ADDIS ABABA UNIVERSITY

Office of Research and Graduate Programs

STUDIES ON FIRST ROWTRANSITION METAL COMPLEXES DERIVED FROM NINHYDRIN AND AMINO ACIDS HAVING POLAR(THREONINE) AND NONPOLAR(LEUCINE) SIDE CHAINS

Negash Getachew

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STUDIES ON FIRST ROWTRANSITION METAL COMPLEXES DERIVED FROM NINHYDRIN AND AMINO ACIDS HAVING POLAR(THREONINE) AND NONPOLAR(LEUCINE) SIDE CHAINS

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By

Negash Getachew

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Studies on First Row Transition Metal Complexes Derived From Ninhydrin and Amino Acids Having Polar (Threonine) and Nonpolar (Leucine) Side Chains

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Department of Chemistry

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To my Lord and Saviour

Jesus Christ

DECLARATION

I, the undersigned declare that this is my original work and has not been submitted for a degree in any other University and all sources of material used for the thesis have been duly acknowledged.

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This thesis has been submitted for examination with my Approval as University advisor

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JESUS, HOWEVER, IS THE RESEON FOR MY EXISTENCE!

Negash Getachew

June 2003

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List of Symbols

R – Ruhmann's purple ($C_{18}NO_4H_8$)

L – Schiff base of Leucine and Ninhydrin ($C_{15}NO_4H_{14}$)

T – Schiff base of Threonine and Ninhydrin ($C_{13}NO_5H_{10}$)

I – The Intermediate amine ($C_9NO_2H_6$)

N – Ninhydrin ($C_9O_3H_4$)

Thr – Threonine ($C_4NO_3H_9$)

Leu – Leucine ($C_6NO_2H_{13}$)

DMSO – Dimethyl sulfoxide

DMF – Dimethyl formamide

μL – Micro liter

mg – milligram

Λ_m– Molar conductance

ν - Stretching frequency (vibration)

$\Omega^{-1}cm^2mol^{-1}$ – per Ohm centimeter square per mol

Mp– Melting Point

Dec.T – Decomposition Temperature

X_m – Molar Susceptibility

X_g – Gram Susceptibility

μ – Effective magnetic moment

BM – Bohr magneton

Studies on First Row Transition Metal Complexes Derived From Ninhydrin and Amino Acids Having Polar (Threonine) and Nonpolar (Leucine) Side Chains

Abstract

. Complexes of Co(II), Ni(II), and Zn(II) with Ruhmann's purple, those of Fe(III) and Mn(II) with an intermediate Schiff base (ketimine and / or aldimine) and Cu(II) with ninhydrin were successfully synthesized. All complexes were distinctly colored and stable to atmospheric conditions. The complexes were characterized by elemental analysis, molar conductance, magnetic susceptibility, infrared and electronic spectral studies.

The ligands were shown to behave as a monobasic tridentate ONO, bidentate ON or OO donor. The complexes of Zn(II), Co(II) and Ni(II) are purely Ruhmann's purple, those of Mn(II) and Fe(III) are Schiff base (ketimine), Schiff base and ninhydrin or Schiff base and Ruhmann's purple complexes. An octahedral geometry is proposed for all of the metal complexes, except Mn(II).

A comparative anti- microbial study of the complexes was undertaken against two gram - negative bacteria, Escherichia coli and one gram - positive bacterium Staphylococcus aureus. It was revealed that except Cu(II) and to some extent Zn(II) complexes most of the complexes exhibit a reduced activity against both bacterium.

KEY WORDS: metal complexes, Schiff base, ninhydrin, Ruhmann's purple, α – Leucine, α–L–Threonine and antimicrobial activity.

1.INTRODUCTION

The physiological activity of organic compounds has been observed to undergo a significant modification on addition of metals. In particular, Schiff bases and their metal complexes have attracted a great deal of attention as anticancer, antitubercular, anticonvulsant, insecticidal, antibacterial, antifungal, antibiotic and anti - inflammability agents.

Proteins are one of the major macromolecules in living systems. The function of a protein is primarily determined by its structure, which in turn is determined by the sequence of amino acids making up the protein. The amino acid sequence is genetically determined and is responsible for the shape, physical characteristics, and biological activity of the protein.

The qualitative and quantitative determination of amino acids, peptides and proteins is of major and growing importance in many areas of biochemical investigations. The most sensitive reaction is that between amino acids and ninhydrin, which produces a characteristic purple colored compound, called Ruhmann's purple or diketo hydrindylene diketo – hydrinde amine.

The reaction however, is not selective enough, because all amino acids, except proline (an imino acid) give the same color. The investigation on the metal complexes (divalent Co, Ni and Zn) of glycine Schiff base indicates that in the presence of the metal ion, Ruhmann's purple is not formed; rather a product with specific color could be isolated.

2. Literature Survey

The α-amino acids in peptides and proteins (excluding proline) consist of a carboxylic acid and an amino functional group attached to the same tetrahedral carbon atom. This carbon is the α-carbon.

Distinct R-groups, that distinguish one amino acid from another, also are attached to the alpha-carbon (except in the case of glycine where the R-group is hydrogen). The fourth substitution on the tetrahedral α-carbon of amino acids is hydrogen.

2.1 The Chemistry of Amino Acids

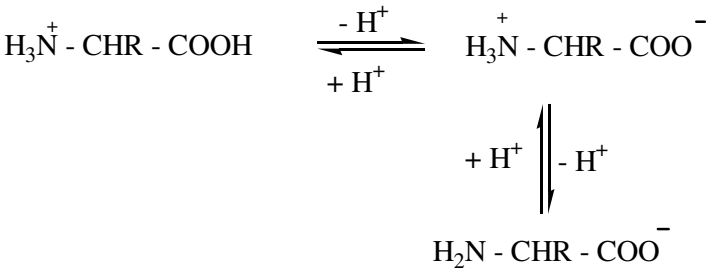
Amino acids are the alphabets for the protein structure. They share the same acidic (-COOH) and basic (- NH_2) groups, but the side chains (R) differ. They are optically active compounds, except glycine. All chiral amino acids obtained from proteins have the L – configuration at their α – carbon atom. Amino acids are classified based on the polarity of their side chains into the following four main classes:

1. Acidic (polar with additional carboxyl group)
2. Basic (polar with additional ionizable N – containing group)
3. Nonpolar and
4. Polar side chains, at physiological pH.

Since amino acids contain two functional groups (amines and carboxylic acids) they undergo the reactions characteristic of those functional groups. Their ionization depends on pH. In aqueous solution, the amino and carboxylic acid groups will ionize to give the zwitterionic form. Hence amino acids are ampholytes having acidic ionization in alkaline solution and basic ionization in acidic solution.

A zwitterion is an internally neutralized ion. The formation of the zwitterion results in:

1. An increase in solubility in water or other ionizing compounds.
2. A decrease in solubility in solvents of low dielectric constant.
3. A high melting point in the solid state.



States of ionization of amino acids in acidic, basic and neutral media.

Amino acids are known to have the following reactivities.

- Carboxyl groups form amides & esters

- Amino groups form Schiff bases and amides and

- Side chains show unique reactivities

Free amino acids at the pH where the species $NH_2 - CHR - COO^-$ is predominant, are good ligands and form stable five membered chelate rings. Under other pH conditions this may not be the case. An amino acid molecule has three donor groups:

- The amine group of the **N** – terminus
- The carboxyl group of the **C** – terminus and
- The functional groups on the side chain are also some times potential candidates.

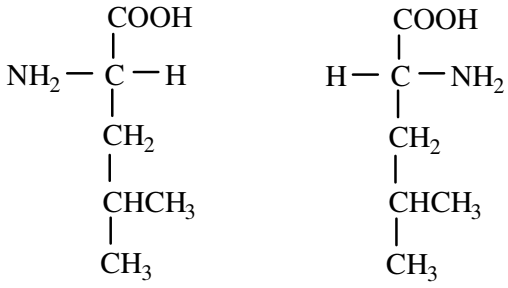
The keto group however, complexes rarely because it forms the less stable four membered rings. The affinity of a metal ion to a certain amino acid is dictated by the hard soft acid base (HSAB) principle.

All peptides and polypeptides are polymers of alpha-amino acids. There are 20 α-amino acids that are relevant to the make-up of organisms' proteins. Several other amino acids are found in the body free or in combined states, i.e. not associated with peptides or proteins.

These non-protein associated amino acids perform specialized functions. Several of the amino acids found in proteins also serve functions distinct from the formation of peptides and proteins, e.g., tyrosine in the formation of thyroid hormones or glutamate acting as a neurotransmitter.

The amino acids under the present investigation being leucine (with non polar side chain) and threonine (with polar side chain); some of their general characteristics are presented here.

Leucine or 2 – Amino – 4 – methyl pentanoic acid or α – aminoisocaproic acid has an empirical formula of $C_6H_{13}O_2N$ with the composition of the constituent elements; carbon 54.94%, Hydrogen 9.99%, nitrogen 10.68% and oxygen 24.39%. This amino acid has a molecular mass of 131.18 g. mol^{-1}. The structure and designation of the stereoisomer is the following:



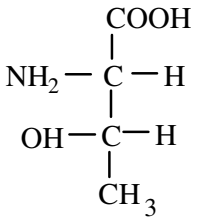
L - Leucine

D - Leucine

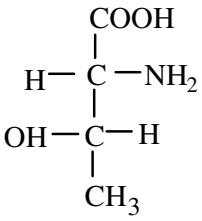
The discovery of leucine is attributed to Proust, who reported in 1899 the separation among the products of the fermentation of milk curds of the crystals of an organic material; which he called 'oxide caseique'. This material was recrystallized three times from hot water [1].

Threonine (2 – amino–3 hydroxy butanoic acid) or α – Amino – β – hydroxyl butanoic acid has a molecular formula of $C_4H_9O_3N$ and the following elemental composition: carbon 40.33%, hydrogen 7.62%, nitrogen 11.76% and oxygen 40.29%.

This amino acid has a molecular mass of 119.12. Threonine with a polar but non ionized side chain has the following structure and designation of stereoisomers.



L - Threonine



D - Threonine

The quantitative estimation of threonine by chemical means may be achieved through oxidation of the amino acid to acetaldehyde, followed by assay of the latter volatile compound with the aid of titrimetric or colorimetric methods.

2.2 The Ninhydrin Reaction

The most important reactions of amino acids are the reactions that are utilized in the formation of peptides and proteins. Protein analysis requires determination of the identity and quantity of each constituent amino acid [2].

The major steps include:

- Hydrolyzing with acids
- Separation with chromatography
- Identification and quantification of the individual amino acids

One popular method developed for the last step is through the ninhydrin reaction. The reaction with ninhydrin (1, 2, 3 - triketo hydridene hydrate) is used as a visual indicator for alpha amino acids, as it gives a characteristic color change [3, 4].

Ninhydrin, which is originally yellow, reacts with free alpha amino groups of primary amines and gives blue / purple product. The blue compound was found to form metal

complexes, the blue usually being converted to red. A 1: 2 metal to ligand ratio was suggested.

But no definite structure was reported except for Mn(II) complex of the blue compound. The product is commonly called Ruhmann's purple, which is formed according to the reaction sequence given on the mechanism given on scheme 1 [5 – 7].

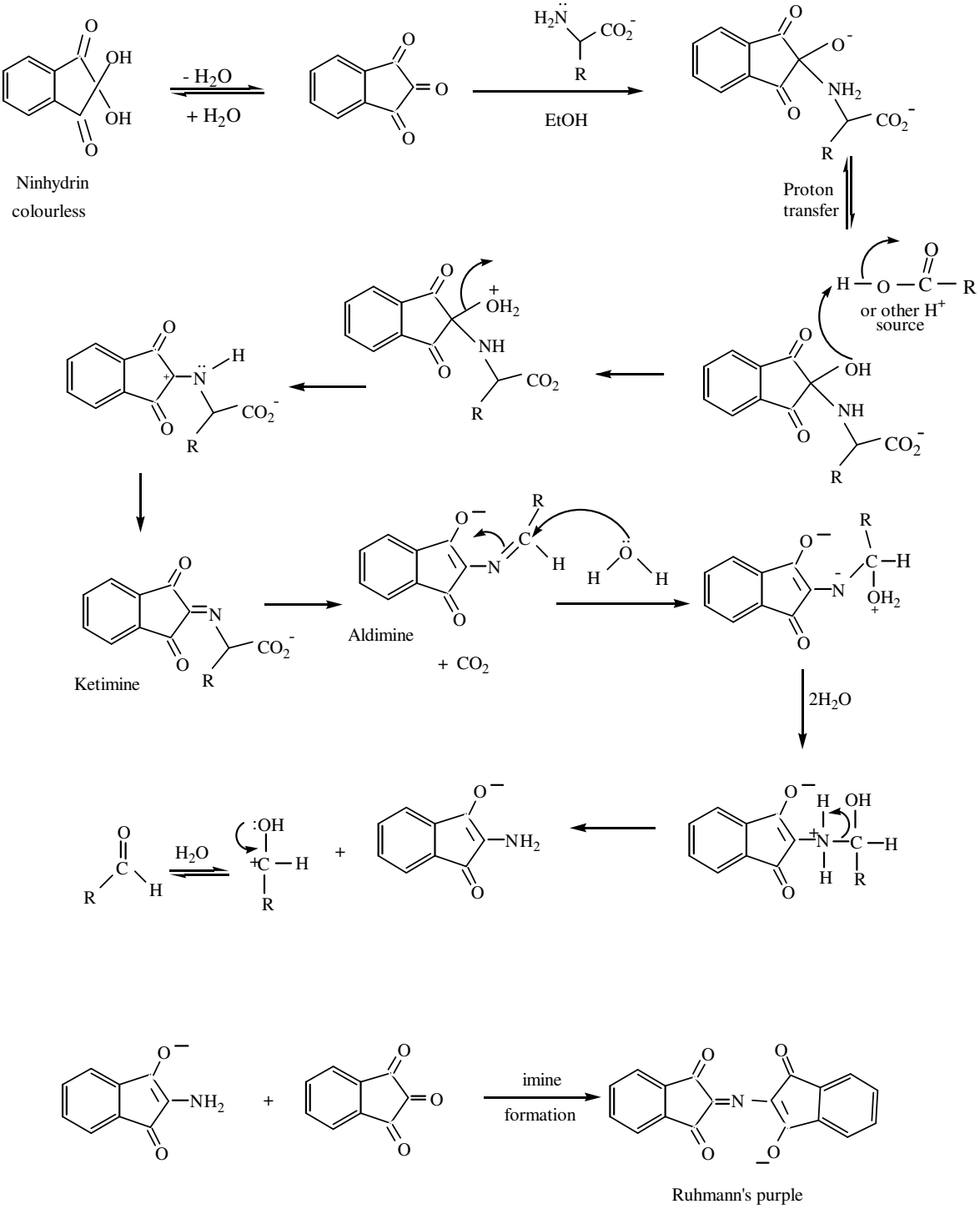
The ninhydrin reaction is a major method in amino acid analysis. However, the reaction is not selective. All amino acids, except proline, and other amine group containing compounds also produce the same product. The final product being the same for all the amino acids, this test does not merit for distinguishing one amino acid from the other. There are four important steps in the above reaction sequence:

1. Condensation – which results in the formation of the ketimine?
2. Decarboxylation – which results in the formation of the aldimine?
3. Hydrolysis – which results in the formation of the intermediate amine and
4. Condensation – which results in the formation of the Ruhmann's purple.

As can be seen in the above scheme, the intermediates (the ketimne, the aldimine and the intermediate amine) and the final product (the Ruhmann's purple) can act as potential Lewis bases towards metal ions.

The ketimine (the Schiff base) being of relatively greater potential because it can act as a tridentate ligand forming two stable five membered rings on complexation with metal ions [8, 9].

Scheme 1 – Mechanism Reaction of Ninhydrin and Amino Acids



Mechanisms: Reaction of alpha amino acids with Ninhydrin

The stability of these complexes decreases with:

- Increase in ring size
- Increase in the length of the side chain
- Increase in distance between the amino and carboxyl group

It also showed that the complexes are distinctly colored, which is important in identifying and quantifying alpha amino acids [10 – 12]. In addition to the above the length of the amino acid side chain has an influence on the complexing properties of the amino acid towards the common transitional metal ions [13].

This is the basis for studying the reaction between ninhydrin and a variety of amino acids in the presence of metal ions, particularly transition metal ions. Ninhydrin has been shown to exhibit a strong antimicrobial activity, which is enhanced upon complexation with metals [14 – 16].

The metal complexes are distinctly colored. A specific correlation of metal ion amino acid color can thus be developed on the results which can form a basis for amino acid identification and determination.

2.3 The Chemistry of Metal ions

The coordination chemistry of transition metal ions considered in the investigation; Mn(II), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) will be presented in terms of d^n configuration.

Manganese(II) Complexes

The ion forms many complexes in which the metal is octahedrally coordinated and most of them are high spin implying the absence of ligand field stabilization energy. Four spin forbidden bands are observed in the visible region, which correspond to $^6A_{1g} \rightarrow {}^4T_{1g}$, $^6A_{1g} \rightarrow {}^4T_{2g}$, $^6A_{1g} \rightarrow {}^4E_g$ and $^6A_{1g} \rightarrow {}^4A_{1g}$ transitions [17].

These transitions are observed around 18800 cm^{-1}, 23000 cm^{-1}, 24900 cm^{-1} and 25100 cm^{-1} respectively. The ε_{max} values are only ~ 0.01 for all these bands, since they are spin forbidden transitions from selection rules point of view.

Other transitions of Mn (II) are $^6A_{1g} \rightarrow {}^4Eg$ (D), $^6A_{1g} \rightarrow {}^4T_{1g}$ (P), $^6A_{1g} \rightarrow {}^4A_{2g}$ (F), $^6A_{1g} \rightarrow {}^4T_{1g}$(F) and $^6A_{1g} \rightarrow {}^4T_{2g}$ (F). These transitions are observed around 28000 cm^{-1}, 32400 cm^{-1}, 35400 cm^{-1}, 36900 cm^{-1} and 40600 cm^{-1}, respectively [18].

Iron(III) complexes

Iron (III) with a d^5 configuration forms mostly octahedral complexes and comparatively less number of square planar complexes. It favors high spin complexes. Iron (III) in octahedral arrangement has been reported to have the following transitions. $^6A_{1g} \rightarrow {}^4T_{1g}$ (G), $^6A_{1g} \rightarrow {}^4T_{1g}$ (G), $^6A_{1g} \rightarrow {}^4A_{1g}$, $^6A_{1g} \rightarrow {}^4E_g$ (G), $^6A_{1g} \rightarrow {}^4T_{2g}$ (D) and $^6A_{1g} \rightarrow {}^4E_g$ (D). [17].

Tetrahedral complexes of Fe(III) generally give more clear spectra with fairly narrow bands but the assignments of the various bands have not been certain. Trigonalbipyramidal Fe(III) exhibits spin forbidden bands between 5000 – 25000cm^{-1} and a charge transfer absorption between 27000 – 33000 cm^{-1} [19].

Cobalt(II) Complexes

Cobalt (II) with a d^7 configuration is known in four coordinate (tetrahedral) and six coordinate(octahedral) stereochemistry. The electronic spectra of tetrahedral cobalt (II) complexes are more intense than those of the octahedral ones [20].

In octahedral cobalt (II) complexes $^4T_{1g}$ and $^2A_{1g}$ are the spin free and spin paired ground state respectively. For high spin octahedral geometry, a band near 8000 – 10000 cm^{-1} can be assigned to $^4T_{1g} \rightarrow {}^4T_{2g}$ transitions. A multiple band observed around 20000 cm^{-1} is attributed to $^4T_{1g} \rightarrow {}^4T_{1g}$ (P) transition.

The $^4T_{1g} \rightarrow {}^2E_g$ transition is interesting in that it represents configurationally $t_{2g}^5e_g^2 \rightarrow t_{2g}^6eg^1$ and should be broad and its maximum should shift to lower frequencies with decreasing temperature, since its energy curve plotted against Dq has a larger negative slope than the curve for the ground term.

Some other transitions of Co (II) are $^4T_{1g} \rightarrow {}^4T_{2g}$ (F), $^4T_{1g} \rightarrow {}^2E_g$, $^4T_{1g} \rightarrow {}^4A_{2g}$ (F) and $^4T_{1g} \rightarrow {}^4T_{1g}$ (P) which are observed at 8000 – 9000 cm^{-1} ,11000 cm^{-1}, 16000 – 18000 cm^{-1} and 20000 – 21000 cm^{-1}, respectively [21].

Tetrahedral complexes of Co (II) with $^4A_2$ ground state are expected to have three transitions: $^4A_2 \rightarrow {}^4T_2$, $^4T_2 \rightarrow {}^4T_1(F)$, and $^4A_2 \rightarrow {}^4T_2$ (P). Low spin square planar complexes exhibit a narrow band near 8500cm^{-1} and a stronger broader band near 20000 cm^{-1}.

Nickel(II) Complexes

Octahedral Ni(II) complexes with $^3A_{2g}$ ground state are expected to have three spin allowed transitions. $^3A_{2g} \rightarrow {}^3T_{2g}$ (F) (7000 – 13000cm^{-1}), $^3A_{2g} \rightarrow {}^3T_{1g}$ (F) (1000 – 20000cm^{-1}) and $^3A_{2g} \rightarrow {}^3T_{1g}$ (P) (19000 – 27000 cm^{-1}).

The octahedral spectra usually consist of a band in the IR at 8600 cm^{-1} with $\varepsilon \sim 2.5$, a close pair of bands in the red (~ 14000 cm^{-1} with $\varepsilon \sim 1.8$) followed by a weaker band ($\varepsilon < 1$) at 18500 cm^{-1} and a some what stronger ($\varepsilon \sim 4$) transition in the blue at 25500 cm^{-1}

In addition, two spin forbidden transitions $^3A_{2g} \rightarrow {}^1E_g$ and $^3A_{2g} \rightarrow {}^1T_{2g}$ are also observed; the first near the second spin allowed transition and the other band between second and third spin allowed transitions. That is in approximations around 15400 cm^{-1} and 18500 cm^{-1} respectively.

Tetrahedral Ni(II) complexes with $^3T_1$ ground state, generally exhibit four transitions. They are $^3T_1 \rightarrow {}^3A_2$, $^3T_1 \rightarrow {}^1E$, $^3T_1 \rightarrow {}^3T_1(P)$ and $^3T_2 \rightarrow {}^1T_1$. The band $^3T_1 \rightarrow {}^3T_1(P)$ is a strong band of high intensity when compared with others [22].

Square planar Ni(II) complexes have three spin allowed d – d bands corresponding to $^1A_{1g} \rightarrow {}^1A_{2g}$, $^1A_{1g} \rightarrow {}^1B_{1g}$ and $^1A_{1g} \rightarrow {}^1E_g$ transitions are expected. The square planar Ni(II) complexes do not have any absorption band below 10,000cm^{-1}, due to large crystal field splitting. Hence, they can be clearly distinguished from octahedral and tetrahedral complexes [23].

Copper(II) Complexes

The Copper(II) ion with its d^9 configuration in octahedral and tetrahedral environment is highly susceptible to distortions. Octahedral complexes without any distortions are expected to have only one d – d absorption band corresponding to $^2E_g \rightarrow {}^2T_{2g}$ transition. Tetrahedral complexes are expected to give a single, broad band corresponding to $^2T_2 \rightarrow {}^2E$ transition in the near IR region [24].

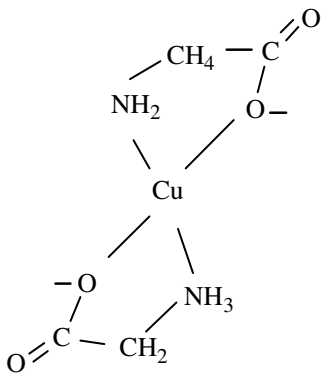
Zinc(II) complexes

Bivalent zinc forms numerous complexes of various stereo chemical types in which tetrahedral complexes are predominant. Monomeric and polymeric zinc (II) complexes have been reported. Square planar geometry is less common in zinc (II) complexes. These complexes do not possess any d – d transition due to the d^{10} configuration [25].

2.4 Metal Complexes of Amino Acids

When α-amino acids form salts with d-block metals the α–amino and carboxylate groups form dative bonds to the metal ion. The effect is to wrap up (chelate) the metal ion with

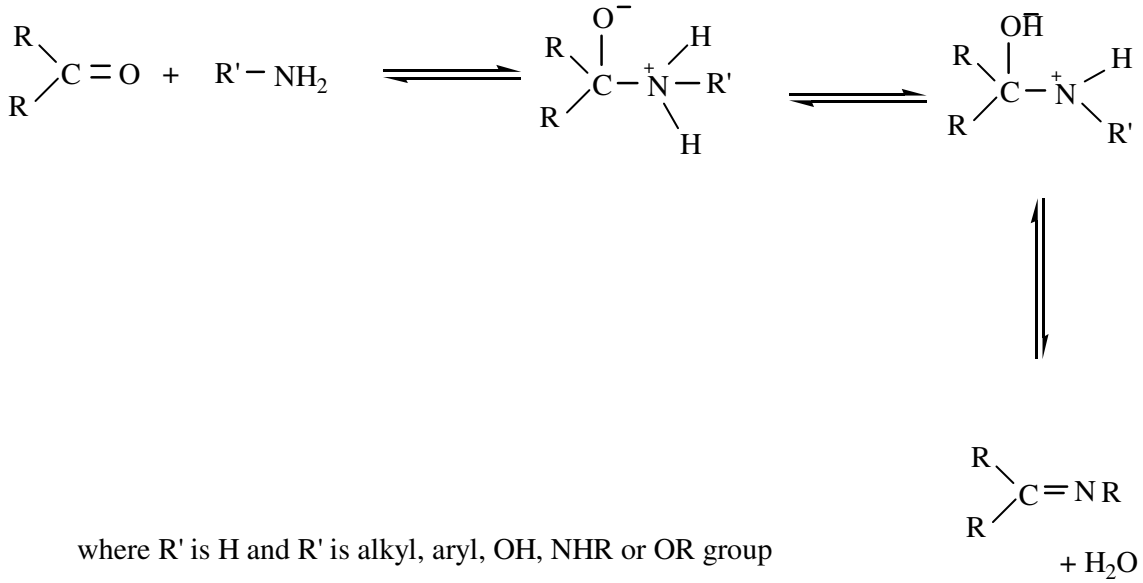
α–amino acid molecules. These salts often have characteristic colors - copper diglycinate (shown below) has a deep blue color [26].



If the nitrogen centers are due to azomethine groups, the ligands can exhibit excellent synthetic flexibility and can form stable metal complexes. Ligands possessing > C = N or azomethine grouping are known as Schiff bases. They are named after Hugo Schiff who first reported them in 1864 [19].

They are compounds containing an imino or azomethine group (R – C = N–) and are usually formed by the condensation of a primary amine with an active carbonyl compound. The reaction to prepare Schiff base is reversible, progressing through a carbinol amine intermediate and requires the removal of water.

Schiff bases which are effective as coordinating ligands have a functional group OH, NH_2, SH, etc, sufficiently near the site of condensation so as to form five or six membered chelated ring on reaction with metal ions.



where R' is H and R' is alkyl, aryl, OH, NHR or OR group

Scheme 2 – Reaction mechanism for the formation of a Schiff base.

Schiff bases have played an important role in the development of coordination chemistry as they readily form stable complexes with most of the transition metals.

In the area of bio – inorganic chemistry, interest on Schiff base metal complexes has been very keen due to the role such complexes play in providing synthetic models for the metal containing sites in metalloproteins and enzymes.

A wide variety of ligands may be obtained via the Schiff base condensation reactions which vary in denticity, flexibility, nature of donor atoms, and in electronic properties. Metal complexes of Schiff bases have varied geometries and magnetic properties.

Multidentate ligands having oxygen and nitrogen donor systems reveal a number of 'ONN' and 'ONO' donor sequences which have resulted in the formation of multinuclear metal chelates.The ligands are of significant synthetic interest from chelation point of view.

As the chelating functions are found in different environment, they are likely to provide a variety of donor systems, like ONN, OO and ONO for efficient metal binding reactions. Furthermore the ligands can behave as symmetric and unsymmetric bis - bidentate and multidentate systems [27].

2.5 Magnetic properties

The measurement of the magnetic susceptibilities and calculations of magnetic moments of a d – block metal complex can usually be interpreted in terms of the number of unpaired electrons which can help in distinguishing high spin complexes from low spin complexes and also relating the geometries of the complexes [28].

The measurement upon temperature variation can provide valuable information of various magnetic characteristics (Para magnetism, diamagnetism etc.) As an example, a d^6 metal ion in high spin / $t_{2g}^4 e_g^2$ / has four unpaired electrons with a magnetic moment of 4.90 BM and the same metal ion in low spin / t_{2g}^6 / has no unpaired electrons and hence its magnetic moment is zero (diamagnetic) [29].

In a free atom or ion, both the orbital and spin angular momenta give rise to a resultant magnetic moment and contribute to Para magnetism. When the atom or ion is part of a

complex, the orbital angular momentum may be eliminated (quenched) partially or fully as a result of the interaction of the electrons with their non spherical environment.

However, the electron spin angular momentum survives, and gives rise to spin – only Para magnetism, which is characteristic of many 3d – metal complexes. The spin only magnetic moment, μ , of a complex with total spin quantum number S is given by the relation:

$$\mu = 2\ \{S(S+1)\}^{1/2}\mu_B$$

Where: $\mu_B = 9.274\ 10^{-24}$ J/T

For most 3d complexes, experimental values lie reasonably close to spin – only predictions. Experimental distinction between high spin and low spin complexes is based on the determination of their magnetic properties.

Complexes are classified as diamagnetic if they tend to move out of a magnetic field and paramagnetic if they tend to move into a magnetic field. The extent of Para magnetism is commonly reported in terms of magnetic dipole moment possessed. The higher the magnetic dipole moment of the complex, the higher the Paramagnetism of the sample is [30].

3. OBJECTIVES AND SCOPE OF THE PRESENT INVESTIGATION

Amino acids are ubiquitous ligands in biology and are the basic building units of proteins. For instance, copper is an essential element, particularly for oxygen transportation, (hemocyanin) existing widely in organisms such as mollusks and shellfish. Therefore the

study of the interaction between copper and amino acids may be useful in understanding the action of metal ions in organisms [31].

Literature survey reveals that metal complexes of ligands derived from the condensation of ninhydrin with α, L – Leucine or α, L – Threonine have not been studied. From the above reaction sequence of amino acids with ninhydrin, it can be observed that ketimine, the aldimine, intermediate amine and Ruhmann's purple are potential ligands that can form stable complexes with transitional metal ions.

It was aimed to synthesize first row transition metal complexes with the ligands derived from the reaction of ninhydrin and amino acids (Leucine and Threonine). The metal ions proposed for the work include Mn(II), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II).

The complexes were characterized on the basis of elemental analysis, melting/ decomposition temperature, molar conductivity measurements, spectral (IR, UV – Vis and AAS) studies and magnetic susceptibility measurements.

Finally, the application of the complexes was studied with respect to their antimicrobial activities in order to assess some structure to activities relations. Some useful analytical procedures can be developed to identify a specific amino acid with better accuracy using ninhydrin in the presence of metal ions. Hence, the general objectives of the present work are:

1. Synthesis of some transition metal (3d) complexes of Schiff bases, one derived from ninhydrin and leucine and another from ninhydrin and threonine.

2. Structural studies of the metal complexes
3. Investigation of possible application (antimicrobial studies)

4. EXPERIMENTAL

4.1 Materials and Methods

Ninhydrin (Pharmacos), α, L – Leucine and α, L – Threonine both Aldrich were used. Metal salts (chlorides) of Mn(II), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) all from BDH. Ethanol (BDH) was used as the solvent for synthesis and purification.

Other solvents; DMSO (BDH), DMF (Riedel – de Haen), acetonitrile (Aldrich), chloroform (BDH), methanol, diethyl ether, nitric acid (BDH), perchloric acid (Fluka), NaOH (Waglechi international), Dichloromethane (Aldrich) and distilled water were also used as solvents.

4.2 Spectroscopic Analysis

UV – visible spectrometric studies were done in the range 200 – 1100 nm using a SPECTRONIC GENESYC 2PC with a 1cm cell at a concentration of 1.0×10^{-3} M solutions in DMSO at room temperature.

Infrared spectra as KBr disks were recorded using a Pye – Unicam SP 2000 Infrared spectrometer in the range 4000 – 600 cm^{-1}. Electrical conductivities of the complexes

were studied at room temperature with freshly prepared 1mM solutions in DMSO using a Philip Harris conductometer.

The complexes were analyzed for metal concentration, using Flame Atomic Absorption (Buck Scientific model) Spectrophotometer. Magnetic susceptibility was measured using MSB – Auto (Sherwood Scientific), Elemental Analysis using Flash EA 1112 (Thermo Quest) and melting points determinations with Electro thermal IA 9200, Digital Melting Point apparatus were done.

The presence of chloride in the samples was determined as AgCl by the sodium fusion method using standard procedures [32].

4.3 Synthesis

4.3.1 Synthesis of Metal (3d) Complexes using Ninhydrin and Leucine

Complexes of the metals were synthesized using a general procedure. 0.005 Mole of ninhydrin (0.89gram) was dissolved in minimum possible amount of absolute ethanol (~ 10 – 12 mL) similarly, 0.005 mole of the metal salt was dissolved in the minimum possible amount of absolute ethanol; and the two solutions were mixed. The pH and the formation of any precipitate were noted and the mixture was refluxed for 45 minutes.

0.005 mole of leucine (0.6gram) was dissolved in a 50:50 mixture of absolute ethanol and distilled water (~ 10 mL of each) and added to the above solution while refluxing.

The pH and the presence of any precipitate were noted and the mixture was refluxed for another two hours.

The resulting colored precipitate was then filtered off while hot and washed first with distilled water and then with absolute ethanol. The product was then left in open air for drying, weighed and stored in a desiccator.

4.3.2 Synthesis of Metal (3d) Complexes of Ninhydrin and Threonine

The same procedure as described under 4.3.1 was followed to synthesize the 3d metal complexes from threonine.

4.3.3 Synthesis of Zinc Complex of Ruhmann's Purple

This was attempted for comparison purpose in view of the existing literature on Ruhmann's purple complexes. To synthesize the Ruhmann's purple complex a general procedure was followed [20]. 0.001 Mole of amino acid (~ 0.6 gram) and 0.002 mole of ninhydrin were separately dissolved in minimum amount of absolute ethanol.

The solutions were mixed and left overnight, while stirring followed by the addition of 0.001 mole of the metal salt (to the deep blue solution) and refluxed for 2.5 hours. The resulting precipitate was filtered while hot and washed with absolute ethanol, dried in open air and stored in a desiccator.

4.3.4 Attempt to Synthesize Copper (II) Ninhydrin Complex

The complexes of copper – on structural investigation indicated the formation of copper (I) – ninhydrin complexes, on an attempt made to separately synthesize copper(II) – ninhydrin complex.

0.005 mole of ninhydrin dissolved in minimum amount of absolute ethanol and 0.005 mole of cupric chloride dissolved in minimum amount of absolute ethanol were mixed and refluxed for longer hours. Refluxing was continued for several hours, which didn't ensure any precipitation or color change.

This probably suggests that copper(II) – ninhydrin complexes do not separate as solids, under the conditions identical to the formation of Schiff base complexes of the metal ions.

4.3.5 Anti - microbial Studies

This was done by the disc – diffusion method in nutrient agar medium. The bacteria were incubated on a nutrient agar slant in separate test tubes for 48 hours. 0.5mL of the exponentially growing cultures of each bacterium was then diluted further with sterile nutrient broth. These were seeded on a nutrient agar/broth media by the top and bottom technique.

The metal complexes and controls (ninhydrin and metal salts) were dissolved in DMSO to make a solution of 25 mg / mL concentration. Two different concentrations of each sample

were studied. 10µL (250mg) and 20µL (500mg) of each sample, controls and solvent were absorbed on paper discs (Antiotic – Assay disc, diameter 0.25 inch) using eppendrof.

The soaked disks were then placed on the inoculated agar plates at relatively regular intervals and incubated in the inverted fashion at 37 ^{0} C for 24h. Any inhibition zones were looked for their diameters measured using a ruler.

5. RESULTS AND DISCUSSION

5.1 General

The metal complexes isolated from both amino acids namely threonine and leucine are all distinctly colored, stable to atmospheric condition and are soluble in solvents of high dielectric constant (DMSO, DMF) but only partially soluble in solvents like methanol and insoluble in petroleum ether.

The complexes of Zn(II), Ni(II) and Co(II) neither melt nor decompose up to 400 ^{o}C, while the complexes of Fe(III) and Mn(II) melt(decompose) at a lower temperature around 284 ^{o}C. Zn(II), Co(II) and Ni(II) favored the formation of Ruhmann's purple complexes, while Schiff bases complexes are formed in the presence of Fe(III) and Mn(II). Cu(II) is favored by the formation of ninhydrin complex.

The compositions of the complexes are indicated in each case by metal and R or N or L or T or a combination in which R = Ruhmann's purple, N = Ninhydrin, L = The Schiff base of ninhydrin and leucine and T = The Schiff base of ninhydrin and threonine. The compositions

are judged from the analytical and spectral data which will be presented and discussed in the following sections.

5.2 Metal Complexes Derived from Ninhydrin and Leucine

5.2.1 Physical Characteristics

The complexes are of two types, those formed with Zn(II), Co(II), Ni(II) and Mn(II) are

Table -1 Physical Characteristic of Metal Complexes of Leucine s

Complex	Color and Appearance	Yield (%)	mp(Dec.T) ^{0}C
ZnR_2	Red brown, powder	78.5	> 400
CuN_3	Brick red, fluffy crystalline	18.2	285
NiR_2	Dark green, powder	81.7	> 400
CoR_2	Grey, powder	75.3	> 400
$FeLN_3$	Brick red, fluffy crystalline	24.1	285
MnL_2R	Golden brown, powder	18.3	282.5

Where: **R** is Ruhmann's purple ($C_{18}NO_4H_8$)

N is Ninhydrin ($C_9O_3H_4$)

L is the Schiff base of Ninhydrin and Leucine ($C_{15}NO_4H_{14}$)

powders and with good yield; while those formed from Fe(III) and Cu(II) are fluffy crystalline substances and with lower percentage yield.

5.2.2 Analytical and Molar Conductance Studies

The analytical data matches with 1:2 metal to Ruhmann's purple complexes of Co(II), Ni(II) and Zn(II). Fe(III) complexes are derived from a Schiff base and ninhydrin while Mn(II) from the Schiff base and Ruhmann's purple. Cu(II) is favored by the formation of a ninhydrin complex.

The elemental analysis data of the complexes are given in Table -2. The deviation in carbon analysis appears to be attributed to experimental limitations. The metal, hydrogen and nitrogen analysis are in good match with the proposed compositions. All the complexes match with an octahedral geometry except Mn(II), which has pentagonal bipyramidal structure.

The molar conductance (Λ_m) values were calculated from conductivity measurements in DMSO. The molar conductance values of the products obtained from all the complexes are very small, in the range of 24 - 39 Ω^{-1} cm^{-2} mol^{-1} This shows that they are non – electrolytes [18].

The complexes were subjected to chloride analysis by sodium fussion and precipitation as AgCl [25]. The result indicates the absence of chloride in the complexes.

Table 2.Analytical and Molar Conductance data for metal complexes of Leucine

Complex	%H calc (found)	%N calc (found)	%C calc (found)	% Metal calc (found)	Λm DMSO $\Omega^{-1}cm^2mol^{-1}$
ZnR_2	2.39 (2.21)	4.18 (3.70)	64.54 (59.36)	9.77 (9.74)	24.15
CuN_3	2.49 (2.81)	0.00 (0.03)	57.70 (59.03)	11.68(11.15)	37.70
NiR_2	2.41 (2.16)	4.23 (4.98)	65.19 (58.18)	8.86 (8.65)	27.77
CoR_2	2.41 (2.41)	4.22 (3.68)	65.16 (63.84)	8.90 (8.47)	28.75
$FeLN_3$	3.22 (3.21)	1.73 (1.47)	62.38 (71.67)	6.93 (6.72)	32.26
MnL_2R	3.99 (3.78)	4.66 (4.79)	63.93 (70.48)	6.10 (5.82)	32.99

Where: **R** is Ruhmann's purple ($C_{18}NO_4H_8$)

N is Ninhydrin ($C_9O_3H_4$)

L is the Schiff base of Ninhydrin and Leucine ($C_{15}NO_4H_{14}$)

5.2.3 Infrared spectral data

From the data the sharp NH stretching band of the free amino acids at 3200 – 3000 cm^{-1} and the OH stretching frequency of ninhydrin are not observed in these spectra, indicating the derivatization of these groups.

The nitrogen may have been converted into tertiary nitrogen that doesn't show any peak in the region [33]. 1768 cm^{-1} band is characteristic of the intermediate carbonyl in the tricarbonyl species, which is in equilibrium with the dihydroxy species [34, 35]. The infrared spectral data are given in Table –3

Table-3 The IR spectral data of the metal complexes of Leucine and the starting materials

Compound	υOH/NH	νC=O free	νC=O cord.	νC=C aromatic	νC=N	νaCOO$^-$	νs'COO$^-$	ν_{O-C}	ν_{M-X}
Ninhyd-rin	3200-2900	1768(s) 1754 1720	----	1590	----	-----	-----	-----	---
α - L- Leu	3000	-----	-----	----	----	1600 (s)	1400(m)	----	---
MnL_2R	----	1740-1720(s)	1640(s)	1610(w)	1530(m)	1660 (m)	1320	1250	700 470
$FeLN_3$	----	1730(s)	1650(m)	1610(w)	1520(m)	1650 (s)	1310	1220	720 560
CoR_2	----	1730-1720(w)	1650(s)	1605(s)	1505(m)	-----	----	1250	750 540
NiR_2	----	1735 - 1725(w)	1640(s)	1610(m)	1510(s)	-----	----	1225	750 630
CuN_3	----	1720 - 1715(s)	1645(m)	1580(m)	-----	-----	-----	1230	740 680
ZnR_2	----	1725 - 1710(w)	1650(s)	1610(s)	1510(m)	-----	-----	1230	735 600

Where: **ν** stands for stretching vibration; **m** is for medium and **s** for strong

R is Ruhmann's purple ($C_{18}NO_4H_8$), N is Ninhydrin ($C_9O_3H_4$)

L is the Schiff base of Ninhydrin and Leucine ($C_{15}NO_4H_{14}$), **Leu** for Leucine

X is halogen, oxygen or nitrogen while **M** is the metal ion

a for antisymmetric and **s'** for symmetric stretching frequencies

Ninhydrin shows three bands in the carbonyl stretching region: 1768, 1754 and 1720 cm^{-1} The bands at 1754 and 1720 cm^{-1} are characteristics of its 1,3 – dicarbonyl functional group. From the infrared spectral data the complexes can be classified into three classes:

1 - The complexes of **Zn(II), Co(II)** and **Ni(II).** These complexes show a new, strong and sharp peak in the region of 1530 – 1505 cm^{-1}, which is characteristic to the coordinated azomethine group.

The C = N stretching frequency of the free aromatic azomethine groups occur between 1640 – 1620 cm^{-1} (1637 cm^{-1} in Ruhmann's purple) but with complexes it occurs at 1540 – 1500 cm^{-1}. A negative shift of about 100 cm^{-1} is a strong evidence of the coordination of the azomethine group via nitrogen [36].

This is one indication for the condensation reaction that occurred between the amine group of the amino acid and the carbonyl group of the ninhydrin, and further the absence of peaks due to such groups from the starting material infers that condensation reaction has taken place.

Leucine and all free amino acids show a strong carboxyl anti symmetric stretching peak at 1600 – 1590 cm^{-1} and a weaker symmetric stretching peak at around 1400 cm^{-1}. In the complexes the antisymmetric stretching mode appears between 1665 – 1630 cm^{-1}, while the symmetric stretching mode appears in the range of 1330 – 1310 cm^{-1}. (See Table –3)

The absence of the strong carboxyl antisymmetric stretching and the weaker symmetric stretching peaks in these complexes is an indication of the absence of the first condensation product or the ketimine.

2 – The complexes of **Mn(II)** and **Fe(III)** also show a new, strong and sharp peak in the region of 1530 – 1520 cm^{-1}, which indicates the presence of a coordinated azomethine group.

In addition to the above data, these complexes indicate a strong carboxylic antisymmetric stretching in the region of 1660 – 1650 cm^{-1} and a weaker symmetric carboxylic stretching in the region of 1320 - 1310 cm^{-1}, this indicates the presence of a coordinated carboxyl group through ionized carboxyl oxygen [37].

Comparing to the ninhydrin, in the spectrum of these products only two carbonyl stretching peaks at a decreased wave numbers exist, which shows the derivatization of the higher frequency (1768 cm^{-1}) carbonyl group in ninhydrin [35]. A close look at the data probably reveals the formation of the Schiff base (ketimine) and ninhydrin complexes with these metal ions.

3 – The complex of **Cu(II)** lacks the characteristic bands of the azomethine and the carboxyl groups, which probably infer the absence of condensation reaction in the presence of these metal ions. (Figure - 4)

The complex shows two carbonyl bands in the region of 1740 – 1715 cm^{-1}, which is characteristic to the uncoordinated carbonyl groups of ninhydrin. The carbonyl group at

1768 $cm^{-1}$is the free ninhydrin however is observed at relatively at lower frequencies, 1645 cm^{-1}, for the Cu (II) complex. This implies that the metal ninhydrin complex formation is favored.

Generally the infrared spectral data shows that the Ruhmann's purple is coordinated via oxygen and nitrogen, in the complexes of Zn(II), Co(II), Ni(II), Fe(III) and Mn(II) complexes [23]. The carbonyl stretching frequency of uncoordinated Ruhmann's purple occurs near 1739cm^{-1} [33]. A negative shift to lower frequency in the complex indicates the coordination of the carbonyl group to metal ions.

These complexes show weak bands in the region 1740 – 1710 cm^{-1}. These are the bands of the uncoordinated carbonyl groups in the complexes. The complexes show M-N and M-O bonds in the region 750 – 200 cm^{-1}.

Other supporting bands include a M- O – C stretching vibration that appears between 1245 – 1200 cm^{-1}. CH bending vibration of CH, CH_2 and CH_3 group that occurs in the range of 1480 – 1350 cm^{-1}, in aromatic C = C stretching near 1590 cm^{-1} and a strong band near 750 cm^{-1} due to ortho disubistitution of an aromatic ring [43].

In the presence of some of the first row transition metal ions Mn(II) and Fe(III) the reaction does not lead to the formation of Ruhmann's purple but stops at the first condensation step and forming a stable Indane – 1, 3 – dione – 2- imine – N – 4 – methyl pentanoate (IDIMP), the ketimine, which act as a tridentate (ONO) donor ligand.

5.2.4 ELECTRONIC AND MAGNETIC SUSCEPTIBILITY SPECTRAL STUDIES

Ninhydrin and Leucine form a deep blue / purple colored compound known as the Ruhmann's purple, which maximally absorbs at 24691.36 and 17543.36 cm^{-1} [38]. The compound is formed via four steps; condensation, decarboxylation, hydrolysis, and further condensation. (See scheme 1). The electronic spectral data of the metal complexes are given in Table – 4.

Table – 4 Elecronic spectral data of metal complexes of leucine and their assignment

Complex	Non ligand electronic spectral bands (cm^{-1})	Assignment	μ / BM
ZnR_2	23696.68, 16528.93	Bands of the coordinated azomethine group	----
CuN_3	34843.21, 27173.91	Bands of ninhydrin	----
NiR_2	23364.49, 16528.93, 11086.47 9900.99	Bands of the coordinated azomethine and aromatic group	2.96
CoR_2	23696.68, 16528.93, 11123.47, 9900.99	Bands of the coordinated azomethine and aromatic group	2.58
$FeLN_3$	34843.21, 27173.91, 29618.56	Bands of ninhydrin and azomethine groups	1.71
MnL_2R	28328.61, 22421.52	Bands of ninhydrin and azomethine groups	1.75
R	24691.36, 17543.36	Bands of uncoordinated azomethine group	-----

Where: **R** is Ruhmann's purple ($C_{18}NO_4H_8$)

N is Ninhydrin ($C_9O_3H_4$)

L is the Schiff base of Ninhydrin and Leucine ($C_{15}NO_4H_{14}$)

μ is the experimental magnetic moment

A very strong band near 20000 cm^{-1} in the Ruhmann's purple is assigned to a combination of л → л* and n → л* transition of the azomethine cromophore which in the free state absorbs between 25000 – 23256 cm^{-1}. The observed bathocromic shift gives a strong support for the coordination of the azomethine nitrogen [39].

This band, because it is very strong, is thought to obscure the d – d bands of the complexes in the region. For this reason the assignments are not given in accordance of their transitions. The bands around 32000cm^{-1} and are assigned to the n → л* transition of the carbonyl group [40,41].

The electronic spectrum of the Zn (II), Co (II) and Ni (II) complexes show common bands at 23696.68 cm^{-1} and 16528.93cm^{-1} which are the characteristic band of the exocyclic azomethine cromophore in the coordination, which in the free state absorbs at 24691.36 cm^{-1} and 17543.36 cm^{-1} in Rhumann's purple.

Such bands of the coordinated azomethine group are also observed at 22421.52 cm^{-1} in the Mn (II) complex and at 20618.56 cm^{-1} in the Fe (III) complexes, which indicate the presence and coordination of the azomethine group. These are assigned to the n → Л* and Л → Л* transitions of the azomethine cromophore.

From the magnetic susceptibility data, it can be observed that most of the complexes prefer a low spin configuration, which is exhibited due to the presence of strong field ligands, such as the azomethine and carbonyl groups.

The Ni (II) complex exhibited an octahedral geometry with two unpaired electrons. The value magnetic moment obtained corresponds to the literature value, indicating the coordination of the metal in an octahedral geometry.

In the complex of Co (II), the magnitude of the magnetic moment found was slightly less than the theoretical value, indicating the coordination of the metal in an octahedral geometry with a low spin configuration. The values of magnetic moment obtained for the complex of Fe(III) similarly indicates the spin only value of the metal coordinated in a low spin octahedral geometry, which is in a good agreement to the literature value [46].

In the case of Mn(II) however the metal is coordinated in a pentagonal by pyramidal geometry, which in a low spin configuration has only one unpaired electron. With this number of unpaired electrons the value obtained matches with literature value taking into account the orbital contribution of the magnetic moments.

The values of magnetic moment obtained for Cu is zero, indicating the metal ion in the complex is diamagnetic. This result shows the reduction of the Cu(II) by other reducing agents into the Cu(I) state, which exhibits no magnetic moment

From the mononuclear complexes of the metal ions of the first transition series, the orbital splitting patterns derived from ligand field theory provide a reliable first order

interpretation (most importantly, in understanding the number of unpaired electrons) of the measured magnetic susceptibilities.

Values of the magnetic susceptibility based on electron spin require only small corrections for the effects of orbital angular momentum. This approach is inadequate where:

1. Spin – orbit coupling is larger
2. In all bi – and polynuclear complexes when spin – spin coupling takes place, either by pathways involving bridging ligands, or by magnetic dipole coupling.
3. Spin – state crossovers

The origin of spin – state crossovers can be expressed in terms of ligand field theory by referring to the magnitude of the orbital splitting. The above values are calculated following the general relation to calculate molar susceptibility values:

$$\mathbf{Xm = Xg \,.\, Mm}$$

$$\mathbf{\mu = 2.82 \{ Xm \,.\, T\,(K)\}^{1/2}}$$

Where: **Xg** is the measured gram susceptibility, **Xm** is molar susceptibility

T (K) is temperature in Kelvin at which the value was recorded and

μ is the magnetic moment in Bohr Magneton

5.2.5 COCLUSION

From the above data and correlating it with the literature report it can be drawn that the ninhydrin – amino acid reactivity is modified in the presence of metal ions. However, this behavior is variable with different metal ions.

The sequence of reactions between ninhydrin and amino acid certainly show preeminence while the binding of metal ions at different stages makes further significant contribution. The notable species preferentially bonded to be the Schiff base, Ruhmann's purple and the hydrolyzed fragments of each of them, in some cases with ninhydrin, probably giving the final products.

Even though some more detail spectrokinetic investigations are necessary to substantiate this conclusion, it is worthwhile to note, that the metal ions play a significant role in influencing the widely studied ninhydrin – amino acid reaction.

Based on the elemental analysis, infrared, conductivity and electronic spectral data the following structures were proposed for the reaction between leucine and ninhydrin in the presence of some of the 3d metal ions.

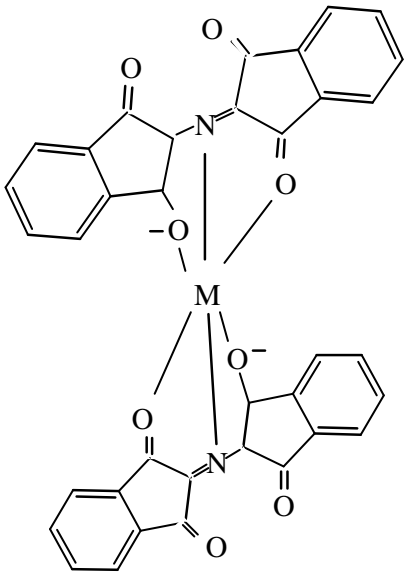


Fig 3 Proposed structure for the Ruhmann's purple complexes of Zn(II), Co(II) and Ni(II)

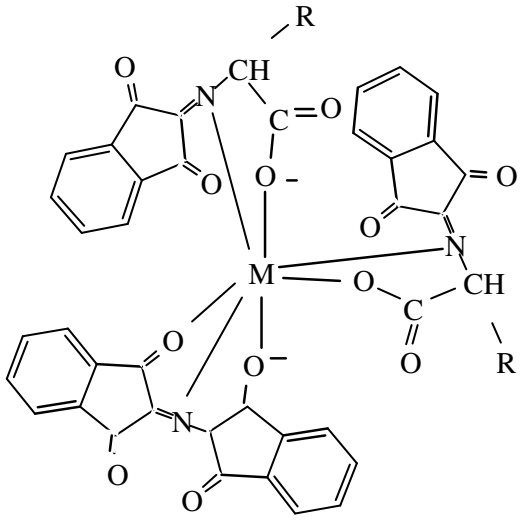


Fig 4 Schiff base and Ruhmann's purple complex of Mn(II)

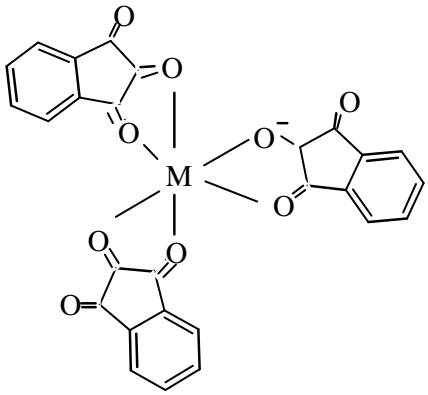


Fig 7 Ninhydrin Complex of Cu(I)

5.3 Metal Complexes of Ninhydrin and Threonine

5.3.1 Physical Characteristics

The complexes are of two types, those formed with Zn(II), Co(II), Ni(II) and Mn(II) are powders in nature and obtained in good yield; while those formed from Fe (III) and Cu (II) are fluffy crystalline substances and obtained in lower percentage yield.

Table-5 Physical Characteristics of Threonine ($C_4H_{19}O_3$) Metal Complexes

Complex	Color and Appearance	Yield (%)	mp(DecT) ^{0}C
ZnR_2	Peat grey, Powder	71.0	> 400
CuN_3	Brick red, Fluffy Crystalline	47.6	228
NiR_2	Greenish, Powder	69.2	> 400
CoR_2	Light green, Powder	63.5	> 400
FeT_2R	Brick red, Fluffy Crystalline	21.6	289
MnI_2	Cloudy grey, Powder	17.6	> 282

Where: **R** - Is Ruhmann's purple ($C_{18}NO_4H_8$)

N - Is Ninhydrin ($C_9O_4H_4$)

T - Is the Schiff base of Ninhydrin and Threonine ($C_{13}NO_5H_{10}$)

I – Is the intermediate amine ($C_9NO_2H_6$)

As can be seen from the above table, the complexes are distinctly colored; they have different percentage yields and appearances.

5.3.2 Analytical and Molar Conductance Studies

The elemental analysis data of the complexes are given in Table 6. The analytical data matches (though carbon value deviates) with 1:2 metals to Ruhmann's purple complexes in an octahedral geometry for the complexes of Co (II), Ni (II) and Zn (II).

The complex of Fe (III) is composed of a Schiff base and Ruhmann's purple with a pentagonal bipyramidal structure. Similarly an intermediate amine complex can be proposed for the Mn(II), with a square planar geometry; while the elemental analysis values that of Cu (II) matches with the ninhydrin complex, of an octahedral geometry.

The molar conductance (Λ_m) values were calculated from conductivity measurements of the solvent (DMSO) and a metal complex in DMSO. The molar conductance values of the products obtained from all the complexes are very small, in the range of 14 - 39 Ω^{-1} cm^{-2} mol^{-1}. These show their non – electrolytic nature [42].

Table 6.Analytical and Molar Conductance data for metal complexes of Ninhydrin and Threonine

Complex	% H calc (found)	% N calc (found)	% C calc (found)	% Metal calc (found)	Λm DMSO $\Omega^{-1}cm^2mol^{-1}$
ZnR_2	2.39 (2.10)	4.18 (4.18)	64.54 (60.98)	9.77 (9.70)	17.67
CuN_3	2.49 (2.48)	0.00 (0.36)	57.70 (56.23)	11.68(11.37)	37.53
NiR_2	2.41 (2.33)	4.23 (4.03)	65.19 (62.84)	8.86 (8.64)	16.76
CoR_2	2.41 (2.33)	4.22 (3.91)	65.16 (63.88)	8.90 (8.61)	14.87
FeL_2R	3.19 (3.53)	4.78 (4.76)	60.14 (72.45)	6.38 (5.94)	37.52
MnI_2	3.20 (2.82)	7.48 (7.58)	57.60 (68.)	4.68 (4.51)	38.41

Where: **R -** Ruhmann's purple ($C_{18}NO_4H_8$)

N - Ninhydrin ($C_9O_3H_4$)

L - The Schiff base of Ninhydrin and Threonine ($C_{13}NO_5H_{10}$)

I – The Intermediate amine ($C_9NO_2H_6$)

The complexes were subjected to chloride analysis by sodium fussion and treatment with $AgNO_3$ [32]. The results indicate the absence of chloride in the complexes.

5.3.3 Infrared spectral data

The infrared data are given in Table 7. A similar general explanation as given in section 5.2.3 can be given for the starting materials.

From the infrared spectral data the complexes can be classified into four classes:

1 - The complexes of **Zn(II), Co(II)** and **Ni(II).** These complexes show a new, strong and sharp peak in the region of 1520 – 1510 cm^{-1}, which is characteristic to the coordinated azomethine group. (Fig - 3)

The C = N stretching frequency of the free aromatic azomethine groups occur between 1640 – 1620 cm^{-1} (1637 cm^{-1} in Ruhmann's purple) but in the complexes it occurs at 1515 – 1510 cm^{-1}. A negative shift of about 100 cm^{-1} is therefore a strong evidence of the coordination of the azomethine group via nitrogen [36].

This is one indication for the condensation reaction that occurred between the amine group of the amino acid and the carbonyl group of the ninhydrin, and also the disappearance of such peaks, from the spectrum assignable to such groups of the starting materials, is a further evidence for the undergoing reaction.

Free amino acids show a strong carboxyl anti symmetric stretching peak at 1600 – 1590 cm^{-1} and a weaker symmetric stretching peak at 1400 cm^{-1}. In the complexes the antisymmetric stretching mode appears between 1665 – 1630 cm^{-1},while the symmetric stretching mode appears in the range 1330 – 1302 cm^{-1} [33].

The absence of the strong carboxyl antisymmetric stretching and the weaker symmetric stretching peaks in these complexes is an indication of the absence of the first condensation product which is ketimine.

2 – The complex of **Fe(III)** also shows a new, strong and sharp peak at 1520 cm^{-1}, which indicates the presence of a coordinated azomethine group (See figure -7)

In addition to the above data, the complex indicates a strong carboxylic antisymmetric stretching at 1660 cm^{-1} and a weaker symmetric carboxylic stretching at 1360 cm^{-1}. This indicates the presence of a coordinated carboxyl group through ionized carboxyl oxygen [38].

Comparing with ninhydrin, in the spectrum of this complex only two carbonyl stretching peaks at a decreased wave numbers (1740 – 1720 cm^{-1}) are found, which shows the derivatization of the higher frequency (1768 cm^{-1}) carbonyl group in ninhydrin [35]. The above IR data analysis is consistent with the complexation of the Schiff base (ketimine) and ninhydrin with the Fe(III) metal ion.

3 – The complex of **Cu (II)** lacks the characteristic band of the azomethine and the carboxyl groups, which probably indicate the absence of condensation reaction in the presence of this metal ion. (Fig - 3)

The complex shows two carbonyl bands in the region of 1740 – 1715 cm^{-1}, which is characteristic to the uncoordinated carbonyl groups of ninhydrin. The carbonyl group at 1768 cm^{-1}, however is, observed at relatively lower frequencies, (1650 cm^{-1}) in the Cu (II) complex. The data clearly shows that the metal ions are favored by the formation of ninhydrin complex

Table-7 IR spectral data of the metal complexes threonine and the starting material

Cpd	νOH/NH	νC=O free	νC=O cord.	νC=C aromatic	νC=N	νaCOO⁻	νs'COO⁻	ν_{O-C}	ν_{M-X}
Ninhyd.	3200 - 2900	1768(s) 1754 1720	----	1590	-----	-----	-----	-----	---
α -L-Thr	3000(b)	-----	----	----	-----	1610(s)	1405(s)	----	----
MnI_2	3500-3400(B)	1730-1720(s)	1620(w)	1600(w)	-----	-----	-----	1240	750 500
$FeTN_3$	----	1740-1720(m)	1620(m)	1610(m)	1520(s)	1660 (s)	1360	1220	710 550
CoR_2	----	1730-1710(w)	1640(s)	1600(s)	1510(s)	----	----	1220	735 550
NiR_2	----	1740 - 1710(w)	1630(s)	1600(m)	1515(s)	----	----	1230	740 605
CuN_3	----	1730 (vs)	1620(m)	1570(m)	-----	----	----	1250	720 650
ZnR_2	----	1750 - 1710(w)	1635(s)	1600(m)	1510(m)	----	----	1220	740 610

Where: **ν** stands for stretching frequency;**δ** stands for bending or deformation; **T** for Threonine; **s**for strong; **w** for weak; **vs** for very strong and **Thr** for threonine

R - Is Ruhmann's purple ($C_{18}NO_4H_8$), N is Ninhydrin ($C_9O_3H_4$)

T- Is the Schiff base of Ninhydrin and Threonine ($C_{13}NO_5H_{10}$)

I – Is the intermediate amine ($C_9NO_2H_6$), **X** = N, O or halogen

a for antisymmetric and **s'** for symmetric stretching frequencies

4 – The complex of **Mn(II).** This complex has the stretching vibrations of the amine groups, which is observed around 3000 cm^{-1} in addition to this the complex indicates the bands of a coordinated and uncoordinated carbonyl groups in their corresponding regions.

However the complex lacks the characteristic peaks of the azomethine or carboxyl groups, which indicates the absence of the intermediate ketimine or the final product, the Ruhmann's purple.

In the presence of Fe(III) the reaction does not lead to the final product but stops at the first condensation step forming Indane – 1, 3 – dione – 2- imine – N – 3 – hydroxyl butanoate (IDIHB), the ketimine, which forms a stable complex with the metal ions, acting as a tridentate (ONO) donor ligand. Other bands can be interpreted in a similar way as described in leucine complexes.

5.3.4 ELECTRONIC AND MAGNETIC SUSCEPTIBILITY SPECTRAL STUDIES

Ninhydrin and Threonine or Leucine form a deep blue / purple colored compound known as the Ruhmann's purple, which maximally absorbs at 24691.36, 17543.36 cm^{-1}(42). The compound is formed via four steps; condensation, decarboxylation, hydrolysis and further condensation, as demonstrated in Scheme - 1.

This azomethine band of the Ruhmann's purple because is very strong, is thought to obscure some d – d bands of the complexes in the region. The electronic spectral data of

the metal complexes are given in Table - 8. The bands observed at 39000 cm^{-1} and 35000 cm^{-1} in the complexes are assigned to the л → л* transition of the benzene moiety [44].

The electronic spectrum of the Zn (II), Co (II) and Ni (II) complexes show common bands around 23000 cm^{-1} and 16000 cm^{-1} which are characteristic bands of the exocyclic azomethine cromophore, while in the Free State it absorbs at 24691.36 cm^{-1} and 17543.36 in Ruhmann's purple.

Table-8.Electronic spectral data of metal complexes of threonine and their assignment

Complex	Electronic spectral bands (cm^{-1})	Assignment	μ / BM
ZnR_2	23696.68, 16528.93	Bands of the azomethine group	Diamagnetic
CuN_3	34843.21, 27173.91	Bands of ninhydrin	Diamagnetic
NiR_2	39840.64, 23310.02, 15576.32 11086.47, 9900.99	Bands of the coordinated azomethine and aromatic groups	2.76
CoR_2	39840.64, 23474.18,16155.09, 11123.47, 9900.99	Bands of the coordinated azomethine and aromatic groups	2.85
FeT_2R	34843.21, 27173.91, 23618.56	Bands of the coordinated azomethine and aromatic groups	1.81
MnI_2	31545.74, 21978.02, 15384.62	Bands of the coordinated carbonyl group and some d-d bands of the metal	1.67
R	24691.36, 17543.36	Bands of the uncoordinated azomethine transitions	-----

Where: **R** - Ruhmann's purple ($C_{18}NO_4H_8$), N is Ninhydrin ($C_9O_3H_4$)

T - The Schiff base of Ninhydrin and Threonine ($C_{13}NO_5H_{10}$)

I – The intermediate amine ($C_9NO_2H_6$)

μ – The magnetic moment

From the magnetic susceptibility data, it can be observed that all the complexes prefer a low spin configuration, which is exhibited due to the presence of strong field ligands, such as the azomethine and carbonyl groups.

The Ni(II) complex exhibited an octahedral geometry with two unpaired electrons. The value of magnetic moment obtained corresponds to the literature value, indicating the coordination of the metal in a low spin octahedral geometry [25)].

In the complex of Co(II), the magnitude of the magnetic moment found was less than the theoretical value, indicating the geometry of the complex is octahedral with a low spin configuration. Hence the value obtained correlates with the orbital momentum corrections being undertaken into considerations.

The value of the magnetic moment obtained for the complex of Fe(III) indicates a low spin octahedral geometry, which is in good agreement with the literature value of magnetic moments [25].

In the case of Mn(II), however, the metal is coordinated in a square planar geometry to the intermediate amine, which in a low spin configuration has only one unpaired electron. The

value obtained is lower than the expected value due to spin orbit coupling and some other diamagnetic effect [30].

The value of magnetic moment obtained for Cu(II) indicates that the metal ion in the complex is diamagnetic. This result shows the reduction of the metal by other reducing agents into the Cu(I) state, which exhibits no magnetic moment [45].

5.3.5 CONCLUSION

An identical conclusion as given in section 5.2.5 can be drawn from the available data. Based on the elemental analysis, Infrared, Conductivity and electronic spectral data the following structures were proposed for the reaction between threonine and ninhydrin in the presence of some of the 3d metal ions.

The structures for Zn(II), Co(II), Ni(II) and Cu(II) being the same with that of the leucine complexes, the structures for Mn(II) and Fe(III) are given below, for the threonine complexes.

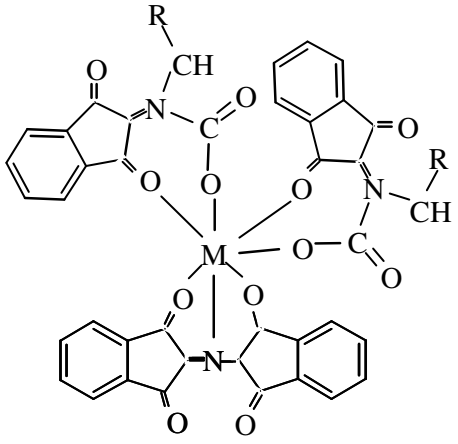


Fig 7.

Schiff base of Threonine and Ruhmann's purple complex of Fe(III) complex, where R is -CH(OH)(CH_3)

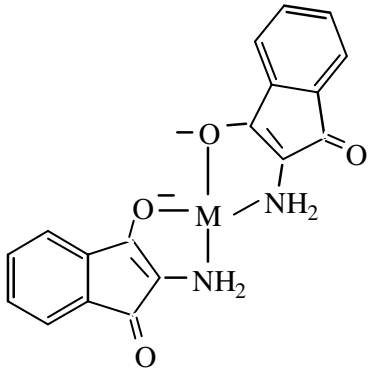


Fig 8. Intermediate amine complex of Mn(II)

6. Comparative Studies

6.1 Comparison of Ruhmann's purple complexes formed under Different procedures

The following comparison is presented in view of the existing literature on Ruhmann's purple complexes. This is given to substantiate the conclusion made above in case of Ruhmann's purple complexes.

Ruhmann's purple was formed upon mixing of the ninhydrin and any amino acid in absolute ethanol, which was then made to react with this zinc metal ion. This was generalized with this complex obtained by mixing ninhydrin, amino acid and zinc metal ion (Ruhmann's purple procedure) [45].

Comparison of the metal the products obtained with the two procedures is presented on Table 9. The Ruhmann's purple thus formed can be reacted with metal ions, like zinc (II) to form complexes. In the above investigation Ruhmann's purple complexes were obtained

in a different procedure, i.e. reacting ninhydrin and amino acid in the presence of metal ion (Schiff base or general procedure)

The products isolated with Ruhmann's purple and the general procedure do not show any difference in color; which implies that in both cases, zinc metal ion forming complex with Ruhmann's purple [47]. A similar conclusion can be drawn that the Co(II) and Ni(II) complexes of both leucine and threonine are also Ruhmann's purple.

Table 9 – Comparison of Zinc complexes synthesized by two procedures

Characteristics	**Ruhmann's purple Procedure**			**General Procedure**		
Color	Reddish			Brick red		
Appearance	Powder			Powder		
mp(Decomp. Temp)	Greater than 400^{o}C			Greater than 400^{o}C		
Character UV – vis Λ_{max}	408nm, 570nm			410nm, 569nm		
Elemental Analysis	%	Calc.	found	%	Calc.	found
	H	2.40	2.10	H	2.40	2.16
	N	4.18	4.98	N	4.18	4.18
	C	64.54	58.18	C	64.54	60.98

General procedure is the one described in sections 4.3.1(4.3.2) and Ruhmann's purple procedure is that described in the section 4.3.3

6.2 COMPARISON OF ZINC COMPLEXES OF THREONINE AND SERINE

The complexes of serine and ninhydrin were investigated earlier in these laboratories. Presently the complexes of threonine and serine were analyzed for carbon, nitrogen and hydrogen composition, and the following comparison was found.

The two amino acids have similar side chains (polar) except that the hydrogen of serine is replaced by methyl group in threonine. The data clearly indicates that the two amino acids have similar composition which implies the formation of Ruhmann's purple complexes with both amino acids, serine and threonine. The comparison of the two amino acids is given in Table 10.

Table 10 – Comparison of Zinc complexes of Serine and Threonine from elemental Analysis data

% composition	Calculated	Threonine	Serine
Hydrogen	2.39	2.21	2.45
Nitrogen	4.18	4.18	4.36
Carbon	64.54	59.36	59.50

7. Anti – microbial Studies

Ninhydrin was found to be strongly active against the two bacteria studied. The activities of the metal complexes, ninhydrin and Ruhmann's purple against the two test organisms are given in table - 11

Table - 11.Results of anti – microbial studies on ninhydrin, the metal complexes

No.	Complex	Test Organism	
		Staphylococcu Aureus	Escherichia coli
		250µg 500µg	250µg 500µg
1	Ninhydrin	++ ++	++ ++
2	RP	- - - -	+ +
3	Zn / Leu	++ ++	- - - -
4	Cu / Leu	++++ ++++	++ ++
5	Ni / Leu	- - - -	- - - -
6	Co / Leu	- - - -	- - - -
7	Fe / Leu	- - - -	- - - -
8	Zn / Thr	++ ++	++ ++
9	Cu / Thr	++ ++	+++ +++
10	Ni / Thr	- - - -	- - - -
11	Co / Thr	+ +	++ +
12	Fe / Thr	- - - -	- - - -
13	Zn(II)	++ ++	++ ++
14	Co(II)	++ +	++ +++
15	Ni(II)	+ +	++ +
16	Fe(III)	++ ++	++ ++
17	Cu(II)	+ ++	++ +

Where : ++++ = very high activity; +++ = high activity; ++ = moderate activity; + = some activity - - = no activity. T stands for Threonine, L for Leucine Complexes

Thus, the observed enhancement of activity of those complexes that were found to be more active than ninhydrin must be due to a synergistic or combination effect associated with the derivatization and complexation of the ninhydrin and the presence of the side group of the amino acid.

These effects may also be associated with the electronic distribution and stereochemistry of the ligand due to complexation. The type of the bacteria upon which the complexes are applied has also an effect on the antimicrobial activity of the complex. The antimicrobial activity data is given on table 11.

Compared to the inactivity of the metal complexes of the Schiff base made with glycine, IDIA, against these two bacteria, the activity of the current new complexes may be mainly due to the side groups and / or their structural variations.

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