Nucleic Acid Base Pair and Mispair Interactions with Metal Ions—A Thermodynamic Aspect

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Potentiometric equilibrium measurements were made for some metal ions (M(II) = Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Ca(II), Sr(II), and Ba(II)) with guanine (A) in a 1:1 (M(II):A) ratio and with cytosine, cytidine, 5-bromocytosine, 5-azacytosine, and 5-fluorocytosine as primary ligands (L) and guanine as secondary ligand in a 1:1:1 (M(II):L:A) ratio at (25.0, 35.0, and 45.0) °C and I = 0.1 mol·dm^{-3} $NaNO_3$ in aqueous solution. The experimental pH-titration data were analyzed by using a BEST computer program in order to evaluate the formation constants of various intermediate species and their relative distribution. The experimental conditions were selected in such a way that the self-association of the nucleobases and their complexes due to stacking interaction was negligibly small, so that only the neutral monomeric and hydroxo ternary complexes were studied. The enthalpy ($\Delta_f H°$) and entropy ($\Delta_f S°$) changes for the formation of binary and ternary complexes were calculated from temperature coefficient data. The $\delta\Delta_f S°$ values are positive for all the metal ligand systems. The negative $\delta\Delta_f H°$ values indicate the extra stabilization of most of the ternary complexes by the exothermic enthalpy change ($\delta\Delta_f S° = \Delta_T S° - \Delta_B S°$ and $\delta\Delta_f H° = \Delta_T H° - \Delta_B S°$ where $\Delta_T S°$, $\Delta_T H°$ and $\Delta_B S°$, $\Delta_B H°$ are the entropy and enthalpy values associated with the ternary and binary complexes, respectively). On the basis of IR data for metal complexes with the 5FC−G mispair, it has been proposed that the guanine is bonded to metal ions through N1/C6═O and N7, whereas cytosine and its derivatives are bonded through N3 atoms in ternary complexes.

Introduction

Guanine, a purine base found in both RNA and DNA, exhibits interesting biological properties. When combined with the sugar ribose in a glycosidic linkage, guanine forms a derivative called guanosine, which in turn can be phosphorylated with from one to three phosphoric acid groups, yielding the three nucleotides GMP (guanosine monophosphate), GDP (guanosine diphosphate), and GTP (guanosine triphosphate). GTP acts as a coenzyme in carbohydrate metabolism and in the biosynthesis of proteins. It can readily donate one of its phosphate groups to adenosine diphosphate (ADP) and form adenosine triphosphate (ATP), an extremely important intermediate in the transfer of chemical energy in living systems. GTP is the source of the guanosine found in RNA, and deoxyguanosine triphosphate (dGTP) is the source of the deoxyguanosine in DNA. Thus, guanine is intimately involved in the preservation and transfer of genetic information. Guanine and 8-azaguanine produced a marked inhibition of tumor and other cancer growth in mice.[1,2] The 5-halogenated pyrimidines also show antitumor properties,[3] and their antitumor properties have been found to be significantly enhanced by the coadministration of guanosine in any combination.[4] Hence, the studies of the formation of metal complexes of cytosine and its derivatives in the presence of its complementary purine base (guanine) are of considerable interest.

Ternary complexes of various metal ions with nucleobases, nucleosides, nucleotides, and other ligands have been reported by several workers,[5−21] as they provide the model for various metal activated reactions in biological systems. Considerably less is known on the interaction between metal ions and the nucleic acid base pairs and mispair.[22−24] But no literature is available on the interaction of metal ions with the guanine-cytosine base pair and mispair.

The present paper reports the studies of the stability constants of binary complexes (1:1) of guanine (A) with metal ions, viz., Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Ca(II), Ba(II), and Sr(II), and the corresponding ternary complexes (1:1:1) with cytosine (C), cytidine (CD), 5-bromocytosine (5BrC), 5-azacytosine (5AC), and 5-fluorocytosine (5FC) potentiometrically at different temperatures.

Experimental Section

Materials and Solutions. The ligands guanine ($C_5H_5N_5O$), cytosine ($C_4H_5N_3O$), and cytidine ($C_9H_{13}N_3O$) were purchased from SRL, Mumbai, whereas 5-bromocytosine ($C_4H_4BrN_3O$), 5-azacytosine ($C_3H_4N_4O$), and 5-fluorocytosine ($C_4H_4FN_3O$) were from Fluka, Switzerland. A stock solution of guanine (1×10^{-2} mol·dm^{-3}) was prepared by dissolving it in 5 cm^3 of (1 mol·dm^{-3}) NaOH solution, with the final alkali concentration being (5×10^{-2} mol·dm^{-3}). Stock solutions of the other ligands (1×10^{-2} mol·dm^{-3}) were prepared by dissolving the required amount of ligands in the minimum volume of freshly double-distilled CO_2-free water with vigorous shaking at 40 °C and subsequently diluting to make the final volume.

Copper nitrate ($Cu(NO_3)_2 \cdot 6H_2O$), nickel nitrate ($Ni(NO_3)_2 \cdot 6H_2O$), cobalt nitrate ($Co(NO_3)_2 \cdot 6H_2O$), zinc nitrate ($Zn(NO_3)_2 \cdot 6H_2O$), cadmium nitrate ($Cd(NO_3)_2 \cdot 6H_2O$), calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$), strontium nitrate ($Sr(NO_3)_2$), and barium nitrate ($Ba(NO_3)_2$) (from E Merck, India) were used to prepare metal solutions (1×10^{-2} mol·dm^{-3}) and were standardized by EDTA titrations using

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Table 1. Proton Dissociation Constants (pK_{na})[a] and the Corresponding Thermodynamic Parameters ($\Delta_f H°$, $\Delta_f G°$, and $\Delta_f S°$) at I = 0.1 mol·dm^{-3} $NaNO_3$ in Aqueous Solution

	pK_{na} at the following t/°C					
ligand	25	35	45	$\Delta_f H°$ (kJ.mol^{-1})	$\Delta_f G°$ (kJ.mol^{-1})	$\Delta_f S°$ (kJ.mol^{-1})
guanine						
pK_{1a}	9.25 ± 0.10	9.13 ± 0.07	8.80 ± 0.08	−2.36 ± 0.06	−3.11 ± 0.05	+2.50 ± 0.08
pK_{2a}	12.20 ± 0.03	11.80 ± 0.02	11.84 ± 0.07	−2.02 ± 0.05	−4.10 ± 0.04	+6.99 ± 0.07
cytosine						
pK_{1a}	4.22 ± 0.04	4.09 ± 0.08	4.00 ± 0.11	−1.13 ± 0.02	−1.37 ± 0.04	+0.81 ± 0.03
pK_{2a}	11.58 ± 0.03	11.50 ± 0.05	11.05 ± 0.08	−2.83 ± 0.03	−3.77 ± 0.08	+3.14 ± 0.06
cytidine						
pK_{1a}	4.21 ± 0.10	4.05 ± 0.07	3.99 ± 0.08	−1.12 ± 0.06	−1.37 ± 0.05	+0.83 ± 0.08
pK_{2a}	11.22 ± 0.03	11.08 ± 0.02	10.70 ± 0.07	−2.70 ± 0.05	−3.65 ± 0.04	+3.17 ± 0.07
5-bromocytosine						
pK_{1a}	3.48 ± 0.08	3.32 ± 0.14	3.30 ± 0.05	−0.88 ± 0.09	−1.13 ± 0.03	+0.83 ± 0.07
pK_{2a}	11.05 ± 0.04	10.74 ± 0.09	10.54 ± 0.06	−2.62 ± 0.05	−3.59 ± 0.06	+3.26 ± 0.06
5-azacytosine						
pK_{1a}	3.45 ± 0.08	3.32 ± 0.05	3.28 ± 0.06	−0.87 ± 0.06	−1.12 ± 0.03	+0.86 ± 0.07
pK_{2a}	10.85 ± 0.09	10.72 ± 0.05	10.36 ± 0.11	−2.55 ± 0.09	−3.53 ± 0.04	+3.29 ± 0.05
5-fluorocytosine						
pK_{1a}	3.38 ± 0.05	3.30 ± 0.03	3.22 ± 0.09	−0.83 ± 0.11	−1.10 ± 0.05	+0.91 ± 0.08
pK_{2a}	10.75 ± 0.03	10.50 ± 0.08	10.70 ± 0.06	−2.40 ± 0.03	−3.49 ± 0.07	+3.68 ± 0.04

[a] $pK_{1a} = -\log K_{1a}$; $K_{1a} = [HA^-][H^+]/[H_2A]$. $pK_{2a} = -\log K_{2a}$; $K_{2a} = [A^{2-}][H^+]/[HA^-]$.

suitable indicators.[25] HNO_3 and NaOH were from Merck. Stock solutions of NaOH (CO_2-free) and HNO_3 were prepared and standardized by a literature method.[25] All the reagents used were of AR grade.

Apparatus. The titrations were performed at (25, 35, and 45) °C with control of ±0.1 °C in a double-walled cell, fitted with a thermostat (Julabo F-10). In all titrations, oxygen-free N_2 gas was passed into the solution before and during the pH measurements. A Schott CG 841 pH meter using a glass electrode (Schott Gerate 6280) was used to monitor the pH changes.

Procedure. The titrant (CO_2-free standard NaOH) was added to the titration cell, and the pH changes were monitored with the pH meter. The pH meter was calibrated with standard buffer solutions (pH 4.0 and 10.0) before the pH measurements. The pH region below 3.5 and above 10.5 was calibrated using standard HCl and NaOH solutions, respectively. To determine the proton dissociation constants (pK_{na}) of the free ligands, the following mixtures (a and b) were prepared for each ligand and titrated separately with the standard alkali solution (0.2 mol·dm^{-3}): (a) HNO_3 (2×10^{-3} mol·dm^{-3}) + $NaNO_3$ (1×10^{-1} mol·dm^{-3}); (b) HNO_3 (2×10^{-3} mol·dm^{-3}) + $NaNO_3$ (1×10^{-1} mol·dm^{-3}) + A (1×10^{-3} mol·dm^{-3}) + HNO_3 (5×10^{-3} mol·dm^{-3}). In the study of binary (1:1) and ternary (1:1:1) systems, the following mixtures (c and d) were prepared for each metal ion and titrated as above: (c) HNO_3 (2×10^{-3} mol·dm^{-3}) + $NaNO_3$ (1×10^{-1} mol·dm^{-3}) + A (1×10^{-3} mol·dm^{-3}) + M(II) (1×10^{-3} mol·dm^{-3}) + HNO_3 (5×10^{-3} mol·dm^{-3}); (d) HNO_3 (2×10^{-3} mol·dm^{-3}) + $NaNO_3$ (1×10^{-1} mol·dm^{-3}) + L (1×10^{-3} mol·dm^{-3}) + A (1×10^{-3} mol·dm^{-3}) + M(II) (1×10^{-3} mol·dm^{-3}) + HNO_3 (5×10^{-3} mol·dm^{-3}). L = C, CD, 5BrC, 5AC, or 5FC. The ionic strength (I) of all the titration mixtures was adjusted to 0.1 mol·dm^{-3} by adding the requisite volume of $NaNO_3$ (1 mol·dm^{-3}). Nitric acid was used to lower the pH of the initial solution mixture. The pH titrations were terminated when either the pH meter readings became unstable, showing a downward drift, or visual precipitation occurred. In analyzing the titration data for the determination of the proton dissociation constants of the free ligands and the formation constants of binary and ternary metal ligand complexes in solution, Bjerrum−Calvin's pH titration technique,[26,27] as adopted by Irving and Rossotti[28,29] for binary systems and by Chidambaram and Bhattacharya[30] for ternary systems, has been used at (25, 35, and 45) °C. Standard deviations were also evaluated for the corresponding equilibrium constants. Several complex equilibria were studied by using computer p^{KAS} and BEST programs.[31]

Enthalpy and entropy values associated with various proton−ligand and metal−ligand equilibria were calculated[32] by eqs i−iii:

$$\Delta_f H° = \frac{2.303 R T_1 T_2 \log(K_2 K_1)}{T_2 - T_1} \quad \text{(i)}$$

$$\Delta_f G° = -RT \ln K \quad \text{(ii)}$$

$$\Delta_f S° = \frac{\Delta_f H° - \Delta_f G°}{T} \quad \text{(iii)}$$

where $\Delta_f H°$ = standard heat content change, R = gas constant, T = absolute temperature, K = equilibrium constant, $\Delta_f G°$ = standard free energy change, and $\Delta_f S°$ = standard entropy change.

Preparation of M(II)−5FC−A Complexes and IR Measurements. The hydrated metal nitrate (1.0 mmol) was refluxed for about 10 h in a mixture of 35.0 cm^3 of ethyl alcohol and 15.0 cm^3 of triethyl orthoformate. Then, 1.0 mmol of 5-fluorocytosine as well as guanine was added to each metal solution separately, and the resultant mixtures (1:1:1) were refluxed for several hours. The volume of the resulting mixture was reduced to about one-third of its original volume, and the pH of the solution was adjusted to about 7 by adding sodium hydroxide solution (0.5 N) with continuous stirring. The solid complexes were separated by filtration and washed several times with ethanol and finally with ether. The complexes were dried at 50−60 °C in oven, and infrared spectra were obtained on a Perkin-Elmer 1600 Series FT-IR spectrometer in the 4000−400 cm^{-1} region using KBr.

Results and Discussion

The proton ligand dissociation constants for guanine have been evaluated pH-metrically at (25, 35, and 45) °C and tabulated in Table 1. They are in good agreement with reported values. The titration curve of the guanine (curve b) shows two inflections, at a = 1 and 2 (Figure 1),

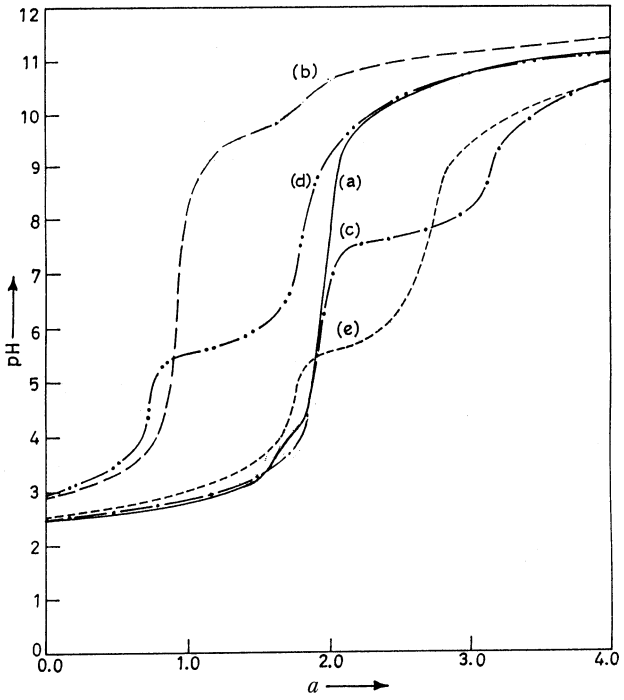

Figure 1. pH against a volume of 0.2 mol·dm^{-3} NaOH for the Cu(II) + 5FC + A system at (25 ± 0.1) °C and I = 0.1 mol·dm^{-3} $NaNO_3$: (a) 2.0 × 10^{-3} mol·dm^{-3} HNO_3 + 1.0 × 10^{-3} mol·dm^{-3} 5FC; (b) 2.0 × 10^{-3} mol·dm^{-3} HNO_3 + 1.0 × 10^{-3} mol·dm^{-3} A + 5.0 × 10^{-3} mol·dm^{-3} HNO_3; (c) 2.0 × 10^{-3} mol·dm^{-3} HNO_3 + 1.0 × 10^{-3} mol·dm^{-3} 5FC + 1.0 × 10^{-3} mol·dm^{-3} Cu(II); (d) 2.0 × 10^{-3} mol·dm^{-3} HNO_3 + 1.0 × 10^{-3} mol·dm^{-3} A + 1.0 × 10^{-3} mol·dm^{-3} Cu(II) + 5.0 × 10^{-3} mol·dm^{-3} HNO_3; (e) 2.0 × 10^{-3} mol·dm^{-3} HNO_3 + 1.0 × 10^{-3} mol·dm^{-3} 5FC + 1.0 × 10^{-3} mol·dm^{-3} Cu(II) + 1.0 × 10^{-3} mol·dm^{-3} A + 5.0 × 10^{-3} mol·dm^{-3} HNO_3. Where a is the number of moles of alkali per mole of ligand.

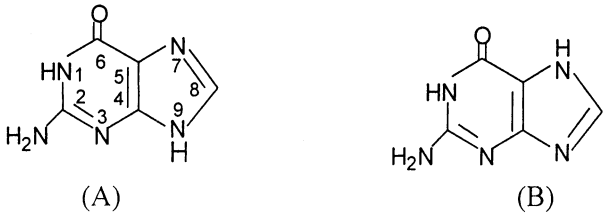

Figure 2. Tautomeric structures of guanine.

indicating that the two acidic groups of guanine dissociated in two steps. The values for proton dissociation constants have been calculated by using the following equations:

$$H_2A \rightleftharpoons HA^- + H^+; \quad K_{1a} = \frac{[HA^-][H^+]}{[H_2A]} \qquad \text{(iv)}$$

$$HA^- \rightleftharpoons A^{2-} + H^+; \quad K_{2a} = \frac{[A^{2-}][H^+]}{[HA^-]} \qquad \text{(v)}$$

The formation curve of the guanine shows that equilibria iv and v exist independently in the pH ranges 5.0 to 8.1 and 8.3 to above, respectively. Guanine exists in a mixture of the tautomeric forms A and B[33] (Figure 2). In guanine, the first deprotonation occurs from the N1−H position (Figure 3) and the second deprotonation occurs from the N7−H group. The same has been reported by other workers.[8] The enthalpy and entropy change associated with the second dissociation constant are less exothermic and more positive than the first dissociation constant (Table 1) though enthalpy changes are less exothermic for the

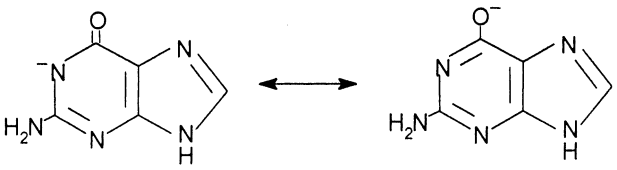

Figure 3. N1−H ionized resonate structures of guanine.

dissociation of the first proton. Weak dissociation of the second proton is due to the enthalpy factor.

The deprotonation constants of guanine were utilized for the evaluation of the stability constants of binary (1:1) M(II)−A as well as ternary (1:1:1) M(II)−L−A complexes at a constant ionic strength (I = 0.1 mol·dm^{-3} $NaNO_3$) and (25, 35, and 45) °C. Here guanine acts as secondary ligand, represented as A, and C, CD, 5BrC, 5AC, and 5FC act as primary ligands, represented as L. The interactions of purine and pyrimidine bases with metal ions separately as well as in the presence of each other (purine−pyrimidine base pairs) are evident from the titration curves (Figure 1). The stability constants of the ternary complexes along with the stability constants of binary (M(II)−guanine) systems for various metal ions are summarized in Table 2.

When a solution contains two different ligands and a metal ion, there may exist equilibria in which either (i) both the ligands may combine with the metal ion simultaneously or (ii) the two ligands may be combined one by one at different pH.

As is evident from the titration curves in the present study, the addition of two ligands is stepwise in the buffer region at a = 2 and a = 3. The formation of ternary complexes takes place according to the following equilibria (Charges are omitted for clarity).

$$M + HL \rightleftharpoons ML + H; \quad K^{M}_{ML} = \frac{[ML][H]}{[M][HL]} \qquad \text{(vi)}$$

$$ML + HA \rightleftharpoons MLA + H; \quad K^{ML}_{MLA} = \frac{[MLA][H]}{[ML][HA]} \qquad \text{(vii)}$$

K^{ML}_{MLA} represents the formation constant of the ternary complexes.

Depending upon the chelation processes, the formations of various stable complex species in the solution were altered. The pH versus a curves for all metal ligand ternary systems studied indicated several inflections and suggested the formation of nonprotonated binary (ML/MA) or ternary (MLA) and monohydroxo ternary species (MLA(OH)$^-$).

$$MLA(H_2O)_2 \rightleftharpoons MLA(OH)(H_2O) + H;$$

$$K_{MLA(OH)(H_2O)} = \frac{[MLA(OH)(H_2O)][H]}{[MLA(H_2O)_2]} \qquad \text{(viii)}$$

The formation constants for the binary and ternary complexes have been evaluated as described previously[22] and are tabulated in Table 3 as log K. Stability constants data revealed the relative stability order of metal ligand binary and ternary complexes as M(II)−L−A > M(II)−A > M(II)−L. This is because of the lesser tendency of secondary ligand (A) toward $M(H_2O)_n^{2+}$ as compared to ML. Comparable values of stability constants for various (1:1:1) systems indicate the same coordination mode of ligands with metal ions.[34]

Species distribution for various possible species in solution has been performed for all the metal ligand systems reported in the present paper. Analysis of the species

Table 2. Formation Constants for the Binary M(II) + Guanine (A) Ligand Complexes Together with the Corresponding Mixed-Ligand Complexes M(II) + L + A at Different Temperatures and I = 0.1 mol·dm^{-3} $NaNO_3$ in Aqueous Solution

t/°C	Co(II)	Ni(II)	Cu(II)	Zn(II)	Cd(II)	Ca(II)	Sr(II)	Ba(II)
				M(II) + G (log K^{M}_{MA})[a]				
25	10.92 ± 0.06	11.27 ± 0.12	11.36 ± 0.08	10.92 ± 0.05	10.58 ± 0.11	7.41 ± 0.08	7.03 ± 0.14	6.71 ± 0.16
35	10.90 ± 0.03	11.06 ± 0.05	11.05 ± 0.09	10.65 ± 0.08	10.33 ± 0.16	7.55 ± 0.06	6.80 ± 0.06	6.50 ± 0.11
45	10.57 ± 0.09	10.90 ± 0.08	10.98 ± 0.11	10.56 ± 0.02	10.23 ± 0.07	7.44 ± 0.07	7.05 ± 0.08	6.73 ± 0.12
				M(II) + C + G (log K^{ML}_{MLA})[b]				
25	11.48 ± 0.08	12.05 ± 0.11	12.34 ± 0.05	11.85 ± 0.02	11.12 ± 0.07	8.12 ± 0.04	7.88 ± 0.05	7.44 ± 0.02
35	11.81 ± 0.04	10.75 ± 0.05	11.78 ± 0.09	10.98 ± 0.11	11.10 ± 0.03	8.80 ± 0.07	7.58 ± 0.17	7.58 ± 0.06
45	11.14 ± 0.06	11.59 ± 0.08	11.92 ± 0.04	11.43 ± 0.05	10.77 ± 0.12	8.22 ± 0.13	7.92 ± 0.08	7.53 ± 0.04
				M(II) + CD + G (log K^{ML}_{MLA})[b]				
25	11.34 ± 0.06	11.68 ± 0.04	12.11 ± 0.12	11.48 ± 0.08	11.02 ± 0.02	7.95 ± 0.05	7.66 ± 0.03	7.22 ± 0.09
35	10.85 ± 0.05	10.20 ± 0.08	12.54 ± 0.05	10.44 ± 0.04	11.00 ± 0.03	8.80 ± 0.11	7.24 ± 0.05	7.58 ± 0.05
45	10.96 ± 0.11	11.24 ± 0.10	11.76 ± 0.07	11.07 ± 0.03	10.67 ± 0.05	8.04 ± 0.03	7.25 ± 0.16	7.60 ± 0.07
				M(II) + 5BrC + G (logK^{ML}_{MLA})[b]				
25	11.22 ± 0.08	11.56 ± 0.05	12.02 ± 0.03	11.42 ± 0.02	10.88 ± 0.09	7.88 ± 0.04	7.57 ± 0.11	7.01 ± 0.04
35	11.12 ± 0.11	10.98 ± 0.07	12.11 ± 0.06	11.00 ± 0.06	10.58 ± 0.15	8.05 ± 0.07	6.72 ± 0.14	6.00 ± 0.06
45	10.88 ± 0.06	11.18 ± 0.13	11.65 ± 0.08	11.05 ± 0.08	10.52 ± 0.03	7.93 ± 0.05	7.55 ± 0.18	6.99 ± 0.09
				M(II) + 5AC + G (log K^{ML}_{MLA})[b]				
25	11.01 ± 0.08	11.47 ± 0.13	11.96 ± 0.04	11.22 ± 0.06	10.78 ± 0.05	7.78 ± 0.08	7.22 ± 0.16	7.05 ± 0.05
35	10.98 ± 0.04	11.02 ± 0.11	11.45 ± 0.09	10.00 ± 0.11	10.00 ± 0.09	6.70 ± 0.03	7.50 ± 0.12	6.40 ± 0.08
45	10.67 ± 0.03	11.08 ± 0.03	11.55 ± 0.07	10.80 ± 0.08	10.40 ± 0.13	7.74 ± 0.11	7.27 ± 0.14	7.04 ± 0.03
				M(II) + 5FC + G (log K^{ML}_{MLA})[b]				
25	10.98 ± 0.07	11.34 ± 0.13	11.44 ± 0.11	11.00 ± 0.05	10.68 ± 0.12	7.65 ± 0.02	7.40 ± 0.18	6.98 ± 0.21
35	10.76 ± 0.11	11.22 ± 0.08	10.90 ± 0.18	10.88 ± 0.09	10.00 ± 0.08	6.48 ± 0.07	7.00 ± 0.16	6.89 ± 0.08
45	10.63 ± 0.17	10.98 ± 0.03	11.05 ± 0.14	10.65 ± 0.16	10.31 ± 0.09	7.60 ± 0.04	7.40 ± 0.12	7.01 ± 0.17

[a] log K^{M}_{MA} is calculated using following equilibrium: M(II) + A ⇌ M(II)(A); [M(II) = Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Ca(II), Sr(II), Ba(II); L = cytosine (C), cytidine (CD), 5-bromocytosine (5BrC), 5-azacytosine (5AC), 5-fluorocytosine (5FC); A = guanine]; ± uncertainties refer to 3 times the standard deviation (3S). [b] log K^{ML}_{MLA} is calculated using following equilibrium: M(II) + L + A ⇌ M(II)(L)(A). The definitions from footnote *a* apply.

distribution curves reveals the existence of the free metal and ternary species along with the neutral binary complex species for both primary and secondary ligands in the solution at various pH. Figure 4 indicates the presence of free metal and 1:1 neutral M(II)−L complexes (L = C, CD, 5BrC, 5AC, and 5FC) in solution around pH 2.0. Their concentration decreases with the increase of the concentration of neutral species of M(II)−A and M(II)−L−A, and they totally disappear at pH ∼ 8.0. From the species distribution curve it can be stated further that the monohydroxo species (i.e., 100% at pH 10.0) of ternary complexes predominate the neutral species, which are being formed comparatively in small amounts in the lower pH range (∼3.0). Due to the difference between the pK values of the primary and secondary ligand, various complex species are formed. At pH 2.0, free metals are present in 75% to 91%. The association of L with each metal ion starts at very low pH range (∼2.0), resulting in the formation of M(II)−L species (8.2−14%) at pH ∼ 2.0. Furthermore, the association of A with each metal ion and M(II)−L species started at pH ∼ 3.0 and the formations of M(II)−A (9−37%) and M(II)−L−A (28−83%) species were observed at pH = 5.7−7.7 and 5.2−7.9, respectively. Finally, the total metal ion distributed into M(II)−L, M(II)−A, and M(II)−L−A species started converting into the quaternary hydroxo species after successive deprotonation of coordinated water molecules at pH > 6.0. In the case of alkaline earth metal, the quaternary hydroxo species dominate neutral ternary species to a larger extent as compared to the transition metal ions. Again, the species distribution curves clearly show that the ternary complexes are more stable than the binary complexes of primary as well as secondary ligands. In the ternary systems where the solution contains a metal ion and two ligands, the formation of ternary complexes in the solution occurs in a situation where either there are interligand interactions, viz., electrostatic, hydrophobic, and stacking interactions, or there are no such interactions.

The Δ log K (Table 4) and Δ log K' (Table 5) are the differences between the stabilities of the binary and ternary metal ligand complexes with respect to both primary and secondary ligands. These provide an insight into the various factors responsible for the formation and stabilization of ternary complexes in the solution. Values of Δ log K are affected by the charge neutralization, the π-accepting capacity of the secondary ligand, and stacking interactions. They are derived from two different constants and vary with temperature. The positive value of Δ log K indicates that the ternary complexes are more stable than the binary complexes and involve interligand interaction.[22] Its negative value implies that binary complexes are more stable than the ternary complexes, but it does not preclude the formation of ternary complexes in the solution.[35,36] Δ log K values are also influenced by the stability constants of the simple binary complexes.[37] The extent to which the two ligands influence the metal ion in the presence of each other as compared to simple 1:1 parent complexes, affecting the coordination modes of the ligands and the geometry of the complexes as a whole, can be assessed by the value of Δ log K. It is well evident fromTables 4 and 5 that the Δ log K values are positive and the metal ligand complexes formed in different systems followed the Irving−William's order[38] in more or less extent. The Δ log K values show that the ternary complexes are more stable than the corresponding binary systems. Stacking interaction is appreciable only in those aromatic ligands that have comparatively free rotation about the metal ligand bond and can take up a parallel configuration for maximum molecular interaction. This interaction is responsible for extra stabilization of ternary complexes, since cytosine is mostly unidentate, involving only the N3[37] binding site in metal coordination, and can take up a configuration paral-

Table 3. Equilibrium Constants (log $K \pm 3S$[a]) for Reactions[b] of (1:1:1) Ternary Complexes M(II) + L + A[c] at (25 ± 0.1) °C and I = 0.1 mol·dm^{-3} $NaNO_3$ in Aqueous Solution

species	C	CD	5BrC	5AC	5FC
			Co(II)		
ML	2.10 ± 0.04	2.13 ± 0.03	2.18 ± 0.03	2.17 ± 0.09	2.25 ± 0.03
MA	14.13 ± 0.03	14.18 ± 0.09	14.24 ±0.05	14.25 ± 0.03	14.22 ± 0.06
MLA	17.96 ± 0.07	18.05 ± 0.05	18.11 ± 0.04	18.12 ± 0.05	18.23 ± 0.05
MLA(OH)$^-$	11.04 ± 0.16	11.18 ± 0.15	11.24 ± 0.08	11.21 ± 0.11	11.36 ± 0.11
			Ni(II)		
ML	2.15 ± 0.02	2.18 ± 0.12	2.26 ± 0.04	2.21 ± 0.08	2.24 ± 0.03
MA	14.28 ± 0.04	14.21 ± 0.18	14.28 ± 0.02	14.33 ± 0.05	14.32 ± 0.09
MLA	18.33 ± 0.02	18.41 ± 0.15	18.38 ± 0.14	18.45 ± 0.03	18.47 ± 0.11
MLA(OH)$^-$	10.78 ± 0.05	10.88 ± 0.12	10.75 ± 0.25	10.83 ± 0.07	10.93 ± 0.10
			Cu(II)		
ML	2.18 ± 0.08	2.21 ± 0.03	2.32 ± 0.03	2.28 ± 0.02	2.27 ± 0.04
MA	14.33 ± 0.11	14.31 ± 0.02	14.28 ± 0.07	14.42 ± 0.07	14.45 ± 0.02
MLA	18.56 ± 0.16	18.88 ± 0.07	18.85 ± 0.04	18.92 ± 0.03	18.95 ± 0.09
MLA(OH)$^-$	10.64 ± 0.05	10.62 ± 0.01	10.68 ± 0.03	10.81 ± 0.01	10.83 ± 0.14
			Zn(II)		
ML	2.13 ± 0.02	2.11 ± 0.01	2.18 ± 0.04	2.21 ± 0.06	2.24 ± 0.16
MA	14.21 ± 0.06	14.45 ± 0.09	14.28 ± 0.09	14.33 ± 0.02	14.37 ± 0.06
MLA	18.12 ± 0.03	18.22 ± 0.04	18.31 ± 0.13	18.45 ± 0.14	18.38 ± 0.03
MLA(OH)$^-$	10.92 ± 0.05	11.08 ± 0.07	11.09 ± 0.02	10.83 ± 0.18	11.18 ± 0.07
			Cd(II)		
ML	2.11 ± 0.14	2.18 ± 0.06	2.15 ± 0.11	2.21 ± 0.01	2.28 ± 0.09
MA	14.06 ± 0.08	14.18 ± 0.19	14.21 ± 0.18	14.27 ± 0.06	14.31 ± 0.12
MLA	17.81 ± 0.09	17.85 ± 0.10	17.89 ± 0.12	17.91 ± 0.06	17.98 ± 0.05
MLA(OH)$^-$	11.11 ± 0.08	11.21 ± 0.06	11.18 ± 0.15	11.24 ± 0.02	11.27 ± 0.18
			Ca(II)		
ML	2.00 ± 0.09	2.05 ± 0.02	2.11 ± 0.03	2.08 ± 0.03	2.21 ± 0.04
MA	14.12 ± 0.04	14.21 ± 0.14	14.22 ± 0.07	14.27 ± 0.05	14.35 ± 0.03
MLA	17.75 ± 0.07	17.92 ± 0.18	17.86 ± 0.04	18.02 ± 0.02	18.12 ± 0.02
MLA(OH)$^-$	10.15 ± 0.11	10.22 ± 0.21	10.21 ± 0.06	10.35 ± 0.01	10.42 ± 0.16
			Sr(II)		
ML	2.05 ± 0.04	2.11 ± 0.02	2.07 ± 0.15	2.06 ± 0.03	2.11 ± 0.04
MA	14.08 ± 0.03	14.12 ± 0.19	14.18 ± 0.06	14.15 ± 0.03	14.17 ± 0.03
MLA	17.71 ± 0.14	17.73 ± 0.04	17.78 ± 0.04	17.77 ± 0.18	17.85 ± 0.04
MLA(OH)$^-$	10.28 ± 0.03	10.22 ± 0.07	10.35 ± 0.05	10.32 ± 0.14	10.42 ± 0.07
			Ba(II)		
ML	2.11 ± 0.08	2.16 ± 0.06	2.14 ± 0.01	2.28 ± 0.04	2.21 ± 0.04
MA	14.05 ± 0.21	14.18 ± 0.04	14.07 ± 0.04	14.10 ± 0.09	14.21 ± 0.05
MLA	17.62 ± 0.03	17.76 ± 0.09	17.68 ± 0.05	17.72 ± 0.02	17.82 ± 0.09
MLA(OH)$^-$	10.21 ± 0.13	10.28 ± 0.06	10.25 ± 0.10	10.32 ± 0.11	10.38 ± 0.11

[a] S = standard deviation. [b] Reactions: M + HL ⇌ ML + H; M + HA ⇌ MA + H; ML + HA ⇌ MLA + H; $MLA(H_2O)_2$ ⇌ $MLA(OH)(H_2O)$. [c] M(II) = Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Ca(II), Sr(II), Ba(II); L = cytosine (C), cytidine (CD), 5-bromocytosine (5BrC), 5-azacytosine (5AC), 5-fluorocytosine (5FC); A = guanine.

lel to guanine (Figure 5). Guanine coordinated to metal ions directly via N7,[14] involving the possible contribution from N1/C6═O in both binary and ternary systems.

It is also clear from Table 3 that, among the various systems under investigation, Cu(II) ternary complexes are least stable. It is a well established fact that purine and pyrimidine bases, nucleosides, and nucleotides are associated extensively in aqueous solution by a mechanism involving vertical stacking of the bases.[35,36,39] The stabilization of ternary complexes in the present work may be due to the fact that guanine and cytosine are taking part in stacking interactions. The stacking interactions are supposed to occur between the pyrimidine moieties of A and L.

Since the value of $\Delta \log K$ varies with temperature, there is a need to measure the extra stability in ternary complexes by other parameters, such as $\delta\Delta_f H°$ and $\delta\Delta_f S°$, which directly measure the extent of extra interaction in these systems. $\delta\Delta_f H°$ and $\delta\Delta_f S°$ are defined as

$$\delta\Delta_f H° = \Delta_T H° - \Delta_B H°$$

$$\delta\Delta_f S° = \Delta_T S° - \Delta_B S°$$

Here $\Delta_T H°$, $\Delta_T S°$ and $\Delta_B H°$, $\Delta_B S°$ are the enthalpy and entropy values associated with the ternary and binary complexes, respectively. Hence, the thermodynamic parameters associated with metal ligand complexes are evaluated by studying the stability constants of binary and ternary systems of guanine at (25, 35, and 45) °C. In both binary and ternary complexes, stability constants decrease with an increase in temperature. The enthalpy changes for the formation of M(II)−ligand (1:1) (except Ca(II), Sr(II), and Ba(II)) systems are exothermic, and the entropy values are positive (Table 6). The complexes of Ca(II), Sr(II), and Ba(II) also exhibit large positive entropy values. The higher entropy values in all the metal−ligand systems further supported the bidentate nature of the ligands in the binary complexes. The Ca(II)−ligand systems have positive enthalpy values as compared to those of other alkaline earth metals. This may be due to the smaller ionic radius of calcium.[40] The enthalpy changes for Ca(II), Sr(II), and Ba(II) complexes are in accord with the corresponding heats of hydration of these ions.

The entropy values of ternary systems are higher than those of the binary M(II)−A systems. The enthalpy changes for the 1:1:1 mixed ligand complex formation of M(II)−L−A

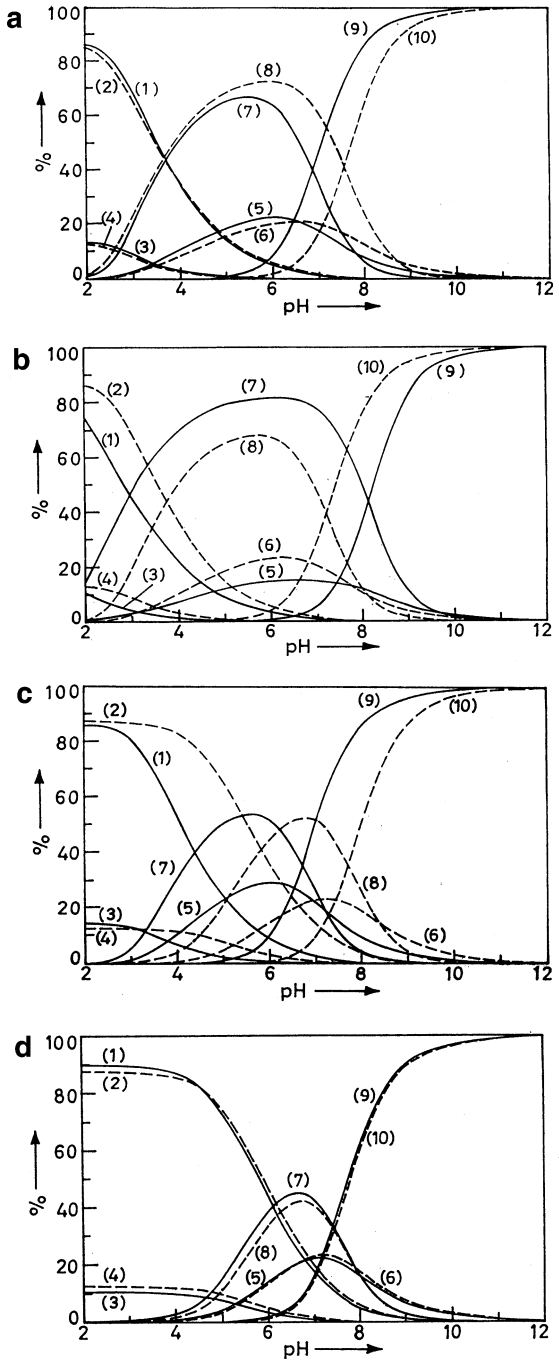

Figure 4. 4. (a) Species distribution curve of the M(II) + 5FC + G (1:1:1) ternary system: (1) Co(II); (2) Ni(II); (3) Co(5FC); (4) Ni(5FC); (5) Co(G); (6) Ni(G); (7) Co(5FC)(G); (8) Ni(5FC)(G); (9) Co(5FC)(G)(OH); (10) Ni(5FC)(G)(OH). (b) Species distribution curve of the M(II) + 5FC + G (1:1:1) ternary system: (1) Cu(II); (2) Zn(II); (3) Cu(5FC); (4) Zn(5FC); (5) Cu(G); (6) Zn(G); (7) Cu(5FC)(G); (8) Zn(5FC)(G); (9) Cu(5FC)(G)(OH); (10) Zn(5FC)(G)(OH). (c) Species distribution curve of the M(II) + 5FC + G (1:1:1) ternary system: (1) Cd(II); (2) Ca(II); (3) Cd(5FC); (4) Ca(5FC); (5) Cd(G); (6) Ca(G); (7) Cd(5FC)(G); (8) Ca(5FC)(G); (9) Cd(5FC)(G)(OH); (10) Ca(5FC)(G)(OH). (d) Species distribution curve of the M(II) + 5FC + G (1:1:1) ternary system: (1) Sr(II); (2) Ba(II); (3) Sr(5FC); (4) Ba(5FC); (5) Sr(G); (6) Ba(G); (7) Sr(5FC)(G); (8) Ba(5FC)(G); (9) Sr(5FC)(G)(OH); (10) Ba(5FC)(G)(OH).

are more negative (more exothermic) than those of the corresponding 1:1 M(II)−A, indicating that the higher negative enthalpy changes favor 1:1:1 mixed ligand complex formation and are responsible for their higher stabilities.

Table 4. Δ log K_M[a] Values for the 1:1:1 M(II)[b] + Primary Ligand (L)[c] + Guanine (A) Ternary Complexes As Determined by Potentiometric pH Titration at Different Temperatures and I = 0.1 mol·dm^{-3} $NaNO_3$ in Aqueous Medium

	Δ log K_M							
t/°C	Co(II)	Ni(II)	Cu(II)	Zn(II)	Cd(II)	Ca(II)	Sr(II)	Ba(II)
				M(II)−C−G				
25	+0.95	+1.30	+1.54	+1.30	+0.84	+1.20	+1.38	+1.42
35	+1.66	+0.33	+1.26	+0.80	+1.21	+1.00	+1.58	+1.08
45	+1.14	+1.49	+1.72	+1.33	+0.87	+1.22	+1.42	+1.43
				M(II)−CD−G				
25	+3.36	+2.09	+1.13	+1.63	+2.76	−0.80		−1.53
35	+3.04	+0.65	+1.79	+2.04	+2.90	+1.22	+1.84	−1.22
45	+3.18	+1.92	+1.83	+1.60	+2.62	+0.42	+1.76	
				M(II)−5BrC−G				
25	+3.33	+3.20	+2.44	+3.52	+3.43	+1.81	+1.75	+1.36
35	+3.43	+2.83	+2.81	+3.29	+3.28	+2.16	+1.07	+0.58
45	+3.01	+3.06	+2.53	+3.48	+3.24	+1.82	+1.67	+1.26
				M(II)−5AC−G				
25	+4.12	+4.36	+3.07	+3.94	+4.56	+1.73	+1.57	+1.40
35	+4.24	+4.06	+2.81	+2.87	+4.00	+0.70	+1.88	+0.70
45	+2.77	+4.46	+2.80	+3.69	+4.30	+1.66	+1.55	+1.32
				M(II)−5FC−G				
25	+4.26	+4.34	+3.52	+4.22	+4.74	+1.85	+2.53	+2.46
35	+3.96	+4.30	+3.00	+3.98	+4.46	+0.48	+2.28	+2.99
45	+4.06	+4.16	+4.05	+4.03	+4.51	+1.78	+2.53	+2.51

[a] Δ log K_M = log $K^{M(L)}_{M(L)(A)}$ − log $K^{M}_{M(A)}$. [b] M(II) = Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Ca(II), Sr(II), Ba(II). [c] L = cytosine (C), cytidine (CD), 5-bromocytosine (5BrC), 5-azacytosine (5AC), 5-fluorocytosine (5FC).

Table 5. Δ log K_M[a] Values for the 1:1:1 M(II)[b] + Primary Ligand (L)[c] + Guanine (A) Ternary Complexes As Determined by Potentiometric pH Titrations at Different Temperatures and I = 0.1 mol·dm^{-3} $NaNO_3$ in Aqueous Medium

	Δ log K_M							
t/°C	Co(II)	Ni(II)	Cu(II)	Zn(II)	Cd(II)	Ca(II)	Sr(II)	Ba(II)
				M(II)−C−G				
25	+0.56	+0.78	+0.98	+0.93	+0.54	+0.71	+0.85	+0.73
35	+0.91	−0.31	+0.73	+0.33	+0.77	+1.25	+0.70	+1.08
45	+0.57	+0.69	+0.94	+0.87	+0.54	+0.78	+0.87	+0.80
				M(II)−CD−G				
25	+0.42	+0.41	+0.76	+0.56	+0.44	+0.54	+0.63	+0.51
35	−0.05	−0.86	+1.49	−0.21	+0.67	+1.25	+0.44	+1.08
45	+0.39	+0.34	+0.78	+0.51	+0.44	+0.60	+0.21	+0.87
				M(II)−5BrC−G				
25	+0.30	+0.29	+0.67	+0.50	+0.30	+0.47	+0.54	+0.30
35	+0.22	−0.08	+1.06	+0.35	+0.25	+0.50	−0.08	−0.50
45	+0.32	+0.28	+0.67	+0.49	+0.29	+0.49	+0.50	+0.26
				M(II)−5AC−G				
25	+0.09	+0.20	+0.61	+0.30	+0.20	+0.37	+0.19	+0.34
35	+0.08	−0.04	+0.40	+0.57	−0.33	−0.85	+0.70	−0.10
45	+0.10	−0.18	+0.57	−0.24	+0.17	+0.30	+0.22	+0.31
				M(II)−5FC−G				
25	+0.08	+0.07	+0.09	+0.08	+0.10	+0.20	+0.37	+0.27
35	−0.14	+0.16	−0.15	+0.23	−0.33	−1.02	+0.20	+0.39
45	+0.06	+0.08	+0.07	+0.09	+0.08	+0.17	+0.35	+0.28

[a] Δ log K_M = log$_)K^{M(L)}_{M(L)(A)}$ − log $K^{M}_{M(L)}$. [b] M(II) = Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Ca(II), Sr(II), Ba(II). [c] L = cytosine (C), cytidine (CD), 5-bromocytosine (5BrC), 5-azacytosine (5AC), 5-fluorocytosine (5FC).

Mixed ligand complexes involving cytosine and cytidine show more positive Δ log K values. The higher stability of the above systems is supported by more exothermic values of $\delta\Delta_f H°$ (Table 7). Values are more endothermic for 1:1:1 systems than for 1:1 binary systems. This may be due to the fact that some more water molecules may still be

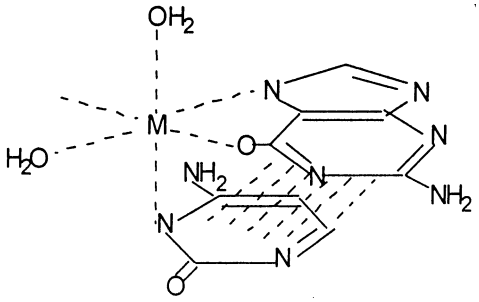

Figure 5. Proposed structure of the ternary complexes of cytosine and guanine showing the stacking interaction in the solution.

Table 6. Thermodynamic Quantities Associated with Binary (1:1) M(II) + Guanine (A) and Ternary (1:1:1) M(II)[a] + Primary Ligand (L)[b] + (A) [$\Delta_f H°$ and $\Delta_f G°$ Values in kJ·mol^{-1}; $\Delta_f S°$ Values in J·mol^{-1}·K^{-1}] Complexes at (25 ± 1) °C and I = 0.1 mol·dm^{-3} $NaNO_3$ in Aqueous Medium

	Co(II)	Ni(II)	Cu(II)	Zn(II)	Cd(II)	Ca(II)	Sr(II)	Ba(II)
				M(II)−G				
$\Delta_f H°$	−1.80	−1.90	−1.90	−1.80	−1.80	+0.11	+0.16	+0.18
$\Delta_f G°$	−3.67	−3.78	−3.82	−3.67	−3.56	−2.49	−2.36	−2.26
$\Delta_f S°$	+6.10	+6.36	+6.41	+6.20	+5.93	+8.71	+8.46	+8.17
				M(II)−C−G				
$\Delta_f H°$	−2.00	−2.00	−2.10	−1.90	−1.90	+0.31	+0.32	+0.44
$\Delta_f G°$	−3.85	−3.92	−4.01	−3.73	−3.62	−2.64	−2.56	−2.42
$\Delta_f S°$	+6.38	+6.36	+6.58	+6.07	+5.88	+9.89	+9.67	+9.59
				M(II)−CD−G				
$\Delta_f H°$	−1.90	−1.90	−2.00	−1.90	−1.90	+0.22	+0.21	+0.23
$\Delta_f G°$	−3.70	−3.80	−3.90	−3.70	−3.60	−2.60	−2.40	−2.50
$\Delta_f S°$	+6.13	+6.36	+6.46	+6.26	+5.78	+9.42	+8.87	+9.34
				M(II)−5BrC−G				
$\Delta_f H°$	−1.80	−1.80	−2.00	−1.90	−1.80	+0.18	+0.18	+0.23
$\Delta_f G°$	−3.60	−3.80	−3.90	−3.70	−3.50	−2.60	−2.50	−2.30
$\Delta_f S°$	+6.09	+6.43	+6.37	+6.23	+5.76	+9.22	+8.86	+8.44
				M(II)−5AC−G				
$\Delta_f H°$	−1.80	−1.90	−2.00	−1.80	−1.80	+0.16	+0.17	+0.19
$\Delta_f G°$	−3.70	−3.90	−4.00	−3.80	−3.60	−2.60	−2.40	−2.40
$\Delta_f S°$	+6.29	+6.49	+6.63	+6.51	+6.15	+9.31	+8.73	+8.59
				M(II)−5FC−G				
$\Delta_f H°$	−1.80	−1.90	−1.90	−1.80	−1.80	+0.12	+0.16	+0.19
$\Delta_f G°$	−3.69	−3.81	−3.84	−3.70	−3.59	−2.57	−2.49	−2.35
$\Delta_f S°$	+6.27	+6.39	+6.49	+6.27	+6.16	+9.04	+8.88	+8.52

[a] M(II) = Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Ca(II), Sr(II), Ba(II). [b] L = cytosine (C), cytidine (CD), 5-bromocytosine (5BrC), 5-azacytosine (5AC), and 5-fluorocytosine (5FC).

attached to the metal ion in 1:1 systems and, when a 1:1:1 complex is formed, more energy is needed in the bond breaking process. There is no regular trend in the $\delta\Delta_f H°$ values that could be compensated by the positive values of $\delta\Delta_f S°$. This offers an evidence for the role of entropy as the primary factor favoring the formation of the neutral metal chelate species in the solution. The positive value of $\delta\Delta_f S°$ and the negative value of $\delta\Delta_f H°$ indicate the extra stabilization of ternary complexes due to the stacking interaction in the ternary systems. Most of the systems show a negative value of $\delta\Delta_f H°$ that can be associated with the energy of stacking, which is purely a van der Waals−London type of interaction.[31]

In conclusion, we can say that all the ligands studied in the present work dissociate two protons. The pK_{1a} and pK_{2a} values of cytosine and its 5-substituted derivatives have been assigned to the N3 and N1/C2=O groups whereas, in the case of cytidine, the first and the second proton dissociation sites are N3 and the sugar moiety, respectively. The species distribution data clearly show that the ternary complexes are more stable than the binary complexes of primary as well as secondary ligands. The stability of the

Table 7. $\delta\Delta_f H°$ and $\delta\Delta_f S°$ Values Associated with Binary (1:1) M(II) + Guanine (G) and Ternary (1:1:1) M(II)[a] + Primary Ligand (L)[b] + Guanine (A) Complexes [$\delta\Delta_f H°$ Values in kJ·mol^{-1}; $\delta\Delta_f S°$ Values in J·mol^{-1}·K^{-1}] at (25 ± 1) °C and I = 0.1 mol·dm^{-3} $NaNO_3$ in Aqueous Medium

	Co(II)	Ni(II)	Cu(II)	Zn(II)	Cd(II)	Ca(II)	Sr(II)	Ba(II)
				M(II)−C−G				
$\delta\Delta_f H°$	−0.10	−0.13	−0.15	−0.10	−0.07	+0.20	+0.16	+0.26
$\delta\Delta_f S°$	+0.28	+0.00	+0.18	−0.13	−0.05	+1.18	+1.20	+1.41
				M(II)−CD−G				
$\delta\Delta_f H°$	−0.01	−0.01	−0.11	−0.05	−0.07	+0.12	+0.06	+0.06
$\delta\Delta_f S°$	+0.02	+0.00	+0.06	+0.06	−0.15	+0.71	+0.41	+1.16
				M(II)−5BrC−G				
$\delta\Delta_f H°$	+0.02	+0.05	−0.10	−0.03	−0.03	+0.08	+0.02	+0.06
$\delta\Delta_f S°$	−0.01	+0.07	−0.03	+0.03	−0.18	+0.51	+0.40	+0.26
				M(II)−5AC−G				
$\delta\Delta_f H°$	+0.02	−0.03	−0.14	−0.01	−0.01	+0.06	+0.02	+0.01
$\delta\Delta_f S°$	+0.18	+0.13	0.22	+0.31	+0.22	+0.60	+0.27	+0.42
				M(II)−5FC−G				
$\delta\Delta_f H°$	+0.03	−0.02	−0.01	−0.01	+0.03	+0.02	+0.01	+0.01
$\delta\Delta_f S°$	+0.17	+0.03	+0.08	+0.07	+0.23	+0.33	+0.42	+0.35

[a] M(II) = Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Ca(II), Sr(II), Ba(II). [b] L = cytosine (C), cytidine (CD), 5-bromocytosine (5BrC), 5-azacytosine (5AC), and 5-fluorocytosine (5FC).

ternary complexes may be attributed to the interligand interaction along with several other factors, like the nature of the metal ion, the geometry of the metal complexes, and solvent effects. Stacking interaction is appreciable only in those aromatic ligands that have a parallel configuration for maximum molecular interaction. Cytosine is mostly unidentate, involving only the N3 group in metal coordination, and can take part in stacking interaction. Guanine is coordinated to metal ions indirectly through N7, involving the possible contribution from N1/C6=O in both binary and ternary systems. The proposed binding sites of primary and secondary ligands in ternary complexes have been further confirmed by IR spectral data of synthesized M(II)−5FC−A complexes. The band corresponding to the δN1−H in-plane bend of 5FC at 1535 cm^{-1} remains almost at the same position in metal complexes, suggesting that the N1 of 5FC was not involved in the bonding. The bands corresponding to νN−H (3135 cm^{-1}) and νC−NH$_2$ + νC=N (1280 cm^{-1}) of 5FC show considerable shifts in complexes, suggesting the participation of N3 in coordination with metal ions, which is supported by the shifting of the νC2=O (1650 cm^{-1}) band of 5FC to the higher frequency side due to nontransfer of electrons from the N3 position to the C2=O group. There are several possible binding sites in guanine, that is, N1, N3, N7, and N9.[41] The νC_6=O frequency (1722 cm^{-1}) of guanine is lowered to an appreciable extent upon the metal complex formation. The infrared spectral studies of the metal complexes exhibit the lowering in the frequency of the νC_5−N$_7$−C$_8$ band (1255 cm^{-1}) in comparison with the νC_2−N$_3$−H and νN$_9$−H bands. This suggests that the guanine coordinates to the metal ions via N1/C6=O and N7 atoms in ternary complexes. It is important to mention here that in the present study guanine and cytosine interact with metal ions at different pH due to their different protonation constants. On the basis of this study, it may be suggested that the different purine and pyrimidine bases of DNA interact with metal ions present in biological systems at different physiological pH in order to maintain the helical structure of DNA.

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