Thermodynamic Considerations in Co-ordination. Part X.[1] A Potentiometric and Calorimetric Investigation of Copper(II) Histidine Complexes in Solution

By **David R. Williams**, Department of Chemistry, University of St. Andrews, St. Andrews, Fife, Scotland

The copper(II)–histidine (histH) system has been investigated at 25·0 °C, I = 3·00M $(Na^+)ClO_4^-$ (*a*) potentiometrically, by use of normalised curve analysis of copper amalgam data and least-squares analyses of glass electrode data, and (*b*) calorimetrically. If (*p*,*q*,*r*) represents the general complex $(hist^-_p\ Cu^{2+}_q\ H^+_r)$, the complexes found between pH 3·0 and 10·4 were (1,1,0), (2,1,0), (1,1,1), (2,1,1), (2,1,2), and (1,1,−1). Enthalpies of formation were measured for the first five complexes and structures are suggested for all six.

FORMATION constants for the copper(II)–histidine system were first reported by Maley and Mellor [2] and complications were noted by Albert [3] in that the system could not be adequately expressed in terms of AB- and A_2B-type constants. The possibilities of histidine being uni- or bi-dentate and of protonated com-

[1] Previous Parts of this series are I, II, and III, F. Holmes and D. R. Williams, *J. Chem. Soc.*, 1967, 729, 1256, 1702; IV and V, I. Grenthe and D. R. Williams, *Acta Chem. Scand.*, 1967, **21**, 341, 347; VI and VII, D. R. Williams, *J. Chem. Soc.* (*A*), 1968, 2965; 1970, 1550; VIII and IX, A. D. Jones and D. R. Williams, *ibid.*, 1970, 3138; 1971, 3159.

[2] L. E. Maley and D. P. Mellor, *Nature*, 1950, **165**, 453.

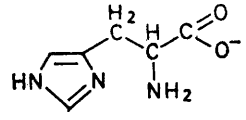
[3] A. Albert, *Biochem. J.*, 1952, **50**, 690.

plexes being formed were considered.[4,5] Later, graphical methods of data analysis were supplemented by the use of computers which permitted not only a closer scrutiny of the protonated complexes but also of hydroxy-complexes.[6-8] More recently Bauman *et al.* studied the system by using calorimetry to split formation constants into their enthalpy and entropy contributions.[9]

Wilson and Martin[10] used an alternative approach. They used a multi-method attack (u.v., charge transfer, visible c.d., absorption spectra, titrations, and formation constants) and our present study follows their advice that calorimetry has been incompletely exploited in the copper–histidine system.

If A, B, and H refer to the total molar concentrations of histidyl$^-$, cupric ion, and ionisable protons, and if we define a general complex $A_pB_qH_r$, the problems are to decide which sets of values of p, q, and r apply to the system, to express the system in terms of the minimum number of (p, q, r) constants, and to measure the heats of formation of these complexes. Although both glass-electrode potentiometry and calorimetry have been used before, this study was designed to be more precise (*a*) by using an amalgam electrode to measure the extent of polynuclearity amongst the triplets (p, q, r) rather than only searching for complexes ' which might reasonably be expected to be present ',[11] (*b*) by limiting the experimental variables in the potentiometry through holding A and B or [H$^+$] and B constant throughout a titration, and (*c*) by using a wider range of A, B, and H in both the potentiometry and calorimetry. The notation is summarised in Table 1.

TABLE 1



(I)

Notation

A = histidyl$^-$ (I)
B = Cu^{2+}
H = H^+

A = total molar concentration of histidyl$^-$
B = total molar concentration of cupric ions
H = total molar concentration of ionisable protons

a = free molar concentration of histidyl$^-$
b = free molar concentration of cupric ions
h = free molar concentration of ionisable protons

E refer to e.m.f.s measured by the glass or metal amalgam electrodes respectively denoted by subscripts g or m. E_0 are the standard electrode potentials and superscripts denote concentrations of ions in solution under test, *e.g.*, E_m^b.

$Y = \frac{(H - h)}{A}$ = average number of H$^+$ bound per histidyl$^-$

[4] S. P. Datta, R. Leberman, and B. R. Rabin, *Biochem. J.*, 1958, **68**, 22.
[5] R. Leberman and B. R. Rabin, *Trans. Faraday Soc.*, 1959, **55**, 1660.
[6] D. D. Perrin, *Nature*, 1959, **184**, 1868.
[7] D. D. Perrin, *J. Chem. Soc.* (*A*), 1967, 724.
[8] H. C. Freeman and R.-P. Martin, *J. Biol. Chem.*, 1969, **244**, 4823.

EXPERIMENTAL

Chemicals were as in Parts VII and VIII,[1] except that L-histidine was used instead of the hydrochloride because chloride ions are suspected of forming complexes at the amalgam. L-Histidine (E. Merck, m.p. 276 °C; lit. 270—280°) was recrystallised twice, dried, and analysed (Found: C, 46·3; H, 5·90; N, 27·0. Calc. for $C_6H_9N_3O_2$: C, 46·4; H, 5·84; N, 27·1%).

Nitrogen was obtained from an oxygen-free cylinder and purified by passage through five thermostatted wash-bottles (2 of chromous chloride, 1 of 0·1M-NaOH, and 2 of ionic background solution). After it had been passed through the titration vessel or the amalgam reservoir it was released into the atmosphere *via* two more thermostatted wash bottles which acted as traps to prevent back-diffusion of oxygen and carbon dioxide.

Glass electrodes were obtained from Activion, platinum electrodes were freshly cleaned before use, and the Wilhelm bridge reference electrode system contained three silver–chloride electrodes (prepared by Brown's method [12]).

The copper amalgam was prepared by electrolysis of an acidified copper(II) solution with a mercury pool as cathode. The constitution was *ca.* 3—4% (w/w), and the amalgam was stored under nitrogen in a container that was so arranged that the amalgam could be blown into the titration vessels by a stream of nitrogen. The amalgam was aged for 2 months at pH 2 before use.

E.m.f. readings were taken on Radiometer digital voltmeters (pHM52 ± 0·05 mV). Calculations were performed on the Atlas computer at Chilton and the IBM 360/44 at St Andrews.

Burettes and pipettes were calibrated and, along with the titration vessels, flushed through with nitrogen before use.

Potentiometry.—The reactions between ligand, metal ion, and protons can be represented (charges omitted) by equation (1) and the equilibrium constant for this generalised reaction is β_{pqr}. Clearly it is necessary to determine the prevailing values of triplets (p, q, r) before accurate values for the constants themselves can be computed. Following Österberg,[13,14] we require a series of data having known values for four of the six quantities A, B, H, a, b, and h, and so employ a cell (2) to provide (A, B, H, h) data and a cell (3) to provide (A, B/b, B, h) data.

$$pA + qB + rH \rightleftharpoons A_pB_qH_r \qquad (1)$$

Glass electrode|equilibrium solution|reference electrode (2)

Cu-Hg|equilibrium solution|reference electrode (3)

Data from cell (3) provides information concerning the values of the triplets and some formation constants. Data from (2) can yield accurate values of all the constants.

Amalgam Electrode Measurements for (A, B/b, B, h) *Data.*

[9] J. L. Meyer and J. E. Bauman, *J. Amer. Chem. Soc.*, 1970, **92**, 4210.
[10] E. W. Wilson, jun., M. H. Kasperian, and R. B. Martin, *J. Amer. Chem. Soc.*, 1970, **92**, 5365.
[11] C. W. Childs and D. D. Perrin, *J. Chem. Soc.* (*A*), 1969, 1039.
[12] A. S. Brown, *J. Amer. Chem. Soc.*, 1934, **56**, 646.
[13] R. Österberg and B. Sjöberg, *J. Biol. Chem.*, 1968, **243**, 3038.
[14] R. Österberg and B. Sjöberg, *Acta Chem. Scand.*, 1968, **22**, 689.

—The vessel and electrodes (glass, amalgam, and reference) were clamped in a thermostat bath at 25·00 ± 0·05 °C. Standard solutions of known concentration of H and B were introduced to determine E_{0g} and E_{0m} respectively from the e.m.f.s measured in the glass electrode and amalgam electrode cells from relationships (4) and (5).

$$E_g = E_{0g} - 59{\cdot}155 \log h \quad (4)$$

$$E_m = E_{0m} - 29{\cdot}577 \log b \quad (5)$$

Liquid-junction potential considerations are encompassed in these E_0 values. This procedure was repeated at the conclusion of the titrations about to be described.

For $-\log h$ values of 3·00, 5·00, 7·00, and 9·00 e.m.f. readings were taken for a series of titrations for B held constant at 2, 5, 10, and 20 mM. Solubility difficulties prevented higher values of B being used. The log h range was chosen on the basis of hydroxy-precipitates occurring at $-\log h > 9{\cdot}00$ and very little complexing occurring below $-\log h = 3{\cdot}00$. For each point in the titration, a concentrated solution of A (B = constant) was added to the vessel (already at constant B). The $-\log h$ was adjusted by adding acid or alkali from Agla syringes and then a small amount of B was added from another syringe to bring the solution back to the constant B currently being used. When both potentiometers showed constant readings, the values were noted, the amalgam surface disturbed by rotating the platinum contact in its socket and the readings retaken when steady. Coincident readings before and after stirring the amalgam were taken as signifying equilibrium. If the system failed to reach equilibrium after 1 h, a drop of amalgam was pumped in from the reservoir to replenish the surface. The resultant data were E_m, A, h (constant for each series of titrations), and B (constant for each titration). Finally, the E_{0g} and E_{0m} values were rechecked at the end of each titration, each titration taking *ca.* 2 days. The possibility that copper might be withdrawn from the amalgam was ruled out by occasionally analysing the resultant titration solution by electrodeposition. Such analyses agreed to within 0·5%. E_m values of repeated titrations agreed to better than 0·3 mV.

Graphs of log $(B/b) = (E_m{}^b - E_m{}^B)/29{\cdot}577$ against A were constructed for each constant log h value.

Glass Electrode Measurements for (A, B, Y, h) Data.—Cell (2) was arranged under similar thermostatting and nitrogen atmosphere conditions to those described for the amalgam measurements. E_{0g} was obtained from e.m.f. measurements on solutions of known h concentration.

From B values of 2, 5, 10, and 19 mM a series of titrations were performed for A held constant and H being varied as h is monitored from equilibrium E_g values. As in the amalgam measurements the ranges of A, B, and H used were limited by solubility, liquid-junction potential interference, and sodium ion correction considerations.

Data Treatment.—A, B/b, B, h data were analysed by curve normalisation to investigate the extent of polynuclearity of the system. The approach is one of *assuming* a set of complexes and relating their βs to experimental data in terms of normalised variables (denoted with a superscript *). Fresh sets are assumed in turn until the data are well fitted.[15]

Experimental points for $-\log h$ = 9·00, 7·00, and 5·00 are shown in Figure 1 and curves drawn through these points will have a unique pattern if only mononuclear complexes are present and this pattern is solely dependent on $-\log h$, pK, and B, *i.e.*, results at a constant $-\log h$ ought to be independent of influence from hydroxy- or protonated mononuclear complex formation.

The mathematical relationships are (charges omitted) as in equations (6) and (7). Histidyl has $\beta_{101} = 4{\cdot}266 \times 10^9$ l mol^{-1}, $\beta_{102} = 3{\cdot}981 \times 10^{16}$ l^2 mol^{-2}, $\beta_{103} = 7{\cdot}620 \times 10^{18}$ l^3 mol^{-3}. For constant $-\log h$, (i) the term in parentheses is constant, K_H say, and (ii) h^r and h^s are constant so that $\beta_{11r} \equiv \beta_{110} \times$ *a constant, etc.* Hence the normalised constants β^*_{11} and β^*_{21} may be used, as in equation (8). When $b \ll B$, we obtain equation (9).

$$A = a + \mathrm{AH} + \mathrm{AH_2} + \mathrm{AH_3} + \mathrm{ABH}_r + 2\mathrm{A_2BH}_s \quad (r \text{ and } s \text{ may be positive, negative, or zero}) \quad (6)$$

$$= a(1 + \beta_{101} \cdot h + \beta_{102} \cdot h^2 + \beta_{103} \cdot h^3) + \beta_{11r} \cdot a \cdot b \cdot h^r + 2\beta_{21s} \cdot a^2 \cdot b \cdot h^s \quad (7)$$

$$A = aK_H + \beta^*_{11} \cdot a \cdot b + 2\beta^*_{21} \cdot a^2 \cdot b \quad (8)$$

$$B = \beta^*_{11} \cdot a \cdot b + \beta^*_{21} \cdot a^2 \cdot b \quad (9)$$

Österberg [13] has treated these two relationships using the normalised definitions of A^*, a^*, B^*, and b^* in equations (10)—(13) to give the normalised relationships (14) and (15). These equations are solved for B^*/b^* by using the known K_H and a set of values for B^*. Each of these B^* is combined with a range of a^* and a normalised curve of log B^*/b^* against log A is constructed (see Figure 1).

$$A^* = A \cdot \beta^*_{21}/\beta^*_{11} \quad (10)$$

$$B^* = B \cdot \beta^*_{21}/\beta^*_{11} \quad (11)$$

$$a^* = a \cdot \beta^*_{21}/\beta^*_{11} \quad (12)$$

$$b^* = b \cdot \beta^*_{11} \quad (13)$$

$$B^* = a^* \,.\, b^* + a^{2*} \,.\, b^* \quad (14)$$

$$A^* = K_H \,.\, a^* + a^* \,.\, b^* + 2 \,.\, a^{2*} \,.\, b^* \quad (15)$$

If only mononuclear complexes are present, it is possible to slide the normalised curves along the vertical and horizontal axis of the experimental points and to obtain a neat fit. In the position of best fit it may be seen, from our definitions of normalised ligand and metal concentrations, that the vertical axis displacement is given by equation (16) and the horizontal axis displacement by (17).

$$= \log \frac{B^*}{b^*} - \log \frac{B}{b} = \log \frac{\beta^*_{21}}{\beta^*_{11} \cdot \beta^*_{11}} \quad (16)$$

$$= \log A^* - \log A = \log\beta^*_{21} - \log \beta^*_{11} \quad (17)$$

Hence the axis displacement may be solved for β^*_{11} and β^*_{21}.

A knowledge of these constants also permits a values to be calculated from $a^* = a(\beta^*_{21}/\beta^*_{11})$ and so a more conventional $\bar{Z}$–(−log a) formation curve can be plotted. If only AB mononuclear complexes were present, formation curves from different B and $-\log h$ values ought to be superimposable.

The normalised curves in the position of best fit are shown in Figure 1. There is no detectable polynuclearity at log h = 9·00 or 7·00. Some was possible for lower B/b at $-\log h$ = 5·00 but only in the lower B/b region where the assumptions in deriving this set of normalised curves become somewhat tentative (*e.g.*, b approaches B). In the positions shown, the normalised constants are as in Table 2.

[15] F. J. C. Rossotti and H. Rossotti, ' The Determination of Stability Constants,' McGraw-Hill, London, 1961.

Thus q(max) appears to be 1, and p(max) may be assumed = 3 from molecular models, co-ordination number, and size considerations. Having three electron-donor groups,

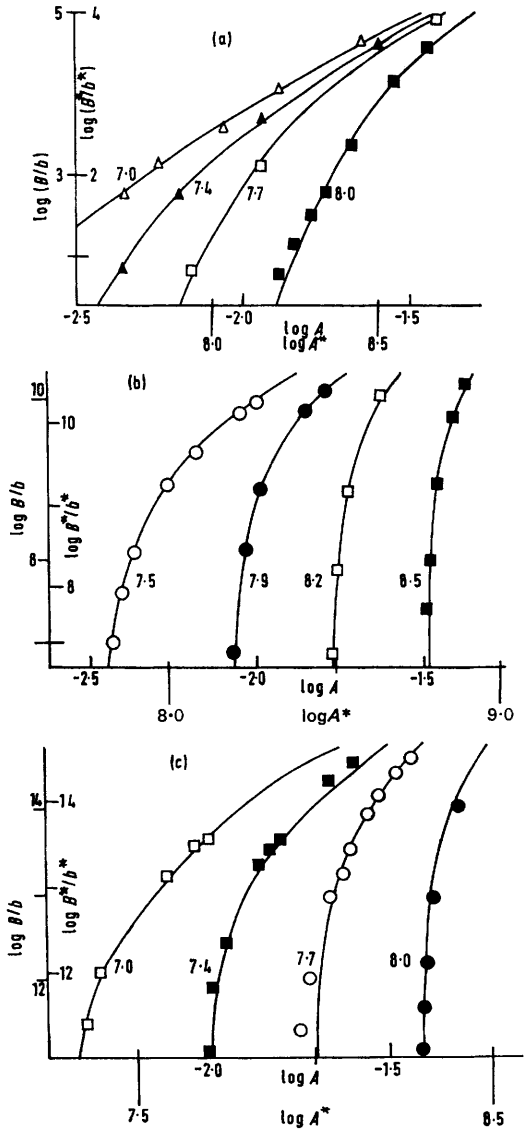


FIGURE 1 Experimental [log (B/b)–(log $A)_{B,h}$] data compared with normalised curves generated for [log (B^*/b^*)/log $A^*]_{B^*K_H}$. From left to right the curves refer to B = 2, 5, 10, and 20 mM respectively; values of log B^* are marked on the curves; (a) $-\log h$ = 5·0 (b) $-\log h$ = 7·0; (c) $-\log h$ = 9·0

AH might reasonably be expected to complex with B, and we must now estimate the maximum value of r by asking whether AH_2 can complex with B. Here we consider the most likely set of data for forming protonated complexes, *i.e.*, $-\log h$ = 3·00 and make the working assumptions (i),

$$\text{(i)}\ A = a + \text{AH} + \text{AH}_2 + \text{AH}_3 \doteqdot a \cdot K_H$$
$$\text{(ii)}\ B = b + \text{AB} + \text{ABH} + \text{ABH}_2$$

from which $a \doteqdot A/K_H$, in the most acid, least complexing region, and (ii). From these assumptions we obtain

$$\frac{(B-b)}{b} = a(\beta_{110} + \beta_{111} \cdot h + \beta_{112} \cdot h^2) \qquad (18)$$

$$\frac{B}{b} \doteqdot \frac{A}{K_H}(\beta_{110} + \beta_{111} \cdot h + \beta_{112} \cdot h^2) + 1 \qquad (19)$$

equations (18) and thence (19) which is the equation of a straight line of B/b against A that passes through B/b = 1.

Such lines, when plotted, had slopes of magnitude $(\beta_{110} + \beta_{111} \cdot h)/K_H$ rather than $(\beta_{110} + \beta_{111} \cdot h + \beta_{112} \cdot h^2)/K_H$ and so it was assumed that AH_2 does not complex with free b. Hence, $r_{max.}$ = one per A present in each complex = $p_{max.}$. (This argument presupposes that an estimate of β_{112} is available. This was obtained from extrapolations of the amalgam β_{110}, β_{210}, β_{111}, and β_{211} values given in the next paragraph.)

For $-\log h$ = 9·00, the amalgam electrode normalised β^*s gave log β_{110} = 9·6, log β_{210} = 19·3 and these values when combined with the $-\log h$ = 5·00 data gave log β_{111} = 16·0 and log β_{211} = 26·1. The maximum values are p = 3, q = 1, and r = 3.

A, B, H, h Data.—These β values and p, q, and r ranges were then used in the computational searching for the 'best' set of formation constants to describe the A, B, H, h data. The two most important programs for performing such calculations are LETAGROP VRID [16,17] and SCOGS.[18] Both use a non-linear least-squares computation based upon the Taylor series but SCOGS considers only first-order terms whereas LETAGROP VRID considers first- and second-order terms and also avoids slow or incorrect convergence by using a twist matrix to negotiate skew pits (the least-squares sum U_0 occurs at the minimum of these pits). Both programs aim to minimise the residuals in titres as defined as $U = \Sigma(\text{titre}_{calc}\text{-titre}_{exptl})^2$. The glass electrode results were searched as in Table 3.

TABLE 2

$-\log h$	log β^*_{11}	log β^*_{21}
9·00	9·63	19·34
7·00	9·96	20·22
5·00	11·09	21·18

TABLE 3

1. $(Y/\log h)_B$ data suggested constants similar to Perrin's [7] and Bauman's [9] when subjected to SCOGS but not to LETAGROP
2. Bauman's series tried (AB, A_2B, ABH, A_2BH) on $(Y/\log h)_{A,B}$ data
3. Perrin's series tried (AB, A_2B, ABH, A_2BH, ABH_{-1}, $A_2B_2H_{-2}$ but $A_2B_2H_{-2}$ was not really necessary; SCOGS converged better without it, see 4
4. AB, A_2B, ABH, A_2BH, ABH_{-1}: converged best by both least-squares programs
5. AB, A_2B, ABH, A_2BH, ABH_{-1} and Freeman's [8] A_2BH_2: 'best' yet by both SCOGS and LETAGROP, *i.e.*, A_2BH_2 is present and this has previously been reported by only Freeman and Martin; [8] A_2BH_{-2} was not found, but it was suitable for 1. Hence, holding A constant removes the necessity of having a dihydroxy-product below pH = 9

   In an endeavour not to presuppose complexes formed we offered the computer all protonated and hydroxy-possibilities up to A_3BH_n and set 5 was invariably the best outcome.

Calorimetry.—The procedure was fundamentally as described in Part VIII and involved repeating the (A, B, H, h) potentiometric titrations in the calorimeter. An example of the enthalpic curves is shown in Figure 2,

[16] N. Ingri and L. G. Sillén, *Arkiv Kemi*, 1964, **23** : **10**, 97.
[17] A. Vacca, modification of ref. 16, personal communication.
[18] I. G. Sayce, *Talanta*, 1968, **15**, 1397.

TABLE 4

Overall Gibbs free energy changes, enthalpies/kJ mol^{-1}, and entropies/J K^{-1} mol^{-1} for histidyl–copper(2+)–proton complexes at 25 °C, I = 3·00 mol l^{-1} in $NaClO_4$

A	B	H	Present work					Other work
			$-\Delta G°$	$-\Delta H°$	$\Delta S°$	$-\Delta H°$ [b]	$\Delta S°$ [b]	$-\Delta H°$
p	q	r						
1	0	1	54·9 [a]	40·42 ± 0·46 [a]	48·9 ± 1·7 [a]	43·6	29·7	
1	0	2	94·8 [a]	77·03 ± 0·88 [a]	59·4 ± 3·4 [a]	73·0	49·4	
1	0	3	107·8 [a]	78·07 ± 1·30 [a]	99·6 ± 5·0 [a]	76·0	77·8	
1	1	0	57·6	43·89 ± 0·71	45·9 ± 4·2	48·4	29·3	$-\Delta H°$ (A_2B)
2	1	0	108·6	83·93 ± 0·82	82·8 ± 3·6	89·2	46·0	87·9 [c]
1	1	1	89·1	66·98 ± 1·01	74·2 ± 4·2	90·5	43·5	87·4 [d]
2	1	1	147·7	107·6 ± 0·98	134·7 ± 4·1	153·1	59·6	92·5 [e]
2	1	2	175·5	129·7 ± 2·07	153·6 ± 8·4	—	—	83·6 [f]
1	1	−1	197·6	—	—	—	—	—

[a] Part VII. [b] Ref. 9. [c] Ref. 23. [d] Ref. 24. [e] Ref. 25. [f] Ref. 26.

TABLE 5

Log β for the species $A_pB_qH_r$ for the present (25 °C and I = 3M-ClO_4^-) and other work. Computations 1—5 gave progressively smaller standard deviations in both the constants and in the titres. The ' best ' constants are shown in bold face and were computed from 24 titrations involving 356 readings. [ ] are 3 × standard deviations in the log constants

			Present work. Calculations nos. 1—5 Further details are given in Table 3					Other workers, ref., temp. and I					
								8, 25 °C 0·1M-	7, 37 °C 0·15M-	9, 25 °C, 0·16M-	21, 25 °C, 0·10M-	4, 25 °C, 0·01M	22, 25 °C, 0·2M-
p	q	r	1	2	3	4	5	KNO_3	KNO_3	KNO_3	KCl		KNO_3
1	1	0	10·94	10·54	10·07	10·09	**10·086** [0·080]	10·13	9·79	10·01	10·21	10·56	10·30
2	1	0	18·35	18·99	19·03	19·03	**19·026** [0·042]	18·10	17·41	18·02	18·53	18·81	—
1	1	1	15·92	15·89	15·61	15·61	**15·615** [0·031]	14·06	14·03	13·58	—	—	—
2	1	1	25·66	25·92	25·88	25·90	**25·884** [0·033]	23·64	23·05	23·71	—	—	—
2	1	2	—	—	—	—	**30·750** [0·048]	26·85	—	—	—	—	—
1	1	−1	4·53	—	3·63	3·38	**3·462** [0·177]	2·13	2·17	—	—	—	—
2	2	−2	6·57	—	→0	—	—	8·01	6·97	—	—	—	—

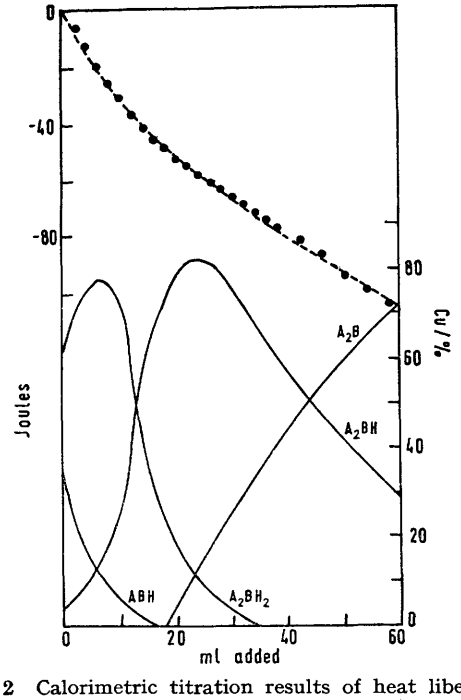


FIGURE 2 Calorimetric titration results of heat liberated (in joules) when 50·00 ml of A = 60·00 mM, B = 19·14 mM, H = 97·78 mM was titrated with a solution of A = 60·00 mM, B = 19·14 mM, H = −18·00 mM. The experimental points are shown as full circles and are plotted above species distribution plots for the four major copper complexes present during this titration. The curves depict the percentage of total copper present in each complex. The broken line is the thermogram calculated from such species distribution plots and the heats of formation listed in Table 5

the reaction heats being some of the most complicated analysed by the RWSOLV program. $\Delta H°$ for ABH_{-1} could not be determined as it occurs at only low concentrations compared with those of the other complexes present. The resultant enthalpies are given in Table 4. The complete experimental data are available from the National Lending Library under Supplementary Publications No. SUP 20363 (11 pp., 1 microfiche).*

DISCUSSION

Formation Constants.—Qualitatively, the results agree with those of Freeman *et al.*[8] and Perrin *et al.*[7] except that for our data $A_2B_2H_{-2}$ is not required for the ' best ' set of constants. This dichotomy possibly arises because moderate changes in the experimental conditions, when dealing with systems of this complexity, are sufficient to require an additional ' constant '. For example, in Table 5 we might compare column 1, where B is constant throughout a titration, to column 5 where both A and B are held constant: Column 5 requires fewer formation constants. Further, Perrin reports *practical* constants obtained over a narrower pH range (3·3—7·0) than ours and at 37 °C. Our constants are *concentration* constants (pH range 3·0—10·4) at 25 °C.

Except for $A_2B_2H_{-2}$, we also agree with the *concentration* constants reported by Freeman *et al.* who used a selected pH range for their least-squares treatment. Both Perrin and Freeman employed a narrower concen-

* For details of Supplementary Publications see Notice to Authors No. 7 in *J. Chem. Soc.* (*A*), 1970, Issue No. 20.

tration range than in the present study. Freeman echoes Sillén's concept that computational methods are most desirable as supplements to graphical methods. We have used graphical methods to indicate the boundaries within which to search for β values and also to

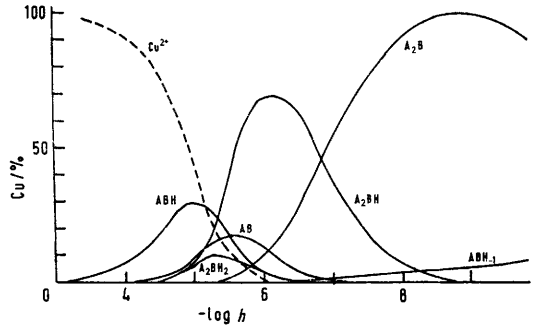


FIGURE 3 Distribution of complexes between Cu^{2+} (B), histidyl$^-$ (A), and the proton (H) expressed as a percentage of the total copper present. The conditions refer to blood plasma total concentrations (B = 18 μM, A = 74 μM). $[Cu_2(OH)_2]$ and $[CuOH^+]$ comprise less than 0·1% of B

yield initial values of the βs. Less rigorous approaches can fail to produce all the 'best' constants; for example, Meyer and Bauman [9] found only four constants out of five searched for.

Numerically, the constants of column 5 of Table 5 agree with those of Freeman and Perrin recalling our previous observation that 3M-ClO_4^- enlarges log β values (Part VII).

Sillén's HALTAFALL program [19] and our 'best' constants were combined to calculate the complexes present in a histidyl$^-$–copper(II) solution of blood plasma total concentrations because 98% of amino-acid bound copper(II) is involved in histidine complexes.[20] The results are shown in Figure 3. From peak positions it may be seen that as $-\log h$ increases, complex formation proceeds as in reactions (20). Extrapolation of this

$$ABH \longrightarrow A_2BH_2 \longrightarrow AB \longrightarrow A_2BH \longrightarrow A_2B \longrightarrow ABH_{-1} \quad (20)$$

series suggests that $A_2B_2H_{-2}$ can only be expected to be present at higher pHs than those shown.

Thermodynamic Parameters for Complex Formation.— Our values for the overall enthalpies and entropies of formation are listed in Table 5 along with previously reported calorimetric results.[21-26] The thermodynamic values for the individual steps leading to these complexes are given in Table 6 and Figure 4 compares the enthalpies of forming AB and A_2B complexes between iron(II), cobalt(II), nickel(II), copper(II), and zinc(II) and histidyl, histamine, and tryptophyl. Clearly, $-\Delta H°$(AB) for A = histidyl > histamine ≫ tryptophyl, implying terdentate bonding between histidyl and

TABLE 6

Stepwise enthalpies and entropies for the histidyl–copper–proton system at 25 °C, I = 3·00 mol l^{-1} in (Na)ClO_4

	Reaction	$-\Delta H°$/ kJ mol^{-1}	$\Delta S°$/ J K^{-1} mol^{-1}
(a)	A + B ⟶ AB	43·9 ± 0·7	45·9 ± 4·2
(b)	AB + A ⟶ A_2B	40·0 ± 1·5	36·9 ± 7·8
(c)	AB + H ⟶ ABH	23·1 ± 1·7	28·3 ± 8·4
(d)	AH + B ⟶ ABH	26·6 ± 1·4	25·3 ± 5·9
(e)	ABH + A ⟶ A_2BH	40·6 ± 1·9	60·5 ± 8·3
(f)	A_2B + H ⟶ A_2BH	23·7 ± 1·8	51·9 ± 7·7
(g)	AB + AH ⟶ A_2BH	23·3 ± 2·1	39·9 ± 10·0
(h)	A_2BH + H ⟶ A_2BH_2	22·1 ± 3·0	18·9 ± 12·5
(i)	2AH + B ⟶ A_2BH_2	48·9 ± 3·0	55·8 ± 11·8
(j)	ABH + AH ⟶ A_2BH_2	22·3 ± 3·5	30·5 ± 14·3

copper. However, models show that all three bonds cannot be of normal length and strength if the copper ion is distorted octahedral. Secondly, $-\Delta H°$(AB ⟶ A_2B) > $-\Delta H°$(AB) for A = histidyl and B = Fe, Co, Ni, or Zn^{II} and for A = tryptophyl or phenylalanyl and B = Cu^{II} [27] (presumably because more energy is necessary to dehydrate B_{aq} than AB_{aq} upon adding an A [28]) whereas for histidyl–Cu^{II} the trend is inverted. This suggests that the second histidyl ligand is less firmly bound than the first. This agrees with Barnes

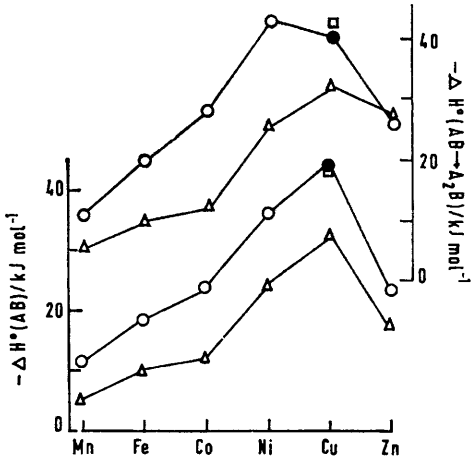


FIGURE 4 $-\Delta H°$ (AB) and $-\Delta H°$ (A_2B) + $\Delta H°$ (AB) for first transition series histidyl (○) tryptophyl (△), and histamine (□) complexes. The full circles represent results taken from this study. Units = kJ mol^{-1}. Conditions = 3M-(Na)ClO_4^- at 25·0 °C

and Pettit's observation [26] that Cu(L-hist)$_2$ was 1 kJ mol^{-1} more stable than Cu(L-hist,D-hist) whereas the converse order applies to the Ni^{II} and Zn^{II} stereoisomers.

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Our entropies of formation have been calculated from $\Delta H°$ and β by use of $\Delta S°_{pqr} = T^{-1}(\Delta H°_{pqr} + RT \ln \beta_{pqr})$ and are larger for $I = 3{\cdot}00$M than for $0{\cdot}15$M[9] and histidyl–copper are larger than histamine–copper (Part III) since the histidyl involves charge neutralisation and a consequent greater loss of water molecules.

Structural Conclusions suggested by the Thermodynamic Parameters.—The histidyl ligand has three readily available co-ordinating groups * and the cupric ion prefers distorted octahedral co-ordination but models show that three *normal* histidyl–copper bonds cannot occur. Further, the potentiometric investigation has shown that copper–histidyl complexes are pH-dependent. Such a balance of bonding between competing donor–proton and donor–metal ion bonds suggests a possibility of tautomerism. However, the ensuing discussion, based upon the stepwise enthalpies and entropies of formation [(a)—(j) of Table 6], suggests that the complexes existing in aqueous solution are of one tautomer only. Literature reports of other aqueous investigations support this suggestion whereas studies involving other temperatures or phases (for example, e.p.r. or crystallography) sometimes suggest a second isomer.

The structure of each complex is considered in turn, conclusions from the present study and other aqueous ambient temperature methods being mentioned first followed by those from other conditions.

AB appears to have structure (II) involving histamine-type planar nitrogen donors and a weaker apical carboxylate.[10] The original suggestion for imidazole–copper bonding came from formation-constant comparisons, *e.g.*, β_{AB} for histidine $\doteqdot \beta_{AB}$ for histamine and $\gg\beta_{AB}$ for phenylalanine ($1{\cdot}22 \times 10^{10}$, $3{\cdot}63 \times 10^{9}$, and $1{\cdot}57 \times 10^{8}$ l mol^{-1} respectively). The enthalpies, $\Delta H°$(AB), that are now available conform to this pattern ($-43{\cdot}89$, $-43{\cdot}05$, and $-21{\cdot}47$ kJ mol^{-1} respectively). Wilson *et al.* have reported structural evidence for (II) [10] and our enthalpies indicate the bonding involved: Doran *et al.* noted that $pK_{\text{histamine}} > pK_{\text{histidine}}$ and yet β_{AB} for histamine $< \beta_{AB}$ for histidine and suggested that all three donor groups are involved. Our $\Delta H°$ values are more specific proof [for histamine, $\Delta H°(AH_2) = -81{\cdot}71$, $\Delta H°(AB) = -43{\cdot}05$, and, for histidine, $\Delta H°(AH_2) = -77{\cdot}03$, $\Delta H°(AB) = -43{\cdot}89$ kJ mol^{-1}] since β arguments include the entropy contribution from histidine having an additional carboxylate group compared with histamine.

Crystal dimensions also exhibit histamine-type bonding with a weakly bound carboxylate subject to considerable strain [29] (N–Cu = 201 and 195 pm, O–Cu = 258 pm and $\angle$N–Cu–O = 68·3°) and i.r. results have indicated terdentate bonding in D_2O.[30] Even the suggestion that imidazole was *not* bonded (*i.e.*, glycine-type co-ordination) [31,32] was later modified to include imidazole involvement.[33]

Structure (III) is suggested for A_2B as the major species. *i.e.*, double histamine-type bonding and one carboxylate and a water apically bonded. As for structure (II), the histamine-type bonding was originally suggested by comparative formation constant

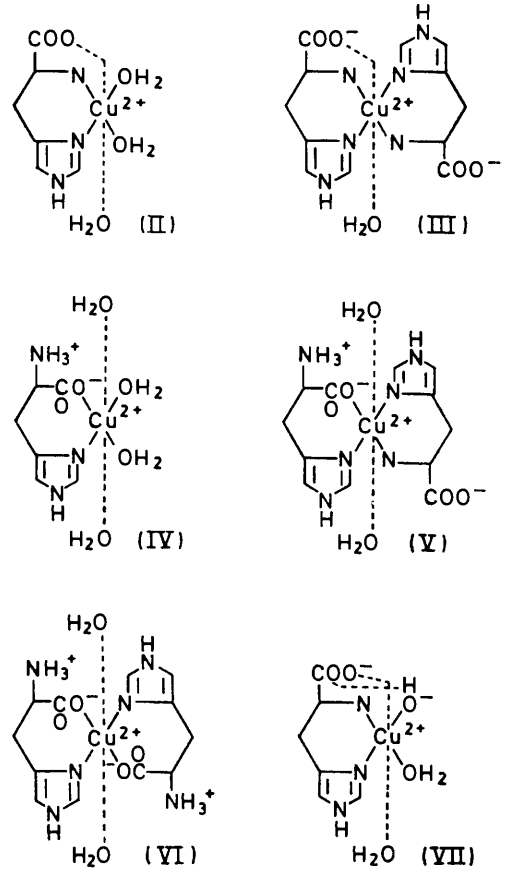

FIGURE 5. Suggested structures for the copper–histidyl complexes present in aqueous solution at 25 °C. Full lines represent planar bonds to copper(II), broken lines are axial bonds, with the exception of the O - - - H in (VII) which is a hydrogen bond.

studies [3,7,22,25,34] and can now be reinforced by use of enthalpy values: $\Delta H°(A_2B)$ for histidine $\doteqdot$ histamine $>$ phenylalanine ($-83{\cdot}93$, $-85{\cdot}10$, and $-57{\cdot}53$ [27] kJ mol^{-1} respectively). Further, the lower than expected position for reaction (b) in Figure 4 suggests that *both* carboxylates cannot be bonded although, in all probability, the *one* strained, carboxylate–copper bond of AB is still present in A_2B. Wilson *et al.*[10] have published spectral evidence for carboxylate–copper bonding and Morris and Martin [35] found that A_2B was non-stereoselective and so concluded that the second ligand is bidentate.

* Apart from the possibility of ionisations at pH > 11, the imidazole NH group is not considered capable of complexing (see Part VI).

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The ΔH° for reaction (b) reinforces this view even more by being lower than that for adding a histamine to copper (40·0 and 43·05 kJ mol^{-1} respectively). Structure (II) depicts the imidazole rings as being *trans* because Barnes and Pettit have shown that such an arrangement gives the least interligand interference.[26]

Structure (III) is supported by the following non-aqueous ambient temperature evidence: crystal studies L-histidinato-L-threoninatoaquocopper(II) gave one apical carboxylate and one water; [29] i.r. indicated carboxylate involvement [31] although o.r.d. did not.[36] N.m.r. indicates that both pyridine-type imidazole nitrogens were involved [37] and the line broadening was explained by ambivalent co-ordination between Cu(4N donors) $\rightleftharpoons$ Cu(3N and 1O donors).[38,39] E.p.r. and spectral shifts were also consistent with such a tautomeric equilibrium.[40]

ABH has suggested structure (IV) and a heat of formation (−66·98 kJ mol^{-1}) which is close to the sums of either forming (i) copper–imidazole, copper–carboxylate and histidine's primary amine–proton bonds (−28·62 − 4·35 − 49·42 = −73·39) or (ii) copper–tryptophyl and histidine's imidazole–proton bonds (−32·09 − 36·61 = −68·70), rather than copper–histamine and histidine's carboxylate–proton bonds (−43·06 − 1·05 = −44·10). Entropy arguments can now be invoked to distinguish between (i) and (ii): ΔS° for reaction (c) is closer to ΔS°(1,3-diaminopropane–proton) − ΔS°(1,3-diaminopropane–copper) (48·9 − 20·1 = 28·8 J K^{-1} mol^{-1}) rather than ΔS°(imidazole–proton) − ΔS°(imidazole–copper) (5·4 − −5·6 = 11·0 J K^{-1} mol^{-1}) (entropies are from Parts III and VI [1]). Hence (i), *i.e.* $-NH_2-H^+$, is the suggested structure. This disagrees with the spectral and formation constant conclusions of Wilson *et al.*[10] but Perrin and Sharma's conclusions from β do infer (i). Further evidence in support of the thermodynamic conclusions is that Carlson and Brown [30] found that the $-NH_2$ was deuteriated in D_2O.

A_2,BH, as shown in structure (V), merely involves adding a second histamine-like ligand to ABH and so ΔH° for reaction (e) $\doteqdot$ ΔH° for reaction (b). Sigel's n.m.r. results agree with this structure.[37]

For the more minor species A_2BH_2 evidence is sparse. Nevertheless, there are enthalpy reasons for postulating structure (VI): $-\Delta H(A_2BH_2) = 129{\cdot}7$ kJ mol^{-1} = 2 × ΔH° (imidazole–copper) + 2 × ΔH°(AH) + 2 × ΔH°-(carboxylate–copper) (−2 × 28·62 − 2 × 40·42 − 2 × 4·35 = −146·78 kJ mol^{-1}). There has been previous n.m.r., pK,[37] and i.r. [31] evidence for this structure. However, it is challenging that the crystal structure of $[Cu(L\text{-HistH})_2(H_2O)_2](NO_3)_2$ has a protonated imidazole nitrogen and glycine-type bonding.[41]

ABH_{-1} probably has structure (VII) since pK measurements indicate that the complex is more stable than Cu(histamine)H_{-1}^+.[7] This occurs because (i) extra carboxylate–copper interaction is introduced when the ligand is histidyl and (ii) hydrogen bonding can occur between the hydroxide ion and the carboxylate group ($-O-H\cdots O=\overset{\cdot}{C}-O-Cu^{2+}$).

I thank Drs. R. Österberg, B. Sjöberg, and A. D. Jones for assistance and advice concerning copper amalgam analysis and to Dr. A. Vacca for assistance with the LETAGROP programme.

[1/1176 *Received*, 9th *July*, 1971]

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