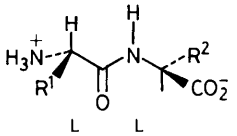
Potentiometric, Calorimetric and Spectroscopic Study of Complexation between Copper(II), Nickel(II),and Cobalt(II) and L,L-Dipeptides containing Weakly or Non-co-ordinating Side Chains†

L. Xiao, M. Jouini, B. T. Fan, and G. Lapluye*
Laboratoire de Chimie Physique, Université Paris VII, 2, Place Jussieu, 75251 Paris Cedex 05, France
J. Huet
Laboratoire de Coordination Bioorganique, Université Paris Sud, 91405 Orsay, France

Complexation of Cu^{II}, Ni^{II}, and Co^{II} with four L,L-dipeptides containing weakly or non co-ordinating side chains (Phe-Leu, Leu-Phe, Phe-Met, and Met-Phe) was studied by potentiometric, calorimetric, and spectroscopic measurements. The results show that the stability of the complexes may be influenced by different factors according to the chemical composition of the species formed in aqueous solution. For species $[MH_{-1}A]$ (A denotes conjugate base form of the ligand) an increase in stability is observed with respect to glycylglycine or to dipeptides containing one non-glycine residue, and this effect is attributed to the hydrophobic interactions between the non-co-ordinating side chains. Another stabilising effect for this species is observed with a C-terminal Phe residue. This phenomenon is attributed to the interaction between the metal ion and the aromatic ring. The enthalpy of this non-covalent effect is evaluated as -9.5 kJ mol^{-1}. This stabilising effect is not found with N-terminal Phe residues. Comparison of the complex formation constants for these copper(II)–dipeptide systems shows a marked difference in the interactions between the benzyl ring and the alkyl chain and between the benzyl ring and the S-methyl alkyl group. Spectroscopic measurements (e.s.r. and u.v.-visible) suggest the presence of a CuN_2O_2 chromophore in $[CuH_{-1}A]$ species. The ease of complex formation is in the order $Cu^{II} > Ni^{II} > Co^{II}$. Cobalt(II) does not deprotonate the peptide below pH 8. For a given species (for example [MA]), the complexes with the three transition metals appear to adopt a common structure.

Studies on proton or metal-ion complexes of dipeptides containing weakly or non-co-ordinating side chains have been carried out from a stereoselectivity point of view, and show large differences between diastereoisomeric pairs L,L and L,D (pure or mixed).[1–6]

Generally, the first ionisation constant is more acidic and the second one (corresponding to the zwitterion form) is more basic for the L,D- as compared with the L,L-dipeptides. This fact has been explained by assuming the β-type conformation of dipeptides in their acidic, neutral, and basic species.[5]



Thermodynamic studies of complexation between transition-metal ions and dipeptides have also been carried out, generally focusing on the stereoselectivity between diastereoisomeric dipeptide pairs.[5] Considerable stability was reported for the complex $[MH_{-1}A]$ of Cu^{II}–Gly-Y (Y = Phe, Tyr, or Met).[7–9] This was attributed to the metal ion–side chain interaction. Moreover, a stability increase of this species with increasing length of the side chain was observed in metal ion-L,L-dipeptide complexes.[3,5] This phenomenon is explained by non-covalent side chain–side chain interactions.

Sigel and Martin[10] reported great differences in copper(II)–, nickel(II)–, and proton–dipeptide complexes having only one non-co-ordinating or weakly co-ordinating side chain. These authors showed a significant stability decrease for the species [MA] in copper(II)– and nickel(II)–dipeptide complexes containing one non-glycine N-terminal residue. In agreement with Rabin's co-ordination model and conclusions,[11] Pasternack *et al.*[12] on the one hand and Sigel[13] on the other conclude that the more basic the zwitterion form of the ligand HA the more stable is the complex MA in Cu^{II}–Gly-X (X = Ala, Met, Leu, *etc.*).

Pettit and Hefford[3] proposed that the following different effects may be discussed in a qualitative manner in order to predict the stability variation in metal ion–dipeptide complexes: (*i*) side chain donor effect; (*ii*) hydrophobic side chain–side chain interaction; (*iii*) steric effects between the side chains themselves (generally destabilising effects); (*iv*) effects on the surrounding solvent sphere, caused by the positioning of the hydrophobic and hydrophilic parts of the total complex; (*v*) π–*d* interaction between the metal ion and the aromatic ring, which may be important in complexes containing aromatic ring moieties.

From all these studies, it appears that non-covalent interactions (ring stacking, solvophobic, hydrophobic, *etc.*) are important factors governing the stability of complexes, but are difficult to quantify and do not act in the same way on the stability.

In this work, we deal with the study of metal-ion complexation by four pure (L,L) dipeptides, containing either Phe (phenylalanine) and Leu (leucine) residues or Phe and Met (methionine) residues: L-phenylalanyl-L-methionine, L-methionyl-L-phenylalanine, L-phenylalanyl-L-Leucine, and L-Leucyl-L-phenylalanine. This will allow us to emphasize the

† *Non-S.I. unit employed:* G = 10^{-4} T.

importance of the non-covalent interactions between the phenyl ring and the alkyl chain on the one hand, the phenyl ring and the S-methylalkyl chain on the other, whether the phenyl ring is on the N or C terminal residue. We study also the difference in stability of the metal ion [copper(II), cobalt(II), and nickel(II)]–dipeptide complexes containing alkyl side chains with those containing an S-methylalkyl one, knowing *a priori* that the methionyl sulphur atom does not co-ordinate the metal ion (Cu^{II}) in its equatorial plane.[14]

Another purpose of this work concerned the metal ion–aromatic ring interaction in complexes with dipeptides containing a N-terminal phenylalanyl residue. Recently, Yamauchi *et al.*[7] observed an abnormal circular dichroism (c.d.) spectrum for the active (L,L) dipeptides with three C-terminal amino acids containing an aromatic ring, L-Tyr-L-X (X = Tyr, Trp, or Phe), accompanying the dimer formation. This anomaly is taken to imply distortion by the dimeric structure of the C-terminal side chain orientation, favouring the copper(II)–aromatic ring interaction.

The structures of nickel(II) complexes are different when complexation occurs with di- or tri-glycine ligands at high pH values. While Cu^{II} forms mainly square-planar complexes, Ni^{II} forms complexes of two different stoicheiometries. In the case of diglycine at pH > 10 the resulting blue solid complex of six-co-ordinate Ni^{II} contains two tridentate Gly-Gly ligands chelated *via* the amino and deprotonated amide nitrogens and a carboxylate oxygen.[15,16] In contrast, other higher peptides (tri- or tetra-glycine) form generally planar complexes with Ni^{II}.[10,17] There are few equilibrium constant values for other dipeptide complexes of nickel. Some results show a great difference between the complexing behaviour of nickel(II) and that of copper(II) at high pH values, but not at low pH.[3,18,19]

The complexation of Co^{II} with oligopeptides depends largely upon the presence of oxygen. In general, the formation of cobalt(II) complexes with peptides occurs at higher pH values compared with copper(II), and even with nickel(II)

The results reported here allow us to compare the complexation behaviours of different transition-metal ions with the same L,L-dipeptides containing weakly or non-co-ordinating side chains.

It should be noted that although some authors have investigated nickel(II) complexes thermodynamically,[20,21] there is much less information available about nickel(II)–dipeptide complexes than for copper(II)–dipeptide ones. Thus investigations with the same ligands allow us to compare the complexes of Cu^{II} and Ni^{II}.

Furthermore, some important structural information concerning the complexes formed was obtained from spectroscopic measurements (e.s.r. and u.v.-visible).

This paper is also devoted to a preliminary investigation of metal ion-Enkephalin complexation. The Phe group is present as the fourth amino acid in Enkephalins. The fifth one is either Leu (in leucine enkephalin) or Met (in methionine enkephalin).*

All the dipeptides used in this work are L,L-dipeptides. The equilibria in the present systems and the relevant equilibrium constants β_{pqr} are defined by equations (1)—(4) (charges are

$$pM + rA + qH \overset{\beta_{pqr}}{\rightleftharpoons} M_pA_rH_q \quad (1)$$

$$\beta_{pqr} = [M_pA_rH_q]/[M]^p[A]^r[H]^q \quad (2)$$

$$M_pA_rH_q \overset{K_{M_pA_rH_q}}{\rightleftharpoons} M_pA_rH_{q-1} + H^+ \quad (3)$$

$$K_{M_pA_rH_q} = [M_pA_rH_{q-1}][H^+]/[M_pA_rH_q] \quad (4)$$

omitted for simplicity) where p,q, and r are the stoicheiometric numbers. A negative value for q denotes a deprotonated or hydroxylated form of the species considered; A denotes the conjugate base of the ligand, HA the zwitterion form, and M the metal ion.

Experimental

Potentiometric Measurements.—All experiments were carried out in potassium nitrate (0.2 mol dm^{-3}). The metal-ion nitrate stock solution was prepared by dissolution of crystals (Merck, analytical grade), and was made slightly acidic by adding nitric acid (Normadose Merck). Potassium hydroxide solution was prepared with freshly twice distilled water, boiled, and then saturated with nitrogen.

The dipeptides (all from Sigma Chemical Co.) were stored under vacuum and used without further purification.

The investigation of the protonation and metal complex equilibria was performed at 25.0 ± 0.1 °C under an atmosphere of purified nitrogen using previously described apparatus and procedure.[22] The concentration of copper(II), nickel(II), and cobalt(II) ions was $c_M = 2 \times 10^{-3}$ mol dm^{-3}, prepared by dissolving the salts directly in twice distilled water under pure nitrogen gas, then acidified with HNO_3 to the required pH values, *i.e.* pH 2 for Cu^{II}, pH 3.5 for Ni^{II} and Co^{II}. The ligand concentrations were 2×10^{-3} and 4×10^{-3} mol dm^{-3}. The treatment of the potentiometric data was detailed elsewhere.[23] Every titration carried out in the presence of metal ions was stopped whenever a steady drift occurred in the mV-meter readings, this drift denoting the formation of a precipitate in the solution. In the case of copper complexation, the precipitate is formed only at a ratio $c_A/c_M = 1:1$ at about pH 5.0. However, in the case of nickel(II) and cobalt(II) the precipitates appear at about pH 8.5, for both ratios $c_A/c_M = 1$ and 2:1. This phenomenon was more evident for dipeptides containing a Met residue.

Calorimetric Measurements.—The calorimetric study was performed at 25.000 ± 0.001 °C under purified nitrogen. The apparatus was described elsewhere.[24] This system allows the concurrent determination of formation constants and enthalpies of complexes by coupling two techniques: potentiometry and isothermal calorimetry. The dipeptide was dissolved in 0.5 mol dm^{-3} KNO_3 solution (50 cm^3), acidified by HNO_3 to pH 2. Both calorimetric and potentiometric measurements were carried out by titrating with 0.5 mol dm^{-3} KOH the acidified solution containing the ligand in the absence of metal ions, in order to determine the deprotonation enthalpies.

To determine formation enthalpies of complexes, the metal ions were added to the solutions containing ligands, according to the ratios $c_A/c_M = 1$ and 2:1 (c_A = concentration of ligand c_M = concentration of metal ion), where $c_A = 0.020$ mol dm^{-3}. The measurements were carried out by titration with KOH from pH 2 to 9.5 in the case of copper(II) complexation, and from pH 4 to 8.5 in the case of nickel(II).

Spectroscopic Measurements.—Electronic absorption spectra were recorded at room temperature (25 °C) using a Beckman model 25 spectrophotometer. The e.s.r. spectra were recorded on a Varian C.S.E. 109 spectrometer operating at *X*-band frequencies and modulated at 100 kHz. Temperature control was achieved using the Varian liquid-nitrogen accessory and the Varian e.s.r. heater control unit (temperature accurate to ±1 °C). The magnetic field was calibrated with a n.m.r. proton probe. A sample of diphenylpicrylhydrazyl (dpph), placed in a dual cavity, was used as g marker (g = 2.0036). Quartz tubes of

* *N*-{*N*-[*N*-(*N*-L-Tyrosylglycyl)glycyl]-L-phenylalanyl}-L-leucine and -methionine.

thickness 1 mm and internal diameter 2 mm were used for spectra recorded at low temperature, while quartz tubes of internal diameter 1 mm were employed for those recorded at high and room temperature. Samples of copper(II)–dipeptide systems were studied at pH 5.8 and 10. At pH 5.8 the $[MH_{-1}A]$ species is formed, which is the major one for such systems in the physiological pH range. Low-temperature measurements were made at −180 °C with the same samples.

Freshly prepared solutions were rigorously deoxygenated under a nitrogen stream prior to use in e.s.r. measurements.

Calculations.—Calculations concerning the complexation constants were carried out by computer programs ACBA,[25] SCOGS,[26] and MINIQUAD.[27] The enthalpies were calculated from experimental heat effects using the method reported previously.[28] In all calculations, the enthalpy value employed for the formation of water was −56.4 kJ mol^{-1},[28] which is in agreement with values reported under similar experimental conditions.[29,30]

Results and Discussion

Dissociation Constants.—The dissociation constants determined in this work for the four dipeptides are given in Table 1 along with some literature values required for the discussion.

Small differences are observed with the pK values for Met-Phe of Pettit and Hefford[3] and that for Leu-Phe of Bonomo *et al.*,[5] probably due to the ionic strength difference. In the case of Phe-Met and Phe-Leu our data are the first reported.

Assuming the β-type conformation[5] for the ligand in solution, the side chain–side chain interactions are at a minimum level in the L,L-dipeptides. In Table 1 the first ionisation constants (pK_1) are less acidic for the ligands studied than in the case of glycylglycine (except for Met-Phe), whilst the second ionisation constants (pK_2) are more acidic. The observed pK_1 sequence for the dipeptides is Met-Phe < Gly-Gly < Leu-Phe < Phe-Met < Phe-Leu, and the observed pK_2 sequence is Met-Phe < Phe-Met < Phe-Leu < Leu-Phe < Gly-Gly.

Table 1. Dissociation constants of dipeptides at 25 °C, I = 0.2 mol dm^{-3} KNO_3

Dipeptides	pK_1	pK_2	Ref.
Gly-Gly	3.12 ± 0.02	8.01 ± 0.02	28
Gly-L-Phe	2.99 ± 0.02	8.08 ± 0.02	34
L-Phe-L-Leu	3.41 ± 0.01	7.30 ± 0.01	*
L-Phe-L-Met	3.24 ± 0.01	7.27 ± 0.01	*
L-Met-L-Phe	3.08 ± 0.01	7.14 ± 0.01	*
	3.14	7.29	3
L-Leu-L-Phe	3.16 ± 0.01	7.67 ± 0.01	*
	3.18	7.69	5

* This work.

The pK_1 values of X-Phe are close together and so are the pK_2 values of Phe-X where X is Gly, Met, or Leu. From these results we can assume that in L,L-dipeptides the most important effect which influences the pK values is the donor effect of the substituent on the nearest asymmetric carbon.

The small pK_2 difference for Phe-X systems (or pK_1 difference for X-Phe) probably results from other effects (electrostatic, steric, *etc.*) which are at a minimum when the β-type conformation for the L,L-dipeptides is considered. The case of diglycine is rather different because of the absence of a side chain.

Our calorimetric results show that the deprotonation enthalpies of the ligands are comparable with those of similar dipeptides[31–33] (see Table 2).

Copper(II)–Dipeptide Complexes.—E.s.r. measurements and electronic absorption spectra. (*a*) *Frozen-state e.s.r.* Spectra were recorded at −180 °C for the ratio c_A/c_M = 8. Preliminary studies have shown that for $c_A = 10^{-3}$ mol dm^{-3} and for ligand: Cu^{II} ratios respectively of 1 and 2:1 a broad featureless transition was observed for the species $[MH_{-1}A]$ of the four copper(II)–dipeptide systems studied. This means that dipolar interactions between intermolecular paramagnetic centres are of greater magnitude than copper hyperfine interactions in these systems. At c_A/c_M = 8:1 relatively poor resolution occurs, but this could be improved by addition of $NaClO_4$. The ClO_4^- anion does not affect the complex structure in aqueous solution, since there is no association between this anion and Cu^{II},[34] but provides marked improvement in the resolution of the e.s.r. spectra, as outlined elsewhere.[35] As pointed out for other systems,[35,36] the maximum amounts of the different species are not observed at the same pH values in potentiometric titrations as in low-temperature e.s.r. experiments, because the equilibria are temperature dependent. Nevertheless, the two main species of the copper(II)–dipeptide systems, $[MH_{-1}A]$ and $[MH_{-1}(OH)A]$, could be unambiguously characterised. Spectra are identical for the two copper(II)–dipeptide systems and exhibit parallel and perpendicular features, typical of Cu^{II} in an axial ligand field with tetragonal distortion by elongation along the axial direction, the limit being the square-planar arrangement characterised by the sequence $g_{\|} \gg g_{\perp} > 2.00$ and $A_{\|}$ values in the range 150—200 G.[37] Hence, the $g_{\|}$ and $A_{\|}$ values are the pertinent parameters to provide structural assignments.

From the spectrum (Figure 1) of the species $[MH_{-1}A]$ in the Cu^{II}-L-Leu-L-Phe system, recorded at pH 6, we get $g_{\|}$ = 2.221 ± 0.003 and $A_{\|}$ = 180 ± 4 G. The same species in the other system yields identical values within experimental error (see Table 3). The $g_{\|}$ and $A_{\|}$ values suggest a CuN_2O_2 in-plane donor set according to the Peisach–Blumberg plots.[38]

From the spectrum (Figure 2) of the $[MH_{-1}(OH)A]$ species of the Cu^{II}-L-Leu-L-Phe system, observed in the range pH 7—10, we obtained $g_{\|}$ = 2.212 ± 0.003 and $A_{\|}$ = 172 ± 3 G (nearly identical values were measured for the other system) and a CuN_2O_2 in-plane donor set is also suggested.[38] Moreover, the smaller value of $A_{\|}$ with respect to that measured for

Table 2. Thermodynamic parameters of proton complex formation of L,L-dipeptides at 25 °C, I = 0.5 mol dm^{-3} KNO_3

	$-\Delta H^{\circ}$/kJ mol^{-1}		$-\Delta G^{\circ}$/kJ mol^{-1}		ΔS°/J K^{-1} mol^{-1}		
Ligand	NH_2	CO_2^-	NH_2	CO_2^-	NH_2	CO_2^-	Ref.
L,L-Phe-Leu	46.6 ± 1.4	0.0 ± 0.5	41.7	19.7	−16.4	66.2	*
L,L-Leu-Phe	46.7 ± 1.2	−0.3 ± 0.3	44.2	18.4	−8.5	62.7	*
	43.6	−0.3	43.9	18.1	1.3	59.8	33
L,L-Leu-Leu	42.7	−1.5	45.1	19.7	7.9	71.0	5
L,L-Val-Phe	43.9	−0.2	43.8	18.2	4.2	61.4	5
Gly-Gly	45.6	2.1	45.7	17.8	0.4	52.7	18

* This work.

Table 3. E.s.r. parameter values for the Cu^{II}–L,L-Leu-Phe system in frozen solution (−180 °C)

	pH				
Parameter	2—3 $[Cu(H_2O)_6]^{2+}$	4 [MA] + [$MH_{-1}A$]	5 [$MH_{-1}A$]	6 [$MH_{-1}A$] + [$MH_{-1}A_2$]	7—10 [MH_{-1}(OH)A]
$A_{\parallel}$	141 + 2	160 ± 2	182 ± 5	*	170 ± 3
$g_{\parallel}$	2.374 ± 0.003	2.300 ± 0.003	2.221 ± 0.004	*	2.214 ± 0.003

* Parameters of [$MH_{-1}A_2$] not discernible from those of [$MH_{-1}A$].

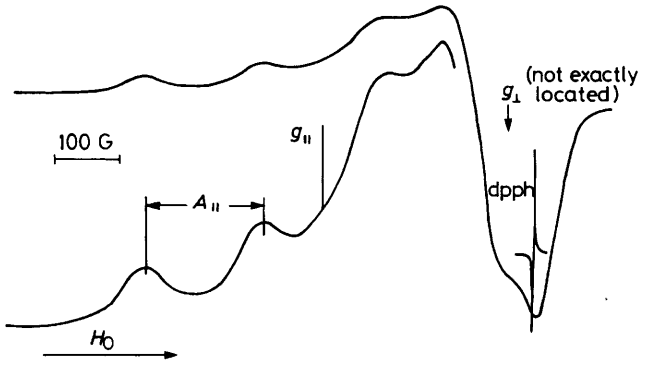


Figure 1. Frozen-state e.s.r. spectrum (−180 °C) of the species [$MH_{-1}A$] for the Cu^{II}–L-Leu-L-Phe system, pH 6, c_A/c_M = 8:1

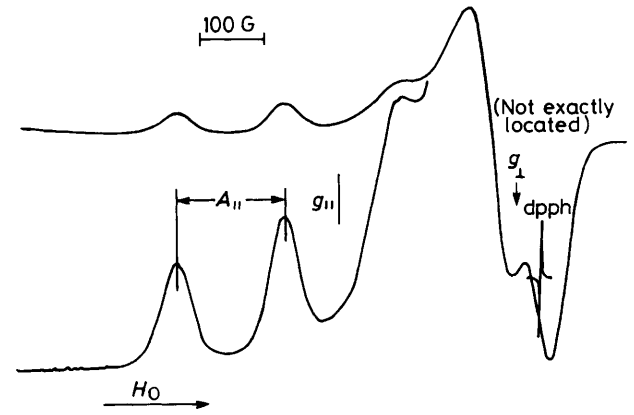


Figure 2. Frozen-state e.s.r. spectrum (−180 °C) of the species [MH_{-1}(OH)A] for the Cu^{II}–L-Leu-L-Phe system, pH 10, c_A/c_M = 8:1

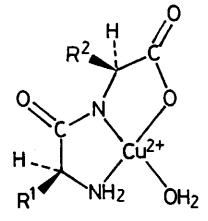
[$MH_{-1}A$] may be interpreted in terms of a slight deformation of the square-planar arrangement towards a more tetrahedral environment.[39]

Spectra recorded between pH 6 and 8 showed drastic overlap between the lines of the [$MH_{-1}A_2$] species and those of [$MH_{-1}A$]. As a consequence, no precise measurement could be made for the [$MH_{-1}A_2$] species.

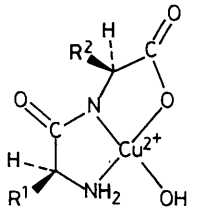
(*b*) *Liquid-state e.s.r.* Three selected spectra belonging to the [$MH_{-1}A$] species of the Cu^{II}–Phe-Leu system are depicted in Figures 3—5 and are also typical of the three other copper(II)–dipeptide systems. The liquid-state e.s.r. spectrum does not vary at a given temperature, whatever the ratio ligand/Cu^{II} used. At room temperature (Figure 3) the expected four-line hyperfine pattern was observed. However, the two low-field components are broadened to such an extent that no accurate measurements could be made. It is known that the line shape of liquid-state e.s.r. spectra may be strongly dependent on the tumbling of a complex,[40] which is temperature and molecular weight dependent. An increase in temperature yields better resolution of the classical four-line hyperfine splitting. This is illustrated in Figure 4 from which the isotropic parameters g_0 and A_0 could be obtained with satisfactory precision. For the whole series, the g_0 factor was about the same and equal to 2.130 ± 0.004, while the hyperfine coupling constant A_0 is 73 ± 3 G. These values suggest the presence of a CuN_2O_2 in-plane chromophore,[41] in agreement with the conclusions of the frozen-state e.s.r. investigation. However, a definite proof of the structure of the [$MH_{-1}A$] species was drawn from a careful study of the high-field line of the spectrum in Figure 5.

The discrepancies in the high- and low-field parts of this hyperfine component can be explained by line broadening due to non-coincidence of peaks from the two isotopes of copper, because their magnetic moments are not exactly identical.[42] The shoulders observed are produced by the superhyperfine interaction of the unpaired electron of Cu^{II} with the nuclear spin of two co-ordinated nitrogen atoms.

To summarise, e.s.r. investigation enables us to propose the structures depicted for the two species [$MH_{-1}A$] and [MH_{-1}(OH)A]. A square-planar arrangement around the copper atom is also assumed for the other complexes described in this paper.



$CuH_{-1}A$



CuH_{-1}(OH)A

(*c*) *Visible absorption spectra.* The visible absorption of copper complexes exhibits a broad *d–d* band[35,43] which shifts to shorter wavelengths as the pH increases (Figure 6). The wavelength of the absorption maximum ($\lambda_{max.}$ = 630 nm) at pH 5 and 10, at which the most important species are [$MH_{-1}A$] and [MH_{-1}(OH)A] respectively, show the presence of a chromophore CuN_2O_2, in agreement with e.s.r. measurements.

Thermodynamic results. The formation constants for the systems studied are given in Table 4, and the thermodynamic parameters ΔH°, ΔG°, and ΔS° in Tables 5 and 6.

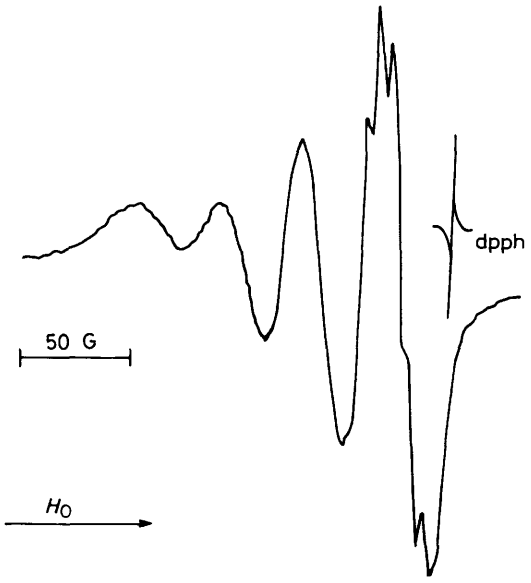


Figure 3. Liquid-state e.s.r. spectrum of the Cu^{II}–L-Leu-L-Phe system at room temperature, pH 5.8; amplitude modulation used = 5 G

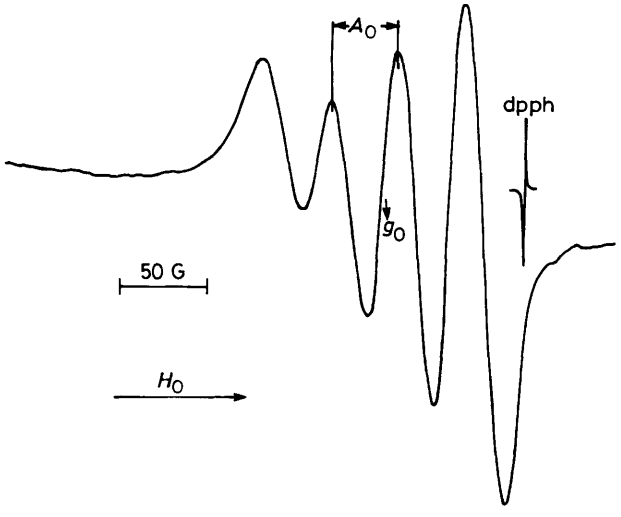


Figure 4. Liquid-state e.s.r. spectrum of the Cu^{II}–L-Leu-L-Phe system at 50 °C, pH 5.6; amplitude modulation used = 20 G.

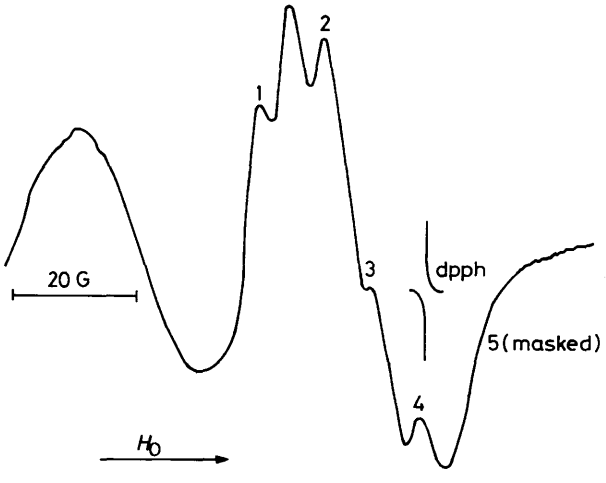


Figure 5. Liquid-state e.s.r. spectrum of the Cu^{II}–L-Leu-L-Phe system at room temperature, pH 6; amplitude modulation used = 5 G

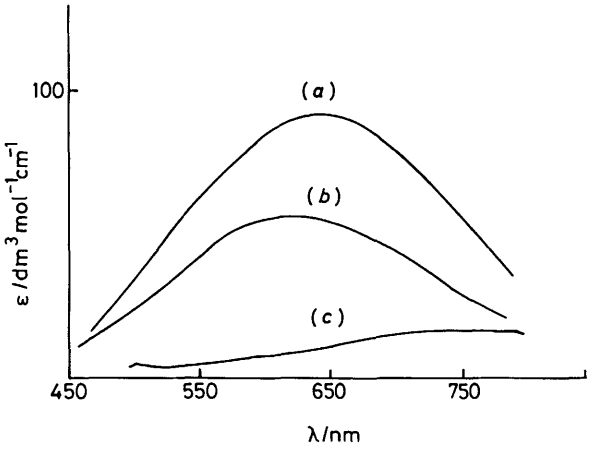
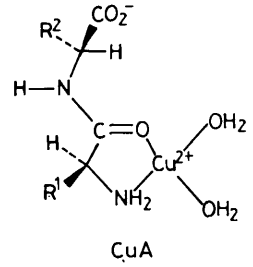


Figure 6. Visible spectra of the Cu^{II}–L-Leu-L-Phe system (*a*) pH 10, $[MH_{-1}(OH)A]$; (*b*) pH 5, $[MH_{-1}A]$; (*c*) pH 3, $[Cu(H_2O)_6]^{2+}$

Rizzarelli and co-workers [5] have published data for $[MH_{-1}A]$ species of the Cu^{II}–L-Leu-L-Phe system at 25 °C and I = 0.1 mol dm^{-3} (KNO_3). The thermodynamic functions reported there are in agreement with our results. Other results that we report in this paper are original.

[MA] *species.* Several authors [12] attempted to correlate the stability of the [MA] complex and the basicity of HA in dipeptides containing one or two non-glycine residues. We did not observe a similar correlation (Table 4). However, the log β_{101} values of Phe-X-Cu^{II} systems (Table 4) (X = Gly, Met, or Leu) appear to be very close to each other. Such a result is also observed for [MA] in the Met-Y-Cu^{II} (Y = Gly or Phe) systems and also in the Leu-Y-Cu^{II} systems. This suggests a similar effect on stabilisation (or destabilisation) of the [MA] species whenever the N-terminal residue in the dipeptides is the same. Assuming once more the β-type conformation for the ligand in solution, the proposed structure for [MA] species is as shown, where the two side chains lie on opposite sides in the L,L



CuA

isomers, and present a large end-to-end distance and minimum side chain–side chain interactions.[3] The two major stabilising (or destabilising) effects on the [MA] species are likely the HA basicity (side chain donor effect) and the steric effect of the N-terminal residue side chain.

The side chain–side chain interactions in [MA] species seem to be at a minimum when the L,L-dipeptides considered contain weakly or non-co-ordinating side chains. The differences in the log β_{101} values are as follows: log β_{101} (Leu-Phe-Cu^{II}) − log β_{101} (Leu-Gly-Cu^{II}) = 0.16, log β_{101} (Met-Gly-Cu^{II}) − log β_{101} (Met-Phe-Cu^{II}) = 0.13, log β_{101} (Phe-Met-Cu^{II}) − log β_{101} (Phe-Gly-Cu^{II}) = 0.04, and log β_{101} (Phe-Met-Cu^{II}) − log β_{101} (Phe-Leu-Cu^{II}) = 0.17. This is also confirmed by the pK_{HA}^{MA} values (Table 4) which denote the copper(II) affinity towards amine nitrogen. They are of the same order for the Phe-X-Cu^{II} systems studied.

Table 4. Formation constants[a] of copper(II)–dipeptide complexes at 25 °C, I = 0.2 mol dm^{-3} KNO_3

Dipeptide	$\log \beta_{111}$	$\log \beta_{101}$	$\log \beta_{1-11}$	$\log \beta_{1-12}$	$\log \beta_{1-21}$	pK_a	pK_b	$\log K_c$	Ref.
Gly-Gly	9.9	5.8	1.4	5.0	−8.8				28[b]
Gly-L-Phe		5.59	1.93	5.23	−6.55				54[c]
L-Phe-Gly		4.93	1.26	4.04	−8.00	3.67	6.74	2.78	28
		±0.04	±0.01	±0.04	±0.01				
L,L-Phe-Leu	8.21	4.70	0.93	3.54	−8.28	3.77	7.35	2.61	*d*
	±0.02	±0.01	±0.01	±0.02	±0.01				
L,L-Phe-Met	9.04	4.97	1.61	4.26	−7.42	3.36	5.81	2.65	
	±0.03	±0.04	±0.01	±0.03	±0.01				
L-Met-Gly		4.67	1.48			3.19			12
		±0.01							
L,L-Met-Phe	8.94	4.54	1.70	4.27	−7.42	2.84	5.72	2.57	
	±0.09	±0.05	±0.01	±0.04	±0.02				
L,L-Met-Phe		4.76	1.76			3.00			3
		±0.04	±0.01						
L-Leu-Gly		4.75	1.49			3.26			3
		±0.22							
L,L-Leu-Leu		5.21	1.33			3.88			5
L,L-Leu-Phe	8.90	4.91	1.88	4.20	−7.40	3.03	5.52	2.32	*d*
	±0.01	±0.02	±0.01	±0.02	±0.01				
L,L-Leu-Phe		4.96	1.89			3.07			5
		±0.06	±0.01						

[a] K_a, K_b, and K_c correspond to the equilibria $[MA] \rightleftharpoons [MH_{-1}A] + H^+$, $[MH_{-1}A] \rightleftharpoons (MH_{-2}A) + H^+$, and $[MH_{-1}A] + A \rightleftharpoons [MH_{-1}A_2]$ respectively. [b] I = 0.15 mol dm^{-3} KNO_3. [c] I = 0.20 mol dm^{-3} KCl. [d] I = 0.5 mol dm^{-3} KNO_3.

Table 5. Thermodynamic functions for the complex formation of copper(II) with L,L-Phe-Leu at 25 °C, I = 0.5 mol dm^{-3} KNO_3

	ΔH^*/kJ mol^{-1}	$-\Delta G^*$/kJ mol^{-1}	ΔS^*/J K^{-1} mol^{-1}
[MA]	−28.0 ± 0.6	26.8	−4.3
$[MH_{-1}A]$	7.4 ± 0.3	5.3	42.6
$[MH_{-1}(OH)A]$	48.5 ± 3.7	−47.2	4.5
$[MH_{-1}A_2]$	−23.6 ± 0.9	20.2	−11.4
$M + HA \rightleftharpoons [MA] + H^+$	18.4	−14.9	11.8
$[MA] \rightleftharpoons [MH_{-1}A] + H^+$	35.4	−21.5	46.6

Table 6. Thermodynamic functions for the complex formation of copper(II) with L,L-Leu-Phe at 25 °C, I = 0.5 mol dm^{-3} KNO_3

	ΔH^*/kJ mol^{-1}	$-\Delta G^*$/kJ mol^{-1}	ΔS^*/J K^{-1} mol^{-1}
[MA]	−27.5 ± 1.5	28.0	1.7
$[MH_{-1}A]$	−2.1 ± 0.2	10.7	29.0
$[MH_{-1}(OH)A]$	39.6 ± 2.3	−42.2	−8.8
$[MH_{-1}A_2]$	−25.1 ± 0.8	24.0	−3.8
$M + HA \rightleftharpoons [MA] + H^+$	18.2	−16.1	6.9
$[MA] \rightleftharpoons [MH_{-1}A] + H^+$	25.4	−17.3	27.6

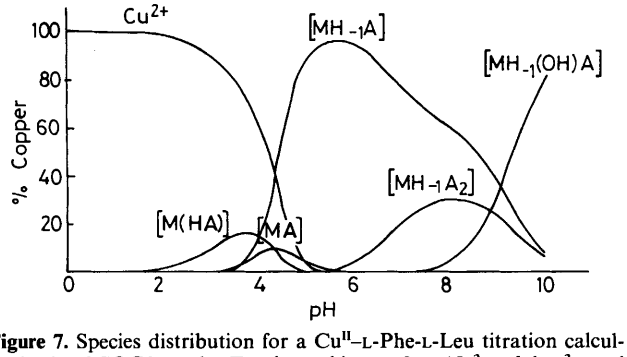


Figure 7. Species distribution for a Cu^{II}–L-Phe-L-Leu titration calculated using SCOGS results. Total metal ion = 2 × 10^{-3} mol dm^{-3}, total ligand = 4 × 10^{-3} mol dm^{-3}, 25 °C, I = 0.2 mol dm^{-3} KNO_3

In agreement with the potentiometric results, the thermodynamic parameters (see Tables 5 and 6) show that the stability of the [MA] species is generally not dependent on the C-terminal residue side chain. In other words, the binding sites are entirely located in the N-terminal amino-acid moiety. This fact can be verified by comparing the formation enthalpy of the [MA] species with that of corresponding species in the copper(II)–glycylglycine system. The formation enthalpy obtained for [MA] in Cu^{II}–L-Phe-L-Leu is comparable with that of Cu^{II}–Gly-Gly according to refs. 28, 31, and 32. The structure of the [MA] species in the Cu^{II}–Gly-Gly system was as shown for the present systems. Moreover, because of the negligible formation enthalpy of the Cu–O bond, when oxygen atom is provided by a carboxylate group, a carbonyl group, or H_2O,[44] the enthalpy for [MA] may be considered as that for Cu–NH_2 binding. This means that in the Cu^{II}-L-Phe-L-Leu system, the formation enthalpy of the Cu–NH_2 bond is about −28 kJ mol^{-1}.

The Cu^{II}–L-Leu-L-Phe system deserves the same consideration, as the formation enthalpy of the [MA] species is directly comparable with that found in the Cu^{II}–L-Phe-L-Leu system.

$[MH_{-1}A]$ *species.* The $[MH_{-1}A]$ species is the major complex for copper(II)–dipeptide systems containing weakly or non-coordinating side chains, from pH 5 to 8, as shown in Figure 7.

As shown in Table 4, the pairs L-Leu-L-Phe ($\log \beta_{1-11}$ = 1.88) and L-Phe-L-Leu ($\log \beta_{1-11}$ = 0.93) display with Cu^{II} two different formation constants. Pettit and Hefford[3] reported approximatively the same differences between the constants of the species (1) L-Val-L-Phe ($\log \beta_{1-11}$ = 1.845) and L-Phe-L-Val ($\log \beta_{1-11}$ = 1.155) and (2) L-Leu-L-Tyr ($\log \beta_{1-11}$ = 1.77) and L-Tyr-L-Leu log (β_{1-11} = 1.07). These data indicate an increase in stability when the aromatic ring is on a C-terminal residue, even in tyrosine. Pettit and Hefford[3] and recently Kiss and Szücs[45] suggested that, in this complex, the phenolic oxygen atom is not involved in copper ligation. Quite different formation constants are found for copper(II) with the pairs L-Phe-Gly ($\log \beta_{1-11}$ = 1.26) and Gly-L-Phe ($\log \beta_{1-11}$ = 1.93), and L-Tyr-Gly ($\log \beta_{1-11}$ = 1.29) and Gly-L-Tyr ($\log \beta_{1-11}$ = 1.70).[45] This difference is not due to side chain donor effects. The Phe and Tyr residues give the same results with glycine or leucine in dipeptide–copper(II) $[MH_{-1}A]$ complexes, whether

N- or C-terminal. In view of these results we conclude that the enhanced C-terminal stabilisation effect of Phe residues compared to the N-terminal one is essentially due to the interaction between the metal ion and the aromatic ring moiety; this was suggested previously by several authors and recently by Bonomo *et al.*[5] In the structure of the $[MH_{-1}A]$ species in copper(II)–L,L-dipeptide solutions[46] the two side chains remain on the same side of the equatorial copper(II) plane. There are three bonds between the copper(II) and the amine and amide nitrogens and the carboxylic oxygen. This last bond is energetically weak[28] and can probably be distorted by metal ion–aromatic ring interaction when the phenyl ring is C-terminal. This probably cannot happen when the phenyl ring is N-terminal, because the copper(II)–amine bond is strong[31,47] and cannot be displaced.

The log β_{1-11} values for Cu^{II}–L-Met-L-Phe and Cu^{II}–Phe-L-Met systems are very similar. It seems that these two dipeptides do not conform to the described phenomenon. The two values are slightly greater than that of the Cu^{II}–Gly-Gly system, and the ionisation constant pK_a $[MA] \rightleftharpoons [MH_{-1}A] + H^+$) is more acidic here than for glycylglycine. Compared to Cu^{II}–L-Leu-L-Phe (log $\beta_{1-11} = 1.88$), Cu^{II}–L-Met-L-Phe possesses a smaller stability constant (log $\beta_{1-11} = 1.70$). However, the formation constant for Cu^{II}–L-Phe-L-Met (log $\beta_{1-11} = 1.61$) is more important than that for Cu^{II}–L-Phe-L-Leu (log $\beta_{1-11} = 0.93$), possibly a result of interaction between the S atom and copper (S-Cu axial interaction).[48] The two interactions [phenyl ring–copper(II) and sulphur–copper] have stabilising effects. This may be the reason why the formation constants are similar for $[MH_{-1}A]$ of both Cu^{II}–L-Phe-L-Met and Cu^{II}–L-Met-L-Phe and could explain in addition the more acidic pK_a value (see Table 4) of the [MA] species, with these two dipeptides, compared with Cu^{II}–Gly-Gly.

The enthalpy obtained for the $[MH_{-1}A]$ species includes in general three components:[49] deprotonation of the peptidic nitrogen and formation of the Cu–N and Cu–O bonds. As seen above, the formation enthalpy of the Cu–O bond can be neglected, as a consequence the enthalpy of $[MH_{-1}A]$ may be attributed to two components only. The difference in the formation enthalpies of the $[MH_{-1}A]$ and [MA] species may reflect the transformation (5) from MA to $MH_{-1}A$, where ΔH_t is the enthalpy for this transformation. In the case of Cu^{II}–L-Phe-L-

$$[CuA]^+ \overset{\Delta H_t}{\rightleftharpoons} [CuH_{-1}A] + H^+ \qquad (5)$$

Leu, $\Delta H_t = 37.5$ kJ mol^{-1}, and $\Delta H_t = 25.5$ kJ mol^{-1} for the Cu^{II}–L-Leu-L-Phe system. These values show a significant difference between the two systems. The $pK_{MA}^{MH_{-1}A}$ (pK_a in Table 4) values which describe the acidity of the [MA] species show the same large difference between the two systems. This difference is due not only to the direct influence of the side-chain groups upon the deprotonation of the peptidic nitrogen, but also is a consequence of different stabilities principally of the $[MH_{-1}A]$ complex. Moreover, the enthalpies of the $[MH_{-1}A]$ species (Cu^{II}–L-Leu-L-Leu, 6.7;[5] Cu^{II}–L-Phe-L-Leu, 7.4; and Cu^{II}–L-Leu-L-Phe; −2.1 kJ mol^{-1}) show the sequence: L-Leu-L-Phe < L-Leu-L-Leu < L-Phe-L-Leu corresponding to the increasing order of the formation constants (see Table 4), L-Phe-L-Leu < L-Leu-L-Leu < L-Leu-L-Phe. Generally for L,L-dipeptides containing two large non-covalent side-chain groups, the stability of the $[MH_{-1}A]$ species is greater than that of the corresponding L,D- or -D,L-dipeptides due to hydrophobic interaction between two side-chain groups.[5] However, this hydrophobic interaction is less evident when the dipeptides contain a benzyl group, because the interaction between the alkyl group and the aromatic ring is less effective.[5] This interpretation is verified for L-Phe-L-Leu, which possesses a smaller formation constant and exhibits a more endothermic effect than L-Leu-L-Leu. However, it does not explain the fact that the $[MH_{-1}A]$ species of L-Leu-L-Phe is more stable than that of L-Leu-L-Leu. We have proposed that this increase in stability for L-Leu-L-Phe can be attributed to interaction between the aromatic ring and the copper ion, as suggested also by other authors for similar systems.[7,50–52]

Furthermore, the results obtained for the systems Cu^{II}–L-Phe-L-Leu and Cu^{II}–L-Leu-L-Phe allow us to quantify the stabilising enthalpy of the metallic ion–aromatic ring interaction. A marked difference in the stability and in the formation enthalpy of the $[MH_{-1}A]$ species was observed for these two systems. Several authors[3] have pointed out that three general factors should be considered for the stability of metal complexes with dipeptides containing non-co-ordinating side chains.

(*i*) *Electrodonor and steric effects.* For $[MH_{-1}A]$ species these effects are the results of the influences of two substituents upon the chelating atoms: amino nitrogen, peptidic nitrogen, and carboxyl oxygen. The two side chains $(CH_3)_2CHCH_2$ and $C_6H_5CH_2$ are different, but according to the structural model of the $[MH_{-1}A]$ species the global effect of two side chains should be comparable when they are located N- or C-terminal. For these two systems, this means that the influence upon the stability of the $[MH_{-1}A]$ complex of the electrodonor and steric effects may be comparable.

(*ii*) *Hydrophobic interaction between two side chains.* The structure established for the $[MH_{-1}A]$ species according to the e.s.r. studies shows a CuN_2O_2 in-plane chromophore, which requires two side chains located on the same side, with respect to this basal plane when L,L-dipeptides are considered. Such an arrangement results in an interaction giving rise to a hydrophobic micelle, so decreasing the solvation enthalpy. This effect is obviously comparable in dipeptide systems containing the same amino acid residues, whatever the sequences. For the systems Cu^{II}–L-Phe-L-Leu and –L-Leu-L-Phe this remains valid.

The above discussions show that the great difference in formation enthalpy and in stability of $[MH_{-1}A]$ species for the two systems cannot be explained by the electrodonor and steric effects, nor by hydrophobic interactions. Moreover, the fact that the entropy change for the Cu^{II}–L-Leu-L-Phe system ($\Delta S^\circ = +28$ J K^{-1} mol^{-1}) is much lower than for Cu^{II}–L-Phe-L-Leu ($\Delta S^\circ = +42$ J K^{-1} mol^{-1}) indicates that, in the $[MH_{-1}A]$ species, the ligand is better ordered around the metal ion in the first system than in the second one. These observations lead us to consider the following factor.

(*iii*) *Interaction between the metal ion and the aromatic ring.* The presence of an aromatic ring in the C-terminal residue gives generally a more stable $[MH_{-1}A]$ species, even in dipeptides containing one non-glycine residue. This stabilising effect, reported by several authors,[7,50–52] has never been quantified. It was often attributed to an interaction between the metal ion and the aromatic ring. This interaction is found only when the aromatic ring is located in the C-terminal residue, because the Cu–O bond is weak and easily distorted. However, the rigid Cu–N bond formed when the aromatic ring is in the N-terminal residue does not favour this interaction.

Taking into account factors (*i*)—(*iii*), we can quantify the stabilising enthalpy due to the metal ion–aromatic ring interaction (ΔH_s), which amounts to the difference in the formation enthalpies of the $[MH_{-1}A]$ species in the two systems, Cu^{II}–L-Leu-L-Phe and –L-Phe-L-Leu, $\Delta H_s = -2.1 - 7.4 = -9.5$ kJ mol^{-1}. This value reported here for the first time appears comparable to the stabilising enthalpies for other non-covalent effects often encountered (*e.g.* ring stacking, about −7.2 kJ; hydrophobic effect, about −7.2 kJ mol^{-1}.[53]

$[MH_{-1}(OH)A]$ *species.* This complex results from the deprotonation of a co-ordinated water molecule in the $[MH_{-1}A]$ species [equation (6)]. The different pK_b values of this

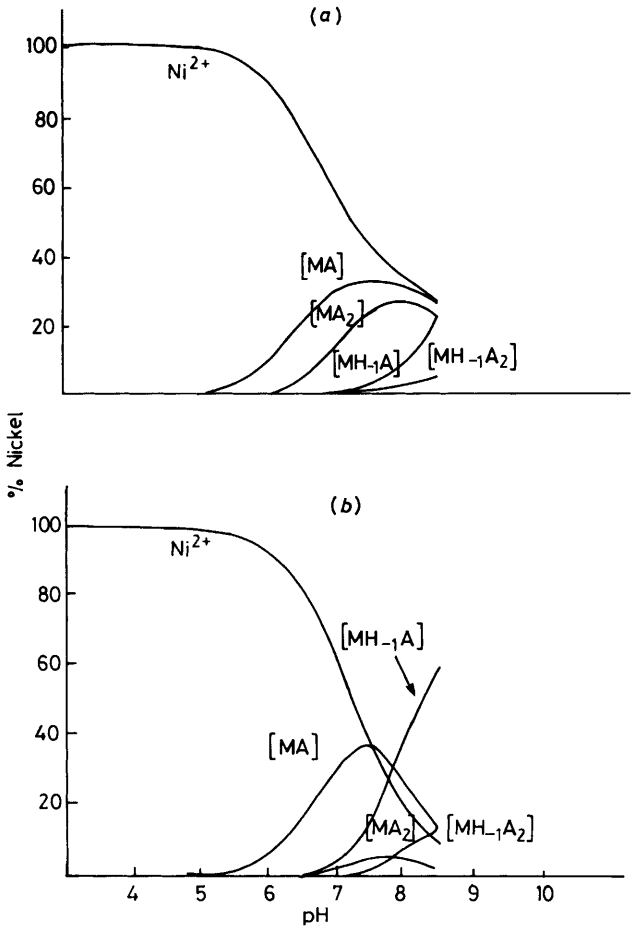


Figure 8. Species distribution for nickel(II)–L,L-dipeptide titrations calculated using SCOGS results. Conditions as in Figure 7. Dipeptides: (*a*) L-Phe-L-Leu; (*b*) L-Leu-L-Phe.

Table 7. Formation constants of nickel(II)–dipeptide complexes at 25 °C, I = 0.2 mol dm^{-3} KNO_3

Species	L,L-Phe-Leu	L,L-Phe-Met	L,L-Leu-Phe	L,L-Met-Phe
[MA]	2.69 ± 0.03	2.68 ± 0.01	2.94 ± 0.02	3.13 ± 0.01
[MA_2]	5.36 ± 0.04	5.24 ± 0.02	5.04 ± 0.13	5.18 ± 0.07
[$MH_{-1}A$]	−5.95 ± 0.03	−5.50 ± 0.01	−4.98 ± 0.01	−5.10 ± 0.02

Table 8. Thermodynamic functions for the complex formation of nickel(II) with L,L-dipeptides at 25 °C, I = 0.5 mol dm^{-3} KNO_3

Dipeptide		ΔH^*/kJ mol^{-1}	$-\Delta G^*$/kJ mol^{-1}	ΔS/J K^{-1} mol^{-1}
L-Phe-L-Leu	[MA]	−15.9 ± 0.2	15.1	−2.8
	[$MH_{-1}A$]	43.6 ± 2.6	−33.9	32.4
	[MA_2]	−28.1 ± 0.4	30.5	8.1
L-Leu-L-Phe	[MA]	−18.1 ± 0.5	16.5	−5.2
	[$MH_{-1}A$]	23.1 ± 2.3	−21.6	0.6
	[MA_2]	−37.3 ± 2.8	31.3	−24.2

$$[MH_{-1}A(H_2O)] \overset{pK_b}{\rightleftharpoons} [MH_{-1}(OH)A] + H^+ \quad (6)$$

equilibrium (Table 4) indicate very little differences for the copper(II) dipeptide systems except for Cu^{II}–L,L-Phe-Leu. This indicates identical solvent accessibility in the [$MH_{-1}A$] species.

The enthalpy found for this species (Tables 5 and 6) includes the formation of [$MH_{-1}A$], the dissociation of H_2O, and the formation of a M–OH bond. Taking into account the dissociation of H_2O, we can calculate the formation enthalpy for the M–OH bond from the relation (7). In the case of Cu^{II}–L-Phe-L-

$$\Delta H(\text{Cu–OH}) = \Delta H[CuH_{-1}(OH)A] - \Delta H(CuH_{-1}A) - \Delta H(H_2O) \quad (7)$$

Leu, we obtain ΔH(Cu–OH) = −16.4 kJ mol^{-1} and for Cu^{II}–L-Leu-L-Phe, ΔH(Cu–OH) = −15.6 kJ mol^{-1}. These values are in satisfactory agreement with that (−17.85 kJ mol^{-1}) obtained directly from the formation of the soluble complex $[Cu_2(OH)_2]^{2+}$.[54] It was shown that there is no stereoselectivity between copper(II) complexes of L,L-or L,D-dipeptide for this species.[1-4,10]

[$MH_{-1}A_2$] *species.* The log K_c values of reaction (8) are presented in Table 4 together with the formation constants for [$MH_{-1}A_2$].

$$[MH_{-1}A] + A \overset{K_c}{\rightleftharpoons} [MH_{-1}A_2] \quad (8)$$

This species can be considered as the addition of a ligand molecule A^- to the complex [$MH_{-1}A$]. Therefore the formation enthalpy of [$MH_{-1}A_2$] is also as the sum of enthalpies of the complexes [$MH_{-1}A$] and [MA] [equation (9)]. Using this

$$\Delta H(MH_{-1}A_2) = \Delta H(MH_{-1}A) + \Delta H(MA) \quad (9)$$

relation, we obtain $\Delta H(MH_{-1}A_2)$ = −20.6 kJ mol^{-1} for Cu^{II}–L-Phe-L-Leu and −29.6 kJ mol^{-1} for Cu^{II}–L-Leu-L-Phe. These values are quite different from those obtained experimentally (−23.6 kJ and −25.1 kJ mol^{-1} respectively). The discrepancies originate probably in an interaction between the side chains of the two ligand molecules.

Nickel(II)*–Dipeptide Complexes.*—Nickel(II)–dipeptide complexes are to date much less well documented than copper(II)–dipeptide ones. The results reported here for these ligands are as yet unpublished, except for the Met-Phe–Ni^{II} system.[4]

For each nickel(II)–ligand system investigated, among all possible species, four remained after the computer calculation: [MA], [MA_2], [$MH_{-1}A$], and [$MH_{-1}A_2$]. The formation constants are shown in Table 7, and Figure 8 shows the species distribution curves for the two systems.

The complexation of Ni^{II} begins much later than that of the Cu^{II}. With all four ligands, the first complexation occurs at about pH 4.5. Under the same experimental conditions, complexation of Cu^{II} starts between pH 2 and 3. Deprotonation of the peptidic nitrogen is delayed until pH 6.5 to give [$MH_{-1}A$] species. Differing from copper(II), where the major species in the physiological pH range is [$MH_{-1}A$], for Ni^{II}, the major species is [MA] in the same pH range, because of the lower ability of Ni^{II} to deprotonate the peptidic nitrogen.

For comparison with the copper(II) systems, the same analysis was performed for the nickel(II)-dipeptide systems. Table 8 displays the thermodynamic parameters for the nickel(II) complexes with L-Phe-L-Leu and L-Leu-L-Phe respectively.

[MA] *species.* The formation constants log β_{101} of this species for the four systems studied are shown in Table 7. For identical N-terminal residues, for example Phe, the formation constants are similar (Ni^{II}–L-Phe-L-Met, log β = 2.68; Ni^{II}-Phe-Leu, log β = 2.69). These results suggest that the complexation of Ni^{II} with dipeptide occurs at the N-terminal amino-acid residue, as found for Cu^{II}. The C-terminal residues of these L,L-dipeptides are not involved in the formation of [MA] species. The proposed structural model for such species in the copper(II) systems may also be valid in the case of the nickel(II) complexes. This is shown by comparing the formation constants of

Table 9. Formation constants of cobalt(II)–dipeptide complexes at 25 °C, I = 0.2 mol dm^{-3} KNO_3

Species	L,L-Phe-Leu	L,L-Phe-Met	L-L-Leu-Phe	L,L-Met-Phe
[MA]	2.37 ± 0.02	2.43 ± 0.03	2.10 ± 0.11	1.91 ± 0.10
[MA_2]	3.28 ± 0.10	3.82 ± 0.11	3.93 ± 0.19	3.57 ± 0.17

Ni^{II}–Gly-X (X = Gly, Phe, or Met)[18,55] (log β = 3.97, 4.03, and 3.96 respectively). The largest difference between them is 0.07 pK unit.

Furthermore, in the first example described in this discussion, the side-chain group of the N-terminal residue has a great influence upon the formation constant. Compared with a glycyl residue, the substitution of hydrogen by a non-co-ordinating side chain group decreases the complexation ability of dipeptides with Ni^{II} ion as encountered in the case of copper(II). Because the side chain in C-terminal residues does not modify the formation constants, we can class these residues according to their stabilising ability for the [MA] species as follows: glycyl > methionyl > leucyl > phenylalanyl. These side-chain groups have an electronic donor effect. It is expected that such a side-chain group hinders complexation.

The enthalpy of Ni–O bond formation is negligible for the oxygen atom provided by a carboxylate, a carbonyl, or H_2O group (for Ni^{2+}–O, ΔH = −0.6 kJ mol^{-1}).[56] The enthalpy of [MA] is approximatively equal to that of the Ni–NH_2 bond. We determined −15.9 kJ mol^{-1} for Ni^{II}–L-Phe-L-Leu, and −18.1 kJ mol^{-1} for Ni^{II}-L-Leu-L-Phe.

Swash and Pettit.[20,21] obtained ΔH = −13.1 kJ mol^{-1} for Ni^{II}-L-Met and ΔH = −13.2 kJ mol^{-1} for Ni^{II}-D-Met. However, the formation of the (butane-2,3-diamine)nickel(II) species is accompanied by an enthalpy ΔH = −32.5 kJ mol^{-1}. Since there are two amine groups in the ligand, the enthalpy for the Ni–NH_2 bond is evaluated to be ΔH = −16.3 kJ mol^{-1}. This value is comparable with our results.

Compared with the corresponding values for the copper(II) complexes, the exothermic effect is weaker in the nickel(II) complexes, although the enthalpy is favourable to the formation of [MA] species. The stability of the [MA] species of Cu^{II} is about 100 times higher than that of Ni^{II} (see Tables 4 and 7). This result agrees with those obtained for the corresponding complexes of (±)-butanediamine. For the copper(II) complex the Cu^{II}–NH_2 bond formation enthalpy is ΔH = −26.4 kJ mol^{-1}.[57]

[MA_2] *species.* The distribution curves (Figure 8) show that this species appears after [MA]; moreover it is a minor species. The bonding sites of both ligand molecules are the same as in [MA] (*i.e.* the amino and carbonyl groups). The log K_d values show the sequence Met-Phe (2.05) < Leu-Phe (2.54) < Phe-Met (2.56) < Phe-Leu (2.71), which contrasts with that of the log β_{101} values. In the [MA_2] species, although the side chain–side chain interactions do not exist within the same ligand molecule, they are certainly important between two different ones. This results in a small decrease in the [MA] formation constant values. Secondly, the larger is the side chain the more important are the interactions. In the four systems studied, the side chain of the Met residue is the longest, and it gives the most important steric perturbation.

The calorimetric results (Table 8) show that the formation enthalpy of [MA_2] is approximatively twice that of the [MA] species [for L-Phe-L-Leu, $2\Delta H$(MA) = −31.8 kJ mol^{-1}; for L-Leu-L-Phe, $2\Delta H$(MA) = −36.2 kJ mol^{-1}]. These values are comparable with ΔH(MA_2). This trend was also observed in copper(II)–amino acid complexes.[57–59] Thus the structure of [NiA_2] is probably similar to that of bis(amino acidato)copper(II) complexes.

[$MH_{-1}A$] *species.* The species [$MH_{-1}A$] is the major copper(II)–dipeptide complex in the range pH 5—8. This is at variance with the nickel(II)–dipeptide systems (for the latter, as the pH approaches 8.5, a precipitate appears, which prevents further potentiometric investigation).

Comparing copper(II)–dipeptide systems with the nickel(II)–dipeptide ones, we observe that the formation constants (log β_{1-11}) of [$MH_{-1}A$] species for the Ni^{II}–X-Phe systems are greater than those of the Ni^{II}-Phe-X systems (X = Leu or Met). The log β_{1-11} difference between the pair Ni^{II}-L-Leu-L-Phe and Ni^{II}-L-Phe-L-Leu amounts to almost 1 pK unit, but reduces to 0.4 pK units for the pair Ni^{II}–L-Met-L-Phe and Ni^{II}–L-Phe-L-Met. As with copper(II), when the Phe residue is C-terminal the stability of the complex is higher than that in which the Phe residue is N-terminal. These features suggest that when M = Ni^{II} the [$MH_{-1}A$] species adopts a similar structure to that when M = Cu^{II}. The discussion for the copper(II)–dipeptide species is also valid in the case of nickel(II). The metal ion–aromatic ring interaction can also be used to explain the stability increase.

Enthalpy values (Table 8) show that the formation of this species is endothermic for two nickel(II)–dipeptide systems. However, the enthalpy is much less important for the Leu-Phe system than for the Phe-Leu one. This indicates, as in the copper case, that the formation of [$MH_{-1}A$] is more difficult in the L-Phe-L-Leu system than in the L-Leu-L-Phe one. This result suggests that, in the case of nickel(II), the metal ion–aromatic ring interaction influences also the stability of the [$MH_{-1}A$] complex, as discussed above for the two copper(II)–dipeptide systems.

Cobalt(II)–Dipeptide Complexes.—Cobalt(II) is a less readily chelated compared with Ni^{II}. Complexation begins at about pH 5.2 for the Co^{II}–Phe-X systems, and at about pH 5.8 for Co^{II}–X-Phe. Abello *et al.*[23] showed that for Co^{II}–Gly-Met only two species are found. In the pH range investigated, we also find two species only for each cobalt(II)–dipeptide system (Table 9). The weak complexing ability of Co^{II} makes deprotonation of the peptidic nitrogen impossible below pH 8.

[MA] *species.* The species [MA] is the main complex in the range pH 7—8.5, as in the case of Ni^{II}. The formation constants log β_{101} of the MA species increase in the following order for the four systems studied: Met-Phe (1.91) < Leu-Phe (2.10) < Phe-Leu (2.37) < Phe-Met (2.43). We notice, as in the nickel(II)–dipeptide systems, that when the N-terminal residue contains the same side-chain group (Phe-X) the formation constants are similar. Between the Co^{II}–L-Phe-L-Leu and Co^{II}–L-Phe-L-Met systems, the difference in log β_{101} is only 0.06 pK unit. The difference between the log β_{101} values for the Co^{II}–L-Met-L-Phe and Co^{II}–L-Leu-L-Phe systems is 0.19 pK unit. This observation allows us to suggest that for Co^{II} the species [MA] has probably the same structure as when M = Cu^{II} or Ni^{II}. The binding sites should be found in the N-terminal residue, between the amino nitrogen and the carbonyl oxygen.

[MA_2] *species.* The formation constants calculated (log K_d) from the equilibria (10) are as follows: Phe-Leu (0.91), Leu-Phe

$$[MA] + A \rightleftharpoons[K_d] [MA_2] \qquad (10)$$

(1.83), Phe-Met (1.39), and Met-Phe (1.66). An important decrease is observed compared with the log β_{101} values. The same explanation as in the case of Ni^{II} seems to be valid: the side chain group interaction between two ligand molecules decreases the stability of the complex by a steric effect.

References

1 R. Nakon and R. J. Angelici, *J. Am. Chem. Soc.*, 1974, **96**, 4178.
2 A. Kaneda and A. E. Martell, *J. Am. Chem. Soc.*, 1977, **99**, 1586.

3 L. D. Pettit and R. J. W. Hefford, 'Metal Ions in Biological Systems,' ed. H. Sigel, 1979, vol. 9, p. 173.
4 R. J. W. Hefford and L. D. Pettit, *J. Chem. Soc., Dalton, Trans.*, 1975, 2302.
5 R. P. Bonomo, R. Coli, V. G. I. Cucinotti, and E. Rizzarelli, *Inorg. Chem.*, 1986, **24**, 1641.
6 P. I. Vestues and R. B. Martin, *J. Am. Chem. Soc.*, 1980, **102**, 7906.
7 O. Yamauchi, K. Tsujide, and A. Odani, *J. Am. Chem. Soc.*, 1985, **107**, 659.
8 L. Sportelli, H. Neubacher, and W. Z. Lohman, *Z. Naturforsch., Teil C*, 1977, **32**, 643.
9 L. Abello, A. Ensuque, R. Touiti, and G. Lapluye, *J. Chim. Phys.*, 1979, **76**, 602.
10 H. Sigel and R. B. Martin, *Chem. Rev.*, 1982, **82**, 385.
11 B. R. Rabin, *Trans. Faraday. Soc.*, 1956, **52**, 1130; S. P. Datta and B. R. Rabin, *ibid.*, p. 1123.
12 R. F. Pasternack, L. Gipp, and H. Sigel, *J. Am. Chem. Soc.*, 1972, **94**, 8031.
13 H. Sigel, *Inorg. Chem.*, 1975, **14**, 1535.
14 C. A. Bear and H. C. Freeman, *Acta Crystallogr., Sect. B*, 1976, **32**, 2534.
15 H. C. Freeman, J. M. Guss, and R. C. Sinclair, *Chem. Commun.*, 1968, 485.
16 H. C. Freeman and J. M. Guss, *Acta., Crystallogr., Sect. B*, 1978, **34**, 2451.
17 R. B. Martin, M. Chamberlin, and J. T. Edsall, *J. Am. Chem. Soc.*, 1960, **82**, 485.
18 M. Jouini, Thesis of Dr. Sci., Université Paris VII, Paris, 1985.
19 R. J. W. Hefford and L. D. Pettit, *J. Chem. Soc., Dalton Trans.*, 1981, 1331.
20 J. L. M. Swash and L. D. Pettit, *Inorg. Chim. Acta*, 1976, **19**, 19.
21 L. D. Pettit and J. L. M. Swash, *J. Chem. Soc., Dalton Trans.*, 1977, 697.
22 L. Abello, A. Ensuque, and G. Lapluye, *J. Chim. Phys.*, 1976, **73**, 268.
23 L. Abello, A. Ensuque, R. Touiti, and G. Lapluye, *J. Chim. Phys.*, 1981, **78**, 615; L. Abello, A. Ensuque, M. Jouini and G. Lapluye, *ibid.*, 1980, **77**, 537.
24 L. Abello, R. Poisson, M. Jouini, and G. Lapluye, *J. Chim. Phys.*, 1985, **82**, 993.
25 G. Arena, E. Rizzarelli, S. Sammartano, and C. Rigano, *Talanta*, 1979, **26**, 1.
26 I. G. Sayce, *Talanta*, 1968, **15**, 1397; 1971, **18**, 653; 1972, **19**, 831.
27 P. Gans, A. Vacca, and A. Sabatini, *Inorg. Chim. Acta*, 1976, **18**, 237.
28 L. Abello, M. Jouini, M. K. Oulaalou, R. Poisson, and G. Lapluye, *J. chim. Phys.*, 1985, **82**, 1001.
29 O. Enea, G. Berthod, M. Croner-Morin, and J. P. Scharff, *Thermochim. Acta*, 1980, **36**, 329.
30 J. J. Christensen, L. D. Hansen, and R. M. Izatt, in 'Handbook of Proton Ionization Heats and Related Thermodynamic Quantities,' Wiley, 1976, p. 209 and refs. therein.
31 A. Gergely and S. Nagypal, *J. Chem. Soc., Dalton Trans.*, 1977, 1104.
32 A. P. Brunetti, E. J. Burke, M. C. Lim, and G. M. Nancollas, *J. Solution Chem.*, 1972, **1**, 153.
33 G. Impellizzeri, R. P. Bonomo, and R. Cali, *Thermochim. Acta*, 1984, **80**, 275.
34 H. Sigel, *J. Chem. Soc., Dalton Trans.*, 1980, 1022.
35 J. Huet, M. Jouini, L. Abello, and G. Lapluye, *J. Chim. Phys.*, 1984, **81**, 505.
36 K. E. Falk, H. C. Freeman, T. Jansson, B. G. Malmstrom, and T. Vannagard, *J. Am. Chem. Soc.*, 1971, **89**, 6071.
37 B. J. Hathaway and D. E. Billing, *Coord. Chem. Rev.*, 1970, **1**, 5.
38 J. Peisach and W. E. Blumberg, *Arch. Biochem. Biophys.*, 1974, **165**, 691.
39 D. M. Dooley, J. Rawlings, J. H. Dawson, P. J. Stephens, L. E. Andresson, B. G. Malmstrom, and H. B. Grey, *J. Am. Chem. Soc.*, 1979, **101**, 5038.
40 R. Wilson and J. Kivelson, *J. Chem. Phys.*, 1966, **44**, 154.
41 A. Rossi, M. Ptak, and P. Grenouillet, *J. Chim. Phys.*, 1974, **71**, 1371.
42 G. F. Bryce, *J. Phys. Chem.*, 1966, **70**, 3549.
43 R. P. Agarwall and D. D. Perrin, *J. Chem. Soc., Dalton Trans.*, 1976, 89.
44 A. McAuley, G. H. Nancollas, and K. Torrance, *J. Inorg. Nucl. Chem.*, 1966, **28**, 917.
45 T. Kiss and Z. Szücs, *J. Chem. Soc., Dalton Trans.*, 1986, 2443.
46 H. C. Freeman, *Adv. Protein Chem.*, 1967, **22**, 336.
47 A. Gergely and E. Farkas, *J. Chem. Soc., Dalton Trans.*, 1982, 381.
48 A. G. Lappin, in 'Metal ions in Biological Systems,' Marcel Dekker, New York, Basel, 1981, vol. 13, p. 15 and refs. therein.
49 A. P. Brunetti, M. C. Lim, and G. H. Nancollas, *J. Am. Chem. Soc.*, 1968, **90**, 5120.
50 W. L. Kwik, K. P. Ang, and G. I. Chen, *J. Inorg. Nucl. Chem.*, 1980, **42**, 303.
51 P. I. Vestues and R. B. Martin, *J. Am. Chem. Soc.*, 1980, **102**, 7906.
52 S. H. Kim and R. B. Martin, *J. Am. Chem. Soc.*, 1984, **106**, 1707.
53 E. Frieden, *J. Chem. Educ.*, 1975, **52**, 754.
54 G. Arena, R. Cali, E. Rizzarelli, and S. Samartano, *Thermochim. Acta*, 1976, **16**, 315.
55 G. Brookes and L. D. Pettit, *J. Chem., Soc. Dalton Trans.*, 1975, 2106.
56 S. J. Ashchroft and C. T. Mortimer, in 'Thermochemistry of Transition Metal Complexes,' Academic Press, London, New York, 1970.
57 K. P. Anderson, W. O. Greenhalgh, and R. M. Izatt, *Inorg. Chem.*, 1966, **5**, 2106.
58 R. M. Izatt, J. J. Christensen, and V. Kothari, *Inorg. Chem.*, 1964, **3**, 1565.
59 A. Gergely and I. Sovago, *J. Inorg. Nucl. Chem.*, 1973, **35**, 4355.

Received 16*th January* 1989; *Paper* 9/00267G