

A SPECTROPHOTOMETRIC STUDY OF THE FORMATION OF  
NICKEL(II) ION COMPLEXES WITH CEFACTOR

**Key words:** Cefactor, Ultraviolet Spectroscopy, Nickel Complexes, Stability  
Constant.

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ABSTRACT

The formation of Ni(II) ion complexes with cefactor has been investigated by ultraviolet spectroscopy. This study has shown that cefactor and  $\text{Ni}^{2+}$  interact in weak acidic medium to give a  $\text{Ni}(\text{CEF})^+$  complex which easily hydrolyzes giving a hydroxo complex  $\text{Ni}(\text{OH})(\text{CEF})$ . This complex species at  $\text{pH} = 8.70$  shows

absorption bands in the range from 400 to 294 nm, with a maximum at 316 nm. Using the Job method, the molar ratios method and the method of relative recovery of complex species the stoichiometric ratio cefactor : Ni(II) = 1:1 was found. The molar absorptivity value,  $\epsilon = 1.12 \pm 0.02 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$  at 316 nm and the concentration constant of the hydroxo complex  $\log K_2 = 8.3 \pm 0.1 \text{ l / mol}$ , were determined at pH = 8.70 and at an ionic strength of  $\mu = 0.01$ .

### INTRODUCTION

Cefactor (Fig. 1), an orally active cephalosporin in clinical practice, belongs to the group of  $\beta$ -lactam antibiotics.

It is well known that  $\beta$ -lactam antibiotics and metal ions form complexes with different compositions in aqueous media<sup>1-4</sup>.

Interactions between these antibiotics and metal ions may change their *in vitro* stability<sup>5,6</sup>. These interactions may also change the physico-chemical properties of drugs and as a result of that, their *in vivo* storage and fate may be affected<sup>1</sup>.

The metal complexes of some semisynthetic  $\beta$ -lactam antibiotics have been investigated by electroanalytical and spectrophotometric methods<sup>7-13</sup>. The stoichiometric composition of complexes and their stability constant values have been established.

In our paper we report optimal conditions (the influence of pH, temperature, ionic strength and the stability of complex with time) of the formation of complex ions between Ni(II) and cefactor. The molar absorptivity, the stoichiometric ratio

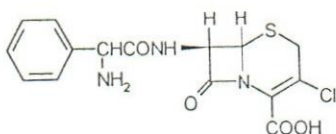


FIG. 1: Chemical structure of cefaclor

and the stability constant have been determined by methods of ultraviolet spectroscopy.

### EXPERIMENTAL

Cefaclor monohydrate (953  $\mu\text{g}/\text{mg}$ ) was obtained from production lots (Ely Lilly and Company) and used as such. Nickel(II) perchlorate hexahydrate and sodium perchlorate monohydrate were obtained from Aldrich Chemical Company. All the other reagents were analytical grade chemicals. Water was deionized before use.

Standard cefaclor solution was freshly prepared by exact weighing of cefaclor monohydrate. The 0.01 M Ni(II) perchlorate solution was prepared by dissolving the proper amount in water. The solution was standardized by a complexometric method<sup>14</sup>. The ionic strength of the solutions was adjusted at 0.01 with sodium perchlorate. The solution pH was maintained by the use of a pH-meter.

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

### RESULTS AND DISCUSSION

The interaction between cefaclor (HCEF) and Ni(II) ions was followed in the pH range from 8.20 to 11.25, by changes in the absorption spectrum in the range from

400 to 290 nm. The optimal conditions of the formation of the complex ions were also evaluated.

The absorption band of cefaclor in 0.1 M HCl shows a maximum absorption at 264 nm ( $\epsilon_{265 \text{ nm}} = 8.4 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$ ). This band is probably a  $\pi, \pi^*$  absorption with some admixture of intramolecular charge-transfer character<sup>15</sup>. In the UV-spectrum of an aqueous solution of cefaclor at pH = 8.70 and at  $\mu = 0.01$ , the same band with a maximum at 264 nm appears.

The instability of cefaclor in alkaline medium could be explained mainly by intermolecular nucleophilic attack at the  $\beta$ -lactam ring. The rate constant of degradation of cefaclor at pH = 9.00 and  $t = 30^\circ \text{C}$  is  $k = 7.782 \times 10^2 / \text{h}^{-1}$ <sup>16</sup>. So, the recording of the UV-spectrum of cefaclor at pH = 8.70 was performed immediately after the preparing of the solution.

In the UV-spectrum of the reaction mixture of cefaclor and Ni(II) perchlorate with equimolar concentrations of  $3 \times 10^{-4} \text{ M}$ , at pH = 8.70 and at  $\mu = 0.01$  a broad absorption band in the range from 400 to 294 nm with a maximum at 316 nm appears. This confirms the formation of complex ions between Ni(II) and cefaclor. The Ni(II) perchlorate solution ( $3 \times 10^{-4} \text{ M}$ ) does not absorb in this range of wavelengths, under the same conditions. The cefaclor solution with the same concentration as in the reaction mixture was used as a blank. The "cut off" point for the blank solution appears at 294 nm and after that the absorbance could be measured.

J.M. Moratal et al.<sup>7</sup> established, by potentiometric methods, that cephalexin (HCEX) and Ni(II) form two moderately stable complexes, Ni(CEX)<sup>+</sup> and Ni(CEX)<sub>2</sub> in weak acidic medium. In addition, the 1:2 complex is easily hydrolyzed to give a species Ni(CEX)<sub>2</sub>(OH) that is predominant for pH > 7.00. Because of the similar structure of cefaclor and cephalexin it could be expected that in acidic medium cefaclor and Ni(II) form complex ions with similar composition.

The UV-spectra of the Ni(II) perchlorate solution (a) and the reaction mixture of cefaclor and Ni(II) perchlorate (b) at pH = 6.13 and at  $\mu = 0.01$  are shown in Fig. 2.

The absorbance at 394 nm, where Ni(II) perchlorate shows maximum absorption, decreases by adding cefaclor to produce the solution with equimolar concentrations of both components. This decrease of the absorbance proves the formation of complex ions between cefaclor and Ni(II) perchlorate in weak acidic medium. The experiment with higher concentration of cefaclor wasn't performed because of its limited solubility in water (10.0 mg/ml)<sup>15</sup>.

#### Optimum Conditions for Complex Formation

The influence of pH in forming the complex ions was followed by changing the UV spectrum of the reaction mixture between cefaclor and Ni(II) perchlorate in equimolar concentrations of  $3 \times 10^{-4}$  M (Fig. 3).

The increasing of intensity of the absorption band in the range from 450 to 294 nm, at different pH values, is followed by shifting of the maximum absorption to longer wavelengths up to the pH = 11.25 ( $\lambda_{\text{max}} = 358$  nm). The appearance of an

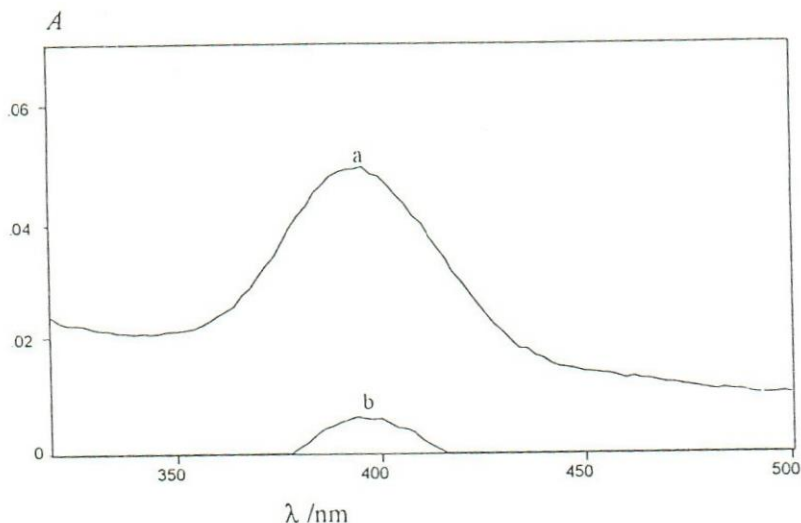


FIG. 2: UV spectra of Ni(II) perchlorate ( $5 \times 10^{-3}$  M) (a) and reaction mixture of cefactor and Ni(II) perchlorate with equimolar concentration ( $5 \times 10^{-3}$  M) (b), at pH = 6.13.

isobestic point at  $\lambda = 338$  nm points out the existence of two components in the solution which are in equilibrium. At pH < 9.50, two inflections on the UV spectra of the reaction mixture appear at  $\lambda = 374$  nm and 400 nm. The inflection at 374 nm is also observed in the reflectance spectrum of  $[\text{Ni}(\text{CEX})(\text{H}_2\text{O})_4]\text{BPh}_4$  complex by J.M. Moratal et al<sup>7</sup>. The spectrum of the aqueous solution of the 1:1 complex was identical to the reflectance spectrum of the solid.

In Fig. 4 and 5 are shown the plots of  $\lambda_{\text{max}}$  and absorbance of the complex ions versus pH values.



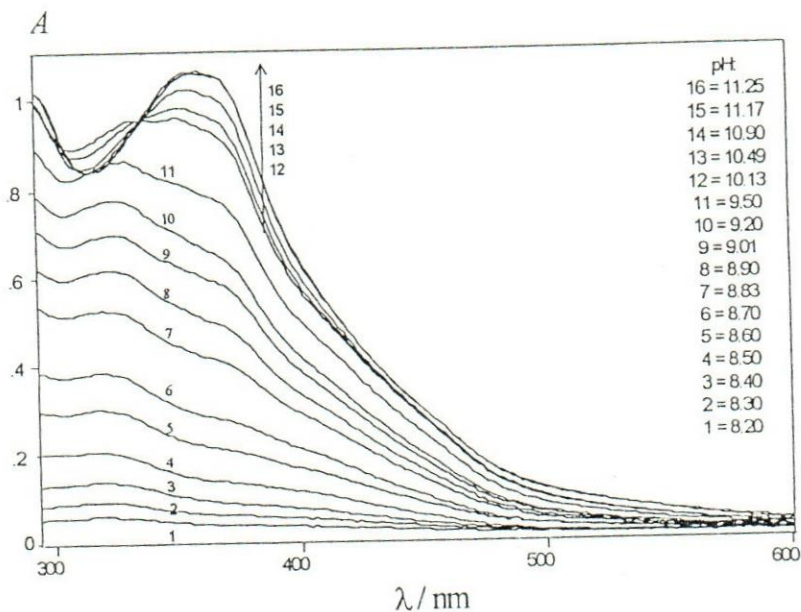
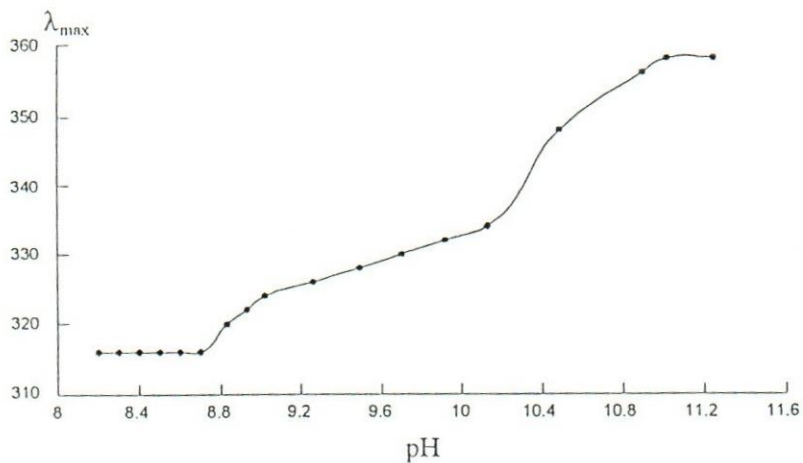
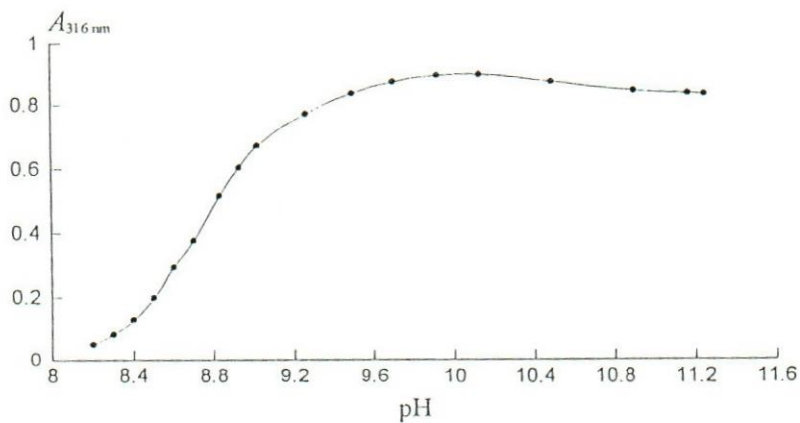


FIG. 3: UV spectra of the reaction mixture of cefaclor and Ni(II) perchlorate in molar ratio 1:1, at different pH values.

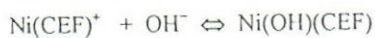
The appearance of a plateau in the pH range from 8.20 to 8.70 at 316 nm (Fig. 4) and increasing of the absorbance in the same pH range (Fig. 5) could be explained by increasing of the concentration of the hydroxo complex  $\text{Ni}(\text{OH})(\text{CEF})$ , which is in agreement with the literature data<sup>7</sup>. The pH = 8.70 was determined as useful because of the same maximum of the absorption band at 316 nm in the range from 8.20 to 8.70 and the maximum value of the absorbance in this range at pH = 8.70 as well as the absence of a plateau on the curve  $A_{316 \text{ nm}} = f(\text{pH})$ . Shifting of  $\lambda_{\text{max}}$  to longer wavelengths at pH > 8.70 and increasing of the absorbance in the range from

FIG. 4: Plots of  $\lambda_{\max}$  of the complex ions *versus* pH.FIG. 5: Plots of absorbance of the complex ions *versus* pH, at 316 nm.



8.70 to 11.25, shows that secondary reaction in the system has occurred (formation of soluble complex ions with degradation products of cefaclor). The appearance of an isobestic point at 338 nm (Fig. 4) confirms this secondary reaction. The color of the reaction solution is also changed from yellow to orange at  $\text{pH} > 10.50$ .

$\text{Ni}(\text{OH})_2$  will start to precipitate in aqueous solution at  $\text{pH} = 8.60$  and  $[\text{Ni}^{2+}] = 3 \times 10^{-4} \text{ M}$  without the presence of cefaclor and according to the data of Nikolbski<sup>17</sup> that  $K_{\text{sp}}^\ominus$  of  $\text{Ni}(\text{OH})_2$  is  $1.6 \times 10^{-14}$ . This did not occur in our case. This fact confirms the existence of soluble complex ions between Ni(II) and cefaclor. The reactions of formation of the complex species, according to the pH dependence, could be proposed by the following equilibria:



The range of maximum absorption of the reaction mixture Ni(II) : cefaclor = 1:1 and particularly the inflection on the absorption band at 374 nm, which also appears in the spectrum of the 1:1 complex between cephalixin and Ni(II) in the solid phase and in the solution, shows that coordination of Ni(II) with cefaclor occurs in a way similar to cephalixin<sup>1,7</sup>, e.g. it takes place through the carbonyl and amino group in the side chain.

The dependence of the absorbance of the hydroxo complex  $\text{Ni}(\text{OH})(\text{CEF})$  on the ionic strength of the solution was examined in the range  $\mu = 0.01 - 0.1$ , at  $\text{pH} = 8.70$ . A linear increase of the complex absorbance by decreasing the ionic strength was determined.

The stability of the hydroxo complex *versus* time was investigated at  $\text{pH} = 8.70$  and at  $\mu = 0.01$ . The hydroxo complex reaches maximum absorption after 20 minutes of preparing the reaction solution and remains stable for one hour at  $23^\circ\text{C}$ .

In the range from 23 °C to 45 °C a linear dependence between the absorbance of the hydroxo complex and the temperature was determined. The change of the absorbance for each degree is  $1.18 \times 10^{-3}$  and  $1.54 \times 10^{-3}$  for solutions which contain equimolar concentrations of Ni(II) and cefaclor  $3 \times 10^{-4}$  M and  $5 \times 10^{-4}$  M, respectively. The obtained results point out the small influence of the temperature in moving of the equilibrium in the direction of formation of the hydroxo complex.

### Stoichiometric Ratio of the Complexes

The Job method<sup>18</sup> was used for determining the stoichiometric ratio between cefaclor and Ni(II) perchlorate reaction. The solutions were prepared by mixing both components with equimolar concentration ( $6 \times 10^{-3}$  M) in ratios from 1:9 to 9:1. Absorbances were measured at 316 nm, pH = 8.70 and at  $\mu = 0.01$  using cefaclor solution of the corresponding concentration as the blank (Fig. 6).

The maximum of Job's curve appears at a molar fraction of  $n = 0.5$  and shows the extent of the reaction Ni(II) : cefaclor = 1:1 or 2:2 or 3:3, etc.

The method of relative recovery of complex species<sup>18</sup> was used for elimination of the reaction in the ratio 2:2 or 3:3 etc. The UV spectra of the reaction mixture with concentration of Ni (II) perchlorate ( $5 \times 10^{-4}$  M) and variable concentrations of cefaclor from  $5 \times 10^{-5}$  M to  $6 \times 10^{-4}$  M, at pH = 8.70 and  $\mu = 0.01$  were used for this method. Fig. 7 shows the dependence  $A / c_{(\text{HCF})} = f(A / A_s)$ , where  $A$  is absorbance of hydroxo complex,  $c_{(\text{HCF})}$  initial concentration of cefaclor and  $A_s$  is the mean value of absorbances of hydroxo complex on the curve's plateau (saturated). The plots on Fig. 7 are based on the data from Table 1.

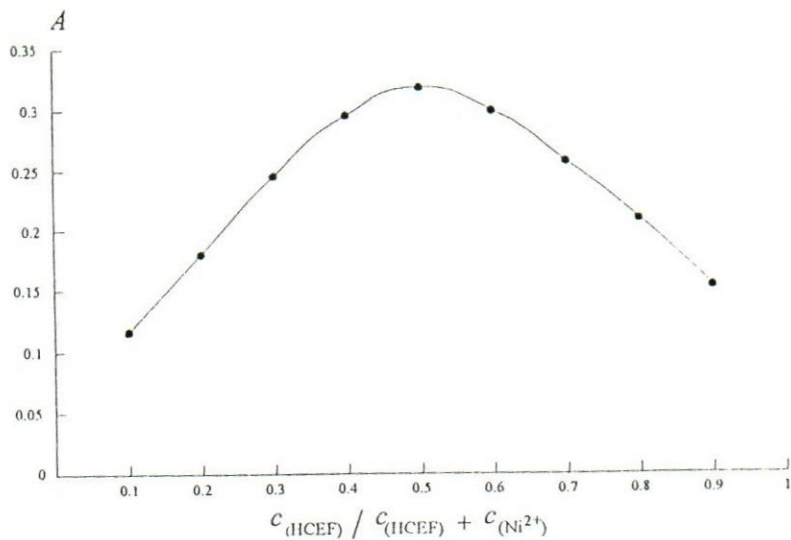


FIG. 6: Job's curve obtained at 316 nm and pH = 8.70.

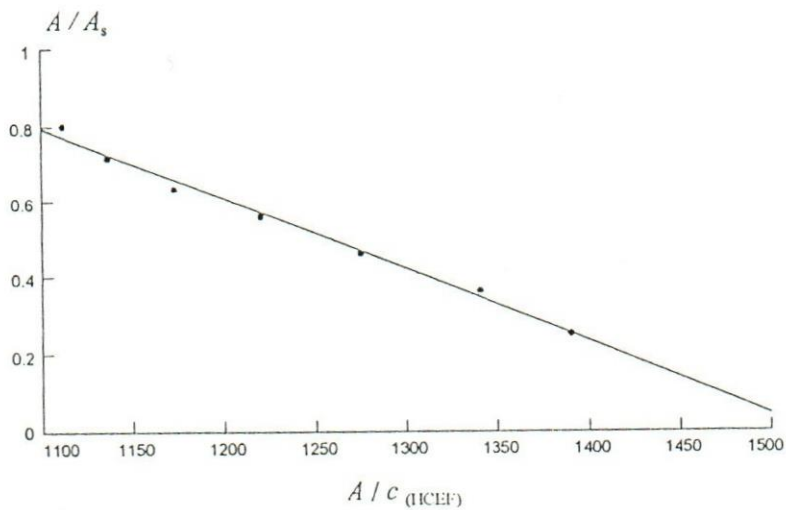
FIG. 7: Plots of  $A / c_{(\text{HCEf})}$  versus  $f (A / A_s)$ , at constant concentration of cefaclor.

TABLE I

Data for construction the curve of relative recovery of complex species

$c_{(\text{HCEF})}$	$A$	$A / c_{(\text{HCEF})}$	$A / A_s$
$1 \times 10^{-4}$	0.139	$1.390 \times 10^3$	0.2504
$1.5 \times 10^{-4}$	0.201	$1.340 \times 10^3$	0.3621
$2 \times 10^{-4}$	0.255	$1.275 \times 10^3$	0.4594
$2.5 \times 10^{-4}$	0.305	$1.220 \times 10^3$	0.5587
$3 \times 10^{-4}$	0.352	$1.173 \times 10^3$	0.6342
$3.5 \times 10^{-4}$	0.398	$1.137 \times 10^3$	0.7171
$4 \times 10^{-4}$	0.445	$1.112 \times 10^3$	0.8012

The equation  $A = 2.851 - 1.871 \times 10^{-3} c$  was obtained by regression analysis, where the correlation coefficient is  $r = 0.9957$ .

The absence of a maximum on the above presented dependence  $A / c_{(\text{HCEF})} = f(A / A_s)$  shows that the stoichiometric coefficient of the component with variable concentration (cefactor) is equal to 1, which confirms the formation of complex species  $\text{Ni}_2(\text{HCEF})$  or  $\text{Ni}(\text{HCEF})$ . The formation of complex  $\text{Ni}_2(\text{HCEF})$  is probably excluded because there is no deviation of linear dependence. These results indicate that the composition of the already formed complex is  $\text{Ni}(\text{HCEF})$  and not  $\text{Ni}_2(\text{HCEF})_2$  or  $\text{Ni}_3(\text{HCEF})_3$  etc, as Job's curve indicates.

The molar ratios method<sup>18</sup> was also used for determining the stoichiometric ratio of the reaction. For this method the same UV spectra as in the previous method were used (Fig. 8).

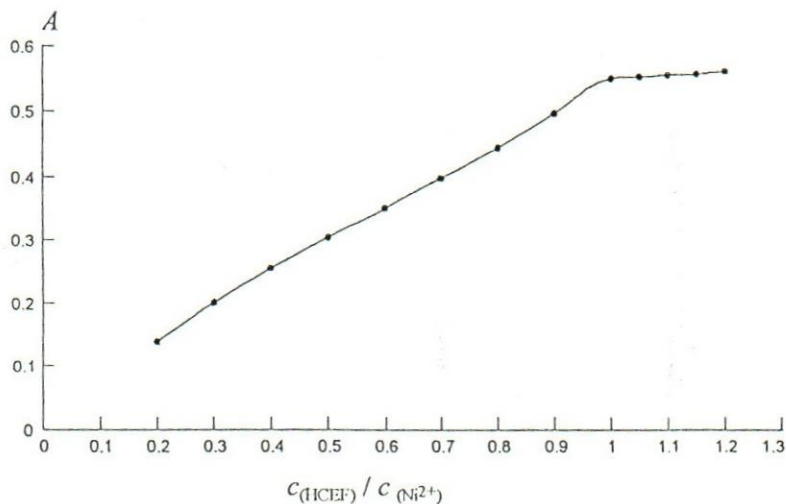


FIG. 8: Plots of absorbance of the complex ions versus the molar ratio of the components ( $5 \times 10^{-4}$  M  $Ni(ClO_4)_2$  and  $5 \times 10^{-5}$  M to  $6 \times 10^{-4}$  M cefaclor)

The intersection of straight lines obtained at molar ratio  $n = 1$ , confirms the fact about the reaction with stoichiometry  $Ni(II) : \text{cefaclor} = 1:1$ .

#### The determination of molar absorptivity and stability constant of hydroxo complex

The molar absorptivity value and the consecutive concentration constant of the reaction of the formation of the hydroxo complex were estimated using the method described by M.C. Cattopadhyaya and R.S. Singh<sup>19</sup>. This method describes determination of the stability constant as well as the molar absorptivity of complexes of the type  $ML_n$  ( $n = 1, 2, 3, \dots$ ) from spectrophotometric data, using the

two solutions with different initial concentrations of metal ions and ligand. The solutions were prepared by mixing Ni(II) perchlorate and cefaclor in the molar ratio 1:1. Equimolar concentration of both components was  $3 \times 10^{-4}$  M in one solution and  $5 \times 10^{-4}$  M in the other solution.

The calculated value of the molar absorptivity is  $\varepsilon = 1.12 \pm 0.02 \times 10^3$  l mol<sup>-1</sup> cm<sup>-1</sup> at 316 nm and the values of concentration constant of the hydroxo complex are  $\log K_2 = 8.3 \pm 0.1$  l / mol and  $\log K_2 = 8.02 \pm 0.1$  l / mol ( $n = 10$ ) for the solutions with equimolar concentrations of cefaclor and Ni(II) perchlorate  $3 \times 10^{-4}$  M and  $5 \times 10^{-4}$  M, respectively.

### CONCLUSION

The interaction between Ni(II) ion and cefaclor in weak acidic medium results in the formation of Ni(CEF)<sup>+</sup> complex which easily hydrolyzes giving a hydroxo complex Ni(OH)(CEF). It was found by methods of ultraviolet spectroscopy that the stoichiometric ratio between cefaclor and Ni(II) is 1:1. The molar absorptivity  $\varepsilon = 1.12 \pm 0.02$  l mol<sup>-1</sup> cm<sup>-1</sup> at 316 nm and the concentration constant of the hydroxo complex  $\log K_2 = 8.3 \pm 0.1$  l / mol, calculated at pH 8.70 and at  $\mu = 0.01$ , were obtained by spectrophotometric data.

Our studies are in progress to investigate the possibility of using Ni(II) perchlorate as a reagent for determination of cefaclor in pure form and in pharmaceutical preparations by UV spectroscopy.

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