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Research Article

ASSESSMENT OF ELECTROANALYTICAL BEHAVIOR OF FAMOTIDINE IN PRESENCE OF ZINC BY DIFFERENTIAL PULSE POLAROGRAPHY

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ABSTRACT

Famotidine (3-[[[2-13(Aminoiminomethyl) amino] - 4thiazolyl] methyl] thio]-N-(aminosulfonyl) propanimidamide) is a histamine H2-receptor antagonist that is a highly potent inhibitor of gastric and acid secretion in humans. The differential pulse polarographic method (DPP) was used for investigation of the electrochemical properties and the quantitative analysis of Famotidine at the mercury electrode. The optimum experimental parameters for the differential pulse polarography (DPP) method were: current range 10uA, data acquisition fast, scan rate: 6mV/sec, drop time: 1sec, scan type: forward, pulse amplitude: 100mV. The linearity for famotidine in HPLC and DPP was found in the concentration range of 1 to 8 µM and 10 to 100 µM respectively. The correlation coefficient values for HPLC and DPP were 0.998 and 0.996 respectively. Limit of detection values for HPLC and DPP were found to be 0.3659 µM and 9.7615 µM respectively. Limit of quantification for HPLC and DPP was found 1.2196 µM and 32.23 µM respectively. From the statistical analysis of the proposed technique, the differential pulse polarography method is more sensitive than HPLC method.

Keywords: Metallopharmaceutical, Famotidine, polarography,

I. INTRODUCTION

Famotidine (FAM) (**Fig. 1**) is chemically (3-[[[2-[(Aminoiminomethyl) amino] - 4thiazolyl] methyl] thio]-N-(aminosulfonyl) propanimidamide). It is commonly used in the treatment of peptic ulcer disease and gastro oesophageal-reflux disease. Famotidine is histamine H_2-receptor antagonist which blocks the action of histamine on stomach cells and reduces acid production. Several techniques including HPLC[1], HPTLC[2], capillary electrophoresis[3], differential pulse voltammetry[4], potentiometry[5], polarography[6], chemiluminescence spectrometry[7] and spectrofluorometry[8] have been employed for determining famotidine in pharmaceutical dosage forms. Some of these methods have enough sensitivity to determine lower concentrations of a drug; however, many of these techniques are deficient in simplicity, cost-effectiveness, and accessibility. Differential pulse polarography is characterized by its speed and simplicity, accuracy and inexpensive instrument needed. Hence it is an important alternative to other analytical techniques with clear advantages in terms of cost of analysis.

The literature review shows that the electro-analytical behavior of the Zn (II) - famotidine complexes has not been studied by DPP till today. Considering the great advantage of DPP, we have studied the electrochemical characterization of famotidine in presence of zinc in pharmaceutical preparation by DPP.

II. MATERIALS AND METHODS

A. Chemicals

Analytical reagent grade famotidine was used for the preparation of solutions in HCl using double distilled water. The purity of reference standards was 99.9%. All other reagents employed were of analytical grade and used without further purification.

B. HPLC studies famotidine

About 40 mg of famotidine was accurately weighed and transferred to a 25 ml volumetric flask. It was dissolved in 15 ml of methanol and made up to the volume with methanol and sonicated for 8 min. From this, a working standard solution of 100 µg/ml of strength was prepared. From this dilution 10, 20, 40, 60 & 80 µg/ml were prepared in 10 ml volumetric flasks with methanol. From each diluted solution, 20 µl of sample was injected into the column at a flow rate of 1.0 ml/min. Each sample was injected 3 times into the column and the corresponding chromatograms were obtained.

C.DPP studies of metal - famotidine complex

To measure the complexation of Zn (II) with famotidine, the electrochemical cell was assembled with 10 mL of sodium acetate buffer having pH 5.00 ±0.10, containing 0.1M KCl in deionized water. Then, the solution was cleaned thoroughly with pure nitrogen for 10 minutes. The polarograms were recorded in the following order: pure supporting electrolyte, after Metal (II) addition, and after addition of each aliquot of famotidine.

III. Method optimization for famotidine-metal complex

The optimization steps for the study of the famotidine-metal complex were carried out using two different concentrations of ligand and metal. The background polarogram was obtained using the following run conditions for differential pulse polarography (DPP); current range: 10µA, data acquisition: fast, scan rate: 6mV/sec, drop time: 1sec, scan type: forward, pulse amplitude: 100mV.

General procedure for polarographic analysis was employed. The Ip and Ep values were observed. The same procedure was repeated with the same parameters. For the optimization steps, the influence of each parameter on the Ip and Ep of each complex was studied. In each experiment, one of the parameters was varied while others were kept constant.

A. Effect of pH

The effect of pH on Ip and Ep of the famotidine-metal complex was studied in the pH range 2-10 pH units containing fixed [Zn^{+2}] and [famotidine] in acetate buffer as a supporting electrolyte and graph of peak current Ip vs. pH and peak potential vs. pH was plotted.

B. Effect of scan Rate (v)

The scan rate (mVs^{-1}) plays an important role in the peak resolution. After the best condition of pH was chosen, the effect of v was carried out. The v were varied from 3 mVs^{-1} to 12 mVs^{-1} while maintaining other parameters constant.

C. Effect of pulse amplitude

The best conditions of other parameter were chosen and the pulse amplitude was optimised as the final step in this optimisation procedure. The effect of pulse amplitude was carried out with a variation of 5 to 100 mV. A graph of Ip against pulse amplitude was then plotted.

IV. Instrumentation

For DPP measurements, a polarographic analyzer model CL-362 supplied by an Elico Ltd, Hyderabad was used. A dropping mercury as a working electrode, saturated calomel as reference and platinum wire as auxiliary electrodes were used.UV-VIS spectrophotometer, PerkinElmer Lambda 25, in 1 cm quartz cell was used for Spectrophotometric analysis. All measurements were made at room temperature. The pH measurements were carried out with the help of Elico pH meter. The specifications of HPLC system used for the study are given below. Quantitative HPLC was performed on a gradient high pressure liquid chromatography (Perkin Elmer HPLC 1100) with one LC-10 AT VP pumps, with UV/VIS detector SPD-10A VP, CTO-10 AS VP column oven (Perkin Elmer), SCL-10AVP system controller (Perkin Elmer), a disposable guard column LC-18 (PELLIGUARD)TM, LC-18, 2 cm, supelco,inc., Bellefonte, and a Reverse Phase C-18 Column (25cm x 4.6 mm i.d; particle size 5 µm) was used. The total chromatographic navigator (Perkin Elmer) software was used for HPLC analysis.

V. RESULTS AND DISCUSSION

A. High-performance liquid chromatography (HPLC)

An RP-HPLC method was proposed as a suitable method for the estimation of famotidine in pharmaceutical dosage form. A good separation was achieved using a C18 column. The chromatographic conditions were

adjusted in order to provide a good performance of the assay. The method involved a mobile phase consisting of methanol-water (80:20, v/v) accomplished at 270 nm. The retention time was 3.3 min at a flow-rate of 1 mL min^{-1} and the injection volume was 10µl. The total run time for an assay was 10 min. The mobile phase was chosen after several trials with other solvent combinations. Mobile phase selection was based on peak parameters (symmetry, tailing), run time, ease of preparation and cost. **Fig. 2** shows a typical chromatogram obtained from the analysis of a standard famotidine using the proposed method.

B. Differential pulse polarography

Before quantitative determination, the electrochemical behavior of famotidine and zinc on DME was studied by differential pulse polarography. DPP curves were recorded in the different supporting electrolytes. The results that were obtained **(Fig. 3)** showed that acetate buffer pH 5.0 containing 1.0 M KCl was the best medium for detection and quantification of famotidine and zinc on the mercury electrode.

C. Calibration of famotidine by HPLC Method

Various mobile phases containing different ratios of methanol and water were examined. The methanol: water (8:2 v/v) mobile phase was selected as optimal for obtaining well defined and resolved peaks. The optimum wavelength for detection and quantitation used was 290 nm. The retention factors for famotidine were found to be 0.23±0.102. Representative chromatogram of famotidine standard solution is as shown in **Fig.2**.The linearity for famotidine in HPLC was found in the concentration range of 1 to 8 µM with the correlation coefficient value 0.998 as shown in **Fig.4 (Table 1).**

D. Calibration of famotidine by DPP Method

The prepared stock solution of famotidine in acetate buffer was further diluted to 10mL to get working standard solution of concentration range 10-100 **µ**M. The Peak current of the solution was measured at its peak potential -1.372 V against acetate buffer as a blank. Calibration curve of famotidine the drug was then plotted by taking the peak current obtained on the y-axis and the concentration of the solution on the x-axis (**Fig. 5).** The curve showed linearity in the range of 10-100 **µ**M with correlation coefficient 0.996. **(Table 2).**

E. Calibration of Zinc by DPP Method

The prepared stock solution of Zn in acetate buffer pH 5.0 was further diluted to 10mL to get working standard solution of concentration range 10-100 **µ**M. The Peak current of Zn solution was measured at its peak potential -1.006 V against acetate buffer as a blank. The linearity curve and DPP polarogram of Zn are as shown in **Fig. 6** with correlation coefficient 0.998. **(Table 3).**

F. CONCLUSION

Both proposed techniques are successfully developed and applied in the determination of famotidine in the pharmaceutical formulation. The results of both techniques were compared; HPLC is more sensitive technique than DPP method. However, the HPLC techniques have disadvantages, such as long analysis time; consume a lot of reagents and expensive, complicated operation, a high cost of maintenance, expensive apparatus and requiring well-controlled experimental conditions.

The DPP method has a great potential as an alternative method for this application in the future and successfully developed for the determination of famotidine, zinc, and their metal complexes in pharmaceuticals **(Table 4).**

Table 1: Linearity of famotidine by HPLC in methanol-water (80:20, v/v)

Conc. (µM)	Peak area	Yi=mx+b	Y-Yi	$(Y-Yi)^2$
1	109,526.40	102064	7,462.40	55687414
2	199,661.00	189188	10,473.00	1.1E+08
4	369,077.00	363436	5,641.00	31820881
6	528,616.40	537684	-9,067.60	82221370
8	712,358.50	711932	426.50	181902.3
			$\sum(Y-Yi)^2$	2.8E+08
		LOD		0.3659 µM
		LOQ		1.2196 µM
		Regression equation : y = 87124x + 14940		
		Regression Coefficient : R^2 = 0.998		

Table 2: Linearity of famotidine by DPP in acetate buffer pH 5.0

Conc. (µM)	Ip=Y	Yi=mx+b	Y-Yi	$(Y-Yi)^2$
10	0.150	0.15	-0.006	0
20	0.244	0.25	-0.007	3.6E-05
30	0.343	0.35	0.008	4.9E-05
40	0.458	0.45	0.035	6.4E-05
50	0.585	0.55	0.051	0.001225
60	0.701	0.65	0.048	0.002601
70	0.798	0.75	0.019	0.002304
80	0.869	0.85	0	0.000361
90	0.950	0.95	0.019	0
100	1.069	1.05	0	0.000361
			$\sum(Y-Yi)^2$	0.007001
		SD		0.02958
		LOD		9.7615 µM
		LOQ		32.23 µM
		Regression equation : y= 0.010x + 0.050		
		Regression Coefficient : R^2 = 0.996		

Table 3: Linearity of zinc by DPP in acetate buffer pH 5.0

Conc. (µM) Zn	Ip=Y	Yi=mx+b	Y-Yi	$(Y-Yi)^2$
10	0.701	0.674	0.027	0.000729
20	1.419	1.364	0.055	0.003025
30	2.161	2.054	0.107	0.011449
40	2.713	2.744	-0.031	0.000961
50	3.328	3.434	-0.106	0.011236
60	4.012	4.124	-0.112	0.012544
70	4.801	4.814	-0.013	0.000169
80	5.612	5.504	0.108	0.011664
90	6.288	6.194	0.094	0.008836
100	6.972	6.884	0.088	0.007744
			$\sum(Y-Yi)^2$	0.068357
		SD		0.008543
		LOD		0.4086 µM
		LOQ		1.362 µM
		Regression equation : y = 0.069x - 0.016		
		Regression Coefficient : R^2 = 0.998		

Table 4: Analytical parameters for calibration curves for famotidine and zinc by DPP and HPLC

Method	Material	Regression Equation	Linearity µM	Ep (V)	LOD µM	LOQ µM	R^2
HPLC	Famotidine	y = 87124x + 14940	1-8	-	0.3659	1.2196	0.998
DPP	Famotidine	y= 0.010x + 0.050	10-100	-1.390	9.7615	32.23	0.996
DPP	Zinc	y = 0.069x - 0.016	10-100	-1.006	0.4086	1.362	0.998

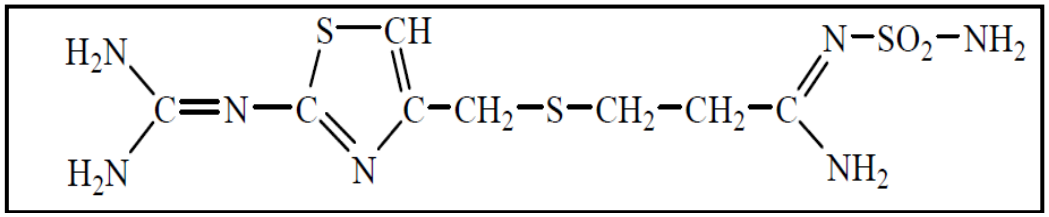

Fig. 1: Chemical structure of famotidine

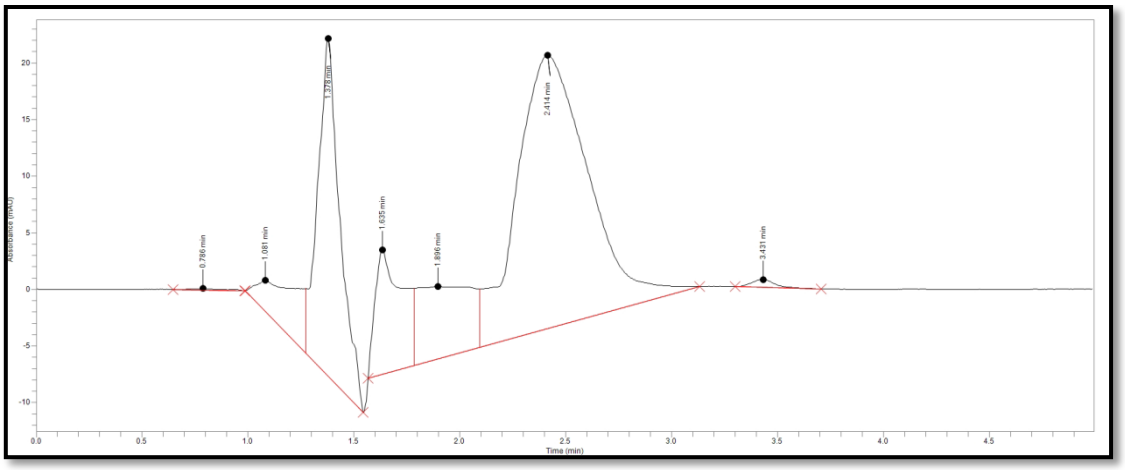

Fig. 2: Chromatogram of famotidine in methanol-water (80:20, v/v)

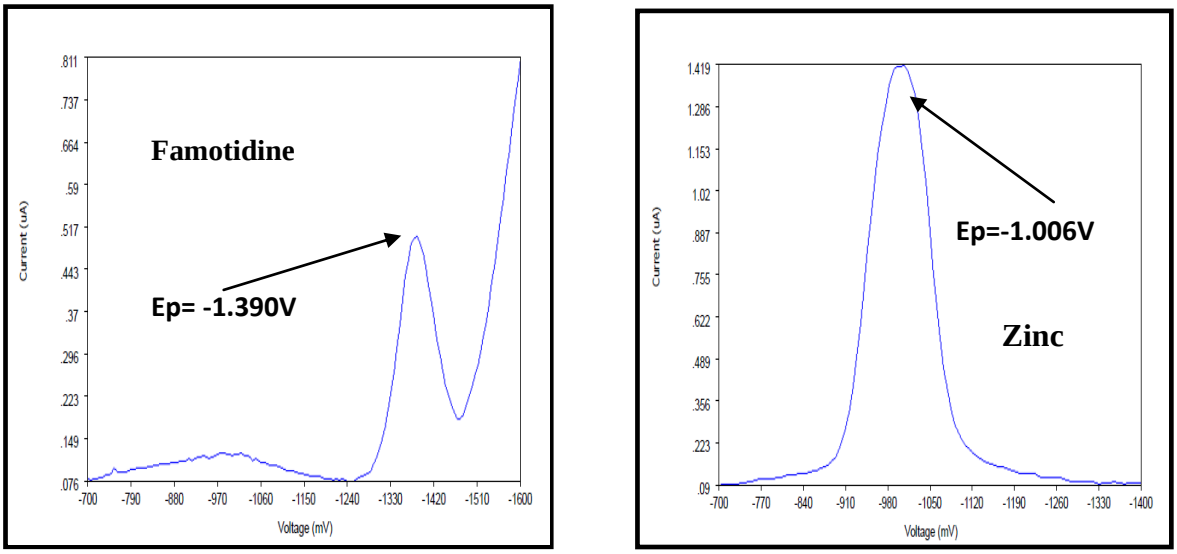

Fig. 3: DPP Polarogram of famotidine and zinc in acetate buffer pH 5.0 obtained at current range 10µA, data acquisition fast, scan rate 6mV/sec, drop time 1sec, scan type forward, pulse amplitude 100mV.

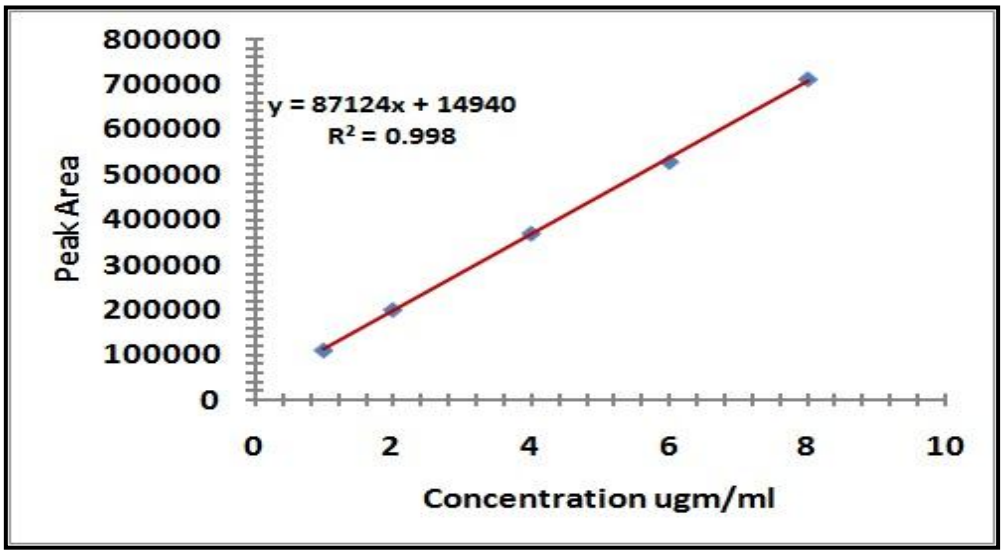

Fig. 4: Linearity curve of famotidine by HPLC in methanol-water (80:20, v/v)

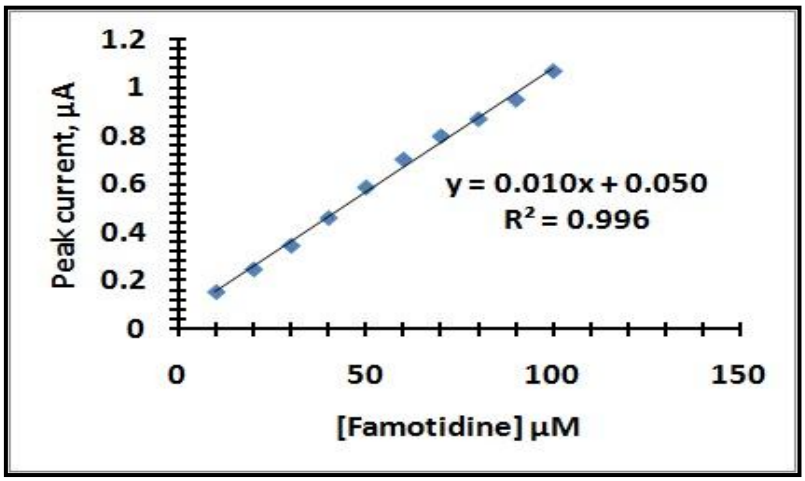

Fig. 5: Linearity curve of famotidine by DPP in acetate buffer pH 5.0

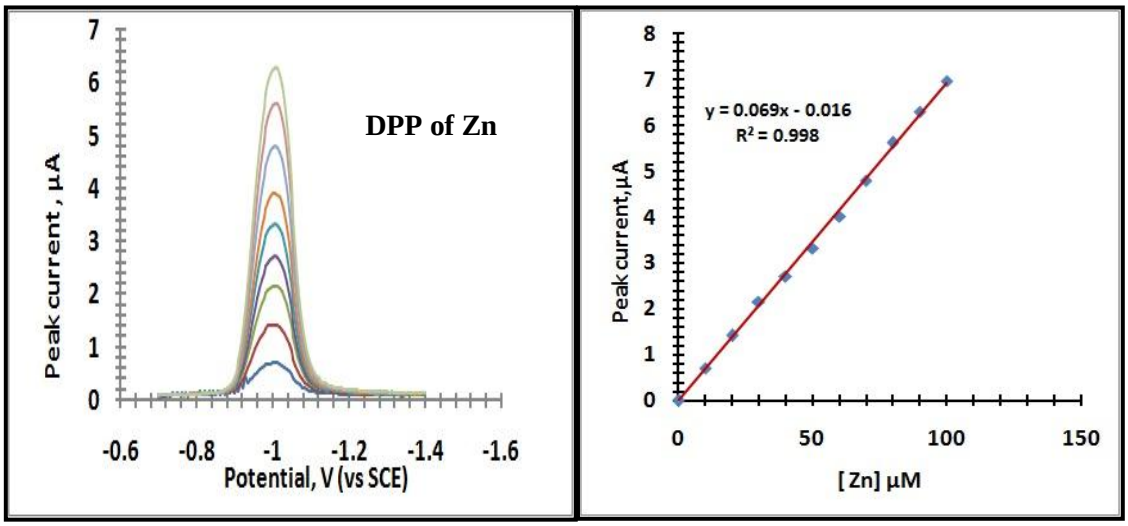

Fig. 6: Differential pulse polarogram and linearity of increasing concentration of zinc at pH 5.0 in acetate buffer solution containing 1.0 M KCl as a supporting electrolyte obtained at 10.0 µM -100 µM. DPP Parameter is current range 10uA, scan rate 6mV/sec, and drop time 1sec.

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