Role of Biologically Important Zwitterionic Buffer Secondary Ligands on the Stability of the Mixed-Ligand Complexes of Divalent Metal Ions and Adenosine 5′-Mono-, 5′-Di-, and 5′-Triphosphate

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Potentiometric equilibrium measurements have been performed at (25.0 ± 0.1) °C and ionic strength I = 0.1 mol dm^{-3} KNO_3 for the interaction of the purine nucleotides adenosine 5′-mono, 5′-di, and 5′-triphosphate and Cu(II), Co(II), Ni(II), Mn(II), Zn(II), Ca(II), and Mg(II) with the biologically important secondary ligand zwitterionic buffers 3-(*N*-morpholino)propanesulfonic acid, 3-[(1,1-dimethyl-2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid, *N*-(2-hydroxyethyl)piperazine-*N*′-2-hydroxypropanesulfonic acid, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), and piperazine-*N*,*N*′-bis(2-hydroxypropanesulfonic acid) in a 1:1:1 ratio and the formation of various 1:1:1 normal and protonated mixed-ligand complex species was inferred from the potentiometric pH titration curves. The experimental conditions were selected such that self-association of the purine nucleotides and their complexes was negligibly small; i.e., the monomeric normal and protonated ternary complexes were studied. Initial estimates of the formation constants of the resulting species and the acid dissociation constants of adenosine 5′-mono-, 5′-di-, and 5′-triphosphate and the zwitterionic buffer secondary ligands have been refined with the SUPERQUAD computer program. In some M(II) mixed-ligand systems, the interligand interactions between the coordinate ligands, possibly H bond formation, have been found to be most effective in deciding the stability of the mixed-ligand complexes formed in solutions.

Introduction

The interactions of metal ions with nucleotides and related compounds is a fertile field in bioinorganic chemistry. An understanding of the quantitative origins of the stability of such complexes and of their structure in solution can lead to a better understanding of important biological processes and may lead to new drugs.[1] For the standardization of pH and control of acidity in the physiological region of pH 7−9, Good and co-workers[2] and Ferguson and co-workers[3] have listed hydrogen ion buffers which are zwitterionic amino acids which are N-substituted taurines, N-substituted glycines, or zwitterionic N-substituted aminosulfonic acids. These compounds are all ampholytes (with zwitterionic structures) and are useful buffers compatible with most media of physiological interest. Potentially useful zwitterionic buffers for use in biochemistry now include 3-(*N*-morpholino)propanesulfonic acid (MOPS), 3-[(1,1-dimethyl-2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid (AMPSO), *N*-(2-hydroxyethyl)piperazine-*N*′-[2-hydroxypropanesulfonic acid] (HEPPSO), piperazine-*N*,*N*′-bis[2-ethanesulfonic acid] (PIPES), and piperazine-*N*,*N*′-bis[2-hydroxypropanesulfonic acid] (POPSO) because of their low toxicity. Ternary complexes containing metal ions and two different types of biologically important ligands, namely, nucleotides (AMP, ADP, or ATP) and zwitterionic buffer ligands, may be considered as models for ternary interactions in which a metal entity cross-links a protein and a nucleic acid. Alternative ternary interactions include metal binding to a protein which subsequently associates with a nucleic acid and initial binding of metal ion to a nucleic acid, which then causes a protein to bind to the metalated nucleic acid. Biologically relevant examples for all three cases are known.[4] Artificial chemical DNA nucleases frequently are based on metal−protein conjugates,[5] thereby representing an application of ternary complex formation in molecular biology.

Ternary complexes of transition divalent metal ions with AMP, ADP, ATP, and other secondary ligands have been investigated.[6−13] For an improved understanding of the mechanism leading to mixed-ligand complexes, the systems M(II) + NU + Z, where NU = AMP, ADP, or ATP, Z = MOPS, AMPSO, HEPPSO, PIPES, or POPSO, and M(II) = Cu(II), Co(II), Ni(II), Mn(II), Zn(II), Ca(II), or Mg(II), have been investigated by potentiometric pH titration. The stability constants of the normal and protonated mixed-ligand complexes formed in solution have been determined. These systems mimic many biological reactions [M(II) + buffer + substrate interactions] and also may be considered as models for protein + M(II) + nucleic acid complexes. Metal ions such as Mg(II), Mn(II), or Zn(II) have an important role in virtually every stage of gene expression involving deoxyribonucleic acid (DNA) replication, transcription, and messenger ribonucleic acid (RNA) translation.[14]

Experimental Section

Material and Solutions. $Na_2AMP·H_2O$, $Na_2ADP·2H_2O$, and $Na_2ATP·3H_2O$, were purchased from Sigma Chemical Co. and were used without purification. The amount of free phosphates initially present in the nucleotides was determined.[15] Our experimental values were found to be 2% for ATP and 3% for ADP and AMP. To account for this and to prepare metal ion nucleotide solutions in a 1:1 molar ratio, we determined the molar mass of these purine nucleotides by potentiometric pH titrations. MOPS, AMPSO, HEPPSO, PIPES, and POPSO

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10.1021/je0001779 CCC: $20.00
Published on Web 02/14/2001

were from Sigma. We determined the molecular weight of MOPS, AMPSO, HEPPSO, PIPES, and POPSO by potentiometric pH titration to determine the purity, especially for acidic/basic contaminants. The purity averages 99.5% for the five biological buffers, with a standard deviation of 0.04%. The nitrate salts of the metal ions, nitric acid, and KOH were from Merck. Stock solutions were prepared using distilled, CO_2-free water. The concentration of KOH used for the titrations was determined by titration with a standard solution of potassium hydrogen phthalate (Merck AG). HNO_3 solutions were prepared and standardized potentiometrically with tris(hydroxymethyl)aminomethane. The concentrations of the metal ion stock solutions were determined by titration with ethylenediaminetetraacetic acid (EDTA).

Apparatus. Potentiometric pH measurements were performed on the solutions in a double-walled glass vessel at (25.0 ± 0.1) °C with a commercial Fisher combined electrode and a Fisher Accumet pH/ion meter model 825 MP. Purified nitrogen[16] was bubbled through the solutions during titrations and a magnetic stirrer was used.

Procedure. The test solution was titrated with standard CO_2-free KOH. The pH range (3.0−10.0) was covered for the calculation for each system. The electrode was calibrated, in both the acidic and alkaline regions, by titrating 0.01 mol dm^{-3} nitric acid with standard KOH under the same experimental conditions. The concentration of the free hydrogen ion, C_{H^+}, at each point of the titration is related to the measured emf, $E°$, of the cell by Nernest equation

$$E = E° + Q \log C_{H^+} \tag{1}$$

where $E°$ is a constant which includes the standard potential of the glass electrode and Q is the slope of the glass electrode response. The value of $E°$ for the electrode was determined from a Gran plot derived from a separate titration of nitric acid with a standard KOH solution under the same temperature and medium conditions. The results obtained were analyzed by the nonlinear least-squares computer program ESAB2M[17] to refine $E°$ and the autoprotolysis constant of water, K_w. During this calculation, the K_w was refined until the best value for Q was obtained. The results obtained indicated the reversible Nernstian response of the glass electrode used. The solutions titrated can be presented according to the following scheme: HNO_3 (4×10^{-3} mol dm^{-3}) + nucleotide (1×10^{-3} mol dm^{-3}) (a); HNO_3 (4×10^{-3} mol dm^{-3}) + nucleotide (1×10^{-3} mol dm^{-3}) + M(II) (1×10^{-3} mol dm^{-3}) (b); HNO_3 (4×10^{-3} mol dm^{-3}) + zwitterionic buffer ligands (1×10^{-3} mol dm^{-3}) (c); HNO_3 (4×10^{-3} mol dm^{-3}) + zwitterionic buffer ligands (1×10^{-3} mol dm^{-3}) + M(II) (1×10^{-3} mol dm^{-3}) (d); HNO_3 (4×10^{-3} mol dm^{-3}) + nucleotide (1×10^{-3} mol dm^{-3}) + zwitterionic buffer ligands (1×10^{-3} mol dm^{-3}) + M(II) (1×10^{-3} mol dm^{-3}) (e). A constant ionic strength was obtained with 0.1 mol dm^{-3} KNO_3, and the total initial volume was kept constant at 25 cm^3. At least four titrations were performed for each system. Titrations of solutions containing 4×10^{-3} mol dm^{-3} HNO_3 + 1×10^{-3} mol dm^{-3} nucleotide (AMP, ADP, or ATP) + 1×10^{-3} mol dm^{-3} zwitterionic buffer ligand (MOPS, AMPSO, HEPPSO, PIPES, or POPSO) under the same exprimental conditions confirmed that the zwitterion buffer ligand and the nucleotide were not directly interacting with each other. For both ligand protonation and metal complex formation equilibria, data were recorded over the largest possible pH interval, although a number of experimental points were frequently discarded for the final stability constant calculations, especially within the range where the complexation observed was insignificant. Typically about 50 data points were collected for each system. To avoid hydrolysis prior to the potentiometric measurements, a known mass of the nucleotides as solid was added to the reaction vessel just prior to performing the titration.

Initial estimates of the formation constants of the normal and protonated ternary complexes and the stability constants of the binary 1:1 complexes have been refined using the SUPERQUAD computer program.[18] During this refinement the stability constant for the species $M_p(NU)_qZ_rH_s$, β_{pqrs}, is defined by the equation (eq 2; charges are omitted for clarity)

$$pM + qNU + rZ + sH \rightleftharpoons M_p(NU)_qZ_rH_s \tag{2}$$

$$\beta_{pqrs} = \frac{[M_p(NU)_qZ_rH_s]}{[M]^p[NU]^q[Z]^r[H]^s} \tag{3}$$

where p, q, r, and s are the moles of M, NU, Z, and H in $M_p(NU)_qZ_rH_s$, respectively. NU = nucleotide (AMP, ADP, or ATP), Z = zwitterionic buffer ligand (MOPS, AMPSO, HEPPSO, PIPES, or POPSO), and M = Cu(II), Co(II), Ni(II), Mn(II), Zn(II), Ca(II), or Mg(II). In addition, the protonation and complexation reactions of the free phosphate initially present in solutions have been included in the calculations to get better conditional stability constants. The constants were refined by minimizing U, defined by

$$U = \sum W_i(E_{obs} - E_{calc})^2 \tag{4}$$

where E_{obs} and E_{calc} refer to the measured and calculated potentials. The weighting factor W_i is defined as the reciprocal of the estimated variance of measurement.

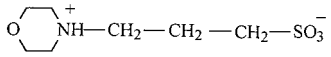
$$W_i = 1/\sigma^2 = 1/[\sigma_E{}^2 + (\delta E/\delta V)^2\sigma_V{}^2] \tag{5}$$

where σ_E and σ_V are the estimated variances of the potential and volume readings, respectively. The quality of fit was judged by the values of the sample standard deviation, S, and the goodness of fit, X^2 (Pearson's test). At σ_E = 0.1 mV (0.001 pH error) and σ_V = 0.005 mL, the values of S in different sets of titrations were between 1.0 and 1.8 and X^2 was between 12.0 and 13.0. The scatter of residuals (E_{obs} − E_{calc}) vs pH was reasonably random, indicating a good fit of the experimental data.

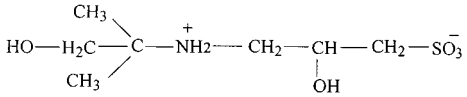
Results and Discussion

The second acid formation constants determined at (25.0 ± 0.1) °C for MOPS (pK_{a2} = 7.16 ± 0.02), AMPSO (pK_{a2} = 9.05 ± 0.03), HEPPSO (pK_{a2} = 7.85 ± 0.02), PIPES (pK_{a2} = 6.94 ± 0.02), and POPSO (pK_{a2} = 7.79 ± 0.03) were in good agreement with those found in the literature.[2,3,19−21] The two acid formation constant values for AMP (pK_{a1} = 3.81 ± 0.03 and pK_{a2} = 6.24 ± 0.03), ADP (pK_{a1} = 3.94 ± 0.03 and pK_{a2} = 6.38 ± 0.04), and ATP (pK_{a1} = 4.05 ± 0.03 and pK_{a2} = 6.51 ± 0.03) and the stability constants of their M(II) complexes were determined from the titration curves, and the results were found to agree well with those reported in the literature.[22] The pK values for primary ionizations of the adenine nucleotides AMP, ADP, and ATP have been estimated[23] as follows: AMP (one primary phosphate hydrogen), 1.0; ADP (two primary phosphate hydrogens), 1.0 and 2.0; ATP (three primary phosphate hydrogens), 1.0, 1.0, and 2.0. Given these pK values at the lower pH initial conditions of the experiments described in this work (pH 2.3−2.5), roughly 10% of the final primary

Chart 1



(MOPS)



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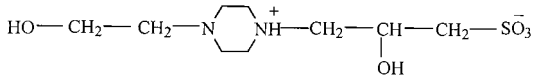
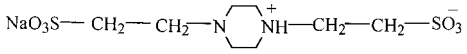
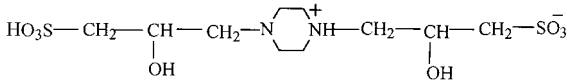


(HEPPSO)



(PIPES)



(POPSO)

phosphate hydrogen would be un-ionized for ATP and ADP. At lower pH values even more primary phosphate hydrogen would be un-ionized, including the pK = 1.0 phosphate ionizations.

MOPS, AMPSO, HEPPSO, PIPES, and POPSO possess the zwitterionic structures shown in Chart 1.

The second dissociation step involves the deprotonation of the cationic group $-N^+H$ of MOPS, HEPPSO, PIPES, and POPSO. For AMPSO, this step involves the deprotonation of the cationic group $-N^+H_2$.

It is evident that the calculated pK's of PIPES and MOPS are lower than that of the parent compound taurine ($^-O_3S(H_2C)_2NH_3^+$), pK_2 = 9.06. This enhancement of acid strength of the NH^+ group in MOPS and PIPES is probably due to the inductive effects of the oxygen atoms and steric hindrance of piperazine groups. The substitution of hydroxyethyl, hydroxymethyl, morpholino, or piperazine groups on the nitrogen atom in taurine usually lowers the value of pK_2 for the isoelectric dissociation processes.

At the experimental pH values used in the calculation of the formation constants of normal ternary complexes in this work, the interfering effects of hydroxy complexes are negligible. Thus, the secondary ligand, Z, combines with the binary 1:1 M^{II}NU {[M^{II}AMP], [M^{II}ADP]$^-$, and [M^{II}ATP]$^{2-}$} complex in a manner similar to its interaction with aquated metal ions "[$M^{II}(H_2O)_6]^{2+}$" in solutions. Thus, the initial estimates of the stability constants of the normal ternary complexes formed in solution have been determined using the Irving and Rossotti formula.[24,25] The side effects due to hydrolysis of metal ions which may be occur at higher pH values have been included during the refinement of the calculated formation constants of the different ternary complexes formed in solution.

In Figures 1−6, representative sets of experimental titration curves obtained according to the sequence described in the Experimental Section for the different M(II) + NU + Z systems studied are displayed. With respect to the titration curves of the M(II) + Z binary

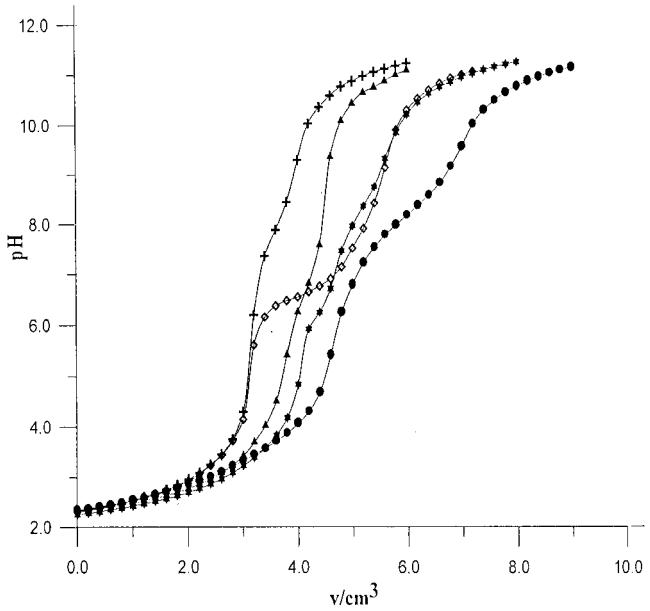


Figure 1. pH against a volume of 0.0330 mol dm^{-3} KOH for the Cu(II) + ATP + HEPPSO system at (25.0 ± 0.1) °C and I = 0.1 mol dm^{-3} KNO_3: (a) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} ATP (▲); (b) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} Cu(II) + 0.001 mol dm^{-3}ATP (★); (c) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} HEPPSO (+); (d) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} Cu(II) + 0.001 mol dm^{-3} HEPPSO (◇); (e) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} Cu(II) + 0.001 mol dm^{-3} ATP + 0.001 mol dm^{-3} HEPPSO (●).

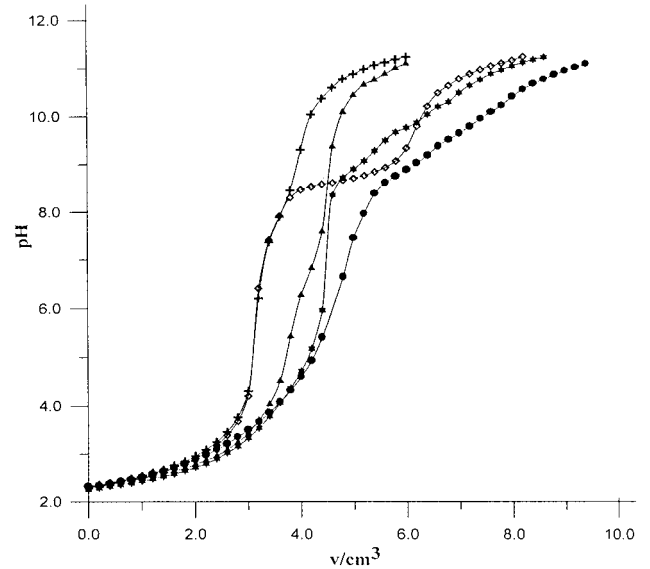


Figure 2. pH against a volume of 0.0330 mol dm^{-3} KOH for the Co(II) + ATP + HEPPSO system at (25.0 ± 0.1) °C and I = 0.1 mol dm^{-3} KNO_3: (a) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} ATP (▲); (b) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} Co(II) + 0.001 mol dm^{-3} ATP (★); (c) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} HEPPSO (+); (d) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} Co(II) + 0.001 mol dm^{-3} HEPPSO (◇); (e) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} Co(II) + 0.001 mol dm^{-3} ATP + 0.001 mol dm^{-3} HEPPSO (●)

complex solutions studied, one may deduce that these complexes begin to form in the pH range of 3.2−8.3 for Cu(II) + HEPPSO, Co(II) + HEPPSO, Ni(II) + HEPPSO, Zn(II) + HEPPSO, and Mg(II) + HEPPSO systems. In all cases, no calculations have been performed beyond the precipitation point; hence, the hydroxy species likely to be

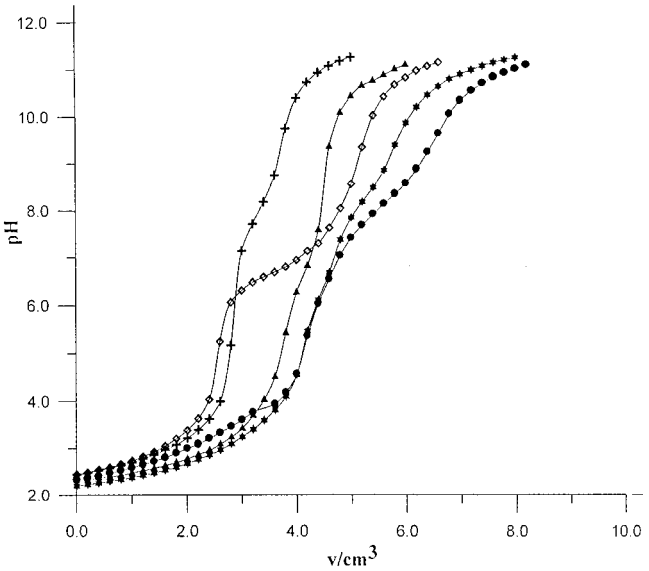


Figure 3. pH against a volume of 0.0330 mol dm^{-3} KOH for the Cu(II) + ATP + POPSO system at (25.0 ± 0.1) °C and I = 0.1 mol dm^{-3} KNO_3: (a) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} ATP (▲); (b) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} Cu(II) + 0.001 mol dm^{-3} ATP (★); (c) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} POPSO (+); (d) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} Cu(II) + 0.001 mol dm^{-3} POPSO (◇); (e) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} Cu(II) + 0.001 mol dm^{-3} ATP + 0.001 mol dm^{-3} POPSO (●).

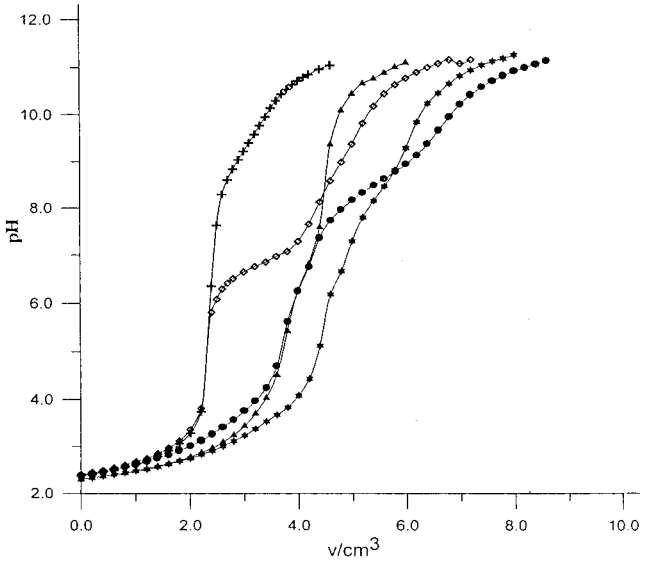


Figure 4. pH against a volume of 0.0330 mol dm^{-3} KOH for the Cu(II) + ATP + AMPSO system at (25.0 ± 0.1) °C and I = 0.1 mol dm^{-3} KNO_3: (a) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} ATP (▲); (b) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} Cu(II) + 0.001 mol dm^{-3} ATP (★); (c) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} AMPSO (+); (d) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} Cu(II) + 0.001 mol dm^{-3} AMPSO (◇); (e) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} Cu(II) + 0.001 mol dm^{-3} ATP + 0.001 mol dm^{-3} AMPSO (●).

formed after this point could not be studied. The binary complex Mn(II) + HEPPSO could not be studied because of precipitation at lower pH values.

For the titration curves of the ternary systems M(II) + NU + HEPPSO, it was observed that complexation starts at a pH > 4.8 for Cu(II) + AMP + HEPPSO, at a pH > 6.8 for Co(II) + AMP + HEPPSO, at a pH > 6.6 for Ni(II) + AMP + HEPPSO, at a pH > 7.6 for Mn(II) + AMP + HEPPSO, at a pH > 6.0 for Ca(II) + AMP + HEPPSO,

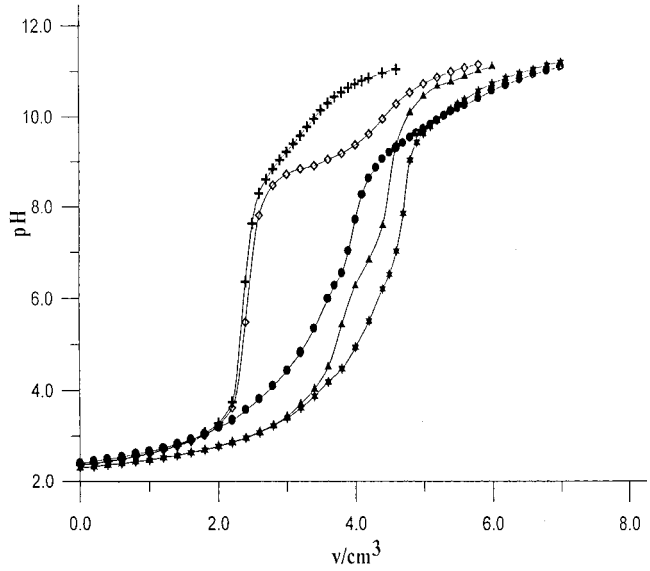


Figure 5. pH against a volume of 0.0330 mol dm^{-3} KOH for the Ni(II) + ATP + AMPSO system at (25.0 ± 0.1) °C and I = 0.1 mol dm^{-3} KNO_3: (a) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} ATP (▲); (b) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} Ni(II) + 0.001 mol dm^{-3} ATP (★); (c) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} AMPSO (+); (d) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} Ni(II) + 0.001 mol dm^{-3} AMPSO (◇); (e) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} Ni(II) + 0.001 mol dm^{-3} ATP + 0.001 mol dm^{-3} AMPSO (●).

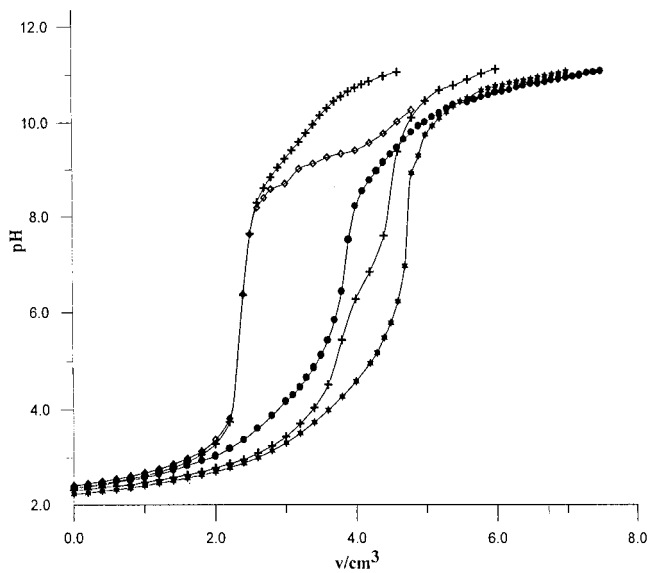


Figure 6. pH against a volume of 0.0330 mol dm^{-3} KOH for the Mn(II) + ATP + AMPSO system at (25.0 ± 0.1) °C and I = 0.1 mol dm^{-3} KNO_3: (a) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} ATP (▲); (b) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} Mn(II) + 0.001 mol dm^{-3} ATP (★); (c) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} AMPSO (+); (d) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} Mn(II) + 0.001 mol dm^{-3} AMPSO (◇); (e) 0.004 mol dm^{-3} HNO_3 + 0.001 mol dm^{-3} Mn(II) + 0.001 mol dm^{-3} ATP + 0.001 mol dm^{-3} AMPSO (●).

and at a pH >5.7 for Mg(II) + AMP + HEPPSO. For Cu(II) + ADP + HEPPSO, Co(II) + ADP + HEPPSO, Ni(II) + ADP + HEPPSO, Mn(II) + ADP + HEPPSO, Zn(II) + ADP + HEPPSO, Ca(II) + ADP + HEPPSO, and Mg(II) + ADP + HEPPSO systems, complexation starts at the pH range of 4.00−7.30. For Cu(II) + ATP + HEPPSO, Co(II) + ATP + HEPPSO, Ni(II) + ATP + HEPPSO, Mn(II) + ATP + HEPPSO, Zn(II) + ATP +

Table 1. Formation Constants for the Binary Cu(II) + Nucleotide (NU), or Zwitterionic Buffer (Z) Ligand Complexes Together with the Corresponding Mixed-Ligand Complexes Cu(II) + NU + Z at (25.0 ± 0.1) °C and I = 0.1 mol dm^{-3} KNO_3[a]

ligand	$\log K^{Cu(II)}_{Cu(II)(NU)}$ or $\log K^{Cu(II)}_{Cu(II)(Z)}$	$\log K^{Cu(II)(AMP)}_{Cu(II)(AMP)(Z)}$ or $\log \beta^{Cu(II)}_{Cu(II)(AMP)(Z)}$	$\log K^{Cu(II)(ADP)}_{Cu(II)(ADP)(Z)}$ or $\log \beta^{Cu(II)}_{Cu(II)(ADP)(Z)}$	$\log K^{Cu(II)(ATP)}_{Cu(II)(ATP)(Z)}$ or $\log \beta^{Cu(II)}_{Cu(II)(ATP)(Z)}$
AMP	3.18 ± 0.03			
ADP	5.90 ± 0.04			
ATP	6.01 ± 0.03			
PIPES	3.75 ± 0.04	6.30 ± 0.03[b]	6.87 ± 0.04[b]	2.55 ± 0.04
			4.06 ± 0.03[c]	8.56 ± 0.03
MOPS	4.04 ± 0.03		2.26 ± 0.02	2.18 ± 0.04
			8.16 ± 0.03	8.19 ± 0.03
POPSO		3.35 ± 0.02	2.44 ± 0.04	3.79 ± 0.04
		6.53 ± 0.03	8.34 ± 0.04	9.80 ± 0.04
HEPPSO	5.04 ± 0.03	5.55 ± 0.04	4.20 ± 0.04	5.60 ± 0.04
		8.73 ± 0.03	10.10 ± 0.04	11.61 ± 0.03
AMPSO	5.30 ± 0.04	3.80 ± 0.03	3.48 ± 0.03	3.59 ± 0.03
		6.98 ± 0.03	9.38 ± 0.04	9.60 ± 0.03

[a] $\log \beta^{Cu(II)}_{Cu(II)(NU)(Z)} = \log K^{Cu(II)(NU)}_{Cu(II)(NU)(Z)} + \log K^{Cu(II)}_{Cu(II)(NU)}$. [b] $\log K^{Cu(II)}_{Cu(II)(NU)(HZ)}$. [c] $\log K^{Cu(II)(NU)(HZ)}_{Cu(II)(NU)(Z)}$ [calculated using the following equilibria: $Cu^{II}(NU)(HZ) \rightleftharpoons Cu^{II}(NU)(Z) + H^+$, $Cu(II) + NU + Z \xrightleftharpoons{K^{Cu(II)(NU)(HZ)}_{Cu(II)(NU)(Z)}} Cu^{II}(NU)(Z)$] ± uncertainities refer to 3 times the standard deviation (3S).

Table 2. Formation Constants for the Binary Co(II) + Nucleotide (NU) or Zwitterionic Buffer (Z) Ligand Complexes Together with the Corresponding Mixed-Ligand Complexes Co(II) + NU + Z at (25.0 ± 0.1) °C and I = 0.1 mol dm^{-3} KNO_3[a]

ligand	$\log K^{Co(II)}_{Co(II)(NU)}$ or $\log K^{Co(II)}_{Co(II)(Z)}$	$\log K^{Co(II)(AMP)}_{Co(II)(AMP)(Z)}$ or $\log \beta^{Co(II)}_{Co(II)(AMP)(Z)}$	$\log K^{Co(II)(ADP)}_{Co(II)(ADP)(Z)}$ or $\log \beta^{Co(II)}_{Co(II)(ADP)(Z)}$	$\log K^{Co(II)(ATP)}_{Co(II)(ATP)(Z)}$ or $\log \beta^{Co(II)}_{Co(II)(ATP)(Z)}$
AMP	2.53 ± 0.03			
ADP	4.20 ± 0.04			
ATP	4.66 ± 0.05			
PIPES	3.30 ± 0.03	5.83 ± 0.03[b]	6.29 ± 0.04[b]	1.60 ± 0.03
		3.12 ± 0.02[c]	2.53 ± 0.03[c]	6.26 ± 0.04
MOPS	3.39 ± 0.02	4.28 ± 0.02[b]	1.57 ± 0.03	1.88 ± 0.03
		2.25 ± 0.03[c]	5.77 ± 0.04	6.54 ± 0.04
POPSO		2.61 ± 0.03	2.83 ± 0.02	3.07 ± 0.02
		5.14 ± 0.03	7.03 ± 0.03	7.73 ± 0.04
HEPPSO	3.50 ± 0.03	3.84 ± 0.03	3.91 ± 0.03	4.72 ± 0.02
		6.37 ± 0.03	8.11 ± 0.04	9.38 ± 0.03
AMPSO	3.52 ± 0.04		3.02 ± 0.03	3.50 ± 0.02
			7.22 ± 0.04	8.16 ± 0.03

[a] $\log \beta^{Co(II)}_{Co(II)(NU)(Z)} = \log K^{Co(II)(NU)}_{Co(II)(NU)(Z)} + \log K^{Co(II)}_{Co(II)(NU)}$. [b] $\log K^{Co(II)}_{Co(II)(NU)(HZ)}$. [c] $\log K^{Co(II)(NU)(HZ)}_{Co(II)(NU)(Z)}$ [calculated using the following equilibria: $Co^{II}(NU)(HZ) \rightleftharpoons Co^{II}(NU)(Z) + H^+$, $Co(II) + NU + Z \xrightleftharpoons{K^{Co(II)(NU)(HZ)}_{Co(II)(NU)(Z)}} Co(II)(NU)(Z)$] ± uncertainities refer to 3 times the standard deviation (3S)].

HEPPSO, Ca(II) + ATP + HEPPSO, and Mg(II) + ATP + HEPPSO systems, complexation starts at the pH range of 3.6−7.8

Different values for log K and log β, which have been calculated and refined using the experimental data, are given in Tables 1−7. Examination of the different formation constant values listed in these tables clearly reveals that the order of the stability constants of the different, normal ternary complexes in the system M(II) + NU + HEPPSO in terms of the metal ion follows generally the trend Cu(II) > Co(II) > Zn(II) > Ni(II) > Mn(II) for M(II) + ATP + HEPPSO systems, and for M(II) + AMP + HEPPSO and M(II) + ADP + HEPPSO systems, the stability constant values decrease in the order Cu(II) > Co(II) > Ni(II) > Mn(II) and Zn(II) > Cu(II) > Co(II) > Mn(II) > Ni(II), respectively. Taking into consideration the factors which affect metal−nucleotide interactions which include binding such as pH, temperature, and metal ion concentration as well as factors associated with metal ion chemistry, one can account for the trend observed for the stability constants of the different ternary complexes of the type M(II) + NU + Z. Certain ligand associations and interactions may be favored via the formation of mixed-ligand complexes, and thus distinct structures may be created in a way that involves only small changes in energy. Purine nucleotides form macrochelates with some divalent metal ions such as Mn^{2+}, Cu^{2+}, and Zn^{2+} involving N7 of the nucleobase.[1] The extent of macrochelate formation is different in each case. Thus, the different nature of the binary complexes existing in solution may affect the stability constants of the different ternary complexes formed in solution depending on the nature of the metal ion. To the authors' knowledge, no data for the ternary complexes of the newer buffers MOPS, AMPSO, HEPPSO, PIPES, or POPSO with the purine nucleotides AMP, ADP, or ATP are available in the literature for comparison.

During SUPERQUAD[18] refinement, the titration data of the ternary complexes Cu(II) + ADP + PIPES, Co(II) + AMP + PIPES, Co(II) + ADP + PIPES, Co(II) + AMP + MOPS, Ni(II) + AMP + PIPES, Ni(II) + ADP + AMPSO, Mn(II) + AMP + PIPES, Mn(II) + AMP + MOPS, Mn(II) + ATP + MOPS, Mn(II) + ADP + AMPSO, Mn(II) + ATP + AMPSO, Zn(II) + ADP + PIPES, Ca(II) + AMP + PIPES, Ca(II) + ATP + PIPES, Ca(II) + ADP + PIPES, Ca(II) + ADP + MOPS, Ca(II) + ATP + MOPS, Mg(II) + ATP + PIPES, and Mg(II) + AMP + AMPSO fit satisfac-

Table 3. Formation Constants for the Binary Ni(II) + Nucleotide (NU) or Zwitterionic Buffer (Z) Ligand Complexes Together with the Corresponding Mixed-Ligand Complexes Ni(II) + NU + Z at (25.0 ± 0.1) °C and I = 0.1 mol dm^{-3} KNO_3[a]

ligand	$\log K^{Ni(II)}_{Ni(II)(NU)}$ or $\log K^{Ni(II)}_{Ni(II)(Z)}$	$\log K^{Ni(II)(AMP)}_{Ni(II)(AMP)(Z)}$ or $\log \beta^{Ni(II)}_{Ni(II)(AMP)(Z)}$	$\log K^{Ni(II)(ADP)}_{Ni(II)(ADP)(Z)}$ or $\log \beta^{Ni(II)}_{Ni(II)(ADP)(Z)}$	$\log K^{Ni(II)(ATP)}_{Ni(II)(ATP)(Z)}$ or $\log \beta^{Ni(II)}_{Ni(II)(ATP)(Z)}$
AMP	2.84 ± 0.03			
ADP	4.50 ± 0.04			
ATP	4.83 ± 0.03			
PIPES	3.39 ± 0.02	5.54 ± 0.03[b]	1.80 ± 0.03	1.59 ± 0.03
		3.42 ± 0.02[c]	5.51 ± 0.04	6.42 ± 0.03
MOPS	3.45 ± 0.03	1.69 ± 0.03	1.52 ± 0.04	1.10 ± 0.03
		6.19 ± 0.03	5.23 ± 0.04	5.93 ± 0.03
POPSO		2.67 ± 0.03	2.72 ± 0.03	3.11 ± 0.03
		7.17 ± 0.03	6.43 ± 0.04	7.94 ± 0.03
HEPPSO	3.59 ± 0.02	3.73 ± 0.04	3.40 ± 0.03	3.55 ± 0.02
		8.23 ± 0.02	7.11 ± 0.04	8.38 ± 0.03
AMPSO	3.62 ± 0.02		6.77 ± 0.04[b]	2.66 ± 0.02
			4.44 ± 0.03[c]	7.49 ± 0.03

[a] $\log \beta^{Ni(II)}_{Ni(II)(NU)(Z)} = \log K^{Ni(II)(NU)}_{Ni(II)(NU)(Z)} + \log K^{Ni(II)}_{Ni(II)(NU)}$. [b] $\log K^{Ni(II)}_{Ni(II)(NU)(HZ)}$. [c] $\log K^{Ni(II)(NU)(HZ)}_{Ni(II)(NU)(Z)}$ [calculated using the following equilibria: $Ni^{II}(NU)(HZ) \rightleftharpoons Ni^{II}(NU)(Z) + H^+$, $Ni(II) + NU + Z \xrightleftharpoons{K^{Ni(II)(NU)(HZ)}_{Ni(II)(NU)(Z)}} Ni^{II}(NU)(Z)$] ± uncertainities refer to 3 times the standard deviation (3S).

Table 4. Formation Constants for the Binary Mn(II) + Nucleotide (NU) or Zwitterionic Buffer (Z) Ligand Complexes Together with the Corresponding Mixed-Ligand Complexes Mn(II) + NU + Z at (25.0 ± 0.1) °C and I = 0.1 mol dm^{-3} KNO_3[a]

ligand	$\log K^{Mn(II)}_{Mn(II)(NU)}$ or $\log K^{Mn(II)}_{Mn(II)(Z)}$	$\log K^{Mn(II)(AMP)}_{Mn(II)(AMP)(Z)}$ or $\log \beta^{Mn(II)}_{Mn(II)(AMP)(Z)}$	$\log K^{Mn(II)(ADP)}_{Mn(II)(ADP)(Z)}$ or $\log \beta^{Mn(II)}_{Mn(II)(ADP)(Z)}$	$\log K^{Mn(II)(ATP)}_{Mn(II)(ATP)(Z)}$ or $\log \beta^{Mn(II)}_{Mn(II)(ATP)(Z)}$
AMP	2.35 ± 0.04			
ADP	4.16 ± 0.03			
ATP	4.70 ± 0.04			
PIPES		5.82 ± 0.04[b]	2.08 ± 0.03	2.23 ± 0.04
		3.19 ± 0.03[c]	6.24 ± 0.03	6.93 ± 0.04
MOPS		4.57 ± 0.03[b]	1.97 ± 0.04	5.42 ± 0.02[b]
		2.34 ± 0.02[c]	6.13 ± 0.03	5.09 ± 0.02[c]
POPSO		2.94 ± 0.03	3.23 ± 0.03	3.05 ± 0.02
		5.29 ± 0.04	7.39 ± 0.03	7.75 ± 0.03
HEPPSO		3.34 ± 0.03	3.87 ± 0.04	3.40 ± 0.03
		5.69 ± 0.04	8.03 ± 0.03	8.10 ± 0.03
AMPSO			6.77 ± 0.04[b]	8.42 ± 0.03[b]
			3.77 ± 0.03[c]	5.47 ± 0.04[c]

[a] $\log \beta^{Mn(II)}_{Mn(II)(NU)(Z)} = \log K^{Mn(II)(NU)}_{Mn(II)(NU)(Z)} + \log K^{Mn(II)}_{Mn(II)(NU)}$. [b] $\log K^{Mn(II)}_{Mn(II)(NU)(HZ)}$. [c] $\log K^{Mn(II)(NU)(HZ)}_{Mn(II)(NU)(Z)}$ [calculated using the following equilibria: $Mn^{II}(NU)(HZ) \rightleftharpoons Mn^{II}(NU)(Z) + H^+$, $Mn(II) + NU + Z \xrightleftharpoons{K^{Mn(II)(NU)(HZ)}_{Mn(II)(NU)(Z)}} Mn^{II}(NU)(Z)$] ± uncertainities refer to 3 times the standard deviation (3S).

torily on the basis of the monoprotonated ternary complexes which dissociate to give normal complexes.

The higher values of stability constants of the ternary complexes containing HEPPSO as a secondary ligand compared with the other zwitterionic buffer ligands in the present study despite the interaction between two negatively charged species M(NU) + Z = [M(NU)(Z)] may lead to the conclusion that the hydroxy groups of HEPPSO may participate in the ternary complexes with AMP, ADP, or ATP.

Unexpectedly, the ternary complex Ca(II) + ATP + HEPPSO is more stable than Mg(II) + ATP + HEPPSO. This may be explained by the "cage-like" orientation of several hydroxo groups and the nitrogen, a structural arrangement into which Ca(II) fits well, while the ionic radius of Mg(II) is too small to allow an optimal interaction with the hydroxo groups.

The order of formation constants of the different normal mixed-ligand complexes of the type M(II) + NU + POPSO in terms of metal ion generally follows the trend Cu(II) > Zn(II) > Ni(II) > Co(II) > Mn(II) for M(II) + ATP + POPSO systems, Mn(II) > Co(II) > Ni(II) > Cu(II) > Zn(II) for M(II) + ADP + POPSO systems, and Cu(II) > Mn(II) > Ni(II) > Co(II) for M(II) + AMP + POPSO systems, respectively.

This behavior may be attributed to the nature of the interaction of the metal ions Cu(II), Ni(II), Co(II), Mn(II), and Zn(II) during their binding to the purine nucleotides AMP, ADP, or ATP. As stated previously,[26] these ions favor mixed oxygen and nitrogen donors. Although N1 is more basic than N7, it was confirmed that macrochelate formation with N1 of the purine moiety is not possible for steric reasons. Such an evaluation, which of course reflects the metal ion binding properties of N7 during the formation of the binary nucleotide complexes, is also significant for ternary complexes. Taking into consideration the equilibria which may exist between the open and closed forms of the formed ternary complexes and the effect of the nature of the metal ion coordination in this equilibria, one can account for the observed trend for the stability constants of the different ternary complexes of the type M(II) + NU + POPSO.

It is worth indicating that in the case of the ternary complexes of the type M(II) + AMP + AMPSO, where M(II) = Ni(II), Co(II), Mn(II), or Zn(II), precipitation was observed during titration. This can be attributed to the hydrolysis of these complexes and the probable formation of hydroxo complex species. With respect to the titration

Table 5. Formation Constants for the Binary Zn(II) + Nucleotide (NU) or Zwitterionic Buffer (Z) Ligand Complexes Together with the Corresponding Mixed-Ligand Complexes Zn(II) + NU + Z at (25.0 ± 0.1) °C and I = 0.1 mol dm^{-3} KNO_3[a]

ligand	$\log K^{Zn(II)}_{Zn(II)(NU)}$ or $\log K^{Zn(II)}_{Zn(II)(Z)}$	$\log K^{Zn(II)(AMP)}_{Zn(II)(AMP)(Z)}$ or $\log \beta^{Zn(II)}_{Zn(II)(AMP)(Z)}$	$\log K^{Zn(II)(ADP)}_{Zn(II)(ADP)(Z)}$ or $\log \beta^{Zn(II)}_{Zn(II)(ADP)(Z)}$	$\log K^{Zn(II)(ATP)}_{Zn(II)(ATP)(Z)}$ or $\log \beta^{Zn(II)}_{Zn(II)(ATP)(Z)}$
AMP	2.72 ± 0.04			
ADP	4.28 ± 0.03			
ATP	4.85 ± 0.04			
PIPES	3.42 ± 0.03		5.68 ± 0.02[b]	2.09 ± 0.02
			3.37 ± 0.03[c]	6.94 ± 0.03
MOPS	3.47 ± 0.04		2.48 ± 0.03	2.30 ± 0.03
			6.76 ± 0.04	7.15 ± 0.03
POPSO			2.20 ± 0.02	3.30 ± 0.03
			6.48 ± 0.03	8.15 ± 0.04
HEPPSO	4.13 ± 0.03		4.19 ± 0.03	3.77 ± 0.02
			8.47 ± 0.04	8.62 ± 0.03
AMPSO	4.42 ± 0.04		4.72 ± 0.03	2.86 ± 0.02
			9.00 ± 0.03	7.71 ± 0.03

[a] $\log \beta^{Zn(II)}_{Zn(II)(NU)(Z)} = \log K^{Zn(II)(NU)}_{Zn(II)(NU)(Z)} + \log K^{Zn(II)}_{Zn(II)(NU)}$. [b] $\log K^{Zn(II)}_{Zn(II)(NU)(HZ)}$. [c] $\log K^{Zn(II)(NU)(HZ)}_{Zn(II)(NU)(Z)}$ [calculated using the following equilibria: $Zn^{II}(NU)(HZ) \rightleftharpoons Zn^{II}(NU)(Z) + H^+$, $Zn(II) + NU + Z \xrightleftharpoons{K^{Zn(II)(NU)(HZ)}_{Zn(II)(NU)(Z)}} Zn^{II}(NU)(Z)$] ± uncertainities refer to 3 times the standard deviation (3S).

Table 6. Formation Constants for the Binary Ca(II) + Nucleotide (NU) or Zwitterionic Buffer (Z) Ligand Complexes Together with the Corresponding Mixed-Ligand Complexes Ca(II) + NU + Z at (25.0 ± 0.1) °C and I = 0.1 mol dm^{-3} KNO_3[a]

ligand	$\log K^{Ca(II)}_{Ca(II)(NU)}$ or $\log K^{Ca(II)}_{Ca(II)(Z)}$	$\log K^{Ca(II)(AMP)}_{Ca(II)(AMP)(Z)}$ or $\log \beta^{Ca(II)}_{Ca(II)(AMP)(Z)}$	$\log K^{Ca(II)(ADP)}_{Ca(II)(ADP)(Z)}$ or $\log \beta^{Ca(II)}_{Ca(II)(ADP)(Z)}$	$\log K^{Ca(II)(ATP)}_{Ca(II)(ATP)(Z)}$ or $\log \beta^{Ca(II)}_{Ca(II)(ATP)(Z)}$
AMP	1.85 ± 0.02			
ADP	2.86 ± 0.03			
ATP	3.97 ± 0.03			
PIPES		5.71 ± 0.03[b]	5.08 ± 0.03[b]	9.78 ± 0.03[b]
		3.11 ± 0.04[c]	1.56 ± 0.02[c]	4.43 ±0.02[c]
MOPS		1.35 ± 0.02	5.17 ± 0.04[b]	5.42 ± 0.04[b]
		3.20 ± 0.02	2.06 ± 0.02[c]	4.66 ±0.03[c]
POPSO		2.82 ± 0.02	3.05 ± 0.02	3.03 ± 0.02
		4.67 ± 0.02	5.91 ± 0.03	7.00 ± 0.03
HEPPSO		3.89 ± 0.02	3.72 ± 0.03	3.42 ± 0.02
		5.74 ± 0.02	6.58 ± 0.03	7.39 ± 0.03
AMPSO		2.35 ± 0.03	2.16 ± 0.02	2.62 ±0.03
		4.20 ± 0.02	5.02 ± 0.03	6.59 ± 0.03

[a] $\log \beta^{Ca(II)}_{Ca(II)(NU)(Z)} = \log K^{Ca(II)(NU)}_{Ca(II)(NU)(Z)} + \log K^{Ca(II)}_{Ca(II)(NU)}$. [b] $\log K^{Ca(II)}_{Ca(II)(NU)(HZ)}$. [c] $\log K^{Ca(II)(NU)(HZ)}_{Ca(II)(NU)(Z)}$ [calculated using the following equilibria: $Ca^{II}(NU)(HZ) \rightleftharpoons Ca^{II}(NU)(Z) + H^+$, $Ca(II) + NU + Z \xrightleftharpoons{K^{Ca(II)(NU)(HZ)}_{Ca(II)(NU)(Z)}} Ca^{II}(NU)(Z)$] ± uncertainities refer to 3 times the standard deviation (3S).

curves of the 1:1:1 ternary complexes of Zn(II) + ADP + AMPSO and Cu(II) + AMP + AMPSO, dissolution of the precipitate which occurred at lower pH values was observed at higher pH's, probably because of the complete formation of ternary complexes in solution.

The weaker binding of the AMPSO-ate anion to the binary M(II) nucleotide complexes as compared with that of the HEPPSO-ate anions was observed. The fact that the AMPSO-ate anion is more basic tends to make it more strongly bound. The effect from the poorer structural matching between the secondary ligand and M(II) nucleotide complex prevails over that from the basicity, and the binding of the AMPSO-ate anion secondary ligand by a M(II) nucleotide complex is weaker than the bonding between the HEPPSO-ate anion with the same binary M(II) nucleotide complex.

The most unexpected result for the ternary systems of the type M(II) + ADP + AMPSO is certainly the high relative stability of the ternary Zn(II) + ADP + AMPSO complex compared to Cu(II) + ADP + AMPSO and Co(II) + ADP + AMPSO. This means that the equilibrium (6) favors the products

$$Zn(ADP) + Zn(AMPSO) \rightleftarrows Zn(ADP)(AMPSO) + Zn \quad (6)$$

It seems that the explanation for this behavior is linked to the varying coordination sphere of Zn(II), which apparently can easily switch from coordination number 6 to 4 or 5.[27,28]

This behavior may explain if the primary ligand ADP binds to an octahedral Zn(II) with a very high probable structure. Further binding of the AMPSO-ate anion should reduce the coordination to 4 or 5 with two water molecules released upon the coordination of the secondary ligand. Such a process is entropically favored and would therefore explain the dissolution of the precipitate in the titration curve of the ternary system Zn(II) + ADP + AMPSO at pH > pK_{a2} of AMPSO, where the coordination of the secondary ligand occurred. Clearly, Co(II) and Ni(II), which have well-defined octahedral coordination spheres, would be less probable to undergo such a process.

To quantify the stability of ternary complexes relative to the stability of the binary parent complexes,[29] one may consider the equilibrium (7)

$$M(NU) + M(Z) \rightleftarrows M(NU)(Z) + M \quad (7)$$

Table 7. Formation Constants for the Binary Mg(II) + Nucleotide (NU) or Zwitterionic Buffer (Z) Ligand Complexes Together with the Corresponding Mixed-Ligand Complexes Mg(II) + NU + Z at (25.0 ± 0.1) °C and I = 0.1 mol dm^{-3} KNO_3[a]

ligand	$\log K^{Mg(II)}_{Mg(II)(NU)}$ or $\log K^{Mg(II)}_{Mg(II)(Z)}$	$\log K^{Mg(II)(AMP)}_{Mg(II)(AMP)(Z)}$ or $\log \beta^{Mg(II)}_{Mg(II)(AMP)(Z)}$	$\log K^{Mg(II)(ADP)}_{Mg(II)(ADP)(Z)}$ or $\log \beta^{Mg(II)}_{Mg(II)(ADP)(Z)}$	$\log K^{Mg(II)(ATP)}_{Mg(II)(ATP)(Z)}$ or $\log \beta^{Mg(II)}_{Mg(II)(ATP)(Z)}$
AMP	1.97 ± 0.03			
ADP	3.17 ± 0.04			
ATP	3.99 ± 0.04			
PIPES		7.52 ± 0.04[b]	2.38 ± 0.04	4.72 ± 0.03[b]
			5.55 ± 0.04	4.56 ± 0.04[c]
MOPS	3.51 ± 0.03	1.31 ± 0.03	2.89 ± 0.04	6.27 ± 0.03[b]
		3.28 ± 0.03	6.06 ± 0.04	5.35 ± 0.04[c]
POPSO		2.90 ± 0.02	2.84 ± 0.04	1.46 ± 0.03
		4.87 ± 0.03	6.01 ± 0.03	5.45 ± 0.04
HEPPSO	3.69 ± 0.04	4.01 ± 0.04	4.83 ± 0.03	3.05 ± 0.03
		5.98 ± 0.03	8.00 ± 0.04	7.04 ± 0.04
AMPSO		7.88 ± 0.03[b]	3.00 ± 0.02	2.22 ± 0.04
		3.32 ± 0.02[c]	6.17 ± 0.03	6.21 ± 0.03

[a] $\log \beta^{Mg(II)}_{Mg(II)(NU)(Z)} = \log K^{Mg(II)(NU)}_{Mg(II)(NU)(Z)} + \log K^{Mg(II)}_{Mg(II)(NU)}$. [b] $\log K^{Mg(II)}_{Mg(II)(NU)(HZ)}$. [c] $\log K^{Mg(II)(NU)(HZ)}_{Mg(II)(NU)(Z)}$ [calculated using the following equilibria: $Mg^{II}(NU)(HZ) \rightleftharpoons Mg^{II}(NU)(Z) + H^+$, $Mg(II) + NU + Z \xrightleftharpoons{K^{Mg(II)(NU)(HZ)}_{Mg(II)(NU)(Z)}} Mg^{II}(NU)(Z)$] ± uncertainities refer to 3 times the standard deviation (3S).

Table 8. Δ log K_M[a] Values for the 1:1:1 M(II) + Nucleotide (NU) + Zwitterionic Buffer (Z) Ternary Complexes, As Determined by Potentiometric pH Titrations at (25.0 ± 0.1) °C and I = 0.1 mol dm^{-3} KNO_3

	Δ log K_M						
$M^{II}(NU)(Z)$	Cu(II)	Ni(II)	Co(II)	Mn(II)	Zn(II)	Ca(II)	Mg(II)
				$M^{II}(AMP)(Z)$			
PIPES							
MOPS		−1.76					−2.20
POPSO							
HEPPSO	+0.51	+0.14	+0.34				+0.32
AMPSO	−1.5						
				$M^{II}(ADP)(Z)$			
PIPES		−1.59					
MOPS	−1.78	−1.93	−1.82		−0.99		−0.62
POPSO							
HEPPSO	−0.84	−0.19	+0.41		+0.06		+1.14
AMPSO	−1.82		−0.50		+0.30		
				$M^{II}(ATP)(Z)$			
PIPES	−1.20	−1.80	−1.70		−1.33		
MOPS	−1.86	−2.35	−1.51		−1.17		
POPSO							
HEPPSO	+0.56	−0.04	+1.22		−0.36		−0.64
AMPSO	−1.71	−0.96	−0.02		−1.56		

[a] $\Delta \log K_M = \log K^{M(II)(NU)}_{M(II)(NU)(Z)} - \log K^{M(II)}_{M(II)(Z)}$.

The corresponding equilibrium constant is defined by eq 8. Values for Δ log K_M may be calculated according to eq 9.

$$10^{\Delta \log K_M} = \frac{[M(NU)(Z)][M(II)]}{[M(NU)][M(Z)]} \quad (8)$$

$$\Delta \log K_M = \log K^{M(NU)}_{M(NU)(Z)} - \log K^{M}_{M(Z)} \quad (9)$$

The results are given in Table 8. Some Δ log K_M values are positive for some of the ternary complexes studied, with the higher values for the formation constants of ternary complexes compared with the binary systems being attributed to the interligand interactions or some cooperativity between the coordinate ligands such as H-bond formation. This also may be explained on the basis of the π-acceptor qualities of the adenine base, causing strengthening of the M(II)−N bonds through the interaction of the M(II) ion to the antibonding π^* orbitals of the heteroaromatic N base. Because of the π-acceptor qualities of the adenine base (i.e., back-donation from metal to ligand), the d-electron content on the metal decreases, rendering the metal more electrophilic. The interaction of the p electrons of the phosphate O atoms with the metal will increase to a greater extent and consequently influence the stability of ternary complexes. The effect of back-bonding does not apply to Ca and Mg ions.

The ternary complexes of the type M(II) + NU + Z may be considered as relatively simple models from which information may be gained about the properties of purine nucleotides and their base moieties regarding the stability constants of the complexes which may be formed during their interactions with the biologically important zwitterionic buffer ligands (MOPS, AMPSO, HEPPSO, PIPES, or POPSO) and M(II) ions. Our investigation confirmed the formation of mixed-ligand complexes of the type M(II) + NU + Z (where Z = MOPS, AMPSO, HEPPSO, PIPES, or POPSO) and M(II) = Cu(II), Co(II), Ni(II), Mn(II), Zn(II), Ca(II), or Mg(II)) in solution. This must be taken into account when studying these biologically important zwitterionic buffer ligands in aqueous solutions containing the above-mentioned metal ions and the purine nucleotides AMP, ADP, or ATP. The likelihood for the formation of ternary complexes is also rather high, as was demonstrated in the present study, affecting the properties of these purine nucleotides in various ways when they are used as substrates.

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Received for review June 12, 2000. Accepted December 4, 2000.

JE0001779