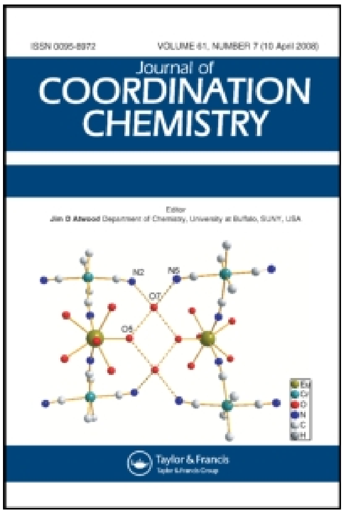


Journal of Coordination Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455674

Complexes of cadmium(II) and mercury(II) ions with cytidine or cytidine-5'-monophosphate in ternary systems including tetramines

Romualda Bregier-Jarzebowska[a]; Lechoslaw Lomozik[a]
[a] Faculty of Chemistry, A. Mickiewicz University, 60-780 Poznan, Grunwaldzka 6, Poland

To cite this Article Bregier-Jarzebowska, Romualda and Lomozik, Lechoslaw(2006) 'Complexes of cadmium(II) and mercury(II) ions with cytidine or cytidine-5'-monophosphate in ternary systems including tetramines', Journal of Coordination Chemistry, 59: 12, 1413 — 1426

To link to this Article: DOI: 10.1080/00958970500512906

URL: http://dx.doi.org/10.1080/00958970500512906

Journal of Coordination Chemistry
Vol. 59, No. 12, 5 August 2006, 1413–1426

Complexes of cadmium(II) and mercury(II) ions with cytidine or cytidine-5′-monophosphate in ternary systems including tetramines

ROMUALDA BREGIER-JARZEBOWSKA and LECHOSLAW LOMOZIK*

Faculty of Chemistry, A. Mickiewicz University, 60-780 Poznan, Grunwaldzka 6, Poland

(*Received in final form 10 October 2005*)

Interactions between Hg(II) and Cd(II) ions in binary systems with 3,3,3-tet (1,11-diamino-4,8-diazaundecane) and in ternary systems with Cyd (cytidine) or CMP (cytidine-5′-monophosphate) and 3,3,3-tet or Spm (1,12-diamino-4,9-diazadodecane, spermine) have been studied. The presence of the following species has been detected: MH_x(3,3,3-tet), M(3,3,3-tet), M(3,3,3-tet)$(OH)_x$ and M(Cyd or CMP)(PA), M(Cyd or CMP)(H_xPA) (M = Cd or Hg, PA = 3,3,3-tet or Spm). Overall stability constants (log β) have been determined and equilibrium constants for complex formation (log K_e) calculated. The mode of coordination in these species has been proposed on the basis of the equilibrium and ^{13}C NMR, ^{31}P NMR and IR studies. Unlike the biogenic amine Spm, its homologue 3,3,3-tet coordinates with Cd(II) and Hg(II) ions with the involvement of all available donor nitrogen atoms. In Cd(Cyd)(3,3,3-tet) the {N5} chromophore is formed, while in Cd(Cyd)(Spm) the {N4} chromophore is involved (only three nitrogen atoms from Spm are involved in metallation). Introduction of the polyamine into the system Cd(II)/CMP (at pH below 7) leads to changes in coordination mode in the anchoring Cd(CMP) complex and the phosphate group of CMP is involved in bonding. In ternary systems, Hg(II)/Cyd(or CMP)/tetramine, the nitrogen atom N(3) and the phosphate group of the nucleotide take part in coordination. The presence of PA in the inner coordination sphere does not change the mode of metal–CMP coordination with respect to that in the binary system.

Keywords: Cadmium(II); Mercury(II); Cytidine; Cytidine-5′-monophosphate; Tetramines; Stability constants; Spectroscopy

1. Introduction

One of the important and still insufficiently explained problems of bioinorganic chemistry is the mode of coordination of complexes formed in ternary systems between metal ions, nucleosides or nucleotides, and polyamines. Reactions of metal ions with nucleic acids, nucleotides or nucleosides are fundamental for many chemical processes in living organisms [1, 2] and hence are important from the biological and clinical points of view [3, 4]. Nucleotides and metal ions take part in many enzymatic reactions

---

*Corresponding author. Email: lomozik@amu.edu.pl

Journal of Coordination Chemistry
ISSN 0095-8972 print: ISSN 1029-0389 online © 2006 Taylor & Francis
DOI: 10.1080/00958970500512906

as co-factors [5]. Moreover, reactions of metal ions with polyamines and nucleotides in biological systems influence genetic information transfer [6–14]. Biogenic amines, Put (putrescine), Spd (spermidine) or Spm (spermine) occur in practically all cells of living organisms [15] at relatively high concentrations, while concentrations of simple structural homologues tn (1,3-diaminopropane), cadaverine, homospermidine, 3,3,3-tet (1,11-diamino-4,8-diazaundecane) or thermospermine are much lower [12, 16, 17]. Because of the high basicity of polyamines they are protonated under physiological conditions and in such form interact with negatively-charged fragments of other biomolecules. The latter include the donor nitrogen atoms of purine and pyrimidine bases of nucleosides and nucleotides, phosphate groups of nucleic acids and components of biological membranes such as acidic phospholipids or protein residues [18]. It has been proven that biogenic amines stabilise nucleic acids [19–21] by inhibiting their thermal denaturation [22] and enzymatic degradation [23]. Recently, particular interest has been shown in the interactions of heavy metal ions like cadmium(II) and mercury(II) with nucleic acids [24–27], because of risk of deformation of the DNA structure accompanied by a permanent change in gene expression, which can lead to neoplasmic changes [23].

The results presented below are a continuation of earlier studies of systems consisting of Hg(II) or Cd(II) with Cyd (cytidine) or CMP (cytidine-5′-monophosphate) and polyamines [28–30] and concerns Hg(II) or Cd(II) in binary systems with 3,3,3-tet and in ternary systems with *Cyd* or CMP and Spm or 3,3,3-tet investigated by potentiometric and spectroscopic methods.

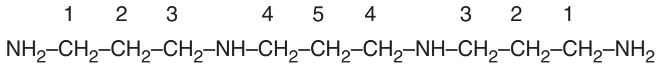
2. Experimental

1,11-Diamino-4,8-diazaundecane (3,3,3-tet) and 1,12-diamino-4,9-diazadodecane (spermine, Spm) were purchased from Merck. Tetramine nitrates were obtained by dilution of an apppropriate amount of free amine and addition equimolar amounts of HNO_3. The white powder obtained was recrystallized, washed with methanol, dried over P_2O_5 or in air. Satisfactory elemental analyses were obtained (±0.5%). Cytidine (Cyd) and cytidine-5′-monophosphate (CMP) were purchased from Sigma. Cytidine nitrate was prepared in the same way as cytidine hydrochloride [31]. Cadmium(II) and mercury(II) salts used were $Cd(NO_3)_2 \cdot 4H_2O$ (Merck) and $Hg(NO_3)_2 \cdot H_2O$ (Aldrich). Concentrations of Hg(II) ions were determined by chloride solution using diphenylcarbazone as indicator, while concentrations of Cd(II) ions were determined complexometrically using EDTA and pyrocatechol violet as indicator. Potentiometric measurements were performed on Metrohm Titrino 702 with a glass electrode (Metrohm 60233 100, 3 M KCl) and calibrated in terms of hydrogen ions concentration [32]. The concentration of ligands in the titrated systems was in the range 1.3×10^{-3} to 2.7×10^{-3} M, the concentration of metal ions was 1.3×10^{-3}–1.4×10^{-3} M, the molar ratio $M:L:L'$ ($M =$ metal, $L =$ nucleoside or nucleotide, $L' =$polyamine) was either 1 : 1 : 1 or 1 : 2 : 2. Ionic strength was maintained at $\mu = 0.1$ M (KNO_3), at $T = 20 \pm 1°C$ in a helium atmosphere. Titrations used CO_2-free $\sim 0.2\,\mathrm{mol\,dm^{-3}}$ NaOH solutions; addition of the NaOH solution did not change the ionic strength (measurements were performed starting from totally protonated polyamine so the $–NH_x^+$ cations were replaced by equivalent amounts of Na^+).

Calculations were made for 100–150 points for each run in the pH range in which no precipitation occurred. Protonation constants of ligands and stability constants of complexes formed were determined using the SUPERQUAD program [33], while the distribution of particular species was obtained by using the HALTAFALL program [34]. Model selection and verification was made according to a documented description [35]. Samples for NMR and IR studies were prepared by dissolving an appropriate amount of the ligand and metal ions in D_2O, adjusting the pH by addition of NaOD or DNO_3. The pH reading (Mera-Tronik N517) was corrected according to the formula pD = pH (reading) + 0.40 [36]. The ligand concentration in the NMR samples was 0.1 M. The ratio of the metal ion concentration to that of the ligand was 1 : 10 : 10 to 1 : 50 : 50. ^{13}C NMR spectra were recorded on a Varian Gemini 300VT spectrometer in the range 20–170 ppm, using dioxane as internal standard. ^{31}P NMR spectra were recorded on a Varian Unity 300 spectrometer with H_3PO_4 as reference. IR spectra were recorded using a Bruker IFS-113v spectrophotometer with a KRS5-50 cell.

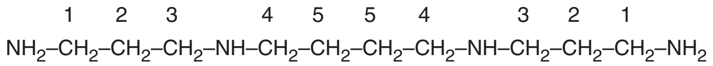
3. Results and discussion

The ligands studied have the following structures.



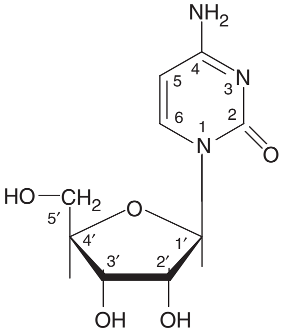
$NH_2\text{–}\overset{1}{CH_2}\text{–}\overset{2}{CH_2}\text{–}\overset{3}{CH_2}\text{–}NH\text{–}\overset{4}{CH_2}\text{–}\overset{5}{CH_2}\text{–}\overset{4}{CH_2}\text{–}NH\text{–}\overset{3}{CH_2}\text{–}\overset{2}{CH_2}\text{–}\overset{1}{CH_2}\text{–}NH_2$

3,3,3-tet

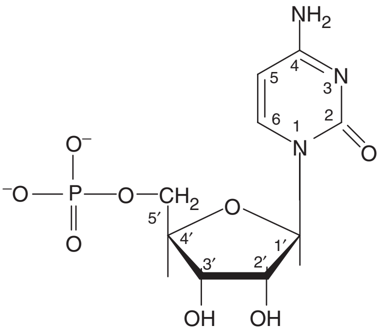


$NH_2\text{–}\overset{1}{CH_2}\text{–}\overset{2}{CH_2}\text{–}\overset{3}{CH_2}\text{–}NH\text{–}\overset{4}{CH_2}\text{–}\overset{5}{CH_2}\text{–}\overset{5}{CH_2}\text{–}\overset{4}{CH_2}\text{–}NH\text{–}\overset{3}{CH_2}\text{–}\overset{2}{CH_2}\text{–}\overset{1}{CH_2}\text{–}NH_2$

Spm



Cyd



CMP

Computer analysis of pH-metric titrations was performed taking into regard previously determined (under the same conditions) protonation constants of the ligands, stability constants of the complexes formed in binary systems, and equilibrium constants of the hydrolysed mercury(II) and cadmium(II) species $Hg(OH)^+$, $Hg(OH)_2$, $Cd(OH)^+$ and $Cd(OH)_2$ [37]. As discussed earlier, no reduction of Hg(II) to Hg(I) was observed [37, 38].

3.1. *Cd(II)/3,3,3-tet system*

Overall stability constants (log β) of cadmium(II) complexes with 3,3,3-tet, a homologue of the biogenic amine Spm, are presented in table 1. As reported earlier, in the interactions with di- and triamines, cadmium(II) ions show a tendency to coordinate of all available nitrogen atoms from the PA molecule. In systems with Spm, the metal ion is coordinated only by 3 nitrogen atoms in the ML species [37]. The composition of Cd($H_3$3,3,3-tet), the pH range of its occurrence (pH 5.0–8.5; figure 1a) and the protonation constants (table 1) indicate that only one nitrogen atom of the polyamine is available for coordination. As comparison of the stability constants of complexes with different degrees of protonation is meaningless, the effectiveness of interactions was assessed on the basis of the equilibrium constants of formation. The log K_e value of the triprotonated species is 2.79 ($\log K_e = \log \beta_{Cd(H_3 3,3,3\text{-tet})} - \log \beta_{H_3(3,3,3\text{-tet})}$, table 1) and is significantly lower than $\log K_e = 3.98$ of an analogous complex with 1,3-diaminopropane, Cd(tn) [37], in which two nitrogen atoms of triamine are involved in metallation, confirming the conclusion concerning nature of Cd($H_3$3,3,3-tet).

The ^{13}C NMR spectrum of Cd($H_3$3,3,3-tet) at pH 7, (C_1 0.150, C_2 0.090, C_3 0.083, C_4 0.107, C_5 0.043 ppm chemical shifts of carbon atoms relative to the spectrum of free ligand) suggests the occurrence of isomers with coordination through the first or second amine group. Deprotonation of the ligand at higher pH and participation of the additional two nitrogen atoms of 3,3,3-tet in cadmium(II) coordination results in an increase in the equilibrium constant of complex formation ($Cd + H3,3,3\text{-tet} \leftrightarrows Cd(H3,3,3\text{-tet})$; $\log K_e = \log \beta_{Cd(H3,3,3\text{-tet})} - \log \beta_{H(3,3,3\text{-tet})} = 5.0$), relative to that of the triprotonated species (table 1). The value $\log K_e = 5.0$ is similar to that calculated for Cd(Spd), $\log K_e = 4.82$, in which coordination was found to involve 3 nitrogen atoms [37].

The fully deprotonated complex Cd(3,3,3-tet) forms in the pH range 7.5–11 (figure 1a), binding at most about 65% of the metal at pH 9.0. The equilibrium constant of formation of the species $Cd + 3,3,3\text{-tet} \leftrightarrows Cd(3,3,3\text{-tet})$ is $\log K_e = 7.66$, table 1, and its increase by 2.66 of log K_e relative to that of Cd(H3,3,3-tet) indicates the involvement of the fourth nitrogen atom of PA in coordination. Such increase is a consequence of the involvement of one more nitrogen atom in coordination, in agreement with $\log K_e = 2.79$ of formation of Cd($H_3$3,3,3-tet); in this case only one

Table 1. Overall stability constants (log β) and equilibrium constants (log K_e) for complex formation in binary Cd(II), Hg(II)/3,3,3-tet systems ($T = 20 \pm 1$°C).

System	Species	log β	log K_e
3,3,3-tet [39]	H_4(3,3,3-tet)	36.39(2)	
	H_3(3,3,3-tet)	29.01(3)	
	H_2(3,3,3-tet)	20.38(3)	
	H(3,3,3-tet)	10.36(2)	
Cd(II)/3,3,3-tet	Cd($H_3$3,3,3-tet)	31.80(3)	2.79
	Cd(H3,3,3-tet)	15.36(5)	5.00
	Cd(3,3,3-tet)	7.66(7)	7.66
	Cd(3,3,3-tet)(OH)	−2.28(2)	
Hg(II)/3,3,3-tet	Hg(3,3,3-tet)(OH)	8.63(5)	
	Hg(3,3,3-tet)$(OH)_2$	−0.62(6)	

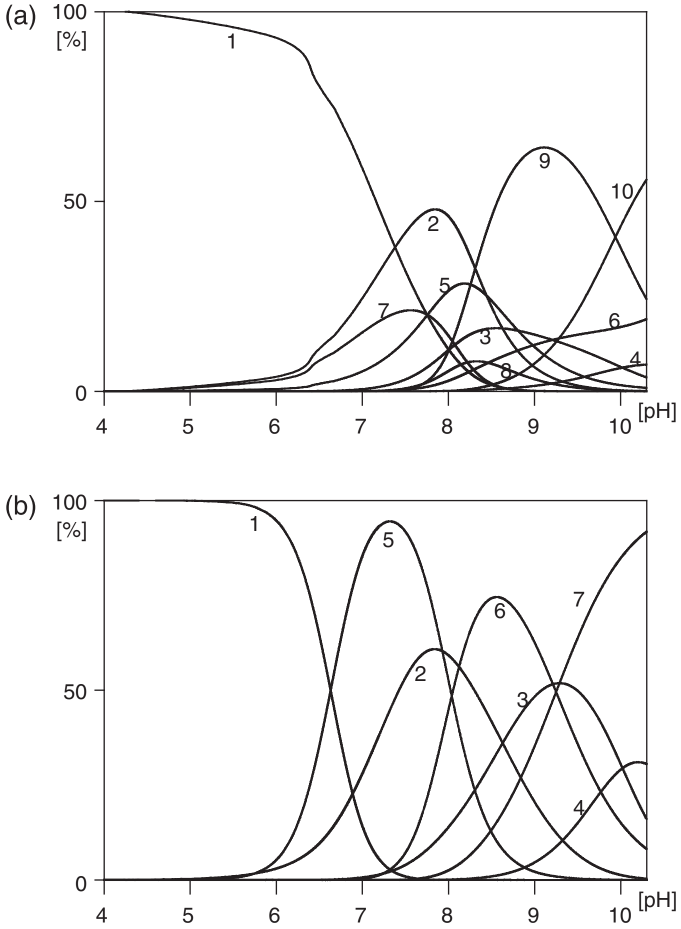


Figure 1. Distribution diagrams for the Cd(II)/3,3,3-tet and Hg(II)/3,3,3-tet systems; percentages of the species refer to total metal; (a) Cd(II)/3,3,3-tet: 1, $H_4$3,3,3-tet; 2, $H_3$3,3,3-tet; 3, $H_2$3,3,3-tet; 4, H3,3,3-tet; 5, $Cd(OH)^+$; 6, $Cd(OH)_2$; 7, Cd($H_3$3,3,3-tet); 8, Cd(H3,3,3-tet); 9, Cd(3,3,3-tet); 10, Cd(3,3,3-tet)(OH); $C_{Cd^{2+}} = 1.32 \times 10^{-3}$ M; $C_{3,3,3\text{-tet}} = 1.32 \times 10^{-3}$ M; (b) Hg(II)/3,3,3-tet: 1, $H_4$3,3,3-tet; 2, $H_3$3,3,3-tet; 3, $H_2$3,3,3-tet; 4, H3,3,3-tet; 5, $Hg(OH)_2$; 6, Hg(3,3,3-tet)(OH); 7, $Hg(3,3,3\text{-tet})(OH)_2$; $C_{Hg^{2+}} = 1.41 \times 10^{-3}$ M; $C_{3,3,3\text{-tet}} = 5.34 \times 10^{-3}$ M.

nitrogen atom takes part in coordination. At pH 9.0, where Cd(3,3,3-tet) dominates, the pattern of signal shifts of carbon atoms neighbouring the donor nitrogen atoms of the polyamine (C_1, C_2, C_3, C_4, C_5; 0.168, 0.360, 0.107, 0.029, 0.085 ppm, respectively) confirms the participation of all nitrogen atoms. It should be noted that because of fast exchange on the NMR time scale, the signals are averaged and only NMR measurements in the range of the observed complex domination permit conclusions on the mode of coordination. Above pH 9.0, figure 1(a), the hydroxocomplex Cd(3,3,3-tet)(OH) is formed. ^{13}C NMR shifts in the positions of the signals assigned to C_1, C_2, C_3, C_4 and C_5 neighbouring the nitrogen atoms of 3,3,3-tet at pH 10.5 are 0.051, 0.116, 0.048, 0.042 and 0.125 ppm, pointing to the participation of all nitrogen atoms of the tetramine in coordination.

3.2. *Hg(II)/3,3,3-tet system*

Interactions of 3,3,3-tet with Hg(II) leads to formation of hydroxocomplexes Hg(3,3,3-tet)(OH) and Hg(3,3,3-tet)$(OH)_2$, which corresponds to the strong tendency of Hg(II) to hydrolysis (overall stability constants are given in table 1). These complexes start forming above pH 7 (figure 1b), binding a maximum of 75% of the metal ion at pH ~ 8.5 in the species Hg(3,3,3-tet)(OH) and 95% pH ~ 10.5 in Hg(3,3,3-tet)$(OH)_2$. ^{13}C NMR spectra of both species show significant changes in the positions of the signals assigned to all carbon atoms of the polyamine, C_1, C_2, C_3, C_4 and C_5. In the spectrum of Hg(3,3,3-tet)(OH) at pH = 8.5 the shifts are 0.990, 1.220, 0.893, 1.023 and 0.628 ppm, respectively, while in Hg(3,3,3-tet)$(OH)_2$ at pH = 10.0 they are 1.242, 1.642, 1.688, 0.428 and 0.227 ppm, indicating the participation of all nitrogen atoms of 3,3,3-tet in the bonding (besides the OH group, which gives {N4O} or {N4O2} chromophores, respectively).

3.3. *Cd(II)/Cyd/tetramine systems*

Complexes found in the systems Cd(II)/Cyd/3,3,3-tet (or Spm) are Cd(Cyd)(H3,3,3-tet), Cd(Cyd)(3,3,3-tet) and Cd(Cyd)(Spm). Overall stability constants are given in table 2. The protonated complex forms in the pH range 7.0–9.5, binding about 10% of the metal ion at pH 8.0 (figure 2a). The value of $\log K_e$ for polyamine reaction with anchoring Cd(Cyd), Cd(Cyd) + H(3,3,3-tet) $\leftrightarrows$ Cd(Cyd)(H3,3,3-tet), is 5.93 and comparison with the equilibrium constant of formation of the binary complex Cd(H3,3,3-tet), $\log K_e = 5.00$, in which 3 nitrogen atoms of PA participate in bonding, imply that the mode of PA coordination in Cd(Cyd)(H3,3,3-tet) is the same. As follows from results concerning other species, introduction of the fourth donor nitrogen atom of 3,3,3-tet into the inner coordination sphere should cause an increase in $\log K_e$ by about 2.7.

Table 2. Overall stability constans ($\log \beta$) and equilibrium constans ($\log K_e$) for complex formation in Cd(II) and Hg(II)/Cyd or CMP/tetramine systems [$\mu = 0.1$ M (KNO_3); $T = 20 \pm 1°C$].

System	Species	$\log \beta$	$\log K_e$
Cd/Cyd/3,3,3-tri	Cd(Cyd)(H3,3,3-tet)	18.72(7)	5.93
	Cd(Cyd)(3,3,3-tet)	10.69(3)	8.26
Cd/Cyd/Spm	Cd(Cyd)(Spm)	8.30(6)	5.87
Cd/CMP/3,3,3-tri	Cd(CMP)($H_4$3,3,3-tet)	42.52(6)	3.73
	Cd(CMP)($H_3$3,3,3-tet)	34.91(8)	3.50
Cd/CMP/Spm	Cd(CMP)(H_4Spm)	45.00(8)	4.23
	Cd(CMP)(H_3Spm)	36.48(11)	3.77
Hg/Cyd/3,3,3-tri	Hg(Cyd)($H_2$3,3,3-tet)	36.86(5)	9.77
	Hg(Cyd)(H3,3,3-tet)	30.85(2)	13.78
	Hg(Cyd)(3,3,3-tet)	23.72(2)	17.01
Hg/Cyd/Spm	Hg(Cyd)(H_2Spm)	38.41(11)	10.41
	Hg(Cyd)(Spm)	22.54(10)	15.83
Hg/CMP/3,3,3-tri	Hg(CMP)($H_2$3,3,3-tet)	39.06(8)	6.88
	Hg(CMP)(H3,3,3-tet)	32.53(3)	10.37
	Hg(CMP)(3,3,3-tet)	24.78(3)	12.98
Hg/CMP/Spm	Hg(CMP)(H_3Spm)	47.07(10)	4.96
	Hg(CMP)(HSpm)	32.38(8)	9.47
	Hg(CMP)(Spm)	23.20(12)	11.40

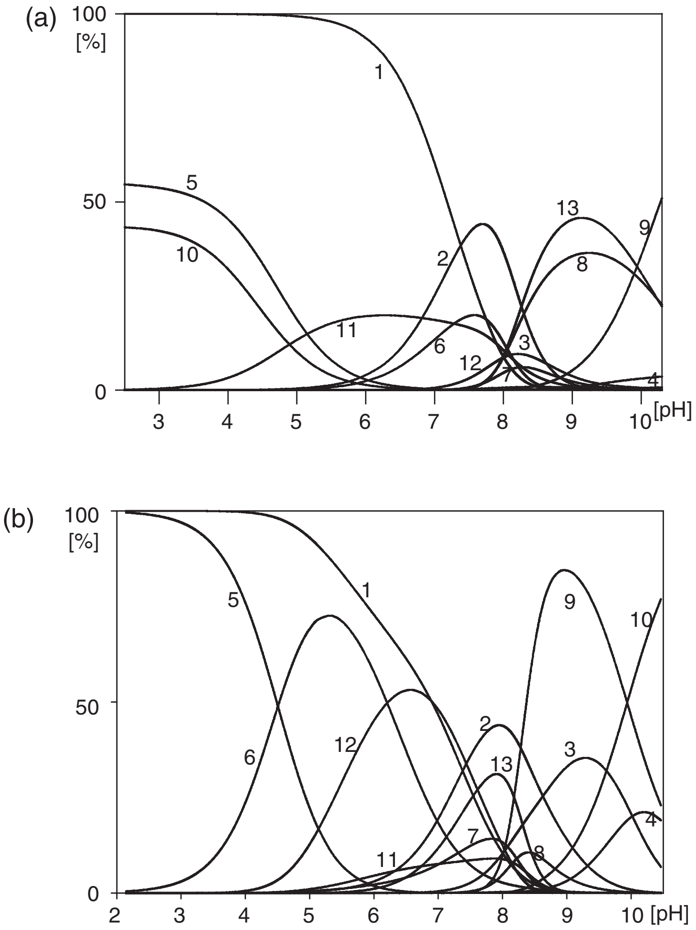


Figure 2. Distribution diagrams for the Cd(II)/Cyd/3,3,3-tet and Cd(II)/CMP/3,3,3-tet systems; percentages of the species refer to total metal; (a) Cd(II)/Cyd/3,3,3-tet: 1, $H_4$3,3,3-tet; 2, $H_3$3,3,3-tet; 3, $H_2$3,3,3-tet; 4, H3,3,3-tet; 5, HCyd; 6, Cd($H_3$3,3,3-tet); 7, Cd(H3,3,3-tet); 8, Cd(3,3,3-tet); 9, Cd(3,3,3-tet)(OH); 10, Cd(HCyd); 11, Cd(Cyd); 12, Cd(Cyd)H(3,3,3-tet); 13, Cd(Cyd)(3,3,3-tet); $C_{Cd^{2+}} = 1.32 \times 10^{-3}$ M; $C_{Cyd} = 2.64 \times 10^{-3}$ M; $C_{3,3,3\text{-tet}} = 2.64 \times 10^{-3}$ M; (b) Cd(II)/CMP/3,3,3-tet: 1, $H_4$3,3,3-tet; 2, $H_3$3,3,3-tet; 3, $H_2$3,3,3-tet; 4, H3,3,3-tet; 5, H_2CMP; 6, HCMP; 7, Cd($H_3$3,3,3-tet); 8, Cd(H3,3,3-tet); 9, Cd(3,3,3-tet); 10, Cd(3,3,3-tet)(OH); 11, Cd(CMP); 12, Cd(CMP)($H_4$3,3,3-tet); 13, Cd(CMP)($H_3$3,3,3-tet); $C_{Cd^{2+}} = 1.32 \times 10^{-3}$ M; $C_{CMP} = 2.64 \times 10^{-3}$ M; $C_{3,3,3\text{-tet}} = 2.64 \times 10^{-3}$ M.

The ternary complex Cd(Cyd)(3,3,3-tet) starts to form from pH 7.5 and reaches a maximum concentration at pH ~ 9, binding about 45% of the metal (figure 2a), while Cd(Cyd)(Spm) forms from a pH of about 8.5 and at pH 10.0 binds about 45% of Cd(II) ions. Significant differences in the equilibrium constants for Cd(Cyd)(3,3,3-tet) formation, $\log K_e = 8.26$, and for Cd(Cyd)(Spm), $\log K_e = 5.87$ (table 2), indicate a different mode of coordination of these tetramines to Cd(Cyd). Moreover, a comparison of $\log K_e = 8.26$ for Cd(Cyd)(3,3,3-tet) and $\log K_e = 7.66$ for Cd(3,3,3-tet) as well as information on the coordination mode in the binary complex suggest that in Cd(Cyd)(3,3,3-tet) all nitrogen atoms from the PA molecule participate in coordination.

This mode is also supported by the pattern of ^{13}C NMR chemical shifts assigned to the carbon atoms of 3,3,3-tet (at pH 9, shifts are 0.197, 0.479, 0.116, 0.076 and 0.178 ppm, relative to positions of signals for C_1, C_2, C_3, C_4 and C_5 in the spectrum of the free ligand), table 3.

The equilibrium constant of Cd(Cyd)(Spm) formation, 2.39 $\log K_e$ units lower than that of formation Cd(Cyd)(3,3,3-tet) (table 2) indicates the involvement of only 3 nitrogen atoms from Spm. This mode of interaction of this bioamine with Cd(II) has already been noted in binary systems [37]. It is confirmed by similar values of $\log K_e = 5.87$ for the formation of Cd(Cyd)(Spm) compared to $\log K_e = 5.93$ for Cd(Cyd)(H3,3,3-tet), table 2, and to $\log K_e = 5.22$ for Cd(Spm) (results from [37]); one of the donor nitrogen atoms of PA is outside the inner coordination sphere. Changes of ^{13}C NMR signals assigned to Spm carbon atoms relative to those of the free ligand are C_1 0.206, C_2 0.652, C_3 0.197, C_4 0.077 and C_5 0.172 ppm at pH 10 (table 3) and do not indicate conclusively which of the amine groups takes part in complex formation. The shifts of signals of C(2) and C(4) atoms localised near N(3) of Cyd are 0.160 and 0.161 ppm for Cd(Cyd)(3,3,3-tet) at pH 9, as well as 0.072 and 0.128 ppm for Cd(Cyd)(Spm) at pH 10, table 3. These values indicate that nitrogen atom N(3) of the pyrimidine ring of the nucleoside is also involved in formation of the {N4} or {N5} chromophores in Cd(Cyd)(Spm) and Cd(Cyd)(3,3,3-tet), respectively.

In IR spectra, no changes were observed in the position of the 1650 cm^{-1} band assigned to the stretching vibration of the C=O group of the nucleoside (similar to the spectra of other Hg(II) and Cd(II) heteroligand complexes including Cyd or CMP and tetramines). This indicates that the oxygen atom of the carbonyl group is not involved in coordination in any of these species.

3.4. *Cd(II)/CMP/tetramine systems*

In systems involving Cd(II), cytidine-5′-monophosphate and tetramines, besides the complexes Cd(CMP)($H_3$3,3,3-tet) and Cd(CMP)(H_3Spm), the adducts (molecular complexes) Cd(CMP)($H_4$3,3,3-tet) and Cd(CMP)(H_4Spm) are formed. Overall stability constants of these species are given in table 2. The adducts start forming from pH close to 4, binding a maximum of 50% of the metal ions at pH near 6.5 in the species with 3,3,3-tet (figure 2b) and 80% of the metal ions in the species with Spm (pH about 7). The pH ranges of formation of the above adducts coincide with the pH range in which the tetramines are fully protonated. This implies that the coordinatively blocked polyamines in $ML \cdots L'$ species (L = nucleotide, L′ = polyamine) are not directly bonded to Cd(II). They are located in the outer coordination sphere and are involved in non-covalent interactions with the phosphate group of the anchoring Cd(CMP) species. Moreover, the phosphate group of the nucleotide, in contrast to the Cd(CMP) complex, binds the metal ion as indicated by the formation constants of the adducts being higher than those of Cd(CMP)($H_3$3,3,3-tet) and Cd(CMP)(H_3Spm) (table 2). The conclusion is confirmed by considerable shifts of signals arising from C(5′) and the phosphorus atom (0.309 and 0.207 ppm, respectively, for Cd(CMP)($H_4$3,3,3-tet) and 0.168 as well as 0.168 ppm, respectively, for Cd(CMP)H_4(Spm), table 3). The value of the formation constant for Cd(CMP)($H_4$3,3,3-tet), $\log K_e = 4.23$, higher than that

Table 3. Differences between ^{13}C NMR and ^{31}P NMR chemical shifts for the ligands in the Cd(II)/*CMP*/triamine systems in relation to metal-free systems (ppm).

		Cytidine or Cytidine 5′-monophosphate										Polyamine				
Systems	pH	C(2)	C(4)	C(5)	C(6)	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	P	C(1)	C(2)	C(3)	C(4)	C(5)
Cd/Cyd/3,3,3-tet	9.0	0.160	0.161	0.015	0.123	0.101	0.036	0.059	0.039	0.085		0.197	0.479	0.116	0.076	0.178
Cd/Cyd/Spm	10	0.072	0.128	0.024	0.021	0.050	0.033	0.082	0.041	0.010		0.206	0.652	0.195	0.077	0.172
Cd/CMP/3,3,3-tet	7.0	0.103	0.055	0.010	0.088	0.081	0.087	0.059	0.148	0.309	0.207	0.077	0.085	0.037	0.009	0.052
	8.0	0.282	0.228	0.087	0.108	0.082	0.078	0.030	0.010	0.037	0.023	0.284	0.061	0.364	0.077	0.301
Cd/CMP/Spm	7.0	0.064	0.016	0.000	0.064	0.039	0.100	0.048	0.122	0.262	0.168	0.016	0.041	0.040	0.065	0.016
	9.0	0.077	0.036	0.030	0.026	0.099	0.118	0.124	0.100	0.046	0.031	0.113	0.340	0.105	0.097	0.202
Hg/Cyd/3,3,3-tet	6.5	2.159	0.072	0.273	0.094	0.019	0.019	0.103	0.112	0.116		0.347	0.240	0.477	0.334	0.214
	9.0	0.160	0.161	0.015	0.137	0.128	0.049	0.059	0.025	0.085		1.532	0.453	1.986	1.452	0.138
Hg/Cyd/Spm	7.6	0.029	0.101	0.042	0.003	0.056	0.034	0.040	0.023	0.032		0.232	0.577	0.168	0.175	0.469
	10	0.088	0.020	0.003	0.010	0.025	0.027	0.046	0.038	0.021		0.254	1.000	0.203	0.123	0.310
Hg/CMP/3,3,3-tet	9.0	0.060	0.008	0.036	0.029	0.163	0.092	0.050	0.065	0.197	0.099	0.125	0.052	1.195	0.744	0.116
Hg/CMP/Spm	7.0	0.129	0.025	0.040	0.148	0.464	0.076	0.027	0.190	0.262	0.124	0.098	0.014	0.084	0.125	0.109
	8.0	0.357	0.153	0.046	0.141	0.458	0.069	0.241	0.110	0.308	0.107	0.014	0.054	0.121	0.158	0.161
	10.0	0.404	0.202	0.073	0.151	0.258	0.071	0.115	0.022	0.180	0.073	0.217	0.564	0.144	2.015	0.149

for (CMP)H_4(3,3,3-tet), log $K_e = 2.70$ [9], also indicating participation of the phosphate group in coordination.

With increasing deprotonation of the ligands, at pH above 6, the complexes Cd(CMP)($H_3$3,3,3-tet) and Cd(CMP)(H_3Spm) start to form. As follows from analysis of the changes in ^{13}C NMR signals (table 3), the nitrogen atom N(3) of the pyrimidine ring of CMP and the unblocked nitrogen atom of the tetramine are involved in metallation. The signal changes assigned to C(5′) in the ^{13}C NMR spectrum and to the phosphorus atom in the ^{31}P NMR spectrum of Cd(CMP)H_3(3,3,3-tet) and Cd(CMP)H_3(Spm) species are 0.037, 0.023 ppm and 0.046, 0.031 ppm, respectively, suggesting low binding effectiveness of the phosphate group. A comparison of the equilibrium constants for species formation (log K_e for Cd(CMP)($H_3$3,3,3-tet) and Cd(CMP)(H_3Spm) are 3.50 and 3.77, respectively) with a higher formation constant for Cd(CMP)(H_2Spd), (log $K_e = 4.81$ [28]), in which the phosphate group is involved in bonding, confirms this conclusion for the triprotonated complexes.

3.5. *Hg(II)/Cyd/tetramine systems*

As in ternary systems with di- and triamines [30], all the tetramines studied form ternary MLL′ complexes (L′ = nucleoside, L′ = polyamine with both ligands in the inner coordination sphere) with Hg(II) and Cyd. The presence of the protonated complexes Hg(Cyd)($H_2$3,3,3-tet), Hg(Cyd)(H_2Spm), Hg(Cyd)(H3,3,3-tet) was also detected. The ML(H_2L′) species containing 3,3,3-tet occurs in the pH range 5.5–7.5 and at pH close to 7.0 binds over 20% of the metal ion (figure 3a), while the species containing Spm forms in the pH range 5–9 and reaches a maximum concentration at pH near 7.5 (40%). Interestingly, results of model selection in the computer analysis of potentiometric titration data and observation of reactions indicate that the presence of both ligands in systems with Hg(II) clearly inhibits hydrolysis of mercury ions.

Values of log $K_e = 9.77$ for Hg(Cyd)($H_2$3,3,3,-tet) formation (table 2) and log $K_e = 10.41$ for Hg(Cyd)(H_2Spm) formation compared with the equilibrium constants of formation of Hg(Cyd)(Hdien), log $K_e = 10.80$, and Hg(Cyd)(HSpd), log $K_e = 11.55$ [40], in which only one nitrogen atom of PA is involved in coordination, imply that in both diprotonated complexes only one nitrogen atom of the tetramine is involved in coordination, despite the presence of two free nitrogen atoms. Moreover, the equilibrium constants of attachment of $H_2$3,3,3-tet or H_2Spm to Hg(Cyd) are 4.58 and 4.05 log K_e units lower, respectively, than the equilibrium constants for formation of Hg(H3,3-tri), log $K_e = 14.35$, and Hg(HSpd), log $K_e = 14.46$, in which two nitrogen atoms of the polyamine take part in bonding [37].

Hg(Cyd)(H3,3,3-tet) forms above pH 5.5 and at about pH 6.5 it binds about 60% of Hg(II), figure 3(a). Involvement of another nitrogen atom of PA, relative to the diprotonated species, causes an increase in the equilibrium constant of formation by about 4 log K_e units (table 2). This enhanced value is comparable to the equilibrium constants of formation of Hg(HSpd) [37], Hg(Cyd)(tn) and Hg(Cyd)(Put) [40], 14.46, 12.84 and 13.79, respectively, in which the participation of two amine groups in the interactions has been established on the basis of equilibrium and spectroscopic studies. The mode of polyamine interaction is confirmed by analysis of ^{13}C NMR spectra. The signal shifts of carbon atoms of PA, C_1, C_2, C_3, C_4, and C_5, relative to their positions

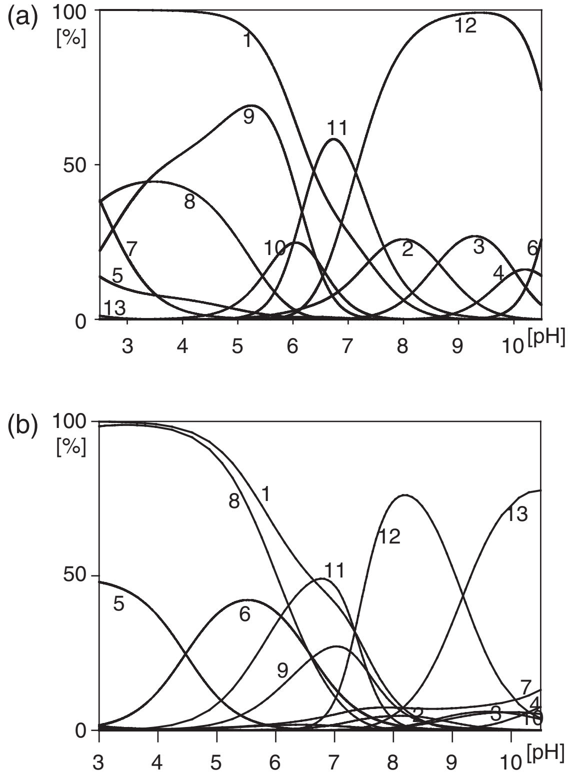


Figure 3. Distribution diagrams for the Hg(II)/Cyd/3,3,3-tet and Hg(II)/CMP/Spm systems; percentages of the species refer to total metal; (a) Hg(II)/Cyd/3,3,3-tet: 1, $H_4$3,3,3-tet; 2, $H_3$3,3,3-tet; 3, $H_2$3,3,3-tet; 4, H3,3,3-tet; 5, HCyd; 6, Hg(3,3,3-tet)$(OH)_2$; 7, Hg(Cyd); 8, $Hg(Cyd)_2$; 9, Hg(Cyd)(OH); 10, Hg(Cyd)($H_2$3,3,3-tet); 11, Hg(Cyd)(H3,3,3-tet); 12, Hg(Cyd)(3,3,3-tet); 13, Hg^{2+}; $C_{Hg^{2+}} = 1.32 \times 10^{-3}$ M; $C_{Cyd} = 2.64 \times 10^{-3}$ M; $C_{3,3,3\text{-tet}} = 2.64 \times 10^{-3}$ M; (b) Hg(II)/CMP/Spm: 1, H_4Spm; 2, H_3Spm; 3, H_2Spm; 4, HSpm; 5, H_2CMP; 6, HCMP; 7, $Hg(OH)_2$; 8, Hg(CMP); 9, Hg(CMP)(OH); 10, Hg(Spm); 11, Hg(CMP)H_3(Spm); 12, Hg(CMP)(HSpm); 13, Hg(CMP)(Spm); $C_{Hg^{2+}} = 1.35 \times 10^{-3}$ M; $C_{Cyd} = 1.35 \times 10^{-3}$ M; $C_{3,3,3\text{-tet}} = 1.35 \times 10^{-3}$ M.

in the spectrum of the free ligand, at pH 6.5 (pH range of Hg(Cyd)(H3,3,3-tri domination) are 0.347, 0.240, 0.477, 0334 and 0.214 ppm, respectively. However, because of the symmetric structure of this polyamine it is impossible to conclude which nitrogen atoms take part in coordination.

The log K_e value, 17.01, for Hg(Cyd)(3,3,3-tet) is higher than that for the analogous species with Spm, 15.83. Moreover, as has been established earlier [37], the equilibrium constants of formation of Hg(HSpm) and Hg(HSpd) are 15.15 and 14.46, respectively ({N2} chromophore). A comparison of these values indicates the involvement of three nitrogen atoms of the tetramine in bonding both in Hg(Cyd)(3,3,3-tet) and Hg(Cyd)(Spm). However, participation of the third nitrogen atom of Spm is weaker. As follows from changes in the positions of the ^{13}C NMR signals of the carbon atoms in the vicinity of the donor nitrogen atom N(3) (pyrimidine ring of cytidine; table 3), in all the species in the systems Hg(II)/Cyd/tetramine the N(3) atom takes part in coordination. For example, in the spectrum of species Hg(Cyd)(3,3,3-tet) the chemical shifts at C(2) and C(4) are 2.159 and 0.072 ppm, respectively.

3.6. *Hg(II)/CMP/tetramine systems*

Interactions of Hg(II) with CMP and tetramines leads to formation of the protonated species $ML(H_3L')$, $ML(H_2L')$, $ML(HL')$ and the ternary species MLL' ($L =$ nucleotide, $L' =$ polyamine). Taking into consideration the stoichiometric composition of $Hg(CMP)(H_3Spm)$ determined from computer analysis of pH-metric data, only one nitrogen atom of the spermine ligand is involved in coordination. This conclusion is supported by a comparison of the equilibrium constant of $Hg(CMP)(H_3Spm)$ formation ($\log K_e = \log \beta_{Hg(CMP)H3(Spm)} - \log \beta_{Hg(CMP)} - \log \beta_{H3(Spm)} = 4.96$, table 2), with the value of $\log K_e = 5.80$ for the complex Hg(CMP)(Htn) [29]. $Hg(CMP)(H_3Spm)$ occurs in the pH range 4–8.5, and dominates at a pH of about 7.0, at which it binds almost 50% of Hg(II), figure 3(b). Unfortunately, analysis of the chemical shifts of the signals assigned to the carbon atoms (0.098, 0.014, 0.084, 0.125 and 0.109 ppm at C_1, C_2, C_3, C_4 and C_5, respectively, of the Spm molecule) does not permit unambiguous conclusions as to which of the nitrogen atoms of PA binds. The chemical shifts at C(2) and C(4) in the neighbourhood of N(3) of the nucleotide of 0.129 and 0.025 ppm, are evidence of the involvement of the endocyclic nitrogen atom N(3) in coordination. On the other hand, the shift of the ^{13}C NMR signal assigned to C(5′) adjacent to the phosphate group and that of the ^{31}P NMR signal assigned to the phosphorus atom (0.262 and 0.124 ppm, respectively, relative to positions in the free ligand spectrum, table 3) indicate that the metal is bonded by the phosphate and {N2O} chromophore formation occurs.

Similar values of equilibrium constants for Hg(CMP)(H3,3,3-tet) and Hg(CMP)(HSpm) formation ($\log K_e = 10.37$ and 9.47, respectively), as well as for Hg(CMP)(HSpd), $\log K_e = 9.80$ (chromophore {N3O}, N(3) atom and phosphate group from CMP [28]), suggests involvement of two nitrogen atoms of the amine in bonding. This is confirmed by the spectroscopic study. For example, changes of C_1, C_2, C_3, C_4 and C_5 signals of Spm in the ^{13}C NMR spectra of Hg(CMP)(HSpm) (pH 8) are 0.014, 0.054, 0.121, 0.158 and 0.161 ppm, respectively, relative to positions in the spectrum of the free ligand. As greater shifts attend C_3, C_4 and C_5, the secondary amine groups are involved in bonding. Similar to the above case, the endocyclic nitrogen atom N(3) of the pyrimidine ring of CMP and the phosphate group (table 3) participate in coordination.

Mixed complexes of the type MLL', dominate above pH 8 for Hg(CMP)(3,3,3-tet) and above pH 9.5 for Hg(CMP)(Spm), binding nearly 75% of Hg(II) at pH close to 9.5 and 10.5, respectively. An increase in the $\log K_e$ values for the above complexes in relation to the monopotonated species (table 2) indicates that an additional nitrogen atom from a polyamine ligand is involved in coordination. However, the pattern of changes in ^{13}C NMR signals assigned to carbon atoms of these symmetric polyamines (table 3) does not allow one to conclude which nitrogen atoms are located in the outer coordination sphere.

Equilibrium constants values for Hg(CMP)(3,3,3-tet) and Hg(CMP)(Spm) formation, similar to those for Hg(CMP)(Spd) and to Hg(CMP)(dien), $\log K_e = 11.52$ and $\log K_e = 12.47$, respectively, point to {N4O} chromophore formation (three nitrogen atoms from PA and one nitrogen atom and a phosphate oxygen atom from CMP).

A similar mode of spermine coordination has been observed in the binary Hg(II)/Spm system [37].

4. Conclusions

In complexes formed in the Cd(II)/3,3,3-tet system all nitrogen atoms of PA are involved in coordination, in contrast to the case with the biogenic amine Spm. In the binary system involving Hg(II) and 3,3,3-tet only hydroxocomplexes are formed, while in the analogous system with Spm ML and MHL type species appear. The main binding groups in the ternary complexes are deprotonated nitrogen atoms of the tetramine, nitrogen atom N(3) of the pyrimidine ring of Cyd or CMP and oxygen atoms of the phosphate group of the nucleotide. Molecular complexes of the type $ML \cdots L'$ are formed only in ternary Cd(II) systems including nucleotides. In the mixed complexes MLL', the mode of coordination observed in the Cd(Cyd)(3,3,3-tet) and Cd(Cyd)(Spm) species is different. In the system with 3,3,3-tet an {N5} chromophore with four nitrogen atoms of polyamine involved in bonding is formed, while with Spm an {N4} chromophore forms. At pH below physiological values, introduction of a polyamine changes the coordination character of the nucleotide in the systems Cd(II)/CMP/tetramine. The phosphate group, ineffective in the complex Cd(CMP), is involved in bonding in the, mixed complex. In analogous systems with Hg(II) ions, the phosphate group takes part in coordination in binary Hg(II)/CMP or ternary systems over the whole pH range analysed. Not all available deprotonated nitrogen atoms of 3,3,3-tet and Spm take part in anchoring Hg(Cyd) in the species formed. The presence of both ligands (nucleoside or nucleotide and polyamine) in the ternary systems with Hg(II) clearly inhibits the characteristic hydrolysis of mercury ions.

Acknowledgements

This work was supported by Grant No. 3 T09A 086 27 of the Polish State Committee for Scientific Research (KBN).

References

[1] M.M.C.A. Castro. *J. Inorg. Biochem.*, **28**, 319 (1986).
[2] G. Borghensani, F. Pulidori, M. Remelli, R. Purrello, E. Rizzarelli. *J. Chem. Soc., Dalton Trans.*, 2095 (1990).
[3] P. Köpf Maier, H. Köpf. *Chem. Rev.*, **87**, 1137 (1987).
[4] S.E. Sherman, S.J. Lippard. *Chem. Rev.*, **87**, 1153 (1987).
[5] A.T. Tu, M.J. Heller, In *Metal Ions in Biological Systems*, H. Sigel (Ed.), Vol. 1, p. 1, Marcel Dekker, New York (1974).
[6] P.M. Vertino, R.J. Bergeron, P.F. Cavanaugh Jr, C.W. Porter. *Biopolymers*, **36**, 691 (1987).
[7] B.G. Feuerstein, N. Pattabiraman, L.J. Mormon. *Nucleic ACIDS Res.*, **18**, 1271 (1990).
[8] G.E. Plum, P.G. Arsscott, V.A. Bloomfield. *Biopolymers*, **30**, 631 (1990).
[9] A. Gasowska, L. Lomozik, R. Jastrzab. *J. Inorg. Biochem.*, **78**, 139 (2000).
[10] L. Lomozik, R. Jastrzab, A. Gasowska. *Polyhedron*, **19**, 1145 (2000).
[11] A. Gasowska, R. Jastrzab, R. Bregier-Jarzebowska, L. Lomozik. *Polyhedron*, **20**, 2305 (2001).

[12] L. Lomozik. Metal complexes with polyamines. In *Handbook of Metal-Ligand Interactions in Biological Fluids, Bioinorganic Chemistry*, G. Berthon (Ed.), Vol. 1, p. 686, Marcel Dekker, New York (1995).
[13] C.W. Tabor, H. Tabor. *Ann. Rev. Biochem.*, **53**, 749 (1984).
[14] D.M. Templeton, B. Sarkar. *Can. J. Chem.*, **63**, 3112 (1985).
[15] S.S. Cohen. *Introduction to the Polyamines*, Prentice-Hall, Englewood Cliffs (1979).
[16] A. Raina, J. Jänne. *Med. Biol.*, **53**, 121 (1975).
[17] B. Tadolini, G. Hakim. Interactions of polyamine with phospholipids. In *Progress in Polyamines Research*, V. Zappia, A.E. Pegg (Eds), Plenum Press, New York (1988).
[18] F. Schuber. *Biochem. J.*, **260**, 1 (1989).
[19] T.J. Thomas, V.A. Bloomfield. *Biopolymers*, **22**, 1097 (1983).
[20] G.E. Plum, P.G. Arscott, V.A. Bloomfield. *Biopolymers*, **30**, 631 (1990).
[21] T.J. Thomas, Z.N. Parthasarathy. *J. Mol. Biol.*, **175**, 313 (1994).
[22] P.M. Vertino, R.J. Bergeron, P.F. Cavanaugh Jr, C.W. Porter. *Biopolymers*, **36**, 691 (1987).
[23] U. Bachrach, G. Eilon. *Biochim. Biophys. Acta*, **197**, 494 (1969).
[24] G. De Munno, S. Mauro, T. Pizzino, D. Viterbo. *J. Chem. Soc., Dalton Trans.*, 1113 (1993).
[25] Y. Katono, Y. Inou, R. Chújô. *Polym. J.*, **9**, 471 (1977).
[26] H.P. Srivastava, D. Tiwari. *J. Indian Chem. Soc.*, **70**, 499 (1993).
[27] M.D. Walker, D.R. Williams. *J. Chem. Soc., Dalton Trans.*, 1186 (1974).
[28] L. Lomozik, R. Bregier-Jarzebowska. *J. Coord. Chem.*, **57**, 1251 (2004).
[29] L. Lomozik, R. Bregier-Jarzebowska. *Polish J. Chem.*, **78**, 529 (2004).
[30] L. Lomozik, R. Bregier-Jarzebowska, A. Gasowsk. *J. Coord. Chem.*, **56**, 203 (2003).
[31] L. Lomozik, A. Gasowska. *J. Inorg. Biochem.*, **62**, 103 (1996).
[32] H.M. Irving, M.G. Miles, L.D. Petit. *Anal. Chim. Acta*, **38**, 475 (1967).
[33] P. Gans, A. Sabatini, A. Vacca. *J. Chem., Soc., Dalton Trans.*, 1195 (1985).
[34] N. Ingi, W. Kakolowicz, L.G. Sillen, B. Wargvist. *Talanta*, **14**, 1261 (1967).
[35] L. Lomozik, M. Jaskolski, A. Wojciechowska. *Polish J. Chem.*, **65**, 1797 (1991).
[36] P.K. Glasoe, F.A. Long. *J. Phys. Chem.*, **64**, 188 (1960).
[37] L. Lomozik, R. Bregier-Jarzebowska. *Polish J. Chem.*, **73**, 927 (1999).
[38] G. Schwarzenbach, I. Szilard. *Helv. Chim. Acta*, **45**, 1222 (1962).
[39] A. Gasowska, L. Lomozik. *Polyhedron*, **21**, 745 (2002).
[40] L. Lomozik, R. Bregier-Jarzebowska. *J. Coord. Chem.*, **56**, 203 (2003).