Metal ion/buffer interactions. Stability of binary and ternary metal ion complexes containing the anion of *N,N*-bis(2-hydroxyethyl)-glycine (Bicine) and adenosine 5'-triphosphate (ATP)

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Abstract

The acidity constants of protonated *N,N*-bis(2-hydroxyethyl)-glycine ($H_2(Bic)^+$) have been measured by potentiometric pH titrations in aqueous solution at $I = 0.5$ M and 1.0 M (KNO_3) at 25 °C. The interaction of Bicinate (Bic^-) with Co^{2+}, Ni^{2+} and Cu^{2+} ($= M^{2+}$) was studied by spectrophotometry and the stability constants of the binary $M(Bic)^+$ complexes were determined: $\log K^{Co}_{Co(Bic)} = 5.08$, $\log K^{Ni}_{Ni(Bic)} = 6.02$, $\log K^{Cu}_{Cu(Bic)} = 8.24$ ($I = 1.0$ M, KNO_3; 25 °C). These results are compared with the corresponding values of the parent ligand, glycinate, and of other amino acids. It is concluded that the hydroxy groups of Bicinate are heavily involved in metal ion binding in the $M(Bic)^+$ complexes. Ternary complexes containing in addition adenosine 5'-triphosphate (ATP) were also studied: $\log K^{Co(ATP)}_{Co(ATP)(Bic)} = 4.53$, $\log K^{Ni(ATP)}_{Ni(ATP)(Bic)} = 5.44$, $\log K^{Cu(ATP)}_{Cu(ATP)(Bic)} = 6.57$. It is evident that these mixed ligand complexes are also still of a rather high stability. The structure of these complexes is discussed, as is the use of Bicine as a buffer substance in the presence of metal ions and other ligating systems.

1. Introduction

N,N-Bis(2-hydroxyethyl)-glycine (Bicine)†† is widely used as a pH regulator in the pH range 7.6 to 9 [1] and it is known [1] as one of the 'Good's buffers' [2]. In recent years Bicine has been used as buffer for many purposes (e.g. refs. 3–5) and it has also been recommended as part of buffer combinations, e.g. for column isoelectric focusing [1]. Furthermore, it has been employed in color photographic processes [6], in analytical methods [7], as a stabilizing agent [8], etc. Many of the properties of Bicine have been studied

[2], including its acid–base behavior in sea water [9] and its reaction volume for protonic ionization [10].

From the structures shown in Fig. 1 it is evident that Bicinate, i.e. the monoanion of Bicine, will form chelate complexes with metal ions; just as it is well known for its parent compound, i.e. glycinate, the anion of the amino acid glycine [11]. Indeed, the stability constants of many metal ion complexes of Bicinate have been determined over the years [12–16]. However, a
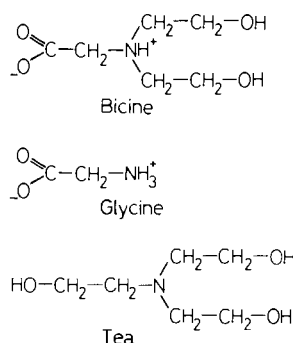
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††Abbreviations: ATP^{4-}: adenosine 5'-triphosphate; ATP (without charge) represents at many instances in this study the sum of $HATP^{3-}$ and ATP^{4-} (see also Section 3.5); Bicine: *N,N*-bis(2-hydroxyethyl)-glycine; Bicinate or Bic^- (the latter is used in formula): monoanion of Bicine; Bistris: 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)-1,3-propanediol; $CH_3N \cdot Gly^-$: *N*-methylglycinate; $(CH_3)_2N \cdot Gly^-$: *N,N*-dimethylglycinate; $C_2H_5N \cdot Gly^-$; *N*-ethylglycinate; $(C_2H_5)_2N \cdot Gly^-$: *N,N*-diethylglycinate; Gly^-: glycinate; L^-: bidentate amino acid monoanion; M^{2+}: divalent metal ion; Tea: triethanolamine.

Fig. 1. Chemical formula of *N,N*-bis(2-hydroxyethyl)-glycine (Bicine), Glycine (H(Gly)) and triethanolamine (Tea).

view of the structure of triethanolamine (Tea) in Fig. 1 indicates that complex formation with Bicinate may occur not only in a glycinate-like way; for Tea it is well known [17] that the hydroxy groups participate in metal ion binding and the same may be anticipated to some extent also for Bicinate complexes. Therefore, it is one of the aims of this study to see if an enhanced complex stability [18] attributable to hydroxy group participation can be observed, and if so, to try to quantify this effect as far as possible in order to draw conclusions regarding Bicinate complexes in solution.

For many years it has been recognized [2] that using Bicine as a buffer in systems also containing metal ions requires that the interactions of Bicine with these metal ions must be considered. However, the awareness that buffer substances may form not only binary but also ternary metal ion complexes is of much more recent origin [19, 20], and, indeed, the formation of such complexes containing both Bicinate and 1,10-phenanthroline has already been described [21]. Considering that Bicine is probably most often used as buffer in biochemical studies, we have now — as the second aim of this work — also endeavored to search for the formation of mixed ligand complexes containing Bicinate and adenosine 5'-triphosphate (ATP). This nucleotide was selected for the studies of the ternary systems as ATP is of high biological relevance [22, 23].

2. Experimental

2.1. Materials

The nitrate salts of Co^{2+} and Ni^{2+} were obtained from Guangdong Taishan Chemical Factory, China, that of Cu^{2+} from Guangzhou Chemical Reagent Factory, China, and that of K^+ from Jinan Chemical Reagent Factory Shangdong, China. N,N-Bis(2-hydroxyethyl)-glycine (Bicine) was from Sigma Chemical Co., St. Louis, MO, USA, and the disodium salt of adenosine 5'-triphosphate (ATP) was from Serva Feinbiochemica GmbH, Heidelberg, FRG. NaOH was purchased from the Chinese Medicine Company Beijing, China, and also (Titrisol) from Merck AG, Darmstadt, FRG. The disodium salt of ethylenediamine-N,N,N',N'-tetraacetic acid (Na_2H_2EDTA) was from Shantou Chemical Reagent Factory, China; its purity was checked via Zn (99.99%), which was obtained from Shanghai Chemical Reagent Factory, China. All reagents were of analytical grade.

Throughout all experiments deionized water, which was in addition distilled, was used. The metal concentrations of the metal(II) nitrate stock solutions were determined by titrations with EDTA using metal indicators [24].

The buffers employed for calibrating the pH-meter or the potentiograph were from Shanghai Aijian Reagent Factory, China (pH 4.003 and 6.864) and from Metrohm AG, Herisau, Switzerland (pH 4.64, 7.00 and 9.00). The direct pH meter readings were used and the acidity constants given in the work are therefore so-called practical or mixed constants. If so desired, the given negative logarithms of the mixed acidity constants may be converted into the corresponding concentration constants by subtracting 0.02 log unit [25].

2.2. Potentiometric measurements

The titrations were carried out with a Metrohm E536 potentiograph, E665 dosimat and an EA120 macro glass electrode (25 °C) (see also the preceding paragraph). The acidity constants $K^H_{H_2(Bic)}$ and $K^H_{H(Bic)}$ were determined by titrating 50 ml aqueous 2.0 mM HNO_3 and KNO_3 ($I=0.5$ or 1.0 M) in the presence and absence of Bicine (0.96 mM) under N_2 with 1 ml 0.1 M NaOH. The evaluations were carried out using the differences in NaOH consumption between such a pair of titrations [25].

For the calculations a curve-fit procedure with a Newton-Gauss non-linear least-squares program in the pH range 2.8–10.0 on a MS-DOS desk computer (Vectra 60PC) connected with a printer (Brother M 1509) and a plotter (Graphtec 3100) was used. All results are the average of at least two sets of independent experiments.

2.3. Spectrophotometric measurements

The stability constants $K^M_{M(Bic)}$ of the binary M(Bicinate)$^+$ complexes and the stability constants $K^{M(ATP)}_{M(ATP)(Bic)}$ of the ternary M(ATP)(Bicinate)$^{2-/3-}$ complexes (see Section 3.5) were determined by recording difference spectra of the visible region between 350 and 900 nm on a Shimadzu MPS 2000 instrument equipped with a graphic printer PR3 and a thermo bath TB 85 in aqueous solutions at 25 °C and $I=1.0$ M (KNO_3).

For both the binary and ternary systems the adjustment of an exact pH value of the solution to be used in the spectrophotometric experiment is crucial (see below in eqn. (2)); therefore special care was taken in measuring the pH. Two different buffer sets for calibration were used (see above) and checked against each other.

The desired pH of each solution of an experimental series was adjusted to an exact value, i.e. ±0.005, by dotting with relatively concentrated HNO_3 or NaOH. The pH was measured with an Orion SA 720 pH-meter using an Orion 91-02 combined glass electrode.

Bicine up to a concentration of 0.1 M has no significant absorbance in the wavelength range used, neither the zwitterionic species (measured at pH 5.9) nor the monoanion (measured at pH 10.3) (see also ref. 2). Similarly, ATP also does not absorb in the whole visible region.

2.4. Measurements with the binary systems

The stability constants $K^M_{M(Bic)}$ of the M(Bicinate)$^+$ complexes for Co^{2+}, Ni^{2+} and Cu^{2+} were measured by recording difference spectra between a quartz cell (1 cm) in the reference beam which contained the metal(II) nitrate ([Co^{2+}] = 0.06–0.32 M; [Ni^{2+}] = 0.08–0.32 M; [Cu^{2+}] = 0.02–0.2 M) and KNO$_3$ to maintain $I = 1.0$ M, and a cell in the sample beam which contained in addition a constant concentration of Bicine that was 0.03 M for the Co^{2+}, 0.04 M for the Ni^{2+} and 0.012 M for the Cu^{2+} experiments.

The difference spectra were evaluated by using the difference in absorbance (ΔA) at several constant wavelengths, i.e. normally at a peak and a valley: for Co^{2+} the differences at 485/390, 485/760 and 505/760 nm were taken; for Ni^{2+} at 373/500, 620/500 and 620/770 nm; for Cu^{2+} at 730/460, 680/460 and 640/460 nm (the wavelengths 680 and 640 nm are at the slope of the peak at 730 nm).

The stability constants were calculated from these spectrophotometric data by an evaluation method based on eqn. (1) [20], similar to the one developed by Benesi–Hildebrand [26]:

$$\frac{[M^{2+}]_{tot}}{\Delta A} = \frac{1}{\Delta A_{max}}[M^{2+}]_{tot} + \frac{1}{\Delta A_{max} K_{app}} \qquad (1)$$

The plots of $[M^{2+}]_{tot}/\Delta A$ versus $[M^{2+}]_{tot}$ resulted in straight lines confirming the formation of 1:1 complexes (see Fig. 2, Section 3.2). The straight lines were calculated by the least-squares regression method. From the intercepts with the x axes, x_0, and the y axes, y_0, one obtains the apparent pH-dependent stability constant for the M(Bicinate)$^+$ complex, i.e. $-1/x_0 = K_{app}$ (M^{-1}), as well as the maximum absorption difference, i.e. $-x_0/y_0 = \Delta A_{max}$. From this latter value one may calculate the absorption coefficient of the difference spectrum, $\Delta \epsilon$ (M^{-1} cm^{-1}), for the M(Bicinate)$^+$ complex, i.e. $\Delta \epsilon = \Delta A_{max}/(d[L]_{tot})$ (for further details see ref. 20).

The apparent stability constants obtained with eqn. (1) are valid only for the pH values of the given experiments (see Table 2, vide infra). These K_{app} values have to be transformed into the corresponding pH-independent stability constants, $K^M_{M(Bic)}$, by taking into account the competition between the proton and the metal ion [27] for the two basic sites of Bicinate. This is achieved with the acidity constants for H$_2$(Bic)$^+$, $K^H_{H_2(Bic)} = 10^{-2.13}$ and $K^H_{H(Bic)} = 10^{-8.33}$ (see Table 1, Section 3.1) and eqn. (2):

$$K^M_{M(Bic)} = K_{app}\left(1 + \frac{[H^+]}{K^H_{H(Bic)}} + \frac{[H^+]^2}{K^H_{H(Bic)}K^H_{H_2(Bic)}}\right) \qquad (2)$$

It is apparent that different K_{app} values valid for different pH values of a given M(Bicinate)$^+$ complex, if transformed into $K^M_{M(Bic)}$, have to give the same value. Indeed, for the present study this is the case within the experimental error limits of the individual values. It may be added that for the selection of adequate pH values for the experiments plots of the absorbance maxima of the difference spectra ([M^{2+}] = 0.3 M, [Bicine] = 0.02 M) versus pH were used as a guide.

The difference absorption coefficients obtained from the experiments are: (i) for Co(Bicinate)$^+$ $\Delta \epsilon_{485/390}$ = 4.53 ± 0.29, $\Delta \epsilon_{485/760}$ = 3.99 ± 0.12, $\Delta \epsilon_{505/760}$ = 4.49 ± 0.24; (ii) for Ni(Bicinate)$^+$ $\Delta \epsilon_{373/500}$ = 4.61 ± 0.21, $\Delta \epsilon_{620/500}$ = 2.63 ± 0.22, $\Delta \epsilon_{620/770}$ = 2.39 + 0.05; (iii) for Cu(Bicinate)$^+$ $\Delta \epsilon_{730/460}$ = 22.80 + 0.40, $\Delta \epsilon_{680/460}$ = 19.80 ± 0.24, $\Delta \epsilon_{640/460}$ = 13.69 ± 0.11 M^{-1} cm^{-1}. All error limits refer to one standard deviation (1σ). The size of all the above $\Delta \epsilon$ values is very similar to those observed previously [20] for the corresponding M(Bistris)$^{2+}$ complexes.

2.5. Measurements with the ternary systems

The ternary systems were studied analogously to the binary ones, but 1:1 ratios of [M^{2+}]:[ATP] were employed; the concentrations used are: [Co^{2+}] = 0.0375–0.15 M with [Bicine] = 0.02 M; [Ni^{2+}] = 0.025–0.15 M with [Bicine] = 0.012 M; [Cu^{2+}] = 0.0075–0.06 M with [Bicine] = 0.003 M. The degree of formation of the ATP complexes is large; i.e. at least 85% of [M^{2+}]$_{tot}$ = [ATP]$_{tot}$ exist under the present conditions in the form of M(ATP)$^{2-}$ and M(H·ATP)$^-$ complexes (calculated with the equilibrium constants given in ref. 28). In other words, the reaction studied is that between M(ATP) complexes and Bicinate (see also below and Section 3.5). It may be mentioned that the hydrolysis of ATP is metal ion promoted [29]; therefore ATP was added to the solutions just prior to the pH adjustment and then the difference spectra were immediately recorded.

The quartz cell (1 cm) in the reference beam contained the 1:1 molar ratio of metal(II) nitrate and ATP. The cell in the sample beam contained in addition Bicine in a constant concentration (see above). Both cells also contained KNO$_3$ to maintain the ionic strength at 1.0 M. The pH of both cells was adjusted to the same value (± 0.005). Again experimental series were carried out at various pH values (see Table 4, Section 3.5). The difference spectra were evaluated at the following wavelengths; i.e. normally again the difference in absorption between a peak and a valley was taken: for Co^{2+} the values at 500/570, 480/570 and 460/570 nm were taken; for Ni^{2+} at 375/412, 375/460, 610/460 and 610/765 nm; and for Cu^{2+} at 660/380 and 620/380 nm (the wavelength 620 nm is at a slope).

A potential problem in the evaluation procedure could be that in the upper part of the concentration
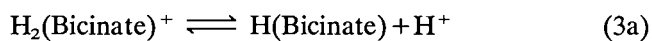
range used some self-association of $M(ATP)^{2-}$ occurs [30]. However, this appears not to be significant as in the evaluation according to eqn. (1) straight lines were observed (see also Fig. 2).

The difference absorption coefficients obtained from the experiments are: (i) for Co(ATP)(Bicinate) $\Delta\epsilon_{500/570}=1.69\pm0.35$ (1σ throughout), $\Delta\epsilon_{480/570}=1.69\pm0.21$, $\Delta\epsilon_{460/570}=1.46\pm0.21$; (ii) for Ni(ATP)-(Bicinate) $\Delta\epsilon_{375/412}=4.52\pm0.69$, $\Delta\epsilon_{375/460}=3.83\pm0.63$, $\Delta\epsilon_{610/460}=2.33\pm0.42$, $\Delta\epsilon_{610/765}=2.43\pm0.45$; (iii) for Cu(ATP)(Bicinate) $\Delta\epsilon_{660/380}=18.13\pm0.55$, $\Delta\epsilon_{620/380}=17.50\pm1.26$ M^{-1} cm^{-1}. These results, if compared with those for the binary systems, are important in a twofold way: (i) they show that the difference absorption spectra of the ternary systems are distinctly different from those of the binary systems, and (ii) the $\Delta\epsilon$ values obtained now for the ternary systems are comparable in size to those of the binary systems. This confirms the above-mentioned conclusion that indeed the reaction between M(ATP) complexes and Bicinate is studied (and not a reaction between traces of free M^{2+} and Bicinate as one might theoretically consider).
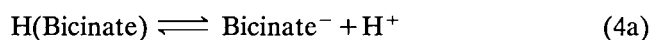
3. Results and discussion

3.1. Acidity constants of monoprotonated Bicine

Bicine as a derivative of glycine shows the normal behavior of α-amino acids, i.e. the amino and carboxylate groups each can accept a proton and consequently the following two equilibria have to be considered:

$$H_2(Bicinate)^+ \rightleftharpoons H(Bicinate)+H^+ \quad (3a)$$

$$K^H_{H_2(Bic)}=[H^+][H(Bic)]/[H_2(Bic)^+] \quad (3b)$$

$$H(Bicinate) \rightleftharpoons Bicinate^- +H^+ \quad (4a)$$

$$K^H_{H(Bic)}=[H^+][Bic^-]/[H(Bic)] \quad (4b)$$

The corresponding acidity constants were determined by potentiometric pH titrations; the results are listed in Table 1. It may be added that in the pH range up to 10, which is the highest value reached in our experiments, no sign for the deprotonation of one of the hydroxy groups of Bicine (cf. Fig. 1) was observed.

TABLE 1. Negative logarithms of the acidity constants of $H_2(Bicinate)^+$ (eqns. (3) and (4)) in aqueous solution at 25 °C and various ionic strengths $(I)^a$

I (M)	$pK^H_{H_2(Bic)}$	$pK^H_{H(Bic)}$	Reference
0.2 (NaClO$_4$)		8.22	31
0.5 (KNO$_3$)	1.95±0.05	8.25±0.01	this work
1.0 (KNO$_3$)	2.13±0.06	8.33±0.03	this work

^{a}The error limits given are three times the standard error or the sum of the probable systematic errors, whichever is larger.

The acidity constants given in Table 1 are in the order of previous results [12–16, 31], which refer to various temperatures and ionic strengths; for example, Good et al. [2] determined for 20 °C $pK^H_{H(Bic)}=8.35$ (at an ionic strength probably close to 0.1 or 0.2 M). This latter value is evidently in fair agreement with those in Table 1, especially if one considers that an increasing temperature facilitates the release of the proton from the N-site [2].

Similarly, an increasing ionic strength generally inhibits ionization of the carboxylic acid group [9], as well as of $H^+(N)$-sites [20, 32]. This is nicely confirmed by the present results for $H_2(Bicinate)^+$ (Table 1). Needless to emphasize, the large ΔpK_a separation of the two acidity constants guarantees that 'neutral' Bicine exists in the zwitterionic form (Fig. 1).

3.2. Stability constants of binary $M(Bicinate)^+$ complexes

The absorption spectra of Co^{2+}, Ni^{2+} or Cu^{2+} are altered by the presence of Bicine as can be seen immediately by the difference in color between the sample (with Bicine) and the reference solution (without Bicine). This alteration can best be quantified by recording difference spectra [20] (see 'Experimental'), and from these the pH-dependent apparent stability constants, which are valid only at the pH of the experiment, for the Co(Bicinate)$^+$, Ni(Bicinate)$^+$ and Cu(Bicinate)$^+$ complexes may be determined with eqn. (1) as shown for some examples in Fig. 2. The apparent stability constants, K_{app} (eqn. (1)), can be transformed into pH-independent stability constants by taking into account the competition of the proton for the two metal ion binding sites [27] by using eqn. (2) (see 'Experimental').

Indeed, all observations may be fully characterized by the following equilibrium:

$$M^{2+} +(Bicinate)^- \rightleftharpoons M(Bicinate)^+ \quad (5a)$$
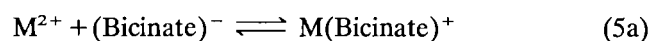
$$K^M_{M(Bic)}=[M(Bic)^+]/([M^{2+}][Bic^-]) \quad (5b)$$

This means, application of eqn. (2) to the log K_{app} values results in log $K^M_{M(Bic)}$ stability constants (eqn. (5)) which are independent of pH within experimental error. All these details are summarized in Table 2, together with the final results obtained for the Co(Bicinate)$^+$, Ni(Bicinate)$^+$ and Cu(Bicinate)$^+$ complexes.

The results of Table 2 agree fairly well with the stability constants available in the literature [2, 12–16, 21] for the corresponding M(Bicinate)$^+$ complexes. This is especially true if one considers the differences of the various studies in temperature and ionic strength:
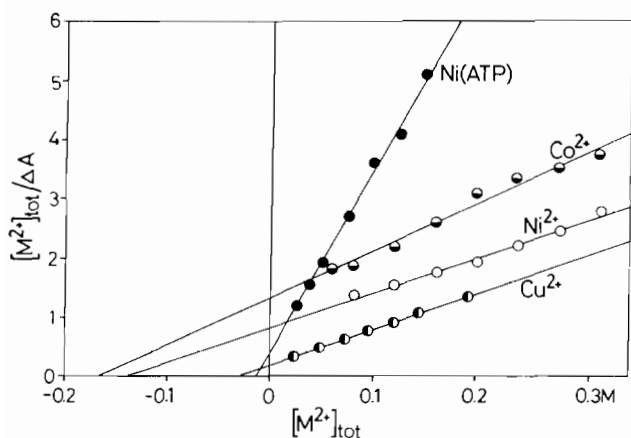
Fig. 2. Graphical determination of the apparent stability constant K_{app} in aqueous solution for Co(Bicinate)$^+$ ($\ominus$), Ni(Bicinate)$^+$ (O), Cu(Bicinate)$^+$ ($\mathbb{O}$) and Ni(ATP)(Bicinate) ($\bullet$) at pH 4.00, 3.20, 1.99 and 4.85, respectively. K_{app} was obtained by plotting $[M^{2+}]_{tot}/\Delta A$ vs. $[M^{2+}]_{tot}$. The conditions were $I=1.0$ M (KNO$_3$) and 25 °C with [Bicine]=0.012 M for the Cu^{2+} and Ni^{2+}/ATP systems, as well as with [Bicine] = 0.03 and 0.04 M for the Co^{2+} and Ni^{2+} systems, respectively. The straight lines were drawn according to the least-squares regression method. The difference spectra (all measured with 1-cm quartz cells) of the Co^{2+} system were evaluated at the maximum absorption (505 nm) and at the minimal absorption (760 nm), i.e. $\Delta A = A_{505} - A_{760}$; the intercept with the x axis corresponds for Co^{2+}/Bicine to $x_0 = -1/K_{app} = -0.166 \pm 0.0152$, i.e. $K_{app} = 6.028 \pm 0.552$, hence log $K_{Co(Bic)}^{Co} = 0.780 + 4.336 = 5.116$ (cf. eqn. (2)). For the Ni^{2+}/Bicine system, $\Delta A = A_{373} - A_{500}$, $x_0 = -0.139 \pm 0.0123$ and $K_{app} = 7.189 \pm 0.636$, hence log $K_{Ni(Bic)}^{Ni} = 0.857 + 5.165 = 6.022$; for the Cu^{2+}/Bicine system, $\Delta A = A_{640} - A_{460}$, $x_0 = -0.0311 \pm 0.00133$ and $K_{app} = 32.154 \pm 1.373$, hence log $K_{Cu(Bic)}^{Cu} = 1.507 + 6.716 = 8.223$; and for the Ni(ATP)/Bicine system, $\Delta A = A_{610} - A_{765}$, $x_0 = -0.0128 \pm 0.0027$ and $K_{app} = 78.064 \pm 16.637$, hence log $K_{Ni(ATP)(Bic)}^{Ni(ATP)} = 1.892 + 3.481 = 5.373$.

e.g. in an early study [33] at 30 °C and $I=0.1$ M (KCl) the results were log $K_{Co(Bic)}^{Co} = 5.27$, log $K_{Ni(Bic)}^{Ni} = 6.38$, and log $K_{Cu(Bic)}^{Cu} = 8.15$. This result confirms also the suitability of the employed spectrophotometric method and adds further confidence in the results described in Section 3.5 for the mixed ligand systems.

3.3. Comparison of the stability of M(Bicinate)$^+$ with some related complexes

There is no doubt that the core coordination of Bicinate to metal ions occurs in a glycinate-type mode via the tertiary amino nitrogen and the carboxylate group (Fig. 1). The question is: to what extent do the two hydroxy groups participate in metal ion binding?

To find at least a tentative answer to this question some relevant equilibrium data have been collected from the literature [34–37] and are given in Table 3. It is evident from the data in the first three rows that variation of the ionic strength affects the equilibrium constants somewhat; however, the stability differences considered below are clearly far beyond this effect.

TABLE 2. Logarithms of the pH-dependent apparent stability constants, log K_{app} (eqn. (1)), of binary M(Bicinate)$^+$ complexes with Co^{2+}, Ni^{2+} and Cu^{2+}, and their transformation into the corresponding pH-independent stability constants, log $K_{M(Bic)}^M$ (eqn. (5)), valid for 25 °C and $I=1.0$ M (KNO$_3$)[a]

M^{2+}	log K_{app}[b]	pH	Individual log $K_{M(Bic)}^M$	Average[a] for log $K_{M(Bic)}^M$
Co^{2+}	0.341	3.60	5.085	
	0.445	3.60	5.189	
	0.751	3.99	5.097	5.08 ± 0.13
	0.806	4.00	5.142	
	0.747	4.00	5.083	
	0.740	4.20	4.874	
Ni^{2+}	0.433	2.83	6.012	
	0.542	2.83	6.121	
	0.894	3.20	6.059	6.02 ± 0.09
	0.837	3.20	6.002	
	1.052	3.50	5.900	
	1.171	3.51	6.009	
Cu^{2+}	0.986	1.59	8.376	
	1.213	1.81	8.223	
	1.388	1.81	8.398	
	1.257	1.81	8.267	
	1.477	1.99	8.194	8.24 ± 0.09
	1.484	2.00	8.185	
	1.793	2.20	8.190	
	1.755	2.20	8.152	
	1.796	2.20	8.193	

[a]The error limits given are three times the standard error of the mean value (3σ). [b]The value listed for log K_{app} at a given pH is the average of several evaluations (cf. Fig. 2) at different wavelengths (see 'Experimental').

Substitution of a single hydrogen by a methyl group at the N-site of glycinate affects complex stability somewhat as the comparison of the data in rows 1–4 of Table 3 shows. However, the same substitution by a single ethyl group has a remarkably stronger negative effect on complex stability: ignoring the slight differences in the pK_{HL}^H values, complex stability is reduced by 1 log unit (cf. row 5 with rows 1–3). Interestingly, this described steric inhibition of a single ethyl group corresponds very closely to that of two methyl groups, as are present in N,N-dimethylglycinate (rows 6 and 7).

Most pronounced is the steric effect clearly in N,N-diethylglycinate (row 8 of Table 3), and it is this ligand which is structurally closest to Bicinate (row 9), if the latter coordinates only in a glycinate-like way. Neglecting the differences in basicity between these two glycinate derivatives, it is quite evident that the hydroxy groups must contribute to metal ion binding in M(Bicinate)$^+$ complexes: Ni(Bicinate)$^+$ is about 1.8 log units more stable than Ni[(C$_2$H$_5$)$_2$N·Gly]$^+$ and the same stability increase may be assumed for Co(Bicinate)$^+$, as a comparison between the values in rows 2, 8 and 9 suggests. The corresponding stability increase for Cu(Bicinate)$^+$ (rows 8 and 9) amounts 'only' to about 1.35 log units.

TABLE 3. Stability constants of Co^{2+}, Ni^{2+} and Cu^{2+} 1:1 complexes of glycinate and of some of its derivatives (analogous to eqn. (5)). The acidity constants of the monoprotonated amino acids (analogous to eqn. (4)) are given for further comparisons. All equilibrium constants refer to aqueous solution and 25 °C

Row no.	Ligand (L$^-$)	I (M)	pK_{HL}^H	log K_{ML}^M for M^{2+} =			Reference
				Co^{2+}	Ni^{2+}	Cu^{2+}	
1	Gly$^-$	0.1	9.62		5.86	8.38	34
2	Gly$^-$	0.1	9.68	4.63	5.83	8.27	35
3	Gly$^-$	1.0	9.75		5.69	8.30	36
4	$(CH_3)N \cdot Gly^-$	0.1	10.01		5.50	7.94	34
5	$(C_2H_5)N \cdot Gly^-$	0.1	10.10		4.81	7.34	34
6	$(CH_3)_2N \cdot Gly^-$	0.1	9.80		4.82	7.30	34
7	$(CH_3)_2N \cdot Gly^-$	0.15	9.80		4.77	7.26	37
8	$(C_2H_5)_2N \cdot Gly^-$	0.1	10.47		4.21	6.88	34
9	Bic$^-$	1.0	8.33	5.08	6.02	8.24	a

^{a}This work; see Tables 1 and 2.

This observation reflects the inability of the tetragonal Cu^{2+} to bind ligating atoms strongly in an apical position [38] though weak interactions are possible [39]. Overall, these comparisons demonstrate clearly, in agreement with an early conclusion [33], that the hydroxy groups participate in metal ion binding of Bicinate.

3.4. Extent of the stability enhancement due to the participation of the hydroxy groups in metal ion binding of Bicinate

The reasonings given in the preceding paragraph lead to the conclusion that the following intramolecular equilibrium must be far on its right side:

$$M(Bicinate)_{op}^+ \rightleftharpoons M(Bicinate)_{cl}^+ \qquad (6)$$
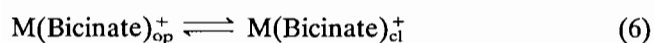
The 'open' isomer in which Bicinate coordinates only in a glycine-type mode is designated as $M(Bicinate)_{op}^+$, while $M(Bicinate)_{cl}^+$ represents the sum of the concentrations of the two isomers in which one and two hydroxy groups participate in metal ion binding, respectively.

It is evident that the presence of any 'closed' species in equilibrium (6) will enhance complex stability [18]; in other words, it will be reflected in the experimentally measured stability constant, $K_{M(Bic)}^M$, as defined by eqn. (5). Such an increased complex stability may be quantified [18] via the so-called stability enhancement factor $(1+E)$, which is defined in eqn. (7):

$$1+E = K_{M(Bic)}^M / K_{M(Bic)_{op}}^M \qquad (7)$$

The constant $K_{M(Bic)_{op}}^M$ characterizes the stability of the open isomer in eqn. (6), and it is evident that disappearance of any stabilizing effect by a further binding site equalizes the two stability constants in eqn. (7), which means then $E = 0$.

As stability constants are often presented as their logarithms, the mentioned stability enhancement factor is also often expressed as $10^{\log\Delta}$ [40–42], which leads

to eqn. (8):

$$\log \Delta = \log K_{M(Bic)}^M - \log K_{M(Bic)_{op}}^M = \log (1+E) \qquad (8)$$

The difficulty one faces with this equation is the need to obtain a value for $\log K_{M(Bic)_{op}}^M$. If one assumes a slope of 0.5 for $\log K_{ML}^M$ versus pK_{HL}^H plots [18] one may estimate values for $\log \Delta$ from the data given in rows 8 and 9 of Table 3; i.e. $\log \Delta_{Ni} \simeq 2.9$ and $\log \Delta_{Cu} \simeq 2.3$. Roughly speaking: we observe a stability enhancement of about 2 to 3 log units due to the participation of the hydroxy groups in metal ion binding of Bicinate. This result confirms (see ref. 18) the above conclusion that equilibrium (6) lies to its right hand side.

3.5. Stability constants of ternary M(ATP)(Bicinate) complexes

The use of Bicine as a buffer substance in biochemical studies, which are normally carried out under multiligand conditions and which often also contain metal ions, shows the need to consider also the formation of mixed ligand complexes involving Bicinate as a second ligand. Therefore we studied as an example the ternary system with ATP, as this nucleotide is of major interest in many biochemical studies [22, 23].

As it turned out during the experiments, the corresponding ternary complexes are still rather stable and therefore relatively low pH values had to be used in the experiments. As a consequence, there are two ATP complexes present, i.e. $M(H \cdot ATP)^-$ and $M(ATP)^{2-}$; together they reach a formation degree of at least 85% under the experimental conditions and it is the reaction between these two ATP complexes and Bicinate that is spectrophotometrically observed as pointed out in 'Experimental'.

This means, the stability constants determined refer to equilibrium (9a):

M(ATP) + Bicinate $\rightleftharpoons$ M(ATP)(Bicinate) (9a)
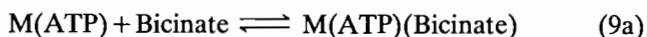
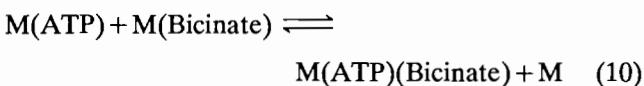$K^{M(ATP)}_{M(ATP)(Bic)} = [M(ATP)(Bic)]/([M(ATP)][Bic^-])$ (9b)

It should be emphasized again that the term 'ATP' includes the ATP^{4-} and $H(ATP)^{3-}$ species; therefore in the above equilibrium, as well as in another one to come (eqn. (10)), the charges are omitted from the species. The above-mentioned definition is used throughout, except in a few cases where the composition of a species is exactly defined via its charge.

An example of the spectrophotometric determination of the apparent stability constant, K_{app}, of one of the ternary complexes is shown in Fig. 2. As described before ('Experimental') these apparent stability constants, which are valid only for the pH of the experiment, may be transformed into pH independent constants (eqn. (9)) by using eqn. (2). The detailed results are summarized in Table 4.

From the individual values given for log $K^{M(ATP)}_{M(ATP)(Bic)}$ it is evident that the results are within the experimental error limits indeed independent of pH. It should be pointed out that the measurements are delicate and therefore the error limits of the final results (note: 3 σ are given in Table 4) are relatively large. Moreover, the most important result of these experiments is not so much the provision of exact stability constants, but that they prove the existence of these ternary M(ATP)(Bicinate) complexes.

3.6. Some comments on the stability and structure of the ternary M(ATP)(Bicinate) complexes

One way to quantify the stability of ternary complexes is to consider their stability relative to the stability of the binary parent complexes [43]; this means, to consider equilibrium (10):

M(ATP) + M(Bicinate) $\rightleftharpoons$

M(ATP)(Bicinate) + M (10)

The corresponding equilibrium constant is defined by eqn. (11):

$$10^{\Delta \log K_M} = \frac{[M(ATP)(Bicinate)][M^{2+}]}{[M(ATP)][M(Bicinate)^+]}$$ (11)

Values for $\Delta \log K_M$ may be calculated [43] according to eqn. (12):

$$\Delta \log K_M = \log K^{M(ATP)}_{M(ATP)(Bic)} - \log K^M_{M(Bic)}$$ (12a)

$$= \log K^{M(Bic)}_{M(Bic)(ATP)} - \log K^M_{M(ATP)}$$ (12b)

Since the stability constants that appear in eqn. (12a) have been determined (Tables 2 and 4), we use this equation to calculate the equilibrium constants $\Delta \log K_M$. The results are given in Table 5.

In Section 3.5 it was already discussed that M(ATP) represents the sum of the species $M(H \cdot ATP)^-$ and $M(ATP)^{2-}$. However, in both these complexes the metal ion is coordinated to the terminal γ phosphate group at ATP (which for most $M(H \cdot ATP)^-$ complexes carries also the proton [28]), its neighboring β phosphate group, and also either to the α phosphate group or to N-7 of the purine moiety (for details see refs. 23 and 28); this means ATP^{4-} and $H(ATP)^{3-}$ act to a first approximation as tridentate ligands. Hence, upon coordination of ATP to a metal ion with a regular octahedral coordination sphere there remain three positions for binding of a further ligand: assuming Bicinate coordinates initially with its bidentate glycinate-type core it has from a statistical point of view [43] three possibilities for binding if ATP is facially coordinated and two possibilities if ATP is equatorially bound. In contrast, the formation of the binary M(Bicinate)$^+$ complex may occur via twelve possibilities. As in both the binary

TABLE 4. Logarithms of the pH-dependent apparent stability constants, log K_{app} (eqn. (1)), of ternary M(ATP)(Bicinate) complexes with Co^{2+}, Ni^{2+} and Cu^{2+}, and their transformation into the corresponding pH-independent stability constants, log $K^{M(ATP)}_{M(ATP)(Bic)}$ (eqn. (9)), valid for 25 °C and $I = 1.0$ M (KNO$_3$)[a]

M(ATP)	log K_{app}[b]	pH	Individual log $K^{M(ATP)}_{M(ATP)(Bic)}$	Average[a] for log $K^{M(ATP)}_{M(ATP)(Bic)}$
Co(ATP)	1.088	5.00	4.419	
	1.641	5.30	4.672	4.53 ± 0.22
	1.467	5.30	4.498	
Ni(ATP)	1.600	4.40	5.532	
	1.938	4.79	5.479	5.44 ± 0.19
	1.835	4.85	5.316	
Cu(ATP)	2.307	4.00	6.643	
	2.324	4.30	6.357	6.57 ± 0.32
	2.978	4.60	6.710	

[a, b]See the corresponding footnotes in Table 2, and also the comments with regard to eqn. (9) in Section 3.5.

TABLE 5. Logarithms of the stability constants for the binary M(Bicinate)$^+$ (eqn. (5))^a and ternary M(ATP)(Bicinate) complexes (eqn. (9))^b, together with the stability differences $\Delta \log K_M$ (eqn. (12)) for aqueous solutions at $I = 1.0$ M (KNO$_3$) and 25 °C^c

M^{2+}	$\log K^{M(ATP)}_{M(ATP)(Bic)}$	$\log K^M_{M(Bic)}$	$\Delta \log K_M{}^c$
Co^{2+}	4.53 ± 0.22	5.08 ± 0.13	-0.55 ± 0.26
Ni^{2+}	5.44 ± 0.19	6.02 ± 0.09	-0.58 ± 0.21
Cu^{2+}	6.57 ± 0.32	8.24 ± 0.09	-1.67 ± 0.33

^{a}Values from Table 2. ^{b}Values from Table 4. ^{c}The error limits given are three times the standard error of the mean value (3σ), and these for $\Delta \log K_M$ were calculated according to the error propagation after Gauss.

and ternary complexes only one Bicinate ligand is bound the probability for dissociation is identical, i.e. one. The statistical value for $10^{\Delta \log K_M}$ according to eqn. (12a) is thus $K_{ternary}/K_{binary} = (3/1)/(12/1) = 0.25$ (facial ATP) or $(2/1)/(12/1) = 0.167$ (equatorial ATP); i.e. one obtains on average for the statistical value of $\Delta \log K_M$ in an octahedral coordination sphere: $\Delta \log K_0 \simeq -0.7$.

Comparison of this statistical value with the results listed in Table 5 for $\Delta \log K_M$ of the Co^{2+} and Ni^{2+} systems indicates a surprisingly large stability of the corresponding ternary complexes. This is especially true if one considers further that in equilibrium (9a) two negatively charged species are reacting with each other, what is evidently not a favorable situation. These reasonings lead then to the conclusion that the hydroxy groups of Bicinate participate somehow also in the ternary Co^{2+} and Ni^{2+} complexes with ATP: in fact, one of the two hydroxy groups may still directly coordinate to the metal ion, while participation of the other is possible only if either N-7 or the α phosphate group of ATP is forced to leave the coordination sphere. A further possibility, which has been indicated before [20] for ternary M(ATP)(Bistris)$^{2-}$ complexes, is that within the ternary complex intramolecular hydrogen bonds are formed between the hydroxy group(s) and, for example, oxygens of the triphosphate chain.

To assess a statistical value for Cu^{2+} regarding $\Delta \log K_{Cu}$ in eqn. (12a) is rather difficult [43] due to the distorted coordination sphere of this metal ion [38, 39]. However, that even the ternary Cu(ATP)(Bicinate) complex is relatively stable despite its low $\Delta \log K_M$ value (i.e. $\Delta \log K_{Cu} = -1.67$; Table 5) is evident from the difference, $\log K^{Cu}_{Cu(Bic)} - \log K^{Cu(Bic)}_{Cu(Bic)_2} = -2.95$ [33]; even if the latter difference is corrected for the statistical effect that favors Bicinate dissociation in the Cu(Bicinate)$_2$ complex, one is left with a difference of -2.65 log units. The most likely structure of Cu(ATP)(Bicinate) is probably a β, γ phosphate coordination of ATP together with a glycinate-type binding

of Bicinate to the equatorial sites of Cu^{2+}. The two apical positions of Cu^{2+} could then (if at all) be occupied by other ligating groups of the two ligands (e.g. the α phosphate group, N-7, or the hydroxy groups). Certainly, in addition there is also here the above-mentioned possibility of intramolecular hydrogen bonding.

Conclusions

In using Bicine as a buffer in aqueous solutions under multi-ligand conditions and in the presence of metal ions one should be aware of at least the following points. (i) The stability of binary M(Bicinate)$^+$ complexes is rather large. (ii) Consequently, Bicine may reduce rather drastically the concentration of the available metal ions under certain conditions in a certain reaction. (iii) The likelihood for the formation of mixed ligand complexes is also rather high, as was demonstrated in this study with ATP; this will affect the properties of ATP in various ways, if it is used as a substrate. (iv) Finally it should be mentioned that it is known [3] that Bicine may interact also directly with proteins. Hence, it is evident that in employing Bicine as a buffer substance great care should be exercised.

Acknowledgements

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Note added in proof

Since this manuscript was submitted a further paper dealing with binary Bicinate complexes has appeared: C. R. Krishnamoorthy and R. Nakon, *J. Coord. Chem., 23* (1991) 233.