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Determination of Trace Levels of Diosmin in a Pharmaceutical Preparation by Adsorptive Stripping Voltammetry at a Glassy Carbon Electrode

M. S. EL-SHAHAWI,*† A. S. BASHAMMAKH,* and T. EL-MOGY**

**Department of Chemistry, Faculty of Science, King Abdulaziz University, P. O. Box 80203, Jeddah 21589, Kingdom of Saudi Arabia*
***Department of Chemistry, Faculty of Science at Damietta, Mansoura University, Mansoura 34517, Egypt*

A systematic study on the electrochemical behavior of diosmin in Britton–Robinson buffer (pH 2.0 – 10.0) at a glassy carbon electrode (GCE) was made. The oxidation process of the drug was found to be *quasi*-reversible with an adsorption-controlled step. The adsorption stripping response was evaluated with respect to various experimental conditions, such as the pH of the supporting electrolyte, the accumulation potential and the accumulation time. The observed anodic peak current at +0.73 V *vs*. Ag/AgCl reference electrode increased linearly over two orders of magnitude from 5.0×10^{-8} M to 9.0×10^{-6} M. A limit of detection down to 3.5×10^{-8} M of diosmin at the GCE was achieved with a mean recovery of 97 ± 2.1%. Based on the electrochemical data, an open-circuit accumulation step in a stirred sample solution of BR at pH 3.0 was developed. The proposed method was successfully applied to the determination of the drug in pharmaceutical formulations. The results compared favorably with the data obtained *via* spectrophotometric and HPLC methods.

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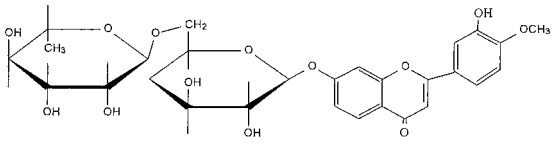
Introduction

Diosmin, which is chemically named 3′,5,7-trihydroxy-4′-methoxy flavone 7, rutinoside (I), is a newly developed flavonoid in *Rutaceae* plants. Flavonoids are widely used for their phlebotonic and antioxidant properties, and also as vascular protectors.[1-8] Recent clinical studies have demonstrated that the diosmin can be used to treat venous leg ulcers and hemorrhoids.[3] Diosmin drug has been successfully used as chemopreventive agents in urinary-bladder[9] and colon carcinogenesis.[10] The drug shows good tolerability and is quite safe and nontoxic.[3] Diosmin has certain biological activities, including an anti-inflammatory effect and an inhibition of prostaglandin synthesis.[11] In view of increasing interest in these bioflavonoids, especially that used in treating chronic venous, chronic hemorrhoids and as antioxidants, several methods have been reported for the determination of diosmin in plant extracts,[12,13] biological fluids,[13,14] and pharmaceutical formulations.[12] A survey of the literature reveled that there are few methods for diosmin determination in pharmaceutical formulations or biological fluids. These methods include: spectrophotometry, spectrodensitometry,[15] and liquid chromatography[16] as well as HPLC methods.[17] Recently, an excellent HPLC method for the simultaneous determination of diosmin in flavonoid extracts and soft gelatin capsules has been published.[17] Currently, no literature data could be found on the electrochemical behavior of diosmin, in general, or its voltammetric determination, in particular.

Therefore, the aim of the present study is to investigate the oxidative behavior of the diosmin at GCE using cyclic voltammetry (CV) and differential pulse voltammetric (DPV) techniques, and also to optimize the experimental conditions for the determination of this compound in pharmaceutical dosage forms. A methodology for the direct and simple determination of the drug in spiked human serum at a very low level is also included.



(I)

Experimental

Reagents and solutions

Diosmin and Dioven® tablets were obtained from Amriya Rhone-poulenc Pharmaceutical Industries Co., Alexandria, Egypt. The stated composition of each tablet contains 150 mg diosmin. A diosmin stock solution (1×10^{-3} M) of diosmin was prepared daily by dissolving an appropriate amount in 2 ml of NaOH (0.02 M) and completed to 10 ml with double-distilled water. Dilute solutions were then prepared by diluting the stock solution with water in calibrating measuring flasks, transferred to polyethylene bottles, and were finally kept in a refrigerator. A series of Britton–Robinson (BR) universal buffer solutions of various pH values ranging from pH 2.0 – 10.0 were prepared and used as supporting electrolytes. The BR buffer solutions were prepared by mixing an acid mixture containing acetic (0.04 M), orthophosphoric (0.04 M), and boric acids (0.04 M), and adjusting the pH with an appropriate volume of sodium

† To whom correspondence should be addressed.
E-mail: mohammad_el_shahawi@yahoo.co.uk

hydroxide (0.2 M) solution to the required pH. All solutions were then prepared from Analar-grade reagents in double-distilled water, and were kept in a refrigerator.

Apparatus

Cyclic, linear sweep and differential pulse voltammetric (DPV) experiments were performed using an AEW2 electrochemical workstation controlled by the EC prog3 electrochemistry software (Sycopel, UK). A thermostated three-compartment electrochemical cell system incorporating a glassy carbon disc electrode (BAS Model MF-2012, Φ = 3 mm) as a working electrode, an Ag/AgCl (3 M KCl) (BAS Model MF-2063) as a reference electrode and a platinum-wire (BAS Model MW-1032) as an auxiliary electrode was used. The solution was deaerated with pure nitrogen before making contact with GCE and the commencement of electrochemical cell. The operating conditions for the DPV were a pulse amplitude of 50 mV, a pulse width of 30 ms, and a scan rate of 10 mV s^{-1}. A Schott Geräte CG 808 digital pH-meter with a H-61 pH combination electrode (Mainz, Germany) with an accuracy of ±0.02 pH was used for pH measurements.

Procedures

Adsorptive stripping differential pulse voltammetry. The transfer of a known volume (5 ml) of the BR buffer electrolyte solution was made into a 10 ml voltammetric cell deaerated under a nitrogen flow for 10 min, and the electrodes were then immersed in the tested solution. All scans were then initiated in the positive direction with an applied potential scan from +0 V to +1.2 V. After measuring the blank solution, an appropriate amount of the standard diosmin solution was introduced into the cell, while the solutions were purged with nitrogen, and then the deposition and stripping steps were repeated. An anodic potential sweep was then carried out under different operational parameters. Before each measurement, the glassy carbon electrode was polished manually with alumina (0.5 mm) dispersed in bidistilled water on a smooth polishing cloth, and gently dried with tissue paper. All measurements were then carried out at room temperature (25 ± 1°C) under a nitrogen atmosphere. The peak current heights were evaluated by means of the tangent method.[18,19]

The influence of the scan rate (ν = 10, 20, 50, 100 and 200 mV s^{-1}) on the cyclic voltammetry of diosmin was investigated using the same solution. At each scan rate, the initial conditions at the electrode surface were restored. The preconcentration step on the analysis of diosmin was critically examined by immersing the polished glassy carbon electrode in a stirred solution (5 ml) of BR buffers containing a known concentration of the drug at a selected period of time. During this period, no potential was applied to the electrode. The stirring was then stopped for an equilibrium time of 15 s and a differential pulse measurement of the surface species was then recorded. The accumulation step was accomplished in the potential range from 0 to +1.2 V. The surface of the glassy carbon electrode was regenerated before each experiment. The electrode was then immersed in a cell solution containing the blank electrolyte until the voltammogram corresponding to the minimum of residual current was obtained. The electrode was then transferred and immersed in the sample solution.

An experimental procedure involving adsorptive stripping voltammetry medium-exchange[20] preconcentration was carried out for the analysis of diosmin. The effect of the pH of the preconcentration solution on the current signal was studied for 5.0 × 10^{-5} M diosmin at the glassy carbon electrode in BR buffer solutions over the pH range 2.0 – 10.0

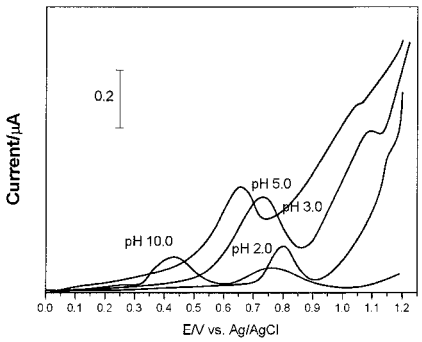


Fig. 1 DPVs for diosmin (5 × 10^{-5} M) in BR buffers at GCE. Scan rate, 10 mV s^{-1}; pulse amplitude, 50 mV; pulse width, 30 ms.

Tablets assay procedures. In the analysis of diosmin in pharmaceutical dosage, one Dioven tablet was ground to a homogeneous fine powder in a mortar. An amount of this powder corresponding to a 1 × 10^{-3} M stock solution of diosmin was accurately weighed, transferred into a 10 ml calibrated flask containing 2 ml of NaOH (0.02 M) and finally completed to the mark with distilled water. The contents of the flask were sonicated for 10 min to achieve complete dissolution. The analyzed solutions were then prepared by diluting aliquots of the clear supernatant with the BR buffer at the optimum pH. Voltammograms of the standard solutions of diosmin were also accomplished in a similar way as for the unknown sample solutions of diosmin by adding increasing amounts of the standard diosmin solution to the voltammetric cell. The diosmin content (mg) in the tested sample solution was finally calculated from the prepared standard calibration plot.

Results and Discussion

Electrochemical behavior of diosmin

No previous electrochemical data were available concerning the redox mechanism of diosmin. Thus, preliminary DPV experiments of diosmin (5.0 × 10^{-5} M) in BR buffer over a wide range of pH 2.0 – 10.0 at GCE were carried out. The DPV showed one well-defined anodic peak accompanied by an ill-defined peak at the employed pH range. Therefore, the analytical application in the present study was focused mainly on the first oxidation peak. A representative differential pulse voltammogram recorded for diosmin (5.0 × 10^{-5} M) at GCE in a BR buffer of pH 3.0 is shown in Fig. 1. Upon increasing the solution pH, the anodic peak potential of diosmin shifted to less-positive values with a slope of 48.8 mV/pH unit (Fig. 2a). This shift is quite close to the theoretical value expected for an electrode reaction involving a 1:1 ratio of electrons/protons.[21,22] The effect of the solution pH on the peak current is also shown in Fig. 2b. At pH 3, an excellent signal enhancement accompanied by a sharp response was obtained. Thus, in subsequent work, a supporting electrolyte of pH 3.0 was chosen.

A typical cyclic voltammogram recorded from 0.0 to +1.2 V *versus* Ag/AgCl for diosmin (5.8 × 10^{-4} M) at GCE in BR buffer of pH 3.0 is shown in Fig. 3. In the reverse scan, a cathodic counter part of the main peak was observed. At various scan rates, the CV showed that the main anodic peak current (i_{p1}) is directly proportional to the sweep rate (ν). The dependence of the cathodic and anodic peak currents on the scan rate; ν, and $\nu^{1/2}$ was critically investigated. The relationship between $i_{p,c}$ or $i_{p,a}$ and ν is a straight line. The current due to the reduction and

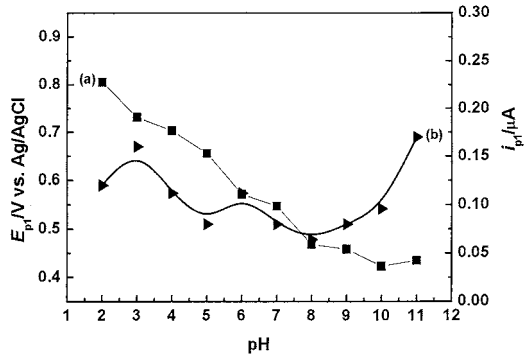


Fig. 2 Effect of the pH on the peak potential (a) and peak current (b) in BR buffers using DPV at GCE diosmin concentration, 5×10^{-5} M; scan rate, 10 mV s^{-1}; pulse amplitude, 50 mV; pulse width, 30 ms.

oxidation is expected to very linearly with v, rather than with $v^{1/2}$. These results indicate that the oxidation of diosmin depends predominately on the adsorbed molecules at the changed interface. This trend is in accord with the adsorption as the rate-limiting step. This wave is *quasi*-reversible; because the peak–peak separation potential (E_{pa}–E_{pc}) was found to be 0.1 V, which is greater than 0.059/n V, and increased upon increasing the scan rate. Thus, the heterogeneous electron transfer rate is relatively slow and the redox process is *quasi*-reversible.[21,23,24] The ratio of the cathodic to the anodic peak currents (i_{pa}/i_{pc}) depends on the sweep rate, and is less than unity, indicating the presence of coupled chemical (CE) reactions.[22,25] The electrochemical oxidation of diosmin appears to be a complex process, and different reaction pathways are possible. The first oxidation process can be postulated as being an overall one-electron/one proton oxidation of the anisole moiety of the diosmin molecule (peak A), which is reduced in the reverse scan. Upon the reverse scan, the chromone moiety is then reduced (peak B) and reoxidized to the chromone moiety (peak C). The surface coverage attained maximum adsorption obtained for 3.0×10^{-7} mol/l diosmin after a 120 s accumulation period (Fig. 4). Thus, subsequently, an accumulation of 120 s was selected.

The effect of the accumulation potential at the open-circuit potential was investigated. The adsorptive behavior at GCE is independent of the accumulation potential due to the non-electrochemical nature of the adsorptive process. Considering these data, an open-circuit condition for stripping analysis was selected. The peak current *versus* the accumulation time plots for 5×10^{-4} (a) and 3.0×10^{-7} (b) M diosmin is shown in Fig. 4. The intersections of these graphs with the peak current axis may be attributed to the fact that the adsorption took place during the equilibrium time, which was fixed at 150 s. The breaks at certain stripping times *i.e.* 120 s for 3.0×10^{-7} M and 5.0×10^{-4} M diosmin, mean that the oxidation of the adsorbed diosmin molecules yields a well-defined anodic peak at +0.73 V *versus* Ag/AgCl. The peak height of this peak increased upon increasing the preconcentration time, indicating an enhancement of the concentration of diosmin molecules at the electrode surface, and a linear relationship was observed for a 2-min accumulation time. Also, for a 2-min preconcentration period, an approximately 4-fold enhancement of the peak current was observed over that attained by the conventional solution-phase pulse voltammetry (0 min). Above 2 min, the accumulation of the peak current started to decrease, suggesting saturation coverage of the electrode surface, although such sensitivity is important. The method also

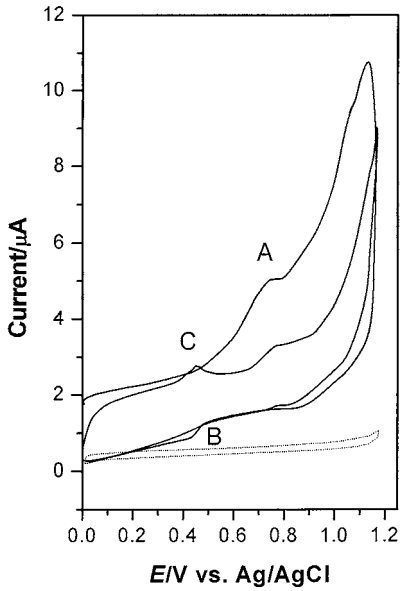


Fig. 3 CVs of diosmin (2.8×10^{-4} M) solution at GCE and 50 mV s^{-1} scan rate. The dotted line represents the blank solution.

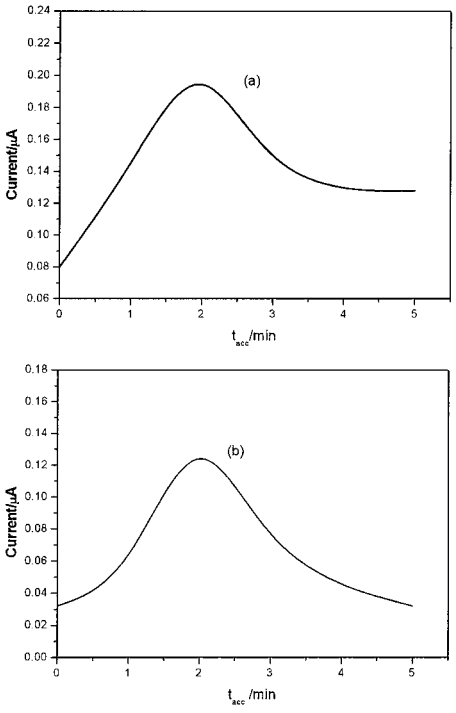


Fig. 4 Effect of the accumulation time (t_{act}) on the peak current of diosmin at 5×10^{-4} M (a) and 3×10^{-7} M (b) concentration levels.

offers good selectivity based on the medium-exchange steps *i.e.* the electrode was transferred after preconcentration had been finished into another cell at the same pH. Thus, in the subsequent experiments, an open-circuit potential was chosen at a 2-min preconcentration time. Under the optimum experimental conditions, the peak current at a potential of +0.73 V increased linearly over two orders of magnitude of concentration from 5.0×10^{-8} M to 9.0×10^{-6} M (Fig. 5).

The characteristics of the calibration graph of the peak current *versus* the concentration calculated from the linear regression,

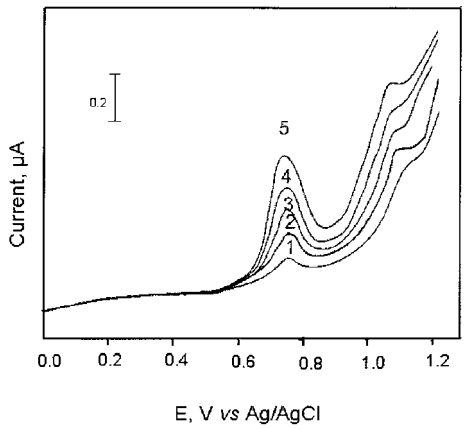


Fig. 5 DPVs of diosmin at various concentrations: 5.8×10^{-8} (1), 1.6×10^{-7} (2), 2.3×10^{-7} (3), 3.3×10^{-7} (4) and 4.4×10^{-7} M (5). Scan rate, 10 mV s^{-1}; pulse amplitude, 50 mV; pulse width, 30 ms. The dotted lines represent the blank solution.

were: slope, 0.03085 μA/μM; current intercept, 0.1118 μA; and correlation coefficient, 0.9998. At concentrations higher than 1.0×10^{-7} M diosmin, a deviation from linearity was observed due to adsorption saturation of the electrode surface. Shortening the preconcentration period or diluting the sample also extended the linearity. A detection limit of approximately 3.5×10^{-8} M was obtained[26] with a 120-s preconcentration time. The reproducibility was determined for 10 replicate experiments with 5.0×10^{-8} M diosmin using DASV; the obtained relative standard deviation was 2.5%.

Analysis of diosmin in dosage forms and in serum

The proposed procedures were successfully applied for the diosmin assay in pharmaceutical formulations. One Dioven tablet (150 mg) was ground to a homogeneous fine powder in a mortar. Voltammograms of the standard solutions of diosmin were then recorded in a similar way as for the unknown sample solutions of diosmin. The concentration was then calculated from a calibration plot of the standard diosmin solution. The diosmin content (mg) in the tested sample solution obtained (4×10^{-6} M) by the proposed method (98.7 ± 1.8%) was found, and is in accord with the reported (99.6 ± 2.1%) and the official (99.2 ± 2.4%) assay methods.[16,17]

The possibility of applying the preconcentration/medium exchange methodology for the determination of diosmin in human serum was tested. The drug determination in spiked serum samples (100 μl) was critically determined. Three replicate samples containing diosmin at concentrations of 1.0×10^{-6}, 2.0×10^{-6} and 3.0×10^{-6} M were recorded employing the recommended procedures. A recovery percentage in the range 99.2 ± 1.6% was successfully achieved.

Conclusions

The proposed voltammetric methods at GCE provide the advantages of simplicity, precision and accuracy for the analysis of diosmin in compared with the reported method. The methods are also free from any interference of commonly used excipients and additives in the formulations of the drug. The method was successfully substantially improved for the analysis of diosmin by allowing the drug to accumulate interracially at the GCE. A preconcentration/medium exchange methodology involving the transfer of the electrode to a blank solution between the accumulation and measurement steps has been successfully applied for diosmin analysis. The method compares favorably in both sensitivity and selectivity with most of the unpublished methods for the determination of diosmin, and it can thus certainly be placed among the sensitive ones.

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