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Potentiometric Studies on Binary and Ternary Complexes of Di- and Trivalent Metal Ions Involving Some Hydroxamic Acids, Amino Acids, and Nucleic Acid Components

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Summary. Formation of binary and ternary complexes of Cu^{II}, Co^{II}, Ni^{II}, Zn^{II}, Fe^{III}, Al^{III}, and Cr^{III} metal ions with some selected aliphatic and aromatic hydroxamic acids and some biologically important amino acids or nucleic acid components was investigated using the potentiometric technique at 25°C and $I = 0.10\,\text{mol}\,\text{dm}^{-3}$ $NaNO_3$. The acid-base properties of the ligands were investigated and discussed. The acidity constants of the ligands were determined and used for determining the stability constants of the complexes formed in aqueous medium under the experimental conditions. The ternary complex formation was found to occur in a stepwise manner. The stability constants of these binary and ternary systems were calculated. The order of stability of the ternary complexes in terms of the nature of hydroxamic acid, amino acid, nucleic acid component and metal ions was investigated and discussed as well as the values of $\Delta \log K$ and $\log X$ for the ternary systems. The concentration distribution of the various complex species in solution was evaluated. In addition, evaluation of the effect of temperature of the medium on the stability of the ternary system M^{III} – benzohydroxamic acid – L-histidine or adenine ($M^{III} = Fe^{III}$, Al^{III}, and Cr^{III}) has been studied. The thermodynamic parameters were calculated and discussed.

Keywords. Amino acids; Binary and ternary complexes; Hydroxamic acids; Nucleic acid components; Potentiometric studies; Stability constants.

Introduction

Hydroxamic acids and their derivatives play an important role in living systems. They have been found as constituents of therapeutics, mostly related with the

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microbial transport of iron and the iron-overload chelating therapy [1]. Recently, hydroxamic acids have also been extensively studied as metal binding complexing agents in the field of zinc and nickel metalloenzyme inhibition, namely as inhibitors of matrix metalloproteinase (*MMP*) [2, 3] and urease [4, 5], respectively. Most of them have even advanced into human clinical trials for the treatment of diseases such as cancer and arthritis. However, patients treated with hydroxamic acid drugs such as siderphore desferrioxamine B (*DF*), a trihydroxamic acid, frequently experienced problems during clinical treatment. These problems include agranurocytosis, joint pains, lupus-like syndromes as well as gastric intolerance [6]. More recently, a new oral iron chelator, salicylhydroxamic acid (*Sham*) has been developed and found to have a promising advantage, since no toxicity has, as yet, been recorded [7]. The iron chelating property is due to presence of the hydroxamic acid moiety (−CO–NOH), which it shares with desferrioxamine B and another lower molecular weight iron chelator, acetohydroxamic acid (*Aha*), which may also have potential as iron chelator [8]. A detailed knowledge of the complexation behavior of hydroxamic acids with metal ions, often existing in biological fluids, has confirmed the importance of these analytical reagents in medicine.

Solution equilibria involved in the formation of binary and ternary complexes of transition metal^{II} ions involving salicylhydroxamic acid and other biologically relevant aminopolycarboxylates, such as N-(2-acetamido)iminodiacetic acid (*ADA*), iminodiacetic acid (*IDA*), and nitrilotriacetic acid (*NTA*), have been studied by us [9] using the *pH*-metric technique. Recently, stabilities of binary and ternary complexes of some transition and alkaline earth metal^{II} ions with ribonucleotides (*AMP*, *ADP*, and *ATP*) and salicylhydroxamic acid were also investigated by us [10] using the same technique.

In continuation of our published work oriented toward the study of complexation equilibria and the determination of stability constants of biologically important complexes [9–16], the present work concerns a study of the solution equilibria involved in the formation of binary and ternary complexes of divalent and trivalent metal ions containing some selected aliphatic and aromatic hydroxamic acids (acetohydroxamic acid (*Aha*), benzohydroxamic acid (*Bha*), and salicylhydroxamic acid (*Sham*)) and other biologically relevant ligands (α-amino acids and nucleic acid components), to investigate the complexation behavior of these systems and to determine the stability constants of the complexes formed in solution, using the *pH*-metric titrations, as these systems mimic many biological reactions (amino acid or nucleic acid component – metal ion – drug interactions). The study adopts the *Irving* and *Rossotti* technique [17] for the determination of stability constants of the different proton-ligands as well as the binary and ternary complexes.

Results and Discussion

Acidity Constants of the Free Ligands

Acetohydroxamic acid and benzohydroxamic acid can each release one proton in the measurable *pH*-range. The pK_a value for benzohydroxamic acid (8.63 ± 0.03)

is found to be lower than that for acetohydroxamic acid (9.35 ± 0.05), which can be attributed to the electron withdrawing effect of the phenyl moiety in the former hydroxamic acid.

Salicylhydroxamic acid contains two acidic groups, the hydroxamic acid and the phenolic OH groups for which pK_a values of 7.40 ± 0.02 and 9.75 ± 0.07, respectively, were obtained at 25°C and $I = 0.1\ \text{mol dm}^{-3}$ $NaNO_3$. The greater acidity of the hydroxamic acid OH group in salicylhydroxamic acid relative to that of benzohydroxamic acid is due to stabilization of the conjugate base by intermolecular hydrogen bonding with the phenolic OH group. The results obtained are in good agreement with literature values [9, 18, 19].

The acid formation constant values for glycine ($pK_{a_2} = 9.63 \pm 0.06$), valine ($pK_{a_2} = 9.65 \pm 0.04$), lysine ($pK_{a_2} = 9.25 \pm 0.04$), serine ($pK_{a_2} = 9.20 \pm 0.03$), and histidine ($pK_{a_2} = 6.05 \pm 0.04$, $pK_{a_3} = 9.10 \pm 0.08$) were also determined under the same experimental conditions from the *pH*-metric titration curves, and the results agree fairly well with data reported previously in the literature [20], after allowing for changes in experimental conditions as well as methods of calculation. It is worth mentioning that the pK_{a_1} values of the amino acids investigated are too low (≤ 2.30) [21] and exist only in strongly acidic solutions. Therefore, these values are not used in our calculations, since the *pH*-metric data are measured in the range $3 \leq pH \leq 10.5$.

As indicated earlier [22] the N_9H^+ group has been established as the site of proton ionization for neutral adenine. The pK_a value (9.75 ± 0.03) agrees quite well with that determined previously using the calorimetric technique. The dissociation constant of cationic inosine and uridine could not be calculated potentiometrically using the glass electrode because of the low pK_a values, $pK_a = 1.2$ [23] for inosine and $pK_a < 0.5$ for uridine [24]. The first proton association constant of neutral inosine, H_2L, was determined potentiometrically by us. Proton dissociation was assigned to the N_1H group with the oxygen at the 6 position bearing the negative charge. Our pK_a value (8.65 ± 0.03) agrees well with that previously reported using the spectrophotometric technique [23]. The first proton association constant of neutral uridine was also determined under the same experimental conditions. The value obtained from this work ($pK_a = 9.20 \pm 0.05$) has been compared with that of the protonation of the analogous N_1H grouping in inosin ($pK_a = 8.65 \pm 0.03$). Inosine is slightly more acidic than uridine, a property which can be attributed to the existence of a higher number of resonance forms in the anion of the purinic inosine derivative due to the presence of two condensed rings in this ligand. Based on the existing data, uridine is ligating in the deprotonated form, through the N_3 atom. It is worth mentioning that the second dissociation of the proton from the ribose OH group of both inosine and uridine, takes place at higher *pH* (>12) and hence could not be measured using the *pH*-metric technique.

Stability Constants of Binary Metal Complexes

Potentiometric *pH* titrations of Cu^{II}, Co^{II}, Ni^{II}, Zn^{II}, Fe^{III}, Al^{III}, and Cr^{III} were performed at both 1:1 and 1:2.5 metal/ligand molar ratios. Representative potentiometric titration curves for the various metal ion-ligand complexes with 1:2.5

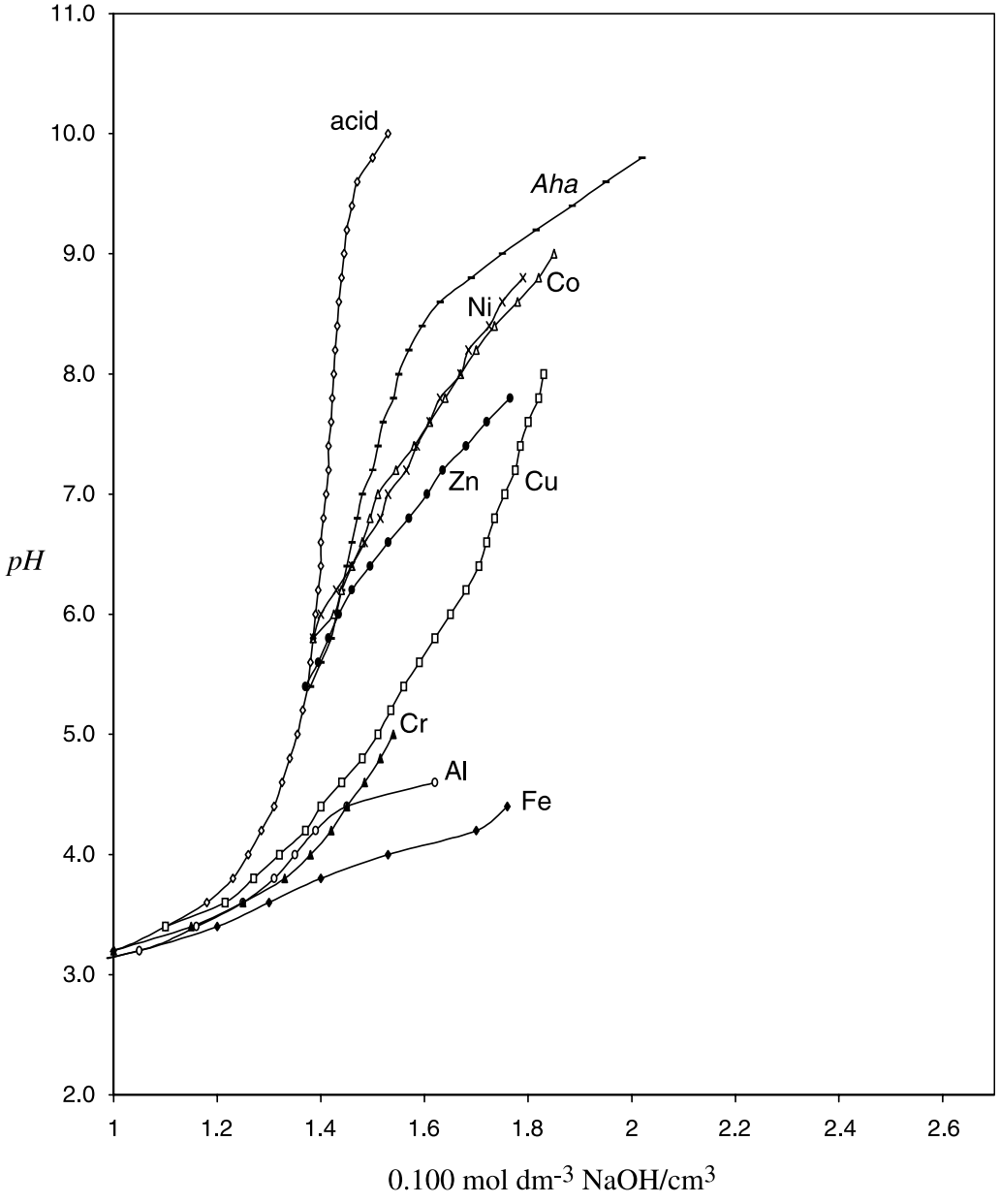

Fig. 1. Potentiometric *pH* titration curves for acetohydroxamic acid, and its metal M^{II} and M^{III} complexes (M^{II} = Cu, Co, Ni, and Zn; M^{III} = Fe, Al, and Cr); $C_L/C_M = 2.5$, $C_L = 1 \times 10^{-3}$ mol dm^{-3} at 25°C and $I = 0.1$ mol dm^{-3} $NaNO_3$

stoichiometry are shown in Fig. 1 for acetohydroxamic acid (*Aha*) systems together with the titration curves for the strong acid (HNO_3) and the free ligand.

Analysis of the complexed ligand curves (Fig. 1) indicates that the addition of metal ion to the free ligand solutions shifts the buffer region of the ligand to lower *pH* values. This shows that complex formation reactions proceed by releasing of protons from such ligands.

Generally, it is observed that the binary metal^{II} and metal^{III} complexes of hydroxamic acids begin to form in the *pH* range of 3.6–6.4 and 2.6–3.2, respectively. With respect to the titration curves of the binary metal^{II}-amino acid or nucleic acid component complexes, one may deduce that these complexes start to form in the *pH* range 3.4–6.8, except in the case of histidine binary complex systems titration curves, where the corresponding complexes begin to form in the *pH* range 3.0–5.0. For metal^{III}-amino acid or nucleic acid component titration curves, it can be deduced that the complexes are formed in the *pH* range 2.8–3.4.

The stability constants of both 1:1 and 1:2 binary complexes of the considered ligands have been determined at 25°C and $I = 0.1\ \text{mol dm}^{-3}$ $NaNO_3$. It can be observed that the stability constants of the different 1:2 metal-ligand complexes are lower than those of the corresponding 1:1 systems, as expected from statistical considerations. The $\Delta \log K$ ($\log K_2 - \log K_1$) values are negative (Tables 1–7). This is the normal trend in neutral ligands where the enthalpy is more favourable for a 1:1 species (exothermic) as compared to a 1:2 species.

Table 1. Stability constants for Cu^{II} binary and ternary complexes at 25 ± 0.1°C and $I = 0.1\ \text{mol dm}^{-3}$ $NaNO_3$

	$\log K_1$	$\log K_2$	$\log K^{MA}_{MAL}$		
			Aha	*Bha*	*Sham*
Aha	8.03 ± 0.02	5.95 ± 0.04			
Bha	7.63 ± 0.04	3.26 ± 0.03			
Sham	13.06 ± 0.06	3.40 ± 0.02			
Gly	7.93 ± 0.03	6.93 ± 0.06	7.62 ± 0.03	8.30 ± 0.02	9.94 ± 0.02
Val	7.88 ± 0.05	4.73 ± 0.08	7.50 ± 0.07	8.26 ± 0.02	8.96 ± 0.05
Lys	7.33 ± 0.02	3.98 ± 0.02	7.34 ± 0.02	8.20 ± 0.04	8.87 ± 0.02
Ser	7.05 ± 0.06	3.50 ± 0.04	7.17 ± 0.05	8.00 ± 0.02	8.65 ± 0.06
His	10.03 ± 0.03	4.74 ± 0.06	10.05 ± 0.03	10.09 ± 0.07	10.20 ± 0.02
Adn	7.70 ± 0.02	5.56 ± 0.02	8.27 ± 0.04	8.33 ± 0.03	10.20 ± 0.06
Urd	4.35 ± 0.05	3.60 ± 0.04	8.15 ± 0.05	8.24 ± 0.08	9.45 ± 0.02
Ino	5.05 ± 0.08	4.13 ± 0.05	7.72 ± 0.03	7.86 ± 0.04	9.23 ± 0.03

	$\log \beta^{M}_{MAL}$			$\Delta \log K$			$\log X$		
	Aha	*Bha*	*Sham*	*Aha*	*Bha*	*Sham*	*Aha*	*Bha*	*Sham*
Gly	15.65	15.93	23.00	−0.31	0.37	2.01	2.46	6.11	14.68
Val	15.53	15.89	22.02	−0.38	0.38	1.08	4.47	8.28	14.97
Lys	15.37	15.63	21.93	0.01	0.67	1.54	5.45	9.06	16.09
Ser	15.20	15.83	21.71	0.12	1.15	1.60	5.87	10.22	16.41
His	18.08	17.72	23.26	0.02	0.06	0.17	7.41	9.78	15.29
Adn	16.30	15.96	23.26	0.57	0.63	2.50	5.36	7.77	16.80
Urd	15.75	15.87	22.51	3.80	3.89	5.10	10.43	12.9	20.61
Ino	16.30	15.49	22.29	2.67	2.81	4.08	8.34	10.91	18.94

Table 2. Stability constants for Co^{II} binary and ternary complexes at $25 \pm 0.1°C$ and $I = 0.1$ mol dm^{-3} $NaNO_3$

	$\log K_1$	$\log K_2$	$\log K^{MA}_{MAL}$		
			Aha	*Bha*	*Sham*
Aha	4.93 ± 0.02	3.50 ± 0.04			
Bha	4.75 ± 0.04	3.26 ± 0.03			
Sham	6.59 ± 0.03	4.52 ± 0.06			
Gly	5.45 ± 0.02	4.03 ± 0.04	5.15 ± 0.06	5.30 ± 0.02	6.33 ± 0.04
Val	5.22 ± 0.05	3.73 ± 0.03	5.12 ± 0.07	5.24 ± 0.02	6.17 ± 0.03
Lys	4.45 ± 0.03	4.20 ± 0.07	4.98 ± 0.02	5.07 ± 0.04	5.60 ± 0.05
Ser	4.38 ± 0.06	3.52 ± 0.04	4.73 ± 0.06	4.91 ± 0.02	5.48 ± 0.06
His	6.96 ± 0.04	5.76 ± 0.02	6.35 ± 0.03	6.50 ± 0.07	7.58 ± 0.02
Adn	6.32 ± 0.02	5.45 ± 0.02	6.82 ± 0.05	6.89 ± 0.03	7.18 ± 0.08
Urd	4.12 ± 0.05	3.96 ± 0.03	5.21 ± 0.04	5.32 ± 0.08	5.93 ± 0.02
Ino	4.04 ± 0.07	3.40 ± 0.05	4.38 ± 0.02	4.62 ± 0.04	5.68 ± 0.06

	$\log \beta^{M}_{MAL}$			$\Delta \log K$			$\log X$		
	Aha	*Bha*	*Sham*	*Aha*	*Bha*	*Sham*	*Aha*	*Bha*	*Sham*
Gly	10.08	10.05	12.92	−0.03	−0.15	0.79	2.25	2.61	5.16
Val	10.05	9.99	12.76	−0.10	0.02	0.95	2.72	3.02	5.46
Lys	9.91	9.82	12.19	0.53	0.62	1.15	2.74	2.98	4.62
Ser	9.66	9.66	12.07	0.35	0.53	1.10	2.99	3.41	5.13
His	13.31	13.46	14.54	1.42	1.75	0.99	5.47	6.19	5.25
Adn	11.75	11.64	13.77	0.50	0.57	0.86	3.30	3.50	4.66
Urd	10.14	10.07	12.52	1.09	1.20	1.81	3.77	4.05	5.85
Ino	9.31	9.37	12.27	0.34	0.58	1.64	2.75	3.29	5.99

Stability Constants of the Ternary Complexes

A representative set of experimental titration curves obtained according to the sequence described in the experimental section for Cu^{II}-benzohydroxamic acid-serine and Ni^{II}-salicylhydroxamic acid-histidine systems under investigation are displayed in Figs. 2 and 3.

The existence of a ternary complex is proved by comparison of the mixed ligand titration curve with the composite curve obtained by graphical addition of the secondary ligand titration data to that of the 1:1 metal-primary ligand titration curve. Therefore, it is assumed that, in the presence of both ligands, hydroxamic acid (*A*) interacts first with the metal ion forming a 1:1 *MA* binary complex which is then followed by interaction of the amino acid or nucleic acid component (the ternary system involving Cu^{II}-benzohydroxamic acid-serine, taken as a representative, is shown in Fig. 2), *i.e.*, the ternary complex formation could be considered in stepwise equilibria (Eqs. (1) and (2)).

$$M + A \rightleftharpoons MA \tag{1}$$

$$MA + L \rightleftharpoons MAL \tag{2}$$

$$K^{MA}_{MAL} = \frac{[MAL]}{[MA][L]} \tag{3}$$

Table 3. Stability constants for Ni^{II} binary and ternary complexes at $25 \pm 0.1°C$ and $I = 0.1$ mol dm^{-3} $NaNO_3$

	$\log K_1$	$\log K_2$	$\log K_{MAL}^{MA}$		
			Aha	*Bha*	*Sham*
Aha	5.70 ± 0.03	4.73 ± 0.04			
Bha	5.05 ± 0.07	3.60 ± 0.05			
Sham	6.02 ± 0.03	3.95 ± 0.06			
Gly	6.82 ± 0.03	4.13 ± 0.04	6.18 ± 0.02	7.25 ± 0.06	7.75 ± 0.02
Val	6.68 ± 0.05	4.55 ± 0.03	5.98 ± 0.07	6.40 ± 0.02	6.95 ± 0.03
Lys	5.67 ± 0.04	3.89 ± 0.02	5.59 ± 0.04	5.60 ± 0.04	5.64 ± 0.05
Ser	5.46 ± 0.07	3.65 ± 0.04	5.26 ± 0.02	5.40 ± 0.03	5.58 ± 0.08
His	8.46 ± 0.04	6.73 ± 0.03	6.82 ± 0.06	7.50 ± 0.06	7.92 ± 0.02
Adn	7.13 ± 0.06	6.25 ± 0.02	6.93 ± 0.05	6.94 ± 0.02	7.18 ± 0.06
Urd	3.97 ± 0.05	3.70 ± 0.06	5.40 ± 0.02	5.50 ± 0.03	5.97 ± 0.02
Ino	3.68 ± 0.04	3.34 ± 0.05	5.05 ± 0.04	5.30 ± 0.08	5.73 ± 0.02

	$\log \beta_{MAL}$			$\Delta \log K$			$\log X$		
	Aha	*Bha*	*Sham*	*Aha*	*Bha*	*Sham*	*Aha*	*Bha*	*Sham*
Gly	11.88	12.29	13.77	−0.64	0.42	0.93	2.38	4.98	6.62
Val	11.68	11.45	12.97	−0.70	−0.28	0.27	1.70	3.02	4.74
Lys	11.29	10.65	11.66	−0.08	−0.07	−0.03	2.59	3.09	3.79
Ser	10.96	10.45	11.60	−0.2	−0.06	0.12	2.38	3.14	4.12
His	15.28	15.96	16.38	1.12	2.19	2.45	4.94	8.08	7.60
Adn	12.63	11.99	13.20	−0.20	−0.19	0.05	1.45	1.95	3.05
Urd	11.10	10.55	11.99	1.43	1.53	2.00	4.10	4.78	6.34
Ino	10.75	10.35	11.75	1.37	1.62	2.05	4.05	5.03	6.51

The overall stability constant β_{MAL}^{M} may be represented by Eq. (4).

$$M + A + L \rightleftharpoons MAL \tag{4}$$

$$\beta_{MAL}^{M} = \frac{[MAL]}{[M][A][L]} = K_{MAL}^{MA} \times K_{MA}^{M} \tag{5}$$

However, in the case of ternary systems involving the metal ions Co^{II}, Ni^{II}, and Zn^{II}, it was observed from the titration curves that the amino acid histidine acts as a primary ligand (A) whereas hydroxamic acids act as secondary ligands (L), as shown in Fig. 3 for the ternary system Ni^{II}-salicylhydroxamic acid-histidine.

The average number of moles of the secondary ligand coordinated to the 1:1 binary complex involving the primary ligand, $\bar{n}_{\text{mix}}$, is calculated as in the original paper [17]. From the values of $\bar{n}_{\text{mix}}$ so obtained, free secondary ligand exponent, pL_{mix} was calculated. Formation curves for Ni^{II}-salicylhydroxamic acid-amino acid or nucleic acid component system, taken as a representative, are shown in Fig. 4.

Table 4. Stability constants for Zn^{II} binary and ternary complexes at 25 ± 0.1°C and $I = 0.1$ mol dm^{-3} $NaNO_3$

	$\log K_1$	$\log K_2$	$\log K_{MAL}^{MA}$		
			Aha	*Bha*	*Sham*
Aha	5.75 ± 0.05	4.70 ± 0.04			
Bha	4.95 ± 0.04	4.26 ± 0.08			
Sham	5.88 ± 0.03	3.98 ± 0.05			
Gly	6.31 ± 0.05	5.47 ± 0.04	5.76 ± 0.06	5.92 ± 0.05	6.32 ± 0.07
Val	5.91 ± 0.05	5.45 ± 0.02	5.74 ± 0.02	5.80 ± 0.02	5.98 ± 0.03
Lys	5.76 ± 0.03	4.95 ± 0.07	5.64 ± 0.02	5.70 ± 0.04	5.93 ± 0.02
Ser	5.26 ± 0.06	4.53 ± 0.04	5.12 ± 0.06	5.28 ± 0.05	5.68 ± 0.05
His	7.15 ± 0.04	6.10 ± 0.02	6.10 ± 0.06	6.70 ± 0.03	7.34 ± 0.02
Adn	6.92 ± 0.04	6.85 ± 0.05	6.50 ± 0.05	6.57 ± 0.06	6.73 ± 0.06
Urd	3.84 ± 0.05	3.40 ± 0.03	5.00 ± 0.03	5.73 ± 0.02	5.90 ± 0.04
Ino	3.82 ± 0.06	3.35 ± 0.02	4.28 ± 0.02	5.22 ± 0.04	5.34 ± 0.03

	$\log \beta_{MAL}^{M}$			$\Delta \log K$			$\log X$		
	Aha	*Bha*	*Sham*	*Aha*	*Bha*	*Sham*	*Aha*	*Bha*	*Sham*
Gly	11.51	10.87	12.20	−0.55	−0.39	0.01	0.79	0.75	2.76
Val	11.49	10.75	11.86	−0.17	−0.11	0.07	1.17	0.93	2.50
Lys	11.39	10.65	11.81	−0.12	−0.06	0.17	1.62	1.38	3.05
Ser	10.87	10.23	11.56	−0.14	0.02	0.42	1.50	1.46	3.47
His	13.25	13.85	14.49	0.35	1.75	1.46	2.80	5.24	5.87
Adn	12.25	11.52	12.61	−0.42	−0.35	−0.19	0.28	0.06	1.59
Urd	10.75	10.68	11.78	1.16	1.89	2.06	3.81	4.91	6.46
Ino	10.03	10.17	11.22	0.46	1.40	1.52	2.44	3.96	5.41

Based on the binary and ternary complex stability constants values, the following conclusions could be drawn:

(a) Stabilities of binary or ternary complexes involving glycine are higher than those containing valine. This behaviour does not follow the basicities as expected, probably due to the similarities of the pK_a values of the considered amino acids. Therefore, it is suggested that the steric effect, caused by the presence of two methyl groups on the carbon atom bearing the amino group (valine), is the responsible factor for lowering the stability of its complexes compared with the simplest amino acid, glycine.

(b) The overall stability constants of the same metal ion binary or ternary complexes decrease according to the following order: *M*-histidine > *M*-lysine > *M*-serine; *M*-histidine-*B* > *M*-lysine-*B* > *M*-serine-*B*. This behaviour can mainly be explained in terms of the decrease in basicity of the amino acid in the same direction. The high stability of the histidine complexes indicates that it acts as a tridentate ligand, coordinated through the carboxylic oxygen, amino nitrogen, and imino nitrogen atoms of the imidazole ring [25]. Lysine behaves as a bidentate ligand and coordinates through adjacent carboxylic and amino groups, thus forming a 5-membered chelate ring. The coordination of the

Table 5. Stability constants for Fe^{III} binary and ternary complexes at $25 \pm 0.1°C$ and $I = 0.1\,mol\,dm^{-3}$ $NaNO_3$

	$\log K_1$	$\log K_2$	$\log K_{MAL}^{MA}$		
			Aha	*Bha*	*Sham*
Aha	11.35 ± 0.03	11.12 ± 0.05			
Bha	11.25 ± 0.02	10.35 ± 0.03			
Sham	14.80 ± 0.08	11.96 ± 0.06			
Gly	10.83 ± 0.03	9.65 ± 0.04	9.40 ± 0.05	9.95 ± 0.06	10.18 ± 0.02
Val	10.25 ± 0.06	8.97 ± 0.03	9.38 ± 0.03	9.68 ± 0.02	10.05 ± 0.03
Lys	9.56 ± 0.04	8.92 ± 0.06	9.37 ± 0.05	9.65 ± 0.04	9.73 ± 0.05
Ser	9.24 ± 0.02	8.90 ± 0.04	8.53 ± 0.02	8.76 ± 0.02	9.45 ± 0.06
His	12.90 ± 0.05	11.96 ± 0.02	12.5 ± 0.03	13.14 ± 0.07	13.19 ± 0.03
Adn	9.32 ± 0.02	9.05 ± 0.07	9.92 ± 0.05	10.00 ± 0.03	10.31 ± 0.07
Urd	9.20 ± 0.05	8.94 ± 0.03	9.45 ± 0.04	9.74 ± 0.08	9.85 ± 0.03
Ino	8.97 ± 0.06	8.53 ± 0.02	8.86 ± 0.07	9.10 ± 0.04	9.98 ± 0.06

	$\log \beta_{MAL}^{M}$			$\Delta \log K$			$\log X$		
	Aha	*Bha*	*Sham*	*Aha*	*Bha*	*Sham*	*Aha*	*Bha*	*Sham*
Gly	20.75	21.20	24.98	−1.43	−0.88	−0.65	−1.45	−0.32	2.72
Val	20.73	20.93	24.85	−0.87	−0.57	−0.20	−0.23	−1.04	3.72
Lys	20.72	20.90	24.53	−0.19	0.09	0.17	0.49	1.72	3.82
Ser	19.88	20.01	24.25	−0.71	−0.48	0.21	−0.85	0.28	3.60
His	23.85	24.39	27.99	−0.40	0.24	0.29	0.37	2.32	4.36
Adn	21.27	21.25	25.11	0.60	0.68	0.99	1.70	2.53	5.09
Urd	20.80	20.99	24.65	0.25	0.54	0.65	0.99	2.24	4.40
Ino	20.21	20.35	24.78	−0.11	0.13	1.01	0.45	1.60	5.30

second amino group is excluded, due to steric hindrance caused by the lysine chain length. For serine, the position of the three donor groups renders them sterically unfavourable for effective tridentate coordination. This is in agreement with the lower stability of its complex than that of histidine-metal complexes.

(c) With respect to the metal binary and ternary complexes involving nucleic acid component, one can deduce that the great stability of adenine metal complexes may be attributed to less steric hindrance in the complexation process. The presence of sugar residues imposes steric crowding in nucleosides (inosine and uridine) for their complexation with metal ions and reduces the overall basicity. The combined effect lowers the stability of metal complexes of nucleosides considerably.

(d) The observed order of stability of ternary systems with respect to the ligand hydroxamic acid is *Sham* > *Bha* > *Aha*. The increased stability of the salicylhydroxamate ternary complexes relative to those of the other ligands may be ascribed to an additional interaction of the phenolic group with the metal ion.

(e) The complex stability of the ternary complexes with respect to the metal ion present follows the order: $Fe^{III} > Al^{III} > Cr^{III} > Cu^{II} > Ni^{II} > Co^{II} > Zn^{II}$, except

Table 6. Stability constants for Al^{III} binary and ternary complexes at $25 \pm 0.1°C$ and $I = 0.1\,mol\,dm^{-3}$ $NaNO_3$

	$\log K_1$	$\log K_2$	$\log K_{MAL}^{MA}$		
			Aha	*Bha*	*Sham*
Aha	8.26 ± 0.06	7.93 ± 0.05			
Bha	9.88 ± 0.02	6.95 ± 0.03			
Sham	12.34 ± 0.03	8.25 ± 0.06			
Gly	5.92 ± 0.03	4.35 ± 0.04	8.55 ± 0.03	8.66 ± 0.06	8.87 ± 0.02
Val	5.83 ± 0.05	3.75 ± 0.03	8.52 ± 0.07	8.63 ± 0.02	8.77 ± 0.03
Lys	5.76 ± 0.07	3.72 ± 0.05	7.92 ± 0.02	8.10 ± 0.04	8.30 ± 0.05
Ser	5.67 ± 0.06	3.64 ± 0.02	7.90 ± 0.06	8.02 ± 0.02	8.24 ± 0.06
His	10.37 ± 0.02	5.33 ± 0.04	8.62 ± 0.03	8.80 ± 0.07	9.30 ± 0.03
Adn	9.13 ± 0.03	7.90 ± 0.02	8.57 ± 0.05	8.69 ± 0.02	9.00 ± 0.08
Urd	8.48 ± 0.05	7.80 ± 0.06	8.07 ± 0.04	8.22 ± 0.08	8.50 ± 0.02
Ino	7.44 ± 0.04	7.24 ± 0.02	7.50 ± 0.06	7.68 ± 0.04	7.92 ± 0.06

	$\log \beta_{MAL}^{M}$			$\Delta \log K$			$\log X$		
	Aha	*Bha*	*Sham*	*Aha*	*Bha*	*Sham*	*Aha*	*Bha*	*Sham*
Gly	16.81	18.54	21.21	2.63	2.74	2.95	7.16	9.98	11.56
Val	16.78	18.51	21.11	2.69	2.80	2.94	7.79	10.61	12.05
Lys	16.18	17.98	20.64	2.16	2.34	2.54	6.69	9.65	11.21
Ser	16.16	17.90	20.58	2.23	2.35	2.57	6.82	9.66	11.26
His	16.88	18.68	21.64	−1.75	−1.57	−1.07	1.87	4.83	6.99
Adn	16.83	18.57	21.34	−0.56	−0.44	−0.13	0.44	3.28	5.06
Urd	16.33	18.10	20.84	−0.41	−0.26	0.02	0.19	3.09	4.81
Ino	15.76	17.56	20.26	0.06	0.24	0.48	0.65	3.61	5.25

for the ternary systems involving Cu^{II} and salicylhydroxamic acid. The order of stability is that which is expected on the basis of the high affinity of Fe^{III} ion for hydroxamate ligands [19], and in the case of the other 3d metal ions on the basis of their positions in the *Irving-Williams* series [26]. The high stability of the Cu^{II} ternary systems of salicylhydroxamic acid can be attributed to the high affinity of Cu^{II} for interaction with salicylhydroxamic acid ($\log K_1 = 13.06 \pm 0.06$) as shown in Table 1.

(f) It is worth mentioning that the stability constants obtained for the interactions of Al^{III} with simple bidentate amino acids (glycine, valine, lysine, and serine) are in the range $\log K_1 = 5.67$–5.92 (Table 6), which is in good agreement with the value derived from LFER (Linear free-energy relation) calculations [27], indicating that such amino acids are weaker Al^{III} binders. The weakening effect can be explained in terms of the electrostatic repulsive effect of the $-NH_3^+$ group [28].

One way to quantify the stability of ternary complexes is by comparison with the stability of the binary complex, and can be expressed in terms of $\Delta \log K$, which

Table 7. Stability constants for Cr^{III} binary and ternary complexes at $25 \pm 0.1°C$ and $I = 0.1\,mol\,dm^{-3}$ $NaNO_3$

	$\log K_1$	$\log K_2$	$\log K^{MA}_{MAL}$		
			Aha	*Bha*	*Sham*
Aha	7.65 ± 0.04	6.22 ± 0.04			
Bha	7.30 ± 0.04	6.12 ± 0.03			
Sham	8.54 ± 0.02	7.00 ± 0.06			
Gly	9.14 ± 0.02	7.55 ± 0.04	8.34 ± 0.03	8.40 ± 0.06	8.65 ± 0.05
Val	8.91 ± 0.05	7.61 ± 0.03	7.93 ± 0.07	8.29 ± 0.02	8.34 ± 0.06
Lys	8.51 ± 0.05	6.92 ± 0.05	7.65 ± 0.05	7.74 ± 0.02	7.92 ± 0.05
Ser	7.92 ± 0.02	7.10 ± 0.04	7.53 ± 0.06	7.71 ± 0.02	7.90 ± 0.06
His	9.45 ± 0.04	6.12 ± 0.02	8.52 ± 0.03	8.53 ± 0.07	8.82 ± 0.02
Adn	8.30 ± 0.02	6.27 ± 0.02	8.40 ± 0.05	8.45 ± 0.03	8.83 ± 0.08
Urd	7.90 ± 0.03	6.93 ± 0.03	7.92 ± 0.04	7.96 ± 0.08	8.34 ± 0.02
Ino	7.80 ± 0.06	6.94 ± 0.05	7.35 ± 0.06	7.35 ± 0.04	7.82 ± 0.05

	$\log \beta^{M}_{MAL}$			$\Delta \log K$			$\log X$		
	Aha	*Bha*	*Sham*	*Aha*	*Bha*	*Sham*	*Aha*	*Bha*	*Sham*
Gly	15.99	15.70	17.19	−0.80	−0.74	−0.49	1.42	1.29	2.15
Val	15.58	15.59	16.88	−0.98	−0.62	−0.57	0.77	1.24	1.70
Lys	15.30	15.04	16.46	−0.86	−0.77	−0.59	1.30	1.23	1.95
Ser	15.18	15.01	16.44	−0.39	−0.21	−0.02	1.47	1.58	2.32
His	16.17	15.83	17.36	−0.93	−0.92	−0.63	2.90	2.67	3.61
Adn	16.05	15.75	17.37	0.10	0.15	0.53	3.66	3.51	4.63
Urd	15.57	15.26	16.88	0.02	0.06	0.44	2.44	2.27	3.39
Ino	15.00	14.70	16.36	−0.45	−0.40	0.02	1.39	1.24	2.44

represents the difference in stabilities for the addition of the secondary ligand *L* to the 1:1 binary complex *MA* and to the aquated metal ion as shown in Eq. (6).

$$\Delta \log K = \log K^{MA}_{MAL} - \log K^{M}_{ML} \tag{6}$$

The value of $\Delta \log K$ is the logarithm of the equilibrium constant of Eq. (7).

$$MA + ML \rightleftharpoons MAL + M \tag{7}$$

A comparison of stability constants of binary complexes (Tables 1–7) indicates that $K_1 > K_2$. Thus, in binary systems, $\Delta \log K$ values are generally negative which indicates the formation of 1:2 species. Similarly, in the case of ternary complex formation, $\Delta \log K$ should display the same trend. This behavior can be explained on the basis of the presence of a fewer number of coordination sites on the *MA* monocomplexes than on the aquated metal ion. Thus the secondary ligand (*L*) is expected to bind the *MA* complex with a smaller stability constant than that with an aquated metal ion. Therefore, $\Delta \log K$ should be negative, generally between −0.5 and −2.0 [29–31] depending on the geometry of the complex. However, in our case, positive $\Delta \log K$ values are generally obtained, indicating a significant stabilization of the ternary systems (Tables 1–7).

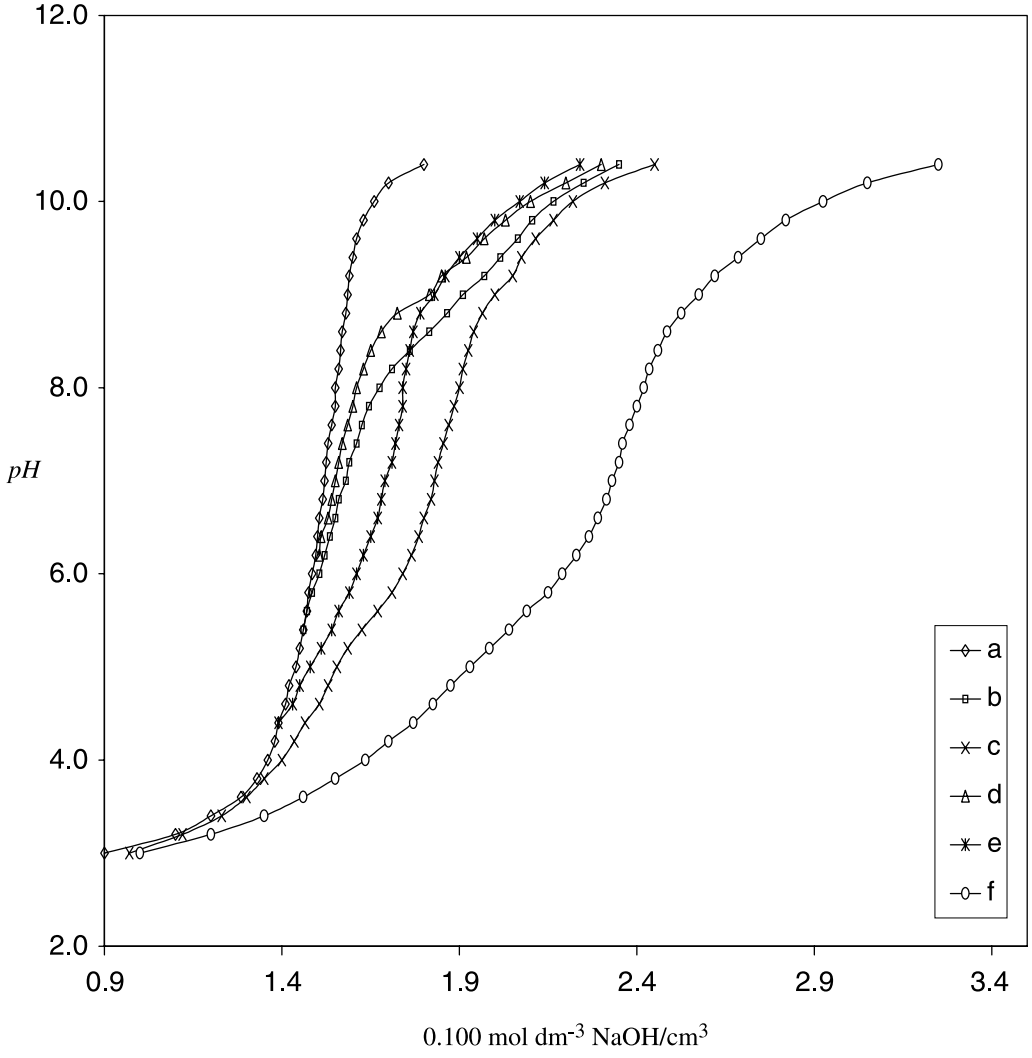

Fig. 2. Potentiometric *pH* titration curves for the Cu^{II} – benzohydroxamic acid – serine system at 25°C and $I = 0.1$ mol dm^{-3} $NaNO_3$: (a) 0.003 mol dm^{-3} HNO_3; (b) solution a + 0.001 mol dm^{-3} benzohydroxamic acid; (c) solution b + 0.0004 mol dm^{-3} Cu^{II}; (d) solution a + 0.001 mol dm^{-3} serine; (e) solution d + 0.0004 mol dm^{-3} Cu^{II}; (f) solution e + 0.001 mol dm^{-3} benzohydroxamic acid

The higher values of $\Delta \log K$, for the ternary systems involving the aromatic hydroxamic acids (*Sham* or *Bha*) than the aliphatic hydroxamic acid, *Aha*, may be attributed to the presence of an aromatic ring [32, 33] which alters the binding properties of these ligands.

Another parameter, known as $\log X$, is frequently used for characterization of the stability of a ternary complex. It measures the tendency of one mole each of the binary complexes MA_2 and ML_2 to disproportionate giving two moles of *MAL*, *i.e.* as shown in Eq. (8).

$$MA_2 + ML_2 \rightleftharpoons 2MAL, \qquad X = \frac{[MAL]^2}{[MA_2][ML_2]} \tag{8}$$

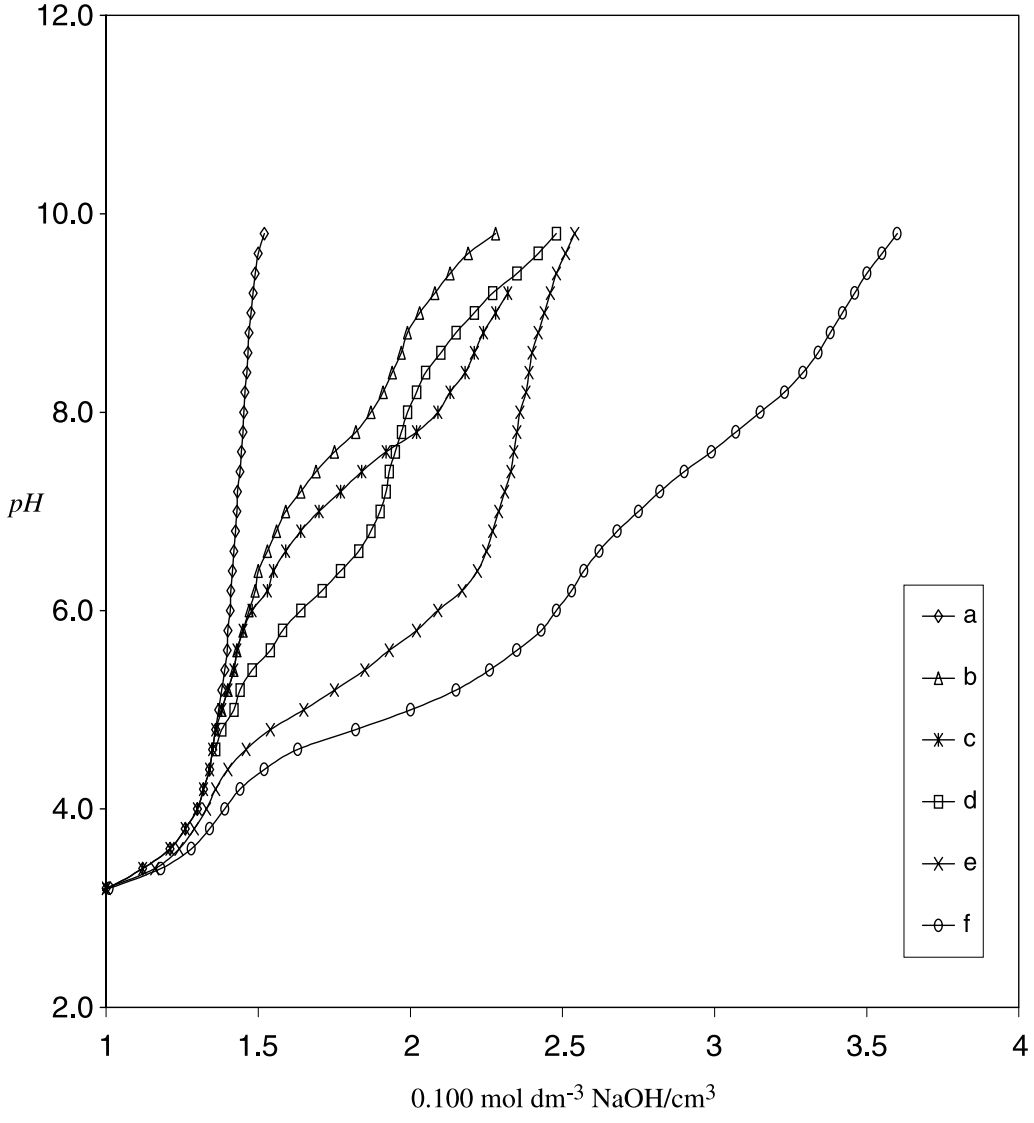

Fig. 3. Potentiometric *pH* titration curves for the Ni^{II} – salicylhydroxamic acid – histidine system at 25°C and $I = 0.1\,mol\,dm^{-3}$ $NaNO_3$: (a) $0.003\,mol\,dm^{-3}$ HNO_3; (b) solution a + $0.001\,mol\,dm^{-3}$ salicylhydroxamic acid; (c) solution b + $0.0004\,mol\,dm^{-3}$ Ni^{II}; (d) solution a + $0.001\,mol\,dm^{-3}$ histidine; (e) solution d + $0.0004\,mol\,dm^{-3}$ Ni^{II}; (f) solution e + $0.001\,mol\,dm^{-3}$ histidine

It is therefore calculated by:

$$\log X = 2\log \beta^{M}_{MAL} - (\log \beta^{M}_{MA_2} + \log \beta^{M}_{ML_2})$$
$$= (\log K^{MA}_{MAL} - \log K^{ML}_{ML_2}) + (\log K^{ML}_{MLA} - \log K^{MA}_{MA_2}) \quad (9)$$

The values of $\log X$ expected from statistical reasons is +0.6 [31, 34] for all geometries. More positive values than those expected statistically are obtained (Tables 1–7) indicating marked stabilities of our ternary complexes.

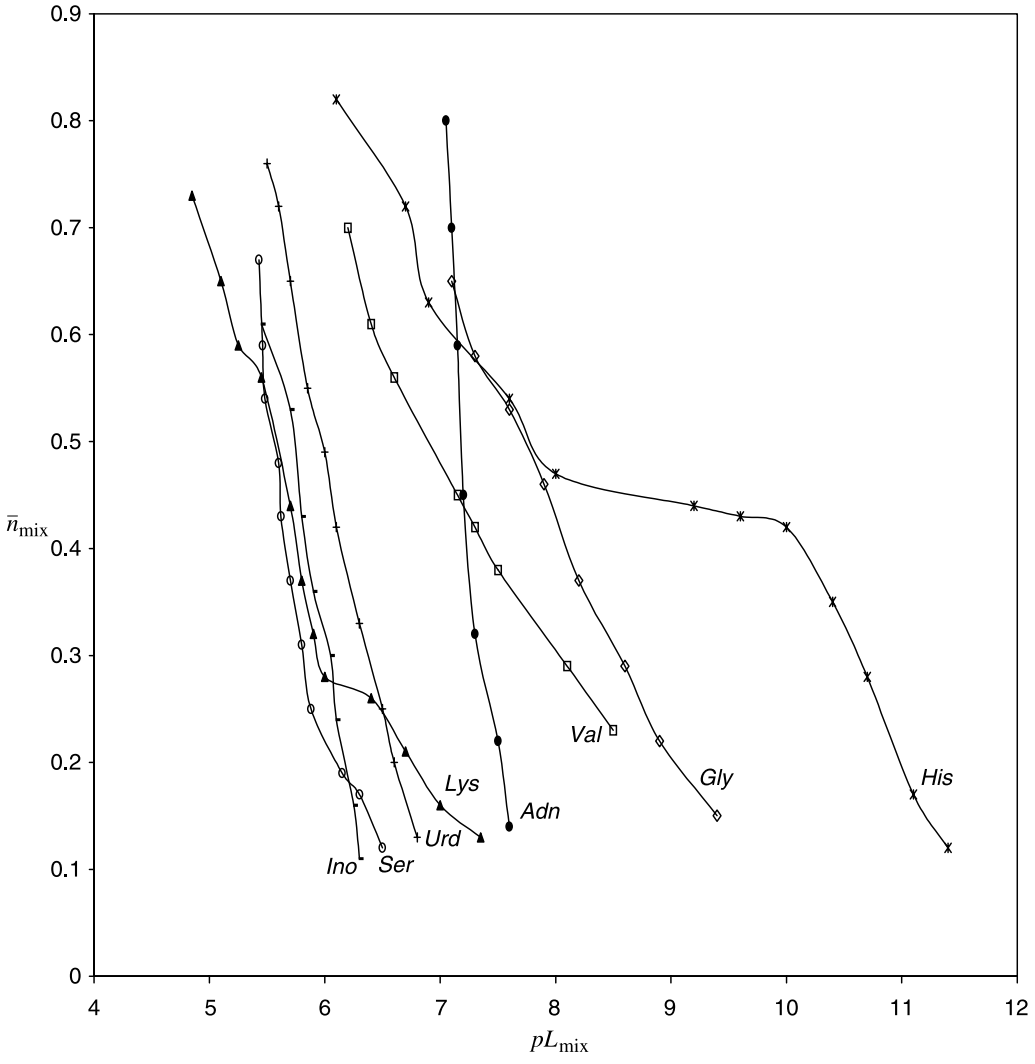

Fig. 4. Formation curves for the Ni^{II} – salicylhydroxamic acid – amino acid or nucleic acid component systems

Estimation of equilibrium concentration of various complex species as a function of *pH* provides a useful picture of metal ion binding in the biological systems. In all of the species distributions, the concentration of the ternary complexes increases with increasing *pH*, thus making the complex formation more favored in the physiological *pH* range. The species distribution pattern for the Cu^{II}-salicylhydroxamic acid-amino acids systems indicates that the ternary complex starts to form at $pH > 6$ and reaches the maximum concentration at $pH \sim 10$. The ternary complexes formed with nucleic acid components reach the maximum concentration ($\sim$40%) at $pH \sim 9$. Thus, the amino acid ternary complexes are the most favored ones whereas those of the nucleic acid components are the least favored, *i.e.* the amino acid will compete with the nucleic acid component for the reaction with Cu^{II}-salicylhydroxamic acid complex.

Thermodynamic Studies

The ternary system M^{III}-benzohydroxamic acid-*L*-histidine or adenine (where $M^{III} = Fe$, Al, and Cr) was selected for further study of the effect of temperature of the medium on dissociation of the mentioned ligands as well as the stability of both 1:1 binary and 1:1:1 ternary complexes. The plot of $\log K^{M(Bha)}_{M(Bha)(His)}$ and $\log K^{M(Bha)}_{M(Bha)(Adn)}$ *vs.* $-1/T$ is linear as shown in Fig. 5.

The thermodynamic parameters associated with the deprotonation of the ligands and the formation of different complexes formed were studied at the constant ionic strength $I = 0.10\,\text{mol dm}^{-3}$ $NaNO_3$. The equilibrium constants have been evaluated at different temperatures (25, 35, 45, and 55°C), along with the

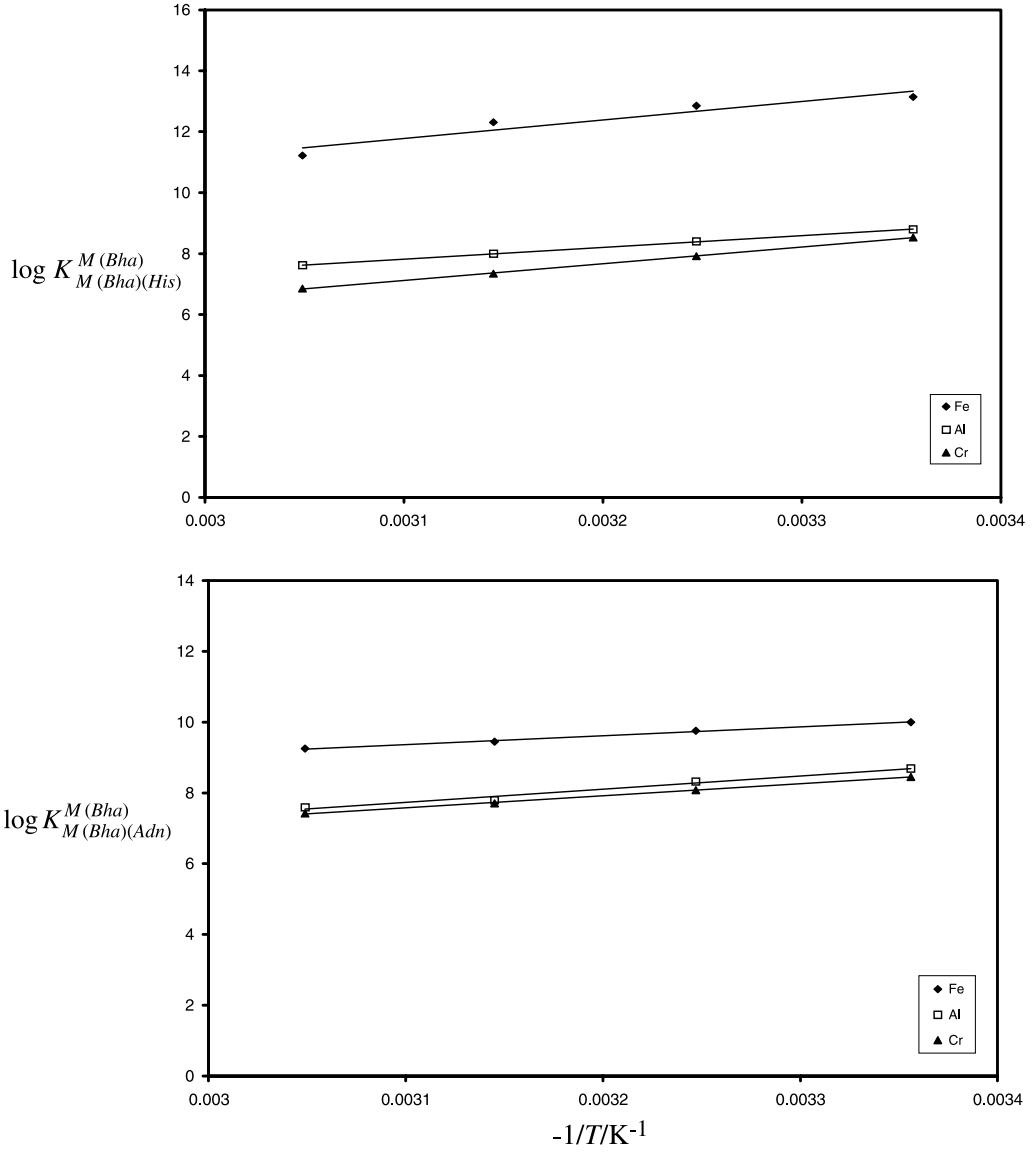

Fig. 5. Plot of $\log K^{M(Bha)}_{M(Bha)(His)}$ and $\log K^{M(Bha)}_{M(Bha)(Adn)}$ *vs.* $-1/T$ at $I = 0.10\,\text{mol dm}^{-3}$ $NaNO_3$

Table 8. Thermodynamic quantities associated with the dissociation of the ligands studied, the interaction of metal ion with the ligands at 1:1 molar ratio, and the interaction of metal(III) ion with the ligands at a 1:1:1 molar ratio, $I = 0.10\ \text{mol dm}^{-3}$ $NaNO_3$

Ligand or complex	Cation	pK_{a_2} or log K			
		t/°C = 25	35	45	55
Benzohydroxamic acid	H	8.63 ± 0.04	8.58 ± 0.04	8.55 ± 0.02	8.50 ± 0.04
L-Histidine	H[(a, b)]				
Adenine	H	9.750 ± 0.02	9.55 ± 0.05	9.40 ± 0.06	9.20 ± 0.02
(1:1) binary complex of *Bha*	Fe^{III}	11.25 ± 0.02	10.60 ± 0.03	10.51 ± 0.04	10.47 ± 0.03
	Al^{III}	9.88 ± 0.02	8.60 ± 0.07	8.04 ± 0.02	7.96 ± 0.05
	Cr^{III}	7.30 ± 0.04	6.66 ± 0.02	6.36 ± 0.05	5.98 ± 0.02
(1:1) binary complex of *His*	Fe^{III}	12.90 ± 0.02	11.86 ± 0.05	11.60 ± 0.06	11.48 ± 0.05
	Al^{III}	10.37 ± 0.02	10.10 ± 0.03	9.85 ± 0.04	9.50 ± 0.04
	Cr^{III}	9.45 ± 0.04	9.25 ± 0.02	8.77 ± 0.04	8.50 ± 0.05
(1:1) binary complex of *Adn*	Fe^{III}	9.32 ± 0.02	9.15 ± 0.04	8.95 ± 0.02	8.82 ± 0.04
	Al^{III}	9.13 ± 0.03	8.80 ± 0.02	8.45 ± 0.03	8.16 ± 0.06
	Cr^{III}	8.30 ± 0.02	8.01 ± 0.06	7.72 ± 0.08	7.25 ± 0.03
(1:1:1) ternary complex of *His*	Fe^{III}	13.14 ± 0.07	12.85 ± 0.04	12.31 ± 0.02	11.22 ± 0.05
	Al^{III}	8.80 ± 0.07	8.40 ± 0.03	8.00 ± 0.07	7.62 ± 0.02
	Cr^{III}	8.53 ± 0.02	7.92 ± 0.05	7.34 ± 0.02	6.85 ± 0.04
(1:1:1) ternary complex of *Adn*	Fe^{III}	10.0 ± 0.03	9.75 ± 0.02	9.45 ± 0.06	9.25 ± 0.07
	Al^{III}	8.69 ± 0.02	8.32 ± 0.07	7.80 ± 0.03	7.59 ± 0.03
	Cr^{III}	8.45 ± 0.07	8.08 ± 0.03	7.70 ± 0.04	7.42 ± 0.02

Ligand or complex	Cation	ΔH° kJ.mol^{-1}	ΔG° kJ.mol^{-1}	ΔS° J.mol^{-1} K^{-1}
Benzohydroxamic acid	H	94.53	48.63	−160.71
L-Histidine	H[(a, b)]	94.42	51.21	−165.80
		26.10	34.04	−105.70
Adenine	H	34.03	54.86	−171.85
(1:1) binary complex of *Bha*	Fe^{III}	−161.45	−63.30	190.21
	Al^{III}	−153.62	−55.60	131.03
	Cr^{III}	−78.00	−41.80	180.72
(1:1) binary complex of *His*	Fe^{III}	−111.18	−72.59	204.20
	Al^{III}	−56.72	−58.35	176.79
	Cr^{III}	−157.88	−53.25	101.63
(1:1) binary complex of *Adn*	Fe^{III}	−166.01	−52.51	30.72
	Al^{III}	−151.26	−51.44	59.09
	Cr^{III}	−49.60	−46.71	138.97
(1:1:1) ternary complex of *His*	Fe^{III}	−155.99	−73.49	204.58
	Al^{III}	−141.81	−49.58	75.63
	Cr^{III}	−124.22	−48.06	59.10
(1:1:1) ternary complex of *Adn*	Fe^{III}	−173.00	−56.34	47.27
	Al^{III}	−139.92	−48.96	70.90
	Cr^{III}	−136.51	−47.61	63.81

For histidine: (a) $pK_{a_2} = 9.10$, 9.00, 8.90, and 8.85; (b) $pK_{a_3} = 6.05$, 5.80, 5.70, and 5.65, at 25, 35, 45, and 55°C, respectively

thermodynamic quantities and the values obtained are given in Table 8. It is shown that the enthalpy changes for the ionization of the ligands (*Bha*, *His*, and *Adn*) are positive indicating that the deprotonation process is endothermic in nature. The positive values of ΔG° for the dissociation processes of the ligands denote that such processes are not spontaneous. Also, the negative values of ΔS° are pointing to increased ordering due to association. The values of stability constants for both the binary and ternary systems studied decrease with increasing temperature which can be mainly attributed to the thermal hydrolysis of the metal complexes [35].

It is of great interest to note that the values of enthalpy changes (ΔH°) for the ternary systems investigated are more negative as compared to those of the corresponding binary complexes, and ensure that despite the steric hindrance due to the primary ligand, benzohydroxamic acid, the bond is stronger in the ternary complex formation [36]. In addition, the relatively high negative values of ΔH° for ternary systems may be attributed to less competition faced by a secondary ligand at this step from a water molecule [36]. However, the complex formation process is spontaneous in nature, as characterized by the negative ΔG° values. The values of ΔS° substantiate the suggestion that the different binary and ternary complexes are formed due to the coordination of the ligand anion to the metal cation. Furthermore, the positive values of ΔS° suggest also a desolvation of the ligands, resulting in weak solvent-ligand interactions, to the advantage of the metal ion-ligand interaction [37].

Experimental Section

Materials and Solutions

Acetohydroxamic acid (*Aha*) and benzohydroxamic acid (*Bha*) were Sigma products. Salicylhydroxamic acid (*Sham*) was purchased in pure form from Nasr Pharmaceutical Chemicals Co., Egypt. Amino acids were provided by Fluka and used without further purification. Anhydrous nucleosides and their bases obtained from Sigma Chemicals (USA) were analytically pure. Their purity was further checked by potentiometric titration with standard sodium hydroxide. Fresh solid nucleosides were weighed out for each titration to avoid any possible hydrolysis or photochemical decomposition. The metal salts were provided by BDH as nitrate or chlorides. Stock solutions of the metal salts were prepared in bidistilled water, and the metal concentration was obtained by standard analytical methods [38]. Carbonate-free sodium hydroxide (titrant, prepared in 0.1 mol dm^{-3} $NaNO_3$ solution) was standardized potentiometrically with KH phthalate (Merck AG). A nitric acid solution ($\approx$0.03 mol dm^{-3}) was prepared and used after standardization. Sodium hydroxide, nitric acid, and sodium nitrate were from Merck p.a.

Apparatus and Procedure

The *pH* titrations were performed using a Metrohm 702 titroprocessor equipped with a 665 dosimat (Switzerland). The titroprocessor and electrode were calibrated with standard buffer solutions, based on the scale of the U.S. National Bureau of Standards [39]. The *pH*-metric titrations were carried out at the desired temperature in a purified nitrogen atmosphere.

The following solutions were prepared (total volume 50 cm^3) and titrated potentiometrically against standard carbonate-free NaOH (0.10 mol dm^{-3}) solution:

a: HNO_3 (0.03 mol dm^{-3}, 5 cm^3) + 0.50 mol dm^{-3} $NaNO_3$ (10 cm^3)
b: solution a + 0.01 mol dm^{-3} hydroxamic acid (5 cm^3)
c: solution b + 0.01 mol dm^{-3} metal ion (2 cm^3)

d: solution a + 0.01 mol dm^{-3} amino acid or nucleic acid component (5 cm^3)
e: solution d + 0.01 mol dm^{-3} metal ion (2 cm^3)
f: solution a + 0.01 mol dm^{-3} metal ion (5 cm^3) + 0.01 mol dm^{-3} hydroxamic acid (5 cm^3) + 0.01 mol dm^{-3} amino acid or nucleic acid component (5 cm^3)

Each of the above solutions was thermostated at the required temperature with an accuracy of ±0.1°C and the solutions were left to stand at this temperature for about 15 min before titration. Magnetic stirring was used during all titrations. The titration was repeated at least four times for each titration curve.

Calculations

Part of the information required for determining the metal complex stability constants is the acid dissociation constant (pK_a). Therefore, the pK_a values were calculated from Eq. (10) [17] where β is the proton-ligand formation constant (K_a) of the ligand and $\bar{n}_H$ is the average number of protons associated per mole of ligand at several pH values.

$$\bar{n}_H = \beta[H^+](1 + \beta[H^+])^{-1} \tag{10}$$

Eq. (11) [17] was used for calculation of the $\bar{n}_H$ values from the titration curves corresponding to solutions a, b, and d, where y is the number of dissociable protons ($y = 1$ in case of acetohydroxamic acid, benzohydroxamic acid, glycine, valine, lysine, serine, adenine, uridine, and inosine, and $y = 2$ in case of salicylhydroxamic acid, and histidine).

$$\bar{n}_H = \left\{ yC_L + \frac{[(V_a - V_b)\ or\ (V_a - V_d)]C_b}{V_o} \right\}(C_L)^{-1} \tag{11}$$

V_a, V_b, and V_d are the volumes of NaOH consumed to reach the same pH values in curves a, b, and d, respectively. C_b and C_L are the concentrations of NaOH and ligand, respectively, and V_o is the original volume (50 cm^3). Titration curves b, c, and d, e (Figs. 2 and 3) were used to calculate the stability constants of the binary metal complexes of hydroxamic acids and amino acids or nucleic acid components, respectively.

The average number of ligand molecules (n_b) coordinated to the metal ion and the free ligand exponent (P_L) at several pH values were calculated according to Eqs. (12) and (13) [17], where V_c and V_e are the volumes of NaOH consumed to reach the same pH values in curves c and e, respectively.

$$n_b = \frac{[(V_c - V_b)\ or\ (V_e - V_d)][C_a + C_b + C_L(y - \bar{n}_H)]}{[(V_o + V_b)\ or\ (V_o + V_d)]\bar{n}_H C_M} \tag{12}$$

$$P_L = \log \left\{ \frac{\sum_{y=0}^{y=1\ or\ 2} \beta_Y^H \left(\frac{1}{10^B}\right)}{C_L - n_b C_M} \frac{V_o + (V_c\ or\ V_e)}{V_o} \right\} \tag{13}$$

C_a is the concentration of HNO_3, and C_M is the initial concentration of the metal ion used. β_Y^H represents the proton-ligand dissociation constants of the ligands, and B is the pH value. $\bar{n}_H$ values were available from the determination of the proton-ligand formation constant. It is worth mentioning that the values of n_b exceed 1.5 indicating the formation of both 1:1 and 1:2 binary complexes.

On the other hand, the titration curves c and f were used to calculate the number of secondary ligands attached to one binary 1:1 MA complex molecule ($\bar{n}_{mix}$) for a mixed ligand ternary complex. The equation used for the calculation of $\bar{n}_{mix}$ (Eq. (14)) was the same as that reported elsewhere [17], where C_M is the concentration of the binary complex involving the primary ligand, which equals the concentration of M^{II} or M^{III} used, C_L is the concentration of the secondary ligand, y is the number of

dissociable protons per molecule of the secondary ligand, and V_f is the volume of NaOH consumed to reach the same *pH* values in curve f.

$$\bar{n}_{mix} = \frac{(V_f - V_c)[C_b + C_a + C_L(y - \bar{n}_H)]}{(V_o + V_c)\bar{n}_H C_M} \tag{14}$$

In this case, the $\bar{n}_H$ values are the average number of protons associated with the secondary ligand at different *pH* values. The $\bar{n}_{mix}$ values do not exceed unity indicating that only one secondary ligand molecule combines with the complex *MA*, forming a 1:1:1 *MAL* ternary complex.

The free secondary ligand exponent, pL_{mix}, was calculated from the obtained values of $\bar{n}_{mix}$ using Eq. (15) [17] where β_Y^H are the proton-ligand dissociation constants of the secondary ligand.

$$pL_{mix} = \log\left\{\frac{\sum_{y=0}^{y=1}\beta_Y^H\left(\frac{1}{10^\beta}\right)}{C_L - \bar{n}_{mix}C_M}\frac{(V_o + V_f)}{V_o}\right\} \tag{15}$$

All other terms have the same meaning as defined above.

A computer program based on unweighted linear least-squares fit was used for all calculations.

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