4. Transition Metal Ions and Amides. VIII.[1]) Discrimination between Different Models for the Complexation of Cu^{2+} with *N, N'*-Diglycyl-1,2-ethanediamine, N,N'-Diglycyl-1,3-propanediamine and Glycine Ethylamide by Potentiometric or by Spectrophotometric Titration

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Summary

The complexation of Cu^{2+} with 1,8-diamino-3,6-diaza-2,7-octanedione (= *N, N'*-diglycyl-1,2-ethanediamine, DED) and with 1,9-diamino-3,7-diaza-2,8-nonanedione (= *N, N'*-diglycyl-1,3-propanediamine, DPD) has been studied by potentiometric and by spectrophotometric titration. With both ligands L the complexation to Cu^{2+} leads to relatively complicated equilibria with $CuLH^{3+}$, CuL^{2+}, $CuLH_{-2}$, and dimeric $Cu_2L_2^{4+}$ complexes. With DED, another dimeric species, $Cu_2L_2H_{-2}^{2+}$, is formed in addition. Independent numerical treatment of spectrophotometric and potentiometric titrations was used to obtain a satisfactory model for the complexation and to test the relative discriminatory power of the two methods. Titrations of glycine ethylamide (GEA) were used as an additional test and as a model for DED and DPD. It was shown that in each case spectrophotometric titrations give results of similar reproducibility and have a discriminatory power equal to or better than potentiometric titrations, provided that optimum mathematical algorithms are used in the numerical treatment.

Introduction. – The complexation of Cu^{2+} by 1,8-diamino-3,6-diaza-2,7-octanedione (= *N, N'*-diglycyl-1,2-ethanediamine; $H_2NCH_2CONHCH_2CH_2NHCOCH_2NH_2$, DED) has been studied by several groups [3–6]. To explain their experimental data, more and more complicated equilibrium models were necessary. In the course of our studies on the formation and dissociation mechanisms of amide complexes [7] we have investigated the kinetics of the Cu^{2+} complexation by the homologue 1,9-diamino-3,7-diaza-2,8-nonanedione (= *N, N'*-diglycyl-1,3-propanediamine; DPD) [8]. The corresponding data for DED indicated a very complicated rate law which in addition could not be explained on the basis of the equilibrium models available at the time.

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With the construction of a simple cell for automatic spectrophotometric titration and on-line data acquisition, a large body of high precision spectrophotometric data can be obtained in our laboratory [9]; moreover, efficient methods of data reduction and least-squares estimation of equilibrium constants and molar absorptivities have been developed [10] [11]. It was our hope that the intriguing problem of the complexation of Cu^{2+} by DED could be successfully tackled with the new instrumental and computational facilities in hand. Moreover, the complexation of Cu^{2+} to DED and DPD were considered to be suitable test which should allow to estimate the relative power of potentiometric and spectrophotometric titrations in the discrimination between a series of models for the description of complicated equilibrium systems. Included into the study are corresponding experiments with glycine ethylamide ($H_2NCH_2CONHCH_2CH_3$, GEA), a ligand which can be considered, to a very good approximation, to represent one half of either DED or DPD.

Experimental Part. – 1. *Materials and Instrumentation.* *N*-Benzyloxycarbonylglycine cyanomethylester (Z–Gly–OCH_2CN) was prepared according to *Schwyzer et al.* [12] from Z–Gly *(Fluka, puriss.)* and chloroacetonitrile ('for synthesis', *Merck*). *N*-Benzyloxycarbonyl-glycine ethylamide (Z–Gly–EA) [13] [14] was obtained by dissolving 4.5 g of Z–Gly–OCH_2CN in 40 ml of ethyl acetate and adding 9.5 ml of 70% aq. ethylamine ('for synthesis', *Merck*). After standing for 24 h at RT., evaporation of the solvent, and recrystallization from CCl_4, 3.28 g (73%) of Z–Gly–EA with m.p. 375–376 K ([13]: 375–377 K; [14]: 374 K) were collected. The protecting group was removed by catalytic hydrogenation as described previously for DED and DPD [5]. After evaporation of acetic acid, conversion into the free amide by an anion exchange resin, acidification to pH 2.5 with 37% HCl-solution, evaporation, and recrystallization from ethanol/acetone, crystals of glycine-ethylamide hydrochloride were obtained. The product was identified by its molecular weight (potentiometric titration), ^{1}H-NMR. in D_2O [1.12 (*t*, $NHCH_2CH_3$); 3.26 (*qa*, $NHCH_2CH_3$); 3.76 (*s*, 2 H–C(2)], and m.p. 414–415 K ([13]: 407 K; [14]: 413.5–414.2 K). The dihydrochlorides of DED and DPD were synthesized according to [5]. The other reagents $CuSO_4$, KCl, and NaOH *titrisol* (all *Merck*) were of analytical grade and used without further purification.

Potentiometric titration curves were obtained on a E 600 ion meter *(Metrohm)* with the microprocessor controlled data acquisition system described previously [16]. Numerical treatment of the data was done on a *HP* 9835 with the aid of a new program TITFIT [17] using analytical derivatives in the iterative refinement of the stability constants.

Spectrophotometric titration curves were obtained using the instrumentation described in [9], but the spectrophotometer was controlled by an *APPLE II* desk-top computer, and the same calculator was used for data reduction and numerical treatment [10] [11].

2. *Measurements.* All experiments were performed at 298 K with $I = 0.5$ (KCl). Twice distilled water was used throughout.

Potentiometric titrations were performed under N_2 with 0.4M NaOH. The acidity constants of the tetradentate ligands DED and DPD were obtained by titrating 25 ml of 0.0064M and 100 ml of 0.0016M solutions of the corresponding dihydrochlorides. For the complex equilibrium constants the analytical ligand and metal ion ($CuSO_4$) concentrations c_L and c_M were: a) $c_L = 0.0032$, $c_M = 0.00144$ or 0.00288M (25 ml samples); b) $c_L = 0.0004$M, $c_M = 0.00036$ or 0.00072M (100 ml samples). For GEA the conditions were: c) $c_L = 0.0064$M, $c_M = 0.0016$, 0.00307 or 0.0063M (25 ml); d) $c_L = 0.0016$M, $c_M = 0.0004$, 0.00077 or 0.0015M (100 ml). In the titrations of GEA with the highest metal to ligand ratios (0.0063 and 0.0015M Cu^{2+}, respectively) precipitates formed after neutralization of one proton per ligand. The corresponding curves were discarded for the final calculations. For the titration of GEA in the absence of metal ion, NaOH was added in 0.01 ml portions up to a total of 0.5 ml. In all other cases 0.02 ml additions up to 0.9 ml (DED, DPD) or 1.0 ml (GEA) were used.

Spectrophotometric titrations were performed with 2.3 ml samples of suitable ligand/metal ion solutions using NaOH-solution as titrant. Thirty spectra per experiment were obtained, and NaOH-solution was added in 0.01 ml portions in each case. The composition of the titration mixtures was as follows: For *N,N′-diglycyl-1,2-ethanediamine* (DED): e) $c_L = 0.0025$M, $c_M = 0.0022$M, NaOH = 0.1M;

f) $c_L = 0.0044$ M, $c_M = 0.0011$ M, NaOH = 0.1 M; g) $c_L = 0.017$ M, $c_M = 0.007$ M, NaOH = 0.5 M. For *N,N'-diglycyl-1,3-propanediamine* (DPD): h) $c_L = 0.0024$ M, $c_M = 0.0023$ M, NaOH = 0.1 M; i) $c_L = 0.0125$ M, $c_M = 0.0113$ M, NaOH = 0.5 M; k) $c_L = 0.0337$ M, $c_M = 0.0023$ M, NaOH = 0.1 M. For *glycine ethylamide* (GEA): l) $c_L = 0.0113$ M, $c_M = 0.0057$ M, NaOH = 0.2 M; m) $c_L = 0.0116$ M, $c_M = 0.0029$ M, NaOH = 0.2 M. For DED and DPD, spectra were obtained between 520 and 680 nm at 10 nm intervals, and for GEA between 460 and 780 nm at 20 nm intervals. In each case, the absorbance at a given wavelength was calculated as the mean of ten individual readings of the digital voltmeter.

All experiments were run in duplicates. For each ligand, two complete sets of potentiometric or spectrophotometric data from Cu^{2+}-containing mixtures were combined for numerical treatment and evaluated independently. Thus, the final stability constants were obtained as the weighted means of the results from four independent sets of experiments. Their uncertainties $\sigma_{\log K}$ in turn were computed from the corresponding standard errors using *Eqn. 1* (absolute error) or *2* (relative error), whichever gave the larger value,

$$\sigma^2_{\log K} = \frac{1}{\Sigma_i 1/\sigma^2_{i,\log K}} \quad \text{(absolute errors)} \tag{1}$$

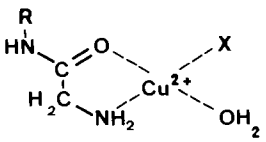
$$\sigma^2_{\log K} = \frac{\Sigma_i 1/\sigma^2_{i,\log K} \cdot (\log K_i - \log \bar{K})^2}{\Sigma_i (N-1)/\sigma^2_{i,\log K}} \quad \text{(relative errors)} \tag{2}$$

with $\sigma_{i,\log K}$ = standard error calculated from one set of experiments, $\log \bar{K}$ = weighted mean, and N = number of independent sets = 4.

The absorption maxima and molar absorptivities of the complexes were calculated by quadratic interpolation of the three highest points of the digitized spectra. For all welldefined species this gave an agreement between the two independent sets within ±3 nm. The majority of results even differed by ±1 nm or less.

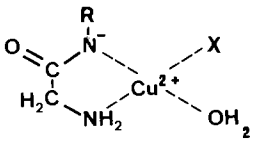
Results and discussion. – As a consequence of the coordinating properties of the amide group, which binds by the carbonyl O-atom in the neutral, but by the N-atom in the deprotonated form, the formation of the six complex species CuL^{2+}, $CuLH^{+}_{-1}$, $CuLH_{-2}$, CuL_2^{2+}, $CuL_2H^{+}_{-1}$, and CuL_2H_{-2} has to be expected with glycine ethylamide (GEA). The corresponding structures Ia, Ib and IIa, IIb, and III–V are conceivable *(Scheme)*. All the species mentioned have been observed previously in investigations with the analogous ligands glycinamide (GA) [18–20] or L-alanin amide (AA) [1]. However, the complete set of complexes has not been detected so far for neither ligand. Thus, in [18] only 1:1 complexes were studied, in [19] [20] only CuL^{2+} and CuL_2^{2+} were found, and in [1] no evidence was obtained for $CuLH_{-2}$.

With the potentially tetradentate diamides DED and DPD, the complexes with structures I–V mentioned above are of course equally possible. However, these species could all have a neutral amino group or a positively charged ammonio group in the substituent R, which would more than double the number of conceivable species. In addition, for DED and DPD the complex $CuLH_{-2}$ would more likely have a tetracoordinated structure VI [5] [6] with two deprotonated amide groups rather than being a hydroxo complex IIb. As described below, dimeric species $Cu_2L_2^{4+}$ (VII) and $Cu_2L_2H^{2+}_{-2}$ (VIII) are also formed with DED and DPD. Therefore, in the relatively narrow pH range of 5–9 the presence of well over a dozen different complex species is expected, and it seems quite unrealistic to obtain a complete analysis of such a system. On the other hand, the formation of chelate complexes like VI, VII, or VIII at relatively low pH may successfully suppress the formation



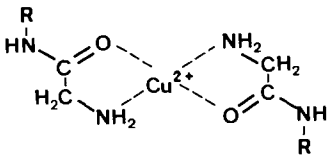
Ia: X = H_2O

Ib: X = OH^-

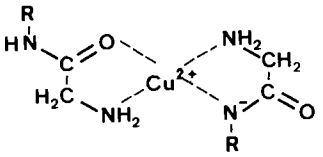


IIa: X = H_2O

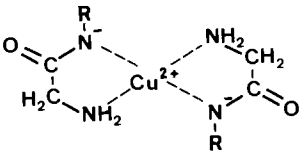
IIb: X = OH^-



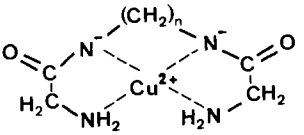
III



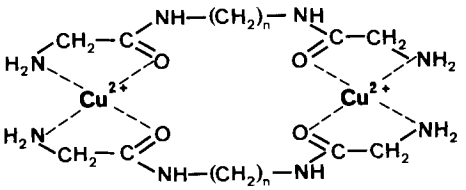
IV



V



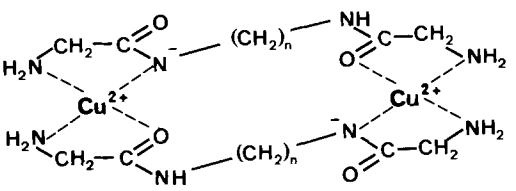
VI



VII

DED: n = 2

DPD: n = 3



VIII

of many complexes analogous to those found with glycine ethylamide, making the system tractable again.

The stability constants determined by potentiometric and spectrophotometric titrations are given in *Table 1,* together with some data for glycinamide (GA) and L-alaninamide (AA) taken from the literature. In addition the molar absorptivities and the absorption maxima of the complex species are summarized in *Table 2.*

Clearly, GEA in many respects is a very close model for the tetradentate diamides DED and DPD. 'Corresponding' species are first of all CuL^{2+} (*Table 1,* equilibrium No. 2) for GEA, GA, and AA, and $CuLH^{3+}$ (No. 10) for DED and DPD (structure Ia) as well as CuL_2^{2+} (GEA, GA, AA, No. 3) and $CuL_2H_2^{4+}$ (DED, DPD, No. 11) with structure III. In both cases the stability constants are identical within ± 0.4 log units for GEA, DED, and DPD. For the 1:2 complexes for which all spectral data are available, the spectral characteristics (λ_{max}, ε) also are practically identical *(cf. Table 2).* For DED and DPD, $CuL_2H_2^{4+}$ must contain two

Table 1. *Logarithms of equilibrium constants for the Cu^{2+} complexes of glycine ethylamide* (GEA), *N,N'-diglycyl-1,2-ethanediamine* (DED), *and N,N'-diglycyl-1,3-propanediamine* (DPD), *together with data for glycinamide* (GA), *and L-alaninamide* (AA) *taken from the literature*

No.	Equilibrium	$\log K$[a]) ($\sigma_{\log K}$[b])		
		GEA	GA	AA[c])
1	$L + H^+ \rightleftharpoons LH^+$	8.19 (0.01)[d])	8.04[e]) 8.06[f])	8.18
2	$Cu^{2+} + L \rightleftharpoons CuL^{2+}$	5.50 (0.03)	5.40[e]) 5.51[f])	5.07
3	$CuL^{2+} + L \rightleftharpoons CuL_2^{2+}$	4.36 (0.02)	4.21[f])	3.92
4	$CuLH_{-1}^{\pm} + H^+ \rightleftharpoons CuL^{2+}$	7.40 (0.17)[g])	7.01[e])	7.22
5	$CuLH_{-2} + 2H^+ \rightleftharpoons CuL^{2+}$	16.50 (0.03)[h])	15.08[e])	–
6	$CuL_2H_{-1}^{\pm} + H^+ \rightleftharpoons CuL_2^{2+}$	8.09 (0.02)	–	6.97
7	$CuL_2H_{-2} + H^+ \rightleftharpoons CuL_2H_{-1}^{\pm}$	10.23 (0.04)	–	8.16
		DED		DPD
8	$L + H^+ \rightleftharpoons LH^+$	8.33 (0.01)[d])		8.42 (0.01)[d])
9	$LH^+ + H^+ \rightleftharpoons LH_2^{2+}$	7.67 (0.01)[d])		7.75 (0.01)[d])
10	$Cu^{2+} + LH^+ \rightleftharpoons CuLH^{3+}$	5.13 (0.01)		5.36 (0.07)
11	$CuLH^{3+} + LH^+ \rightleftharpoons CuL_2H_2^{4+}$	4.15 (0.04)		4.30 (0.03)
12	$Cu^{2+} + L \rightleftharpoons CuL^{2+}$	7.68 (0.20)[g])		7.78 (0.13)
13	$Cu^{2+} + L \rightleftharpoons CuLH_{-2} + 2H^+$	−6.37 (0.02)		−3.13 (0.07)
14	$2Cu^{2+} + 2L \rightleftharpoons Cu_2L_2^{4+}$	18.84 (0.02)		19.26 (0.16)
15	$Cu_2L_2H_{-2}^{2+} + 2H^+ \rightleftharpoons Cu_2L_2^{4+}$	12.79 (0.06)		–

a) Weighted mean from 2 complete sets of potentiometric titrations and 2 complete sets of spectrophotometric titrations. Mixed constants with $[H^+] = 10^{-pH}$ are given. Concentration constants are obtained with activity coefficient a ($[H^+]$) = 0.87.

b) Standard error.

c) From [1].

d) From 4 individual titration curves.

e) From [18].

f) From [19].

g) From spectrophotometric titrations only.

h) Spectrophotometric data, potentiometry included gives 16.53 (0.13).

Table 2. *Absorption maxima (nm) and molar absorptivities ($mol^{-1} \cdot l \cdot cm^{-1}$) of 'corresponding' complexes with GEA, DED, and DPD*

Species	GEA		DED		DPD		Species
	λ_{max}	ε	λ_{max}	ε[a]	λ_{max}	ε[a]	
CuL^{2+}	758	35	$\geqslant 680$[b]	–	$\geqslant 680$[b]	–	$CuLH^{3+}$
$CuLH^{+}_{-1}$	700	23[c]	–[d]	–	696	35	CuL^{2+}
CuL_2^{2+}	665	48	660	47	670	56	$CuL_2H_2^{4+}$
CuL_2^{2+}	665	48	662	42	670	52	$Cu_2L_2^{4+}$
$CuL_2H^{+}_{-1}$	597	59	597	54	–[e]	–	$Cu_2L_2H^{2+}_{-2}$
CuL_2H_{-2}	535	69	513	163	505	62	$CuLH_{-2}$

[a]) Per mol of Cu^{2+}.
[b]) Experimental data only up to 680 nm.
[c]) Minor species, $\lambda_{max} \pm 20$ nm.
[d]) No reproducible value obtained.
[e]) Species not observed.

protonated amino groups ($-NH_3^+$) in the residue R. Complexes like CuL_2H^{3+} or CuL_2^{2+} with the same structure but with neutral amino end groups are not observed.

A next set of corresponding complexes is given by CuL_2H_{-2} (GEA, No. 7, structure V) and $CuLH_{-2}$ (DED, DPD, No. 13, structure VI). Here, the metal ion is coordinated by two amino and two deprotonated amide N-atoms as indicated by the absorption maxima at very short wavelength, *i.e.* between 505 and 535 nm. The difference of some 30 nm between the twodentate GEA and the tetradentate DED and DPD is a reflection of the blueshift expected for the additional chelate ring in VI relative to V. This is wellknown from the series $Cu(NH_3)_4^{2+}$ ($\lambda_{max} = 590$ nm) [22], $Cu(en)_2^{2+}$ ($\lambda_{max} = 550$ nm; en = ethylenediamine) [23], and $Cu(DND)^{2+}$ ($\lambda_{max} = 528$ nm; DND = 3,7-diaza-1,9-nonanediamine) [24], as well as from several analogous systems [25].

A final case among the monomeric species is the pair $CuLH^{+}_{-1}$ (GEA, No. 4) and CuL^{2+} (DED, DPD, No. 12). These species are never present in dominant concentrations and their properties are not overly welldefined. As to the structure, a hydroxo complex Ib or a species IIa with a deprotonated amide group are both possible. But for Ib a maximum near 750 nm would be expected. However, the equilibrium between the two isomers Ib and IIa may not be completely shifted towards IIa, as it is suggested by the low maximum absorptivities and the broad spectra calculated.

It must be noted that neither with DED nor with DPD any indication for the intermediate state of deprotonation, $CuLH^{+}_{-1}$, was obtained. The potentiometric and the spectrophotometric data for DED and DPD could however not be explained with models based exclusively on the monomeric species discussed so far. The two dimeric complexes $Cu_2L_2^{4+}$ and $Cu_2L_2H^{2+}_{-2}$ (DED only) are formed in addition. The latter has already been postulated some time ago [6]. The spectral data given in [6] ($\lambda_{max} = 590$ nm, $\varepsilon = 108$ per mol of complex) agree well with those found in the present study *(cf. Table 2)*. Stability constants obviously do not help in assigning structures to $Cu_2L_2^{4+}$ or $Cu_2L_2H^{2+}_{-2}$. The spectral data, however, proved to be most useful. 'Corresponding' pairs are a) CuL_2^{2+} (GEA, $\lambda_{max} = 665$ nm,

structure III) and $Cu_2L_2^{4+}$ (DED, DPD, $\lambda_{max} = 660$ and 670 nm, respectively), and b) $CuL_2H_{-1}^{+}$ (GEA, 597 nm, structure IV) and $Cu_2L_2H_{-2}^{2+}$ (DED, 597 nm). As indicated in *Table 2*, not only the absorption maxima but also the absorptivities (per mol of Cu^{2+}) of the related species agree very well. $Cu_2L_2^{4+}$ thus contains a $2N,2O$-chromophore (metal bounded to 2 N- and 2 O-atoms) as in III, and its structure is given by VII. $Cu_2L_2H_{-2}^{2+}$ is described by a $2N,N^{-},O$-configuration as in IV, and the corresponding structure would be VIII. The authors of [6] have given a choice of 4 possible structures for $Cu_2L_2H_{-2}^{2+}$ among which VIII is also found. However, they preferred another species with $2N,2N^{-}$-coordination and two bridging deprotonated amide groups. In our opinion, this possibility is rather unlikely in the light of the spectral data, and also because all resonance stabilization would be lost in an amide group with a deprotonated N-atom as a bridging ligand. In fact, a bridging amide N-atom has not yet been observed in copper coordination chemistry.

One might be wondering why $Cu_2L_2H_{-2}^{2+}$ is formed only with DED and not with DPD; in fact, structure VIII would be equally feasible for DPD. Most likely, the reason lies in the much higher stability (more than 3 orders of magnitude) of $CuLH_{-2}$ (VI) for DPD compared to $CuLH_{-2}$ for DED in agreement with the general preference for 5,6,5-rings over 5,5,5-rings in tetradentate linear chelators. As a consequence, with DPD the fully deprotonated $CuLH_{-2}$ is already formed in the pH region where otherwise $Cu_2L_2H_{-2}^{2+}$ would occur.

As can also be seen from *Table 1*, that glycinamide (GA) and alaninamide (AA) would have been misleading models for DED and DPD, even though the stabilities of the complexes CuL^{2+} and CuL_2^{2+} (equilibria 2 and 3) are similar to those of GEA. On the other hand, the stability constants of the complexes with deprotonated amide groups, $CuLH_{-2}$, $CuL_2H_{-1}^{+}$ and CuL_2H_{-2} (equilibria 5–7) differ by 1.4, 1.1 and 2.1 log units. If the data for AA and GA were taken as reference, the corresponding structures IIb, IV, and V (R = H) should be present in significant amounts also for the tetradentate ligands DED and DPD ($R = (CH_2)_nNHCOCH_2NH_2$). However, the stability of these structures is greatly reduced for GEA by steric hindrance (*cf.* [2]). The same is true for DED and DPD, and therefore these species cannot successfully compete with the chelates VI–VIII which are of course only possible with the tetradentate ligands.

We are confident that using the combination of potentiometric and spectrophotometric titrations along with the model ligand GEA two highly complex systems have been successfully treated, at least as far as all major species are concerned. Another central aspect of this work was the comparison of the relative discriminatory power of potentiometric and of spectrophotometric data in determining the model for the description of complicated equilibrium systems. So far, potentiometry has been preferred in general, while spectrophotometry has been considered somewhat unsafe for multicomponent systems even though in principle the information content of spectrophotometric data obtained at many wavelengths is much higher [26].

The choice of the correct or 'best' model was by no means obvious in our case, and the question of significance of additional parameters was nontrivial, too. To deal with these problems the potentiometric and the spectrophotometric data were

treated separately, and the variances obtained with different models subjected to the *F*-test (*cf. e.g.* [21]). The results for the most successful models and for those based on literature claims [5] [6] are summarized in *Table 3*. For each model *Table 3* contains the degrees of freedom (n), the *F*-value, and its probability P of being significantly worse than the best one. The probabilities are roughly subdivided into 4 categories: ++highly significant, model 'out of question'; +*ca.* 99–99.9% probability; ±marginally significant, *ca.* 90–99%; −unsignificant or doubtful significance. It is quite obvious from the results presented in *Table 3* that for each system the significance of the spectrophotometric data is at least equal to that of the potentiometric data. In 6 out of 13 cases the probabilities fall into the same category, in the other 7 the spectrophotometric data excel the potentiometric by one, two or three classes. The effects are most dramatic with DPD where two models (IV, V) are rejected with the highest significance (++) based on spectrophotometric data, but not at all based on potentiometry (−). It may be added, that with three exceptions the higher level of significance of the spectrophotometric data

Table 3. *Relative discriminatory power of spectrophotometric and of potentiometric titrations*

No.	Model	Potentiom.			Spectrophotom.		
		n[a])	F[b])	P[c])	n[a])	F[b])	P[c])
N,N'-Diglycyl-1,2-ethanediamine (DED)							
I	CuL^{2+}, $CuLH_{-2}$, $Cu_2L_2H_{-2}^{2+}$[d])	177	43.3	++	1459	3.38	++
II	$CuLH^{3+}$, CuL^{2+}, $CuLH_{-1}^{+}$, $CuLH_{-2}$[e])	176	38.4	++	1441	20.6	++
III	$CuLH^{3+}$, CuL^{2+}, $CuLH_{-2}$, $Cu_2L_2H_{-2}^{2+}$	176	26.0	++	1441	2.55	++
IV	$CuLH^{3+}$, $CuLH_{-2}$, $Cu_2L_2^{4+}$, $Cu_2L_2H_{-2}^{2+}$	176	1.30	±	1441	1.49	+
V	$CuLH^{3+}$, CuL^{2+}, $CuLH_{-2}$, $Cu_2L_2^{4+}$, $Cu_2L_2H_{-2}^{2+}$	175	1.15	−	1423	1.17	±
VI	$CuLH^{3+}$, $CuLH_{-2}$, $Cu_2L_2^{4+}$, $Cu_2L_2H_{-2}^{2+}$, $CuL_2H_2^{4+}$	175	1.13	−	1423	1.32	+
VII	$CuLH^{3+}$, CuL^{2+}, $CuLH_{-2}$, $Cu_2L_2^{4+}$, $Cu_2L_2H_{-2}^{2+}$, $CuL_2H_2^{4+}$	174	1.00	−−	1405	1.00	−−
N,N'-Diglycyl-1,3-propanediamine (DPD)[f])							
I	CuL^{2+}, $CuLH_{-2}$[d])	178	17.4	++	1477	52	++
II	$CuLH^{3+}$, CuL^{2+}, $CuLH_{-2}$[e])	177	3.86	++	1459	39	++
IV	$CuLH^{3+}$, $CuLH_{-2}$, $Cu_2L_2^{4+}$	177	1.16	−	1459	51	++
V	$CuLH^{3+}$, CuL^{2+}, $CuLH_{-2}$, $Cu_2L_2^{4+}$	176	1.01	−	1441	39	++
VI	$CuLH^{3+}$, $CuLH_{-2}$, $Cu_2L_2^{4+}$, $CuL_2H_2^{4+}$	176	1.15	−	1441	1.37	+
VII	$CuLH^{3+}$, CuL^{2+}, $CuLH_{-2}$, $Cu_2L_2^{4+}$, $CuL_2H_2^{4+}$	175	1.00	−−	1423	1.00	−−
Glycine Ethylamide (GEA)							
VIII	CuL^{2+}, CuL_2^{2+}, $CuL_2H_{-1}^{+}$, CuL_2H_{-2}	196	7.74	++	965	57	++
IX	CuL^{2+}, $CuLH_{-2}$, CuL_2^{2+}, $CuL_2H_{-1}^{+}$, CuL_2H_{-2}	195	1.88	+[g])	947	7.2	++
X	CuL^{2+}, $CuLH_{-1}^{+}$, $CuLH_{-2}$, CuL_2^{2+}, $CuL_2H_{-1}^{+}$, CuL_2H_{-2}	194	1.00	−−	929	1.00	−−

[a]) Degrees of freedom.
[b]) *F*-value, relative variance, *cf. e.g.* [21].
[c]) Level of significance, probability of unsufficient model: ++indicates ≫99%, +indicates >99%, ±indicates 90–99% and −indicates not significant.
[d]) Model suggested in [6].
[e]) Suggested in [5].
[f]) No formation of $CuLH_{-1}^{+}$ or its dimer, models II and III identical.
[g]) Second series of measurements gives unsignificant *F*-value (1.06).

is not a consequence of the larger number of degrees of freedom for the latter experiments, but is directly reflected by a higher F-value. With potentiometric measurements alone, the complexes CuL^{2+} and $CuL_2H_2^{4+}$, although 'reasonable' species and although expected from the results with the 'model' GEA, could not have been considered significant for either DED or DPD.

For the systems described in the present work numerical treatment of data from spectrophotometric titrations has yielded stability constants which compare very well with those obtained potentiometrically. The results are of similar reproducibility, and the standard errors extracted from the nonlinear least squares treatment do not differ significantly. Moreover, spectrophotometric titrations have been superior in their discriminatory power between different models, and they yield additional informations in the form of the absorption spectra of the complex species, providing the possibility of a most valuable check on the choice of a correct model. We are sure that spectrophotometric titrations of the type described in this work will prove to be equally useful for many other complicated systems. However, it must be clearly stated that this critically depends on the acquisition of very high precision data [9] [27] at many wavelengths and on rigorous numerical treatment.

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