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Short communication

Square-wave adsorptive stripping voltammetric determination of danazol in capsules

Ahmed H. Alghamdi [a], Fatallah F. Belal [b], Mohamed A. Al-Omar [c,*]

[a] Department of Chemistry, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
[b] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Mansoura, Mansoura, Egypt
[c] Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

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Abstract

Based on the interfacial adsorptive character of danazol onto the hanging mercury drop electrode (HMDE), a simple and sensitive square-wave adsorptive stripping voltammetric (SW-AdSV) procedure for the electrochemical analysis of this drug in pharmaceutical formulations has been developed and validated. Cyclic and SW-AdSV voltammograms showed a single well-defined irreversible cathodic peak. Various chemical and instrumental parameters affecting the monitored electroanalytical response were investigated and optimized for the danazol determination. Under these optimized conditions the SW-AdSV peak current showed a linear dependence on drug concentration over the range 7.5×10^{-8}–3.75×10^{-7} mol l^{-1} ($r = 0.999$) with estimated detection limit (at a S/N ratio of 3) of 5.7×10^{-9} mol l^{-1} (1.78 ng ml^{-1}). A mean recovery of $100.9 \pm 1.2\%$ and relative standard deviation of 1.07% were achieved. Possible interferences by substances usually present in the pharmaceutical tablets and formulations were also evaluated. The proposed electrochemical procedure was successfully applied for the determination of danazol in pharmaceutical capsules (Danol^{TM} 100 mg) with mean recoveries of $100.48 \pm 0.87\%$. Results of the developed SW-AdSV method were comparable with those obtained by reported analytical procedures.

Keywords: Square wave voltammetry; Adsorptive stripping voltammetry; Danazol; Pharmaceutical formulations

1. Introduction

Over the last decade, adsorptive stripping voltammetry (AdSV) has been established as a very reliable analytical technique, which widely recognized as one of the most sensitive methods in electrochemical chemistry. Hence, the interest in utilizing square-wave adsorptive stripping voltammetry (SW-AdSV) technique in the determination and monitoring of a wide range of drugs has been raised considerably. Adsorptive stripping voltammetric approach has been considered as a universal and highly useful voltammetric approach due to its remarkable sensitivity, selectivity, its multi-elements/components applicability, low cost and stability to on-line measurements. Adsorption of numerous species on the surface of mercury, other metal and modified electrodes lead to the preconcentration of these analytes, a process ensured outstanding sensitive for the electroanalytical determinations [1–4]. The applicability of the adsorptive accumulation procedure in the determination of pharmaceutical drugs in biological fluids and drug dosage forms have been reviewed elsewhere [5–7].

Danazol is a synthetic derivative of the progestogen ethisterone that inhibits the release of gonadotrophins from pituitary gland, thus in turn preventing the release of sex hormones [8]. Clinically, danazol has been used for the treatment of a variety of conditions such as endometriosis, fibrocystic breast disease, gynaecomastia and hereditary angioedema [9]. Additionally, danazol is also used in doping field as other anabolic steroids to increase muscle development and strength, decrease healing time and diminish fatigue [10].

This drug has been determined in many pharmaceutical preparations and biological fluids with various analytical methods. However, most of the reported methods for the analysis of danazol rely deeply on the use of chromatographic techniques. HPLC [11–17], GC [18,19] and TLC [20] chromatographic procedures have been described for identification and determination of danazol. However, although danazol predominantly analysed

* Corresponding author. Tel.: +9661 4677343; fax: +9661 4676220.
E-mail address: drmohammed1423@yahoo.co.uk (M.A. Al-Omar).

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and studied by these chromatographic approaches, yet other analytical techniques such as chemiluminescent immunoassay [21] voltammetry [22] were also applied for similar analytical task.

To date no adsorptive stripping nor square-wave voltammetric procedures for the assay and quantification of danazol are reported in literature. Hence, the current electroanalytical research aimed to study the square-wave voltammetric behavior of danazol and its interfacial adsorptive accumulation onto the hanging mercury drop electrode. Based on the results obtained, a simple, sensitive and low cost SW-AdSV procedure was developed for the direct determination of danazol in pharmaceutical capsules.

2. Experimental

2.1. Apparatus

All adsorptive stripping and cyclic voltammetric measurements were carried out with 757 VA computrace (Metrohm, Herisau, Switzerland) in connection with Dell computer and controlled by (VA computrace 2.0) control software. Stripping and cyclic voltammograms were obtained via a Hewlett-Packard laser jet printer. A conventional three-electrode system was used in the hanging mercury drop electrode (HMDE) mode.

2.2. Reagents

All chemicals used were of analytical reagent grade and were used without further purification. Danazol stock solution of 1×10^{-2} mol l^{-1} was prepared by dissolving the appropriate amount in methanol in 10 ml volumetric flask. Britton–Robinson (B–R) supporting buffer (pH $\approx$ 2, 0.04 M in each constituent) was prepared by dissolving 2.47 g of boric acid in 500 ml distilled water containing 2.3 ml of glacial acetic acid and then adding 2.7 ml of *ortho*-phosphoric acid and diluting to 1 l with distilled water. The carbonate buffer was 0.1 mol l^{-1} in both sodium hydrogen carbonate and disodium carbonate, while phosphate buffer was prepared from 0.1 mol l^{-1} in both phosphoric acid and sodium phosphate.

2.3. Procedure

The general procedure adopted for obtaining adsorptive stripping voltammograms was as follows: a 20 ml aliquot of B–R supporting buffer at desired pH was pipetted in a clean and dry voltammetric cell and the required standard solutions of danazol were added. The test solutions were purged with nitrogen for 5 min initially, while the solution was stirred. The accumulation potential of 0.0 V versus Ag/AgCl was applied to a new mercury drop while the solution was stirred for 90 s (unless otherwise stated). Following the preconcentration period, the cathodic scans were carried out over the range 0.0 to −1.4 V. All measurements were made at room temperature.

3. Results and discussion

3.1. The electrochemical behavior of danazol

The cyclic voltammetric behavior of 1×10^{-4} mol l^{-1} danazol in Britton–Robinson buffer pH 2 at the hanging mercury drop electrode monitored in the cathodic direction yielded a single well-defined peak at −1091 mV probably attributed to the cathodic reduction of the isoxazole ring present in the analyte molecule [22]. No oxidation peak was observed in the positive scanning half-cycle, indicating the irreversible nature of he electrode process. The interfacial accumulation of the drug was designated from repetitive cyclic voltammograms for danazol recorded following stirring for 60 s at 0.0 V prior to the first scan produced considerable cathodic peak (scan 1). As can be seen from Fig. 1, a substantial decrease of the monitored electrochemical signal was observed in subsequent repetitive scans. Such behavior indicated rapid adsorption of danazol from the working electrode surface. The voltammetric cycles carried out for increasing scan rate values over the range 50–500 mV s^{-1} gave rise to an electrochemical response with increased peak current intensities. The plot of log i_p versus log ν, gave a straight line with slope value of 0.82, which is to some degree close to the theoretical value of 1.0 that is expected for an adsorption-controlled process [23], indicating the interfacial adsorptive character of danazol onto the surface electrode. In addition, the observed peak potential shift to a more negative values on the increase of scan rate confirmed the irreversible nature of the studied cathodic reduction process.

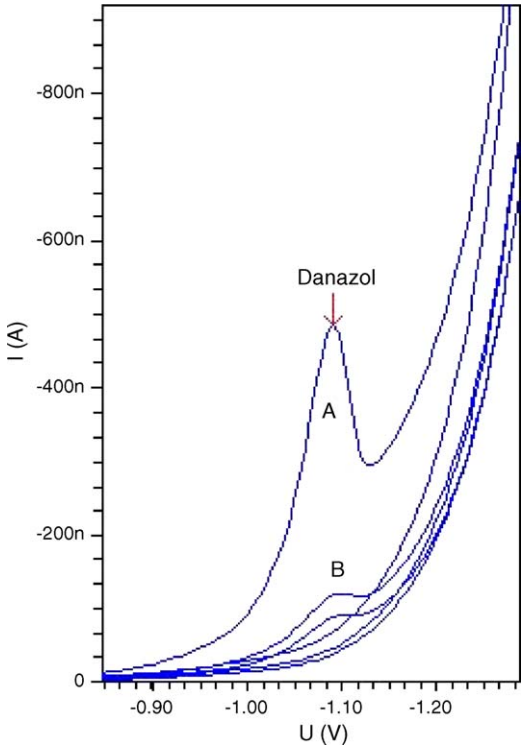

Fig. 1. Repetitive cyclic voltammograms for 1×10^{-4} mol l^{-1} danazol in pH 2 B–R buffer, scan rate 100 mV^{-1}, accumulation potential 0.0 V and preconcentration time 60 s (scan A) and 0 s (scan B).

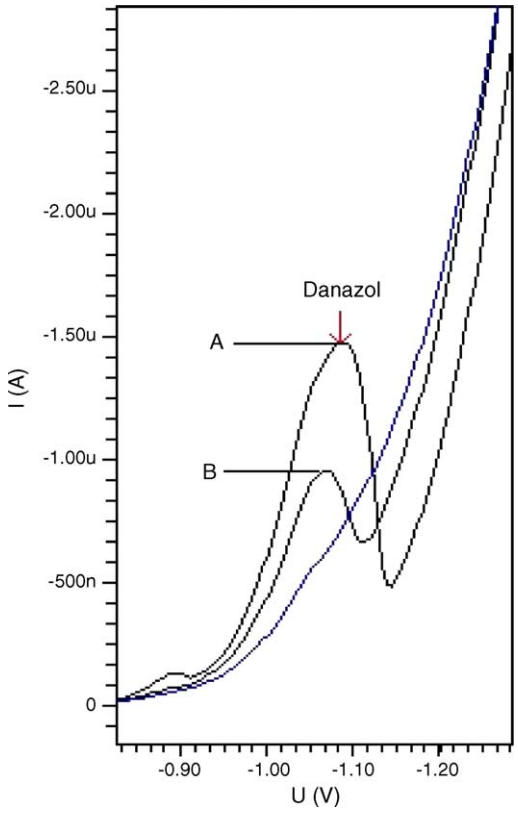

Fig. 2. SW-AdSV voltammogram for danazol drug in B–R buffer (pH 2.6), $\underline{T}_{acc}$: 60 s, E_{acc}: 0.0 V, scan rate: 300 mV s^{-1}, SW frequency: 50 Hz and pulse amplitude: 80 mV. danazol concentration: (A) 5.0×10^{-7} and (B) 2.5×10^{-7} mol l^{-1}.

The strong adsorption phenomenon of danazol can be used as an effective preconcentration step prior to the actual voltammetric quantification of the analyte. The adsorptive stripping voltammetric response of danazol at HMDE was examined in Britton–Robinson buffer pH 2.6 using the differential pulse (DP) and square wave (SW) excitation waveforms. The electrochemical current intensity for the cathodic reduction of danazol recorded by the square wave voltammetric technique was nearly 10 times higher than that generated by the differential pulse excitation mode. Due to its intense sensitivity, therefore SW-AdSV approach was used in all the subsequent experiments. Fig. 2 shows a square-wave adsorptive stripping voltammogram for danazol after 60 s accumulation period at 0.0 V, which illustrates a single well-defined AdSV peak at −1062 mV versus Ag/AgCl reference electrode.

3.2. Optimum parameters and experimental conditions

3.2.1. Effect of supporting electrolyte and pH

Since adsorption of danazol on the surface of the HMDE was used as a convenient accumulation step prior to its voltammetric determination, various preliminary experiments were carried out in various supporting buffers at different pH values in order to assess their impact on the monitored electroanalysis signal. Among the investigated supporting electrolytes (acetate, phosphate, carbonate and Britton–Robinson buffers), the ideal stripping voltammetric peak, its sensitivity and resolution have been observed when using B–R buffer solution. In addition, the observed SW-AdSV signal was vitally pH-dependent since intense electrochemical currents were only observed at mild acidic media. When the influence of buffer acidity on the observed analytical signal for danazol was investigated over the pH range 1–9, it was noticed that very weak analytical signals were obtained if buffer solutions with pH values out of the range 2–5. After 90 s accumulation time for 2×10^{-7} mol l^{-1} danazol test solution at 0.0 V preconcentration potential, the highest AdSV peak height was achieved at pH 3 and beyond this optimum pH value the monitored adsorptive stripping response decreased gradually. Furthermore, the danazol AdSV peak potential was found to be dependent on the pH of the buffer solution. A gradual shift to more negative potential was observed from −1065 to −1309 mV when varying pH value over the range 2–9, indicating consumption of hydrogen ions in the electrochemical reduction process.

3.2.2. Effect of accumulation time and potential

For 2×10^{-7} mol l^{-1} danazol solution, variation of accumulation time parameter over 0–13 s adsorption period at $E_{acc} = 0.0$ V preconcentration potential initiated a remarkable enhancement for the AdSV peak current up to 90 s accumulation time and then it became virtually curved due to the saturation of the surface of the working electrode. For further AdSV quantitative studies for danazol, an accumulation time of 90 s was selected as optimal value since it provided relatively high peak current with adequate practical time. In addition, when the influence of preconcentration potential on the observed stripping voltammetric signal was examined over the range of −0.6 to +0.2 V at 90 s accumulation time, the peak current increased steadily over the positive direction till it reached its maximum value at $E_p = 0.0$ V where it decreased sharply thereafter. Hence, for optimal analytical sensitivity this experimental parameter was maintained at 0.0 V.

3.2.3. Effect of potential sweep conditions

The observed stripping voltammetric signal can be further maximized by adjusting the way the applied potential was scanned. Hence, when the scan rate parameter was varied over the range 100–900 mV s^{-1}, virtually a linear enhancement for the SW-AdSV peak current was observed when the scan rate was varied over the range 100–600 mV s^{-1}. However, the monitored cathodic peak current started to leveled off when scan rate values faster than 70 mV s^{-1} were used. Accordingly, 600 mV s^{-1} scan rate value was recommended for the subsequent work. In addition, the impact of varying the square-wave frequency on the SW-AdSV current intensity was also evaluated. The effect of this operating variable was studied over the range 20–90 Hz and it was concluded that in order to assure maximum peak current, 40 Hz square-wave frequency was the ideal choice for this operational parameter. Furthermore, varying the value of excitation wave pulse amplitude also plays an important role for the measured stripping voltammetric current intensity. Increasing this parameter over the range 20–100 mV, resulted in a substantial enhancement of the voltammetric peak current. Accordingly, for

Table 1
Application of the proposed and reference methods to the analysis of commercial capsules of danazol

Labeled content	AdSV method % Recovery	References methods % Recovery	
		Conventional voltammetry [12]	HPLC [15]
Danol^{TM} capsules 100 mg	101.2 99.6 100 101.6 100	$n=5$	
Mean standard deviation	100.48 ± 0.87	100 ± 1.0	$100 \pm .08$

future work 80 mV excitation wave pulse amplitude value was adopted.

3.3. Analytical performance of the developed procedure

3.3.1. Calibration graph and detection limit

Under the optimum experimental conditions a good linear correlation was obtained between danazol electrochemical response and its concentration in the range 7.5×10^{-8}–3.75×10^{-7} mol l^{-1}. The parameters of the concentration-current straight line were calculated by the least-squares method. The standard curve was linear with correlation coefficient (r) not less than 0.998. The regression analysis data was calculated at 95% confidence level for the slope ($b \pm ts_b$) and intercept ($a \pm ts_a$). The results were:

$$i_p(\text{nA}) = (60.1 \pm 0.017) + (3.7 \times 10^9 \pm 1.38 \times 10^8)C$$

$$r = 0.998$$

where i_p is the SW-AdSV peak current, b the slope, s_b standard deviation for the slope, t the t-value at 95% confidence level for (n-2), s_a the standard deviation for the intercept and C is the danazol concentration in mol l^{-1}. The detection limit defined as three times the signal-to-noise ratio (S/N = 3) monitored at the optimum conditions for danazol was calculated and was found to be 5.7×10^{-9} mol l^{-1} (1.78 ng ml^{-1}).

3.3.2. Accuracy, precision and stability

The accuracy of the proposed method was checked by calculating the recovery of known amount of danazol (1×10^{-7} mol l^{-1}) added to B–R buffer solution and analysed via the optimized stripping voltammetric procedure. The value of the mean recovery obtained by the standard addition method was 100.9% with standard deviation of 1.2% (the analytical measurements repeated five times). The analytical precision of the developed method was verified from the reproducibility of 10 determinations of 2×10^{-7} mol l^{-1} danazol and the estimated relative standard deviation (R.S.D.%) was 1.07%. Finally, When the SW-AdSV signal of 2×10^{-7} mol l^{-1} danazol solution was monitored every fifteen minutes, it was found to be nearly stable for a period of at least 2 h.

3.3.3. Interferences

The competitive co-adsorption interference was evaluated in the presence of various substances that are usually found in the pharmaceutical tablets and formulations. For these investigations, the interfering species were added at different concentrations (5-, 25- and 50-fold) higher than the concentration of danazol (2×10^{-7} mol l^{-1}). The additions of filling materials (sucrose, lactose and cellulose), disintegrate agent (starch) and lubricants such as magnesium stearate caused no significant effects on the SW-AdSV response of danazol. Hence, this compound may need not to be extracted from these tablet ingredients or additives prior to its determination in tablets.

3.4. Analytical applications

Following the electroanalysis procedure described above, the validity of the developed method for the determination of danazol in pharmaceutical formulation. The danazol content of commercially available capsules (Danol^{TM} 100 mg) was determined directly by the proposed SW-AdSV method after the required dissolving and filtration steps. Five aliquot of the dissolved sample were diluted to the required concentration level. As can be seen from Table 1, the analytical results achieved by the proposed SW-AdSV procedure were in good agreement with those reported in the literature for the analysis of the same pharmaceutical capsules. The agreement of the obtained result was tested by the paired F-test statistical approach [24]. The variances of both analytical methods were found to be not differ significantly, since the calculated F-test value (1.18) was less than the critical value (5.05) at the 95% confidence level ($P = 0.05$).

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