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Histamine as a Ligand in Blood Plasma. Part 4.† Potentiometric Investigation of the Complex Formation in the Copper(II)–Cystinate, Copper(II)–Lysinate, Copper(II)–Histamine–Cystinate and Copper(II)–Histamine–Lysinate Systems

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In our attempt to simulate the distribution of the metal histamine complexes in human blood plasma, the present paper reports the potentiometric investigation of the equilibria between copper(II), cystinate and lysinate, as well as those in the respective ternary systems with histamine.

No mixed-ligand complex could be found in the copper–histamine–cystinate system, but two very stable species, Cu–hsn–lys and Cu–hsn-lys-H, have been characterised in the copper–histamine–lysinate system.

Introduction

Except in organs such as the stomach and the gastrointestinal tract, the greater part of the histamine which is present in the human body is stored in mast cells in tissues, and in basophils in blood [1]. Histamine is released explosively from these specific sites, by an energy-dependent mechanism involving calcium ions [2], in response to immunological (IgE-antigen-mediated immune reactions) or non immunological (trauma, toxins, and by histamine-liberator compounds) stimuli [3]. If generalized, for instance following the intravenous injection of a substance inducing either an immunological response or a direct degranulation of basophils, the release may cause profound changes in the cardiovascular system and induce respectively anaphylactic or anaphylactoid shock [4]. In the latter case, it has recently been demonstrated that the physiological effects of histamine depend closely on its level in blood plasma; a concentration of about hundred times higher than normal would cause death [5].

Two recent approaches which involve the use of tracemetal ions have been initiated to oppose histamine toxicity. The first is aimed at blocking the histamine release itself. Zinc has been shown to be particularly efficient in this respect [6], presumably owing to its property of stabilizing the basophil membrane [7]. This can be related to the paralysing effect on plasma cell membranes reported elsewhere [8, 9]. The second tends to alleviate the effects of histamine when it has been released. In view of this, physiological experiments were carried out on mice. These experiments clearly established that zinc can exert a beneficial effect on anaphylactic disorders, and antagonize the aggravating influence of copper ions [10]. The histamine catabolism in mice is of the same type as in man, essentially consisting of methylation [11, 12]. Thus it would appear that this beneficial effect of zinc might also be applicable to humans.

Before such an application can be envisaged, it was necessary to understand the action pathway of this metal ion with respect to histamine catabolism. Indeed, histamine exists mainly in its protonated forms in blood plasma [13]. Moreover, its highly polar molecule itself cannot cross readily cell membranes to reach the tissues in which it can be catabolised [14]. Metal complexation could hence be reasonably expected to play a part in its passive diffusion process. It was thus decided to investigate the possible effects of zinc and copper upon histamine catabolism, on the basis of the computer simulation of its distribution among its various metal complexes in normal blood plasma.

Nevertheless, the reliability of such a simulation depends on the reliability of the formation constants on which the model is based [15, 16]. Accordingly, the previous parts of this series were devoted to the determination of the formation constants for:

(i) the binary systems consisting of histamine and the metal ions normally present in blood plasma [13],

†Part 3 is reference 18.

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TABLE I. Summary of the Titration Data used for Calculating Stability Constants. Initial total concentrations of copper (C_M), first ligand histamine (C_L), second ligand (C_X), strong acid, (C_H), and −log [H] range.

System	C_M	C_L	C_X	C_H	−log [H] Range
	(mmol dm^{-3})				
Proton-cystinate			10.00	68.90	1.2–9.8
			5.00	34.30	1.6–10.1
			2.50	12.00	1.8–9.8
			7.50	36.75	1.4–9.8
			5.00	34.41	1.5–10.0
			5.00	34.41	1.5–10.0
Copper–cystinate	4.96		15.00	104.43	1.6–3.2
	4.96		15.00	104.43	1.7–3.4
	4.96		5.07	34.63	2.1–3.2
	9.93		5.07	35.07	1.9–2.8
	4.96		9.92	68.73	1.9–3.2
	2.48		9.92	68.52	1.6–4.0
	1.99		9.92	68.47	1.9–4.7
	0.99		9.92	68.39	1.5–4.5
Copper–lysinate	4.96		18.72	28.48	2.4–8.9
	9.93		9.36	23.31	2.0–5.9
	4.96		9.36	22.87	2.0–7.8
	4.96		4.68	11.65	2.2–6.3
	9.93		4.68	12.09	2.2–7.4
Copper–histamine–cystinate	4.96	4.55	5.00	34.75	1.7–3.2
	2.48	2.27	2.50	17.38	1.9–3.6
	4.96	2.27	2.50	17.59	1.9–3.5
	2.48	4.55	5.00	34.54	1.7–3.4
	2.48	4.55	2.50	17.38	2.1–3.5
	2.48	2.27	5.00	34.54	1.6–3.5
Copper–histamine–lysinate	4.96	9.10	9.50	40.15	2.5–10.5
	9.93	9.10	9.50	39.87	2.5–10.1
	4.96	9.10	4.75	24.81	2.7–10.2
	9.93	4.55	9.50	25.24	2.3–6.7
	4.96	4.62	9.36	22.87	2.6–10.5
	4.96	4.62	4.68	17.26	2.6–10.1

(ii) the ternary systems resulting from the combination of these metal–histamine complexes with some of the main low-molecular-weight ligands in plasma [17, 18]. As no definite conclusion can be drawn until the level of confidence* of the simulated distribution of the histamine complexes reaches the realistic percentage of 95%, a number of determinations was still necessary. The present paper reports the potentiometric investigation of the copper–histamine–cystinate and copper–histamine–lysinate systems. The preliminary study of the copper–cystinate and copper–lysinate parent binary systems is also described.

*Defined as the ratio of the concentration of metal-complexed histamine derived from our experimental determinations under plasma conditions, to the corresponding total concentration.

Experimental

Reagents

Histamine was supplied in sealed ampoules by Sigma Chemical Co, in the form of crystalline free base. Lysine was also obtained as crystalline free base from Sigma Chemical Co. Cystine was purchased from Merck (Biochemical grade). All of these ligands were potentiometrically Gran titrated and were consequently used without further purification.

The stock solution of copper perchlorate was prepared from crystals supplied by G. Frederick Smith Chemical Co and was made slightly acid by adding perchloric acid Prolabo (Normatom grade) in order to prevent hydrolysis and absorption of carbon dioxide. The metal content of the solution was determined by complexometric titrations against EDTA using murexide as an indicator [19]. Its mineral acid content was deduced from direct potentiometric readings.

Sodium perchlorate solutions were prepared from Merck reagent grade crystals, as previously described [16]. Sodium hydroxide solutions were prepared by diluting the contents of BDH concentrated volumetric solution vials with freshly boiled deionised water, saturated in nitrogen. They were standardized as previously described [18].

Experimental Procedure

All potentiometric titrations used a Beckman model 4500 digital voltmeter, equipped with a Beckman S 39301 glass electrode and a saturated sodium chloride Ingold calomel electrode. Ionic strength and isotonicity with blood plasma were ensured by sodium perchlorate 0.15 mol dm^{-3}, temperature being maintained at 37.00 ± 0.02 °C in the reaction cell. The electrode system was calibrated in terms of hydrogen-ion concentrations and the pK_w value was considered to be 13.38, as previously determined by one of us [16]. Every titration carried out in the presence of copper was stopped as soon as a precipitate appeared in the solution, as confirmed by a steady drift in the voltmeter readings.

Values of copper, ligands and strong acid concentrations, as well as –log [H] ranges used in calculating the formation constants are summarized in Table I. Larger intervals of –log [H] than those shown in Table I were actually explored in the presence of copper, but the corresponding experimental data was not employed in the computations whenever complexation was judged insignificant.

Calculation of Formation Constants

The MINIQUAD programme [20] was used throughout all the formation constant calculations.

For the binary systems, the composition of the species possibly existing under the investigated concentration conditions was derived from the shape of the formation curve of the system. The average number of ligands bound to each metal ion was obtained from the equation [1]

$$\bar{q} = \{C_L - ([L] + [HL] + [H_2L] + \dots)\}/C_M \qquad (1)$$

in which C_L and C_M respectively represent the total ligand and the total metal concentrations. As this relation is established independently of the nature of the metal complexes existing in the solution, the formation curves obtained for the various total ligand/total metal concentration ratios are superimposable as long as simple mononuclear species are present. Consequently, any specific deviation from the classical shape of the formation curve can be interpreted in terms of the existence of protonated, hydrolysed or polynuclear species, as will be discussed later. Therefore, besides the usual examination of the numerical fittings (squared residuals, R factor) corresponding to the different possible combinations of the formation constants in a given system, the selection of the 'best' set was finally based on the graphical comparisons made between the experimenal $\bar{q}$ functions and the theoretical ones, as simulated by means of the PSEUDOPLOT programme [21].

For the mixed ligand ternary systems, the same kind of graphical comparisons was used, which comparisons were now based on the average number of protons bound to both of the ligands studied, in accordance with the relation [2]

$$\bar{s} = (C_H + NDP_L \cdot C_L + NDP_X \cdot C_X - C_{OH} + [OH] - [H])/(C_L + C_X) \qquad (2)$$

in which C_H, C_L, C_X and C_{OH} represent the total concentrations of strong acid, first ligand L, second ligand X and sodium hydroxide, and NDP the number of dissociable protons of the related ligands. The simulation of the $\bar{s}$ function was carried out with

TABLE II. Stability Constants $\beta_{pqrs} = [M_rL_pX_qH_s]/[M]^r[L]^p[X]^q[H]^s$ of Parent Complexes of Histamine and Lysinate at 37 °C and I = 0.15 mol dm^{-3} $NaClO_4$, and Copper(II) Hydroxides as Used in the Calculations (L = histamine, X = lysinate).

System	p	q	r	s	log β	Ref.
Proton–histamine	1	0	0	1	9.426	13
	1	0	0	2	15.315	
Copper-histamine	1	0	1	0	9.163	13
	2	0	1	0	15.475	
	1	0	1	1	12.576	
	2	0	1	1	21.024	
	2	0	1	–1	4.219	
	2	0	2	–2	7.059	
	2	0	1	–2	–5.948	
Proton-lysinate	0	1	0	1	10.296	24
	0	1	0	2	19.183	
	0	1	0	3	21.330	
Copper-hydroxide	0	0	1	–1	–6.80	25
	0	0	2	–2	–10.52	

TABLE III. Stability Constants obtained from These Studies*. The Formula of the general complex is the same as in Table II; n = number of experimental observations; S = sum of squares of residuals; L = histamine; X = cystinate or lysinate.

System	p	q	r	s	log β	S	n
Proton–cystinate	0	1	0	1	8.596 ± 0.004	0.667 E – 06	255
	0	1	0	2	16.451 ± 0.005		
	0	1	0	3	18.541 ± 0.012		
	0	1	0	4	19.901 ± 0.011		
Copper-cystinate	0	1	1	1	16.081 ± 0.011	0.109 E – 05	240
	0	1	2	0	14.860 ± 0.095		
	0	2	2	0	28.241 ± 0.023		
Copper-lysinate	0	1	1	0	10.850 ± 0.065	0.101 E – 05	211
	0	1	1	1	17.985 ± 0.010		
	0	2	1	1	25.623 ± 0.035		
	0	2	1	2	34.797 ± 0.019		
	0	1	1	2	20.640 ± 0.052		
Copper–histamine–lysinate	1	1	1	0	16.908 ± 0.014	0.232 E – 05	341
	1	1	1	1	26.550 ± 0.009		

*No ternary complex could be characterised in the copper–histamine–cystinate system under the conditions mentioned in Table I.

a modified version of the PSEUDOPLOT programme [22].

All the available parent protonation and binary complex formation constants which were used in the present calculations are given in Table II.

Results

Protonation of Cystinate

The protonation of cystinate had already been studied at 20 °C by Hawkins and Perrin [25], who had pointed out their failure to obtain the stability constants of the two most acidic steps. In addition, they noted they were able to establish an evaluation of the two basic constants relative to the amino groups by back titration of cystinate previously dissolved in two equivalents of alkali. This resulted in a low accuracy on the corresponding values, due to the supersaturation of the solution.

In a more recent study, Hallman, Perrin and Watt [26] mentioned the determination of these two basic constants and the rough estimation of the first of the two acidic ones at 37 °C, but did not supply any detail on their mode of investigation.

Unlike Hawkins and Perrin [25], we dissolved cystine in just sufficient excess of perchloric acid and titrated the initial solution obtained with sodium hydroxide in the classical way. This enabled us to reach a relatively high limit for $\bar{s}$ (3.5), at the lowest $-\log[H]$ value (1.4). Under these conditions, it must nonetheless be noted that (i) the saturation of the solution in acid may cause changes in the activity coefficients of the reactants, the more so as the ionic strength is fixed at 0.15 mol dm^{-3}. This kind of remark however is often valid – even if to a lesser degree – for biological conditions [27], (ii) the glass electrode response may deviate from the theoretical linearity.

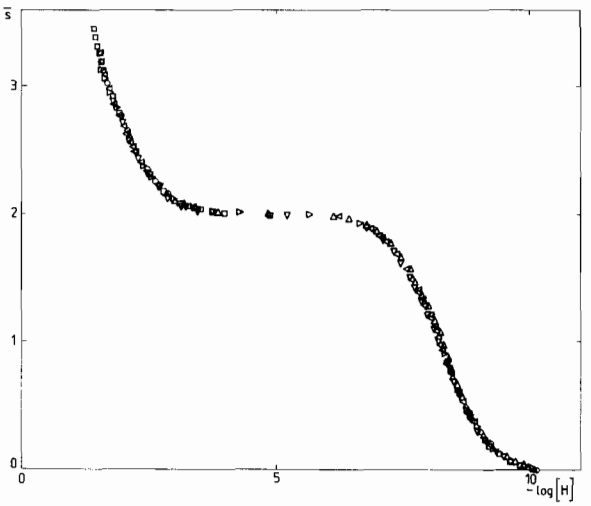

Fig. 1. Cystinate protonation curve at 37 °C in aqueous medium $NaClO_4$ 0.15 mol dm^{-3}. The following symbols correspond respectively to the order of the experiments as given in Table I: ○, △, ▽, □, ▷, ◁.

On account of these special conditions, we ran a great number of titrations to test the reliability of the measurements in the more acid range, *i.e.* below

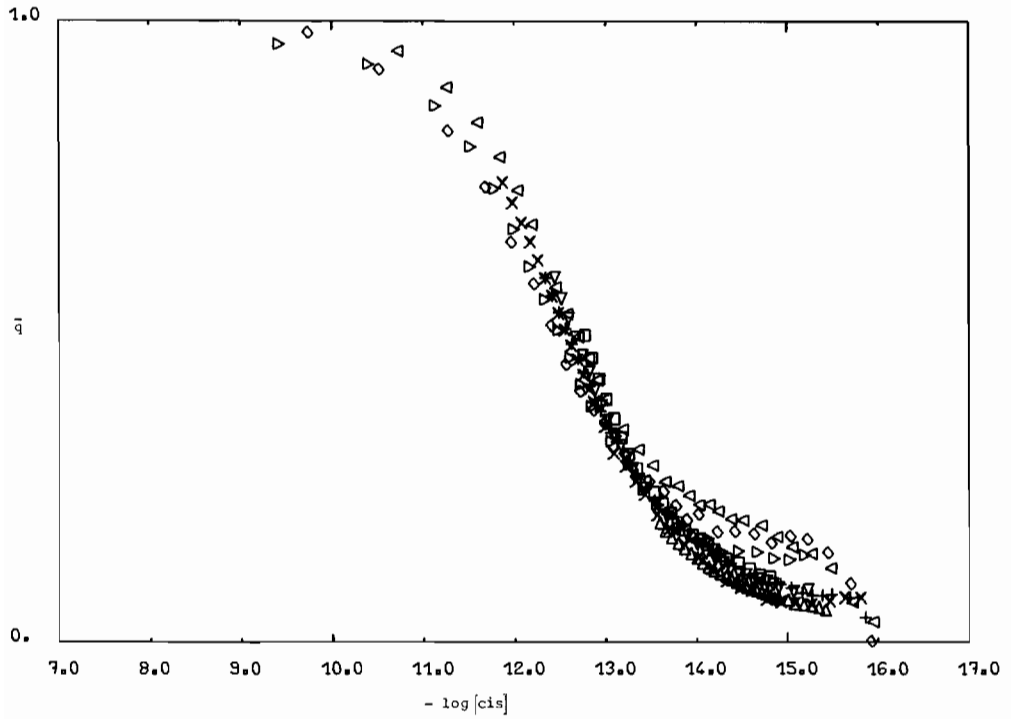

Fig. 2. Copper(II)–cystinate experimental formation curve at 37 °C in aqueous medium $NaClO_4$ 0.15 mol dm^{-3}. The following symbols correspond respectively to the order of the experiments as given in Table I: +, X, □, △, ▽, ▷, ◁, ◇.

–log[H] = 2.5, and did not take into consideration the experimental points systematically removed from the average curve obtained (Fig. 1). Moreover, we Gran titrated perchloric acid and cystine with the help of the very e.m.f. measurements obtained for every protonation curve.

As mentioned earlier [25], the cystine zwitterion is not soluble in aqueous solution, so we had to run the titrations in such a way that the –log[H] inflexion range was crossed as quickly as possible, 'jumping' from –log[H] ~ 4 to –log[H] ~ 6. Every time this transition was not quick enough, a slight precipitate occurred in the solution, which redissolved beyond –log[H] ~ 8, but we succeeded in avoiding significant precipitation in several of our titrations, which were predominantly used for the calculations. Thus we could determine all of the four protonation constants of cystinate, the logarithmic values of which are shown in Table III.

Copper Cystinate

Very few investigations on the complex formation in the copper–cystinate system were to be found in the literature; in particular, no quantitative study of the formation constants was reported but those of the above mentioned authors [25, 26]. Hawkins and Perrin [25] had suggested the formation of the series of complexes MXH, M_2X_2H, MX_2H_2, $M_2X_3H_2$, M_2X and M_2X_2, but had specified that MXH, MX_2H_2 and M_2X_2 accounted for 97% of all the copper present, M_2X_2 being the predominant species among these three. Later, Hallman, Perrin and Watt [26] mentioned only the species M_2X_2 and MXH.

In earlier studies, various authors [28–30] had postulated the formation of the mononuclear species MX, in which copper was assumed to be coordinated via both amino and carboxylate groups, but Hawkins and Perrin demonstrated, using atomic models, that simultaneous attachment of both ends of the cystinate anion to the same copper(II) ion was sterically impossible. Concerning this MX complex, other authors [31] hau also suggested that it was present in dilute solution, but unfolded on concentration, to give rise to the chelation of two different cystinate molecules to each copper ion, thus resulting in a polymer of infinite chain length.

In order to investigate very carefully the different complex stoichiometries possibly occurring in this system, we carried out our experiments in the widest range of metal to ligand ratios, as indicated in Table I. Furthermore, the strong acid concentrations were necessarily high and even the slightest inaccuracy on these could have a bad effect on the calculations of the complexed fractions of cystinate; we thus used for the copper complexation study the concentrations of these two reactants already titrated under the same conditions during the

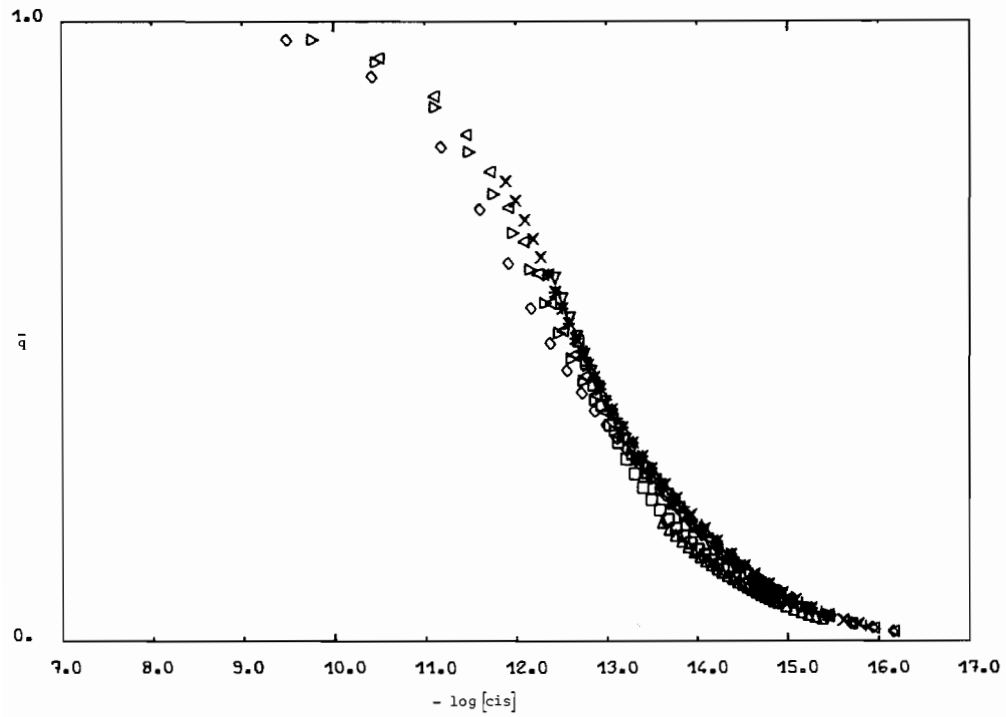

Fig. 3. Copper(II)–cystinate formation curve as obtained from PSEUDOPLOT simulation based on results in Table III.

protonation experiments, or titrated them separately under the adequate conditions.

The formation curve shown in Fig. 2 revealed a regular shape with an upper limit of $\bar{q} = 1$. Due to considerations developed in the above paragraph concerning protonation, some inconsistent data were obtained under the most acidic conditions, corresponding to $-\log[cis]$ values higher than 16; hence they were disregarded in the formation constant computations. Apart from the upper limit just mentioned, two other main features could be distinguished on the formation curve: (i) a systematic parallel spreading, characteristic of the existence of polynuclear species, (ii) the slight rising of the lower points which did not reach $\bar{q} = 0$, possibly indicating the formation of protonated complexes.

Taking into account these observations together with the indications given by the above mentioned authors, we tried to refine different sets of formation constants. First, we ran the model suggested by Hallman, Perrin and Watt [26], consisting of only M_2X_2 and MXH. Then the refinement of Hawkins and Perrin's constants [25] produced negative values for M_2X_2H, MX_2H_2 and M_2X_3H, the consequent addition of M_2X to M_2X_2 and MXH resulting in a slightly lower sum of squares (respectively 0.1086 E – 05 instead of 0.1197 E – 05), which indicated that M_2X was only a minor species. In spite of this, the PSEUDOPLOT approach (Fig. 3) revealed that M_2X should not be regarded as negligible, so we considered the results in Table III as the 'best' for this system.

An attempt was made to replace M_2X_2 by MX in the 'best' model, but the fitting was less acceptable. Moreover the simultaneous refinement of MX together with M_2X_2 resulted in a negative constant for MX. Similarly, we tested to which extend the polymerisation formerly mentioned could be regarded as realistic. We tried to refine MXH together with M_2X_2 and M_3X_3: this resulted in a negative formation constant for M_2X_2 and a sum of squares of 0.1024 E – 05, but the PSEUDOPLOT simulation of the formation curve was worse than the previous one shown in Fig. 3. Finally, we tried to refine MXH, M_3X_3 and M_4X_4 simultaneously: the M_4X_4 constant became negative. We can thus assert the results in Table III to be the 'best' for this system under the present experimental conditions.

Copper Lysinate

Except Kaczmarek *et al.* [32] who had formerly reported the existence of the only species MX and MX_2, the various authors who had successively studied the copper lysinate system agreed on the stoichiometries of the complexes MXH, MX_2H, MX_2H_2 and MX_2, which were characterised whatever were the respective conditions investigated [33–36]. We nevertheless tried to refine a number of sets of constants derived from several combinations of the MX, MX_2, MXH, MXH_2, MX_2H and MX_2H_2 which

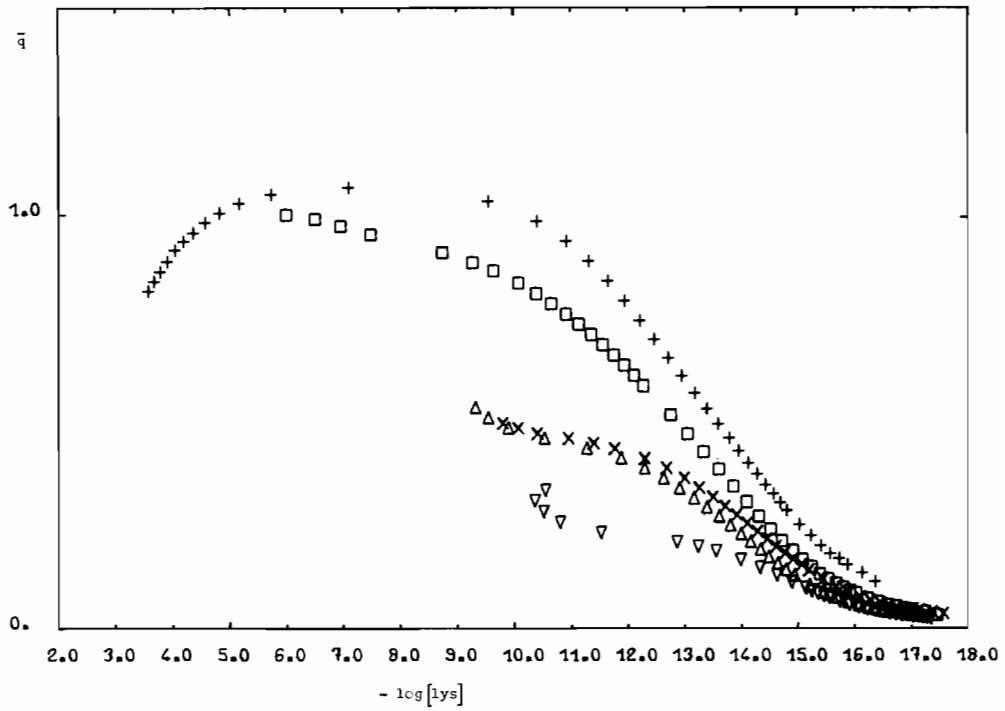

Fig. 4. Copper(II)–lysinate experimental formation curve at 37 °C in aqueous solution $NaClO_4$ 0.15 mol dm^{-3}. The following symbols correspond respectively to the order of the experiments as given in Table I: +, ×, □, △, ▽.

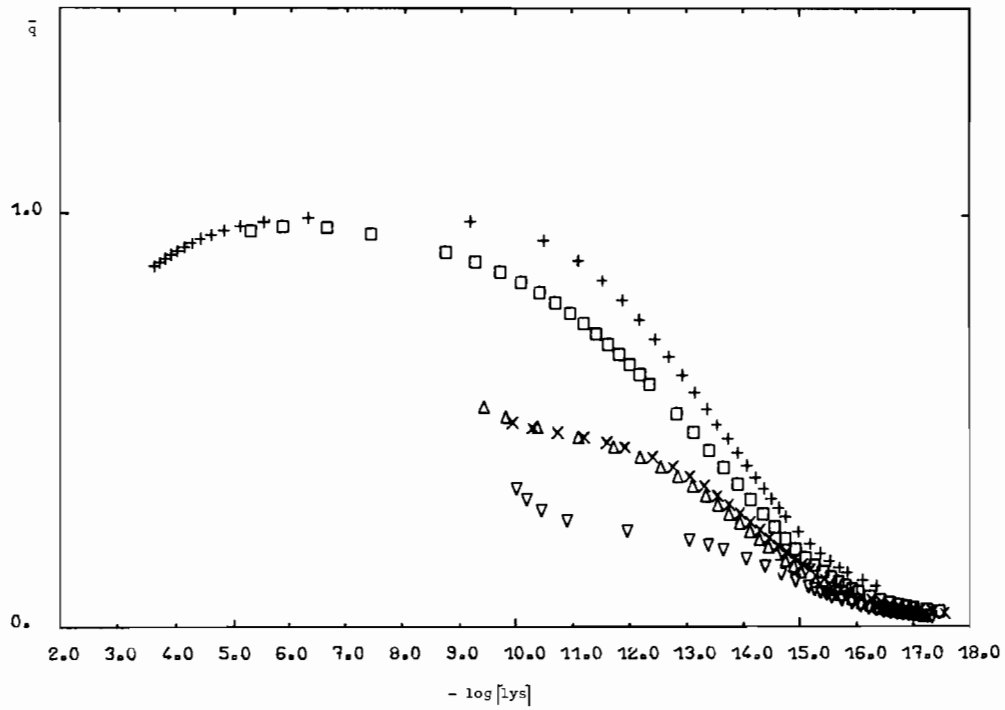

Fig. 5. Copper(II)–lysinate formation curve as obtained from PSEUDOPLOT simulation based on results in Table III.

we could expect to obtain from the formation curve (Fig. 4).

Every time MX_2 appeared in our calculations, even in the simplest model MX, MX_2 suggested by Kaczmarek *et al.* [32], its formation constant turned out to be negative. This observation does not seem very surprising, however. In fact, Gergely *et al.* [36] as well as Nakasuka *et al.* [34] had described

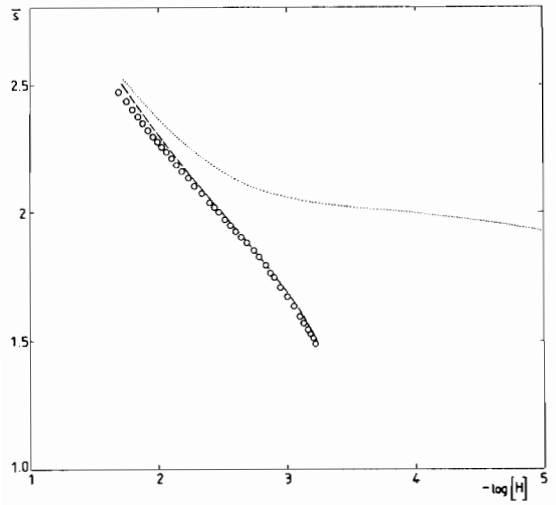

Fig. 6. Protonation curve of a mixture of histamine and cystinate in the presence of copper(II), at the respective overall concentrations: C_{hsn} = 4.55, C_{cis} = 5.00, C_{Cu} = 4.96 mmol dm^{-3}. The dotted line simulates the curve assuming no metal complexation; the broken line simulates the curve assuming no mixed-ligand species formation; experimental points are materialized.

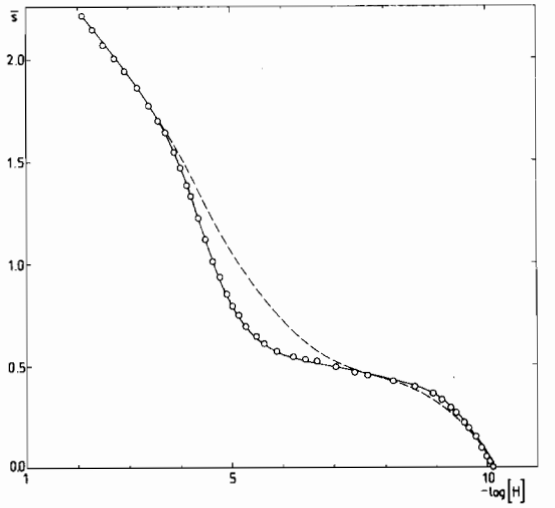

Fig. 7. Protonation curve of a mixture of histamine and lysinate in the presence of copper(II), at the respective overall concentrations: C_{hsn} = 9.10, C_{lys} = 9.50, C_{Cu} = 9.93 mmol dm^{-3}. The broken line simulates the curve assuming no mixed-ligand species formation; the solid line takes into account the Cu–hsn–lys and Cu–hsn–lys–H as given in Table III; experimental points are materialized.

the formation of MX_2 as significant only for –log [H] > 10. As can be seen in Table I, the highest –log[H] value that could be reached in our study was 8.9, due to the occurring of precipitates in the solutions. The formation of MX_2 could thus be ruled out under the present experimental conditions.

Among the various combinations of constants examined, the previous authors' set was reduced to MXH, MX_2H and MX_2H_2, the sum of squares being 0.199 E – 05 in that case. The adding of MX to this model improved both the sum of squares (0.150 E – 05) and the standard deviations relative to these first three constants, but further introduction of MXH_2 into the resulting set still improved the sum of squares, the standard deviations and the PSEUDOPLOT simulations as well (Fig. 5). Corresponding results are shown in Table III.

Copper–Histamine–Cystinate

As no complex had been found in the copper cystinate system with any stoichiometry based on the 1:2 metal to ligand ratio, the formation of mixed-ligand species between copper, cystinate and histamine was not expected to be favoured.

Accordingly, the PSEUDOPLOT-simulated protonation curves of both ligands, assuming no ternary species, fitted fairly well with the experimental determinations, as can be seen on Fig. 6, giving one of the experiments reported in Table I as an example. Obviously, the deviation observed in Fig. 6 between the simulated protonation curves respectively in the absence and in the presence of copper could be accounted for by binary complexes only. Nevertheless, we tried to estimate and refine equilibrium constants for MLX, MLXH, $MLXH_2$, and also M_2LX and M_2LXH although less likely to occur from the chemical point of view. But, as expected above, no ternary species could be characterised, all of these constants being made negative during MINIQUAD refinements.

Copper–Histamine–Lysinate

Unlike cystinate, lysinate gave rise to the formation of 1:2 metal to ligand ratio stoichiometries. Indeed, MX_2H was characterised as a significant species as well as ML_2H in the copper–histamine system. To a lesser extent, the occurrence of MX_2, even though at high –log[H] values, together with ML_2 could result in the formation of a significant MLX species.

In this respect, the difference observed between the experimental overall protonation curve of both ligands and the simulated one assuming no ternary species (Fig. 7) clearly suggested the existence of such species. Accordingly, MLX and MLXH whose formation constants can be seen in Table III were unequivocally characterised. These values were confirmed by the protonation curves simulated on their basis, which produced remarkable fits with the corresponding experimental ones, as is shown in Fig. 7 given as an example.

Discussion

In spite of the insolubility of the cystine zwitterion in aqueous solution, the dissolving of cystine in an excess of acid and the use of the experimental procedure described above enabled us to determine the whole protonation curve of the cystinate anion. The two most basic protonation constants derived from this curve (Fig. 1) confirm essentially the values already mentioned by Hallman, Perrin and Watt under similar experimental conditions [26]; our finding of slightly lower values is in line with our previous observations on such protonation constant determinations [16–18, 22, 23]. Furthermore, the two most acidic constants were determined for the first time, and with good accuracy. This is of some importance with regard to the evaluation of the complexation of metal ions in the acidic range, when based on proton selective electrode measurements.

For the reasons above, the investigation of the cupric ion complexation with cystinate could be monitored on the basis of the various experimental formation curves, depending on the different reactant concentrations and metal to ligand ratios used. Advantage was taken of the features of these curves in (i) the preliminary assessment of the complex stoichiometries, (ii) the final choice of the 'best' set of formation constants for the system, based on graphical comparisons as developed above. In that respect, two main remarks can be made: (i) we confirmed the existence of the major species MXH and M_2X_2 mentioned by Perrin *et al.* in their more recent study [26], but also unequivocally characterised M_2X as a minor species, on both numerical and graphical grounds; (ii) PSEUDOPLOT graphical comparisons enabled us to establish definitely the existence of M_2X_2 rather than either MX or M_3X_3, although the numerical fit was better for the latter.

The very high stability of M_2X_2, probably due to the specific stericity suggested by Hawkins and Perrin [25], is apparently the main reason why no MX_2 species can be formed between copper(II) and cystinate, as clearly appears from Fig. 2. For the same reason, no MLX could be characterised in the copper–histamine–cystinate system either.

Concerning copper(II) lysinate interactions, apart from our characterization of MX and MXH_2 as minor species which had not been mentioned by previous authors [33–36], the major discrepancy of our results with the former lies in the absence of MX_2 in our 'best' set of constants. Besides our usual approach to choose this best set, we thus checked that the introduction into it of the MX_2 constant found by Agarwal [33] under similar experimental conditions did not improve the PSEUDOPLOT simulations, which were actually worsened. Moreover, we ran a COMICS [37] distribution of the species formed by the pertinent reactants under plasma conditions and checked that the MX_2 species, already shown to be insignificant below $-\log[H] = 10$ [35, 36], could definitely be disregarded for the plasma applications in view.

As for the copper(II)–histamine–lysinate system, the comparison of the experimental formation constants of MLX and MLXH with those calculated on purely statistical bases [38] can throw light on the ability of the reactants to give rise to mixed-ligand ternary species. In accordance with the following equation:

$$\log \beta_{MLXH_s} = \tfrac{1}{2} \log \beta_{ML_2H_s} + \tfrac{1}{2} \log \beta_{MX_2H_s} + \log 2$$

using $\beta_{ML_2H_s}$ values as obtained by two of the present authors [13], β_{MX_2H} as taken from this study and β_{MX_2} from ref. 33, statistical values of the formation constants for MLX and MLXH are respectively 15.34 and 23.62 which are to be compared with the experimental values in Table III.

The sharp increase of stability observed in the ternary complexation of this system could result in a greater involvement than originally expected for the corresponding species in the distribution of the metal histamine complexes in blood plasma [13]. This will be examined in our next paper in this series.

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