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Complexes of Uracil (2,4-Dihydroxypyrimidine) Derivatives. Part I. Cu(II), Ca(II) and Mg(II) Coordination with Uracil and Related Compounds in Aqueous Solution

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Potentiometric pH studies were carried out on proton and metal (Cu(II), Ca(II) and Mg(II)) complexes of HL = uracil, thymine (5-methyluracil), 6-chloromethyluracil, 5-hydroxymethyluracil, 6-methyluracil and a diethyl 6-uracilmethylphosphonate derivative. For Cu(II) additional spectroscopic (absorption and EPR) measurements were carried out. The results have been compared with the literature data accessible only for uracil and thymine. A deprotonation of the ML complexes in a more basic solution was indicated, thus, extending the coordination mode proposed up to now for uracil and thymine. An electron withdrawing effect of substituents has been found for 6-chloromethyluracil and diethyl 6-uracilmethylphosphonate.

Key words: copper, calcium, magnesium, uracil ligands, coordination modes, substituent effects

Pyrimidine bases are ligands of biological interest as fragments of nucleosides and nucleotides [1,2]. Moreover, some of them exhibit anti-tumor activity, *e.g.* 5-fluorouracil [3,4], widely used for the treatment of tumors alone or in combination chemotherapy with platinum drugs (for instance oxaliplatin/5-FU in the case of colorectal cancer) [5]. Metal complexes of phosphonate ligands of heterocyclic systems have recently attracted interest due to their significant antitumor activity [6]. Platinum complexes incorporating pyridylmethyl phosphonate ligands showed *in vivo* cytostatic activity against (Sa 180 sarcoma) solid tumors [7,8].

In spite of the high biological relevance of pyrimidine derivatives, the metal-ligand interactions in aqueous solutions were reported only for uracil and thymine [9]. We describe herein the investigation of complex equilibria in copper(II), calcium(II) and magnesium(II) – ligand systems, where HL = uracil, thymine (5-methyluracil), and other commercially available compounds (5-chloromethyluracil, 6-chloromethyluracil, 5-hydroxymethyluracil, 6-methyluracil) but also the new phosphonate derivative [10]: diethyl 6-uracilmethylphosphonate (Figure 1).

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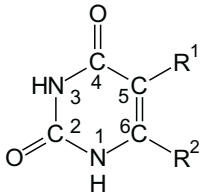

R^1 = H ; R^2 = H : uracil (2,4 dihydroxypyrimidine)
R^1 = Me ; R^2 = H : thymine (2,4 dihydroxy-5-methylpyrymidine)
R^1 = H ; R^2 = CH_2Cl : 6-chloromethyluracil
R^1 = CH_2OH ; R^2 = H : 5-hydroxymethyluracil
R^1 = H ; R^2 = Me : 6-methyluracil
R^1 = H ; R^2 = $CH_2P(O)(OEt)_2$: 6-umpe; diethyl 6-uracilmethylphosphonate

Figure 1. Chemical structure of uracil system derivatives.

Among the metals selected here, copper(II) is one of the trace metals involved in the living systems in numerous electron transfer and redox processes, whereas calcium(II) and magnesium(II) belong to the most abundant metals of essential biological significance.

Previously [11] the stability constants of the Mg^{2+} and Ca^{2+} complexes with 5-umpa^{2-} and 6-umpa^{2-} (where 5-umpa^{2-} = 5-uracilmethylphosphonate, 6-umpa^{2-} = 6-uracilmethylphosphonate) in aqueous solution were determined by potentiometric pH titrations. In order to broaden the scope of this work and to determine the effects of the additional substituents, *i.e.* Me, CH_2Cl, CH_2OH, $CH_2P(O)(OEt)_2$, we have now extended our studies to some derivatives of uracil system. The results have been compared with the literature data (accessible only for uracil and thymine but different in the proposed coordination modes) [12–20].

EXPERIMENTAL

Reagents: Uracil and thymine, both pure, were purchased from Sigma; 6-chloromethyluracil, 5-hydroxymethyl uracil and 6-methyluracil, also pure, were obtained from Aldrich. Diethyl 6-uracilmethylphosphonate (6-umpe) was synthesized as described [10]. The purity of the ligands was checked potentiometrically. The stock copper(II), calcium(II) and magnesium(II) nitrates solutions were standardized complexometrically using disodium salt of ethylenediaminetetraacetic acid in presence of murexide. Sodium hydroxide, Merck, 0.1 M solution was ppa – prior to the titrations involved in calculations of formation constants the content of carbonates was controlled by the Gran method [21] incorporating the data of EMF = f(–log [H^+]) acid – base calibrations.

Potentiometric studies: The protonation constants of the ligands and the stability constants of the copper(II) complexes were determined by pH-metric titration of 3 and 4 cm^3 samples, at temperature 25 ± 0.1°C. The total concentration of the metal in each sample ranged within 2.0–6.0×10^{-3} mol dm^{-3} and the ligand to metal ratio was 2:1, 3:1 and 4:1. The ionic strength was adjusted to 0.1 mol dm^{-3} with KNO_3. The titrations were carried out with carbonate-free NaOH solution of known concentration (0.1 mol dm^{-3}). The value of pK_w = 13.77 resulting from our acid – base calibrations was in full agreement with the one reported in [9] for the same conditions.

The pH was measured with a Molspin Ltd (Newcastle upon Tyne, England) automatic titration set and combined CMAWL/4/5/S7 electrode (Russell pH Limited, Auchtermuchty, Scotland). The total volume of the Hamilton microsyringe in the autoburette was 250 μl, the volume increments amounted to 0.0025 ml. The titrations were performed by using MOLSPIN.EXE software.

The electrode was calibrated in the $-\log [H^+]$ scale by titration of a 0.005 M HNO_3 (adjusted to I = 0.1 M by KNO_3) with 0.1 M NaOH, temp. 25°C. Then concentration overall stability constants $\beta_{mlh} = [M_mL_lH_h]/[M]^m[L]^l[H]^h$ were calculated by the SUPERQUAD computer program [22].

Spectroscopic studies: The electronic absorption spectra of thermostated solutions were recorded on a Pye Unicam UV-4 double beam spectrophotometer operated by VISION Software. Silica cells (Philips) of 4 cm path length were used. The samples of total initial volume 10 cm^3 (I = 0.1 M; KNO_3, $C_M = 5.0\times10^{-3}$ mol dm^{-3}, $C_L = 1.0\times10^{-2}$ mol dm^{-3}) were titrated by adding equal portions of 0.1 M NaOH. Then the absorption data files were used in the HYPERQUAD computer program [22]. An analogous titration was carried out in absence of the ligand but under the same conditions.

Electron paramagnetic resonance (EPR) spectra were performed at 77 K on a Bruker ESP 300E spectrometer at the X-band frequency (about 9.45 GHz) and equipped with the Bruker NMR gauss meter ER 035M and the Hewlett-Packard microwave frequency counter HP 5350B. The spectra were analysed by a computer simulation program WINEPR SimFonia Version 1.25 provided and copyrighted by Bruker Instruments, Inc.

The EPR spectra were performed in water solutions containing the total metal concentrations and the ligand to metal concentration ratios comparable with those described for the potentiometric experiments.

RESULTS AND DISCUSSION

Potentiometric determination of the protonation constants: Prior to measurement of the metal–ligand systems, titrations of the ligands alone were carried out in solution. As a result it followed, in agreement with [18,19], that all of them behave as monoprotonated ligands. The described up to now spectroscopic data for uracil and thymine indicate the N3 hydrogen as the dissociating proton in the predominating dioxo tautomer [23].

Table 1. Protonation constants [a] of uracil and its derivatives at 25°C, I = 0.1 mol×dm^{-3} (KNO_3). Solvent: H_2O.

Ligand	log K	Number of points	Fitting [b]		Literature [c]
			σ	χ^2	
uracil	9.349(3)	190	5.13	27.49	9.46 [15]
thymine	9.669(3)	220	6.82	24.81	9.85 [15]
6-chloromethyluracil	8.475(3)	190	7.72	12.56	–
5-hydroxymethyluracil	9.265(1)	180	2.37	23.32	–
6-methyluracil	9.606(1)	160	2.03	27.31	–
6-umpe	8.194(1)	160	3.05	14.33	–

[a] log K(N3H); [b] data for each ligand included in one refinement; [c] referring to the same temperature and medium.

The protonation constants reported here (Table 1) are in reasonable agreement with those of [15], since the differences do not exceed 0.2 log unit. As seen from Table 1, among all the ligands under study the most acidic ones are evidently 6-chloromethyluracil and 6-umpe. In each of them R^2 protons are substituted by either a chloromethyl or ethylphosphonate group. Hence, probably due to the known inductive and electron-withdrawing effect of such groups [24,25] the ligands become more acidic.

Copper(II)

Potentiometric data: The titrations with copper(II), just like for the other metals, were carried out at a small excess of hydrogen ions, which means that the total amount of protons consisted of ligand N3H as well as of $0.75–1.0\times10^{-3}$ mmole of HNO_3 per sample volume 3 or 4 cm^3. Thus the titrations started at pH ~3.5. In the case of copper(II) above pH 6.5 a precipitate was observed – probably, as it follows from other similar studies [12,19], copper(II) hydroxide. In spite of that the SUPERQUAD refinements lead to convergent results within the pH range ~5.5 – ~6.5 (Table 2). Apart of the known aquo-ion hydrolysis constant ($\log \beta_{10\text{-}1} = -7.223$) [26], the model providing the best fit of experimental data included the formation of monomeric ML (*i.e.* 110) and MLH_{-1} (*i.e.* 11-1) species, where the 11-1 may be obtained from 110 after the loss of a proton of one water molecule in the metal coordination sphere – promoted by the bipositively charged metal ion. Since:

$$\beta_{11\text{-}1} = \frac{[MLH_{-1}][H]}{[M][L]} \qquad \text{and} \qquad \beta_{110} = \frac{[ML]}{[M][L]}$$

the difference $\Delta \log \beta_{mlh} = \log \beta_{110} - \log \beta_{11\text{-}1}$, denotes the exponent of deprotonation constant $pK^{ML}_{MLH_{-1}}$ of reaction (1) and also the approximate pH of equal concentrations [CuL] and $[CuLH_{-1}]$ in equilibrium. The values $pK^{ML}_{MLH_{-1}}$ were similar for all the ligands – on the average 6.0 ± 0.1:

$$ML \rightleftarrows MLH^{-}_{-1} + H^{+} \qquad (1)$$

The ML_2 complexes were accepted only for few, single titration files. Moreover, other species, *e.g.* the protonated MLH and dimeric M_2L_2, were rejected in every case.

Table 2. Formation constants of Cu(II) complexes with uracil and its derivatives at 25°C, $I = 0.1$ mol×dm^{-3} (KNO_3). Solvent: H_2O. $L = L^-$ denotes the ligand deprotonated at N3H. Standard deviations in parentheses.

Ligand	Species ($Cu_mL_lH_h$)	$\log \beta_{mlh}$	$pK^{ML}_{MLH_{-1}}$	Number of titrations	Literature
uracil	1 1 0 [a]	4.21(6)	6.12	17	4.55 [c], 4.97 [d]
	1 1 -1 [b]	–1.91(5)			–
thymine	1 1 0 [a]	4.23(6)	5.85	16	5.80 [e]
	1 1 -1 [b]	–1.62(3)			–
6-chloromethyluracil	1 1 0 [a]	3.43(6)	6.00	10	–
	1 1 -1 [b]	–2.57(7)			–
5-hydroxymethyluracil	1 1 0 [a]	4.07(7)	6.00	16	–
	1 1 -1 [b]	–1.93(6)			–
6-methyluracil	1 1 0 [a]	4.11(9)	5.88	12	–
	1 1 -1 [b]	–1.77(7)			–
6-umpe	1 1 0 [a]	2.94(6)	5.88	11	–
	1 1 -1 [b]	–2.94(9)			–

[a] equilibrium: $Cu^{2+} + L^- \rightleftarrows CuL^+$; [b] equilibrium: $Cu^{2+} + L^- \rightleftarrows CuLH_{-1} + H^+$;
[c] 37°C, 0.15 M $NaNO_3$ [12]; [d] 25°C, 0.10 M $NaNO_3$ [13]; [e] 45°C, 0.10 M KNO_3 [19].

The log β_{110} values of uracil and thymine in Table 2 may be compared with the literature data obtained under somewhat different conditions. In the case of uracil the stability constant is in some agreement with those of Casassas *et al.* [12] and M'Boungu *et al.* [13], but for thymine it is significantly lower than in [19]. As seen in Table 2, the CuL formation constants determined by us are the lowest for 6-chloromethyluracil and 6-umpe. At the same time our overall β_{11-1} constants for those ligands are the highest in absolute values. This result mentioned here clearly corresponds to the observed reduced basicity of 6-chloromethyluracil and 6-umpe (Table 1). On the other hand, it should be stressed that the β_{11-1} formation constants were not reported up to now in the literature for Cu(II) – pyrimidine ligand interactions, although Casassas *et al.* [12] confirmed the formation of such MLH_{-1} species for Zn(II).

The species distribution curves as function of pH indicate that the content of the CuL and $CuLH_{-1}$ species in the equilibrium mixture was relatively low even at the highest attainable pH (*e.g.* as in Figure 2) but the statistical parameters were acceptable ($\sigma < 3$ and $\chi^2 < 12.60$ at significance level 0.05).

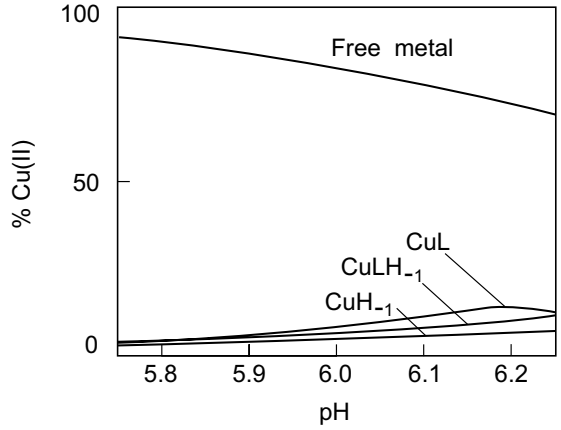

Figure 2. Species distribution diagram for the Cu(II)–thymine system. $C_{Cu} = 0.005$ mol dm^{-3}, $C_{ligand} = 0.015$ mol dm^{-3}; $\sigma = 1.90$, $\chi^2 = 4.00$

Spectroscopic measurements: The spectrophotometric titrations of Cu(II) species evidenced an increase in absorbance and a blue shift of the band maximum from *ca* 800 nm up to *ca* 760 nm. Owing to the precipitation observed at pH > 6 the molar neutralization coefficient was limited to $a \sim 0.1–0.2$, *i.e.* pH = 5.82–6.03, as seen for instance in Figure 3a.

At the same time the measured absorbances appeared to be relatively low as the solubility of the ligand limited the used concentration of copper(II). In spite of unsatisfying statistical parameters, formation of the CuL complex has been confirmed, however, for all the ligands by attaining convergence in the HYPERQUAD refinements. From the other assumed complexes the deprotonated $CuLH_{-1}$ species could also be indicated by the spectrophotometric method but only in some experiments.

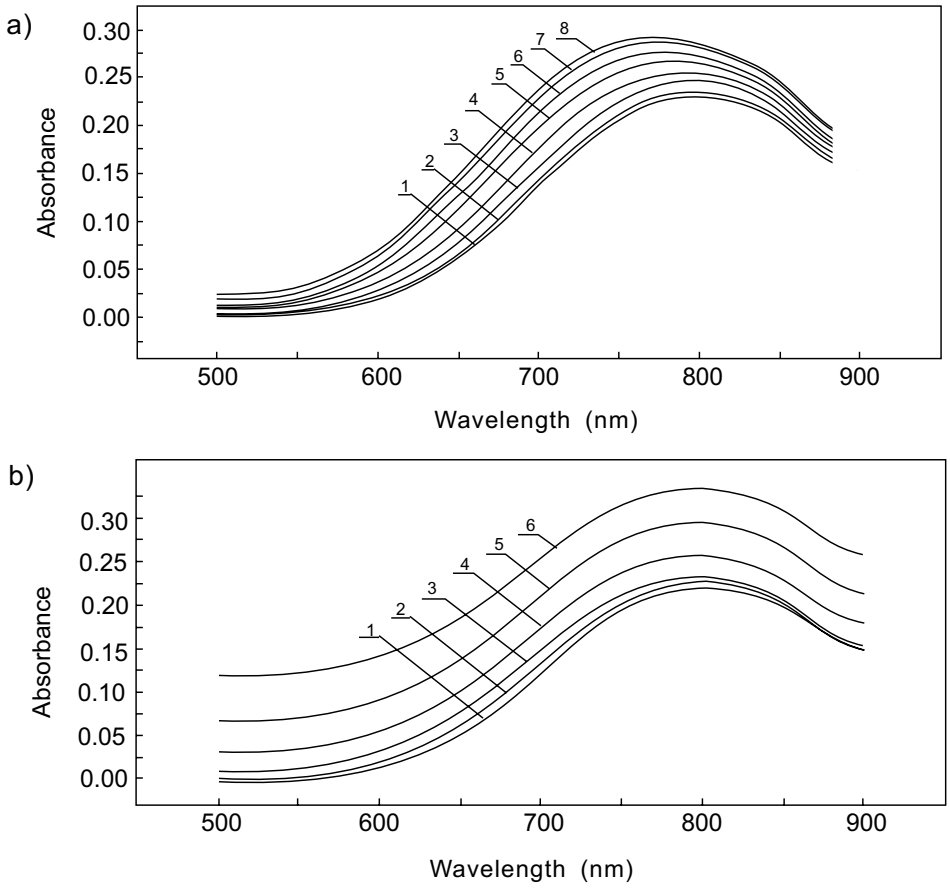

Figure 3. a) Spectrophotometric titration of Cu(II)-5-hydroxymethyluracil. C_{Cu} = 0.005 mol dm^{-3}, C_{ligand} = 0.010 mol dm^{-3}. Path length l = 4 cm. Molar neutralization coefficient a = mmole of base / mmole of ligand and pH: 1– 0.01; 5.82, 2 – 0.02; 5.86, 3 – 0.04; 5.92, 4 – 0.06; 5.95, 5 – 0.09; 5.98, 6 – 0.11; 6.0, 7 – 0.13; 6.02, 8 – 0.14; 6.03
b) Spectrophotometric titration in absence of ligand. C_{Cu} = 0.005 mol dm^{-3}. Path length l = 4 cm. pH: 1 – 5.13, 2 – 5.56, 3 – 5.75, 4 – 5.78, 5 – 5.76, 6 – 5.73

This result may be easily explained on the basis of the described potentiometric results as the deprotonated complex predominates in a relatively higher pH, where the rising concentration of low soluble copper(II) hydroxides makes the absorption measurements impossible. The lack of splitting in the observed consecutive *d-d* absorption spectra (Figure 3a) and the shift towards higher energies during alkalization corresponds to a transformation of the $[Cu(aqua)]^{2+}$ ion to complex species of similar, tetragonal symmetry but higher ligand field power [27]. Comparatively a titration in absence of the ligand but with the same portions of 0.1 M NaOH has been performed (Figure 3b). In this case the precipitation became clearly visible already at pH ~ 5.7 and the hypsochromic shift in maximum was not observed. Further addition of base caused a decrease in pH.

The formation of the complexes between Cu(II) ions and uracil or its derivatives as ligands is distinctly evidenced by the characteristic change of the EPR spectra of frozen solutions (containing Cu(II) ions and the ligands) as a function of pH in comparison with the spectrum of Cu(II) ions surrounded by water molecules (Figure 4, Table 3). The axial character of the EPR spectra with $g_{II} >> g_{\perp} > 2.0023$ and $A_{II} >> A_{\perp}$ is typical for a tetragonal geometry of Cu(II) complexes and the $d_{x^2-y^2}$ orbital of copper unpaired electron.

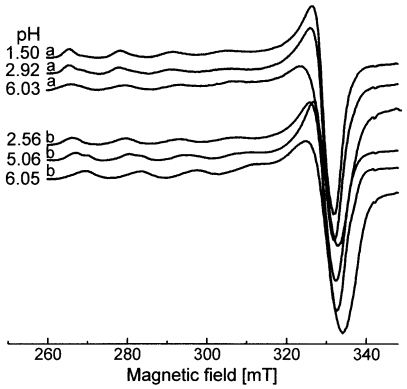

Figure 4. EPR spectra of the complexes formed in water solutions containing Cu(II) ions and uracil at pH ≤ 6.03 (a) or tymine at pH ≤ 6.05 (b) as a function of pH at 77 K.

Table 3. EPR parameters of Cu(II) complexes with uracil or uracil derivatives [a), b)].

Ligand	pH	g_{II}	$g_{\perp}$	A_{II}	$A_{\perp}$
uracil	1.19–2.24	2.403	2.077	140	10
	2.92	2.403	2.077	140	10
		(2.38)	(2.08)	(150)	(10)
	5.26–6.02	2.385	2.074	149	10
thymine	2.56–4.55	2.387	2.074	149	10
	5.08	2.387	2.074	149	10
		2.354	2.068	152	10
	5.42–6.43	2.354	2.068	152	10
6-methyluracil	2.00	2.400	2.076	142	10
		(2.365)	(2.076)	(145)	10
	5.5–5.8	2.404	2.076	140	10
		2.363	2.074	146	10
	6.2–6.5	2.363	2.074	146	10
6-chloromethyluracil	1.92	2.404	2.077	142	10
	2.54	2.384	2.077	147	10
		2.357	2.073	149	10
	5.38	2.384	2.073	147	10
		2.357	2.064	149	10
5-hydroxymethyluracil	1.65–3.88	2.404	2.075	140	10
	4.87	2.383	2.065	145	10
6-umpe	1.57–2.70	2.404	2.074	142	10
	6.08	2.404	2.074	142	10
		2.368	2.068	146	10

[a)] The A parameters are given in $10^{-4}cm^{-1}$ units. [b)] The second set of the parameters corresponds to the complex in equilibrium; the parentheses indicate much smaller content of the second complex.

$[Cu(aqua)]^{2+}$ with the parameters $g_{II} = 2.404$ and A_{II} about 140×10^{-4} cm^{-1} is observed at the lowest pH about 2 as dominant. The additional signals present in the spectra of the acidic solutions suggest formation of the first complex of a very small content. For Cu-thymine and Cu-6-chloromethyluracil systems, owing to the high sensitivity of the EPR spectroscopy, already at pH about 2.5 the spectra correspond to some contribution of the complex species. A change of the spectra upon pH rising is consistent with an increasing concentration of the complexes. Further decrease of g_{II} and a slight increase of A_{II} parameters suggest the formation of a new complex, except of Cu-uracil and Cu-6-hydroxymethyluracil systems, where under the reached pH spectra remain unchanged. At pH above 5.5 the spectra reveal the equilibrium between two complexes in the case of Cu-thymine and Cu-6-chloromethyluracil systems, a significant domination of the first complex over $[Cu(aqua)]^{2+}$ for the Cu-uracil and Cu-5-hydroxymethyluracil systems and a predominance of the second complex over the first one for Cu-6-methyluracil system. Taking into consideration the attained pH, the formation of two complexes detected in the EPR spectra strongly supports the results of analysis of the potentiometric titration data. The values of the EPR spectral parameters assigned to the complexes suggest that a weak ligand field is provided by only one ligand involved in Cu(II) coordination. The parameters observed for the first complex (g_{II} about 2.38 and A_{II} within the range $147–149\times10^{-4}$ cm^{-1}) are comparable to those reported for 1:1 Cu(II) complex with adenosine [28]. Moreover, the spin Hamiltonian parameters are similar to those evidenced for Cu(II) complexes with one substituted imidazole ligand ($g_{II} = 2.360$ and $A_{II} = 145\times10^{-4}$ cm^{-1}) [29] and are significantly different from those found previously for the complex containing two substituted imidazoles in Cu(II) coordination sphere ($g_{II} = 2.325$ and $A_{II} = 154\times10^{-4}$ cm^{-1}) [30]. Hence, the EPR data imply that only one nitrogen atom is coordinated in Cu(II) plane in both the complexes, leading to a weaker Cu(II) binding than that provided by the imidazole nitrogen. A decrease of g_{II} and a slight increase of A_{II} for the second complex is most likely produced by the substitution of water molecule by OH^- group [31] in accordance with the complex formulae predicted by the analysis of potentiometric titration data.

Calcium(II) and magnesium(II)

The interactions with Ca(II) and Mg(II) were investigated potentiometrically. The titrations with calcium and magnesium indicated a much higher degree of complexation (*i.e.* the complexed metal to total metal concentration ratio) than the one with Cu(II) – up to 90% and up to 80%, respectively (Figures 5 and 6). Moreover, except of HL = 6-umpe, for both metals the ML_2 complexes have been confirmed besides the ML and MLH_{-1} species.

The pH range of complexation, where precipitation had not been observed, was much wider than that in the case of Cu(II) and amounted to 7.5–9.5 for Ca(II) and 8–10.5 for Mg(II). The values of formation constants could be related to the literature data only for $\log \beta_{110}$ – Tables 4 and 5. As can be seen in comparison with Table 2, they were of two orders of magnitude lower than for copper(II). Hence, for 6-umpe not all of the metal – ligand interactions found for the other ligands were detectable.

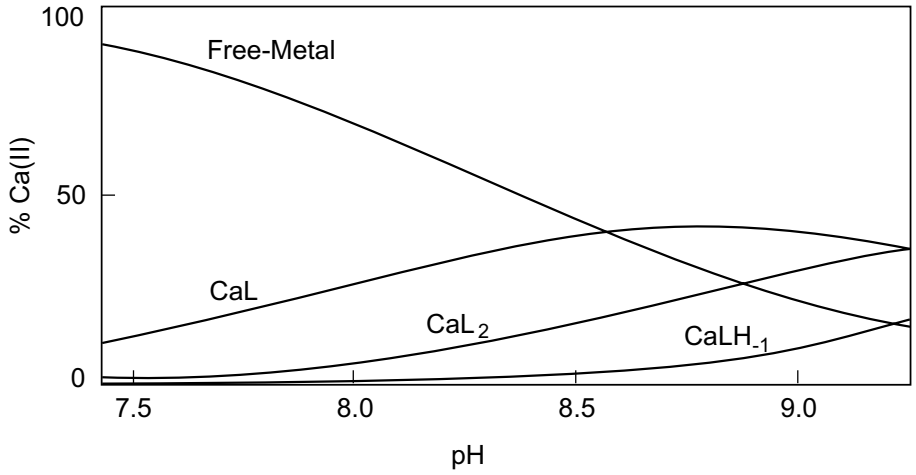

Figure 5. Species distribution diagram for the Ca(II)–diethyl 6-uracilmethylphosphonate system. C_{Ca} = 0.003 mol dm^{-3}, C_{ligand} = 0.009 mol dm^{-3}; σ = 2.95, χ^2 = 7.33.

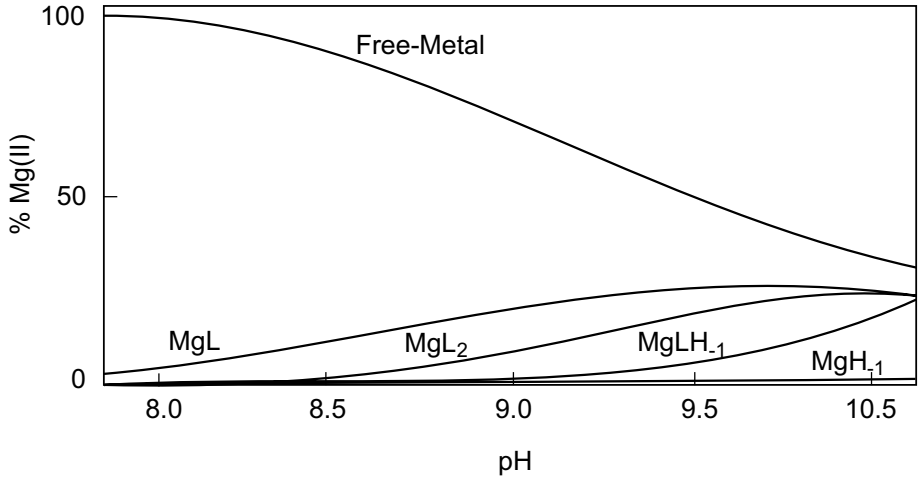

Figure 6. Species distribution diagram for the Mg(II)–methyluracil system. C_{Mg} = 0.003 mol dm^{-3}, C_{ligand} = 0.006 mol dm^{-3}; σ = 2.82, χ^2 = 4.70.

The refinements confirmed only the ML and MLH_{-1} species in the case of Ca(II) and MLH_{-1} for Mg(II). Quite a good agreement, however, may be observed when comparing the value of log β_{110} for Ca(II) – 1.1 ± 0.1 with the corresponding constant reported in [11] for 6-umpa^{2-}: 1.40 ± 0.05. This result supports the lack of O4 – phosphonate group chelation stated for 6-umpa^{2-} on contrary to 5-umpa^{2-}, where the formation of a seven-membered ring was found [11].

Table 4. Formation constants of Ca(II) complexes with uracil and its derivatives at 25°C, $I = 0.1$ mol×dm^{-3} (KNO_3). Solvent: H_2O. L = L^- denotes the ligand deprotonated at N3H. Standard deviations in parentheses.

Ligand	Species ($Ca_mL_lH_h$)	log β_{mlh}	$pK^{ML}_{MLH_{-1}}$	Number of titrations	Literature
uracil	1 1 0 [a]	2.19(2)	9.68	6	2.4 [d]
	1 1 -1 [b]	–7.49(5)			–
	1 2 0 [c]	4.32(5)			–
thymine	1 1 0 [a]	2.40(8)	10.04	11	2.92 [e]
	1 1 -1 [b]	–7.64(5)			–
	1 2 0 [c]	4.51(5)			–
6-chloromethyluracil	1 1 0 [a]	2.54(3)	8.32	10	–
	1 1 -1 [b]	–5.78(9)			–
	1 2 0 [c]	4.72(9)			–
5-hydroxymethyluracil	1 1 0 [a]	2.26(3)	9.35	8	–
	1 1 -1 [b]	–7.09(8)			–
	1 2 0 [c]	4.37(7)			–
6-methyluracil	1 1 0 [a]	2.34(4)	9.51	12	–
	1 1 -1 [b]	–7.17(8)			–
	1 2 0 [c]	4.44(7)			–
6-umpe	1 1 0 [a]	1.1(1)	8.8	6	–
	1 1 -1 [b]	–7.7(1)			–
	1 2 0 [c]	–			–

[a] equilibrium: $Ca^{2+} + L^- \rightleftharpoons CaL^+$; [b] equilibrium: $Ca^{2+} + L^- \rightleftharpoons CaLH_{-1} + H^+$; [c] equilibrium: $Ca^{2+} + 2\,L^- \rightleftharpoons CaL_2$; [d] 45°C, 0.1 M KNO_3 [19]; [e] 25°C, 0.1 M KNO_3 [15].

Table 5. Formation constants of Mg(II) complexes with uracil and its derivatives at 25°C, $I = 0.1$ mol×dm^{-3} (KNO_3). Solvent: H_2O. L = L^- denotes the ligand deprotonated at N3H. Standard deviations in parentheses.

Ligand	Species ($Mg_mL_lH_h$)	log β_{mlh}	$pK^{ML}_{MLH_{-1}}$	Number of titrations	Literature
uracil	1 1 0 [a]	2.30(3)	9.68	7	2.6 [d]
	1 1 -1 [b]	–7.38(9)			–
	1 2 0 [c]	4.23(3)			–
thymine	1 1 0 [a]	2.71(6)	9.31	10	2.8 [e], 3.06 [f]
	1 1 -1 [b]	–6.6(1)			–
	1 2 0 [c]	5.30(9)			–
6-chloromethyluracil	1 1 0 [a]	2.33(3)	8.72	10	–
	1 1 -1 [b]	–6.37(5)			–
	1 2 0 [c]	4.57(7)			–
5-hydroxymethyluracil	1 1 0 [a]	2.13(7)	9.67	10	–
	1 1 -1 [b]	–7.54(9)			–
	1 2 0 [c]	4.1(1)			–
6-methyluracil	1 1 0 [a]	2.41(2)	9.78	10	–
	1 1 -1 [b]	–7.37(5)			–
	1 2 0 [c]	4.70(3)			–
6-umpe	1 1 0 [a]	–	–	6	–
	1 1 -1 [b]	–8.3(1)			–
	1 2 0 [c]	–			–

[a] equilibrium: $Mg^{2+} + L^- \rightleftharpoons MgL^+$; [b] equilibrium: $Mg^{2+} + L^- \rightleftharpoons MgLH_{-1} + H^+$; [c] equilibrium: $Mg^{2+} + 2\,L^- \rightleftharpoons MgL_2$; [d] 45 °C, 0.1 M KNO_3 [19]; [e] 45 °C, 0.1 M KNO_3 [19]; [f] 35 °C, 0.1 M KNO_3 [17].

The values of $pK^{ML}_{MLH_{-1}} = \Delta \log \beta_{mlh} = \log \beta_{110} - \log \beta_{11\text{-}1}$ *ca* 8–10 (Tables 4 and 5) were distinctly lower than the corresponding aqua-ion hydrolysis constants under close to our conditions ($\log \beta_{10\text{-}1}$ = 11.7 for Ca and 11.5 for Mg) [32], which may explain the lack of precipitation within the main pH range of complexation. As it follows then the titrations could be carried out without precipitation to relatively high pH values and the ML and ML_2 complexes were detected in more significant amounts than it was possible for Cu(II). Thus, the part of complexed metal was predominating, leading to a higher degree of complexation despite lower formation constants.

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