ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2015, *60*(5-6), 491-499

INVESTIGATION OF VOLTAMMETRIC BEHAVIOR AND ELECTROANALYTICAL DETERMINATION OF ANTICANCER EPIRUBICIN *VIA* GLASSY CARBON ELECTRODE USING DIFFERENTIAL PULSE AND SQUARE WAVE VOLTAMMETRY TECHNIQUES

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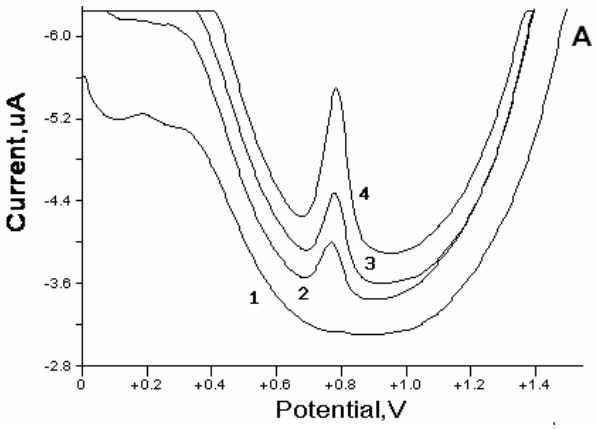
Received November 10, 2014

The electrochemical oxidation of epirubicin was investigated using cyclic, differential pulse and square wave voltammetry at glassy carbon electrode. The aim of the study was to determine epirubicin levels in pharmaceuticals via electrochemical methods. The oxidation process was quasi-reversible over the pH range studied and exhibited diffusion controlled electrode process. All experimental parameters have been optimized under the optimum conditions. The oxidation peak current was linearly proportional to the concentration of epirubicin in the range of 2×10^{-7}-3.6×10^{-5} M with a detection limit of 4.1×10^{-8} M and 4.9×10^{-8} M by differential pulse and square wave voltammetry, respectively. These unique properties make the sensor suitable for the analysis of the trace amounts of epirubicin in pharmaceutical preparations. The proposed methods were applied to commercial preparations. Differential pulse and square wave voltammetry techniques were compared with t-test and F-test.



INTRODUCTION

Epirubicin (EPR), (Scheme 1a) is antineoplastic agent, used for breast, pancreatic, lung and ovarian cancers.[1-4] Moreover, there are studies showing that it is active in non-Hodgkin's lymphoma and soft-tissue sarcomas. It was clinically developed in France and Italy at the beginning of the 1980s after its synthesis and it has a better cardiac tolerability compared to other anthracyclines. Its anti-tumor effects are exerted by interference with synthesis of DNA; through intercalating between the DNA strands, it inhibits replication and transcription. It is metabolized by the liver and eliminated in the urine with a half life of 30 to 40 hours.[5,6]

The voltammetric studies on epirubicin generally focus on epirubicin-DNA interaction.[7-10] There are two voltammetric studies in the literature which examine determination of epirubicin without referring to DNA interaction.[11,12] One of them developed single walled carbon nanotubes modified glassy carbon electrode (GCE) for determination.[11] The second study was undertaken by our group in which surfactant effect on the EPR response was examined by using diamond electrode. In doing that detailed oxidation mechanism was studied using diamond electrode.[12]

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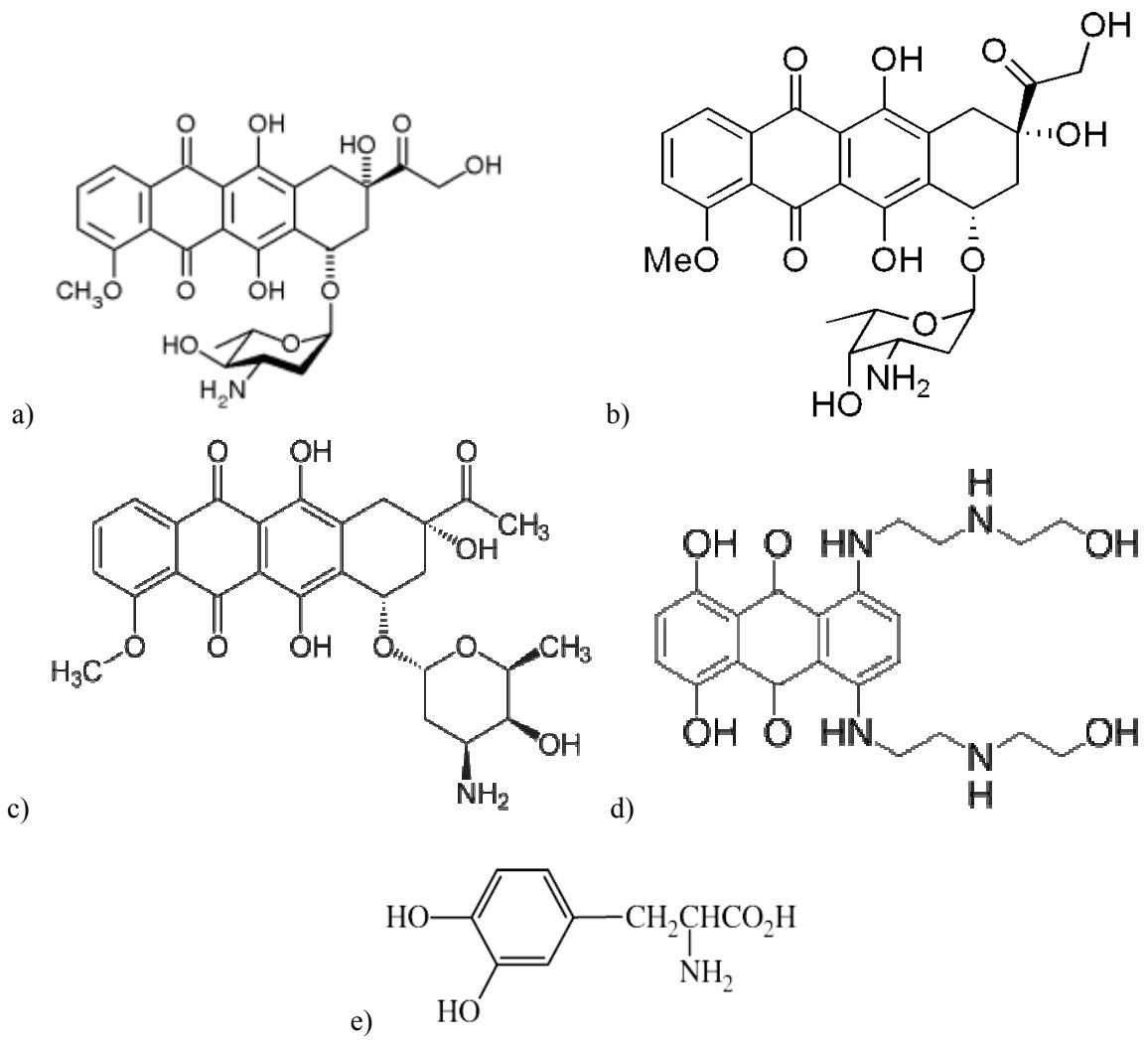

Scheme 1 – The chemical structure of a) epirubicin, b) doxorubicin, c) daunorubicin, d) mitoxantrone, e) L-dopa.

Electroanalytical methods emerge with the interplay between electricity and chemistry; in other words they were used to measure electrical quantities, such as current, potential, or charge and their relationship with the chemical parameters. These methods are widely used in fields like environmental monitoring, industrial quality control or biomedical analysis.[13] Another field in which electrochemical methods are extensively used is drug analysis and these methods have proved to be highly sensitive due to the straight forwardness, low cost and relatively short analysis time.[14] GCE is the most common carbon-based electrode because of its excellent mechanical and electrical properties, wide potential range, chemically inert nature and impermeability to gases. They are easily mounted, polishable and compatible with all common solvents. They allow many applications in many different areas, since their performances are relatively reproducible.[15]

The aim of this work is to develop simple, sensitive, rapid, low cost and reliable determination method for EPR in bulk materials and pharmaceutical dosage forms with a detailed investigation on the voltammetric behavior and possible oxidation mechanism of EPR on GCE using cyclic (CV), differential pulse (DPV), and square wave voltammetric (SWV) techniques.

RESULTS AND DISCUSSION

Cyclic Voltammetric Behavior of EPR

The voltammetric behavior of EPR at GCE was examined as details. Fig. 1 shows the repetitive cyclic voltammograms of 1×10^{-4} M EPR in 0.5 M H_2SO_4 solution, in pH 7.75 phosphate buffer and in pH 5.5 acetate buffer with a scan rate of 100 mV s^{-1}. EPR exhibited one distinct well

defined and well defined anodic peak at nearly +0.9 V and a small peak in the reverse scan at nearly +0.6 V in 0.5 M H_2SO_4 solution (Fig. 1A), suggesting that the electrochemical reaction was the quasi-reversible nature of the oxidation process. In the second and third sweeps, the peak currents decreased and remove to more positive potential values (Fig. 1). This phenomenon may be due to the fact that the adsorption of EPR or its oxidative products occurs at the electrode surface and weakly adsorbed.

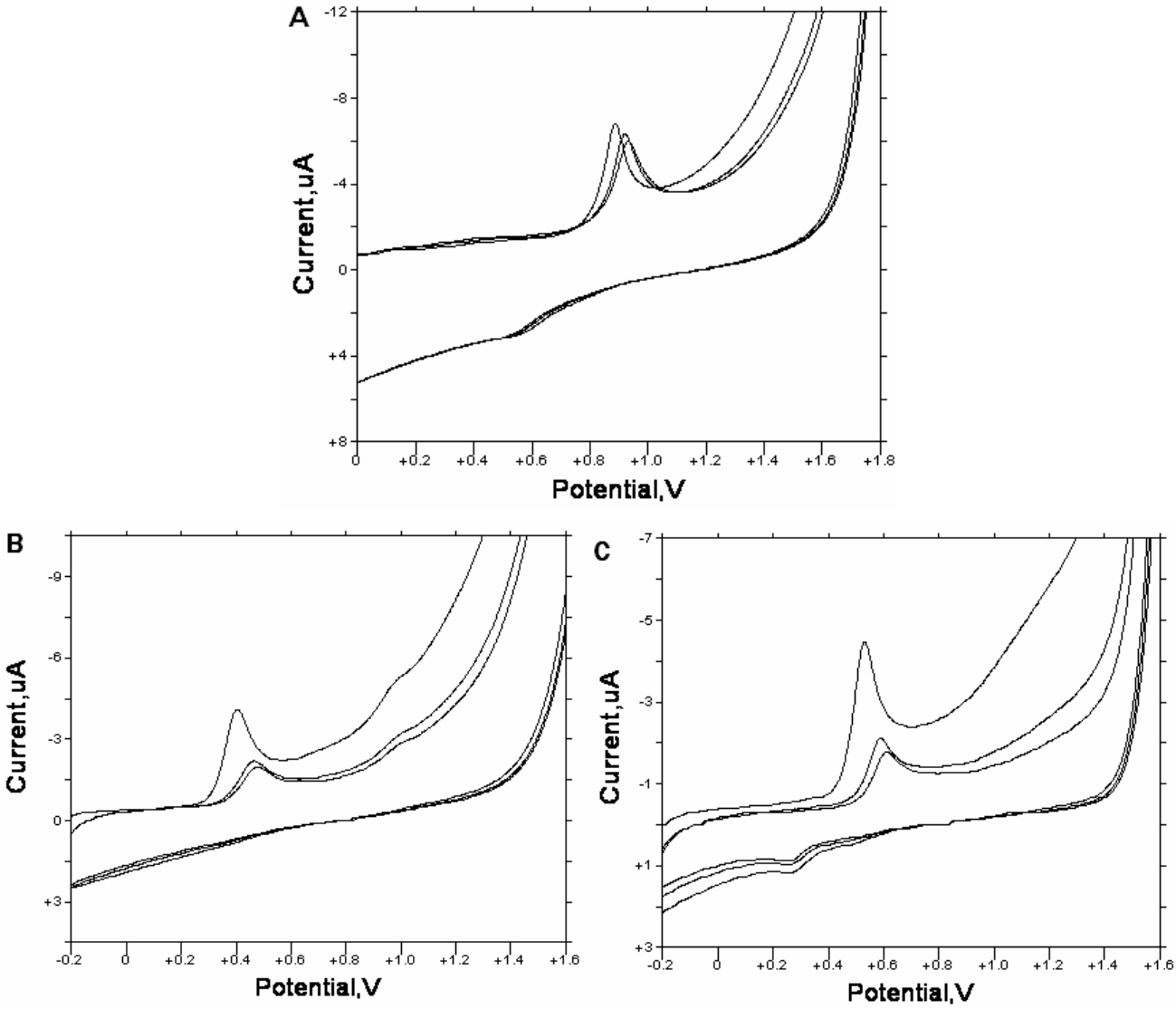

Fig. 1 – The repetitive cyclic voltammograms of 1×10^{-4} M EPR with a scan rate of 100 mV s^{-1} in A) 0.5 M H_2SO_4, B) pH 7.75 phosphate buffer, C) pH 5.5 acetate buffer.

Influence of pH

Within the range between pH 3.0 and 10.0, the peak potential shifted to less positive values, together with a decrease in peak currents with increasing the pH of the buffer solution as shown in Fig. 2. For the DPV and SWV responses in all working media, the relationships between the peak potential and pH can be expressed by the following equations:

$$Ep\ (mV) = -819.2 + 61.926\ pH;\quad r = 0.998\ n = 18\ \text{for DPV} \qquad (1)$$

$$Ep\ (mV) = -858.5 + 62.395\ pH;\quad r = 0.989\ n = 18\ \text{for SWV} \qquad (2)$$

The potential values remain pH dependent. The slopes of these equations were around 62 mV/pH. According to the obtained slope values of these equations, equal amounts of electrons and protons

($2H^+$ and $2e^-$) are involved in the rate-determining steps. Also the experimental results showed that shapes of the curves and maximum peak currents were better in acidic pH values From Ip-pH graphs, maximum current was obtained in 0.5 M H_2SO_4, pH 7.75 phosphate and pH 5.5 acetate buffers. Therefore these mediums were used further experiments.

Influence of scan rate

Useful information involving electrochemical mechanism can usually be acquired from the relationship between peak current and scan rate. Therefore, the electrochemical oxidation behavior of EPR at different scan rates from 5-1000 mV s^{-1} was also studied in different buffer solutions. 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 solution. The equations are:

$$I_p\ (\mu A) = 0.47\ (\upsilon)^{1/2} - 0.66 \quad r=0.995\ n=7 \quad \text{for 0.5 M } H_2SO_4 \qquad (4)$$

$$I_p\ (\mu A) = 0.59\ (\upsilon)^{1/2} - 2.37 \quad r=0.992\ n=7 \quad \text{for pH 5.5 acetate buffer} \qquad (5)$$

$$I_p\ (\mu A) = 0.38\ (\upsilon)^{1/2} - 1.34 \quad r=0.992\ n=7 \quad \text{for pH 7.75 phosphate buffer} \qquad (6)$$

In addition, there was a linear relation between log I_p and log υ, corresponding to the following equations:

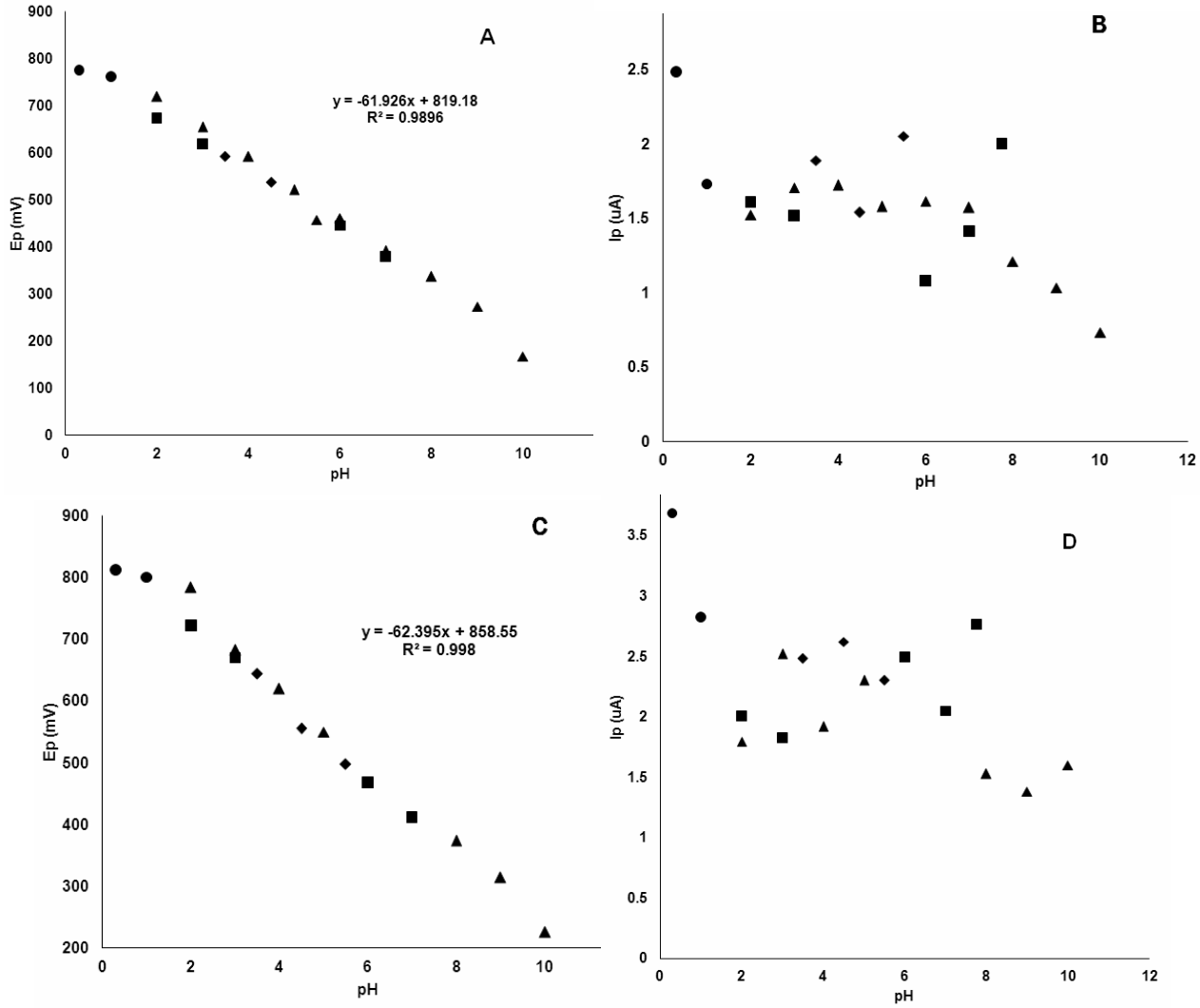

Fig. 2 – a) Plot of E_p *vs* pH of EPR solution for DPV b) Plot of I_p *vs* pH of EPR solution for DPV c) Plot of E_p *vs* pH of EPR solution for SWV d) Plot of I_p *vs*. pH of EPR solution for SWV (●) 0.1 M H_2SO_4, (▲) 0.04 M BR buffer, (■) 0.2 M phosphate buffer, (♦) 0.2 M acetate buffer.

$$\log I_p = 0.64 \log \upsilon - 0.67 \quad r=0.996\ n=9 \text{ for } 0.5\ M\ H_2SO_4 \quad (7)$$

$$\log I_p = 0.64 \log \upsilon - 0.84 \quad r=0.996\ n=7 \text{ for pH } 5.5 \text{ acetate buffer} \quad (8)$$

$$\log I_p = 0.66 \log \upsilon - 0.87 \quad r=0.993\ n=5 \text{ for pH } 7.75 \text{ phosphate buffer} \quad (9)$$

The slope of about 0.6 is very close to the theoretically expected value of 0.5 for diffusion controlled process in selected supporting electrolytes.[16]

The peak potential shifted to more positive values with increasing the scan rate linear relation between peak potential and logarithm of scan rate can be expressed as:

$$E_p\ (mV) = 61.919 \log \upsilon\ (mV\ s^{-1}) + 734.71 \quad r=0.997 \quad (10)$$

Oxidation pathway

To investigate the redox behavior of the EPR, cyclic voltammetric method was used which is the most suitable method for giving insights into its metabolic fate.[17-19] EPR contains highly electroactive hydroxyl groups on the benzene rings which makes it suitable for electrochemical detection. EPR structure like drug active compounds such as Daunorubicin, Doxorubicin, Mitoxantrone and L-Dopa was used to understand the mechanism of EPR. In previous study with diamond electrode, reversible or quasi-reversible electron process leading to formation of quinonic structure of EPR was obtained.[12] Similar to this behavior, 1×10^{-4} M EPR has same oxidation peak with daunorubicin and doxorubicin at 0.5 M H_2SO_4, (Fig.4A, D) pH 7.75 phosphate buffer (Fig. 4B, E) and at pH 5.5 acetate buffer (Fig.4C, F). As it is well known, L-dopa have hydroxyl groups on benzene ring. It has oxidation and reduction peaks followed very well by CV in acidic media (Fig. 4G) and only oxidation peak at pH 7.75 phosphate buffer and pH 5.5 acetate buffer (Fig. 4H, I). Related with the previous results[12] and current results (Fig. 4) initial oxidation with two electrons may be occurs and the hydroxyl group converts to quinone.

Analytical characterization

According to the obtained results, quantitative analysis of EPR was achieved via DPV and SWV techniques. 0.5 M H_2SO_4 solution was selected as the supporting electrolyte for the quantification as EPR gave maximum peak current at pH 0.3. DP and SW voltammograms obtained with increasing amounts of EPR. The plots of I_p versus concentration showed linearity over the EPR concentration in the range of 2×10^{-7}-3.6×10^{-5} M with and equation I_p (μA)=0.0329+0.2766 C r=0.999 n=6 for DPV and I_p (μA)=0.0392+0.2563 C r=0.998 n=6 for SWV. Deviation from linearity was observed for more concentrated solutions, due to the adsorption of EPR or its oxidation product on the electrode surface.[20] LOD and LOQ values were calculated from the equations of LOD=3.3s/m, LOQ=10s/m[15] using standard deviation of the response and the slope of the calibration curve. Low values of both LOD and LOQ values confirmed the sensitivity of the proposed methods and are showed in Table 1. The low values of standard error of the slope, intercept and also greater correlation coefficient than 0.99 confirm the precision of the proposed voltammetric methods.

Parenteral preparation analysis

The DPV and SWV techniques were applied to Epirubicin "Ebewe® parenteral preparation" claim to contain 100 mg epirubicin per 50 mL of the solution in order to prove the applicability of the proposed methods. In order 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. 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 label. The detected content was 2.005 mg with 100.27% recovery for DPV, 2.006 mg with 100.31% recovery for SWV. The results obtained were compared statistically using student's t-test and variance ratio F test (Table 2). Statistical

analysis of the results of proposed methods using the student's t-test and F-test show no significant difference between the performance of two methods regarding the accuracy and precisions.

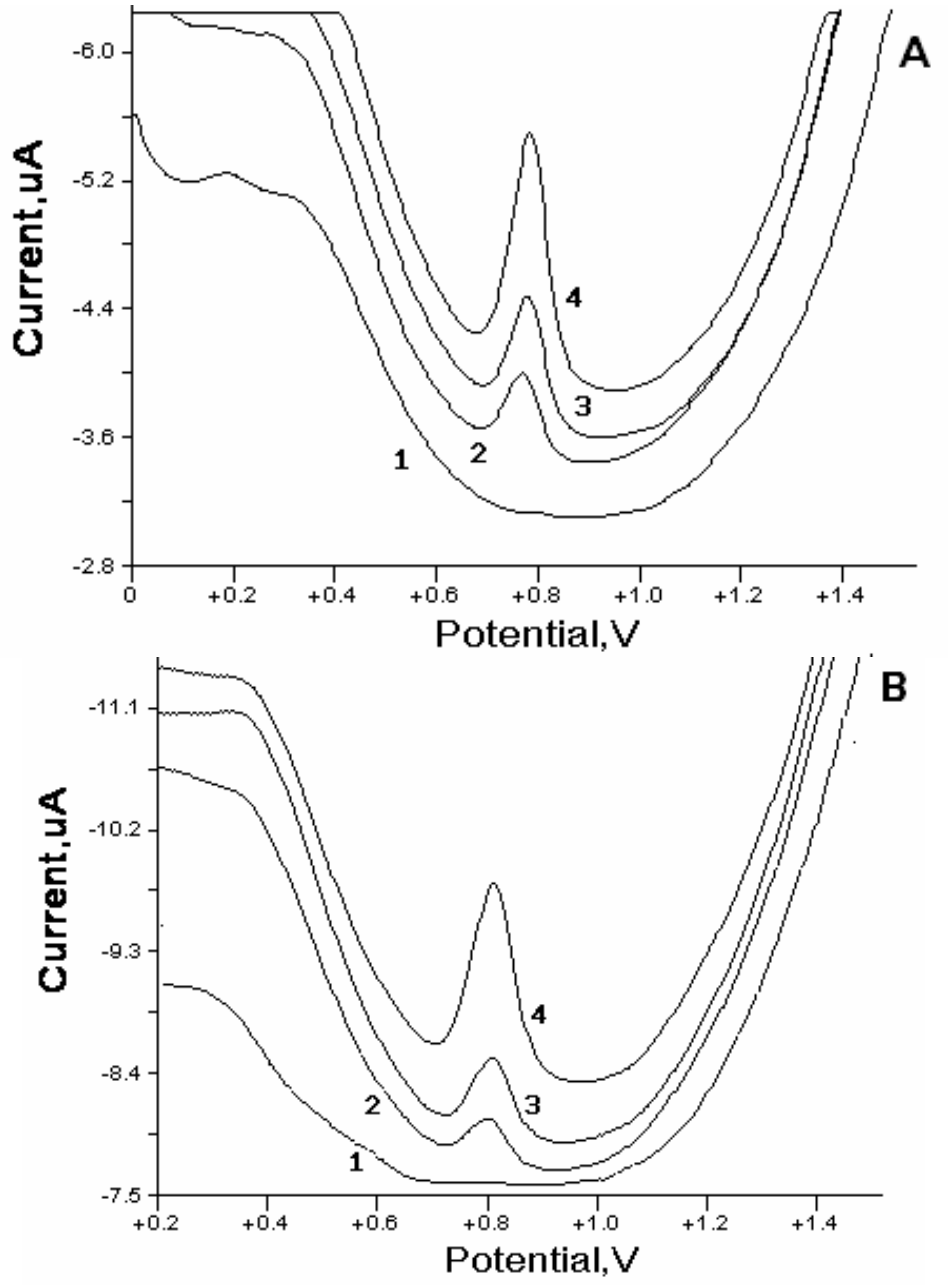

Fig. 3 – A) DPV and B) SWV results obtained for the determination in 0.5 M H_2SO_4 with GCE; (1) blank solution; (2) 1×10^{-6} M; (3) 2×10^{-6} M; (4) 1×10^{-5} M EPR.

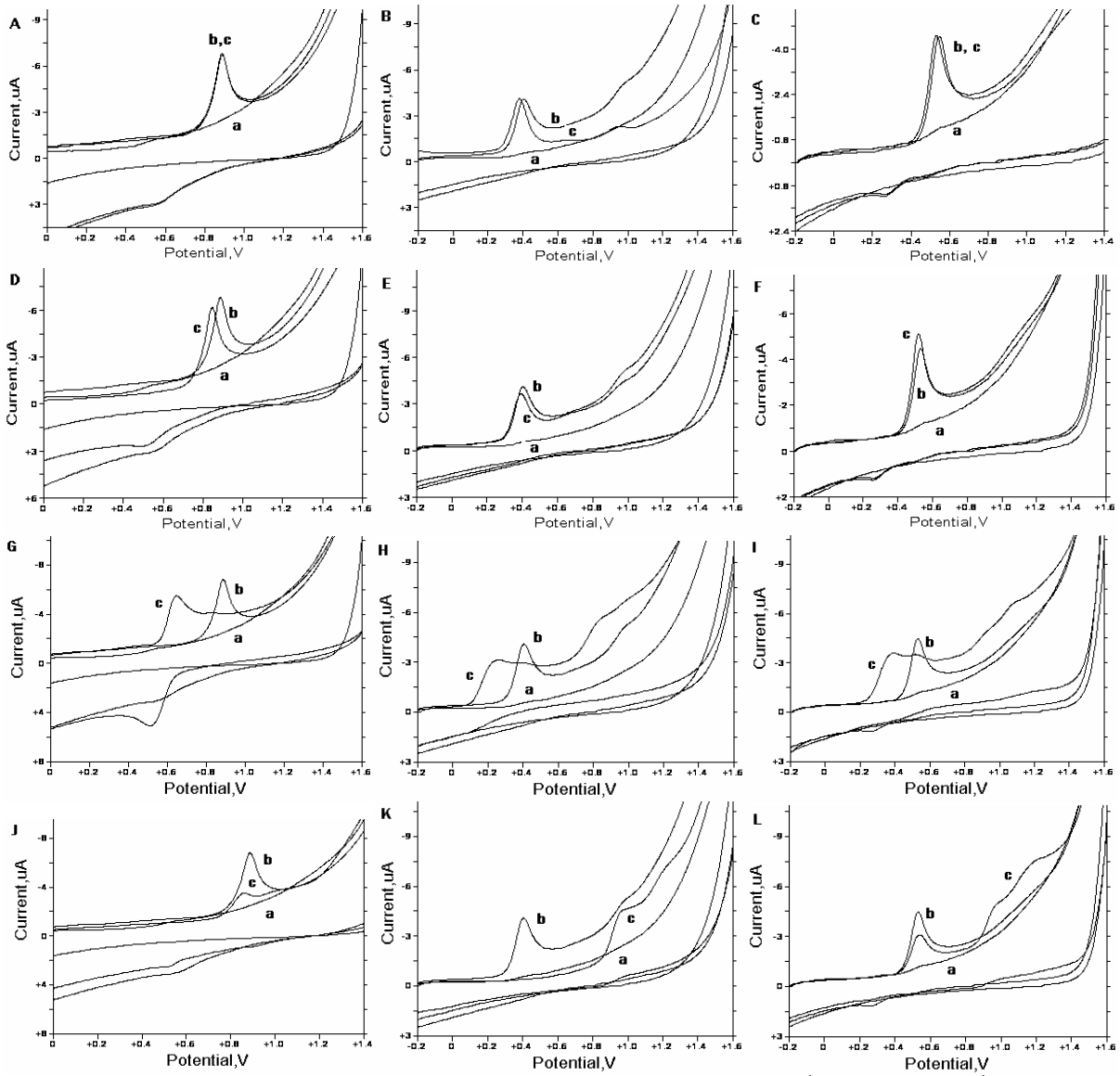

Fig. 4 – Cyclic voltammograms of selected model compounds with EPR a) blank, b) 1×10^{-4} M EPR, c) 1×10^{-4} M selected model compound A) Daunorubicin in 0.5 M H_2SO_4, B) Daunorubicin in pH 7.75 phosphate buffer, C) Daunorubicin in pH 5.5 acetate buffer, D) Doxorubicin in 0.5 M H_2SO_4, E) Doxorubicin in pH 7.75 phosphate buffer, F) Doxorubicin in pH 5.5 acetate buffer, G) L-Dopa in 0.5 M H_2SO_4, H) L-Dopa in pH 7.75 phosphate buffer, I) L-Dopa in pH 5.5 acetate buffer, J) Mitoxantrone in 0.5 M H_2SO_4, K) Mitoxantrone in pH 7.75 phosphate buffer, L) Mitoxantrone in pH 5.5 acetate buffer.

Table 1

Statistical evaluation of the calibration data for quantitative determination of EPR by voltammetry

Technique	**DPV**	**SWV**
Measured Potential (mV)	798	818
Linearity Range (M)	2×10^{-7}-3.6×10^{-5}	2×10^{-7}-3.6×10^{-5}
Slope	0.277	0.256
Intercept	0.033	0.039
Correlation Coefficient	0.998	0.999

Table 1 (continued)

SE of slope	3.08×10^{-3}	5.15×10^{-3}
SE of intercept	6.93×10^{-3}	1.16×10^{-3}
Limit of Detection (M)	4.1×10^{-8}	4.9×10^{-8}
Limit of Quantification (M)	1.4×10^{-7}	1.6×10^{-7}
Within-day precision (RSD %)	0.68	0.71
Between-day precision (RSD %)	0.97	0.80

Table 2

Results of the assays and the recovery analysis of EPR in pharmaceutics via voltammetry

Technique	**DPV**	**SWV**
Labeled claim (mg)	100.00	100.00
Amount found (mg)*	100.04	100.17
RSD (%)	0.93	0.60
Bias (%)	-0.042	-0.169
t value (t_{theo}: 2.310)	t_{calc}: 0.583	0.290
F value (F_{theo}: 6.39)	F_{calc}: 0.023	0.023
Added (mg)	2.000	2.000
Found (mg)*	2.005	2.006
Recovery (%)	100.272	100.310
RSD % of recovery	0.300	0.119
Bias (%)	-0.272	-0.309

*Obtained from 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 GCE (BAS: Φ = 3 mm, diameter) as working electrode, a platinum wire counter electrode, and an Ag/AgCl saturated KCl reference electrode. GCE 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.

Reagents

EPR and its pharmaceutical dosage form (Epirubicin "Ebewe® parenteral preparation") were supplied from EBV Pharm. Inc. (Istanbul, Turkey). The compounds which were used in mechanism study; daunorubicin, doxorubicin, mitoxantron and L-dopa were supplied from different pharmaceutical companies in Turkey and Sigma. Stock solutions were prepared daily by dissolution of methanol solutions. Standard solutions were prepared by dilution of stock solution using methanol: water (20:80 v/v) mixture. H_2SO_4 solutions (0.1 and 0.5 M), phosphate buffer (0.2 M, pH 2.0-8.0), acetate buffer (0.2 M, pH 3.4-5.5) and Britton-Robinson (BR) buffer (0.04 M, pH 2.0-12.0) were used as supporting electrolytes. Sodium hydroxide was purchased from Merck. All solutions were protected from light and used within 24 h to avoid decomposition.

Validation of the analytical procedure

For the validation of the proposed methods, precision and accuracy were examined by assaying five replicate samples as

individual days (within day) and intermediate precision (between days) over a week period with using the concentration of 2×10^{-6} M EPR. Relative standard deviations (RSD %) and bias % were calculated to check the precision of the method. After statistical evaluation the results indicate that method is analytically acceptable from the view point of precision.[15, 21-23] Accuracy of the proposed method was tested and for checking 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 percentage error was calculated by using the formula below;

$$\frac{(Found\ concentration - spiked\ concentration)}{Spiked\ concentration} x100$$

Voltammograms of the sample solutions recorded a week after preparation did not show any appreciable change in assay values. The calibration equations for DPV and SWV techniques were constructed by plotting the peak current against EPR concentration.

Analysis of EPR from Dosage Forms

1.25 mL of Epirubicin "Ebewe® parenteral preparation" claim to contain 100 mg epirubicin per 50 mL of the solution was dissolved in 25 mL of methanol (*ca.* 2×10^{-4} M). Working solutions including EPR and 20% of methanol were used for the voltammetric investigations and were prepared by dilution of the stock solution with supporting electrolytes. The nominal content of the solution was determined from corresponding regression equations.

CONCLUSIONS

DP and SW voltammetric techniques utilized in this study are easy to be carried out for the reliable analysis of EPR both in the bulk form and in commercial dosage forms in 0.5 M H_2SO_4 at GCE. Some model compounds were also studied to clarify the oxidation mechanism of EPR. The oxidation pathway of EPR was observed through two electron-two proton mechanism, quasi-reversible and diffusion controlled mechanisms. The advantages of the proposed methods included accuracy and time-saving as well as simplicity of experiment and reagents. When this study compared to our previous work[12] nearly same LOD values were obtained without surfactant effect for both techniques. According to recovery studies, developed techniques were seemed to be free from interferences of the excipients in the tablet dosage forms.

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