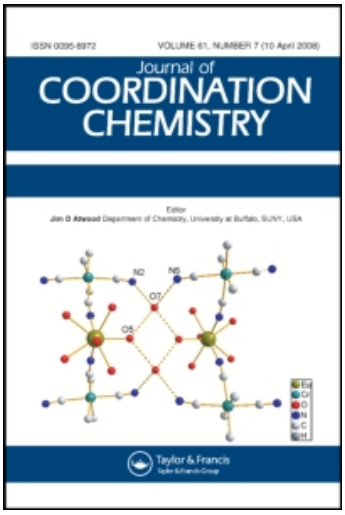

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Interactions of cadmium(II) ions with adenosine as well as adenosine-5'-monophosphate and diamine or triamines in the ternary systems

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Interactions of cadmium(II) ions with adenosine as well as adenosine-5′-monophosphate and diamine or triamines in the ternary systems

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The mode of coordination in complexes formed in the systems Cd(II)/Ado/di- or triamine and Cd(II)/AMP/di- or triamine has been established on the basis of the equilibrium and spectral investigation. The overall stability constants ($\log \beta$) and equilibrium constants of formation ($\log K_e$) of the complexes formed in the above systems have been determined. In the species, the main interaction centres are the nitrogen atoms N(1) or N(7) of the purine ring of Ado and AMP, while in the nucleotide also the oxygen atoms of the phosphate group and donor nitrogen atoms of the polyamine (PA) molecule. The effect of polyamine on the coordination dichotomy has been ascertained. For example, the presence of polyamine in the ternary system is responsible for involvement of only N(7) of the nucleoside in the metallation in Cd(Ado)H(Put), while in the binary system Cd(II)/Ado, the N(1)/N(7) dichotomy occurs in the whole pH range studied. Polyamine can also affect the character of the phosphate group of the nucleotide. The phosphate group, inactive in the binary system Cd(II)/AMP, after introduction of the polyamine becomes engaged in the interactions, e.g. in the complex Cd(AMP)H(dien). In Cd(AMP)H(en) and Cd(AMP)H(Put) no participation of the oxygen atom of the phosphate group has been found. This group is involved in weak interactions with a protonated amine group PA located in the outer coordination sphere. On the other hand, in the Cd(AMP)H(3,3-tri) complex the phosphate group is the only site of Cd(II) bonding. In this species the donor nitrogen atoms N(1) and N(7) of the nucleotide are outside the inner coordination sphere and involved in noncovalent interactions with protonated amine group from 3,3-tri.

Keywords: Cadmium; Nucleosides; Nucleotides; Polyamines; Coordination mode

1. Introduction

Interactions of metal ions with nucleic acids and their fragments (nucleosides and nucleotides) [1–3] and biogenic amines (e.g. putrescine and spermidine) [4, 5] change the character of many bioprocesses. In physiological conditions polyamines (PA) occur in the protonated form and enter into electrostatic interactions with the negative fragments of other biomolecules (e.g. nucleic acids or components of biological membranes) [6–11]. These organic polycations [12] are involved e.g. in the growth and

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proliferation of cells [8, 12, 13], genetic information transfer [14–16] and stabilisation of the DNA conformation by linking directly to the negatively charged major groove of the double helix [8, 17, 18]. The preferred bonding sites of DNA and PA (i.e. the base residues as well as phosphate groups) have been established [19–22]. The release of polyamines in biological systems is regulated mainly by enzymes containing Cu ions such as amine oxidase, which catalyses oxidation of the amino group to aldehyde by reducing the positive charge of these polycations [23]. Certain polyamines are substrates of amine oxidases also when bound to various polyphosphates, such as ADP or ATP [24]. The donor nitrogen atoms N(1) and N(7) of purine ring and the oxygen atoms from the phosphate group are the main sites of interaction with metal ions as well as with bioligands occurring in living organisms (e.g. amine acids or polyamines) [25–27]. Even small changes in the ligand's structure (e.g. in the length of the polyamine chain) have a significant effect on reactions with metal ions or other molecules in living cells [28, 29].

In a number of systems of Cu(II) ions with the nucleoside or nucleotide ligands, the occurrence of a mixture of isomers (coordination dichotomy) with monofunctional bonding of metal ions *via* N(1) or N(7) atoms of a purine ring is observed. Simultaneous coordination with involvement of both nitrogens is impossible for steric reasons [26, 30–34]. Similar interactions have been established in the systems with the ions Ni(II), Co(II), Zn(II), Cd(II) and Hg(II) [34–37]. The presence of a polyamine can change the character of coordination dichotomy [31, 32].

This article is a continuation of our earlier studies of the systems Cd(II)/Cyd (CMP)/PA [38–40] and reports results of the equilibrium and spectral study of the complexation reactions in ternary systems of cadmium(II) ions, adenosine (Ado), adenosine-5′-monophosphate (AMP) and polyamines: 1,2-diaminoethane (en), 1,3-diaminopropane (tn), 1,4-diaminobutane (Put), 1,5-diamino-3-azapentane (dien), 1,6-diamino-3-azahexane (2,3-tri), 1,7-diamino-4-azaheptane (3,3-tri) and 1,8-diamino-4-azaoctane (Spd).

2. Experimental

1,2-Diaminoethane (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, 1,5-diamino-3-azapentane (dien), 1,6-diamino-3-azahexane (2,3-tri), 1,7-diamino-4-azaheptane (3,3-tri) – from Aldrich. The appropriate nitrates were prepared by dissolving a proper amount of free amine and addition of an equimolar amount of HNO_3. The obtained white precipitate was recrystallized, washed with methanol and dried in a desiccator over P_4O_{10} or in the air. Adenosine (Ado) and adenosine 5′-monophosphate – sodium salt (AMP) were purchased from Sigma. Adenosine as nitrate salt was prepared in the same way as nucleoside hydrochlorides [31, 32]. The ligands used as nitrate salts were subjected to elemental analysis and the results (%C, %N, %H) were in agreement with the theoretically calculated values ($\pm 0.5\%$). $Cd(NO_3)_2 \cdot 4H_2O$ was purchased from Merck. The concentration of Cd(II) ions was determined complexometrically using EDTA and pyrocatechol violet as an indicator. Potentiometric studies were performed on a DTS Radiometer 800 Multi-Titration System with a GK-2401C electrode calibrated in terms of hydrogen

ions concentration [41] with a preliminary use of borax (pH = 9.225) and phthalate (pH = 4.002) standard buffers. The concentration of the ligands in the titrated systems varied from $1.3 \cdot 10^{-3}$ to $3.4 \cdot 10^{-3}$ M, the concentration of metal ions – from $1.3 \cdot 10^{-3}$ to $1.7 \cdot 10^{-3}$ M, the ratio of M : L : L′ 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 ± 1°C under helium, using as a titrant CO_2-free NaOH solution (about 0.2 M). Addition of NaOH solution did not change the ionic strength, because the measurements were performed, starting from fully protonated polyamines, so $-NH_x^+$ cations were replaced by equivalent amounts of Na^+. The calculations were performed using 100–350 points for each job, taking into account only this part of the titration curve, when there was no precipitate in the solution. The selection of the models and the determination of the stability constants of the complexes were made using the SUPERQUAD program [42], whereas the distribution of particular forms was determined by the HALTAFALL program [43]. The criteria used for verification of results are given in [44]. Samples for ^{13}C NMR investigation were prepared by dissolving appropriate amounts of the ligands and metal nitrates in D_2O and adjusting pH by addition of NaOD and DNO_3, correcting pH-readings (a pH meter N517 made by Mera-Tronik) according to the formula: $pD = pH_{readings} + 0.40$ [45]. The concentration of ligands in samples for NMR studies was 0.1 M. The metal ion to ligands concentration ratio was 1 : 10 : 10 to 1 : 50 : 50. Measurements of ^{13}C NMR and ^{31}P NMR were taken on a NMR Gemini – 300VT Varian and on a NMR Unity-300 Varian spectrometer with dioxane or H_3PO_4 as internal standards, respectively. The ligands studied are presented in scheme 1.

3. Results and discussion

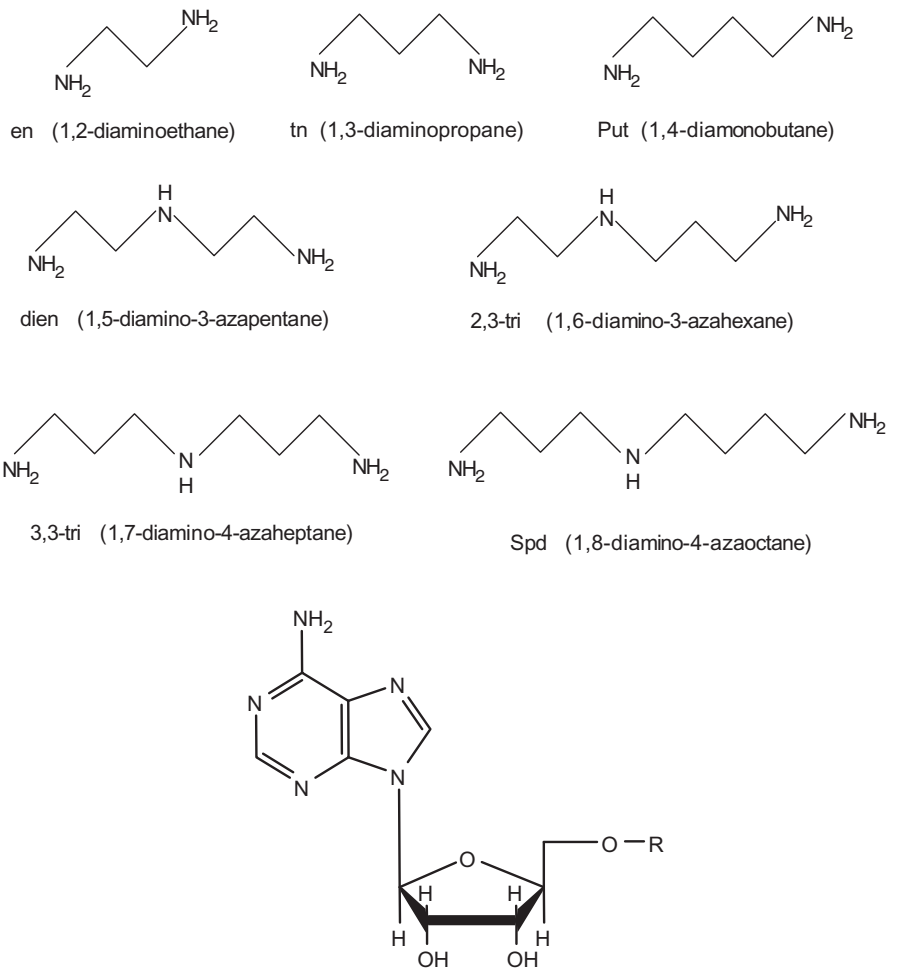
Computer analysis of the pH metric titration results was performed taking into account the protonation constants of the ligands studied, overall stability constants ($\log \beta$) of complexes formed in binary systems: Cd(II)/Ado, Cd(II)/AMP, Cd(II)/diamine and Cd(II)/triamine and the equilibrium constants of the formation of cadmium hydroxocomplexes $Cd(OH)^+$ and $Cd(OH)_2$; $M^{2+} + xH_2O \rightleftarrows M(OH)_x^{2-x} + xH^+$, determined earlier at the same conditions [46].

3.1. *Ternary Cd(II)/nucleoside/polyamine systems*

3.1.1. Cd(II)/Ado/diamine systems. Table 1 presents the overall stability constants ($\log \beta$) and equilibrium constants ($\log K_e$) of formation of mixed complexes formed in ternary systems of Cd(II) ions with adenosine (Ado) and diamines (PA), calculated from the equation:

$$\log K_e^{HxPA}{}_{Cd(Ado)Hx(PA)} = \log \beta_{Cd(Ado)(PA)} - \log \beta_{Cd(Ado)} - \log \beta_{HxPA}$$

In the Cd(II)/Ado/diamine systems only putrescine (Put) shows a tendency towards formation of monoprotonated complexes MLHL′ (where: M = metal; L = nucleoside;



Scheme 1. The structures of the ligands studied.

L′ = polyamine). In the systems with polyamines of a shorter carbon chain (en and its homologue tn) the mixed ligand complexes MLL′ are formed. The same magnitude order of the equilibrium constant for binary Cd(en) complex, ({N2} chromophore), $\log \beta_1 = \log K_e = 5.31$ [46] and those of formation of the species Cd(Ado)(en) and Cd(Ado)(tn), $\log K_e$ of 5.70 and 4.98, respectively, indicate the participation of two donor nitrogen atoms of PA ($\log K_e$ corresponds to the energy of the bond between polyamine and Cd(II) as well as between polyamine and Cd(II) ion of the anchoring Cd(Ado) complex, respectively). However, coordination with formation of a thermodynamically preferred five-membered chelate ring, as found in the en complex and less preferred six-membered ring in tn complex results in different $\log K_e$.

Table 1. Overall stability constans ($\log\beta$) and equlibrium constants ($\log K_e$) for complexes in Cd(II)/Ado/diamine and Cd(II)/Ado/triamine ternary systems.

System	Equlibrium	$\log\beta$	$\log K_e$	Chromophor
CdII/Ado)/en	Cd(Ado) + en ⇌ Cd(Ado)(en)	8.02 (15)	5.70	{N3}
Cd(II)/Ado/tn	Cd(Ado) + tn ⇌ Cd(Ado)(tn)	7.30 (8)	4.98	{N3}
Cd(II)/Ado/Put	Cd(Ado) + HPut ⇌ Cd(Ado)H(Put)	16.96 (14)	4.09	{N2}
Cd(II)/Ado/dien	Cd(Ado) + dien ⇌ Cd(Ado)(dien)	10.28 (8)	7.96	{N4}
	Cd(Ado) + dien + H_2O ⇌ Cd(Ado)(dien)(OH) + H^+	2.63 (6)	–	{N4}
Cd(II)/Ado/2,3-tri	Cd(Ado) + 2,3-tri ⇌ Cd(Ado)(2,3-tri)	9.69 (10)	7.37	{N4}
Cd(II)/Ado/3,3-tri	Cd(Ado) + 3,3-tri ⇌ Cd(Ado)(3,3-tri)	10.20 (5)	7.88	{N4}
	Cd(Ado) + $H_2$3,3-tri ⇌ Cd(Ado)($H_2$3,3-tri)	26.59 (8)	3.75	{N2}
Cd(II)/Ado/Spd	Cd(Ado) + H_2Spd ⇌ Cd(Ado)H_2(Spd)	26.08 (9)	2.98	{N2}

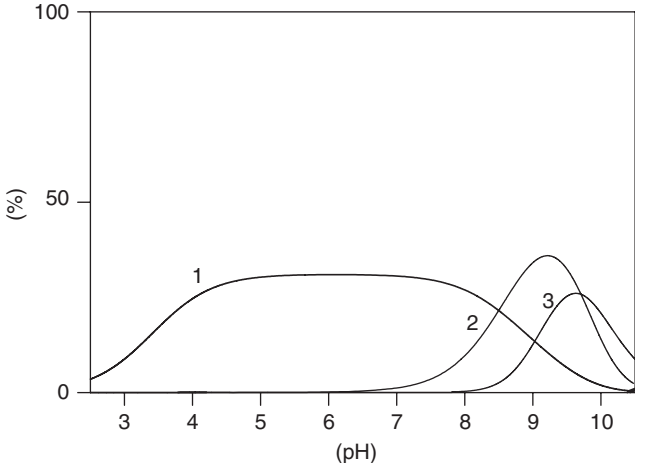
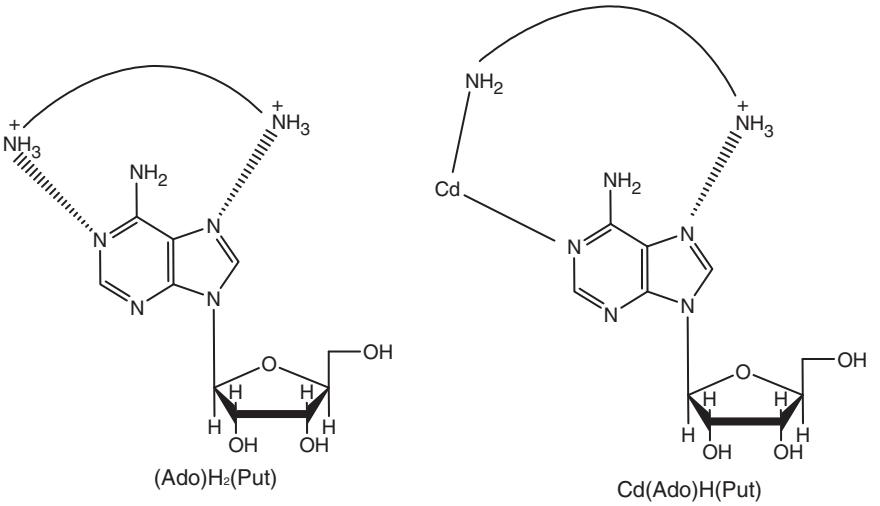


Figure 1. Distribution diagrams for the system Cu(II)/Ado/Put (percentage refers to the total metal); 1, Cd(Ado); 2, Cd(Ado)H(Put); 3, Cd(Put) $C_{Cd2+} = 1.4 \times 10^{-3}$mol dm^{-3}; $C_{Ado} = 2.7 \times 10^{-3}$mol dm^{-3}; $C_{Put} = 2.7 \times 10^{-3}$mol dm^{-3}.

The process of formation of mixed complexes in the system Cd(II)/Ado/en starts for pH close to 7.0, while in the system Cd(II)/Ado/tn – from pH close to 8.0. Both heteroligand complexes bind ca 20% of metal ions, at pH of about 9. In the system with putrescine Cd(II)/Ado/Put (figure 1), at low pH there is a binary complex Cd(Ado), while the mixed complex with monofunctional bonding of putrescine Cd(Ado)H(Put) is present at pH above 7.0. This mode of coordination can be understood taking into regard the pH range of its formation and the pK of adenosine. The donor nitrogen atom from the nucleoside is deprotonated and the protonated HPut molecule is located in the inner coordination sphere of the metal ion. The equilibrium constant of Cd(Ado)H(Put) formation: $\log K_e = \log\beta_{Cd(Ado)H(Put)} - \log\beta_{Cd(Ado)} - \log\beta_{H(Put)} = 16.96 - 2.32 - 0.55 = 4.09$, smaller by 1.61 and 0.89 $\log K_e$ units smaller than those of Cd(Ado)(en) and Cd(Ado)(tn), in which two donor nitrogen atoms from the diamine take part in metallation.

In the metal-free system Ado/Put studied earlier [32] formation of the (Ado)H_2(Put) adduct characterised by the stability constant $\log\beta = 22.04$ and equilibrium constant $\log K_e = 1.51$ was observed in the pH range 4–10. In this adduct the presence



Scheme 2. Mode of interactions in (Ado) H_2 (Put) and Cd (Ado) H (Put).

Table 2. Differences between ^{13}C NMR chemical shifts for the ligands in the Cd(II)/Ado/diamine systems in relation to the free ligands [ppm].

		Ado					PA	
System	pH	C(2)	C(4)	C(5)	C(6)	C(8)	C(1)	C(2)
Cd(II)/Ado/en	9.0	0.023	0.040	0.055	0.030	0.082	0.132	–
Cd(II)/Ado/tn	9.4	0.063	0.040	0.488	0.052	0.093	0.149	0.270
Cd(II)/Ado/Put	6.0	0.291	0.078	0.147	0.145	0.209	0.006	0.002

of intermolecular noncovalent ligand–ligand interactions has been established, with the deprotonated N(1) and N(7) nitrogen atoms from the nucleoside (these of high electron density) and protonated donor nitrogen atoms of putrescine as the interaction centres. In the Cd(II)/Ado/Put system the presence of Cd(II) changes the character of interactions (noncovalent into covalent) between the bioligands, as shown in scheme 2. Due to these changes the value of $\log K_e = 4.09$ for Cd(Ado)H(Put) is by a few orders of magnitude increased with respect to that of the adduct (Ado)H_2(Put) ($\log K_e = 1.51$).

Analysis of the ^{13}C NMR spectra (table 2) suggests that in the Cd(Ado)(en) complex the metal is bonded exclusively by a donor nitrogen atom N(7). The shifts of signals assigned to the carbon atoms C(5) and C(8) located in the neighbourhood of N(7) from the purine ring are 0.055 and 0.082 ppm, respectively, while the signals assigned to carbon atoms C(2) and C(6) localised in the vicinity of N(1) are clearly lower, 0.023 and 0.030 ppm, respectively. This fact confirms that despite a stronger basic character of N(1) from purine nucleoside, N(7) is the preferred interaction site in coordination compounds [47]. As concluded from results of NMR investigation (table 2), the coordination character of nucleoside in the Cd(Ado)(en) complex is different than that in the binary system of Cd(II) ions with Ado [46], in which there is a coordination dichotomy (a mixture of isomers with the coordination either by N(1) or by N(7) of the

nucleoside, as a simultaneous coordination to both of them N(1) and N(7) with formation of chelate is impossible for the steric reasons). The introduction of en into the system Cd/Ado leads to disappearance of the coordination dichotomy, as has been observed in the Cd/Ado/Put system. The influence of polyamines on the character of interaction has been recently observed in Cu(II)/Ado/PA systems, as well [31, 32].

The presence of 1,3-diaminopropane was found to have no effect on the dichotomy found in the Cd(Ado) complex. In the Cd(Ado)(tn) complex this nucleoside interacts with metal ions either through N(1) or N(7), as concluded from NMR study (table 2). The involvement of the $-NH_2$ groups in coordination in the complexes is confirmed by shifts of the signals assigned to carbon atoms from the vicinity of the donor nitrogen atoms of the polyamine, relative to the positions of the corresponding signals in the spectra of free ligands. For example in the spectrum of Cd(Ado)(tn) at pH = 9.4, at which the complex is dominant, the chemical shifts of C(1) and C(2) are 0.149 ppm and C(2) 0.270 ppm, respectively. In all systems discussed (with the nucleoside and nucleotide) the involvement of the carbonyl groups or the donor atoms from ribose was not observed, as reported [32, 36, 46, 48, 49].

3.1.2. Cd(II)/Ado/triamine systems. In the systems of Cd(II) with Ado and triamines the occurrence of the following species was established: protonated of MLH_2L' type, mixed of MLL′ type and the hydroxocomplex Cd(Ado)(dien)(OH). The protonated complexes Cd(Ado)H_2(3,3-tri) and Cd(Ado)H_2(Spd) start forming from pH close to 6 and disappear at pH of 9 and 10, respectively, binding a maximum of 35% and 25% of the metal at pH 8 and 9, respectively. The MLL′ complexes with dien, 2,3-tri and 3,3-tri form in the pH range from about 7 and bind a maximum of 30%, 25% and 65% of the metal, respectively. Taking into regard the protonation constants, the pH range of the protonated MLH_2L' complexes formation and their stoichiometry indicate participation of only one donor nitrogen atom of the polyamine in the coordination, besides the endocyclic nitrogen atoms of the nucleoside. This conclusion is supported by the equilibrium constants of formation of Cd(Ado)H_2(3,3-tri) and Cd(Ado)H_2(Spd), $\log K_e = 3.75$ and $\log K_e = 2.98$, respectively (table 1). These values are lower than $\log K_e$ of Cd(Ado)(en) and Cd(Ado)(tn), table 1 and than that of the binary complex CdH(3,3-tri) [46] $\log K_e = 4.69$, in which two nitrogen atoms of PA take part in the metallation. In the ^{13}C NMR spectra of Cd(Ado)H_2(3,3-tri) and Cd(Ado)H_2(Spd) the chemical shifts of the signals assigned to C(2) and C(6) neighbouring N(1) as well as C(5) and C(8) neighbouring N(7) of the nucleoside are respectively: 0.148; 0.118; 0.113; 0.121 ppm and 0.357; 0.414; 0.284; 0.263 ppm (table 3) which also strongly indicates the involvement of either N(1) or N(7) in the coordination (coordination dichotomy).

Table 3. Differences between ^{13}C NMR chemical shifts for the ligands in the Cd(II)/Ado/triamine systems in relation to the free ligands [ppm].

		Ado					PA						
System	pH	C(2)	C(4)	C(5)	C(6)	C(8)	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)	C(7)
Cd(II)/Ado/dien	8.5	0.303	0.058	0.232	0.312	0.222	0.055	0.673					
Cd(II)/Ado/2,3-tri	8.0	0.004	0.028	0.077	0.019	0.021	0.236	0.176	0.096	0.209	0.183		
Cd(II)/Ado/3,3-tri	8.0	0.148	0.057	0.113	0.118	0.121	0.603	1.424	0.401				
	10.0	0.160	0.027	0.617	0.089	0.016	0.247	1.893	0.164				
Cd(II)/Ado/Spd	9.0	0.357	0.023	0.284	0.414	0.263	0.013	0.220	0.060	0.080	0.209	0.106	0.011

The insignificant chemical shifts of the C(1) and C(7) atoms located at the terminal amine groups of Spd (asymmetric biogenic amine) exclude participation of the primary nitrogens in the interactions. On the other hand, significant changes in the chemical shift of the carbon atoms from the neighbourhood of the secondary nitrogen atom, $-NH_x$ group, (table 3) suggest the involvement of this atom of Spd in the metallation.

The equilibrium constants for MLL′ species Cd(Ado)(dien), Cd(Ado)(2,3-tri) and Cd(Ado)(3,3-tri) which are 7.96, 7.37 and 7.88, respectively (table 1) are much higher than those for the analogous ternary complexes with diamines, e.g. for Cd(Ado)(tn), $\log K_e = 4.98$ (table 1). This observation testifies to the participation of three nitrogen atoms of triamine in the coordination and chelate ring closure with formation of chromophore {N4} (the fourth atom from the nucleoside). The formation of such a chromophore with a system of coupled rings 5/5 for dien, 5/6 for 2,3-tri and 6/6 for 3,3-tri is also indicated by values of the equilibrium constants of formation of these complexes similar to those of the corresponding binary species being $\log K_e = 7.68$ for Cd(dien); $\log K_e = 7.37$ for Cd(2,3-tri) and $\log K_e = 6.90$ for Cd(3,3-tri) [46]. Moreover, such a mode of coordination of the polyamine with respect to the anchoring Cd(Ado) is confirmed by ^{13}C NMR spectra analysis (table 3). For example, the chemical shifts assigned to the carbon atoms C(1), C(2), C(3), C(4) and C(5) for a mixed complex with 2,3-tri, relative to those of the free ligand are 0.236, 0.176, 0.096, 0.209 and 0.183 ppm, respectively. In the species studied also the donor nitrogen atoms of the nucleoside are involved in coordination. In the ^{13}C NMR spectra of the Cd(II)/Ado/dien system at pH 8.5 (the pH range of Cd(Ado)(dien) complex domination) the chemical shifts of C(2), C(6), C(5) and C(8) are 0.303 0.312 ppm, 0.232 and 0.222 ppm. This pattern of changes means that in the systems of Cd(II) ions with Ado and triamines there is coordination dichotomy.

3.2. *Ternary Cd(II)/nucleotide/polyamine system*

3.2.1. Cd(II)/AMP/diamina systems. Complexation reactions of complex formation in the systems with nucleotides start at pH values lower than in the systems with nucleosides, a consequence of the involvement of the deprotonated phosphate group in the metallation. In the Cd(II)/AMP/diamine systems, the $ML\cdots L'$ type complexes: $Cd(AMP)H_2(en)$, $Cd(AMP)H_2(tn)$ and $Cd(AMP)H_2(Put)$ and the protonated complexes Cd(AMP)H(en) and Cd(AMP)H(Put) were observed to form.

The pH range of occurrence for $Cd(AMP)H_2(en)$, $Cd(AMP)H_2(tn)$ (figure 2) and $Cd(AMP)H_2(Put)$ (pH above 5, in the conditions of deprotonated nucleotide and fully protonated diamine), composition of the complexes and the equilibrium constants of their formation ($\log K_e$) 3.45, 3.71 and 3.66 (table 4), are similar to those obtained for the analogous species occurring in the system of cytidine-5′-monophosphate (e.g. for $Cd(CMP)H_2(tn)$ $\log K_e = 3.96$, while for $Cd(CMP)H_2(Put)$ $\log K_e = 3.52$ [39]) suggests that the protonated species are molecular complexes with noncovalent interaction between fully protonated amine and Cd(AMP) complex.

The equilibrium constants of formation of molecular complexes are lower than those of formation of Cd(Ado)(en) and Cd(Ado)(tn), $\log K_e = 5.70$ and 4.98, respectively (table 1), which indicates a different mode of interaction of diamine and confirms formation of a molecular complex with the nucleotide, as described earlier for a similar system [50]. Analysis of the chemical shifts of the AMP carbon atoms in the ^{13}C NMR

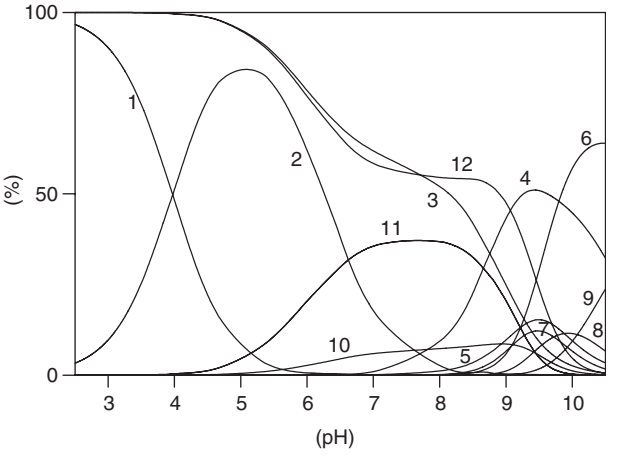


Figure 2. Distribution diagrams for the system Cd(II)/AMP/tn (percentage refers to the total metal); 1, H_2AMP; 2, HAMP; 3, H_2tn; 4, Htn; 5, $Cd(OH)^+$; 6, $Cd(OH)_2$; 7, Cd(tn); 8, $Cd(tn)_2$; 9, $Cd(tn)(OH)_2$; 10, Cd(AMP); 11, $Cd(AMP)H_2(tn)$; 12, Cd^{2+}; $C_{Cd2+} = 1.4 \times 10^{-3}$mol dm^{-3}; $C_{AMP} = 1.4 \times 10^{-3}$mol dm^{-3}; $C_{tn} = 1.4 \times 10^{-3}$mol dm^{-3}.

Table 4. Overall stability constans ($\log\beta$) and equlibrium constants ($\log K_e$) for complexes in Cd(II)/AMP/diamine and triamine ternary systems.

Systems	Equlibrium	$\log\beta$	$\log K_e$	Chromophor
Cd(II)/AMP/en	$Cd(AMP) + H_2en \rightleftharpoons Cd(AMP)H_2en$	23.50 (8)	3.45	Molecular complex
	$Cd(AMP) + Hen \rightleftharpoons Cd(AMP)Hen$	16.52 (11)	3.75	{N2}
Cd(II)/AMP/tn	$Cd(AMP) + H_2tn \rightleftharpoons Cd(AMP)H_2tn$	25.53 (5)	3.71	Molecular complex
Cd(II)/AMP/Put	$Cd(AMP) + H_2Put \rightleftharpoons Cd(AMP)H_2Put$	26.32 (3)	3.66	Molecular complex
	$Cd(AMP) + HPut \rightleftharpoons Cd(AMP)HPut$	17.26 (4)	4.23	{N2}
Cd(II)/AMP/dien	$Cd(AMP) + Hdien \rightleftharpoons Cd(AMP)Hdien$	17.97 (9)	5.53	{N3,O}
Cd(II)/AMP/2,3-tri	$Cd(AMP) + H_2(2,3\text{-tri}) \rightleftharpoons Cd(AMP)H_2(2,3\text{-tri})$	26.14 (5)	3.95	{N2,O}
	$Cd(AMP) + H(2,3\text{-tri}) \rightleftharpoons Cd(AMP)H(2,3\text{-tri})$	18.59 (5)	5.71	{N3,O}
Cd(II)/AMP/3,3-tri	$Cd(AMP) + H_3(3,3\text{-tri}) \rightleftharpoons Cd(AMP)H_3(3,3\text{-tri})$	35.37 (8)	4.04	Molecular complex
	$Cd(AMP) + H(3,3\text{-tri}) \rightleftharpoons Cd(AMP)H(3,3\text{-tri})$	19.22 (9)	5.99	{N2,O}
Cd(II)/AMP/Spd	$Cd(AMP) + H_3Spd \rightleftharpoons Cd(AMP)H_3Spd$	35.49 (10)	3.71	Molecular complex

and ^{31}P NMR spectra of the species studied (table 5) suggests that in the molecular complexes with en and Put interaction centres are N(1) or N(7) atoms of nucleotide and oxygen atoms of the phosphate group.

However, in $Cd(AMP)H_2(tn)$ only N(7) takes part in the interactions (table 4). Moreover, in the ^{31}P NMR spectra of all molecular complexes the chemical shifts of the phosphorus atom are changed, which, together with the results of the equilibrium study, point to participation of phosphate groups in reaction. As follows from the above analysis, the introduction of diamine into the Cd(II)/AMP system changes the mode of metal ion interaction with AMP. The phosphate group of the nucleotide, inactive in a binary system (as it has been found earlier [46]), is now involved in coordination. The totally protonated PA is in the outer coordination sphere and engaged in noncovalent interactions with anchoring Cd(AMP), as indicated by the changes in the NMR signals. For instance at pH = 8, where the $Cd(AMP)H_2(Put)$ species dominates, the chemical shifts of the carbon atoms C(1) and C(2) of putrescine are 0.182 and 0.463 ppm, respectively.

Table 5. Differences between ^{13}C NMR chemical shifts for the ligands in the Cd(II)/AMP/triamine systems in relation to the free ligands [ppm].

		AMP						PA						
Systems	pH	C(2)	C(4)	C(5)	C(6)	C(8)	P	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)	C(7)
Cd(II)/AMP/en	6.0	0.008	0.005	0.064	0.065	0.041	0.207	0.148						
	8.0	0.002	0.027	0.238	0.024	0.030	0.030	0.534						
Cd(II)/AMP/tn	7.0	0.032	0.010	0.033	0.031	0.133	0.138	0.053	0.015					
Cd(II)/AMP/Put	8.0	0.169	0.027	0.101	0.154	0.113	0.122	0.182	0.463					
	10.0	0.168	0.026	0.005	0.066	0.133	0.038	1.643	0.433					
Cd(II)/AMP/dien	6.0	0.096	0.009	0.066	0.032	0.012	0.366	0.097	0.049					
Cd(II)/AMP/2,3-tri	7.0	0.269	0.045	0.138	0.156	0.160	0.236	0.602	0.242	0.223	0.674	0.298		
	8.0	0.281	0.069	0.182	0.253	0.195	0.100	0.163	0.020	0.231	0.086	0.060		
Cd(II)/AMP/3,3-tri	6.0	0.083	0.027	0.059	0.044	0.096	0.626	0.104	0.187	0.170				
	9.0	0.023	0.029	0.012	0.029	0.031	0.095	0.267	0.754	0.304				
Cd(II)/AMP/Spd	8.0	0.059	0.021	0.031	0.014	0.026	0.092	0.146	0.204	0.161	0.158	0.224	0.116	0.129

The Cd(AMP)H(en) complex starts forming at pH above 6, while Cd(AMP)H(Put) at pH above 7. As follows from the values of the equilibrium constants of the above species (table 4), higher than those of molecular complexes, the stoichiometric composition and the pH range of complex formation, the polyamine binds metal ions, however only by one $-NH_2$ group of diamine. This conclusion has been drawn from the ^{13}C NMR study. For example, at pH 10, at which the complex Cd(AMP)H(Put) dominates the changes in signals assigned to the C(1) and C(2) carbon atoms of Put are 1.643 and 0.433 ppm, similar to en species. Moreover, the signals assigned to the carbon atoms in the vicinity of the N(7) atom from AMP are also observed to be shifted (table 5). Therefore, in the species Cd(AMP)H(en), as in Cd(Ado)(en), coordination is realised only through the N(7) atom, while in Cd(AMP)H(Put) a coordination dichotomy N1/N7 is observed (table 5). In the two protonated complexes no efficient interaction of the phosphate group was observed as concluded from analyses of chemical shifts in the ^{31}P NMR spectra of Cd(AMP)H(en) and Cd(AMP)H(Put) at the phosphorus atom (they are only 0.030 and 0.038 ppm, respectively). The lack of participation of the phosphate group is confirmed by similar values of $\log K_e = 4.23$ obtained for the complex Cd(AMP)H(Put) and $\log K_e = 4.09$ obtained for Cd(Ado)H(Put), where a phosphate group does not exist (table 1). Moreover, according to some authors in similar metal/polyamine/nucleotide systems the metal ions binds endocyclic nitrogen atoms, while the phosphate group is engaged in noncovalent interactions with protonated polyamines [51].

3.2.2. Cd(II)/AMP/triamine systems. The overall stability constants ($\log \beta$) and equilibrium constants for the molecular complexes of $ML \cdots L'$ type and protonated ones of MLH_xL' type formed in the systems Cd(II)/AMP/triamine are presented in table 4. The molecular complex $Cd(AMP)H_3$(3,3-tri) appears in the pH range from 4.5 to 9.0, while $Cd(AMP)H_3$(Spd) in the pH range from 4.5 to pH 9.5 (figure 3), and the diprotonated species $Cd(AMP)H_2$(2,3-tri) is present in the pH range 5.0–9.0.

The monoprotonated complexes Cd(AMP)H(dien), Cd(AMP)H(2,3-tri) and Cd(AMP)H(3,3-tri) start forming from pH 5.5, 6.5 and 7.5, respectively. Except for Cd(AMP)H(2,3-tri), these complexes are dominant species in the system and bind about 75% of metal ions. The values of $\log K_e$ of the molecular complexes $Cd(AMP)H_3$ (3,3-tri) and $Cd(AMP)H_3$(Spd) are similar to those determined for $Cd(AMP)H_2$(en), $Cd(AMP)H_2$(tn) and $Cd(AMP)H_2$(Put) (table 4), which indicates a similar mode of interaction. The totally protonated triamine located in the outer coordination sphere is engaged in noncovalent interactions with the anchoring Cd(AMP). This mode of interaction is supported by changes in the signal positions in the ^{13}C NMR spectra (table 5). In both complexes of $ML \cdots L'$ type, (similarly as in the systems with diamines), the oxygen atoms of the nucleotide phosphate groups are involved in the coordination sphere of Cd(II) ions, as the ^{31}P NMR spectra of these complexes reveal significant shifts of the phosphorus signals relative to their positions in the spectra of free ligands, table 5. Moreover, the ^{13}C NMR spectra suggest the coordination dichotomy in the species $Cd(AMP)H_3$(3,3-tri) (the involvement of the donor N(1) or N(7) in the metallation), while in the analogous species with Spd the metal ion is bound only by N(7), (table 5).

The diprotonated MLH_2L' species is present only in the Cd(II)/AMP/2,3-tri system, reaching the maximum concentration at pH close to 7.0. Analysis of the NMR data

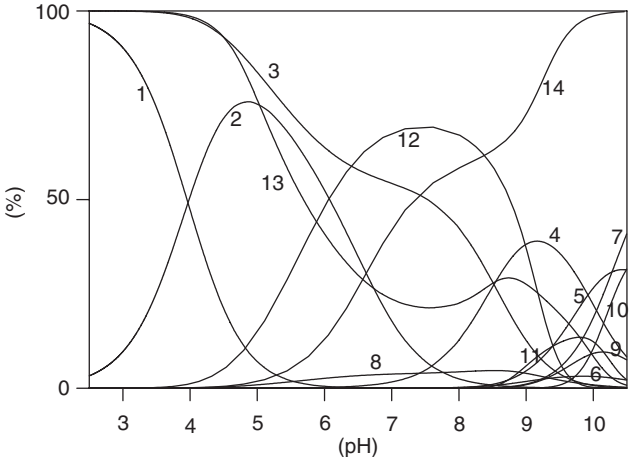


Figure 3. Distribution diagrams for the system Cd(II)/AMP/Spd (percentage refers to the total metal); 1, H_2AMP; 2, HAMP; 3, H_3Spd; 4, H_2Spd; 5, Spd; 6, $Cd(OH)^+$; 7, $Cd(OH)_2$; 8, Cd(AMP); 9, Cd(Spd); 10, $Cd(Spd)_2$; 11, $Cd(HSpd)_2$; 12, $Cd(AMP)H_3(Spd)$, 13, Cd^{2+}; 14, AMP $C_{Cd2+} = 1.7 \times 10^{-3}$mol dm^{-3}; $C_{AMP} = 3.4 \times 10^{-3}$mol dm^{-3}; $C_{Spd} = 3.4 \times 10^{-3}$mol dm^{-3}.

leaves no doubt about the involvement of the N(1) or N(7) nitrogen atoms and the oxygen atom from the purine nucleotide phosphate group in coordination in this species. On the other hand, according to the stoichiometry analysis and changes in the positions of the carbon signals from 2,3-tri (table 5), one of the $-NH_x$ groups of amine takes part in the metallation, while the other protonated groups are involved in the noncovalent interactions with the purine ring of the nucleotide.

The Cd(AMP)H(dien), Cd(AMP)H(2,3-tri) and Cd(AMP)H(3,3-tri) species (with monoprotonated triamines) are characterised by $\log K_e$ of 5.52, 5.71 and 5.99, respectively, and are similar to the value ($\log K_e = 5.31$) determined for the binary Cd(en) complex [46], indicating a similar coordination with the involvement of two $-NH_x$ groups from the polyamine. As a result of the reaction of triamine, five-membered chelate rings are formed. Analysis of the ^{13}C NMR spectra at pH = 9.0 in the system Cd/AMP/3,3-tri (table 5) has shown that in Cd(AMP)H(3,3-tri) the donor nitrogen atoms from the AMP purine ring do not take part in the metallation. The only sites of interactions in this bioligand are the oxygen atoms of the phosphate group, which is indicated by the shift of the phosphorus signal in the ^{31}P NMR spectrum by 0.095 ppm with respect to its position in the free ligand spectrum (table 5). Moreover, two amine groups of 3,3-tri are involved in the coordination, while the third protonated group is engaged in noncovalent interactions with the nitrogen atoms of the nucleotide purine ring. The untypical interactions with respect to those of the other ternary triamine species, corresponds to the high value of the equilibrium constant of this species formation, $\log K_e = 5.99$, in the complex in which the {N2, O} chromophore is present.

4. Conclusions

The main sites of metallation in the Cd(II)/Ado/polyamine systems are the donor nitrogen atoms N(1) or N(7) of the nucleotide purine ring and the nitrogen atoms from the polyamine.

In the systems of Cd(II) with Ado and tn, dien, 3,3-tri and Spd, the coordination dichotomy N(1)/N(7) has been established in the whole pH range studied. This situation is different than that in the Cd(II) systems with Ado, en, Put and 2,3-tri in which the coordination dichotomy, present in the binary complex Cd(Ado) disappears and the main interaction centre in the nucleotide is only N(7).

Similarly, as in the adenosine systems, in MLL′ complexes formed in the Cd(II)/AMP/polyamine systems, all donor nitrogen atoms from polyamines are involved in metallation. The introduction of the triamines into the system Cd(II)/AMP activates the phosphate group from the nucleotide (additional centre of interactions), which does not take part in coordination in the binary complex Cd(AMP). Such an activating effect on this group is not observed in the protonated complexes with diamines. In the molecular complexes of the ML $\cdots$ L′ type, with either di- or triamines, the polyamine is in the outer coordination sphere and engaged in noncovalent interactions with the anchoring Cd(AMP) complex.

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