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Medium Effect and Thermodynamic Studies for the Proton−Ligand and Metal−Ligand Formation Constants of the Ternary Systems M^{II} + Adenosine-5′-triphosphate (ATP) + Asparagine

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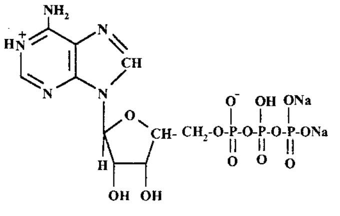
Proton−ligand and metal−ligand stability constants formed in the ternary system Ni^{II} + adenosine-5′-triphosphate (ATP) + asparagine are determined pH-metrically in different aqueous−organic solvent mixtures at 25 °C and at a constant ionic strength $I = 0.10$ mol dm^{-3} (KNO_3), and are discussed in terms of both the proportion and solvent characteristics. The organic solvents used are ethanol, DMF, dioxane, and acetonitrile (AN). It was concluded that solvent effects such as stabilization of the proton by ion−solvent interaction as well as the basicity of the medium, in addition to the electrostatic effect, have a profound influence on the proton−ligand formation constants for ATP and asparagine. On the other hand, the electrostatic effect of the medium and the hydrogen-bonding interaction seem to play the major role in the formation of the binary and ternary metal complexes. Moreover, the thermodynamic functions ΔH, $\Delta G°$, and $\Delta S°$ associated with the formation of proton−ligand and the 1:1 binary as well as the 1:1:1 ternary metal complexes in the system M^{II} + (ATP) + asparagine (where M^{II} = Cu^{II}, Ni^{II}, and Co^{II}) in pure aqueous media were also determined and discussed.

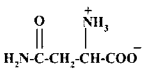
Introduction

Adenosine-5′-triphosphate (ATP) is a biologically important ligand that plays a key role in the metabolism of organisms providing transphosphorylation in the presence of metal ions.[1] Further, numerous metal ions form ATP adducts which could be involved in many enzymatic processes;[2] hence, the complex formation of transition metals with this compound is of fundamental interest for bioinorganic chemistry. Thus, a considerable interest has been focused on the study of the binary metal complexes formed with ATP. On the other hand, ternary complexes of some transition metal ions with ATP and some secondary ligands have been studied using several techniques.[1,3−9] Recently, we have reported a study on the formation of the binary and ternary metal complexes of some divalent and trivalent transition metal ions with ATP and some mono- and dicarboxylic amino acids.[10] Although a little attention[11] has been given to studying the medium effect on the ionization processes of ATP, a scanning of the literature reveals that no studies appear to have been made on the medium effect on the binary as well as the ternary metal complex formation containing the biologically important ligand ATP. Moreover, the literature is lacking studies concerning the thermodynamic properties of the formation of proton−ligand and metal−ligand complexes containing ATP. Therefore, this article is devoted to study the medium effect on the formation constants of the proton−ligand as well as the 1:1 binary and 1:1:1 ternary mixed ligand complexes formed between Ni^{II} ion, ATP, and asparagine in different aqueous solutions containing varying proportions of organic solvents at 25 ± 0.1 °C at the constant ionic strength $I = 0.10$ mol dm^{-3} of KNO_3. The organic solvents used are ethanol as an amphiprotic, DMF as a dipolar aprotic, acetonitrile as a low basic dipolar aprotic, and dioxane as a low basic, low polarity solvent. The study adopts the Irving and Rossotti technique[12] for the determination of stability constants of the different proton−ligand as well as the binary and ternary mixed ligand complexes. The stabilities of the different proton−ligand, binary, and mixed ligand complexes have been discussed in terms of both the nature and the proportion of the organic solvent used. Moreover, the thermodynamic functions ΔH, $\Delta G°$, and $\Delta S°$ associated with formation of the proton−ligand as well as the 1:1 binary and 1:1:1 ternary mixed ligand complexes in the ternary systems M^{II} + ATP + asparagine (where M^{II} = Co^{II}, Ni^{II}, and Cu^{II}) in pure aqueous media at the constant ionic strength $I = 0.10$ mol dm^{-3} (KNO_3) were also determined and discussed.

The structures of the ligands used in this study are



Adenosine-5`-triphosphate disodium salt (ATP)



Asparagine

Experimental Section

Materials and Solutions. Adenosine-5′-triphosphate tetrahydrate (ATP) in the form of the disodium salt and

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asparagine were analytical grade (BDH) products with a high purity. The organic solvents utilized were of a high purity and were distilled before use by standard methods.[13] All other chemicals employed were of A. R. grade. Due to the possible hydrolysis of ATP, a stock solution could not be prepared and kept for a period of time. Thus, the desired concentration was prepared by weighing an accurate mass and dissolving it in the appropriate volume of CO_2-free doubly distilled water before each titration. A stock solution of asparagine was also prepared in a similar way. Solutions of Cu^{II}, Ni^{II}, and Co^{II} were prepared from their nitrate salts and were standardized as recommended.[14] Carbonate-free KOH was prepared and standardized by a standard solution of potassium hydrogen phthalate. A solution of HNO_3 was prepared and used after standardization. A stock solution of KNO_3 was also prepared by dissolving the required mass in the appropriate volume of doubly distilled water.

pH-Metric Titration. A solution made from metal ion, primary ligand ATP, and/or secondary ligand asparagine in a molar ratio of 1:1:1 was titrated with a relatively concentrated standard CO_2-free KOH solution. A constant ionic strength was maintained at 0.10 mol dm^{-3} KNO_3, and a total volume 25 cm^3 was used for each titration. The pH-measurements were carried out with an Orion 701A digital pH-meter (accurate to ±0.005 pH unit) with a glass–calomel electrode. The accuracy of the pH-meter was checked using standard buffer solutions with pH values of 4.01 and 9.18 at 25 ± 0.1 °C. The temperature was adjusted using a Fisher Scientific Isotemp Refrigerated Circulator model 9000 water thermostat accurate to ±0.1 °C. The various solutions titrated were as follows: (a) (0.0015 to 0.00327) mol dm^{-3} of HNO_3; (b) solution a + 0.001 mol dm^{-3} of asparagine; (c) solution b + 0.001 mol dm^{-3} of M^{II}; (d) solution a + 0.001 mol dm^{-3} of ATP; (e) solution d + 0.001 mol dm^{-3} of M^{II}; (f) solution e + 0.001 mol dm^{-3} of asparagine. The above solutions a–e contain the same proportion (mass/mass) of each organic solvent used. However, the titration of the solutions containing ATP was completed in the shortest time to avoid the possibility of hydrolysis of ATP.

To account for the differences in acidity, basicity, relative permittivity, and ion activities for a water + organic solvent mixture relative to pure water, where the pH-meter is standardized using aqueous buffers, the pH values in the former medium were corrected according to the method described by Douhéret,[15] where the meter reading pH_R obtained in a partially aqueous medium differs by an amount δ from the corrected reading pH*:

$$\mathrm{pH}^* = \mathrm{pH}_R - \delta \tag{1}$$

Values of δ for the various proportions of each organic solvent were determined as recommended by Douhéret.[15]

Procedure. From the titration curves corresponding to solutions a, b, and d the average number of protons associated per one mole of a ligand ($\bar{n}_H$) at several pH values was calculated using the following equation:[12]

$$\bar{n}_H = \left\{ yC_L + \frac{(V_a - V_b \text{ or } V_a - V_d)C_b}{V_0} \right\}(C_L)^{-1} \tag{2}$$

The pK_a values were calculated from the following relationship:[16]

$$\bar{n}_H = \beta[H^+](1 + \beta[H^+])^{-1} \tag{3}$$

where β is the formation constant of the ligand.

Titration curves b, c and d, e were used for calculating the stability constants of the binary metal complexes of asparagine and ATP, respectively. The average number of ligand molecules (ATP or asparagine) coordinated to the metal ion ($\bar{n}$) and the free ligand exponent at several pH values (pL) were calculated by the following equations:[12]

$$\bar{n} = \frac{[(V_c - V_b) \text{ or } (V_e - V_d)][C_b + C_a + C_L(y - \bar{n}_H)]}{[(V_0 + V_b) \text{ or } (V_0 + V_d)]\bar{n}_H C_M} \tag{4}$$

$$\mathrm{p}L = \log\left\{ \frac{\sum_{y=0}^{y=1\mathrm{or}2} \beta_y^H \left(\frac{1}{10^B}\right)}{C_L \bar{n} C_M} \frac{V_0 + (V_c \text{ or } V_e)}{V_0} \right\} \tag{5}$$

where y is the number of dissociable protons ($y = 2$ and 1 in the case of ATP and asparagine, respectively). C_a, C_b, and C_L are the concentrations of HNO_3, KOH, and a ligand, respectively, and V_0 is the original volume (25 cm^3). V_a, V_b, V_c, V_d, and V_e are the volumes of KOH consumed to reach the same pH values in curves a–e, respectively. $\bar{n}_H$ values, the average number of protons associated with a ligand at different pH values, were available from the determination of its proton–ligand formation constant. C_M is the initial concentration of the metal ion used. It is worth mentioning that the values of ($\bar{n}$) do not exceed unity, revealing the formation of only 1:1 binary complexes.

On the other hand, the horizontal distance between curves e and f was measured and used for calculating the number of asparagine anions as a secondary ligand attached to one binary $[M(ATP)]^{2-}$ complex molecule ($\bar{n}_{mix}$) for a mixed ligand ternary complex. The equation used for the calculation of ($\bar{n}_{mix}$) was the same as that reported elsewhere.[10,12]

$$\bar{n}_{mix} = \frac{[(V_f - V_e)][C_b + C_a + C_L(y - \bar{n}_H)]}{[V_0 + V_e]\bar{n}_H C_M} \tag{6}$$

Here C_M is the concentration of $[M(ATP)]^{2-}$, which is equal to the concentration of M^{II} used, C_L is the concentration of the secondary ligand, y is the number of dissociable protons of asparagine as a secondary ligand, and V_f is the volume of KOH consumed to reach the same pH values in curve f. In this case, the $\bar{n}_H$ values are the average number of protons associated with the secondary ligand asparagine at different pH values. The $\bar{n}_{mix}$ values do not exceed unity, indicating that only one secondary ligand molecule combines with the complex $[M(ATP)]^{2-}$, forming a 1:1:1 ternary complex. The free secondary ligand exponent, pL_{mix}, was calculated from the obtained values of $\bar{n}_{mix}$ using the following equation:[10,12]

$$\mathrm{p}L_{mix} = \log\left\{ \frac{\sum_{y=0}^{y=1} \beta_y^H \left(\frac{1}{10^B}\right)}{C_L \bar{n}_{mix} C_M} \frac{V_0 + V_f}{V_0} \right\} \tag{7}$$

where β_y^H are the proton–ligand dissociation constant values of asparagine, and B is the pH value. All other terms have the same meaning as defined above. All calculations were performed using a computer program based on unweighted linear least-squares fits.

Results and Discussion

Medium Effect on the Proton–Ligand Stability Constants. Part of the information required for determining the metal complex stability constants is the proton–

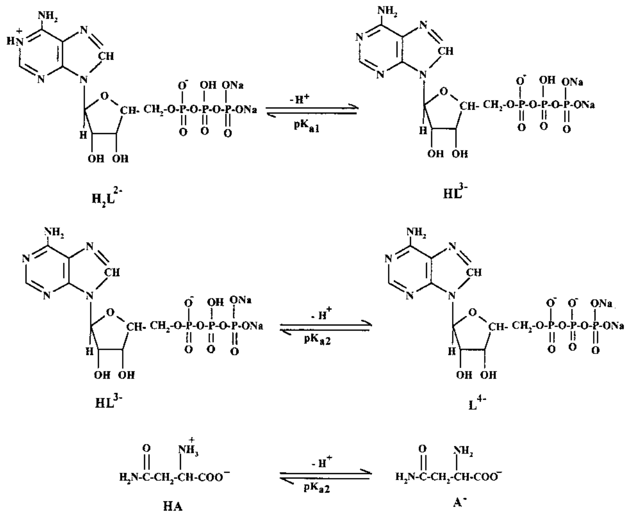
Table 1. Proton−Ligand Stability Constant Values for Asparagine and Adenosine-5′-triphosphate (ATP) in Different Water (1) + Organic Solvent (2) Mixtures at 25 °C and at the Constant Ionic Strength $I = 0.01$ mol dm^{-3} KNO$_3$

			ATP		asparagine
$100w_2$[a]	x_2[a]	$(\epsilon_r)_m$	pK_{a1}	pK_{a2}	pK_{a2}
			Ethanol		
00	00	78.40	4.08 ± 0.01	6.57 ± 0.01	8.82 ± 0.02
20	0.089	73.59	3.87 ± 0.02	6.82 ± 0.02	9.04 ± 0.03
30	0.143	70.66	3.68 ± 0.01	7.03 ± 0.02	9.32 ± 0.02
40	0.206	67.26	3.57 ± 0.03	7.12 ± 0.04	9.53 ± 0.04
50	0.281	63.20	3.52 ± 0.02	7.34 ± 0.03	9.69 ± 0.03
			DMF		
00	00	78.40	4.08 ± 0.01	6.57 ± 0.01	8.82 ± 0.02
20	0.058	75.98	3.84 ± 0.03	6.67 ± 0.03	8.98 ± 0.02
30	0.095	74.44	3.68 ± 0.03	6.79 ± 0.01	9.08 ± 0.02
40	0.141	72.52	3.59 ± 0.02	6.97 ± 0.02	9.27 ± 0.05
50	0.197	70.19	3.52 ± 0.01	7.09 ± 0.04	9.35 ± 0.04
			Dioxane		
00	00	78.40	4.08 ± 0.01	6.57 ± 0.01	8.82 ± 0.02
20	0.048	74.74	4.17 ± 0.03	6.85 ± 0.03	9.15 ± 0.02
30	0.080	72.30	4.46 ± 0.04	7.18 ± 0.03	9.48 ± 0.03
40	0.120	69.26	4.62 ± 0.02	7.24 ± 0.02	9.65 ± 0.03
50	0.170	65.45	4.83 ± 0.01	7.59 ± 0.03	9.84 ± 0.04
			Acetonitrile		
00	00	78.40	4.08 ± 0.01	6.57 ± 0.01	8.82 ± 0.02
20	0.099	74.20	4.21 ± 0.03	7.05 ± 0.03	9.33 ± 0.01
30	0.158	71.70	4.58 ± 0.01	7.28 ± 0.02	9.51 ± 0.02
40	0.226	68.82	4.74 ± 0.03	7.38 ± 0.04	9.73 ± 0.02
50	0.305	65.47	4.92 ± 0.02	7.61 ± 0.03	9.84 ± 0.03

[a] w and x are the mass fraction and the mole fraction of the solvent, respectively.

ligand stability constants. Therefore, prior to studying the metal−ligand complexes, the stability constants of the protonation equilibria of the ligands under investigation are determined in various water + organic solvent mixtures. Previous studies provide evidence that the first ionization step (pK_{a1}) of ATP is associated with the formation of the protonated form of the adenosine moiety,[17] where it involves the aromatic $^+$NH group. The second proton ionization step was attributed to the phosphate groups.

The following equations represent the acid−base equilibria of the first and second proton−ligand formation steps (pK_{a1} and pK_{a2}) of ATP (H_2L^{2-}) as well as that of the second step of asparagine, HA (pK_{a2}):



It is worth mentioning that the first ionization step (pK_{a1}) of asparagine is very low, occurring only in a strongly acidic solution; therefore, it was not determined under our experimental conditions. The pK_a values for ATP and asparagine, in different aquo-organic solvent mixtures at 25 ± 0.1 °C and at the constant ionic strength $I = 0.10$ mol dm^{-3} KNO$_3$, were obtained by using two different methods (average value and straight line methods).

The mean pK_a values along with the errors as estimated by applying the least-squares fits are listed in Table 1. The values obtained of pK_{a1} and pK_{a2} for ATP and pK_{a2} for asparagine in pure aqueous media are in good agreement with the literature values.[10] The data in Table 1 clearly indicate that the proton−ligand stability constant values depend largely on both the nature and the proportion of the organic solvent used. Generally, the first proton−ligand formation constant for ATP decreases as the amount of the amphiprotic solvent ethanol or the dipolar aprotic solvent DMF in the aqueous medium is increased, whereas it increases with increasing the proportion of the low basic dipolar aprotic solvent (acetonitrile) or the low basic, low polarity solvent (dioxane). On the other hand, increasing the amount of the organic solvent in the medium leads to an increase in the second proton−ligand formation constants of both ATP and asparagine.

The two steps illustrating the proton−ligand formation processes for ATP and the second step of the proton−ligand formation process for asparagine can be represented, in general, by the following eqs 8−10, respectively:

$$H_2L^{2-} \Leftrightarrow HL^{3-} + H^+ \qquad (8)$$

$$HL^{3-} \Leftrightarrow L^{4-} + H^+ \qquad (9)$$

$$HA \Leftrightarrow A^- + H^+ \qquad (10)$$

The proton−ligand formation constants in pure water (K_a) corresponding to the above steps are related to that in a partially aqueous medium (K_a') by relations 11−13, respectively.[18]

$$K_{a1} = K_{a1'}\gamma_{HL^{3-}}\gamma_{H^+}/\gamma_{H_2L^{2-}} \qquad (11)$$

$$K_{a2} = K_{a2'}\gamma_{L^{4-}}\gamma_{H^+}/\gamma_{HL^{3-}} \qquad (12)$$

$$K_{a2} = K_{a2'}\gamma_{A^-}\gamma_{H^+}/\gamma_{HA} \qquad (13)$$

where γ is the activity coefficient of the respective species in a partially aqueous medium relative to that in pure water. It is known that the electrostatic effect, resulting from the change in the relative permittivity of the medium, affects the activity coefficient of the charged species,[18] the magnitude of the electrostatic effect being inversely proportional to the radius of the ionic species considered.[19] Consequently, the magnitude of this effect on the proton exceeds that on the other ionic species. Further, the electrostatic effects on the activity coefficient of the species H_2L^{2-} and HL^{3-} in eq 11 as well as HL^{-3} and L^{4-} in eq 12 cancel to some extent.[19] Thus, increasing the amount of the organic solvent in the medium will increase the activity coefficient of the proton H^+. Consequently, according to eqs 11−13, the proton−ligand stability constants of ATP and asparagine should increase on increasing the content of the organic solvent (i.e., pK_a is high). This is consistent with the results obtained for the pK_{a1} value of ATP in the aqueous mixtures of the low basic dipolar aprotic solvent (acetonitrile) or the low basic, low polarity solvent (dioxane), as well as for the pK_{a2} values of ATP and asparagine in the aqueous mixtures of all the organic solvents used. However, in light of the relation[20] $pK_a = e^2/[2.303a(\epsilon_r)_m kT]$,

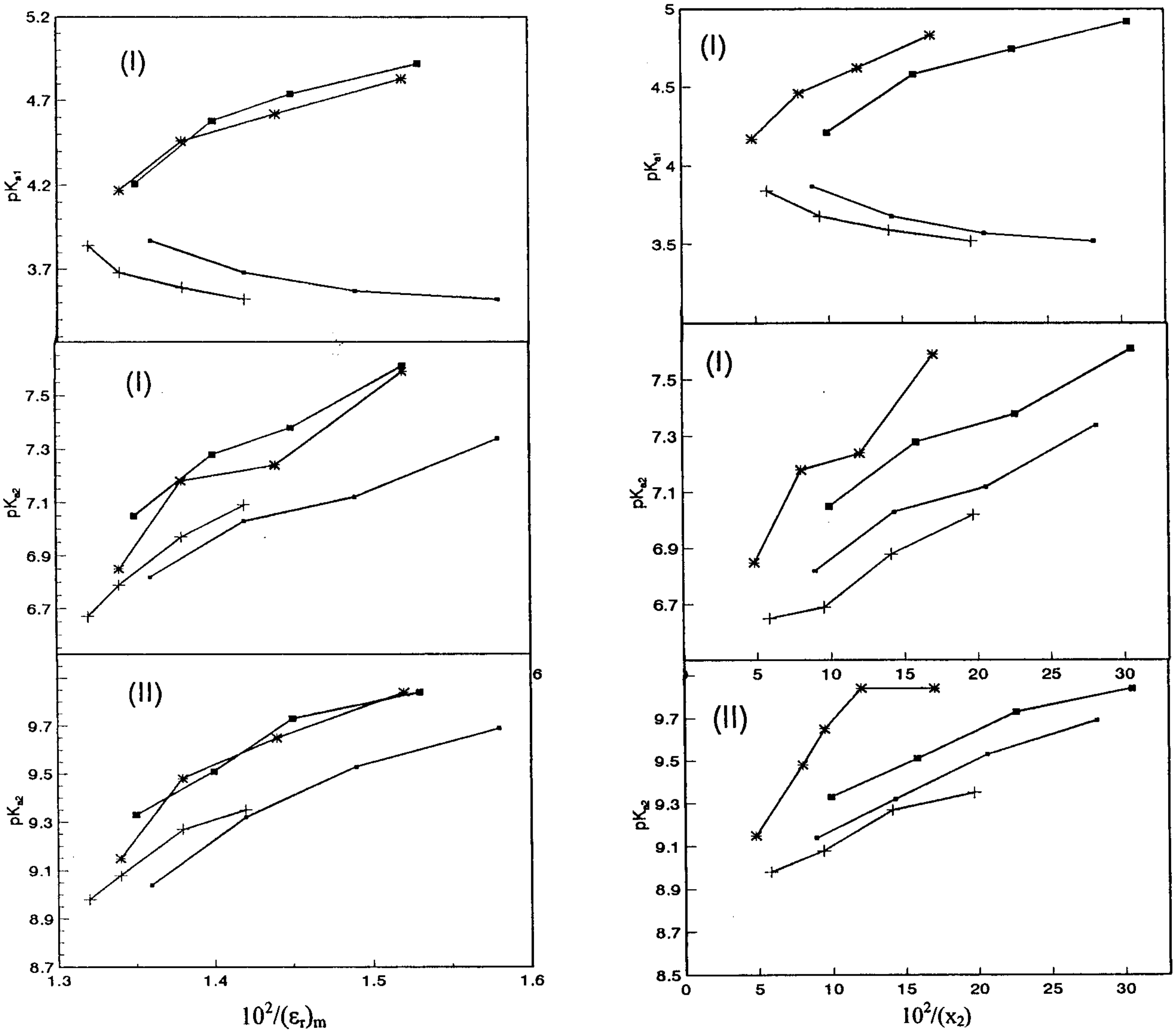


Figure 1. (a) Variation of pK_a values of adenosine-5′-triphosphate (ATP) (I) and asparagine (II) with $1/[(\epsilon_r)_m]$ of the medium at 25 °C at the constant ionic strength $I = 0.10$ mol dm $^{-3}$ (KNO_3): (■) acetonitrile; (*) dioxane; (●) ethanol; and (+) DMF. (b) Variation of the proton−ligand stability constants (pK_a) of the ligands with the mole fraction (x_2) of the organic solvents at 25 °C: (I) ATP; (II) asparagine.

which relates the variation of pK_a values with the relative permittivity of the medium $(\epsilon_r)_m$, the plots of pK_a values against $\{1/(\epsilon_r)_m\}$ are not linear (Figure 1a), where the $(\epsilon_r)_m$ is obtained from the relation

$$(\epsilon_r)_m = (\epsilon_r)_w\chi_{(w)} + (\epsilon_r)_s\chi_{(s)} \qquad (14)$$

Here $(\epsilon_r)_m$ and χ are the relative permittivity and mole fraction, and the subscripts w and s refer to water and solvent, respectively. Further, since DMF and acetonitrile have similar relative permittivities (ϵ_r = 36.7 and 36.0, respectively, at 25 °C), the electrostatic effects in aqueous solutions containing the same mole fraction of the two solvents should be similar (i.e., the pK_a values for each ligand in such solutions should be the same). However, the pK_a values in solutions containing the same mole fraction of these solvents are markedly different (see Table 1 and Figure 1b). This indicates that other solvent effects, in addition to the electrostatic effect, strongly influence the proton−ligand formation process in the aqueous mixtures.

On the other hand, the stability constant of the formation of the $^+$NH group (pK_{a1}) in the adenosine moiety of ATP is decreased in the ethanol + water and DMF + water mixtures. Accordingly, factors rather than the electrostatic one are likely playing the major role in determining the effect of the amphiprotic solvent (ethanol) or the dipolar aprotic solvent (DMF) on the formation of $^+$NH group. Generally, the basicity of pure water is slightly influenced by the addition of an amphiprotic solvent (pK_w is changed from 14 to 14.9 for the 1:1 ethanol−water mixture).[17] Further, pure water and ethanol have approximately the donor ability (DN = 18 and 19, respectively).[21] Accordingly, it is deduced that changing the medium basicity by addition of ethanol to the aqueous medium has little influence on the proton−ligand stability constants of ATP and asparagine. Furthermore, water molecules are characterized by a greater tendency to donate hydrogen bonds compared with those of other solvents.[22] Thus, the conjugate base of ATP will be less stabilized by the hydrogen bond interaction on increasing the amount of the organic solvent in the medium (i.e., high pK_{a1}). However, this is not the case. Therefore, the difference in the stabilization of the conjugate base by hydrogen bonding in partially aqueous media relative to that in pure water appears to be not important in the determination of pK_{a1} for ATP in ethanol−water or DMF−water mixtures. It was recognized that properties of solvents such as relative permittivity, basic or acidic strength, and hydrogen-bonding interaction play a major

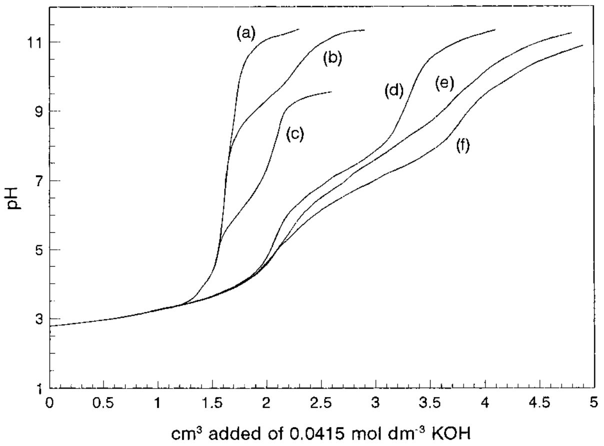


Figure 2. pH-Metric titration curves for the [Ni^{II} + (ATP) + (asparagine)] system in a 20% (mass/mass) water + DMF mixture at 25 °C and at the constant ionic strength $I = 0.10$ mol dm^{-3} (KNO$_3$): (a) 0.0027 mol dm^{-3} HNO$_3$; (b) solution a + 0.001 mol dm^{-3} asparagine; (c) solution b + 0.001 mol dm^{-3} Ni^{II}; (d) solution a + 0.001 mol dm^{-3} ATP; (e) solution d + 0.001 mol dm^{-3} Ni^{II}; (f) solution e + 0.001 mol dm^{-3} asparagine.

but not exclusive role.[18] This indicates that other solvent effects such as stabilization of the ionic species by ion−solvent interaction strongly influenced the formation of the $^{+}$NH moiety in the aqueous mixtures of ethanol and DMF. Accordingly, the observed decrease in the pK_{a1} value for ATP as the proportion of ethanol or DMF in the aqueous media is increased can be mainly ascribed to the high stabilization of the proton by its interaction with organic solvent and water molecules in water + organic solvent mixtures (ion−solvent interaction) compared with that of water molecules alone in pure water. Comparing the pK_a values obtained in different aqueous mixtures having the same solvent composition (Figure 1b) clearly reveals that the proton−ligand formation constants vary with the nature of the organic solvent used according to the sequence dioxane > acetonitrile > ethanol > DMF. This is expected as a result of the decreasing of the solvent polarizability on going from DMF to dioxane within the above sequence.[23] Moreover, examination of the results obtained reveals that the pK_a value in the aqueous mixtures of DMF is less than those obtained in the presence of the same mole fraction of other solvents (Figure 1b). This can be ascribed to the high basic character of DMF, which reflects itself in the construction of a strong hydrogen bonding; thereby, the ionization process is facilitated.

Medium Effects on the Metal−Ligand Stability Constants. Figure 2 displays a representative set of the experimental titration curves obtained for the different Ni^{II} + ATP + asparagine systems according to the sequence described in the Experimental Section in different water + organic solvent mixtures.

Titration curve c of the 1:1 binary Ni^{II} + asparagine complex solution reveals that this complex begins to form, in different aqueous−organic solvent mixtures, at a pH range of 4.20−5.00. The titration solutions of Ni^{II} + asparagine complex in various water + organic solvent mixtures do not show any precipitate in the pH range of complex formation, denoting no interference of the hydrolysis reactions in the formation of this binary complex. However, depending largely on the nature of the organic solvent used, the complex solution of the 1:1 binary Ni^{II} + asparagine shows a precipitate at a relatively high pH range (8.50−9.50). This is attributed to the hydrolysis of this complex, forming hydroxo complex species. Thus, a study beyond the hydrolysis point could not be possible for this complex.

Titration curve e obtained for the 1:1 binary Ni^{II} + ATP solution diverges from curve d, corresponding to the free ligand ATP, at a lower pH value (3.2−4.5), depending on the nature of the organic solvent used, indicating the formation of the binary Ni^{II} + ATP complex. However, titration curve e shows a buffer zone after the addition of two moles of base per mole of ATP. This indicates the formation of a protonated 1:1 binary complex [NiH(ATP)]$^{1-}$ at the addition of one mole of base per mole of ATP, which acts as a strong acid and accordingly dissociates readily to form the unprotonated 1:1 binary complex [Ni(ATP)]$^{2-}$. This conclusion is substantiated by the absence of such a buffer zone in titration curve e, corresponding to the addition of one mole of base per mole of ATP. The 1:1 binary [Ni(ATP)]$^{2-}$ complex is quite stable, in the different compositions of aqueous−organic solvent mixtures studied, up to high pH values where hydroxo complex species could not be observed.

Titration curve f obtained for the 1:1:1 ternary Ni^{II} + ATP + asparagine indicated that the 1:1 binary Ni^{II} + ATP is first formed and it subsequently coordinates with asparagine, HA, as a secondary ligand, affording the mixed ligand 1:1:1 complex [Ni(ATP)(A)]$^{3-}$ in a stepwise manner, as represented as follows:

$$\mathrm{Ni^{II}} + (\mathrm{ATP}) \leftrightarrow [\mathrm{Ni(ATP)}]^{2-} + 2\mathrm{H}^{+} \tag{15}$$

$$[\mathrm{Ni(ATP)}]^{2-} + \mathrm{A}^{1-} \leftrightarrow [\mathrm{Ni(ATP)(A)}]^{3-} \tag{16}$$

(ATP and A^{1-} are the divalent anion of adenosine-5′-triphosphate (H$_2$L)$^{2-}$ and the monovalent anion of asparagine, respectively).

Except in the high percentage of ethanol, the ternary complex solution does not show any precipitation in the different aquo-organic solvent mixtures, denoting that this complex has no tendency to undergo a hydrolysis reaction. However, in an ethanol−water mixture (50% mass/mass), a precipitate was formed at low pH value due to the formation of hydroxo complex species resulting from the possible hydrolysis of the formed complex. Moreover, small divergences are observed for the titration curves of the ternary complex solutions f on using high percentages of acetonitrile as an organic cosolvent, characterizing the formation of a weak complex. In general, studies could not be possible in such water + organic solvent mixtures. Further, no buffer zone is observed in titration curve f, corresponding to the ternary complex solution pointing to the formation of an unprotonated (normal) ternary complex.

The mean formation constant log K values of the different 1:1 binary and 1:1:1 ternary complexes, in different aqueous−organic solvent mixtures obtained from the corresponding titration curves using the average value and straight line methods, along with the error as obtained by applying the least-squares fits are listed in Table 2. The stability constant values of the 1:1 binary Ni^{II} + ATP (4.93) or Ni^{II} + asparagine (5.61) and that of the 1:1:1 ternary complex Ni^{II} + ATP + asparagine (4.66) reported previously in a pure aqueous medium[10] are reproduced in our own experiments. The data cited in Table 2 reveal that the stability of the binary and ternary complexes is largely dependent upon both the nature and the proportion of the organic solvent used. Generally, increasing the proportion of the organic solvent in the aqueous medium led to an increase in the stability of the complexes. This behavior could be mainly explained on the basis of the electrostatic

Table 2. Mean log *K* Values for 1:1 Binary and 1:1:1 Ternary Metal Complexes Formed in the System Ni + (ATP) + Asparagine in Different Water (1) + Organic Solvent (2) Mixtures at 25 °C and at the Constant Ionic Strength $I = 0.01$ mol dm^{-3} KNO$_3$

			log K^b		
$100w_2{}^a$	$x_2{}^a$	$(\epsilon_r)_m$	(Ni + Asp)	(Ni + ATP)	(Ni + ATP + Asp)
			Ethanol		
00	00	78.40	5.61 ± 0.02	4.93 ± 0.04	4.66 ± 0.07
20	0.089	73.59	5.83 ± 0.03	5.29 ± 0.06	5.01 ± 0.09
30	0.143	70.66	6.01 ± 0.05	5.43 ± 0.04	5.32 ± 0.07
40	0.206	67.26	6.32 ± 0.04	5.72 ± 0.05	5.89 ± 0.11
50	0.281	63.20	6.54 ± 0.03	5.89 ± 0.05	*
			DMF		
00	00	78.40	5.61 ± 0.02	4.93 ± 0.04	4.66 ± 0.07
20	0.058	75.98	5.78 ± 0.04	5.12 ± 0.05	4.98 ± 0.08
30	0.095	74.44	5.99 ± 0.04	5.39 ± 0.07	5.26 ± 0.10
40	0.141	72.52	6.18 ± 0.03	5.57 ± 0.04	5.78 ± 0.09
50	0.197	70.19	6.34 ± 0.02	5.76 ± 0.05	6.11 ± 0.05
			Dioxane		
00	00	78.40	5.61 ± 0.02	4.93 ± 0.04	4.66 ± 0.07
20	0.048	74.74	6.29 ± 0.04	5.45 ± 0.08	5.69 ± 0/0.08
30	0.080	72.30	6.49 ± 0.02	5.73 ± 0.04	6.20 ± 0.06
40	0.120	69.26	6.94 ± 0.04	5.98 ± 0.06	6.63 ± 0.09
50	0.170	65.45	7.26 ± 0.03	6.32 ± 0.05	6.89 ± 0.08
			Acetonitrile		
00	00	78.40	5.61 ± 0.02	4.93 ± 0.04	4.66 ± 0.07
20	0.099	74.20	6.50 ± 0.04	5.63 ± 0.06	5.68 ± 0.06
30	0.158	71.70	6.71 ± 0.03	5.97 ± 0.08	**
40	0.226	68.82	7.01 ± 0.03	6.21 ± 0.06	**
50	0.305	65.47	7.29 ± 0.04	6.64 ± 0.04	**

[a] *w* and *x* are the mass fraction and the mole fraction of the solvent, respectively. [b] ATP and Asp refer to adenosine-5′-triphosphate and asparagine, respectively. *, precipitate was formed; **, very weak complex.

effect, where increasing the amount of the organic solvent in the aqueous medium will result in decreasing its relative permittivity [$(\epsilon_r)_m$]. Consequently, the fraction of associated ions to form ion pairs and higher aggregates such as triple ions and dipole aggregates will increase.[17] Thus, the concentration of free ions is very low and the complexes are governed largely by ionic association reactions. On the other hand, according to Coetzee and Ritchie,[18] the change in the relative permittivity of the medium influences the activity coefficient of the charged species. Thus, the activity coefficient of the Ni^{II} ion and the ligand anions will increase on increasing the amount of the organic solvent in the aqueous medium. Accordingly, the complex formation constants are increased on increasing the proportion of the organic solvent content in the medium. Therefore, one can conclude that the electrostatic effects, established from the change in the relative permittivity of the medium, play the major role in the magnitude of the complexes stability, and the interactions between Ni^{II} and the ligands are predominately ionic. This conclusion is substantiated by the observation that the stability constants of the complexes increase, generally, on increasing the value $1/(\epsilon_r)_m$. However, in light of the relation given elsewhere,[20] the variation of log *K* values with $1/(\epsilon_r)_m$ is not linear (Figure 3). This behavior indicates that the increase in the stability constant of the formation of the metal ligand complex, though mainly governed by the electrostatic effect, is influenced also by other solvent effects. Previous studies[24] provide evidences suggesting the presence of the probable importance interactions with solvent in the overall reactions of complex formation. Water molecules have a high tendency to develop hydrogen bonds compared with other solvent.[22] Thus, the ligand anions of ATP and asparagine are less stabilized by hydrogen-bonding interactions as the amount of the organic solvent in the medium is increased. This will lead to an increase in the association of these anions with the positive metal ion (Ni^{II}), forming the metal complexes.

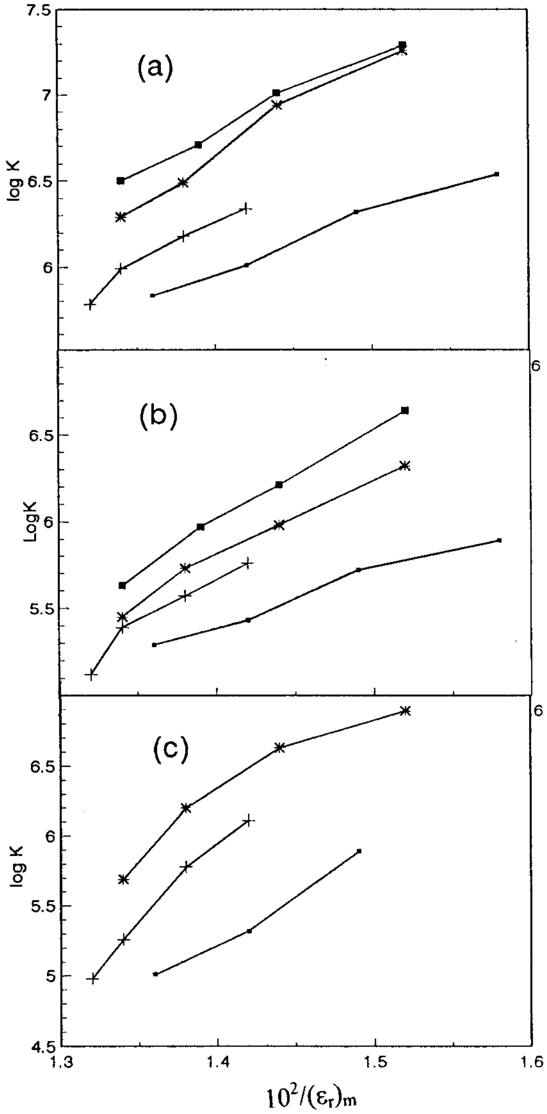


Figure 3. Variation of log *K* values of Ni^{II} complexes in the system [Ni^{II} + (ATP) + (asparagine)] with $1/[(\epsilon_r)_m]$ of the medium at 25 °C at the constant ionic strength $I = 0.10$ mol dm^{-3} (KNO$_3$): (a) binary asparagine; (b) binary ATP; (c) ternary complex; (■) acetonitrile; (∗) dioxane; (+) DMF; (●) ethanol.

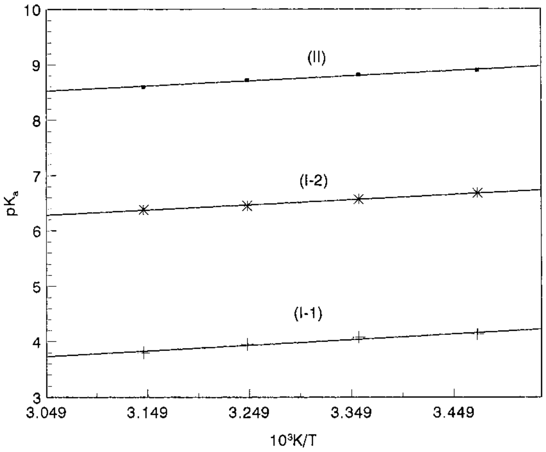


Figure 4. Plots of pK_a values of ATP and asparagine against $1/T$ at the constant ionic strength $I = 0.10$ mol dm^{-3} (KNO$_3$); (I-1) pK_{a1} of ATP; (I-2) pK_{a2} of ATP; (II) pK_{a2} of asparagine.

Table 3. Thermodynamic Functions Associated with the Formation of the Proton−Ligand Process of Asparagine and ATP[a] in a Pure Aqueous Medium and at the Constant Ionic Strength $I = 0.01$ mol dm^{-3} KNO$_3$

						$-\Delta H$	ΔG^b	$-\Delta S^b$
compound		t/°C = 15	t/°C = 25	t/°C = 35	t/°C = 45	kJ mol^{-1}	kJ mol^{-1}	J mol^{-1} K^{-1}
asparagine	pK_{a2}	8.90	8.82	8.72	8.60	17.51	50.33	227.65
ATP	pK_{a1}	4.13	4.08	3.95	3.80	19.55	23.28	143.72
	pK_{a2}	6.68	6.57	6.45	6.38	17.95	37.49	186.04

[a] ATP refers to adenosine-5′-triphosphate. [b] Values calculated at 25 °C.

Table 4. Thermodynamic Functions Associated with the Formation of 1:1 Binary and 1:1:1 Ternary Metal Complexes Formed in the System [M^{II} + (ATP) + (Asparagine)] in a Pure Aqueous Medium and at the Constant Ionic Strength $I = 0.01$ mol dm^{-3} (KNO$_3$)

		log K				$-\Delta H$	$-\Delta G^{\circ\,b}$	$\Delta S^{\circ\,b}$
complex	metal ion	t/°C = 15	t/°C = 25	t/°C = 35	t/°C = 45	kJ mol^{-1}	kJ mol^{-1}	J mol^{-1}
(1:1) binary complex of ATP[a]	Co^{II}	4.48	4.40	4.35	4.30	10.39	25.11	49.40
	Ni^{II}	4.99	4.93	4.83	4.76	13.86	28.13	47.89
	Cu^{II}	6.12	6.00	5.93	5.80	18.07	34.24	54.26
(1:1) binary complex of asparagine	Co^{II}	4.42	4.37	4.30	4.22	11.73	24.94	44.33
	Ni^{II}	5.70	5.61	5.50	5.37	19.26	32.01	42.78
	Cu^{II}	8.14	8.05	7.82	7.79	22.54	45.93	78.49
(1:1:1) ternary complex	Co^{II}	4.17	4.09	4.00	3.94	13.71	23.33	32.28
	Ni^{II}	4.75	4.64	4.51	4.40	20.71	26.48	19.36
	Cu^{II}	6.70	6.62	6.43	6.30	24.32	37.77	45.13

[a] ATP refers to adenosine-5′-triphosphate. [b] Values calculated at 25 °C.

Thermodynamic Studies. The thermodynamic functions **ΔH**, **$\Delta G°$**, and **$\Delta S°$** associated with the formation of the proton−ligand and metal−ligand complexes in the ternary systems M^{II} + ATP + asparagine (where M^{II} = Co^{II}, Ni^{II}, and Cu^{II}) were determined in a pure aqueous medium at the constant ionic strength $I = 0.10$ mol dm^{-3} (KNO$_3$). The proton−ligand stability constants for ATP and asparagine (pK_{a1}, pK_{a2}, and pK_{a2}, respectively) and the stability constants of its 1:1 binary as well as 1:1:1 ternary metal complexes with Co^{II}, Ni^{II}, and Cu^{II} have been evaluated at 15, 25, 35, and 45 °C. By plotting pK_a and log K values at different temperatures versus $1/T$ (Figures 4 and 5) and equating the gradient with $-\Delta H/19.15$, the enthalpy change ΔH for the proton−ligand or metal−ligand complex formation process was determined. The standard free energy changes ($\Delta G°$) associated with the formation of proton−ligand and metal−ligand complexes at 25 °C were calculated using the equation $\Delta G° = -RT \ln K$. From the free energy change ($\Delta G°$) and the enthalpy change (ΔH) values, the entropy change ($\Delta S°$) was also deduced by the equation $\Delta S° = (\Delta H - \Delta G°)/T$. The proton−ligand and metal−ligand formation constants for the system under investigation along with the different thermodynamic parameters (ΔH, $\Delta G°$, and $\Delta S°$) are given in Tables 3 and 4, respectively.

The obtained results (Table 3) indicate that the proton−ligand stability constant decreases with increasing temperature, demonstrating that the proton−ligand stability constant is more pronounced at lower temperature. This is consistent with the exothermic nature of the proton−ligand formation process; that is, ΔH is negative. Otherwise, the acidity constant is more pronounced with increasing temperature. The positive values of $\Delta G°$ for the proton−ligand formation processes denote that such processes are not spontaneous. Moreover, the values of entropy changes ($\Delta S°$) for the different proton−ligand formations are negative, pointing to increased ordering due to association. The small negative value observed for the entropy change corresponding to the pK_{a1} compared to that of pK_{a2} for ATP indicates less ordering of the ionic species upon association to form the protonated form ($^+$NH) of the adenosine moiety (pK_{a1}). This is consistent with the relatively high ΔH value for this process, indicating that the formation of the protonated form ($^+$NH) of ATP is more exothermic in nature.

On the other hand, the results in Table 4 reveal that the different metal−ligand complexation processes are exothermic and more favored at lower temperatures, where ΔH values are negative. The ΔH values for ternary complexes are more negative and ensure that, despite the steric hindrance due to the primary ligand, the bond is stronger in the ternary complex formation.[25] Moreover, the relatively high negative values of ΔH for ternary complexes may also be due to less competition faced by a secondary ligand at this step from a water molecule.[25] However, the metal−ligand complex formation is spontaneous in nature, as characterized by the negative $\Delta G°$ value. The $\Delta G°$ values for M^{II} + asparagine complexes are more negative than those for the binary M^{II} + ATP or the ternary M^{II} + ATP + asparagine complexes, indicating that the binary M^{II} + asparagine complexes are more thermodynamically favored. This is consistent with the higher stability constant of the binary M^{II} + asparagine complexes relative to those for the binary M^{II} + ATP and ternary M^{II} + ATP + asparagine complexes. Furthermore, the metal−ligand complex formation process is entropy favored where the values of the entropy changes ($\Delta S°$) are positive. This behavior is in harmony with the positive value of the entropy change observed for the formation reaction involving positive and negative ions to give a complex of a different charge in solution.[26] Therefore, the positive $\Delta S°$ values obtained substantiate the suggestion that the different 1:1 binary and 1:1:1 ternary metal complexes are formed due to the coordination of the ligand anions to the positive metal ions. Further, the positive value of the entropy change accompanying complex formation suggests also a desolvation of the ligands, resulting in weak solvent−ligand interactions, to the advantage of the ligand−metal ion interaction.[27]

The magnitude of the $\Delta G°$ and ΔH values for all the complexes, in terms of the nature of the metal ion, increases in the order Cu^{II} > Ni^{II} > Co^{II}. This behavior is

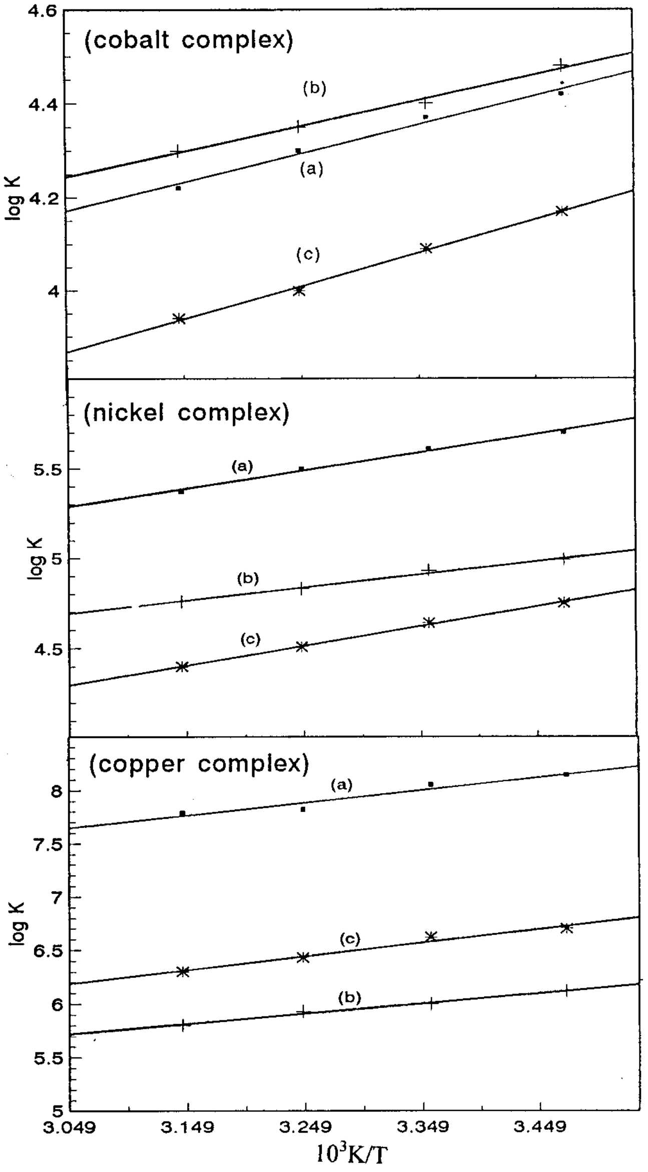


Figure 5. Plots of log K values of M^{II} complexes against $1/T$ at the constant ionic strength $I = 0.10$ mol dm^{-3} (KNO_3): (a) binary asparagine; (b) binary ATP; (c) ternary complex.

consistent with the order of increasing the stability constants for the complexes of such metal complexes,[10] which is in good agreement with the order preconditioned by binding affinities, as revealed in the Irving–Williams series.[28] This series was confirmed recently,[29] where it was reported that the stability constants are increased with increasing ionic radii, electronegativity, and ionization potentials of the metal ions.[27] This behavior is consistent with the results obtained for the ΔH and ΔG° values as well as the stability constants of the different complexes studied.

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