Equilibrium Studies of Cytidine 5'-triphosphate & Nicotinic acid with Metal-cations [Co(II), Ni(II), Cu(II), Cd(II), Mg(II) and Ca(II)]

RAJENDRA KUMAR[1], BRAJESH KUMAR SHUKLA[1],
SHALINI VERMA[1] and V. KRISHNA[2]

[1]Department of Chemistry, University of Allahabad, Allahabad-211002, India.
[2]Department of Chemistry, Ewing Christian College, Allahabad-211003, India.
Corresponding author e-mail vkrishnaalld@rediffmail.com

Abstract

In this study, equilibrium experiments on the recovery of nicotinic acid (0.01M) were performed using Cytidine5-triphosphate (0.01M) & metal-cations {Co(II), Ni(II), Cu(II), Cd(II), Mg(II) and Ca(II)} with double distilled water. The chemical equilibrium of acid and complexes is interpreted as a result of the formation of 1:1binary complex. The formation of various 1:1:1ternary mixed ligand complexes were inferred from the potentiometric titration curves under experimental conditions (constant ionic strength I =0.1 mol/dm^3, at 37±0.1^{0}C). Initial estimates of the formation constants of the resulting species and the acid dissociation constants of nicotinic acid and Cytidine 5'-triphosphate with metal-cations as said above, have been refined through Stability Constant of Generalized Species (SCOGS) computer program.

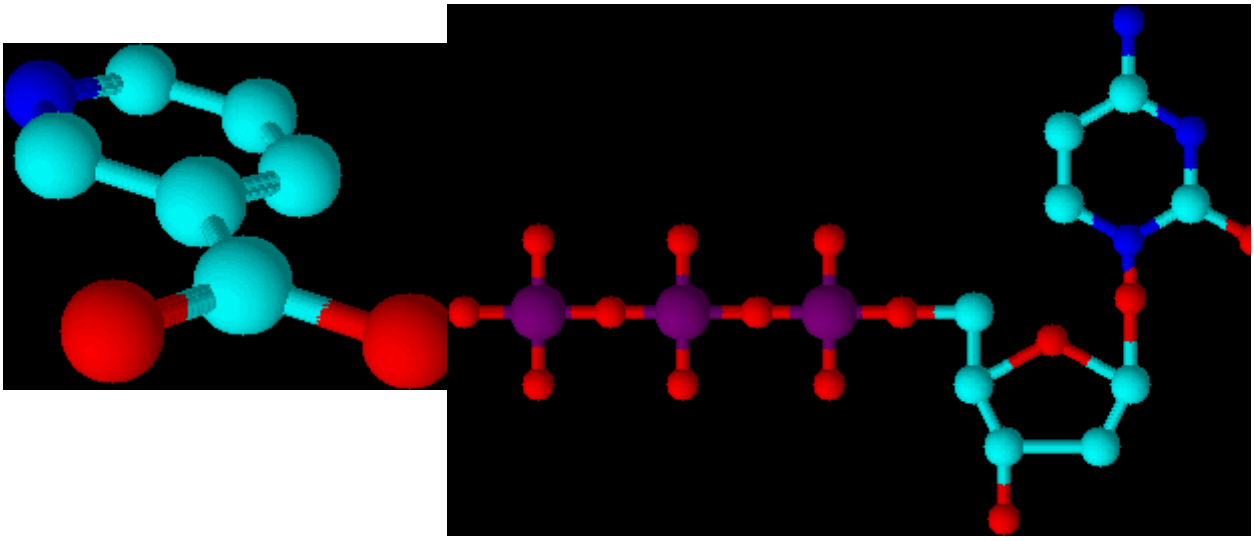
Keywords–Metal-cat ions, Nicotinic acid, Cytidine 5'-triphosphate, pH-meter, SCOGS.

Introduction

Pyridine carboxylic acids (picolinic, nicotinic, and iso-nicotinic acids) are attractive because of their potential use and importance in metabolic reactions. In particular, their natural and synthetic derivatives are used in medicinal chemistry because of their physiological behaviour (1-3). Nicotinic acid (niacin) is a water-soluble vitamin (B-3), which is used for the treatment of schizophrenia, hypercholesterolemia, diabetes, osteoarthritis, autoimmune diseases and pellagra (4)

The effective centres of coordination with metal ions {Co(II), Ni(II), Cu(II), Cd(II), Mg(II) and Ca(II)} are the donor nitrogen atoms N(3) from pyrimidine bases and the oxygen atoms from the phosphate groups of the cytidine 5'-triphosphate (nucleotide). These centres are also the sites of non- covalent interactions with the other bio-ligands present in living organisms, such as small organic, polyamines, polycations or amino acids (5, 6). To quantify the metal ion-base interaction in $M(CTP)^{2-}$ the intramolecular equilibrium between an "open" isomer, $M(NTP)^{2-}$ open and a "closed" species, $M(NTP)^{z-}$, has to be considered. Use of the terms open and closed (macrochelate) does not imply a single fixed structure fitting each description. The term ***open*** refers to a family of conformations, though several structures may also occur in the closed form. The distinction between the terms should be viewed operationally the greater stability constant observed with $M(CTP)^{2-}$ implies structures not occurring with $Cu(UTP)^{-}$ and $Cu(TTP)^{2-}$ these additional structures are termed ***closed.*** N-3 is known to be an effective binding site in cytidine complexes (7-8,9-11). Apparently, Cu^{2+} is the only metal ion with a large enough

affinity for N-3 to force part of CTP^{4-} in $M(CTP)^{2-}$ into a syn-conformation. $Cu(CTP)^{-}$ is, by about 0.17 log unit, more stable than expected for a pure triphosphate coordination (12).



3D- Structure of Nicotinic acid

3D- Structure of Cytidine 5'-triphosphate

Material and Methods

Stock solutions of Nicotinic Acid and cytidine 5'- triphosphate were used as such Carbonate free sodium hydroxide solution which is prepared by standard method (13). Metal nitrate solutions were prepared and standardized by EDTA titration. Solution of NaOH and HNO_3 were prepared in double distilled water and standardized against standard oxalic acid as usual.

The ionic strength of all mixture solutions was kept 0.01M $NaNO_3$.The free acid concentration was kept 0.02M in each case. The following sets of solutions were prepared keeping the total volume 50 ml in each case. The molar ratio of binary and ternary system was taken in 1:1 & 1:1:1 ratio respectively (14).

5ml $NaNO_3$ (1.0M) + 5ml HNO_3 (0.02M) + H_2O
5ml $NaNO_3$ (1.0M) + 5ml HNO_3 (0.02M) + 5ml L_1 (0.01M) + H_2O
5ml $NaNO_3$ (1.0M) + 5MLHNO_3 (0.02M) + 5ml L_2 (0.01M) + H_2O
5ml $NaNO_3$ (1.0M) + 5ml HNO_3 (0.02M) + 5ml M (0.01M) + H_2O
5ml $NaNO_3$ (1.0M) + 5ml HNO_3 (0.02M) + 5mlM (0.01M) + 5ml L_1 (0.01M) + H_2O
5ml $NaNO_3$ (1.0M) + 5ml HNO_3 (0.02M) + 5ml M (0.01M) + 5ml L_2 (0.01M) + H_2O
5ml $NaNO_3$ (1.0M) + 5ml HNO_3 (0.02M) + 5ml M (0.01M) + 5ml L_1 (0.01M) + 5ml L_2 (0.01M) + H_2O

Where M is {Co(II), Ni(II), Cu(II), Cd(II), Mg(II) and Ca(II)} metal ions and L_1, L_2 are Nicotinic Acid and Cytidine 5'-triphosphate respectively. The titration curves of pH solution verses volume of NaOH for each set of solutions were plotted for potentiometric behaviour of solution at different pH. The species distribution curves were obtained by plotting % concentration vs. pH of the species through ORIGIN61 software.

Results and Discussion

New computer program SCOGS (Stability Constant of Generalized Species) developed by Sayce which employs the conventional non linear least square approach. The program is written in FORTRAN IV (15). It is capable of calculating simultaneously or individually , association constants for any of the species formed in the system containing up to two metals and two ligands, provided that the degree of complex formation is pH dependent. Thus, SCOGS may be utilized to analyse appropriate pH titration data to yield metal-ion hydrolytic constants, stability constants of simple complexes (ML_1, ML_2 and ML_1L_2 etc.). SCOGS may also be used to calculate constants for "mixed" complexes containing two different metals and two different ligands. The protonation constants of the ligand were calculated from the potentiometric pH titration data of solutions according to Irving and Rossetti's method (16) and the acid dissociation constants for the sodium salt of Cytidine 5'-tri phosphate anion (H_3L_1-) are related to the dissociation equilibrium as follows:

$H_3L_1^- \Leftrightarrow H_2L_1^{2-} + H^+$ .....(1)
$H_2L_1^{2-} \Leftrightarrow H L_1^{3-} + H^+$ .....(2)
$H L_1^{3-} \Leftrightarrow L_1^{4-} + H^+$ .....(3)

The experimental data of the potentiometric pH titration for both Mg (II) and Ca(II) binary systems are completely described by the following equations:

$M^{2+} + HL_1^{3-} \Leftrightarrow MHL_1^-$ .....(4)
$K_1 = [MHL_1^-]/[M^{2+}][HL_1^{3-}]$
$M^{2+} + L_1^{4-} \Leftrightarrow ML_1^{2-}$ .....(5)
$K_2 = [ML_1^{2-}]/[M^{2+}][L_1]$
$ML_1(qa)^{2-} \Leftrightarrow [ML_1(OH)]^{3-} + H+$ .....(6)
$K_3 = [ML_1(OH)^{3-}][H+]/[ML_1(aq)^{2-}]$

The formation of ternary complexes in an aqueous solution may be conveniently expressed by the equilibrium:

$pM + rL_1 + sL_2 + tOH \Leftrightarrow (M)p(L_1)r(L_2)s(OH)t$ .....(7)

The overall stability constant is given by the equation:

$\beta prst = [(M)p(L_1)r(L_2)s(OH)t] / [M]^p[L_1]^r[L_2]^s[OH]^t$

Where L_1 stands for the Cytidine 5'-triphosphate and L_2 stand for nicotinic acid ($C_6H_5NO_2$) and the stoichiometric numbers p, r, s are either zero or positive integer and't' is a negative
-integer for a protonated species, positive values for a hydroxo or deprotonated species and zero for a neutral species.
The overall stability constant (βprst) defined may be used to calculate the species distribution curves that provides the clues for the formation equilibria of the complexes. After the description of species distribution curve through SCOGS, following equilibria have been proposed:

$L_2^- + M^{2+} \Leftrightarrow ML_2^+$ .....(8)

The formation of ternary complex may be explained as per the following equilibrium:

$ML_1 + L_2H \Leftrightarrow MHL_1L_2$ ..... (9)

$ML_1 + L_2H^- \Leftrightarrow ML_1L_2^{2-} + H^+$ ..... (10)

Where M = Metal ions, L_1 represents the sodium salt of cytidine 5'-triphosphate and L_2 represents the Nicotinic acid ($C_6H_5NO_2$) . The Ternary (1:1:1) complexes have been used in this study to ensure the exclusive formation of the $[M(C_6H_5NO_2)(CTP)]^{3-}$ type complex,

Table1: Binary complex potentiometric titration data of (1) Acid (2) Acid+Ligand (3)Acid+Ligand+Mg (4) Acid+Ligand+Ca (5) Acid+Ligand+Cu (6) Acid+Ligand+Cd of binary (1:1) systems.

NaOH of the solu -tion	Acid	Acid + ligand	Acid+ Ligand + Mg	Acid+ Ligand+ Ca	Acid+ Ligand+ Cu	Acid+ Ligand+ Cd
0	2.42	2.48	2.73	2.75	2.68	2.7
0.2	2.5	2.54	2.78	2.8	2.77	2.75
0.4	2.56	2.61	2.88	2.88	2.85	2.82
0.6	3.6	2.68	3.03	3.04	3.01	2.92
0.8	3.72	2.76	3.2	3.23	3.21	3.15
1	3.81	3.86	3.77	3.78	3.54	3.67
1.2	4.02	4.1	4.91	5.48	4.41	4.97
1.3	4.3	4.34	6.78	6.59	5.68	6.5
1.6	4.32	4.96	10.53	8.01	6.9	8.24
1.8	4.48	6.2	10.76	10.02	8.63	10.12
2	6.36	9.32	11 10.49	10.31	10.65	
2.2	9.35	9.48	11.09	10.77	10.67	10.84
2.3	11.56	11.52	11.11	10.93	10.9	10.94
2.6	--	11.98	11.17	11.02	11.06	11.04
2.8	--	--	11.22	11.11	11.17	11.17
3	--	--	11.27	11.17	11.3	11.23

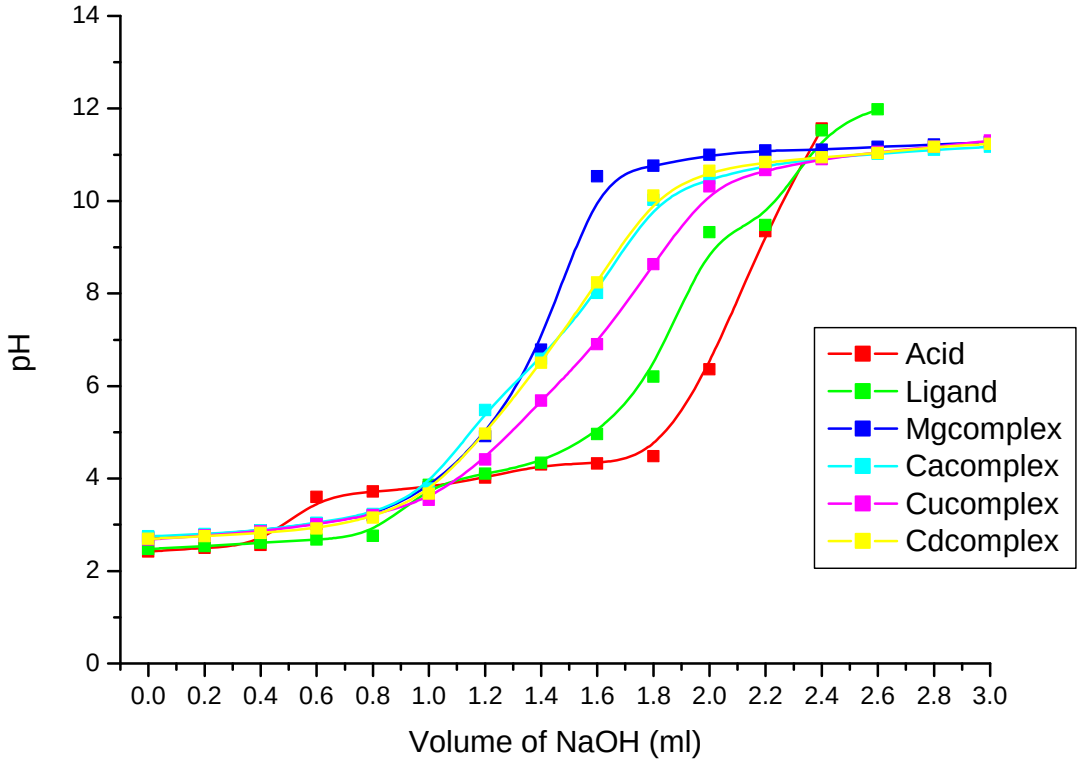


Fig.1 Experimental titration curve of (1) Acid, (2) Acid+ligand (3) Acid+Ligand+Mg (4) Acid+Ligand+Ca (5) Acid+Ligand+Cu (6) Acid+Ligand+Cd

Table-2 Proton ligand formation constant, Stability constant and other related constants of the binary and ternary species of metal-cation Mg (II) complexes with cytidine 5'-triphosphate and nicotinic acid at different pH

pH	H_3L_1	H_2L_1	HL_1	HL_2	ML_1	ML_2	ML_1L_2
2.72	4.31	-	0.45	95.1	4.42	-	4.88
2.77	11.0	0.16	0.84	87.8	11.4	0.22	12.2
2.88	12.1	0.29	1.27	85.7	12.7	0.54	14.3
3	11.1	0.47	1.80	85.5	9.58	1.13	14.5
3.24	5.74	0.73	2.82	87.6	7.47	3.08	12.4
3.77	0.32	0.47	3.85	88.7	8.09	6.68	11.3
4.51		0.10	4.19	87.7	8.90	7.98	12.3
5.70			4.42	86.7	9.70	8.89	13.3
6.95			4.63	85.7		9.70	14.3

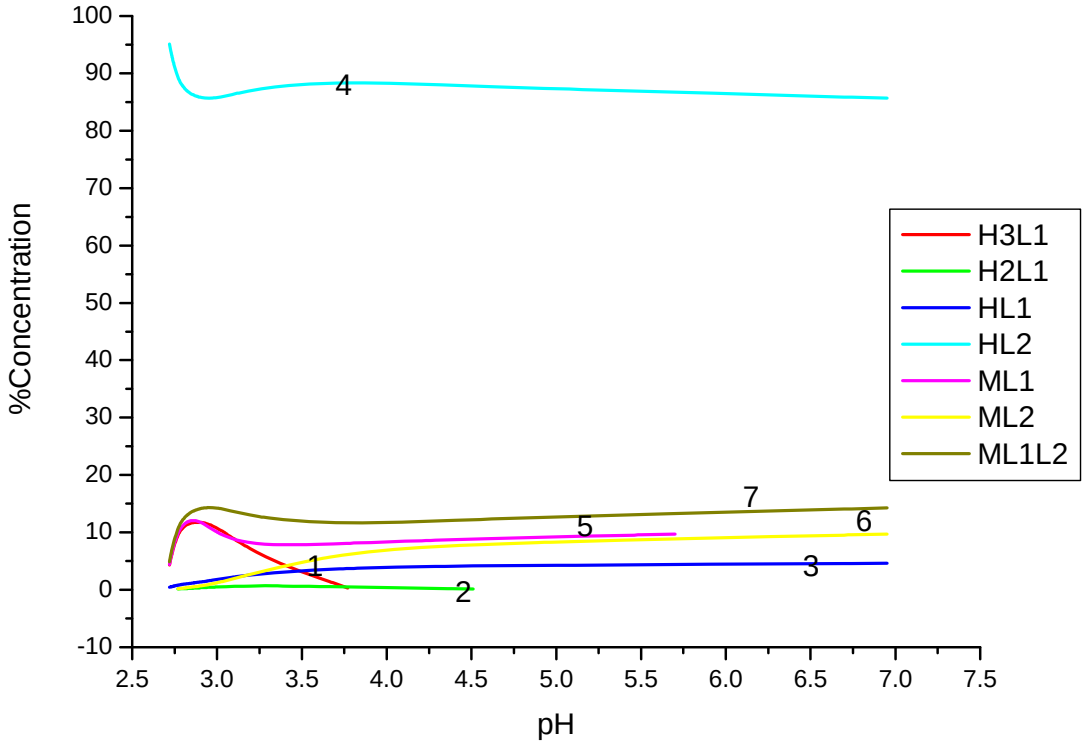


Fig.2 Species distribution curve of 1:1:1 ternary Mg(II)L_1L_2-system (1)H_3L_1 (2)H_2L_1 (3)HL_1(4)HL_2 (5)Mg(II)L_1 (6)Mg(II)L_2 (7)Mg(II)L_1L_2

Table-3 Proton ligand formation constant, Stability constant and other related constants of the binary and ternary species of metal-cation Ca (II) complexes with cytidine 5'-triphosphate and nicotinic acid at different pH

pH	H_3L_1	H_2L_1	HL_1	HL_2	ML_1	ML_2	ML_1L_2
2.67	56.1	0.52	88.7	39.5	7.31	3.2	0.58
2.75	71.9	0.97	95.7	25.2	2.73	0.53	1.31
2.87	66.5	1.56	96.3	28.7	1.94	0.44	2.77
3.01	53.9	2.41	96	37.2	1.63	0.54	5.91
3.80	21.4	3.63	93.7	56.3	1.59	1.21	17.4
3.73	1.92	2.36	86.2	62.7	2.51	2.69	30.4
4.49	0.48	54.4	56.4	6.97	7.39	35.7	
5.83		5.55	48.6	12.3	12.3	39.1	
6.70		0.8	47.7	12.4	11.4	40.9	

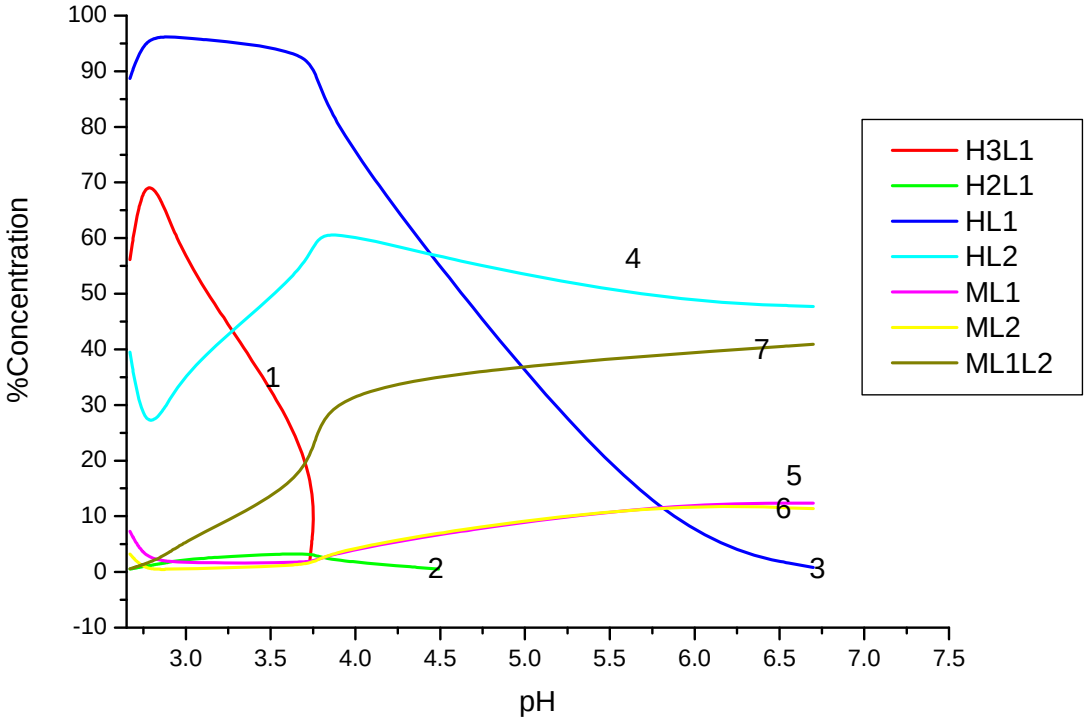


Fig.3 Species distribution curve of 1:1:1 ternary Ca(II)L_1L_2-system (1)H_3L_1 (2)H_2L_1 (3) HL_1 (4)HL_2(5)Ca(II)L_1 (6)Ca(II)L_2 (7)Ca(II)L_1L_2

Conclusion

The tendency of complex formation in binary and ternary system of metal and ligand has represented their coordination behaviour in terms of stability. This study, describes the relative stability constants and possible species concentration of metal complexes in binary and ternary system. The highest species percent concentration of (1:1) binary complexes of Ca(II)-L_1, Mg(II)-L_1,Ca(II)-L_2 and Mg(II)-L_2 systems were found to be 12.3, 12.7, 40.9 and 9.70% at different pH range from 5.53-5.83, 2.73-2.88, 6.64-6.70 and 6.92-6.95 respectively, while (1:1:1) ternary system L_2-Ca(II)-L_1 is 30.4% at (3.70-3.73) pH & L_2-Mg(II)-L_1 is 14.5% at pH =3.

Acknowledgement

The authors are thankful to the Head, Department of Chemistry, University of Allahabad, Allahabad for their continuous support and providing all necessary facilities for the research work.

References

[1] C.L.Broadhurst, P. Domenico, Clinical Studies on Chromium Picolinate Supplementation in Diabetes Mellitus焜A Review. Diabetes.Technol. Ther. 2006, 8, 677.

[2] G. D. Yadav, S. S.Joshi, P. S. Lathi, Enzymatic Synthesis of Isoniazid in Non-Aqueous Medium. Enzyme Microb. Technol. 2005, 36, 217.

[3] F.Wu, Z. Q.Huang, N. Y. Hu, Electrochemical Synthesis of 4- Picolinic Acid. J. Guangdong Chem. Eng. 1991, 4, 21.
[4] M. Cantarella, L. Cantarella, A.Gallifuoco, R.Intellini, O. Kaplan, A.Spera, L. Martınkova, Amidase-Catalyzed Production of Nicotinic Acid in Batch and Continuous Stirred Membrane Reactors. Enzyme Microb. Technol. 2008, 42, 222.
[5] R. B. Martin, "Nucleoside sites for transition metal ion binding," Accounts of Chemical Research, 1985, vol. 18, no. 2, pp. 32-38.
[6] B. de Castro, J. Pereira, P. Gameiro, and F. C. Castro, "Multinuclear NMR and potentiometric studies on the interaction of zinc and cadmium with cytidine and glycylglycine. The effect of the anion," Journal of Inorganic Biochemistry, 1992. vol. 45, no. 1, pp. 53–64.
[7] Helmut Sigel,*Ia Roger Tribolet,la Raman Malini-Balakrishnan,'" and R. Bruce Martin, American Chemical Society, 1987, Vol. 26, No. 13.
[8] IbMartin, R. B. Acc. Chem. Res. 1985, 18, 32-38.
[9] Mariam, Y. H.; Martin, R. B. Inorg. Chim. Acta 1979, 35, 23-28.
[10] Scheller, K. H.; Hofstetter, F.; Mitchell, P. R.; Prijs, B.; Sigel, H. J. Am. Chem. SOC. 1981, 103, 247-260.
[11] Martin, R. B. Fed. Proc., Fed. Am. SOC. Exp. Biol. 1961, 2O(No. 3, Suppl. lo), 54-59.
[12] Kim, S. H.; Martin, R. B. Inorg. Chim. Acra 1984, 91, 19-24.
[13] Vogel AI, A Text Book of Quantitative Analysis, Longman, London, 1961,241.
[14] R. Kumar, B.K. Shukla, S. Verma and V. Krishna, International Journal of Chemical Studies, 2015, 3(4), 20-23.
[15] I.G. Sayce, Talanta, 1968,15,1397.
[16] A.F. Parisi, and B.L. Valleem, Am J Clin Nutr, 1969,22,1222.