where βn (overall stability constant) is the product of stepwise formation constant ($K_1, K_2, K_3, \ldots\ldots. K_n$).

The protonation of the ligand L occurs in steps exactly in the same way as complexation reaction consequently following proton ligand equilibria may be considered.

$$L + H \rightleftharpoons HL$$

$$HL + H \rightleftharpoons H_2L$$

$$H_2L + H \rightleftharpoons H_3L$$

$$H_{n-1}L + H \rightleftharpoons H_nL \quad \ldots\ldots\ldots\ldots (5)$$

The proton ligand stability constants thus are given by,

$$K_1^H = \frac{[HL]}{[H]\,[L]}$$

$$K_2^H = \frac{[H_2L]}{[HL]\,[H]}$$

$$K_3^H = \frac{[H_3L]}{[H_2L]\,[H]}$$

$$K_n^H = \frac{[H_nL]}{[H_{n-1}L]\,[H]} \quad (6)$$

Hence βn^H, the overall proton ligand stability constant is determined by,

$$\beta_n^H = \frac{[H_nL]}{[H]^n\,[H_{n-1}L]} \quad \ldots\ldots\ldots\ldots\ldots (7)$$

The formation of mixed ligand (Ternary systems) and mixed metal-mixed ligand Quaternary systems can be considered to take place as follows:

Mixed ligand ternary systems

$$M + L_1 + L_2 \rightleftharpoons ML_1L_2$$

$$\beta_{ML1L2} = \frac{[ML_1L_2]}{[M]\,[L_1]\,[L_2]} \quad \ldots\ldots\ldots\ldots (8)$$

Mixed metal-mixed ligand quaternary systems

$$M_1L_1L_2 + M_2 \rightleftharpoons M_1M_2L_1L_2$$

$$\beta_{M1M2L1L2} = \frac{[M_1M_2L_1L_2]}{[M_1L_1L_2]\,[M_2]} \quad \ldots\ldots\ldots\ldots (9)$$

$$M_1 + M_2 + L_1 + L_2 \rightleftharpoons M_1M_2L_1L_2$$

$$\beta_{M1M2L1L2} = \frac{[M_1M_2L_1L_2]}{[M_1]\,[M_2]\,[L_1]\,[L_2]} \quad \ldots\ldots\ldots\ldots (10)$$

2.2 pH- METRIC TITRATION

In the present paper a comprehensive study has been done on mixed ligand MAB (ternary) in 1:1:1 and 1:2:1 molar ratio and mixed metal–mixed ligand M_1M_2AB (quaternary) systems in 1:1:1:1 molar ratio of Hg (II) and Pb (II) with 2-Aminosuccinic acid (A) and 5-methyluracil (B) by potentiometry in aqueous medium using Bjerrum's [7] method modified by Irving and Rossotti [8]. All the solutions were prepared in double distilled water. The pH measurements were done by an electric digital pH meter (Eutech 501) with a glass electrode supplied with the instrument and working on 220V/50 cycles stabilized by A.C. mains. The pH meter has a reproducibility of ±0.01 pH. Following solution mixtures were prepared keeping the total volume 50 ml in each case:

Solution A: 5 ml $NaNO_3$ (1.0 M) + 5ml HNO_3 (0.02M) + 40 ml water

Solution B: 5 ml $NaNO_3$ (1.0 M) + 5ml HNO_3 (0.02M) + 5ml A (0.01M) + 35 ml. water

Solution C: 5 ml $NaNO_3$ (1.0 M) + 5ml HNO_3 (0.02M) + 5ml A (0.01M) + 5 ml M (II) (0.01M) + 30 ml water.

Mixed ligand ternary system (1: 1: 1) (M: A: B)

Solution D: 5 ml $NaNO_3$ (1.0 M) + 5ml HNO_3 (0.02M) + 5ml A (0.01M) + 5 ml M (II) (0.01M) + 5 ml B (0.01M) + 25 ml water

Mixed ligand ternary system (1:2: 1)(M:A: B)

Solution D: 5 ml $NaNO_3$ (1.0 M) + 5ml HNO_3 (0.02M) + 10ml A (0.01M) + 5 ml M (II) (0.01M) + 5 ml B (0.01M) + 20 ml water

Mixed metal- mixed ligand quaternary system (1: 1: 1: 1) (M_1: M_2: A: B)

Solution E : 5 ml $NaNO_3$ (1.0 M) + 5ml HNO_3 (0.02M) + 5ml A (0.01M) + 5 ml M_1(II) (0.01M) + 5ml B(0.01M)+ 5ml M_2(II) (0.01M) + 20 ml water

M_1 (II) and M_2 (II) are Hg and Pb.

A = 2-Aminosuccinic acid (2-ASA),

B = 5-methyluracil (5- MU)

All the metal salts used were of Analar Grade and were standardized volumetrically by titration with the disodium salt of EDTA is presence of suitable indicators, as described by Schwarzenbatch [9].Each set of solution was then titrated against alkali (NaOH). The pH meter reading with progressive additionof alkali to the titration mixtures were noted, when the reading of pH meter stabilized. The pH values were plotted against the volume of NaOH and the titration curves were obtained. The titrations were continued until the appearance of turbidity.

2.3 Computer Programs for Evaluation of Stability Constant

Many computer programs have been developed for calculating the stability constants of metal complexes, from pH titration data. In 1962 Ingri and Sillen [10] described the program KUSKA for calculating the compositions of solutions containing one kind of metal ion and one kind of ligand. I.G.Sayce [11-13] developed a new computer program SCOGS (Stability constant of generalized species) which employs the conventional non linear least square approach. The program is written in FORTRAN IV. 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 (MA, MB and MA_2 etc.). SCOGS may also be used to calculate constants for "mixed" complexes containing two different metals and two different ligands. This program has been used successfully in the refinement of ternary and quaternary system's data studied in the present work.

3. Results and Discussion

The overall stability constants (β_{pqrst}) of investigated complexes were evaluated by SCOGS and are expressed by the general equation in aqueous solutions as follows:

$$pM_1+qM_2+rA+sB+t(OH) \rightleftharpoons (M_1)_p(M_2)_q(A)_r(B)_s(OH)_t$$

$$\beta_{pqrst} = \frac{[(M_1)_p(M_2)_q(A)_r(B)_s(OH)_t]}{[M_1]^p[M_2]^q[A]^r[B]^s[OH]^t} \quad \text{.................... (11)}$$

In above equation the p, q, r and s are either the zero or positive integer and t is a negative integer for a protonated species, positive integer for a hydroxo or a deprotonated species and zero for a neutral species.

3.1 Species Distribution Curve

The species distribution curves are obtained by plotting percent (%) concentration of the species obtained through SCOGS computer programme against pH. The pH titration curves and species distribution curves are finally sketched by running the computer program ORIGIN 4.0.

3.1.1 Hg (II)- 2-ASA(A)- 5-MU (B) Ternary (1:1:1) System

The species distribution curves of Hg AB are represented in fig. 1. In this system Hg^{+2}, H_3A, H_2A, HA, BH, Hg $(OH)^+$, Hg A, Hg B and Hg AB species are existing in remarkable values. Binary complex of HgA exist at ~3.7pH having maximum concentration ~ 26% Another binary complex Hg B shows its presence with maximum concentration of ~71% at very start of the titration and showing the decline trend as the pH increased.

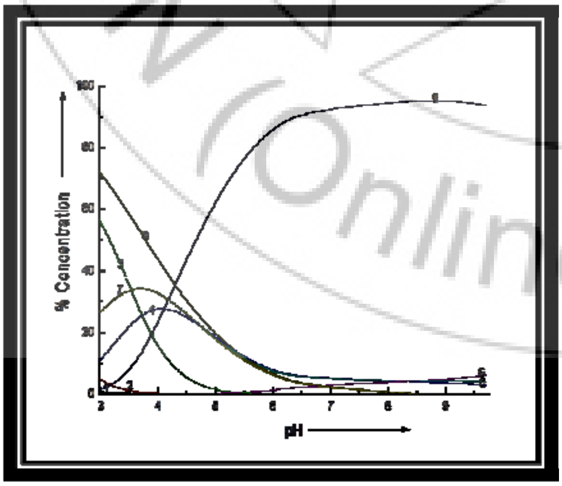


Figure 1: Species Distribution Curves of 1:1:1 Hg (II)-2-ASA (A) 5-MU (B) System
(1) Hg^{2+} (2) H_3A (3) H_2A (4) HA (5) BH (6) $Hg(OH)^+$ (7) Hg A (8) HgB (9)HgAB

In this system the ternary complex HgAB existed very high pH ~9.5 with maximum concentration of 98%.

3.1.2 Hg (II) - 2-ASA (A)- 5-MU(B) Ternary (1:2:1) System

Distribution curve are represented in fig. 2.

In this system protonated ligand species H_3A, H_2A, HA and BH, free metal ion species Hg^{2+} (aq.), Hydroxo species: Hg $(OH)_2$, Binary species Hg A, Hg B and Ternary species: Hg AB are identified. From the species distribution curves it is clear that the binary complex of Hg B exist in good concentration ~ 73% at start of the titration and gradually decreases with increase in pH range. Another binary complex shows its value~ 37% concentration at the pH ~3.3. H_2A have the maximum concentration ~ 57% at the start of titration. Ternary complex of Hg AB exist with higher value having maximum concentration ~ 93% at higher pH ~ 9.5.

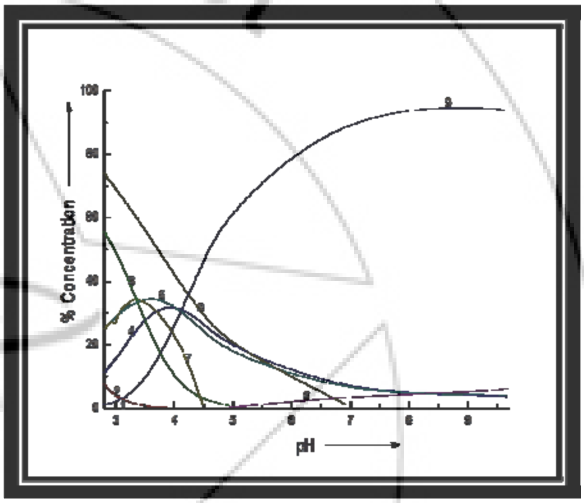


Figure 2: Species Distribution Curves of 1:2:1 Hg (II)-2-ASA (A) - 5-MU (B) System
(1) Hg^{2+} (2) H_3A (3) H_2A (4) HA (5) BH (6) $Hg(OH)_2$ (7)Hg A (8)HgB (9) HgAB

3.1.3 Hg (II)-Pb (II) - 2-ASA (A) and 5-MU (B) Quaternary (1:1:1:1) System

The distribution diagram of present system is represented in fig. 3. Species distribution diagram provide evidence for existence of following species Hg^{2+},$Pb^{2+}$$H_3A$, H_2A HA, BH, Hg $(OH)_2$,Hg A, HgB,PbA ,PbB, HgAB, PbAB and Hg Pb AB .Binary complex of Hg with ligand A have their existence very low while the another binary complex Hg B shows amount ~12% at the pH ~ 7.5. Complex PB- A attains maximum concentration ~2.0% at the start of pH titration.

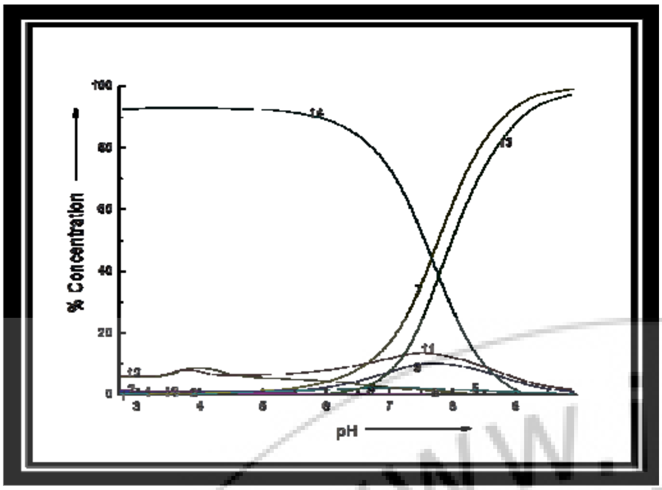


Figure 3: Species Distribution Curves of1:1:1:1 Hg (II)–Pb (II) –2-ASA (A) - 5-MU (B) System
(1) Hg^{2+}(II) (2) Pb^{2+}(II) (3) H_3A (4) H_2A (5) HA (6)BH (7)$Hg(OH)_2$(8)Hg A (9)Hg B (10)Pb A (11)Pb B (12)Hg AB (13)Pb A B(14)Hg Pb AB

Pb B complex attain the maximum concentration ~17% at the ~ 7.5 pH value. Ternary complexes of Hg AB existed with maximum concentration ~10% at the ~ 4.2 pH while the PbAB existed with maximum concentration ~92% at the ~ 9.5 pH. The major species which is quaternary complex of Hg Pb AB attain the maximum concentration ~92% at the ~ 4.8 pH.

3.1.4 Overall stability constants and other related constants of binary, ternary and quaternary complexes for M (II) 2-ASA (A) - 5-MU (B) (1:1:1), (1:2:1) and M_1II) - M_2 (II) 2-ASA (A) - 5-MU (B) (1:1:1:1) System

- Proton-ligand formation constant (log β_{00r0t}/ log β_{000st}) of 2-ASA - 5-MU at 37 ± 1^0C I = 0.1 $NaNO_3$

Complex	*log β_{00r0t}/ log β_{000st}*
H_3A	15.26
H_2A	13.33
HA	9.63
BH	9.94

- Hydrolytic constants (log β_{p000t}/ log β_{0q00t}) M^{2+} (aq.) ions.

Complex	*Hg*	*Pb*
$M(OH)^+$	-3.84	-9.84
$M(OH)_2$	-6.38	-15.54

- Metal-Ligand constants (log β_{p0r00}/ log β_{0qr00}/ log β_{p0s0}/ log β_{0q0s0}) Binary System

Complex	*Hg*	*Pb*
MA	13.09	11.61
MB	13.45	13.33

- Metal-Ligand constants (log β_{p0rs0}/ log $\beta_{0qrs)}$: Ternary System(1:1:1)

Complex	*Hg*	*Pb*
MAB	21.18	19.25

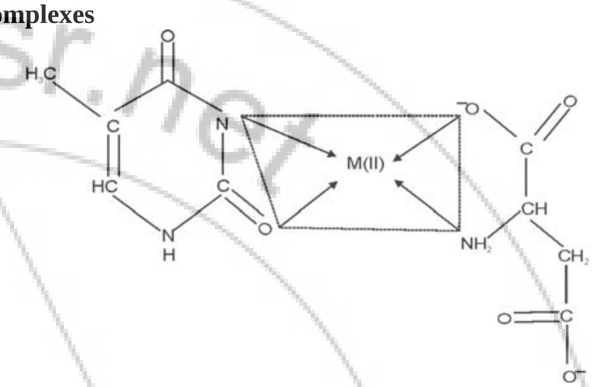
- Metal-Ligand constants (log β_{p0rs0}/ log β_{0qrs0}/): Ternary System (1:2:1)

Complex	Hg	Pb
MAB	21.45	19.84

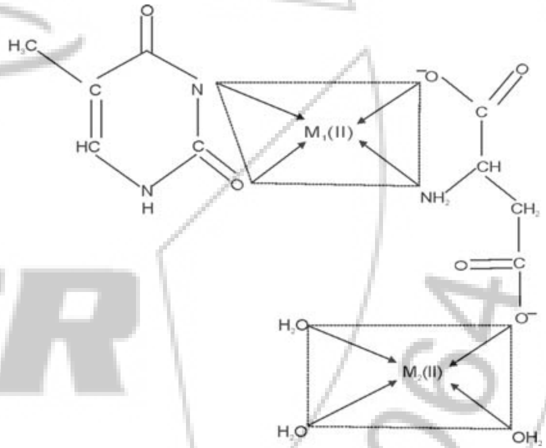
- Metal-Ligand constants (Log β_{pqrst}) : Quaternary System (1:1:1:1)

Complex	Hg-Pb
M_1 M_2-A-B	28.85

3.1.5 Proposed Structures of Ternary and Quaternary complexes



M (II)-2-ASA-5-MU 4- Coordinated



M_1 (II) - M_2 (II)-2- ASA-5-MU 4- Coordinated

4. Conclusions

Human life is precious, so its prevention is essential and challenging. Various factors are present in our surrounding which are hazardous for human health and other living beings. Metal toxicity is one of them. Metal are widely used by human beings direct or indirect way. Some metals are fruitful while some others are harmful. In these metals some are poisonous which causes various types of diseases and disorder in human body. The toxic metal ions inevitably exist as metal complexes in biological systems by interaction with the numerous molecules possessing groupings capable of complexation or chelation. The word, to chelate, means to grab onto something. Thus, chelating agents are substances that have a strong ability to grab onto toxic metals and dislodge them from the tissues so they can be removed. The 2- ASA and 5- MU are very good chelating agent having strong ability to bind metal ions by metal – ligand interaction and thus form metal chelates or complexes of biological significance and hence the toxicity will be removed. The stability of investigated complexes can be explained on the basis of increased number of fused rings and the extra stabilization caused by ligand-ligand interactions.

Stability Order of Ternary Complexes:

Mixed ligand ternary (1: 2: 1) System:

- Hg (II) / Pb (II) -2-ASA (A) – 5-MU (B) Hg A B (1: 2: 1) > Pb A B (1: 2: 1)

Mixed ligand ternary (1: 1: 1) System:

- Hg(II) / Pb (II)-2-ASA (A) – 5-MU (B) Hg AB (1: 1: 1) > Pb AB (1: 1: 1)

Mixed metal-mixed ligand quaternary (1: 1: 1:1) System:
Hg Pb A B

Overall Stability Order of Investigated Complexes:

Hg Pb AB (Quaternary) > Hg AB (Ternary) > Pb AB (Ternary)

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