ANALYTICAL INVESTIGATIONS OF CEPHALOSPORINS PART 15. POLAROGRAPHICAL COMPARISON OF TWO 3-[(AMINOCARBONYL) - OXY-METHYL] SUBSTITUTED CEPHALOSPORINS AND THE DETERMINATION OF CEFOXITIN IN PHARMACEUTICAL FORMULATIONS

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Summary Polarographical properties of cefoxitin were investigated and compared with cefuroxime by using CRP and DPP. The electroactive group present in cefoxitin is R' leaving group of CH_2-R' which is located at C-3. Then the electrochemical method developed was applied to cefoxitin in pharmaceutical formulations 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 (CRP and DPP) were varying between ±0,39 % — ± 0,42 % while it is ± 0,60 % for Hg (II) - imidazole - EDTA method and ± 1,54 % for Ni (II) - hydroxylamine method.

The purpose of our investigations was to develop electroanalytical methods which would enable to determine cephalosporins in vitro and in vivo. Polarographical properties of cefuroxime having 3-[(Aminocarbonyl)-oxy-methyl] group as R_2 substituent have been investigated by using DPP and this substance in pharmaceutical formulations has been assayed, as well[1].

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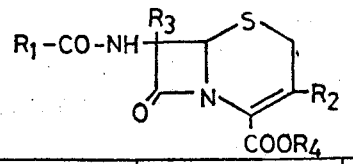
In the present study, polarographical properties of cefuroxime (C_1) which has the same R_2 substituent have been examined and a comparison was made between cefuroxime and cefoxitin (C_2). Furthermore, the electroanalytical method developed was applied to C_2 in pharmaceutical formulations and the results obtained were compared with the results of Hg (II)-imidazole-EDTA[2] and Ni (II)-hydroxylamine[3] methods.

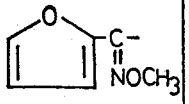
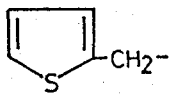
Chemical structure of the investigated cephalosporins is shown in Table 1.

Microbiological techniques have been proposed for C_2 [4], besides HPLC methods [5] and automatized colorimetric hydroxylamine method which is stated in USP XX [6].

Studies concerned with chemical analysis methods developed by making use of voltammetric determinations have gained great importance in recent years. Especially, the application of electrochemical methods to in vitro and in vivo determination of the substances having electroactive groups is very advantageous since the indicated substances undergo no chemical change in the determination.

Table 1 — Chemical structure of cefuroxime and cefoxitin



Substance	R_1	R_2	R_3	R_4
C_1 Cefuroxime		$-CH_2OCONH_2$	H	Na
C_2 Cefoxitin			CH_3O-	Na

EXPERIMENTAL

Apparatus

Polarographic measurements were carried out using a differential 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. Conditions of measurement for CRP are as the following :

Sweep amplitude : 1000 mVs^{-1}
Sweep rate : 400 mVs^{-1}
Delay time : 8 s

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

Cefoxitin sodium working standard and Mefoxitin^R vials were kindly supplied by Sharp and Dohme GmbH/West Germany.

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

Procedure

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

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* 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.

Pharmaceutical formulations containing 1 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 50 ml with DMF. From these solutions, 2 ml was transferred into a waterjacketted polarographic cell kept at 20 ± 0,1°C and 18 ml of acetate buffer solution of pH 4.0 was added. The height of the mercury reservoir was held at 60 cm and determinations were performed by DPP and CRP.

RESULTS AND DISCUSSION

DPP and CRP determinations of C_2 were performed in the pH range of 1.0-6.0, using various buffer solutions and 10 % DMF as the supporting electrolyte. It has been observed that this substance gave a polarographic wave with a peak potential varying between 1.050 V - 1.132 V due to the substituted 3-methyl group which we had previously observed in other similar cephalosporin derivatives.[7] Furthermore C_1 has a methoxyimino group which is polarograp-

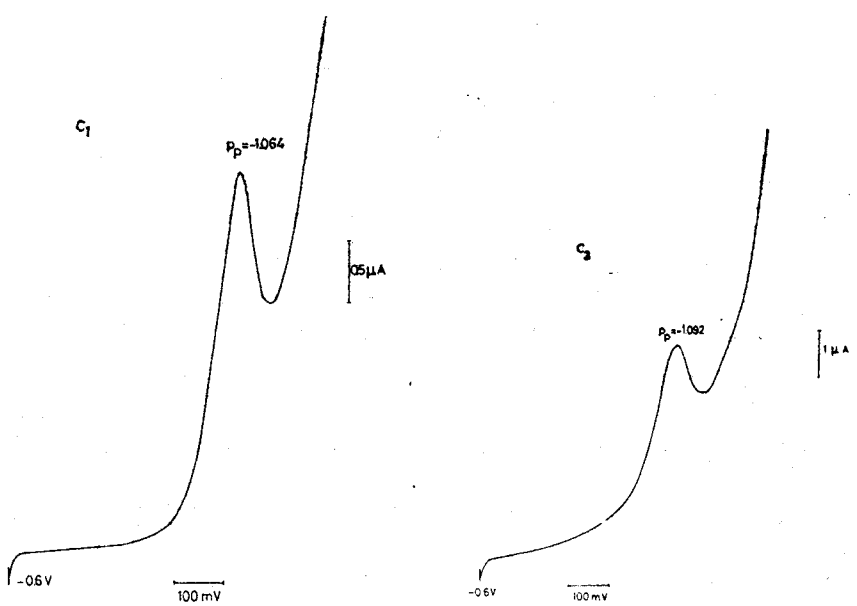

Fig. 1. CRP polarograms of C_1 (100 μg/ml) and C_2 (90 μg/ml) in acetate buffer of pH 4.0 and 10 % DMF

hically active in addition to the leaving group at C-3 and the reduction peak related to this group appears at a more positive potential (-0.362 V) as the second peak. The polarographical properties of the indicated group have been explained in detail in our previous study and therefore the two reduction peaks of C_1 have been taken into consideration only for comparison purposes[8]. Fig. 1 shows CRP polarograms of C_1 and C_2 in acetate buffer of pH 4.0.

No reduction peak is observed for C_1 and C_2 at pH > 6.0 This is probably due to the interference of the reduction peak regarding the leaving group with the reduction potential of the cations of the supporting electrolyte. The dependence of peak height (i^sp) and peak potential (p_p) on pH is shown in Fig. 2 and 3, respectively.

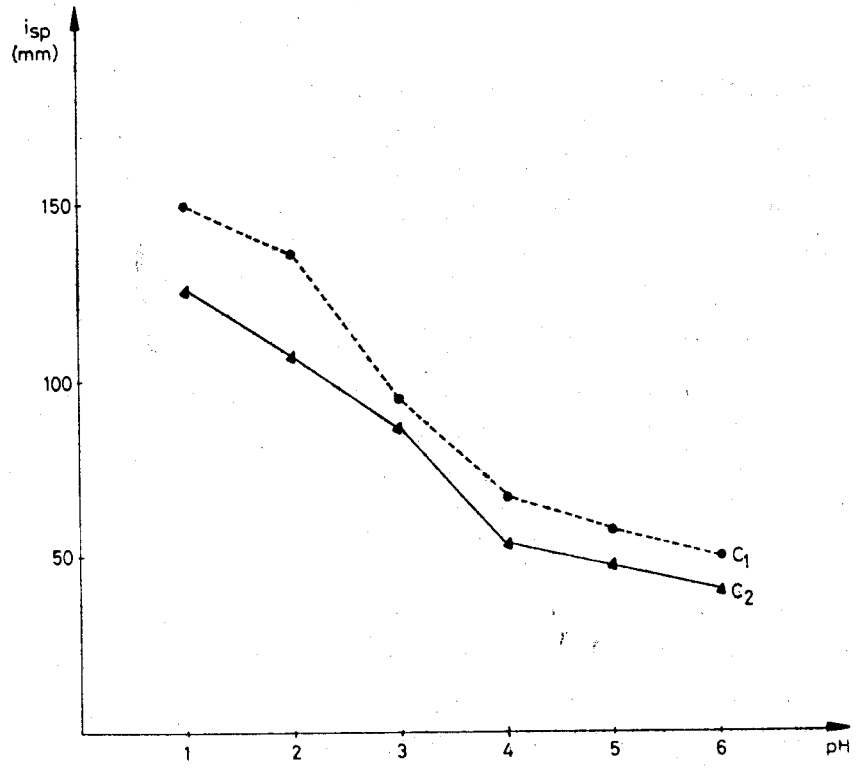

Fig. 2. The dependence of peak height on pH of C_1 (100 μg/ml) and C_2 (90 μg/ml) with 10 % DMF

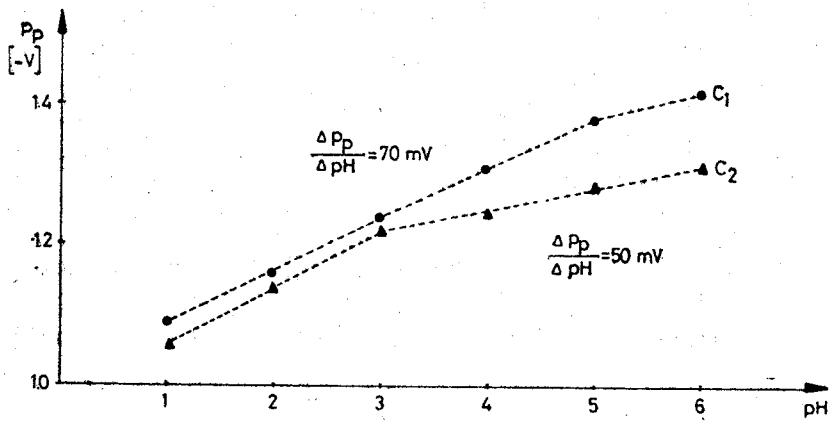

Fig. 3. The dependence of peak potential on pH of C_1 (100 μg/ml) and C_2 (90 μg/ml) with 10 % DMF

For both of the substances, the peak potentials which are highly dependent on pH shift to the negative potential and some differences are observed for the peak heights also.

The differences in peak heights are probably due to the different diffusion constants that the substances have. In the measurements it was seen that the second peak height in C_1 and the first in C_2 are linear with the concentration and this evidence enables the determination of these substances in dosage forms.

The reduction mechanism in Fig. 5 is proposed for the investigated cephalosporins according to the results obtained after the application of correlation to the substances having similar reduction mechanisms under the same conditions as well as coulometry and controlled potential electrolysis.

The polarographic properties determined were utilized in the assay of cefoxitin in vials, then a comparison was made with Hg - (II) - imidazole-EDTA and Ni (II) - hydroxylamine methods(9).

Hg (II)-imidazole method has been 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 encountered in the determination of the pre-

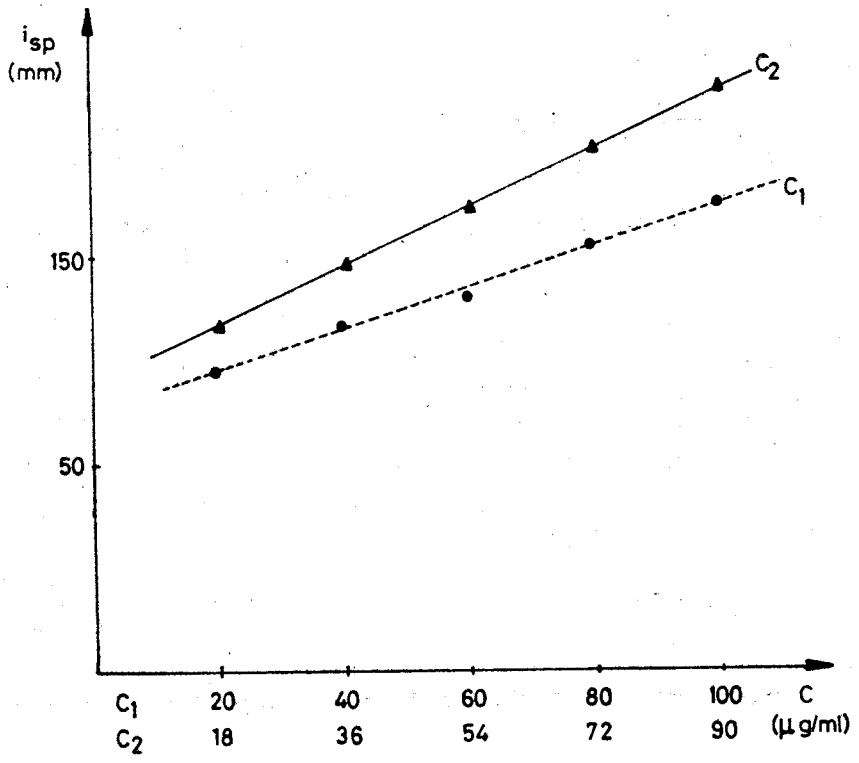

Fig. 4. The linear relationship between peak heights and concentrations of the investigated cephalosporins in acetate buffer of pH 4.0 and 10 % DMC

cipitation of Hg (II) salts, the method was modified by adding some EDTA into the reagent. In our previous studies, several amino cephalosporins, cephaloridin, cephazolin, cefoxitin, cefmetazole, cefotiam and cefsulodin[10] have been determined by using this modified method.

Ni (II)-hydroxylamine method developed by D.L. Mays and his friends is a modified form of the method stated in USP XX and Code of Federal Regulations and uses Ni (II)-ion as a catalyst and stabilizer [11].

Statistical values obtained from the evaluation of the results of the methods are shown in Table 2.

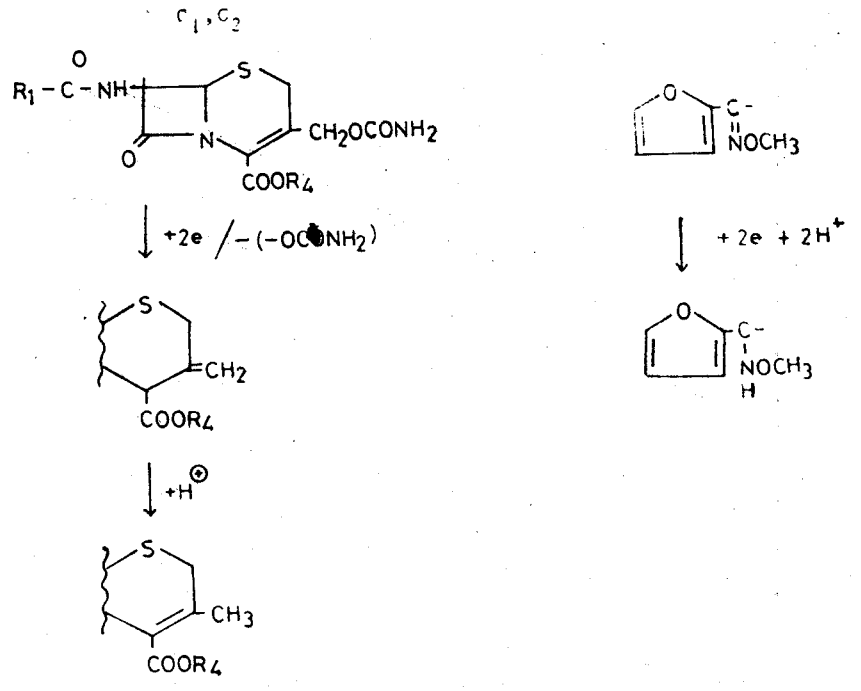

Fig. 5. Proposed reduction mechanism for the investigated cephalosporins

Table 2. Statistical values for determination results of cefoxitin in mefoxitin^R vials (number of determinations n = 5)

The amount of cefoxitin				
Found x mg ± s_{rel} %				
Polarographic			Spectrophotometric	
Labelled	DPP	CRP	Hg(II)-imidazole EDTA	Ni(II)-hydroxylamin
1000 mg Cefoxitin	99,7 ± 0,39	1000, 4 ± 42	1002,2 ± 0,66	995,6 ± 1,54

As it is seen from the table, the relative standard deviation value for DPP is ± 0,39 % and for CRP is ± 0,42 % whereas it is ± 0.66 % for Hg (II)-imidazole-EDTA and ± 1.54 % for Ni (II) - hydroxylamine methods.

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