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Complexes of Cadmium(II) and Mercury(II) with Cytidine 5′-Monophosphate and Diamines in Ternary Systems

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Formation of Cd(II) and Hg(II) complexes in ternary systems with cytidine 5′-monophosphate (*CMP*) and diamines (*en*, *tn*, *Put*) has been studied. In Hg(II)/CMP/diamine systems the formation of heteroligand compounds is accompanied by the occurrence of non-covalent interactions and the formation of molecular complexes. The presence of polyamine in ternary systems does not change the metal-nucleotide mode of coordination. Similarly as in the binary Hg(II)/CMP system, the metallation involves the donor endocyclic nitrogen N(3) atom and *CMP* phosphate group. On the other hand, in the Cd(II)/CMP system, the introduction of a polyamine changes the coordination mode of the nucleotide. Phosphate group, inactive in binary systems, takes part in the complexation in ternary systems. In systems of cadmium(II) ions, *CMP* and diamines, only molecular complexes are formed, in which the polyamine is in the outer coordination sphere. In contrast to Cd(CMP)(H_2tn) and Cd(CMP)(H_2Put) compounds, in Cd(CMP)(H_2en) species the phosphate group of nucleotide does not take part in the metallation, *i.e.* the metal ion coordinates with *CMP*, through the atom N(3) of the nucleotide, and the phosphate group is involved in non-covalent interactions with *en*.

Key words: cadmium(II), mercury(II), cytidine 5′-monophosphate, diamine, complexes

Metal ions present in living organisms significantly affect the functions of particular components of the biological system [1–3], including processes of transcription and replication of nucleic acids [4–6]. While interacting with bioligands, metal ions induce changes in their conformation [7,8], and should, therefore, be treated as factors interfering in the character of molecular complex formation processes [9].

The increased content of heavy metals (among which mercury and cadmium belong to the most threatening [10–13]) in the natural environment may lead to their increased level in living organisms, disturbing natural functions of such compounds as nucleic acids, which in combination with a permanent change in the expression of genes may lead to neoplasmic changes [10]. In biological systems, metal ions bind donor atoms of bioligands, according to the theory of hard and soft acids and bases [14–16]. Cd^{2+} and Hg^{2+} similarly as Pd^{2+} and Pt^{2+} ions show greatest affinity to the nitrogen atoms of purine or pyrimidine bases in molecules of nucleic acids. Biogenic

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amines, present in all biological tissues [17–22] under physiological conditions, occur in protonated form, in which they can be involved in non-covalent interactions with the negative phosphate groups of nucleic acids and/or donor endocyclic nitrogen atoms or oxygen atoms of purine or pyrimidine bases [23], playing a significant role in genetic information transfer [24–26]. As shown in the clinical study, changes in concentration of some polyamines may be helpful in making a cancer diagnosis [21,27] or for monitoring the treatment [21,28–30].

The reported study is a continuation of our earlier works on interactions between nucleic acid fragments and polyamines and the metallation of these bioligands [31–33]. This paper presents results of potentiometric and spectral studies of reactions in ternary systems of Cd(II) or Hg(II) ions with *CMP* and diamines.

EXPERIMENTAL

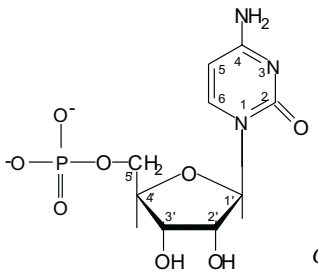
Ethylenediamine (*en*) was purchased from Merck, 1,3-diaminopropane (*tn*) and 1,4-diaminobutane (putrescine, *Put*) – from Sigma. Appropriate nitrates were prepared by dissolving in water a proper amount of free amine and adding an equimolar amount of HNO_3. The obtained white precipitate was recrystallized, washed out with methanol, and dried in a dessicator over P_4O_{10} or in air. Nitrates of polyamines were subjected to elemental analysis and the results (%C, %N, %H) were in agreement with the theoretically calculated values (±0.5%). Cytidine 5′-monophosphate (*CMP*) (as acid) was purchased from Sigma. Cadmium(II) and mercury(II) ions were applied in the $Cd(NO_3)_2 \cdot 4H_2O$ form (from Merck) and $Hg(NO_3)_2 \cdot H_2O$ (was purchased from Aldrich). In order to avoid hydrolysis, a strictly determined amount of HNO_3 (taken into account in a computer analysis) has been added to a stock solution of $Hg(NO_3)_2$. Concentration of Hg(II) ions was determined by precipitation titration with NaCl solution, using diphenylcarbazone as an indicator, while 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 ion concentration [34] and using borax (pH = 9.225) and phthalate (pH = 4.002) buffers. Concentration of ligands in the titrated systems varied from $1.3 \cdot 10^{-3}$ to $2.6 \cdot 10^{-3}$M, concentration of metal ions – from $1.3 \cdot 10^{-3}$ to $1.7 \cdot 10^{-3}$ M; the ratio of M:L:L' (M = metal, L – nucleotide, L' – PA) 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 T = 20 ± 1°C under helium, using CO_2-free NaOH solution (about 0.2 M) as a titrant. 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^+. Calculations were performed using 100–150 points for each run, taking into account only that part of the titration curve corresponding to no precipitate in the system. Selection of models and determination of protonation constants of ligands and stability constants of complexes were made using the SUPERQUAD program [35], whereas distribution of particular forms was determined by the HALTAFALL program [36]. Selection and verification of models was performed as described in [37]. Samples for NMR and IR studies were prepared by dissolving appropriate amounts of ligand and metal nitrates in D_2O and adjusting acidity by addition of NaOD and DNO_3, correcting pH-readings (a pH meter N517 made by Mera-Tronik), according to the formula: $pD = pH_{readings} + 0.40$ [38]. The ligand concentration in the samples for NMR studies was 0.1 M. The metal ion to ligand ratio was 1:10:10 to 1:100:100 (at lower concentration ratio a precipitate has occurred). ^{13}C NMR spectra were recorded on a Varian Gemini – 300VT in the range of 20–170 ppm, using dioxane as an internal standard. ^{31}P NMR spectra were recorded on a Varian Unity 300 spectrometer using H_3PO_4 as a standard (H_3PO_4:D_2O = 1:10). IR measurements were carried out using an IFS-113v Bruker spectrophotometer with a KRS5-50 cell.

The ligands studied

1 1
NH_2-CH_2-CH_2-NH_2 *en* (1,2-diaminoethane)

1 2 1
NH_2-CH_2-CH_2-CH_2-NH_2 *tn* (1,3-diaminopropane)

1 2 2 1
NH_2-CH_2-CH_2-CH_2-CH_2-NH_2 *Put* (1,4-diaminobutane)



CMP

RESULTS AND DISCUSSION

Computer analysis of the pH-metric titration data was made taking into account earlier determined (under identical experimental conditions) protonation constants of the bioligands, stability constants of complexes formed in binary systems, and stability constants of mercury(II) and cadmium(II) hydroxocomplexes: $Hg(OH)^+$, $Hg(OH)_2$, $Cd(OH)^+$ and $Cd(OH)_2$ [39]. As reported in [39,40], no reduction of Hg(II) to Hg(I) ions was observed.

The system Hg(II)/CMP/diamine: Table 1 presents overall stability constants ($\log\beta$) and equilibrium constants ($\log K_e$) of complex formation in ternary systems of Hg(II) with *CMP* and diamines.

Table 1. Overall stability constants ($\log\beta$) and equilibrium constants ($\log K_e$) of complex formation and the mode of coordination in Hg(II) and Cd(II)/CMP/diamine ternary systems [μ = 0.1 M (KNO_3)].

Species	$\log\beta$	$\log K_e$	Chromophore	Number of polyamine nitrogen atoms involved in coordination
Hg(CMP)(en)	20.15 (3)	8.35	{N3,O}	2N
Hg(CMP)(tn)	20.06 (7)	8.26	{N3,O}	2N
Hg(CMP)(Htn)	28.18 (9)	5.80	{N2,O}	1N
Hg(CMP)(H_2tn)	34.51 (12)	3.37	{N1,O}	molecular complex
Hg(CMP)(HPut)	28.94 (8)	6.59	{N2,O}	1N
Hg(CMP)(H_2Put)	35.36 (11)	3.38	{N1,O}	molecular complex
Hg(CMP)$(HPut)_2$	43.26 (11)			
Cd(CMP)(H_2en)	22.79 (8)	2.82	{N1}	molecular complex
Cd(CMP)(H_2tn)	25.70 (12)	3.96	{N1,O}	molecular complex
Cd(CMP)(H_2Put)	26.10 (7)	3.52	{N1,O}	molecular complex

Close values of equilibrium constants $\log K_e$ for formation of Hg(CMP)(en) and Hg(CMP)(tn) species:

$$Hg(CMP) + en \leftrightarrows Hg(CMP)(en)$$

$$Hg(CMP) + tn \leftrightarrows Hg(CMP)(tn)$$

of 8.35 and 8.26, respectively, (Table 1) ($\log K_e = \log\beta_{Hg(CMP)(PA)} - \log\beta_{Hg(CMP)}$), suggest the same mode of coordination of *en* and *tn* to the anchoring Hg(CMP) complex. Comparing the equilibrium constants with that determined for a binary

complex: Hg + Hen ⇆ Hg(Hen), in which only one nitrogen atom of *en* is involved in the coordination (log K_e = 6.66 [39]), indicates a contribution of the second nitrogen atom in diamine metallation in the mixed complexes (in binary Hg/PA systems, mono- as well as bidentate coordination is observed [39]). This conclusion is confirmed by the results of the ^{13}C NMR spectral study. For the species Hg(CMP)(en), the signal shift assigned to C(1) atom of polyamine is 0.622 ppm at pH 7.0, and the changes in the positions of the signals assigned to C(1) and C(2) atoms of *tn* in the Hg(CMP)(tn) species are 0.412 and 1.898 ppm, at pH 9.0 (relative to those of the free ligand), Table 2.

The results of spectral measurements have also indicated the involvement of the N(3) atom and oxygen atoms from the phosphate group of the nucleotide. For example, in the pH range of Hg(CMP)(tn) species domination, *i.e.* close to pH of 9, the shifts in the signals assigned to C(2) and C(4) atoms, adjacent to N(3), are 0.045 and 0.115 ppm, respectively. The change in signal coming from C(5') atom (the atom next to the phosphate group) is 0.122 ppm and the shift in the ^{31}P NMR signal is 0.075 ppm. However, in the IR spectrum, no changes have been observed in the position of the 1650 cm^{-1} band, assigned to the stretching vibrations of the C=O group of the nucleotide (similarly as in the spectra of other Hg(II) and Cd(II) heteroligand complexes including *CMP* and diamines), which means that the oxygen atom from the carbonyl group is not involved in metallation. Therefore, in the observed MLL' type species, a {N3,O$_x$} chromophore is formed, including two nitrogen atoms of the diamine, the N(3) atom of *CMP* and the oxygen atoms of nucleotide phosphate group.

Equilibrium constants of formation of protonated complexes:

$$\text{Hg(CMP)} + \text{Htn} \leftrightarrows \text{Hg(CMP)(Htn)}$$

$$\text{Hg(CMP)} + \text{HPut} \leftrightarrows \text{Hg(CMP)(HPut)}$$

are 5.80 and 6.59, respectively, ($\log K_e = \log \beta_{\text{Hg(CMP)(HPA)}} - \log \beta_{\text{Hg(CMP)}} - \log \beta_{\text{(HPA)}}$, Table 1). There is a good relation between the above values and the log K_e = 6.66 for the binary species Hg(Hen) formation [39], in which only one PA nitrogen atom is involved in metallation. The shifts in the ^{13}C NMR signals, assigned to C(1) and C(2) atoms of *Put* (in the range of Hg(CMP)(HPut) domination, pH = 8, Figure 1) are 0.070 and 1.146 ppm, respectively (relatively to free ligand).

Under the same condition, the changes in the signals assigned to C(2) and C(4) atoms of the pyrimidine ring by 0.240 and 0.102 ppm, as well as the changes in the signals coming from C(5') and from the phosphorus atoms by 0.154 and 0.183 ppm, suggest the involvement of N(3) atom, as well as oxygen atoms from the *CMP* phosphate group (formation of {N2,O} chromophore). In contrast to analogous systems with *Cyd* [41] in *CMP* systems, the presence of molecular complexes has been detected. The pH range of molecular species formation (ML⁞⁞⁞⁞L' type, *e.g.* Hg(CMP)(H$_2$Put), pH 2.5–7.5, Figure 1) coincides with the range, in which the diamine is totally protonated, which indicates that all the donor nitrogen atoms are coordinatively blocked. This observation suggests, that PA does not bind Hg(II) ion and located in the outer coordination sphere is surprisingly enough involved in

Table 2. Differences between ^{13}C NMR and ^{31}P NMR chemical shifts for the ligands in the Hg(II) and Cd(II)/CMP/diamine systems in relation to metal-free systems [ppm].

Systems	PA	pH	cytidine 5′-monophosphate										diamine	
			C(2)	C(4)	C(5)	C(6)	C(1')	C(2')	C(3')	C(4')	C(5')	P(α)	C(1)	C(2)
Hg(II)/CMP/diamine	*en*	7.0	0.065	0.080	0.045	0.015	0.053	0.022	0.030	0.137	0.197	0.061	0.622	
	tn	4.0	0.055	0.089	0.033	0.017	0.054	0.023	0.032	0.122	0.132	0.098	0.075	0.088
		7.0	0.073	0.131	0.042	0.023	0.034	0.021	0.043	0.098	0.110	0.101	0.076	0.134
		9.0	0.045	0.115	0.030	0.015	0.037	0.016	0.090	0.091	0.122	0.075	0.412	1.898
	Put	4.0	0.323	0.373	0.243	0.130	0.043	0.015	0.032	0.032	0.099	0.084	0.031	0.038
		8.0	0.240	0.102	0.009	0.092	0.099	0.017	0.037	0.137	0.154	0.183	0.070	0.146
		9.4	0.015	0.061	0.016	0.015	0.076	0.016	0.061	0.046	0.076	0.076	0.009	0.078
Cd(II)/CMP/diamine	*en*	6.5	0.121	0.093	0.035	0.055	0.121	0.024	0.056	0.045	0.030	0.038	0.060	
	tn	7.0	0.097	0.065	0.049	0.032	0.211	0.032	0.097	0.162	0.162	0.198	0.040	0.241
	Put	8.0	0.033	0.051	0.025	0.082	0.162	0.056	0.086	0.049	0.092	0.084	0.051	0.082

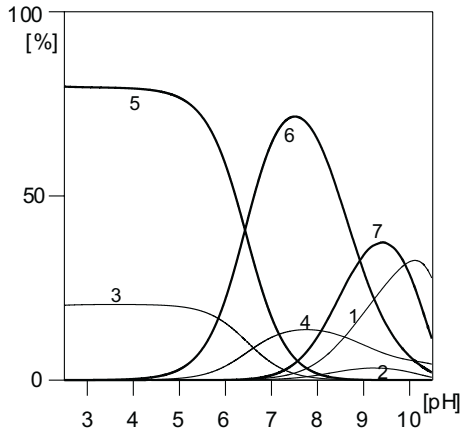


Figure 1. Distribution diagrams for the Hg(II)/CMP/Put system; percentages of the species refer to total metal: 1. Hg(Put); 2. $Hg(HPut)_2$; 3. Hg(CMP); 4. Hg(CMP)(OH); 5. $Hg(CMP)(H_2Put)$; 6. Hg(CMP)(HPut); 7. $Hg(CMP)(HPut)_2$. $C_{Hg} = 1.29 \cdot 10^{-3}$ M; $C_{CMP} = 2.66 \cdot 10^{-3}$ M; $C_{put} = 2.66 \cdot 10^{-3}$ M.

non-covalent interactions with a metallated phosphate group of the anchoring Hg(CMP). This mode of interaction is also suggested by results of the ^{13}C and ^{31}P NMR studies, Table 2. Significant shifts in the signals assigned to C(2) and C(4) atoms of the pyrimidine ring, observed in the spectra of the $Hg(CMP)(H_2Put)$ complex, recorded at pH 4.0, by 0.323 and 0.373 ppm, and the changes in the signal coming from the phosphorus atom (0.084 ppm), point to an involvement of the nitrogen atom N(3) and phosphate group of the nucleotide in interactions with Hg(II). At the same pH, small, however, systematic shifts in the signals assigned to C(1) and C(2) atoms of *Put* are 0.031 and 0.038 ppm, Table 2. This type of non-covalent interaction of the metallated phosphate group with polyamines has been observed earlier, *e.g.* in systems with Cu(II) ions [42,43]. The log K_e values for molecular complexes formation:

$$Hg(CMP) + H_2tn \leftrightarrows Hg(CMP)(H_2tn)$$

$$Hg(CMP) + H_2Put \leftrightarrows Hg(CMP)(H_2Put)$$

equal to 3.37 and 3.38 ($\log K_e = \log \beta_{Hg(CMP)(H_2PA)} - \log \beta_{Hg(CMP)} - \log \beta_{(H_2PA)}$) and are lower by 2.43 and 3.31 than the equilibrium constants characterising the Hg(CMP)(Htn) and Hg(CMP)(HPut) species formation (Table 1) and lower by 11.05 and 13.56, when compared to those of binary species Hg(tn) and Hg(Put), in which two nitrogen atoms of PA are involved in interactions with the metal ion [39], which clearly testifies to a different character of interactions in molecular complexes. Formation of molecular complexes is also confirmed by the coincidence of the titration curves, obtained experimentally and those simulated by computer assuming an adduct formation (with the use of the determined β values; an example is given in Figure 2). There is an excellent agreement between the pH range of the curves divergence (when the adduct formation is not taken into account) and the pH range of the adduct formation.

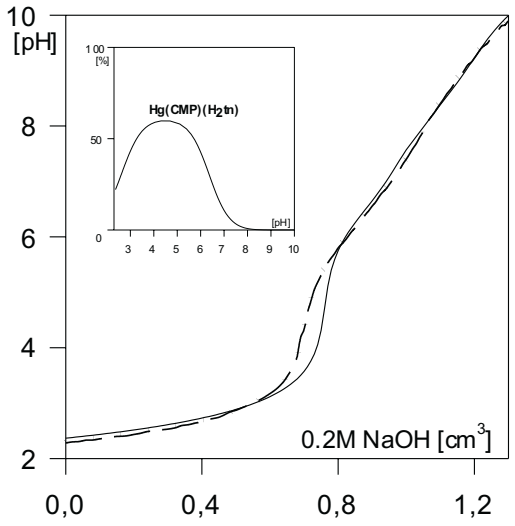
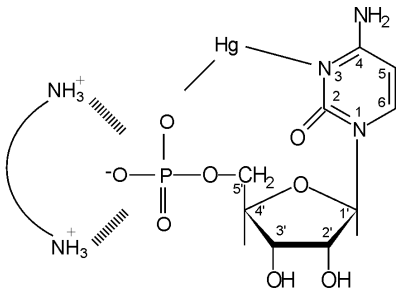


Figure 2. Experimental and simulated titration curves for the Hg/CMP/tn systems: - - - - experimental curves; ..... simulated curve (adduct formation taken into account); —— simulated curve (adduct formation not taken into account); $C_{Hg} = C_{CMP} = C_{tn} = 1.3 \cdot 10^{-3}$ M.

Scheme 1 presents the proposed tentative structure of molecular complexes of Hg(CMP)(H_2diamine).



Scheme 1. Tentative mode of interaction in the Hg(CMP)(H_2diamine) complex.

The system Cd(II)/CMP/diamine: Table 1 presents overall stability constants ($\log\beta$) and equilibrium constants ($\log K_e$) for complexes formed in ternary systems of Cd(II) with *CMP* and diamines. The stoichiometry of Cd(CMP)(H_2en), Cd(CMP)(H_2tn) and Cd(CMP)(H_2Put) complexes and the pH ranges of their occurrence (an example of distribution is given in Figure 3) indicate the formation of molecular complexes, in which one of the ligands (diamine) is located in the outer coordination sphere (metallation sites of protonated amine groups are blocked) and is involved in non-covalent interactions with Cd(CMP).

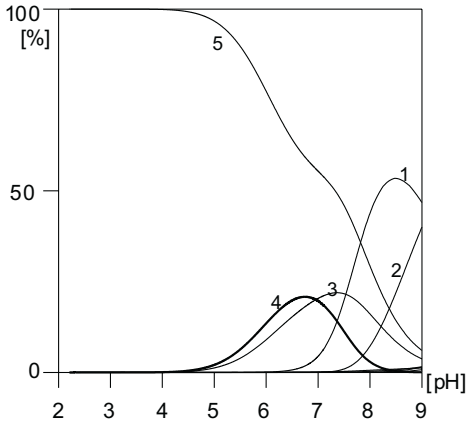


Figure 3. Distribution diagrams for the Cd(II)/CMP/en system; percentages of the species refer to total metal: 1. Cd(en); 2. $Cd(en)_2$; 3. Cd(CMP); 4. $Cd(CMP)(H_2en)$; 5. Cd^{2+}.
$C_{Cd} = 1.99 \cdot 10^{-3}$ M; $C_{CMP} = 2.68 \cdot 10^{-3}$ M; $C_{en} = 2.69 \cdot 10^{-3}$ M.

In the binary system Cd(II)/CMP, cadmium(II) coordinates the *CMP* molecule only through the N(3) atom of the pyrimidine ring of the nucleotide. The phosphate group is not directly involved in coordination, as it can be concluded from comparison of log K_e for *CMP* and cytidine species ($\log\beta_{Cd(Cyd)} = 2.43$, $\log\beta_{Cd(CMP)} = 2.40$, [39]). However, in certain ternary complexes studied, introduction of polyamine is the reason why also the phosphate group, apart from the nitrogen N(3) atom, participates in the interaction. This conclusion is based on the analysis of the shifts of the signals, coming from C(2), C(4) and C(5') atoms located close to the potential sites of interaction, as well as on the changes in the signals, assigned to the phosphorus atom and the C(1) and C(2) atoms of tn or Put, (Table 2, the range of molecular complex domination). Activation of the phosphate group after introduction of *tn* or *Put* into the Cd(II)/CMP binary system is confirmed by the results of the equilibrium study. Comparison of the log K_e values for $Cd(CMP)(H_2tn)$ and $Cd(CMP)(H_2Put)$ species (log K_e = 3.96 and 3.52, respectively) with the value for $Cd(CMP)(H_2en)$ complex (log K_e = 2.82), Table 1, indicates a different mode of interaction in the *en* species. NMR studies of $Cd(CMP)(H_2en)$ species point to the metallation only through the N(3) atom. The shifts in the signal, coming from C(2) and C(4) atoms of the pyrimidine ring of *CMP*, are 0.121 and 0.093 ppm respectively. On the other hand, the shifts in the signal, assigned to C(5') of ribose and changes in the signal coming from phosphorus atom, are significantly smaller than those in the spectra of $Cd(CMP)(H_2tn)$ and $Cd(CMP)(H_2Put)$ species, Table 2.

CONCLUSIONS

In all species formed in the systems Hg(II)/CMP/diamine, the endocyclic nitrogen atoms N(3) and phosphate groups from the nucleotide are involved in the interactions. In the MLL' and ML(HL') type species, the chromophore {N3,O} and {N2,O}, respectively, occurs. In molecular complexes (adducts) ML ⁞⁞⁞⁞ L', the diamine is in the outer coordination sphere and surprisingly enough takes part in the non-covalent interactions with the metallated phosphate group of the nucleotide in the anchoring Hg(CMP).

In the ternary systems Cd(II)/CMP/diamine, the presence of the polyamine changes the coordination character of the nucleotide. The phosphate group, inactive in the binary complex Cd(CMP), is involved in the metallation in the ternary one. The presence of *en* has no effect on the change in the character of the interactions in the ternary systems, relative to that in the binary ones, which confirms the significance of the structural factor (the polyamine length) on the coordination effectiveness of the group $–O–PO_3$.

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REFERENCES

1. Ebashi S., *J. Biochem*., **50**, 236 (1961).
2. Hasselbach W. and Makinose M., *Biochemistry,* **2**, 333 (1961).
3. Cohen M., *Biochemistry,* **2**, 623 (1963).
4. Sánchez-Cortés S., Molina M., Garcia-Ramos J.V. and Carmona P. *J. of Raman Spectroscopy*, **22**, 819 (1991).
5. Zappia V., Progress in Polyamine Research, Ed. Pegg A.E., Plenum Press, NY 1988.
6. Diebler H., Secco F. and Venturi M., *Bioph. Chem.*, **26**, 193 (1987).
7. Kopf-Maier P. and Kopf H., *Chem. Rev.*, **87**, 1137 (1987).
8. Sherman S.E. and Lippard S.J., *Chem. Rev.*, **87**, 1153 (1987).
9. Łomozik L., *Wiad. Chem*., **48**, 439 (1994).
10. De Munno G., Mauro S., Pizzino T. and Viterbo D., *J. Chem. Soc. Dalton Trans.*, 1113 (1993).
11. Katono Y., Inoue Y. and Chűjô R., *Polym. J.*, **9**(5), 471 (1977).
12. Srivastawa H.P. and. Tiwari D., *J. Indian. Chem. Soc*., **70**, 499 (1993).
13. Walker M.D. and Williams D.R., *J. Chem. Soc. Dalton Trans.*, 1186 (1974).
14. Martin R.B., Metal ions binding to nucleoside and nucleotides, Ed. A.V. Xavier, Frontiers in Bioinorganic Chemistry, VCH, Weinheim, 1986.
15. Marzilli L.G., Metal complex of nucleic acid derivatives and nucleotides; Binding sites and structures, Eds. Eichhorn G.L., Marzilli L.G., Metal Ions in Genetic Information Transfer, Elsevier/North-Holland, Amsterdam, 1981.
16. Sigel H., *Eur. J. Biochem*., **65**, 165 (1987).
17. Tabor H. and Tabor C.W., *Farmacol. Rev.*, **16**, 245 (1964).
18. Raina A., *Acta Physiol. Scand*., **60**, 1 (1963).
19. Theoharides T.C., *Life Sciences,* **27**, 703 (1980).
20. Tabor C.W. and Tabor H., *Ann. Rev. Biochem*., **45**, 285 (1976).
21. Seiler N., *J. Chromatog*., **379**, 157 (1986).

22. Schuber F., *Biochem. J*., **260**, 1 (1989).
23. Tabor C.W. and Tabor H., *Annu. Rev. Biochem*., **53**, 749 (1984).
24. Vertino P.M., Bergeron H.J., Cavanaugh P.F. Jr and Porter C.W., *Biopolymers*, **26**, 691 (1987).
25. Feuerstein B.G., Pattabiraman N. and Marton L.J., *Nucleic Acids Res*., **18**, 1271 (1990).
26. Plum G.E., Arscott P.G. and Bloomfield V.A., *Biopolymers*, **30**, 631 (1990).
27. Williams H.G. and Canellakis Z.N., *Perspec. Biol. Med*., **22**, 421 (1973).
28. Inne J., Pösö H. and Raina A., *Bioch. Bioph. Acta*, **473**, 241 (1978).
29. Russell D.H., Lewy C.C., Schimpff S.C. and Hawk I.A., *Cancer Res*., **32**, 1555 (1971).
30. Horn Y., Stuart L.B., Walach N., Lubich W.P., Spigel L. and Marton L.J., *Cancer Res.*, **42**, 3248 (1982).
31. Gasowska A. and Lomozik L., *Polish J. Chem*., **73**, 465 (1999).
32. Lomozik L., Jastrzab R. and Gasowska A., *Polyhedron*, **19**, 1145 (2000).
33. Gasowska A., Lomozik L. and Jastrzab R., *J. Inorg. Biochem*., **78**, 139 (2000).
34. Irving H.M., Miles M.G. and Pettit L.D., *Anal. Chim. Acta*, **38**, 475 (1967).
35. Gans P., Sabatini A. and Vacca A., *J. Chem. Soc. Dalton Trans*., 1195 (1985).
36. Ingri N., Kakolowicz W., Sillen L.G. and Wargvist B., *Talanta*, **14**, 1261 (1967).
37. Lomozik L., Jaskolski M. and Wojciechowska A., *Polish J. Chem*., **65**, 1797 (1991).
38. Glasoe P.K. and Long F.A., *J. Phys. Chem*., **64**, 188 (1960).
39. Lomozik L. and Bregier-Jarzebowska R., *Polish J. Chem*., **73**, 927 (1999).
40. Schwarzenbach G. and Szilard I., *Helv. Chim. Acta*, **45**, 1222 (1962).
41. Lomozik L., Bregier-Jarzebowska R. and Gasowska A., *J. Coord. Chem.*, **56**, 203 (2003).
42. Lomozik L. and Gasowska A., *J. Inorg. Biochem*., **72**, 37 (1998).
43. Lomozik L. and Jastrzab R., *J. Inorg. Biochem*., **93**, 132 (2003).