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Potentiometric Studies on Some Cephalosporin Complexes

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Summary. The interaction of Ca(II), Cu(II), Zn(II), Pb(II), and La(III) ions with the antibiotics cephalexin, cefadroxil, cephaloridine, and cefoperazone as secondary ligands was investigated potentiometrically. The formation constants were determined for a ligand-to-metal ratio of 1:1 at 25°C and $I = 0.1\ \text{mol} \cdot \text{dm}^{-3}$ KNO_3. The protonation constants of the complexes were evaluated for the system M : cephalosporin : glycine = 1 : 1 : 1. The order of stability of the binary and ternary complexes were examined. It was found that glycine adds preferably [M(II)-cephalosporin] rather than to the aqueous complexes of M(II). In all cases 1:1:1 complexes were formed.

Keywords. Potentiometry; Glycine; Cephalosporins; Complexes.

Introduction

Among the most important broadband antibiotics are the cephalosporins, a series of compounds containing a β-lactam moiety fused with a six-membered dihydrothiazine ring and an acetoxymethyl group at position 3. They have been used in medicine in the treatment of some diseases [1]. Recently, much attention has been paid to the study of binary and ternary complexes of transition metals with molecules of biological and pharmaceutical interest [2, 3]. Furthermore, it has been suggested that the presence of metal ions in biological fluids could have a significant effect on the therapeutic action of drugs [4, 5].

Semi-synthetic cephalosporin antibiotics have structures similar to that of penicillins, both groups of compounds being characterized by similar properties and determined by the same methods [6]. Little informaion is available on complexes containing cephalosporin derivatives. The formation of a complex between cephadroxil and Cu(II) ion as well as the coordination compounds of ceftazidine, ceftrioxone, ceftrizoxime, and cefoperazone with Pb(II) have been studied spectrophotometrically [7, 8]. Also, the dissociation constant of cephalexin in different solvents (H_2O and/or H_2O-*DMF*) has been determined [9–11]. In this paper, we report for the first time on metal complexes of cephalosporins of the type

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M(II)-cephalosporin (cephalexin, cefadroxil, cephaloridine, cefoperazone) and *M*(II)-cephalosporin-glycine (cephalexin) with $M = Ca(II)$, Cu(II), Zn(II), Pb(II), and La(III). The dissociation constants of the ligands and the stability constants of their complexes were determined.

Results and Discussion

Proton-ligand systems

The titration curves obtained for cephalexin, cefadroxil, cephaloridine, and cefoperazone are similar in behaviour; representative examples are shown in Fig. 1. The values of $\bar{n}A$ as determined according to *Irving* and *Rossotti* [12] were complied from the titration data using solutions *a* and *b*. Calculations of proton-ligand association constants were carried out by plotting $\bar{n}A$ against *pH* (Figs. 2, 3). The values of $\log K_1H$ and $\log K_2H$ (the first and second proton association constants of the studied cephalosporins) are the pH values corresponding to $\bar{n}A = 0.5$ and 1.5, respectively.

The SUPERQUAD computer program [13] was used to refine the overall protonation or formation constants by a least squares fit. The pK_a values obtained by treatment of several sets of potentiometric data are quoted in Table 1. The result for glycine shows good agreement with literature data after allowing for changes in experimental conditions as well as methods of calculations. It should be mentioned, however, that the pK_a value for glycine is too low (≤ 2.35) [14]; thus, it is not used in our calculations.

Binary metal-ligand systems

The titration curves of the metal-ligand solutions *c* differ well separated from those of solutions *b* (Fig. 1), demonstrating replacement of H^+ due to complexation. The

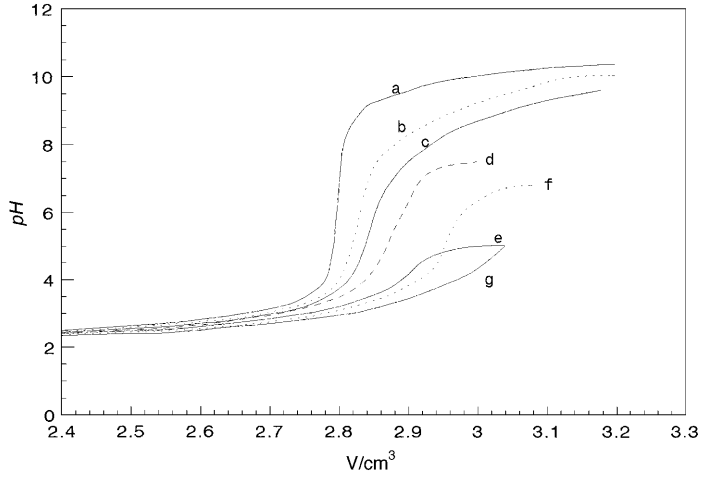

Fig. 1. Potentiometric titration curves of cephaloridine; *a*: 0.01 *M* HNO_3, *b*: *a* + 0.001 *M* cephaloridine, *c*: *b* + 0.001 *M* Ca(II), *d*: *b* + 0.001 *M* Zn(II), *e*: *b* + 0.001 *M* Pb(II), *f*: *b* + 0.001 *M* La(III), *g*: *b* + 0.001 *M* Cu(II)

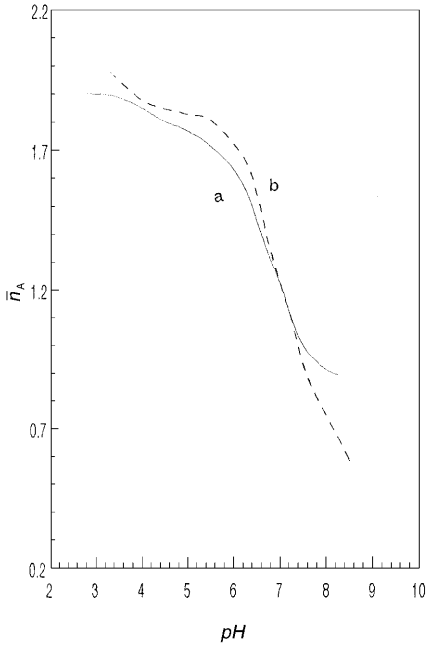

Fig. 2. Proton formation curves of a) Cephalexin b) Cefadroxil

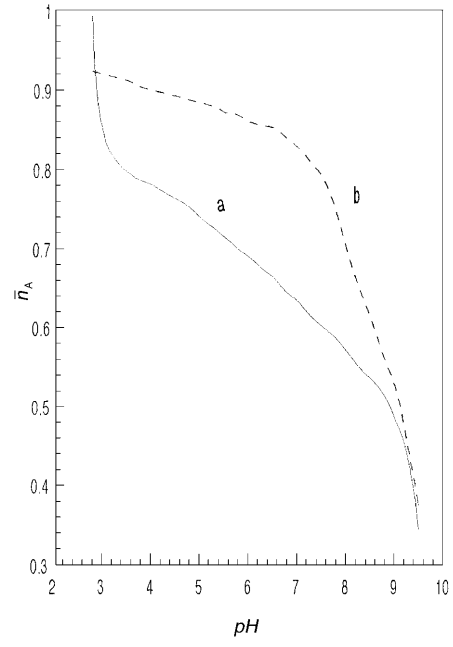

Fig. 3. Protonation curves of a) cefoperazone, b) cephaloridine

$\bar{n}$ values were plotted against the corresponding pL values to obtain the formation curves of the complexation equilibria (Fig. 4). From these curves the values of the stability constants were computed using standard procedures based on the calculations of the average number of ligands bound per metal ion, $\bar{n}$, and the free ligand exponent, pL, as described previously [12]. Only 1:1 ligand/metal

Table 1. Formation constants of metal complexes with cephalexin cefadroxil, cephaloridine, and cefoperazone

Central ions	Cephalexin		Cefadroxil		Cephaloridine	Cefoperazone
	logK_1	logK_2	logK_1	logK_2	log$K$	log$K$
H^+	6.3	9.7	6.5	8.7	9.1	8.9
Ca(II)	3.47	–	3.6	–	3.75	4.27
Zn(II)	4.41	–	4.81	–	5.25	5.97
Pb(II)	4.98	–	5.67	–	8.00	6.7
La(III)	5.57	–	5.87	–	8.55	7.01
Cu(II)	6.06	–	9.2	–	8.70	7.5

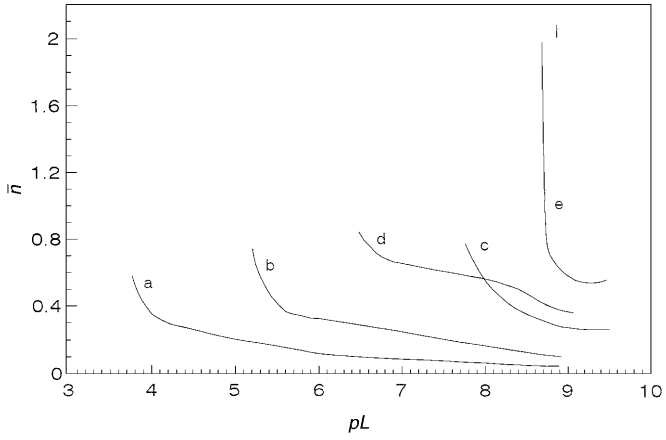

Fig. 4. Metal ion-cephaloridine formation curves; (a) Ca(II), (b) Zn(II), (c)Pb(II), (d) La(III), (e) Cu(II)

complexes were formed between Ca(II), Cu(II), Zn(II), Pb(II), and La(III) and the investigated cephalosporins.

The stability constants of the complexes formed with cephalexin, cefadroxil, cephaloridine, and cefoperazone decrease in the order Cu(II) > La(III) > Pb(II) > Zn(II) > Ca(II), which is in agreements with the decrease in the ionic potential (charge per ions radius) of metal ions. On the other hand, the results indicate that metal-glycine complexes are more stable than those of cephalosporins. This behaviour may be interpreted based on the bidentate nature of glycine which coordinates through the α-amino nitrogen and the carboxylic oxygen atoms forming stable five-membered chelate rings, unlike the cephalosporin ligands which may be coordinated through the carboxylate group, the 8-carbonyl group, and the amino nitrogen atom, thus leading to the formation of six-membered rings. It is well known that 3d metal ions prefer five-membered to six-membered rings in their chelates. The order of stabilities of the above complexes in terms of the cephalosporins is cephaloridine > cefoperazone > cefadroxil > cephalexin. This can be interpreted taking into account the basicity of these compounds. Potentiometric titration curves for some binary complexes with cephalexin in a 1:4 metal:ligand ratio afforded the same stability constants those of the 1:1

Table 2. Formation constants of metal complexes with chephalexin (1:4)

Central ion	$\log K_1$	$\log K_2$
H^+	6.7	9.5
Ca(II)	5.6	–
Cu(II)	9.3	–

compounds (Table 2). The stability constant of copper chelats with cefadroxil was also determined potentiometrically using the procedure described by *Sarin* [15]. According to this method, cefadroxil reacts with copper(II) forming two types of complexes with ligand-to-metal ratios of 1:1 and 2:1.

Ternary metal-ligand systems

The stability constants of the ternary complexes containing cephalexin and glycine were calculated from the data obtained from *pH*-metric titrations according to Eq. (1).

$$M(\text{gly}) + \text{cephalexin} \rightleftharpoons M(\text{gly})(\text{cephalexin}),$$
$$K^{M(\text{gly})}_{M(\text{gly})(\text{cephalo})} = \frac{[M(\text{gly})(\text{cephalexin})]}{[M(\text{gly})][\text{cephalexin}]} \tag{1}$$

Similarly, the constants of the binary complexes were also determined (Eqs. (2) and (3)).

$$M + \text{cephalo} \rightleftharpoons M(\text{cephalo})$$
$$K^{M}_{M(\text{cephalo})} = \frac{[M(\text{cephalo})]}{[M][\text{cephalo}]} \tag{2}$$

$$M + \text{gly} \rightleftharpoons M(\text{gly})$$
$$K^{M}_{M(\text{gly})} = \frac{[M(\text{gly})]}{[M][\text{gly}]} \tag{3}$$

It is assumed, for convenience, that complexation of the secondary ligand (cephalexin) starts after formation of the *M*(II)-glycine 1:1 complex. Thus, the overall stability constant $\beta^{M}_{M(\text{gly})(\text{cephalo})}$ may be represented as given in Eq. (4).

$$M + \text{gly} + \text{cephalo} \rightleftharpoons M(\text{cephalo})(\text{gly})$$
$$\beta^{M}_{M(\text{gly})(\text{cephalo})} = \frac{[M(\text{gly})(\text{cephalo})]}{[M][\text{gly}][\text{cephalo}]} = K^{M}_{M(\text{gly})(\text{cephalo})} \times K^{M}_{M(\text{gly})} \tag{4}$$

The difference $(V_4 - V_3) - (V_2 - V_1)$, where V_1, V_2, V_3, and V_4 are the volumes of NaOH required to reach the same *pH* value on curves a, b, e, and f, respectively, can be used for the calculation of $\bar{n}_{\text{mix}}$ (average number of secondary ligand molecules associated with one $[M(\text{gly})]^{n+}$ ion) according to Eq. (5) [16].

$$\bar{n}_{\text{mix}} = \frac{(V_{\text{f}} - V_{\text{e}}) - (V_{\text{b}} - V_{\text{a}})((E + N) + (Y - \bar{n}_{\text{H}}))}{(V_{\text{o}} + V_{\text{c}})T^{\circ}_{M}\bar{n}_{\text{H}}} \tag{5}$$

In Eq. (5), T_M° is the concentration of [M(gly)] which is equal to the concentration of M(II) used, Y is the number of dissociable protons of glycine ($Y = Z$) and values of $\bar{n}_H$ for the secondary ligand at different pH were calculated from the amino acid formation curve; all other symbols have their usual meanings. From the values of $\bar{n}_{mix}$, the free secondary ligand exponent pL_{mix} was calculated using the equation (6) where β is the second formation constant of glycine and B is the pH-meter reading.

$$pL_{mix} = \log \left(\frac{\sum_{n=0}^{i} \beta_n^H \left(\frac{1}{10^B} \right)^n}{T_L^0 - \bar{n}_{mix} \cdot T_M^0} \cdot \frac{V_0 + V_f}{V_0} \right) \tag{6}$$

The second dissociation constant of glycine was determined from the titration curves a and d using the formula of *Rossotti* and *Irving* [12]. The obtained value (9.55) is in good agreement with literature data [17]. Formation curves corresponding to the various M(II)-cephalexin-glycine systems were obtained by polotting $\bar{n}_{mix}$ *vs.* pL_{mix}; representative results are shown in Fig. 5. The corresponding formation constants $\log^M_{M(gly)(cephalexin)}$ obtained by the average value method are reported in Table 3. $\Delta \log K$, as defined by Eq. (7) is a measure of the stability of the ternary complexes relative to the binary complexes and was interpreted on the basis of statistical considerations and the nature of species formed in solution. It was found that Ca(II), Cu(II), Zn(II), and Pb(II) each form a single mixed complex (1:1:1) with cephalexin. The importance of such mixed complexes can be ascribed to their application as models for metalo-enzyme substrate complexes and also as components of multi metal-multi ligand systems in biological fluids. Accordingly, the study of such mixed ligand complexes is considered as an effort to understand the nature of metal-ion complexation in biological systems.

$$\Delta \log K = \log K^M_{M(gly)(cephalexin)} \cdot \log K^M_{M(cephalexin)} \tag{7}$$

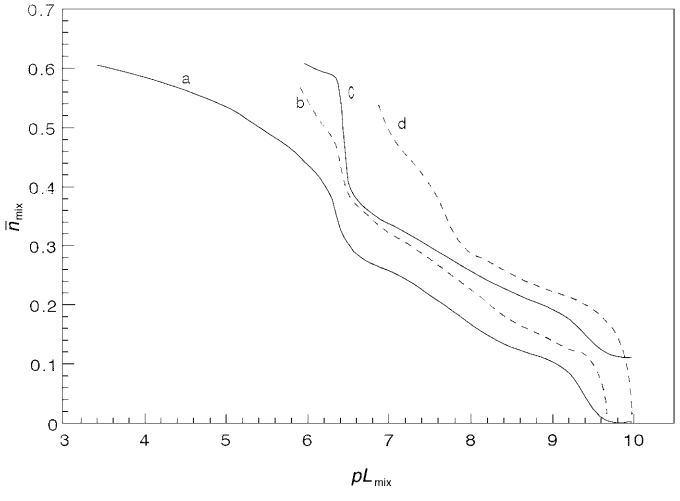

Fig. 5. Metal ion-cephalexin-glycine formation curves; (a) Ca(II), (b) Zn(II), (c) Pb(II), (d) Cu(II)

Table 3. Proton-ligand association constants of cephalexin (L_2) and stability constants of binary and ternary complexes at 25°C, $I = 0.1\,\mathrm{mol}\cdot\mathrm{dm}^{-3}$ (KNO_3); $\Delta\log K = \beta_{\mathrm{mix}} - \beta_{\mathrm{gly}}$

Cation	$\log K_1^{\mathrm{H}}$	$\log K_2^{\mathrm{H}}$	$M(L_2)$ $\log K_1$	M(gly) $\log K_1$	$\log K^{M(\mathrm{gly})}_{M(\mathrm{gly})(L_2)}$	$\log \beta^{M}_{M(\mathrm{gly})(L_2)}$	$\log K$
H^+	6.3	9.7	–	–	–	–	–
Ca(II)	–	–	3.47	3.85	5.50	8.97	1.65
Zb(II)	–	–	4.41	4.90	6.20	10.61	1.30
Pb(II)	–	–	4.98	5.75	6.42	11.40	0.67
Cu(II)	–	–	6.06	6.22	7.05	13.11	0.83

$\Delta\log K = \beta_{\mathrm{mix}} - \beta_{\mathrm{gly}}$.

The best least squares fits [13] for the investigated ternary systems were obtained for 1:1:1 systems. Generally, it is worth mentioning that cephalosporin ligands show no precipitation during titration. Thus, they are not hydrolyzed under the experimental conditions, even in the high *pH* region. This behaviour may be explained on the basis that the electron density of the metal-ligand bonds in ternary chelates is redistributed in such a way that the ternary chelates are more polar than the binary chelates and hence are not easily hydrolyzed at high *pH* values.

The 1:1 binary complexes of *M*(II)-glycine begin to form at lower *PH*s than the corresponding complexes with cephalexin. The $\log K^{M(\mathrm{gly})}_{M(\mathrm{gly})(\mathrm{cephalexin})}$ values (Table 3) found to decrease in the order Cu(II) > Pb(II) > Zn(II) Ca(II) as was already observed for the binary complexes. The relative stability of the ternary complexes as compared to the corresponding binary complexes can be expressed quantitatively in terms of $\Delta\log K$ (Table 3).

Conclusions

The intrinsic antimicrobial activity of natural cephalosporins is low, but the attachment of various functional groups (*e.g.* acetoxymethyl) has yielded drugs of good therapeutic activity and low toxicity. Their binary and ternary complexes are of importance with respect to their application, as models for metal-enzyme-substrate complexes, and as components of multi metal-multi ligand systems in biological fluids. Accordingly, this study considered as an effort to understand the nature of metal-ion complexation in biological systems. The order of stabilities of the complexes is cephaloridine > cefoperazone > cefadroxil > cephalexin. Cephalosporins react with metal ions forming one type of complexes of molar ratios 1:1 (ligand-to-metal). The stability constants of the ternary complexes containing cephalexin and glycine were also calculated. It was found that each metal ions forms a single mixed complex (1:1:1) with cephalexin and glycine.

Experimental

Calvin-Bjerrum's technique as adopted by *Irving* and *Rossotti* [12] and *Katkar* and *Munshi* [16] was used to determine the protonation constants of the ligands (cephalosporins and glycine) and the formation constants of their metal complexes at 25 ± 0.1°C in aqueous solutions.

pH Measurements were carried out with an Accumet *pH*-meter Model 825 MP. During the titrations, oxygen-free nitrogen was bubbled through the solutions. The electrode system was calibrated in terms of hydrogen ion concentrations instead of activities; thus, all constants determined in this work are concentration constants. The solutions of Ca(II), Cu(II), Zn(II), Pb(II), and La(III) (AR, BDH) as nitrates were prepared and titrated complexometrically by *EDTA* [2]. Potassium nitrate and cephalosporin solutions (cephalexin, cefadroxil cephaloridine, and cefoperazone; Sigma) were prepared in bidistilled water as fresh solutions.

In the binary systems studied, the following solutions were titrated potentiometrically with $0.2\,\mathrm{mol\cdot dm^{-3}}$ standard carbonate-free sodium hydroxide solutions standardized against standard potassium hydrogen phthalate: *a*: $0.01\,\mathrm{mol\cdot dm^{-3}}$ HNO_3, *b*: $a + 0.001\,\mathrm{mol\cdot dm^{-3}}$ cephalosporin, and *c*: $b + 0.001\,\mathrm{mol\cdot dm^{-3}}$ metal nitrate solution. In the ternary systems, numerous titrations of different *M*(II)-cephalexin and glycine mixtures in a 1:1:1 molar ratio ($1 \times 10^{-3}\,\mathrm{mol\cdot dm^{-3}}$ each) were performed according to the following scheme: *a*: $0.01\,\mathrm{mol\cdot dm^{-3}}$ HNO_3, *b*: $a +$ cephalosporin ($1 \times 10^{-3}\,\mathrm{mol\cdot dm^{-3}}$), *c*: $b + 1 \times 10^{-3}\,\mathrm{mol\cdot dm^{-3}}$ *M*(II), *d*: a + glycine ($1 \times 10^{-3}\,\mathrm{mol\cdot dm^{-3}}$), *e*: $d + 1 \times 10^{-3}\,\mathrm{mol\cdot dm^{-3}}$ *M*(II), and *f*: 0.01 *M* HNO_3 $+1 \times 10^{-3}\,\mathrm{mol\cdot dm^{-3}}$ cephalosporin $+1 \times 10^{-3}\,\mathrm{mol\cdot dm^{-3}}$ glycine $+1 \times 10^{-3}\,\mathrm{mol\cdot dm^{-3}}$ *M*(II). The total volume was adjusted to $50\,\mathrm{cm^3}$ by adding doubly-distilled water in each case. The titrations were performed at $25 \pm 0.1^{\circ}\mathrm{C}$ and $I = 0.1\,\mathrm{mol\cdot dm^{-3}}$ KNO_3.

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