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Ternary Complexes in Solution. Comparison of the Coordination Tendency of Some Biologically Important Zwitterionic Buffers Toward the Binary Complexes of Cu(II) and Adenosine 5′-Mono-, 5′-Di-, and 5′-Triphosphate

H. A. Azab* and **A. M. El-Nady**

Chemistry Department, Faculty of Science, Assiut University, Assiut, Egypt

Summary. Potentiometric equilibrium measurements have been made at 25.0 ± 0.1 °C and ionic strength $I = 0.1$ mol dm^{-3} KNO_3 for the interaction of adenosine 5′-mono-, 5′-di-, and 5′-triphosphate (*AMP*, *ADP* and *ATP*) and Cu(II) with biologically important secondary ligand zwitterionic buffers (N,N-*bis*-(2-hydroxyethyl)-2-aminoethanesulphonic acid (*BES*), N-*tris*-(hydroxymethyl)-methyl-2-aminoethanesulphonic acid (*TES*), N,N-*bis*-(2-hydroxyethyl)-glycine (Bicine) and *tris*-(hydroxymethyl)-methylaminopropane sulphonic acid (*TAPS*)) in a 1:1:1 ratio and the formation of various 1:1:1 mixed ligand complex species inferred from the potentiometric *pH* titration curves. Initial estimates of the formation constants of the resulting species and the acid dissociation constants of *AMP*, *ADP*, *ATP*, and secondary ligands have been refined with the SUPERQUAD computer program. Negative and positive $\Delta \log K$ values were obtained for the ternary systems studied. In some Cu(II) ternary systems studied the interligand interactions or some cooperativity between the coordinate ligands, possibly H bond formation, has been found to be most effective in deciding the stability of the ternary complexes formed in solution. Stabilities of mixed ligand complexes increase in the order *AMP* < *ADP* < *ATP*. The trend in stability constants of the mixed-ligand complexes of the title zwitterionic buffer ligands is found to be *TAPS* > *Bicine* > *TES* > *BES*.

Keywords. Ternary complexes; Coordination tendency; Adenosine; Copper; Potentiometric titration.

Ternäre Komplexe in Lösung. Vergleich der Koordinationstendenz einiger biologisch wichtiger zwitterionischer Puffer an binäre Komplexe von Cu(II) und Adenosin-5′-mono-, -5′-di- und -5′-triphosphat

Zusammenfassung. Die Wechselwirkungen zwischen Adenosin-5′-mono, -5′-di- und -5′-triphosphat (*AMP*, *ADP* und *ATP*), Cu(II) und biologisch wichtigen zwitterionischen Puffern mit Sekundärligandeigenschaften (N,N-*bis*-(2-Hydroxyethyl)-2-aminoethansulfonsäure (*BES*), N-*tris*-(Hydroxymethyl)-methyl-2-aminoethansulfonsäure (*TES*), N,N-*bis*-(2-Hydroxyethyl)-glycin (Bicin) und *tris*-(Hydroxymethyl)-methylaminopropansulfonsäure (*TAPS*) im Verhältnis 1:1:1 wurden mittels potentiometrischer Gleichgewichtsmessungen bei 25.0 ± 0.1 °C und $I = 0.1$ mol·dm^{-3} KNO_3 untersucht. Die Titrationskurven lassen auf verschiedene Komplexe mit gemischten Liganden im Verhältnis von 1:1:1 schließen. Erste Abschätzungen der Bildungskonstanten der entstehenden Produkte und der Dissoziationskonstanten von *AMP*, *ADP*, *ATP* und der Sekundärliganden wurden mit Hilfe des

Programms SUPERQUAD verfeinert. Für die untersuchten ternären Systeme wurden negative und positive Werte für $\Delta \log K$ erhalten. In einigen der Cu(II)-Komplexe wird die Stabilität des ternären Systems in Lösung hauptsächlich durch Wechselwirkungen zwischen den Liganden, möglicherweise durch die Ausbildung von Wasserstoffbrückenbindungen, bestimmt. Die Stabilität der Komplexe steigt in der Reihenfolge *AMP* < *ADP* < *ATP*. Der entsprechende Trend der Stabilitätskonstanten bei den gemischten Komplexen mit den im Titel genannten zwitterionischen Puffern lautet *TAPS* > *Bicin* > *TES* > *BES*.

Introduction

Metal ion complex formations are among the prominent interactions in nature [1–3], and the zwitterionic buffers are important hydrogen ion buffers for biological research, while the ribonucleotides adenosine 5′-mono, 5′-di-, and 5′-triphosphates (*AMP*, *ADP*, and *ATP*) are equally important as substrates for many enzymic reactions [4–7]. Ternary complexes of transition divalent metal ions with *AMP*, *ADP*, and *ATP* and other secondary ligands such as the catechols, ethanolamines, 2,2′-bipyridyl, ethylenediamine, pyrocatecholate, biogenic amines, 1,10-phenanthroline, tyrosine, phenylalanine, glycine, histidine, imidazole, ammonia, aliphatic dipeptides, and monocarboxylic acids have been investigated using several techniques [8–25] (*pH*-potentiometry, spectrophotometry, and calorimetry). For an improved understanding of the driving forces leading to mixed ligand complexes of the type Cu(II)-nucleotide-zwitterionic buffer ligands (Cu(II)-*Nu-L*), where nucleotide = *AMP*, *ADP*, or *ATP*, and zwitterionic buffer ligands = *BES*, Bicine, *TAPS* or *TES*, have been investigated by potentiometric *pH* titration to determine the stability constants of the complexes formed as these systems mimic many biological reactions (Cu(II) ion-buffer-substrate interactions) and also may be considered as models for enzyme-Cu(II)-substrate complexes.

Experimental

Materials and Solutions. Adenosine 5′-monophosphoric acid disodium salt $C_{10}H_{12}N_5Na_2O_7P \cdot H_2O$ ($Na_2AMP \cdot H_2O$), adenosine 5′-diphosphoric acid disodium salt $C_{10}H_{13}N_5Na_2O_{10}P_2 \cdot 2H_2O$ ($Na_2ADP \cdot 2H_2O$), and adenosine 5′-triphosphoric acid disodium salt $C_{10}H_{14}N_5Na_2O_{13}P_3 \cdot 3H_2O$ ($Na_2ATP \cdot 3H_2O$), were purchased from Sigma Chemical Co. and were used without purification. The amount of free phosphates initially present in the neucleotides was determined [26]. It was found to be 2% for *ATP* and 3% for *ADP* and *AMP*. To account for this and to prepare metal ion nucleotide solutions of exactly 1:1 ratio, we also determined (by potentiometric *pH* titrations) the molecular weight of these nucleotides. N,N-*bis*-(2-hydroxyethyl)-2-aminoethanesulphonic acid (*BES*), N-*tris*-(hydroxymethyl)-methyl-2-aminoethane-sulphonic acid, (*TES*), N,N-*bis*-(2-hydroxyethyl)-glycine (Bicine) and *tris*-(hydroxymethyl)-methylaminopropane sulphonic acid (*TAPS*) were analytical grade (Merck) with a purity of 99% and were further purified according to *Perrin* [27]. $Cu(NO_3)_2 \cdot 6H_2O$, nitric acid, and NaOH were of p.a. grade. The concentration of NaOH 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 volumetrically with *tris*-(hydroxymethyl)-aminoethane. The concentrations of the metal ion stock solutions were determined with ethylenediaminetetraacetic acid (*EDTA*).

Apparatus. Potentiometric *pH* measurements were made on solutions in a double-walled glass vessel using a Beckman Model 4500 digital *pH* meter with a precision of ± 0.1 mV. The potentiometric system

was connected to a glass electrode (Metrohm 1028) connected to a double junction reference electrode (Orion 9020). The titrant was delivered by an Amel 882 dispenser, readable to 1 µl. The measurement cell was kept at a temperature constant within ± 0.1 °C, and a magnetic stirrer was used. Purified nitrogen was bubbled through the solutions during titrations.

Procedure. The test solution was titrated with standard CO_2-free NaOH. The electrodes were calibrated, in both the acidic and alkaline regions, by titrating 0.01 *M* nitric acid with standard potassium hydroxide under the same experimental conditions. Carbonate-free KOH was standardized against standard potassium hydrogen phthalate with the aid of a *Gran* plot.

The concentration of free hydrogen ion, C_{H^+}, at each point of the titration is related to the measured emf, E°, of the cell by the *Nernst* equation:

$$E = E^\circ + Q \log C_{H^+} \tag{1}$$

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 standard KOH solution under the same temperature and medium conditions as for the test solution titration. The results so obtained were analyzed by the nonlinear least-squares computer program ESAB2M [28] to refine E° and the autoprotolysis constant of water, K_w.

In order to avoid hydrolysis prior to the potentiometric measurements, samples of the nucleotides were weighed out as the solid and added to the reaction vessel just prior to performing the titration. The solutions titrated can be presented according to the following scheme: HNO_3 (a); HNO_3 + nucleotide (b); HNO_3 + nucleotide + Cu(II) (c); HNO_3 + zwitterionic buffer ligand (d); HNO_3 + zwitterionic buffer ligand + Cu(II) (e); HNO_3 + nucleotide + zwitterionic buffer ligand + Cu(II) (f) (cf. Figs.). A constant ionic strength was obtained with 0.1 *M* KNO_3, and the total volume was kept constant at 25 cm^3.

Results and Discussion

To calculate the initial estimates of the stability constants of the ternary complexes of Cu(II) with *AMP*, *ADP*, *ATP*, and *BES*, Bicine, *TAPS* or *TES* the following equations were used:

$$\mathrm{Cu(II)}(Nu) + L \rightleftharpoons \mathrm{Cu(II)}(Nu)(L) \tag{2}$$

$$K^{\mathrm{Cu(II)}(Nu)}_{\mathrm{Cu(II)}(Nu)(L)} = \frac{[\mathrm{Cu(II)}(Nu)(L)]}{[\mathrm{Cu(II)}(Nu)][L]} \tag{3}$$

$$\mathrm{Cu(II)} + Nu \rightleftharpoons \mathrm{Cu(II)}(Nu) \tag{4}$$

$$K^{\mathrm{Cu(II)}}_{\mathrm{Cu(II)}(Nu)} = \frac{[\mathrm{Cu(II)}(Nu)]}{[\mathrm{Cu(II)}][Nu]} \tag{5}$$

$$\mathrm{Cu(II)} + L \rightleftharpoons \mathrm{Cu(II)}(L) \tag{6}$$

$$K^{\mathrm{Cu(II)}}_{\mathrm{Cu(II)}(L)} = \frac{[\mathrm{Cu(II)}(L)]}{[\mathrm{Cu(II)}][L]} \tag{7}$$

[$I = 0.1$ mol dm^{-3} (KNO_3), T = 25 °C]

where L = zwitterionic buffer ligands (*BES*, Bicine, *TAPS* and *TES*) and Nu = nucleotide (*AMP*, *ADP* and *ATP*). 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 overall stability constant $\beta^{Cu(II)}_{Cu(II)(Nu)(L)}$ may be represented by Eq. 8.

$$Cu(II) + Nu + L \rightleftharpoons Cu(II)(Nu)(L) \tag{8}$$

$$\beta^{Cu(II)}_{Cu(II)(Nu)(L)} = \frac{[Cu(II)(Nu)(L)]}{[Cu(II)][Nu][L]} = K^{Cu(II)(Nu)}_{Cu(II)(Nu)(L)} \cdot K^{Cu(II)}_{Cu(II)(Nu)} \tag{9}$$

Formation constants and protonation constants were refined with the SUPERQUAD computer program [29]. All the calculations were performed on an IBM XT 286 personal computer. The constants were refined by minimizing the error-square sum, U, of the potentials:

$$U = \Sigma W_i (E_{obs} - E_{calc})^2 \tag{10}$$

where E_{obs} and E_{calc} refer to the measured potential and that calculated from Eq. 1. The weighting factor W_i is defined as the reciprocal of the estimated variance of the measurement:

$$W_i = 1/\sigma^2 = 1/[\sigma_E^2 + (\partial E/\partial V)^2 \sigma_v^2] \tag{11}$$

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 $\sigma_E = 0.1$ mV (0.001 *pH* error) and $\sigma_v = 0.005$ 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, without any significant systematic trends, thus indicating a good fit of the experimental data.

At the experimental *pH* values used in the calculation in this work the interfering effects of hydroxy complexes are negligible. Thus, the secondary ligand, L, combines with the binary 1:1 Cu(II)(*Nu*)([Cu(II)(*AMP*)], [Cu(II)(*ADP*)$^-$], and [Cu(II)(*ATP*)]$^{2-}$) complex in a manner similar to its interaction with aquated metal ions $[Cu(H_2O)_6]^{2+}$ in solutions. Therefore, the initial estimates of the stability constants of the ternary complexes formed in solution have been determined using the *Rossotti* and *Irving* formula [30]. These values were then refined using the SUPERQUAD computer program [29].

The determined acidity constants of *BES* ($pK_a = 7.09 \pm 0.02$), Bicine ($pK_a = 8.29 \pm 0.02$), *TAPS* ($pK_a = 8.35 \pm 0.03$) and *TES* ($pK_a = 7.40 \pm 0.03$) and the stability constants of their binary Cu(II) complexes are in good agreement with those found in literature [31, 32].

The two acid formation constant values for *AMP* ($pK_{a1} = 3.81 \pm 0.03$, $pK_{a2} = 6.24 \pm 0.03$), *ADP* ($pK_{a1} = 3.94 \pm 0.03$, $pK_{a2} = 6.38 \pm 0.04$), and *ATP* ($pK_{a1} = 4.05 \pm 0.03$, $pK_{a2} = 6.51 \pm 0.03$) and the stability constants of their Cu(II) complexes were determined from the titration curves, and the results were found to agree well with those reported in the literature [33–36].

In the case of *ADP* and *ATP* the monoprotonated complexes, *i.e.* Cu(*HADP*) and Cu(*HATP*)$^-$, were taken into consideration. The calculated values $\log K^{Cu}_{Cu(HADP)} = 2.61$ and $\log K^{Cu}_{Cu(HATP)^-} = 3.33$ agree also favorably with the literature [36].

Early researchers [37–40] found pK_{a1} values of 3.5–4.2 to be associated with proton ionization from the protonated forms of *AMP*, *ADP*, and *ATP*. Calorimetric

work [41] provides evidence that proton ionization from protonated adenine and adenosine is from the N_1H^+ group. The second proton ionization was attributed to the phosphate groups.

The purine bases have two high electron density centers which are possible sites for metal ion chelation, *viz.* C_6NH_2/N_7 and N_3–N_9. Chelation of Cu^{2+} by both sites has been suggested [42–45].

Potentiometric [46, 47], ^{31}P NMR [46, 48] and aqueous solution infrared absorption data [48] confirm the binding of Cu^{2+} to the phosphate portion of *AMP*, *ADP*, and *ATP*. These studies are in essential agreement that Cu^{2+} binds the available phosphate group in the mono- and dinucleotides but only the α- and β-phosphates in *ATP*. This latter behavior has been attributed to the square-planar stereochemical requirements of Cu^{2+}.

On the basis of the observed lack of reaction (from *pH* titration data) between Cu^{2+} and adenosine and the increased stability of Cu^{2+} complexes in the order *AMP* < *ADP* < *ATP*, the suggestions has been made that Cu^{2+} did not react with the basic moiety of *ATP* [47]. However, proton NMR studies have demonstrated binding of Cu^{2+} to the N_7 positions of the adenine base in d*AMP* [46].

Berger and *Eichhorn* [46] conclude that, in general, Cu^{2+} can bind to multiple sites on the adenine base, with preference for a given site influenced by molecular associations which in the different *AMP* isomers are governed by the position of the phosphate on the ribose. In the case of 2′-*AMP* a chelate involving N_3 and a phosphate group of the same molecule is proposed. It is expected that Cu^{2+} ions would interact more strongly with the electron donor groups of the purine base of mononucleotides than they do with the phosphate groups.

Differences in the nature of binding sites for Cu^{2+} ions on purine nucleotides have been characterized by different *g* values and hyperfine splittings, as measured by the EPR spectra of the complexes formed under conditions close to physiological

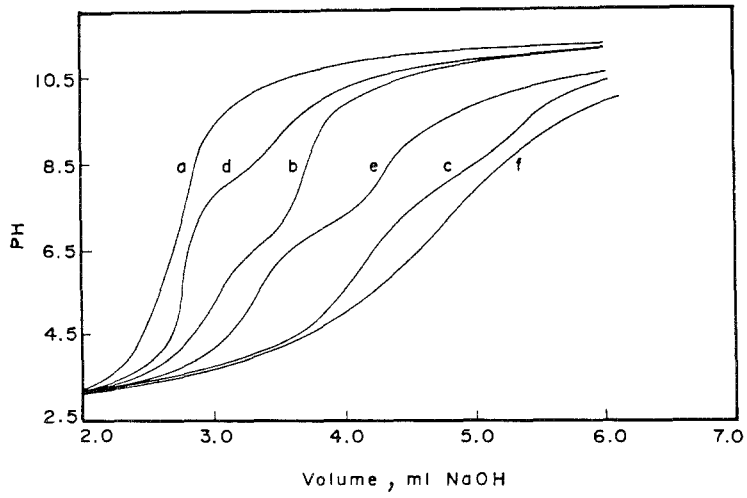

Fig. 1. *pH* against volume of 0.0495 mol·dm^{-3} NaOH for the Cu(II)-*ATP*-bicine system at 25 °C and $I = 0.1$ mol·dm^{-3} KNO_3. (a) 0.0048 mol·dm^{-3} HNO_3; (b) solution (a) $+1 \times 10^{-3}$ mol·dm^{-3} *ATP*; (c) solution (b) $+1 \times 10^{-3}$ mol·dm^{-3} Cu(II); (d) solution (a) $+1 \times 10^{-3}$ mol·dm^{-3} bicine; (e) solution (d) $+1 \times 10^{-3}$ mol·dm^{-3} Cu(II); (f) solution (e) $+1 \times 10^{-3}$ mol·dm^{-3} *ATP*

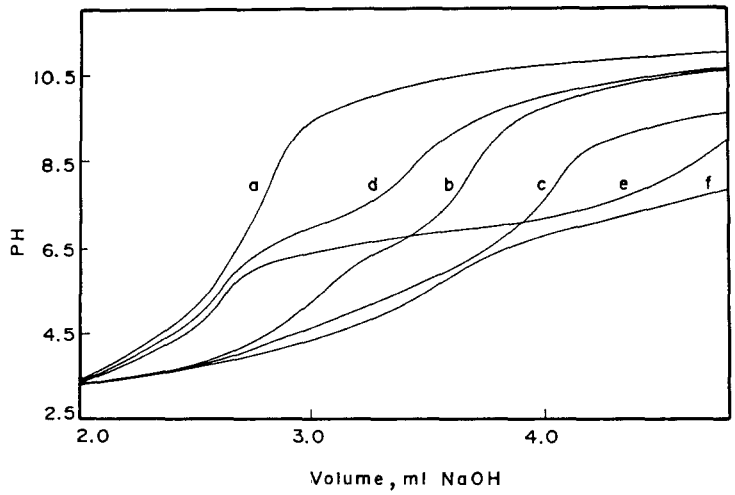

Fig. 2. *pH* against volume of 0.0495 mol·dm^{-3} NaOH for the Cu(II)-*ATP-BES* system at 25 °C and $I = 0.1$ mol·dm^{-3} KNO_3. (a) 0.0048 mol·dm^{-3} HNO_3; (b) solution (a) $+1 \times 10^{-3}$ mol·dm^{-3} *ATP*; (c) solution (b) $+1 \times 10^{-3}$ mol·dm^{-3} Cu(II); (d) solution (a) $+1 \times 10^{-3}$ mol·dm^{-3} *BES*; (e) solution (d) $+1 \times 10^{-3}$ mol·dm^{-3} Cu(II); (f) solution (e) $+1 \times 10^{-3}$ mol·dm^{-3} *ATP*

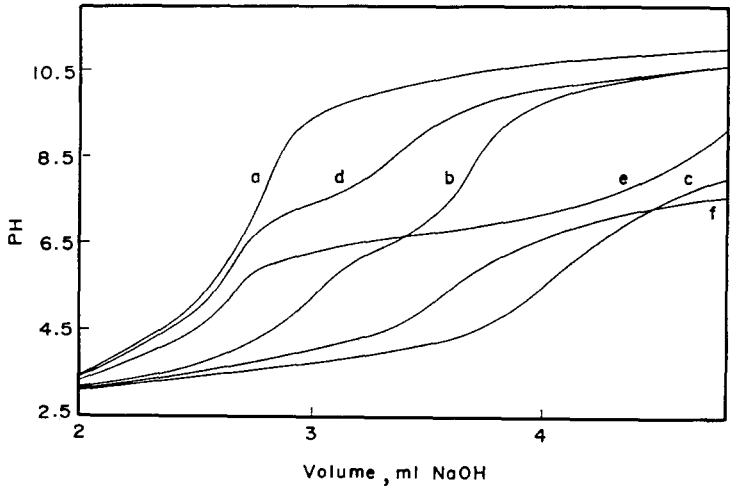

Fig. 3. *pH* against volume of 0.0495 mol·dm^{-3} NaOH for the Cu(II)-*ATP-TES* system at 25 °C and $I = 0.1$ mol·dm^{-3} KNO_3. (a) 0.0048 mol·dm^{-3} HNO_3; (b) solution (a) $+1 \times 10^{-3}$ mol·dm^{-3} *ATP*; (c) solution (b) $+1 \times 10^{-3}$ mol·dm^{-3} Cu(II); (d) solution (a) $+1 \times 10^{-3}$ mol·dm^{-3} *TES*; (e) solution (d) $+1 \times 10^{-3}$ mol·dm^{-3} Cu(II); (f) solution (e) $+1 \times 10^{-3}$ mol·dm^{-3} *ATP*

ones [51]. Thus, there is lack of agreement as to the assignment of the site of coordination. Our opinion on this point will be discussed later.

In Figs. 1–3, representative sets of experimental titration curves obtained according to the sequence described in the experimental section for the different Cu(II)-*Nu-L* systems studied are displayed. It is observed that the Cu(II)-*Nu* titration curve (c) diverges from the nucleotide curve (b) in the lower *pH* range ($pH \sim 3.5$ for Cu(II)-*ATP-BES* and $pH \sim 2.5$ for Cu(II)-*ATP*-Bicine or Cu(II)-*ATP-TES* system), denoting the formation of the Cu(II)-*Nu* complex.

Table 1. Formation constant values for the binary Cu(II)-nucleotide or zwitterionic buffer ligand complexes and those for the mixed ligand complexes Cu(II)-nucleotide-zwitterionic buffer ligand at 25 °C and $I = 0.1\ \text{mol}\cdot\text{dm}^{-3}\ KNO_3$

Ligand	$\log K^{Cu(II)}_{Cu(II)(Nu)}$ or $\log K^{Cu(II)}_{Cu(II)(L)}$	$\log K^{Cu(II)}_{Cu(II)(AMP)(L)}$	$\log K^{Cu(II)(ADP)}_{Cu(II)(ADP)(L)}$	$\log K^{Cu(II)(ATP)}_{Cu(II)(ATP)(L)}$	$\log \beta^{Cu(II)}_{Cu(II)(AMP)(L)}$	$\log \beta^{Cu(II)}_{Cu(II)(ADP)(L)}$	$\log \beta^{Cu(II)}_{Cu(II)(ATP)(L)}$	$\Delta \log K_1$[a]	$\Delta \log K_2$[a]	$\Delta \log K_3$[a]
AMP	3.20 ± 0.02	–	–	–	–	–	–	–	–	–
ADP	6.05 ± 0.03	–	–	–	–	–	–	–	–	–
ATP	6.40 ± 0.03	–	–	–	–	–	–	–	–	–
BES	3.51 ± 0.02	4.19 ± 0.02	4.60 ± 0.03	4.75 ± 0.02	7.39	10.65	11.15	0.68	1.09	1.24
Bicine	8.12 ± 0.03	4.89 ± 0.03	5.28 ± 0.02	5.60 ± 0.01	8.09	11.33	12.00	−3.23	−2.84	−2.52
TAPS	5.62 ± 0.02	5.21 ± 0.02	5.54 ± 0.03	5.72 ± 0.02	8.41	11.59	12.12	−0.41	−0.08	0.10
TES	3.22 ± 0.03	4.58 ± 0.01	4.96 ± 0.02	5.05 ± 0.03	7.78	11.01	11.45	1.36	1.74	1.83

[a] $\Delta \log K_1 = \log K^{Cu(II)(AMP)}_{Cu(II)(AMP)(L)} - \log K^{Cu(II)}_{Cu(II)(L)}$; $\Delta \log K_2 = \log K^{Cu(II)(ADP)}_{Cu(II)(ADP)(L)} - \log K^{Cu(II)}_{Cu(II)(L)}$; $\Delta \log K_3 = \log K^{Cu(II)(ATP)}_{Cu(II)(ATP)(L)} - \log K^{Cu(II)}_{Cu(II)(L)}$

Generally, the complex titration curves show an inflection after addition of 2 mol of base per 1 mol of the nucleotide (*AMP*, *ADP*, or *ATP*). This indicates the simultaneous dissociation of two protons from *AMP* while in the case of *ADP* and *ATP* the complex species Cu(*HADP*), Cu(*ADP*), Cu(*HATP*)$^-$, and Cu(*ATP*) have been formed in solution. Cu(II)-*Nu* species are quite stable up to high *pH* value, *i.e.* they have no tendency to form hydroxy complexes. With respect to the titration curves of the Cu(II)-*L* binary complex solutions studied, one may deduce that these complexes begin to form at $pH > 5.0$ for the Cu(II)-*BES* system and at $pH > 3.30$ for the Cu(II)-Bicine and Cu(II)-*TES* systems. Generally, for all Cu(II)-*L* complexes studied precipitation occurred at $pH > 10.5$. In all cases no calculations have been performed beyond the precipitation point; hence, the hydroxy species likely to be formed after this point could not be studied.

For the titration curves of the ternary systems studied (Cu(II)-*Nu-L*) one observes that C and F are well separated at a $pH > 3.5$ for the Cu(II)-*ATP-BES* and Cu(II)-*ATP*-Bicine systems and a $pH > 6.8$ for the Cu(II)-*ATP-TES* system. This behavior reveals that in these *pH* ranges coordination of the secondary ligand, the zwitterionic buffer, with Cu(II)-*Nu* starts.

Examination of the different formation constant values listed in Table 1 clearly reveals that the formation constant of the mixed ligand complexes increases in the order *AMP* < *ADP* < *ATP*. Though many studies in solution favored the phosphate group rather than the base as the primary metal binding site, the simultaneous binding of Cu(II) ion to the N_7 site of the adenine residue [52, 53] and phosphate may also be reported in the mixed ligand complexes formed in the present work. Thus, the Cu(II) bound to the base moiety may promote intramolecular base-phosphate interactions. Thus, the mixed ligands studied may be considered as relatively simple models from which information may be gained about the properties of nucleotides and their base moieties regarding the strength of their intractions with the biologically important zwitterionic buffer ligads (*BES*, Bicine, *TAPS* and *TES*) and even insight into the factors which influence the strength of these systems with respect to their ability to mimic enzyme-metal ion-substrate complexes. Our investigation confirmed the formation of mixed ligand complexes of the type Cu(II)-*Nu-L* (where *L* = *BES*, Bicine, *TAPS* and *TES*) in solution, hence great reservations should be exercized in employing *BES*, Bicine, *TAPS* or *TES* as buffers in systems containing Cu(II) ions and *AMP*, *ADP* or *ATP*.

With respect to the secondary ligands, the formation constants of the mixed ligand complexes decreases in the following order: *TAPS* > Bicine > *TES* > *BES*. This behavior can be interpreted in terms of the basicities of the secondary ligand zwitterionic buffer used. It is well known that the increase in basicity of a ligand increases the stability of its metal complexes. The relation between basicity of ligands and the stability constants of complexes has been extensively discussed [54, 55].

$\Delta \log K$ as defined by Eq. 12 is a measure of the stability of the ternary complexes with respect to the binary complexes.

$$\Delta \log K = \log K^{\mathrm{Cu(II)}(Nu)}_{\mathrm{Cu(II)}(Nu)(L)} - \log K^{\mathrm{Cu(II)}}_{\mathrm{Cu(II)}(L)} \tag{12}$$

$\Delta \log K$ values are positive for some of the investigated ternary complexes, (cf. Table 1). The higher stability constants of ternay complexes compared with binary systems may be attributed to the interligand interactions or some cooperativity between the

coordinate ligands, possibly H-bond formation. This may also be explained on the basis of the π acceptor qualities of the adenine base. Thus, the π-electron-donating tendency of the Cu(II) ion to the antibonding π^* orbitals of hetero aromatic *N*-base, such as adenine base, cause strengthening of the Cu(II)-*N* bond. Due to the π-acceptor qualities of the adenine base (*i.e.* back-donation from metal to ligand), the d-electron content on the metal decreases, which renders 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.

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