Electron Spin Resonance Determination of Formation Constants of Copper(II) Dipeptide Complexes †

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Formation constants for complexes between copper(II) and a series of aliphatic dipeptides have been determined by e.s.r. spectroscopy. An iteration method for the evaluation of digitized e.s.r. spectra is introduced. The e.s.r. spectra and formation constants of all copper species obtained by this method are compared with the results of potentiometric titrations; thus the ambiguous formation of the complexes $[CuL_2H_2]$ and $[CuL_2]^{2-}$ (H_2L = ^+H_3N-CHR-CO-NH-CHR′-COO^-; R,R′ = alkyl) could be excluded. The existence of the dimer species $[Cu_2L_2(OH)]^-$ was confirmed. The influence of the side chains on the spectra of all complexes is discussed.

During the last fifteen years the system copper–glycylglycine has been investigated extensively by means of e.s.r. spectroscopy.[1-10] Most studies were carried out at low temperatures using frozen solutions, powder, or single crystals.[2-7] Gould and Mason [1] attempted to interpret the e.s.r. spectra using potentiometric results. They found only three distinct e.s.r. spectra for the species Cu^{2+}, [CuL], and $[CuL(OH)]^-$ (H_2L = ^+H_3N-CHR-CO-NH-CHR′-COO^-; R,R′ = alkyl), varying pH and the metal–ligand ratio, whereas at least five species are clearly shown by potentiometric titrations: Cu^{2+}, $[CuLH]^+$, [CuL], $[CuL(OH)]^-$, and $[CuL_2H]^-$.[11-21] These five species should also be detectable by e.s.r. spectroscopy. Besides the mentioned complexes, further species $[CuL_2H_2]$, $[CuL_2]^{2-}$, and $[Cu_2L_2(OH)]^-$ have been proposed by some authors.[12,13,17-21] A very careful analysis of solutions containing copper and aliphatic dipeptides has been performed, therefore, in order to find consistent results for e.s.r. spectroscopy as well as potentiometric titrations.

Experimental

Materials.—The compound $CuCl_2{\cdot}2H_2O$ was dried at 130 °C to constant weight. The concentration of copper stock solution was examined by complexometric titration. The dipeptides glycylglycine (Gly-Gly), DL-alanylglycine (Ala-Gly),‡ glycyl-DL-alanine (Gly-Ala),‡ DL-leucylglycine (Leu-Gly),‡ glycyl-DL-leucine (Gly-Leu),‡ L-prolylglycine (Pro-Gly), glycyl-L-proline (Gly-Pro), DL-alanyl-DL-alanine (Ala-Ala),‡ DL-alanyl-DL-leucine (Ala-Leu),‡ L-alanyl-L-proline (Ala-Pro), L-prolyl-L-alanine (Pro-Ala), DL-leucyl-DL-leucine (Leu-Leu),‡ and L-prolyl-L-leucine (Pro-Leu) were obtained from Sigma Chemical Co., generally of Sigma analytical grade.

Physical Measurements.—Solutions for e.s.r. spectroscopy were prepared by potentiometric titrations. During titration a 0.2 cm^3 sample was taken for each e.s.r. spectrum. Titrations were carried out at various metal–ligand ratios from 1 : 1 to 1 : 4. The concentration of copper chloride was 1.00×10^{-2} mol dm^{-3} in all investigations. The pH values of all e.s.r. solutions were measured using a combined glass electrode and a Schott pH meter CG 803. All investigations were carried out at 20 °C and ionic strength 0.20 mol dm^{-3} (KCl).

Small n.m.r. standard tubes were taken for e.s.r. measurements. The n.m.r. standard tubes filled with 0.020 mol dm^{-3} KCl solution display almost no spectrum within the recorded magnetic field (2 830—3 228 G). A small slope is observed from 3 150 to 3 228 G. This part of the spectrum is subtracted, however, as described in the *Method* section leading to straight baselines in all cases. The tube volume was calibrated using copper stock solution as standard. During each series of e.s.r. measurements (recorded with a Varian E-104A spectrometer), three control spectra of copper chloride were obtained to guarantee equal conditions for the whole set of spectra. At the beginning of this work a series of e.s.r. spectra were recorded several times interchanging all tubes and the results were consistent indicating a reproducibility of $\pm 1\%$. The linewidth and the additivity of the digital points of the spectra were examined by the following experiment. A series of e.s.r. spectra were recorded for the system copper–glycylglycine (1 : 1) at pH 6.5. The concentration was changed from 0.01 to 0.001 mol dm^{-3}. The linewidth remained constant within $\pm 1\%$ and the peak heights showed a linear decrease with decreasing copper concentration indicating an accuracy of $\pm 1\%$. For every copper–dipeptide system 30 to 40 spectra were digitized, 200 digital points per spectrum. A FORTRAN program was developed to evaluate the recorded spectra. The main parts of this program are the evaluation of the equations (1)—(6) as given in the following section and the iterative variation of the constants followed by the Newton–Raphson procedure for every new set of constants. All computations were carried out on the CYBER 74 computer of the University of Innsbruck.

Method.—Complex formation of dipeptides with copper is dependent on the pH of the solutions. Variations of the pH value or the metal–dipeptide ratio lead to significant changes in the e.s.r. signals indicating a varying species distribution.[1] If the linewidth of the signals remains constant, the height of the signals is directly proportional to the concentration of the species and the detected e.s.r. spectrum shows the sum of the spectra of all existing copper species.[6,22-25] Such conditions are achieved if the total copper concentration is kept constant for all solutions.[26] In this case the following method is applicable.

Initially the recorded spectra are stored in digitized form (S'_{ij}) and corrected (S_{ij}) according to the tube spectrum (A_{ij}) and the tube capacity (B_j), where i indicates the digital points and j is the sequence number of the spectrum. In order to achieve equivalent conditions all the spectra are normalized to constant metal concentration (M_{con}); M_j is the total metal concentration present in the case of the jth spectrum. See equation (1).

$$S_{ij} = (S'_{ij} - A_{ij})M_{con}/(B_jM_j) \qquad (1)$$

† *Non-S.I. unit employed:* G = 10^{-4} T.
‡ These dipeptides were used in the DL-form. The calculated formation constants are therefore mean values for all present stereoisomers.

The corrected form S_{ij} can be calculated if the spectra of the pure species (R_{ik}, k indicates the different species) and the relative amounts (X_{jk}) of these species are known [equation (2)].

$$S_{ij} = \sum_k (R_{ik} X_{jk}) \tag{2}$$

Taking into account the stability constants (K_k), the total metal (M_j) and total ligand (L_j) concentrations, and the pH value, it is possible to evaluate X_{jk} using the Newton–Raphson method,[20] if the protonation constants of the ligand are known.

If only one complex is formed, the recorded e.s.r. spectra are the sum of the spectra of the free metal ion and the complex. An initial formation constant (K_r^1) has to be assumed in order to lead to X_{jk}^1. The spectrum of the complex (R_{ir}^1) is then evaluated by subtraction of the free metal ion spectrum from any spectrum (S_{im}), where m denotes the spectrum used and r the complex species considered, in which the complex concentration should be high [equation (3)].

$$R_{ir} = [S_{im} - \sum_{k \neq r} (R_{ik} X_{mk})]/X_{mr} \tag{3}$$

All the variables of equation (2) are now known and all further measured spectra (S_{ij} $j \neq m$) are computed. The sum of the differences of calculated and measured spectra is stored in F.

$$f_j^1 = \sum_i \left[\sum_k (R_{ik}^1 X_{jk}^1) - S_{ij} \right]^2 \qquad F^1 = \sum_{j \neq m} f_j^1 \tag{4}$$

The formation constant K_r is varied logarithmically by an increment D according to equation (5) leading to X_{jk}^{2-5},

$$pK_r^{2,3} = pK_r^1 \pm D \qquad pK_r^{4,5} = pK_r^1 \pm 2D \tag{5}$$

R_{ir}^{2-5}, and F^{2-5}. The pK value yielding the smallest F is used as a new starting value and the variation of the constant is continued with smaller increments. It is not necessary to have a good approximation for the initial constant, if the first increment (D) is large enough.

Equation (3) can be extended, if two or more complexes are formed simultaneously. Two recorded spectra (S_{im} and S_{in}) are needed, for example, to calculate the spectra of two complexes (R_{ir} and R_{is}) [equation (6); n and m denote the spectra used and r and s the complex species considered]. In general,

$$\begin{aligned} X_{mr}R_{ir} + X_{ms}R_{is} &= S_{im} - \sum_{k \neq r.s} (R_{ik} X_{mk}) \\ X_{nr}R_{ir} + X_{ns}R_{is} &= S_{in} - \sum_{k \neq r.s} (R_{ik} X_{nk}) \end{aligned} \tag{6}$$

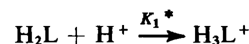
for ν simultaneously formed complexes, ν equations have to be used. In the case of the system copper–aliphatic dipeptides it is possible to find pH ranges and metal–ligand ratios, where only one unknown complex is formed to a considerable extent. Thus equation (3) is sufficient (ν = 1).

After this procedure the first set of spectra of pure species and formation constants (νpK values and νR_{ik}) are known and stored. Now the next set of species can be considered and their pK and R_{ik} values are obtained as above.

If one of the assumed complexes does not exist, its formation constant decreases continuously. The standard deviation reaches the experimental error only if all the existing species have been considered. The maximum concentration of the complexes has to exceed 10% of the total metal concentration, otherwise these species cannot be detected. Although the accuracy is lower than that using potentiometric titrations, this method provides some advantage. If two complexes show a similar species distribution and pH dependency, for example $[CuL_2H_2] \leftrightarrow [CuL]$ or $[CuL_2]^{2-} \leftrightarrow [CuL(OH)]^-$, these species cannot be distinguished by means of potentiometric titrations,[18,21,23] whereas the e.s.r. method allows their distinction. Only if two different species exhibit almost the same spectrum and their species distribution is equivalent at the same time, the complexes cannot be distinguished even using this method.

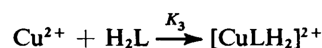
Results and Discussion

Model.—The following equilibria have been considered for the simulation of the experimental e.s.r. spectra (H_2L denotes the zwitterionic dipeptide ^+H_3N-CHR-CO-NH-CHR′-COO^-).

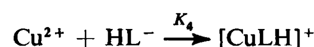


$$H_2L + H^+ \xrightarrow{K_1^*} H_3L^+$$

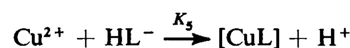
$$H_2L \xrightarrow{K_2^*} HL^- + H^+$$



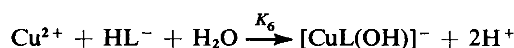
$$Cu^{2+} + H_2L \xrightarrow{K_3} [CuLH_2]^{2+}$$



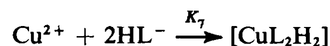
$$Cu^{2+} + HL^- \xrightarrow{K_4} [CuLH]^+$$



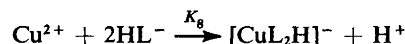
$$Cu^{2+} + HL^- \xrightarrow{K_5} [CuL] + H^+$$



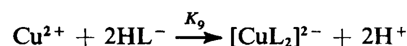
$$Cu^{2+} + HL^- + H_2O \xrightarrow{K_6} [CuL(OH)]^- + 2H^+$$



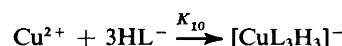
$$Cu^{2+} + 2HL^- \xrightarrow{K_7} [CuL_2H_2]$$



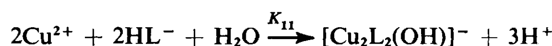
$$Cu^{2+} + 2HL^- \xrightarrow{K_8} [CuL_2H]^- + H^+$$



$$Cu^{2+} + 2HL^- \xrightarrow{K_9} [CuL_2]^{2-} + 2H^+$$



$$Cu^{2+} + 3HL^- \xrightarrow{K_{10}} [CuL_3H_3]^-$$



$$2Cu^{2+} + 2HL^- + H_2O \xrightarrow{K_{11}} [Cu_2L_2(OH)]^- + 3H^+$$

At first, only the 1 : 1 metal–ligand ratio was considered. The species $[CuLH]^+$, [CuL], $[CuL(OH)]^-$, and $[Cu_2L_2(OH)]^-$ could be detected, whereas $[CuLH_2]^{2+}$ was not found in any case. It was impossible to reach a good simulation neglecting or replacing any of these four species. As the e.s.r. spectrum of the dimer complex $[Cu_2L_2(OH)]^-$ is not detectable in aqueous solutions at room temperature (although this species is being formed),[6,24] all digital points for this species were set to zero. After this measure is taken, good simulation can be obtained. Figure 1(*b*) shows the apparent decrease of the total copper concentration, which is caused by the formation of $[Cu_2L_2(OH)]^-$.

At increasing ligand concentrations only poor simulation is obtained using the same set of species [Figure 1(*c*)]. Neither $[CuL_2H_2]$ nor $[CuL_2]^{2-}$ or $[CuL_3H_3]^-$ reduce the error significantly. Only the addition of the complex $[CuL_2H]^-$ yields a satisfactory simulation of all recorded e.s.r. spectra. The species $[CuL_2]^{2-}$ and $[CuL_3H_3]^-$ could not be detected in any case [Figure 1(*c*) and (*d*)]. The dipeptides without a peptide proton like glycylproline and alanylproline only form the species $[CuLH]^+$ and $[CuL_2H_2]$;[19,20] all recorded spectra could be constructed using these two complexes.

While potentiometric titrations could not prove whether

* The protonation and dissociation constants of the dipeptides were taken from our previous work.[20]

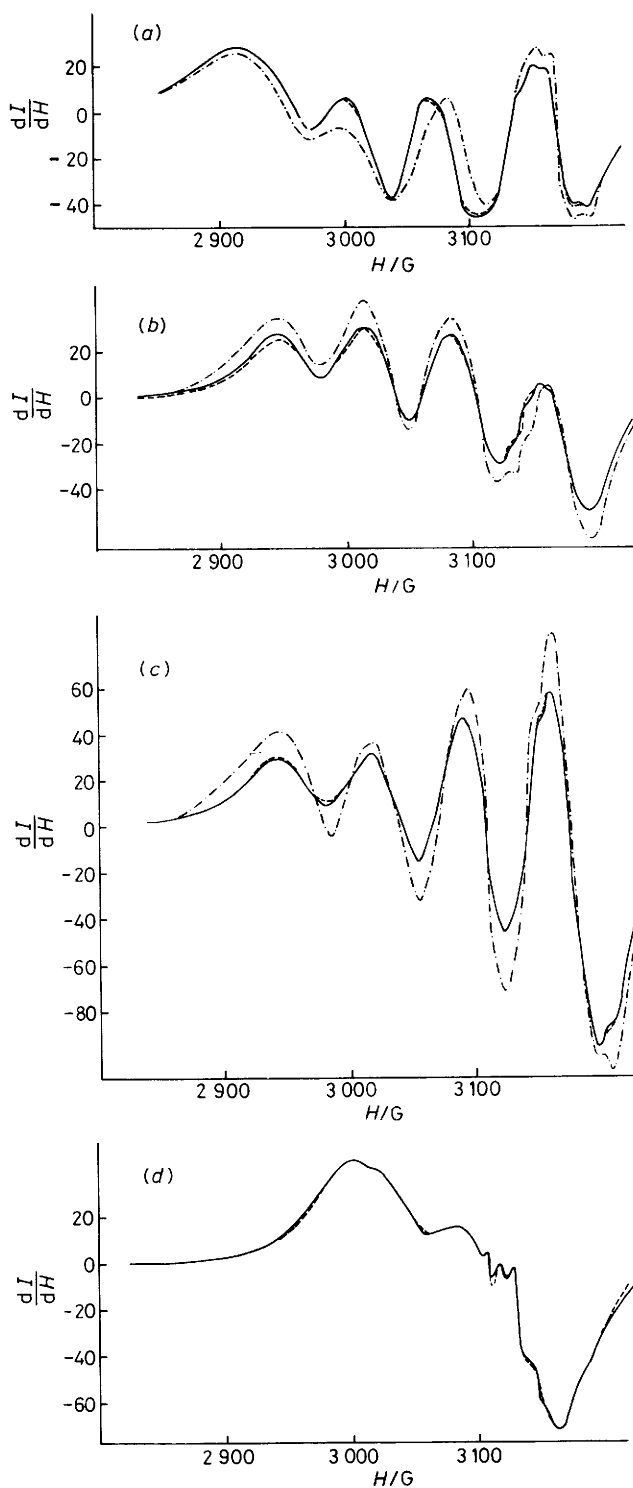


Figure 1. Test of the Method by comparison of calculated and recorded e.s.r. spectra. (*a*) [Cu^{2+}] = 9.8 × 10^{-3}, [Gly-Gly] = 9.9 × 10^{-3} mol dm^{-3}, pH 4.63; (*b*) [Cu^{2+}] = 9.54 × 10^{-3}, [Ala-Gly] = 9.73 × 10^{-3} mol dm^{-3}, pH 9.26; (*c*) [Cu^{2+}] = 9.49 × 10^{-3}, [Ala-Leu] = 2.99 × 10^{-2} mol dm^{-3}, pH 7.42; (*d*) [Cu^{2+}] = 9.0 × 10^{-3}, [Ala-Gly] = 3.53 × 10^{-2} mol dm^{-3}, pH 10.97. Spectra shown are recorded (——); calculated (- - - -) with the species (*a*) Cu^{2+}, [CuL], [CuLH]$^+$; (*b*) [CuL], [CuL(OH)]$^-$, [Cu_2L_2(OH)]$^-$; and (*c*),(*d*) Cu^{2+}, [CuLH]$^+$, [CuL], [CuL(OH)]$^-$, [Cu_2L_2(OH)]$^-$, [CuL_2H]$^-$; and calculated (— · — · —) using the species (*a*) Cu^{2+}, [CuL]; (*b*) CuL, [CuL(OH)]$^-$; and (*c*) Cu^{2+}, [CuLH]$^+$, [CuL], [CuL(OH)]$^-$, [Cu_2L_2(OH)]$^-$. The height of e.s.r. signals is represented by d*I*/d*H* in arbitrary units

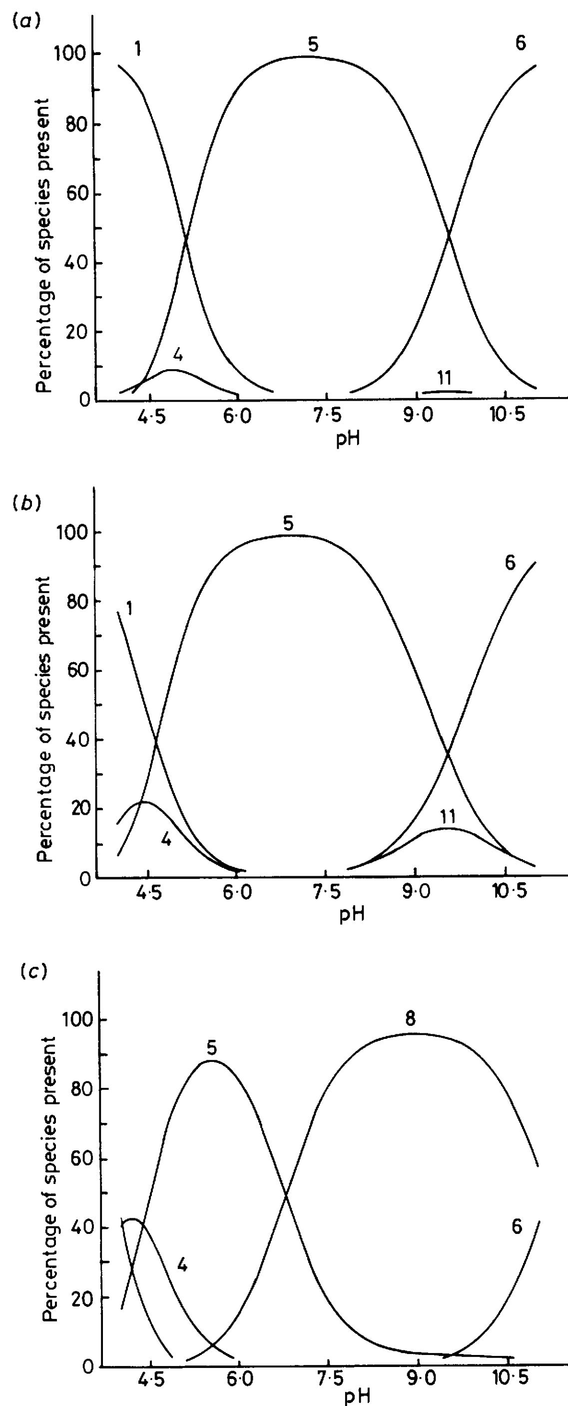


Figure 2. Species distribution depending on copper–ligand ratio and pH. (*a*) [Cu^{2+}] = 1.0 × 10^{-3} and [Gly-Ala] = 1.0 × 10^{-3} mol dm^{-3}, (*b*) [Cu^{2+}] = 1.0 × 10^{-2} and [Gly-Ala] = 1.0 × 10^{-2} mol dm^{-3}, (*c*) [Cu^{2+}] = 1.0 × 10^{-2} and [Gly-Ala] = 4.0 × 10^{-2} mol dm^{-3}. 1 = Cu^{2+}, 4 = [CuLH]$^+$, 5 = [CuL], 6 = [CuL(OH)]$^-$, 8 = [CuL_2H]$^-$, and 11 = [Cu_2L_2(OH)]$^-$. The species distribution was plotted taking into account the evaluated formation constants

[CuL_2H_2] and [CuL_2]$^{2-}$ exist or not, the e.s.r. method can exclude both species for dipeptides containing a peptide proton within the studied pH range (pH 4—11). The existence of the dimer species [Cu_2L_2(OH)]$^-$ in solution was rather ambiguous on the basis of potentiometric titrations [20,21] only, whereas e.s.r. spectroscopy could detect this complex. This is due to the different metal concentration (Figure 2),

Table 1. Complex formation constants given as log K of copper with aliphatic dipeptides (H_2L) and $\sqrt{F}$ values (in dI/dH per point of spectra)

Dipeptide	$[CuLH]^+$	[CuL]	$[CuL(OH)]^-$	$[CuL_2H_2]$	$[CuL_2H]^-$	$[Cu_2L_2(OH)]^-$	$\sqrt{F}$
Gly-Gly	5.62 [a]	1.45	−8.09		4.56	−4.63	±1.2
	5.67 [b]	1.44	−7.75		4.77		
	5.67 [c]	1.46	−7.78		4.30	−4.17	±1.4
Gly-Ala	5.90	1.53	−8.00		4.76	−4.42	
	5.83 [b]	1.59	−7.92		4.77		
	5.76 [d]	1.55	−7.94		4.63	−4.18	
	5.74 [e]	1.69	−7.72	11.16	3.76	−3.75	
Gly-Leu	6.03	0.99	−8.53		4.39	−5.27	±1.4
	5.94 [b]	1.10	−8.30		4.65		
Gly-Pro	6.70			11.72			
	6.49 [b]			11.62			
	6.44 [e]			11.53			
Ala-Gly	5.28	1.22	−8.41		3.83	−5.18	±1.6
	5.10 [b]	1.31	−8.37		3.92		
	5.25 [d]	1.35	−8.16		3.95	−4.66	
Ala-Ala	5.72	1.24	−8.26		3.97	−5.14	±1.7
	5.57 [b]	1.74	−7.55		4.83		
	5.33 [d]	1.43	−8.01		4.13	−4.39	
Ala-Leu	5.44	0.94	−8.59		3.49	−5.27	±1.1
	5.47 [b]	1.14	−8.26		4.18		
Ala-Pro	6.09			10.42			±1.1
	5.86 [b]			10.36			
Leu-Gly	5.11	1.28	−8.50		3.59	−5.24	±1.5
	5.19 [b]	1.32	−8.29		3.92		
Leu-Leu		0.87	−8.79		3.22	−5.69	±2.0
	4.99 [b]	0.80	−8.67		4.24		
Pro-Gly	6.08	2.43	−7.05		5.02	−3.76	±1.4
	5.97 [b]	2.67	−6.70		5.95		
	6.40 [f]	2.45	−6.89		6.41	−2.56	
Pro-Ala	6.09	2.67	−6.99		5.31	−3.97	±1.2
	5.83 [b]	2.94	−6.79		6.31		
Pro-Leu	5.83	2.27	−7.24		5.09		
	5.47 [b]	2.22	−7.63		5.70		±1.9

A change of pK_k by ΔpK_k leads to a doubled F (see text): $\Delta pK_4 = \pm 0.3$; $\Delta pK_5 = \pm 0.2$; $\Delta pK_6 = \pm 0.1$; $\Delta pK_8 = \pm 0.3$; $\Delta pK_{11} = \pm 0.3$; $\Delta pK_7 = \pm 0.2$. Footnotes *b*—*f* refer to potentiometric titrations.

[a] This work, 0.20 mol dm^{-3} KCl, 20 °C. [b] Ref. 20, 0.20 mol dm^{-3} KCl, 20 °C; all titration data were evaluated again, neglecting the species $[CuL_2H_2]$ and $[CuL_2]^{2-}$. [c] Ref. 13, 0.10 mol dm^{-3} KNO_3, 25 °C. [d] Ref. 18, 0.20 mol dm^{-3} KCl, 25 °C. [e] Ref. 21, 0.10 mol dm^{-3} KNO_3, 25 °C. [f] Ref. 19, 0.16 mol dm^{-3} KNO_3, 25 °C.

Table 2. g Values and coupling constants [a] of all detected complexes [b]

	$[CuLH]^+$		[CuL]		$[CuL(OH)]^-$		$[CuL_2H]^-$	
Dipeptide	g	A_{Cu}	g	A_{Cu}	g	A_{Cu}	g	A_{Cu}
Gly-Gly	2.162 [c]	52	2.121	69	2.121	41	2.118	57
Gly-Ala	2.164	52	2.121	69	2.118	44	2.115	61
Gly-Leu	2.162	52	2.119	73	2.115	47	2.114	64
Ala-Gly	2.161	56	2.121	70	2.118	42	2.115	59
Ala-Ala	2.162	56	2.118	71	2.117	46	2.114	62
Ala-Leu	2.161	56	2.118	73	2.113	49	2.113	65
Leu-Gly	2.159	55	2.119	69	2.118	42	2.113	62
Leu-Leu			2.119	73	2.113	49	2.110	65
Pro-Gly	2.159	57	2.118	71	2.117	45	2.117	60
Pro-Ala	2.158	62	2.118	74	2.115	50	2.113	66
Pro-Leu	2.160	60	2.117	74	2.113	52	2.112	69
	$[CuLH]^+$		$[CuL_2H_2]$					
Gly-Pro	2.163	52	2.138	55				
Ala-Pro	2.161	56	2.137	55				

[a] The coupling constant A_N = 12—12.5 G for all species, where splitting is detectable (see text); A_{Cu} is the coupling constant for the interaction with the copper nucleus ($I_{Cu} = \frac{3}{2}$) in G. [b] The g, A_{Cu}, and A_N values were estimated graphically from the calculated spectra. [c] ±0.001; the magnetic field was calibrated using $PPh_2CH_2CH_2PPh_2$ as standard.

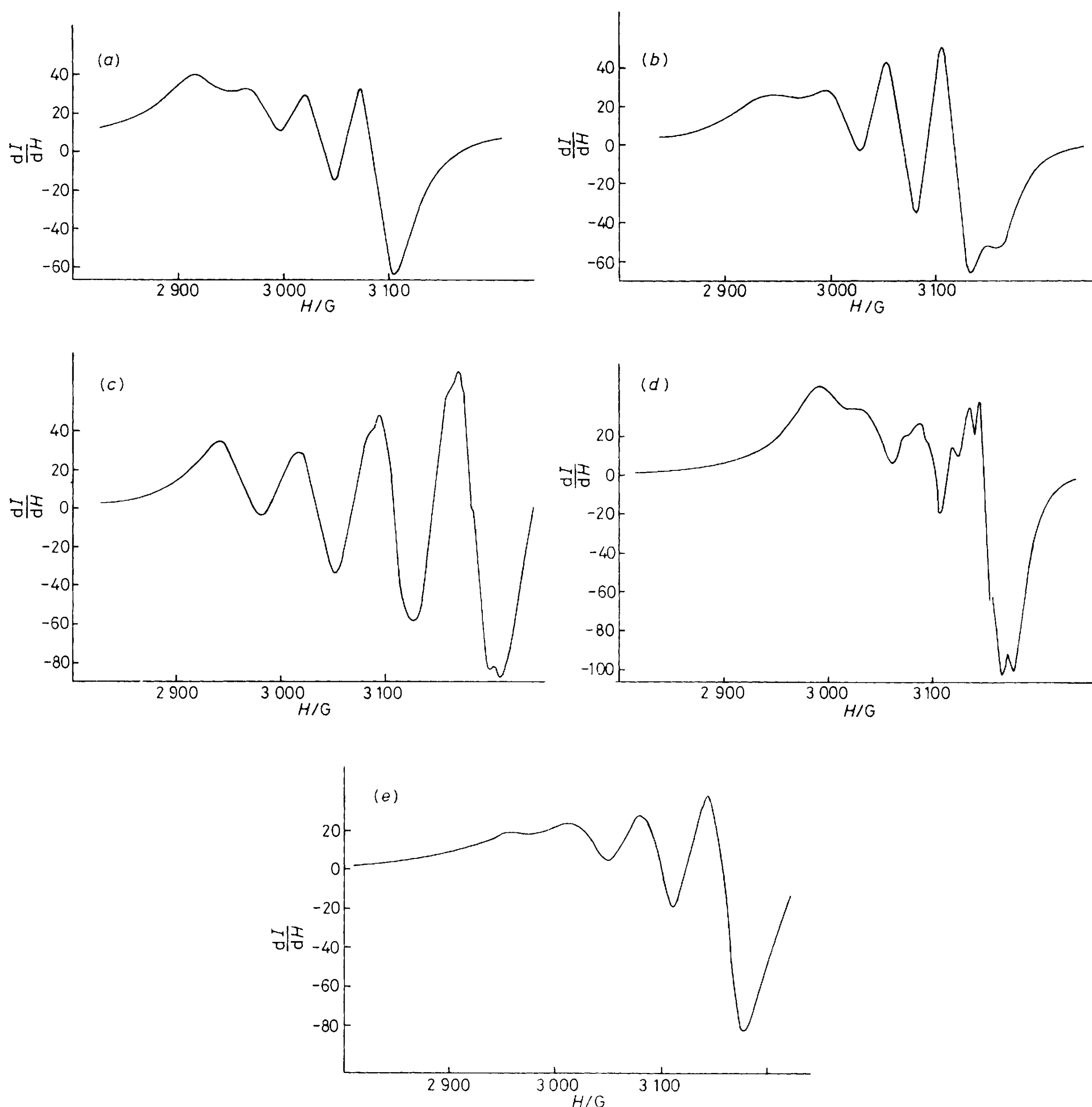


Figure 3. The calculated e.s.r. spectra of the copper–dipeptide complexes (*a*) $[CuLH]^+$ (H_2L = Gly-Pro), (*b*) $[CuL_2H_2]$ (H_2L = Gly-Pro), (*c*) [CuL] (H_2L = Ala-Leu), (*d*) $[CuL(OH)]^-$ (H_2L = Ala-Leu), and (*e*) $[CuL_2H]^-$ (H_2L = Ala-Leu)

because at low copper concentrations, as used for the titrations, the dimer species is not formed to a detectable extent.

The calculated formation constants are listed in Table 1 and are compared with the constants obtained by means of potentiometric titrations. Small differences can be attributed to the different experimental conditions and to the lower accuracy of the e.s.r. method.

E.S.R. Spectra of the Complexes (*see Table* 2).—$[CuLH]^+$. The e.s.r. spectrum shows four signals caused by the nuclear spin of copper ($I_{Cu} = \frac{3}{2}$). The shape of the spectrum is rather asymmetrical, the heights of the peaks increasing with increasing field strength. The use of various dipeptides does not lead to any significant changes in the form of the spectrum. The coupling constants are only dependent on the *N*-terminal side chain, indicating co-ordination of the amino-group and the carbonyl oxygen according to the series Gly-X $<$ Ala-X $\approx$ Leu-X $<$ Pro-X and X-Gly $\approx$ X-Ala $\approx$ X-Leu $\approx$ X-Pro.

$[CuL(OH)]^-$. An additional hyperfine coupling with the high-field lines can be observed besides the copper induced one. This type of splitting should be caused by two nitrogen atoms simultaneously,[1] since the assumption, that only one nitrogen atom is interacting with the copper ion, leads to a very unreasonable coupling constant A_{Cu}. The A_{Cu} values increase with the size of the side chains. The *C*-terminal amino-

acid induces a stronger influence than the *N*-terminal one. A comparison of the dipeptides Ala-Gly ⟷ Gly-Ala or Leu-Gly ⟷ Gly-Leu confirms this finding. The resolution of the nitrogen hyperfine coupling is improved if one or both side chains are enlarged. Thus, the dipeptides Pro-Leu, Leu-Leu, and Leu-Gly show the best resolution.

[CuL]. This species also displays a second hyperfine coupling at the highest field line and the resolution of this splitting is dependent on the side chains. Three sets with increasing resolution can be established: Gly-Gly $\approx$ Gly-Ala $\approx$ Ala-Gly $<$ Ala-Ala $\approx$ Gly-Leu $\approx$ Pro-Gly $\approx$ Ala-Leu $<$ Leu-Gly $\approx$ Pro-Ala $\approx$ Pro-Leu $\approx$ Leu-Leu. Changes in g and A_{Cu} values are rather small, especially if the influence of the *N*-terminal side chain is almost insignificant. The high resolution of Leu-Gly demonstrates, however, the interaction of the *N*-terminal amino-acid with the copper ion.

[CuL_2H_2]. Only the dipeptides Gly-Pro and Ala-Pro form this species in a detectable amount. Both spectra are very similar (Figure 3). Again the fourth peak shows a further splitting. This splitting {40—50 G as in the case of [$CuL(OH)]^-$} may be caused by the coupling of two nitrogen atoms. The resolution of this coupling is rather poor, thus, no further information can be obtained. The series of recorded spectra, where this complex is formed from 0 to 90%, show that the spectrum in Figure 3(*b*) represents a spectrum of a pure species.

[$CuL_2H]^-$. The e.s.r. spectrum exhibits ordinary hyperfine coupling, only the dipeptides Pro-Gly, Pro-Ala, Pro-Leu, and Leu-Leu show a further splitting of the highest field line, which may be attributed to interactions with more than one nitrogen atom. An exact interpretation is impossible due to insufficient resolution. The influence of the side chains on the A_{Cu} values is similar to that for the complex [$CuL(OH)]^-$.

Conclusions

The described evaluation of digitized e.s.r. spectra leads to results consistent with potentiometric titrations. The ambiguity of species replacing each other can be reduced significantly. For this reason the e.s.r. method is suitable to examine models obtained by potentiometric titrations, if the metal ion and complex species are e.s.r. detectable. Within the investigated systems the species [CuL_2H_2] and [$CuL_2]^{2-}$ could be excluded, which had been impossible so far. On the other hand, this method yields the digitized e.s.r. spectra of all pure complexes and thus ion–ligand interaction can be investigated. Further, this method is also applicable for systems which are pH independent and cannot be evaluated by means of potentiometric titrations, therefore representing a supplementary tool for the investigation of complex equilibria.

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