Investigation of Mixed Ligand Complex Equilibria of ternary complexes of Cu(II), Ni(II), Co(II) and Zn(II) metal ions with Glutamine and Thymine in solution by Potentiometric Method

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ABSTRACT

The study of metal complexes with physiologically active ligands has both scientific and practical implications. In this work, we investigated the formation of binary and ternary complexes involving metal ions such as Cu (II), Ni (II), Co (II) and Zn (II), specifically with two physiologically relevant ligands: glutamine (A) as the major ligand and thymine (B) as the secondary ligand. To evaluate the complexation behaviour of these species in aqueous environments, a potentiometric method was used at $37\pm1°C$ and ionic strength, $I = 0.1$ M $NaNO_3$. The complexes' stability constants were found using Irving and Rossetti's approach, which was subsequently improved using the SCOGS computer software. The findings demonstrated that in most cases, mixed-ligand ternary complexes formed concurrently, often at higher pH levels, resulting in a steady decline in binary complex concentrations for the corresponding metal ions. The species distribution curve displays the binary and ternary species that were investigated. The ligands' protonation constants and the stability constants of their complexes with metal ions followed the order of MA, MB, and MAB.

KEYWORDS: Potentiometric studies, Transition metals, Biomolecules, Formation constant, SCOGS, Chelation

INTRODUCTION

Chelation transports metals to susceptible areas and increases their carcinogenic potential. In the other direction, metals can scavenge ligands through complexation or mixed complex formation, with the latter occurring as a result of contact with binary and ternary complexes. Mixed ligand complex formation is important in analytical chemistry, allowing for more selective and sensitive methods, as well as biological, industrial, and medicinal applications (M. Singh et al., 2021).

Chelation of the coordination complex is a crucial process in chemistry. It is an intravenous medication designed particularly to combat the accumulation of heavy metals in the body. The basic goal of chelation treatment is to bind and eliminate toxic substances from the body. This treatment technique employs chelating drugs, that have ligands capable of interacting with an individual metal ion via two or more

donor atoms(S. Singh et al., 2000). Chelating ligands are those that attach to a metal ion and form a structure that is cyclic with the metal ion in the centre. A metal chelate is the chemical generated when the chelating ligand interacts with a metal ion. This work will contribute to changing the coordination environment of hazardous metal ions and increasing their excretion from organisms(Barman & Mukherjee, 2008).

The analysis of the potentiometric behaviour of coordination complexes has sparked researchers' interest in the speciation and stability constant of mixed ligand complexes, particularly the ternary form, in which the mixed ligands promote the discovery of new techniques with increased sensitivity and selectivity. The link between chelation treatment and ternary chelate potentiometry is gaining attention due to the prevalence of mixed chelation in physiological fluids (Marzotto et al., 1993). Numerous possible ligands compete for the remaining metal ions. The competition of ligands for metal ions is a complicated process that can have a substantial impact on the state of equilibrium and functioning of metal-chelate complexes. Essential metal ions, such as, Cu (II)(Xiao et al., 1990), Ni (II)(Alabbası et al., 2023), Co (II)(Abdel-Rahman et al., 1996) and Zn (II) (M. Singh et al., 2022)develop metal chelates with the amino acids like glutamine and thymine, and they play an important role in metal ion complexation within biological systems. These metal ions frequently form complexes with amino acids and peptides, serving as models for studying metal-amino acid equilibria in enzymatic activities. As a result, it would be beneficial to explore the formation constants of these metal ions in combination with the ligands often found in biological fluids (Ahmed, 2007).

Glutamine Fig. 1(a)(M. Singh et al., 2010) is a non-essential amino acid, sometimes known as L-glutamine. It is one among the basic amino acids that make up proteins. Glutamine is synthesised in the body from other amino acids, most notably glutamate and ammonia, and can also be received by food. Glutamine is essential for a variety of biological functions and metabolic pathways. It is particularly plentiful in skeletal muscles and is thought to be a major source of energy for cells that divide, including immune system cells, intestinal cells, and some cancer cells(Newsholme et al., 2003). The enzyme glutamine synthetase converts glutamate and ammonia into glutamine. The most important glutamine-producing tissue is muscle mass, which accounts for approximately 90% of all glutamine synthesised. The lung and brain also emit modest quantities of glutamine. Although the liver is capable of significant glutamine synthesis, its involvement in the metabolism of glutamine is more regulatory than production, as the liver absorbs vast quantities of glutamine from the gut (Bender, 2012).

Thymine Fig. 1(b)(Ahmed, 2007), commonly known as 5-methyluracil, is an organic base from the pyrimidine family. In 1893-94, thymine was extracted from calf thymus and beef spleen, making it the very first pyrimidine to be purified naturally. Thymine may be produced by methylation of uracil at the 5th carbon, as the name implies. RNA often replaces thymine with uracil. Thymine is one of four nucleobases of DNA, symbolised by the letters G-C-A-T(M. Singh et al., 2022). The others are cytosine, guanine, and adenine. Thymine (T) typically pairs with adenine. In DNA, thymine (T) forms two hydrogen bonds with adenine (A) to stabilise nucleic acid structures. 5-fluorouracil (5-FU) may target thymine during cancer therapy. 5-FU can function as a metabolic analogue of thymine in DNA synthesis and uracil in RNA synthesis. Substituting this analogue decreases DNA synthesis in actively dividing cells. Thymine is present in all cells, both eukaryotic and prokaryotic, and plays a crucial role in the transmission of genetic information (except in RNA viruses). This highlights the interconnectedness of life (M. Singh et al., 2010).

Understanding the aforementioned factors, the current work focusses on the formation equilibria, stability constant, speciation curve, as well as coordination of certain ternary chelates involving Glutamine and Thymine with Cu(II), Ni(II), Co(II), and Zn(II) metal ions under biologically relevant conditions.

(a)   **(b)**
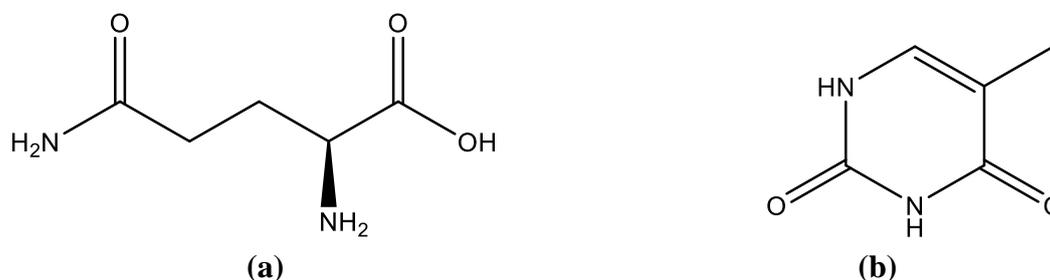
Fig.1 Structure of ligands (a) Glutamine, (b) Thymine

MATERIALS AND METHOD

Materials and Reagents – The ligands used in the study, glutamine and thymine, were analytical grade and commercially available, thus no additional purification processes were required. Metal nitrates and ligands dissolve in double-distilled water which had been carbon dioxide-free. Aqueous metal nitrate solutions were standardised in the presence of an appropriate indicator. Similarly, stock glutamine and thymine solutions were compared to a standard oxalic acid solution. This stage involves calculating the concentrations of the ligand solutions using a known concentration of oxalic acid as a reference. Potentiometric titrations were performed at 37 ± 1ºC with a glass electrode and an electric digital pH meter (Systronics). The addition of NaOH altered the pH of the solution, and the resulting electrode potential measurements revealed information regarding the complexation behaviour and stability of metal-ligand complexes (Shankar et al., 2016). The tests were carried out in the presence of a sodium nitrate ($NaNO_3$) solution with a concentration of 0.1 M. Overall, the standardised solutions, controlled conditions, and potentiometric measurements using a pH meter with a glass electrode all helped to accurately assess the complexation behaviour and stability of the metal-ligand complexes formed between metal nitrates and glutamine/adenine ligands in the study(Shalini et al., 2015).

APPARATUS AND MEASURING TECHNIQUES

Potentiometric titrations of both binary and ternary solutions were carried out at room temperature with an electric digital pH meter fitted with a glass electrode. The pH meter was set to 220 V/50 cycles, which was stabilised by the alternating current mains. The pH meter's electrode was conditioned regularly with a saturated potassium chloride solution from BDH to preserve its performance and precision, with a repeatability of 0.01 pH (Shankar et al., 2016).

For potentiometric titrations, a standardised NaOH solution with a concentration of 0.01 M was utilised. Solution mixes including binary and ternary systems were titrated using a standardised NaOH solution. The use of a standardised NaOH solution as a titrant enables the monitoring of pH variations during the titration procedure (Amin Mir et al., 2021).

The pH readings at various phases of the titration can be used to determine the complexation behaviour and stability of binary and ternary systems (Türkel, 2015b). In this work, many solution combinations were produced for potentiometric titrations, and the potentiometric behaviour of the solutions at various pH levels was investigated. The solution mixtures have the following compositions:

5 ml NaNO$_3$ (1.0M) + 5 ml HNO$_3$ (0.02 M) + H$_2$O

2. 5 ml NaNO$_3$ (1.0M) + 5 ml HNO$_3$ (0.02 M) + 5 ml M (0.01) + H$_2$O

3. 5 ml NaNO$_3$ (1.0M) + 5 ml HNO$_3$(0.02 M) + 5 ml M (0.01) + 5 ml A (0.01M) + H$_2$O

4. 5 ml NaNO$_3$ (1.0M) + 5 ml HNO$_3$ (0.02 M) + 5 ml M (0.01) + 5 ml A (0.01M) + 5 ml B (0.01M) H$_2$O

In these mixes, M denotes the metal ions Cu(II), Co(II), Ni(II), or Zn(II); A denotes glutamine; and B denotes thymine. The volume of each component and the amount of water were varied to produce the appropriate concentrations and total quantities of 50 ml for the titration tests. The pH of each solution was determined using a potentiometric approach, and the results were used to create titration curves that depicted the variation in pH as a function of the amount of NaOH supplied throughout the titration. These graphs show the potentiometric behaviour of the solutions as well as the pH variations caused by the addition of NaOH (Sayce, 1968).

Furthermore, species distribution curves were obtained using the stability constant of any generalised species (SCOGS) computer program(Türkel, 2015a). The program calculated the percentage concentrations of different species formed and plotted them in opposition to pH to obtain the species distribution curves, which provided insight into the distribution and relative concentrations of the various species formed in the solution (Aljahdali et al., 2013).

RESULT AND DISCUSSION

Irving and Rossetti's approach uses potentiometric pH titration data to derive the ligands' protonation constants. The protonation of the corresponding ligand involved in complex formation causes the equilibria of metal-ligand complex formation to be governed by the pH of the solution. At higher pH, the ternary complex titration curve overlaps with the binary titration curve. Table 1 shows the protonation constants of both ligands as well as the hydrolytic constants of chosen metal ions from previously published literature(Gollapalli Nageswara Rao, 2012). The ionisation equilibria of the ligands, glutamine and thymine, were studied, and the following equilibria were proposed.
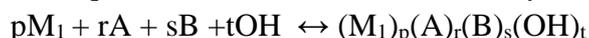
$H_2A \leftrightarrow H^+ + HA^-$

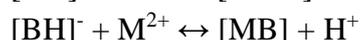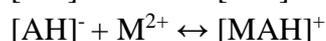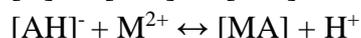$K_1 = [H^+][HA^-]/ [H_2A]$

$HA^- \leftrightarrow H^+ + A^{2-}$
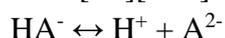
$K_2 = [H^+][A^{2-}]/[HA^-]$

The equilibria of the formation of ternary complex has been shown as follows:

$pM_1 + rA + sB + tOH \leftrightarrow (M_1)_p(A)_r(B)_s(OH)_t$
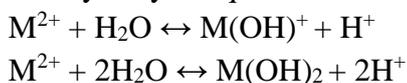
$ß_{prst} = (M_1)_p(A)_r(B)_s(OH)_t/[M_1]^p[A]^r[B]^s[OH]^t$

where, A represents the main ligand, glutamine, and B for the secondary ligand, thymine. Stochiometric numbers p, r, and s can be zero or positive integers, whereas t is a negative integer for protonated species. Based on the description of species distribution curves acquired by the SCOGS computer tool, the following equilibria should be expected for the ternary system:

$[A]^{--} + [M^{2+}] \leftrightarrow [MA]$

$[AH]^- + M^{2+} \leftrightarrow [MA] + H^+$

$[AH]^- + M^{2+} \leftrightarrow [MAH]^+$

$[BH]^- + M^{2+} \leftrightarrow [MB] + H^+$

$[MA] + [BH]^- \leftrightarrow [MHAB]^-$

$[MAH]^+ + [BH]^- \leftrightarrow [MAB]^{--} + H^+$
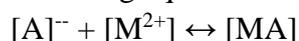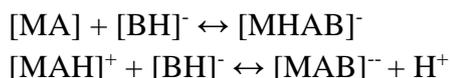
The hydrolytic equilibria are as follows:

$M^{2+} + H_2O \leftrightarrow M(OH)^+ + H^+$

$M^{2+} + 2H_2O \leftrightarrow M(OH)_2 + 2H^+$

Table 1 - Proton ligand formation constant of Glutamine as a primary ligand and Thymine as secondary ligand and hydrolytic constant of some important metal ions:

Species	Protonation constant (Log ß prst)			
	Ligands		Metal Hydrolytic	
	Primary	Secondary	$M(OH)^+$	$M(OH)_2$
H_2A	11.18			
HA	9.01			
HB		9.94		
Cu(II)			-6.29	-13.10
Ni(II)			-8.10	-16.87
Co(II)			-8.23	-17.83
Zn(II)			-7.89	-14.92

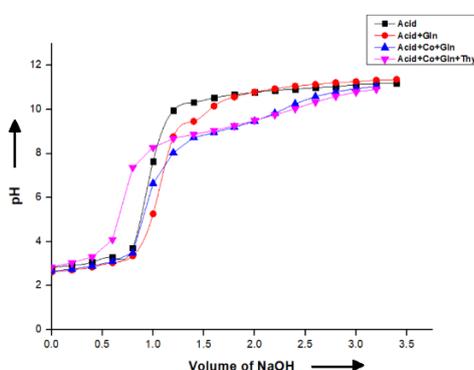**Potentiometric Titration Curves of Complexes**

The ternary complex's (1:1:1) potentiometric titration curve should be graphed against pH and alkali volume, as illustrated in Fig. 2, 3, 4, and 5. This graph displays the titration curves for acid, ligand, binary complex, and ternary complex. The potentiometric titration curve indicates that curve 'C' represents the binary complex formation, which is then followed by curve 'B'. In a similar manner, the ternary complex formation occurs after the binary complex has formed.
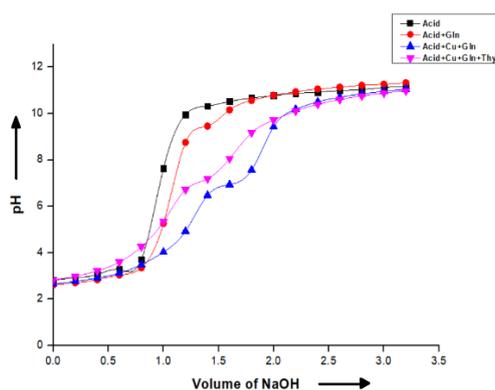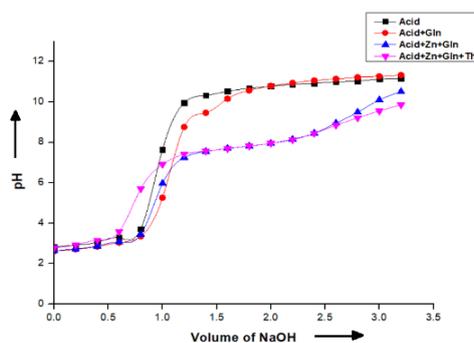

Fig. 2 - Potentiometric Titration Curves of Ternary (1:1:1) Cu(II)- Gln(A)- Thy(B) System
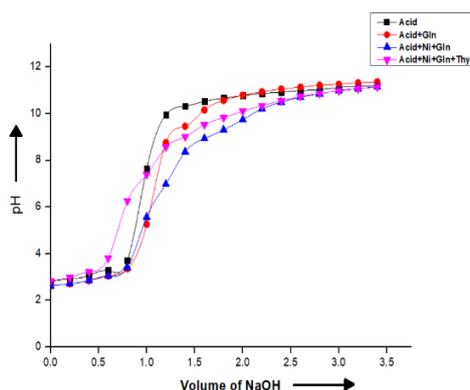
Fig. 3 - Potentiometric Titration Curves of Ternary (1:1:1) Ni(II)- Gln(A)- Thy(B) System

Fig. 4 - Potentiometric Titration Curves of Ternary (1:1:1) Co(II)- Gln(A)- Thy(B) System

Fig. 5 - Potentiometric Titration Curves of Ternary (1:1:1) Zn(II)- Gln(A)- Thy(B) System

Species Distribution Curves of Complexes

1. Ternary Copper(II) complex formation equilibria involving glutamine and thymine

The distribution of species for the Cu(II)- Glutamine- Thymine system is illustrated in Fig. 6. Significant concentrations of ternary complex species, protonated ligand species (AH_2, AH, BH), binary complex species (Cu-A and Cu-B), and free metal ions are observed. The speciation curves demonstrate a decrease in protonated ligand species concentrations within the pH range of ~2.82-9.72, indicating metal-ligand complex formation. Binary species Cu-A and Cu-B reach peak concentrations of ≈43.57% and ≈50.15%

respectively between pH ~5.0-5.6, subsequently declining as pH increases. The Cu(II)- Glutamine - Thymine concentration profile shows mixed ligand complex formation from pH ~5.2–10.4, peaking at ≈90.1% at pH ~10.11. The ternary complex species concentration rises as protonated ligand species and Cu^{2+}(aq.) metal ions decrease, suggesting stepwise complex formation.
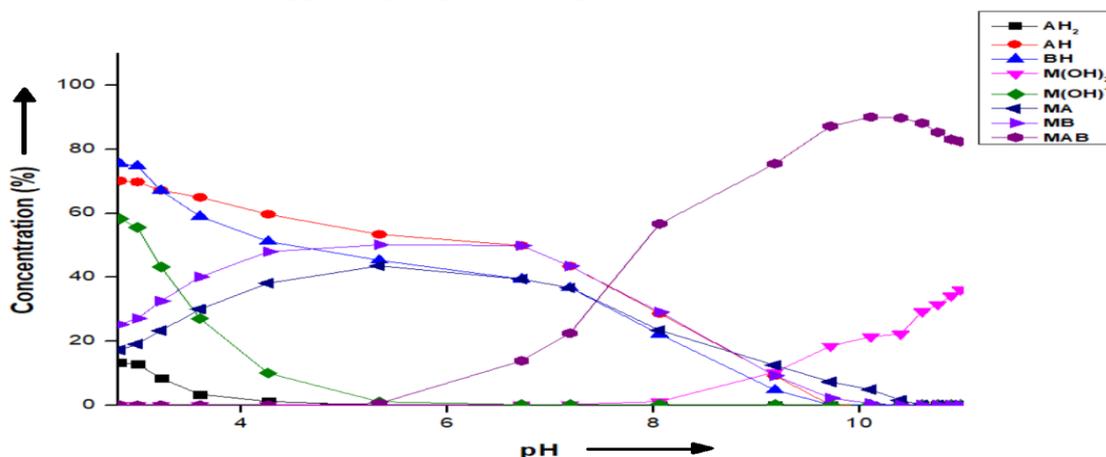

Fig. 6 – Species distribution curve of Cu (II) – Glutamine (A) – Thymine (B) system (1:1:1)

2. Ternary Nickel(II) complex formation equilibria involving glutamine and thymine

Fig. 7 displays the species distribution curves for the Ni(II)- Glutamine -Thymine system. The speciation curve clearly shows a declining trend for all protonated ligand species (AH_2, AH, BH). The free metal ion concentration also decreases with increasing pH, indicating its involvement in complex formation. AH initially increases to a maximum of ≈97% at pH ~3.81, while BH reaches ≈98.8% at the initial pH ~2.84, before both declines, signalling mixed ligand complex formation. The binary complex Ni(II)-A exists at 16.6% at pH ~6.26, while another binary complex Ni(II)- B reaches a maximum concentration of ≈69.2% at the same pH. Their concentrations gradually decrease with further pH increase, likely due to Ni-A-B ternary complex formation. Hydroxo species $Ni(OH)_2$ and $Ni(OH)^+$ are also observed at higher pH levels. The Ni(II)- Glutamine-Thymine complex species concentration gradually increases from pH ~6.26, reaching a maximum of ≈84.7% at pH ~9.83. The speciation curves suggest stepwise formation of the ternary complex.
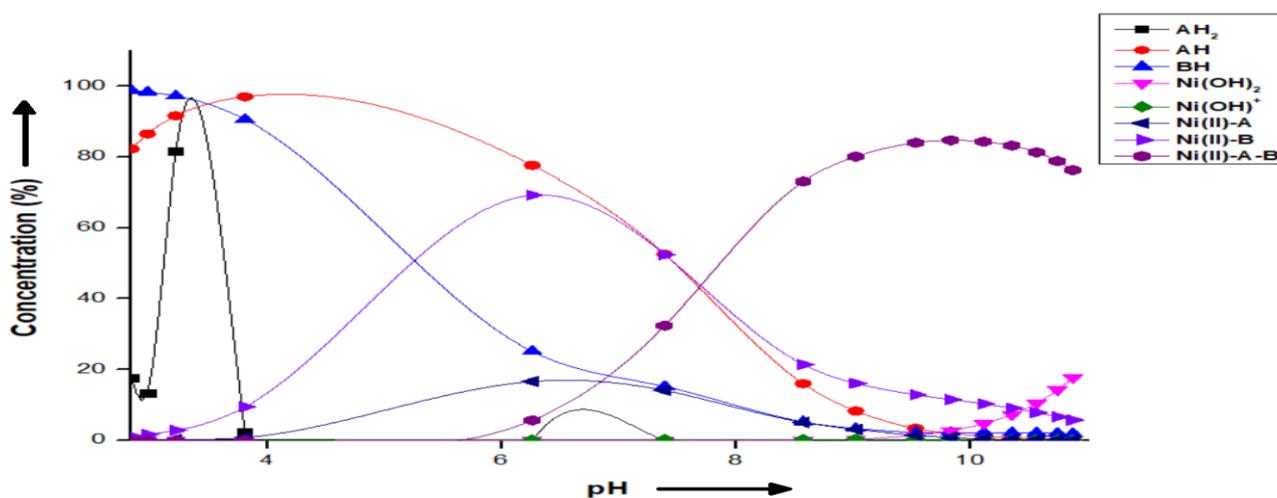

Fig. 7 – Species distribution curve of Ni (II) – Glutamine (A)– Thymine (B) system (1:1:1)

3. Ternary Cobalt(II) complex formation equilibria involving glutamine and thymine

Figure 8 illustrates the formation curves for the Co (II)- Glutamine-Thymine system. The mixed ligand complexation equilibrium involves ternary complex species, with AH_2, AH, BH, and binary Co-A and Co-B present in significant concentrations. The graph clearly shows that as pH increases, the concentration of AH_2 and BH species decreases, while AH species initially increase and then decrease, indicating complexation. The speciation curves demonstrate that the Co(II)-B binary complex species exists in the pH range of approximately 2.9-10.0, reaching a maximum value of about 60% at pH 7.37. Complexation begins with the addition of alkali, and as pH further increases, the concentration of Co(II)-B binary complex gradually decreases. Conversely, Co(II)-A is predominant in the lower pH range of 4.0-10.0, reaching 13.99% at pH 7.37. The distribution diagram reveals that the Co (II)- Glutamine-Thymine species occurs throughout the pH range of 4.09-10.92, confirming complex formation. As the binary complexes Co-A and Co-B decrease in concentration, the ternary species reaches its maximum presence of approximately 65% at pH 10.04.
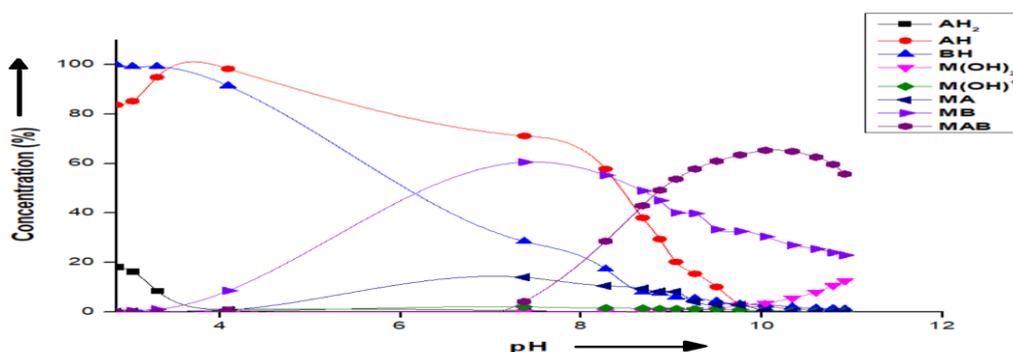

Fig. 8 – Species distribution curve of Co (II) – Glutamine (A) – Thymine (B) system (1:1:1)

4. Ternary Zinc(II) complex formation equilibria involving glutamine and thymine

Figure 9 displays the formation curves for the Zn (II)- Glutamine-Thymine system. The ternary complex species and protonated ligand species (AH_2, AH, BH) exist in substantial concentrations: approximately 18.2% at pH 2.78, 82.3% at pH 2.78, 94.5% at pH 3.5, and 99.9% at pH 2.78, respectively. The binary complex species is present throughout the entire pH range of 2.78-10.16. The speciation curves indicate that the mixed ligand complex species is predominant in this system. Ternary complexation initiates at pH 5.68 and gradually increases, peaking at pH 9.21. The rise in ternary complex species concentration is facilitated by the decline in free metal ion Zn^{2+}(aq) concentration and all protonated ligand species (AH_2, AH, and BH), demonstrating their involvement in complex formation. The speciation curves show that the Zn(II)-Thymine binary complex species is the major species, with a maximum concentration of about 65% at pH 5.7, which gradually decreases as pH increases. In contrast, the Zn(II)-Gln species reaches 8.87% at pH 6.91 in the system. The metal hydroxo species $Zn(OH)^+$ and $Zn(OH)_2$ are found to be protonated in the higher pH region.
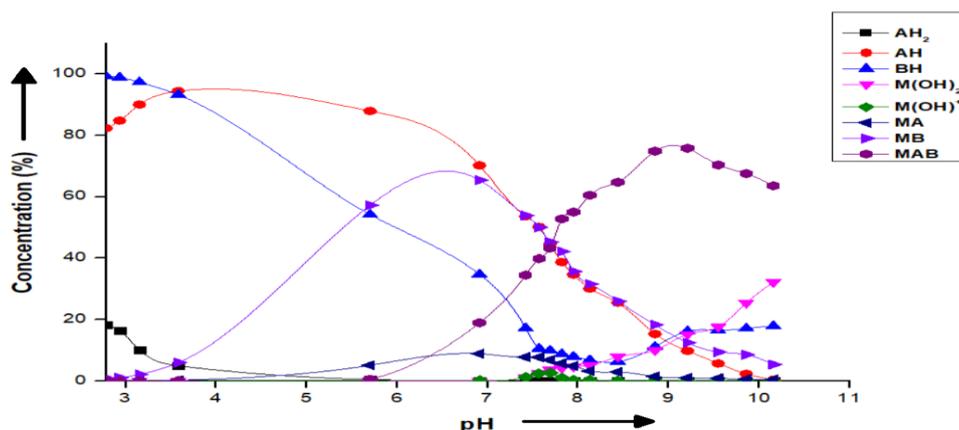
Fig. 9 – Species distribution curve of Zn (II) – Glutamine (A) – Thymine (B) system (1:1:1)

From the structural point of view, the solution structure for the mixed ligand ternary complex of 1:1:1 Copper(II)-Glutamine-Thymine are as follows:
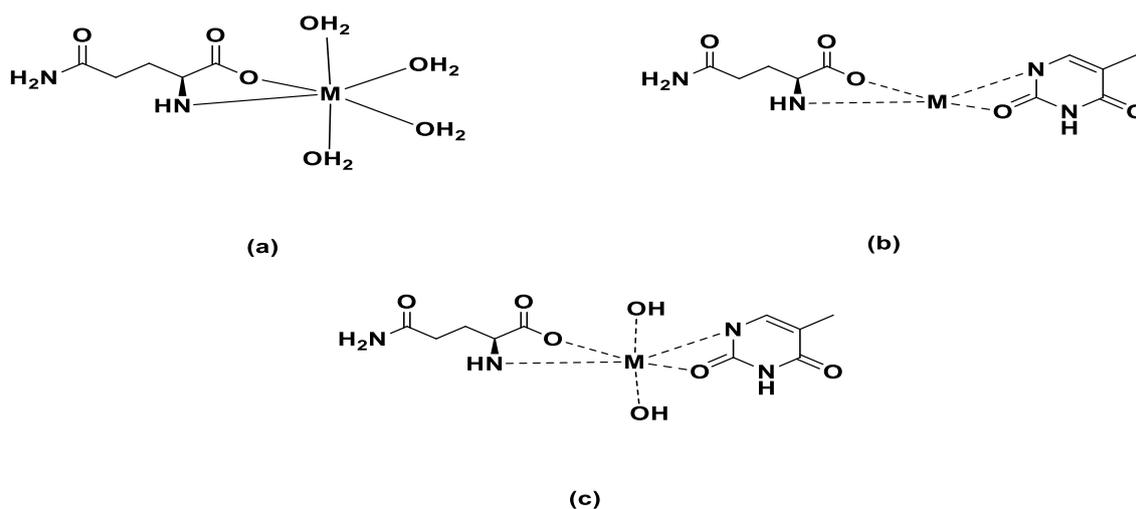

Fig. 10 Schematic representation of (a) binary complex, (b)tetra-coordinated ternary complex and (c) hexa-coordinated ternary complex

In the binary system depicted in Fig. 10(a), two protonated entities are released from glutamine's binding site and contribute to complex formation. The N_3H group of thymine undergoes proton dissociation. Given that thymine is thymidine's nucleoside, it can be presumed that the initial dissociation from thymine occurs at the N_3H group (Kiraz et al., 2019). The enhanced stability of ternary complexes likely results from hydrophobic or stacking interactions between ligands. In the ternary system shown in Fig. 10(b & c), glutamine binds to the metal ion via nitrogen and oxygen, while thymine functions as a bidentate ligand in tetra-coordinated and hexa-coordinated ternary complex (Newsholme et al., 2003).

CONCLUSION

This investigation suggests that ternary complex formation occurs at higher pH levels in each system. As pH increases, the titration curves of the ternary complex (1:1:1) merge with the binary complex curve (1:1) due to dissociation and involvement in ternary complex formation. Tables 2 and 3 present the

stability constants for binary and ternary species of metal ions with Glutamine (A) and Thymine (B)(Sujatha et al., 2017). The formation of ternary complexes is influenced by the nature of both ligands. Using the Irving-Rossetti method, the estimated percentages of ternary species formation are 90% for Cu(II)-Gln-Thy at pH 10, 84% for Ni(II)-Gln-Thy at pH 9, 65% for Co(II)-Gln-Thy at pH 10, and 70% for Zn(II)-Gln-Thy at pH 9.72. The overall stability constants for mixed ligand ternary systems of Glutamine (A) and Thymine (B) follow this order: A-Cu(II)-B > A-Ni(II)-B > A-Co(II)-B > A-Zn(II)-B. The study concludes that thymine generally acts as a bidentate ligand. In Cu(II) and Co(II) complexes, coordination occurs through the ring nitrogen and carboxyl oxygen (Aljahdali et al., 2013). In Ni(II) complexes, bonding takes place from both ring nitrogens. For Zn(II) complexes, bonding also involves the ring nitrogen and one carboxyl oxygen, but one thymine coordinates at the C_2O position, while the other coordinates at C_4O.

Table 2 - Stability constant of the binary species of some essential metal complexes with Glutamine as a primary ligand and Thymine as secondary ligand:

S.No.	Metal ions	Ligands	
		Glutamine (A)	Thymine (B)
1.	Cu(II)	7.75	8.83
2.	Ni(II)	5.56	7.20
3.	Co(II)	4.40	6.34
4.	Zn(II)	4.62	7.06

Table 3 - Stability constant ternary species of some essential metal complexes with Glutamine as a primary ligand and Thymine as secondary ligand:

S.No.	Ternary Metal Complex	
	Complexes	Stability Constants
1.	Cu-A-B	12.74
2.	Ni-A-B	10.08
3.	Co-A-B	9.08
4.	Zn-A-B	10.68

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