combination electrode was used to determine the hydrogen ion concentration. The electrode system was calibrated by titrating acetic acid directly and comparing the observed pH-meter reading with the actual hydrogen ion concentration as calculated from the data tabulated by Harned and Owen [6]. Values below a pH of 3.5 and above 10.5 were calibrated by measuring the pH in hydrochloric acid and sodium hydroxide solutions respectively. The experimental method employed consisted of potentiometric titration of various ligands involved in this investigation with standard sodium hydroxide solution. The ionic strength was maintained constant at 0.10 mol.dm^{-3} using potassium nitrate solution as supporting electrolyte. A stream of nitrogen gas was passed over the solution during the course of the titration to eliminate the adverse effect of atmospheric carbon dioxide.

The acid dissociation constant K_a and K_{2a} of thiodipropionic acid, thiodiacetic acid, oxalic acid, malonic acid and succinic acid were calculated by graphical method as reported earlier [7]. When simultaneous dissociation of the protons takes place, the dissociaton constants can be calculated using the following relationships.

$$A = \frac{(a - 1)\,T_L + [H^+]}{\{(2 - a)/[H^+]\}T_L - 1} \qquad \ldots (1)$$

$$B = \frac{\{(a - 1)\,T_L + [H^+]\}}{\{[H^+]\,a\,T_L + [H^+]} \qquad \ldots (2)$$

$$C = K_{2a}\,\frac{B}{A} - \frac{1}{K_a} \qquad \ldots (3)$$

where 'a' is moles of base added per mole of the ligand; T_L is total concentration of the ligand species present in the solution.

A plot of B/A *vs* C gives a straight line, the intercept of which gives $1/K_a$ and slope corresponds to K_{2a}.

The pK_a values of the ligands were calculated by direct algebraic method with the help of suitable material balanced equations. The final form of the equation to calculate the dissociation constants by algebraic method are:

$$K_a = \frac{[H^+]\,\{(a - 1)\,T_L + [H^+] - [OH^-]\}}{T_L - \{(a - 1)\,T_L + [H^+] - [OH^-]\}} \qquad \ldots (4)$$

$$\text{and } K^{ML}_{MLA} = \frac{[MLA]}{[ML]\,[A]} \qquad \ldots (5)$$

RESULTS AND DISCUSSION

Titration curves of Cu(II)-guanosine - thiodiacetic acid system is presented in Fig 1. Titration curves with other metal ions with guanosine and acidic ligands - oxalic acid, malonic acid, succinic acid, thiodipropianic acid are all similar. It may be seen that titration curves of metal-acid binary system and metal-guanosine-acid systems are overlaping till m = 2, they diverge after m=2. It appears that guanosine is added in the complex as a secondary ligand after m = 2.

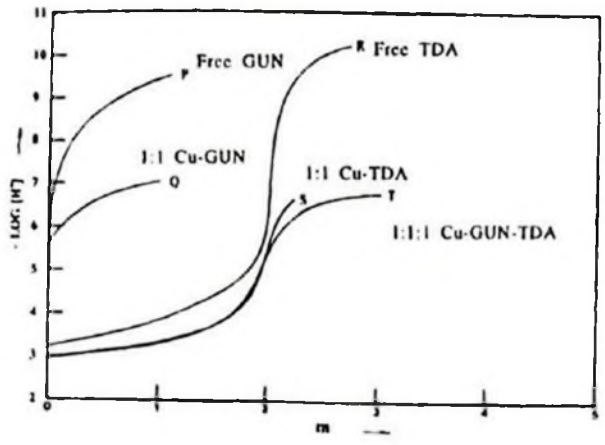


Fig1. Potiontiometric Titration Curves

The data between m = 2 and m = 3 are used to calculate K^{ML}_{MLA}.

Titration curves of metal-guanosine with imidazoles (IMD, BIMD, 5,6- DMBIMD) are similar to the above except that the binary and ternary titration curves overlap till m = 1 and diverge later. Here in these cases, the data between m = 1 and m = 2 are used to calculate K^{ML}_{MLA}.

Titration curves of metal-guanosine-NTA system differs from the above two, in that the binary and ternary curves overlap till m = 3. Here the data between m = 3 and m = 4 are used to calculate K^{ML}_{MLA}.

Site of Coordinate Bonding

Guanosine is a weak base. In presence of equimolar hydrochloric acid, N_7 is the only site available for protonation. This is confirmed by the X-ray diffraction studies of guanosine hydrochloride [8], IR studies of the guanosine cation [9] and a comparison of the UV spectra of the cations of 7,9-dimethyl and 1,7,9-trimethyl guanine with those of 7-methyl guanosine and 1,7-dimethyl guanosine [10]. Guanosine, Xanthosine and Inosine are structurally similar but for the C_2 position. The pK_a values of guanosine are very close to those of guanine and hypoxanthine [11]. It is of interest to compare the second dissociation constant of guanosine with dissociation constant of inosine [12]. The similarity of these ionization constants indicates that the proton ionization may be from the same site N_1H/C_6O. ^1H NMR studies of Pt(II) - guanosine complex [13] shows the signals due to the C_8H proton at δ = 8.86 ppm. The C_8H signal shifted to down field compared to that of free guanosine (δ = 7.80 ppm) indicating N_7 of guanosine as the site of coordination.

The influence of hard and soft metals on the ^{13}C NMR spectrum of guanosine and inosine under both neutral and basic conditions in DMSO has been determined by Marzilli *et. al.* [14]. Based on the ^{13}C chemical shifts of C_6 [14], it is confirmed that guanosine binds to metal through C_6-O of the guanosine. It could be argued that the metal is binding at a combination of N_1, O_6 or N_7, O_6. Based on ^{13}C chemical shift data, these authors proposed that guanosine binds to metals through N_7 and C_6-O. Sigel [15] has also mentioned N_7 as the most prominent site for binding. ^{15}N NMR chemical shifts are highly sensitive to site-selective diamagnetic metal ion binding for guanosine. Buchanan and Stothers reported large chemical shift changes

for the ^{15}N spectra of nucleosides on addition of $HgCl_2$ or $Zn(NO_3)_2$ to the nucleoside [16]. The induced ^{15}N up-field shifts show that N_7 is the preferred nitrogen for Hg and Zn complexes. This is further supported by pronounced up-field shift of N_7 upon protonation [17] whereas minor changes are seen for the remaining four nitrogens. The electron density calculations [18] showed that in guanosine C_6-O and N_7 are the metal binding sites. Based on this, we also propose N_7 and C_6-O as the probable binding sites in guanosine.

The stabilities of the 1:1 metal guanosine complexes decreased in the order $Cu^{2+} > Zn^{2+} > Ni^{2+} > Co^{2+}$. Similar trends were observed in the corresponding nucleotides GTP and GMP [19].

The stability constants of 1:1 system are in the order $Cu^{2+} > Ni^{2+} > Zn^{2+} > Co^{2+}$. A comparison of stabilities of these systems (Table 1) indicates that the stabilities decrease in the order; metal^{2+} -Ethylene diamine > metal^{2+} -IMD >metal^{2+} -DMBIMD > metal^{2+} -BIMD. They are in accordance with basicity order. Ethylenediamine acts as a bidentate ligand whereas others are monodentate and having a capacity to participate in $d\pi$-$p\pi$ interations. Hence, metal^{2+} -EN complexes are more stable than metal^{2+} -IMD or BIMD or DMBIMD complexes.

Ternary complexes of guanosine are more stable in some systems and less stable in some others depending on the nature of secondary ligand. Then the question arises that what are the factors or the driving force, responsible for the formation of stable ternary complex in solution. Some of these factors can be outlined as 1) statistical reasons, 2) charge neutralization in the mixed ligand complex, 3) steric factors (bulky groups and ring size of chelate), 4) formation of π bonds and 5) stacking interaction.

One or more of these factors may be responsible for overall stability of the complex. The enhanced or reduced stability of the ternary complex is usually measured in terms of Δ log K. This is the difference in the stabilities of ternary and corresponding binary complexes. If the Δ log K values are

Table 1

Dissociation Constant of Ligands and Stability Constants of Metal(II)- Ligand Binary Complex

T = 35°; μ = 0.1 M KNO_3

Ligand (A)	pK^H of Ligand 1	2	3	log K$^M_{MA}$ of metal ions Cu^{2+}	Ni^{2+}	Co^{2+}	Zn^{2+}
GUN	3.09	9.02	-	5.50	3.39	3.04	4.08
IMD	6.88	-	-	4.28	3.07	2.42	2.69
DMBI	5.96	-	-	3.45	3.05	2.35	2.60
BIMD	5.57	-	-	3.05	2.84	2.10	2.40
PYDCA	2.48	4.75	-	5.68	5.15	4.94	4.78
TDA	2.84	4.24	-	4.53	4.24	3.60	3.70
TDPA	3.95	4.71	-	3.35	3.14	2.46	2.75
IMDA	2.90	9.13	-	10.56	8.76	7.83	7.79
NTA	1.35	2.70	9.55	13.37	11.57	10.41	10.85
OXA	1.03	3.88	-	5.09	4.92	4.21	4.35
MAA	3.17	5.35	-	5.05	3.51	3.08	3.23
SUA	4.13	5.22	-	3.23	2.89	2.37	2.85

positive, ternary complexes are more stable than the binary complexes and if the values are negative, the ternary complexes are less stable. The inter ligand interactions have been found to be most effective in deciding the stability of the ternary complexes in solution along with other factors like the nature of the metal ion and the geometry of the metal complexes.

In 1:1:1 ternary M^{2+} -GUN-L (L=TDA, TDPA, IMDA, NTA, OXA, MAA, SUA) systems, the complex formation is step wise; Ligand 'L' forms 1:1 complex in the lower buffer region followed by a formation of a mixed ligand complex in the higher buffer region by guanosine. The stability constants for the 1:1:1 metal A-guanosine systems are less stable than the corresponding 1:1 systems Table 1. This is in accord with the statistical considerations. As TDA, TDPA, NTA, IMDA, OXA, MAA and SUA have two negative charges, it can be expected that the tendency of N,O donor (guanosine) to combine with MA should be much less than the tendency to combine with M^{2+} (aq). This results in more negative Δlog K values. In these cases, no hydrophobic or stacking interactions are possible between nucleoside and dinegative ligand.

It is observed that ternary complexes of guanosine with oxalates are more stable (less

Table 2

Stability Constants of Ternary Complexes of Bivalent Metal Ions and Δlog K values

GUN as the primary Ligand; T = 35°; μ = 0.1 M KNO_3

Complex of metal ions	log K^{MA}_{MAL}				Δlog K = log K^{MA}_{MAL} - log K^{M}_{MA}			
	Cu	Ni	Co	Zn	Cu	Ni	Co	Zn
Metal(II)-GUN-IMD	5.65	3.45	3.16	4.18	+0.13	+0.06	+0.12	+0.10
Metal(II)-GUN-DMBI	5.80	3.64	3.18	4.90	+0.28	+0.25	+0.14	+0.82
Metal(II)-GUN-BIMD	5.70	3.55	3.06	4.72	+0.18	+0.16	+0.04	+0.64
Metal(II)-GUN-PYDCA	5.15	3.05	2.85	3.85	+0.07	+0.11	-0.02	-0.01
Metal(II)-GUN-TDA	5.36	3.38	2.80	3.87	-0.17	-0.01	-0.24	-0.21
Metal(II)-GUN-TDPA	5.33	3.36	2.95	4.02	-0.19	-0.03	-0.09	-0.06
Metal(II)-GUN-IMDA	4.33	2.52	2.19	3.62	-1.19	-0.87	-0.85	-0.46
Metal(II)-GUN-NTA	3.44	2.29	1.81	1.92	-2.08	-1.10	-1.23	-2.16
Metal(II)-GUN-OXA	4.60	2.98	2.67	3.62	-0.92	-0.41	-0.37	-0.46
Metal(II)-GUN-MAA	4.93	3.23	3.15	4.09	-0.59	-0.16	-0.04	-0.10
Metal(II)-GUN-SUA	5.06	3.38	3.19	4.08	-0.46	-0.01	-0.02	-0.02

negative Δ log K values) than malonate and succinate complexes. In oxalate ternary complexes, both ligands form 5 membered rings whereas latter complexes have 5 & 6 membered rings and 5 & 7 membered rings respectively. Two 5 membered rings are more stable than 5 & 6 or 5 & 7 membered rings.

The 1:1:1 metal^{2+} -GUN-L (L = IMD or BIMD or DMBIMD) complexes form step-wise complexation. The stability constants for the 1:1:1 metal - guanosine-L system and the Δ log K values are given in Table 2. The Δ log K values show that metal guanosine-L systems are more stable than the corresponding M-L system. This is contrary to statistical considerations.

It is worthwhile to compare the relative stabilities of ternary complexes containing OXA, SUA, IMDA and NTA with ternary complexes of IMD, BIMD, PYDCA etc. The Δ log K values of ternary complexes involving IMD, BIMD and GUN are more positive relative to the corresponding complexes containing IMDA, NTA, TDPA, TDA, OXA etc. The higher stability of IMD or BIMD is due to favourable electrostatic interaction between the positively charged (M-IMD^{2+} or M-BIMD^{2+}) complexes and the mononegative guanosine. Similar stabilization does not occur with neutral complexes (M-IMDA or TDA or oxalic acid etc.) or negatively charged (M-NTA)$^{-}$ complexes. Metal-ligand back bonding in binary (M-IMD) complexes causing the metal center to become more positive and hence favouring the interaction with anionic guanosine. In binary (M-IMDA) or (M-NTA) and (M-TDA or OXA) complexes, the absence of such back bonding effects allows electron density to build upon metal center and the interaction with secondary ligand guanosine is not favoured.

In addition, ternary M^{2+} with IMD or BIMD or DMBIMD (which possesses aromatic side chains) and guanosine are considerably stabilized due to intra molecular metal ion mediated stacking interaction between the aromatic moieties of the two ligands. M-NTA-GUN systems are less stable than M-IMDA-GUN due to statistical factor (IMDA occupies two sites on the metal ion whereas NTA occupies three sites).

The Δ log K values of the M^{2+}-PYDCA-GUN are less positive compared to mixed ligand complexes involving M-IMD or BIMD. PYDCA is mononegatively charged (NO^{-}), whereas IMD, BIMD and DMBIMD are neutral N donor ligands, but compared to dinegative IMDA or TDA & trinegative NTA, the Δlog K values of ternary M^{2+}-PYDCA-GUN are less negative. The higher stability can be attributed to the π acidic character of PYDCA which stabilizes the ternary complex and makes Δ log K values less negative.

As an aid in visualizing the significance and implication of the equations used for the calculation of stability constants of the binary and ternary systems, species distribution diagram for free ligands, 1:1 metal ligand (ML) and 1:1:1 ternary (MAL) and free metal ion as a function of pH were drawn up using

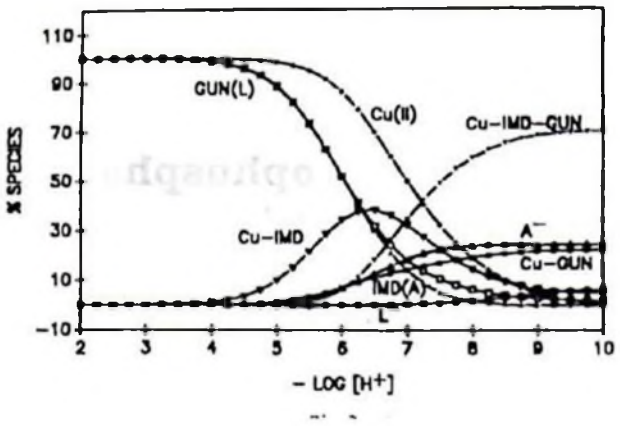


Fig.2 Species Distribution Curves

1:1 Cu-GUA GUN and 1:1:1 Cu-GUA-IMD

program SPE (BEST). As the pH is varied from 2 to 12, the uncomplexed M^{2+} changes to complexed species. In Fig 2, the distribution curves of Cu^{2+}-GUN-IMD are given. These curves support the formation of mixed ligand complexes. The free Cu^{+2} ion concentration decreases with increase in the pH. The ternary Cu^{2+}-IMD-GUN are more stable than their corresponding binary complexes and hence its % species is much higher than binary species. Up to 6 pH, the ternary complex formation is negligible. Later, it reaches to 75% as the pH increases, whereas in Cu^{2+}-PYDCA-GUN, it reaches to 85%. The ternary sysems reported in this investigation clearly show the dominant influence which the primary ligand already attached to a metal ion exerts upon the interaction of the metal ion with an incoming secondary ligands. The factors operating in the stabilization of model ternary systems may also play an important role in determining the stability of enzyme-catalyzed reactions in biological systems.

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