ELECTROCHEMICAL BEHAVIOR OF DISOPYRAMIDE AND ITS ADSORPTIVE STRIPPING DETERMINATION IN PHARMACEUTICAL DOSAGE FORMS AND BIOLOGICAL FLUIDS

Funda ÖZTÜRK[a1], Ibrahim Hüdai TAŞDEMIR[b], Zehra DURMUŞ[a2] and Esma KILIÇ[a3,*]

[a] *Ankara University, Faculty of Science, Department of Chemistry, Ankara 06100, Turkey; e-mail: [1] fozanozturk@yahoo.com, [2] zdurmus@science.ankara.edu.tr; [3] ekilic@science.ankara.edu.tr*

[b] *Ahi Evran University, Faculty of Arts and Science, Department of Chemistry, Kirşehir 40100, Turkey; e-mail: ihtasdemir@ahievran.edu.tr*

Received January 27, 2010
Accepted February 24, 2010
Published online June 17, 2010

Electrochemical behavior of disopyramide (DPA) and optimum conditions to its quantitative determination were investigated using voltammetric methods. Some electrochemical parameters such as diffusion coefficient, surface coverage of adsorbed molecules, electron transfer coefficient, standard rate constant and number of electrons were calculated using the results of cyclic and square-wave voltammetry. All studies were based on the quasi-reversible and adsorption-controlled electrochemical reduction signal of DPA at about –1.60 V vs Ag|AgCl at pH 10.0 in Britton–Robinson buffer. This adsorptive character of molecule was used to develop fully validated, new, rapid, selective and simple square-wave cathodic adsorptive stripping voltammetric (SWCAdSV) method to the direct determination of DPA in pharmaceutical dosage forms and biological samples without time-consuming steps prior to drug assay. Peak current of electrochemical reduction of DPA was found to change linearly with the concentration in the range from 7.15×10^{-8} mol l^{-1} (0.024 mg l^{-1}) to 1.43×10^{-6} mol l^{-1} (0.49 mg l^{-1}). Limit of detection (LOD) and limit of quantification (LOQ) were found to be 5.65×10^{-8} mol l^{-1} (0.019 mg l^{-1}) and 1.88×10^{-7} mol l^{-1} (0.064 mg l^{-1}), respectively. The method was successfully applied to assay the drug in tablets, human serum and human urine with good recoveries at about 100%.

Keywords: Adsorption; Electrochemistry; Electron transfer; Voltammetry; Disopyramide; Square wave cathodic adsorptive stripping voltammetry; Pharmaceuticals; Human serum; Human urine; Hanging mercury drop electrode.

Disopyramide (DPA) chemically known as α-[2-(diisoproylamino)ethyl]-α-phenyl-2-pyridineacetamide is an antiarrhythmic medication. Chemical structure of DPA is shown in Fig. 1. It is categorized as a class IA antiarrhythmic drug according to the Vanghan and Williams classification

doi:10.1135/cccc2010010

which is widely used in clinic trials. It shows an effective prophylaxis and treatment of ventricular and super ventricular arrhythmias and it has also been shown to reduce gradient and improve symptoms in patients who suffer from obstructive hypertrophic cardiomyopathy[1–4].

Several analytical techniques including liquid chromatography[5], high-performance liquid chromatography[6–14], capillary gas chromatography[15], gas chromatography[16], stable isotope dilution method[17] and ion-selective electrode[18,19] have been devised for the determination of DPA in pharmaceutical samples or biological fluids. All these reported methods are neither sufficiently sensitive nor tedious and they require highly sophisticated instrumentation. Although DPA is an electroactive molecule on different electrodes, there is only one study dealing with electrochemical behavior of the DPA based on oxidation[20] in the literature. This study was carried out on GC electrode and there is no analytical application to assay of DPA from different samples such as tablets and biological media. Furthermore, reviewing the literature revealed that, up to the present time, there is no electrochemical study dealing with reduction properties of DPA and voltammetric method using hanging mercury drop electrode for the assay of DPA in pharmaceutical formulation and biological samples.

The voltammetric techniques, such as cyclic voltammetry, differential pulse voltammetry and square-wave voltammetry have been proved to be very sensitive for the determination of organic molecules including drugs and related molecules in pharmaceutical dosage forms and biological fluids. These methods are faster, easier to be operated and cheaper than spectroscopic and chromatographic methods. The sensitivity increases when the stripping voltammetry is employed. Adsorptive stripping voltammetry has been shown to be an efficient electroanalytical technique for determination of sub-nanomolar level of a wide range of drugs which have interfacial adsorptive character onto the working electrode surface. Its remarkable sensitivity is attributed to the combination of an effective accumulation step

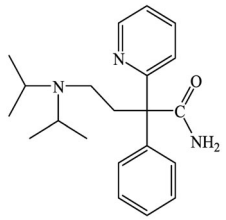


FIG. 1
Chemical structure of DPA

with an advanced measurement procedures that generates an extremely favorable signal to back ground ratio. It usually involves a simple deposition step and most of the excipients used do not interfere in the subsequent determination of the drugs. There are many applications of stripping voltammetric methods[21–25].

One aim of present study is the investigation of electrochemical reduction behaviors of DPA using voltammetric methods. Current study was also aimed to propose a tentative reaction mechanism. Development of new validated square-wave adsorptive stripping voltammetric assay method for the direct determination of DPA in different samples including pharmaceutical preparations, human serum and human urine was one of the other goals of present study.

EXPERIMENTAL

Apparatus

All voltammetric measurements such as cyclic voltammetry (CV), controlled potential coulometry (CPC), square-wave cathodic adsorptive stripping voltammetry (SWCAdSV) were carried out using a CH-instrument electrochemical analyzer (CHI 760). A three-electrode cell system incorporating the hanging mercury drop electrode (HMDE BAS CGME 1108) as a working electrode, platinum wire (BAS MW-1034) as an auxiliary electrode and an Ag|AgCl reference electrode stored in 3.0 M KCl solution (MF-2052 RE-5B) were used in all experiments.

A three-electrode combination system for bulk electrolysis was consisted of mercury pool (55.4 cm^2) as a working electrode, coiled platinum wire (23 cm) as an auxiliary electrode (BAS MW-1033) and Ag|AgCl (in 3.0 M KCl) reference electrode (BAS MF-2052 RE-5B).

All pH measurements were made with Thermo Orion Model 720A pH ion meter having an Orion combined glass pH electrode (912600) which had been calibrated with pH 4.13 and 8.20 stock buffer solutions before measurements.

Double-distilled deionized water was supplied from Human Power I^+, Ultra Pure Water System (Produced by ELGA as PURELAB Option-S). All the data were obtained at ambient temperature.

FT-IR measurements were carried out using IRPrestige-21 (IRAffinity-1, FTIR-84005; from Shimadzu Corporation). These measurements were taken before and after bulk electrolysis of 25 ml of 1.20×10^{-3} M DPA solution prepared in Britton–Robinson buffer (BR) at pH 10.0 and extracted into CCl_4 (from Merck).

Reagents and Solutions

Standard sample of DPA (99.0%, from Aldrich) was used to prepare the stock solution of DPA. Stock solution of DPA was prepared by dissolution of precisely weighed amounts of DPA in water in order to get the DPA concentration of 4.0×10^{-3} mol l^{-1} (1.36 g l^{-1}). Calibration solutions were prepared diluting the stock solution with BR buffer and pH value of

these solutions to desired one (such as 7.0, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0 and 11.5) for pH studies were adjusted using 0.2 M NaOH solutions.

All chemicals used in preparation of BR solution, such as phosphoric acid (Riedel), boric acid (Riedel), acetic acid (Merck) and sodium hydroxide (Merck) to adjust the pH of supporting electrolyte, were of analytical reagent grade. Double-distilled deionized water was used in preparations of all the solutions.

All DPA solutions were protected from light and were used within 24 h to avoid decomposition. However, electrochemical response of sample solutions recorded after preparation did not show any significant change in following studies.

Preparation and Analysis of Samples

Rythmodan tablets were used as pharmaceutical dosage form which contains 250 mg of DPA and some amount excipients per tablet. To prepare the solutions of tablets, initially the drug content of ten tablets was weighed, finely powdered and mixed. The average mass per tablet was determined. A sample equivalent to one tablet was weighed and transferred into the calibrated flask of 250.0 ml volume and completed to the mark with water. The contents of the flask were sonicated for 30 min to achieve complete dissolution of DPA. After solution step, the content of flask was centrifuged at 1500 rpm for 30 min. An amount of 1.0 ml of sample from the clear supernatant liquor was withdrawn and quantitatively diluted to 250.0 ml with BR buffer. This solution was kept at refrigerator and given the name stock tablet solution. Sufficient volumes (such as 0.125, 0.250, 0.500, 0.750 and 1.00 µl) from stock tablet solution were transferred to electrochemical cell containing 10.0 ml of BR buffer, pH was adjusted to desired value and performed determination of DPA in tablets using the calibration curve method.

Similarly, spiked human serum and urine samples were analyzed. Serum and urine samples, obtained from healthy individuals were stored frozen until assay. After gentle thawing, 1.0 ml aliquot volumes of serum (or urine) was added to electrochemical cell containing 9.0 ml of BR buffer and then sufficient volumes (such as 125, 250, 500 and 750 µl) from stock tablet solution were transferred to this cell. After deareation with argon, measurements were performed to determine DPA content of cell using the calibration curve method.

Voltammetric Procedure

In cyclic voltammetry, 9.0 ml of DPA solution in BR buffer was placed into the electrochemical cell for each time. The solution was deoxygenated with purified argon (99.99% purity) for 2 min before the first running and for 30 s between runnings. After deareation, a hanging mercury drop was formed, and then the voltammograms were recorded applying a negative-going scan from –1.00 to –1.75 V.

RESULTS AND DISCUSSION

Electrochemical Behavior of DPA

The electrochemical behavior, diffusion and adsorption properties of DPA were studied using cyclic voltammetry, square-wave voltammetry and con-

trolled potential electrolysis. In cyclic voltammetric studies, a single well-defined reduction peak was observed at a potential of about –1.60 V at pH 10.0 (Fig. 2). There is no peaks when a blank BR solution was scanned at the same conditions, and peak intensity increases linearly with increasing concentration of DPA, concluded that this cathodic reduction peak is due to the reduction of DPA molecules. As can be seen from Fig. 2, there is also an anodic peak at reverse scan.

The influences of the potential scan rate on cathodic peak potential ($E_{p,c}$) and cathodic peak current ($i_{p,c}$) at HMDE were investigated for 5.0×10^{-5} M DPA in the 0.005–10.0 V s^{-1} range. The peak potential shifts to more cathodic values with increasing scan rate and there is an anodic peak (Fig. 3). When the scan rate varied from 0.005 to 10.0 V s^{-1} in 5.0×10^{-5} M solution of DPA, a linear dependence of the cathodic peak current $i_{p,c}$ (in μA) upon the scan rate (in V s^{-1}) was found as given equation $i_{p,c} = 1.06\,\nu - 0.02$ with $R^2 = 0.996$, confirmed an adsorption behavior. Also a plot of logarithm of peak current (in A) versus logarithm of scan rate gave a straight line with a slope of 0.944 for DPA, very close to the theoretical value of 1.0 for adsorbed species, which expressed that electrode process is controlled mainly by adsorption[21]. Also the plot of peak current versus square root of scan rate was constructed and this graph is not linear even if scan rate extremely

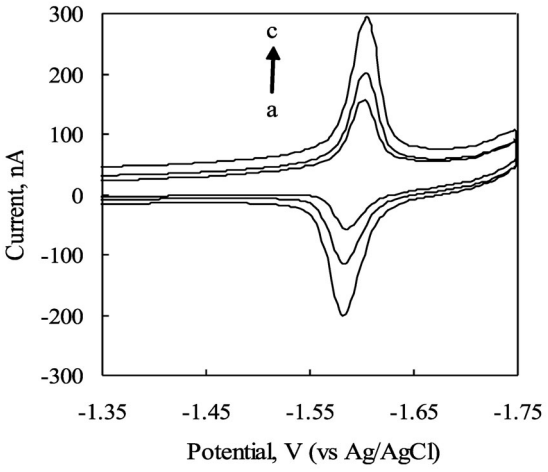


FIG. 2
Cyclic voltammograms of 5.0×10^{-5} (*a*), 2.5×10^{-4} (*b*) and 5.0×10^{-4} M DPA (*c*) solutions in BR buffer at pH 10.0 and scan rate of 0.10 V s^{-1}

low or extremely high. These results show us that electrode reaction is controlled by adsorption.

There is also an anodic peak indicating the reversible nature of DPA/electrode interaction. In fact for an ideal reversible electrochemical mechanism, ratio of anodic peak currents to cathodic peak current is unity and peak potential is not affected by scan rate[21]. In the present study, peak potential shifts to more negative values with increasing scan rate, and ratio of anodic peak current to cathodic peak current is not unity. Anodic peak is wider than cathodic one but they both have approximately the same area. This behavior may show the strong adsorption of product and weak adsorption of reactant. Some extra studies were carried out to control the adsorption phenomena according to literature[26,27]. As a result, the value of the ratio of cathodic peak current to concentration ($i_{p,c}/C$) decreases with the increasing concentration, the value of the ratio of cathodic peak current to multiplication of concentration and scan rate ($i_{p,c}/Cv$) is nearly constant with the increasing scan rate, and the value of the ratio of cathodic peak current to multiplication of concentration and square root of scan rate ($i_{p,c}/Cv^{1/2}$) increases with the increasing scan rate. According to these investigations, a quasi-reversible charge transfer reaction that includes the adsorption of product and reactant with different strength to electrode surface may be proposed.

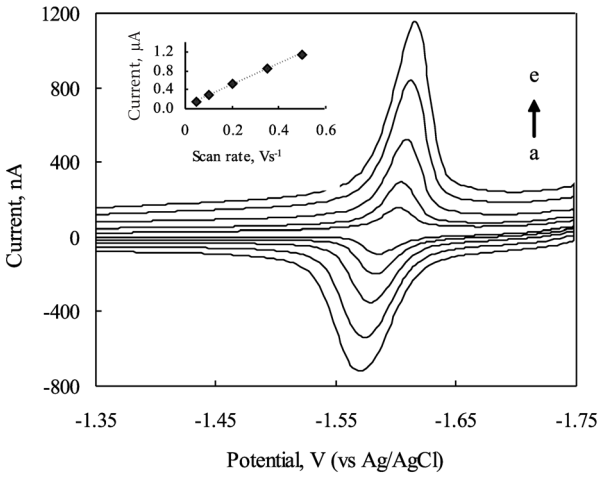


FIG. 3

Cyclic voltammograms of 5.0×10^{-4} M DPA in BR buffer at pH 10.0 with different scan rates 0.05 (*a*), 0.10 (*b*), 0.20 (*c*), 0.30 (*d*) and 0.50 V s^{-1} (*e*). Inset: i_p versus scan rate

In electrochemical studies, pH is one of the variables that commonly and strongly influence the electrochemical behavior of molecules. Therefore, the electrochemical behavior of DPA was studied as a function of pH in the pH 8.5–11.5 range. As can be seen from the square-wave voltammograms at different pH in Fig. 4, the potential of the cathodic peak shifts to more negative values and peak current decreases with the pH decrease (Fig. 4). This unusual behavior may be explained as the amount of adsorbed DPA increases with increasing pH and this increasing in the amount of adsorbed molecule (i.e. alkaline catalyzed mechanism) shows an effect to increase the peak current. At pH values lower than 8.0, peak potential may be in the more cathodic region than hydrogen evaluation region on HMDE, therefore effect of lower pH values could not be studied. In square-wave voltammetric (SWV) studies, peak potential changes linearly with the pH value as given in equation E_p = 0.068 pH – 2.21 with R^2 = 0.984. The experimental value of the peak potential slope against pH curves in SWV studies was found to be 68 mV per unit pH value in the given pH range. The slope is higher than the theoretical value of 60 mV per unit pH required for the assumed 2 e/2 H^+ or 4 e/4 H^+ process[28,29] of the electro reduction of DPA.

Based on literature, Eq. (*1*) was used in SWV to find the number of protons in electrode mechanism[30].

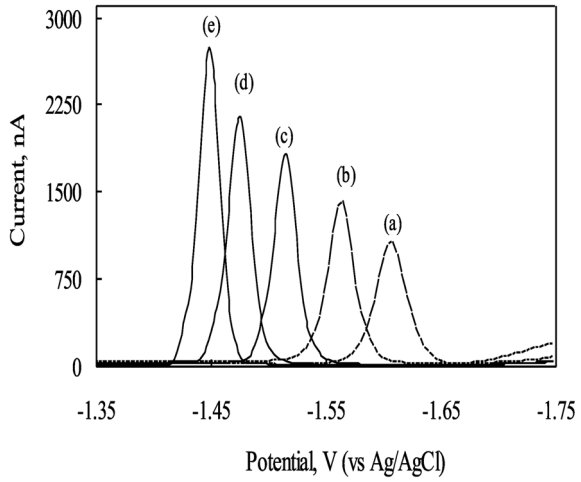


FIG. 4
Influence of pH 9.0 (a), 9.8 (b), 10.5 (c), 11.0 (d) and 11.4 (e) on square-wave voltammograms of 1.2×10^{-6} M DPA

$$E_p = E^0 + \frac{RT}{nF}\ln\frac{[Q]}{[R]} - \frac{\partial RT}{nF}\ln[H^+] \quad (1)$$

In this equation, ∂ is the number of protons participated in reaction mechanism and others are usual constants with their known values. Number of protons involved in reaction mechanism was found to be 2 from the slope value of the plot of E_p vs pH value.

To find out the number of electron(s), following relations proposed for adsorption process[21] were used in cyclic voltammetry

$$i_p = \frac{n^2 F^2 \Gamma A \nu}{4RT} \quad (2)$$

and the relation

$$Q = nFA\Gamma \quad (3)$$

where i_p is the peak current (in A), Q is the charge (in C) consumed by the surface process as calculated by the integration of the area under the peak, n is the total number of electrons transferred in electrode reaction, Γ is the surface coverage of adsorbed substance (in mol cm^{-2}), A is the working mercury electrode area (0.0145 cm^2), F is the Faraday constant (96485 C mol^{-1}) and ν is the scanning rate (in V s^{-1})[21,30]. Substituting the Γ term of Eq. (*3*) into Eq. (*2*), it is easy to get a new relation for n

$$n = \frac{4i_p RT}{FQ\nu} . \quad (4)$$

In the scan rate range from 0.005 to 0.500 V s^{-1}, number of electron(s) transferred in electrode reaction (n) was calculated using directly an equation given above for an each scan rate and using the slope of peak current versus scan rate. As a result of both methods, calculation and graphical method, number of electrons in electrochemical step was found to be 2.03 ± 0.12.

The surface coverage of adsorbed substance was calculated from the slope of the curve of the peak current versus scan rate according to Eq. (*2*) and it was found to be 2.50×10^{-11} mol cm^{-2} if $0.005 \le \nu \le 0.500$ V s^{-1}. So, from this value it is easy to say that each DPA molecule at electrode surface occupies an area of 6.64 nm^2.

The following equation, which expresses adsorption phenomena validated by Garrido[31], was used to calculate the diffusion coefficient of DPA

$$i_p = 1.06 \times 10^6 n^2 ACvD^{1/2}t_p^{1/2} . \quad (5)$$

The mean of the diffusion coefficient calculated from this equation was obtained as 3.31×10^{-8} cm^2 s^{-1}.

Using data from CV studies and Eq. (*6*) given below, electron transfer coefficient (α) was calculated[21].

$$E_p = k + \frac{RT}{n\alpha F} \ln v \qquad (6)$$

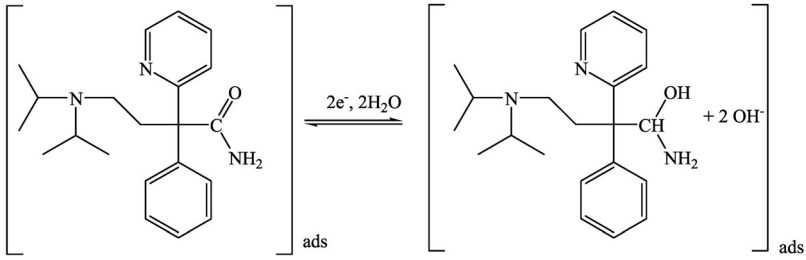
According to this equation, using the slope value of the plot of E_p vs ln v and 2 for n, the value of α was calculated to be 0.69 and the rate constant (k_s) was calculated according to the equation given below[30]

$$\ln k_s = \alpha \ln(1-\alpha) + (1-\alpha)\ln\alpha - \ln\frac{RT}{nFv} - \alpha(1-\alpha)\frac{nF\Delta E_p}{2.3RT} \qquad (7)$$

k_s value was found to be 1.73 s^{-1}.

Proposed Mechanism

To propose a tentative electrode reaction mechanism for DPA, results of voltammetric studies given above and also bulk electrolysis carried out at –1.75 V were evaluated. Both the number of transferred electrons and the number of protons participated in electrode reaction were found to be 2. Furthermore, the solutions of DPA before and after bulk electrolysis were analyzed by IR spectrometry. The peak observed at 1749 cm^{-1} before the bulk electrolysis disappeared after the bulk electrolysis of DPA. On the basis of all experimental results, it can be concluded that carbonyl group of DPA is reduced to corresponding alcohol as given below.



A similar type of mechanism was also given for the reduction of C=ONH$_2$ group in literature[32,33].

Electroanalytical Determination of DPA

Adsorptive stripping method was proposed to assay of DPA on HMDE because its reduction process is controlled by adsorption. Stripping methods are effective and rapid electroanalytical techniques. Especially, adsorptive stripping analysis greatly enhances the scope of stripping measurements toward numerous low amounts of organic compounds. Short adsorption times (1–5 min) result in a very effective interfacial accumulation[21–25]. In the present study, initially instrumental parameters and experimental conditions, such as type and the concentration of supporting electrolyte, pH, DPA concentration, deposition time and deposition potential, were optimized for determination of DPA.

The square-wave (SW) response markedly depends on the parameters of the excitement signal. In order to obtain a well-defined square-wave voltammetric peak shape, the optimum instrumental conditions, such as frequency (f), scan increment (ΔE_i) and pulse-amplitude (ΔE_a), were studied for 5.0×10^{-7} M DPA in a BR buffer of pH 10.0 at a HMDE. The optimum instrumental conditions were found to be f = 25 Hz, ΔE_i = 4 mV and ΔE_a = 15 mV with and without accumulation mode.

The peak responses for the studied drug were affected by the type of supporting electrolytes. Two different supporting electrolytes were examined including BR and NH_3/NH_4Cl buffers. The highest peak current and the best peak shape were obtained in the presence of BR buffer containing 0.04 mol l^{-1} for each component, although peak current increases with increasing buffer concentration in the range between 0.01 and 0.20 mol l^{-1}. In optimization of supporting electrolyte concentration, pH of the medium was held constant at 10.0.

The pH of a solution is a critical factor affecting both the rate and equilibrium state of the accumulation process and the rate of the electrode reaction. The influence of the pH on the SWCAdSV responses was studied at HMDE between pH values from 8.5 to 11.5. In optimization of pH value, not only peak current was chosen as an important parameter, but also peak shape, peak symmetry and linearity range were also chosen as other important parameters. In order to get useful peak shape and larger linearity range, 10.0 was selected as the optimum pH value although peak current values are greater in higher pH values (Fig. 4).

In stripping method, the influence of deposition time on the peak height for 5.0×10^{-7} M DPA was examined at different deposition times over the range from 15 to 150 s. The resulted peak current increases with the increase of the deposition time from 15 to 60 s; then, begins to decrease with

increasing deposition time (Fig. 5). As a result, optimum deposition time was selected to be 60 s for DPA.

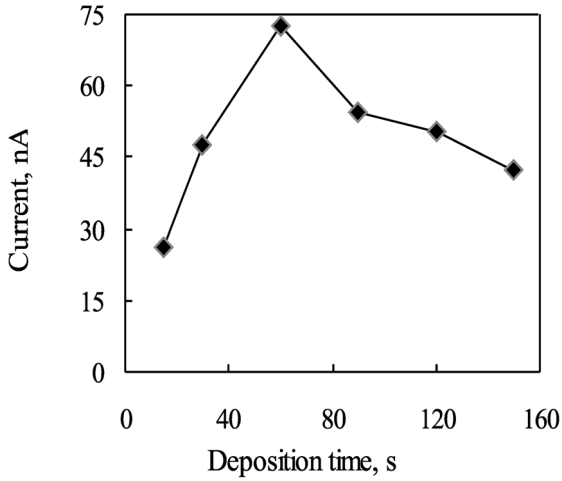


FIG. 5
Effect of deposition time on peak current of 5.0×10^{-7} M DPA at pH 10.0 in SWCAdSV (deposition potential –1.0 V)

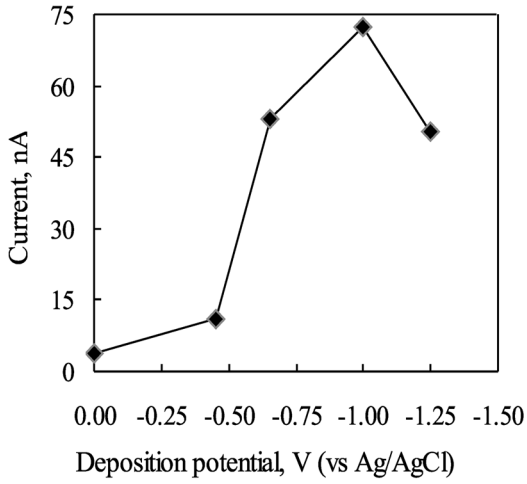


FIG. 6
Effect of deposition potential on peak current of 5.0×10^{-7} M DPA at pH 10.0 in SWCAdSV (deposition time 60 s)

The influence of the deposition potential (from 0.0 to –1.25 V) on the SWCAdSV signal was studied for 5.0×10^{-7} M DPA solution. Variation of the peak current values versus deposition potential for 5.0×10^{-7} M DPA was given in Fig. 6. The dependence of the peak current on the deposition potential showed a decrease at more negative potentials after –1.00 V. The maximum peak current in the deposition step was observed for the deposition potential of –1.00 V in SWCAdSV method.

To establish the linearity range (working concentration range) of DPA in SWCAdSV, different standard solutions were used ranged from 1.64×10^{-8} to 1.02×10^{-5} mol l^{-1}. For each concentration, five reproducible measurements were taken and mean of these measurements was used to plot the calibration curve. Result of concentration studies showed that the average peak current of reduction peak changes linearly with DPA concentration in the range from 7.15×10^{-8} mol l^{-1} (0.024 mg l^{-1}) to 1.43×10^{-6} mol l^{-1} (0.48 mg l^{-1}) (Fig. 7).

The characteristics of the calibration plots were summarized in Table IV.

Application of Method to Dosage Form and Biological Samples

In order to evaluate the adequacy of the proposed method, DPA was determined quantifying commercial pharmaceutical tablets of Rythmodan (labeled as 250 mg of DPA per tablet). No pretreatment such as time-consuming extraction or evaporation step was required for the sample preparation. The proposed SWCAdSV method was applied to the direct determination of

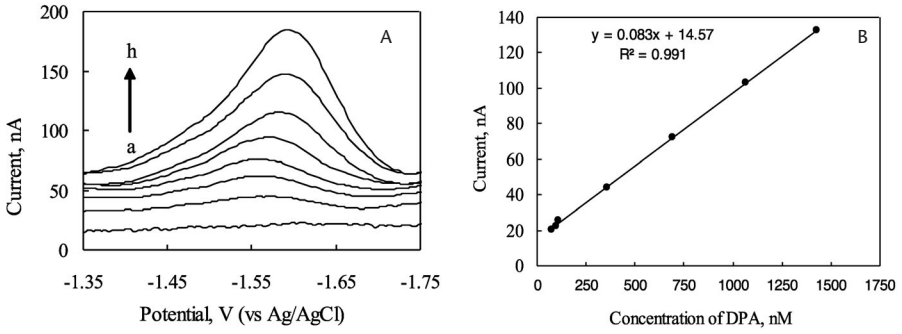


FIG. 7

A SWCAdS voltammograms of DPA at different concentrations (in nM): base line (*a*), 71.5 (*b*), 96.9 (*c*), 110 (*d*), 354 (*e*), 692 (*f*), 1065 (*g*) and 1430 (*h*). B Calibration curve for corresponding solutions of DPA given in A

DPA in pharmaceutical dosage forms using calibration curve method. The results of analysis found using the proposed method for pharmaceutical preparations were given in Table I, for spiked human urine and spiked human serum were given in Tables II and III. The accuracy of the proposed method was determined by its recovery values.

It can be seen from these tables that average recovery values are in good agreement with the RSD values less than 10% for human urine and human serum and less than 7% for tablets, which is good evidence of validity of

TABLE I
Results of DPA amounts in Rythmodan tablets determined using the proposed SWCAdSV method

Sample	Nominal value per tablet, mg	Found values per tablet, mg	Recovery value[a] %	RSD[b] %
I	250	274.51, 243.96, 232.18, 258.30, 266.04	102.0 ± 8.5	6.67
II	250	254.08, 240.66, 239.04, 262.98, 271.26	101.4 ± 6.9	5.50

[a] Results of recovery values are given as mean ± $ts/\sqrt{N}$ (at 95% confidence level). [b] RSD is relative standard deviation.

TABLE II
Results of DPA amounts in human urine spiked by tablet and by standard DPA determined using the proposed SWCAdSV method

Sample	Spiked amount, μg	Found amounts, μg	Recovery value[a] %	RSD[b] %
Tablet in urine I	0.5	0.41, 0.48, 0.48, 0.50, 053	96.0 ± 10.9	9.19
Tablet in urine II	1.0	0.90, 0.95, 0.97, 1.01, 1.05	97.6 ± 7.1	5.87
Tablet in urine III	2.0	1.82, 1.92, 1.94, 2.05, 2.09	97.6 ± 7.2	5.92
Tablet in urine IV	3.0	2.70, 2.85, 2.91, 3.02, 3.16	98.2 ± 6.7	5.49
Standard in urine I	0.5	0.45, 0.46, 0.51, 0.52, 0.55	99.6 ± 10.5	8.45
Standard in urine II	1.0	0.92, 0.93, 0.94, 1.05, 1.11	99.0 ± 10.6	8.60
Standard in urine III	2.0	1.88, 1.91, 1.95, 2.11, 2.19	100.4 ± 8.4	6.72
Standard in urine IV	3.0	2.71, 2.89, 2.97, 3.26, 3.28	100.7 ± 10.2	8.13

[a] Results of recovery values are given as mean ± $ts/\sqrt{N}$ (at 95% confidence level). [b] RSD is relative standard deviation.

method. Thus, the precision is very satisfactory for the analysis of biological samples as well as bulk formulation. These results indicate that the content of DPA in the pharmaceuticals and biological fluids can be safely determined using the proposed voltammetric method without interference with other substances in the samples. The proposed method can be applied to pharmaceuticals, human serum and human urine after a simple dilution step with direct measurements.

Method Validation

Validation of an analytical method is the process by which it is established that the performance characteristics of the method meets the requirements for the intended analytical applications. The elements required for method validation are: linearity range, limits of detection and quantitation, accuracy, reproducibility, stability, selectivity and robustness[34].

To establish the working concentration range (linearity range) of DPA in SWCAdSV, eleven different standard solutions were used ranged from 1.64×10^{-8} to 1.43×10^{-6} mol l^{-1}. A good linearity is evident from the values of correlation coefficient (R^2) of 0.991 (Fig. 7B) thus confirmed validity of the SWCAdSV method for the assay of DPA (Table IV).

TABLE III
Results of DPA amounts in human serum spiked by tablet and by standard DPA determined using the proposed SWCAdSV methode

Sample	Spiked amount, μg	Found amounts, μg	Recovery value[a] %	RSD[b] %
Tablet in serum I	0.5	0.44, 0.46, 0.47. 0.52, 0.52	96.4 ± 9.0	7.54
Tablet in serum II	1.0	0.92, 0.94, 0.98, 1.09, 1.11	100.8 ± 10.8	8.63
Tablet in serum III	2.0	1.82, 1.90, 1.92, 2.09, 2.15	99.0 ± 8.4	6.86
Tablet in serum IV	3.0	2.76, 2.92, 2.95, 3.12, 3.22	97.8 ± 7.4	5.99
Standard in serum I	0.5	0.46, 0.46, 0.49, 0.54, 0.57	100.8 ± 12.3	9.78
Standard in serum II	1.0	0.94, 0.97, 1.00, 1.06, 1.10	101.4 ± 8.1	6.45
Standard in serum III	2.0	1.89, 1.92, 1.97, 2.05, 2.09	99.2 ± 5.3	4.27
Standard in serum IV	3.0	2.79, 2.85, 2.94, 3.02, 3.10	97.8 ± 5.2	4.23

[a] Results of recovery values are given as mean ± $ts/\sqrt{N}$ (at 95% confidence level). [b] RSD is relative standard deviation.

Limit of detection (LOD) and limit of quantitation (LOQ) values were calculated using the relations: LOD = 3 s/m and LOQ = 10 s/m (ref.[35]). The abbreviation of s is the standard deviation of intercept of calibration curve and m is the slope of the related calibration curve. LOD and LOQ values were found 5.65×10^{-8} mol l^{-1} (0.019 mg l^{-1}) and 1.88×10^{-7} mol l^{-1} (0.064 mg l^{-1}), respectively. Both LOD and LOQ values confirmed that the proposed method could be used to determine the DPA content of highly diluted biological samples such as serum and urine.

The accuracy of measurements by means of the described procedure was checked calculating the recovery of a known concentration of DPA following the proposed method at optimum instrumental and experimental conditions. Recovery values range between 101.4 and 102.0% for tablet analysis, found between 96.0 and 100.7% for urine analysis and between 96.4 and 101.4% for serum analysis (Tables I–III). From these recovery values it is concluded that the proposed method is highly accurate.

The high sensitivity of an analytical method is usually accompanied by a very good reproducibility. This analytical performance was evaluated from five repeated measurements of electrochemical signal of different DPA solutions following the proposed method. The precision of the proposed procedure is excellent because the relative standard deviation of recovery

TABLE IV
Regression data of the calibration curve for assay of DPA by SWCAdSV

Calibration parameter	Value
Linearity range, mol l^{-1}	7.15×10^{-8}–1.43×10^{-6}
Slope of calibration curve (m), A l mol^{-1}	0.0834
Intercept, A	1.46×10^{-8}
Standard deviation of calibration, A	3.18×10^{-9}
Standard deviation of slope, A l mol^{-1}	0.00289
Standard deviation of intercept (s), A	1.57×10^{-9}
Limit of detection (LOD), mol l^{-1}	5.65×10^{-8}
Limit of quantification (LOQ), mol l^{-1}	1.88×10^{-7}
Regression coefficient (R^2)	0.991
Repeatability of peak current (RSD[a], %)	1.57
Repeatability of peak potential (RSD[a], %)	0.54

[a] RSD is relative standard deviation.

values ranges between 4.23 and 9.78% for all measurement, including tablets, urine and serum samples (Tables I–III).

The stability of DPA in the BR buffer of pH 10.0 was evaluated under the optimal procedural conditions monitoring the changes in both the cathodic peak potential and the cathodic peak current of standard DPA solution. Relative standard deviations of peak current and peak potentials for five series of measurements were found to be 1.57 and 0.54%, respectively (Table IV). As a result, no significant change in peak potential and peak current confirms the stability of DPA over the time period of measurements. Furthermore, DPA solution was found to be stable at least 2 months when kept in a refrigerator.

During an application of the proposed method to biological samples and tablets, before adding a standard solution of DPA, voltammetric base line of biological medium was measured applying the same procedures as applied to calibration studies with standard samples. In such applications, no extra voltammetric signal in studying potential window indicates that there is no significant interference of various inorganic cations, anions and some organic substances found in pharmaceutical preparations (tablets) and biological mediums (human urine and human serum). These results show that reduction peak is specific to DPA and this peak can be used selectively to determine the DPA in biological fluids.

The robustness[36] of the measurements by means of the described SWCAdSV procedure to assay of DPA was examined studying the effect of small variation of some important procedural conditions such as pH value, accumulation potential, accumulation time and room temperature of different days. Small changes (±1%) in such conditions do not affect the recovery of procedure.

CONCLUSION

In this study, electrochemical reduction behavior of DPA was studied on HMDE for the first time. Electrochemical behaviors of pharmaceutical compounds may have valuable findings in either understanding of the mechanism of their action or determining their concentration in living organisms at various times after the intake.

Developed methods provide a sensitive, fast, cost-effective, high-throughput and simple approach to the determination of DPA in tablet dosage forms, spiked human serum and spiked human urine samples. As applied to serum and urine samples, the proposed method offers an advantage that no prior extraction procedure is required. Furthermore, the pro-

posed methods have distinct advantages over the other existing methods regarding sensitivity, time-consuming, lower detectability and no excipients as interfering with the analysis, avoiding a separation step. The results of *t*- and F-tests, the variances between two methods (HPLC[10] and SWCAdSV) were found to be insignificant at 95% confidence level indicating that no significant differences exist between the performances of these two methods regarding their accuracy, precision and recoveries (Table V). As a result, the proposed method might be alternative to the HPLC techniques.

TABLE V
Statistical analysis of the results obtained by the SWCAdSV and HPLC methods for DPA

Method	Mean recovery, %	RSD[a], %	*N*
SWCAdSV	99.25	5.5	10
HPLC	100.34	3.2	8
F-Test signifikance	2.95	F (tabulated) = 6.39	
t-Test signifikance	0.92	*t* (tabulated) = 2.23	

[a] RSD is relative standard deviation.

REFERENCES

1. Mayer E., Krämer B. K., Ress K. M., Kühlkamp V., Liebich H. M., Risler T., Seipel L.: *J. Chromatogr.* **1991**, *572*, 39.
2. Barac M. S. I., Maron B., Casey S., Chojnowska L., Dickie S., Elliot P., McKenna W.: *Cardiac Function and Heart Failure*, 170 A.
3. Abdellatef H. E.: *Spectrochim. Acta, Part A* **2007**, *66*, 1248.
4. Delgado J. N., Remers W. A.: *Wilson and Gisvold's Text Book of Organic Medicinal and Pharmaceutical Chemistry*, p. 598. Wolters Kluwer, Lippincott, Williams/Wilkins, Philadelphia 1998.
5. Lecorre P., Gibassier D., Sado P.: *J. Chromatogr. Biomed. Sci. Appl.* **1988**, *424*, 424.
6. Wang I. H., Kushida K., Ishizaki T.: *Ther. Drug Monit.* **1986**, *8*, 85.
7. Norris R. L. G., Ahokas I. T., Ravenscroft P. I.: *Clin. Exp. Pharmacol. Physiol.* **1982**, *9*, 452.
8. Norris R. L. G., Ahokas J. T., Ravenscroft P. J.: *J. Chromatogr.* **1982**, *230*, 433.
9. Witek A., Zawisza P., Przyborowski L.: *J. Pharm. Biomed. Anal.* **1994**, *12*, 425.
10. Bortocan R., Lanchote V. L., Cesarino E. J., Bonato P. S.: *J. Chromatogr., B* **2000**, *744*, 299.
11. Hanada K., Akimoto S., Mitsui K., Hashiguchi M., Ogata H.: *J. Chromatogr., B* **1998**, *710*, 129.
12. Le Corre P., Gibassier D., Sado P., Le Verge R.: *J. Chromatogr.* **1988**, *450*, 211.
13. Ahokas J. T., Davies C., Ravenscroft P. J.: *J. Chromatogr., B: Biomed. Sci. Appl.* **1980**, *183*, 65.

14. Charette C., McGilveray I. J., Mainville C.: *J. Chromatogr., B: Biomed. Sci. Appl.* **1983,** *274*, 219.
15. Kwong T., Keller J., Kalburgi A.: *Clin. Chem.* **1983**, *29*, 1249.
16. Rao G. V. K., Ritzmann S. E.: *Clin. Chem.* **1981**, *27*, 1087.
17. Haskins N. J., Ford G. C., Palmer R. F.: *Biomed Mass Spectrom.* **1980**, *7*, 74.
18. Katsu T., Mori Y.: *Anal. Chim. Acta* **1997**, *343*, 79.
19. Stefan R. I.: *Anal. Chim. Acta* **1997**, *350*, 105.
20. Diculescu V. C., Enache T. A., Oliveira-Brett A. M.: *Anal. Lett.* **2007**, *40*, 2860.
21. Wang J.: *Analytical Electrochemistry*, p. 37. Wiley–VCH, New York 2000.
22. Barek J., Pecková K., Vyskočil V.: *Curr. Anal. Chem.* **2008**, *4*, 242.
23. Ignjatović L. M., Barek J., Zima J., Stević M. C.: *Collect. Czech. Chem. Commun.* **2008**, *73*, 97.
24. Němcová L., Zima J., Barek J.: *Collect. Czech. Chem. Commun.* **2009**, *74*, 1477.
25. Jemelková Z., Zima J., Barek J.: *Collect. Czech. Chem. Commun.* **2009**, *74*, 1503.
26. Wopschall M. S., Shain I.: *Anal. Chem.* **1969**, *39*, 1514.
27. Hulbert M. H., Shain I.: *Anal. Chem.* **1970**, *42*, 162.
28. Eriksson A., Nyholm L.: *Electrochim. Acta* **1999**, *44*, 4029.
29. Montoya M. R., Mellado J. M. R.: *J. Electroanal. Chem.* **1996**, *417*, 113.
30. Wang L., Zhang Z., Ye B.: *Electrochim. Acta* **2006**, *51*, 5961.
31. Garrido J. A., Rodriguez R. M., Bastida R. M., Brillas E.: *J. Electroanal. Chem. Interfacial Electrochem.* **1992**, *324*, 19.
32. Galvin M. R., Mellado J. M. R., Blanco F. G: *J. Electroanal. Chem.* **1988**, *251*, 163.
33. Angulo M., Montaya M. R., Galvin R. M., Mellado J. M. R.: *Electroanalysis* **1997**, *9*, 345.
34. Beltagi A. M., Abdallah O. M., Ghoneimc M. M.: *Talanta* **2008**, *74*, 851.
35. Guzmán A., Agüí L., Pedrero M., Yáñez-Sedeño P. J. M.: *Electroanalysis* **2004**, *16*, 1763.
36. Al-Majed A. A., Belal F., Abadi A., Al-Obaid A. M.: *Farmaco* **2000**, *55*, 233.