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Aspartic acid (amino acid)-Cu(II) complex via potentiometric method and study of its stability constant, thermodynamic parameters and antimicrobial effect

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Abstract: The Aspartic acid-Cu(II) complex is a coordination compound formed by the binding of copper(II) ions with aspartic acid, an essential amino acid, exhibiting potential biological and pharmacological applications. This study investigates the complexation behavior between aspartic acid and Cu(II) ions using potentiometric titration methods at varying temperatures (303K, 308K, and 313K) under controlled ionic strength conditions. The stability constants of the Cu(II)-aspartic acid complex were determined using the modified Irving-Rossotti technique. Thermodynamic parameters including Gibbs free energy (ΔG), enthalpy change (ΔH), and entropy change (ΔS) were calculated to understand the spontaneity and nature of the complexation process. The proton-ligand stability constants (pKa) and metal-ligand stability constants (log K) were evaluated at different temperatures. Additionally, the antimicrobial activity of the synthesized complex was assessed against selected bacterial strains using standard protocols to explore its potential therapeutic applications. The complex formation was confirmed through various physicochemical techniques, and the results indicate the formation of stable coordination compounds under the experimental conditions.

Keywords: *Aspartic acid-Cu(II) complex, Complexation, Potentiometric method, Stability constant, Antimicrobial effect etc.*

I.INTRODUCTION:

Aspartic acid, a non-essential amino acid, features a distinctive structure characterized by a carboxylic acid group and an amine group attached to a four-carbon backbone. In biological systems, aspartic acid plays a crucial role in various metabolic processes, serving as a building block for protein synthesis and functioning as a neurotransmitter in the central nervous system(Usiello et al., 2020). It is involved in the urea cycle and the synthesis of other amino acids, such as asparagine and methionine. The unique properties of aspartic acid contribute to its diverse roles in cellular signaling, metabolic pathways, and overall homeostasis within living organisms (Gorgoglione et al., 2022).

The complexation of metal ions with various compounds has gained significant attention in recent years, due to its implications in biological and environmental applications. A study demonstrated the formation of complexes between transition metals like Cu(II), Zn(II), and Ru(III) with heterocyclic compounds such as pyrazoles and aziridines. These complexes exhibited a range of biological activities, including anti-inflammatory, antioxidant, and anticancer properties, highlighting their potential in medicinal chemistry (Malinowska et al., 2019). The properties of these complexes have often been dictated by their coordination geometry and ligand structure, which enhanced their biological efficacy while minimizing toxicity.

On the other hand, the complexation of metal ions with amino acids is another significant area of research, revealing various synthesized complexes with potential applications in biomedicine and environmental science. Some studies have reported the formation of complexes between transition metals such as Cu(II), Co(II), and Ni(II) with amino acids like aspartic acid and histidine (Lawal, 2019). For instance, a study synthesized Co(II), Ni(II), and Cu(II) Schiff base complexes using 2-[(4-{[(4 methylphenyl)sulfonothioyl]oxy}phenyl)methylene]aminobenzoic acid, demonstrating their antibacterial and antifungal activities against various microorganisms, thus highlighting their therapeutic potential (Mumtaz et al., 2020). These metal-amino acid complexes exhibited unique properties that not only facilitated metal ion detection but also presented promising avenues for therapeutic applications in combating microbial resistance and environmental monitoring.

In the aspect of study of the structure of these complexes the stability constant and thermodynamic parameter assist in understanding their functioning. The stability constant (K) is a quantitative measure of the stability of a metal-ligand complex, reflecting the affinity between the metal ion and the ligand, such as an amino acid(Murphy et al., 2020). Thermodynamic parameters, including enthalpy (ΔH), entropy (ΔS), and Gibbs free energy (ΔG), provide insights into the energetics of complex formation. These parameters help elucidate the interactions at play during complexation, influencing the stability and reactivity of the resulting species. Besides these two, other properties which the complex exhibit is antimicrobial activity which is often attributed by the enhanced penetration ability of these complexes into microbial cells, disrupting cellular functions. The significance of stability constants and thermodynamic parameters lies in their ability to inform structural studies of complexes, allowing researchers to predict functionality based on stability profiles. Understanding these relationships is crucial for developing new antimicrobial agents and optimizing existing therapies in light of rising antibiotic resistance(Garda et al., 2020).

The transition metal of interest for our study is copper (Cu) and the complexation of copper (Cu) ions with amino acids has been extensively studied, revealing significant insights into their stability, thermodynamic parameters, and potential applications. A study utilized the Irving-Rossotti method to synthesize ternary

complexes involving Cu(II) with L-asparagine and β-lactam antibiotics like amoxicillin. The formation constants for these complexes were found to be log b (Cu(II)-ASP-Amox) = 15.83 and log b (Cu(II)-Glut-Amox) = 20.76, indicating strong stability in the presence of amino acids, which enhances the bioavailability of the antibiotics against bacterial strains (Karaderi et al., 2024). Another notable example involved potentiometric studies of Cu(II) complexes with L-valine and paracetamol, demonstrating binary and ternary complex formations with stability constants indicating that Cu(II) complexes are more stable than their Ni(II) counterparts (Alabbasi et al., 2023). The thermodynamic parameters, such as enthalpy and entropy changes during complex formation, were also assessed, providing insights into the energetics of these interactions.

Furthermore, Cu-amino acid complexes have shown promising antimicrobial activities. For instance, Cu-acetate complexes exhibited significant inhibitory effects against Pseudomonas species at low concentrations, showcasing their potential as antimicrobial agents(Damena et al., 2022). The stability constants and thermodynamic parameters are crucial for understanding the structural integrity and functionality of these complexes, as they correlate with their biological activity and effectiveness in therapeutic applications(Murphy et al., 2020). Overall, the research underscores the importance of Cu-amino acid complexes in developing novel antimicrobial strategies while providing a framework for further exploration in medicinal chemistry.

Although the study regarding complexation of copper ions with various compounds have already been done the complexation between copper and aspartic acid and further study regarding its structural integrity or stability constant and about its property of antimicrobial activity was still an unexplored area and thus in this study, we have synthesized complex between aspartic acid and copper ions. And further determined its stability constant, thermodynamic parameters and antimicrobial activity. This study was essential because of the significant implications these complexes in biological systems and their potential therapeutic applications.

II.EXPERIMENTAL

1.1 Material

Aspartic acid, Copper (II) nitrate, NaOH, HCl and NaCl - can be obtained from reputable Indian manufacturers like Merck India, SRL Pvt Ltd (Sisco Research Laboratories), Hi-Media Laboratories, Loba Chemie, Thomas Baker, and SD Fine Chemicals Ltd at analytical grade purity. The 50% aqueous methanol solution can be prepared using methanol from the same suppliers. Laboratory equipment like pH meters, electrodes and thermostats are available from Indian companies like Systronics, Labtronics, and VSI Electronics.

Glassware and reaction vessels can be sourced from Borosil Glass Works Ltd.

1.2 Method

1.2.1 Formation of Cu(II)- aspartic acid Complex

The Cu(II)-aspartic acid complex forms when Cu^{2+} ions coordinate with the amino acid aspartic acid under controlled pH and temperature conditions. During complexation, the copper(II) ion typically binds to aspartic acid through the α-amino group (-NH2) and the α-carboxylate group (-COO-) forming a five-membered chelate ring. The β-carboxylate group of aspartic acid can also participate in coordination, leading to the formation of stable complexes. The complex formation is confirmed by changes in the absorption spectra and can be monitored using potentiometric titration methods at different temperatures (typically 303-313K) while maintaining constant ionic strength. The stability constants and thermodynamic parameters of the complex can be determined using the Irving-Rossotti method. The resulting Cu(II)-aspartic acid complex typically exhibits a blue-green color characteristic of copper coordination compounds.

1.2.2 Potentiometric determination of stability constant

One accurate way to calculate stability constants is to use the pH meter to measure the concentration of hydrogen ions. Based on the observation that complex formation, which is followed by the release of protons from acidic ligands, directly affects the pH of the solution, it is constructed. It is possible to determine the stability constant of the complex generated by using the quantity of alkali needed to neutralize the H+ ions or the extent of the observed pH shift.

Potentiomtric titration of different solutions against sodium hydroxide solution at 0.1 M NaNO3 ionic strength is the experimental method used to establish stability constants.

This allows for the determination of the complexes' pKa, $\bar{n}$, and pL values. In each set, water and methanol (50 v/v) were added to keep the total volume of solution constant, and the necessary quantity of NaNO3 was added to maintain a constant ionic strength of 0.1M. To ensure repeatability, every titration was carried out in triplicate, and all potentiometric titrations were conducted throughout the pH range of 2–10 to prevent the development of hydroxy complexes.

Plotting the pH meter values for titrations (i), (ii), and (iii) against the amount of NaOH used in each instance was done. Because hydrogen ions are released when the ligand complexes with metal, more NaOH is needed to achieve the same pH value.

when metal ions are present. The absence of hydrolysis in the solution was demonstrated by the quick titration equilibrium and the lack of any discernible pH meter drift.

1.2.3 Calculation of acid dissociation constant (pKa) of ligand

A solution of mineral acid alone with concentration E' is titrated against a base, BOH. The following will be a point on the titration curve:

$$[H] = E' + [OH] - B \quad \text{.....(3.1)}$$

Likewise, the following is a point on the pH titration plot of a solution that contains the ligand TL- and the mineral acid E″:

$$[H]'' = E'' + [OH]'' - B'' + yT_L'' - \bar{n}T_L''$$

……(3.2)

When the two solutions' pH meter readings are the same, that is, $[H]' = [H]$, and $[OH]' = [OH]$, and the solutions both have the same ionic strengths, it follows that,

$$\bar{n}H = \frac{(E'' - E') - (B'' - B') + yT_L''}{T_L''}$$

……(3.3)

Assuming that each titration has the same starting volume (V_O), mineral acid concentration (EO), and total ligand concentration (T^o), and that volume V' and V'' of base of concentration (N') are added to achieve the same pH point, then

$$E'' = V_0 E_0/(V_0 + V''),\ B'' = V''N_B/(V_0 + V'') \text{ and } T_L'' = V_0 T_L^0/(V_0 + V'')$$

….(3.4)

For E' and B', comparable relationships may be found. Next, using equation (ii)

$$\bar{n}_H = \left[\frac{yT_L^0 (V'-V'')(N_B+E_0)}{(V_0 + V')}\right] / T_L^0$$

……(3.5)

The value of $\bar{n}H$ can be obtained using the equation, where y represents the number of dissociable protons. The pKa value of the ligand is obtained from the plot of ($\bar{n}$H / 1- $\bar{n}$H) Vs pH. If the ligand has two ionizable hydrogen atoms, pKa2 is calculated by plotting log (2-$\bar{n}$H / $\bar{n}$H – 1) Vs pH. The Bjerrum half integral method is used to calculate pKa, with pKa1 representing the pH value at $\bar{n}H$ = 0.5 and pKa2 representing the pH value at $\bar{n}H$ = 1.5.

1.2.4 Calculation of, $\bar{n}$ and pL

The pH titration of a solution that contains the ligand, mineral acid, and metal ion TM is now taken into consideration. anywhere along the curve,

$$[H]'' = E'' + yT_L''' + [OH]''' - B''' + nH''' T_L''' + n_H''' T_M$$

……(3.6)

If this solution's ionic strength and pH values match those found in equation (ii), then

$$[H]'' = [H]''' ,\ [OH]'' = [OH]''' \text{ and } \bar{n}_H'' = \bar{n}_H'''$$

……(3.7)

So that,

$$\bar{n} = \frac{(E'' - E'') + (T_L'' - T_L''')(y\bar{n}_H''') - (B'' - B''')}{\bar{n}_H'''' T_M}$$

……(3.8)

Once more, if the starting volume (V_O), the concentration of the acid (E_O), and the ligand (T^O) are the same in every solution, and if the volume V″ and V‴ of the base of concentration (NB) are added to get to points B″ and B‴, we obtain

$$\bar{n} = \frac{(V''' - V'') + [(N_B + E_0 + T_L^0)(y - \bar{n}_H)]}{(\bar{V}_0 + V''\bar{n}_H T_M}$$

……..(3.9)

The values of the $\bar{n}$ may thus be computed using the knowledge of the difference in the volume of base required to get the same pH meter reading for the two systems, namely one containing metal ions and the other containing none. Equation (3.6) may be used to get the equivalent value of pL from the values of $\bar{n}$ at any pH.

Applying volume correction, the above equation becomes

$$pL = -\log[L] = \log\left[\frac{\Sigma_0^y y\beta_y^H [H]y}{T_L - nT_M} \cdot \frac{V_0 + V'''}{V_0}\right]$$

……..(3.10)

Where β^Hy is proton ligand stability constant. Now the stability constant can be calculated by using the following method:

2.2.3.1 Least square method:

Numerous techniques, such as curve fitting, spreading factor, half $\bar{n}$ method, correction term method, extrapolation methods, and least squares program, can be used to assess the stability constants of mononuclear complexes. Bjerrum's approach is used to interpret the majority of literature data.The following fundamental formulas and definitions are used in the general aspect of calculating consecutive stability constants of the mononuclear complexes using a weighted least squares approach.

[M] = concentration of the uncomplexed central ion or molecules

[L] = concentration of the free ligand

[MLn] = concentration of the nth complex species

A representation of the metal-ligand equilibrium reaction would be

$$M + L \leftrightarrow ML$$
$$M + 2L \leftrightarrow ML_2$$
$$M + 3L \leftrightarrow ML_3$$
$$M + nML \leftrightarrow ML_n$$

The overall stability constant is given

$$\beta n = \frac{[MLn]}{[M][L]^n} = \Sigma^N_{n=1} K_n$$

……..(3.11)

Where the K's stand for the stability constants for the stepwise creation of the various complexes. The concentration of all central metal ions, or TM, equals

$$T_M = [M] + \Sigma^N_{n=1}[ML_n] \quad \ldots\ldots\ldots\ldots(3.12)$$

Additionally, the ligand concentration total (TL) can be expressed as

$$T_L = [L] + \Sigma^N_{n=1}\, n[ML_n] \quad \ldots\ldots\ldots\ldots(3.13)$$

Where N represents the most ligands that may be attached to a single metal atom. The average number of the ligand coupled with each central atom is known as the ligand number, or $\bar{n}$.

$$\bar{n} = \frac{T_L - [L]}{T_M} = \frac{\Sigma^N_{n=1}\, n\beta_n\, [L]^n}{\Sigma^N_{n=1}\, \beta_n\, [L]^n} \quad \ldots\ldots\ldots..(3.14)$$

The assumption that a 1:2 complex is not formed until the 1:1 complex is completely formed, during the titration led to the following simplified equation as,

For,

$$n = 1, \quad \bar{n} = \frac{K_1\,[L]}{1 + K_1[L]} \quad \ldots\ldots\ldots..(3.15)$$

By rearrangement

$$k_1 = \frac{[\bar{n}]}{1 - \bar{n}[L]} \quad \ldots\ldots\ldots(3.16)$$

$$\log K_1 = \log \frac{[\bar{n}]}{1-\bar{n}} + pL \quad \ldots\ldots\ldots(3.17)$$

$$\log \frac{[\bar{n}]}{1-\bar{n}} = \log K_1 - pL \quad \ldots\ldots\ldots\ldots.(3.18)$$

Thus, a plot of $\log \frac{[\bar{n}]}{1-\bar{n}}$ Vs pL is a straight line. The intercept of which is logK1

$$\bar{n} = \frac{K_1[L] + 2K_1K_2\,[L^2]}{1 + K_1\,[L] + K_1K_2\,[L]} \quad \ldots\ldots\ldots\ldots(3.19)$$

$$\frac{[\bar{n}]}{1 - \bar{n}[L]} = \frac{[2 - \bar{n}] + l\,K_1K_2}{[\bar{n} - 1]} - K_1 \quad \ldots\ldots\ldots\ldots(3.20)$$

$$K_2 = \frac{1}{[L]}\left[\frac{\bar{n} + (\bar{n} - 1)K_1[L]}{(2 - \bar{n})\,K_1\,[L]}\right] \quad \ldots\ldots\ldots\ldots(3.21)$$

$$\log K_2 = pL + \log\left[\frac{\bar{n} + (n - 1)K_1[L]}{(2 - \bar{n})\,K_1\,[L]}\right] \quad \ldots\ldots\ldots..(3.22)$$

$$\log K_2 = pL + \log \frac{[(\bar{n} - 1)]}{(2 - \bar{n})} \quad \ldots\ldots\ldots(3.23)$$

$$\log \frac{[(\bar{n} - 1)]}{(2 - \bar{n})} = \log K_2 - pL \quad \ldots\ldots\ldots(3.24)$$

Thus, a plot of log $[(\bar{n}-1)] / (2-\bar{n})$ Vs pL gives a straight line with intercept equal to $\log K_2$.

In the case where the formation curves are incomplete in the sense that they do not reach the value of $\bar{n} = 1.5$ and in the cases in which the formation curve are not wave like indicating the formation of the second complexes starts before the complex of the 1:1 complex, logK values are calculated by the least square method.

2.2.3.2 Bjerrum's half-integral method

Based on the formation curves spanning a range of $0 < \bar{n} < 2$, the values of pL at $\bar{n} = 0.5$ and $\bar{n} = 1.5$ were determined as logK1 and logK2, respectively, in the Bjerrum half integral technique.

For,

$$\log K_1 = \log \frac{[\bar{n}]}{1 - \bar{n}} + pL \quad \ldots\ldots\ldots(3.25)$$

At

$$\bar{n} = 0.5, \qquad \log K_1 = \frac{0.5}{1 - 0.5} + pL = pL \quad \ldots\ldots\ldots(3.26)$$

For,

$$\log K_2 = \log \frac{[(\bar{n} - 1)]}{(2 - \bar{n})} + pL \quad \ldots\ldots\ldots..(3.27)$$

At

$$n = 1.5, \qquad \log K_2 = \log \frac{1.5 - 1}{2 - 1.5} + pL$$

…….(3.28)

$$= \log \frac{0.5}{0.5} + pL$$

……..(3.29)

$$= pL$$

…….(3.30)

i.e., a graph plotted between $\bar{n}$ and pL corresponds to $\log K_1$ and $\log K_2$ at pL value of 0.5 and 1.5 respectively.

The production of polynuclear complexes has been considered to be extremely unlikely due to the extremely low concentration of metal ions utilized in the titrations. Additionally, since the stability constants have been established in the low pH range, the development of hydroxy complexes may be disregarded.

2.2.4 Calculation of thermodynamic quantities

Understanding the thermodynamic behavior of metal complexes requires a comprehensive analysis of key energy parameters, including Gibbs free energy, enthalpy, and entropy changes. To fully comprehend the fundamental forces at work during complex formation in solution, it's essential to quantify these energy transformations. While stability constants provide direct insight into the free energy changes of complexation, they tell only part of the story. The determination of enthalpy changes becomes crucial as it enables scientists to calculate the entropy changes associated with complex formation. This complete thermodynamic picture is particularly valuable when studying how temperature influences complex stability. Temperature-dependent studies yield valuable insights into the interplay between these thermodynamic parameters and help predict the behavior of metal complexes under various conditions

2.2.5 Calculation of free energy

The stability constant (K) of the complex is related to the change in free energy ΔG by the reaction,

$$\Delta G = -2.303\ RT\ \log K$$

…….(3.31)

The equation is used (Y. Yastsimirskii, 1961) to evaluate the ΔG value.

2.2.6 Calculation of enthalpy

The change in enthalpy (ΔH) is given by the thermal effects on the stability constant as found by studies at different temperature (Crow D. R., 1979). Enthalpy change can be found directly from the isobaric equation.

$$\Delta H = -2.303R\ \frac{T_2 T_1}{T_2 - T_1} \log \frac{K_2}{K_1}$$

..……..(3.32)

Where K_1 and K_2 are stability constants at temperature T_1 and T_2 respectively. T is the absolute temperature and (in Kelvin) and R is the gas constant (8.314 J $mol^{-1}K^{-1}$).

2.2.7 Calculation of Entropy

The change in entropy ΔS can be calculated by the equation,

$$\Delta S = \frac{\Delta H - \Delta G}{\Delta T}$$

……..(3.33)

2.2.8 Antibacterial studies

Escherichia coli (NCIM 2065), a Gram-negative bacterium, was procured and sub-cultured on Nutritive Agar Media (NAM) to ensure optimal growth and viability. The sub-cultured bacteria were then stored at a refrigerated temperature of 4°C to maintain their viability for future use. Prior to the antibacterial activity test, the microbes were allowed to grow for 24 hours in NAM at optimal temperatures, reaching their logarithmic phase and ensuring maximum sensitivity to the test compound.

A novel Cu(II) complex with aspartic acid, a naturally occurring amino acid, was synthesized and tested for its antibacterial properties against Escherichia coli using the well diffusion method on NAM. This method involved creating wells in the agar medium and filling them with varying concentrations of the Cu(II)-aspartic acid complex. The plates were then incubated at 37°C for 24 hours, allowing the complex to diffuse into the surrounding agar.

By employing a millimeter-scale measurement to determine the zone of inhibition (ZOI) surrounding each well, the antibacterial activity was assessed. The ZOI is a clear, bacteria-free zone that forms around the well, indicating the effectiveness of the Cu(II)-aspartic acid complex in inhibiting bacterial growth. The size of the ZOI was directly correlated with the antibacterial potency of the complex, with larger zones indicating greater inhibitory effects.

III. RESULT AND DISCUSSION:

3.1 Potentiometric determination of stability constant

This study investigates the complexation of Cu(II) with aspartic acid, an amino acid, at temperatures 303, 308, and 313K. Three sets of test solutions were prepared, and solution (i) was placed in a titration vessel and thermally equilibrated at 303K for 15-20 minutes. A combination electrode was dipped into the solution and connected to a calibrated pH meter. The solution was then titrated with 0.01M NaOH, stirred with a magnetic stirrer, and pH readings were noted.

Subsequent calculations determined $\bar{n}$ and pL values, presented in table 4 indicating the formation of 1:1 and 1:2 complexes. Figures 1, 2, and 3 show formation curves plotted from pL and $\bar{n}$ values. Stability constants were calculated using least squares treatment and Bjerrum's half-integral method, with results shown in table 5.

Thermodynamic parameters, including overall changes in free energy (ΔG), enthalpy (ΔH), and entropy (ΔS), were determined from stability constants and presented in table 6. The study's findings demonstrate favorable complexation between Cu(II) and aspartic acid, with stability constants and thermodynamic

parameters supporting the formation of 1:1 and 1:2 complexes. These results contribute to understanding the interactions between metal ions and amino acids.

Table: 1 Complexation of Cu (II) with Aspartic acid (amino acid)

$[H]^+$ =7.5×10^{-4}mol dm^{-3} [L] = 2.5×10^{-3}mol dm^{-3} [M] = 2.5×10^{-4} mol dm^{-3} [NaOH] = 1×10^{-2}mol dm^{-3} Temperature = 303K I = 0.1 mol dm^{-3} KNO_3

No. of Observation Solution	(i) (with acid) Solution		(ii) (with ligand) Solution		(iii) (with ligand + metal)	
	No.of drops of NaOH	pH	No.of drops of NaOH	pH	No.of drops of NaOH	pH
1.	0	2.97	0	4.37	0	3.96
2.	4	3.9	4	4.65	4	4.01
3.	8	4.22	8	4.77	8	4.23
4.	12	4.86	12	4.89	12	4.54
5.	16	5.23	16	4.96	16	4.86
6.	20	5.89	20	5.02	20	4.99
7.	24	6.32	24	5.11	24	5.02
8.	28	6.9	28	5.23	28	5.09
9.	32	7.44	32	5.31	32	5.11
10.	36	7.99	36	5.44	36	5.16
11.	40	8.5	40	5.51	40	5.21
12.	42	8.9	42	5.58	42	5.24
13.	44	9.05	44	5.63	44	5.28
14.	46	9.11	46	5.65	46	5.32
15.	48	9.15	48	5.67	48	5.36
16.	50	9.21	50	5.69	50	5.38
17.	52	9.26	52	5.73	52	5.41
18.	54	9.30	54	5.79	54	5.44
19.	56	9.35	56	5.86	56	5.49
20.	58	9.41	58	5.91	58	5.56
21.	60	9.46	60	5.96	60	5.59
22.	62	9.52	62	6.01	62	5.64
23.	64	9.57	64	6.09	64	5.69
24.	66	9.63	66	6.13	66	5.75
25.	68	9.68	68	6.19	68	5.80
26.	70	9.74	70	6.23	70	5.85
27.	72	9.79	72	6.28	72	5.90
28.	74	9.83	74	6.34	74	5.94
29.	76	9.88	76	6.41	76	5.99
30.	78	9.99	78	6.48	78	6.02
31.	80	10.06	80	6.51	80	6.1

Table: 2 Complexation of Cu (II) with Aspartic acid (amino acid)

$[H]^+$ =7.5×10^{-4}mol dm^{-3} [L] = 2.5×10^{-3}mol dm^{-3} [M] = 2.5×10^{-4} mol dm^{-3} [NaOH] = 1×10^{-2}mol dm^{-3} Temperature = 308K I = 0.1 mol dm^{-3} KNO_3

No. of Observation Solution	(i) (with acid) Solution		(ii) (with ligand) Solution		(iii) (with ligand + metal)	
	No.of drops of NaOH	pH	No.of drops of NaOH	pH	No.of drops of NaOH	pH
1.	0	2.90	0	4.53	0	4.43
2.	4	3.42	4	4.89	4	4.76
3.	8	4.61	8	4.99	8	4.89
4.	12	5.43	12	5.09	12	4.98
5.	16	6.28	16	5.25	16	5.11
6.	20	6.93	20	5.33	20	5.27
7.	24	7.42	24	5.46	24	5.39
8.	28	7.79	28	5.59	28	5.51
9.	32	8.11	32	5.68	32	5.63
10.	36	8.33	36	5.79	36	5.71
11.	40	8.59	40	5.87	40	5.79

12.	42	8.71	42	5.95	42	5.83
13.	44	8.84	44	6.01	44	5.89
14.	46	8.93	46	6.08	46	5.91
15.	48	9.03	48	6.12	48	5.93
16.	50	9.19	50	6.14	50	5.95
17.	52	9.31	52	6.18	52	5.97
18.	54	9.46	54	6.23	54	5.99
19.	56	9.55	56	6.27	56	6.02
20.	58	9.64	58	6.31	58	6.04
21.	60	9.73	60	6.36	60	6.06
22.	62	9.81	62	6.40	62	6.08
23.	64	9.88	64	6.46	64	6.11
24.	66	9.93	66	6.52	66	6.13
25.	68	9.99	68	6.56	68	6.16
26.	70	10.03	70	6.61	70	6.19
27.	72	10.10	72	6.65	72	6.22
28.	74	10.13	74	6.67	74	6.25
29.	76	10.17	76	6.69	76	6.29
30.	78	10.20	78	6.73	78	6.32
31.	80	10.20	80	6.76	80	6.35

Table: 3 Complexation of Cu (II) with Aspartic acid (amino acid)

$[H]^+$ =7.5×10^{-4}mol dm^{-3} [L] = 2.5×10^{-3}mol dm^{-3} [M] = 2.5×10^{-4} mol dm^{-3} [NaOH] = 1×10^{-2}mol dm^{-3} Temperature = 313K I = 0.1 mol dm^{-3} KNO3

No. of Observation Solution	(i) (with acid) Solution		(ii) (with ligand) Solution		(iii) (with ligand + metal)	
	No.of drops	pH	No.of drops	pH	No.of drops	pH
	of NaOH		of NaOH		of NaOH	
1.	0	2.91	0	4.56	0	4.43
2.	4	4.14	4	4.83	4	4.78
3.	8	5.17	8	4.99	8	4.93
4.	12	6.33	12	5.18	12	5.11
5.	16	7.54	16	5.29	16	5.29
6.	20	8.27	20	5.37	20	5.41
7.	24	8.59	24	5.49	24	5.59
8.	28	8.85	28	5.57	28	5.71
9.	32	9.11	32	5.70	32	5.80
10.	36	9.29	36	5.83	36	5.93
11.	40	9.40	40	5.96	40	5.99
12.	42	9.49	42	6.03	42	6.02
13.	44	9.56	44	6.12	44	6.04
14.	46	9.61	46	6.19	46	6.06
15.	48	9.67	48	6.24	48	6.09
16.	50	9.73	50	6.30	50	6.13
17.	52	9.81	52	6.34	52	6.16
18.	54	9.87	54	6.37	54	6.19
19.	56	9.94	56	6.42	56	6.22
20.	58	9.99	58	6.47	58	6.25
21.	60	10.05	60	6.51	60	6.27
22.	62	10.09	62	6.56	62	6.30
23.	64	10.14	64	6.61	64	6.34
24.	66	10.19	66	6.65	66	6.37
25.	68	10.27	68	6.68	68	6.41
26.	70	10.34	70	6.71	70	6.45
27.	72	10.39	72	6.75	72	6.48
28.	74	10.43	74	6.79	74	6.52
29.	76	10.49	76	6.82	76	6.57
30.	78	10.51	78	6.85	78	6.61
31.	80	10.54	80	6.88	80	6.65

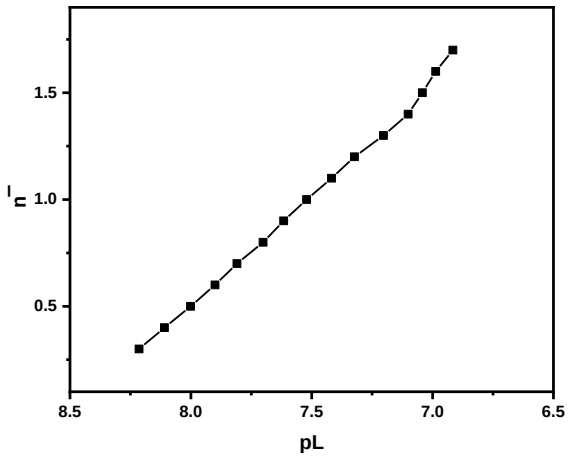

Figure 1: Formation curve of Cu (II) - Aspartic acid (amino acid) complexes in 50% (v/v) methanol water medium at temperature 303K

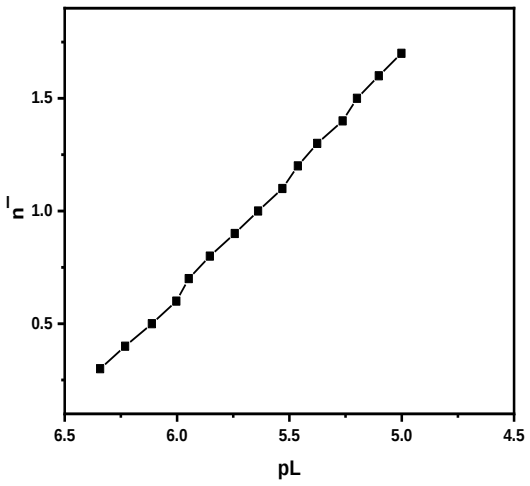

Figure 2: Formation curve of Cu (II) - Aspartic acid (amino acid) complexes in 50% (v/v) methanol water medium at temperature 308K

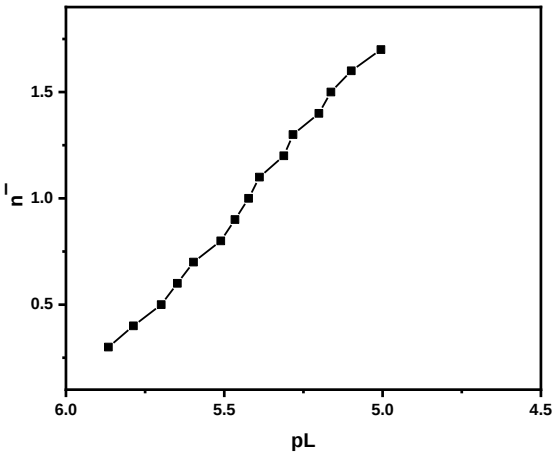

Figure 3: Formation curve of Cu (II) - Aspartic acid (amino acid) complexes in 50% (v/v) methanol water medium at temperature 313K

Table :4 $\bar{n}$ and pL values of Cu (II) with Aspartic acid (amino acid) at temperature 303, 308, and 313K

303K (pKa= 5.3)		308K (pKa= 5.6)		313K (pKa= 6.0)	
$\bar{n}$	pL	$\bar{n}$	pL	$\bar{n}$	pL
0.1		0.1		0.1	
0.2		0.2		0.2	
0.3	8.214	0.3	6.342	0.3	5.866
0.4	8.109	0.4	6.231	0.4	5.787
0.5	8.001	0.5	6.112	0.5	5.699
0.6	7.900	0.6	6.003	0.6	5.648
0.7	7.809	0.7	5.948	0.7	5.597
0.8	7.701	0.8	5.854	0.8	5.511
0.9	7.616	0.9	5.743	0.9	5.466
1.0	7.521	1.0	5.639	1.0	5.423
1.1	7.418	1.1	5.531	1.1	5.389
1.2	7.323	1.2	5.462	1.2	5.312
1.3	7.203	1.3	5.376	1.3	5.283
1.4	7.101	1.4	5.263	1.4	5.201
1.5	7.042	1.5	5.199	1.5	5.163
1.6	6.987	1.6	5.102	1.6	5.099
1.7	6.916	1.7	5.001	1.7	5.005

Table :5 Stability constant values for Complexation of Cu (II) with Aspartic acid (amino acid) in 50 % (v/v) water- methanol

Temperature K	Bjerrum half integral method			Weighted least square method		
	$\log K_1$	$\log K_2$	$\log\beta_2$	$\log K_1$	$\log K_2$	$\log\beta_2$
303	8.001	7.042	15.043	8.147	6.285	14.432
308	6.112	5.199	11.311	6.283	4.426	10.709
313	5.699	5.163	10.862	5.814	4.102	9.916

Table :6 Thermodynamic parameters for Cu (II) - Aspartic acid (amino acid) Complex at temperatures 303, 308, and 313K

Temperature K	Gibbs Energy change (kJmol^{-1})			Enthalpy change (303-313K kJmol^{-1})			Entropy change at 308K (kJmol^{-1}k^{-1})		
	$-\Delta G_1$	$-\Delta G_2$	$-\Delta G\beta_2$	$-\Delta H_1$	$-\Delta H_2$	$-\Delta H\beta_2$	$-\Delta S_1$	$-\Delta S_2$	$\Delta S\beta_2$
303	46.49	35.87	82.36						
308	36.47	25.69	62.16	92.83	87.45	180.28	50.85	43.21	94.11
313	34.36	24.25	58.61						

3.2 Antibacterial studies

Gram-negative Escherichia coli (*E. coli*) was significantly inhibited by the Cu(II)-aspartic acid combination.The complex's inhibitory effects were attributed to its ability to disrupt the bacterial cell membrane, interfere with metabolic processes, and induce oxidative stress. Specifically, the Cu(II) ion's coordination with aspartic acid enhanced its ability to penetrate the bacterial cell wall, causing damage to cellular components and ultimately leading to cell death. The complex's antibacterial potency was evaluated using the well diffusion method, revealing a notable zone of inhibition (ZOI) around the well. The ZOI's size correlated directly with the complex's concentration, indicating a dose-dependent antibacterial response. These findings suggest that the Cu(II)-Aspartic acid complex may serve as a promising antimicrobial agent against E. coli infections.

Figure 4: Antibacterial of Cu(II)-Aspartic acid complex against *E. Coli*

Table 7: Measurements of Zone of inhibition (mm) of Cu(II)-Aspartic acid complex

Bacteria	Cu(II)-Aspartic acid complex	
	10 µg/ml	20 µg/ml
E. coli	0.8	1.9

IV.CONCLUSION:

The experimental findings demonstrate successful formation of Cu(II)-aspartic acid complex with well-defined stability constants that vary with temperature. The negative ΔG values confirm the spontaneous nature of complex formation, while positive ΔH and ΔS values suggest an endothermic process driven by entropy. The stability constants show that the complexation reaction is exothermic as the temperature rises. The antimicrobial screening revealed that the Cu(II)-aspartic acid complex exhibits enhanced activity compared to free aspartic acid, suggesting potential applications in pharmaceutical research. The potentiometric method proved to be reliable for studying metal-amino acid interactions, providing valuable insights into the coordination chemistry of copper with aspartic acid. These results contribute to the understanding of metal-amino acid interactions and their biological implications, opening avenues for future research in bioinorganic chemistry and drug development.

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