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Voltammetric and HPLC determination of dorzolamide hydrochloride in eye drops

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The behaviour of dorzolamide hydrochloride (DOR) was investigated at a glassy carbon electrode in different buffer systems using cyclic (CV), linear sweep (LSV) and differential pulse voltammetry (DPV). The oxidation process was found to be irreversible over the pH range studied (2.0–8.0) and was shown to be diffusion controlled. An analytical method with adequate precision and accuracy was developed for the determination of DOR in Britton-Robinson buffer (BRb) at pH = 3.06 containing 10% methanol as supporting electrolyte. The peak current varied linearly with DOR concentration in the range 4.0×10^{-5}–6.0×10^{-4} M. Furthermore, a HPLC method with diode array detection was developed. A calibration graph was established for 1.1×10^{-6}–1.9×10^{-4} M of DOR. The procedures were successfully applied for the determination of the drug in eye drops.

1. Introduction

Dorzolamide hydrochloride (DOR), [(−)-(*SS*)-4-ethylamino-5,6-dihydro-6-methyl-7,7-dioxide-4*H*-thieno (2,3-*b*) thio-pyran-2-sulfonamide], is a carbonic anhydrase inhibitor. It is used topically in the management of open-angle glaucoma and ocular hypertension, either alone or as adjunctive therapy with a topical beta blocker [1].

The methods available for the analysis of DOR in eye drops and biological fluids are based on HPLC assay with UR detection [2–6]. Spectrophotometric [7, 8] and capillary electrophoresis [9] methods also have been used for the analysis of DOR in eye drops.

In the literature, no example of the electrochemical determination of DOR in eye drops was found. In the recent years, modern electroanalytical methods, such as cyclic voltammetry (CV), linear sweep voltammetry (LSV) , and differential pulse voltammetry (DPV), have been applied for the determination of compounds of pharmaceutical interest [10–14]. In general, these methods are faster, easier, cheaper and more sensitive than spectrometric and HPLC methods.

The aim of the present work was to investigate the determination of DOR at a glassy carbon electrode using CV, LSV and DPV. An electroanalytical procedure for the determination of DOR in eye drops was optimized. The voltammetric techniques data were compared with the HPLC method chosen as reference.

2. Investigations, results and discussion

2.1. Voltammetry

The electrooxidation of DOR was investigated in 0.2 M H_2SO_4, BRb and phosphate buffers (Pb).

A cyclic voltammogram of 1.0×10^{-4} M DOR in BRb at pH = 3.06 containing 10% methanol at a scan rate of 100 mV · s^{-1} is shown in Fig. 1. One well-defined anodic peak was observed at 943.5 mV. The fact that no peak was observed in the reverse scan suggests that the oxida-

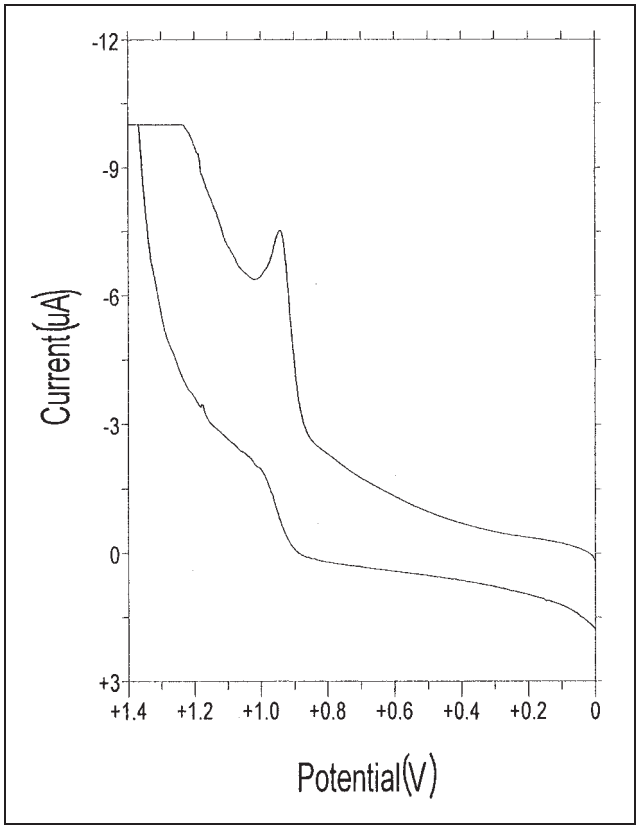

Fig. 1: Cyclic voltammogram of DOR (1.0×10^{-4} M) in BRb of pH: 3.06, containing 10% methanol, at scan rate of 100 mV · s^{-1}

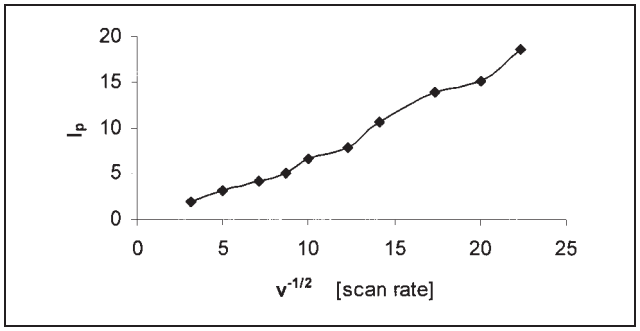

Fig. 2: Variation of peak current, i_p (μA), with scan rate, $v^{1/2}$ (Vs^{-1})

tion process is an irreversible one. The effect of potential scan rate on the peak current and the peak potential of DOR was evaluated. Fig. 2 shows the influence of the square root of the scan rate on the peak current. A linear relationship was observed between 10 and 500 $mV \cdot s^{-1}$ which is typical for diffusion-controlled currents.

The logaritm of peak current - logarithm of scan rate (log i_p-log v) relationship was also linear (log $i_p = 0.49$ log v + 0.14). The slope means that the reaction is diffusion controlled. No important surface reaction effect occurs under the given conditions. From the curves obtained in BRb of pH = 3.06 with different concentrations of DOR the currents at 943.5 mV were recorded. Log i-log C relationship was obtained as log i = 0.99 log C +0.47. It can be seen from the slope that the reaction order was found to be 1.

The peak potential shifted to less positive values on increasing scan rate, which confirms the irreversible nature of the oxidation process at the glassy carbon electrode using CV.

The value of αn, the product of transfer coefficient and number of electrons transferred in the rate determining step, was determined from a Tafel plot (log i vs E) of the voltammetric curves. The Tafel plot was obtained with a scan rate of 5 $mV \cdot s^{-1}$ beginning from a steady-state potential in BRb of pH = 3.06. From the slope of the linear part αn was found to be 0.31. The αn value obtained shows the total irreversibility of the electron transfer process.

The effect of pH was investigated in phosphate and BR buffers. In Fig. 3 the peak potential vs pH plot is presented. The potential of the anodic peak decreases linear with pH. In the range pH = 5.0–8.0, it was constant. In Fig. 4, differential pulse voltammograms obtained in BR buffers of different pH values are shown. Best results were obtained at pH = 3.06. The effect of the DOR concentration on the peak current at +1012.0 mV shows a linear relationship between peak current and concentration. The results showed that quantitative determination of DOR could be made by DPV. The optimum conditions were found as pH = 3.06 BRb. 9 : 1 v/v methanol : water;

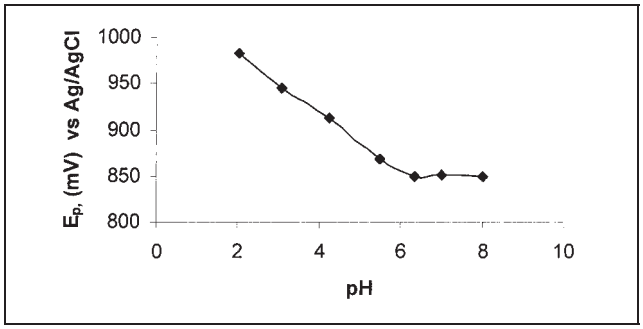

Fig. 3: Effect of pH on peak potentials for 1.0×10^{-4} M DOR solutions in BRb at pH: 3.06 by means of DPV at a glassy carbon electrode

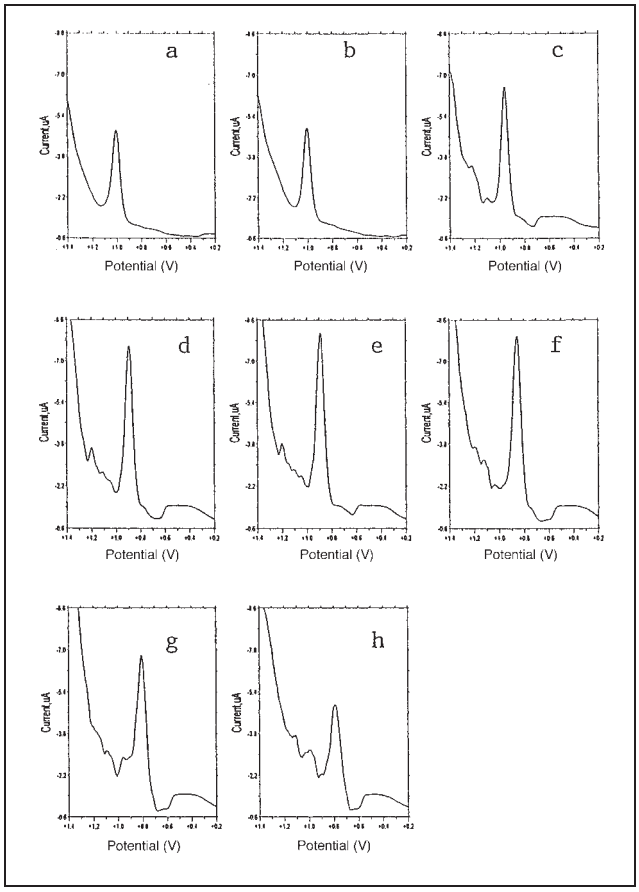

Fig. 4: Differential pulse voltammograms recorded in BR buffers of different pH containing 1.0×10^{-4} M DOR, scan rate 20 mVs^{-1}. a) pH: 1.53; b) pH: 2.02; c) pH: 2.87; d) pH: 3.06; e) pH: 5.00; f) pH: 6.01; g) pH: 7.24; h) pH: 8.16. Scan rate 20 $mV \cdot s^{-1}$, pulse amplitude 50 mV; sample width 17 ms, pulse width: 50 ms, pulse period 200 ms.

20 $mV \cdot s^{-1}$ scan rate, 50 mV pulse amplitude, 17 ms sample width, 50 ms pulse width, 200 ms pulse period.

On the basis of the electrochemical oxidation of DOR at a glassy carbon electrode methods were developed involving CV, LSV and DPV for the determination of the drug. The results are seen in Table 1.

2.2. High-Performance Liquid Chromatography

The reversed-phase HPLC method was developed for the rapid quality control of DOR and as reference method for the developed voltammetric methods.

Mobile phase and flow rate selection were based on peak parameters (height, asymmetry, tailing), baseline drift, run time and ease of preparation of the mobile phase. The system with methanol : acetonitrile : phosphate buffer (8 : 10 : 85 v/v/v) mobile phase and 1.2 $ml \cdot min^{-1}$ flow rate proved to be quite robust. A RP-YMC pack ODS A-132 C_{18} column is recommended because of its ruggedness and reproducibility. Ibuprofen was applied as an internal standard (I.S.). A typical chromatogram for DOR and ibuprofen (I.S.) using RP- YMC pack ODS A-132 C_{18} (150 × 4.6 mm i.d., 5 μm) column at flow rate of 1.2 $ml \cdot min^{-1}$ is shown in Fig. 5. The optimum wavelength for detection was 250 nm . The average retention times for DOR and ibuprofen were found to be 4.1 ± 0.04, and 2.6 ± 0.01 min, respectively. Under the described HPLC parameters, the respective compounds were clearly separated and their corresponding peaks were sharply developed at reasonable retention times.

Table 1: Determination of DOR by CV, LSV, DPV , and HPLC.

Parameters	CV	LSV	DPV	HPLC
Range (M)	$4.0 \times 10^{-5} - 6.0 \times 10^{-4}$	$3.8 \times 10^{-5} - 6.2 \times 10^{-4}$	$3.8 \times 10^{-5} - 6.2 \times 10^{-4}$	$1.1 \times 10^{-6} - 1.9 \times 10^{-4}$
Regression equation (Y)[a]				
Slope (b)	1.83×10^{4}	2.34×10^{4}	8.02×10^{4}	1.32×10^{-2}
Std. dev. on slope (S_b)	1.87×10^{-5}	4.72×10^{-5}	3.81×10^{-4}	2.34×10^{-6}
Intercept (a)	0.15	0.74	0.88	2.63×10^{-2}
Std. dev. on intercept (S_a)	1.89×10^{-7}	4.63×10^{-6}	3.58×10^{-4}	2.14×10^{-6}
Std. error of estimation (S_e)	4.52×10^{-6}	4.21×10^{-6}	2.24×10^{-3}	1.17×10^{-7}
Correlation coefficient (r)	0.9980	0.9984	0.9997	0.9999

a Y = a+bC where C is concentration in M and Y in peak area and current units for HPLC and voltammetric methods, respectively

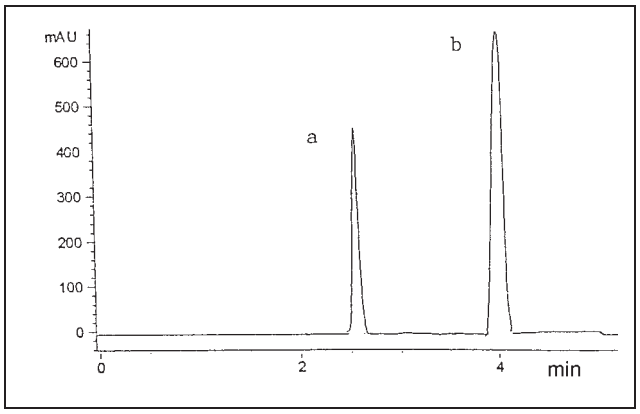

Fig. 5: HPLC chromatogram of a 20 µl injection containing 1.0×10^{-4} M of ibuprofen (I. S.) (a) and (b) 1.0×10^{-4} M of DOR (b)

The calibration curve was linear in the range of 1.1×10^{-6}–1.9×10^{-4} M. The calibation curve equation is $Y = a + bC$, where Y represents the DOR peak area to ibuprofen (I.S) peak area ratio and C represents the DOR concentration. Table 1 represents calibration characteristics for the peak area ratio of varying amounts of DOR to a constant amount of ibuprofen (1.0×10^{-4} M).

2.3. Validation

The validation of the procedures was carried out via the evaluation of the limit of detection (LOD), limit of quantitation (LOQ), repeatability, recovery, specificity, and robustness. The LOD and LOQ were calculated from the calibration curves as kSD/b where $k = 3$ for LOD and 10 for LOQ, SD is the standard deviation of the intercept and b is the slope of the calibration curve. The values of LOD and LOQ were 6.2×10^{-6} M (for CV), 6.4×10^{-6} M (for LSV), 4.4×10^{-6} M (for DPV), 3.5×10^{-7} M (for HPLC) and 8.8×10^{-6} M (for CV), 7.0×10^{-6} M (for LSV), 6.5×10^{-6} M (for DPV), 8.8×10^{-7} M (for HPLC), respectively. Repeatability and recovery were examined by performing five replicate measurements for the concentration of $4.0 \times 10^{-5} - 6.0 \times 10^{-4}$ M DOR (for CV), $3.8 \times 10^{-5} - 6.2 \times 10^{-4}$ M (for LSV), $3.8 \times 10^{-5} - 6.2 \times 10^{-4}$ M (for DPV) and 1.1×10^{-6}–1.9×10^{-4} M (for HPLC), respectively. Mean recoveries of 99.2 ± 0.48 for CV, 98.2 ± 0.63 for LSV, 99.8 ± 0.52 for DPV, and 99.9 ± 0.28 for HPLC were achieved.

Table 2: Comparative studies for DOR formulations

Analysis techniques	CV	LSV	DPV	HPLC
Commercial dosage forms[a]				
Mean[b]	2.1	1.8	1.9	2.1
R.S.D (%)	0.14	0.72	0.17	0.09
Calculated t value	0.96	1.08	1.14	
t, theoretical (p = 0.05)	2.26	2.26	2.26	

[a] 2.0% of DOR per drop (Trusopt®)
[b] Each value is the mean of ten experiments

The intra-day reproducibilty of the methods was evaluated for five independent determinations of 1.0×10^{-4} M solutions, yielding relative standard deviations of 1.75, 0.86 and 0.64%, for CV, LSV and DPV, respectively. The RSD value for intra-day assay reproducibilty at 1.0×10^{-4} M solutions was found to be 1.24, 0.65 and 0.34% for CV, LSV and DPV, respectively, indicating good repeatability and accuracy of the methods.

The applicability of the proposed voltammetric methods and HPLC for the assay of a simple dosage forms was examined by analysing eye drops. The voltammetric results were compared with the HPLC results by means of student's t-test at 95% confidence level. No significant difference was found (Table 2).

Based on the above results, all the developed methods may be recommended for routine and quality control analysis of the investigated drug in pharmaceutical dosage forms.

3. Experimental

3.1. Apparatus

Voltammetric measurements were taken using a BAS 100 W/B electrochemical analyser and a HP 1100 laserjet printer. The three-electrode system was composed of a BAS MF 2012 glassy carbon disc electrode, a BAS MF 1063 type silver/silver chloride/saturated KCl reference electrode and a BAS MV 1032 platinum wire auxiliary electrode. The potentials in the text were given versus silver/silver chloride electrode.

The HPLC system consisted of a membrane degasser, a binary solvent delivery system, a Rheodyne injector equipped with a 20 µl sample loop, and a diode array and multiple wavelength UV/VIS detectors (1100 Series, Agilent Technologies, USA). The detection wavelength was at 250 nm, and the peak areas ratio were integrated automatically with Windows NT based LC ChemStation Software.

3.2. Reagents

DOR was obtained from MSD Pharm. Co. and ibuprofen from Eczacibas Pharm. Co. was obtained and were used without prior purification.

Analytical grade phosphoric acid, HPLC grade methanol and acetonitrile were purchased from Merck Co. All other chemicals were of analytical-reagent grade and were used as received.

3.3. Solution preparation

A stock solution of 1×10^{-4} M DOR was prepared in 9 : 1 (v/v) methanol : water. Diluted working standard solutions were then prepared daily from fresh stock solution and contained 9 : 1 (v/v) methanol:water. Tests were performed in 0.5 M H_2SO_4 and BR buffers. BR buffers were prepared from 0.04 M phosphoric, acetic and boric acids. pH was adjusted by the addition of 6.0 M NaOH solution.

3.4. Pretreatment of the working electrode

The glassy carbon (GC) electrode was polished with 0.5 µm alumina powder on a polishing cloth prior to each electrochemical measurement. Then, it was thoroughly rinsed with methanol and double distilled water, and gently dried with a tissue paper.

3.5. Chromatographic conditions

Reversed phase technique was used. The mobile phases included methanol: acetonitrile: phosphate buffer (8 : 10 : 85 v/v/v). The analytical column was a RP-YMC pack ODS A-132 C_{18} (150 × 4.6 mm i.d., 5 μm) column. All analyses were done under isocratic conditions at a flow rate of 1.2 ml · min^{-1} and at room temperature. A diode array detector was fixed at 250 nm. All solvents were filtered through a 0.45 μm milipore filter and degassed in an ultrasonic bath.

3.6. Procedure

Stock solutions of concentrations $3.8 \times 10^{-5} - 6.2 \times 10^{-4}$ M DOR were prepared in methanol and stored in dark bottles at +4 ^{0}C. The working solutions under voltammetric investigations were prepared by dilution of the stock solution and contained 9 : 1 (v/v) methanol : water. The DOR did not change its concentration with time. BRb (0.04 M) in the pH range 2.0–8.0 was used as supporting electrolyte when studying the influence of pH. The pH was adjusted to the desired value by adding the required volume of a 5.0 M NaOH solution. Phosphate buffer (0.05 M) in the pH range 2.0–8.0 was used as supporting electrolyte when studying the influence of pH. The pH was adjusted to the desired value by adding the required volume of a phosphoric acid solution. On the other hands, working solutions (1.1×10^{-6} to 1.9×10^{-4} M) were prepared by the appropriate dilutions of the stock standard with mobile phase for HPLC. All working solutions were prepared freshly every day.

3.7. Analysis of eye drops

A commercial pharmaceutical preparation (Trusopt® eye drops MSD Pharm. Ind., Turkey, containing 2.0% of DOR and 0.00075% benzalkonium chloride and water q.s. per drop) was assayed. 1.0 ml of eye drops was transferred into a volumetric flask and diluted with methanol. Thus a stock of 1.0×10^{-4} M was prepared. All the test solutions were obtained by diluting this stock solution with the selected supporting electrolyte. Voltammograms were recorded. The HPLC determination of DOR was made by adding an aliquot of the above mentioned solution to the mobile phase. Then these solutions were filtered through 0.45 μm membrane filters. Triplicate 20 μl injections were made for each solution.

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