Thermodynamics of Complex Formation between Hydrogen, Copper(II), and Nickel (II) Ions and Dipeptides containing Non-co-ordinating Substituent Groups†

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Formation constants for the various complexes formed between H^+, Cu^{2+}, and Ni^{2+} and a range of dipeptides which do not contain co-ordinating substituent groups have been measured potentiometrically at 25 °C and I = 0.10M ($K[NO_3]$). The structures of the complexed species are discussed and conclusions drawn on the equilibria present in nickel–dipeptide systems.

THE formation of complexes between dipeptides and transition-metal ions has been studied by a number of techniques using a range of different dipeptides, the metal ions most usually employed being Cu^{2+} and Ni^{2+}. Simple dipeptides have a number of potential donor sites, many of which are pH sensitive. In addition substituent groups on the component amino-acids may provide additional donor centres (*e.g.* the histidyl group, His-) making the elucidation of the particular mode of bonding very complicated and pH dependent.

With Cu^{2+} the donor sites of simple dipeptides as a function of pH have been proposed on the basis of thermodynamic,[1-7] spectral,[2,3] and kinetic studies.[1] As a result there is general agreement on the structures of the complexes although some uncertainties still exist. Many of the complexes of Ni^{2+} are defined less clearly, particularly at high pH values, and there is uncertainty as to the modes of ionization of protons from the complexes, the donor centres and, in some cases, the metal–ligand ratios of the complexed species.

In spite of numerous studies of different metal–dipeptide complexes no systematic study has been made of the complexes formed between a particular metal ion and a range of different dipeptides. We report the results of a detailed study of Cu^{2+} and Ni^{2+} complexes of such a range of dipeptides or related ligands, and draw conclusions as to the probable equilibria taking place and the modes of bonding in the complex species involved. The dipeptides studied fall into two groups. The first group covers a range of simple dipeptides containing non-co-ordinating side chains only which differ in their bulk or aliphatic–aromatic character. Glycyl-β-alanine, Gly-βAla, was also included since this shows the influence of a larger chelate ring. Glycylproline, Gly-Pro, differs from the others in incorporating a secondary nitrogen into the peptide bond. As a result the dipeptide does not have a replaceable hydrogen on the peptide nitrogen. Triglycine, Gly-Gly-Gly, was included for comparison purposes and serine amide, Ser-NH_2, resembles a dipeptide without the carboxyl group, having only the amine and amide nitrogen-donor centres. Results for the second group of dipeptides, consisting of dipeptides containing a His residue which has a potential additional metal-binding site, will be presented later. For all the dipeptides studied, with the exception of those containing Gly residues, there are two assymetric centres. To avoid the complications of possible stereoselectivity between the various isomeric species, the optically homogeneous LL isomers were used in all cases.

The behaviour of Cu^{2+} and Ni^{2+} towards dipeptides is markedly different. It is therefore convenient to consider the complexes separately.

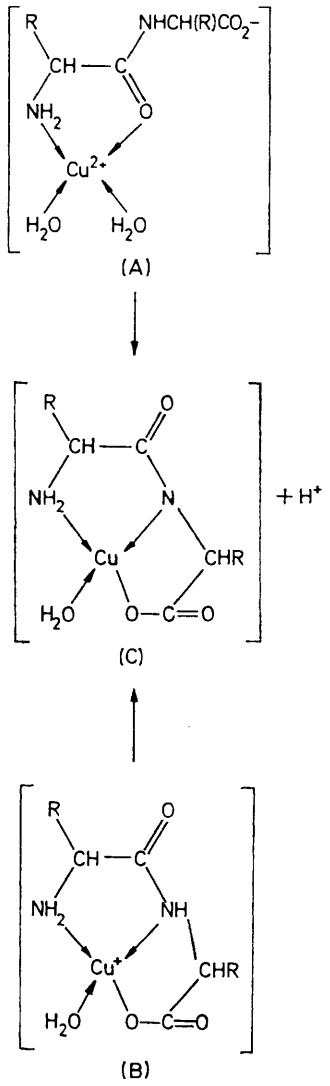
Complexes with Cu^{2+}.—At low pH a simple dipeptide, HL, may bond to Cu^{2+} in two possible ways, (A) and (B), to give the chelated complex $[CuL]^+$. For structure (A) there is no possibility of co-ordination *via* the carboxyl oxygen atom whereas if one assumes that the amide nitrogen bonds as in (B) metal-carboxyl bonding becomes possible. Structure (B) has been suggested from a study of the frequency changes in the i.r. absorption band of the carboxyl group.[2] However, most workers favour structure (A). For example, the enthalpies of formation of the complexes $[Cu(Gly\text{-}GlyO)]^+$ and $[Cu(Gly\text{-}Gly\text{-}GlyO)]^+$ (where GlyO represents loss of a proton from the glycine carboxyl group) are approximately the same,[7] which would not be expected on the basis of (B) where multidentate structures are possible and the number of copper-peptide nitrogen bonds is different for the di- and triglycine complexes. In addition structure (B) requires a non-planar amide nitrogen atom which would destroy the resonance stabilization present in the free dipeptides.[8] *X*-Ray structures of a number of copper(II)–dipeptide complexes confirm that, in the solid state at least, structure (A) is present.[9] Kinetic[10] and thermodynamic[7] data have also been interpreted as supporting bonding through the amino-nitrogen and amide oxygen atoms as in (A).

As the pH of the solution is increased the amide proton ionizes and a rearrangement of donor centres takes

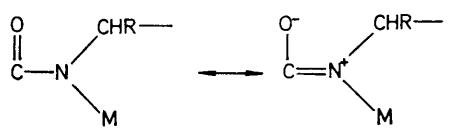
† No reprints available.

[1] R. F. Pasternack, L. Gibb, and H. Sigel, *J. Amer. Chem. Soc.*, 1972, **94**, 8031.
[2] M. K. Kim and A. E. Martell, *J. Amer. Chem. Soc.*, 1966, **88**, 914; 1967, **89**, 5138; *Biochemistry*, 1964, **3**, 1169.
[3] R. B. Martin, M. Chamberlin, and J. T. Edsall, *J. Amer. Chem. Soc.*, 1960, **82**, 495.
[4] W. L. Koltum, M. Fried, and F. R. N. Gurd, *J. Amer. Chem. Soc.*, 1960, **82**, 233.
[5] S. P. Datta, R. Leberman, and R. B. Rabin, *Trans. Faraday Soc.*, 1956, **52**, 1130; 1959, **55**, 2141.
[6] G. F. Bryce, J. M. H. Pinkerton, L. Steinrauf, and F. R. N. Gurd, *J. Biol. Chem.*, 1965, **240**, 3829.
[7] A. P. Brunetti, M. C. Lim, and C. H. Nancollas, *J. Amer. Chem. Soc.*, 1968, **90**, 5120.
[8] R. E. Marsh and J. Donohue, *Adv. Protein Chem.*, 1967, **22**, 235.
[9] M. C. Freeman, *Adv. Protein Chem.*, 1967, **22**, 257.
[10] R. F. Pasternack and M. Angwin, *J. Amer. Chem. Soc.*, 1970, **92**, 5878.

place to give structure (C), which will be referred to as [Cu(H$_{-1}$L)]. This structure is supported by studies on both the crystal [9] and in solution (i.r. and thermodynamic



studies). The lengthening of the C–O bond and shortening of the C–N bond suggests some resonance stabilization of the type (D). The two chelate rings have been



shown to be virtually planar. In crystals of [Cu(Gly-GlyO)] the copper has a co-ordination number of five, *i.e.* structure (C) with the addition of second water molecule as a long axial bond. At higher pH values the equatorial water molecule ionizes to form a monohydroxy complex of formula [Cu(H$_{-2}$L)]$^-$ (or [Cu(H$_{-1}$L)(OH)]$^-$).

Complexes with Ni^{2+}.—These have not been studied as extensively as those of Cu^{2+}. Compared to Cu^{2+} much higher pH values are required to ionize the amide proton in nickel(II)–dipeptide complexes, if it is, in fact, ionized. Potentiometric titrations show that a hydrogen ion can be removed above pH 9 but the source could be an amide hydrogen or the hydration sphere of the Ni^{2+} ion. Prior to this ionization the dipeptide is believed to bond as a bidentate ligand using the terminal amine nitrogen- and peptide oxygen-donor centres as in (A).[5] Hydrolysis experiments indicate that the carboxyl group is not co-ordinated, supporting this structure. The [NiL]$^+$ complexes of Gly-Gly and glycyl-leucine, Gly-Leu, have approximately the same stability but the complex with Leu-Gly is less stable.[1] If model (A) is correct, this observation is understandable since substitution at the carbon atom adjacent to the amino-group would introduce steric hindrance on complex formation. In Gly-Leu the Bu^i group is three atoms removed from the donor centre and so it would have little steric effect. Evidence has been presented to show that the ionization occurring at *ca.* pH 9 is, in fact, from the amide nitrogen atom.[3] This was obtained by comparing the proton-ionization constants of Ni^{2+} complexes of glycylsarcosine, Gly-Sar (which does not contain an amide hydrogen atom), with those of dipeptides which do contain amide hydrogens such as Gly-Gly.

The behaviour of Ni^{2+} towards dipeptides differs from its behaviour towards tri- and tetra-peptides. For example, the [Ni(Gly-GlyO)] complex remains blue as the pH is increased while [Ni(Gly-Gly-GlyO)] is blue at low pH but becomes yellow as the pH is increased and the nickel changes from paramagnetic to diamagnetic indicating a change from octahedral to planar co-ordination.[2] The increase in tetragonal distortion and ligand field necessary to cause this change presumably results from replacement of oxygen-(carbonyl) by nitrogen-(amide) donor atoms in the co-ordination plane when peptides higher than dipeptides are used.

EXPERIMENTAL

Dipeptides were obtained from the Sigma Chemical Co., generally of SIGMA grade.

Metal complex-formation constants were calculated from potentiometric titration curves of the dipeptides in the absence and presence of metal ions at 25 °C and I = 0.10M (K[NO$_3$]).* Changes in pH were followed using a glass electrode and Radiometer PHM64 pH meter calibrated in terms of hydrogen-ion concentrations. Titrations were carried out at a number of metal:ligand ratios (although most complex species were most clearly defined in 1 : 1 mixtures) and with a range of ligand concentrations. Formation constants were calculated from the experimental data with the help of the MINIQUAD computer program.[11] The quoted standard deviations are meaningful for the purposes of internal comparison but do not include systematic errors. The major species are clearly identified in the Tables. Such species are certainly present in the equilibria and their formation constants are virtually independent of the particular model used. Minor species, for which the concentration

* 1M = 1 mol dm^{-3}.

[11] A. Sabatini, A. Vacca, and P. Gans, *Talanta*, 1974, **21**, 53.

never exceeded 5% of the total metal present, were less clearly defined. Attempts were made to fit a large number of different models to the experimental data and that selected gave the best statistical fit over the data available.

Species which did not contribute significantly to this model were neglected.

RESULTS AND DISCUSSION

Complex-formation constants were expressed in terms of the recognised convention, usually in the form of overall formation constants, β. For example, in the reaction (1) where L is the ionized form of the free ligand $[NH_2{\cdot}CH(R){\cdot}CO{\cdot}NH{\cdot}CH(R){\cdot}CO_2^-]$, the overall formation constant is β_{xyz}. Reactions involving ionization of an amide hydrogen atom [*e.g.* (A) ⟶ (C)] cannot be distinguished unambiguously from hydrolysis of coordinated water since they will have the same apparent stoicheiometry. Such reactions were also expressed as overall formation constants as illustrated in equations (2) and (3). Stepwise constants will be referred to similarly,

$$M_x + L_y + H_z \longrightarrow [M_xL_yH_z] \quad (1)$$

$$M + L \xrightarrow{\beta_{11-1}} [M(H_{-1}L)] + H$$
$$\{\text{or } M + L + H_2O \longrightarrow [ML(OH)] + H\} \quad (2)$$

$$\beta_{11-1} = \frac{[M(H_{-1}L)][H]}{[M][L]} \left(\text{or } \frac{[ML(OH)]}{[M][L][H]^{-1}}\right)$$
$$= \frac{[M(H_{-1}L)]K_w}{[M][L][OH]} \left(\text{or } \frac{[ML(OH)]K_w}{[M][L][OH]}\right) \quad (3)$$

e.g. as in (4). For convenience, complexes with Cu^{2+} and Ni^{2+} will be considered separately.

$$[M(H_{-2}L)] + H \xrightarrow{K_{11-1}{}^{11-1}} [M(H_{-1}L)] \quad (4)$$

TABLE 1

Proton-complex formation constants of some dipeptides at 25 °C and I = 0.10M (K[NO_3]). Standard deviations (σ values) are 0.004 or less

Dipeptide	log K_{011}	log $K_{012}{}^{011}$
Gly-Ala	8.216	3.144
	8.19 ± 0.04 [a]	3.34 ± 0.02 [a]
Gly-βAla	8.185	4.020
	8.27 ± 0.02 [b]	4.07 ± 0.03
Gly-PhAla	8.157	2.987
	8.16 ± 0.02 [a]	3.12 ± 0.02 [a]
Gly-Pro	8.562	2.850
	8.48 [c]	2.97 [c]
Gly-Val	8.233	3.154
	8.18 [b]	3.15 [b]
Leu-Leu	7.911	3.455
Val-Ala	8.053	3.429
Val-Val	7.937	3.405
Gly-Gly-Gly	7.935	3.210
	7.87 [d]	3.18 [d]
Ser-NH_2	7.290	

[a] Ref. 12. [b] Ref. 13. [c] Ref. 4. [d] Ref. 7.

Proton complex-formation constants of the free ligands are shown in Table 1 together with some literature values. Protonation reactions of metal complexes are included with the corresponding metal complex-formation constants. In Table 1 K_{011} refers to protonation of the amine nitrogen atom and $K_{012}{}^{011}$ to that of the carboxyl group. Values for K_{011} demonstrate that the amino-nitrogen atom in dipeptides is less basic than in the corresponding amino-acid.

Copper(II) complex-formation constants are shown in Table 2, together with some constants for associated protonation reactions. The most important species in the equilibria is 11—1, with a small contribution from 110 at low pH (below 6) and an increasing contribution from 11—2 as the pH approaches 10. However, in this pH region the situation is complicated by the formation of a binuclear ' dimer ' which is undoubtedly present and is represented as the 22—3 species. In the pH region 8—10 the bis 12—1 complex is also important.

TABLE 2

Copper complex-formation constants at 25 °C and I = 0.10M (K[NO_3]). Standard deviations (σ values) are given in parentheses

	log β_{xyz}							pK_1' (log $K_{110}{}^{11-1}$)	pK_2' (log $K_{11-1}{}^{11-2}$)
Ligand species	110	120	11—1	12—1	11—2	22—3	log K_{Dimer}		
Gly-Ala	5.741(5)	11.16(6)	1.686(5)	4.910(4)	−7.723(2)	−3.76(8)	2.28	4.05	9.41
	5.61 [a]		0.80 [a]	4.64 [a]	−9.62 [a]			4.81 [a]	10.42 [a]
Gly-βAla	5.69(1)		1.122(1)	3.99(1)	−9.009(6)	−4.92(3)	2.97	4.57	10.13
	6.11 [a]		1.42 [a]	5.08 [a]	−8.37 [a]			4.69 [a]	9.79 [a]
Gly-PhAla	5.82(4)		1.934(4)	5.23(4)	−7.45(1)	−3.06(10)	2.46	3.88	9.39
	5.45 [b]		1.59 [b]	4.77 [b]				3.86 [b]	
Gly-Pro	6.440(1)	11.525(8)							
	6.43 [c]	11.45 [c]							
Gly-Val	5.77(1)	11.26(5)	1.122(1)	4.48(1)	−8.167(8)	−4.35(10)	2.70	4.65	9.29
	5.62 [c]		0.87 [c]	3.81 [c]	−8.43 [c]	−5.38 [c]	2.18 [c]	4.75 [c]	9.30 [c]
Leu-Leu	5.24(2)		1.378(1)	4.46(2)	−7.816(3)			3.86	9.20
Val-Ala	5.61(4)		1.816(3)	4.4(1)	−7.75(4)	−2.9(1)?		3.79	9.57
Val-Val	5.20(4)		1.354(2)	4.2(1)	−8.00(5)			3.85	9.35
Gly-Gly-Gly [d]	5.127(9)	9.6(1)	−0.046(2)	2.9(1)	−6.774(3)			5.17	6.73
	5.04 [e]		−0.02 [e]		−6.80 [e]			5.06 [e]	6.78 [e]
Ser-NH_2	4.612(9)	8.21(3)	−1.948(7)	1.81(2)	−9.515(9)	−8.65(3)	2.82	6.56	7.57

$\log K_{Dimer} = \log \beta_{22-3} - \log \beta_{11-1} - \log \beta_{11-2}$.

[a] Ref. 13. [b] Ref. 12. [c] Ref. 4. [d] $\log \beta_{11-3} = -18.18(1)$. [e] Ref. 7.

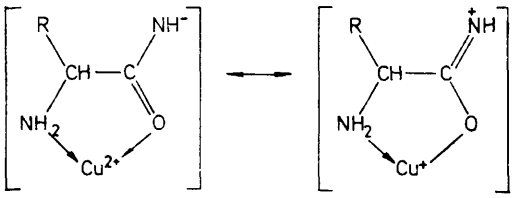
[12] J. L. Biester and P. M. Ruoff, *J. Amer. Chem. Soc.*, 1959, **81**, 657.

[13] U. I. Salakhutdinov, A. P. Borisova, Yu V. Granowskii, I. A. Savich, and V. I. Spitsyn, *Proc. Acad. Sci.* (*U.S.S.R.*), 1967, **177**, 1039 (365).

Examination of the formation constants shown in Table 2 leads to the following observations.

(i) Glycylproline does not contain an ionizable proton on its peptide nitrogen atom, hence this nitrogen is essentially non-co-ordinating. As a result the copper–Gly-Pro equilibrium is much simpler than that with other dipeptides, the only important complexes being the 110 and 120 species.

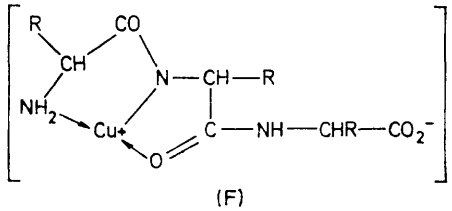
(ii) The side chain of the amino-acid forming the carboxyl half of the dipeptide has little effect on the stability of the $[CuL]^+$ (110) complex. Changing the side chain (particularly changing its bulkiness) of the amino-half of the dipeptide has a more dramatic effect on the stability. For example L-valyl-L-valine, Val-Val, is less stable than Gly-Val by 0.57 log units (110 species). With Ser-NH_2, β_{110} is of the order expected, allowing for the lower basicity of the nitrogen, if it is assumed that the bonding is the same as in the dipeptide complexes. Both these observations support bonding through the amine nitrogen and the carbonyl oxygen atom as in (A), stabilized by the resonance (E). Multidentate chelation,



(E)

as suggested by Kim and Martell,[2] is unlikely since if this were the case the side chain on the carboxyl half of the dipeptide would have a greater influence on the formation constants, the value of β_{110} for the Ser-NH_2 complex would be markedly lower in stability, and the value of β_{110} for the Gly-Gly-Gly complex would be expected to be larger than for the dipeptide complexes.

(iii) pK_1' values for the $[CuL]^+$ complexes (log K_{110}^{11-1}) are too small to refer to ionization of co-ordinated water molecules (metal-ion hydrolysis) and are assumed to refer to ionization of the amide hydrogen atom to give the tridentate species (C). The corresponding complex of Gly-Gly-Gly would be formulated as in (F). The

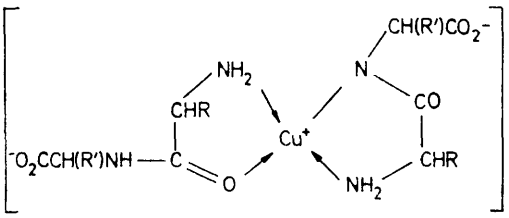


(F)

value of β_{11-1} for Gly-Gly-Gly is less than for the dipeptides as expected since the amide C=O group is a less efficient donor than the CO_2^- group. The value of β_{11-1} for Ser-NH_2 is the lowest of all because only one five-membered chelate ring can be formed.

The difference in stability between the complexes of Gly-Ala and Gly-βAla shows that two five-membered chelate rings are more stable than a five- and six-membered ring. This is in contrast to the situation when a tridentate ligand bonds in a facial rather than a meridional manner.[14]

(iv) In the Ser-NH_2 system log K_{12-1}^{110} is smaller than log β_{11-1} by 0.9 log units; *i.e.* the affinity of the species $[CuL]^+$ for the $H_{-1}L$ ion is less than that of the free metal ion, Cu^{2+}, by about the amount expected on statistical and general environmental grounds, assuming similar bonding in each case. In all other systems the difference is more than 2 log units, suggesting that the deprotonated dipeptides ($H_{-1}L$) bond more strongly to Cu^{2+} than to $[CuL]^+$. This is to be expected if the tridentate nature of the deprotonated dipeptide is accepted. The structure of the 12—1 species may therefore be assumed to be as shown in (G).



(G)

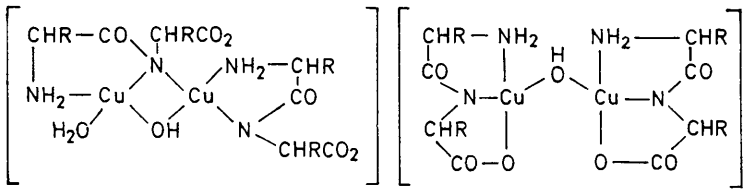
(v) The ionization reaction, pK_2' (log K_{11-1}^{11-2}), refers to deprotonation of a co-ordinated water molecule from the 11—1 species, with the exception of Gly-Gly-Gly where it refers to ionization of a hydrogen ion from the second amide nitrogen atom. With Ser-NH_2 the value of pK_2' is smaller than for the other complexes because there are more ionization sites and the 11—1 complex is positively charged rather than neutral. There appears to be no correlation between the stability of the 11—1 species and the ease of deprotonation of the co-ordinated water molecule (pK_2'), as suggested by Rabin.[5]

(vi) The dimerization reaction to form the 22—3 species is believed to be between the 11—1 and 11—2 species rather than proton displacement of an undetected 22—2 dimer. The 22—3 dimer is only formed in the region of the species distribution when the 11—1 and 11—2 species are in relatively high concentrations. There is no dimerization before the 11—2 species is significant. Dimerization appears to be negligible when both amino-acid residues of the dipeptide possess bulky side chains (*e.g.* with Val-Val).

The structure which has been proposed for the dimer, (H), involves bridging OH^- ion and amide nitrogen.[2] The experimental results reported here do not support this. The values for K_{Dimer} in Table 2 are approximately the same for all the dipeptides and for Ser-NH_2, yet formation of (H) requires the breaking of two Cu←O=C bonds and the sharing of two donor centres between the two copper ions. This would remove any resonance stabilization of the amide nitrogen atom which must be tetrahedral if a bridging atom. If (H) is the correct structure, the value of K_{Dimer} for the Ser-NH_2 complex should be larger than for the dipeptide dimers since there are no Cu←OC bonds to break and the effective charge

[14] G. Brookes, unpublished work.

on the copper ions is more positive allowing formation of stronger bridging bonds. It is therefore reasonable to propose a dimeric structure containing Cu–O as well as Cu–N bonds. However, it is difficult to construct such a ' dimer ' containing two hydroxide bridges while retaining the lack of symmetry implied by the species formula $[Cu_2(H_{-3}L_2)]$. The mono-bridged dimer, (I), is a possible if not immediately obvious structure.



(II) (I)

(vii) The only system which showed any tendency to form the 12—2 species was Cu-Ser-NH_2, presumably because this ligand can only bond in a bidentate manner.

In all the dipeptide systems titration curves for metal: ligand ratios of 1 : 1 and 1 : 2 had virtually the same characteristics. On addition of alkali the pH rose steadily to 4 or 5 when the mol ratio of alkali to metal was *ca.* 2:1. The position of this end-point was approximately independent of the dipeptide concentration, suggesting that the 11—1 species is formed in *ca.* 100% yield before further co-ordination takes place. Only after this end-point, *i.e.* pH >7, did species distributions depend on the metal: dipeptide ratio.

Nickel complex-formation constants were much less clearly defined than those of copper. When a 2 : 1 mixture of, for example, Gly-Ala with Ni^{2+} was titrated there was no sharp end-point at the proton equivalence point. Instead, in the region of the end-point, the system took a long time to reach equilibrium and reproducibility of the data was generally poor. These problems were overcome,

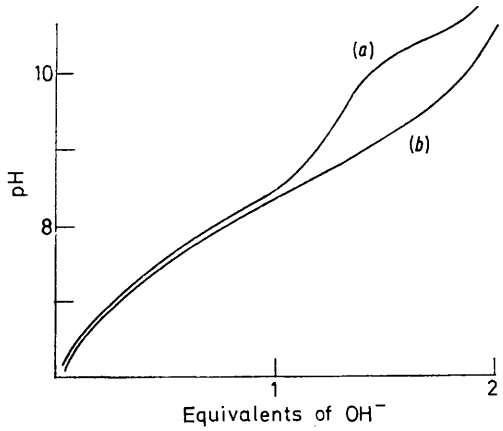
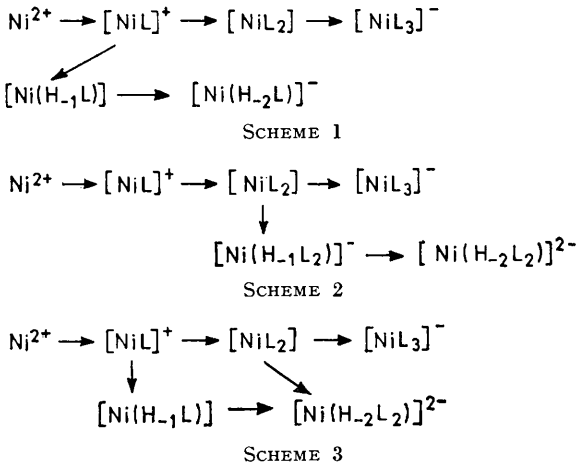


FIGURE 1 Titration curves for 1 : 3 mixtures of Ni^{2+} with Gly-Val (*a*) and Gly-PhAla (*b*)

or reduced, by increasing the ligand to metal ratio in all the systems studied to 3 : 1 and 5 : 1. Titration curves for different dipeptides showed markedly different shapes, those for Gly-Val and glycyl-phenylalanine making the two extremes as shown in Figure 1. Titrations with Val-Val and Val-Ala were restricted to below pH 9.3 as a result of precipitation. Precipitation also occurred at *ca.* pH 10.4 with Gly-βAla.

Computation based on the experimental data revealed that three different models (Schemes 1—3) described the behaviour of the system satisfactorily. On the basis of the sum of the squares of the residuals on the total metal, $c_M\{i.e.\ [c_M(calc.) - c_M(expt.)]^2\}$, total ligand, and total displaceable hydrogen-ion concentrations, and on the



SCHEME 1

SCHEME 2

SCHEME 3

statistical distribution of these residuals,[11] it was impossible to select one model in preference to another. Stepwise formation of the tris complex, $[NiL_3]^-$, is included in each scheme; differences arise in the proton-dissociation reactions. When the complexes $[Ni(H_{-1}L)]$ and $[Ni(H_{-1}L_2)]^-$ were included in the same model for computation one of them was rejected during the refinement, depending on the initial estimates of the formation constants. A similar situation existed with the species $[Ni(H_{-2}L)]^-$ and $[Ni(H_{-2}L_2)]^{2-}$. These two pairs of complexes simulated each other in the data analysis and shared a high degree of correlation. Since selection of a particular model on statistical grounds was unreliable, results for each of the three models are included in Table 3, and chemical arguments will be presented to select one of them.

As with Cu^{2+}, Gly-Pro with Ni^{2+} only forms straightforward stepwise complexed species, each model giving the same limited range of three constants which fitted the data extremely well. Since Gly-Pro has no ionizable protons on its amide nitrogen atom, this result suggests

that the proton-ionization reactions followed by the other dipeptides involve the amide proton rather than hydrolysis of a co-ordinated water molecule. Early precipitation in some cases made detection of such complexes as the 12—2 species impossible so that there was no distinction between models 2 and 3. Ni^{2+}–Gly-Gly-Gly data were only accommodated satisfactorily by model 3. Values for β_{110} and β_{120} were approximately independent of the model selected and represent the most important species below pH 9. Hence the uncertainty introduced by the choice between three models is only significant above this pH.

TABLE 3

Nickel complex-formation constants at 25 °C and I = 0.10M ($K[NO_3]$). Standard deviations are given in parentheses

	log β_{xyz} (model 3)					
Ligand species	110	120	130	11—1	12—2	pK_1' (log K_{110}^{11-1})
Gly-Ala	4.229(3)	7.596(8)	9.7(1)	−4.560(8)	−12.239(2)	8.79
Gly-βAla	4.191(3)	7.529(3)	9.74(5)	−5.04(6)	−11.4(1)	9.24
Gly-PhAla	4.03(5)	7.49(1)	9.8(1)	−4.55(2)	−10.85(2)	8.59
Gly-Pro	4.757(5)	8.645(7)	11.46(4)			
Gly-Val	4.31(1)	7.79(1)	10.38(3)	−5.13(1)	?	9.44
Val-Ala	3.47(3)	6.28(5)	?	−5.36(5)	?	8.83
Val-Val	3.12(3)	5.94(4)	?	−6.04(7)	−12.4(1)	9.16
Gly-Gly-Gly *	3.800(7)	6.88(1)		−4.75(1)		

	12—1 (model 2)	12—2 (model 2)	11—2 (model 1)
Gly-Ala	−1.516(8)	−12.192(6)	−15.22(1)
Gly-βAla	−2.11(6)	−11.5(1)	−14.4(1)
Gly-PhAla	−1.58(2)	−10.86(2)	−13.81(3)
Gly-Val	−2.21(1)	?	?
Val-Ala	−2.37(1)	?	?
Val-Val	−3.08(6)	−12.41(9)	−15.5(1)

* log β_{11-2} = −12.793(4), log β_{11-3} = −24.26(2).

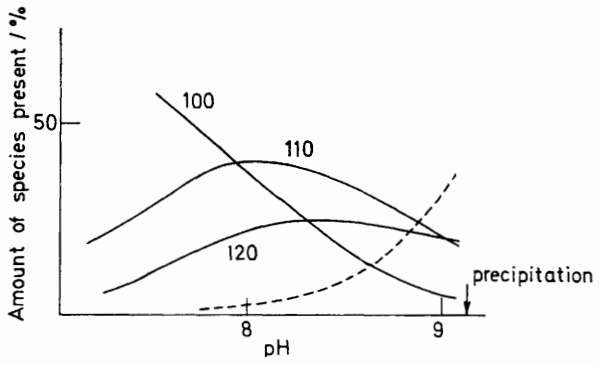


FIGURE 2 Species-distribution plots for the Ni^{2+}–Val–Val system prior to precipitation. For significance of the broken line see text

The observation of precipitation in the Ni^{2+}–Val-Val, –Val-Ala, and –Gly-βAla systems combined with the species distribution plots for each of the possible models gives a clue to the most likely model. It is assumed that the most likely species to precipitate are the neutral complexes $[NiL_2]$ or $[Ni(H_{-1}L)]$. Species-distribution plots were almost identical for each model below pH 9. A typical plot is shown in Figure 2 in which the broken line represents the distribution of either $[Ni(H_{-1}L)]$ (models 1 and 3) or $[Ni(H_{-1}L_2)]^-$ (model 2). The precipitate is unlikely to be $[NiL_2]$ since this species reached a maximum concentration at *ca.* pH 8.5, well before any precipitation took place. The unknown species is therefore likely to be the complex actually precipitating and since $[Ni(H_{-1}L_2)]^-$ is charged the evidence suggests that it is $[Ni(H_{-1}L)]$. Analysis of the precipitate, formed in more concentrated solution, gave a nickel : ligand ratio of 1 : 1 rather than 1 : 2. As a result model 2 may be eliminated and deprotonation of the $[NiL]^+$ species assumed. By analogy the same conclusion may be suggested for the other dipeptides.

Choice between models 1 and 3 is more difficult. Model 1 requires proton ionization of the $[Ni(H_{-1}L)]$ complex while model 3 involves co-ordination of a second dipeptide ion. Assuming model 1, values for pK_2' (log K_{11-1}^{11-2}) were found to be *ca.* 10. If such a proton-ionization reaction does, in fact, take place it must represent ionization of a co-ordinated water molecule. A pK value greater than 10 would be expected for such a reaction. Hence model 3 is to be preferred, although it is possible that 'microequilibria' may include both models, relative contributions depending on the actual dipeptide considered. A species-distribution plot for the Ni^{2+}–Gly-PhAla system, assuming model 3, is shown in Figure 3.

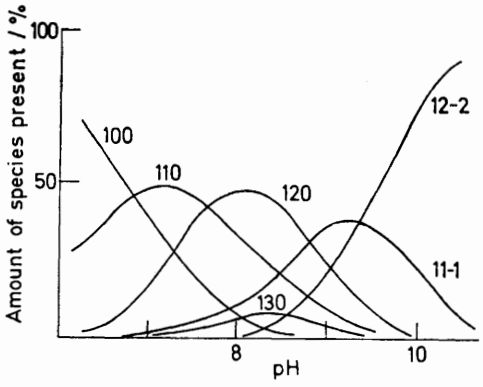


FIGURE 3 Species-distribution plots for a 1 : 3 mixture of Ni^{2+} and Gly-PhAla

The complex represented by $[Ni(H_{-1}L)]^-$ (11—1

species) may result from either hydrolysis of a co-ordinated water molecule {in hydroxo-complex[NiL(OH)]} or a dipeptide with the amide hydrogen atom ionized. The absence of such a species with Gly-Pro suggests the latter structure and this is supported by comparison of pK_1' (log K_{110}^{11-1}) values. These show a surprisingly large variation and explain the different behaviour on titration illustrated in Figure 2. The lowest value for pK_1' (8.55) was found for Gly-PhAla and the highest for Gly-Val (9.44), but all these values are rather low for a Ni^{2+} hydrolysis reaction. The higher values may contain some ' microcontributions ' from a hydrolysis reaction, but the wide spread in values for pK_1' is probably the result of the close proximity of the substituent group to the amide nitrogen atom. Electron-repelling groups (*e.g.* Bu^i) will tend to delay proton ionization, causing an increase in pK_1', while electron-attracting groups (*e.g.* Ph) will encourage such ionization.

There is little variation in stability of the $[NiL]^+$ complexes of the Gly dipeptides, but there is a marked drop in stability of the $[NiL]^+$ complexes of Val-Val and Val-Ala. This suggests that the bonding centres are the amino-nitrogen and carbonyl oxygen atoms as in (A). On deprotonation to form the $[Ni(H_{-1}L)]$ complex a rearrangement of donor centres must take place to introduce amide nitrogen bonding as proposed above. The greater acidity of the Gly-PhAla and, to a smaller extent, Gly-Ala complexes (as measured by pK_1') limits formation of the tris complexes since, in the deprotonated form, the dipeptide ion would be tridentate as in structure (C). Hence the precision of values for log β_{130} for these complexes is low. With Val-Val and Val-Ala early precipitation of nickel mono complexes prevented formation of tris complexes in detectable quantities.

[5/469 *Received*, 10*th March*, 1975]