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ANALYTICAL INVESTIGATIONS OF CEPHALOSPORINS PART 10. COMPARATIVE POLAROGRAPHICAL STUDY OF 3-[[(1-METHYL-1H-TETRAZOLE-5-YL) THIO] METHYL] SUBSTITUTED CEPHALOSPORINS WITH CEFAZOLIN

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Summary: Polarographical properties of Sq-14359, cefmetazole and cefazolin were investigated by using cathode ray polarography (CRP), and differential pulse polarography (DPP). The electroactive group present in the investigated cephalosporins is R′ leaving group, of CH_2-R′ which is located at the 3-position. Then the electrochemical methods developed were applied to the cephalosporins in dosage forms and the results of the developed method were compared with the results of Hg(II)imidazole-EDTA and Ni(II)-hydroxylamine methods. Standard deviation values obtained for the electroanalytical methods were varying between $\pm$ 0.36 % - $\pm$ 0.68 % while it is between $\pm$ 0.61 % $\pm$0.84 % for (Hg(II)-imidazole-EDTA method and between $\pm$ 1.13 % - $\pm$1.29 % for Ni(II)-hydroxylamine method.

Keywords: Determination cephalosporin derivatives, cathode ray polarography, differential pulse polarography.

INTRODUCTION

The purpose of our investigations was to develop electroanalytical methods which would enable to determine cephalosporins. Polarographical properties of cefamandole lithium (C_1), cefamandole nafate (C_2) and cefoperazone (C_3) having 3-[1-methyl-1H-tetrazol-5-yl) thiomethyl] group as R_2 substituent have been determined and the indicated substances in pharmaceutical formulations have been assayed in our previous studies.[1]

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In the present study, our aim was to examine and compare the polarographical properties of Sq-14359 (C_4) and cefmetazole (C_5) which have 3-[1-methyl-1H-tetrazol-5-yl) thiomethyl] groups and cefazolin which has 3-[[(5-methyl,1,3,4-thiadiazole-2-yl) thio[methyl] group as R_2 substituent. Furthermore, the developed method was applied to the determination of cefazolin in pharmaceutical formulations and the results obtained were compared with the results of Hg(II)-imidazole-EDTA[2] and Ni(II)-hydroxylamine methods.[3]

Chemical structure of the investigated cephalosporins is shown in Table I. Several analytical methods have been proposed for C_4 and C_5 besides microbiological techniques.[4] These include titrimetric[5, 6] spectrophotometric[7-10] and HPLC methods[11-16] Meanwhile colorimetric hydroxylamine method is indicated for C_6 in USP XX.[17]

EXPERIMENTAL

Apparatus

Polarographic measurements were carried out using a diferential pulse polarograph Metrohm E 506 and differential electron ray polarograph Amel 448A which has a function generator and a differential vertical amplifier. For DPP operations, a forced drop time of 2 s., a scan rate of 25 mVs^{-1} and a pulse amplitude of 100 mV were used. Sweep amplitude of 1000 mVs^{-1}, sweep rate of 400 mVs^{-1} and delay time of 8 s. is used throughuft.

The dropping mercury electrode which can be regulated electronically has a dropping time of 26 s. Thermostatically controlled microcells (20°C) with saturated calomel electrode were employed.

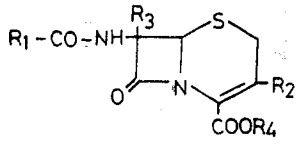
Visible spectrophotometric measurements were performed. With a Beckmann B model spectrophotometer using 1 cm glass cuvettes. The pH measurements were made by using a 7020 Electronic Instruments Limited instrument.

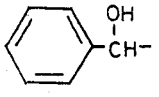
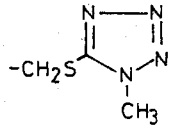
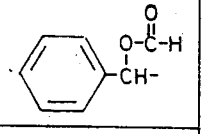
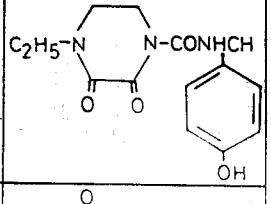
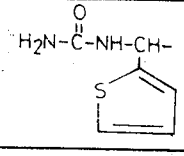
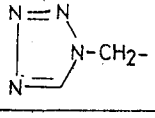
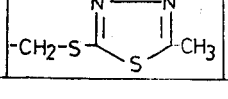
Chemicals and Reagents

Cefazolin sodium, Sq-14359 and cefmetazole sodium working standards were kindly supplied by Eli Lilly and Company Limited /England, E.R. Squibb and Sons., Inc./ABD and Sankyo Company Ltd/Japan, respectively. Kefzol[R] vials, Gramaxin[R] Vials and Maksipor[R] vials were gifts from Eli Lilly and Company Limited/England, Boehringer Mannheim GmbH/West Gèrmany and Fako İlaç Fabrikası/Turkey, respectively.

TABLE I

CHEMICAL STRUCTURE OF THE INVESTIGATED CEPHALOSPIRINS



Substance	R_1	R_2	R_3	R_4
C_1 Cefamandole lithium			H	Li
C_2 Cefamandole nafate			H	Na
C_3 Cefoperazone			H	Na
C_4 Sq-14359			CH_3O-	Na
C_5 Cefmetazole	$N\equiv C-CH_2-S-CH_2-$		CH_3O	Na
C_6 Cefazolin			H	Na

All reagents and solvents used in this study were of analytical grade. The pH of the reaction solutions were maintained at the desired value by appropriate buffer systems. The buffer solutions used are indicated in Table II.

TABLE II

EXPERIMENTAL POLAROGRAPHIC DATA FOR THE INVESTIGATED CEPHALOSPORINS

Compound	Supporting electrolyte	Number of peaks	Number of electrons	Pp	Polarographic current	Cyclovoltammetry	Determinations limits ng/ml DPP	CRP
C_4	10 % DMF	pH 1.0-5.0 1 pH 5.0-10.0 2	1. peak 2	1. peak − 0.776 V 2. peak − 1.228 V in Citrate buffer pH 5.0	1. peak $is_p = kxc$ 2. peak Praenatrium waves	Irreversible	56.23	84.35
C_5	10 % DMF	pH 1.0-10.0 1	2	− 0.772 in Citrate + HCl buffer pH 4.0	$is_p = kxc$	Irreversible	49.35	74.03
C_6	10 % DMF	pH 1.0-4.0 1 pH 4.0-10.0 2	1. peak 2	1. peak − 0.792 2. peak − 1.032 in Citrate + HCl buffer pH 4.0	1. peak $is_p = kxc$ 2. peak Catalytic	Irreversible	47.64	71.46

Procedure

10^{-3} M of cephalosporins in DMF* were used in the polarographic analyses DMF was purified by column chromotography**.

Then from this stock solution, solutions with the desired concentrations were obtained by diluting with appropriate buffer solutions to volume. Stock solutions should be stored below +10°C in dark.

Application of the Polarographic Method, to the Cephalosporin Vials.

Pharmaceutical formulations containing 0.25, 0.50 and 1.0 g of cephalosporin were diluted to volume with sterile water for injection solutions (According to B.P. 1980). Then 0.1 ml aliquots were pipetted into volumetric flasks and were diluted to 10 ml, 25 ml and 50 ml with DMF, respectively. From these solutions, 2 ml was transferred into a water-jecketted polarographic cell kept at 20 ± 0.1°C and 18 ml of appropriate buffer solution was added. The height of the mercury reservoir was kept at 60 cm and determinations were performed by DPP and CRP.

RESULTS AND DISCUSSION

In the DPP and CRP determinations of C_4, C_5 and C_6, it has been observed that these substances gave a polarographic wave with a peak potential which varied between -0,772 C - -0,792 V due to the substituted 3-methyl group. This is in good accordance with our previous findings in which we investigated cephalosporins haveing similar chemical structure.[1]

In our studies, 10 % DMF was used as the supporting electrolyte with the selected buffer solutions. C_4 and C_5 have 3-[[(1-methyl-1H-tetrazole-5-yl)thio]methyl] group as the leaving group wihich is reduced by taking 2é $2H^+$ at the mercury electrode in the pH range of 1-10. Among the investigated cephalosporins, C_5 gave a single peak while C_4 after pH > 5.0 and C_6 after pH > 4.0 gave a second peak as well in the pH range studied respectively. DPP and CRP polarograms of the three investigated cephalosporins are seen in Figure 1.

We suppose that the second peak of C_4 observed about -1.264 V at pH > 5 was the praenatrium wave with the greatest possibility which is due to the tiehnyl group at R_2 substituent.[18] We have met the similar case in the determination of Clotiazepam which has the same group.[18] C_6 gives a catalytical wave because of proton binding of the 3-[[(1-methyl-1H-tetrazole-5-yl)thio]methyl] group at R_2 substituent starting from pH > 4.

* DMF = Dimethylformamide

** Basic Al_2O_3 (Activity degree I) Woelm/West Germany and Silica gel 60 (0.063-0.2 mm) Merck/West Germany were used as the column material.

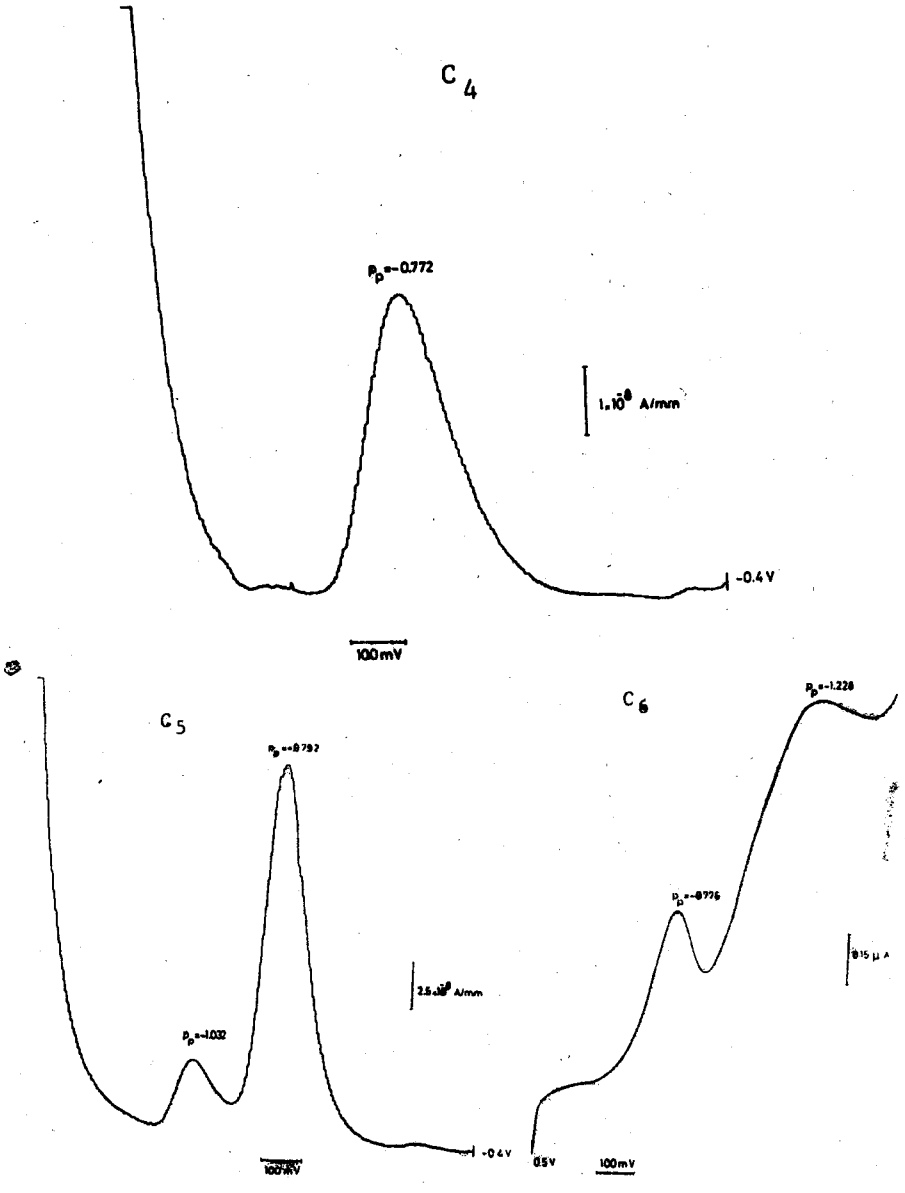


Figure 1

DPP and CRP polarograms of C_4 (32.0 µg/ml), C_5 (60,8 µg/ml) and C_6 (82.4 µg/ml) (conditions of measurement for the studied cephalosporins are indicated in Table II).

Conditions of measurement and experimental data for the studied cephalosporins which give polarographic peaks are summarized in Table II.

The peak heights in all three cephalosporins are linear with the concentration and this evidence enables to determine these subtances in dosage form (Figure 2).

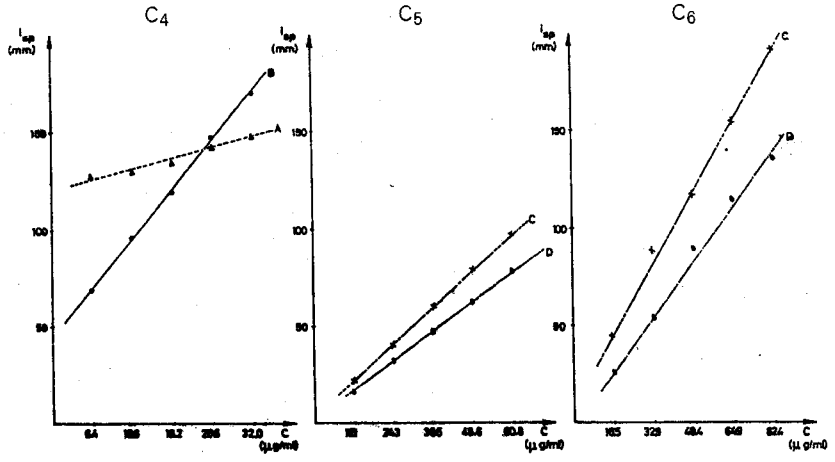


Figure 2

The linear relationship between peak heights and concentrations of the studied cephalosporins under the measurement conditions specified in Table II.

The dependence of peak height (is_p) and peak potential (p_p) on pH due to CH_2-R′ group at the leaving group is shown in Figure 3 and 4, respectively.

The peak potentials which are highly dependent on pH, shift to the negative potential and some differences are observed for the peak heights of the studied cephalosporins as well.

In order to determine the number of electrons transferred, correlation studies were made using substances having similar structures and diffusion coefficients. Furthermore, the results obtained with coulometry and controlled potential electrolysis were compared with the coulometric results which indicate a transfer of $2e^-$. The proposed reduction mechanism 15 given in Figure 5.

The polarographical properties determined were utilized in the assay of C_6 in vials, then a comparison was made with Ni (II)-hydroxylamine(3) and Hg (II)-imidazole-EDTA(2) methods.

Hg (II)-imidazole method has heen used in the determination of penicillins and included in several pharmacopoeiae as an official method. When the indicated method was applied to the determination of a number of cephalosporins, the necessity of working in alkaline pH range was

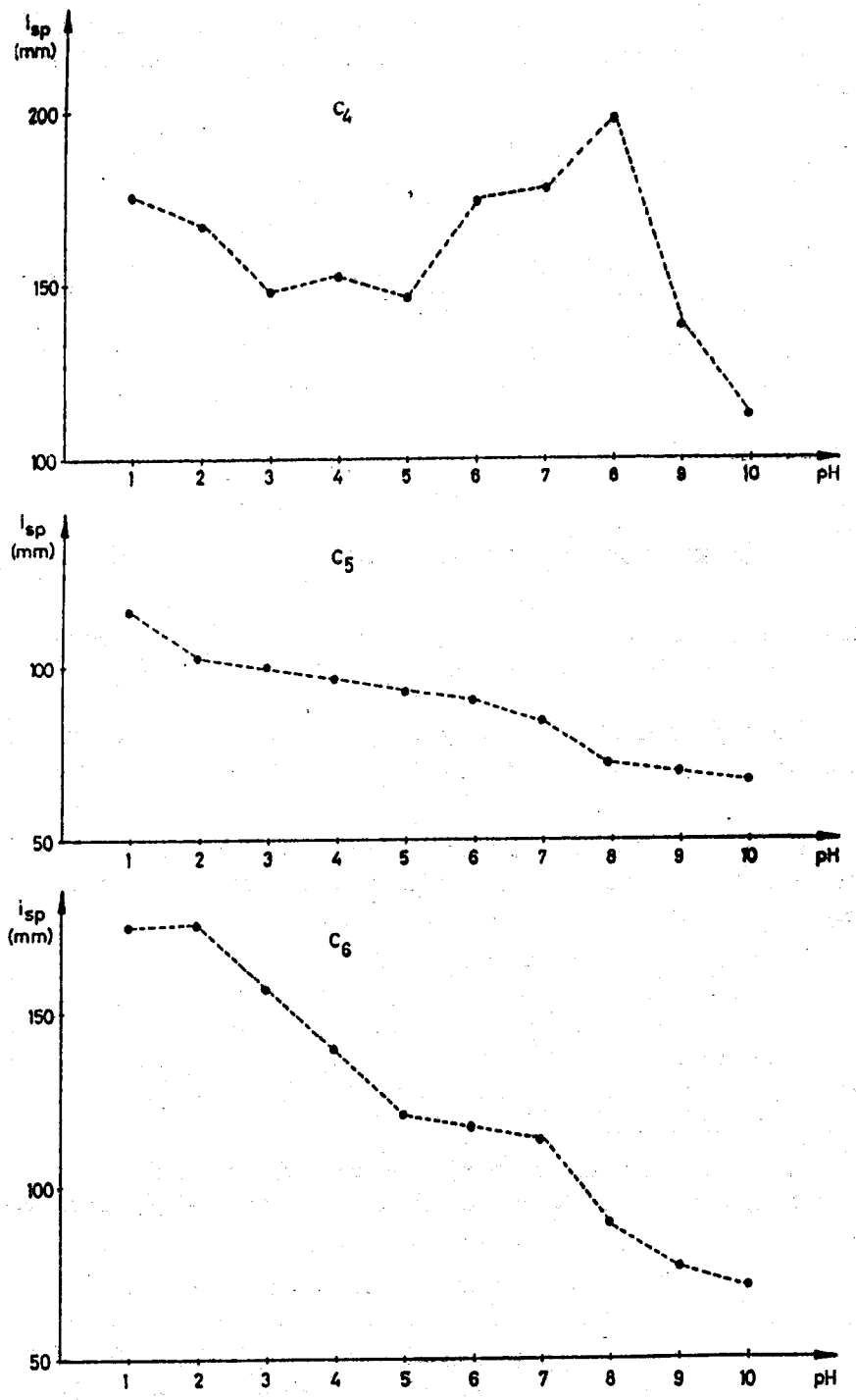


Figure 3

The dependence of peak height on pH of C_4 (32.0 µg/ml), C_5 (60.8 µg/ml) and C_6 (82.4 µg/ml) under the measurement conditions specified in Table II.

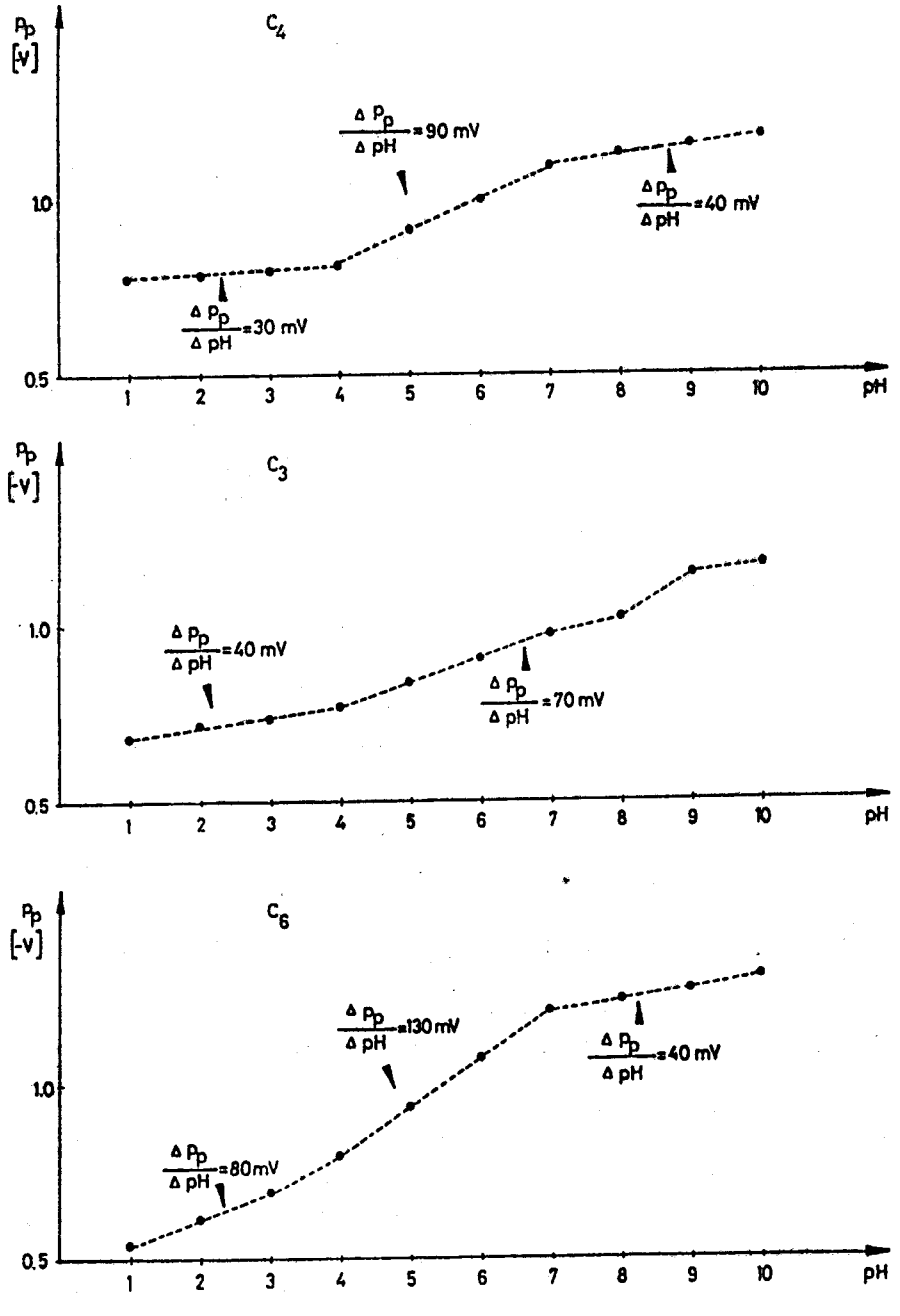


Figure 4

The dependence of peak potential on pH of C_4 (32.0 μg/ml), C_5 (60.8 μg/ml) and C_6 (82.4 μg/ml) under the working conditions specified in Table II.

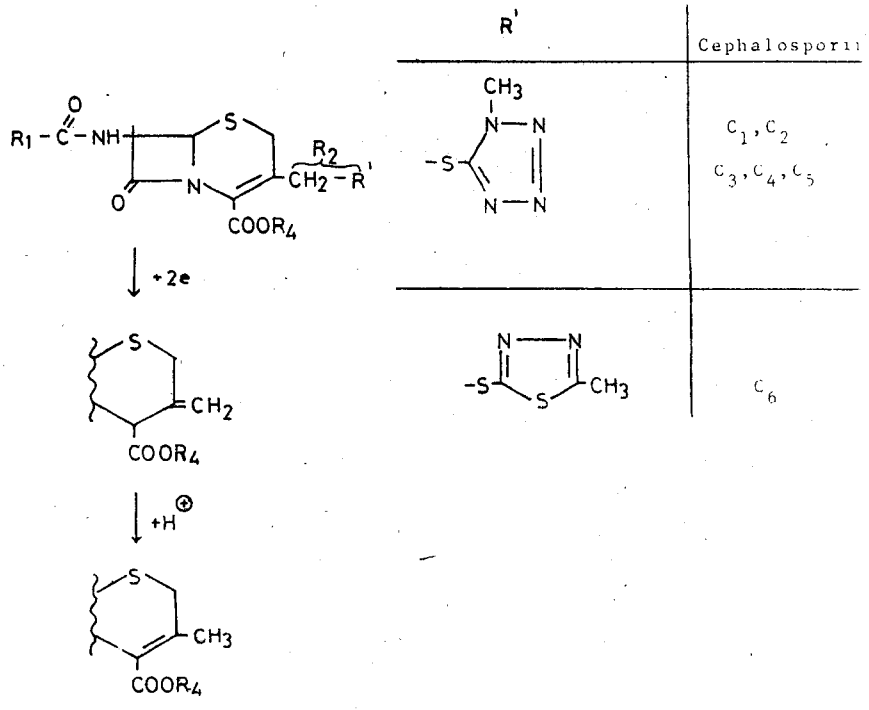


Figure 5

Proposed reduction mechanism for the investigated cephalosporins.

encountered in the determination of the optimum conditions for cephalosporins and for preventing the precipitation of Hg (II) salts the method was modified by adding some EDTA into the reagent. In our previous studies C_5 and C_6 have been determined by using this modified method[2] whereas the method used for the determination of penicillins have been applied to C_4.

Meanwhile, as the dosage forms of C_4 and C_5 are not presented into therapy, the comparisons were made only with C_6. Ni (II)-hydroxylamine method developed by D.L. Mays et al[19] is a modifed form of the method stated in USP XX and Code of Federal Regulations and uses Ni (II)-ion as a catalyst and stabilizer.[17] Statistical values obtained from the evaluation of the results of the methods are shown in Table III.

As it is seen from the table, the relative standard deviation values for DPP and CRP change between ± 0.39 % and ± 0.42 % whereas it is between ± 0.66 % and ± 1.54 % for both of the spectrophotometrical comparison methods.

TABLE III

STATISTICAL VALUES FOR DETERMINATION RESULTS OF CEFAZOLIN (C_6) VIALS POLAROGRAPHICAL ANALYSIS MEDIUM: CITRATE + HCl – BUFFER pH 4.0 WITH 10 % DMF NUMBER OF DETERMINATIONS: n = 5

Pharmaceutical Formulations (Vials)	The amount of cefazolin				
		Found $\bar{x}$ mg ± srel %			
		Polarographic		Spectrophotometric	
	Labelled	DPP	CRP	Hg(II)-imidazole-EDTA	Ni(II)-hydroxylamine
Maksipor[R] 250	250 mg C_6	254,3 ± 0,68	256,4 ± 0,36	251,4 ± 0,72	258,6 ± 1,22
Maksipor[R] 1000	1000 mg C_6	1004,8 ± 0,51	100,5 ± 0,43	1005,8 ± 0,73	998,4 ± 1,13
Kefzol[R]	500 mg C_6	502,7 ± 0,46	500,7 ± 0,44	503,0 ± 0,76	503,9 ± 1,21
Gramaxin[R] 250	250 mg C_6	253,4 ± 0,40	252,1 ± 0,39	251,7 ± 0,61	250,9 ± 1,28
Gramaxin[R] 500	500 mg C_6	499,6 ± 0,41	499,8 ± 0,38	502,6 ± 0,84	505,8 ± 1,29
Gromaxin[R] 1.0	1000 mg C_6	1002,4 ± 0,39	1003,1 ± 0,40	1004,9 ± 0,79	1006,8 ± 1,13

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