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Complex Formation Equilibria between 2-Amino-2(hydroxymethyl)-1,3-propanediol (tris, tham) and Nickel(II), Copper(II), Zinc(II) and Hydrogen Ions in Aqueous Solutions

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The reactions of 2-amino-2(hydroxymethyl)-1,3-propanediol (TRIS, THAM) with H^+, Ni^{2+}, Cu^{2+}, and Zn^{2+} ions have been studied at 298 K in aqueous solutions (0.15 mol dm^{-3} NaCl) using potentiometric techniques. The protonation constant of the ligand and the formation constants of several metal complexes have been calculated with the aid of the program MIQUV. The equilibrium models are selected on the basis of a critical evaluation of the least squares results and of a statistical analysis of the weighted residuals.

Introduction

The compound 2-amino-2(hydroxymethyl)-1,3-propanediol (hereafter abbreviated as *tris*) which is commonly used as a biochemical buffer, acts as a ligand towards many metal cations [1]. The formation of complexes between *tris* and cations of nickel(II), copper(II), silver(I), zinc(II), cadmium(II), mercury(II) and lead(II) was demonstrated in aqueous solution and the formation constants for some complex species were reported [2–6]. Unfortunately, the effect of metal–*tris* interaction has almost always been neglected in determinations of stability constants in *tris*-buffered media.

The previously-determined formation constants of *tris* complexes are referred to different standard states: sometimes the conditions of temperature and/or ionic strength are not even specified. With the aim of obtaining improved information on the subject we have started a systematic study of the thermodynamics of complex formation between *tris* and metal ions at 25 °C and in an aqueous medium containing 0.15 mol dm^{-3} NaCl. This communication reports the results of the investigation on the equilibria involving the ions of nickel(II), copper(II) and zinc(II). The copper(II)–*tris* system has been studied previously with potentiometric, spectrophotometric and polarographic techniques. The nickel(II)–*tris* equilibria have been investigated potentiometrically by Bai and Martell [4] and, more recently, by Forsling [5]: experiments performed under very different conditions of ionic strength, where different complex species were identified. The equilibria between zinc(II) and *tris* were investigated spectrophotometrically [6], but the conclusions were ambiguous.

Experimental

Materials

Tris (Merck, high purity standard) was used without further purification after drying at 100 °C.

Stock solutions of hydrochloric acid, sodium hydroxide and metal chlorides were prepared as previously described [7].

High purity sodium chloride (Merck) was used as supporting electrolyte.

E.M.F. Measurements

Potentiometric titrations were carried out using an ORION mod. 701A pH/mV meter, an ORION 91-01 glass electrode, a saturated calomel electrode and a salt bridge containing a 0.15 mol dm^{-3} NaCl solution. The solution in the titration vessel was stirred by means of a mechanical stirrer. A stream of nitrogen, presaturated with water vapour by bubbling it through a 0.15 mol dm^{-3} NaCl solution, was blown over the surface of the solution. The entire cell was thermostatted at 25.0 ± 0.1 °C.

Solutions containing variable quantitites of *tris*, hydrochloric acid and copper(II) chloride were titrated with a sodium hydroxide solution dispensed from a Mettler DV10 digital burette. In some cases back-titrations were performed with hydrochloric acid solution. The ionic strength was always adjusted to 0.15 mol dm^{-3} by adding solid sodium chloride. The potentiometric measurements were performed in a fully automated mode under the control of a BASIC program, purpose-written for a Rockwell AIM 65 (4K RAM) microprocessor.

The ionic product of water in 0.15 mol dm^{-3} NaCl solution at 25 °C was determined as $pK_w = 13.755 \pm 0.003$, a value in excellent agreement with previous determinations [8].

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The potentiometric cell was calibrated by Gran's method using strong acid–strong base titrations in 0.15 mol dm^{-3} NaCl solution.

As previously reported [4, 5], the reaction between Ni^{2+} and *tris* is extremely slow. Preliminary experiments showed that an ordinary titration may last several days, and even then equilibrium may not be reached at all points. For this reason the measurements were performed in batches, as described by Bai and Martell [4]. A series of solutions of different composition was prepared and kept at 25 °C for 48 hours before measuring the potential. Known volumes of NaOH solution were then added to each of the solutions and new potentiometric measurements were made after 48 hours at 25 °C. This procedure was repeated four times. The nickel(II) ion and ligand *tris* concentrations ranged in the intervals 1.3–12.3 and 4.8–42.3 mmol dm^{-3}, respectively, with molar ratios varying from 1:1 to 1:8.

For the zinc(II)–*tris* system an HCl solution was added to solutions containing the metal ion, the ligand and the amount of hydrochloric acid being sufficient to prevent the precipitation of zinc hydroxide.

The experimental details of the titration curves for the ligand protonation and for the copper(II) and zinc(II) complex formation are given in Table I.

Calculations

The program used for the calculations reported in this paper, MIQUV, was recently developed by us [9] in an attempt to improve the reliability of the species selection process. The program computes the formation constants by minimising the sum of the weighted squared residuals between observed and calculated e.m.f. values. The weighting of the experimental observations takes into account the estimated errors of both e.m.f. and titrant volume. This scheme is statistically sounder than that used by the program MINIQUAD [10], since it attaches correctly weighted errors to the experimental quantities. With MINIQUAD we have selected the most probable equilibrium model by following a strategy [11] based on statistical inferences applied to the R factor, an agreement index based on residuals between observed and calculated total reactant concentrations. There is also a χ^2 test to determine whether the residuals follow a normal distribution. With MIQUV, the residuals are based on the measured potentials and are weighted to take into account the errors on both potential and volume readings. The weighted residuals can more reasonably be expected to follow a normal distribution, and so the χ^2 test becomes more realistic than in MINIQUAD.

In place of the R factor we now use the weighted variance of the residuals, σ^2, a dimensionless quantity which should have a value of unity in the ideal case of correctly weighted residuals belonging to a normal distribution.

$$\sigma^2 = \sum_{i=1}^{n} w_i(E_i^{obs} - E_i^{calc})^2/(n - m)$$

where n is the number of experimental observations, E_i^{obs} and E_i^{calc} are the observed and calculated e.m.f. values, w_i is the weight of the *i*-th observation, and (n – m) is the number of degrees of freedom of the system (the number of observations minus the number of refined parameters). This quantity σ^2 is strongly dependent on the estimated errors in the measurements of e.m.f. and of titrant volume. We estimate these errors to be 0.2 mV and 0.002 cm^3 in our system, and σ^2 values not greater than 9 are acceptable for systems with an appropriate equilibrium model and experimental data of good quality.

Different equilibrium models produce different σ^2 values which can be used to perform an F test [12]. Let us assume that the minimum value of the sample variance, σ_0^2, has been reached for the hypothesis H_0. An alternative hypothesis H_k, which supplies a value of the variance σ_k^2, can be rejected at the α significance level if

TABLE I. Experimental Details of the Potentiometric Measurements.

Curve	Ion	Initial Concentrations (m*M*)			pH range	Number of data points
		MCl_2	*tris*	HCl		
1	Cu^{2+}	1.194	7.319	8.902	5.9–8.9	37
2	Cu^{2+}	0.519	5.559	6.987	6.4–9.3	25
3	Cu^{2+}	1.060	4.343	6.680	6.4–8.8	21
4	Cu^{2+}	3.053	12.104	0	7.7–6.3	36
5	Cu^{2+}	1.456	6.907	0	7.9–6.0	29
6	Zn^{2+}	1.049	9.204	8.057	7.2–6.3	10
7	Zn^{2+}	0.869	9.662	8.614	7.1–6.1	8
8	Zn^{2+}	1.957	14.878	13.050	7.1–6.0	22

$\sigma_k^2/\sigma_0^2 > F_{\alpha,\nu_k,\nu_0}$

where ν_k and ν_0 are the values of the degrees of freedom pertinent to hypotheses H_k and H_0. Tables containing values of F_{α,ν_1,ν_2} where $\alpha = 0.01$ and/or 0.05, for selected values of ν_1 and ν_2 are reported in most textbooks of mathematical statistics. We have calculated some values by numerical integration, as described in ref. 12.

A χ^2 test is performed by MIQUV after the refinement of the formation constants, to ascertain whether the distribution of the weighted residuals in normal or not [10].

For χ^2 values less than 12.6, we can feel confident at the 95% significance level that the weighted residuals follow a normal distribution. In other words, we can exclude the presence of systematic errors, such as errors in the analytical concentrations, standard potentials, or errors in the number or stoichiometry of the complex species present at the equilibrium.

The equilibrium model was selected in successive trials according to the following procedure. The first calculations are performed by adopting the models proposed in previous investigations. Other species are then introduced in addition to, or in place of, those present in the starting models. The least squares refinement of the formation constants provides the values of the standard deviations of the parameters, the agreement factor σ^2 and the goodness of fit statistic χ^2 for each hypothesis.

Models with σ^2 values greater than 9 are considered unsatisfactory and are therefore rejected. If more than one model gives acceptable values of σ^2, the F-test is performed taking as the null hypothesis, the model with the lowest value of σ^2. In the most favourable case this test can lead to the exclusion of all other hypotheses at the chosen significance level, thus leaving only one acceptable model. Where two or more models do not differ significantly from each other, the corresponding values of the χ^2 statistic are considered next. χ^2 should be small if systematic errors, such as an erroneous description of the equilibrium model, are absent. However, χ^2 is rarely found to be less than 12.6 because of the inevitable systematic errors in the analytical concentrations and standard potentials.

It may happen that for models with acceptable σ^2, the χ^2 test aoes not allow a unique choice of the equilibrium model. In this case the model is selected by applying the following considerations, in rough order of priority:

(i) *simplicity:* the model should be made as simple as possible by excluding poorly defined species. A poorly defined species is one for which the standard deviation on the formation constant is high, >33% say, and whose concentration is low throughout the range of the titration data. We take as low a value of less than 2% of the total concentration of complex species;

(ii) *similarity:* the model, which is in agreement with the previous results of reliable measurements on either the same or analogous systems, is generally preferred;

(iii) *chemical significance:* the selected species must have a plausible stoichiometry and should be formed in reasonable reaction pathways.

Results and Discussion

The protonation constant of *tris*, log $\beta = 8.110 \pm 0.003$, at 25 °C in 0.15 mol dm^{-3} NaCl, is in good agreement with the previously published results [13]. The values of σ^2 and χ^2, 3.22 and 4.29 respectively, are within acceptable limits. Since the equilibrium model for this system is unique (only one protonated species) we can be confident that the experimental measurements are not significantly affected by systematic errors. The values of this protonation constant and of the ionic product of water were used in the subsequent calculations on the metal containing systems without further refinement.

The results most relevant to the selection process for the equilibrium model of the nickel(II)–*tris* system are summarized in Table II. Two initial models were tried. In the refinement process two formation constants of the Forsling [4] model (Ni/1) turned out negative and were rejected, whereas all the formation constants of the model proposed [5] by Bai and Martell (Ni/2) were retained. In both cases the σ^2 and χ^2 values are acceptable, being under the limits discussed above. Moreover the two σ^2 values do not differ significantly; their ratio, 1.36, is less than the value of $F_{0.05,45,46} = 1.60$. Thus, the F test does not allow us to reject the model Ni/1. Models Ni/3–Ni/6 cannot be rejected at the 5% significance level, but a dozen or so other models, which are not shown in Table II, failed the F test at this level.

The model Ni/3 initially contained all the species proposed by Bai and Martell and by Forsling. In the refinement three formation constants were rejected but both σ^2 and χ^2 decrease. The other models in Table II are related to Ni/3 by removal of the species with poorly defined formation constants in that model.

The best agreement between experimental and calculated data is obtained for the model Ni/3, but this model is the most complicated one, and the formation constant of one of the species has a high standard deviation. The exclusion of that species leads to the model Ni/6 which has a σ^2 value slightly higher than the model Ni/3. The species retained have low standard deviations on the formation

TABLE II. Logarithms of the Cumulative Formation Constants of the Nickel(II)–*tris* Complexes Obtained for Different Equilibrium Models.[a]

	Ni/1	Ni/2	Ni/3	Ni/4	Ni/5	Ni/6
ML	2.636(7)	2.61(1)	2.636(6)	2.635(6)	2.641(6)	2.641(6)
ML_2	4.75(1)	4.86(1)	4.75(1)	4.76(1)	4.75(1)	4.75(1)
$M_2L_3H_{-3}$	–	-15.1(6)	-14.63(6)	-14.60(6)	-14.60(6)	-14.57(5)
$M_3L_2H_{-3}$	-14.13(9)	–	-14.4(2)	–	–	–
$M_3L_3H_{-5}$	–	-27.02(3)	neg	neg	neg	neg
M_4H_{-4}	neg	–	neg	-25.4(2)	–	–
$M_4L_4H_{-4}$	neg	–	neg	neg	-14.5(6)	–
$M_4L_4H_{-5}$	-22.3(4)	–	-21.82(9)	-21.76(6)	-21.8(1)	-21.65(4)
$M_4L_4H_{-6}$	-29.47(2)	–	-29.70(5)	-29.72(5)	-29.71(6)	-29.75(5)
σ^2	7.43	5.47	5.01	5.04	5.41	5.49
χ^2	10.8	10.8	5.68	9.84	5.04	6.00

[a] M = Ni; L = *tris*, charges are omitted for simplicity; values in parentheses are standard deviations on the last significant figure.

constants. This model can be considered a good representation of the experimental data. The model Ni/6 is to be preferred to the model Ni/2 for two reasons: (i) in the latter model a species is poorly defined and (ii) in the model Ni/6 tetrameric species are retained in place of a trimeric species. The tetrameric hydroxo species was previously found to be the main species formed during the hydrolysis of Ni^{2+} [14] and complexes of this tetramer with *tris* were postulated by Forsling [5]. It is possible that the structure of the tetramer is similar to that found recently in the solid state [15].

The values of the formation constants obtained by us are not in good agreement with those reported by Bai and Martell [4] (25 °C, 0.1 *M* KNO_3), with the exception of the formation constant of the species Ni*tris*$^{2+}$ (log $\beta = 2.63$). Our values are also different from those reported by Forsling (25 °C, 3 M $NaClO_4$), probably on account of the different ionic strength used.

The most relevant results for the system Cu(II)–*tris* are reported in Table III. The model Cu/1 contains the species proposed by Bai and Martell [4]. In the refinement process the formation constants of Cu*tris*$_2^{2+}$ and Cu*tris*$_4^{2+}$ were rejected. The resulting σ^2 value is acceptable whilst the χ^2 value is fairly high. It seems somewhat strange that the Cu*tris*$_2^{2+}$ complex is rejected whereas the complexes Cu*tris*$^{2+}$ and Cu*tris*$_3^{2+}$ are retained in the refined model. The model Cu/2 contains all the species retained in the previous refinement except that the Cu*tris*$_3^{2+}$ complex has been replaced by Cu*tris*$_2^{2+}$. On refinement the latter species was retained but the corresponding formation constant has a high standard deviation. The σ^2 value is appreciably increased with respect to the former model.

Other models were then considered which contain the 1:3 in place of the 1:2 species, and which include other dimeric species one at a time. The model Cu/7 shows the lowest σ^2 value but the χ^2 value is not acceptable. Therefore the model Cu/3 which has a fairly good σ^2 value and the lowest value of the χ^2 statistic is the most reliable. All the formation constants are well defined even if the model is fairly complicated, consisting of seven complex species.

As noted in the experimental section, the titration curves for the Zinc(II)–*tris* system are necessarily limited to a few experimental points in a very narrow pH range. At pH values lower than 6 Zinc(II)–*tris* complexes are not formed while at pH values higher than 7 the precipitation of zinc hydroxide occurs.

Finally, models consisting of the monomeric species Zn*tris*$^{2+}$, Zn*tris*$_2^{2+}$, Zn*tris*$_3^{3+}$, Zn*tris*$_4^{4+}$ and Zn*tris*H_{-1}^{+} have been considered. The only acceptable hypothesis on the basis of selection criteria described above, consists of the single species Zn*tris*$^{2+}$:

$$Zn^{2+} + tris \rightleftharpoons Zn\mathit{tris}^{2+} \qquad \log\beta = 2.271(8)$$

the σ^2 and χ^2 values, 0.87 and 10.0 respectively, support the soundness of the statistical choice. The other models investigated show σ^2 values ranging from 14.6 to 55.2. These models can be rejected on the basis either of the high σ^2 value or the F-test. Finally, the binary hydrolytic species $ZnOH^+$ and Zn_2OH^{3+} were included with fixed values of their formation constants (log $\beta_{01-1} = -9.0$ and log $\beta_{02-1} = -7.3$) [13]. No significant difference was observed with respect to the models in which these species are absent, because of their low formation percentage.

TABLE III. Logarithms of the Cumulative Formation Constants of the Copper(II)–*tris* Complexes Obtained for Different Equilibrium Models.[a]

	Cu/1	Cu/2	Cu/3	Cu/4	Cu/5	Cu/6	Cu/7	Cu/8	Cu/9
ML	4.12(1)	4.01(3)	4.17(2)	4.08(2)	4.14(2)	4.14(1)	4.13(2)	4.09(2)	4.14(1)
MLH_{-1}	−2.35(2)	−2.39(2)	−2.39(2)	−2.31(1)	−2.35(2)	−2.40(2)	−2.44(3)	−2.42(2)	−2.40(2)
ML_2	neg	7.5(1)	–	–	–	–	7.2(2)	7.3(1)	–
ML_2H_{-1}	1.27(1)	1.32(1)	1.28(1)	1.15(3)	1.30(1)	1.30(1)	1.28(2)	1.29(1)	1.30(1)
ML_2H_{-2}	−6.231(5)	−6.263(6)	−6.208(6)	−6.240(6)	−6.222(6)	−6.228(5)	−6.222(7)	−6.236(6)	−6.228(5)
ML_3	11.10(5)	–	11.01(6)	11.21(4)	10.9(1)	10^b	neg	–	10^b
ML_4	neg	–	–	–	–	–	–	–	–
$M_2L_2H_{-2}$	−2.06(6)	−2.3(1)	−1.96(5)	−2.04(5)	−2.08(6)	−1.75(5)	−1.81(6)	−1.88(7)	−1.75(5)
$M_2L_2H_{-3}$	–	–	−9.01(8)	–	–	–	−9.1(1)	–	–
$M_2L_2H_{-4}$	–	–	–	−16.23(8)	–	–	−16.9(4)	–	–
$M_2L_3H_{-2}$	–	–	–	–	1.2(1)	–	–	–	–
$M_2L_4H_{-2}$	–	–	–	–	–	4.82(7)	4.80(5)	4.81(4)	4.82(7)
σ^2	4.82	6.39	4.14	4.18	4.61	4.07	3.54	3.91	4.06
χ^2	22.49	25.19	19.78	33.73	24.11	70.92	71.89	76.22	70.92

[a] M = Cu; L = *tris*; charges are omitted for simplicity; values in parentheses are standard deviations on the last significant figure.
[b] The standard deviation is greater than the value of the constant.

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