J. CHEM. SOC. DALTON TRANS. 1985

The Effect of a Dansyl Group on the Co-ordinative Ability of *N*-Protected Amino Acids. Part 1. Behaviour of the Copper(II) Ion–*N*-Dansylglycinate System in Aqueous and Methanolic Solution *

Giovanna Battistuzzi Gavioli and Giulia Grandi
Istituto di Chimica Fisica, University of Modena, Via Campi 183, 41100 Modena, Italy
Ledi Menabue, Gian Carlo Pellacani, and Marco Sola
Istituto di Chimica Generale e Inorganica, University of Modena, Via Campi 183, 41100 Modena, Italy

Polarographic, potentiometric, and spectrophotometric measurements show that copper(II) and *N*-dansylglycine (5-dimethylaminonaphthalene-1-sulphonylglycine) form the same complexes in aqueous and methanolic solution. Mixed-hydroxy complexes are the prevailing species. In aqueous (methanolic) solution up to pH 5(5) no formation of complexes is observed; in the pH range 7—8.5 (8.5—11) a change in the ligand co-ordination from *O*-co-ordination through the carboxylate group to *N,O*-bidentate co-ordination through the carboxylate oxygen and the deprotonated amide nitrogen is revealed. There is a low tendency for two *N*-dansylglycine ligand molecules to co-ordinate to the Cu^{II} ion, probably due to the steric hindrance of the dansyl group. The equilibria in which the prevailing complexes are involved and the reduction processes at the electrode are proposed. The nucleophilic and electrophilic properties of the two solvents are used to explain the solvent effect on the polarographic parameters and on the stability constants of the complexes.

Some studies on the co-ordinative properties of amino acids *N*-protected by an R–SO$_2$ (R = aryl) group emphasize that Cu^{II} and Pd^{II} ions, unlike Zn^{II}, Co^{II}, or Ni^{II}, are effective in substituting for the nitrogen-bound hydrogen, giving rise to a strong bond.[1] The amino acids bearing an R′–CO (R′ = alkyl) or R–CO protecting group do not exhibit this type of co-ordination.[2–4] This particular behaviour of R–SO$_2$ amino acids is due to the more acidic character of the amide hydrogen of sulphonamides as compared to a peptide hydrogen, and to the presence of the α-carboxylate group which may act as a primary ligating group toward a metal ion.[1,5]

Being interested in the co-ordinative properties of this type of ligand, in this paper we report an investigation of the copper(II)–*N*-dansylglycinate system [dnsgly = *N*-dansylglycine (5-dimethylaminonaphthalene-1-sulphonylglycine); dnsglyO = *N*-dansylglycinate(1 –); dnsglyNO = *N*-dansylglycinate (2 –)] in aqueous and methanolic solution.

The aim of this work was to evaluate the effect of the size of the aromatic group on the type of complexes present in solution and to define the most favourable conditions from which solid complexes may be obtained. The results of the present work will also be compared with those previously found for the copper(II)–*N*-tosylglycinate system.[6,7]

The dansyl group, studied here as a *N*-protecting group for glycine, is widely used as a fluorescent probe to study protein conformational changes[8,9] and to identify *N*-terminal residues of peptides.[10,11]

Experimental

Polarographic analysis in aqueous solution was performed by adding different amounts of dnsgly dissolved in doubly-distilled water to aqueous Cu^{II} perchlorate (10^{-4} mol dm^{-3}) to give metal-to-ligand molar ratios in the range 1:2—1:10. The pH of the solutions was determined by adding aqueous NaOH (0.1 mol dm^{-3}). $NaClO_4$ (0.1 mol dm^{-3}) was used as base electrolyte and kept the ionic strength constant (I = 0.1 mol dm^{-3}). In order to characterize the electronic transfer process, concentrations of copper(II) ion ranging from 10^{-4} to 5×10^{-4} mol dm^{-3} and dropping times of 2, 3, 4, and 6 s were used. The study of the system in methanolic solution was performed under the same experimental conditions described above. Anhydrous methanol (C. Erba) was used as supplied and the salts $NaClO_4$ and $Cu(ClO_4)_2\cdot 6H_2O$ were recrystallized from this solvent before use. Polarographic measurements were carried out using an AMEL Multipolarograph model 472 instrument at 25 ± 0.1 °C. A saturated calomel electrode (s.c.e.) was used as a reference for the aqueous solutions and a $Ag/AgNO_3$ (0.1 mol dm^{-3}) electrode for the methanolic solutions. However, all $E_{\frac{1}{2}}$ values are referred to s.c.e.

The pH measurements were performed with a Beckman select ion 5000 pH-meter using, in aqueous solution, an Ingold HA 405-60-K1 pH combined electrode and, in methanolic solution, an Ingold B7213 glass electrode as indicator electrode and $Ag/AgNO_3$ (0.1 mol dm^{-3}) as reference electrode. For pH measurements in methanolic solution two buffers at pH 5.79 ± 0.05 and pH 7.53 ± 0.05 referred to the pH scale in methanol were used.[12] All pH values in methanolic solution refer to this pH scale.[13]

Electronic spectra were recorded with a Beckman DU-8 spectrophotometer. Solutions for spectrophotometric analysis were prepared keeping the ionic strength constant (KCl, I = 0.1 mol dm^{-3}).

N-Dansylglycine was purchased from the Sigma Chemical Co. All spectrophotometric measurements were performed at 25 ± 0.1 °C.

In aqueous solution, in the pH range 7—10, after nearly 3 h, a colour change of the system from green-blue to dark red was observed, probably due to an intramolecular redox process involving Cu^{II} ion reduction and ligand decomposition.

Results and Discussion

Behaviour of N-*Dansylglycine in Aqueous and Methanolic Solution.*—*N*-Dansylglycine exhibits physical and chemical properties [low melting point (157—158 °C), slight solubility

---

* *Supplementary data available* (No. SUP 56310, 5 pp.): Tables of electrochemical parameters in aqueous and methanolic solution, electronic spectra of dnsgly in methanol in the pH range 1—7, neutralization curve of dnsgly in aqueous solution. See Instructions for Authors, *J. Chem. Soc., Dalton Trans.*, 1985, Issue 1, pp. xvii—xix.

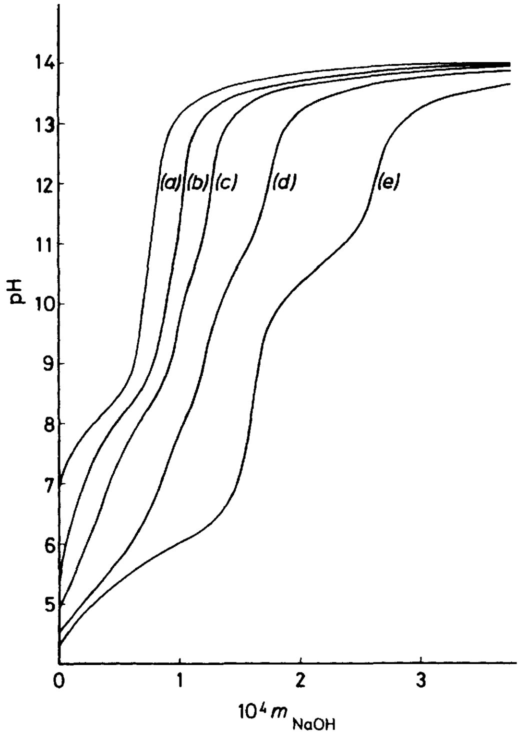


Figure 1. Neutralization curves of dnsgly in the presence of Cu^{2+} in methanolic solution ($m_{dnsgly} = 8 \times 10^{-5}$): $m_{Cu^{2+}}$ = (*a*) 0; (*b*) 10^{-5}; (*c*) 2×10^{-5}; (*d*) 4×10^{-5}; and (*e*) 8×10^{-5}; *m* = number of moles

in water, great solubility in organic solvents] peculiar to a non-zwitterionic character, which is confirmed by spectroscopic data and preliminary structural results.[14] Consequently, its dissociation equilibria at increasing pH are given by equations (1)—(3). The pK_{3a} value, determined spectrophotometrically in

$$(CH_3)_2\overset{+}{N}HC_{10}H_6SO_2NHCH_2COOH \xrightleftharpoons{pK_{1a}} (CH_3)_2NC_{10}H_6SO_2NHCH_2COOH \quad (1)$$

$$(CH_3)_2NC_{10}H_6SO_2NHCH_2COOH \xrightleftharpoons{pK_{2a}} (CH_3)_2NC_{10}H_6SO_2NHCH_2COO^- \quad (2)$$

$$(CH_3)_2NC_{10}H_6SO_2NHCH_2COO^- \xrightleftharpoons{pK_{3a}} (CH_3)_2NC_{10}H_6SO_2N^-CH_2COO^- \quad (3)$$

aqueous and methanolic solution, is 11.7 and 13.7 respectively. Equilibria (1) and (2) are closely dependent on the solvent used. In methanolic solution, pK_{1a}, determined spectrophotometrically, is 3.4 ± 0.2 (the spectra are reported in SUP 56310) and pK_{2a}, determined potentiometrically, is 8.1 ± 0.1 (Figure 1). In aqueous solution, the single step present in the pH-metric titration curve (Figure 2) with an apparent pK_a value of 4.4 ± 0.1 has been resolved into two steps each involving one proton, by titrating a more concentrated dnsgly aqueous solution (4×10^{-3} mol dm^{-3}) with a small amount of HCl previously added in order to obtain the fully protonated form of dnsgly. The pK_{1a} and pK_{2a} values obtained are 3.1 ± 0.1 and 4.8 ± 0.1 respectively (the titration curve is available in SUP No. 56310), in agreement with the most recently reported results).[15] However, conflicting data concerning these pK_a values are reported in ref. 8.

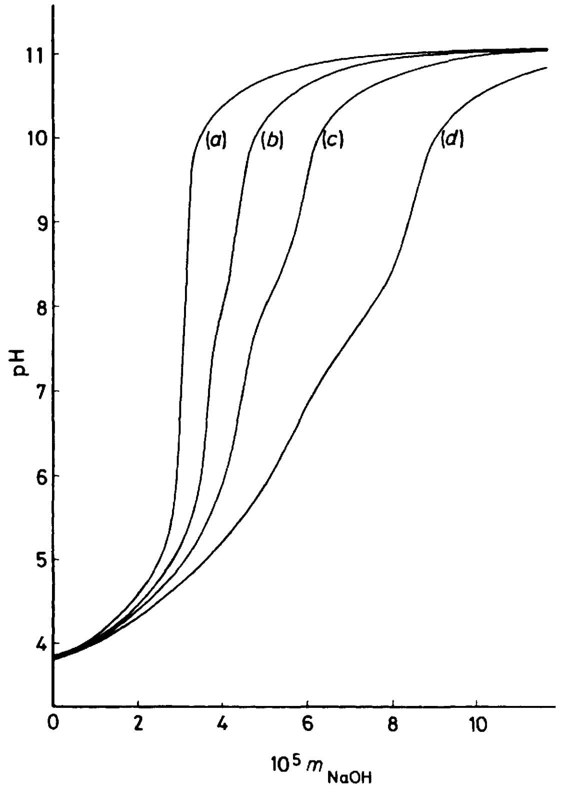


Figure 2. Neutralization curves of dnsgly in the presence of Cu^{2+} in aqueous solution ($m_{dnsgly} = 3 \times 10^{-5}$): $m_{Cu^{2+}}$ = (*a*) 0; (*b*) 0.75×10^{-5}; (*c*) 1.5×10^{-5}; and (*d*) 3×10^{-5}; *m* = number of moles

Potentiometric, Polarographic, and Spectrophotometric Behaviour of the Cu^{II}-N-*Dansylglycinate System.*—The pH-metric titration curves of the Cu^{II}—dnsgly system with different metal-to-ligand molar ratios show two sharp steps in both methanolic (Figure 1) and aqueous (Figure 2) solutions. The amounts of titrating NaOH solution necessary to reach the first and the second equivalent point depend on the metal-to-ligand molar ratio according to equations (4) and (5), respectively,

$$m_{NaOH} = m_{dnsgly} + m_{Cu} \quad (4)$$

$$m_{NaOH} = m_{dnsgly} + 2m_{Cu} \quad (5)$$

where *m* is the number of moles. The first step probably corresponds to the formation of a mixed monohydroxy-copper(II) complex in which dnsgly is co-ordinated through the carboxylate group.[1] This step is closely dependent on the solvent: in methanolic solution, at increasing Cu^{II} ion concentration, a decrease in the initial pH is observed, probably due to Cu^{II} substitution for the carboxylic hydrogen atom of the amino acid.[16] The second step, having a pK_a value (8.0 ± 0.2 in water and 10.5 ± 0.1 in methanol) markedly lower than the pK_{3a} of dnsgly, may be reasonably attributed to the Cu^{II}-promoted amide deprotonation of the co-ordinated amino-acid molecule.[1,6] While from equation (4) no information can be obtained about the number of dnsglyO$^-$ ions directly involved in metal co-ordination, equation (5) suggests the co-ordination with Cu^{II} of only one dnsglyNO^{2-} ion, independent of the metal-to-ligand molar ratio of the solution.

In order to obtain information about the species present in solution, polarographic measurements on the Cu^{II}-dnsgly system were performed at different pH values and ligand concentrations. It is found that in both solvents the system behaves in the same way [Figure 3(*a*) and (*b*)]. In aqueous

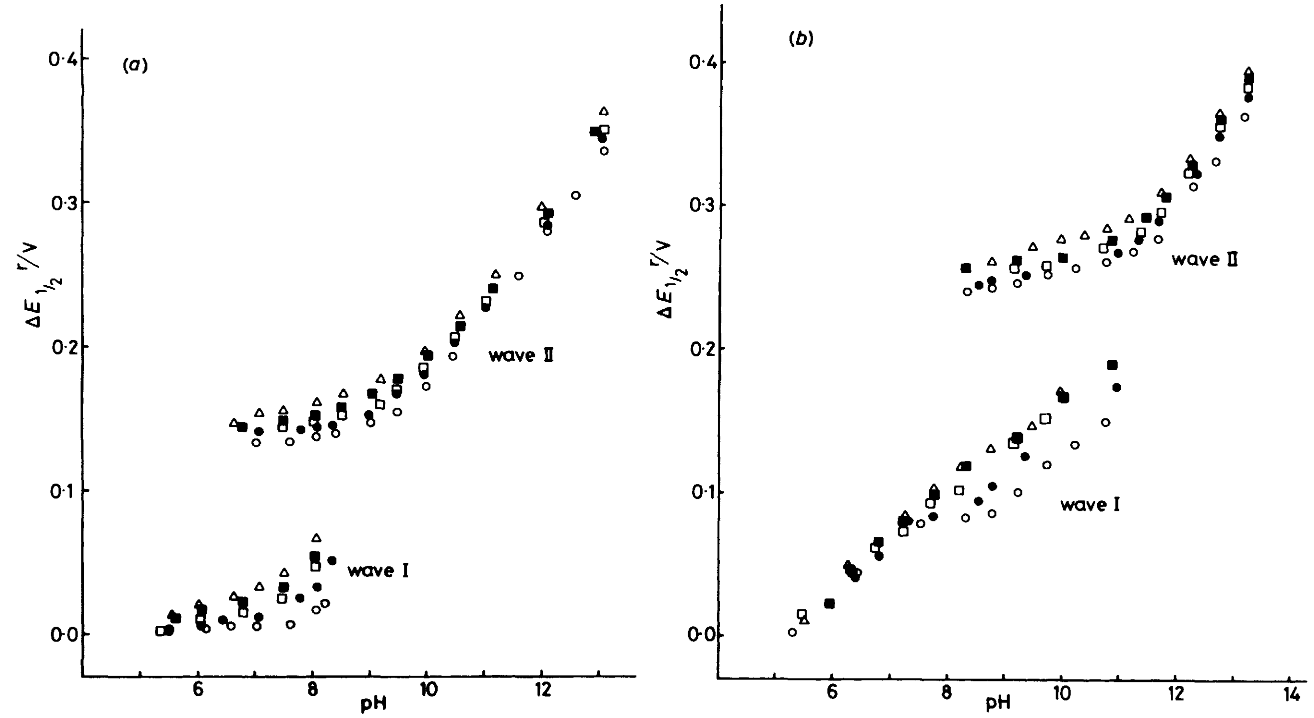


Figure 3. Plots of $\Delta E_{\frac{1}{2}}{}^{r}$ *vs.* pH at increasing dnsgly concentration. (*a*) Aqueous solution; (*b*) methanolic solution. $C_{Cu^{2+}} = 10^{-4}$ mol dm^{-3}; $C_{dnsgly} = 2 \times 10^{-4}$ (○); 4×10^{-4} (●); 6×10^{-4} (□); 8×10^{-4} (■); and 10^{-3} mol dm^{-3} (△) [$\Delta E_{\frac{1}{2}}{}^{r} = (E_{\frac{1}{2}}{}^{r})_M - (E_{\frac{1}{2}}{}^{r})_C$, where $(E_{\frac{1}{2}}{}^{r})_M$ is the reversible half-wave potential of the uncomplexed metal ion and $(E_{\frac{1}{2}}{}^{r})_C$ that of the complexed metal ion]

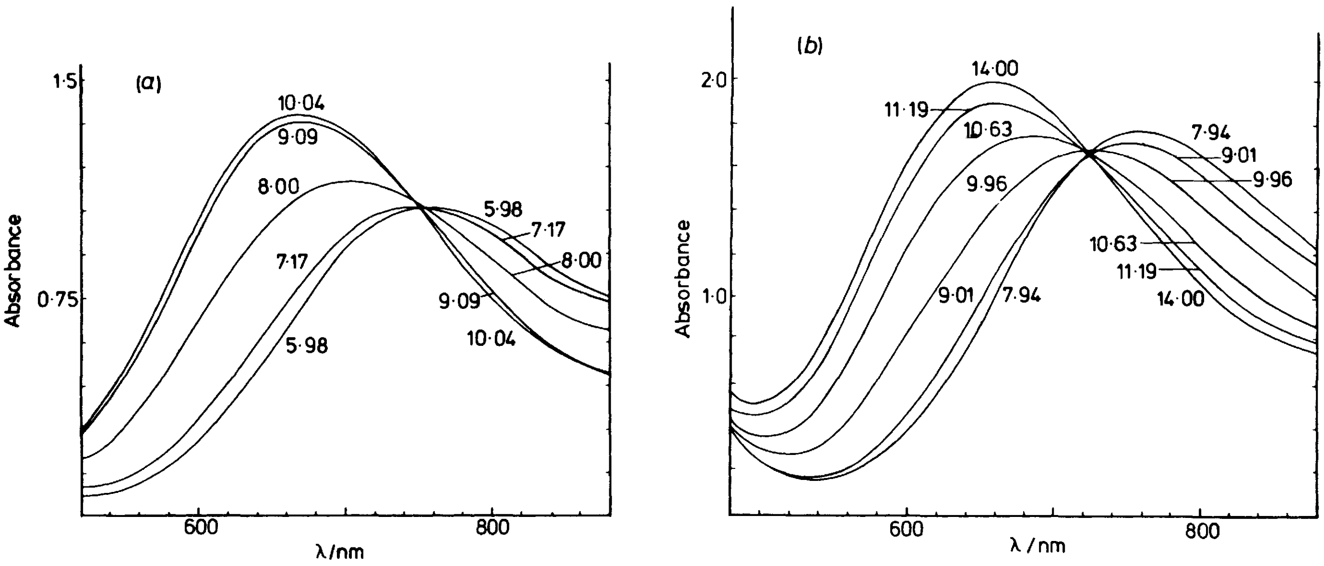


Figure 4. Electronic spectra of the Cu^{II}–dnsgly system in (*a*) aqueous and (*b*) methanolic solution at various pH. $C_{Cu^{2+}} = 2.5 \times 10^{-3}$ mol dm^{-3}; $C_{dnsgly} = 5 \times 10^{-3}$ mol dm^{-3}. Curves are labelled with the appropriate pH values. Cell length = 10 cm

(methanolic) solution, at a pH lower than 5 (5) only one wave (wave I) is present, corresponding to the solvated Cu^{II} ion reduction (reversible, bielectronic, and diffusion controlled). At a pH greater than 5 (5) the $E_{\frac{1}{2}}$ value of wave I decreases on increasing the ligand concentration and the pH of the solution, while i_d (diffusion current) remains constant. At a pH greater than 6.5 (8.5) a new wave (wave II) appears at more negative $E_{\frac{1}{2}}$ values than wave I, showing an increasing i_d value with increasing pH, while wave I decreases until it disappears at a pH greater than 8.5 (11). The sum of their i_d values remains constant. On increasing the pH and ligand concentration of the solution, the $E_{\frac{1}{2}}$ value of wave II decreases. Semi-logarithmic analysis of waves I and II shows that they both correspond to a quasi-reversible, bielectronic reduction process. Since each wave is controlled by the diffusion at the electrode of the electroactive species, the species reduced in wave I give rise to those reduced in wave II in the bulk of the solution. The reversible half-wave potentials ($E_{\frac{1}{2}}{}^{r}$) of both waves, used to characterize the species present in solution and to calculate their overall stability constants, were determined by the Matsuda–

Table. Complexes present in aqueous and methanolic solution at different pH and their overall stability constants (β)*

Solvent	Wave I	Wave II	
H_2O	$5 < pH < 8.5$ $[Cu(dnsglyO)(OH)]$, 1.0×10^{11} $[Cu(dnsglyO)_2(OH)]^-$, 2.5×10^{14}	$7 < pH < 11.5$ $[Cu(dnsglyNO)]$, 4.0×10^{8} $[Cu(dnsglyNO)_2]^{2-}$, 2.5×10^{11} $[Cu(dnsglyNO)(OH)]^-$, 5.0×10^{13} $[Cu(dnsglyNO)_2(OH)]^{3-}$, 1.2×10^{17}	$pH > 11.5$ $[Cu(dnsglyNO)(OH)_2]^{2-}$, 1.4×10^{17} $[Cu(dnsglyNO)_2(OH)_2]^{4-}$, 1.0×10^{20}
CH_3OH	$5 < pH < 11$ $[Cu(dnsglyO)(OH)]$, 3.0×10^{14} $[Cu(dnsgly)_2(OH)]^-$, 1.2×10^{18}	$8.5 < pH < 12$ $[Cu(dnsglyNO)]$, 1.3×10^{12} $[Cu(dnsglyNO)_2]^{2-}$, 2.0×10^{15} $[Cu(dnsglyNO)(OH)]^-$, 4.0×10^{17} $[Cu(dnsglyNO)_2(OH)]^{3-}$, 5.0×10^{20}	$pH > 12$ $[Cu(dnsglyNO)(OH)_2]^{2-}$, 5.5×10^{21} $[Cu(dnsglyNO)_2(OH)_2]^{4-}$, 1.0×10^{25}

* For concentration ranges where single species predominate see text and Figures 5 and 6.

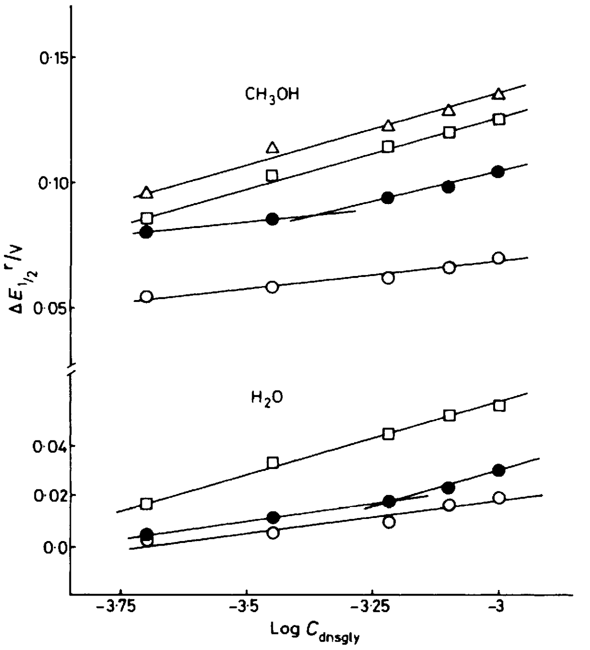


Figure 5. Wave I: plots of $\Delta E_{\frac{1}{2}}^r$ *vs.* log C_{dnsgly} at different pH values. In water: pH = 6.00 (○); 7.00 (●); 8.00 (□). In methanol: pH = 6.75 (○); 7.75 (●); 8.75 (□); 9.00 (△)

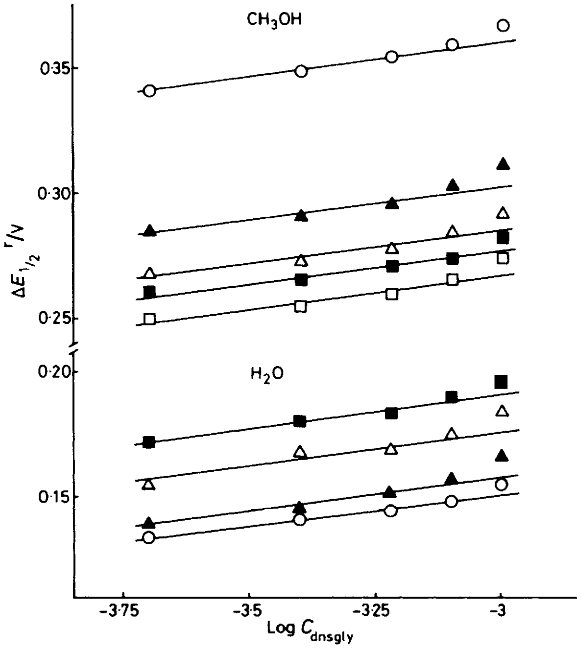
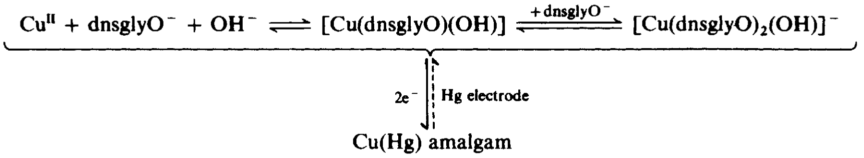


Figure 6. Wave II: plots of $\Delta E_{\frac{1}{2}}^r$ *vs.* log C_{dnsgly} at different pH values. In water: pH = 7.50 (○): 8.50 (▲); 9.50 (△); 10.00 (■). In methanol: pH = 9.75 (□); 10.75 (■); 11.25 (△); 11.75 (▲); 12.75 (○)

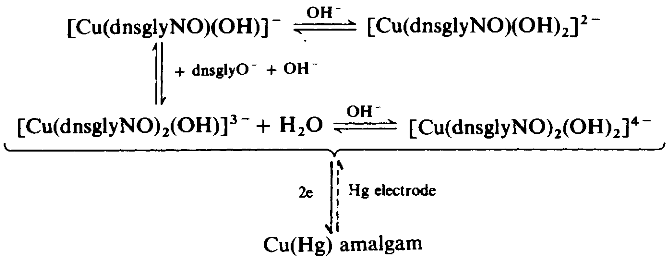
Ayabe method.[17] The polarographic data of the system in both solvents are reported in SUP No. 56310.

It is noteworthy that in both solvents, only wave I is present in the pH range in which the first potentiometric step is observed and only wave II is present at a pH greater than that corresponding to the second step. Both the polarographic waves are simultaneously present at intermediate pH values: in this pH range the electronic spectra of the system [Figure 4(*a*) and (*b*)] show a blue shift of the *d*—*d* band maximum which may be ascribed to the amide nitrogen deprotonation equilibrium (the calculated pK_a values of 7.9 ± 0.2 and 10.2 ± 0.1 in aqueous and methanolic solution, respectively, are in good agreement with those determined potentiometrically). Consequently, the reduction process of wave I must involve species in which the ligand is co-ordinated through the carboxylate group and that of wave II must involve species in which the ligand acts in a bidentate manner through the carboxylic oxygen and deprotonated amide nitrogen atoms. Since the plots reported in Figure 3(*a*) and (*b*) are curves in both solvents, the species involved in the reduction processes of waves I and II are mixed-hydroxy complexes.

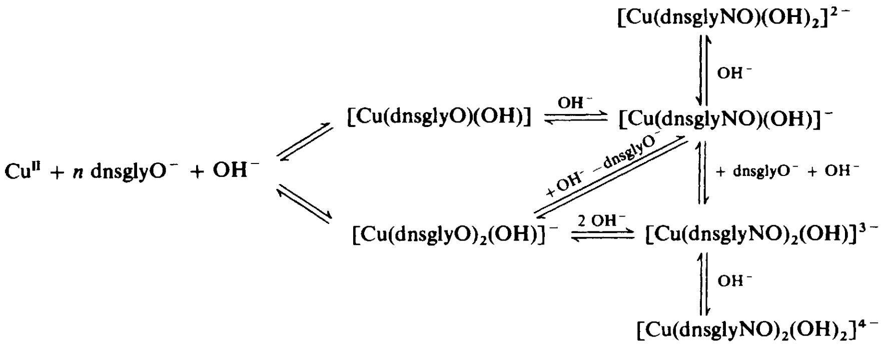
Wave I. Using the Lingane method,[18] the number of ligand molecules co-ordinated to the Cu^{II} ion may be evaluated. Plots of $\Delta E_{\frac{1}{2}}^r$ *vs.* log C_{dnsgly} at different pH values (Figure 5) gives rise to straight or broken lines. From their slopes the maximum co-ordination number of the ligand ($n_{max.}$) could be calculated. Both in aqueous and methanolic solution $n_{max.}$ is found to depend on the pH and ligand concentration: on increasing these two parameters, the co-ordination of two dnsglyO$^-$ ions is favoured. The Shaap–McMasters method,[19] applied by assuming the absence of the two species $Cu(OH)^+$ and $Cu(OH)_2$,[6b] reveals the presence, in both solvents, of the complexes $[Cu(dnsglyO)(OH)]$ and $[Cu(dnsglyO)_2(OH)]^-$; their stability constants (β) are reported in the Table. The co-ordination of Cu^{II} with only one hydroxide ion closely agrees with the potentiometric results. The overall reduction process and the equilibria between the complexes are the same in both solvents and can be represented by Scheme 1.



Scheme 1.



Scheme 2.



Scheme 3.

Wave II. As stated above, this wave involves the reduction of species in which dnsgly acts as a bidentate dianion. In both solvents, the Shaap–McMasters method shows that, over the wide range of ligand-to-metal molar ratios investigated, the following complexes can be present: $[\mathrm{Cu(dnsglyNO)}]$, $[\mathrm{Cu(dnsglyNO)_2}]^{2-}$, $[\mathrm{Cu(dnsglyNO)(OH)}]^-$. $[\mathrm{Cu(dnsglyNO)_2(OH)}]^{3-}$, $[\mathrm{Cu(dnsglyNO)(OH)_2}]^{2-}$, and $[\mathrm{Cu(dnsglyNO)_2(OH)_2}]^{4-}$; the mixed-hydroxy complexes are the prevailing species (see Table). More precisely, the Lingane plots (Figure 6) clearly indicate that up to a ligand-to-metal molar ratio of *ca.* 8:1, only one $\mathrm{dnsglyNO^{2-}}$ ion is co-ordinated to $\mathrm{Cu^{II}}$; the binding of two $\mathrm{dnsglyNO^{2-}}$ ions can only reasonably take place at greater ratios, in agreement with that reported for $\mathrm{Cu^{II}}$–amino acidate systems under similar conditions.[6,20] The overall reduction process can be represented by Scheme 2.

The results of this analysis, summarized in the Table, suggest the amide deprotonation equilibria (6) and (7) of *N*-dansylglycine bonded to $\mathrm{Cu^{II}}$.

$$[\mathrm{Cu(dnsglyO)(OH)}] + \mathrm{OH^-} \rightleftharpoons [\mathrm{Cu(dnsglyNO)(OH)}]^- + \mathrm{H_2O} \tag{6}$$

$$[\mathrm{Cu(dnsglyO)_2(OH)}]^- + \mathrm{OH^-} \rightleftharpoons [\mathrm{Cu(dnsglyNO)(OH)}]^- + \mathrm{dnsglyO^-} + \mathrm{H_2O} \tag{7}$$

The equilibria involving the species with two $\mathrm{dnsglyNO^{2-}}$ ions co-ordinated to $\mathrm{Cu^{II}}$, existing only in a large excess of ligand, can be described by (8) and (9).

$$[\mathrm{Cu(dnsglyO)_2(OH)}]^- + 2\,\mathrm{OH^-} \rightleftharpoons [\mathrm{Cu(dnsglyNO)_2(OH)}]^{3-} + 2\,\mathrm{H_2O} \tag{8}$$

$$[\mathrm{Cu(dnsglyNO)(OH)}]^- + \mathrm{dnsglyO^-} + \mathrm{OH^-} \rightleftharpoons [\mathrm{Cu(dnsglyNO)_2(OH)}]^{3-} + \mathrm{H_2O} \tag{9}$$

Conclusions

Unlike the *N*-tosylglycinate–copper(II) system previously investigated,[6,7] the overall equilibria are the same in both aqueous and alcoholic media and can be represented by Scheme 3. This comparison also shows that the naphthalene moiety of the dansyl group does not influence the type of complexes present in solution, but affects the ligand co-ordination number, the greater steric hindrance of the dansyl group generally favouring the binding of only one ligand molecule.

The copper(II) ion is effective in promoting amide deprotonation in *N*-dansylglycine, markedly lowering its $\mathrm{p}K_{\mathrm{NH}}$ value, as is also found for *N*-tosyl-α-amino acids. It is noteworthy that this behaviour is generally observed only for metal ions such as $\mathrm{Cu^{II}}$ and $\mathrm{Pd^{II}}$ which may form hydroxy species at low pH values.[21]

While in both solvents the same species are present, their stability constants are greater in methanol than in water. This effect may be explained by taking into account the nucleophilic (donor number = dn) and electrophilic (acceptor number = an) properties of the solvents ($\mathrm{dn_{H_2O}} = 33$, $\mathrm{dn_{CH_3OH}} = 19.1$;

$an_{H_2O} = 54.8$, $an_{CH_3OH} = 41.3$).[22,23] The $E_{\frac{1}{2}}{}^{r}$ values of Cu^{II} and of the complexes decrease on increasing the dn of the solvent,[24] because a greater nucleophile character of the solvent strengthens the 'inner' solvation sphere of metal ion and, in the complex, by forming an 'outer' solvation sphere around the amino acid, strengthens its bond to the Cu^{II} ion.[25] Since the strength of this bond depends on the stability of the solvated ions, on the co-ordinative properties of the ligand, and on the electron-pair donor and electron-pair acceptor properties of the solvent, the greater amphoteric character of water leads to a decrease in the stability of the complex owing to the stabilization of both cation and anion.[26]

Acknowledgements

We thank the Ministero della Pubblica Istruzione (Italy) for financial support. The work described in this paper is a part of the research activity of M. S. during the first year of his Dottorato di Ricerca in Scienze Chimiche at the Istituto di Chimica Generale e Inorganica, University of Modena.

References

1 H. Sigel and R. B. Martin, *Chem. Rev.*, 1982, **82**, 385 and refs. therein.
2 L. P. Battaglia, A. Bonamartini Corradi, G. Marcotrigiano, L. Menabue, and G. C. Pellacani, *Inorg. Chem.*, 1981, **20**, 1075 and refs. therein; M. R. Udupa and B. Krebs, *Inorg. Chim. Acta*, 1979, **37**, 1.
3 G. Battistuzzi Gavioli, G. Grandi, G. Marcotrigiano, L. Menabue, G. C. Pellacani, and M. Tonelli, Proceedings XI Congresso Nazionale di Chimica Inorganica, Arcavacata di Rende, Cosenza, Italy, September 1978, p. 4e; R. Andreoli, G. Battistuzzi Gavioli, L. Benedetti, G. Grandi, G. Marcotrigiano, L. Menabue, and G. C. Pellacani, *Inorg. Chim. Acta*, 1980, **46**, 215 and refs. therein.
4 L. Antolini, L. Menabue, P. Prampolini, and M. Saladini, *J. Chem. Soc., Dalton Trans.*, 1982, 2109 and refs. therein.
5 A. Rastelli and P. G. De Benedetti, *J. Chem. Res.*, 1977, 90.
6 (*a*) L. Antolini, L. P. Battaglia, G. Battistuzzi Gavioli, A. Bonamartini Corradi, G. Grandi, G. Marcotrigiano, L. Menabue, and G. C. Pellacani, *J. Am. Chem. Soc.*, 1983, **105**, 4327; (*b*) *ibid.*, p. 4333.
7 L. Antolini, L. Menabue, G. C. Pellacani, G. Battistuzzi Gavioli, G. Grandi, L. P. Battaglia, A. Bonamartini Corradi, and G. Marcotrigiano, *J. Chem. Soc., Dalton Trans.*, 1984, 1687.
8 N. Ikuta, J. Koga, and N. Kuroki, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 228 and refs. therein.
9 G. A. Davis, *J. Am. Chem. Soc.*, 1972, **94**, 5089 and refs. therein.
10 E. M. Koroleva, V. G. Maltsev, B. G. Belenkii, and M. Viska, *J. Chromatogr.*, 1982, **242**, 145.
11 C. De Jong, G. J. Hughes, E. Van Wieringen, and K. J. Wilson, *J. Chromatogr.*, 1982, **241**, 345.
12 D. D. Perrin and B. Dempsey, 'Buffers for pH and Metal Ion Control,' Chapman and Hall, London, 1979, p. 88.
13 R. G. Bates, 'Determination of pH, Theory and Practice,' Wiley, New York, 1973, p. 211.
14 M. Sola, unpublished work.
15 Z. Iškandarani and D. J. Pietrzyk, *Anal Chem.*, 1981, **53**, 489 and refs. therein.
16 P. Souchay and J. Lefebvre; 'Equilibres et Reactivitè des Complexes en Solution,' Masson et Cie, Paris, 1969, p. 75.
17 H. Matsuda and Y. Ayabe, *Z. Elektrochem.*, 1962, **66**, 469.
18 D. R. Crow, 'Polarography of Metal Complexes,' Academic Press, New York, 1969, pp. 56—84 and refs. therein.
19 W. B. Shaap and D. L. McMasters, *J. Am. Chem. Soc.*, 1961, **83**, 4699.
20 G. Berthon, M. Piktas, and M. J. Blais, *Inorg. Chim. Acta*, 1984, **93**, 117; P. Sharrock and R. Haran, *J. Coord. Chem.*, 1981, **11**, 117 and refs. therein.
21 G. Charlot, 'L'Analyse Qualitative et le Reactions en Solution,' Masson et Cie, Paris, 1963, pp. 273, 325.
22 V. Gutmann, *Coord. Chem. Rev.*, 1976, **18**, 225.
23 V. Gutmann, *Electrochim. Acta*, 1976, **21**, 661.
24 G. Gritzner, H. Morauer, and V. Gutmann, *J. Electroanal. Chem. Interfacial Electrochem.*, 1979, **101**, 185.
25 V. Gutmann and R. Schmid, *Coord. Chem. Rev.*, 1974, **12**, 263.
26 R. Andreoli, L. Benedetti, G. Grandi, and G. Battistuzzi Gavioli, *Electrochim. Acta*, 1984, **29**, 227.

Received 21*st November* 1984; *Paper* 4/1982