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Voltammetric analysis of trazodone HCl in pharmaceuticals and biological fluids

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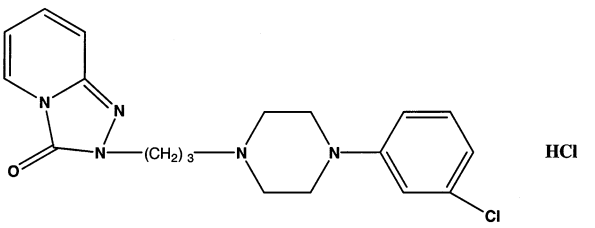
Abstract

The voltammetric behavior of trazodone (TRZ) HCl was studied using direct current (DC_t), differential pulse (DPP) and alternating current (AC_t) polarography. The drug manifests cathodic waves over the pH range of 10–14. The waves were characterized as being irreversible, diffusion-controlled with limited adsorption properties. At pH 10, the diffusion current–concentration relationship was found to be rectilinear over the range 4–32 and 0.8–24 μg ml^{-1} using DC_t and DPP modes, respectively, with minimum detectability ($S/N = 2$) of 0.104 μg ml^{-1} (2.45×10^{-6} M) and 0.314 μg ml^{-1} (7.397×10^{-6} M) using the DPP and DC_t modes, respectively. The diffusion-current constant (I_d) is 4.31 ± 0.02 ($n = 6$). The proposed method was successfully applied to the determination of the studied compound either in pure form or in formulations. The results obtained were favorably compared with those given using a reference method. Furthermore, the proposed method was applied to the determination of TRZ in spiked human urine and plasma adopting the DPP technique. No prior extraction step is needed in case of urine. The percentage recoveries were 98.43 ± 0.79 and 97.44 ± 0.705 ($n = 4$) in spiked human urine and plasma, respectively. A pathway for the electrode reaction was postulated.

Keywords: Trazodone HCl; Dosage forms; Polarography; Voltammetry; Urine; Plasma

1. Introduction

Trazodone (TRZ), 2-{3-[4-(*m*-chlorophenyl)-1-piperazinyl]propyl}-1,2,4-triazolo [4,3-a] pyridine-3(2H) one hydrochloride, is a triazolopyridine derivative with antidepressant effect. The chemical structure of TRZ is given below.



Structural Formula of Trazodone HCl

TRZ is a triazolopyridine antidepressant chemically unrelated to other classes of antidepressant. It blocks the reuptake of serotonin at presynaptic

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neurones and also has an action at 5-HT_1 receptors [1]. Unlike the tricyclic antidepressants, TRZ does not inhibit the peripheral reuptake of noradrenaline, although it may indirectly facilitates neuronal release. TRZ blocks central α_1-adrenoceptors and appears to have no effect on the central reuptake of dopamine [1].

A good guide to the work published for this compound up to 1987 is found in the monograph written by Gorecki and Verbeeck on its analytical profile [2]. The more recent reports include, spectrophotometry [3], liquid chromatography [4], gas chromatography [5–7], potentiometry [8–10], voltammetry using platinum electrode in rotating condition [11] and high performance liquid chromatography [12–22]. Although chromatographic methods offer high degree of specificity, yet, sample clean up and the instrument limitations preclude their use in routine clinical studies. The voltammetric technique offers another possibility for the estimation of this compound. Review of the literature revealed that, up to the present time, nothing has been published concerning the polarographic behavior of this compound. The molecular structure of the studied compound is characterized by the presence of an electroactive carbonyl group; this fact initiated the present study. The most striking advantage of the proposed method is that no extraction step is required in case of analysis of urine samples. About the analysis of the spiked plasma, an extraction with diethyl ether is needed to overcome the interference of plasma protein. The extraction procedure described by Vistelle et al. [19] was adopted.

2. Experimental

2.1. Apparatus

The polarographic study and 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 company. The polarograms were recorded using a potential scan rate of 10 mV s^{-1}. A three-electrode system composed of a dropping mercury electrode (DME), Ag/AgCl reference electrode, and a graphite rod as the auxiliary elctrode was used. Phase selective AC_t polarograms were recorded using the same instrument; the superimposed alternating voltage being 15 mV at a frequency of 75 Hz and a phase angle of 90°. The effect of mercury height was studied using a 506 VA stand of the same company with Ag/AgCl reference electrode and a platinum wire as an auxiliary electrode. The solutions were purged with pure nitrogen gas for 5 min, then polarographed at ambient temperature.

2.2. Materials and reagents

Pure drug was kindly provided by EIPICO, Pharmaceutical Company, Cairo, Egypt and was used as received. Tablets each containing 50 mg of TRZ HCl (Batch Number 000824) and tablets each containing 100 mg of TRZ HCl (Batch Number 994187) were obtained from commercial sources, in the local market. Plasma was kindly provided by Mansoura University Hospital and kept frozen until use after gentle thawing. Urine sample was obtained from healthy volunteers (male around 40 years old).

2.2.1. Reagents

Britton Robinson buffers (0.08 M) covering the pH range 9–12 [23]. Sodium hydroxide: (BDH, UK) 0.1, 1 and 2 M aqueous solutions. Methanol: AR grade (Aldrich-Chemie, Germany). Diethyl ether: AR grade (Aldrich, USA).

2.3. Standard solutions

A stock solution containing 200 μg ml^{-1} of TRZ HCl was prepared in distilled water and was further diluted with the same solvent to give the appropriate concentrations.

2.3.1. Calibration graph

Transfer aliquot volumes of TRZ HCl covering the working range (cited in Table 2) into 25 ml volumetric flasks. Complete to the mark with BRb of pH 10.0. Transfer the whole contents of the measuring flask into the polarographic cell. Pass pure nitrogen gas for 5 min. Record the DC_t

and DPP polarograms over the range −1.0 to −1.6 V versus Ag/AgCl, using a pulse amplitude of 70 mV in case of DPP mode. Plot the final concentration of the drug (μg ml^{-1}) versus the current (μA) to get the calibration graph. Alternatively, derive the corresponding regression equation.

2.3.2. Procedure for tablets

Weigh and pulverize ten tablets. Transfer an accurately weighed quantity of the powder equivalent to 20 mg of the studied compound into a small conical flask, extract the drug three times each with 30 ml of distilled water by sonication for 15 min. Filter the extract into a 100 ml volumetric flask. Wash the conical flask with few milliliters of distilled water and pass the washings into the same volumetric flask, complete to volume with the same solvent. Transfer aliquot volumes covering the working range cited in Table 2 into 25 ml volumetric flask. Complete to the mark with BRb of pH 10, transfer into the polarographic cell and pass nitorgen gas for 5 min. Record the DC_t and DPP polarograms. Calculate the labeled content of the tablets using either calibration graph or the corresponding regression equation.

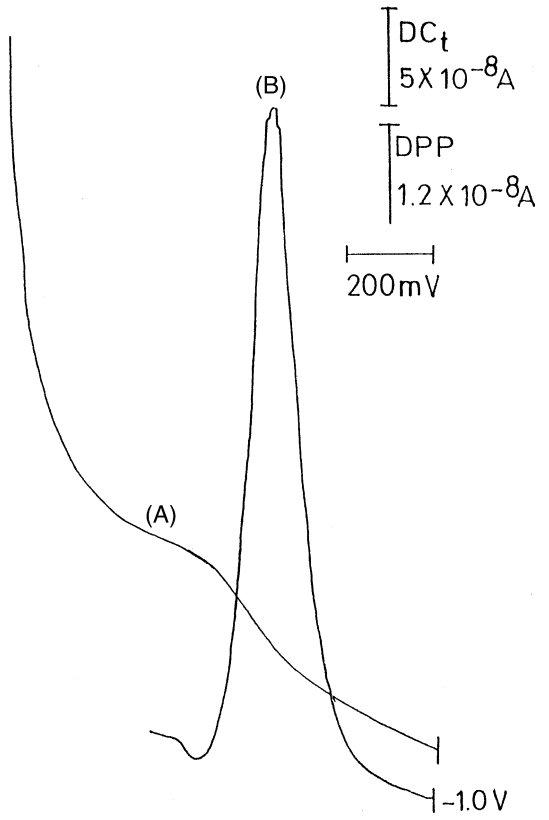


Fig. 1. Typical polarogram of TRZ HCl (16 μg ml^{-1}) in BRb of pH 10.0. (A) DC_t mode, (B) DPP mode.

2.4. Assay of trazodone HCl in biological fluids

2.4.1. Urine

Transfer 1 ml of urine sample spiked with a suitable amount of TRZ HCl into 25 ml volumetric flask. Complete to the volume with BRb of pH 10. Transfer the whole contents of the flask into the polarographic cell. Pass nitrogen gas for 5 min. Determine the nominal content of the drug from the corresponding regression equation.

2.4.2. Plasma

Transfer 1 ml of spiked plasma into a centrifuge tube. Add 0.2 ml of 2 M NaOH. Extract with 3 × 5 ml of diethyl ether and shake well on a vortex mixer for 30 s then centrifuge for 5 min at 12 000 rpm in a microcentrifuge. Transfer the clear supernatant into an evaporating dish and evaporate the combined extracts under nitrogen gas. Dissolve the residue in 5 ml of methanol. Then proceed as described above. Determine the nominal content of the drug from the corresponding regression equation.

3. Results and discussion

Fig. 1 shows typical DC_t and DPP polarograms of TRZ HCl in BRb of pH 10. Reduction of TRZ at the DME was found to be pH dependant as the $E_{1/2}$ values were shifted to more positive values upon increasing the pH (Fig. 2).

Logarithmic analysis of the reduction waves obtained in BRb of different pH values resulted in straight lines. The αn_a values were calculated using the treatment of Meites and Israel [24]. The results are shown in Table 1. Assuming that the rate determining step involves the transfer of two electrons (a free radical, one-electron transfer is

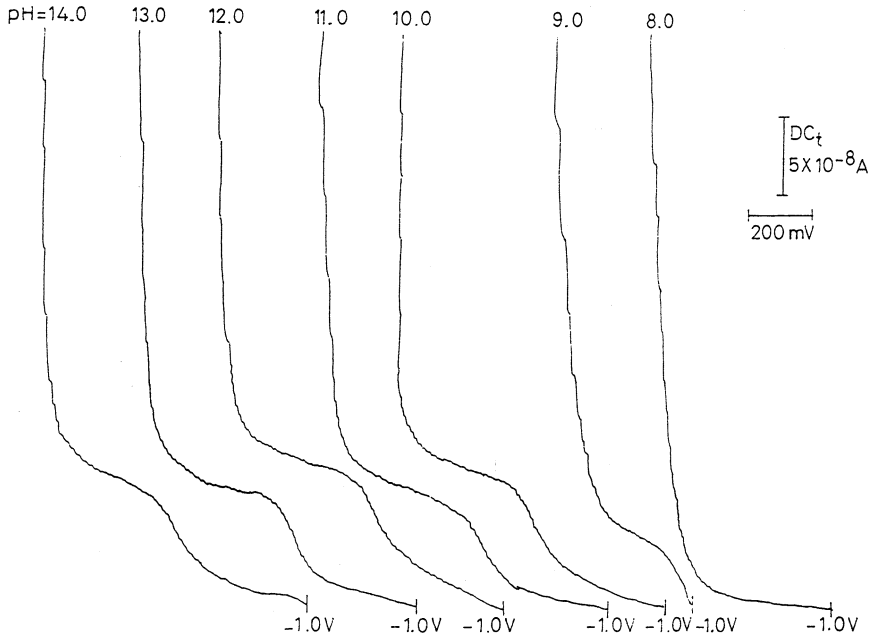


Fig. 2. Effect of pH on the development of the polarographic waves of TRZ HCl (2×10^{-5} M).

Table 1
Effect of pH on the development of the polarographic waves of TRZ HCl

pH	$E_{1/2}$ (V)	$\Delta E_{1/2}/\Delta$pH	Half peak width $W_{1/2}$ (mV)	αn_a
10.0	−1.45	−10	125	0.93
11.0	−1.44	−10	135	0.62
12.0	−1.43	−10	140	0.74
13.0	−1.42	0	160	0.70
14.0	−1.42		140	0.88

$W_{1/2}$ is the half-peak width in DPP mode; α is the transfer coefficient; n_a is the number of electrons transferred at the rate determining step.

not likely to occur), the values of αn_a suggest that the reduction process is irreversible in nature.

3.1. Study of the wave characteristics

Increasing the mercury height (h) resulted in a corresponding increase in the waveheight (w); a plot of $\sqrt{h}$ versus the wave height gave a straight line. A plot of $\log h$ versus $\log w$ gave a straight line, the slope of which was 0.6. Changing the buffer concentration over the range 0.006–0.06 M resulted in a negligible decrease in the wave height. These two characteristics point out to a diffusion controlled nature of the wave.

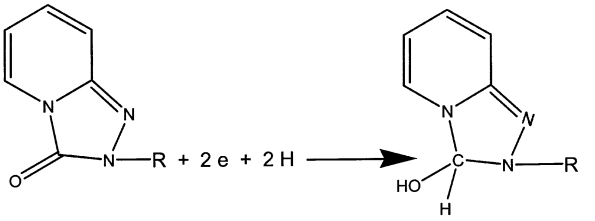
The alternating current-behavior (AC_t) of TRZ was studied using a phase-selective angle of 90°. In BRb of pH 10, the summit potentials (E_s) was shifted to more negative value of 110 mV than the corresponding $E_{1/2}$ value. Fig. 3 demonstrates that no adsorption, neither of the depolarizer nor its reaction product, takes place.

The diffusion current constant [$I_d = i_d/Cm^{2/3} \times t^{1/6}$] was calculated at 25 °C and was found to be 4.31 ± 0.02. The diffusion coefficient (D) of TRZ was also calculated using Ilkovic equation [25] and was found to be 1.26×10^{-5} cm^2 s^{-1} in BRb of pH 10. This small value is attributed to the bulky nature of the compound.

Solutions of the studied compound in BRb of pH 10 were found to be stable for more than 2 h.

3.2. Mechanism of electrode reaction

The electrode reaction and the number of electrons transferred were determined through a comparative experimental study of the waveheight of TRZ HCl with that obtained from an equimolar solution of a previously studied, structurally-related, compound with the same reducible function group, and of nearly identical value of diffusion coefficient; namely fleroxacin [26]. In BRb of pH 10 both compounds gave one wave of the same height, and thus pointing out to a two-electron transfer process. It can be concluded that, only the carbonyl group is involved in the reduction process according to the following mechanism.



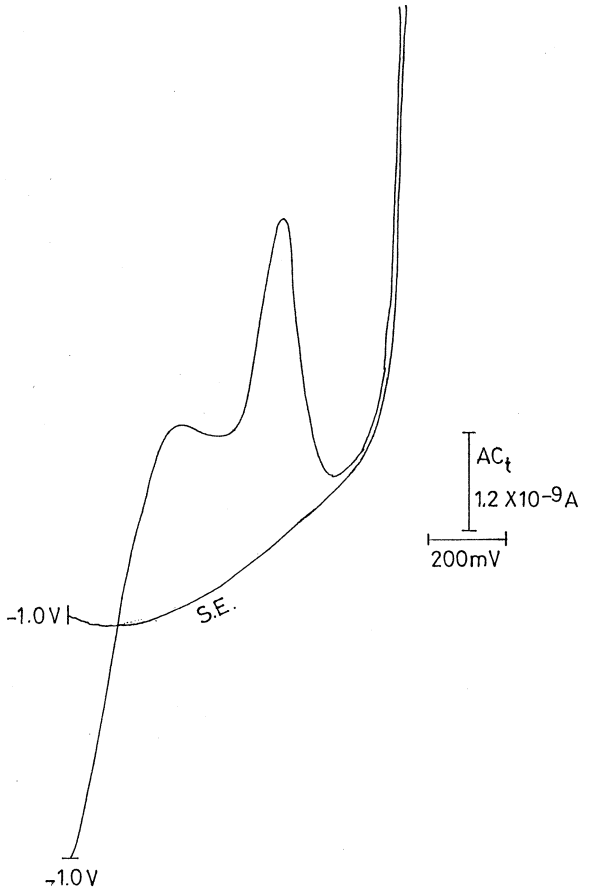


Fig. 3. Alternating current behaviour of TRZ HCl (20 μg ml^{-1}) in BRb of pH 10. Superimposed alternating voltage, 15 mV; frequency, 75 Hz; phase angle 90° (SE, supporting electrolyte).

3.3. Analytical applications

Polarograms of TRZ exhibit well-defined cathodic wave. The current is diffusion-controlled and is proportional to the concentration of the depolarizer over a convenient range of concentration. Both DC_t and DPP modes were successfully applied to the assay of TRZ either per se, in pharmaceutical dosage forms or in spiked biological fluids.

Plots representing the relationship between the concentration of TRZ HCl and diffusion current give straight lines over the concentration range of 4.0–32 and 0.8–24 μg ml^{-1} using DC_t and DPP modes, respectively, with a minimum detectability ($S/N = 2$) of 0.104 μg ml^{-1} (2.45×10^{-6} M) and 0.314 μg ml^{-1} (7.397×10^{-6} M) using the DPP and DC_t modes, respectively. Linear regression analysis of the data gave the following equations:

$$i_d = -2.451 \times 10^{-4} + 0.013C \quad (r = 0.9999)$$

using DC_t mode …… and

$$i_p = 5.209 \times 10^{-5} + 5.633 \times 10^{-3}C \quad (r = 0.9999)$$

using DPP mode, respectively, where C is the concentration in μg ml^{-1}, i_d is the current in μA in the DC_t mode and i_p is the current in μA in the DPP mode (Table 3).

Statistical evaluation of the regression lines regarding the standard deviation of the residuals ($S_{y/x}$), the standard deviation of the intercept (S_a) and the standard deviation of the slope (S_b) is given in Table 2. Statistical analysis [27] of the results obtained by both methods using the Student's t-test and variance ratio F-test, shows no

Table 2
Performance data of the proposed method

Parametrs mode	DC_t	DPP
Concentration range (μg ml^{-1})	4–32	0.8–24
Minimum detectability (M)	7.397×10^{-6}	2.45×10^{-6}
Correlation coefficient (r)	0.9999	0.9999
Slope	0.013	5.633×10^{-3}
Intercept	-2.451×10^{-4}	5.209×10^{-5}
$S_{y/x}$	1.339×10^{-3}	1.961×10^{-4}
S_a	6.546×10^{-4}	9.995×10^{-5}
S_b	5.742×10^{-5}	9.464×10^{-6}
Percent error	0.17	0.200
Applications	Tablets	Tablets, spiked urine and plasma

$S_{y/x}$, is the standard deviation of the residuals; S_a, standard deviation of the intercept of regression line; S_b, standard deviation of the slope of regression line; % error, R.S.D.%/$\sqrt{n}$.

significant difference between the performance of the two methods regarding the accuracy and precision, respectively (Table 4).

Both DC_t and DPP modes were successfully applied to the assay of the studied compound either per se, in pharmaceutical dosage forms or in spiked biological fluids. The percentage recoveries for TRZ in tablets are, 100.00 ± 0.16, 100.10 ± 0.42, respectively, using DC_t mode and 100.66 ± 1.086, 100.24 ± 1.09, respectively, using DPP mode (Table 3).

The results obtained for TRZ in tablets (Table 5) were favorably compared with a reference method [3].

4. Analysis of biological fluids

TRZ HCl is a triazolopyridine derivative with antidepressant effect. It is well-absorbed after oral administration. Peak plasma concentrations fol-

Table 3
Correlation between the concentration of TRZ HCl and the diffusion current in the DC_t mode

Number	Concentration (mM)	Current (μA)	i_d/C (μA mM^{-1})	$I_d = i_d/Cm^{2/3}t^{1/6}$
1	0.0098	0.0512	5.224	4.310
2	0.0196	0.1024	5.224	4.310
3	0.0294	0.1530	5.204	4.294
4	0.0392	0.2038	5.199	4.289
5	0.0588	0.3094	5.262	4.342
6	0.0784	0.4088	5.214	4.302
X			5.221	4.31
±S.D.			0.02	0.02

Each result is the average of three separate determinations.

Table 4
Polarographic analysis of TRZ HCl in pure form using DC_t and DPP modes

Parameters	DC_t mode	DPP mode	Reference method [3]
Number of experiments	6	7	3
Mean found (%) ± S.D.	100.13 ± 0.45	99.74 ± 0.58	100.00 ± 0.90
Variance	0.203	0.336	0.81
Student's t-value	0.30 (2.37)	0.56 (2.31)	
Variance ratio F-test	3.99 (5.79)	2.41 (5.14)	

Figures between parentheses are the tabulated t and F values, respectively, at $P = 0.05$ [27].

Table 5
Polarographic determination of TRZ HCl in dosage forms using DC_t and DPP modes

Preparations	Percent recovery		Reference method [3]
	DC_t mode	DPP mode	
Tritico tablets (TRZ HCl 100 mg per tablet)	99.86	101.00	100.31
	100.18	99.44	99.50
	99.98	101.53	100.08
Mean found (%) ± S.D.	100.00 ± 0.16	100.66 ± 1.086	99.96 ± 0.41
Student's *t*-value	0.16	1.04	
Variance ratio *F*-test	6.46	7.02	
Tritico tablets	101.25	100.95	101.10
(TRZ HCl 50 mg per tablet)	100.63	98.99	100.00
	101.43	100.78	99.81
Mean found (%) ± S.D.	101.10 ± 0.42	100.24 ± 1.09	100.30 ± 0.70
Student's *t*-value	1.69	0.08	
Variance ratio *F*-test	2.78	0.41	

The tabulated values of *t* and *F* are (2.78) and (19.0), respectively, at $P = 0.05$ [27]. All the pharmaceutical preparations are products of EIPICO. Egyptian INT. Pharmaceutical Industries Co. A.R.E.

lowing oral administration of a single 50 mg dose ranges from 0.5 to 1.0 μg ml^{-1} [28]. Following oral ingestion of a single 100 mg TRZ HCl dose average maximum plasma concentration of 1.61 μg ml^{-1} was reported for a capsule formulation [29]. This value lies within the working concentration range of the proposed method.

The DPP mode could be successfully applied to the determination of TRZ in spiked urine and plasma, over the specific concentration range. The results are abridged in Table 6. The mean percentage recoveries for TRZ HCl in spiked urine and plasma at the same day are 98.43 ± 0.79 and 97.44 ± 0.705, respectively, using DPP mode.

Table 6
Polarographic determination of TRZ HCl in spiked urine and plasma using DPP mode

Sample	Added amount (μg)	Amount found (μg)	Percent recovery
1-a-Urine (intra-day precision)	2.0	1.974	98.70
	2.0	1.987	99.35
	2.0	1.950	98.13
	2.0	1.950	97.50
Mean			98.43
±S.D.			0.79
1-b-Urine (inter-day precision)	2.0	2.025	101.25
	2.0	1.961	98.05
	2.0	1.987	99.35
	2.0	1.975	98.75
Mean			99.35
±S.D.			1.37
2-Plasma (intra-day precision)	2.0	1.950	97.50
	2.0	1.933	96.65
	2.0	1.967	98.35
	2.0	1.945	97.25
Mean			97.44
±S.D.			0.705

4.1. Precision and repeatability

The within days precision was evaluated through replicate analysis of urine sample spiked with 2 μg ml^{-1}. The percentage recoveries based on four separate determinations ranged from 97.50 to 99.35 with mean percentage recovery of 98.43 ± 0.79. However, those for spiked plasma ranged from 96.65 to 98.35 with mean percentage recovery of 97.44 ± 0.705, thus indicating the high precision of the method.

The inter-day precision on four successive days was evaluated through replicate analysis of urine sample spiked with 2 μg ml^{-1}. The percentage recoveries based on the average of four separate determinations were 99.35 ± 1.37.

5. Conclusion

On conclusion, simple, rapid and sensitive methods have been developed for the determination of TRZ HCl in formulations and spiked urine and plasma. However, the DPP mode proves a specificity over the DC_t mode regarding its validity for biological fluids, where 0.8 μg ml^{-1} could be determined with a reasonable reproducibility. The DC_t mode could be applied over the concentration range 4–32 μg ml^{-1} which is more applicable for dosage forms. The proposed methods have distinct advantages over other existing methods regarding sensitivity, time saving and minimum detectability, moreover, it can be applied to the determination of TRZ HCl in spiked human urine without prior treatment.

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