Investigations and chemical speciation of ternary complexes of bivalent metal ions with cephalexine as primary ligand and glycine and valine as secondary ligands

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Abstract : Studies on ternary complexes of bivalent metal ions viz. Cu^{II}, Pb^{II}, Ni^{II}, Zn^{II}, Co^{II}, Cd^{II}, Mn^{II}, Mg^{II} and Ca^{II}, hereafter, M(II), with cephalexine (HCEX) as primary ligand and glycine (Gly) and valine (Val) as secondary ligands in 1 : 1 : 1 ratio, have been investigated by potentiometric technique at μ = 0.1 M ($NaClO_4$) and temp. = 25 $\pm$ 0.5° in aqueous medium. The stability constants and S_{min} values of the ternary complexes M(II)-HCEX-Gly (A) and M(II)-HCEX-Val (B) have been calculated. The results have been discussed in terms of electrostatic and metal-ligand interaction. To visualize the nature of equilibrium and to evaluate the calculated stability constants of ternary complexes, species distribution diagrams have been plotted.

Keywords : Ternary complexes, stability constants, bivalent metal ions, cephalexine.

Much interest has been shown in the chemistry of β-lactam antibiotics in relation to their useful biological activities in recent years[1,2]. β-Lactam antibiotics such as penicillins, cephalosporins, oxacephalosporins, represent the most important class of drugs against infectious diseases caused by bacteria[1]. It is known that cephalexine (HCEX) an orally active cephalosporin in clinical practice, belongs to first generation with low β-lactamase stability. Cephalexine exhibits a broad spectrum of antibacterial activity[3] and may be used for the treatment of wide range of bacterial infections particularly urinary tract infections[3,4]. It is known that antibiotic activity is related to the ability of these compounds to form complexes with metal ions[5,6]. Glycine and valine have been preferred as secondary ligands because they are important chelating agents. Glycine and valine forms chelated compounds[7-10] with various bivalent and trivalent metal ions.
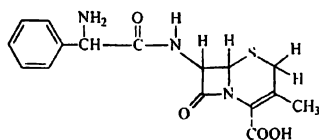
HCEX

In the present investigations, stability constants of ternary complexes of bivalent metal ions with HCEX, here-after, L_1, as primary ligand and Gly and Val, hereafter, L_2, as secondary ligands have been reported by using potentiometric technique at μ=0.1 M $NaClO_4$ and temp. = 25 $\pm$ 0.5° in aqueous medium. The bivalent metal ions used are : Cu^{II}, Pb^{II}, Ni^{II}, Zn^{II}, Co^{II}, Cd^{II}, Mn^{II}, Mg^{II} and Ca^{II}, hereafter, M(II). To visualize the formation of various species at different pH values, species distribution curves have been plotted.

Results and discussion

Cephalexine, HCEX has two ionisable hydrogen ions. Using the method of Irving and Rossotti[11], the pKa values have been found to be pKa_1 = 2.65 and pKa_2 = 9.75. Mode of coordination of HCEX with M(II) is a function of side chain and also of pH[12]. In the present study it has been found that complexation of HCEX with M(II) is maximum after pH ~7 and hence the coordination in HCEX is taking place from the side chain amino group and carbonyl group[12]. Cephalexine (HCEX) acts as primary ligand as the stability constants, of binary complexes it forms with bivalent metal ions, are greater than that of the glycine (Gly) or valine (Val) (Table 1).

$\bar{n}$ and pL values of the ternary complexes have been determined using the equation suggested by Irving-Rossotti[11]. The formation of ternary complexes was confirmed from the shape of pH titration curves, i.e. there is

Table 1. Formation constants and S_{min} values of ternary M(II)-HCEX-L_2 complexes of bivalent metal ions, where L_2 = Gly and Val in aqueous solution $\mu = 0.1\ M$ (NaClO$_4$), temp. = 25 ± 0.5°

(i) 1 : 1 M(II)-ligand binary constants :

M(II)	HCEX		Gly		Val	
	log $K^M_{M(HCEX)}$	S_{min}	log $K^M_{M(Gly)}$	S_{min}	log $K^M_{M(Val)}$	S_{min}
Cu^{II}	7.51	0.1650	6.98	0.0052	6.88	0.0010
Pb^{II}	7.12	0.0001	6.74	0.0002	6.66	0.0025
Ni^{II}	6.93	0.0269	6.72	0.0014	6.52	0.0132
Zn^{II}	6.31	0.0629	6.20	0.0312	6.12	0.0052
Co^{II}	5.64	0.1657	5.42	0.1250	5.58	0.0004
Cd^{II}	5.51	0.0042	5.38	0.0003	5.48	0.0032
Mn^{II}	5.41	0.0007	4.92	0.1105	5.02	0.0105
Mg^{II}	5.13	0.0074	4.36	0.1002	4.70	0.0312
Ca^{II}	4.32	0.0358	4.06	0.0020	4.24	0.0220

(ii) 1 : 1 : 1 M(II)-HCEX-L_2 ternary constants :

M(II)	Gly		Val	
	log $K^M_{M(Gly)-(HCEX)}$	S_{min}	log $K^M_{M(Val)-(HCEX)}$	S_{min}
Cu^{II}	6.53	0.0285	6.80	0.1976
Pb^{II}	6.42	0.0002	6.52	0.0003
Ni^{II}	6.24	0.0803	6.10	0.0015
Zn^{II}	5.81	0.0088	5.86	0.0015
Co^{II}	5.71	0.1320	5.37	0.0025
Cd^{II}	5.15	0.0058	5.12	0.0024
Mn^{II}	5.05	0.0077	5.01	0.0022
Mg^{II}	4.50	0.0055	4.99	0.0022
Ca^{II}	3.83	0.0001	4.30	0.0002

lowering of potentiometric titration curves, representing the mixed system in comparison to the curve for binary complex of the metal with the primary ligand[13].

Titration curves of the solution (iv) and (vi) overlap in lower pH range, indicating that secondary ligand (Gly or Val) does not combine with metal ions in this pH range and only complexation of HCEX takes place. At pH higher than ~4.0, titration curve of solution (vi) diverges from the curve of solution (iv), showing the coordination of the secondary ligand with the species, M(II) : HCEX. The reaction may be represented as follows for M(II), HCEX and Gly system :

M(II) + HCEX → M(II)-HCEX

M(II)-HCEX + Gly → M(II)-HCEX-Gly
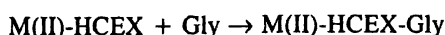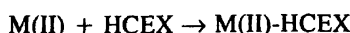
$$K_{M(II)\ :\ HCEX\ :\ Gly} = \frac{[M(II)\text{-HCEX-Gly}]}{[M(II)\text{-HCEX}]\ [Gly]}$$

The values of formation constants along with S_{min} values

are given in Table 1. Results of the present investigations show that the stability constants of ternary complexes formed are less than the stability constants of M(II)-HCEX binary system (Table 1). The lower stability of the ternary complexes can be explained on the basis of steric hindrance, electrostatic considerations, difference in bond type, geometrical structure and non-compatibility of ligand used.

Out of the above factors, steric consideration seems to be the most important because in the present studies of ternary complexes, primary ligand HCEX coordinates with the metal ion in the lower pH range and forms 1 : 1 complex[14]. In solution set (vi), M(II)-HCEX-Gly complex is formed as the titration curve runs below M(II)-HCEX titration curve after pH 4.0. So it is evident that the entry of the secondary ligand faces steric hindrance due to bigger M(II)-HCEX size (as compared to aqua-ion), which tries to restrict the entry of the secondary ligand in the coordination sphere of the metal ion and thus reduces the stability of ternary complex. The stability constant values of ternary complexes are found to follow the order : Cu^{II} > Pb^{II} > Ni^{II} > Zn^{II} > Co^{II} > Cd^{II} > Mn^{II} > Mg^{II} > Ca^{II}, for both M(II)-HCEX-Gly and M(II)-HCEX-Val systems.

On the basis of electrostatic considerations, it can be seen that HCEX reacts first with the bivalent metal ion to form M(II)-HCEX species. The interaction of the incoming secondary ligand (Gly or Val) with M(II)-CEX$^+$ is energetically unfavorable as compared to the interactions between $[M(H_2O)_n]^{2+}$ and Gly or Val. Hence the stability of the mixed ligand complex is reduced considerably. HCEX reacts with the metal ions in lower pH range whereas Gly or Val does not react in this region. The complexation of the secondary ligand takes place only after the reaction of HCEX with metal ions is complete. These facts clearly indicate that HCEX and Gly or Val are non-compatible ligands in the coordination sphere of the bivalent metal ion. According to Fridman[15], if two ligands are compatible in the coordination sphere of the metal ion, the ternary complex formed will be more stable than either of the parent complexes whereas if the two ligands are non-compatible, the ternary complex formed will be less stable.

The stability constants of ternary complexes are also dependent on the type of bonds formed between the metal ion and the ligands. According to Kida[16,17] the stability of the ternary complexes is lowered down if the geometrical structures and the bond types of the parent complexes are different from each other. In the present case,

these factors also appear to contribute to the lowering of the stability of mixed ligand complexes.

Species distribution studies :

To visualize the nature of the equilibria and to evaluate the calculated stability constants of the ternary complexes M(II)-HCEX-Gly (A) and M(II)-HCEX-Val (B), where M(II) = Cu^{II}, Pb^{II}, Ni^{II}, Zn^{II}, Co^{II}, Cd^{II}, Mn^{II}, Mg^{II} and Ca^{II}, species distribution curves have been plotted as a function of pH at temp. = 25 ± 0.5° and μ=0.1 *M* ($NaClO_4$) using the program SPE. Representative SPE curves for Cu^{II}-HCEX-Gly (A) and Cu^{II}-HCEX-Val (B) systems are given in Fig. 1.

SPE distribution curves of ternary system, M(II)-HCEX-Gly (A) showed that the formation of ternary complex started at pH ~6.5; when M(II) = Cu^{II}, Pb^{II}, Ni^{II}, Zn^{II} and Co^{II}, pH ~7.0; when M(II) = Cd^{II}, pH ~8.0; when M(II) = Ca^{II} and pH ~8.5; when M(II) = Mg^{II}. Ternary complexes attain their maximum concentration in the pH range 8.7–9.5, after which the concentration falls to zero in the pH range 10.5–12.0.

For ternary system (B), the SPE distribution curves showed that the formation of ternary complex started at pH ~6.0; when M(II) = Cu^{II} and Ni^{II}, at pH ~6.5; when M(II) = Pb^{II} and Zn^{II}, pH ~7.0; when M(II) = Cd^{II}, pH ~7.5; when M(II) = Co^{II} and Mn^{II}, pH ~8.0; when M(II) = Ca^{II} and pH ~8.5; when M(II) = Mg^{II}. It attains its maximum concentration in the pH range 8.3–9.6 after which the concentration decreased to zero in the pH range 9.5–12.0.

From the SPE distribution curves it is concluded that the formation of ternary complex started only after the metal-primary ligand complex has attained its maximum concentration. This indicates that the metal-primary ligand

complex (M(II)-HCEX) is formed first and then the secondary ligand (Gly or Val) gets attached to it resulting in the formation of ternary complex. Moreover, the maximum percentage of the formation of ternary complexes is less than that of the binary complex; this indicates that the ternary complexes are less stable as compared to the binary complex. In none of the systems studied, hydrolysis was observed.

Experimental

pH-metric titrations were carried out with PHM 83 Autocal pH-meter (Radiometer Copenhagen) with combined glass and calomel electrodes assembly. Cephalexine (HCEX, Sigma), glycine (Gly) and valine (Val) (A.R.) were used. Solutions of cephalexine (HCEX), glycine (Gly) and valine (Val) were prepared by dissolving the requisite amount of weighed samples in double distilled water. All the metal ion solutions were prepared from A.R. (B.D.H.) samples of the corresponding sulphates or nitrates and standardized complexometrically[18].

Tetramethylammonium hydroxide, TMAH (E. Merck) ((CH_3)$_4$NOH) solution in double distilled water was used as the titrant. It was standardized with a standard solution of oxalic acid. Perchloric acid ($HClO_4$) was standardized with standard TMAH solution. To ensure constant ionic strength during the titration an inert electrolyte sodium perchlorate, $NaClO_4$ (Riedel) was added in concentration greater than that of the complexing constituents.

pH Titration procedure :

Using the method of Bjerrum and Calvin as modified by Irving and Rossotti[11], the values of $\bar{n}$ and pL and formation constants of mixed ligand complexes have been calculated by weighted least squares method of Sullivan *et al.*[19]. The titrations have been carried out in a covered double walled glass cell in the nitrogen atmosphere, which was pre-saturated with double distilled water. All measurements were made in aqueous medium at a definite temperature (25 ± 0.5°), which was kept constant by using a Julabo F20 (West Germany) thermostat and at 0.1 *M* ($NaClO_4$) ionic strength. The following sets of solutions have been titrated against standardized TMAH (0.05 *M*) solution.

(i) 2.5 ml $HClO_4$ (0.05 *M*) + 1.0 ml $NaClO_4$ (2.0 *M*) + 0.2 ml K_2SO_4/KNO_3 (0.02 *M*) + 16.3 ml H_2O.

(ii) 2.5 ml $HClO_4$ (0.05 *M*) + 1.0 ml $NaClO_4$ (2.0 *M*) + 0.2 ml K_2SO_4/KNO_3 (0.02 *M*) + 2.0 ml L_1 (0.002 M) + 14.3 ml H_2O.
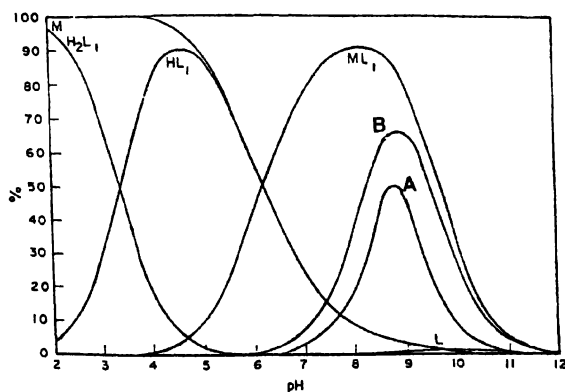

Fig. 1. Species distribution curves of Cu^{II}-HCEX (ML_1), Cu^{II}-HCEX-Gly (A) and Cu^{II}-HCEX-Val (B) systems vs pH.

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(iii) 2.5 ml $HClO_4$ (0.05 M) + 1.0 ml $NaClO_4$ (2.0 M) + 0.2 ml K_2SO_4/KNO_3 (0.02 M) + 2.0 ml L_2 (0.002 M) + 14.3 ml H_2O.

(iv) 2.5 ml $HClO_4$ (0.05 M) + 1.0 ml $NaClO_4$ (2.0 M) + 0.2 ml $MSO_4/M(NO_3)_2$ (0.02 M) + 2.0 ml L_1 (0.002 M) + 14.3 ml H_2O.

(v) 2.5 ml $HClO_4$ (0.05 M) + 1.0 ml $NaClO_4$ (2.0 M) + 0.2 ml $MSO_4/M(NO_3)_2$ (0.02 M) + 2.0 ml L_2 (0.002 M) + 14.3 ml H_2O.

(vi) 2.5 ml $HClO_4$ (0.05 M) + 1.0 ml $NaClO_4$ (2.0 M) + 0.2 ml $MSO_4/M(NO_3)_2$ (0.02 M) + 2.0 ml L_1 (0.002 M) + 2.0 ml L_2 (0.002 M) + 12.3 ml H_2O.

Acknowledgement

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