) L-dopa. Scan rate 100 mVs^{-1}.

Deviation from linearity was observed for more concentrated solutions, due to the adsorption of EPR or its oxidation product on the electrode surface.[35] Low LOD and LOQ values confirmed the sensitivity of the proposed methods and are shown in Table 1. The repeatability and reproducibility of peak potential and peak current were tested by five experiments on 10 μg mL^{-1} of EPR in the presence of SDS and they reported in Table 1. The low values of standard error of the slope, intercept and also greater correlation coefficient than 0.99 confirmed that the precision of the proposed voltammetric methods.



Scheme 2 – The possible electrooxidation pathway of EPR on BDD electrode.

Table 1

Statistical evaluation of the calibration data for quantitative determination of EPR by BDD electrode

	DPV	**SWV**
Measured potential (V)	0.932	0.972
Linearity Range (μg mL^{-1})	0.50-40.00	0.50-40.00
Slope	0.041	0.047
Intercept	-0.020	-0.039
Correlation Coefficient	0.998	0.998
SE of Slope	9.91×10^{-4}	9.96×10^{-4}
SE of Intercept	1.87×10^{-2}	1.74×10^{-2}
Limit of Detection (μg mL^{-1})	0.043	0.073
Limit of Quantification (μg mL^{-1})	0.142	0.245
Repeatability of peak current[a] (RSD %)	0.850	0.385
Repeatability of peak potential[a] (RSD %)	0.234	0.736
Reproducibility of peak current[a] (RSD %)	1.975	1.959
Reproducibility of peak potential[a] (RSD %)	0.472	0.771

[a] Each value is the mean of five experiments.

Parenteral preparation analysis

In order to evaluate the applicability of the proposed methods in pharmaceutical dosage form analysis, a commercial dosage form containing EPR, such as Epirubicin Ebewe® (100 mg/50 mL) was used. To determine whether the excipients show any interference with the analyzed compound, and obtaining the accuracy of the developed method known amount of the pure drugs were added to different pre-analyzed formulation EPR and the mixture were analyzed by DPV and SWV methods using BDD electrode. In the basis of all these results, the voltammetric methods were applied to the direct determination of EPR in commercial dosage form using the related calibration curves. No pretreatment such as precipitation, filtration, extraction and evaporation except adequate dilution were used for the experiments in this study. According to the results in Table 2, both voltammetric methods can easily be used to determine EPR in dosage form. The results are in good agreement with the content marked in the reported label.

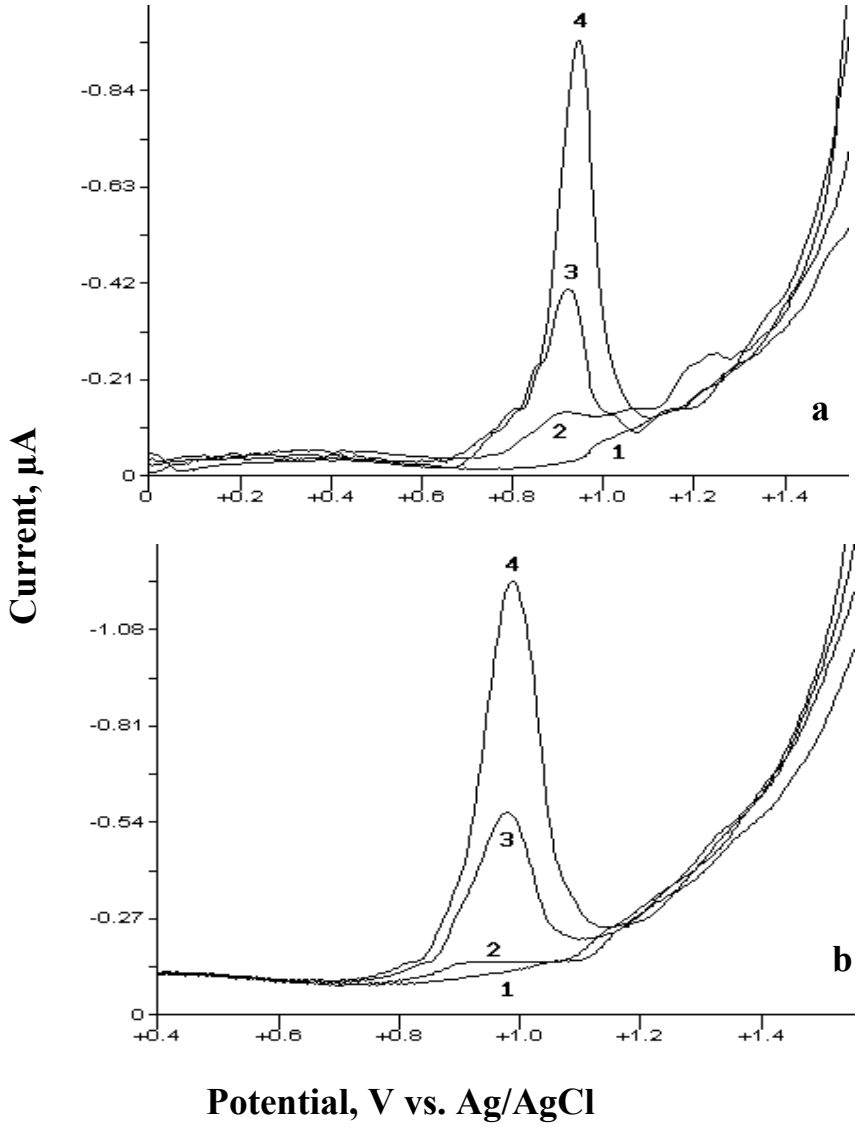


Fig. 5 – (a) DPV and (b) SWV obtained for the determination in 0.1 M H_2SO_4 with BDD electrode; (1) blank solution; (2) 1 µg mL^{-1}; (3) 10 µg mL^{-1}; (4) 20 µg mL^{-1} EPR.

Table 2

Results of the assay and the recovery analysis of EPR in pharmaceutical dosage forms via BDD electrode

	DPV	**SWV**
Labeled claim (100mg/50mL)	100.000	100.000
Amount found (mg)[a]	100.180	101.347
RSD (%)	0.460	1.648
Bias (%)	-0.180	-1.347
Added (mg)	2.000	2.000

Table 2 (continued)

Found (mg)[a]	2.024	2.060
Recovery (%)	101.195	101.603
RSD % of recovery	1.212	0.882
Bias (%)	-1.195	-1.603

[a] Each value is the mean of five experiments.

EXPERIMENTAL

Apparatus

Voltammetric measurements were recorded using BAS 100 W (Bioanalytical System, USA), electrochemical analyzer with a standard three-electrode configuration. The three-electrode system consisted of a BDD electrode (Windsor Scientific Ltd; Φ = 3 mm, diameter) as working electrode, a platinum wire counter electrode, and an Ag/AgCl saturated KCl reference electrode. Working electrode was polished manually with aqueous slurry of alumina powder (Φ = 0.01 μm) on a damp smooth polishing cloth (BAS velvet polishing pad) just before each measurement. All measurements were achieved at room temperature. Operating conditions for DPV were: pulse amplitude, 50 mV; pulse width, 50 ms; scan rate, 20 mV s^{-1}; for SWV were: pulse amplitude, 25 mV; frequency, 15 Hz; potential step, 4 mV. The pH measurements were made using a model 538 WTW with a combined electrode with an accuracy of ± 0.05 pH.

Coulometric experiments were performed AUTOLAB-PGSTAT 30 (Eco Chemie, Utrecht, The Netherlands) electrochemical and electroanalytical instrument in the potentiostatic mode using Pt foil with a large surface area as the working electrode and Pt wire as the counter electrode.

Reagents

EPR and its pharmaceutical dosage form (Epirubicin "Ebewe® parenteral preparation") were supplied from EBV Pharm. Inc. (Istanbul, Turkey). For the model compound study, daunorubicin, doxorubicin, mitoxantrone, L-dopa were supplied from different pharmaceutical companies in Turkey and Sigma. Sodium hydroxide was purchased from Merck. Sodium dodecyl sulphate (SDS) was from Sigma. Stock solutions were prepared daily by dissolution of methanol. Standard solutions were prepared by dilution of stock solution using methanol: supporting electrolyte (20:80 v/v) mixture. Sulphuric acids (0.1 and 0.5 M), phosphate (0.2 M, pH 2.0-8.0), acetate (1.0 M, pH 3.4-5.5) and Britton-Robinson (BR) buffers (0.04 M, pH 2.0-10.0) were used as supporting electrolytes. For surfactant effect analysis, working solutions including EPR and SDS (1×10^{-3} M) and 20 % of methanol were used and prepared by dilution of the stock solution with supporting electrolyte (0.1 M sulphuric acid solution). All solutions were protected from light and used within 24 h to avoid decomposition.

Validation of the analytical procedures

For the validation of the proposed methods, precision and accuracy were checked by assaying five replicate samples on the same day (within day) and the different days (between days) over a week period for 10 μg mL^{-1} of EPR. Relative standard deviations (RSD %) were calculated to check the precision of the method.[37-39] The accuracy and precision of the developed methods are described in a quantitative fashion using relative errors (Bias %).

The calibration equations for DPV and SWV techniques were constructed by plotting the peak current against EPR concentration.

To study the accuracy of the proposed method and to check the interferences from excipients used in the dosage form, recovery experiments were carried out. The concentration of EPR was calculated using standard addition method. For these experiments, known amount of pure EPR were added to the previously analyzed parenteral preparation samples.

The nominal content and recovery of EPR were calculated using regression equation that was previously obtained from calibration plot.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated from the equations of LOD = 3.3 s/m and LOQ = 10 s/m using the standard deviation of response (s) and the slope of the calibration curve (m).[25]

Analysis of EPR from dosage forms

1.25 mL of EPR "Ebewe® parenteral preparation" claim to contain 100 mg EPR per 50 mL of the solution was dissolved in 25 mL of methanol (*ca.* 100 μg mL^{-1}). Working solutions including EPR and SDS (1×10^{-3} M) and 20 % of methanol were used and prepared by dilution of the stock solution to 10 μg mL^{-1} with supporting electrolyte.

CONCLUSIONS

In the present work, BDD electrode was successfully used for the oxidation of EPR in sulphuric acid solutions. Developed DP and SW voltammetric techniques are easy to be carried out for the reliable analysis of EPR either in bulk form or in commercial dosage forms. Taking to the advantage of the SDS effect on voltammetric response of EPR, BDD electrode combined with high sensitive and precise and also accurate DPV and SWV could allow an attractive trace analysis of EPR. A probable oxidation mechanism was

proposed in this study and two electrons two protons mechanism was obtained for the oxidation of EPR. Also, diffusion controlled irreversible mechanisms were observed for the oxidation pathway of EPR. The proposed methods offer the advantages of accuracy and time-saving as well as simplicity of experiment and reagents. High percentage recoveries showed that proposed methods are free from interferences of the commonly used excipients and additives in the drug formulation. These methods suggest analysis of EPR in differently equipped laboratories.

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