XIII_702

ELSEVIER

International Journal of Pharmaceutics 134 (1996) 213–221

international journal of pharmaceutics

Spectrophotometric study of copper(II) ion complexes with cefaclor

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Received 18 October 1995; revised 19 December 1995; accepted 18 January 1996

Abstract

The formation of complex ions between cefaclor and Cu^{2+} has been investigated by ultraviolet spectroscopy. The conditions of formation of complex ions were studied in weak acidic, neutral and alkaline medium in the pH range from 5.60 to 11.05. The interaction between cefaclor and Cu(II) ion in acidic medium results in the formation of $Cu(CEF)^+$ complex and it is supposed that the ligand acts as zwitterion which is coordinated to the metal ion. This complex easily hydrolyzes giving a hydroxo complex Cu(OH)(CEF). The Job method, the molar ratios method and the method of relative recovery of complex species were applied and it was found that the stoichiometric ratio between cefaclor and Cu(II) is 1:1. The molar absorptivity $\epsilon/M^{-1}\ l\ cm^{-1} = 2670 \pm 35$ at 300 nm and the consecutive concentration constant of the hydroxo complex $\log K_2\ /M^{-1}\ l = 9.13 \pm 0.118$, were determined at pH $= 8.00$ and at an ionic strength of $\mu = 0.1$.

Keywords: Cefaclor; Copper complexes; Stability constant; Ultraviolet spectroscopy

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1. Introduction

β-lactam antibiotics interact with metal ions and this interaction is of a complex nature (Page, 1987; Van Krimpen et al., 1988). The investigations show that metal(II) ions catalyze hydrolysis of some penicillins and cephalosporins and the saturation kinetics from the concentration of metal ions was observed (Gensmantel et al., 1980). In the presence of an excess of metal ions, an antibiotic/metal ion complex was formed. It was found also, that the ratio metal: penicillin was 1:1 and the great catalytic effects of Cu^{2+} ions was established in the case of hydrolysis of benzylpenicillin. It was supposed that the metal ion stabilizes the tetrahedral transition complex formed during the hydroxide-ion catalyzed hydrolysis of benzylpenicillin and that the site of coordination with Cu(II) ion is the β-lactam ni-

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PII S0378-5173(96)04459-6

trogen and carboxylate group (Gensmantel et al., 1978, 1980). Cressman et al. (1969) have also investigated the rate of hydrolysis of natural penicillins and their investigation suggests that the nitrogen substituent on the 6 position is involved in a transition complex prior to the ring opening.

Literature data for the metal complexes of semisynthetic penicillins, especially cephalosporins are scarce. Fazakerley and Jackson (1975) have examined the interaction between ampicillin and cephalexin with Cu(II) and Mn(II) ions using ^{1}H NMR and it was found that the metal ion is coordinated through the carbonyl and amino group in the side chain. Coordination between metal ions and cephalexin, which takes place through the side chain, has been also confirmed using infrared spectroscopy and magnetic measurements (Moratal et al., 1989).

The metal complexes of semisynthetic penicillins and cephalosporins and their stability constants have been determined mainly by electroanalytical methods, spectrophotometric and other methods (Veselinovic and Kapetanovic, 1985; Veselinovic and Kapetanovic, 1989; Abdel-Gawad et al., 1987; Kapetanovic et al., 1990).

The aim of our paper is to establish the optimal conditions of forming the complex ions between cefaclor and Cu^{2+} ions and to evaluate the stoichiometric ratio and stability constant by methods of ultraviolet spectroscopy.

2. Experimental

Cefaclor monohydrate (953 μg/mg) was obtained from the production lots (Ely Lilly Company). Copper(II) perchlorate hexahydrate, sodium perchlorate monohydrate (Aldrich Chemical Company) and all other chemicals used were reagent grade. Water was deionized before use.

Cefaclor solution was always prepared by exact weighing of standard substance and used for one day only. Cu(II) perchlorate solution (0.01 M) was standardized by the complexometric method (Schwartzenbach and Flaschka, 1965).

Sodium perchlorate solution was used to maintain constant ionic strength of $\mu = 0.1$.

The reaction solution pH was maintained at the desired value by the use of a pH meter.

The UV-spectra were recorded on HP 8452 A diode array computer •controlled spectrometer (Hewlett Packard), equipped with thermostated cell compartment.

3. Results and discussion

The UV-spectrum of aqueous solution of cefaclor ($5 \cdot 10^{-5}$ M) at pH 8.00 and at $\mu = 0.1$ is shown in Fig. 1.

The UV-spectrum of cefaclor exhibit two bands with a maximum absorption at 264 nm (ϵ /M^{-1} l cm^{-1} = 9180) and 206 nm (ϵ /M^{-1} l cm^{-1} = 16700).

The chemical interaction of the Cu^{2+} with cefaclor was determined by UV spectroscopy following the spectral changes in the range from 400 to 282 nm. The UV-spectra of cefaclor and Cu(II) perchlorate reaction mixture (a) and Cu(II) perchlorate (b), at pH = 8.00 and at $\mu = 0.1$ are shown in Fig. 2.

The appearance of the absorption band in the range from 400 to 282 nm with maximum absorption at 300 nm, indicate formation of complex ions between cefaclor and Cu(II) perchlorate at pH = 8.00. The molar absorptivity of Cu(II) perchlorate (ϵ /M^{-1} l cm^{-1} = 564 at 300 nm) is more less than the molar absorptivity of reaction mixture of cefaclor and Cu(II) perchlorate (ϵ / M^{-1} l cm^{-1} = 2670 at 300 nm). The high molar absorptivity of cefaclor in the wavelength range up to 280 nm (ϵ /M^{-1} l cm^{-1} = 8400 at 265 nm) (Lorenz, 1980) required the use of cefaclor solution with the same concentration as in the reaction mixture, as a blank solution. The 'cut off' point for the blank solution appears at 282 nm and after that the absorbance could be measured.

3.1. Optimum conditions for complex formation

Cefaclor (HCEF) act as an acid and a base. The dissociation constants of cefaclor, determined in an aqueous solution, are pK_{a1} (–COOH) = 1.5 ± 0.2 and pK_{a2} ($-NH_3^+$) = 7.17 (Lorenz, 1980). A cationic species H_2CEF^+ is formed by dissolv-

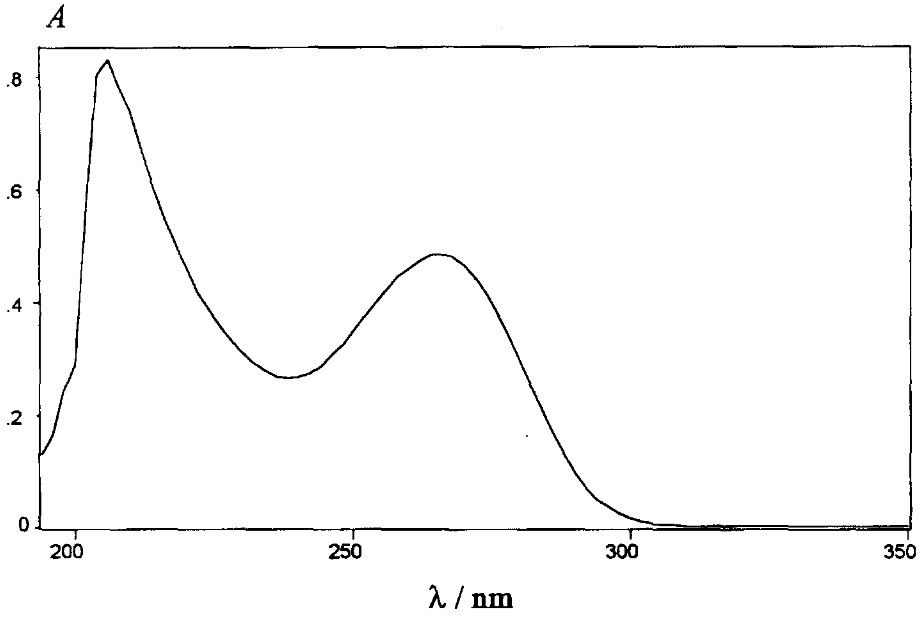

Fig. 1. UV-spectrum of cefaclor ($5 \cdot 10^{-5}$ M) at pH = 8.00.

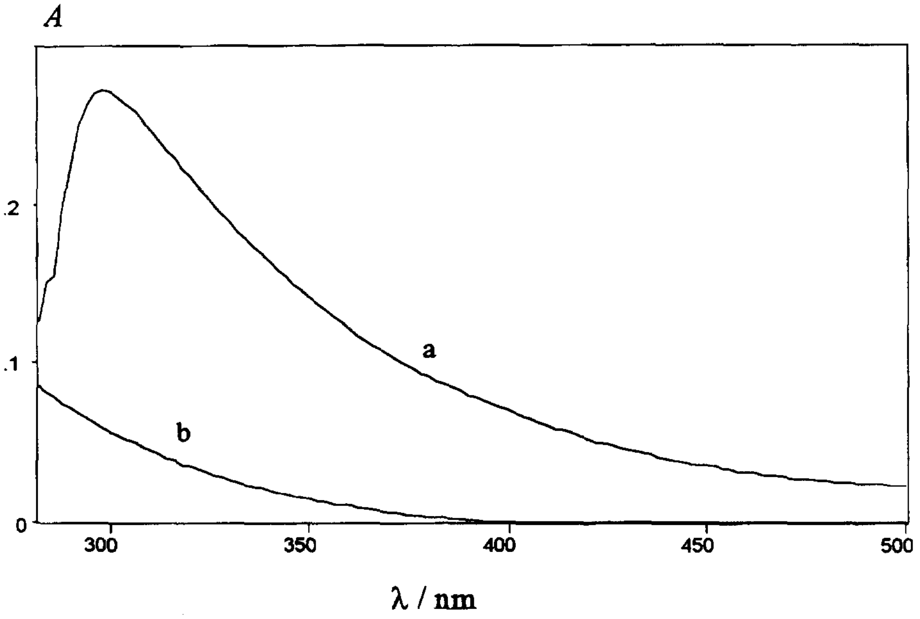

Fig. 2. UV-spectra of cefaclor ($5 \cdot 10^{-4}$ M) and Cu(II) perchlorate ($1 \cdot 10^{-4}$ M) reaction mixture (a), and Cu(II) perchlorate ($1 \cdot 10^{-4}$ M) (b), at pH = 8.00.

ing cefaclor in an acidic medium, which acts as a diprotic acid according to the following equilibria[1]:

$H_2(CEF)^+ \Leftrightarrow HCEF + H^+$

$HCEF \Leftrightarrow CEF^- + H^+$

The zwitterion of cefaclor dominates the pH range between both pK_a values and in this pH

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[1] In this equilibria, the part of the solvent is not shown.

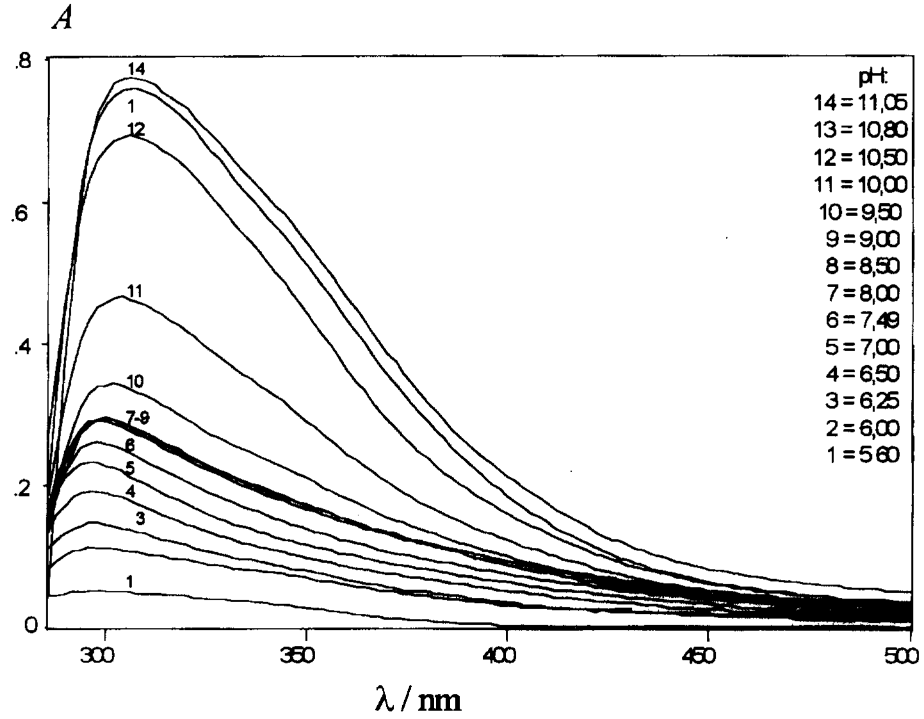

Fig. 3. UV-spectra of cefaclor and Cu(II) perchlorate reaction mixture at different pH values.

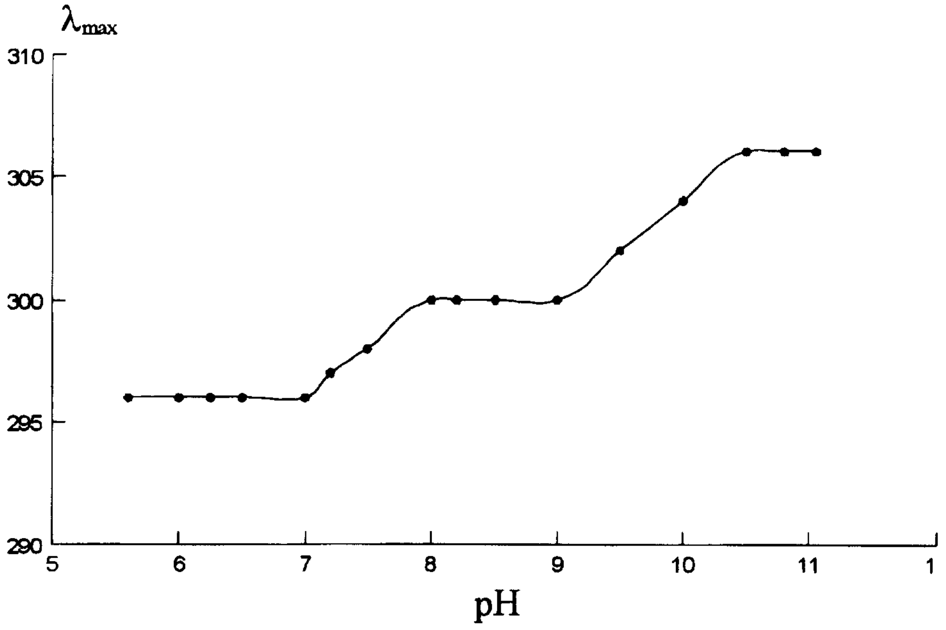

Fig. 4. Plots of λ_{max} of the complex ions versus pH.

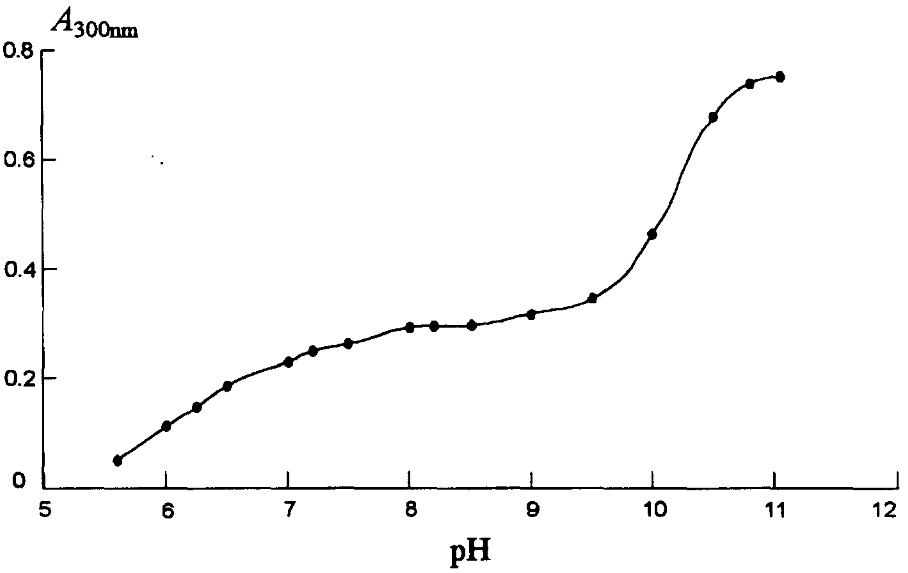

Fig. 5. Plots of absorbance of the complex ions versus pH at 300 nm.

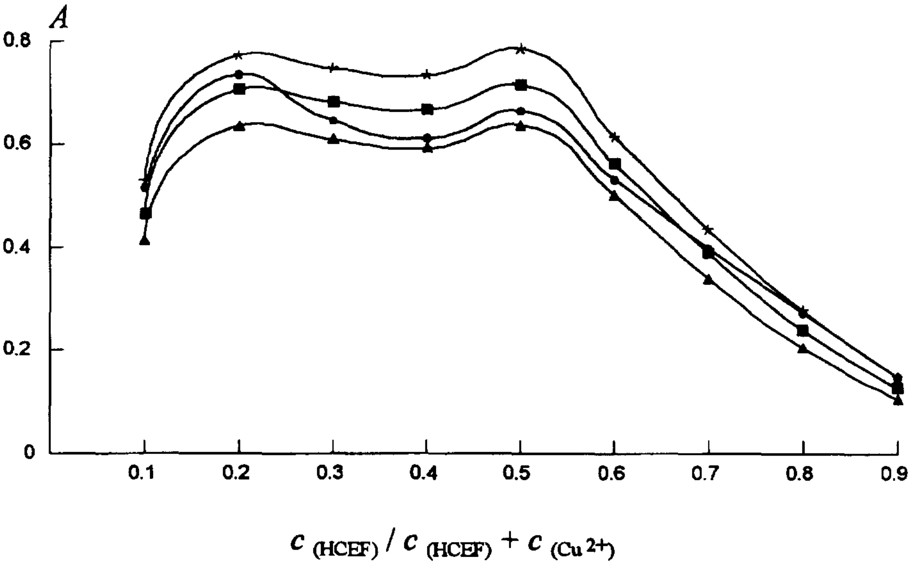

Fig. 6. Job's curves: 290 nm (•), 300 nm (*), 310 nm (■) and 320 nm (▲) and at pH = 8.00.

range the ligand should act as zwitterion which is coordinated to the metal ion (Moratal et al., 1989; Kapetanovic et al., 1990).

The influence of pH in forming the complex ions was followed by changing the UV-spectrum of cefaclor and Cu(II) reaction mixture in a molar ratio 5:1, at different pH and at μ = 0.1 (Fig. 3). The possibility of existing of higher complex ions is excluded by examination the mixture with ratio cefaclor:Cu(II) perchlorate up to 7:1.

The interaction between Cu(II) and cefaclor, in an acidic medium, results in the formation of $Cu(CEF)^+$ complex where the zwitterion of cefaclor takes part. This complex species hydrolyzes in a weak acidic medium (pH = 5.60) to give hydroxo complex Cu(OH)(CEF), which is confirmed by the appearance of the band at this pH

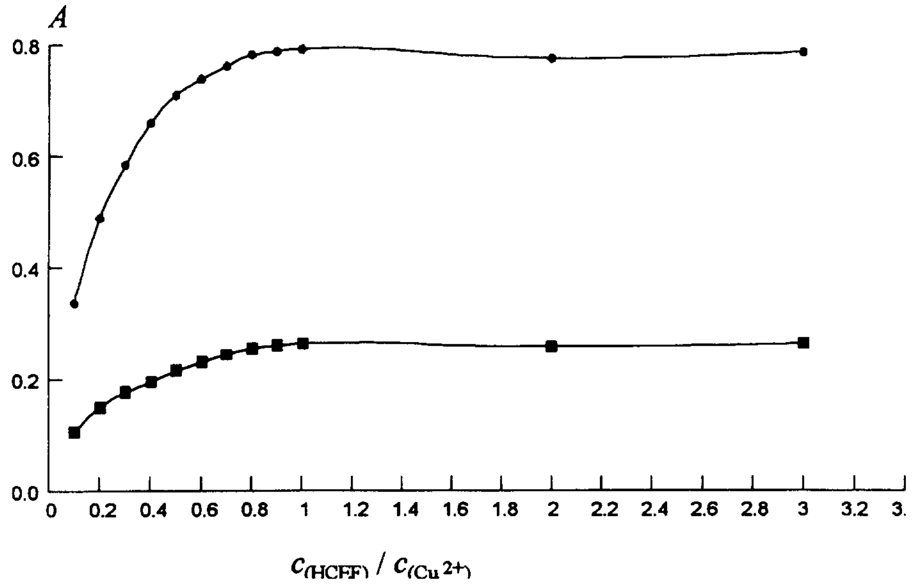

Fig. 7. Plots of absorbances of the complex ions versus the molar ratio of the components: (■) $1 \cdot 10^{-4}$ M Cu(II) and $1 \cdot 10^{-5}$ to $3 \cdot 10^{-4}$ M cefaclor; (•) $3 \cdot 10^{-4}$ M Cu(II) and $3 \cdot 10^{-5}$ M to $9 \cdot 10^{-4}$ M cefaclor).

value (Fig. 3). The band is wide and asymmetrical because of the overlapping possibility of two or more bands with a different intensity, but at very close wavelengths. By adding hydroxide ions in the system the maximum of the band is shifted at a longer wavelength.

The plots of λ_{max} and absorbance of the complex ions versus pH shown in Figs. 4 and 5, give a clearer picture of the changes which have happened in the reaction solution at different pH values.

The appearance of a plateau in the pH range from 5.60 to 7.00 at 294 nm (Fig. 4) and increasing of the absorbance in the same pH range (Fig. 5), could be explained by the presence of both complex species $Cu(CEF)^+$ and Cu(OH)(CEF), because of their similar spectral characteristics. The appearance of another plateau at 300 nm in the pH range from 8.00 to 9.00 (Fig. 4) points out the existence of hydroxo complex Cu(OH)(CEF) only, which is also confirmed by the appearance of a plateau of the absorbances in this pH range (Fig. 5). Shifting the maximum at a longer wavelengths and increasing the absorbance at pH > 9.00 shows that a higher hydroxo complex $Cu(OH)_2(CEF)$ is formed. At pH > 10.70 an intensive yellow-orange coloring of solution is observed, as a result of possible degradation of cefaclor in strong alkaline medium (Yamana and Tsui, 1976).

According to the pH dependence, the reaction of formation of the complex species could be presented by the following equilibria:

$$Cu^{2+} + HCEF \Leftrightarrow Cu(CEF)^+ + H^+$$

$$Cu(CEF)^+ + OH^- \Leftrightarrow Cu(OH)(CEF)$$

The structure similarity between cefaclor and cephalexin is probably reason for similar ability of their complexes formation. According to the literature data (Fazakerley and Jackson, 1975; Moratal et al., 1989) it could be predicted that the coordination of cefaclor with Cu(II) takes place through the carbonyl and amino group in the side chain. In our previous paper (Dimitrovska et al., 1995) the copper(II) ion catalyzed hydrolysis of cefaclor was investigated and no significant effect was found. The obtained results were consistent with the assumption that the metal ion is not involved in the stabilization of the tetrahedral transition complex prior to the β-lactam opening, which confirms the fact that the cefaclor complex occurs in the side chain at pH = 8.00.

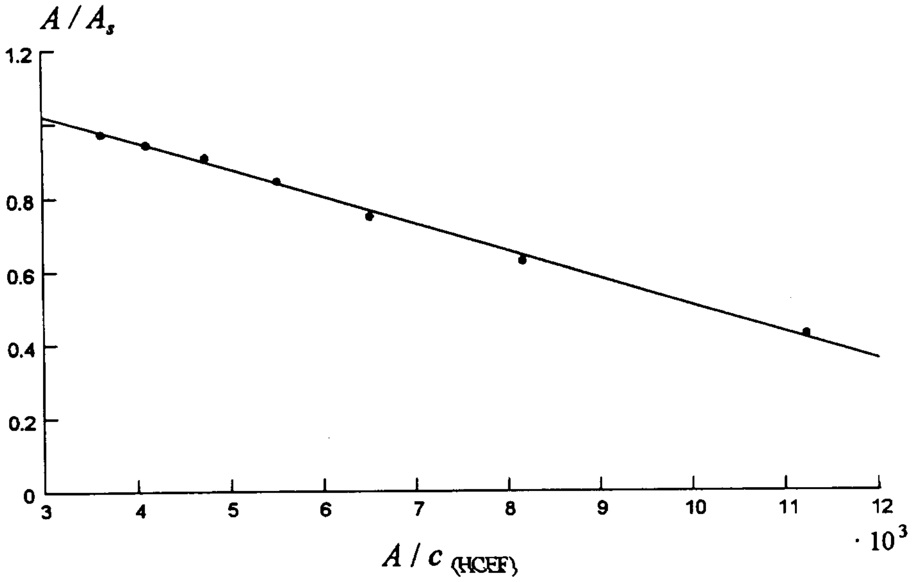

Fig. 8. Plots of $A/c_{(\mathrm{HCEF})}$ versus A/A_s at a constant concentration of Cu(II) perchlorate.

The range of the absorption maximum of the reaction mixture Cu(II)/cefaclor in molar ratio 1:1, at pH = 8.00, is related with the absorption maximum of Cu-complexes with peptides of aromatic amino acids (Koltun et al., 1960).

The intensity of the absorption band of hydroxo complex Cu(OH)(CEF) (ϵ /M^{-1} l cm^{-1} = 2670) shows that most probably there is a electrical transfer of charge Cu → HCEF, e.g. $\sigma_{\mathrm{Cu(II)}} \rightarrow \pi^*_{\mathrm{HCEF}}$.

$Cu(OH)_2$ will start to precipitate at pH = 8.00 and $c\ Cu^{2+} = 1 \cdot 10^{-4}$ M, without presence of cefaclor, according to the data of Savic and Savic (1989) that $K^{\varnothing}_{sp}$ of $Cu(OH)_2$ is $5 \cdot 10^{-20}$ which does not occur in the pH range from 4.00 to 10.70. This is yet another proof of the existence of soluble complex ions between Cu(II) and cefaclor.

The absorbance of the hydroxo complex Cu(OH)(CEF), remains unchanged by varying the ionic strength of the solution. The investigation was performed in the range of $\mu = 0.01-0.5$. The absence of the influence of ionic strength, points out the weak polarity of the band Cu → O.

The stability of the hydroxo complex versus time was examined at pH = 8.00 and at $\mu = 0.1$. The obtained results show that the complex is stable for one hour at 23°C.

The absorbance of this complex species remains the same in the range from 21°C to 45°C. Thermodynamical stability in this temperature range is also confirmed with the fact that there is no change in the appearance of the UV-spectrum of hydroxo complex.

3.2. Stoichiometric ratio of the complexes

The Job method, the molar ratios method and the method of relative recovery of complex species (Bulatov and Kalinkin, 1986) were used for determining the stoichiometry of the reaction between cefaclor and Cu(II) perchlorate. The solutions for Job's method were prepared by mixing both components with equimolar concentration of $5 \cdot 10^{-3}$ M, in ratio from 1:9 to 9:1. Absorbances were measured at 290, 300, 310 and 320 nm, pH = 8.00, t = 23°C and at μ = 0.1 using cefaclor solution of the corresponding concentration as the blank. The Job's curves at different wavelengths are shown in Fig. 6.

According to the appearance of the Job's curves it could be concluded that more than one complex species in the system are formed. The obtained curves have a marked maximum at a molar fraction of n = 0.5 which indicates the course of the reaction Cu(II)/cefaclor = 1:1 or 2:2 or 3:3 etc. The presence of another maximum at a molar fraction of n = 0.2, shows that in the range of these concentration the reaction in the ratio Cu(II)/cefaclor = 4:1 is possible. The analysis of Job's curves shows the presence of mononuclear (polymeric) and polynuclear complex ions whose appearance at pH = 8.00 depends on the relation between metal ions and ligand.

The molar ratios method was used at pH = 8.00 (Fig. 7). Two series of solutions with Cu(II) perchlorate concentration of $1\cdot10^{-4}$ M and $3\cdot10^{-4}$ M were prepared. The concentration of cefaclor was varied from $1\cdot10^{-5}$ M to $3\cdot10^{-4}$ M and from $3\cdot10^{-5}$ M to $9\cdot10^{-4}$ M, respectively. Absorbances were measured at 300 nm and at $\mu = 0.1$.

The presence of more than one complex species in the system simultaneously, is reflected on the shape of the curves shown in Fig. 7, which eliminate the use of molar ratios method for the exact determination of stoichiometric ratio of the reactants.

Both the wide and undefined maximums on Job's curves and the fact that the Job method doesn't determine the exact ratio of the components but their relative ratio, determined the use of the method of relative recovery of complex species.

In this method the spectra from the molar ratios method at concentration of Cu(II) perchlorate $3\cdot10^{-4}$ M were used. From these spectral data the values $A/c_{(\mathrm{HCEF})}$ and A/A_s were calculated, where A is absorbance of hydroxo complex, $c_{(\mathrm{HCEF})}$ initial concentration of cefaclor and A_s is mean value of absorbances of hydroxo complex on curve's plateau (saturated). The plots on Fig. 8 are based on the data from Table 1.

Table 1
Data for construction the curve of relative recovery of complex species

$c_{(\mathrm{HCEF})}$	A	$A/c_{(\mathrm{HCEF})}$	A/A_s
3×10^{-5}	0.377	1.1233×10^4	0.4296
6×10^{-5}	0.490	8.1667×10^3	0.6246
9×10^{-5}	0.586	6.5110×10^3	0.7470
1.2×10^{-4}	0.661	5.5080×10^3	0.8426
1.5×10^{-4}	0.711	4.7400×10^3	0.9064
1.8×10^{-4}	0.739	4.1056×10^3	0.9421
2.1×10^{-4}	0.762	3.6286×10^3	0.9710

The equation $A = 1.239 - 7.321\cdot10^{-5}\ c$ was obtained by regression analysis, where the correlation coefficient is $r = 0.9979$.

The absence of a maximum on the above presented dependence $A/c_{(\mathrm{HCEF})} = \mathrm{f}(A/A_s)$, shows that the stoichiometric coefficient of the component with variable concentration (cefaclor) is equal to 1. It means that the formation of complex species $\mathrm{Cu_2(HCEF)}$ or Cu(HCEF) is possible. In this case the formation forming of complex species $\mathrm{Cu_2(HCEF)}$ is excluded because there is no deviation of linear dependence. So, it follows that the already formed complex species is Cu(HCEF) but not $\mathrm{Cu_2(HCEF)_2}$ or $\mathrm{Cu_3(HCEF)_3}$ etc, as Job's curves suppose.

3.3. *The determination of molar absorptivity and stability constant of hydroxo complex*

The molar absorptivity value and the consecutive concentration constant of the reaction of formation of the hydroxo complex were calculated using the method for calculation of the stability constant and molar absorptivity of complexes of the type ML*n* (n = 1, 2, 3......) from spectrophotometric data, described by Chattopadhyaya and Singh (1974). Two solutions with different initial concentration of metal ion and ligand were used. The solutions were prepared by mixing Cu(II) perchlorate and cefaclor in ratio 1:1. Equimolar concentration of Cu(II) perchlorate and cefaclor was $1\cdot10^{-4}$ M in one solution and $3\cdot10^{-4}$ M in the other solution.

The calculated value of the molar absorptivity and the consecutive concentration constant of the hydroxo complex at pH = 8.00, t = 23°C and at $\mu = 0.1$ are $\epsilon\ /\mathrm{M^{-1}\ l\ cm^{-1}} = 2670 \pm 35$ and $\log K_2\ /\mathrm{M^{-1}\ l} = 9.13 \pm 0.118$ for $n = 10$, respectively.

The value of ϵ was also calculated at different wavelengths near the absorption maximum and all these values give approximately the same value of $\log K_2$.

4. Conclusion

In acidic medium Cu^{2+} interacts with cefaclor to give the $Cu(CEF)^+$ complex which easily hydrolyzed to give Cu(OH)(CEF). The hydroxo complex dominates at $pH > 7.50$. This complex species at $pH = 8.00$ shows absorption band in the range from 400 to 282 nm, with a maximum at 300 nm. It was found that the stoichiometric ratio of cefaclor to Cu(II) is 1:1. The molar absoptivity ($\epsilon/M^{-1}\,l\,cm^{-1} = 2670 \pm 35$ at 300 nm) and the consecutive concentration constant of the hydroxo complex ($\log K_2/M^{-1}\,l = 9.13 \pm 0.118$) calculated at pH 8.00, $t = 23\,^0C$ and at $\mu = 0.1$, were obtained by spectrophotometric data.

Studies are in progress to examine the possibility of using Cu(II) perchlorate as a reagent for determination of cefaclor in drug formulations by UV-spectroscopy.

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