Hacettepe Üniversitesi Eğitim Fakültesi Dergisi
Yıl 1988 / Sayı 3 / ss. 221 – 229

ANALYTICAL INVESTIGATIONS OF CEPHALOSPORINS PART 6. COMPARATIVE POLAROGRAPHICAL STUDY OF CEFOTIAM AND 7-[2-METHOXYIMINO-2-(2-AMINO-1,3-THIAZOL-4-YL) ACETAMIDO] SUBSTITUTED CEPHALOSPORINS

Prof. Dr. İnci MORGİL*, S. ERBAŞ*
Ş. AYCAN, K. ULAŞ***, I. FEDAİ**

Summary: Polarographical behaviour of cefotiam which has 7-[2-(2-amino-1,3-thiazol-4-yl) acetamido] group and ceftizoxime, cefotaxime and ceftriaxone which have 7-(2-methoxyimino-2-(2-amino-1,3-thiazol-4-yl) acetamido] groups as the R_1 substituent were investigated by using cathode ray polarography (CRP) and then the results were compared. The cathodic reduction which is $2e^- + 2H^+$ in case of cefotiam and $4e^- 4H^+$ for ceftizoxime, cefotaxime and ceftriaxone has been calculated and compared. Cefotaxime, ceftriaxon and cefotiam gave an additional cathodic reduction because of the leaving group of $-CH_2-R'$ (R' = leaving group) but ceftizoxime did not give any as it has no R_2 substituent. The dependence of peak height (is_p) and peak potential (p_p) on pH have been investigated for the studied cephalosporins.

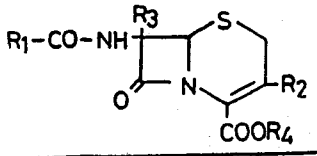
We have developed a new method which enable to determine ceftriaxon, cefuroxime, cefotaxime and ceftizoxime in pharmaceutical formulations by making use of the polarographical behaviours of the given cephalosporins studied by differential pulse polarography (DPP).[1] The results obtained from the polarographical analysis of cefotiam have led us to compare it 7-[2-methoxyimino-2-(2-amino-1,3-thiazol-4-yl)acetamido] substituted cephalosporins.

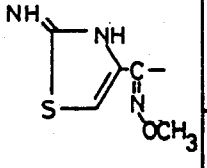
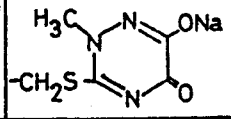
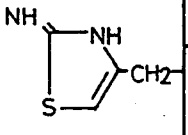
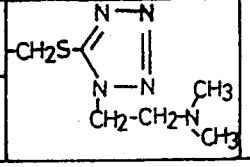
As it is observed in Table I, C_1, C_2 and C_3 having the same R_1 substituent bear different R_2 substituents. Meanwhile, C_4 has 7-[2-(2-amino-1,3-thiazol-4-yl)acetamido] group as R_1 substituent.

* Hacettepe Üniversitesi Eğitim Fakültesi Öğretim Üyesi.
** Yıldız Üniversitesi Fen Edebiyat Fakültesi Öğretim Üyesi.
*** 100. Yıl Üniversitesi Öğretim Üyesi.

TABLE I

CHEMICAL STRUCTURE OF THE INVESTIGATED CEPHALOSPORINS



Substance	R_1	R_2	R_3	R_4
C_1 Ceftizoxime Sodium		–	H	Na
C_2 Cefotaxime Sodium		$-CH_2OCOCH_3$	H	Na
C_3 Ceftriaxone Sodium			H	Na
C_4 Cefotiam Sodium			H	H

EXPERIMENTAL

Material

Ceftizoxime sodium working standard (Boehringer Mannheim GmbH/West Germany), cefotaxime sodium working standard (Hoechst AG/West Germany), ceftriaxone sodium working standard (Hoffmann La Roche and Company/Switzerland) and cefotiam sodium working standard (Ciba-Geigy/West Germany) were kindly supplied for our studies.

Reagents and Apparatus

All reagents and solvents were of analytical grade. The pH of the reaction solutions were maintained at the desired value by appropriate buffer systems. The buffer solutions used were hydrochloric acid - sodium chloride, phosphoric acid - monobasic sodium phosphate, acetic acid - sodium acetate, monobasic - dibasic sodium phosphates and boric acid - sodium borate.

The polarographic experiments were carried out using a differential electron ray polarograph Amel 448A which has a function generator and a differential vertical amplifier. The dropping mercury electrode which can be regulated electronically has a dropping time of 26s. The determinations were carried out in the thermostatitically controlled microcells (20°C) using saturated calomel electrode. Conditions of measurement for CRP are as the following.

Sweep Amplitude	:	1000 mV/s
Sweep Rate	:	400 mV/s
Delay Time	:	8 s

General Procedure

Stock solutions of the investigated cephalosporins were prepared in the concentration of 10^{-4} M. Polarographic determinations were carried out in 10^{-5} M solutions which were prepared by diluting the stock solution to 1:10 with the buffer solutions indicated.

RESULTS AND DISCUSSION

Cathodic reduction peaks according to Randles' Law isp = $Kn^{3/2} V^{1/2} c$ of the cephalosporins were investigated in the pH range of 1-10 by using cathode ray polarography. Cathodic sweeps and first derivative polarograms (FDP) were also recorded. (Figure 1) shows the cathodic and anodic sweeps of the investigated cephalosporins at pH 4.0. Cathodic reduction of C_1, C_2, C_3 and C_4 was between -0.824 and -0.924 V and followed an irreversible pathway A single or a double reduction step was observed in relation with the pH of the analysis medium.[1] C_1 which has no substituent at C-3-position, gave a single peak while the other three which have $-CH_2-R'$ leaving group at R_2 substituent gave two peaks in the pH range studied.

Our literature study is in good agreement with our experimental findings in which the second peak which was observed about -1.1 V is because of $-CH_2-R'$ group at the R_2 substituent of the investigated cephalosporins in which R' behaves as the leaving group.[2-5] C_2 and C_3 and C_4 have acetoxymethyl, [[(2,5-dihydro-6-hydroxy-2-methyl-5-oxo-astriazin-3-yl)-thio methyl], [[[2-2-(dimethylamino)ethyl]-1H-tetrazol-5-yl]thio] methyl groups respectively as the leaving group. The first reduction peak which appears between -0.824 V and -0.924 V is due to the methoxyimino and imino groups present at C-7-position of C_1, C_2 and C_3. Figure 2 and 3 show the relationship of isp versus pH and pp versus pH evaluated from the CRP polarograms of the investigated cephalosporins recorded at a concentration of 10^{-4} M.

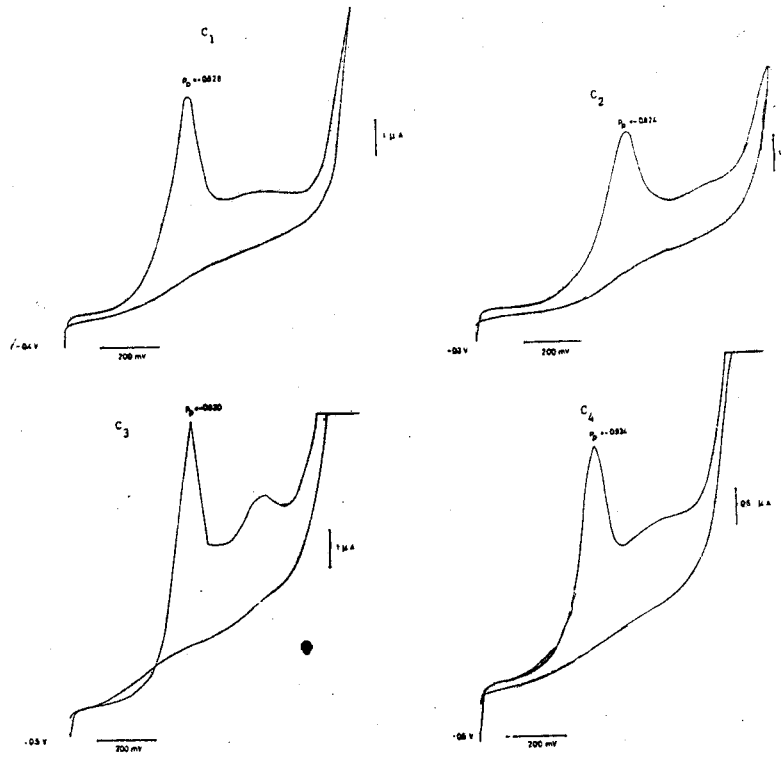


Figure 1

Anodic and cathodic sweeps of the investigated cephalosporins (c = 10^{-4}M, Acetate Buffer pH 4.0).

As it is seen in Figure 2, the first peak height obtained for C_4 is lower than the peak heights obtained for the other cephalosporins studied. Therefore the is_p values calculated from CRP and FDP polarograms of each of 10^{-5} M of cephalosporins in buffer solutions at pH 4.0 and 7.0 were correlated under the same conditions. Results shown in Table II indicated that only -C=N- group of 7-[2-(2-amino-1,3-thiazol -4-yl) acetamido] group in R_1 substituent is responsible for the peak which was observed at -0.924 V for C_4.

TABLE II

CORRELATION OF PEAK HEIGHT (μA) OF C_4 WITH C_1, C_3 AND C_3 AT A CONCENTRATION OF 10^{-5} M

	C_1/C_4		C_2/C_4		C_3/C_4	
Polarographic Method	pH 4.0	pH 7.0	pH 4.0	pH 7.0	pH 4.0	pH 7.0
CRP	1.88	1.17	1.37	0.40	2.20	2.05
FDP	1.82	1.30	2.55	1.51	2.21	1.65

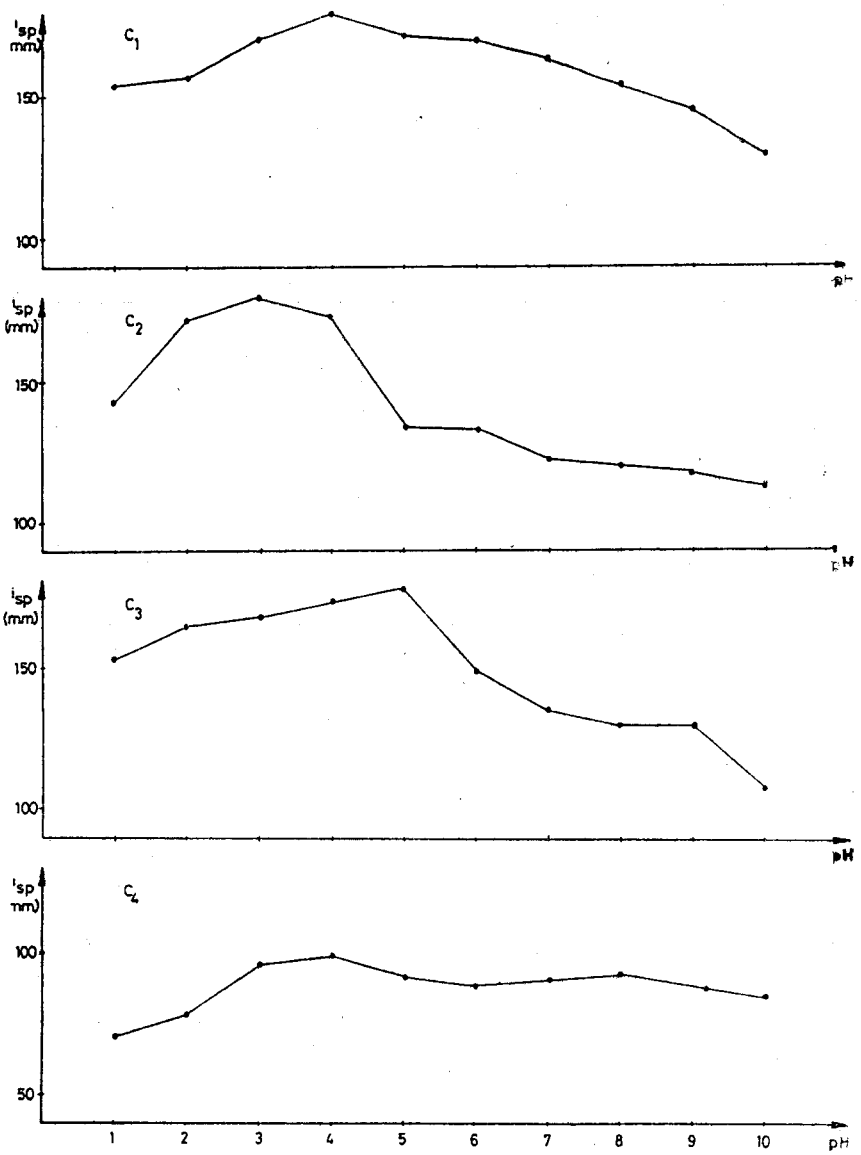


Figure 2
Relationship between is_p and pH for the investigated cephalosporins.

Furthermore, when FDP polarograms of the studied cephalosporins were examined under the same conditions, two peaks were observed only for C_1 which does not have R_2 substituent. This indicates that -C=N- in methoxyimino and -C=N- in (2-amino-1,3 thiazol-4-yl)

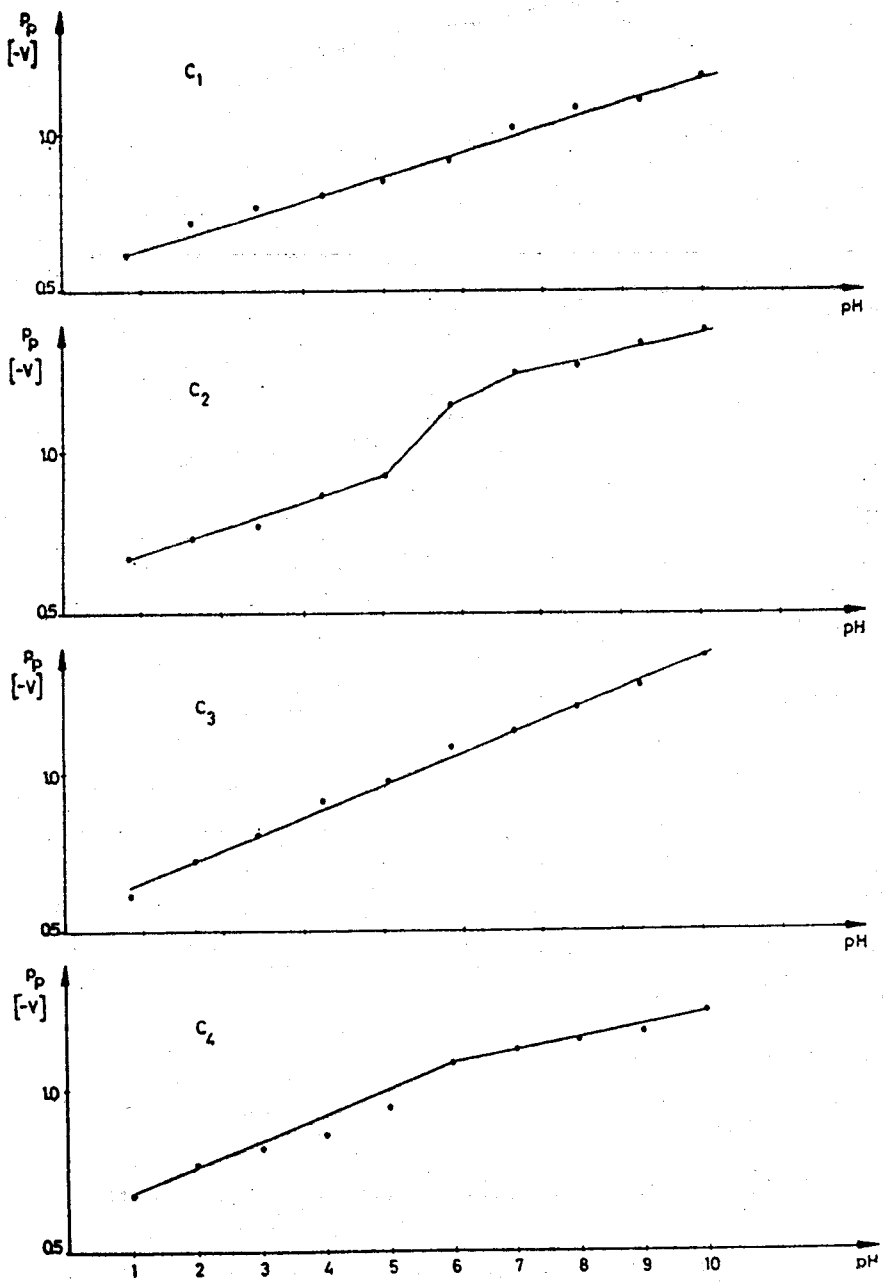


Figure 3

Relationship between p_p and pH for the investigated cephalosporins.

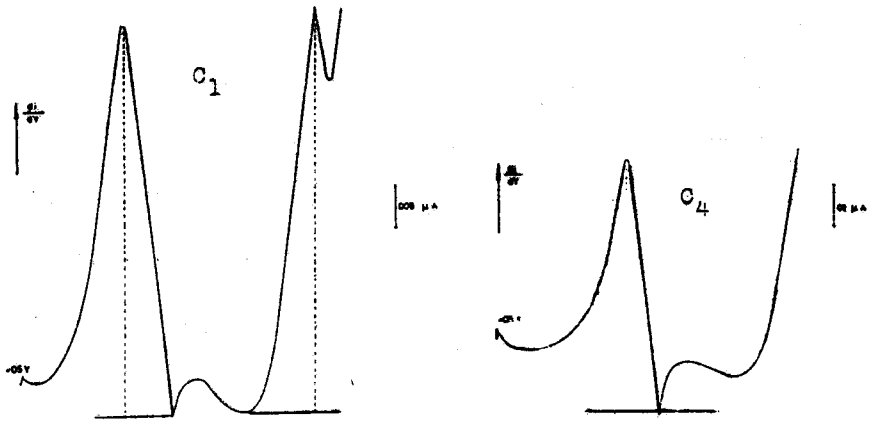


Figure 4

FDP polarograms of C_1 and C_4 ($c = 10^{-5}M$, acetate buffer Hp 4.0).

acetamido groups are responsible for the two peaks which were observed in FDP polarogram of C_1 (Figure 4). In this case each group is reduced by taking $2e^- + 2H^+$. In the other hand the same polarogram which was taken under the same experimental conditions for C_2 and C_3 exhibited a single peak. Meanwhile the peak height of the same peak which was observed for C_4 was approximately half of C_2 and C_3 peaks. These are all shown in Table II. As the peak potentials of the investigated cephalosporins are so close to each other and the leaving group in R_2 substituent of each is found to be polarographically active giving a cathodic reduction around -1.1 V(1-5), the reduction concerning both of the -C=N- groups of C_2, C_3, and C_4 proceeds together while the indicated groups are separated by an application of rapid sweep technique in case of C_1 having no R_2 substituent. Reduction mechanisms proposed for the studied cephalosporins are seen in Figure 5.

The precision of the proposed reaction mechanisms can be followed by using controlled potential electrolysis (CPE) as well as by isolating and elucidating the structure of the reduction products formed as a results of preparative reduction. More studies about this fact will be published soon.

Acknowledgement

Sincere thanks of the authors are to Alexander von Humboldt Foundation in West Germany for the donation of cathode ray polarograph.

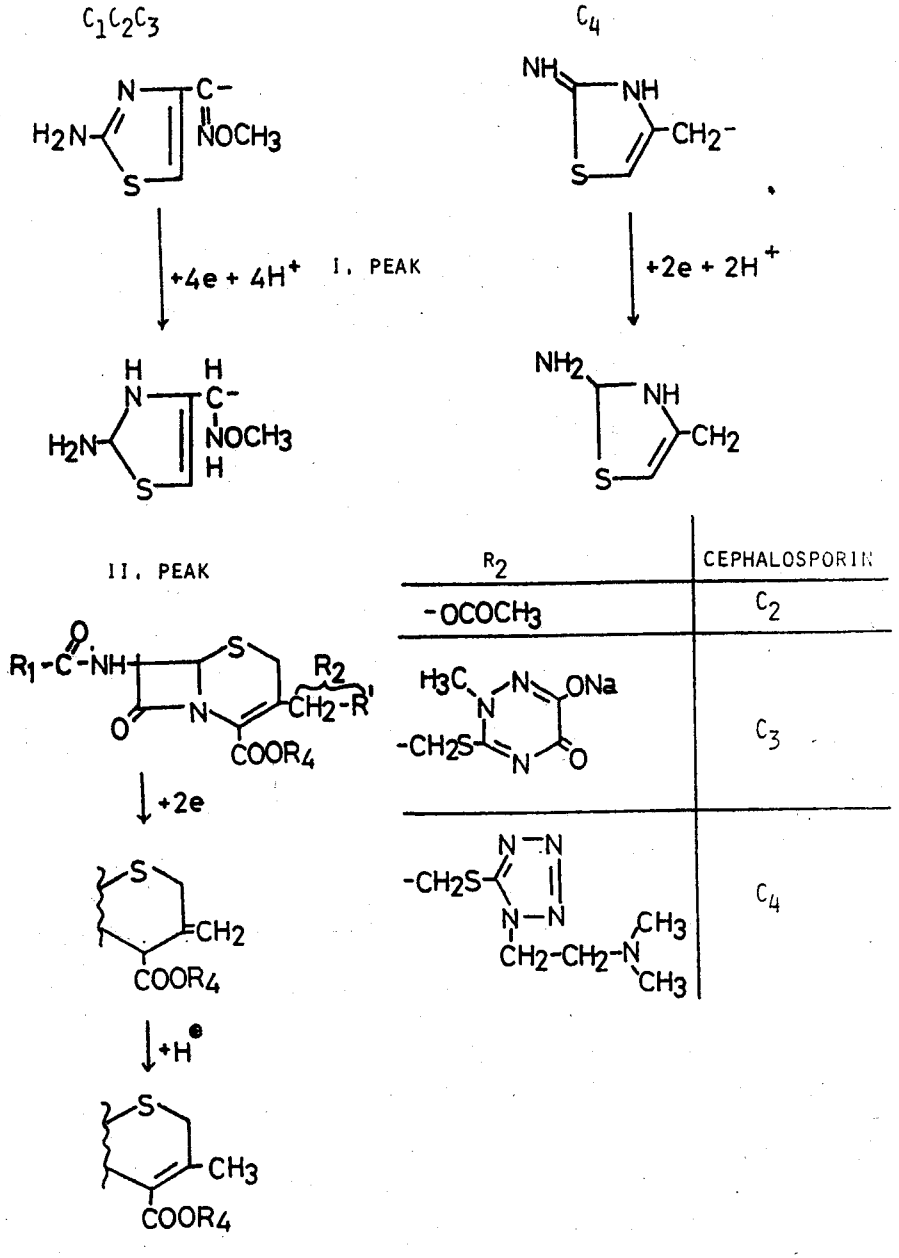


Figure 5
Reduction mechanisms proposed for the cephalosporins studied.