

## Metal complexes of thymidine – a study of binary and ternary systems with bivalent and trivalent metal ions in solution

P RABINDRA REDDY\*, C N KEERTHI and T K ADHARANI

Department of Chemistry, Osmania University, Hyderabad 500 007, India

MS received 30 June 1988; revised 14 October 1988

**Abstract.** The interaction of bivalent [Cu(II), Ni(II), Zn(II), Co(II), Mg(II), Ca(II)] and trivalent [La(III), Pr(III), Nd(III), Sm(III), Gd(III), Dy(III)] metal ions with thymidine both in binary and ternary systems have been investigated by potentiometric pH measurements at 35°C and 0.10 M (KNO<sub>3</sub>) ionic strength. The secondary ligands used in this investigation were glycine, histidine, histamine, *o*-phenanthroline and  $\alpha$ ,  $\alpha'$ -bipyridyl. The various stability constants pertaining to the above interactions were computed and compared. The influence of pH on the concentrations of several species present in aqueous solution, has been assessed. The effects of (i) charge on the metal ions, and (ii) the presence of a methyl group on the pyrimidine ring, on the stabilities of complexes have been assessed. A comparison with the corresponding constants of uridine has been made. Thymidine forms more stable complexes than uridine.

**Keywords.** Metal complexes of thymidine; binary and ternary systems; bivalent and trivalent metal ions.

### 1. Introduction

A number of investigations (Rabindra Reddy *et al* 1976, 1978, 1979, 1983, 1984, 1985; Taqui Khan and Madhusudan Raju 1981; Ramalingam and Krishna Murthy 1982; Rabindra Reddy and Harilatha Reddy 1983; Rabindra Reddy and Venugopal Reddy 1983; Taqui Khan *et al* 1983; Deshpande *et al* 1983; Bruce 1985) have been carried out to study the affinity of metal ions for heterocyclic bases (nucleic bases) attached and unattached to ribose and phosphate moieties. As a part of a broad programme to systematically investigate these interactions we have selected pyrimidine nucleosides for our study. In our previous publication, the interaction of metal ions with uridine (Rabindra Reddy and Malleshwar Rao 1986) has been reported. In the present work an attempt has been made to study the interaction of various metal ions with thymidine. Earlier investigations on thymidine were mostly confined to the Pt(II) and Pd(II) complexes (Inagoki and Kidani 1978; Kim and Bruce 1984; Tang *et al* 1984), kinetic and NMR study of methylated pyrimidine nucleosides (Allore *et al* 1983), preparation of solid complexes (Goodgame and Johns 1978), reaction with hydroxy methyl mercury (France *et al* 1985) and interactions with Cu(II) and Hg(II) (Raoul *et al* 1987).

The nitrogens of thymine are protonated in neutral solution with  $pK_a$  about 9.5 for

---

\* For correspondence

N(3) and about 14 for N(1) in the unsubstituted bases. This indicates that the metal ion binding sites are highly pH-dependent favouring the oxygens in acid solutions (Carrabine and Sundarlingam 1971) and N(3) in slightly basic solutions (Kotowycz and Suzuki 1973; Kosturko *et al* 1974).

In view of this, a detailed physicochemical investigation on the interaction of thymidine with bivalent and trivalent metal ions in the entire pH range have been carried out. We have also extended our study to ternary systems with a view to assess the influence of secondary ligands on the structure and stability of (1:1) metal-nucleoside systems.

The metal ions and various secondary ligands used in this study are alkaline earth metals Mg(II), Ca(II), transition metals Cu(II), Ni(II), Zn(II), Co(II), lanthanons La(III), Pr(III), Nd(III), Sm(III), Gd(III), Dy(III) and glycine, histidine, histamine, ortho-phenanthroline (*o*-phen) and  $\alpha, \alpha'$ -bipyridyl (bipy) respectively. The stability constants of these systems are computed by potentiometric pH titration techniques. The stability constants of binary and ternary systems of bivalent and trivalent metal ions have been compared to determine the charge effects on the stabilities of these complexes in solution. The stabilities of thymidine and uridine complexes in both binary and ternary systems were compared to assess the effect of the methyl group. This type of correlation we hope will provide useful information about the coordinating abilities of the primary ligands and the metal ions.

## 2. Experimental

Thymidine, glycine, histidine, histamine, *o*-phen and bipy were obtained from the Sigma Chemical Company, USA. Transition and alkaline earth metal ions were of AnalaR grade and were standardized volumetrically by titration with the disodium salt of ethylene diamine tetraacetic acid in the presence of a suitable indicator as outlined by Schwarzenbach (1957). All rare earth oxides were of Johnson & Mathey grade and stock solutions were prepared by dissolving known weights of the oxides in pure nitric acid. The lighter lanthanides were standardized with the disodium salt of EDTA (Kolthoft and Elving 1963). Carbonate-free sodium hydroxide was prepared and was standardized by titration with potassium hydrogen phthalate (Schwarzenbach and Bidermann 1948). The method consisted of the potentiometric titration of ligand with standard sodium hydroxide in the absence and presence of metal ions. For every titration fresh solid ligand was weighed out into the reaction cell to avoid possible hydrolysis. The concentrations of ligand and metal ion were of the order of  $1 \times 10^{-3}$  M. The ionic strength was kept constant by using 0.1 M KNO<sub>3</sub> solution as supporting electrolyte. The titrations, binary (1:1) and ternary (1:1:1), were carried out at  $35 \pm 0.1^\circ\text{C}$  temperature. The other details about experimentation can be obtained from our earlier work (Rabindra Reddy *et al* 1984).

## 3. Calculations

### 3.1 Dissociation constants

The acid dissociation constants of the primary ligand thymidine and secondary ligands glycine, histidine, histamine, *o*-phen and bipy were calculated by the usual algebraic method.

## 3.2 Stability constants

In order to calculate the stability constants of binary complexes of Cu(II), Ni(II), Zn(II), Co(II), Mg(II), Ca(II); La(III), Pr(III), Nd(III), Sm(III), Gd(III), Dy(III) with thymidine in a 1:1 ratio in the buffer region between  $m = 0$  and 1 (where  $m =$  moles of base added per mole of metal ion) the following equations were employed (charges omitted for clarity),



with related equilibria,



$$K_{ML}^M = \frac{T_M - [M]}{[M][L]}, \quad (3)$$

where

$T_M =$  total concentration of metal ion species,

$[M] =$  concentration of unbound metal ion

$[L] =$  concentration of unbound ligand.

Equations (4)–(7) were used to calculate stability constants of ternary complexes of Cu(II), Ni(II), Zn(II), Co(II) with thymidine and histidine or histamine; Cu(II), Ni(II), Zn(II), Co(II), Mg(II), Ca(II) with thymidine and *o*-phen or bipy and Cu(II):thymidine:glycine system in a (1:1:1) ratio.

It is assumed that in the buffer region between  $m = 0$  and 2, a simple (1:1) metal:secondary ligand complex is formed and in the buffer region between  $m = 2$  and 3, the ternary complex is formed. Accordingly the equations used were (omitting charges)

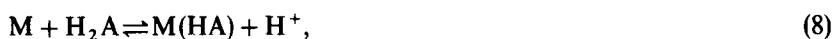


$$K_{MAL}^{MA} = \frac{T_M - [M]}{[MA][L]}, \quad (7)$$

where MA is the (1:1) metal:secondary ligand complex, HL = thymidine,  $H_2A =$  glycine, histidine, histamine, *o*-phen, bipy.

To calculate the stability constants of the ternary systems Ni(II), Zn(II), Co(II), Mg(II) and Ca(II) with thymidine and glycine; Mg(II) and Ca(II) with thymidine and histidine or histamine; lanthanons with thymidine and glycine or histidine in a (1:1:1) ratio, (8) to (13) were employed.

It is assumed that in the buffer region between  $m = 0$  and 1 a monoprotonated metal:secondary ligand complex is formed [(8)–(10)] and in the buffer region between  $m = 1$  and 3, a ternary complex is formed [(11)–(13)].





$$K_{MHA}^M = \frac{T_M - [M]}{[M][HA]}, \quad (10)$$



$$K_{MAL}^{MHA} = \frac{T_M - [M]}{[M][A][L]}, \quad (13)$$

where HL = thymidine; H<sub>2</sub>A = glycine, histidine or histamine, MHA = protonated (1:1) metal secondary ligand complex.

Stability constants were calculated by setting up suitable material balanced equations as dictated by experimental evidence with the help of Computer Programme BEST and the species distribution curves with respect to pH were generated with the program SPC (Motekaitis and Martell 1982).

#### 4. Results

The proton dissociation constants for the ligands thymidine, glycine, histidine, histamine, *o*-phen and bipy are listed in table 1.

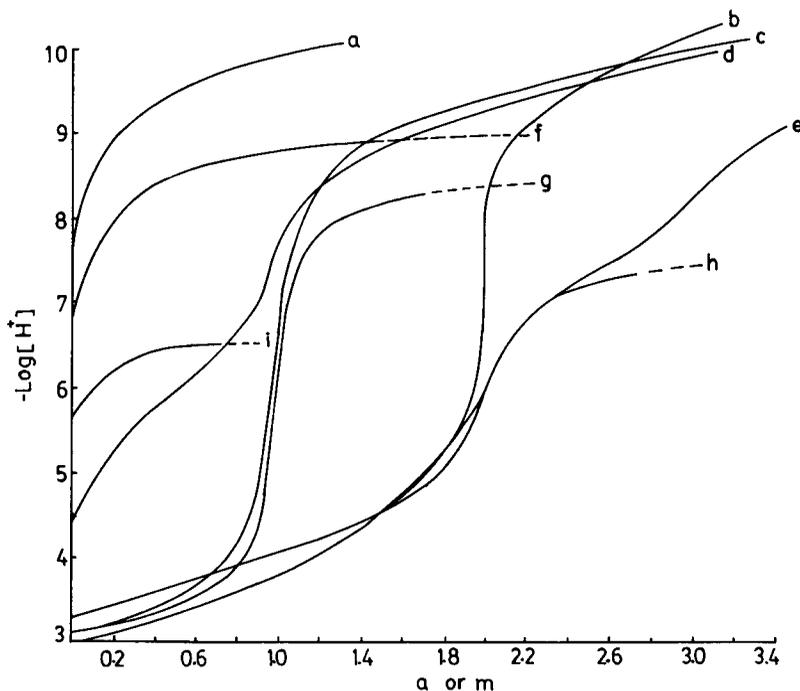
##### 4.1 Metal:thymidine (1:1) systems

The titration curves of Cu(II)/La(III):thymidine in (1:1) ratio are given in figure 1 i, f. A precipitate appeared after  $m = 0.7$  and the stability constants were calculated using (3) taking the experimental points below the precipitation region. Similar results were observed for Ni(II), Co(II), Pr(III), Nd(III), Sm(III), Gd(III), Dy(III) systems. For the other systems [Ca(II), Mg(II), Zn(II)], the constants were calculated in the entire buffer region between  $m = 0$  and 1 and the constants thus calculated are presented in tables 2 and 3.

To assess the influence of pH on the concentrations of several species present in aqueous solutions of the binary (1:1) Cu(II):thymidine systems a species distribution curve (figure 2) has been obtained and it shows that metal complexation begins only

**Table 1.** Acid dissociation constants of ligands.  
Temperature = 35°C;  $I = 0.10 \text{ mol dm}^{-3}$  (KNO<sub>3</sub>)

Ligand	$pK_a$	$pK_{2a}$
Thymidine	$9.54 \pm 0.01$	—
Glycine	$2.50 \pm 0.02$	$0.75 \pm 0.02$
Histidine	$6.00 \pm 0.04$	$9.00 \pm 0.04$
Histamine	$5.87 \pm 0.04$	$9.63 \pm 0.04$
<i>o</i> -Phenanthroline	$2.16 \pm 0.03$	$4.99 \pm 0.03$
$\alpha, \alpha'$ -Bipyridyl	—	$4.36 \pm 0.02$



**Figure 1.** Potentiometric titration curves for the interaction of thymidine with Mg(II), Cu(II) and La(III) in both binary (1:1) and ternary (1:1:1) systems at 35°C,  $I = 0.10 \text{ mol dm}^{-3}$  ( $\text{KNO}_3$ ). (a) Free thymidine; (b) Mg(II):thymidine:bipy; (c) Mg(II):thymidine:glycine; (d) Mg(II):thymidine:histidine; (e) Cu(II):thymidine:histidine; (f) La(III):thymidine; (g) La(III):thymidine:glycine; (h) Cu(II):thymidine:glycine; (i) Cu(II):thymidine.  $m$  = moles of base added per mole of metal ion, for curves b, c, d, e, f and g;  $a$  = mole of base added per mole of ligand, for curves a and i.

around neutral pH and gradually increases with pH. This is consistent with our  $pK_a$  data and pH range.

#### 4.2 Metal:thymidine:glycine (1:1:1) system

The mixed ligand titration curve of Cu(II):thymidine:glycine (figure 1h) shows an inflection at  $m = 2$  followed by a buffer region between  $m = 2$  and 3. In view of the closeness of the  $pK_{2a}$  of glycine and  $pK_a$  of thymidine both the possibilities ( $K_{M(HA)L}^M$  and  $K_{MA}^M$ ) were computed in the buffer region between  $m = 0$  and 2. However, it was confirmed on comparison with (1:1) Cu(II):glycine binary stability data that only 1:1 Cu(II):glycine ( $K_{MA}^M$ ) complex is formed in that buffer region. The ternary constant ( $K_{MAL}^{MA}$ ) was calculated in the buffer region between  $m = 2$  and 3.

The titration curves of Mg(II) (figure 1c) and Ca(II) ternary systems showed an inflection at  $m = 1$  followed by a buffer region, where the simultaneous release of two protons was assumed and the constant  $K_{MAL}^{MHA}$  was calculated between  $m = 1$  and 3. Ni(II), Co(II), Zn(II) ternary systems also showed an inflection at  $m = 1$  followed by a buffer region. However, a precipitate appeared after  $m = 2.4$  and the stability constants were calculated taking the experimental points below the precipitation region.

The mixed ligand titration curve of La(III):thymidine:glycine (figure 1g) showed an

**Table 2.** Stability constants\* of the binary and ternary complexes of thymidine (HL) with glycine (H<sub>2</sub>A), histidine (H<sub>2</sub>A), histamine (H<sub>2</sub>A), o-Phen (HA) and bipy (H<sub>2</sub>A).  
Temperature = 35°C; I = 0.10 mol dm<sup>-3</sup> (KNO<sub>3</sub>)

Metal ion (II)	M(II):Thy (1:1)		M(II):Thy:Gly (1:1:1)		M(II):Thy:histidine (1:1:1)		M(II):Thy:histamine (1:1:1)		M(II):Thy:o-phen (1:1:1)		M(II):Thy:bipy (1:1:1)	
	log K <sub>ML</sub> <sup>M</sup>	log K <sub>MAL</sub> <sup>MA</sup>	log K <sub>MAL</sub> <sup>MA</sup>	log K <sub>MAL</sub> <sup>MHA</sup>	log K <sub>MAL</sub> <sup>MA</sup>	K <sub>MAL</sub> <sup>MHA</sup>	log K <sub>MAL</sub> <sup>MA</sup>	K <sub>MAL</sub> <sup>MHA</sup>	log K <sub>MAL</sub> <sup>MA</sup>	K <sub>MAL</sub> <sup>MHA</sup>	log K <sub>MAL</sub> <sup>MA</sup>	log K <sub>MAL</sub> <sup>MA</sup>
Cu	5.95	—	5.21	—	5.56	—	5.42	—	5.77	—	6.14	—
Ni	3.61	—	—	11.78	—	—	—	—	3.71	—	3.40	—
Zn	5.26	—	—	11.66	—	—	—	—	4.71	—	5.22	—
Co	3.54	—	—	10.52	—	—	—	—	3.12	—	3.62	—
Mg	2.40	—	—	8.29	—	8.43	—	—	2.33	—	2.39	—
Ca	2.84	—	—	8.41	—	8.46	—	—	8.36	—	2.54	—

\* Deviations omitted for clarity.

**Table 3.** Stability constants\* of binary and ternary complexes of thymidine (HL) with glycine (H<sub>2</sub>A) and histidine (H<sub>2</sub>A).  
Temperature = 35°C; I = 0.10 mol dm<sup>-3</sup> (KNO<sub>3</sub>)

Metal ion (III)	M(III):Thy (1:1)		M(III):Thy:Gly (1:1:1)		M(III):Thy:Histidine (1:1:1)	
	log K <sub>ML</sub> <sup>M</sup>	log K <sub>MAL</sub> <sup>MA</sup>	log K <sub>MAL</sub> <sup>MA</sup>	log K <sub>MAL</sub> <sup>MHA</sup>	log K <sub>MAL</sub> <sup>MA</sup>	log K <sub>MAL</sub> <sup>MHA</sup>
La	4.50	—	10.61	—	10.80	—
Pr	4.78	—	12.19	—	12.20	—
Nd	5.20	—	12.29	—	12.33	—
Sm	5.22	—	12.84	—	12.88	—
Cd	5.28	—	13.20	—	13.25	—
Dy	5.36	—	13.84	—	13.76	—

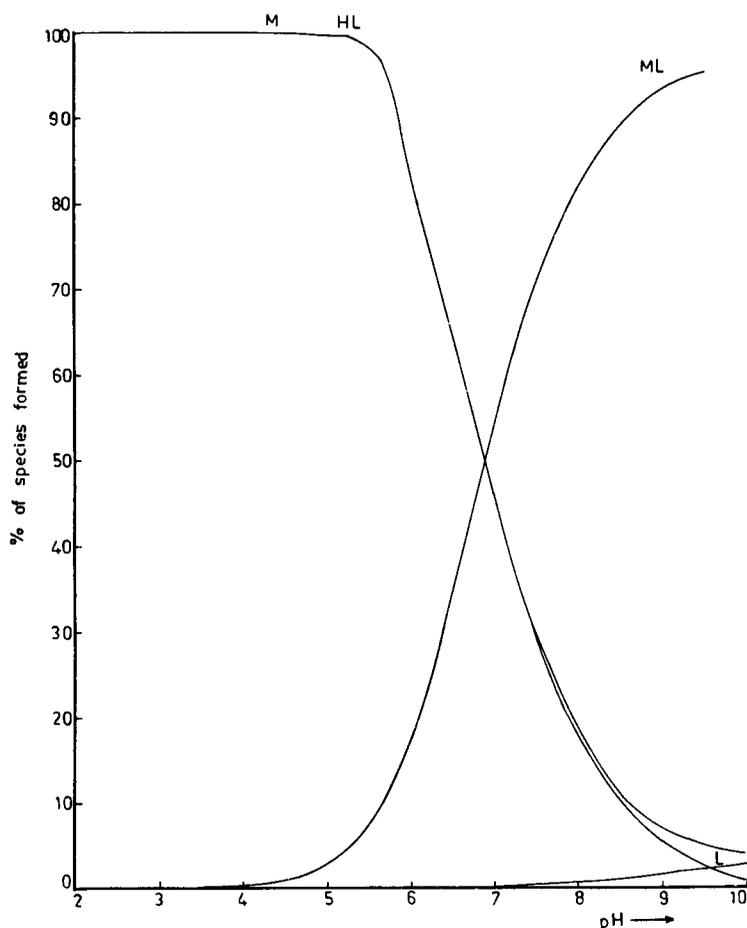


Figure 2. Species distribution curve of the Cu(II):thymidine (1:1) binary system.

inflection at  $m = 1$  followed by a buffer region. A precipitate appeared after  $m = 2.0$ . The constant  $K_{MAL}^{MHA}$  was calculated in that buffer region taking the experimental points below the precipitation region. The titration curves of all other trivalent ternary systems showed similar behaviour.

The stability constants are presented in tables 2 and 3.

#### 4.3 Metal:thymidine:histidine (1:1:1) system

The mixed ligand titration curve of Cu(II):thymidine:histidine system (figure 1e) shows an inflection at  $m = 2$  indicating the formation of a binary (1:1) Cu(II):thymidine complex in the buffer region between  $m = 0$  and 2. This is confirmed on comparison with  $K_{MA}^M$  data of the binary (1:1) metal:histidine system. The ternary constant  $K_{MAL}^{MA}$  was calculated in the buffer region between  $m = 2$  and 3 using (3). A species distribution curve of this ternary system between pH range 2 and 10 is shown in figure 3. Our assumption that the 1:1 metal:histidine complex is formed in the buffer region between  $m = 0$  and 2 is clearly substantiated and shown in the species distribution curve, further the formation of this complex reaches a maximum at

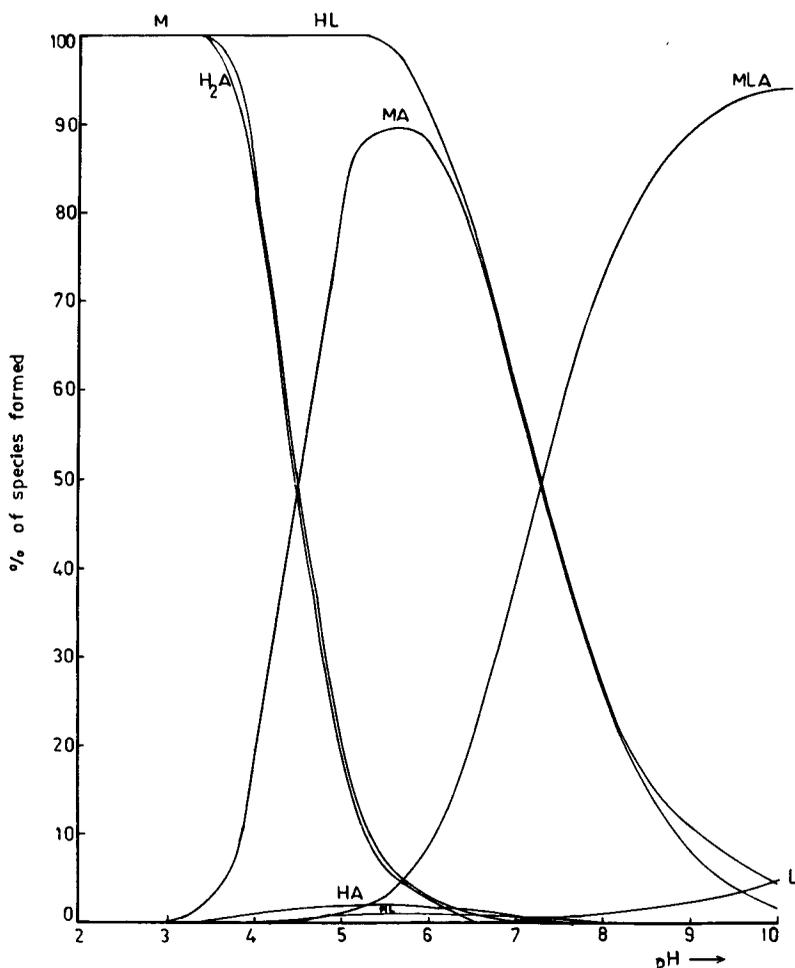


Figure 3. Species distribution curve of the Cu(II):thymidine:histidine (1:1:1) ternary system.

pH = 6. The formation of ternary complex (i.e.  $MA + HL \rightleftharpoons MAL$ ) begins at neutral pH and reaches a maximum around pH 10.

An inflection at  $m = 2$  was also observed for Ni(II), Zn(II), Co(II) ternary systems but the ternary constant  $K_{MAL}^{MA}$  could not be calculated due to precipitation around  $m = 2$ . In the case of Mg(II) (figure 1d) and Ca(II) ternary systems an inflection was obtained at  $m = 1$  indicating the formation of monoprotonated metal:histidine complex in the buffer region between  $m = 0$  and 1 which was confirmed on comparison with 1:1 M(II):histidine data. The ternary constant  $K_{MAL}^{M(HA)}$  was calculated in the buffer region between  $m = 1$  and 3 with the help of (13). The constants thus calculated are presented in table 2.

The titration curve of the Dy(III):thymidine:histidine system shows an inflection at  $m = 1$  indicating the formation of a monoprotonated M(III):histidine complex in the buffer region between  $m = 0$  and 1 and this was confirmed on comparing data in this region with monoprotonated (1:1) metal ligand system stability data. Due to the appearance of a solid phase after  $m = 2$  the constant was calculated taking

experimental points below the precipitation region. Similar trends were also observed for the remaining ternary systems of trivalent metal ions.

#### 4.4 Metal:thymidine:histamine (1:1:1) system

This system shows behaviour similar to the metal:thymidine:histidine system for bivalent metal ions and the constants calculated are listed in table 2. In the case of trivalent metal:thymidine:histamine system ternary stability data could not be computed because of the appearance of a precipitate in the buffer region.

#### 4.5 Metal:thymidine:bipy/o-phen (1:1:1) system

The mixed ligand titration curves of M(II):thymidine:o-phen/bipy show an inflection at  $m = 2$  indicating the formation of a (1:1) metal:o-phen/bipy complex which was confirmed on comparison with binary data. In the buffer region between  $m = 2$  and 3 the formation of a ternary complex is assumed and the constant  $K_{MAL}^{MA}$  was calculated with the help of (7) and the constants presented in table 2.

### 5. Discussion

The  $pK_a$  value of thymidine (9.54) is in fair agreement with the literature data (Levene *et al* 1926; Izatt *et al* 1971). The closeness in the  $pK_a$  values of thymine (9.51) (Taqi Khan and Satyanarayana 1982) and thymidine suggest that the proton ionisation is from the same site i.e., N(3)-H. A comparison of the dissociation constants of thymidine and uridine (9.01) (Rabindra Reddy and Malleshwar Rao 1986) indicate that thymidine is slightly more basic than uridine. This could be due to the presence of electron-donating methyl group in the C-5 position of the pyrimidine ring. A similar increase in the  $pK_a$  value has been observed in the case of 5-alkyl uracil (Kulakowska *et al* 1974).

The stabilities of 1:1 bivalent metal:thymidine complexes are reported in table 2. The constants increase in the order: Mg(II) < Ca(II) < Co(II) < Ni(II) < Cu(II) > Zn(II), which is the Irving-Williams order of stability. The stability constants of 1:1 trivalent metal:thymidine increase in the order: La(III) < Pr(III) > Nd(III) < Sm(III) < Gd(III) < Dy(III) which indicates the dependence of stability constants on the ionic radii, a trend that is observed for a variety of ligands with the lanthanons (Clanwaert and Stockx 1968).

It can be seen from tables 2 and 3 that the lanthanide complexes are more stable than the corresponding bivalent metal complexes. This can be explained on the basis of greater positive charges on the trivalent metal ions which permit closer approach of ligands resulting in better electrostatic interaction.

It is of interest here to compare the 1:1 binary stability constants of uridine (Rabindra Reddy and Malleshwar Rao 1986) with these constants. The slight differences in the stabilities of thymidine and uridine systems are due to the differences in their basicities. However, the closeness in the magnitude of the data suggest that the mode of bonding is similar in both the systems. In the case of uridine it was shown that N(3) and O(4) are involved in metal coordination (Rabindra Reddy and Malleshwar Rao 1986). Based on stereochemistry, basicity and experimental pH range of the systems under investigation we conclude that thymidine also acts as a bidentate

**Table 4.**  $\Delta \log K$  values for metal(II)–ligand systems in solution.  
Temperature = 35°C;  $I = 0.10 \text{ mol dm}^{-3}$  ( $\text{KNO}_3$ )

Metal ion (II)	M(II):Thy:Gly (1:1:1)	M(II):Thy:Histidine (1:1:1)	M(II):Thy:Histamine (1:1:1)	M(II):Thy: <i>o</i> -Phen (1:1:1)	M(II):Thy:bipy (1:1:1)
Cu	– 0.74	– 0.39	– 0.53	– 0.18	+ 0.32
Ni	+ 1.25	—	—	+ 0.10	– 0.21
Zn	+ 0.90	—	—	– 0.55	– 0.04
Co	+ 1.76	—	—	– 0.10	– 0.08
Mg	+ 2.49	—	—	+ 0.34	– 0.01
Ca	+ 1.99	—	—	– 0.58	– 0.30

ligand involving N(3) and O(4) in metal bonding. The earlier suggestion that thymidine binds predominantly through the deoxy ribose unit (Goodgame and Johns 1978) is ruled out as it is possible only at high pH.

The stability constants pertaining to the formation of ternary complexes are listed in tables 2 and 3. It can be seen from the tables that bipy and *o*-phen form similar complexes whereas glycine, histidine and histamine form different types of complexes. This shows the high specificity and selectivity of metal–ligand interactions in solution. Even though direct comparison cannot be made, some generalisations could be made by comparing the absolute values of the stability constants of various ternary systems. The metal:thymidine:bipy system is more stable compared to the *o*-phen system. The lower stability of the latter is due to its greater rigidity. In the case of glycine, histidine and histamine systems, a comparison could be made only for Cu(II), Mg(II) and Ca(II) complexes. Histidine forms more stable ternary complexes followed by histamine and glycine. Similar results were also obtained in the case of trivalent ternary systems. This type of stabilisation in histidine complexes has been observed earlier (Rabindra Reddy and Harilatha Reddy 1985).

The  $\Delta \log K$  [the difference between overall (1:1:1) and (1:1) constants] values of bivalent ternary systems are listed in table 4. These values could not be computed for trivalent ternary systems owing to the formation of different types of complexes in binary and ternary systems. It can be seen from the table that an effective comparison could be made only in the case of Cu(II) system. The  $\Delta \log K$  values follow the order glycine < histamine < histidine < *o*-phen < bipy which are in accordance with their respective reactive tendencies. Based on these data some conclusions can be made about the nature of histidine as a ligand. The data suggest that histidine shows histamine-like behaviour. It is important to note here that histidine is glycine-like if there is only one histidine in the system, and if there are two histidines, the first may be glycine-like and the second histamine-like (Sigel and McCormick 1971). For the rest of the metal ions in case of *o*-phen and bipy systems the  $\Delta \log K$  values are negative compared to the high positive values in the case of the glycine system. This is due to the fact that in the case of glycine the values are calculated in the buffer region between  $m = 1$  and 3 taking into account the  $pK_a$  of thymidine and  $pK_{2a}$  of glycine. In contrast to that only the  $pK_a$  of thymidine was used in the calculation as dictated by experimental observations.

With a view to obtain clear understanding about the nature of bonding in these systems, further studies are in progress in this laboratory.

## Acknowledgements

Financial assistance from the University Grants Commission is gratefully acknowledged.

## References

- Allore B D, Queen A, Blonskii W J and Huiska F E 1983 *Can. J. Chem.* **61** 2397  
Bruce R 1985 *Acc. Chem. Res.* **18** 32  
Carrabine J A and Sundaralingam M 1971 *Biochemistry* **10** 292  
Clanwaert J and Stockx J 1968 *Z. Naturforsch.* **B23** 25  
Deshpande S V, Sharma R K and Srivastava T S 1983 *Inorg. Chim. Acta* **78** 13  
France C and Beauchamp A L 1985 *Can. J. Chem.* **63** 3456  
Goodgame M and Johns K W 1978 *J. Chem. Soc., Dalton Trans.* 1294  
Inagoki K and Kidani Y 1978 *Bioinorg. Chem.* **9** 333  
Izatt R M, Christensen J J and Rytting J H 1971 *Chem. Rev.* **71** 439  
Kim S H and Bruce M R 1984 *Inorg. Chim. Acta* **91** 19  
Kolthoff I M and Elving P J (eds) 1963 *Treatise on analytical chemistry* (New York: Interscience) vol. 8, part II, p. 51  
Kosturko L D, Folzer C and Stewart R F 1974 *Biochemistry* **13** 3949  
Kostowycz G and Suzuki O 1973 *Biochemistry* **12** 18  
Kulakowska I, Geller M, Lesyng B and Wierzychowski K L 1974 *Biochem. Biophys. Acta* **361** 119  
Levene P A, Lawrence W B and Simms H S 1926 *Biochemistry* **70** 229  
Motekaitis J R and Martell A E 1982 *Can. J. Chem.* **60** 2403  
Raoul M B, Michelle P R, Germaine T D, Gerald P and Bernard P 1987 *Can. J. Chem.* **65** 1479  
Ramalingam K and Krishna Murthy C R 1982 *Inorg. Chim. Acta* **67** 167  
Rabindra Reddy P and Harilatha Reddy M 1983 *Polyhedron* **2** 1171  
Rabindra Reddy P and Harilatha Reddy M 1985 *J. Chem. Soc., Dalton Trans.* 239  
Rabindra Reddy P, Harilatha Reddy M and Prasad Reddy T R 1985 *Proc. Indian Acad. Sci (Chem. Sci.)* **95** 547  
Rabindra Reddy P, Harilatha Reddy M and Venugopal Reddy K 1984 *Inorg. Chem.* **22** 974  
Rabindra Reddy P and Malleshwar Rao V B 1986 *J. Chem. Soc., Dalton Trans.* 2331  
Rabindra Reddy P and Venugopal Reddy K 1983 *Inorg. Chim. Acta* **80** 95  
Rabindra Reddy P, Venugopal Reddy K and Taqui Khan M M 1976 *J. Inorg. Nucl. Chem.* **38** 1923  
Rabindra Reddy P, Venugopal Reddy K and Taqui Khan M M 1978 *J. Inorg. Nucl. Chem.* **40** 1265  
Rabindra Reddy P, Venugopal Reddy K and Taqui Khan M M 1979 *J. Inorg. Nucl. Chem.* **41** 423  
Rabindra Reddy P, Venugopal Reddy K and Taqui Khan M M 1983 *Indian J. Chem.* **22** 999  
Schwarzenbach G and Bidermann R 1948 *Helv. Chim. Acta* **31** 311  
Schwarzenbach G 1957 *Complexometric titration* (New York: Interscience) p. 77  
Sigel H and McCormick O B 1971 *J. Am. Chem. Soc.* **93** 2041  
Tang W, Zheng S, Yuan C and Tai A 1984 *Gaodeng Xuexiao Huaxue Xuebao* **5** 1  
Taqi Khan B, Goud N G and Vijaya Kumari S 1983 *Inorg. Chim. Acta* **80** 145  
Taqi Khan B and Madhusudan Raju R 1981 *Indian J. Chem.* **20** 860  
Taqi Khan M M and Satyanarayana S 1982 *Indian J. Chem.* **21** 913