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ELECTROCHEMICAL REDUCTION BEHAVIOUR AND DETERMINATION OF CEFQUINOME

Chenna Rohini Kumar Putta¹, Ramakrishna Naidu Gurijala², Balaji Kodigutta¹,
Sridevi Chadive³ and Suresh Reddy Cirandur^{1*}

¹Department of Chemistry, S.V. University, Tirupati – 517502, Andhra Pradesh, India.

²Department of Environmental Sciences, S.V. University, Tirupati - 517502, Andhra Pradesh, India.

³Department of Chemistry, S.P.W. Degree and PG College, Tirupati - 517502, Andhra Pradesh, India.

ABSTRACT

The electrochemical reduction behaviour of cefquinome has been studied by using dc polarography, cyclic voltammetry, ac polarography, differential pulse polarography, millicolometry and controlled potential electrolysis in universal buffers ranging from pH 2.0 to 12.0 in DMF-water mixtures. Kinetic parameters such as diffusion coefficient (D) and forward rate constant ($k_{f,h}^{\circ}$) values are evaluated and a reduction mechanism is proposed. A simple and rapid differential pulse polarographic method has been developed for the determination of cefquinome in pharmaceutical formulations using the standard addition method. The detection limit is 2.15×10^{-8} M.

Keywords: Cefquinome, Polarography, Reduction mechanism, Kinetic parameters, Pharmaceutical formulations.

INTRODUCTION

Cefquinome((Z)-1-((7-(2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido)-2-carboxy-8-oxo-5-thia-1-aza bicyclo[4.2.0]oct-2-en-3-yl)methyl) - 5,6,7,8- tetrahy dro quinolin -1-ium) is an extended spectrum beta-lactam and member of the fourth-generation cephalosporin Figure 1[1-2]. It is intended for the treatment of bovine respiratory disease. Cefquinome has been used only in veterinary medicine and only for individual treatment, and is safe and well tolerated. It is resistant to β -lactamase and is a zwitter-ionic compound with improved penetration into the periplasmic space of Gram-negative bacilli and enhance binding to penicillin- binding proteins. Like other β -lactams, cefquinome is bactericidal by inhibiting the cell wall synthesis of actively growing bacteria [3].

Various methods have been employed for the determination of different cephalosporins in pharmaceutical preparations including high-performance liquid chromatography [4-6], thin layer chromatography [7-9] and UV spectrometry [10-12].

Very little attention has been paid to the polarographic determination of cefquinome. The purpose of this work is to establish the experimental conditions of the electrochemical behavior of cefquinome by dc polarography, cyclic voltammetry, ac polarography and differential pulse polarography (DPP) and determination of cefquinome in pharmaceutical formulations by DPP.

EXPERIMENTAL

Model 364 polarographic analyzer supplied from PARC coupled with BD 8 Kipp and Zonen X-t recorder was used for d.c. polarographic measurements. Other techniques were carried out using a Metrohm unit: E 506 polarecord coupled with E 612 VA-scanner, E 648 VA-combistand, E 608 VA-controller and a digital electronics 2000 X-Y/t recorder. All electrochemical measurements were carried out using a conventional three-electrode design at $25 \pm 0.1^{\circ}\text{C}$. The dropping mercury electrode (area: 0.0223 cm^2 , flow rate of Hg: 2.73 mg/sec , and mercury

column height: 35 cm) and hanging mercury drop electrode (with an area of 0.0328 cm^2) were used as working electrodes. Reference electrodes employed were Ag/AgCl (s), Cl^- electrode for cyclic voltammetry, a.c. polarography and differential pulse polarography and saturated calomel electrode (SCE) for d.c. polarography. Platinum electrode was used as counter electrode for all the techniques to complete electrolytic current. A modified cell with mercury pool cathode, SCE, platinum wire gauze electrode and spot galvanometer was used for controlled potential electrolysis.

Pure cefquinome was obtained from Glaxo India Limited, Bombay and was used without further purification. Universal buffers of pH 2.0 to 12.0 were prepared by using 0.2 M boric acid, and 0.05 M citric acid and 0.1 M trisodium orthophosphate. All the chemicals used are of pure analytical grade. Stock solution of cefquinome was prepared by dissolving the required amount in dimethyl formamide and making up to volume with the supporting electrolyte to obtain the desired concentration. The test solution was purged with purified nitrogen for 10 min before the voltammograms were run.

RESULTS AND DISCUSSION

Characterization of wave/ peak

The electrochemical behaviour of cefquinome has been examined over the pH range 2.0-12.0. It was found to exhibit a single well defined wave/peak in the entire pH range of 2.0-12.0 in all the techniques. The wave/peak is assigned to the four electron reduction of the azomethine group to the corresponding amino group by a mechanism involving the reductive fission of the N-O bond in the $>\text{C}=\text{N}-\text{O}$ linkage. Typical voltammograms are shown in Figures 2-5.

Nature of the electrode process

The diffusion-controlled nature of the electrode process is evidenced from the linear plots of i_d vs. $t^{1/2}$ and i_p vs. $v^{1/2}$ passing through the origin. Drop time effect was carried out in DPP, which confirmed the absence of adsorption of the electro active species on the electrode surface by the linear plots of i_m vs. $t^{2/3}$ [13]. In Cyclic voltammetry the current function $i_p/Cv^{1/2}$ was found to be fairly constant with scan rate also showing the electrode processes to be free from kinetic complications. The irreversible nature of the wave was confirmed from the absence of anodic peak in the reverse scan of cyclic voltammogram. The reduction potentials were observed to be shifted toward more negative potentials with increasing concentrations of cefquinome also indicating the irreversible nature of the electrode process [14].

The reduction potentials of the cefquinome wave/peak were found to be dependent on pH and to shift toward more negative potentials with an increase in pH of the buffer solutions showing the proton involvement in the electrode process. An increase in pH increases the

dissociation constant of the protonated species, which could affect the protonation rate [15]. As a consequence, the reduction potentials were shifted to more negative values [16].

Identification of the product

The total number of electrons involved during the electrode reaction for cefquinome is determined by millicoulometry and it is found to be four in acidic (pH 2.0) and basic (pH 10.0) medium. Controlled potential electrolysis experiment is conducted at -0.75 V vs. SCE in pH 4.0 for cefquinome. After electrolysis, the reduced product is extracted with ether and ethereal layer was evaporated on water bath. The obtained compound exhibited doublet absorption bands at $3050-2800 \text{ cm}^{-1}$ in IR spectrum due to the presence of amino group. Whereas weak band appeared in IR spectrum of the original compound for $>\text{C}=\text{N}-$ group at $1450-1500 \text{ cm}^{-1}$ is found to disappear indicate the reduction of azomethine functional group. No polarographic wave is noticed after electrolysis of the investigated compound which also confirmed the reduction of $>\text{C}=\text{N}-$ group.

Electrode Mechanism

Based on the results obtained from all the techniques, the electrochemical reduction mechanism of cefquinome can be given as follows:

Kinetic data

The values obtained for diffusion coefficient (D) and heterogeneous forward rate constant ($k_{f,h}^\circ$) at various pH values by different techniques are given in **Table 1**. The diffusion coefficients evaluated from all techniques were in good agreement because no adsorption complications were involved in the electrode process. The reason for a slight decrease in D values with increase in pH may be attributed to the non-availability of protons with the increase in pH. The rate constant values, which were seen to gradually decrease with increase in pH for the reduction of azomethine group, may account for the shift of reduction potentials towards more negative values. The rate constants (k_s) obtained in ac polarography was found to be high when compared to other techniques. The rate constants were evaluated at standard potentials in ac polarography and at potentials $E=0$ by the other techniques. The magnitude and the nature of the charge transfer contribution to frequency in ac polarography played a role in this connection.

Analysis

In the present investigation, differential pulse polarography (DPP) has been used for the quantitative estimation of cefquinome using both calibration and standard addition methods. Cefquinome is found to exhibit well resolved peaks which are suitable for the analysis in

pH 4.0 and 6.0. In basic buffers of pH > 8.0, the reduction of azomethine is difficult due to non-availability of protons and it is not useful for analytical purpose. The peak currents vary linearly with the concentration of cefquinome over the range $1.37 \times 10^{-5} \text{ M} - 2.1 \times 10^{-8} \text{ M}$ with the lower detection limit of $2.15 \times 10^{-8} \text{ M}$. The lower detection limit was calculated using the following expression:

$$dl = 3 \left(\frac{Sd}{m} \right)$$

Where, Sd = Standard division; m = slope of calibration plot

Recommended analytical procedure (Standard addition method)

A standard solution of cefquinome ($1.0 \times 10^{-5} \text{ M}$) was prepared in DMF. One mL of standard solution was transferred into a polarographic cell and made up with 9 ml

of the supporting electrolyte (pH 4.0) and was then deoxygenated with nitrogen for 15 min. After obtaining the polarogram, a small increment of the standard solution of cefquinome was added to the cell and deaerated for 1 min, and a polarogram was again recorded under similar conditions. In the same manner, 10 polarograms were recorded for standard additions. The optimum conditions for the analytical determination were found to be in pH 4.0 with droptime of 2 sec and pulse amplitude of 60 mV. The relative standard deviation and correlation coefficient (for 10 replicants) were 1.18 % and 0.995 respectively.

Cefquinome can be successfully determined in pharmaceutical formulations without any prior separation by using the recommended procedure. **Table 2** gives the assay results of cefquinome injections. The recovery of 98.74-99.92 % obtained with the proposed differential pulse polarographic method indicates its accuracy and reproducibility.

Table 1. Typical Electrode kinetic data for Cefquinome, Concentration = 0.5 mM

pH	DC Polarography (Drop time: 3 sec)			Cyclic Voltammetry (Scan rate: 40 mVs ⁻¹)			AC polarography (Drop time: 3 sec)			Differential pulse polarography (drop time: 2 sec)		
	$\frac{-E_{1/2}}{V}$	$\frac{D \times 10^5}{\text{cm}^2 \text{ s}^{-1}}$	$\frac{k_{f,h}^\circ}{\text{cms}^{-1}}$	$\frac{-E_p}{V}$	$\frac{D \times 10^5}{\text{cm}^2 \text{ s}^{-1}}$	$\frac{k_{f,h}^\circ}{\text{cms}^{-1}}$	$\frac{-E_s}{V}$	$\frac{D \times 10^5}{\text{cm}^2 \text{ s}^{-1}}$	$\frac{k_s}{\text{cms}^{-1}}$	$\frac{-E_m}{V}$	$\frac{D \times 10^5}{\text{cm}^2 \text{ s}^{-1}}$	$\frac{k_{f,h}^\circ}{\text{cms}^{-1}}$
2.0	0.64	7.50	3.20×10^{-9}	0.62	6.32	3.42×10^{-9}	0.61	5.01	9.46×10^{-5}	0.64	6.32	8.62×10^{-8}
4.0	0.69	6.00	4.26×10^{-11}	0.69	6.28	4.36×10^{-11}	0.70	4.87	7.62×10^{-6}	0.70	6.28	6.42×10^{-10}
6.0	0.72	4.10	3.96×10^{-12}	0.74	5.87	3.98×10^{-12}	0.76	3.65	2.21×10^{-7}	0.82	6.12	4.26×10^{-11}
8.0	0.85	2.10	6.42×10^{-13}	0.86	3.59	6.35×10^{-13}	0.85	2.45	5.10×10^{-9}	0.88	3.54	2.64×10^{-12}
10.0	1.06	1.80	4.61×10^{-15}	0.98	3.26	4.64×10^{-16}	1.01	2.38	5.89×10^{-10}	1.06	3.46	5.53×10^{-14}
12.0	1.22	1.70	3.10×10^{-17}	1.21	2.89	3.16×10^{-18}	1.24	2.26	3.18×10^{-13}	1.22	3.28	4.24×10^{-17}

Table 2. Assay of Cefquinome formulations by differential pulse polarography in pH 4.0

Pulse amplitude: 60 mV, Drop Time: 2 Sec

Sample (Injections)	Labelled amount (mg)	Amount found (mg)	Recovery %	Standard deviation
COBACTAN	25	24.82	98.74	0.025
CEPHAQUARD	10	9.98	99.92	0.016
VIRBACTAN	25	24.97	99.89	0.017

Fig 1. Structure of Cefquinome

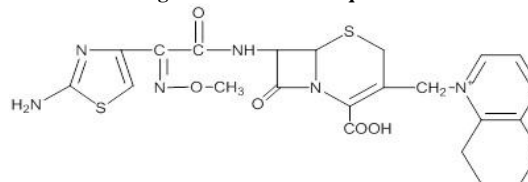
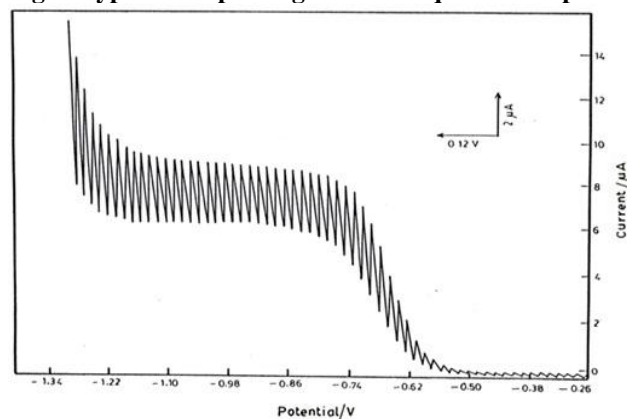
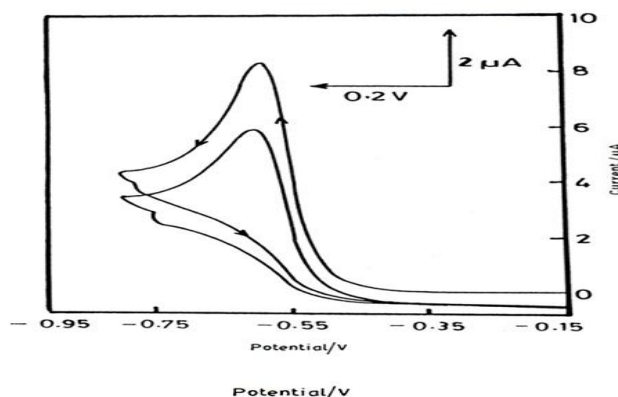


Fig 2. Typical d.c. polarogram of Cefquinome in pH 2.0



Concentration: 0.5 mM, Drop time: 3 Sec.

Fig 3. Typical cyclic voltammogram of Cefquinome in pH 2.0



Concentration: 0.5 mM, Scan rate: 40 mVs⁻¹

Concentration: 0.5 mM, Drop time: 3 Sec a: a.c. peak, b: base line

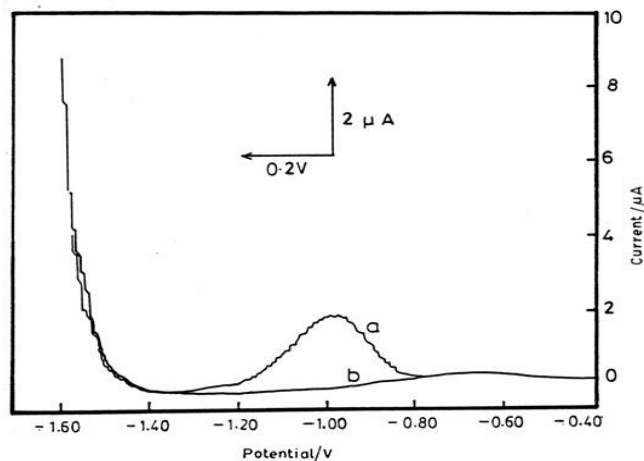
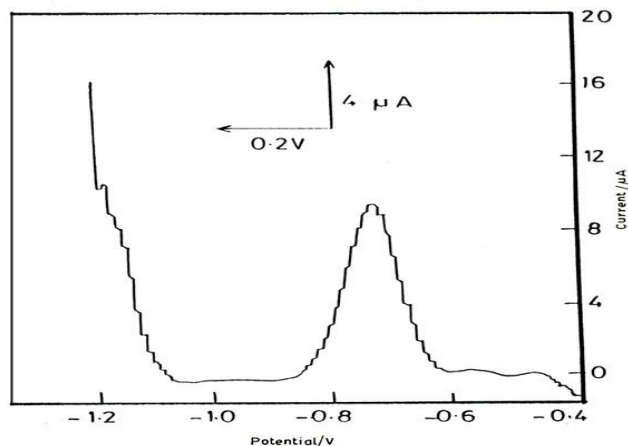
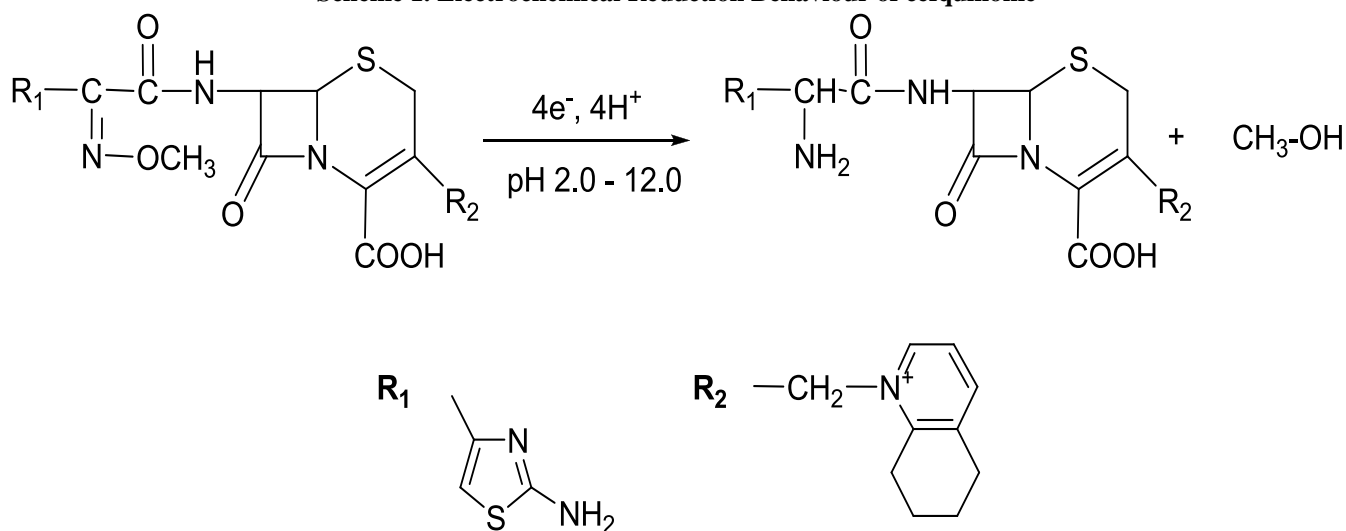


Fig 4. Typical a.c. polarogram of Cefquinome in pH 10.0
Concentration: 0.5 mM, Drop time: 2 Sec



Scheme 1. Electrochemical Reduction Behaviour of cefquinome



CONCLUSION

The work describes the electrochemical behaviour of cefquinome based on the reduction of the azomethine group at dropping mercury electrode and hanging mercury drop electrode. The recovery result shows that differential pulse polarography is a simple, reliable and inexpensive method for the determination of cefquinome in formulations. The main advantage of the proposed method over the other ones is that the excipients do not interfere and a separation procedure is not necessary.

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