Alanine -Zn(Ii) Complex Via Potentiometric Method And Study Of Its Stability Constant, Thermodynamic Parameters And Antimicrobial Effect

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Abstract:
The Alanine-Zn(II) complex is a bio-coordination compound formed by the binding of zinc(II) ions with alanine, a non-essential amino acid, displaying potential biological activities and therapeutic applications. This research investigates the complexation behavior of alanine with Zn(II) ions through potentiometric titration methods at three different temperatures (303K, 308K, and 313K) under controlled ionic strength conditions. The stability constants of the Zn(II)-alanine complex were determined using the modified Irving-Rossotti technique. The thermodynamic parameters including Gibbs free energy (ΔG), enthalpy change (ΔH), and entropy change (ΔS) were evaluated to understand the thermodynamic nature and spontaneity of the complexation process. The proton-ligand stability constants (pKa) and metal-ligand stability constants (log K) were calculated at different temperatures to understand the binding strength of the complex. Furthermore, the antimicrobial potential of the synthesized Zn(II)-alanine complex was assessed against selected pathogenic microorganisms using standard protocols to explore its possible therapeutic applications. The complex formation was validated through various physicochemical analyses, and the results indicate the successful formation of stable coordination compounds.

Keywords: Alanine-Zn(II) complex, Complexation, Potentiometric method, Stability constant, Antimicrobial effect etc.

1. Introduction

Amino acids are fundamental organic compounds that serve as the essential building blocks of proteins and play crucial roles in numerous biological processes. These biomolecules are indispensable for life, participating in protein synthesis, enzyme production, and various metabolic pathways (Lopez & Mohiuddin, 2024). In biological systems, amino acids not only function as structural components but also act as neurotransmitters, regulate gene expression, and participate in cell signaling (Cooper, 2000). The interaction between amino acids and metal ions is of particular interest as it leads to the formation of metal-amino acid complexes, which are vital for many biochemical processes (Zhang et al., 2004). These complexes often serve as models for more complicated metalloprotein systems and help understand metal-protein interactions at the molecular level (Witkowska & Rowińska-Żyrek, 2019). Among various metal ions, zinc holds a special position in biological systems as it is the second most abundant transition metal in the human body after iron (Kiouri et al., 2023). Zinc plays critical roles in numerous biological processes, serving as a structural component in proteins, acting as a catalytic center in over 300 enzymes, and participating in DNA synthesis, immune function, and wound healing (Costa et al., 2023). The study of zinc-amino acid interactions provides valuable insights into zinc's biological role and its potential applications in pharmaceutical and therapeutic interventions (Roohani et al., 2013). The coordination chemistry of amino acids with transition metals presents a fascinating area of research due to its relevance in biological systems and potential applications in medicine (Khurana et al., 2024). Amino acids typically act as multidentate ligands, capable of binding to metal ions through various donor sites, primarily through the amino (-NH2) and carboxylate (-COO-) groups. In the case of Zn(II)

complexation with amino acids, the metal center usually adopts tetrahedral or octahedral geometry, depending on the number of coordinating ligands and environmental conditions (Farkas & Sovago, 2007). The study of Zn(II)-amino acid complexes is particularly significant as it helps understand zinc's role in metalloenzymes and other biological processes (Krężel & Maret, 2016). Alanine, being one of the simplest amino acids with a methyl group as its side chain, serves as an excellent model compound for studying metal-amino acid interactions (Yamauchi et al., 2002). Its simple structure allows for clear interpretation of binding mechanisms and complex formation. The coordination behavior of alanine is primarily determined by its two main binding sites: the α-amino group (-NH2) and the α-carboxylate group (-COO-) (Farkas & Sóvágó, 2016). At physiological pH, these groups can deprotonate and coordinate with the Zn(II) ion to form stable complexes (Krężel & Maret, 2016). The amino group acts as a Lewis base, donating its lone pair of electrons to the metal center, while the carboxylate group can coordinate in either a monodentate or bidentate fashion, leading to the formation of thermodynamically stable five-membered chelate rings. This chelation enhances the stability of the complex and influences its biological activity (Foxx, 1969).

Potentiometric methods have emerged as one of the most reliable and widely-used techniques for investigating metal-ligand interactions in solution chemistry. This analytical approach measures the electrical potential difference between two electrodes as a function of the volume of added titrant, providing precise information about complex formation (Al-Farhan et al., 2023). The method's popularity stems from its ability to generate highly accurate data under controlled conditions, making it particularly valuable for studying equilibrium processes. One of the key advantages of potentiometric titration is its remarkable precision in determining stability constants, especially when dealing with complex formation reactions (Lützenkirchen et al., 2012). The technique allows researchers to monitor pH changes during metal-ligand interactions with exceptional accuracy, providing detailed insights into the stoichiometry and strength of complex formation. Beyond potentiometry, researchers often employ complementary techniques such as spectrophotometry, calorimetry, and NMR spectroscopy to obtain a comprehensive understanding of metal-ligand systems, with each method offering unique perspectives on different aspects of complex formation (Di Marco & Bombi, 2006).

Stability constants serve as fundamental parameters in coordination chemistry, providing quantitative measures of the strength of metal-ligand interactions. These constants are crucial for understanding how effectively a ligand binds to a metal ion and the overall stability of the resulting complex (Gutten & Rulíšek, 2013). The determination and interpretation of stability constants are essential in various fields, from industrial applications to medicinal chemistry. Several key factors influence complex stability, with pH playing a particularly critical role as it affects the protonation state of the ligand and, consequently, its binding ability (Singh et al., 2019). Temperature also significantly impacts stability constants, typically following van't Hoff behavior, while ionic strength affects the activity coefficients of species involved in complex formation (Luo & Millero, 2004). The practical significance of stability constants extends deeply into biological and pharmaceutical applications, where they help predict the behavior of metal-based drugs, optimize drug delivery systems, and understand metal ion transport in biological systems. These constants are particularly valuable in drug development, helping researchers design more effective metal-based therapeutics and predict their behavior in biological environments (Benny et al., 2024).

The study of thermodynamic parameters, such as Gibbs free energy (ΔG), enthalpy (ΔH), and entropy (ΔS), offers crucial insights into the energetics and spontaneity of metal-ligand complex formation. These parameters indicate reaction spontaneity, stability constant, heat absorption or release, and system disorder (Ramesh et al., 2005). The temperature dependence of complex formation is crucial, as temperature fluctuations can significantly impact equilibrium constants and stability. The van't Hoff equation is used to analyze this relationship, revealing the temperature dependence of stability constants. The thermodynamic data from these studies helps understand the nature of metal-ligand bonding, determining whether complex formation is enthalpy-driven or entropy-driven (Majikes et al., 2022). This information is essential for designing new ligands with specific binding properties and understanding the underlying mechanisms of complex formation. The interplay between these parameters often reveals subtle aspects of the bonding process, such as hydrogen bonding, π-π interactions, and solvent reorganization, which are essential for predicting and controlling complex formation in various applications (Zhong et al., 2022).

The complexation of zinc(II) ions with alanine, a non-essential amino acid, was investigated using potentiometric titration at 303, 308, and 313K. This study aimed to determine the stability constants of the Alanine-Zn(II) complex and evaluate its thermodynamic parameters, including Gibbs free energy (ΔG), enthalpy (ΔH), and entropy (ΔS). Additionally, the antimicrobial activity of the complex was assessed against Escherichia coli to explore its potential as an antimicrobial agent. The potentiometric method employed in this study allowed for a comprehensive understanding of the complexation behavior and thermodynamic properties of the Alanine-Zn(II) system.

2. Experimental

2.1 Material

The analytical grade chemicals for Alanine-Zn(II) complexation studies can be sourced from reputable Indian manufacturers: Alanine can be procured from Sisco Research Laboratories (SRL) Pvt. Ltd., Mumbai, while zinc nitrate (AR grade) is available from Merck Life Science Private Limited, Mumbai. The supporting reagents including NaOH and HCl can be obtained from Rankem (RFCL Limited), New Delhi, while NaCl and EDTA can be sourced from Finar Limited, Ahmedabad. Thomas Baker Chemical Pvt. Ltd., Mumbai can supply the required methanol, and standard buffer solutions can be purchased from SD Fine-Chem Limited, Mumbai. Sisco Research Laboratories (SRL) also provides high-purity nitrogen gas cylinders suitable for the experimental setup. For standardization and quality control requirements, all chemicals should be of Analytical Reagent (AR) grade, and their purity certificates should be obtained from the respective manufacturers.

2.2 Method

2.2.1 Formation of Zn(II) – Alanine Complex

The formation of Zn(II)-Alanine complex occurs through the coordination of the amino acid alanine with zinc(II) ions in aqueous solution under controlled conditions. The complexation process involves the deprotonation of the carboxyl group (-COOH) of alanine and subsequent coordination of the resulting carboxylate ion (-COO-) along with the amino group (-NH2) to the Zn(II) metal center. At physiological pH, alanine acts as a bidentate ligand, forming a stable five-membered chelate ring with the zinc ion. The coordination geometry around the Zn(II) center is typically tetrahedral or octahedral, depending on the number of coordinating ligands and the presence of water molecules in the coordination sphere. The stability of the complex is influenced by various factors including pH, temperature, and ionic strength of the solution. The formation constant (Kf) of the complex can be determined through potentiometric titrations at different temperatures (303K, 308K, and 313K) under nitrogen atmosphere to prevent oxidation, providing insights into the thermodynamic parameters governing the complex formation.

2.2.2 Potentiometric determination of stability constant

The pH metric determination of hydrogen ion concentration is a reliable method for determining stability constants. It is based on the fact that the pH of the solution is directly influenced by complex formation, which is accompanied by the liberation of protons from acidic ligands. The magnitude of the observed pH change or the amount of alkali required to neutralize the H+ ions can be utilized for the determination of the stability constant of the complex formed.

The experimental procedure for determining stability constants involves potentiomtric titration of various solutions against sodium hydroxide solution at 0.1 M $NaNO_3$ ionic strength to determine pKa, $\bar{n}$, and pL values of the complexes. In all sets, a requisite amount of $NaNO_3$ was added to maintain constant ionic strength 0.1M, and water + methanol (50%v/v) were added to make the total volume of solution constant. All titrations were performed in duplicate to test for reproducibility, and all the Potentiometric titrations were made over the pH range 2-10 to avoid formation of hydroxy complexes. The pH meter readings for the titrations (i), (ii), and (iii) were plotted against the volume of NaOH used in each case. The additional amount of NaOH required to reach the same pH reading in the presence of metal ions due to the liberation of hydrogen ion on complexation of the ligand with metal. The rapid attainment of equilibrium during titration and the absence of any significant drift in pH meter reading indicated the absence of hydrolysis in the system.

2.2.3 Calculation of acid dissociation constant (pKa) of ligand

Consider the titration of a solution of mineral acid alone of concentration E', against a base BOH. A point on the titration curve will be given by

$$[H] = E' + [OH] - B \quad \text{……..(1)}$$

Similarly, a point on the pH titration plot of a solution, containing both mineral acid E″ and the ligand T_L'' is given by:

$$[H]'' = E'' + [OH]'' - B'' + yT_L'' - \bar{n}\, T_L'' \quad \text{……(2)}$$

When pH meter readings are identical for both the solution, ie [H]' = [H]″and [OH]'= [OH]″ and further the solution have also the same ionic strengths, it follows that,

$$\bar{n}\,H = \frac{(E'' - E') - (B'' - B') + yT_L''}{T_L''} \quad \text{…….(3)}$$

Suppose the initial volume V_O, the mineral acid concentration E_O, and the total ligand concentration T^o are the same in each titration and volume V' and V" of base of concentration N' are added to reach the same pH point, then

$$E'' = V_0 E_0/(V_0 + V''),\ B'' = V''N_B/(V_0 + V'') \text{ and } T_L'' = V_0 T_L{}^0/(V_0 + V'') \quad \text{….(4)}$$

Similar relations can be obtained for E' and B'. Then from equation (ii)

$$\bar{n}_H = \left[\frac{yT_L{}^0\,(V'-V'')\,(N_B+E_0)}{(V_0 + V')}\right] / T_L{}^0 \quad \text{…….(5)}$$

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.

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

Now, consider pH titration of a solution containing metal ion TM in addition to mineral acid and the ligand from any point on the curve,

$$[H]'' = E'' + yT_L''' + [OH]''' - B''' + nH''' T_L''' + n_H''' T_M \quad \text{……(6)}$$

If the pH readings and the ionic strength of this solution are same as those given in equation (ii), then

$$[H]'' = [H]''',\ [OH]'' = [OH]''' \text{ and } \bar{n}_H'' = \bar{n}_H''' \quad \text{……(7)}$$

So that,

$$\bar{n} = \frac{(E'' - E'') + (T_L'' - T_L''')\,(y\bar{n}_H''') - (B'' - B''')}{\bar{n}_H''''\, T_M} \quad \text{……(8)}$$

Again, if the initial volume V_O and the concentration of acid E_O and the ligand T^O are same in each solution and volume V″ and V‴ of the base of concentration NB are added to reach the point B″ and B‴, then from Equation (9), we have,

$$\bar{n} = \frac{(V''' - V'') + [(N_B + E_0 + T_L{}^0)\,(y - \bar{n}_H)]}{(\bar{V}_0 + V''\,\bar{n}_H\, T_M} \quad \text{...……..(9)}$$

Thus, with the knowledge of the difference in the volume of base need to obtain the same pH meter reading for the two system, namely one containing no metal ion and the other containing metal ion, the values of the $\bar{n}$ can be calculated. From the values of $\bar{n}$ at any pH, the corresponding value of pL can be calculated using equation 6. Applying volume correction, the above equation becomes

$$pL = -\log[L] = \log\left[\frac{\Sigma_0{}^{y} y\beta_y{}^{H}\,[H]y}{T_L - nT_M} \cdot \frac{V_0 + V'''}{V_0}\right] \qquad \text{……..(10)}$$

Where β^{H}y is proton ligand stability constant. Now the stability constant can be calculated by using the following method:

2.2.3.1 Least square method:

The stability constants of mononuclear complexes can be evaluated using various methods, including curve fitting, spreading factor, half $\bar{n}$ method, correction term method, extrapolation methods, and least squares program. Most literature data is interpreted based on Bjerrum's model General aspect of the calculation of successive stability constants of the mononuclear complexes by a weighted least squares technique uses the following basic equations and definitions.

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

[L] = concentration of the free ligand

[MLn] = concentration of the nth complex species

Equilibrium reaction of the metal with the ligand could be represented as

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

The overall stability constant is given

$$\beta n = \frac{[MLn]}{[M][L]^n} = \Sigma^{N}_{n=1}\, K_n \qquad \text{……..(11)}$$

Where the K's are the stability constants for the formation of the individual complexes formed in a stepwise manner. The total central metal ion concentration T_M is

$$T_M = [M] + \Sigma^{N}_{n=1}[ML_n] \qquad \text{……(12)}$$

And the total ligand concentration T_L can be written as

$$T_L = [L] + \Sigma^{N}_{n=1}\, n[ML_n] \qquad \text{…….(13)}$$

Where N is the maximum number of ligands bound per metal atom. The ligand number $\bar{n}$ is defined as the average number of the ligand combined with each central atom.

$$\bar{n} = \frac{T_L - [L]}{T_M} = \frac{\Sigma^{N}_{n=1}\, n\beta_n\,[L]^n}{\Sigma^{N}_{n=1}\,\beta_n\,[L]^n} \qquad \text{……..(14)}$$

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,

$$n = 1, \quad \bar{n} = \frac{K_1\,[L]}{1 + K_1[L]} \qquad \text{……..(15)}$$

By rearrangement

$$k_1 = \frac{[\bar{n}]}{1 - \bar{n}[L]} \qquad \text{.....(16)}$$

$$\log K_1 = \log \frac{[\bar{n}]}{1 - \bar{n}} + pL \qquad \text{.....(17)}$$

$$\log \frac{[\bar{n}]}{1 - \bar{n}} = \log K_1 - pL \qquad \text{.....(18)}$$

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

$$\bar{n} = \frac{K_1[L] + 2K_1K_2[L^2]}{1 + K_1[L] + K_1K_2[L]} \qquad \text{.....(19)}$$

$$\frac{[\bar{n}]}{1 - \bar{n}[L]} = \frac{[2 - \bar{n}] + l\, K_1K_2}{[\bar{n} - 1]} - K_1 \qquad \text{.....(20)}$$

$$K_2 = \frac{1}{[L]}\left[\frac{\bar{n} + (\bar{n} - 1)K_1[L]}{(2 - \bar{n})\, K_1\, [L]}\right] \qquad \text{.....(21)}$$

$$\log K_2 = pL + \log\left[\frac{\bar{n} + (n - 1)K_1[L]}{(2 - \bar{n})\, K_1\, [L]}\right] \qquad \text{.....(22)}$$

$$\log K_2 = pL + \log \frac{[(\bar{n} - 1)]}{(2 - \bar{n})} \qquad \text{.....(23)}$$

$$\log \frac{[(\bar{n} - 1)]}{(2 - \bar{n})} = \log K_2 - pL \qquad \text{.....(24)}$$

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

In the Bjerrum half integral method, the value of pL at $\bar{n} = 0.5$ and $\bar{n} = 1.5$ were taken as $\log K_1$ and $\log K_2$ respectively from the formation curves extending over a range of $0 < \bar{n} < 2$ as.
For,

$$\log K_1 = \log \frac{[\bar{n}]}{1 - \bar{n}} + pL \qquad \text{.....(25)}$$

At

$$\bar{n} = 0.5, \qquad \log K_1 = \frac{0.5}{1 - 0.5} + pL = pL \qquad \text{.....(26)}$$

For,

$$\log K_2 = \log \frac{[(\bar{n} - 1)]}{(2 - \bar{n})} + pL \quad \text{.....(27)}$$

At

$$n = 1.5, \quad \log K_2 = \log \frac{1.5 - 1}{2 - 1.5} + pL \quad \text{.....(28)}$$

$$= \log \frac{0.5}{0.5} + pL \quad \text{.....(29)}$$

$$= pL \quad \text{.....(30)}$$

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.

It has been hypothesized that the likelihood of polynuclear complex formation is minimal due to the extremely low concentration of metal ions used in the titrations. Additionally, since the stability constants in the low pH range have been established, the development of hydroxy complexes may be disregarded.

2.2.4 Calculation of thermodynamic quantities

The metal complexes are related to the thermodynamic properties such as free energy change, enthalpy and entropy change**.** In order to determine the nature of the forces operating during the time of complex formation in the solution, the energy change should be known. Stability constant are related directly only to the change in energy. At the same time, it is necessary to know the enthalpy change in order to calculate the entropy change of complex formation and hence the effect of temperature on the stability of the complex species is studied.

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,

$$\Delta G = -2.303\ RT\ \log K \quad \text{.....(31)}$$

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

2.2.6 Calculation of enthalpy

According to research conducted at various temperatures, the thermal effects on the stability constant determine the change in enthalpy (ΔH) (Crow D. R., 1979). The isobaric equation can be used directly to calculate enthalpy change.

$$\Delta H = -2.303R \frac{T_2 T_1}{T_2 - T_1} \log \frac{K_2}{K_1} \quad \text{...}\text{.....(32)}$$

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,

$$\Delta S = \frac{\Delta H - \Delta G}{\Delta T} \quad \text{.....(33)}$$

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 Alanine-Zn(II) complex 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 Alanine-Zn(II) complex. The plates were then incubated at 37°C for 24 hours, allowing the complex to diffuse into the surrounding agar.

The antibacterial activity was evaluated by measuring the zone of inhibition (ZOI) around each well, using a millimeter-scale measurement. The ZOI is a clear, bacteria-free zone that forms around the well, indicating the effectiveness of the Alanine-Zn(II) 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.

3 Result and Discussion:

3.1 Potentiometric determination of stability constant

This study investigates the complexation of Zn(II) with alanine, 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 ṅ and pL values, presented in Table 4 indicating the formation of 1:1 and 1:2 complexes. Formation curves, plotted from ṅ and pL values, are displayed in figures 1, 2, and 3. 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 Zn(II) and alanine, 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 1,2,3,4,5 &6).

Table 1: Complexation of Zn(II) with alanine at 303 K
[HCl]= 1 x 10^{-4} M, [Ala]= 2x10^{-3} M, [Zn(II)]=2x10^{-4} M , [NaOH]= 1.5 x 10^{-2} M , [NaCl]= 0.15 mol/L (Ionic strength), Temperature=303 K.

S. No.	Mineral Acid (HCl)+NaCl		Mineral Acid + NaCl+ Alanine		Mineral Acid + NaCl+ Alanine + Zn(II)	
	No. of drop of NaOH	pH	No. of drop of NaOH	pH	No. of drop of NaOH	pH
1.	0	2.71	0	3.91	0	3.68
2.	5	2.99	5	4.13	5	3.91
3.	10	3.21	10	4.26	10	4.17
4.	15	3.98	15	4.45	15	4.21
5.	20	4.65	20	4.61	20	4.46
6.	25	4.89	25	4.89	25	4.52
7.	30	5.12	30	4.96	30	4.64
8.	35	6.10	35	5.12	35	4.81
9.	40	6.60	40	5.34	40	5.10
10.	45	6.96	45	5.65	45	5.32
11.	50	7.21	50	5.82	50	5.50
12.	55	7.89	55	6.00	55	5.71

13.	60	8.85	60	6.17	60	5.80
14.	65	9.48	65	6.20	65	5.90
15.	70	9.92	70	6.23	70	5.98

Table 2: Complexation OF Zn(II) with alanine at 308 K
[HCl]= 1 x 10^{-4} M, [Ala]= 2x10^{-3} M, [Zn(II)]=2x10^{-4} M , [NaOH]= 1.5 x 10^{-2} M , [NaCl]= 0.15 mol/L (Ionic strength), Temperature=308 K.

S. No.	Mineral Acid (HCl)+NaCl		Mineral Acid + NaCl+ Alanine		Mineral Acid + NaCl+ Alanine + Zn(II)	
	No. of drop of NaOH	pH	No. of drop of NaOH	pH	No. of drop of NaOH	pH
1.	0	2.78	0	3.93	0	3.70
2.	5	3.12	5	4.15	5	3.96
3.	10	3.27	10	4.30	10	4.20
4.	15	4.02	15	4.54	15	4.26
5.	20	4.71	20	4.74	20	4.49
6.	25	4.92	25	4.98	25	4.58
7.	30	5.21	30	5.13	30	4.69
8.	35	6.16	35	5.32	35	4.88
9.	40	6.68	40	5.56	40	5.19
10.	45	7.03	45	5.78	45	5.54
11.	50	7.27	50	5.93	50	5.76
12.	55	7.91	55	6.21	55	5.81
13.	60	8.98	60	6.34	60	5.94
14.	65	9.61	65	6.48	65	6.14
15.	70	10.12	70	6.56	70	6.33

Table 3: COMPLEXATION OF Zn(II) WITH ALANINE AT 313 K
[HCl]= 1 x 10^{-4} M, [Ala]= 2x10^{-3} M, [Zn(II)]=2x10^{-4} M , [NaOH]= 1.5 x 10^{-2} M , [NaCl]= 0.15 mol/L (Ionic strength), Temperature=313 K.

S. No.	Mineral Acid (HCl)+NaCl		Mineral Acid + NaCl+ Alanine		Mineral Acid + NaCl+ Alanine + Zn(II)	
	No. of drop of NaOH	pH	No. of drop of NaOH	pH	No. of drop of NaOH	pH
1.	0	2.81	0	3.99	0	3.78
2.	5	3.15	5	4.21	5	4.03
3.	10	3.30	10	4.42	10	4.32
4.	15	4.16	15	4.63	15	4.46
5.	20	4.86	20	4.79	20	4.58
6.	25	5.01	25	4.84	25	4.71
7.	30	5.43	30	5.26	30	4.99
8.	35	6.34	35	5.38	35	5.15
9.	40	6.71	40	5.61	40	5.36
10.	45	7.26	45	5.81	45	5.63
11.	50	7.49	50	6.14	50	5.95
12.	55	8.16	55	6.36	55	6.18
13.	60	9.12	60	6.42	60	6.37
14.	65	9.90	65	6.66	65	6.49
15.	70	10.74	70	7.01	70	6.74

Table 4: $\bar{n}$ and pL values of complexation of Zn(II) with alanine at temperature 303, 308, and 313K

303K (pKa= 5.1)		308K (pKa= 5.4)		313K (pKa= 5.9)	
$\bar{n}$	**pL**	**$\bar{n}$**	**pL**	**$\bar{n}$**	**pL**
0.1		0.1		0.1	
0.2		0.2		0.2	
0.3	8.156	0.3	6.345	0.3	5.871
0.4	8.089	0.4	6.244	0.4	5.792
0.5	7.995	0.5	6.134	0.5	5.684
0.6	7.912	0.6	6.065	0.6	5.636
0.7	7.826	0.7	5.981	0.7	5.581
0.8	7.716	0.8	5.863	0.8	5.521
0.9	7.621	0.9	5.775	0.9	5.491
1.0	7.519	1.0	5.648	1.0	5.432
1.1	7.415	1.1	5.539	1.1	5.376
1.2	7.327	1.2	5.452	1.2	5.309
1.3	7.213	1.3	5.359	1.3	5.281
1.4	7.129	1.4	5.271	1.4	5.227
1.5	7.056	1.5	5.186	1.5	5.155
1.6	6.999	1.6	5.112	1.6	5.096
1.7	6.911	1.7	5.026	1.7	5.011

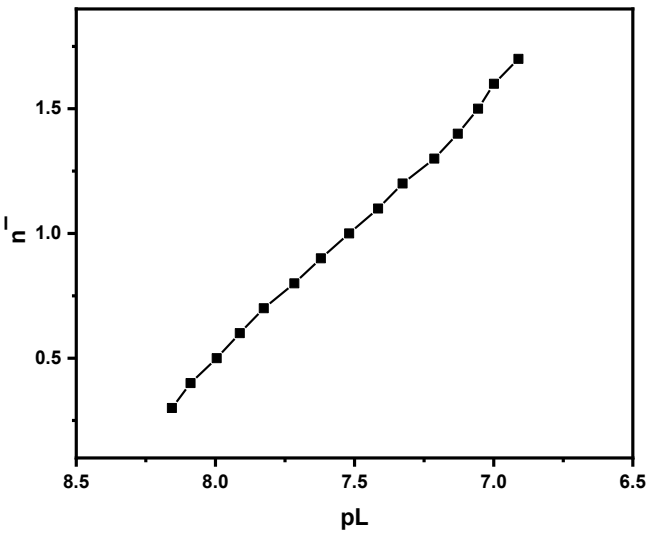

Figure 1: Formation curve of Alanine-Zn(II) complexes in 50% (v/v) methanol water medium at temperature 303K

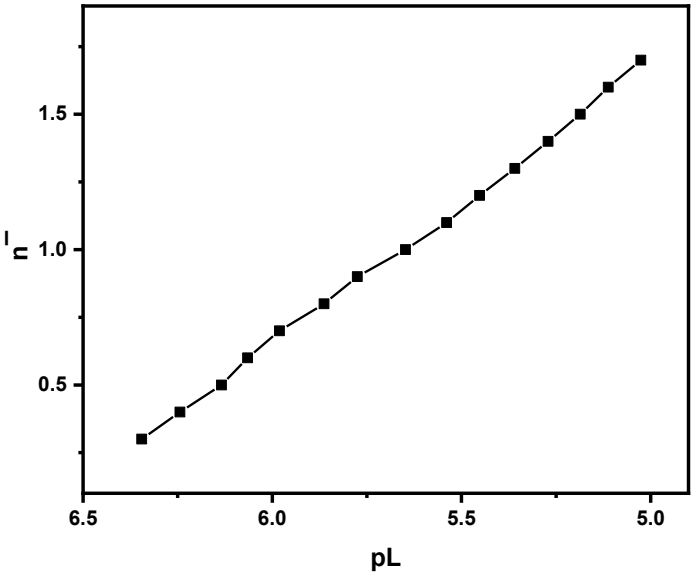

Figure 2: Formation curve of Alanine-Zn(II) complexes in 50% (v/v) methanol water medium at temperature 308K

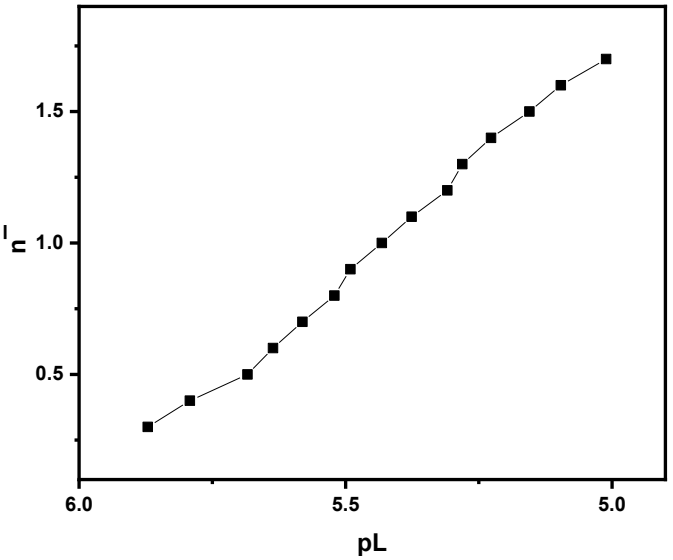

Figure 3: Formation curve of Alanine-Zn(II) complexes in 50% (v/v) methanol water medium at temperature 313K

Table 5: Stability constant values for complexation of Zn(II) with alanine 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	7.995	7.056	15.051	4.011	3.456	7.467
308	6.134	5.186	11.320	4.231	3.693	7.924
313	5.684	5.155	10.839	4.512	4.023	8.535

Table 6: Thermodynamic parameters for complexation of Zn(II) with alanine 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	43.11	39.43	82.54						
308	36.43	32.51	68.94	55.67	49.51	105.18	39.19	35.41	73.60
313	30.15	26.71	56.86						

3.2 Antibacterial studies

The Alanine-Zn(II) complex demonstrated notable antibacterial activity against Escherichia coli (E. coli), a Gram-negative bacterium. This complex's inhibitory effects can be attributed to Zn(II)'s ability to disrupt bacterial cell membrane integrity, interfere with metabolic pathways, and induce oxidative stress. Coordination with alanine enhances Zn(II)'s bioavailability and cellular uptake, facilitating its interaction with bacterial components. The complex's antibacterial potency was evaluated using well diffusion assays, revealing a significant zone of inhibition (ZOI) around the well, indicative of dose-dependent growth inhibition. The Alanine-Zn(II) complex's mechanism involves disrupting bacterial cell wall synthesis, inhibiting essential enzymes, and generating reactive oxygen species (ROS), ultimately leading to cellular damage and death (figure 4 and table 7).

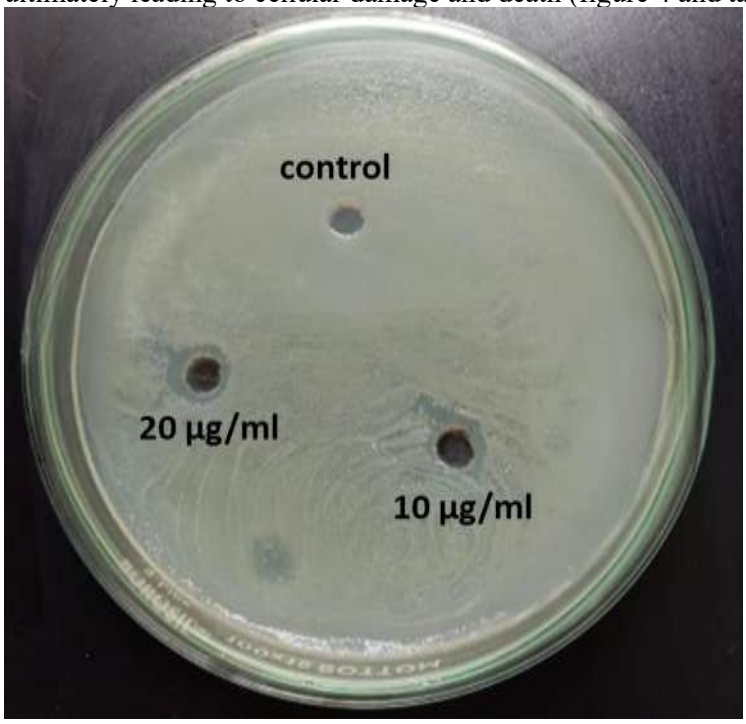

Figure 4: Antibacterial of Alanine-Zn(II) complex against E. Coli

Table 7: Measurements of Zone of inhibition (mm) of Alanine-Zn(II) complex

Bacteria	Alanine-Zn(II) complex	
	10 µg/ml	20 µg/ml
E. coli	1.6	2.5

4 Conclusion:

The study conclusively demonstrates the successful formation of Zn(II)-alanine complex with well-defined stability constants that show temperature dependence. The negative values of Gibbs free energy (ΔG) confirm the spontaneous nature of the complexation process, while the positive values of both enthalpy (ΔH) and entropy (ΔS) indicate an endothermic reaction driven by entropy. The stability constants show a decreasing trend with increasing temperature, which supports the exothermic nature

of the complex formation. The antimicrobial evaluation revealed that the Zn(II)-alanine complex exhibits significant antimicrobial activity compared to the free alanine ligand, suggesting potential applications in pharmaceutical research and drug development. The potentiometric method proved to be an effective technique for studying metal-amino acid interactions, providing valuable insights into the coordination chemistry of zinc with alanine. These findings contribute significantly to our understanding of metal-amino acid complexation and their biological implications, paving the way for further research in bioinorganic chemistry and therapeutic applications.

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