Coordinating Properties of the Cephalexine Antibiotic. A Potentiometric Study of the Complexes Formation between Cephalexine and Co(II), Ni(II) and Cu(II) Metal Ions

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(Received March 8, 1989)

Abstract

The formation of complexes between Co(II), Ni(II) and Cu(II) with Cephalexine has been investigated using potentiometric techniques. The stability constants of the complexes formed were calculated using the non-linear least-squares computer program SUPERQUAD. The obtained values were: Co(II) $\log \beta_1 = 2.40$, $\log \beta_{MLOH} = 8.89$; Ni(II) $\log \beta_1 = 2.80$, $\log \beta_2 = 5.10$, $\log \beta_{ML2OH} = 12.09$; Cu(II) $\log \beta_1 = 4.094$ (25 °C, 0.1 M KNO_3).

The compound $[Ni(CEX)(OH_2)_4]BPh_4$ has been synthesized and characterized by electronic, IR and NMR spectroscopies as well as by magnetic measurements. From these studies it is proposed that the Cephalexinate anion acts as a bidentate ligand and is bound to the metal ion through the carbonyl and the amino-NH_2 groups of the side chain.

Introduction

Much interest has been shown in the chemistry of β-lactam antibiotics in relation to their useful biological activities in recent years [1–4]. β-Lactam antibiotics, such as penicillins, cephalosporins and oxacephalosporins, represent the most important class of drugs against infections diseases caused by bacteria. The biologically active principle of these antibiotics is the β-lactam ring, the reactivity and selectivity of which towards biological substrates can be decisively modified by substituents. These antibiotics show bacteriocidal effects because they disrupt bacterial cell wall biosynthesis by acylating and thereby inactivating transpeptidases and carboxypeptidases [5] that are essential for the synthesis of the stabilizing framework of the bacterial cell wall, the peptidoglycan. Because the antibacterial activity of an antibiotic depends on the acylation of those enzymes by the β-lactam ring of the anti-

biotic, the chemical reactivity of this group is an important factor affecting antibacterial activity. A number of parameters has been proposed as indicators of the β-lactam reactivity, i.e. the IR carbonyl stretching frequency [6–8].

Most of the new antibiotics are cephalosporins and their drugs are now the most prescribed of all antimicrobials. Cephalexine (HCEX) (Fig. 1), an orally active cephalosporin in clinical practice, belongs to the first generation with low β-lactamase stability. The first generation cephalosporins have good gram-positive activity and relatively modest gram-negative activity. There are several indications for which first-generation cephalosporins have been shown to be safe and effective prophylasis.

It is well known that the antibiotics interact with the metal ions and this interaction is of a complex nature. Transition metal ions cause a great increase in the rate of aminolysis and hydrolysis of penicillin and cephalosphorins. Page *et al.* [2, 9, 10] studied the aminolysis and hydrolysis of benzylpenicillin in the presence of several metal ions as Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}. Though all these metal ions produce an enhancement of the hydrolysis rate, the effect of Cu^{2+} was more pronounced. From kinetic and NMR studies [2, 11] it is indicated that copper(II) ion coordinates to the carboxylate group and the β-lactam nitrogen of benzylpenicillin.

Fazakerley and Jackson [11, 12] established that there are several possible sites of coordination for the metal ion on penicillin. The two most likely
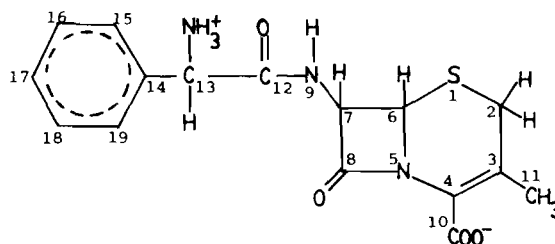
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Fig. 1. Cephalexine (HCEX).

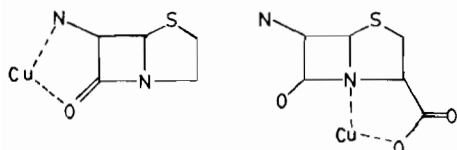Fig. 2. Coordination modes of β-lactamic antibiotics.
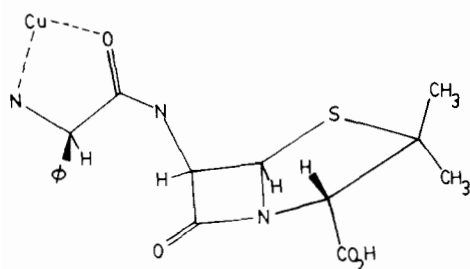

Fig. 3. Coordination mode of CEX⁻ to Cu²⁺.

coordination sites are shown in Fig. 2. From ^{1}H NMR studies, Fazakerley and Jackson [11] proposed that the interaction between Cu^{2+} and Cephalexine takes place through the side chain in a similar form to the ampicillin Cu(II) complex (Fig. 3).

Studies about the interaction between cephalosporins and metal ions are very scarce. Hernández *et al.* [13] carried out a qualitative study on the reactivity of HCEX with the most usual metallic cations, and recently the interaction of HCEX and the metal ions Zn^{2+} and Cd^{2+} has been investigated [14].

We report here a quantitative study of the protonation–deprotonation equilibria of HCEX as well as the formation equilibria of the complexes with Co^{2+}, Ni^{2+} and Cu^{2+} ions.

Experimental

Reagents
HCEX was supplied in powder form. It was purified by recrystallization. Co(II), Ni(II) and Cu(II) solutions, prepared from $Co(NO_3)_2 \cdot 6H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$ and $Cu(NO_3)_3 \cdot 3H_2O$ respectively (Merck reagents), were standardized by atomic absorption spectroscopy. Carbonate-free potassium hydroxide (0.1 M) and nitric acid (0.1 M) solutions were used in the potentiometric study. The 99.7% D_2O and 40% NaOD in D_2O were obtained from the Fluka Chemical Co. All the other reagents were analytical grade chemicals.

Physical Techniques
Potential measurements were performed with a Radiometer 84 pH-meter using a GK2401C combined glass electrode. Dry O_2-free N_2 saturated with water vapour was bubbled through the solution in the

vessel reaction. The titrant was delivered by a Crison burette-738. The titration system was controlled by an Apple IIe microcomputer equipped with a 9-in video display and one minifloppy disk driver. A basic program [15] was used to monitor, for each titration point, the e.m.f. values and the volume of titrant added. When the observed e.m.f. was constant, within user-defined limits, the next volume of titrant was added automatically and the cycle repeated until the predefined total volume of titrant had been added.

Measurements were made at 25.0 ± 0.1 °C and 0.1 M KNO_3. The concentration of hydrogen ion was calculated from the experimental e.m.f. values, E (expressed in mV), by means of the expression

$$[H^+] = \exp(E - E^{o\prime})/25.692$$

The experimental e.m.f. values have not been corrected for the liquid junction potential or for the potential drift of the electrode during the measurements. Preliminary acid–base titrations which were carried out under the same experimental conditions showed that the corrections due to the above effects were negligible over the pH range investigated. The electrode was always calibrated as a hydrogen ion concentration probe, but, as a matter of convenience, pH instead of $-\log[H^+]$ is used throughout the text. Accurate concentration values of the basic titrant, as well as the $E^{o\prime}$ value, were determined with an aqueous nitric acid solution, which was previously standardized, before each titration series. The ionic product of water was determined under the same experimental conditions. The value found ($pK_w = 13.746 \pm 0.002$) is in good agreement with those previously reported [16].

The SUPERQUAD [17] non-linear least-squares computer program was used to calculate acidity and stability constants. The thermal stability of the isolated complex was studied with a Setaram Balance recording simultaneously the TG, DTG and ATD curves. The diffuse reflectance spectrum was recorded with a UV–Vis–NIR Perkin-Elmer Lambda 9 spectrophotometer using magnesium oxide as the reference. IR spectra were registered with a Pye Unicam Spectrophotometer Model 5P 200 on a KBr disk.

Magnetic susceptibility measurements were carried out with a Gouy balance at room temperature. They were corrected for the diamagnetism of the constituent atoms by the use of Pascal's constants. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-200 MHz spectrometer operating in the pulsed Fourier Transform mode and the chemical shifts were measured relative to TMS.

Synthesis of [Ni(CEX)(H₂O)₄]BPh₄
A total of 0.42 g of $Ni(NO_3)_2 \cdot 6H_2O$ was added to a solution of 0.5 g of Cephalexine in 50 ml of

water. Then 3 ml of sodium tetraphenylborate (0.48 M) were added, and a very light green precipitate was formed. The obtained compound was filtered, washed first with water and then with ethanol. Finally, it was dried in an air stream and stored over $CaCl_2$.

Anal. Found: Ni, 7.2; C, 59.23; H, 5.45; N, 5.2. Calc.: Ni, 7.38; C, 60.3; H, 5.53; N, 5.28%.

Results

Acidity Constants of H_2CEX^+

Aqueous solutions containing Cephalexine (4.8 × 10^{-3} M) and nitric acid (5 × 10^{-3} M) were titrated with a standardized KOH solution (~0.09 M). The protonation curve, $\bar{j}$ versus pH, was obtained from the potentiometric data (see Fig. 4). This curve is
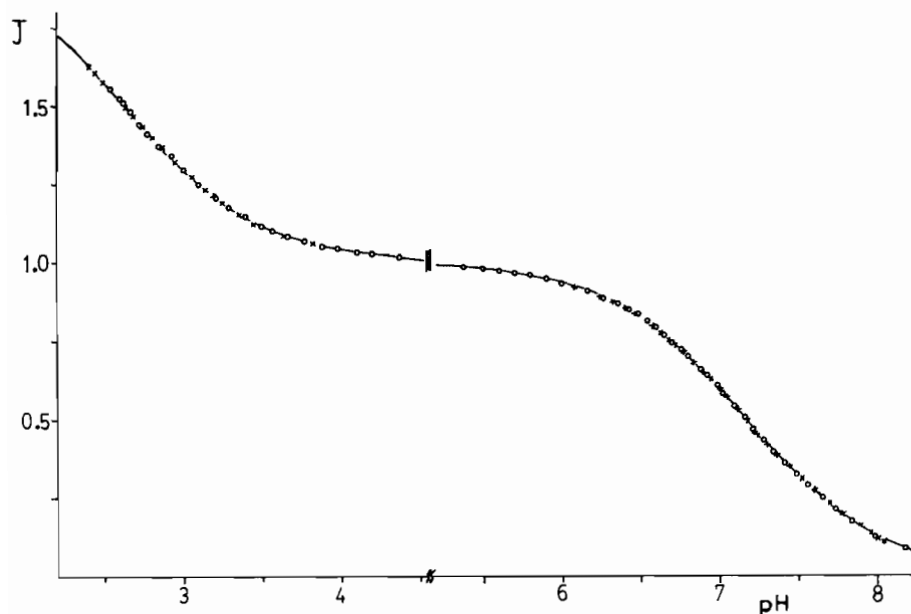

Fig. 4. Protonation curve of Cephalexinate anion (CEX^-). The symbols represent the experimental points. Only a fraction of them is plotted. The solid line is the theoretical curve calculated with the computed constants.
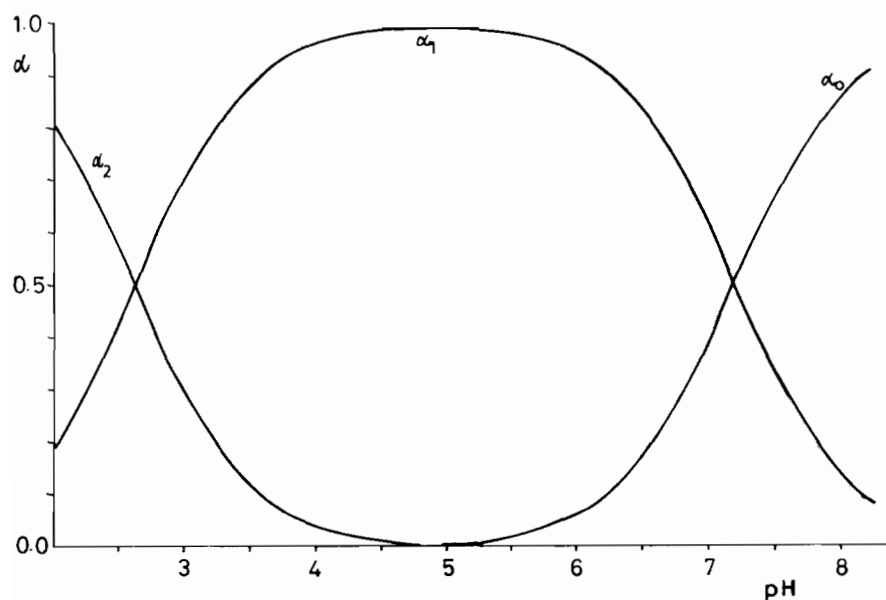

Fig. 5. Distribution diagram α(pH) for the existing species in a solution of HCEX. α_0 = [CEX^-] : (C_M), α_1 = [HCEX] : (C_M), α_2 = [H_2CEX^+] : (C_M).

composed of two distinct sigmoid steps, separated by a long plateau at $\bar{j} = 1$ indicating that the values of pK_{a2} and pK_{a1} are separated by more than four units and the two protonation steps could be treated independently. The deprotonation process of HCEX could not be followed for pH > 8.3 because experimental data were not reproducible. This fact agrees with the observed instability of HCEX in basic medium [18].

The proton association constants, β_j, were determined using the SUPERQUAD [17] computer program. The values obtained were: $\log \beta_{j1} = 7.185 \pm 0.001$, $\log \beta_{j2} = 9.808 \pm 0.002$ (0.1 M KNO$_3$, 25.0 ± 0.1 °C). In the calculations we used 155 experimental points from two different experiments. The theoretical protonation curve calculated from these constants fits the experimental data very well (see Fig. 4). The distribution diagram (α_j, $-\log[H^+]$) for the species in solution is plotted in Fig. 5.

In acid medium HCEX is protonated to give a cationic species H_2CEX^+. This species behaves as a diprotonic acid. Addition of base produces, in a first stage, the deprotonation of the carboxylic group ($pK_{a1} = 2.623$) yielding the zwitterion species HCEX that, a practical consideration, is the only one species existent in the pH range of 4–6 ($\alpha >$ 95%). In a second stage, when pH is increased, the deprotonation of the amino group, $-NH^+_3$, takes place giving an anionic species CEX^- which is predominant at pH > 7.5.

^{1}H NMR Spectrum of Cephalexine

The ^{1}H NMR data for a solution of HCEX in D$_2$O (pD ~ 6.2) are summarized in Table 1. It is easy to assign signals to most protons by interpretation of the integration values, splitting patterns and selective proton irradiation.

Complexes Formation Study

Aqueous solutions containing HCEX (2×10^{-2} M) and Co^{2+} (2×10^{-3} M) were titrated with 0.165 M potassium hydroxide. At pH > 7.3 the potential

measurements were unstable, because of the hydrolytic decomposition of the Cephalexine. From the pH titration curves, the following equilibria may be proposed

$$Co^{2+}aq + CEX^- \rightleftharpoons Co(CEX)^+$$

$$Co(CEX)^+ + OH^- \rightleftharpoons Co(CEX)(OH)$$
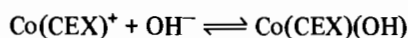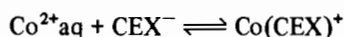
Stability constant calculations were performed by electronic computation using the program SUPERQUAD [17]. The β_n values obtained were: $\log \beta_1 = 2.40 \pm 0.01$, $\log \beta_{MLOH} = 8.89 \pm 0.01$ (25.0 ± 0.1 °C, 0.1 M KNO$_3$). In these calculations 167 experimental points from three independent titrations were used. The distribution diagram for the species in solution is plotted in Fig. 6. In aqueous solution, Co^{2+} interacts with CEX$^-$ to give the Co(CEX)$^+$ complex and this species is a weak complex easily hydrolyzed to give Co(CEX)(OH). Both species coexist in equilibrium in the pH range of 6.5–7.5. The hydroxo species is predominant at pH > 7.3, but irreversible degradation of Cephalexine occurs at this pH.
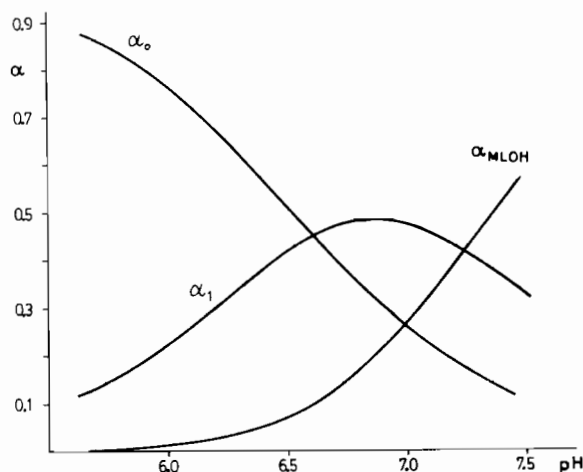

Fig. 6. Distribution diagram α(pH) for Co^{2+}–CEX$^-$ system: $\alpha_1 = [Co(CEX)]^+$: (C_M), $\alpha_{MLOH} = [Co(CEX)(OH)]$: (C_M).

TABLE 1. Chemical shifts of ^{1}H NMR resonances of HCEX in D$_2$O (pD ~ 6.2)

Shifts (ppm)	No. of protons	Multiplicity	J(cps)	Assignment	Chemical shifts	
					δ^b (ppm)	δ^c (ppm)
1.60	3	s		H-11	1.88(s)	2.06(s)
2.74; 3.23	2	ABquartet	17.9	H-2	3.19(AB)	3.30(AB)
4.79	1	d	4.5	H-6	4.93(d)	5.0(d)
4.96	1	s		H-13	4.59(s)	5.32(s)
5.40	1	d	4.5	H-7	5.59(d)	5.68(d)
7.28	5	s		H-15/H-19	7.42(s)	7.61(s)
8.35[a]	2	s		$-NH_2$ (side chain)		
8.85[a]	1	s		$-NH$ (side chain)		

[a]In DMSO-d$_6$ as disolvent.   [b]From ref. 19.   [c]From ref. 20.
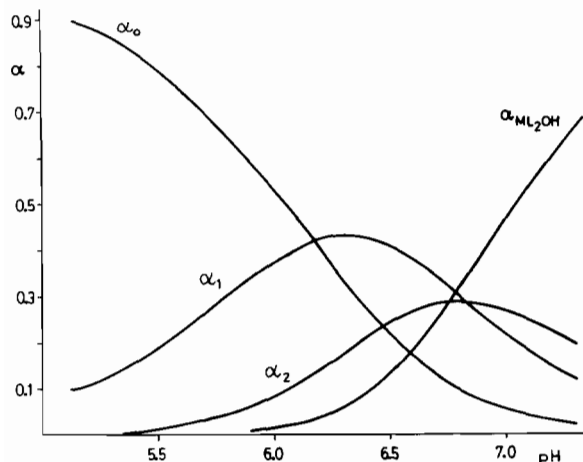
Fig. 7. Distribution diagram α(pH) for Ni^{2+}–CEX^- system: $\alpha_1 = [Ni(CEX)]^+$: (C_M), $\alpha_2 = Ni(CEX)_2$: (C_M), $\alpha_{ML2OH} = [Ni(CEX)_2(OH)]^-$: (C_M).

In a similar way, an aqueous solution of Ni^{2+} and HCEX was titrated with 0.166 M KOH. The metal ion concentration was varied between $(1-2) \times 10^{-3}$ M and the metal:ligand ratio was 1:10. The potential measurements were unstable at pH > 7.5 for the same reasons as those given above.

The calculations were performed with 115 experimental points from three independent titrations, and the β_n values obtained were: $\log \beta_1 = 2.802 \pm 0.008$, $\log \beta_2 = 5.10 \pm 0.02$ and $\log \beta_{ML2OH} = 12.09 \pm 0.01$. The distribution diagram for the species in solution is shown in Fig. 7. Ni^{2+} interacts with CEX^- to give two moderately stable complexes, $Ni(CEX)^+$ and $Ni(CEX)_2$. In addition, the 1:2 complex is easy hydrolyzed to give a species $Ni(CEX)_2$-$(OH)^-$ that is predominant for pH > 7.0.

$[Ni(CEX)(H_2O)_4]BPh_4$ is a paramagnetic, light-green solid with $\mu_{eff} = 2.95$ BM. The study of thermal stability by TG and ATD shows that, in N_2 atmosphere, an endothermic process, corresponding to the dehydration, starts at ~ 70 °C and a exothermic decomposition takes place at $110-120$ °C. The reflectance spectrum of the complex shows two absorption bands at 9200 and 15 380 cm^{-1} and another one at $\sim 26\,600$ cm^{-1} that appeared as a shoulder because of the existence of an intense UV band. The visible spectrum of an aqueous solution of the 1:1 complex is identical to the reflectance spectrum of the solid. The position and relative intensity of these bands fit well with the expected ones for the d–d transitions in a Ni(II) octahedral complex. Analysis of the spectrum allows the following assignments: ${}^3T_{2g} \leftarrow {}^3A_{2g}$ 9175, ${}^3T_{1g}(F) \leftarrow {}^3A_{2g}$ 15 380 and ${}^3T_{1g}(P) \leftarrow {}^3A_{2g}$ 26 670 cm^{-1}, with a Δ_0 value of 9200 cm^{-1}.

The ${}^1H$ NMR spectrum of the 1:1 complex in DMSO-d_6 solution is shown in Fig. 8. The signal at 8.35 ppm, attributed to the amino group of

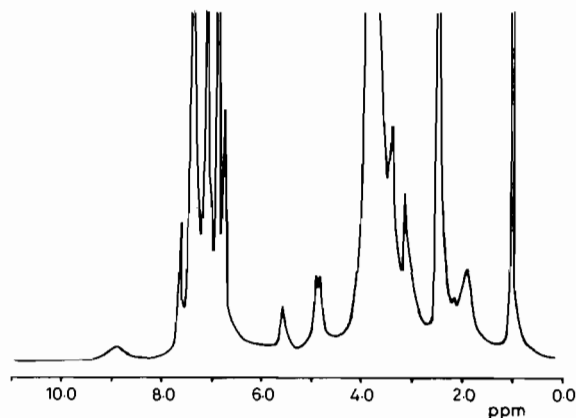Fig. 8. ${}^1H$ NMR spectrum of the complex $[Ni(CEX)(OH_2)_4]$-BPh_4 in DMSO-d_6 solution.

HCEX, does not appear in the ${}^1H$ NMR of the complex. This is consistent with a direct interaction of the amino group with the paramagnetic Ni^{2+} ion.

When IR spectra of the complex and HCEX are compared, the most remarkable differences are the following.

The HCEX IR spectrum shows a broad band at 2600 cm^{-1} attributed to NH_3^+ stretching vibrations [21, 22]. This band does not appear in the complex.

At 3050–2950 cm^{-1} a characteristic band is observed corresponding to ν(=C–H) vibrations of the tetraphenylborate counter-ion. An intense band appears at 1760 cm^{-1} attributed to C=O stretching vibrations of the β-lactam ring. This band is not shifted with respect to the ligand, indicating that the β-lactamic ring is not modified in the complex, and that this group does not interact directly with the metal ion.

The band assigned to the ligand at C=O amide stretching vibrations is shifted in the complex from 1690 to 1600 cm^{-1}, indicating an interaction with the Ni^{2+} ion. The broad band at 1600 cm^{-1} includes the stretching vibration of C=O of the carboxylate group. This band appears in the same range in the ligand [6]. This indicates that the carboxylate group has no direct participation in the bonding linkage with the metal ion.

On the other hand, aqueous solutions containing Cu^{2+} $(2 \times 10^{-3}$ M), HCEX $(10^{-2}$ M) and a known excess of nitric acid, were potentiometrically titrated with KOH (0.126 M). At pH ~ 5 potential measurements were not reliable because a precipitation process occurs. In neutral medium, pH ~ 7, the latter dissolves to give a dark brown solution, but potential measurements were not stable either. The treatment of the potentiometric data for pH < 4.5 indicates the formation of only a single 1:1 complex, $Cu(CEX)^+$, with a stability constant $\log \beta = 4.094 \pm$

0.008. Though the treatment of the potentiometric values for the range of pH = 4.5—5 suggests the existence of a species Cu(CEX)(OH), this species would be formed to such a low extent ($\alpha < 7\%$) that determination of its stability is not possible.

Discussion

Cephalexine, HCEX, behaves simultaneously as an acid and a base. When dissolved in acid medium a cationic species H_2CEX^+ is formed that acts as a diprotic acid according to the following equilibria

$$H_2CEX^+ \rightleftharpoons HCEX + H^+$$

$$HCEX \rightleftharpoons CEX^- + H^+$$
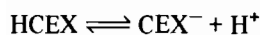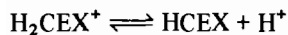
The carboxyl group is the acid centre with pK_{a1}, and the pK_{a2} is assigned to the primary amine nitrogen on the side chain.

The ^1H and ^{13}C NMR spectra of HCEX obtained by us are similar to those published [19, 20, 23, 24]. The small differences observed in the shifts of groups implicated in the acid—base equilibria are due to the different pH conditions of sample preparations. Comparison of the chemical shift values given in Table 1 shows that there is a deshielding of the H-13 of 0.31 ppm upon protonation of the NH_2-group of the side chain. In fact, in our conditions the NH_2-group is essentially protonated and the inductive effect of nitrogen will be more important on H-13. In a similar way, the only remarkable difference in the ^{13}C NMR spectrum obtained by us, at pH $\sim$ 5.5, is due to the carboxylate (C-10) shifted 4.8 ppm with respect to the obtained spectrum at pH < 1 [23].

The stability constants of the complexes formed between Co^{2+}, Ni^{2+} and Cu^{2+} with Cephalexine are shown in Table 2. The three metal ions form 1:1 complexes with CEX^-, and their stability constants β_1 follow the Irving—Williams order $Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^2$. In addition Ni^{2+} forms a 1:2 complex. Cobalt and nickel form rather weak complexes easily hydrolyzed even in neutral medium to give hydroxy species. In the presence of these metal ions the hydrolytic decomposition of HCEX is more easy and the effect is more pronounced for Cu^{2+}.

TABLE 2. Stability constants of Cephalexinate complexes (25 °C, 0.1 M KNO_3)

Complex	M = cobalt	M = nickel	M = copper	M = zinc[a]
ML^+	2.40	2.802	4.094	2.39
ML(OH)	8.89			9.19
ML_2		5.10		
$ML_2(OH)^-$		12.09		

[a]From ref. 14.

Fazakerley and Jackson [11] have studied, by ^1H NMR, the coordination mode of some antibiotics in their Cu^{2+} complexes. From these studies it is indicated that complexation of Cephalexine, at pH 8, occurs at the side chain (Fig. 3). At pH 5.5 it is suggested that the ligand is the zwitterion species, which is coordinated to the metal ion via the carboxyl group and the ring nitrogen, because at this pH the amino group of the side chain is essentially protonated. As indicated by these authors, the ^1H NMR study at pH 5.5 is difficult because of the low solubility of HCEX. In fact, at this pH a precipitate is formed as indicated above (see 'Results'). However, our potentiometric study reveals that the product formed at pH 5.5 is probably a Cu^{2+} hydroxocomplex rather less soluble than HCEX. On the other hand, if the zwitterion species, HCEX, is supposed as the ligand, it is not possible to obtain a fitting of the experimental data.

The reflectance spectrum and the μ_{eff} value for the complex $[Ni(CEX)(OH_2)_4]BPh_4$ fit well with the expected ones for an octahedral geometry. The Cephalexinate anion, CEX^-, acts as a bidentate ligand and is bound to the metal ion through the carbonyl and the amino NH_2 groups of the side chain (see Fig. 3) according to its IR spectrum. The obedience of the Irving—Williams series as indicated above, suggest that the 1:1 complexes of Co(II) and Cu(II) may be octahedral in analogy to the Ni(II) complex.

Acknowledgements

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