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Electrochemical behaviour and determination of acrivastine in pharmaceuticals and human urine

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Abstract

The differential pulse polarography, DC-tast polarography and cyclic voltammetry behaviour of acrivastine was studied in Britton–Robinson buffer solutions (pH 2–11.7). In acidic media, a non-reversible diffusion controlled reduction process involving four electrons takes place. Two reduction waves appear at a $E_{1/2} = -0.6$ and -0.99 V. The reduction mechanism is discussed. The linear relationship between peak current height and acrivastine concentration allowed the differential pulse polarographic determination of acrivastine over a wide concentration range, from 0.35 to 26.1 mg l^{-1}at pH 2.5. The procedure was applied to determination of the drug in pharmaceutical formulations and human urine samples.
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Keywords: Acrivastine determination; Differential pulse polarography; Urine; Pharmaceuticals

1. Introduction

Acrivastine,(E,E)-3-[6-[1-(4-methylphenyl)-3-(1-pyrrolidinyl)-1-propenyl)-2-pyridinyl]-2-propenoic acid] is a short acting histamine H_1-receptor antagonist with a rapid onset of action structurally closely related to triprolidine. The addition of a polar acrylic chain to the pyridine ring decreases lipophilicity and lessens the propensity of acrivastine to enter the central nervous system [1]. Double-blind clinical trials have shown acrivastine (usually 8 mg three times daily) to be an effective and well tolerated antihistamine in the treatment of chronic urticaria and allergic rhinitis [2].

Acrivastine has been studied and determined by several procedures: high-performance liquid chromatography (HPLC) for the determination of acrivastine in capsules using ultraviolet detection [3], gas chromatography mass-spectrometric analysis [4], a sensitive radioimunoassay to measure plasma levels [5], two non-direct spectrofluorimetric methods [6,7] and several spectrophotometric procedures to measure acrivastine in urine and pharmaceuticals [3,6,8,9]. The reported methods show that there are very few methods for the

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determination of acrivastine in biological fluids as it depends on the use of CG-MS [4], involving a lengthy, tedious method that requires a highly sophisticated and expensive instrumentation. The other method depends on the use of derivative spectrophotometry [8] and requires a prior extraction step. This method is also subject to interference from compounds present in urine.

On the other hand, electrochemical studies on the determination of acrivastine are scarce.

In this paper, different electrochemical techniques were used to study acrivastine in aqueous solution, and a rapid direct method was developed for the determination of this compound at low concentration levels. The method was applied to the analysis of pharmaceuticals and human urine samples.

2. Experimental

2.1. Reagents

All reagents were of analytical-reagent grade or better. High-purity water was obtained from a Millipore (Milford, MA) Milli-Q Plus system and used throughout. Acrivastine chlorhydrate was friendly provided by Glaxo-Wellcome (Greenford, Middlesex, UK). A total of 348.0 mg l^{-1} aqueous stock solutions of acrivastine chlorhydrate were freshly prepared weekly. Britton–Robinson buffer of pH 2.5 (prepared by mixing 5 ml of KCl (100 g l^{-1}), 0.9 ml of NaOH 0.8 M and 6.25 ml of an acid solution 0.3 M in each of the following acids: acetic acid, boric acid and ortho-phosphoric acid) was used as the supporting electrolyte in the voltammetric experiments. Voltammetric measurements require the suppression of polarographic maxima. A Triton X-100 aqueous solution (0.8% p/v) was used as suppressor.

2.2. Apparatus

Electroanalytical measurements were carried out by using a Potentiostat PGSTAT10-Autolab of ECO-Chemie B.V. (Utrecht, The Netherlands) in combination with a Metrohm 663 VA polarographic stand and fitted with a Hyundai 486/100 PC provided with the appropriate GPES 4.2 (General Purpose Electrochemical Software) software. A three-electrode combination was used, consisting of a saturated KCl/Ag/AgCl reference electrode, a dropping-mercury electrode (DME) as a working electrode and a platinum wire as auxiliary electrode. The temperature was controlled by using a double-wall polarogarphic cell and a Tectron (Barcelona, Spain) 3000543 thermoregulator. Controlled-potential microcoulometry was performed with the PGSTAT10-Methrom 663VA in the potentiostatic mode at a hanging drop mercury electrode as cathode with continuous stirring. Chronocoulometric plots were used for evaluating the results. A Philips (Eindoven, Netherlands) Model PU-8720 UV/VIS spectrophotometer was used for the absorbance measurements. A Crison (Bacerlona, Spain) Model 2002 pH-meter with a combined glass-calomel electrode was also used.. A HPLC equipment (Merck-Hitachi) consisted of a L-7100 pump, a Rheodyne (Cotati, CA) model 7725i injection valve with a 20 μl sample loop, a 25 cm × 4 mm LichroCART RP-18 5 μm column (Tecknokroma, Barcelona, Spain) and a model L-7455 diode array detector controlled by a Merck-Hitachi D-7000 interface equipped with a HPLC system manager® software.

2.3. Polarographic determination of acrivastine

An aliquot of sample solution containing 9.50–653 μg of acrivastine was placed into a 25 ml calibrated flask, 5 ml of Britton–Robinson buffer (pH 2.5) and 1 ml of triton X-100. The solution was diluted to volume with water and mixed well. The solution was then transferred into a polarographic cell. The differential pulse polarogram was recorded, after deoxygenation with a stream of pure nitrogen during 10 min, from −0.2 to −1.2 V. A calibration plot obtained with known concentration of acrivastine was used to convert peak height into sample concentrations. DC-tast polarography and cyclic voltammetry experiments were carried out under identical conditions. The prepared solutions remain stable for at least 24 h.

2.4. Determination of acrivastine in pharmaceuticals

The proposed procedure for the determination of acrivastine was applied to its direct determination in one pharmaceutical formulation (Semprex®-D capsules), after solubilization in water and filtration through 45 μm filter.

2.5. Determination of acrivastine in urine

The amount of acrivastine in urine samples was determined by the standard addition method.

A total of 4 ml of urine samples were placed into 25 ml calibrated flasks, aliquots of the stock solution of acrivastine, 5 ml of buffer (pH 2.5) and 1 ml of triton X-100 solution were added and the samples were diluted to the mark with water and mixed well. The polarograms were recorded according to the recommended procedure for acrivastine. Electrochemical response was linearly dependent on acrivastine concentration within the dynamic range from 0.35–26.1 mg l^{-1}.

3. Results and discussion

3.1. Reduction waves of the acrivastine

This study was made in the pH range 2.0–11.7 at a target concentration of 3.48 mg l^{-1} aqueous acrivastine solutions. Stock 348.0 mg l^{-1} solution of acrivastine was stable up to several months. DP and DC-tast polarography records show (Fig. 1) two reduction waves with half-wave potentials $E_{1/2} = -0.60$ V and $E_{1/2} = -0.99$ V at pH 2.5.

Plots of peak potential (E_p) versus pH are depicted in Fig. 2. As the pH was gradually increased, the peak potential shifted towards more negative values. Linear pH-dependence of the peak potential, for both waves, in the ranges:

Wave I

pH 2–3.8 E_p (V) = −0.789–0.057 pH; $r = 0.990$
pH 3.8–12 E_p (V) = 0.979−0.007 pH; $r = 0.982$

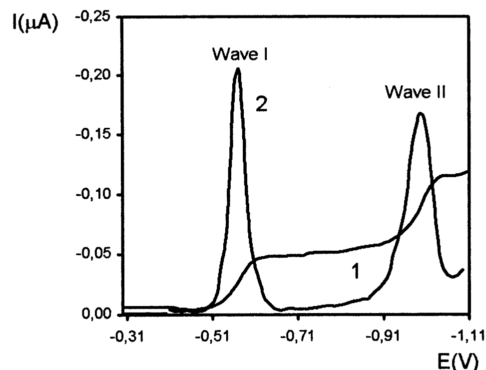


Fig. 1. Polarograms of acrivastine: 3.48 mg l^{-1}, pH 2.5, triton X-100 0.032% (p/v); (1) DC-tast polarography; (2) DP polarography.

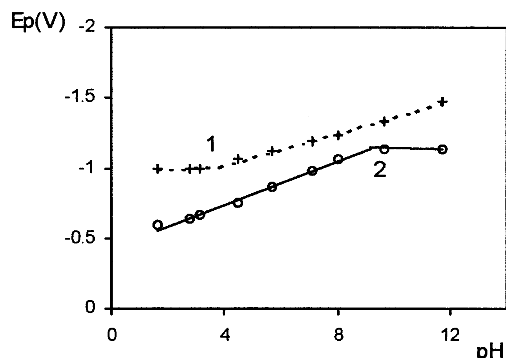


Fig. 2. Effect of pH on the peak potentials of differential pulse polarographic peak obtained for a 3.48 mg l^{-1} solution of acrivastine; (i) *Wave I*, (2) *Wave II*.

Wave II

pH 2–9.3 E_p (V) = −0.436–0.076 pH; $r = 0.988$
pH 9.3–12 E_p (V) = 1.188–0.005 pH; $r = 1$

shows that protons participate directly on the reduction process. The study of the influence of pH on peak currents (Fig. 3) was also carried out to determine whether the electroactive species participate in equilibria involving protons directly and to obtain the pH range for maximum signal. The height of the peak reaches a maximum in the pH range 1.5–3.0 for wave I and 1.5–5 for wave II. From these polarographic data, pH 2.5 was chosen as the optimum one for the determination of acrivastine.

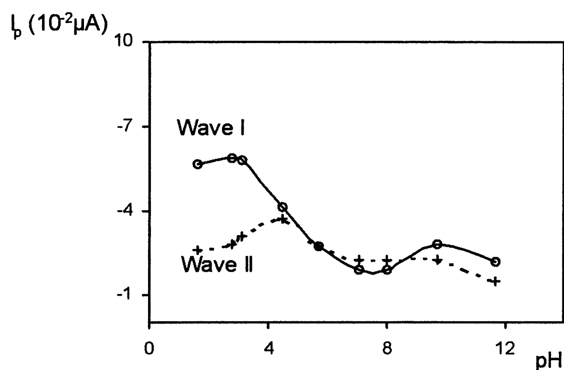


Fig. 3. Effect of pH on the peak current of differential pulse polarographic peak obtained for a 3.48 mg l^{-1} solution of acrivastine.

3.2. Effect of operating parameters

The peak height increased linearly, for both waves, with the increase in pulse amplitude from −15 to −80 mV and the peak potential was displaced towards positive potentials (Table 1).

A linear relationship between temperature and current height was observed for both waves when temperature was increased between 25 and 60 °C. Temperature coefficients values confirm that the processes are controlled mainly by diffusion (Table 2).

3.3. Number of electrons transferred, reversibility of the system and mechanism of reduction

Controlled-potential microcoulometry was used to determine the number of electrons transferred in the reduction process (n). Microelectrolyses of acrivastine solutions of different concentrations at pH 2.5 and pH 5.1 were done for both of the two waves (Table 3). The results obtained ($n_1 = 2$, $n_2 = 2$) supports the olephinic bond reduction hypothesis.

A cyclic voltammetry scan was recorded in order to determine the reversibility of the system. Acrivastine yields two well-defined reduction waves but oxidation waves are not observed (Fig. 4). The reduction waves are displaced to more negative potentials when the scan rate, V_b, increases, what is a characteristic behavior of irreversible processes. When plotting i_p versus $V_b^{1/2}$ a linear relationship was observed as with diffusion-controlled electrode processes, [I_{p1} (μA) $= 1.3\times10^{-2}$–$7.7\times10^{-3}V_b^{1/2}$, $r_1 = 0.9992$ (wave I); I_{p2} (μA) $= 7.8\times10^{-3}$–$3.3\times10^{-3}V_b^{1/2}$, $r_2 = 0.9991$ (wave II)]. The αn_a (electron transfer coefficient) value and the number of protons (p) corresponding to the rate-determining step were calculated at different pH values. In the pH range 2–6, αn_a was found to be 1.14 ± 0.23 (wave I) and

Table 1
Variation of peak intensity as a function of modulation amplitude

ΔE (mV)	i_p (μA) (Wave I)	E_p (V) (Wave I)	i_p (μA) (Wave II)	E_p (V) (Wave II)
−15	−0.009	−0.61	−0.008	−1.01
−20	−0.014	−0.6	−0.012	−1.01
−25	−0.019	−0.59	−0.016	−1.01
−30	−0.030	−0.6	−0.020	−1.01
−35	−0.037	−0.58	−0.026	−1.01
−40	−0.047	−0.57	−0.034	−1.01
−50	−0.060	−0.57	−0.045	−1.01
−60	−0.073	−0.56	−0.059	−0.99
−70	−0.086	−0.56	−0.080	−0.99
−80	−0.097	−0.55	−0.092	−0.99
−90	−0.098	−0.55	−0.010	−0.99
−100	−0.098	−0.53	−0.011	−1.01
−120	−0.098	−0.51	−0.011	−0.96
−140	−0.098	−0.49	−0.011	−0.93
−160	−0.096	−0.47	−0.096	−0.92
−180	−0.090	−0.45	−0.083	−0.9

Table 2
Temperature coefficients

	DC-tast		DP	
	Wave I	Wave II	Wave I	Wave II
Temperature coefficients, %	1.79 ± 0.27	−3.5 ± 0.42	−0.49 ± 0.09	−1.27 ± 0.14

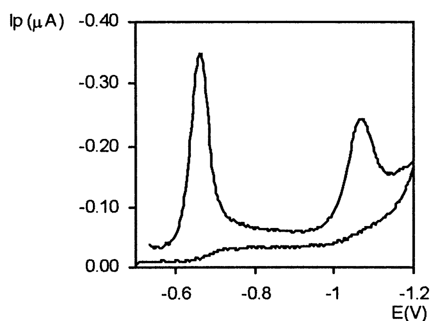


Fig. 4. Current–potential curve for cyclic voltammetry.

1.98 ± 0.36 (wave II). Using the following expression: $\Delta E_{1/2}/\Delta pH = 0.059p/\alpha n_a$, p was found to be 1.78 ± 0.73 (wave I) and 1.60 ± 0.21 (wave II).

Taking into account the mechanism proposed for a similar compound, triprolidine [10] (Fig. 5) that shows a cathodic wave assigned to the reduction of the olephinic bond, a solution of triprolidine was prepared according to the above described procedures to compare the polarogram with that obtained for acrivastine. Fig. 6 shows the DP and DC-tast polarography records, a reduction wave with half-wave potential $E_{1/2} = -1.05$ V that coincide with the second wave of acrivastine. This leads to conclude the following mechanism for the reduction of acrivastine.

The less cathodic wave (−0.60 V) has been assigned to the reduction of the olephinic bond between the pyridinic ring and the carboxylic group.

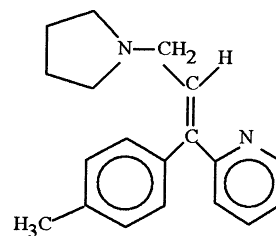


Fig. 5. Chemical formula of triprolidine.

Table 3
Number of electrons calculated using microcoolometry at different pH and concentrations

Concentration (mg ml^{-1})	n			
	pH 2.5		pH 5.1	
	Wave I	Wave II	Wave I	Wave II
2.61	2.04	2.52	2.18	2.38
3.48	1.53	2.26	1.42	1.80
10.44	1.50	2.02	1.63	1.99
17.4	1.60	2.10	1.75	1.87
26.1	1.73	1.80	1.50	2.24
$\bar{n}$	1.68 ± 0.20	2.15 ± 0.25	1.70 ± 0.27	2.06 ± 0.25

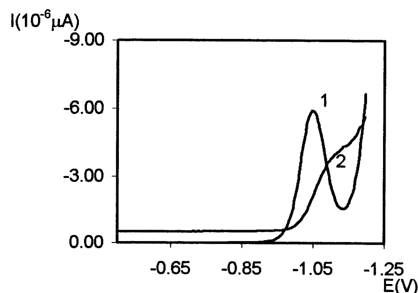
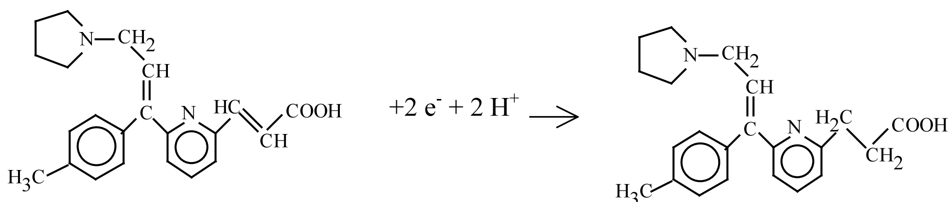
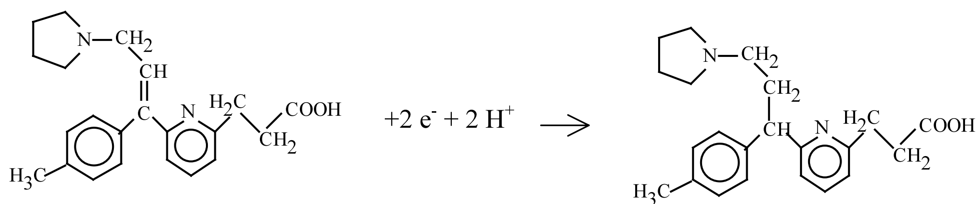


Fig. 6. Polarograms of triprolidine pH 2.5, triton X-100 0.032% (p/v); (1) DC-tast polarography; (2) DP polarography.



The second cathodic wave (−1.00 V) has been assigned to the reduction of the olephinic bond between the three rings.



3.4. *Variation of peak intensity with concentration of acrivastine*

A calibration plot was obtained from known concentrations of acrivastine using the peak current in µA as analytical response. Series of standard solutions (five replicates) of acrivastine were prepared by following the procedures described in recommended procedures section. The calibration graph was found to be linear over the range 0.35–26.1 mg l^{-1} (Eqs. (1) and (2))

$$\text{Wave I} \quad I_p = (0.013 \pm 2.750 \times 10^{-4})C + (0.027 \pm 4.550 \times 10^{-4}) \quad (n = 10, r = 0.9998) \tag{1}$$

$$\text{Wave II} \quad I_p = (7.34 \times 10^{-3} \pm 1.66 \times 10^{-4})C + (6.23 \times 10^{-3} \pm 2.79 \times 10^{-4}) \quad (n = 10, r = 0.9997) \tag{2}$$

The detection limit derived thereof according to

Table 4
Precision results obtained for both waves at different concentrations

	Concentration 1.05 mg ml^{-1}		Concentration 2.61 mg ml^{-1}	
	Wave I	Wave II	Wave I	Wave II
E (%)	2.86	4.40	1.28	1.53

Table 5
Optimisation of variables for analytical purposes

Initial potential	−0.2 V
Modulation amplitude	−50 mV
Scan rate	10 mV s^{-1}
Scan direction	Negative
Current range	0.1–100 μA
Buffer concentration	0.3 M
pH	2.5
Purge time	10 min

Miller and Miller [11] was 0.11 mg l^{-1} for both waves and the quantitation limits were 0.35 mg l^{-1} for wave I and 0.38 mg l^{-1} for wave II. Precision data were obtained for eleven determinations of 1.05 and 2.61 mg l^{-1} acrivastine solutions. The relative standard deviation is shown in Table 4. The optimised parameters for using the DP polarographic method for acrivastine determination are given in Table 5.

3.5. Interference studies

Acrivastine is usually associated with pseudoephedrine for its administration to patients suffering from allergies. Accordingly, the effect of pseudoephedrine on the determination of acrivastine was studied. The criterion for interference was current height (I_p) differing by more than 5% from that of acrivastine alone. It is possible to affirm that pseudoephedrine does not interfere in the determination of acrivastine in a relation lower than 1:12 (w/w).

As pharmaceutical formulations contains excipients, the effect of the following substances: lactose, sacarose, glucose and fructose, was studied. Using the same criterion for interference as with pseudoephedrine it is possible to affirm that the four substances does not interfere in the

Table 7
Total content of acrivastine for urine samples (for details see text)

Proposed method[a] (mg)	Chromatographic method[a] (mg)	F_{test}	t_{test}
12.8 ± 0.2	12.7 ± 0.2	1	0.87
12.4 ± 0.1	12.4 ± 0.2	4	0
13.1 ± 0.1	13.2 ± 0.1	1	1.73
13.3 ± 0.2	13.2 ± 0.2	1	0.87
13.6 ± 0.1	13.6 ± 0.1	1	0
12.5 ± 0.1	12.7 ± 0.2	4	0.77
13.1 ± 0.1	13.1 ± 0.2	4	0
13.4 ± 0.2	13.4 ± 0.2	1	0
13.0 ± 0.1	13.1 ± 0.1	1	1.73
13.3 ± 0.2	13.3 ± 0.1	4	0
		39.1[b]	3.18[b]

[a] Average of five determinations ± S.D.
[b] Critical values for F and t ($P = 0.05$).

Table 6
Results for the analysed pharmaceutical (Semprex-D®)

Label content (mg)	Proposed method[a] (mg)	Chromatographic method[a] (mg)	F_{test}	t_{test}
8	7.9 ± 0.2	8.0 ± 0.2	1	0.79
			9.61[b]	2.31[b]

[a] Average of five determinations ± S.D.
[b] Critical values for F and t ($P = 0.05$).

determination of acrivastine in a relation of 1:1000 (w/w).

3.6. Determination of acrivastine in pharmaceutical formulations

Five capsules of Semprex-D® (Glaxo-Wellcome) with acrivastine label content of 8 mg and pseudoephedrine contents of 60 mg, respectively, were directly analysed, after solubilization in water and filtration through 45 μm filter, with the proposed method from external calibration obtaining a value of 7.9 ± 0.2 mg of acrivastine. The application of an independent method (derivative spectrophotometric determination [9] at 288 nm shows a result of 8.0 ± 0.2 mg; as can be observed in Table 6, there is a good agreement between both methods and the application of Student's t-test and F-test indicate that the method is accurate (null hypothesis accepted) [11].

3.7. Determination of acrivastine in urine

Two capsules of Semprex-D® (Glaxo-Wellcome) with acrivastine label content of 8 mg and pseudoephedrine contents of 60 mg were orally administered to ten volunteers within an 8 h interval. Urine samples were collected over a period of 48 h and treated according to procedure described in the experimental section. Determinations for triplicate of each urine sample using the standard addition method lead the results of Table 7.

The samples were also analyzed by a HPLC method using a LichroCART RP-C18 column, a mixture of water/acetonitrile/methanol/perchloric acid/n-octylamine (500:130:25:13:0.3 v/v) as mobile phase, and UV detection at 260 nm [3]. As can be seen, good agreement was found between the HPLC and the proposed method, statistically proved according to the paired t-test and F-test [11], showed in Table 7. The excreted acrivastine is about of 80% of the total amount administered, which is in good agreement with the data of other authors [8].

4. Conclusions

The results obtained show that the proposed method may be useful to determine acrivastine in human urine at the levels obtained after the administration of normal clinical doses and it would be a method of choice for monitoring this substance in patients, as well as it could be used as a stability-indicating method. The method has been also applied to the determination of the active constituent in a pharmaceutical preparation. The proposed procedure can be an alternative method to the chromatographic methods for the analysis of acrivastine, and allows the direct determination of acrivastine in urine without extraction procedures; however, pseudoephedrine does not interfere at the clinical dosages.

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