

Preparation, Spectroscopic Analysis and Antibacterial Activity of Some Selected Amino Acids Transition Metal Complexes

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Abstract: This study was conducted to prepare, analysis and antibacterial activity of some selected amino acids (Glycine and Phenylalanine) and their transition metal complexes with Ni(II) and Co(II). The solid complexes were synthesized through condensation of amino acids with appropriate amount of chloride salt of Nickel (II) and Cobalt (II) in (1:2) molar ratio of [M-L] in aqueous media at pH (9.6). The Prepared complexes were subjected to spectroscopic analysis by infrared and UV-Vis. The prepared metal complexes were tested against two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtiles*) and two gram negative bacteria (*Escherichia coli*, and *Pseudomonas aeruginosa*) by using diffusion method. The results of infrared spectra of amino acids metal complexes showed several new bonds formed [M-N, M-O] which indicate that the amino acids acts as bidentate ligands involving carboxylic oxygen and nitrogen atom of amino group in coordination and the electronic spectra of amino acids metal complexes showed absorption bands correspond to the $n-\sigma^*$, $n-\pi^*$, and $\pi-\pi^*$. The result of the antibacterial tests indicates that the Co(II) Glycine complex possesses higher activity and other metal complexes Co(II) phenylalanine, Ni(II) Glycine, Ni(II) Phenylalanine possess partial activity.

Keywords: Glycine and Phenylalanine, Nickel (II) and Cobalt (II) Complexes, Antibacterial Activity.

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1. INTRODUCTION

In recent years transition metals amino acid complexes have received much attention because the proved to be useful, antibacterial agent [1]. Twenty natural amino acids comprise the building block of proteins, [2] from these twenty amino acids, eight are an essential cannot be produce by human body, Complexes of transition metal with amino acids in proteins and peptides are utilized in numerous biological processes, such as oxygen conveyer, electron transfer and oxidant. In these processes the enzymatic active site which is very specific, forms complexes with divalent metal ions [3]. A knowledge of the interaction between biological active molecules and metal is needed when preparing biomaterials or considering certain aspects of biocompatibility. The study of model species such as the simple amino acids can assist in the interpretation of

more complex system. Amino acid has the neutral donor N at one end and acidic replaceable length to span two adjacent coordinating sit and the resulting complexes is a non-electrolyte chelate or inner complex compound. Antibodies it has been suggested that valine may be beneficial for those with herpes simplex infection [4].

2. EXPERIMENTAL

Materials and Measurements

All chemicals used in this work were analytical grade and used without further purification. Infrared spectra were recorded by using KBr disc in the range (4000-400 cm^{-1}) on (FT-IR type-1650) spectrophotometer. UV-Visible electronic spectra were recorded on (UV-Visible type-1800) spectrophotometer Shimadzu.

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Synthesis of Amino Acids Complexes

Preparation of Nickel and Cobalt Glycine Complexes

These complexes were prepared by mixing 1.5g (2mmol) of glycine dissolved in 10 ml distilled water for deprotonation of amino acids NaOH was added and the pH was adjusted to 9.6 and then (2.37g) (1mmol) of

Cobalt Chloride or 1.1g (1mmol) Nickel Chloride dissolved separately in 30 ml of distilled water and then added to the deprotonated amino acids solution under stirring the mixture was heated under reflux for 2-3 hours the final product was washed and air dried [5-7].

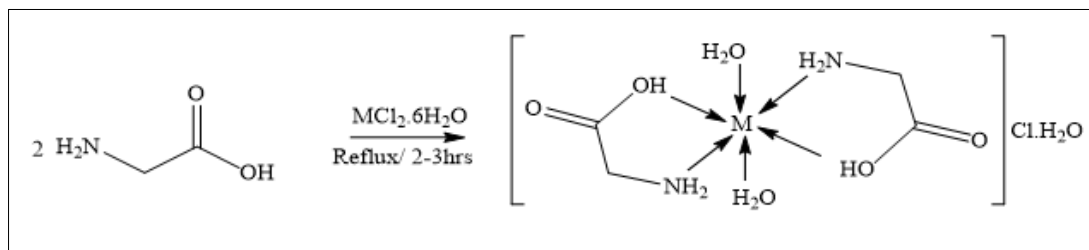


Figure 1: Preparation of Nickel (II) and Cobalt (II) glycine complexes

Preparation of Nickel and Cobalt Phenylalanine Complexes

These complexes were prepared by mixing 1.78g (2mmol) of phenylalanine dissolved in 10 ml of distilled water for deprotonation of amino acids NaOH was added and the pH was adjusted to 9.6 and then 2.37g

(1mmol) of Cobalt Chloride or 1.1g (1mmol) Nickel Chloride dissolved separately in 30ml of distilled water and then added to the deprotonated amino acids solution under stirring the mixture was heated under reflux for 2-3 hours the final product was washed and air dried [5-7].

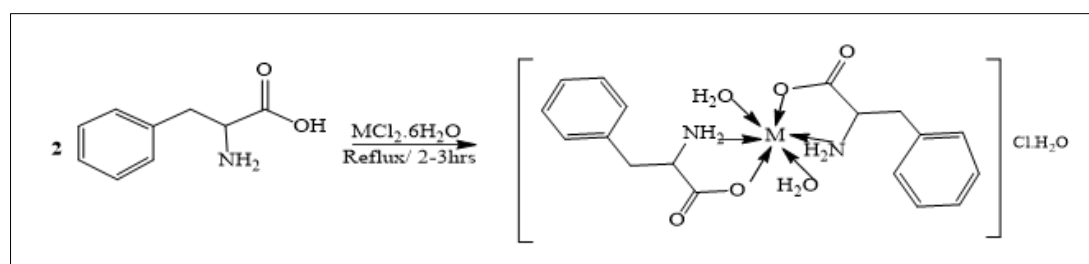


Figure 2: Preparation of Nickel (II) and Cobalt (II) phenylalanine complexes

Preparation of Tested Organisms

Preparation of Bacterial Suspensions

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8 C.F.U/ml. The suspension was stored in the refrigerator at 4°C till used. The average number of viable organisms per ml of the stock. Suspension was determined by means of the surface viable counting technique. Serial dilution of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37°C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension [8]. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant

so that suspensions with very close obtained viable counts would be counts [8].

Testing of Antibacterial Susceptibility

Paper disc diffusion method was used to screen the antibacterial activity of the prepared compounds and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines Bacterial suspension was diluted with sterile physiological solution to 10^8 cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20µl of a solution of each compound [9].

The inoculated plates were incubated at 37°C for 24 h in the inverted. The diameters (mm) of the inhibition zones were measured antibacterial activity results were expressed in term of the diameter of zone of inhibition and. <9 mm zone was considered as inactive, (9-12)mm as partially active while 13-18mm as active and >18mm as very active [9].

3- RESULTS AND DISCUSSION

Characterization of Complexes

The selected amino acids Glycine and Phenylalanine form complexes of Stoichiometric ratio

1:2 (M:L) with Co(II), Ni (II) metal ions The synthesized complexes have been investigated by electronic spectra and infrared spectra.

Table 1: Some physical properties of the prepared complexes

S. No	Complexes	Color	Yield%	Molecular Weight	Abs λ_{max}
1	Ni-(Gly) ₂ (H ₂ O) ₂ Cl.H ₂ O	Green	23	249.14	205, 252
2	Co-(Gly) ₂ (H ₂ O) ₂ Cl.H ₂ O	Purple	25	248.14	206
3	Ni-(Phe) ₂ (H ₂ O) ₂ Cl.H ₂ O	Green	40	429.38	214, 247, 252, 258, 264
4	Co-(Phe) ₂ (H ₂ O) ₂ Cl.H ₂ O	Purple	16	428.38	220,247,258,264

Electronic Spectra of Glycine and Phenylalanine Ni (II) and Co (II) complexes

The electronic spectra of free amino acids Glycine and Phenylalanine, showed strong absorption bands at 217 and 223 nm; these bands assigned to $\pi \rightarrow \pi^*$ transition [5]. The energy of intra ligand bands slightly changed upon complexation due to the involvement of oxygen nitrogen atoms of amino acids in coordination [5, 10]. The intraligand transitions of the Co (II) and the Ni(II) complexes of Glycine and Phenylalanine are observed in the range (205 to 264) nm and these bands are mainly due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions [5-10].

Infrared spectrum of Glycine Co (II) and Ni (II) complexes

The ν (M-N) frequencies Co-Gly and Ni-Gly complexes were observed at 514 and 518 cm^{-1} respectively while ν (M-O) frequencies were identified at 700 and 705 respectively. The symmetric vibrations absorption of ν (COO) appear at 1467 and 1463 cm^{-1} and asymmetric vibration absorption of ν (COO) appear at 1622 and 1622 cm^{-1} respectively. Absorption peaks at

3022 and 3018 cm^{-1} were assigned to ν (NH₂) stretching frequency of Co-Gly and Ni-Gly complexes respectively. All complexes showed additional bands at 3414 and 3415 cm^{-1} attributed to the water molecule. Absorption peaks about 2904 and 2719 cm^{-1} was assigned to ν (C-H) stretching frequency.

Infrared spectrum of Phenylalanine Ni (II) and Co (II) Complexes

The ν (M-N) frequencies for Co-Phe and Ni-Phe complexes were observed at 464 and 468 cm^{-1} while ν (M-O) frequencies were identified at 546 and 526 cm^{-1} respectively. The symmetric vibrations absorption of ν (COO) appear at 1494 and 1492 cm^{-1} and asymmetric vibration absorption of ν (COO) appear at 1560 and 1562 cm^{-1} for Co-Phe and Ni-Phe respectively. Absorption peaks at 3029 and 3024 cm^{-1} were assigned to ν (NH₂) stretching frequency for Co-Phe and Ni-Phe respectively. All complexes showed additional bands at 3747 and 3340 cm^{-1} attributed to water molecules. Absorption peaks at 2956 and 2416 cm^{-1} was assigned to ν (C-H) stretching frequency.

Table 2: IR spectra of Glycine and phenylalanine complexes

S. No	Complexes	ν (COO)	as ν (COO)	ν (NH ₂)	ν (-CH)	ν (H ₂ O)	ν (M-O)	ν (M-N)
1	Ni-(Gly) ₂ (H ₂ O) ₂ Cl.H ₂ O	1463	1622	3018	2719	3415	705	518
2	Co-(Gly) ₂ (H ₂ O) ₂ Cl.H ₂ O	1467	1622	3022	2904	3414	700	514
3	Ni-(Phe) ₂ (H ₂ O) ₂ Cl.H ₂ O	1492	1562	3024	2616	3340	526	468
4	Co-(Phe) ₂ (H ₂ O) ₂ Cl.H ₂ O	1494	1560	3029	2956	3747	546	464

Antibacterial Activity

The Prepared complexes were screened for their in vitro antibacterial activity against, (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*). The antibacterial activity results of metal complexes were presented in Table (3) it

has been observed that most compounds showed antibacterial activity. The results obtained indicated that, the Co-Gly complex shows very good activity against all tested bacteria and the other metal complexes of (Co-Phe, Ni-Gly and Ni-Phe) show partial activity.

Table 3: Inhibition Zones of metal complexes in (mm) at (1mg/ml)

S. No	Complexes	Conc Mg/ml	B.s	S.a	E.coli	Ps.a
1	Ni-(Gly) ₂ (H ₂ O) ₂ Cl.H ₂ O	%10	9	9	10	10
2	Co-(Gly) ₂ (H ₂ O) ₂ Cl.H ₂ O	%10	23	19	16	21
3	Ni-(Phe) ₂ (H ₂ O) ₂ Cl.H ₂ O	%10	12	11	13	10
4	Co-(Phe) ₂ (H ₂ O) ₂ Cl.H ₂ O	%10	9	8	8	8

Gram+ve: B.s. = *Bacillus subtilis*, S.a = *Staphylococcus aureus* Gram-ve: E.C = *Escherichia Coli*, Ps.a = *Pseudomonas aeruginos*. Where: >18 mm sensitive, 14–18 mm intermediate >14 mm resistant.

4- CONCLUSION

The synthesized Nickel (II) and Cobalt (II) complexes with amino acids Glycine and Phenylalanine are characterized by using, infrared and UV Visible Spectroscopy. The IR spectra indicated the presence of amino acid coordinated through nitrogen atom and oxygen atom from carboxylic group. The experimental data suggest that the ligands act as bidentate and adopt on octahedral stereochemistry. And the results of antibacterial activity show that Co-Gly complex possess very good activity and other complexes possess partial activity.

Conflicts of Interest: The author declares that there are no conflicts of interest regarding the publication of this paper.

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