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Voltammetric investigation of oxidation of zuclopenthixol and application to its determination in dosage forms and in drug dissolution studies☆

Zühre Şentürk [a,*], Sibel A. Özkan [b], Yalçın Özkan [c], Hassan Y. Aboul-Enein [d]

[a] *Gazi University, Faculty of Pharmacy, Department of Analytical Chemistry, 06330 Ankara, Turkey*
[b] *Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, 06100 Ankara, Turkey*
[c] *Gülhane Military Medical Academy, Department of Pharmaceutical Technology, 06018 Ankara, Turkey*
[d] *Bioanalytical and Drug Development Laboratory, Department of Biological and Medical Research, King Faisal Specialist Hospital and Research Center, P.O. Box 3354, Riyadh 11211, Saudi Arabia*

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Abstract

The oxidative voltammetric behaviour of zuclopenthixol (ZPT) at a glassy carbon has been studied using cyclic, linear sweep and differential pulse voltammetry. Oxidation of the drug produced three pH dependent anodic steps (representing an irreversible oxidation). Using differential pulse voltammetry, the drug yielded a well-defined voltammetric response in phosphate buffer, pH 5.2 at + 0.82 V (vs. Ag/AgCl). This process could be used to determine ZPT concentrations in the range 8×10^{-7}–2×10^{-4} M. The method was applied, without any interferences from the excipients, to the determination of the drug in tablets and oral drops, and in drug dissolution studies.

Keywords: Zuclopenthixol; Cyclic voltammetry; Differential pulse voltammetry; Glassy carbon electrode; Formulation analysis; Dissolution profile

1. Introduction

Thioxanthene neuroleptics have a more pronounced action than phenothiazines and have gradually replaced them [1]. Zuclopenthixol (ZPT), a *cis*-(*Z*)-isomer of clopenthixol, {(*Z*)-4-[3-[2-chloro-9H-thioxanthene-9-yliden]-propyl]-1-piperazine ethanol}, is a thioxanthene of high potency with general properties similar to the phenothiazine, chlorpromazine [2]. It has a piperazine side-chain as the other structurally related phenothiazine, fluphenazine.

ZPT is used for the treatment of schizophrenia and other psychoses and administered as the hydrochloride, usually by mouth [2]. In animals and

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* Corresponding author. Tel.: + 90-312-2227337; fax: + 90-312-2235018.
E-mail address: ozkan@pharmacy.ankara.edu.tr (S.A. Özkan)

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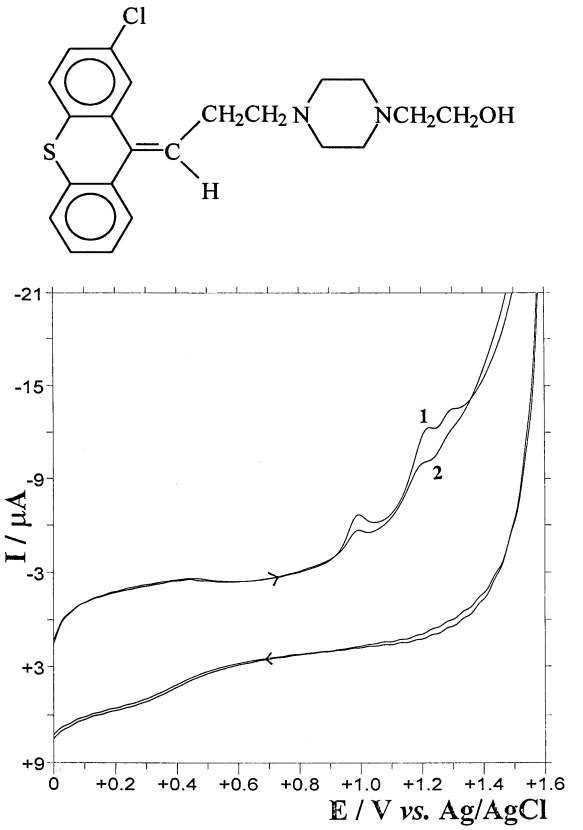

Fig. 1. Cyclic voltammograms of ZPT in 0.1 M H_2SO_4. Scan rate, 100 mV s^{-1}. (1) 6×10^{-5} M ZPT; (2) 2×10^{-5} M ZPT.

humans, *N*-dealkyl-ZPT, ZPT-sulphoxide and ZPT-*N*-oxide were found to be its main metabolites [3].

There have been few reports for the determination of the drug in the formulations or in biological media including spectrophotometry [4], fluorimetry [5,6], high-performance liquid chromatography with UV detection [7,8], fluorescence detection [3], mass spectrometric detection [9,10] and diode array detection [10].

Up to date, no examination by electrochemical oxidation has appeared in the literature. The objective of the work described in this paper was to investigate the electrochemical behaviour of ZPT and devise a suitable differential pulse voltammetric method for the analysis of ZPT in pharmaceutical preparations and to the study the dissolution profile of the drug.

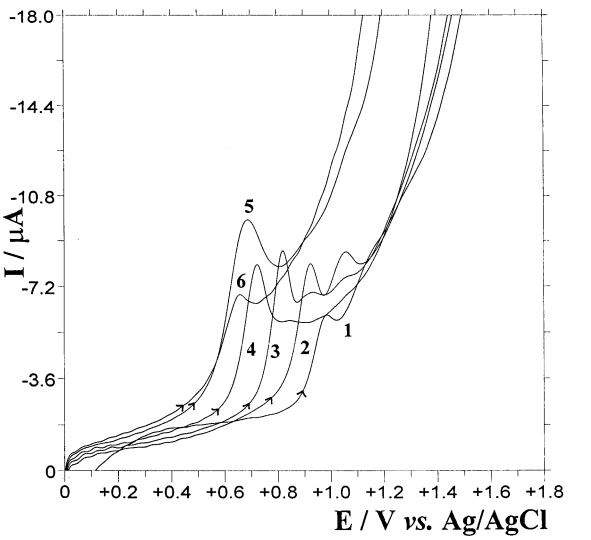

Fig. 2. Linear sweep voltammograms of 2×10^{-5} M ZPT in Britton–Robinson buffer. Scan rate, 100 mV s^{-1}. (1) pH 2; (2) pH 4; (3) pH 6; (4) pH 8; (5) pH 10; (6) pH 11.5.

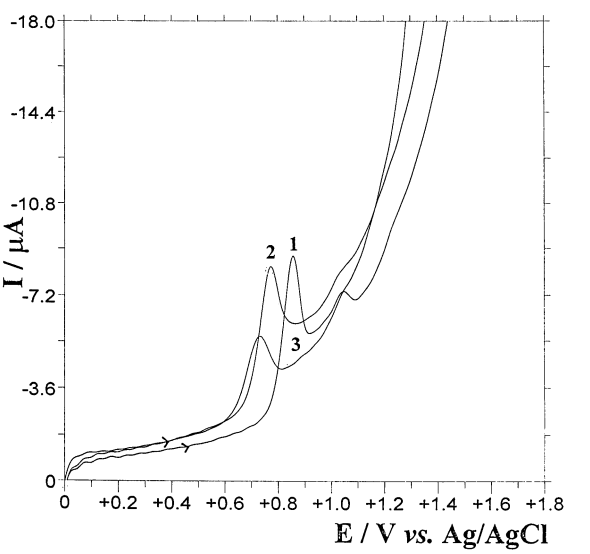

Fig. 3. Linear sweep voltammograms of 2×10^{-5} M ZPT in phosphate buffer. Scan rate, 100 mV s^{-1}. (1) pH 5.2; (2) pH 7; (3) pH 8.3.

2. Experimental

2.1. Apparatus

Electrochemical measurements were made with BAS 100W electrochemical analyser (Bioanalytical System, USA). Voltammetric measurements were utilised a glassy carbon working electrode ($\Phi = 3$ mm, BAS), a platinum wire auxiliary electrode and Ag/AgCl (NaCl 3 M, BAS) reference electrode. Before each experiment, the glassy carbon electrode was polished manually with alu-

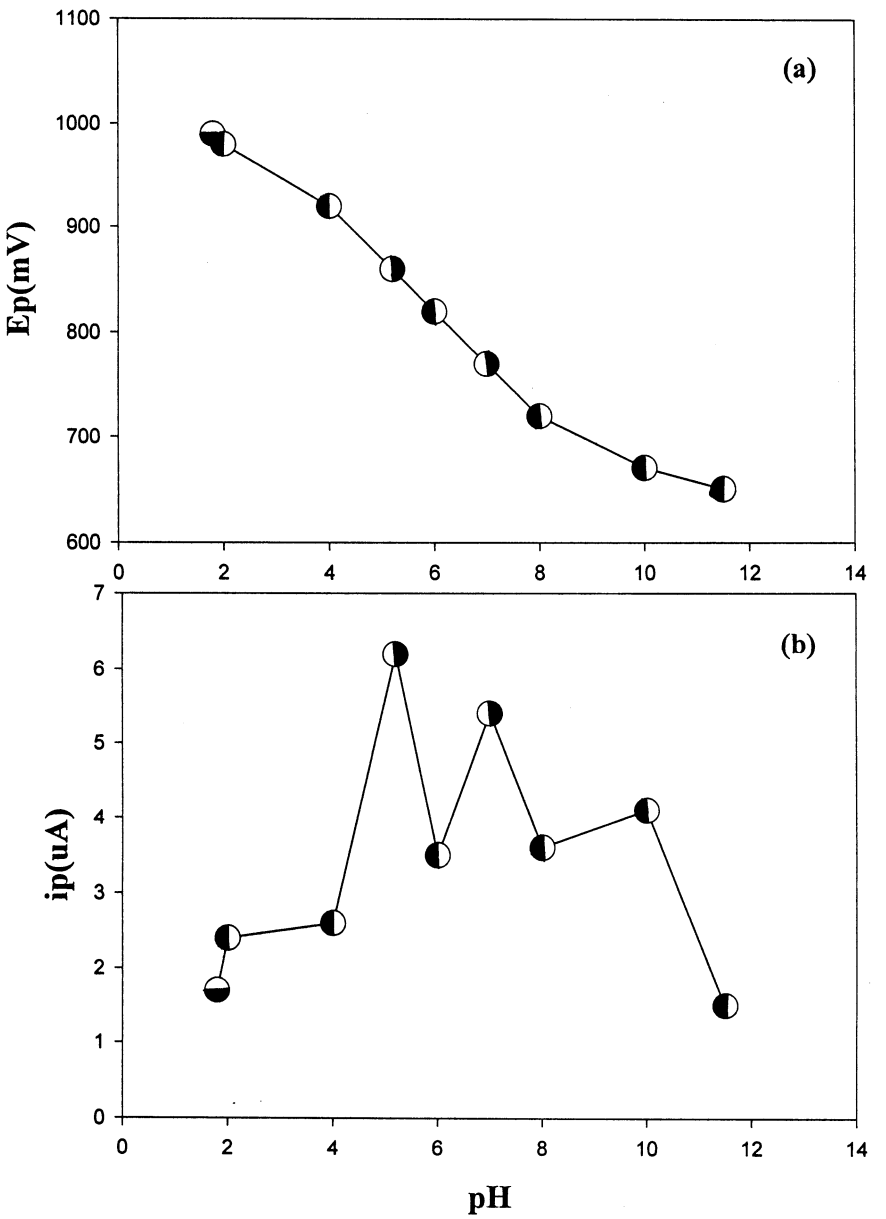

Fig. 4. Effects of pH on ZPT peak potential (a) and peak current (b); ZPT concentration, 2×10^{-5} M; scan rate, 100 mV s^{-1}. (◒) H_2SO_4; (◐) Britton–Robinson buffer; (◑) Phosphate buffer.

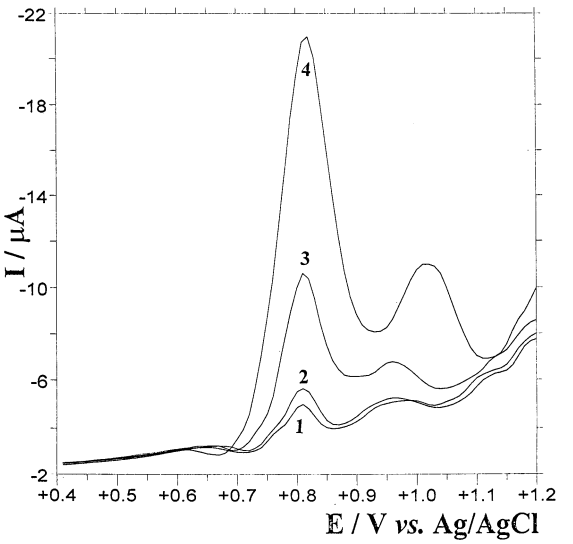

Fig. 5. Differential pulse voltammograms of different concentrations of ZPT in phosphate buffer pH 5.2. Scan rate, 50 mV s^{-1}; pulse amplitude, 50 mV. (1) 4×10^{-6} M; (2) 8×10^{-6} M; (3) 4×10^{-5} M; (4) 8×10^{-5} M.

mina ($\Phi = 0.01$ μm,) in the presence of double distilled water on a smooth polishing cloth.

The dissolution test was performed in a paddle dissolution apparatus recommended by the USP 23 [11].

Spectrophotometric measurements were carried out using a Shimadzu 1601 PC double beam UV–Vis spectrophotometer.

2.2. Reagents

Zuclopenthixol dihydrochloride (kindly provided by Lundbeck, Valby, Denmark) was used without further purification. All other chemicals were of analytical grade (Merck or Sigma) and were used as received.

Although, the results showed that the light did not affect significantly the analysis of ZPT [12], solids and solutions were protected from light.

Stock solutions under voltammetric investigation were prepared daily by direct dissolution in selected supporting electrolytes. Three different supporting electrolytes, namely sulphuric acid (0.1 M), phosphate buffer (pH 5.2, 7.0, 8.3, 0.2 M) and Britton–Robinson buffer (pH 2, 4, 6, 8, 10, 11.5, 0.04 M) were prepared in doubly distilled water.

For dissolution studies, working solutions of hydrochloric acid (0.1 M), which is adequate to physiological conditions in gastric fluids, were used.

2.3. Analysis of dosage forms

Ten tablets were weighed and pulverised. The required amount of sample corresponding to a stock solution of concentration ca. 10^{-3} M was accurately weighed and transferred into a 100 ml standard flask containing 80 ml of phosphate buffer, pH 5.2. The contents of the flask were stirred magnetically for 15 min to effect complete dissolution and then diluted to the mark with the selected supporting electrolyte. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting them with the same supporting electrolyte. Voltammograms were recorded as for pure ZPT.

No sample preparation for the oral drops was used other than dilution with the proposed supporting electrolyte.

2.4. In vitro dissolution studies

The dissolution methodology was carried out according to the USP dissolution procedures for

Table 1
Characteristics of ZPT calibration plots in phosphate buffer pH 5.2 at glassy carbon electrode

Concentration range (M)	Slope (μA M^{-1})	Intercept (μA)	R	RSD of slope	RSD of intercept
8×10^{-7}–2×10^{-4}	1.19×10^{5}	0.14	0.998	0.60	1.54

Table 2
Assay of ZPT pharmaceutical preparations

	Differential pulse voltammetry				Spectrophotometry (BP 1998)
	Oral drop		Tablet		Tablet
	Labelled amount (mg ml^{-1})	Amount found	Labelled amount (mg per tablet)	Amount found	Amount found
	20.00	19.96	25.00	25.20	24.30
	20.00	20.17	25.00	25.00	24.30
	20.00	20.80	25.00	24.50	25.34
	20.00	20.20	25.00	25.20	25.30
	20.00	19.96	25.00	24.80	25.30
Mean		20.23		24.94	24.91
RSD (%)		1.70		1.19	2.23
95% Confidence limit		0.43		0.37	0.69
t-Test of significance				0.114[a]	

[a] P: 0.05; t: 2.306.

Table 3
Characteristics of ZPT calibration plots in 0.1 M HCl

Method	Concentration range (M)	Slope	Intercept	r	RSD of slope	RSD of intercept
Differential pulse voltammetry (+0.78 V)	1×10^{-5}–1×10^{-4} ($n=7$)	2.68×10^{4}	0.685	0.999	0.67	1.17
Spectrophotometry (268.3 nm)	1×10^{-5}–1×10^{-4} ($n=6$)	1.25×10^{4}	2.68×10^{-3}	0.999	0.47	1.74

the single-entity products with use of a USP paddle-stirrer type of apparatus in 900 ml of 0.1 MHCl, at a stirring rate of 75 rpm. The temperature of the cell was controlled at 37 ± 0.5°C by use of thermostatic bath. Using the voltammetric technique, the current values were recorded at appropriate time intervals, and the amount of ZPT released was determined from a calibration graph. The dissolution test data were performed from the average of six parallel studies.

Furthermore, to obtain comparative results, an UV spectrophotometric method at 268.3 nm was also applied. This spectrophotometric method was very similar to those described in the USP for the single-entity products.

3. Results and discussion

ZPT gave three anodic waves at about + 1.0, + 1.2 and + 1.3 V in 0.1 M sulphuric acid using cyclic voltammetry (Fig. 1). A slight shift in the potentials of second and third waves was observed towards more positive values with increasing concentration. This suggests that the slight adsorption occur after the first step. Continued scanning resulted in a negative shift in the half-wave potentials and a decrease in limiting current for all waves. On the reverse sweep, no distinct reduction wave was observed, indicating that the drug is irreversibly oxidised at the glassy carbon electrode.

The electrooxidation of ZPT was also studied over pH range 2.0–11.5 in buffer media (Figs. 2 and 3). The first wave was well developed throughout, but the second wave was poorly defined at pH 2, well developed at pH 4, 5 but less marked above pH 6. The third wave was less pronounced throughout the pH range studied.

The first wave was also in the form of a peak, and was easily measurable. Hence, all subsequent work was based on the measurement of the magnitude of this step.

The effects of potential scan rate between 10 and 1000 mV s^{-1} on the peak potential and the peak current of ZPT were evaluated. The linear increase in the oxidation peak current with the square root of the scan rate with a slope of 0.85 (correlation coefficient 0.9993), showed the diffusion control process. A 117 mV positive shift in the peak potential was observed, which also confirms the irreversibility of the process, with simultaneous increase in diffusion current when the scan rate was increased. A plot of logarithm of peak current versus logarithm of scan rate gave a straight line with a slope of 0.73 (correlation coefficient 0.994). Slopes of 0.50 and 1.0 are expected for ideal reactions of solution and surface species, respectively [13].

The peak shifted towards negative potentials with an increase in pH, in such a way that three straight lines with different slopes could be observed. The positions of the breaks are close to the pK_a1 and pK_a2 values of ZPT at about 3.6 and 7.8 [14] (Fig. 4a). The effect of pH and the nature of the supporting electrolytes on peak current shows a maximum at pH 5.2 in phosphate buffer (Fig. 4b).

By considering the structures of in vivo metabolites of the drug, it can be concluded that the first oxidation wave involves two-electron oxidation to the sulphoxide. Even though the exact oxidation mechanism was not determined, we may assume that the second and third oxidation steps of ZPT are located on the piperazine ring.

3.1. Quantitative determination

The application of the differential pulse waveform (pulse amplitude = 50 mV) yielded voltammograms in which the peak currents were greater than those obtained by linear sweep voltammetry. The peak potential versus pH plot was similar to that obtained by linear sweep voltammetry, showing three linear ranges with different slopes.

A pulse interval of 0.25 s gave rise to the sharpest and symmetrical peak shape. The optimum scan rate was found to be 50 mV s^{-1}. For analytical purposes, best response (with regard to peak current sensitivity and morphology) was obtained with phosphate buffer pH 5.2.

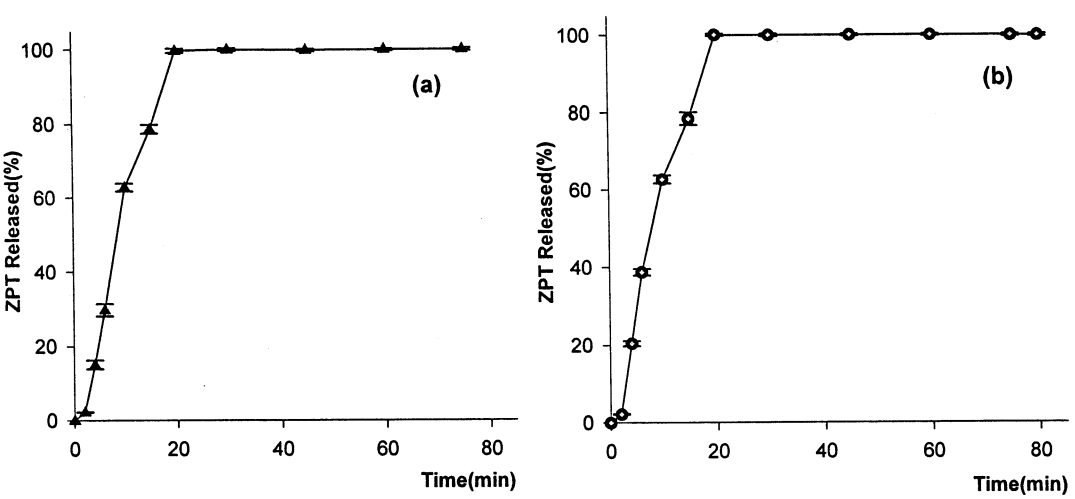

Fig. 6. In vitro dissolution profiles of ZPT tablets (a) UV spectrophotometry, (b) differential pulse voltammetry.

The reproducibility of peak potential and peak current was tested by repeating five experiments on 2×10^{-4} M ZPT. The relative standard deviations were calculated to be 0.12 and 1.5% for peak potential and peak current, respectively.

Using the optimum conditions described in Fig. 5, a linear calibration curve was obtained for ZPT in the range 8×10^{-7}–2×10^{-4} M. Table 1 summarises the characteristics of this graph. The limit of detection of the procedure was found to be 2.2×10^{-7} M, which was calculated as the blank response plus three times the blank standard deviation divided by the slope of the calibration curve.

On the basis of these results, the proposed method was applied to the direct determination of ZPT in tablets and oral drops (Table 2). The drug is recently included in British Pharmacopoeia [15]. ZPT tablets were also determined with the official procedure, which involves a spectrophotometric method [15]. In comparison to the official method, the proposed method is more sensitive. On the other hand, as Table 2 shows, the calculated t-value did not exceed the tabulated value. This result indicates that there is no significant difference between the population means for the two procedures. Moreover, in order to know whether the excipients in the tablet (lactose, iron oxide and titanium dioxide) show any interference with the analysis, known amounts of the pure drug were added to the same aliquot portions of the same powdered tablets and mixtures were analysed by the proposed method. The recovery study shows a recovery average of 97.8% with a RSD of 0.5%, indicating adequate precision and accuracy of the method. The recovery study was also applied to oral drop forms, which contain alcohol as solubiliser. The recovery and its relative standard deviation were found to be 99.7 and 0.3%, respectively.

The principal advantage of the proposed voltammetric method over the already published procedures for ZPT formulations is that it involves no sample preparation other than dissolving and transferring an aliquot to the supporting electrolyte, and does not require separation procedures such as filtration, extraction, and expensive grades of solutions. Consequently, the above presented method is a good analytical alternative for determining ZPT in pharmaceutical formulations.

3.2. Dissolution studies for tablets

The proposed differential pulse voltammetric method was applied to the quantitation of ZPT in dissolution rate samples from the tablets. The results of the linear regression analysis from standard ZPT in 0.1 M HCl obtained by proposed voltammetric and comparison spectrophotometric methods are given in Table 3. The reproducibility of the methods was studied by analysing a solution containing 5.9×10^{-5} M in six replicates. The relative standard deviations were calculated to be 0.2% for differential pulse voltammetric and 0.6% for spectrophotometric method.

The release rate profiles were drawn as the percentage drug dissolved from the tablets versus time for both methods (Fig. 6).

Five different kinetics such as, zero order, first order, Hixson–Crowell, RRSBW, $Q - \sqrt{t}$ were applied to the results obtained from the dissolution studies and the results were evaluated kinetically [16,17]. According to the investigation of the kinetic assessment of the release data, the best released kinetic was found to be RRSBW, because of the highest correlation coefficient and the lowest AKAIKE's information criteria (Table 4). For this kinetic, $T_{63.2\%}$ results were obtained at 12.42 and 12.88 min for differential pulse voltammetric and spectrophotometric methods, respectively. According to the RRSBW kinetic, $\beta > 1$ is characteristic for a slower initial rate followed by an accelerated approach to the final plateau, i.e. an initial upward curvature and a sigmoid overall appearance [16]. In this study, shape factors (β) obtained from RRSBW was found to be 1.41 for voltammetric method and 1.55 for spectrophotometric method. The release of ZPT from tablets was completed after 20 min in both methods.

The release percentages at all the time period are very similar in the two methods investigated, without any significant differences. On the other hand, the voltammetric measurements are more simple and rapid as compared with the UV spectrophotometric method. No treatment of the sample is required (e.g. filtration) before the measurements. Excipients presented in the tablet do not interfere with the analysis.

Table 4
The kinetic assessment of release data from ZPT tablet formulation[a]

Method	Zero order			First order			Hixson–Crowell			RRSBW				$Q-\sqrt{t}$		
	k_r^0	AIC	r	k_r	AIC	r	K	AIC	r	$T_{\%63.2(\min)}$	β	AIC	r	k	AIC	r
Differential pulse voltammetry	13.92	8.71	0.734	2.93	−9.19	0.736	1.072	−10.94	0.78	12.42	1.41	−29.98	0.925	50.55	−6.89	0.843
Spectrophotometry	17.21	6.42	0.744	6.51	−20.51	0.843	1.902	−13.92	0.819	12.88	1.55	−27.21	0.95	55.8	−7.69	0.855

[a] k_r^0, zero order release rate constant; k_r, first order release rate constant; T, value stands for the time for 63.2% release of the drug; β, shape factor; k, rate constant obtained from $Q-\sqrt{t}$ kinetic; K, rate constant obtained from Hixson–Crowell kinetic; r, correlation coefficient; AIC, AKAIKE's information criteria.

References

[1] M.I. Walash, M. Rizk, A. El-Brashy, Analyst 113 (1988) 1309–1312.
[2] M. Bloch, E. Gur, A.Y. Shalev, Psychopharmacology 109 (1992) 377–378.
[3] B.B. Hansen, S.H. Hansen, J. Chromatogr. 658 (1994) 319–325.
[4] F.A. Aly, Mikrochim. Acta 110 (1993) 187–192.
[5] S.M. Hassan, F. Belal, F. Ibrahim, F.A. Aly, Talanta 36 (1989) 557–560.
[6] F. Belal, F. Ibrahim, S.M. Hassan, F.A. Aly, Anal. Chim. Acta 255 (1991) 103–106.
[7] A. Viala, N. Hou, A. Durand, B. Ba, T. Aaes-Jorgensen, A. Jorgensen, J. Pharm. Belg. 38 (1983) 299–303.
[8] A. Viala, N. Hou, B. Ba, A. Durand, H. Dufour, N.D. Agostino, C. Berda, A. Jorgensen, Psychopharmacology 83 (1984) 147–150.
[9] A.C. Tas, J. van der Greef, M.C. ten Noever de Brauw, T.A. Plomp, R.A.A. Maes, M. Hohn, U. Rapp, J. Anal. Toxicol. 10 (1986) 46–48.
[10] A. Tracqui, P. Kintz, V. Cirimele, F. Berthault, P. Mangin, B. Ludes, J. Anal. Toxicol. 21 (1997) 314–318.
[11] The United States Pharmacopeia, 23rd Revision, Rand McNally, Tounton, MA, 1995, pp. 1791–1793.
[12] C.B. Eap, L. Koeb, P. Baumann, J. Pharm. Biomed. Anal. 11 (1993) 451–457.
[13] E. Laviron, J. Electroanal. Chem. 112 (1980) 11–23.
[14] K. Thoma, K. Albert, Pharm. Acta Helv. 55 (1980) 8–12.
[15] The British Pharmacopoeia 1998, Version 2.0, [CD-ROM], The Stationary Office Ltd., March 1998.
[16] F. Langenbucher, Pharm. Ind. 38 (1976) 472–477.
[17] T. Higuchi, J. Pharm. Sci. 52 (1963) 1145–1149.