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Short communication

Voltammetric study of danazol and its determination in capsules and spiked biological fluids

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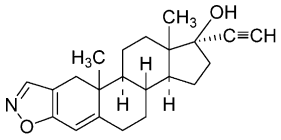
Abstract

The voltammetric behaviour of danazol DZ (antigonadotropin) was studied using cyclic voltammetry, direct current, differential pulse polarography (DPP) and alternating current polarography. Danazol exhibited irreversible cathodic waves over the pH range of 1–5 in Britton Robinson buffers. At pH 1 (the analytical pH), a well-defined wave with E1/2 of −1.04 V versus Ag/AgCl reference electrode was obtained. The diffusion current constant (I_d) was 4.8 ± 0.14 μA.L.m mole^{-1} and the current–concentration plot was rectilinear over the range from 5×10^{-6} to 1×10^{-4} M with correlation coefficient ($n = 11$) of 0.995. The calculated detection limit was 1×10^{-6} M using the DPP mode. The wave was characterized as being irreversible, diffusion-controlled although adsorption phenomenon played a limited role in the electrode process. The proposed method was applied to commercial capsules and the average percentage recovery was in agreement with that obtained by the official USP method. The method was extended to the in vitro determination of DZ in spiked human urine and plasma samples, the percentage recoveries were 96 ± 4 and 97 ± 5, respectively. A proposal of the electrode reaction was postulated.

Keywords: Danazol; Voltammetry; Polarography; Biological fluids; Capsules

1. Introduction

Danazol (DZ) is a nonester synthetic hormone with weak androgenic effects and is structurally related to testosterone and ethisterone. Chemically, it is: 17α-pregna-2,4-dien-20-ynol(2,3-D)-isoxazol-17-ol.



Structural Formula of Danazol.

Clinically, DZ is a gonadotrophin inhibitor indicated for the treatment of endometriosis, hereditary angioedema and fibrocystic breast disease [1]. DZ is also used in doping field as other anabolic steroids to increase muscle development and strength, decrease healing time following injury, diminish fatigue, and increase aggressiveness [2]. The adverse effects associated with the anabolic use of DZ are dependent on the dose and duration of use. These effects have been described with more frequent incidence in females (amenorrhea, breakthrough bleeding or spotting, decreased breast size, irregular menstrual periods, weight gain) and some other effects with less frequency in both females and males such as muscle spasm, unusual weakness and virilism [3].

Danazol is the subject of a monograph in the United States Pharmacopoeia, USP [4], which recommends a direct spectrophotometric measurement at 285 nm for the assay of the raw material and HPLC for the assay of the capsules. Reviewing the literature revealed that few methods have been reported for the determination of DZ whether in formulations or in biological fluids; and most of them rely on the use of chromatography. A quantitative TLC method has been reported for the validation of the purity analysis of DZ and its capsules [5]. HPLC has been frequently applied to the

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analysis of DZ in bulk form [6] pharmaceutical formulations [7–11] and biological fluids [11–17]. Gas chromatography [18,19] and immunoassay [20,21] was also reported.

Nothing was reported on the electrochemistry of danazol in general, nor its voltammetric behaviour in particular. This led us to study the voltammetric behaviour of DZ in an attempt to develop a simple and reliable method for its determination in dosage forms and biological fluids. The proposed method can be considered as an alternative substitute for the chromatographic methods. The results obtained were promising.

2. Experimental

2.1. Apparatus

The cyclic voltammogram was obtained using cyclic voltammograph (757 VA Computrace) from Metrohm Herisau, Switzerland. It consists of a hanging mercury drop electrode in the DPP mode, a saturated Ag/AgCl reference electrode and a platinum wire as the auxiliary electrode. Nitrogen was used for deoxygenation. Scan rate was 2 mV/s and mercury drop size was 0.2 mm^2. Current range was 0–400 nA and potential range was from −0.8 to −1.2 V. A computer-driven HP printer was used to get the output.

The polarographic study and the differential pulse polarographic (DPP) measurements were carried out using the Polorecord E 506 Metrohm (Herisau, Switzerland). The drop-time of 1 s was electronically controlled using a 505 Stand from the same company. The polarograms were recorded using a potential scan rate of 10 mV/s. A three-electrode system composed of a dropping mercury electrode (DME) as the working electrode, Ag/AgCl reference electrode and a platinum wire as the auxiliary electrode was used. The pulse amplitude in the DPP mode was −50 mV. The alternating current (ac_t) behaviour was studied using the same instrument. The superimposed alternating voltage being 15 mV at a frequency of 75 Hz and a phase angle of 90°.

2.2. Reagents and materials

- Danazol was obtained from Sanofi Winthrop, France, as a gift and was used as received. Capsules Danol™ containing 100 mg DZ were obtained from the local market.
- Plasma was kindly provided by King Khalid University Hospital and was kept frozen until use after gentle thawing.
- Urine was obtained from healthy volunteers (40 years old).
- Britton Robinson buffer [22] covering the pH range 2.1–10 (0.04 M).
- Hydrochloric acid (0.1 M).
- *n*-Hexane.

A stock solution of danazol (1×10^{-3} M) was prepared in methanol and was further diluted with the same solvent as appropriate. The solutions were stable for at least one week if kept in the refrigerator protected from light.

Solutions of DZ in methanol were stable for one week if kept away from light in the refrigerator. In 0.1 M HCl containing 20% (v/v) methanol, the solutions were stable for at least 2 h.

2.3. Procedures

2.3.1. Recommended procedure

Transfer aliquots of danazol stock solution into a set of 25 ml volumetric flasks, so that the final concentration is in the range from 5×10^{-6} to 1×10^{-4} M. Add sufficient methanol, so that its concentration is always not less than 20% (v/v). Complete to volume with 0.1 M HCl. Pour the contents of the flask into the polarographic cell. Pass nitrogen gas for 5 min. Record the polarograms over the range from −0.8 to 1.4 V using the DPP mode. Plot the produced current versus the final concentration to get the calibration graph. Alternatively, derive the regression equation.

2.3.2. Procedure for the capsules

Empty and mix the contents of 10 capsules. Transfer a weighed amount of the powder equivalent to 33.74 mg of danazol into a 100 ml volumetric flask; add 80 ml of methanol, and shake well for 10 min. Complete to the volume with the same solvent, mix well and filter. Transfer aliquots of the filtrate into 25 ml volumetric flask then proceed as described under Section 2.3.1. The nominal content of the capsules is obtained from the calibration graph or the regression equation.

2.3.3. Procedure for the spiked urine and plasma

Transfer 1 ml of spiked urine or plasma into a small separating funnel. Extract three times each with 3 ml of *n*-hexane. Evaporate the combined hexane extracts under nitrogen at 60 °C. Dissolve the residue in methanol and transfer quantitatively into 25 ml measuring flask. Proceed as described under Section 2.3.1. Determine the nominal content of danazol in urine or plasma from the corresponding regression equation.

3. Results and discussion

3.1. Study of the wave characteristics

Danazol was found to exhibit cathodic wave over the pH range 1–5. It also developed anodic waves over the same pH range, but those waves were ill-defined and the sensitivity was poor, therefore, our study was limited to the cathodic mode. Fig. 1 shows the typical polarogram of danazol in 0.1 M HCl containing 20% (v/v) methanol as a solubilizer. The wave showed cathodic shift upon increasing the pH (Table 1) up to pH 5 then it became broad and overlapped with the hydrogen-over voltage wave. The relation between the half-wave potential E1/2 (mV) and pH is given by the following regression equation:

$$E_{1/2} = 951 + 71\text{pH} \quad (r = 0.991)$$

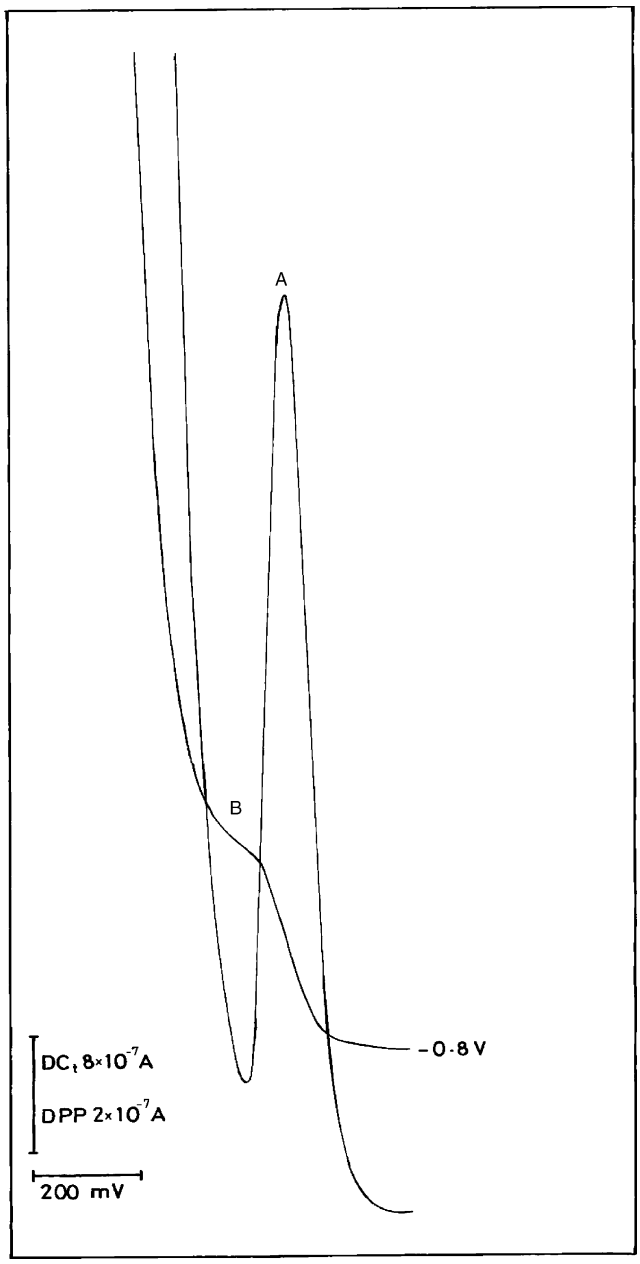


Fig. 1. Typical polarograms of danazol (1×10^{-5} M) in 0.1 M HCl containing 20% (v/v) methanol. (A) DPP polarogram and (B) dc_t polarogram.

Logarithmic analysis of the waves obtained in BRb of different pH values resulted in straight lines with different slopes. Assuming that the rate-determining step involves the transfer of two electrons (a free-radical one electron transfer is not likely to occur because of the polar nature of the medium). The values of the slopes indicate that the reduction process is irreversible in nature. The αn_a values were calculated according to the treatment of Meites and Israel [23] and are listed in Table 1. The irreversibility of the cathodic waves was studied using the cyclic voltammogram of danazol (1×10^{-4} M) in 0.1 M HCl containing 20% methanol and a scan rate of 2 mV/s. Fig. 2 shows that only a cathodic wave is

Table 1
Effect of pH on the development of polarographic waves of danazol

pH	E (mV)	$\Delta E_{1/2}/\Delta$pH	I_d/C	$W_{1/2}$ (mV)	αn_a	Z_{H+}
1	1040		4.8	80	1.35	0.66
		36				
2.1	1080		4.2	90	0.72	0.20
		70				
3	1150		3.9	105	0.69	0.20
		80				
4	1230		3.2	140	0.60	0.15
		90				
5	1320		2.4	160	0.67	0.19

$W_{1/2}$ (mV) is the half-peak width of the wave in the DPP mode; n_a, the number of electrons transferred in the rate-determining step; α, the transfer coefficient; Z_{H^+}, the number of protons transferred in the rate-determining step.

produced over the potential range from −0.8 to −1.2 V, pointing out to the complete irreversibility of waves. The number of protons (Z_{H+}) was calculated using the following formula [24]:

$$\Delta E_{1/2}/\Delta\text{pH} = -\frac{0.059 Z_{H^+}}{\alpha n_a}$$

Increasing the mercury reservoir height (h) resulted in a corresponding increase in wave height (W). A plot of W versus $\sqrt{h}$ gave a straight line. Also a plot of log W versus log h gave a straight line with a slope of 0.61. Changing the ionic strength of the medium (using 0.1 M KCl) had no effect on the value of the current. The temperature coefficient was studied over the range 25–50 °C and was found to be +2.25%/°C. All these facts point to the diffusion-controlled nature of the polarographic wave, although adsorption phenomenon played a limited role in the electrode process as revealed by the slightly high value (0.61) of the slope of the logarithmic plot of the waveheight versus mercury height. This conclusion was supported by studying the alternating current behaviour of danazol in the same supporting electrolyte (0.1 M HCl containing 20%, v/v, methanol) at a phase angle of 90° and superimposed

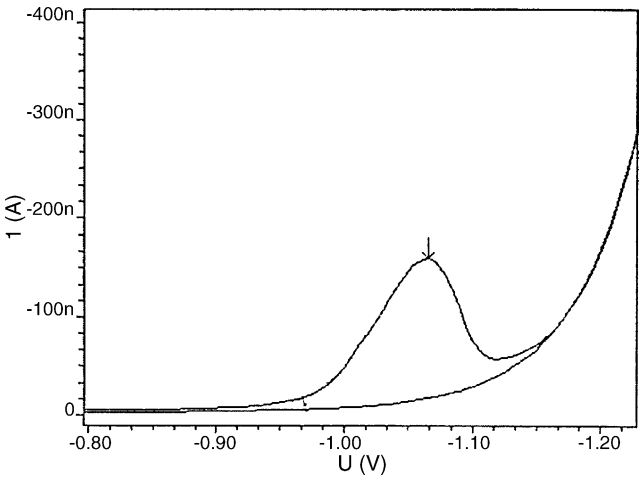


Fig. 2. Cyclic voltammogram of danazol (1×10^{-4} M) in 0.1 M HCl containing 20% (v/v) methanol. Scan rate: 2 mV/s.

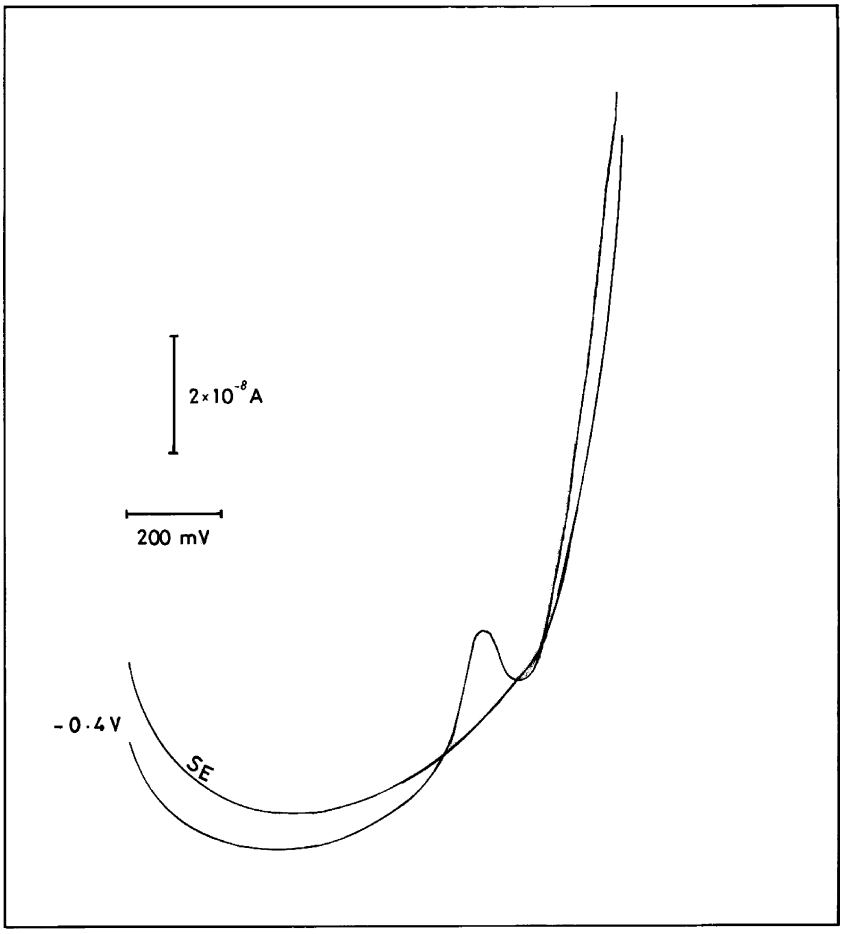


Fig. 3. Alternating current polarogram of danazol (1×10^{-5} M) in 0.1 M HCl containing 20% (v/v) methanol. Phase angle, 90°; superimposed potential, 15 mV; SE, supporting electrolyte.

voltage of 15 mV (Fig. 3). The summit potential (E_s) was shifted 210 mV more negative than the corresponding $E_{1/2}$. The figure demonstrates that the depolarizer (DZ) is adsorbed to the mercury surface while its reduction product is not. The addition of methanol, to the electrolysed solution, however, decreases the effect of adsorption on the electrode process.

Moreover, the value of $E_{1/4}$ to $E_{3/4}$ of −50 mV at pH 1 indicates an irreversible two-electrons reduction process, and the appearance of the ac_t polarographic waves (Fig. 3) indicates that a slow electron transfer is involved in the electrode reaction [25].

3.2. Analytical applications

Polarograms of danazol using dc_t mode in 0.1 M HCl containing 20% (v/v) methanol exhibit well-defined cathodic waves. No polarographic maxima were developed, therefore, no maximum suppressor was needed. The current is diffusion-controlled and is proportional to the concentration over a convenient range. At that pH value, the dc_t wave was the steepest and the DPP peak had the least half-peak width (Table 1).

The relation between the limiting diffusion current in the DPP mode and concentration is rectilinear over the range from 5×10^{-6} to 1×10^{-4} M. Regression analysis of the data gave the following equation:

$$i_p\,(\mu A) = 0.0968 + 1.168 \times 10^4 C \quad (r = 0.995)$$

where i_p is the peak current in the DPP mode and C, the concentration in μg/ml. The lower detection limit is 1×10^{-6} M.

Statistical evaluation of the regression line regarding standard deviation of the residuals ($S_{y/x}$) standard deviation of the slope (S_b) and standard deviation of the intercept (S_a) gave the following values: 0.039 (μA.L.M.), 394 (μA.L.M.) and 0.0138 (μA), respectively. These values point out to the low scattering of the calibration points of the standard curve [26].

The proposed method was applied to the determination of DZ in commercial capsules. The %recovery based on five separate determinations is abridged in Table 2. The results obtained were statistically compared with those given with a reference HPLC method [4]. Statistical analysis of the results obtained by the proposed and the reference method [4], revealed no significant difference between the performance of

Table 2
Application of the proposed method to the analysis of danazol in bulk powder and in capsules

Material	%Recovery	
	Proposed method	Reference method [4]
1. Bulk powder	99 ± 0.6	100 ± 0.6
F	1.26 (3.217)	
t	0.62 (2.262)	
2. Danol capsules		
$\bar{X}$	100	100
±S.D.	1	0.8
F	1.77 (3.217)	
t	0.49 (2.262)	

The figures in parentheses are the tabulated values of *F* and *t* at $p = 0.05$, respectively.

the two method regarding accuracy and precision as revealed by the Student's *t*-test and the variance ratio *F*-test [26].

The sensitivity of the method allowed the determination of the drug in spiked human urine and plasma. Danazol is orally administered in a dose of 200–400 mg daily in two or four divided doses adjusted according to the response. The anticipated blood level concentration will be 4–8 μg/ml (1.18×10^{-5} to 2.36×10^{-5} M) which is within the working concentration range of the proposed method. The results of linear regression analysis of the data relating peak current and concentration in urine and plasma are given in Table 3.

The method was successfully adopted for the determination of DZ in spiked human urine and plasma adopting the standard addition method. The method necessitated extraction with *n*-hexane adopting the method of Nygard et al. [17]. The results abridged in Table 4 are satisfactorily accurate and precise. Typical polarograms of unspiked (A) and spiked urine are shown in Fig. 4.

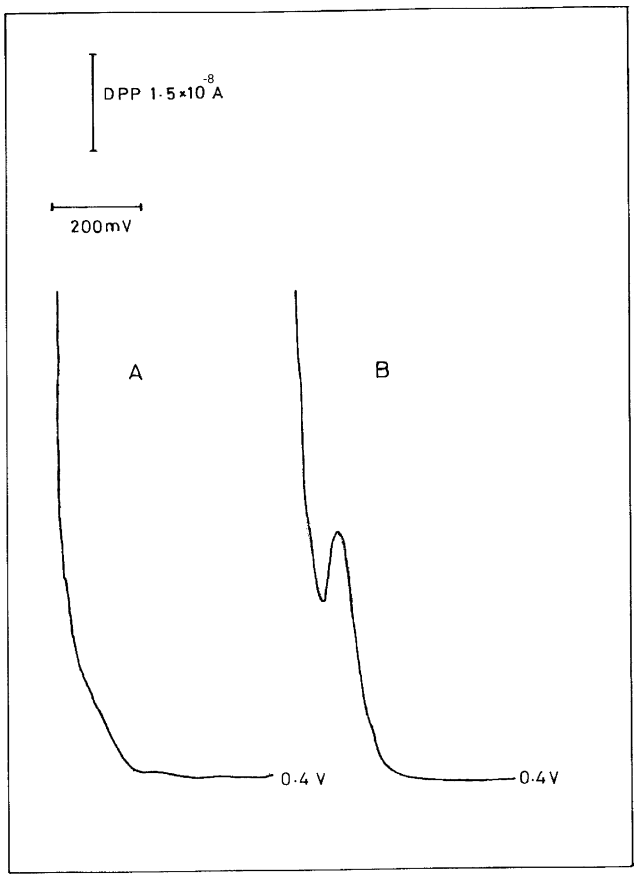


Fig. 4. DPP polarograms of danazol in urine at pH 1. (A) Blank urine and (B) spiked urine (6 μg/ml).

3.3. Interference study

Study of the interference likely to be introduced by some steroidal drugs and some commonly used drugs was studied by recording their polarograms under the same experimental conditions. No interference was encountered from testosterone, diethylstilbesterol, clomiphene citrate, paracetamol, caffeine, ascorbic acid, glycine or cysteine. Ethisterone and hydrocortisone, on the other hand, interfere with the assay.

Table 3
Performance data for the application of the proposed method to biological fluids

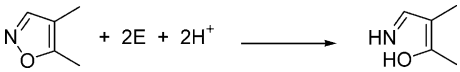
Material	Slope (*b*)	Intercept (*a*)	Correlation coefficient (*r*)	Limit of detection (M)
Urine	$0.04422 \pm 8.8 \times 10^{-4}$	$0.045 \pm 3.9 \times 10^{-3}$	0.9988	9.8×10^{-7}
Plasma	$0.0247 \pm 3.2 \times 10^{-3}$	0.076 ± 0.02	0.9973	6.6×10^{-6}

Table 4
Application of the proposed method to the determination of danazol in spiked human urine and plasma

Urine			Plasma		
Amount added (μg/ml)	Found (μg/ml)	%Recovery	Amount added (μg/ml)	Found (μg/ml)	%Recovery
3.0	2.915	97	4.0	4.135	103
4.0	3.626	91	7.0	6.245	89
5.0	4.621	93			
7.0	7.18	103	10.0	9.958	100
$\bar{X}$		96	$\bar{X}$		97
±S.D.		4	±S.D.		5

3.4. Proposed mechanism of the electrode reaction

The number of electrons transferred during the reduction process was accomplished through comparing the waveheight of DZ with that obtained from an equimolar solution of an earlier studied compound having similar functional group and of nearly identical value of the diffusion-coefficient, namely, azintamide [27]. In 0.1 M HCl containing 20% methanol, both compounds gave one wave. The wave of DZ was double that of azintamide; thus pointing out to the transfer of four electrons (total number). Logarithmic analysis of the waves established that two electrons are transferred in the rate-determining step and the shift in $E_{1/2}$ potentials with increasing pH indicates that two H^+ are consumed in this step. Based on these facts, it is postulated that the isoxazole ring is the site of reduction. The electrode reaction is suggested to proceed as follows:



The separation of the reduction products and the exact mechanism of electrode reaction will be the subject of a further communication.

4. Conclusion

A simple, sensitive, time saving and reliable method was developed for the determination of danazol in dosage forms and spiked human urine and plasma. The method involves a simple cleanup procedure before being applied to biological fluids. The detection limit is comparable to those given by chromatographic methods.

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