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Pulse polarographic (constant and increasing) determinations of doxazosin in pharmaceutical tablets

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Abstract

The optimum conditions using DC polarography and the determination of doxazosin employing SIAP and SCAP polarographic techniques are described in this study. All the experiments were conducted in the supporting electrolyte consisting of 20% ethanol (v/v), 0.2 M KCl and 0.2 M acetate buffer at various pH values in order to examine the optimum conditions, and pH 3.5 for the determination of doxazosin. Well-defined curves were obtained in the pH range of 1.5–7.5. The system was diffusional and irreversible at pH 3.5. The calibration studies were performed by using SIAP and SCAP polarography and satisfactory results were observed for all techniques. Since the sensitivity of SIAP and SCAP techniques were higher than the others, the determination of doxazosin was performed in filtered and unfiltered tablet solutions containing 4 mg active material. In the analysis of a tablet, the relative standard deviations (S_{rel} %) of the techniques are in the filtered solutions ± 0.9 (SIAP), ± 0.8 (SCAP) and in the unfiltered solutions ± 0.7 (SIAP), ± 0.8 (SCAP) and no interference was observed during the analysis. The determination methods proposed in this study appear to be accurate, rapid and practicable. Therefore, these techniques may be suitable for the content uniformity tests.

Keywords: Polarography of doxazosin; Determination of doxazosin; Pharmaceutical application; Quality control assay method

1. Introduction

Doxazosin mesylate [(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(1,4-benzodioxan-2-yl-carbonyl)-piperazine monomethansulphonate] (DOX) is a postsynaptic α-1 adrenoreceptor antagonist. It is structurally similar to prazosin and its chemical structure is demonstrated in Fig. 1.

DOX is a potent antihypertensive agent and it is very effective when administered either orally or intravenously. It is slowly eliminated in man and its long half-life provides the basis for once-daily dosing [1,2].

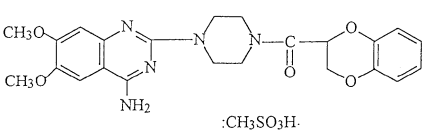

Fig. 1. The chemical structure of DOX.

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To date it appears that only HPLC methods have been employed for the determination of DOX. Previous studies cover the determination of active material in the body fluids depending on the pharmacological evaluations [3–6].

The DOX molecule has a quinazoline group and it has been reported that this group is reduced by two electrons on the mercury electrode [7]. Starting from this point of view, it was thought that it could be achieved the determination of DOX by polarographic techniques.

The aim of this study is to progress a polarographic method by utilizing due to the reduction of quinazoline group of molecule on the surface of mercury electrode. To do this, the optimum analytical conditions and polarographic parameters were found out using direct current polarographic technique. Additionally, the electrocapillary curve and its reversibility reaction of DOX were also elucidated. The calibration studies were performed with the use of optimum conditions employing differential pulse (DP), superimposed increasing amplitude pulse (SIAP) and superimposed constant amplitude pulse (SCAP) polarographic techniques. It was observed that SIAP and SCAP techniques were found to be more sensitive than those of DC and DP. Therefore, the practicability of the techniques was investigated by applying to a pharmaceutical dosage form of 4 mg DOX tablet. The results of the method was compared those of UV-spectrophotometry and they were evaluated by computing statistically.

2. Experimental

2.1. Apparatus

Polarographic system comprising of Polaropulse Model PRG-5; the electrodes dual-function EGMA type cell stand for polarography and voltammetry, with dropping mercury as working, platinum wire as auxiliary and saturated Ag/AgCl as reference electrodes (all Tacussel, Belgium) were used. The polarograms were recorded by BBC Goertz Metrawatt Model SE 790 *x-y* recorder. A Model P 114 pH meter (Consort) was employed for measuring and adjusting the pH of the solution. Spectrophotometric studies were made using a Model 160 A spectrophotometer (Shimadzu, Japan) The system was termostated by MT Lauda M6 circulation thermostat.

2.2. Chemicals

Standard DOX (99.97%) was supplied from Pfizer Ilaç Sanayi A.Ş. (Istanbul) and it was used without further purification. All the other chemicals used in the experiments were the product of Merck Co. (Germany) and they were all analytical grade. Double distilled water for the preparation of the solutions and double distilled mercury for the polarographic studies were employed. However, the commercial preparation of DOX (Cardura® tablet containing 2 or 4 mg active material) is produced, only 4 mg tablets were tested to examine the validation of the method in this study.

2.3. Supporting electrolyte

An aqueous solution containing 20% ethanol (v/v), 0.2 M KCl and acetate or phosphate buffer was the most suitable supporting electrolyte. The pH of the buffers were adjusted by adding 2 M HCl or NaOH solutions.

2.4. Polarographic procedure

A well-developed polarographic stand was used to perform the experiments. It has two sections and one of the sections is related with polarography. Polarographic site has a pressurized mercury tank and its pressure is provided by a nitrogen gas cylinder. There are two manometers between mercury tank and gas cylinder. The manometer on the polarographic stand is accurately adjustable. Thus, the flow of mercury is provided by pressure applied to the tank under controlled conditions. Besides, the drop size growth can be adjusted by means of a needle on the capillary hole and drop growing corresponds to the capillary opening duration.

For the polarographic studies, 10 ml supporting electrolyte containing 6.0×10^{-5} M DOX were

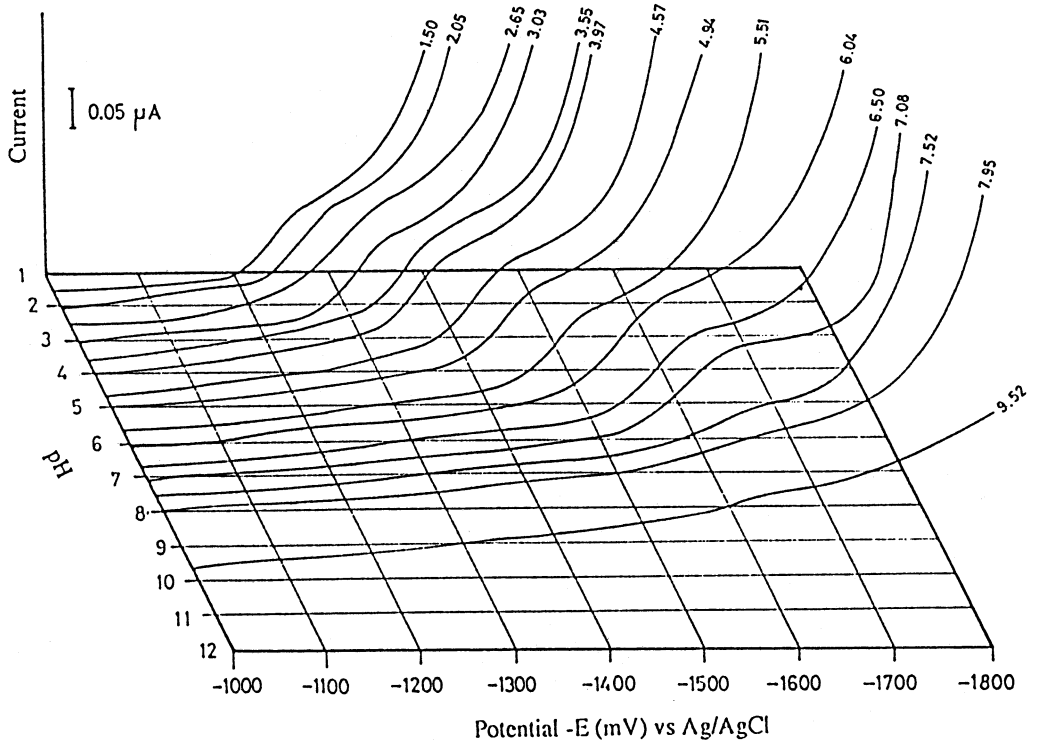

Fig. 2. The polarograms of 6.0×10^{-5} M DOX in the supporting electrolyte consisting of 0.2 M KCl, 20% (v/v) ethanol and 0.2 M buffer at various pH.

put into the polarographic cell and purified nitrogen was passed through the solution for 10 min. The solution of 6.0×10^{-5} M DOX was used to investigate the effect of pH and the other polarographic parameters on the limiting current. Polarographic investigations were carried out by scanning cathodically in the range of -1000– -1800 mV against saturated Ag/AgCl reference electrode potential.

To examine the effect of pressure on the limiting current, drop time and drop growing was kept constant and the pressure in the tank was varied by the manometer of electrode stand. Thus, the growth of mercury drops were linearly changed.

The other parameters used during the experiments are given in the related topics. All the experiments were conducted in ambient temperature.

2.5. Spectrophotometric procedure

The aqueous solution of 1.0×10^{-3} M DOX solution was prepared and a series of standard solution in the concentration range of 1.0×10^{-5} and 5.0×10^{-5} M diluted from the stock solution. The spectrophotometric measurements were made at 330 nm using quartz cells against double distilled water.

2.6. Analysis of pharmaceutical dosage form

For the pharmaceutical analysis, 20 tablets were weighed and powdered in a mortar. The average weight of a tablet was calculated. The powder of Cardura® tablet containing equivalent of 4 mg DOX were weighed accurately, transferred into a 100 ml flask and added some supporting electrolyte to dissolve the active material. It was

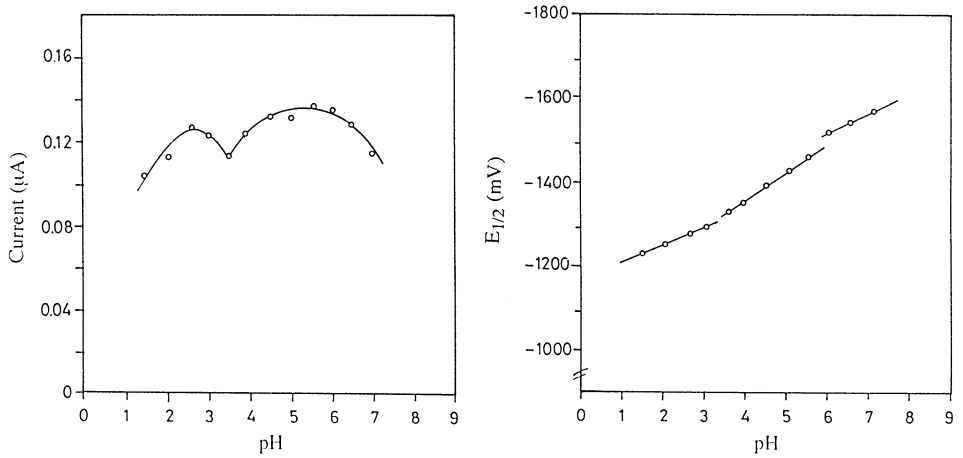

Fig. 3. The variation of the limiting current (a) the half-wave potential (b) versus pH.

stirred magnetically for 10 min and made up to volume of 100 ml with supporting electrolyte. A total of half the solution was left by itself and rough particles of the tablets were precipitated by gravity. The supernatant was diluted by the supporting electrolyte and it was directly used. The second half of the solution was centrifuged at first, then it was filtered. The filtered solution was diluted either with supporting electrolyte for the polarographic or with distilled water for the spectrophotometric determination of the active material.

3. Result and discussion

3.1. Polarographic behavior of DOX

The DC polarograms of 6.0×10^{-5} M DOX in the supporting electrolyte were recorded at various pH and well-defined one-step polarographic waves were appeared in the pH range 1.5–7.5. The polarograms are demonstrated in Fig. 2. As it is seen that well-defined and morphologically good polarograms appeared at around pH 3.5, where the limiting current was -1400 mV.

The variations of the limiting current versus pH were examined and it was observed that it exhibits an m-shaped curve and forms an indent around pH 3.5 and the magnitudes of the limiting currents decrease dramatically below pH 1.5 and above pH 7.0. The pH dependence of half-wave potential shows three straight lines with different slopes. The break at around pH 3.5 can be attributed to the dissociation constant of DOX molecule. The pK_a value of DOX have been reported as 4.8 [8]. In the comparison of these values, it seems that a shift may depend on the chemicals such as a high concentration of glucose and glycerol used during the experiments. At pH 3.5, the molecule is in the protonated form and the reduction of the molecule is probably realized on the azomethin group. The plots of the limiting current and the half-wave potential against pH are demonstrated in Fig. 3a and 3b.

To elucidate the factor influencing the polarographic current, the polarogram of $4.0\text{x}10^{-5}$ M DOX solution having pH 3.5 were recorded utilizing the pressure applied to the mercury reservoir in the range of 400–1200 dyne cm^{-2} employing drop time of 0.8 s, potential rate of 4 mV s^{-1}. The relation of the limiting current versus pressure gives an arc-type curve. These suggest that the reaction was diffusional [9]. The plots of pressure and square-root of pressure versus the limiting current are in Fig. 4. To get the exact decision, the dependence of the limiting current on pressure must be a straight line. The plots of the limiting current versus square-root of pressure is a straight line which corresponds to the equation of $i_{lim}(\mu A) = 1.62 \times 10^{-3}\sqrt{P}(\text{dyne}^{1/2}\text{cm}^{-1})$

-0.0014; $r = 0.9987$. The last plots exhibit a straight line and is conformation of the diffusional character of the polarographic current.

According to the theoretical consideration [9], the variation of the limiting current versus drop time was a straight line when the 1/6 power of function was applied. The latter result also makes certain that the polarographic current is diffusional.

The temperature effect on the limiting current of 4.0x10^{-5} M DOX in the supporting electrolyte at pH 3.5, keeping the potential at -1400 mV was examined in the range of 22–51°C. A straight line corresponds to the equation of $i_{lim}(\mu A) =$

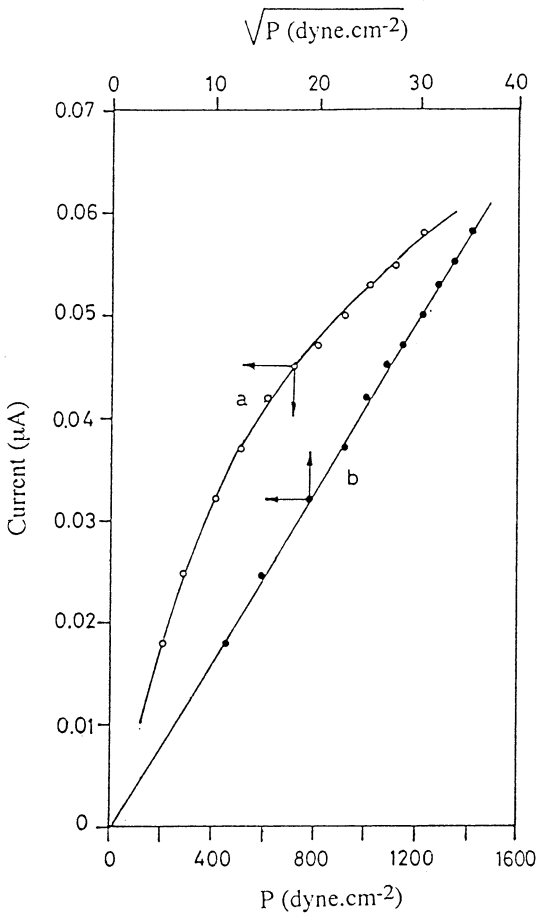

Fig. 4. The variation of the limiting current of 6.0×10^{-5} M DOX versus pressure (○) and square-root (●) of pressure.

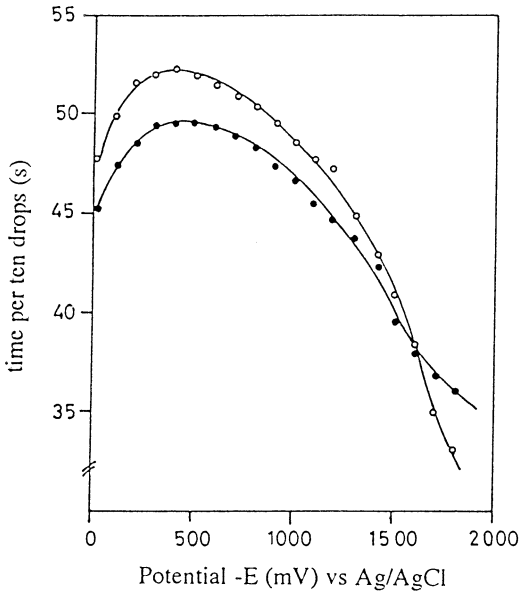

Fig. 5. The electrocapillary curves of supporting electrolyte (○) and with 6.0×10^{-5} M DOX (●).

$0.0037 \times T(°C) - 0.05$; $r = 0.9993$ was obtained between 22 and 40°C. This result also confirms the previous conclusions in this range of temperature mentioned above.

As is seen from the results, the factor affecting the polarographic current is diffusional. However, it was observed that it changes into adsorptional character out of pH 3 and 4.

3.2. Reversibility of the process

Reversibility of the reduction reaction in the supporting electrolyte at pH 3.5 was studied employing the differential pulse technique [10]. At first, the potential was anodically and cathodically scanned in the range -1000– -1500 mV, respectively. Then, $E_p^a - E_p^c$ and i_p^a/i_p^c values were found to decide on the reversibility of the process. The calculated parameters were in the following: $E_p^a = -1290$ mV, $E_p^c = -1400$ mV, $i_p^a = 0.057\,\mu$A and $i_p^c = 0.118\,\mu$A. According to the criterion that were given above, it is concluded that the process is irreversible for the solution of DOX at pH 3.5.

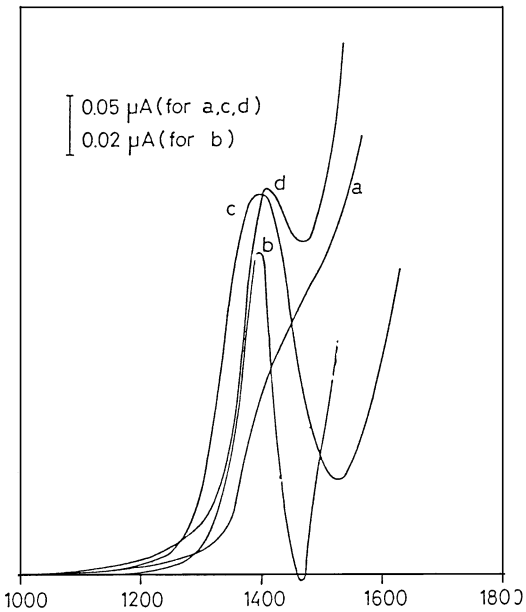

Fig. 6. The polarograms of 6.0×10^{-5} M DOX recorded by DC (a), DP (b) SIAP (c) and SCAP (d) polarographic techniques.

3.3. Electrocapillary curve

Applying 1000 dyne cm^{-2} pressure to the mercury reservoir, the freely dropping time for ten drops of mercury was measured at each 100 mV in the range of 0–1800 mV for only supporting electrolyte and supporting electrolyte containing 4.0×10^{-5} M DOX at pH 3.5. The plots of the freely dropping time of ten drops mercury versus applied potential are demonstrated in Fig. 5. The electrocapillary curve of the solution containing DOX goes beneath the curve of the supporting electrolyte and the curves are superimposed at about −1600 mV. This may be due to the changes of surface tension in the DOX solution.

Table 1
The calibration equations of standard DOX, dublicating the experiments for five concentrations in the range of 2.0×10^{-5}–1×10^{-4} M in the supporting electrolyte at pH 3.5, employing SCAP and SIAP polarographic techniques

C(M)	SIAP i_{lim} (μA)	SCAP i_{lim} (μA)
2.0×10^{-5}	0.090	0.082
4.0×10^{-5}	0.190	0.170
6.0×10^{-5}	0.300	0.255
8.0×10^{-5}	0.390	0.345
1.0×10^{-4}	0.490	0.435

Calibration equations $i_{lim}(\mu A) = 5000.0 \times C(M) - 8.0 \times 10^{-3}$ at −1400 mV ($r = 0.9999$) and $i_{lim.}(\mu A) = 4405.0 \times C(M) - 6.9 \times 10^{-3}$ at peak maximum ($r = 0.9996$).

3.4. The effect of concentration on the limiting current

The effect of concentration was examined by SCAP and SIAP polarographic techniques in the supporting electrolyte at pH 3.5 in the range of 2.0×10^{-5}–1.0×10^{-4} M employing the optimum DC polarographic conditions such as pressure of 1000 dyne cm^{-2}, drop time of 0.8 s and potential rate of 4 mV s^{-1}. Certain polarographic techniques were tested for the examination of concentration effect of DOX and well-correlated relations were calculated for SIAP and SCAP polarographic techniques. The polarograms of the techniques are given in Fig. 6. They are all well defined and analytically available curves. Furthermore, DOX was found to be stable at least 1 week at calibration conditions. The calibration equations, their correlation coefficients and the other explanations are given in Table 1.

These results show that the techniques proposed in this study are equally usable for the determination of DOX. However, SIAP and SCAP polarographic techniques are seemed to be more sensitive than the others.

3.5. Application of the SIAP and SCAP polarographic techniques to the DOX tablets

The SIAP and SCAP polarographic techniques were applied to the tablets (Cardura® tablet) containing 4 mg active material DOX and it was determined in filtered and unfiltered solutions. As it was declared earlier, polarography has many advantages as an analytical method [11]. One of them is its employment in the analysis of pharmaceutical preparations without any separation such as filtration. Since filtration is a time-consuming procedure, the effect of filtration on the determination of DOX was investigated employing opti-

Table 2
The statistical assay results of DOX by SIAP, SCAP polarographic techniques and UV-spectrophotometry in 4 mg Cardura tablet

	SIAP polarography		SCAP polarography		UV-spectrophotometry
	Filtered	Unfiltered	Filtered	Unfiltered	
No. of assay	8	8	8	8	8
Mean recovery (mg) ± S.D.	3.97 ± 0.03	3.98 ± 0.03	3.99 ± 0.03	4.00 ± 0.03	3.98 ± 0.01
S_{rel} % ± confidence limits	0.9 ± 0.02	0.7 ± 0.02	0.8 ± 0.02	0.8 ± 0.02	0.459 ± 0.01
t-test of significance	0.85	0.33	0.88	1.52	2.14*
F-test of significance	3.72	2.45	2.79	3.15	4.12**

*$t_{0.05} = 2.14$ (table).
**$F_{0.05} = 4.12$ (table).

mum analytical and polarographic conditions as in the calibration studies. Almost equal results were obtained for the filtered and unfiltered tablet solutions. These results show that the ingredients in the DOX tablets do not interfere the experiments and its determination can be achieved without processing the filtration procedure that is unusual for most of the analytical methods.

Spectrophotometry was chosen as a comparison method to evaluate the validity of the polarographic techniques. A calibration equation $A = 9084 \times C(M) - 0.003$; $r = 0.999$, for the UV-spectrophotometric method was found at 330 nm, where DOX absorbs the light maximum. The determinations were done in the filtered solutions while the spectrophotometric studies were being conducted.

The results obtained by the SIAP and SCAP polarographic techniques were compared to those of the spectrophotometric method utilizing certain statistical evaluations. The results of the statistical evaluations are demonstrated in Table 2.

4. Conclusions

There are good agreement both the results of polarographic techniques (in the employment of filtered and unfiltered solutions) and spectrophotometry. The pharmaceutical dosage form of DOX utilized in this study provide the official requirements [12]. The results of *F*- and *t*-tests show that there are insignificant differences between the techniques. The polarographic techniques proposed in this study are also equally usable in filtered and unfiltered DOX solutions. Thus, it is concluded that the techniques are practicable, sensitive and accurate and can be proposed for the quality control tests.

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