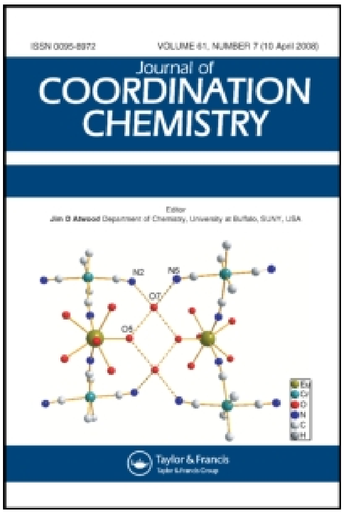

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Cadmium(II) and Mercury(II) Complexes in Ternary Systems with Cytidine and Diamines or Triamines

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CADMIUM(II) AND MERCURY(II) COMPLEXES IN TERNARY SYSTEMS WITH CYTIDINE AND DIAMINES OR TRIAMINES

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The mode of coordination of complexes formed in the systems Cd(II) or Hg(II)/cytidine/di- or triamine is proposed on the basis of equilibrium and spectroscopic studies. Mercury(II) binds much more strongly to cytidine and polyamine (PA) than cadmium. It was found from equilibrium and ^{13}C NMR studies that in the Hg(II) and Cd(II)/*Cyd*/di- or triamine complexes, metallation mainly involves the N(3) atom of the pyrimidine base of the nucleoside and $-NH_x^+$ groups from PA. In MLL′ complexes of both metals with diamines, all available donor nitrogen atoms of the polyamine are involved in coordination. In analogous systems with triamines, interaction of all nitrogen atoms is observed for Cd(II) systems as well as in the Hg(*Cyd*)(2,3-*tri*) species. Only two nitrogen atoms of the polyamine coordinate in ternary Hg(II) complexes with *dien, 3,3-tri* and *Spd*.

Keywords: Hg(II); Cd(II); complexes; cytidine; polyamines

INTRODUCTION

Metal ions significantly affect biological functions of particular components of chemical systems in living organisms [1–3]. Metal complexes are involved in almost all biological processes taking place at the molecular level, including those with nucleic acids, their constituents (nucleosides, nucleotides, free bases), proteins [4,5], and polyamines [6–10]. Reactions of metal ions with these bioligands have long been subject to considerable interest [11–16]. The interactions of biogenic amines, e.g. putrescine (*Put*), spermidine (*Spd*) or spermine (*Spm*) with nucleic acids are particularly important in genetic information transfer processes [7,8,17]. Metal ions are believed to affect the character of non-covalent interactions among bioligands. The stability and character of complexes with polyamines depend mainly on the metal ion type: hard ions like alkali earths interact mainly with hard oxygen atoms, e.g. those from phosphate groups, while soft ions such as transition metals interact mainly with nitrogen atoms of purine or pyrimidine bases [18–21]. Such metals as zinc and cadmium are

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characterised by weaker complex forming ability than typical transition metals making strong bonds with bioligands [22–24].

Much attention has been paid to the interaction of toxic metal ions such as cadmium(II) or mercury(II) [25–28], with nucleic acids, because of possible distortions of the DNA structure, which could lead to a permanent change in gene expression and consequently to neoplasmic changes [25]. It has been established that the complexation ability of nucleosides changes in the series $Guo > Ado \geq Cyd > Urd \geq Thd$ [29–31]. As indicated by the results of a study of the interaction of cytidine with a number of metal ions [11,32–37], the preferred site of coordination of this ligand is the heterocyclic N(3) nitrogen atom of the pyrimidine ring [32,38–43].

The paper reports results of potentiometric and spectroscopic investigations of ternary systems of Hg(II) and Cd(II) ions with cytidine and di-or triamines. The studies are a continuation of our earlier work on complexation in binary systems of Hg(II) and Cd(II) ions with polyamines, nucleosides and nucleotides [44].

EXPERIMENTAL

Ethylenediamine (*en*) and 1,8-diamino-4-azaoctane (spermidine, *Spd*) were purchased from Merck, 1,3-diaminopropane (*tn*) and 1,4-diaminobutane (putrescine, *Put*) – from Sigma, diethylenetriamine (*dien*), 1,6-diamino-3-azahexane (*2,3-tri*), 1,7-diamino-4-azaheptane (*3,3-tri*) – from Aldrich. The appropriate polyamine nitrates were prepared by dissolving an appropriate amount of free amine and addition of an equimolar amount of HNO_3. The white precipitate obtained was recrystallised, washed with methanol and dried in desiccator over P_4O_{10} or in air. Cytidine (*Cyd*) was bought from Sigma. Its nitrate was prepared in the same way as nucleoside hydrochlorides described earlier [35]. In all measurements polyamines and nucleosides were used as nitrate salts. The ligands were subjected to elemental analysis and results were in agreement with theoretically calculated values ($\pm 0.5\%$). $Hg(NO_3)_2 \cdot H_2O$ was purchased from Aldrich and $Cd(NO_3)_2 \cdot 4H_2O$ from Merck. The concentration of Hg(II) ions was determined by a precipitation titration with NaC1 solution using diphenylcarbazone as indicator, while the concentration of Cd(II) ions was determined complexometrically using EDTA and pyrocatechol violet as indicator. Potentiometric studies were performed on a DTS Radiometer 800 Multi-Titration system with a GK-2401C electrode calibrated in terms of hydrogen ion concentration [45]. The concentration of the ligands in the titrated systems varied from 1.3×10^{-3} to 3.4×10^{-3} M, the concentration of metal ions from 1.3×10^{-3} to 1.7×10^{-3} M and the ratio of M:L:L′ (L = Cyd, L′ = polyamine) in the samples studied was 1 : 1 : 1 and 1 : 2 : 2. Potentiometric titrations were performed at ionic strength $\mu = 0.1$ M (KNO_3), at $20 \pm 1°$ C under helium, using CO_2-free NaOH solution. Calculations were performed using 100–150 points for each run taking into account only that part of the titration curve obtained when there was no precipitate in the solution. Selection of models and determination of protonation constants of ligands as well as stability constants of complexes were made using the SUPERQUAD program [46], whereas the distribution of particular forms was calculated by the HALTAFALL program [47]. Selection and verification of models was performed as described earlier [48]. Samples for ^{13}C NMR were prepared by dissolving appropriate amounts of the ligands and metal nitrates in D_2O and adjusting the pH by addition of NaOD and DNO_3, correcting pH-readings (a Mera-Tronik pH meter N517) according to the formula pD = pH readings + 0.40 [49]. The concentration of ligands in samples for NMR studies was 0.1M and the metal ion to ligand ratio varied from 1:10 :10 to 1:100 :100. ^{13}C NMR spectra were recorded Varian Gemini-300VT and Varian Unity 300 NMR spectrometers in the range 20–170 ppm, using dioxane as internal standard.

The ligands studied

$\mathrm{NH_2\text{-}\overset{1}{C}H_2\text{-}\overset{1}{C}H_2\text{-}NH_2}$ *en* (ethylenediamine)

$\mathrm{NH_2\text{-}\overset{1}{C}H_2\text{-}\overset{2}{C}H_2\text{-}\overset{1}{C}H_2\text{-}NH_2}$ *tn* (1,3-diaminopropane)

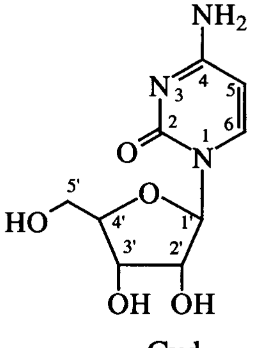
$\mathrm{NH_2\text{-}\overset{1}{C}H_2\text{-}\overset{2}{C}H_2\text{-}\overset{2}{C}H_2\text{-}\overset{1}{C}H_2\text{-}NH_2}$ *Put* (1,4-diamonobutane)

$\mathrm{\overset{a}{N}H_2\text{-}\overset{1}{C}H_2\text{-}\overset{2}{C}H_2\text{-}\overset{b}{N}H_2\text{-}\overset{2}{C}H_2\text{-}\overset{1}{C}H_2\text{-}\overset{a}{N}H_2}$ *dien* (diethylenetriamine)

$\mathrm{\overset{a}{N}H_2\text{-}\overset{1}{C}H_2\text{-}\overset{2}{C}H_2\text{-}\overset{b}{N}H\text{-}\overset{3}{C}H_2\text{-}\overset{4}{C}H_2\text{-}\overset{5}{C}H_2\text{-}\overset{c}{N}H_2}$ 2, 3-*tri* (1,6-diamino-3-azahexane)

$\mathrm{\overset{a}{N}H_2\text{-}\overset{1}{C}H_2\text{-}\overset{2}{C}H_2\text{-}\overset{3}{C}H_2\text{-}\overset{b}{N}H\text{-}\overset{3}{C}H_2\text{-}\overset{2}{C}H_2\text{-}\overset{1}{C}H_2\text{-}\overset{a}{N}H_2}$ 3, 3-*tri* (1,7-diamino-4-azaheptane)

$\mathrm{\overset{a}{N}H_2\text{-}\overset{1}{C}H_2\text{-}\overset{2}{C}H_2\text{-}\overset{3}{C}H_2\text{-}\overset{b}{N}H\text{-}\overset{4}{C}H_2\text{-}\overset{5}{C}H_2\text{-}\overset{6}{C}H_2\text{-}\overset{7}{C}H_2\text{-}\overset{c}{N}H_2}$ *Spd* (1,8-diamino-4-azaoctane)



Cyd

RESULTS AND DISCUSSION

Computer analysis of the pH titration data was performed taking into regard the earlier determined protonation constants of the ligands and equilibrium constants for formation of hydroxocomplexes $\mathrm{Hg(OH)^+}$, $\mathrm{Hg(OH)_2^{\circ}}$, $\mathrm{Cd(OH)^+}$ and $\mathrm{Cd(OH)_2^{\circ}}$ [44]. As concluded earlier [44,50], no reduction of Hg(II) to Hg(I) was observed.

Hg(II)/Cyd//PA systems

Table I presents stability constants (log β) of mixed complexes of Hg(II) with *Cyd* and di-or triamines and formation constants, log K_e.

As observed earlier in binary systems of mercury(II) ions with diamines [44], and also for Hg(II)/nucleoside/diamine systems, an untypical enhancement of the stability of

TABLE I Overall stability constants (log β) and equilibrium constants (log K_e) for complexes in Hg(II)/*Cyd*/di- or triamine ternary systems

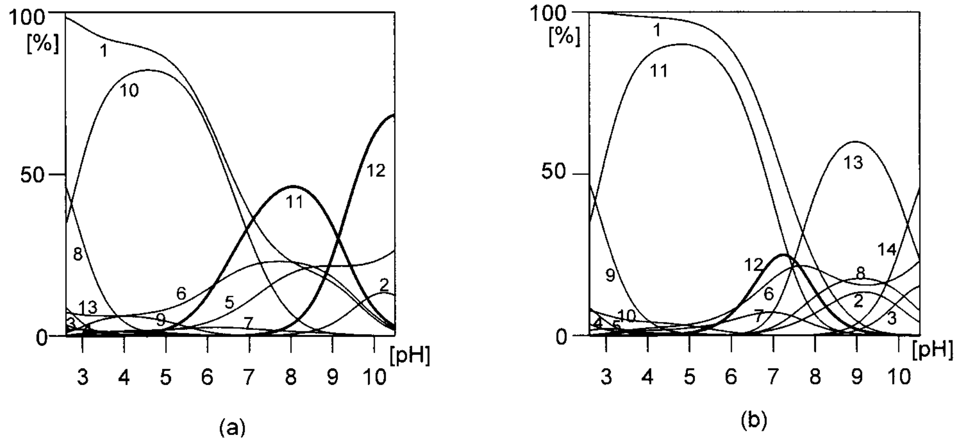
Equilibrium	*log β*	*log K_e*
$\mathrm{Hg}(\mathit{Cyd})+\mathit{en}\rightleftharpoons\mathrm{Hg}(\mathit{Cyd})(\mathit{en})$	18.43 (11)	11.72
$\mathrm{Hg}(\mathit{Cyd})+\mathit{en}+\mathrm{H_2O}\rightleftharpoons\mathrm{Hg}(\mathit{Cyd})(\mathit{en})(\mathrm{OH})+\mathrm{H}^+$	11.07 (8)	
$\mathrm{Hg}(\mathit{Cyd})+\mathit{tn}\rightleftharpoons\mathrm{Hg}(\mathit{Cyd})(\mathit{tn})$	19.55 (8)	12.84
$\mathrm{Hg}(\mathit{Cyd})+\mathit{tn}+\mathrm{H_2O}\rightleftharpoons\mathrm{Hg}(\mathit{Cyd})(\mathit{tn})(\mathrm{OH})+\mathrm{H}^+$	11.07 (8)	
$\mathrm{Hg}(\mathit{Cyd})+\mathit{Put}\rightleftharpoons\mathrm{Hg}(\mathit{Cyd})(\mathit{Put})$	20.50 (16)	13.79
$\mathrm{Hg}(\mathit{Cyd})+\mathit{Put}+\mathrm{H_2O}\rightleftharpoons\mathrm{Hg}(\mathit{Cyd})(\mathit{Put})(\mathrm{OH})+\mathrm{H}^+$	11.36 (16)	
$\mathrm{Hg}(\mathit{Cyd})+\mathit{dien}\rightleftharpoons\mathrm{Hg}(\mathit{Cyd})(\mathit{dien})$	22.08 (5)	15.37
$\mathrm{Hg}(\mathit{Cyd})+\mathrm{H}\mathit{dien}\rightleftharpoons\mathrm{Hg}(\mathit{Cyd})(\mathrm{H}\mathit{dien})$	27.47 (5)	10.80
$\mathrm{Hg}(\mathit{Cyd})+\mathit{dien}+\mathrm{H_2O}\rightleftharpoons\mathrm{Hg}(\mathit{Cyd})(\mathit{dien})(\mathrm{OH})+\mathrm{H}^+$	14.21 (6)	
$\mathrm{Hg}(\mathit{Cyd})+\mathit{2,3\text{-}tri}\rightleftharpoons\mathrm{Hg}(\mathit{Cyd})(\mathit{2,3\text{-}tri})$	28.59 (6)	21.88
$\mathrm{Hg}(\mathit{Cyd})+\mathrm{H}\mathit{2,3\text{-}tri}\rightleftharpoons\mathrm{Hg}(\mathit{Cyd})(\mathrm{H}\mathit{2,3\text{-}tri})$	33.95 (9)	16.84
$\mathrm{Hg}(\mathit{Cyd})+\mathrm{H_2}\mathit{2,3\text{-}tri}\rightleftharpoons\mathrm{Hg}(\mathit{Cyd})(\mathrm{H_2}\mathit{2,3\text{-}tri})$	39.34 (7)	12.91
$\mathrm{Hg}(\mathit{Cyd})+\mathit{3,3\text{-}tri}\rightleftharpoons\mathrm{Hg}(\mathit{Cyd})(\mathit{3,3\text{-}tri})$	23.28 (4)	16.57
$\mathrm{Hg}(\mathit{Cyd})+\mathit{3,3\text{-}tri}+\mathrm{H_2O}\rightleftharpoons\mathrm{Hg}(\mathit{Cyd})(\mathit{3,3\text{-}tri})(\mathrm{OH})+\mathrm{H}^+$	12.52 (11)	
$\mathrm{Hg}(\mathit{Cyd})+\mathit{Spd}\rightleftharpoons\mathrm{Hg}(\mathit{Cyd})(\mathit{Spd})$	21.62 (6)	14.91
$\mathrm{Hg}(\mathit{Cyd})+\mathrm{H}\mathit{Spd}\rightleftharpoons\mathrm{Hg}(\mathit{Cyd})(\mathrm{H}\mathit{Spd})$	29.12 (9)	11.55
$\mathrm{Hg}(\mathit{Cyd})+\mathit{Spd}+\mathrm{H_2O}\rightleftharpoons\mathrm{Hg}(\mathit{Cyd})(\mathit{Spd})(\mathrm{OH})+\mathrm{H}^+$	11.40 (7)	

complexes with increasing size of the PA ring is observed (Table I). This is related to the mercury ion tendency to form linear complexes, which favour large chelate structures with smaller ring strain [37]. A comparison of the equilibrium constants ($\log K_e$) of the parent and ternary complexes suggests the involvement of two donor nitrogen atoms of the diamine. For instance, in the system involving Hg(II) and *en* ({N2} chromophore): $\mathrm{Hg}+\mathit{en}\rightleftharpoons\mathrm{Hg}(\mathit{en})$, $\log\beta_1=\log K_\mathrm{e}=13.85$ [44], while in the ternary system Hg(II), *en* and *Cyd*, the equilibrium constant for the heteroligand complex formation: $\mathrm{Hg}(\mathit{Cyd})+\mathit{en}\rightleftharpoons\mathrm{Hg}(\mathit{Cyd})(\mathit{en})$, $\log K_\mathrm{e}=\log\beta_{\mathrm{Hg(Cyd)(en)}}-\log\beta_{\mathrm{Hg(Cyd)}}=11.72$ (Table I). Undoubtedly, elimination of one of the amine nitrogen donor atoms from coordination would lead to a greater decrease in $\log K_e$, as has been found for the formation of Hg(H*en*) with monodentate amine coordination ($\log K_e=6.66$,) [44]. The difference in the equilibrium constant of formation of Hg(*en*) and Hg(*Cyd*)(*en*), (2.13 $\log K_\mathrm{e}$ units) suggests lower efficiency of interaction in the mixed complex. The involvement of the two nitrogen atoms of the diamine in metal binding in mixed complexes has been confirmed by ^{13}C NMR (Table II).

For example, at pH 6.0 and 10.0, at which the dominant species are Hg(*Cyd*)(*tn*) and Hg(*Cyd*)(*tn*)(OH), respectively, NMR shifts of the diamine carbon atom signals (0.090 ppm for C(1), 0.940 ppm for C(2) and 0.197 ppm for C(1), 2.284 ppm for C(2) in both species, respectively, Table II) indicate the involvement of both nitrogen atoms in coordination. Shifts in signal positions assigned to carbon atoms C(2) and C(4) in the vicinity of N(3) of the nucleoside molecule in Hg(*Cyd*)(*tn*) are 0.408 and 0.543 ppm and in Hg(*Cyd*)(*tn*)(OH) 0.214 and 0.456 ppm (Table II), pointing to the involvement of the endocyclic N(3) atom in coordination. An analogous pattern of changes is observed in other ternary systems with diamines (Table II), which, with regard to the results of the equilibrium study, indicates the formation of {N3} chromophores with the involvement of two diamine donor nitrogen atoms and N(3) from the *Cyd* molecule. MLL′ type mixed complexes (M = metal, L = nucleoside, L′ = polyamine) are formed at pH from ~4.5 for *en* and ~5.0 for *tn* and *Put*, whereas the hydroxocomplexes from pH ~ 5.5 for *en* and ~7.0 for *tn* and *Put*. Figure 1a presents exemplary distributions of the complex forms in the ternary system involving Hg(II) ions with *Put* and *Cyd*.

TABLE II ^{13}C NMR chemical shifts for Hg(II)/*Cyd*/di- or triamine systems and differences (in parantheses) between corresponding chemical shifts of the ligands in ternary systems and metal – free ligands (ppm)

		Hg/Cyd/diamine					*Hg/Cyd/triamine*								
		en	*tn*		*Put*		*dien*			*2,3-tri*		*3,3-tri*	*Spd*		
		pH 9.2	*pH 6.0*	*pH 10.0*	*pH 8.0*	*pH 10.2*	*pH 4.0*	*pH 6.0*	*pH 10.0*	*pH 4.0*	*pH 8.0*	*pH 8.0*	*pH 7.0*	*pH 9.0*	*pH 10.5*
Cyd	C(2)	158.472 (0.074)	158.585 (0.408)	158.592 (0.214)	158.459 (0.061)	158.459 (0.036)	153.772 (2.147)	158.423 (0.346)	158.470 (0.094)	154.071 (0.254)	158.464 (0.081)	158.595 (0.197)	158.611 (0.054)	158.439 (0.098)	158.455 (0.092)
	C(4)	167.005 (0.057)	167.288 (0.543)	167.243 (0.456)	167.018 (0.046)	167.018 (0.489)	163.462 (1.588)	166.981 (0.307)	167.013 (0.226)	163.697 (0.246)	167.021 (0.049)	167.222 (0.250)	167.199 (0.151)	167.013 (0.054)	167.038 (0.041)
	C(5)	97.009 (0.044)	97.168 (0.230)	97.092 (0.137)	97.055 (0.046)	97.055 (0.257)	96.439 (0.270)	97.062 (0.140)	97.062 (0.107)	96.503 (0.047)	97.070 (0.046)	97.091 (0.082)	97.201 (0.151)	97.045 (0.003)	97.038 (0.043)
	C(6)	142.399 (0.064)	142.574 (0.249)	142.627 (0.244)	143.432 (0.015)	142.432 (0.146)	143.710 (0.746)	142.415 (0.063)	142.415 (0.032)	143.621 (0.017)	142.070 (0.055)	142.634 (0.190)	142.573 (0.030)	142.440 (0.007)	142.440 (0.008)
PA	C(1)	40.780 (0.108)	37.579 (0.090)	38.335 (0.197)	39.703 (0.134)	40.538 (0.081)	37.196 (0.640)	39.639 (0.191)	40.100 (0.407)	37.310 (0.018)	39.219 (0.359)	38.081 (0.106)	37.432 (0.006)	38.741 (0.156)	39.154 (0.027)
	C(2)		25.924 (0.940)	28.694 (2.284)	24.724 (0.537)	27.562 (0.288)	45.585 (0.067)	45.973 (0.071)	49.815 (1.210)	45.900 (0.015)	46.919 (0.548)	25.583 (0.376)	24.658 (0.080)	28.000 (0.095)	30.046 (0.293)
	C(3)									45.155 (0.012)	46.369 (0.161)	46.010 (0.087)	45.319 (0.063)	46.402 (0.092)	46.701 (0.012)
	C(4)									24.546 (0.053)	27.134 (0.605)		47.835 (0.070)	48.408 (0.016)	48.731 (0.067)
	C(5)									36.266 (0.098)	38.636 (0.304)		23.447 (0.143)	25.355 (0.046)	25.864 (0.117)
	C(6)												24.510 (0.131)	26.107 (0.144)	28.137 (0.367)
	C(7)												39.640 (0.015)	40.181 (0.078)	40.683 (0.061)



(a) (b)

FIGURE 1 Distribution diagrams for the systems (a) Hg(II)/*Cyd*/*Put*: 1.H$_2$(*Put*), 2.H(*Put*), 3.H(*Cyd*), 4.Hg(OH)$^+$, 5.Hg(OH)$_2$, 6.Hg(*Put*), 7.Hg(H*Put*)$_2$, 8.Hg(*Cyd*), 9.Hg(*Cyd*)$_2$, 10.Hg(*Cyd*)(OH), 11.Hg(*Cyd*)(*Put*), 12.Hg(*Cyd*)(*Put*)(OH), 13.Hg^{2+} $C_{Cyd} = 1.33 \times 10^{-3}$M, $C_{Put} = 1.33 \times 10^{-3}$M, $C_{Hg}{}^{2+} = 1.35 \times 10^{-3}$M; (b) Hg(II)/*Cyd*/*Spd*: 1.H$_3$(*Spd*), 2.H$_2$(*Spd*), 3.H(*Spd*), 4.H(*Cyd*), 5.Hg(OH)$^+$, 6.Hg(OH)$_2$, 7.Hg(H*Spd*), 8.Hg(*Spd*), 9.Hg(*Cyd*), 10.Hg(*Cyd*)$_2$, 11.Hg(*Cyd*)(OH), 12.Hg(*Cyd*)(H*Spd*), 13.Hg (*Cyd*)(*Spd*),14.Hg(*Cyd*)(*Spd*)(OH) $C_{Cyd} = 1.34 \times 10^{-3}$M, $C_{Spd} = 1.38 \times 10^{-3}$M, $C^{+2}_{Hg} = 1.33 \times 10^{-3}$M.

Hg(II)/Cyd/triamines

As in the diamine ternary systems, all triamines studied form MLL′ heteroligand complexes with Hg ions and cytidine. Close values of the equilibrium constants for Hg(*Cyd*)(*Put*) as well as Hg(*Cyd*)(*dien*), Hg(*Cyd*)(*3,3-tri*) and Hg(*Cyd*)(*Spd*) complexes, Table I, suggest that only two polyamine donor nitrogen atoms undergo effective metallation, while the third deprotonated amine group remains outside the inner coordination sphere. Moreover, e.g. the formation constant for spermidine reaction with Hg(*Cyd*), ($\log K_e = 14.91$, Table I) is similar to that of Hg(H*Spd*) complex formation, in which only two amine nitrogen atoms are involved in coordination (Hg + H*Spd*$\rightleftharpoons$Hg(H*Spd*); $\log K_e = \log \beta_{HgSpd} - \log \beta_{HSpd} = 14.46$ [44].

The above mode of polyamine interaction has been confirmed by ^{13}C NMR studies. For instance, in the system Hg(II)/*Cyd*/*Spd*, shifts of the signals from C(1), C(2), C(3), C(4), C(5), C(6) and C(7) atoms at pH 9.0 (at which the dominant species is Hg(*Cyd*)(*Spd*)) are 0.156, 0.095, 0.092, 0.016, 0.046, 0.144 and 0.078 ppm, respectively (Table II), indicating the involvement of two terminal nitrogen atoms in coordination. The value of $\log K_e = 21.88$ for the complex Hg(*Cyd*)(2,3-*tri*) is significantly higher than for other triamines (Table I) and indicates a different coordination mode, with involvement of three donor nitrogen atoms; this corresponds to the shift pattern of ^{13}C NMR signals assigned to all carbons atoms neighbouring nitrogen atoms of the triamine [C(1) 0.359 ppm, C(2) 0.548 ppm, C(3) 0.161 ppm, C(4) 0.605 ppm, C(5) 0.304 ppm]. The above observations confirm the significant influence of structural factors (polyamine chain length) on the mode of coordination in the complexes studied [36,51].

Analysis of the equilibrium (Table I) and spectroscopic (Table II) results suggests that in monoprotonated complexes with *dien*, *Spd* as well as in the species Hg(*Cyd*)(H$_2$2,3-*tri*), only one triamine donor nitrogen atom is engaged in metallation. This follows from the value of the equilibrium constant for formation of the species Hg(*Cyd*)(H*Spd*) ($\log K_e = 11.55$), lower by 3.36 $\log K_e$ units relative to that of the complex Hg(*Cyd*) (*Spd*) ($\log K_e = 14.91$, {N3} chromophore; Table I).

TABLE III Overall stability constants (log β) and equilibrium constants (log K_e) of complexes in Cd(II)/*Cyd*/di- or triamine ternary systems

Equlibrium	*log β*	*log K_e*
Cd(*Cyd*)+*en* ⇌ Cd(*Cyd*)(*en*)	8.43 (11)	6.00
Cd(*Cyd*)+H*tn* ⇌ Cd(*Cyd*)(H*tn*)	16.93 (12)	3.92
Cd(*Cyd*)+H*Put* ⇌ Cd(*Cyd*)(H*Put*)	16.88 (7)	3.90
Cd(*Cyd*)+*dien* ⇌ Cd(*Cyd*)(*dien*)	11.30 (2)	8.87
Cd(*Cyd*)+H*dien* ⇌ Cd(*Cyd*)(H*dien*)	18.14 (7)	5.75
Cd(*Cyd*)+H_2*dien* ⇌ Cd(*Cyd*)(H_2*dien*)	25.42 (2)	3.82
Cd(*Cyd*)+*2,3-tri* ⇌ Cd(*Cyd*)(*2,3-tri*)	10.54 (5)	8.11
Cd(*Cyd*)+*3,3-tri* ⇌ Cd(*Cyd*)(*3,3-tri*)	9.71 (7)	7.28
Cd(*Cyd*)+H_2*3,3-tri* ⇌ Cd(*Cyd*)(H_2*3,3-tri*)	26.28 (10)	3.33
Cd(*Cyd*)+H_2*Spd* ⇌ Cd(*Cyd*)(H_2*Spd*)	26.74 (11)	3.53

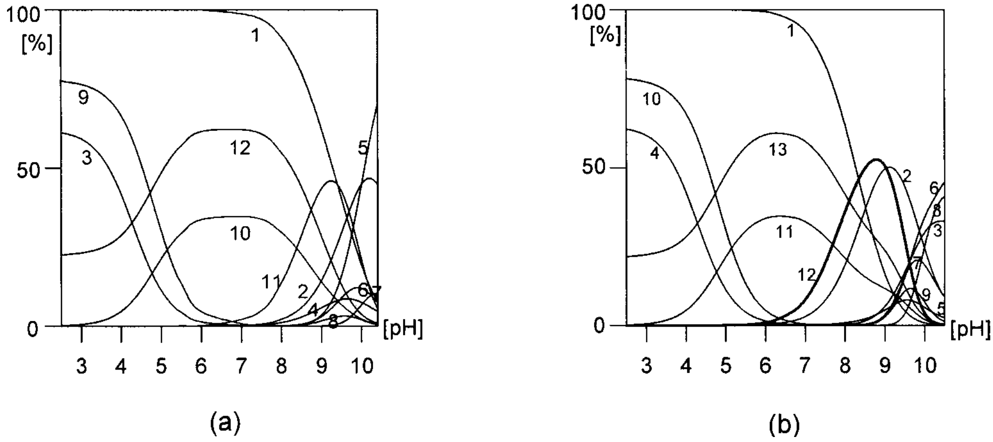


FIGURE 2 Distribution diagrams for the systems (a) Cd(II)/*Cyd*/*Put*: 1.H_2(*Put*), 2.H(*Put*), 3.H(*Cyd*), 4.Cd(OH^+), 5.Cd$(OH)_2$, 6.Cd(*Put*), 7.Cd$(Put)_2$, 8.Cd$(HPut)_2$, 9.Cd(H*Cyd*), 10.Cd(*Cyd*), 11.Cd(*Cyd*)(H*Put*), 12.$Cd^{2+}$$C_{Cyd} = 2.82 \times 10^{-3}$ M, $C_{Put} = 2.64 \times 10^{-3}$ M, $C_{Cd}{}^{2+} = 1.29 \times 10^{-4}$ M; (b) Cd(II)/*Cyd*/*Spd*: 1.H_3(*Spd*), 2.H_2(*Spd*), 3.H(*Spd*), 4.H(*Cyd*), 5.Cd$(OH)^+$, 6.Cd$(OH)_2$, 7.Cd(*Spd*), 8.Cd$(Spd)_2$, 9.Cd$(HSpd)_2$, 10.Cd(H*Cyd*), 11.Cd(*Cyd*), 12.Cd(*Cyd*)(H_2*Spd*), 13.Cd^{2+} $C_{Cyd} = 2.70 \times 10^{-3}$ M, $C_{Spd} = 2.70 \times 10^{-3}$M, $Ccd^{2+} = 1.27 \times 10^{-3}$M.

Shifts of signals assigned to carbon atoms in the neighbourhood of the terminal nitrogen atom N_c (the longer fragment of the chain) in the monofunctional complexes of asymmetric amines are much greater than the others, suggesting the participation of this nitrogen atom in coordination. Moreover, in all ternary systems described, the nitrogen atom N(3) of the pyrimidine ring of the cytidine molecule takes part in coordination as indicated by shifts of ^{13}C NMR signals of carbon atoms in the neighbourhood of the donor (Table II).

In systems with *dien* and *2,3-tri* the mixed complexes start forming at pH ~ 3, while in systems with *3,3-tri* and *Spd* they start appearing at pH ~ 5.0, a consequence of the greater basicity of long-chain amines. Figure 1b presents exemplary distribution curves of the complex species forming in the system Hg(II)/*Cyd*/*Spd*.

TABLE IV ^{13}C NMR chemical shifts for Cd(II)/*Cyd*/di- or triamine systems and differences (in parentheses) between corresponding chemical shifts of the ligands in ternary systems and metal – free ligands [ppm]

		Cd/Cyd/diamine			*Cd/Cyd/triamine*					
		en	*tn*	*Put*	*dien*		*2,3-tri*	*3,3-tri*		*Spd*
		pH 6.0	*pH 9.0*	*pH 9.0*	*pH 6.0*	*pH 9.2*	*pH 8.4*	*pH 8.0*	*pH 9.2*	*pH 9.0*
Cyd	C(2)	158.610 (0.062)	158.284 (0.328)	158.284 (0.328)	158.419 (0.342)	158.292 (0.106)	158.474 (0.091)	158.601 (0.203)	158.277 (0.121)	158.621 (0.223)
	C(4)	167.522 (0.336)	166.836 (0.350)	166.844 (0.342)	167.063 (0.389)	166.836 (0.152)	167.003 (0.031)	167.191 (0.219)	166.821 (0.167)	167.196 (0.208)
	C(5)	97.091 (0.020)	96.987 (0.084)	96.994 (0.077)	97.080 (0.158)	96.994 (0.021)	97.055 (0.031)	97.110 (0.101)	96.987 (0.022)	97.106 (0.097)
	C(6)	142.622 (0.043)	142.319 (0.260)	142.326 (0.253)	142.679 (0.327)	142.311 (0.152)	142.417 (0.061)	142.610 (0.163)	142.311 (0.152)	142.584 (0.121)
PA	C(1)	40.550 (0.132)	38.739 (0.768)	40.128 (0.329)	39.519 (0.093)	39.642 (0.199)	39.233 (0.655)	37.900 (0.075)	39.012 (0.447)	38.202 (0.036)
	C(2)		29.944 (0.053)	26.052 (0.892)	45.908 (0.123)	47.124 (1.617)	46.350 (0.142)	25.565 (0.394)	28.457 (1.183)	26.665 (0.050)
	C(3)						47.064 (0.693)	45.884 (0.039)	46.722 (0.463)	45.976 (0.012)
	C(4)						27.182 (0.653)			48.157 (0.023)
	C(5)						38.656 (0.324)			24.707 (0.030)
	C(6)									25.299 (0.023)
	C(7)									39.890 (0.007)

The system Cd(II)/Cyd/PA

Table III lists stability constants ($\log\beta$) of mixed-ligand complexes of Cd(II) with *Cyd* and di-or triamines and equilibrium constants for complex formation.

Cd(II)/Cyd/diamine

In ternary systems of cadmium (II) ions with *Cyd* and diamines, MLL′ forms only with *en*. The other diamines studied prefer formation of monoprotonated complexes. In the complex Cd(*Cyd*)(*en*), two amine donor nitrogen atoms and N(3) from cytidine ring take part in coordination ({N3} chromophore), as can be concluded from the value for the equilibrium constant for Cd(*Cyd*)(*en*) formation: Cd(*Cyd*) + *en*$\rightleftharpoons$Cd(*Cyd*)(*en*); $\log K_e = \log\beta_{Cd(Cyd)(en)} - \log\beta_{Cd(Cyd)} = 6.0$ and $\log K_e = 5.31$ for the complex Cd(*en*), for which bidentate interaction has been established [44]. Much lower values for monoprotonated mixed complexes: 3.92 and 3.90 for Cd(*Cyd*)(H*tn*) and Cd(*Cyd*)(H*Put*), respectively, Table III, confirm the bidentate character of *en* in the heteroligand complex.

Complexes of cadmium(II) with *Cyd* and diamines start forming from pH ~ 7.0, and their maximum concentrations occur at pH ~ 9.0 for Cd(*Cyd*)(*en*) and Cd(*Cyd*)(H*tn*) and at pH ~ 9.5 for the monoprotonated complex with *Put*. Figure 2a presents distribution curves of complex species forming in the ternary system involving Cd(II), cytidine and putrescine.

Similar to the binary system Cd(II)/*Cyd* [44], in the above ternary Cd(II)/*Cyd*/diamine systems metallation was found to involve N(3) atom from the cytidine ring with formation of {N3} chromophores with *en* and {N2} chromophores with *tn* and *Put*, as indicated by ^{13}C NMR results. Shifts of the C(2) and C(4) carbon atom signals near N(3) are (at pH ~ 6) 0.062 and 0.336 ppm, 0.328 and 0.350 ppm, 0.328 and 0.342 ppm, for the systems with *en*, *tn*, and *Put*, respectively (Table IV) and are significantly greater than shifts of the C(5) and C(6) signals.

Cd(II)/Cyd/triamines

As indicated by the results of the equilibrium study, cadmium(II) ions in ternary systems with cytidine, *dien*, *2,3-tri* and *3,3-tri* form MLL′ mixed-ligand complexes. Stability constants ($\log\beta_1$) of the complexes and equilibrium constants of formation, Table III, are higher than corresponding values determined for the Cd(*Cyd*)(*en*) complex (for which the two diamine nitrogen atoms were found to be involved in coordination; this indicates that with triamines the third donor nitrogen atom from the triamine is also involved in coordination. Confirmation of this mode of coordination lies in the similarity of the equilibrium constants for the Cd(*Cyd*)(PA) heteroligand complexes ($\log K_e = 8.87$, 8.11 and 7.28 for systems with *dien*, *2,3-tri* and *3,3-tri*, respectively) with those for the Cd(*dien*), Cd(*2,3-tri*) and Cd(*3,3-tri*) parent complexes ($\log K_e = 7.68$, 7.44 and 6.90, respectively). In the latter three complexes, three amine nitrogen atoms are involved in coordination [44]. This mode interactions has been clearly confirmed by spectroscopy (Table IV). For instance, the shifts of the ^{13}C NMR carbon signals for the asymmetric triamine *2,3-tri* are C(1) 0.655; C(2) 0.142; C(3) 0.693; C(4) 0.653 and C(5) 0.324 ppm, and point to changes in the vicinity of the nitrogen atoms N_a, N_b and N_c; this further suggests the participation of all donor atoms of PA in the interactions. In the system Hg(II)/*Cyd*/diamine (or triamine) as well as Cd(II)/*Cyd*/diamine and Cd(II)/*Cyd*/triamine, the endocyclic nitrogen atom from the pyrimidine ring is involved in metallation, leading to {N4} chromophore formation. For instance, in the Cd(*Cyd*)(*dien*) complex, ^{13}C NMR carbon atom signal shifts (at pH 9.2 0.106 ppm and 0.152 ppm for C(2) and C(4), respectively), point to the involvement of N(3). In the protonated complex Cd(*Cyd*)(H*dien*) a N(3) atom from *Cyd* (Table IV) as well as two nitrogen atoms from the triamine take part in coordination. This follows from the value for the equilibrium constant for formation of Cd(*Cyd*)(H*dien*), $\log K_e = 5.75$ (Table III), being much lower than that for the species Cd(*Cyd*)(*dien*), with three nitrogen atoms from *dien* in the inner coordination sphere ($\log K_e = 8.87$). In the diprotonated complexes Cd(*Cyd*)(H_2*dien*), Cd(*Cyd*)($H_2$3,3-*tri*) and Cd(*Cyd*)(H_2*Spd*), the equilibrium constants (log K_e) values are 3.82, 3.33, and 3.53, respectively, (Table III) unambiguously indicate the involvement of only one nitrogen atom of the triamine. Shifts of signals assigned to the carbon atom C(1) and C(2) of the asymmetric amine are 0.036 and 0.050 ppm, respectively (Table IV), for the Cd(*Cyd*)(H_2*Spd*) and suggest that the N_a nitrogen atom of spermidine takes part in coordination. The relatively small but systematic shifts can be explained by the low concentrations of the complexes studied. The appearance of precipitates in the ternary systems makes it impossible to prepare samples of higher concentration for NMR studies. As follows from the distribution curves of species, the protonated complexes start forming from pH 3.5 in the system with *dien* and from pH 6.0 in the systems with *3,3-tri* and *Spd*, while MLL′ complexes start forming from pH ~ 7. The diprotonated complex Cd(*Cyd*)(H_2*Spd*) reaches maximum concentration at pH ~ 9.0 and begins to decompose above pH 10.0 (Figure 2b).

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