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Metal Complexes of Cephradine: Synthesis and Equilibrium Studies

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Summary. The complex formation equilibria of the binary and ternary systems *M*(II)-Cephradine and *M*(II)-2,2-Bipyridyl-Cephradine were investigated by a potentiometric technique at 25 °C and an ionic strength of 0.1 *M* $NaNO_3$ (*M* = Cu, Ni, Co, and Zn). The effect of dioxane as a solvent on the protonation constants of cephradine and the formation constants of the Cu-cephradine complex was studied. The relative stability of each ternary complex was compared with that of the corresponding binary complex in terms of $\Delta \log K$. The mode of ternary complex formation was ascertained by conductivity measurements. The copper(II), nickel(II), and cobalt(II) complexes of cephradine were synthesized and characterized by elemental analysis, conductivity measurements, and IR spectra.

Keywords. Cephradine complexes; Equilibrium studies; Synthesis of cephradine complexes.

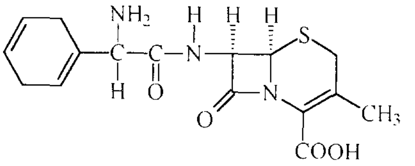
Metallkomplexe von Cephradin: Synthese und Gleichgewichtsstudien

Zusammenfassung. Die Komplexbildungsgleichgewichte der binären und ternären Systeme *M*(II)-Cephradin und *M*(II)-2,2-Bipyridyl-Cephradin (*M* = Cu, Ni, Co und Zn) wurden bei 25 °C und einer Ionenstärke von 0.1 *M* $NaNO_3$ untersucht. Der Einfluß von Dioxan als Lösungsmittel auf die Protonierungskonstanten von Cephradin und die Komplexbildungskonstanten des Cu-Cephradin-Komplexes wurde ermittelt. Die relative Stabilität der ternären gegenüber den entsprechenden binären Komplexen wurde über $\Delta \log K$ verglichen. Die Bildungsweise der ternären Komplexe wurde mittels Leitfähigkeitsmessungen abgesichert. Die Cu(II)-, Ni(II)- und Co(II)-Komplexe von Cephradin wurden synthetisiert und durch Elementaranalyse, Leitfähigkeitsmessungen und Infrarotspektroskopie charakterisiert.

Introduction

Most pharmaceuticals contain electron donor groups likely to bind metal ions occuring naturally [1]. Among these, cephalosporin antibiotics have long been known to behave as relatively efficient chelating agents [2]. Cephradine is one of these antibiotics. They are the most important class of drugs against infectious diseases caused by bacteria [3, 4]. Essential trace metal ions like zinc and copper are present in too low concentration in blood plasma to significantly influence the bioavailability of these drugs [5, 6]. However, the belief that antibiotic action is related to the ability of these compounds to form complexes with metal ions has stimulated investigations of the complexing properties of antibiotics as ligands [7, 8]. Investigation on the binary and ternary complexes of cephradine may therefore help toward understanding the driving forces leading to the formation of such complexes in biological systems.

It is now well established that the "effective" or "equivalent solution" dielectric constants in proteins [9, 10] or active site cavities of enzymes [11] are low compared to that in bulk water. Estimates for the dielectric constants in such locations range from ≈ 30 to 70 [9–11]. Hence, by employing aqueous solutions that contain ≈ 10–50% 1,4-dioxane [12], one may except to simulate to some degree the situation in active site cavities [13] and extrapolate the data to physiological conditions. Cephradine complexes with transition metal ions have not been investigated up to now. According to our studies on binary and ternary complexes of antibiotics [14–16], the present investigation deals with the solution equilibria of transition metal ions with cephradine and the isolation and an investigation of some of its complexes.



Cephradine

Results and Discussion

Acid-base equilibria of cephradine

Cephradine is protonated in acid medium to yield the cationic species $(\mathrm{H}Ceph)^+$. An examination of the potentiometric equilibrium titration curve shows the presence of two buffer regions. In the first one, the carboxylic group is neutralized ($pK_{a1} = 2.42$), yielding the zwitter ion species ($Ceph^{\pm}$) which is the only species existent at pH 5.2 (degree of formation 99%). In the second buffer region, the deprotonation of the ammonium group takes place giving an anionic species $(Ceph\text{-H})^-$ ($pK_{a2} = 7.54$, Table 1) which predominates at pH 9.2.

Effect of solvent

The protonation constants of cephradine and the formation constants of Cu(II) complexes in dioxane-water mixtures of various compositions are given in Table 2. The variation of pK_a is shown as a function of solvent composition in Fig. 1. As shown by the data of Table 2 and Fig. 1, the results confirm those of others which took into account the difference in behaviour of the carboxyl group ionization compared to that of the ammonium group [17]. Thus, for the dissociation of the carboxylic proton, pK_{a1} increases linearly with increasing concentration of the organic solvent. This results from the ability of a solvent of low dielectric constant to increase the electrostatic forces between the ions and to facilitate the formation of molecular species. The pK_{NH}^{+} values decrease with increasing dioxane content.

Table 1. Formation constants of the binary and ternary complexes of cephradine

System	1[a]	p[a]	q[a]	$\log\beta$[b]	S[c]	*pH* range	$\Delta\log K$
H^+-*Ceph*	0	1	1	7.54 (0.00)	1.6×10^{-7}	2.7–9.2	
	0	1	2	9.96 (0.01)			
Cu^{2+}-*Ceph*	1	1	0	4.06 (0.09)	5.2×10^{-7}	2.6–6.1	
	1	1	−1	−1.03 (0.02)			
Ni^{2+}-*Ceph*	1	1	0	3.12 (0.03)	5.6×10^{-7}	2.6–8.1	
	1	1	−1	−5.15 (0.03)			
Zn^{2+}-*Ceph*	1	1	0	2.06 (0.04)	8.7×10^{-7}	2.6–7.8	
	1	1	−1	−6.25 (0.04)			
Co^{2+}-*Ceph*	1	1	0	2.55 (0.04)	7.7×10^{-7}	2.6–8.3	
Cu^{2+}-*bpy*[d]	1	1	0	8.1			
Ni^{2+}-*bpy*[d]	1	1	0	7.1			
Co^{2+}-*bpy*[d]	1	1	0	5.7			
Zn^{2+}-*bpy*[d]	1	1	0	5.2			
Ternary Complexes							
Cu^{2+}-*bpy-Ceph*	1	1	0	4.54 (0.09)	4.6×10^{-6}	2.7–6.5	0.48
Ni^{2+}-*bpy-Ceph*	1	1	0	3.34 (0.05)	6.1×10^{-6}	2.7–8.0	0.22
Co^{2+}-*bpy-Ceph*	1	1	0	2.89 (0.05)	4.2×10^{-6}	2.7–8.1	0.34
Zn^{2+}-*bpy-Ceph*	1	1	0	3.08 (0.06)	6.4×10^{-6}	2.8–7.4	1.02

[a] 1,p and q refer to $[M_1(Ceph)_pH_q]$ for the binary complexes; and 1 refers to $M(bpy)$ for the ternary complexes;

[b] standard deviations in parentheses;

[c] sum of square of residuals; (about 60 data points);

[d] formation constants of bipyridyl complexes are taken from Ref. [14].

This can be interpreted by non-electrostatic forces which could include geometrical aspects, hydrogen bonding, and solvent-solute interactions. This behaviour is in agreement with that proposed for oxines [22].

Binary complexes

Potentiometric equilibrium titration curves of nickel(II)-cephradine, taken as being representative, are shown in Fig. 2. In the titration curve, the first buffer region is identical with that of free cephradine, indicating that no coordination takes place. In the second buffer region there is a significant lowering of the titration curve with respect to that of free cephradine, indicating the formation of a metal complex by release of a proton from the $-NH_3^+$ group. The potentiometric data could be fitted by assuming the formation of the 1:1 complex $[M(Ceph\text{-}H)]^+$ and the corresponding hydrolyzed species. The variation of the formation constant of the Cu(II)-cephradine complex as function of solvent composition is shown by the data of Table 2. The formation constant decreases with increasing dioxane content of the solvent. This behaviour can be explained by the variation of the protonation ability of the amine which is the binding site in the complexes as the organic content increases. The order

Table 2. Solvent effect on the dissociation constant of cephradine and the formation constant of the Cu-cephradine complex

System	% of Dioxane (v/v)	l^{a}	p^{a}	q^{a}	$\log \beta^{b}$	S^{c}
H^+-*Ceph*	20	0	1	1	6.99 (0.02)	1.1×10^{-6}
		0	1	2	10.08 (0.03)	
	40	0	1	1	6.74 (0.03)	2.1×10^{-6}
		0	1	2	10.41 (0.04)	
	60	0	1	1	6.42 (0.03)	2.2×10^{-6}
		0	1	2	10.65 (0.04)	
	75	0	1	1	6.36 (0.02)	9.0×10^{-7}
		0	1	2	11.11 (0.02)	
Cu-Ceph	20	1	1	0	5.12 (0.05)	3.8×10^{-6}
		1	1	−1	−0.39 (0.07)	
	40	1	1	0	5.09 (0.04)	3.0×10^{-6}
		1	1	−1	−0.13 (0.05)	
	60	1	1	0	4.92 (0.05)	5.4×10^{-6}
		1	1	−1	−0.40 (0.07)	
	75	1	1	0	4.20 (0.05)	5.2×10^{-6}
		1	1	−1	−1.59 (0.06)	

For a–c, cf Table 1

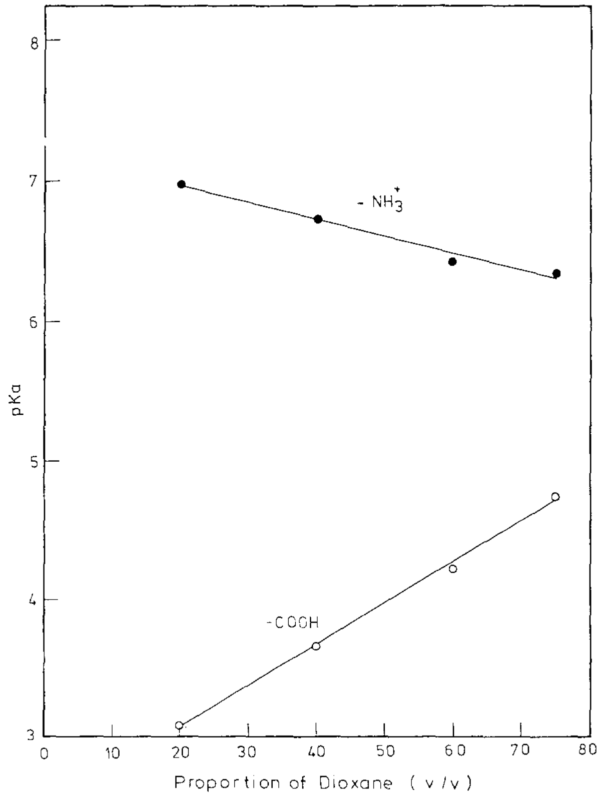


Fig. 1. Effect of solvent on the pK_a values of cephradine

of stabilities for the complexes was found to be Co<Ni<Cu>Zn which is in accordance with *Irving–William's* order [19].

Ternary complexes

The potentiometric titration curve of the ternary system M(II)-*bpy*-cephradine coincides with the 1:1 M(II)-*bpy* curve in the region $0<a<2$ (a = number of moles of base added per mole of ligand). In this region the M(II)-*bpy* complex was formed first, due to its high stability compared to that of M(II)-Cephradine complexes (Table. 1). The formation of a ternary complex was ascertained by comparison of the mixed ligand titration curve with the composite curve obtained by graphical addition of the cephradine titration data to that of the M(II)-*bpy* titration curve. The mixed ligand system was found to deviate considerably from the resultant composite curve indicating the formation of a ternary complex. Thus, formation of ternary complexes can be described by the following equilibria:

$$M^{2+}_{(aq)} + bpy \rightleftharpoons [M(bpy)]^{2+} \tag{1}$$

$$[M(bpy)]^{2+} + Ceph^{-} \rightleftharpoons [M(bpy)(Ceph)]^{+} \tag{2}$$

The relative stability of the ternary complexes, as compared to those of the corresponding binary complexes, can be quantitatively expressed in different ways. The most suitable comparison [20] is in terms of $\Delta\log K$ as defined by equation 3.

$$\Delta\log K = \log K^{M(bpy)}_{M(bpy)(Ceph)} - \log K^{M}_{M(Ceph)} \tag{3}$$

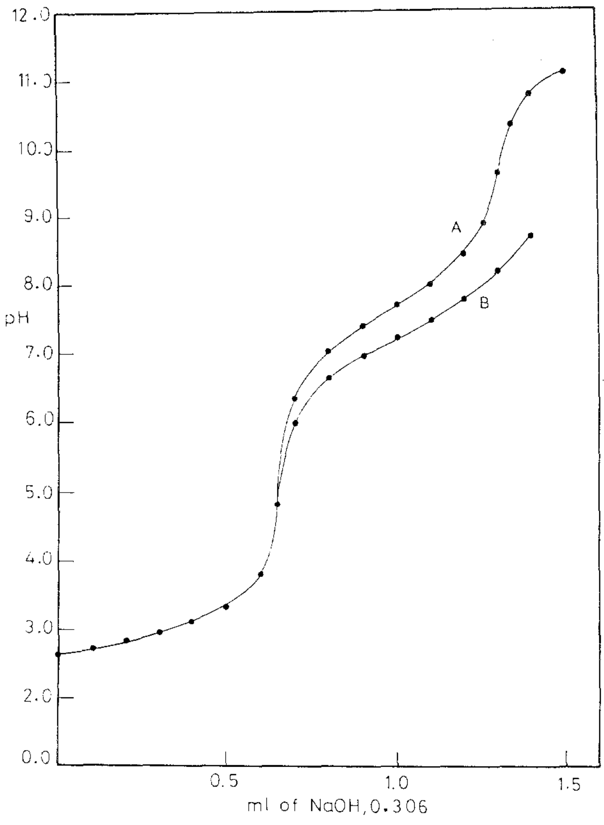


Fig. 2. Potentiometric titration curves of the Ni(II)-cephradine system; (A) cephradine, (B) Ni(II)-cephradine mixture

The $\Delta\log K$ values given in Table 1 are invariably positive. This means that cephradine forms a more stable complex with the *M-bpy* complex than with the free metal ion. Derived from $\Delta\log K$ values, the ease of ternary complex formation is: Zn(II) > Cu(II) > Ni(II) > Co(II). The enhanced stability for Zn(II) has been previously explained [21, 22] on the basis of π-bonding [23]. Therefore, if π-bonding is indeed the major responsible factor, then copper(II) should have a higher positive $\Delta\log K$ value since it is the better π-donor [24]. The observed anomaly can be explained by considering the existence of equilibrium 4 in solution:

$$cis\text{-}[M(bpy)(Ceph)(H_2O)_2]^+ \rightleftharpoons trans\text{-}[M(bpy)(Ceph)(H_2O)_2]^+ \qquad 4$$

In the *cis* isomer, the secondary ligand has to span one equatorial and one axial position keeping the two H_2O molecules at the *cis* position. This creates a strain as a result of the *Jahn–Teller* distortion [25]. For Zn(II) (d^{10} system), there is no distortion and there is no strain in either of the isomers. This proposition could also be supported by the fact that H_2O occupies the *cis* position in $[Cu(bpy)_2(H_2O)_2]^{2+}$ [26]. A similar observation has recently been made in [Cu(*bpy*) (acetohydroxamate)]$^+$ [27].

The concentration distribution of the various complex species formed in solution as a function of *pH* was calculated by means of the MINIQUAD-75 program. The distribution curves of nickel-cephradine, taken as a representative, are shown in Fig. 3. The extent of complex formation was found to be *pH* dependent.

The conductometric titration curve of the ternary complex of nickel(II) (Fig. 4) shows an initial decrease and an inflection at $a = 2$. This probably corresponds to the neutralization of H^+ ions resulting from the formation of the Ni(II)-*bpy* complex.

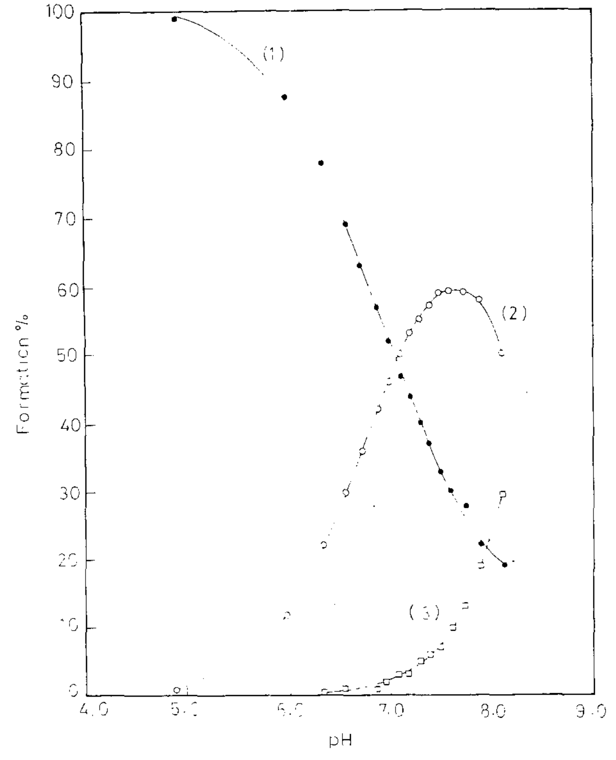


Fig. 3. Concentration distribution of various complex species as a function of *pH* for the Ni(II)-cephradine system; (1) [Ni(II)], (2) $[Ni(Ceph\text{-}H)]^+$, (3) $[Ni(Ceph\text{-}H)(OH)]$

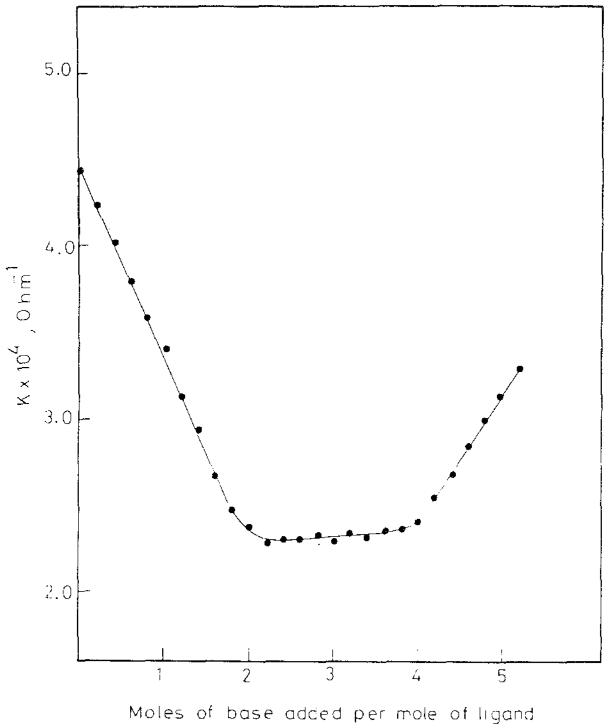


Fig. 4. Conductometric titration curve of the nickel(II)-bipyridyl-cephradine system

Between $a = 2$ and $a = 4$, the conductance increases slightly, supposedly due to the formation of a ternary complex associated with the release of two protons from cephradine. Beyond $a = 4$, the conductance increases more appreciably due to the presence of an excess of NaOH.

Microanalyses of the solid complexes along with IR and molar conductivity data are listed in Table 3. The data indicate that the complexes are of 1:1 stoichiometry. The molar conductance values of 10^{-3} *M* solutions of the complex in *DMF* were found to be in the range of 4–8 $\mathrm{Ohm^{-1} \cdot cm^2 \cdot mol^{-1}}$ which indicates a non-electrolytic nature of the complexes and that the anion (NO_3^-) is coordinated to the metal ion.

With respect to the mode of chelation, IR spectra suggested the most probable binding sites. Cephradine has a broad band around 2600 $\mathrm{cm^{-1}}$, attributed to the

Table 3. Analytical data of cephradine complexes

Compound	% C[a]	% H[a]	% N[a]	% S[a]	Λ_M ($\mathrm{ohm^{-1} \cdot cm^2 \cdot mol^{-1}}$)	$\nu_{CO(amide)}$ ($\mathrm{cm^{-1}}$)
$(C_{16}H_{18}N_3O_4S)Cu(NO_3) \cdot H_2O$	39.5	4.3	11.2	6.7	6.5	1581
	(39.1)	(4.1)	(11.4)	(6.5)		
$(C_{16}H_{18}N_3O_4S)Ni(NO_3) \cdot H_2O$	39.9	4.2	11.3	6.5	4.5	1672
	(39.4)	(4.1)	(11.5)	(6.6)		
$(C_{16}H_{18}N_3O_4S)Co(NO_3) \cdot H_2O$	40.0	4.2	11.2	6.8	8.0	1672
	(39.4)	(4.1)	(11.5)	(6.6)		

[a] Calculated values in parentheses

$-NH_3^+$ stretching vibration [28]. This band did not appear in the spectra of the complexes. A strong band at 1686 cm^{-1} was assigned to the C=O stretching vibration of the amide group. This band is significantly shifted to lower wave numbers upon complexation. Therefore, the amino and carbonyl groups of the side chain participate in the complex formation. This is in agreement with the data of the nickel(II) complex of cephalexine, which has a structure similar to cephradine [29].

Conclusion

The analysis of complex formation equilibria indicates that metal ions form complexes with cephradine. The formation constant of the copper(II) complex decreases with increasing dioxane content of the solvent. The ternary complexes are formed in a stepwise mechanism. The solid copper(II), nickel(II), and cobalt(II) complexes are of stoichiometry 1:1. The amino and carbonyl groups of cephradine participate in complex formation.

Experimental

Materials

Cephradine trihydrate (*Ceph*; Bristol Standard 99%) was supplied by El-Nil Co. According to its elemental analysis, it was sufficiently pure and therefore, used without further purification. Accounting for the instability of antibiotics in aqueous media [30], fresh solutions were prepared immediately before use by dissolving it in aqueous equimolar nitric acid solution. 2,2'-Bipyridyl (*bpy*) was purchased from Sigma. The protonated form was used. The metal nitrates were provided by B.D.H. Concentrations of stock solutions of the metal ions were determined by conventional analytical methods [31]. All solutions were prepared in deionized water.

Apparatus

The potentiometric measurements were performed by a Metrohm 686 titroprocessor equipped with a 665 Dosimat (Switzerland). The electrode was calibrated with standard buffer solutions prepared according to NBS specifications [32]. The conductance of solutions was measured with a WTW LBR conductivity bridge. IR spectra (KBr) were recorded on a Perkin-Elmer 1403 spectrophotometer.

Procedures and techniques

The mixtures A–D were prepared for equilibrium constant determinations:

(A) 0.02 *M Ceph* (10 ml) + 0.13 *M* $NaNO_3$ (30 ml)
(B) 0.01 *M* metal ion (10 ml) + 0.02 *M Ceph* (10 ml) + 0.20 *M* $NaNO_3$ (20 ml)
(C) 0.02 *M* metal ion (10 ml) + 0.02 *M bpy* (10 ml) + 0.20 *M* $NaNO_3$ (20 ml)
(D) 0.02 *M* metal ion (10 ml) + 0.02 *M bpy* (10 ml) + 0.02 *M Ceph* (10 ml) + 0.40 *M* $NaNO_3$ (10 ml)

The following mixture (E) was prepared and titrated conductometrically with 0.306 *M* NaOH:

(E) 0.02 *M* nickel(II) (10 ml) + 0.02 *M bpy* (10 ml) + 0.02 *M Ceph* (10 ml)

Mixtures A–D were titrated potentiometrically against a standard NaOH solution (0.306 *M*) at 25 °C in an atmosphere of purified N_2. A slight opacity and foaming were observed during the titrations. According to our usual protocol, experiments were nonetheless pursued until a steady drift became noticeable in the *pH* reading. This was taken as a criterion for the occurance of precipitation. The acid dissociation constants of cephradine were determined by titrating mixture A. The formation constants of the binary complexes formed in solution were determined by titrating mixture B. The *M*(II):*Ceph.* concentration ratio used was 1:2 to allow the formation of 1:1 and other complexes. The stability constants of the ternary complexes were determined by titration of mixture D, utilizing the data obtained in the *pH* range corresponding to the complete formation of the $[M(bpy)]^{2+}$ complex. The calculations were performed using the computer program MINIQUAD-75 [33]. The stoichiometries and stability constants of the complexes formed were determined by trying various possible composition models for the systems studied. The model selected was that which gave the best statistical fit and which was chemically consistent with the titration data without giving any systematic drifts in the magnitudes of various residuals, as described elsewhere [33]. Tables 1 and 2 list the stability constants together with their standard deviations and the sum of the square of residuals as obtained from MINIQUAD-75 calculations.

Synthesis of the metal complexes

The solid copper(II), nickel(II), and cobalt(II) complexes of cephradine were prepared by dissolving 1 mmol of cephradine in a NaOH solution (5 ml, 0.2 *M*, *i.e.* 1 mmol). This solution was mixed with 1 mmol of the metal salt dissolved in the least amount of water. The solution mixture was refluxed for 1 h, whereby the solid complexes were precipitated. The complexes were filtered, washed with water, ethanol, and finally with ether. The analytical data are given in Table 3.

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