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ARTICLE *in* ELECTROANALYSIS · MAY 1995

Impact Factor: 2.14 · DOI: 10.1002/elan.1140070513

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Polarographic Behavior and Determination of Oxamniquine in Dosage Forms

F. Belal and *F. A. Aly**

Department of Analytical Chemistry, Faculty of Pharmacy, University of Mansoura, Mansoura 35516, Egypt

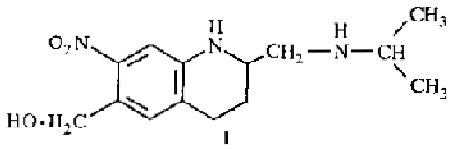
Received: August 2, 1993
Final version: November 3, 1993

Abstract

A highly sensitive method is described for the determination of oxamniquine in its pure form and in capsules. The proposed method depends upon the polarographic activity of oxamniquine in Britton–Robinson buffer, whereby a well-defined cathodic wave, followed by a more negative ill-defined one, was produced over the whole pH range. The first wave was characterized as being irreversible and diffusion-controlled with limited absorption properties. The current–concentration relationship was found to be rectilinear over the ranges 1×10^{-5} to 5×10^{-4} and 1×10^{-6} to 5×10^{-4} M in the direct current (DC_T) and differential pulse polarography (DPP) modes, respectively, with a minimum detectability of 1×10^{-7} M. The number of electrons involved in the reduction process was established through coulometric measurement at an applied potential. The mechanism of the electrode reaction was verified and confirmed through chemical reactions of the electrolyzed solution. The proposed method was successfully applied to the determination of oxamniquine in capsules, and the results obtained were in accordance with those found using the United States Pharmacopeia (USP) XXII method.

Keywords: Oxamniquine, Pharmaceutical analysis, Polarography

1. Introduction



I

Oxamniquine (**I**) is an oral schistosomicidal drug which is widely prescribed in tropical countries. It is the drug of choice for the treatment of *Schistosomia mansoni* infections. Clinical studies show that it gives high cure rates and is well tolerated and effective in all stages of infection, including the early acute and the later chronic and complicated phases [1]. As a consequence of its efficacy, safety, and convenience of treatment, oxamniquine can be administered by paramedical personnel under field conditions, and was the first schistosomicidal drug suitable for mass administration. It has thus made a major contribution towards the control and treatment of *S. mansoni* infections, which is very satisfying for those associated with its discovery and development.

The USP (XXII) [2] recommends a direct spectrophotometric measurement for the quantitation of oxamniquine, both per se and in capsules. A more specific spectrophotometric method based on reduction using Zn/HCl, followed by diazotization and coupling with ethyl acetoacetate, has been used [3]. Other reported methods include nonaqueous titrimetry [4], gas chromatography [5], and high performance liquid chromatography [6, 7]. Reviewing the literature revealed that, up to the present time, nothing has been published concerning its electrochemical reactivity in general nor its polarographic behavior in particular. The presence of a nitro group attached to an aromatic moiety initiated the present study.

2. Experimental

2.1. Reagents

Oxamniquine was kindly provided by Pfizer (Sandwich, UK) and used as received. Vansil capsules (containing 250 mg of oxamniquine) were obtained from commercial sources. The following solutions were used: a 0.05% freshly prepared [8] aqueous solution of gelatin, 0.08 M Britton–Robinson buffers (BRB) [8], covering the pH range 2.09–9.37, and methanol (AR grade, Aldrich).

A stock solution (4×10^{-3} M) of oxamniquine in methanol was prepared. This solution was further diluted with methanol to give the appropriate concentrations for the working solutions. The methanol concentration in the polarographic cell was kept always at 20%. The solutions were purged with pure nitrogen for 5 min then polarographed at ambient temperature.

2.2. Apparatus

The polarographic study and the DPP measurements were carried out using the Polarecord E 506 Metrohm (Herisau, Switzerland). The drop-time of 1 s was electronically-controlled using a 663 VA stand from the same company. The polarograms were recorded using a potential scan of 100 mV for 10 s. A three-electrode system composed of a DME as the working electrode, an Ag/AgCl reference electrode, and a graphite rod as the auxiliary electrode was used. Phase-selective AC_T polarograms of 1×10^{-4} M solution were recorded using the same instrument, the superimposed alternating voltage (AC) being 15 mV at a frequency of 75 Hz and a phase angle of 90°.

The cyclic voltammetry (CV) apparatus consisted of a Wenking LB 75H potentiostat, a Wenking VSG 72 function generator, a Goldstar DM-6135 digital multimeter and a Philips PM-8271 *xyt* recorder. A three-electrode system composed of a graphite working electrode, an Ag/AgCl reference electrode, and a platinum auxiliary electrode was used. The voltammograms of 1×10^{-3} M solution were recorded at different scan rates of 20, 50, 100, 200, 300, 400, and 500 mV/s.

The coulometry measurements at a controlled potential were carried out using the Wenking multifunction assembly, consisting of a Wenking potentiostat Model 70 HVI, a Wenking integrator SSI 70, a multipurpose unit Model E 505, Metrohm (Herisau, Switzerland), and a recorder 626 Metrohm Polarecord. A three-electrode system (comprising a

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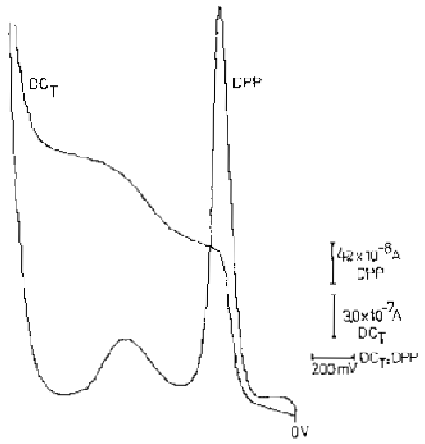

Fig. 1. Typical polarogram of oxamniquine (1×10^{-4} M) in BRB pH 4.77 containing 20% methanol.

stirring mercury pool as the working electrode, an Ag/AgCl reference electrode, and a graphite rod as the auxiliary electrode) was used. Solutions of oxamniquine in acetate buffer of pH 4.2 containing 50% methanol were electrolyzed at −0.4 and −1.0 V.

2.3. Procedure for Capsules

Empty out the contents of 20 capsules as completely as possible. Mix well and transfer a weighed quantity equivalent to 28 mg of oxamniquine. Dissolve in 50 mL methanol and filter. Transfer 1 mL of the solution into the polarographic cell, add 3 mL methanol and 16 mL BRB pH 4.77. Record the DC_T and DPP polarograms. Calculate the nominal content using a calibration graph or the regression equation.

3. Results and Discussion

Figure 1 shows a typical polarogram of oxamniquine (1×10^{-4} M solution) in BRB of pH 4.77. A well-defined cathodic wave followed by a more negative, ill-defined one is produced. The wave height ratio of the first wave to the second one is always 2 : 1. The two waves increase as a linear function of pH (Fig. 2). The relations between $E_{1/2}$ and pH for the first and second waves are expressed by the following equations,

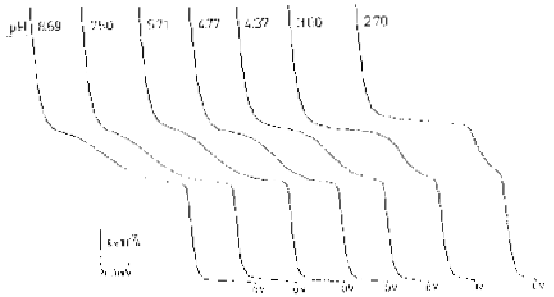

Fig. 2. Effect of pH on the development of the polarographic waves of oxamniquine (2×10^{-4} M).

Table 1. Effect of pH on the development of the polarographic waves of oxamniquine.

pH	$E_{1/2}$ (V)	$\Delta E_{1/2}/\Delta pH$ (mV)	αn_a
2.09	0.17	−49.2	1.102
2.70	0.20	−44.0	1.148
3.60	−0.24	−65.0	1.174
4.37	−0.28	−62.0	1.200
4.77	0.31	−53.0	1.420
5.71	−0.36	−57.0	1.50
6.59	0.41	−55.0	1.543
7.50	−0.46	−50.0	1.688
8.69	−0.51	−44.0	1.862
9.37	−0.55		1.929

respectively

$$E_{1/2} = -0.0538 - 0.0535\,\mathrm{pH} \quad (R^2 = 0.99940) \qquad (1)$$

$$E_{1/2} = -0.0693 - 0.1330\,\mathrm{pH} \quad (R^2 = 0.97549) \qquad (2)$$

Logarithmic analysis of the reduction wave (i.e., the first wave) obtained in BRB of different pH values resulted in straight lines. Assuming that the rate-determining step involves the transfer of two electrons (a free radical, one electron transfer is not likely to occur), the values of the slopes suggest that the reduction process is irreversible in character. The αn_a values were calculated using the treatment of Meites and Israel [9], and are listed in Table 1. It is noticed that the degree of reversibility

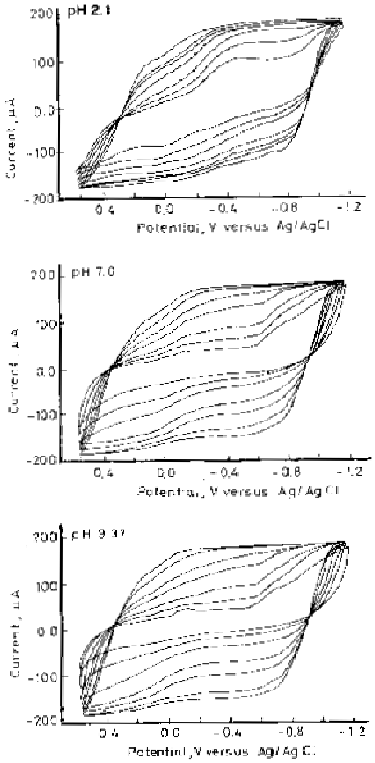

Fig. 3. Cyclic voltammograms of oxamniquine (1×10^{-3} M) in BRB of different pH values. Scan rate = 20, 50, 100, 200, 300, 400, and 500 mV/s.

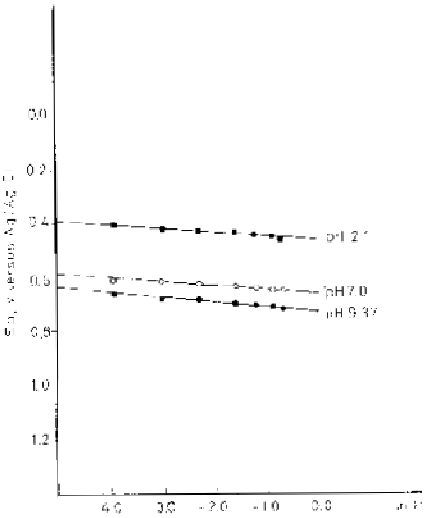

Fig. 4. Plot of peak potential versus ln v.

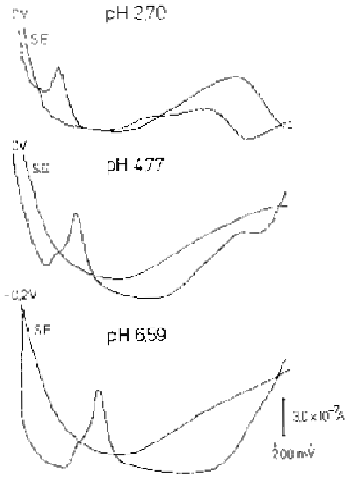

Fig. 5. Alternating current behavior of oxamniquine (1×10^{-4} M) in BRB of different pH values. Superimposed alternating voltage: 15 mV; frequency: 75 Hz; phase angle: 90°. (S.E.: supporting electrolyte.)

increases as the pH increases. This was further confirmed by CV measurements.

Figure 3 shows the voltammograms of oxamniquine (1×10^{-3} M solution) in BRB of pH 2.1, 7.0, and 9.37. Two cathodic peaks are obtained using the different scan rates (20–500 mV/s). The peak potentials of the first one display a cathodic shift on increasing the scan rate, thus revealing the irreversible nature of the reduction process [10].

Applying the relationship that correlates the peak potential (E_p) with the scan rate (v), the αn_a values are evaluated from the following equation [11]

$$E_p = 1.14\frac{RT}{\alpha n_a F} + \frac{RT}{\alpha n_a F}\ln\left(\frac{K_f^0 h}{D^{1/2}}\right) - \frac{RT}{2\alpha n_a F}\ln(\alpha n_a v) \quad (3)$$

Also, on plotting E_p versus ln v (for the first wave) at different pH values (Fig. 4), a linear relationship was obtained. The gradients of the slopes are proportional to αn_a. The gradient of the slope increases as the pH is increased, thus as the value of αn_a increases so does the degree of reversibility increase as the pH increases.

The number of protons, Z, consumed in the electrode reaction is given by [12]

$$\Delta E_{1/2}/\Delta \mathrm{pH} = -0.059\, Z/\alpha n_a \quad (4)$$

where α is the transfer coefficient. The value of αn_a is calculated from the following equation

$$E = E_{1/2} - (0.059/\alpha n_a)\log[i/i_d - i)] \quad (5)$$

where i_d is the limiting current. At pH 4.77 Z was found to be 1.62, i.e., 2 protons are probably consumed in the electrode reaction.

3.1. Study of the Wave Character

Increasing the mercury height (h) resulted in a corresponding increase in the wave height (w); a plot of $\sqrt{h}$ vs. the wave height gave a straight line. A plot of log h vs. log w gave a straight line, the slope of which was 0.5. Changing the buffer concentration over the range 0.01–0.08 M resulted in a negligible increase in the wave height. The wave height showed a linear correlation with increases in the temperature. The temperature coefficient calculated according to Meites [13] was +1.606%/°C over the range 20–50°C. These three characteristics point to a diffusion-controlled process.

At high concentrations (more than 1×10^{-4} M), a sharp maximum of the first kind was produced. This could be suppressed by the addition of a few drops of 0.5% gelatin solution. The addition of gelatin, however, did not affect the steepness of the wave.

The alternating current behavior (ΔC_T) of 1×10^{-4} M solution of oxamniquine was studied using a phase selective angle of 90°. In BRB of pH 2.4, 4.77, and 6.59, the peak potentials were 195, 185, and 205 mV more negative than the corresponding $E_{1/2}$ values, respectively. Figure 5 demonstrates that at pH 4.77 and 6.59, adsorption of the depolarizer and its reduction product occurs. The diffusion coefficient (D) was calculated using the Ilkovic equation [14]

$$i_d = 607\, n C D^{1/2} m^{2/3} t^{1/6} \quad (6)$$

In BRB 4.77, D was found to be 4×10^{-6} cm^2 s^{-1}.

Solutions of oxamniquine in methanol were stable overnight and for three days if kept in the refrigerator. Solutions of oxamniquine in BRB pH 4.77 containing 20% methanol were stable for more than 4 h.

The relation between the limiting current, i_d (μA), and the concentration, C (mM), was found to be rectilinear over the concentration range 1×10^{-5} to 5×10^{-4} M and 1×10^{-6} to 5×10^{-4} M in the DC_T and DPP modes, respectively. Linear regression analysis of the above data gave the following equations

$$C = 0.00065 + 0.09017\, i_d \quad (R^2 = 0.99924) \quad (7)$$

using the DC_T mode, and

$$C = -0.00056 + 0.02254\, i_d \quad (R^2 = 0.99940) \quad (8)$$

using the DPP mode, with a minimum detectability of 1×10^{-7} M. The diffusion-current constant [$I_d = i_d/(C m^{2/3} t^{1/6})$] was calculated at 25°C and found to be 4.71 ± 0.09 (Table 2).

Table 2. Correlation between concentration and limiting current in the DC_T mode.

No.	*Concentration [mM]*	*Current [a] [μA]*	i_d/C *[μA/mM]*	$I_d = i_d/(C m^{2/3} t^{1/6})$
1	1.00×10^{-2}	0.0924	9.24	4.71
2	1.25×10^{-2}	0.114	9.12	4.65
3	2.50×10^{-2}	0.228	9.12	4.65
4	6.25×10^{-2}	0.594	9.25	4.71
5	0.1250	1.132	9.05	4.61
6	0.1875	1.800	9.60	4.89
7	0.2500	2.270	9.08	4.62
8	0.3125	2.920	9.34	4.76
9	0.3700	3.500	9.40	4.82
10	0.4000	3.700	9.25	4.71
11	0.5000	4.600	9.20	4.69
$\bar{X}$			9.24	4.71
SD			0.07	0.09
RSD			1.829%	1.908%

[a] Each result is the average of three separate determinations.

3.2. Number of Electrons Involved in the Electrode Reaction

Two approaches were adopted to determine the number of electrons consumed during the reduction; the first was through comparison of the wave height of oxamniquine with that obtained from an equimolar solution of a previously studied compound, i.e., nitrobenzene. In BRB pH 4.77 both compounds gave two waves. The first waves were of the same height, corresponding to a four-electron transfer process for the first wave and, consequently, two electrons for the second one. The second approach was through coulometric measurement at a controlled potential. The BRB was found to disturb the wave (this disturbance was probably due to interaction between the boric acid content of the buffer and the hydroxyl group of the side chain) and rendered the electrolysis process very slow. Therefore acetate buffer was used instead. Applying a potential of −0.4 V and −1.0 V, the number of coulombs consumed corresponded to the consumption of four and six electrons, respectively.

It is evident from the experimental results that a slow electron-transfer reaction is involved in the reduction of oxamniquine. Logarithmic analysis of the waves established that two electrons are involved in the rate-determining step of the first wave, and the shift in the $E_{1/2}$ potentials with increasing pH indicates that two hydrogen atoms are consumed in this step. However, coulometric measurement at an applied potential established that four electrons are consumed in the overall reduction of the first wave and two more electrons for the second wave, i.e., a total of six electrons for the two waves. Based on these facts the following mechanism [15] may be postulated for the first polarographic wave

$$R\text{-}NO_2 + 2H^+ + 2e \xrightarrow{\text{slow}} R\text{-}NO + H_2O \quad (9)$$

$$R\text{-}NO + 2H^+ + 2e \xrightarrow{\text{fast}} R\text{-}NHOH \quad (10)$$

The second reduction of oxamniquine involves two electrons and is due to a further reduction of the hydroxyl-amino group to the primary amine

$$R\text{-}NHOH + 2H^+ + 2e \xrightarrow{\text{slow}} R\text{-}NH_2 + H_2O \quad (11)$$

This last reduction step is highly irreversible and the polarographic wave was poorly defined.

The proposed mechanism was confirmed by testing for the primary aromatic amine in the electrolyzed solution (at an applied potential of −1.0 V) using the diazocoupling reaction [3], yellow color peaking at 350 nm was produced.

Table 3. Content uniformity test results of oxamniquine in commercial capsules using the proposed and official methods.

No.	*Recovery adopting the proposed methods [%]*	
	DC_T	*DPP*
1	101.53	102.40
2	102.05	102.05
3	101.90	101.35
4	101.85	101.88
5	102.37	101.98
6	101.16	102.10
7	101.80	102.40
8	101.60	102.40
9	101.19	101.15
10	101.17	101.30
$\bar{X}$	101.76	101.80
SD	0.4873	0.5320
RSD	0.4789	0.5230

3.3. Analytical Applications

Polarograms of oxamniquine in BRB pH 4.77 with a few drops (six drops) of 0.5% gelatin solution exhibit a very well-defined cathodic wave. The current is diffusion-controlled and proportional to the concentration over a convenient range. Both DC_T and DPP modes were successfully applied to the content uniformity test of oxamniquine in commercial capsules (250 mg), and the results obtained are shown in Table 3.

Both DC_T and DPP were applied for the determination of oxamniquine in capsules. The percentage recoveries, based on six separate determinations, were 102.5 ± 0.27 and 101.9 ± 0.15, respectively. The result obtained using the official USP method [2] was 102.05 ± 0.42. Statistical analysis [16] of the results obtained by both methods using the student *t*-test and variance ratio *F*-test, shows no significant difference between the performances of the two methods as regards the accuracy and precision, respectively. However, the proposed method is more specific (less subject to interferences) and more sensitive. The proposed method is based on the presence of the nitro group; the latter is essential for the pharmacological activity of oxamniquine [17]. Hence the method measures the biological activity of the compound.

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