ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

# SYNTHESIS AND CHARACTERIZATION OF SOME METAL COMPLEXES OF SCHIFF BASES DERIVED FROM NINHYDRIN AND a, L-VALINE

*By*

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**JULY 2001**

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Scheme 1 The reaction of ninhydrin with a-amino acid

Scheme 2 Reaction of glycine with ninhydrin in the presence of metal (II) ions

## List of Abbreviations and Symbols

| | | |
|---|---|---|
| DMF | - | Dimethyl formamide |
| DMSO | - | Dimethyl sulfoxide |
| niM | - | Millimolar |
| M. pt. | - | Melting point |
| Decomp. temp. | - | Decomposition temperature |
| TR | - | Infrared |
| UV-Vis | - | Ultraviolet-Visible |
| NMR | - | Nuclear magnetic resonance |
| nm | - | Nanometer |
| cm | - | Centimeter |
| $\Lambda_{max}$ | - | Wavelength of maximum absorbance |
| $\varepsilon_{max}$ | - | Molar absorptivity |
| Amax | - | Molar conductance |
| Q | - | Ohm |
| v | - | Stretching vibration |
| 8 | - | Bending or deformation vibration |
| IDIDP | - | Indane-1,3-Dione -2-Imine-N-(3,3 dimethyl )– Propionate, IDIDP |

# ABSTRACT

## Synthesis and Characterization of Some Metal Complexes of Schiff bases Derived from Ninhydrin and a, L-Valine

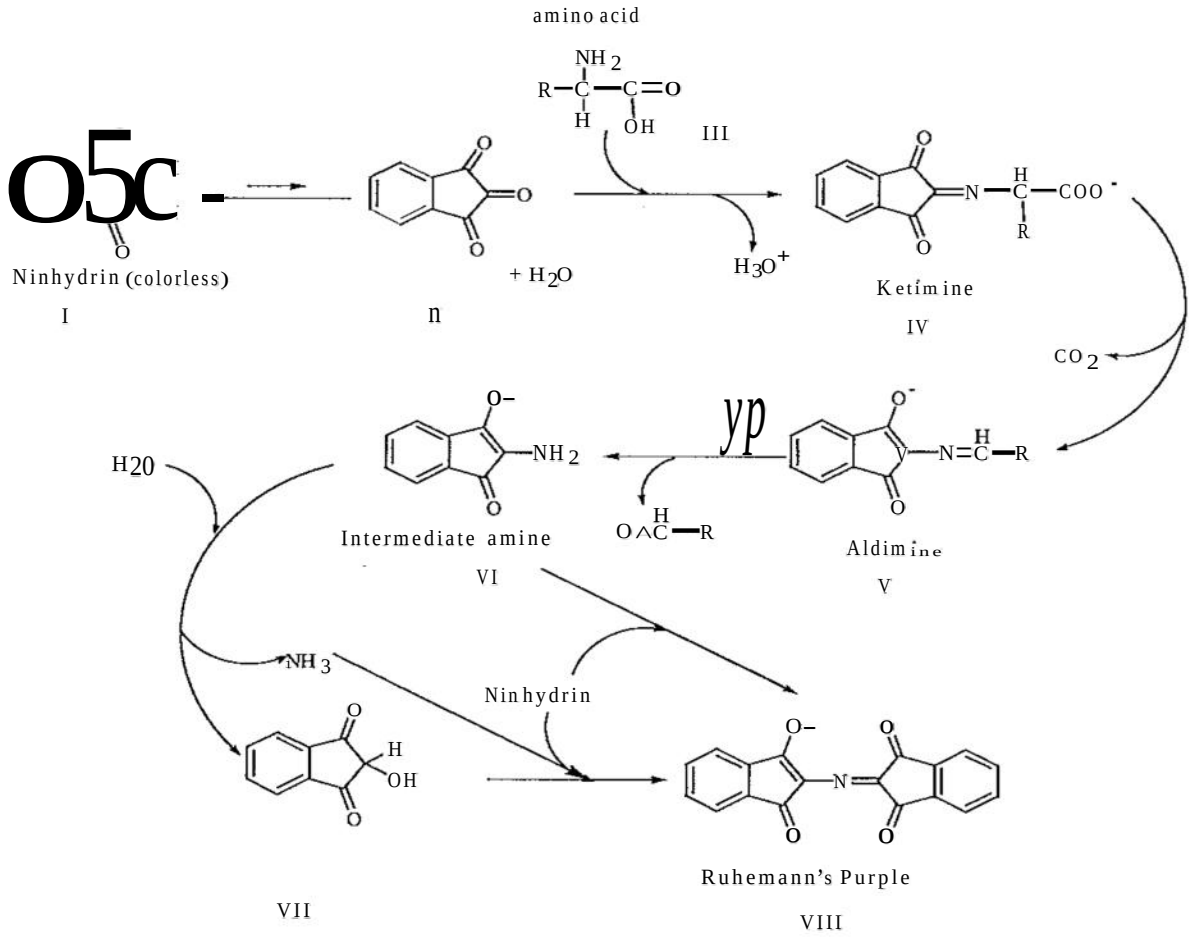
Complexes of Mn (II), Fe (II), Co (II), Co (III), Ni (II) and Zn (II) with Schiff base derived from ninhydrin and valine have been successfully synthesized. The complexes were distinctly colored and were characterized by elemental analysis, molar conductance, Infrared and electronic spectral studies. The ligands were found to act as a mono basic tridentate, ONO donor.

Synthesis of metal complexes of the Schiff base derived from ninhydrin and cysteine was unsuccessful.Variations between amino acids lie in the nature of side chain, which confers individuality upon each amino acids. Thus studies on the side chain,-SH , were done by taking in to consideration the properties of –SH group. With the assumption that acidity of the group had an effect, acidic amino acid (tyrosine) was taken but the result was positive. It showed that acidity was not the reason for cysteine not forming metal complex. The other property considered was the soft sulfur group, since the metals used in the synthesis were hard, other soft metal (Cd(II)) was taken with the cysteine schiff base itself and it has formed a metal complex. Hence, the reason for not forming metal complex with first row transition metal is due to the soft side chain, -SH group.

Comparative anti microbial study of the complexes with the starting material ninhydrin was undertaken against three bacteria. Enhanced activity than ninhydrin on the two bacteria were observed.

## INTRODUCTION

The ninhydrin colour reaction has proved to be very useful in qualitative work and is widely used in the visualization of amino acid bands after electrophoretic or chromatographic separation of mixtures. Several workers have extensively studied the chemistry of the reaction. A simplified form of the mechanism proposed by Fillippovich and McCaldin [1,2] is shown in Scheme 1.




Scheme 1 The reaction of ninhydrin with a-amino acid

The Schiff bases formed due to the reaction of ninhydrin with a-amino acid are potential Lewis bases. Hence, they are good ligands to metal ions as shown in figure 1 below [1].

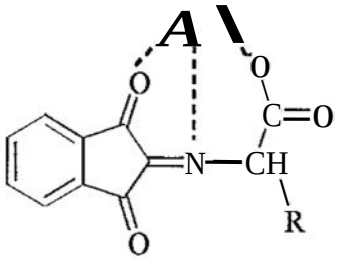



Fig 1 Metal binding sites of a Schiff base derived from ninhydrin and an a- amino acid

The ketimine **IV** (Scheme 1) is a potential ligand to metal ions, that can act as a tridentate ligand forming two stable five membered rings on complexation. A recent study on the reaction of ninhydrin with glycine in the presence of metal ion; Co(II), Ni(II) and Zn(II),indicate that the reaction appears to stop just after the condensation of ninhydrin and glycine forming an intermediate Schiff base [3]. This condensed product after deprotonation results in the formation of an anion, which acts as a potential tridentate metal binding ONO donor, producing stable five-membered metal chelates. The metal complexes were characteristically coloured, and it is anticipated that this can form a basis for studying the reaction between ninhydrin and a variety of amino acids in the presence of metal ions, particularly transition metal ions. A

specific correlation of metal ion - amino acid - colour can thus be developed based on the results and this can form a basis for amino acid identification and determination.

Ninhydrin exhibits bactriostatic, virucidal, fungistatic, antigen-antibody reactive, inhibitive and diabetogenic properties [4]. The activity of ninhydrin is totally lost in metal- bound state of the complexes.

The present investigation is; therefore, to synthesize metal complexes of Schifif bases derived from ninhydrin and the a-amino acids cysteine and valine. The complexes were characterized by analytical methods and anti microbial studies against *Escherichia Coli*, *Proteus mirabilis*, and *Staphylococcus aureus* were undertaken.

# CHAPTER 1 THEORETICAL BACKGROUND

## 1.1. The Chemistry of Amino Acids

Amino acids are organic molecules of low relative molecular mass (approximately 100 - 200), which contain at least one carboxyl ($COO^-$) and one amino ($NH_2$) group and are essential constituents of plant and animal tissues. The variations, which occur between the different amino acids, lie in the nature of their R groups (side chains), a feature that is of fundamental importance and confers individually upon each amino acid [4,5]. At the present time, some 25 different amino acids have been demonstrated to occur in the proteins of mammalian tissues. All possess in common a primary amino substituent, a carboxyl moiety, and hydrogen atom attached to the a - carbon atom. The amino acids, proline and hydroproline, may be considered as a - amino acids whose respective amino groups have become involved in the closure of a pyrrolidine ring [6]. The majority of the amino acids possess at least one asymmetric carbon atom and may therefore exist in at least two optically active forms. Although most of the natural amino acids are of the L - configuration, a number of D - amino acids also occur in nature [7]. The a - amino acids have the following structure (Fig. 2).

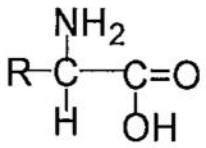


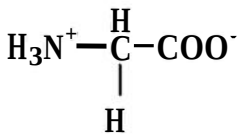

**Fig.** 2 General structure of a- amino acid

The most common and perhaps the most useful way of classifying the 20 “standard” amino acids are according to the polarities of their side chains (R groups). Based on this classification scheme, there are three major types of amino acids: (i) those with non polar R groups (e.g. valine), **(ii)** those with uncharged polar R groups (e.g. cysteine) and **(iii)** those with charged polar R groups (e.g. histidine).

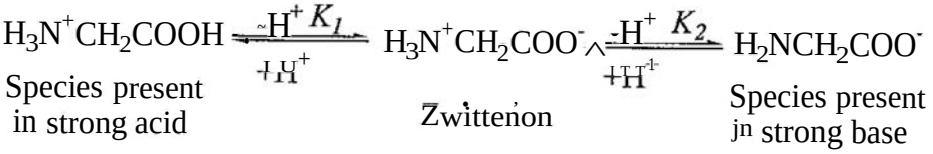
### 1.1 .1 Physicochemical Properties

All the common amino acid components of proteins are white crystalline solids, which are stable in the solid form at ordinary temperature. When heated, amino acids undergo decomposition at relatively high temperature. Amino acids vary considerably in their solubility in water. Cysteine and tyrosine are the least soluble of the common amino acid constituents of protein, while proline and hydroxyproline are extremely soluble.

Amino acids exist in aqueous solution as dipolar ions (zwitterions); for example, glycine may be represented as follows:



Following Bronsted, glycine can react either as an acid or as a base, i.e. as an ampholyte [8]:




$$K_1 = \frac{[H_3N^+CH_2COO^-][H^+]}{[^+H_3NCH_2COOH]}$$

$$K_2 = \frac{[H_2NCH_2COO^-][H^+]}{[^+H_3NCH_2COO^-]}$$

As a consequence of the dipolar ion form of the amino acids, **(i)** an increase in the solubility in water or other ionising compounds including other amino acids **(ii)** a decrease in the solubility in media of low dielectric constant such as alcohol, and **(iii)** a high melting point in the solid state are resulted. All the consequences are seemingly derived from the strong, intramolecular electrical forces, which are characteristics of the dipolar ion form [9,10,11].

The zwitterionic form exists at some specific pH for a specific amino acid. At this pH, the charges are equal and opposite, the molecule carries no net charge and exhibits electrophoretic mobility. This point is called isoelectric point (pi). For most simple amino acids pi is not just a point but a broad zone of pH values over which practically all the ampholyte is composed of dipolar ions. pi may depend on combination of $H^+$ and $OH^-$ [12].

### 1.1.2 Chemical Reactions of Amino Acids

All the amino acids have a series of reactions in common - those, which occur at the amino or carboxyl functions, or both. In addition, if the amino acid contains a reactive side chain, it exhibits a series of specific reactions. Such specific reactions are of interest in two particular respects. First, reactions of those groups that result in the production of colour are frequently useful for the detection and semi quantitative estimation of proteins and of the individual amino acids. Second, reactions that occur at the side chain of amino acids are frequently of use in efforts to modify proteins chemically [13,14].

Many of the reactions at the amino or carboxyl group are alkylation, acylation, ester and amide formation.

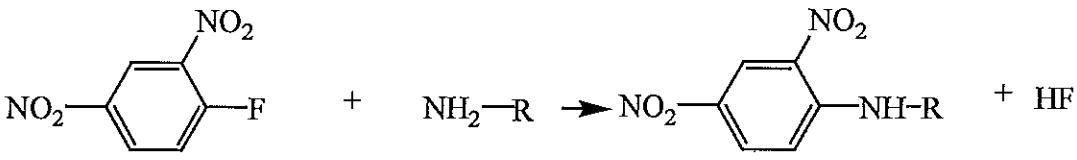
## Ninhydrin Reaction

The reaction of ninhydrin (triketohydrindene hydrate) with amino acids is of particular importance for the detection and quantitative estimation of the latter. Ninhydrin is a powerful oxidising agent and elicits the oxidative deamination of the α- amino group, liberating ammonia, carbon dioxide, the corresponding aldehyde and reduced form of the ninhydrin. The $NH_3$ then reacts with an additional mole of ninhydrin and the reduced ninhydrin to yield a purple substance that absorbs maximally near 570 nm. Because this absorption is nearly a linear function of the amount of amino groups originally present, this reaction provides a convenient

and quantitative calorimetric essay for amines. Although, the ninhydrin reaction is quite general for primary amines, the evolution of $CO_2$ which may be followed manometrically, is reasonably diagnostic for α - amino acids [6,15] In the case of the imino acids an alternate product is formed that is bright yellow (absorption maximum near 440nm). Hence, the ninhydrin reaction serves to distinguish these substances from the bulk of the protein amino acids.

**1 - Fluoro - 2,4 - dinitrobenzene**

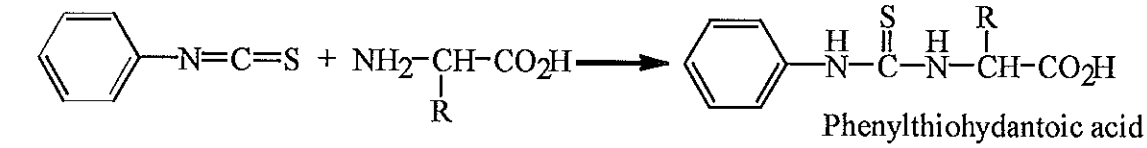
The arylation of amino acids with 1 - fluoro - 2,4 - dinitrobenzene (FDNB) is of great importance in structural protein chemistry [13].



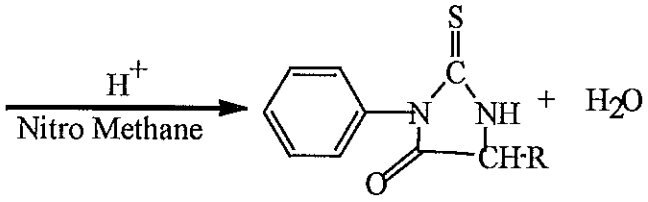

The value of dinitrophenylation lies in the quantitative production of yellow crystalline derivatives which are readily separated by chromatographic technique and which may be quantified spectrophotometrically.

**Phenylisothiocyanate**

Phenylisothiocyanate reacts readily with amino acids to yield the corresponding Phenylthiohydrantoic acids which on treatment with acid in nonhydroxylic solvents, cyclize, yielding phenylthiohydantoins.



Phenylthiohydantoic acid

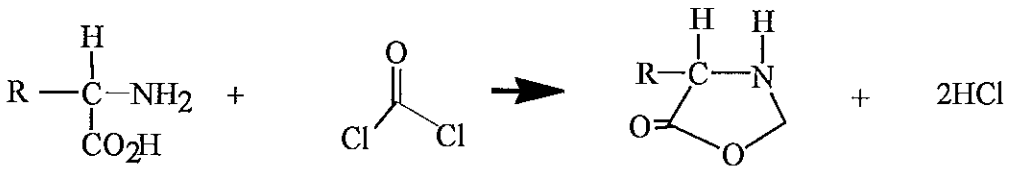


Phenylthiohydantoin

These amino acid derivatives are easily characterized and quantified. This reaction, like that of FDNB, is quite useful in structural studies [16].

**Phosgene**

The reaction of phosgene with α - amino acids yields N – carboxy anhydrides (Leuchs anhydrides).



These compounds react readily with nucleophilic reagents and are important intermediates in the synthesis of protein like polymer [4].

### 1.1.3 Metal Complexes of Amino Acids

$NH_2$-CHR-$COO^-$ has three donor groups; N : $O^-$ and =O. In general, amino acid complexes, involve either a metal carboxylate salt or an amine complex or both; in which case we have a five membered chelating ring.

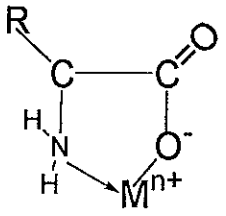


**Fig. 3** A general structure of a five membered chelate ring formed between a metal ion and α - amino acids

Studies on the stability and ease of formation of metal complexes revealed that the stability diminishes as the side chain lengthens and as the distance between amino and the carboxyl

groups increase [8] Thus, with α - amino acids six - membered rings with the diminished stability would be expected to be formed, whereas with γ -, δ- and ε - amino acids still higher membered and quite unstable rings would be in prospect. Among the metal ions the order of stability of complex formation with divalent metal ions was found to be $Hg^{2+} > Cu^{2+} > Ni^{2}+ > Co^{2+} > Cd^{2+} > Fe^{2+} > Mn^{2+} > Mg^{2+} > Fe^{3+}$. Stability was also found to be a function of pH [14,17].

Compounds containing an imino or azomethine group (R-C=N-) are usually formed by the condensation of a primary amine with an active carbonyl compound. They are named after Hugo Schiff who first reported them in 1864 [18]. The reaction to prepare Schiff base is reversible, progressing through a carbinolamine intermediate and requires the removal of water.

Schiff bases, which are effective as co-ordinating ligands have functional groups, -OH, $NH_2$, SH, etc., sufficiently near the site of condensation so as to form five or six membered chelate ring on reaction with metal ions.

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 [19]. A wide variety of ligands may be obtained via the Schiff base condensation reactions, which vary in denticity, flexibility, nature of donor atoms and electronic properties.

## 1.2 Chemistry of Metal Ions

The coordination chemistry of transition metal ions employed in this work is presented briefly, particularly from the stereochemistry viewpoint. The metal ions that have been used are VO(II), Cr(III), Mn(II), Fe(III), Co(III)/Co(II), Ni(II), Cu(II) and Zn(II). The discussion will be presented sequentially in terms of $d^n$ configuration.

### Oxo - Vanadium (IV)/VO(II) Complexes

Vanadium has rich and varied chemistry, which includes compounds of oxidation states from II to +V. VO(II) ion forms a wide variety of stable complexes, which may be cationic, anionic or neutral with square pyramidal or octahedral geometries. The most characteristic feature of the IR spectra of VO (II) complexes is the presence of a strong and sharp band due to V = O at $980 \pm 50$ $cm^{-1}$ [18,20].

Balhausen and Gray first formulated a ligand field picture of VO(II) complexes [18]. In the complexes, the unpaired electron is located in the $d_{xy}$ orbital. The visible spectra of VO(II) complexes are characterized by three spin allowed d - d transitions $^2B_{2g} \rightarrow {}^2E_g$, $^2B_{2g} \rightarrow {}^2B_{1g}$ and $^2B_{2g} \rightarrow {}^2A_{1g}$ . Generally they are observed in the region 10000 - 15000, 15000 - 19000 and 19000 - 26000$cm^{-1}$, respectively [18,21] for octahedral geometry.

**Chromium (III) Complexes**

Chromium (III) with a $d^3$ system forms numerous complexes in which it exhibits a coordination number of six with octahedral geometry. Two quartet states, $^4F$ and $^4P$, are derived from the $d^3$ configuration and in a regular octahedral field the $^4F$ splits in to $^4A_{2\,g}$, and $^4T_{1g}$ and $^4T_{2g}$ states. Octahedral Cr (III) complexes having $^4A_{2g}$ ground state are expected to have three spin allowed transitions $^4A_{2g} \rightarrow {}^4T_{2g}$, $^4A_{2g} \rightarrow {}^4T_{2g}(P)$ and $^4A_{2g} \rightarrow {}^4T_{1g}(P)$. In addition, spin - forbidden transitions $^4A_{2g} \rightarrow {}^2T_{2g}$ and $^2E_g$ are also observed [22].

No simple square planar complexes of Cr(III) are known and there appear to be compounds in which metal is believed to be tetrahedrally coordinated.

**Manganese ( II ) Complexes**

The great majority of Mn(II) compounds are high-spin and in the $^6S$ ground state. A high number of Mn (II) complexes of bidentate, tridentate and quadridentate [23] Schiff base have been reported.

Since the $^6S$ ground state for Mn(II) is orbitally degenerate, it cannot be split by the crystal field of any symmetry. The crystal field spectrum of high-spin $d^5$ complexes is therefore expected to be very weak and also justify the pale colour of Mn(II) salts. Four spin forbidden bands are

observed in the visible region which correspond to $^6A_{1g} \rightarrow {}^4T_{1g}$, $^6A_{2g} \rightarrow {}^4T_{2g}$, $^6A_{1g} \rightarrow {}^4E_g$ and $^6A_{1g} \rightarrow {}^4A_{1g}$ transition in octahedral geometry.

**Iron (III) Complexes**

Iron (III) with a $d^5$ configuration forms mostly octahedral complexes and comparatively less number of octahedral and square planar species.Fe(III) is isolectronic with Mn(II) and magnetically it favours high-spin complexes.

The electronic spectra of octahedral Fe(III) complexes have not been adequately characterized. Fe(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 $^6A_{1g} \rightarrow {}^4E_g(D)$. These assignments are not certain as Fe(III) complexes have charge – transfer bands in the visible and near UV-region, which in many cases obscure the very weak, spin forbidden d-d bands [24].

**Cobalt (II) Complexes**

CO(II) with a $d^7$ configuration is known in four coordinate (tetrahedral) and six coordinate (octahedral) stereochemistry. The electronic spectra of tetrahedral Co(II) complexes are more intense than those of the octahedral ones. In octahedral Co(II) complexes, $^4T_{1g}$ and $^2A_{1g}$ are the spin-free and spin-paired ground state, respectively.

For high spin octahedral geometry, a band observed near 8000- 10000 $cm^{-1}$ can be assigned to $^4T_{1g} \rightarrow {}^4T_{2g}$ transition. A multiple band observed around 20000 $cm^{-1}$ is attributed to $^4T_{1g} \rightarrow {}^4T_{1g}$ (P) transitions, [18,24], which may be in admixture with spin forbidden transitions to a doublet state derived principally from $^2G$ and $^2H$ terms. The transition to $^4A_{2g}$ is very weak, often appearing as a shoulder.

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 8500 $cm^{-1}$ and a stronger broader band near 20000 $cm^{-1}$.

**Cobalt (III) Complexes**

Cobalt (III) has great affinity for nitrogen ligands and forms an enormous number of complexes in which cobalt is bonded to ammonia, an amine, nitro groups or nitrogen atom in NCS groups often in addition to water molecules, halides, carbonate, or hydroxide ions [25].

Co(III) is isoelectronic with Fe(II)($d^6$) and qualitatively the same energy level diagram applies to both species. In contrast to Fe(II), however, almost all cobalt (III) complexes are low spin ($t_{2g}$ configuration) with the $^1A_{1g}$ ground state. For such low spin Co(III) complexes two spin allowed transitions from $^1A_1 \rightarrow {}^1T_{1g}$ (15700-22000) and $^1A_{1g} \rightarrow {}^1T_{2g}$ (22800-25800 $cm^{-1}$) are expected [18].

**Nickel (II) Complexes**

The electronic configuration of the Ni(II) is $d^8$. Octahedral Ni(II) complexes having $^3A_{2g}$ ground state are expected to have three spin allowed transitions $^3A_{2g}\rightarrow{}^3T_{2\,g}$, $^3A_{2g}\rightarrow{}^3T_{1g}(P)$ and $^3A_{2g}\rightarrow{}^3T_{2g}$ in the range of 7000-13000, 11000-20000 and 19000-27000 $cm^{-1}$, respectively. In addition to these three transitions, two spin-forbidden transitions $^3A_{2g}\rightarrow{}^1E_g$ and $^3A_{2g}\rightarrow{}^1T_{2g}$ are also observed one at near the second spin-allowed transition and another band between second and third spin-allowed transitions.

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 $^3T_2\rightarrow{}^1T_1$[26].

**Copper (II) Complexes**

The Cu(II) ion with its $d^9$ configuration in octahedral and tetrahedral environment is highly susceptible to Jahn-Teller distortion. In tetrahedral arrangement also Jahn-Teller distortion is operative, in spite of the fact that large spin-orbit coupling constants might produce sufficient splitting of $^2T_2$ ground state [21].

Octahedral complexes without any distortion 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.

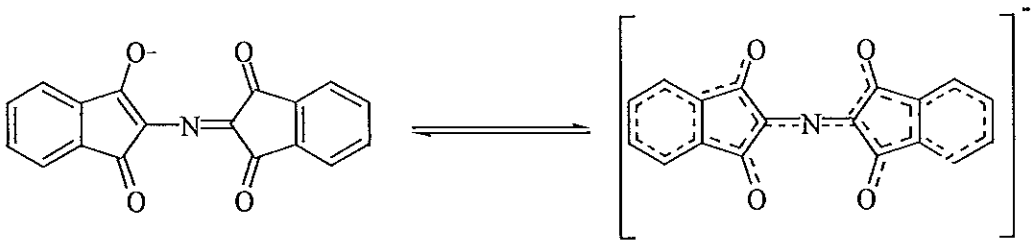
## Zinc (II) Complexes

Bivalent Zinc forms numerous complexes of various stereoeochemical types in which tetrahedral complexes are predominant. Monomeric and polymeric Zn(II) complexes have been reported. Square planar geometry is less common in Zn(II) complexes. These complexes are diamagnetic and do not possess any d-d transitions due to $d^{10}$ configuration [18].

# CHAPTER 2 LITERATURE SURVEY

The reaction of ninhydrin with amino acids has been used extensively for detecting and estimating amino acids. However, the mechanism of the reaction has given rise to a number of theories, and only recently has its nature been well understood. Ruhemann (1911) observed that ninhydrin and a warm aqueous solution of an amino acid gives a deep purple-blue colour when treated with the reagent. He obtained the purple colour with the amino acids that were available at that time, including β-alanine and β-aminopropionic acid, although aromatic amino acids, such as anthralinic acid, were shown to be the exception. There has been much confusion in late years concerning the type of amino acid, which is reactive, and it has sometimes been stated that only α-amino acids undergo this reaction. It has not always been appreciated, when attempt have been made to explain the ninhydrin reaction, that β-,γ-,δ-, and even ε-amino acids do react under the appropriate conditions. This gives an indication of the probable course of the ninhydrin reaction since, if the reaction is as general as is stated above, it is likely that the purple colour observed is the same in each case, and that only a fragment of the amino acid involved is contained in the coloured compound [1,27,28].

Ruhemann who also isolated the intermediate aminoreductone correctly interpreted the overall reaction. The mechanism is outlined by McCaldin[1]. Ruhemann proposed structure [1,29] for the purple coloured reaction product of ninhydrin with α-amino acids, ammonia or related primary amine compounds which was later modified to [27]:



VII IX

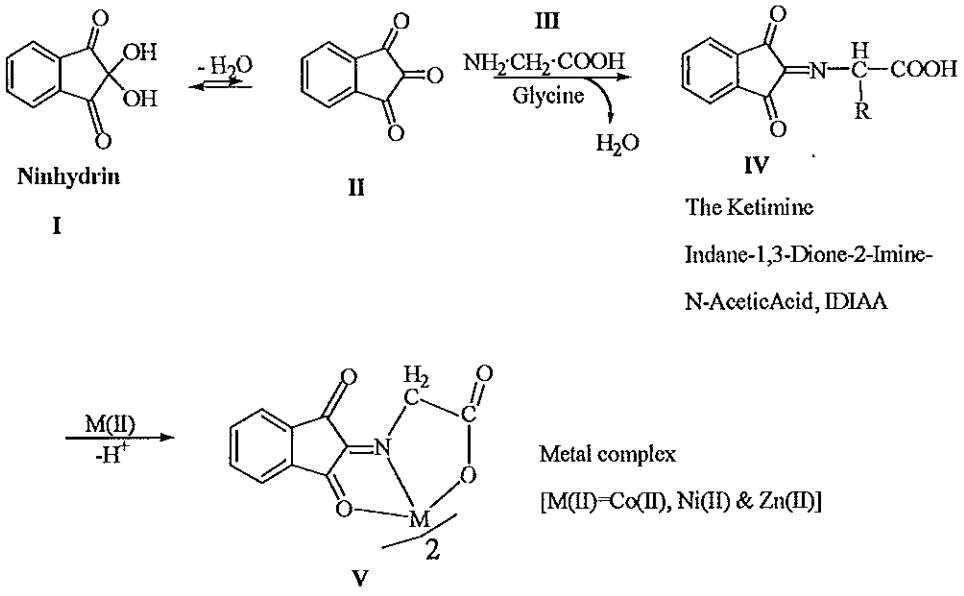
**Fig 4** Structure of Ruhemann`s purple

Several workers [30-35] have also studied the effect of metal ions on the ninhydrin reaction. In the chromatographic studies of ninhydrin/amino acid reactions, the transition metal ions were found to stabilize the bluish violet pigments, Ruhemann's purple [30]. The metal complexes of Schiff bases lend themselves ideally to a study of the effect of metal ions upon the equilibrium between two small molecules and the larger molecule that may be produced from them. Metal ions form complexes with the small molecules, the amine and the aldehyde, and the larger molecule, the Schiff base [36,37].

This mechanism is of importance in biochemistry, because the reactions catalysed by vitamin $B_6$ have been postulated to proceed through the intermediate formation of Schiff bases from pyridoxal and amino acids [38]. Since most of these reactions have been carried out without enzyme, but in the presence of metal ions, by Metzler and Snell, a mechanism for these reactions involving the metal complexes of these Schiff bases has been proposed [38-40] This mechanism has been confirmed for the non-enzymatic reactions by spectrophotometric studies, and some of the Schiff base complexes have been isolated in the solid state [41,42].

The rate of formation of the complex of nickel and copper (II) with the salicylaldehyde-glycine Schiff base is studied for the cases in which one of the organic reactants is added to the metal complex of the other and for the case in which the metal is added to the premixed organic reactants. It is found that the formation of the Schiff base complex is greatly retarded by prior reaction of either nickel or copper with one of the Schiff base components. Ni (II) decreases the amount of Schiff base at equilibrium whereas Cu (II) increases it. Equilibrium is reached much more rapidly with nickel than with copper [36,43,44].

The synthesis and characterization of new complexes of Co (II), Ni (II) and Zn (II) chelates of the Schiff base, indane-1, 3-dione-2-imine-N-acetic acid (IDIAA), formed by the condensation of ninhydrin with glycine was done by Rao and Reddy [45]. In the presence of metal ions (M (II) = Co, Ni, and Zn), the reaction appears to stop just after the condensation of ninhydrin and glycine forming an intermediate Schiff base, indane –1,3-dione-2-imine-N-acetic acid (IDIAA). This condensed product after deprotonation results in the formation of an anion, indane-1, 3-dione-2-imine-N-acetate (IDIA), which acts as a potential tridentate metal-binding ONO donor producing stable five- membered metal chelates (Scheme 2)[45].




**Scheme 2**, Reaction of glycine with ninhydrin in the presence of metal (II) ions

## OBJECTIVES AND SCOPE OF THE PRESENT INVESTIGATION

The physiological activity of organic compounds has been observed to undergo a significant enhancement on addition of metals. In particular, Schiff bases and their metal complexes have attracted a great deal of attentions as anticancer, antitubercular, anticonvulsant, insecticidal, antibacterial, antifugal, antiviral, antibiotic anti-inflammatory agents and plant growth regulators[1,33].

The qualitative and quantitative determination of amino acids, peptides and proteins is of major and growing importance in many areas of biochemical investigation. The most sensitive characteristic reaction in such determination is that between the amino acids and ninhydrin[46] producing characteristic purple-coloured Schiff base, the Ruhemann`s purple (diketo-

hydrindylene diketo – hydrindamine). But the reaction is not selective enough because all amino acids, except proline, give the same colour and other amine group containing compounds, like imino acids, ammonia, urea, β, γ, δ and ε - amino acids, other amines, etc also react very similarly with ninhydrin[47].

The investigation on the metal complex (M = Co, Ni, Zn) of glycine Schiff base indicated [45] that in the presence of the metal ion, Ruhemann`s purple is not formed rather specific coloured products could be isolated. Hence, if such data is available, analytical procedures may be devised to identify a specific amino acid with a better accuracy than the ninhydrin reaction method.

The present work is based on the synthesis and characterization of first raw transition metals VO(II),Cr(III),Mn(II),Fe(III) , Co(II), Co(III) ,Ni(II) , Cu(II) and Zn(II) complexes of two different Schiff bases, one derived from ninhydrin and cysteine and another one derived from ninhydrin and valine ( Indane-1,3-Dione-2-Imine-N-(3,3 dimethyl)-Propionate, IDIDP)

In view of the physical properties of ninhydrin and Schiff base complexes, a comparative study on the antimicrobial activity of ninhydrin and these metal complexes has been undertaken against three bacteria ( *Escherichia Coli, Proteus mirabilis and Staphylococcus aureus).*

# CHAPTER 3 EXPERIMENTAL PART

## 3.1. Materials and Chemicals

Metal salts $CoCl_2 \cdot 6H_2O$, $CrCl_3 \cdot 6H_2O$ (Both from Riedel deHaen), $Ni(NO_3)_2 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$, $FeCl_3$, $VOSO_4$, $ZnCl_2$ and $MnCl_2$, cysteine and valine (all from BDH), $CuCl_2 \cdot 2H_2O$ (Merck) and $[Co(NH_3)_5Cl]Cl_2$ synthesised in the laboratory. Ninhydrin (Pharmacos) were used. Absolute ethanol (Fluka) was used as a solvent throughout the synthesis, purification and analytical work. Other solvents like methanol, DMSO, DMF, petroleum ether, acetone, chloroform, benzene, and water were also used. Other materials used include pre-coated thin layer chromatographic plates (Polygram SIL G/$UV_{254}$, Mackerey-Nagel), nutrient agar, nutrient broth, autoclave, petri dishes, incubator and other common laboratory materials.

## 3.2 Instruments and Experimental Conditions

UV-Visible spectrophotometric studies of $10^{-3}$-$10^{-4}$ M solutions of the compounds in DMSO and chloroform in the range of 200-1100 nm using SPECTRONIC GENESYS 2PC with 1 cm cell at room temperature.

Melting points / decomposition temperatures of the products were determined using Bock-Monoscop ( `M` ) – Werck – NR instrument.

Infrared spectra were recorded using a Pye-Unicam SP 2000 Infrared Spectrophotometer in the range of 4000-200 $cm^{-1}$ and with a Buck Scientific Infrared Spectrophotometer Model 500 in the range of 4000- 600 $cm^{-1}$ as KBr disks.

The metal complexes have been analysed for metal using a Varian SpectrAA-20 Plus Atomic Absorption Spectrometer at the Ethiopian Geological Survey and Quality and Standards Authority of Ethiopia. Chloride contents were determined as AgCl by sodium fusion method using standard method [48]. The electrical conductivities of $10^{-3}$M solutions of the complexes in DMSO were obtained from a Philip Harris condactometer at room temperature.

Anti microbial studies were done by the disk – diffusion method in a nutrient agar medium against the three test organisms already noted, supplied from the Biomedical Laboratory, Department of Biology, Addis Ababa University.

## 3.3 Synthesis

### 3.3.1 Synthesis of Metal Complexes of Schiff Base Derived from Ninhydrin and Valine ( Indane-1, 3-Dione -2-Imine-N- (3,3 dimethyl ) –Propionate, IDIDP )

The Schiff base were prepared in the reaction mixture in the presence of the metal ion Co(II), Ni(II), Cu(II), VO(II), Zn(II), Fe(III), Cr(III), Mn(II) with same procedure and varying the metal ion.

0.01 mole of a metal ion was dissolved with heating when necessary in the possible minimum amount of ethanol. Then, 0.01 mole of ninhydrin was added, shaken and heated to dissolve it all. The solution was then refluxed for an hour and 0.01 mole of the valine was added. In all cases colour of the solution changed immediately upon addition of the amino acid. The mixture then refluxed for two additional hours and filtered in hot conditions through a Whatman #50 filter paper, washed with ethanol, distilled water and 50:50 mixture of ethanol and petroleum ether (50 - 80°C). The product was then dried in open air and stored in a desiccator.

### 3.3.2 Synthesis of Metal Complexes of Schiff Base Derived from Ninhydrin and Cysteine

Exactly the same procedure as 3.3.1 was followed and the metal (first row transition metals) complexes of the Schiff base derived from cysteine and ninhydrin were synthesised. The same procedure was followed to synthesise Cd(II) complex.

### 3.3.3 Synthesis of Metal Complexes of Schiff Base of Tyrosine

Exactly same procedure as 3.3.1 was followed and Zn(II) complex of the Schiff base derived from tyrosine and ninhydrin was synthesised.

## 3.4 Metal Complexes of Ruhemann`s

1:2 ratio of 0.01 mole of valine and ninhydrin was mixed and left overnight in order the reaction to get completed. Then, the metal ion was added and refluxed for 2 hrs. The products were filtered, washed and purified. This was done for the three metals: Zn(II), Cu(II) and Co(II). The complexes were different from the metal complexes of the Shiff base.

Ruhemann`s purple was also prepared by concentrating a 1:2 mixture of amino acid and ninhydrin in ethanol.

## 3.5 Anti-microbial Studies

The study was done by a disc-diffusion method in a nutrient agar medium against the three bacteria (*Escherichia Coli, Proteus mirabilis and Staphylococcus aureus*).

The bacteria were incubated on a nutrient agar slant in separate test tubes for 48 hrs. The exponentially growing cultures (0.5 mL) of each bacterium were 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 some metal salts) were dissolved in DMSO to make 25 mg/mL concentration. Two different concentrations of each sample were studied. 10μL (25 mg) and 20 μL (500 mg) of each sample, controls and solvents were absorbed on paper discs (Antibiotic-Assay disk, diam. 0.25 inch) using Eppendrof. The soaked paper disks were then placed on the inoculated agar plates at relatively regular intervals and incubated in the inverted fashion at 37 ° C for 24 hrs. Any inhibition zones were looked for and their diameters measured using a ruler.

# CHAPTER 4 RESULTS AND DISCUSSION

## 4.1 General

Reaction between α-amino acid and ninhydrin is one of the most commonly used methods for the detection and estimation of amino acid. Ninhydrin with valine or cysteine form the colored compound, Ruhemman`s purple. This purple coloured compound show sharply defined absorption maxima at 570 and 410 nm within 15-20 minutes of mixing [33,49].

Several preliminary experiments were done for Co(II) with different solvents / solvent mixtures and different molar ratio of the reactants to select a suitable solvent and molar ratio of reactants. Solvents studied include absolute ethanol, 95% aqueous ethanol, 50% aqueous dioxane and 50% aqueous methanol. These solvents were selected in light of published work in a similar study [45] and by being common solvents for all the reactants. Molar ratios of metal ion: amino acid: ninhydrin considered were 1:1:1, 1:2:2, 1:1:2 and 2:1:1. The yield and purity of the resulting complexes were then compared. The solvent ethanol and 1:1:1 molar ratio of ninhydrin, metal ion and amino acid were found to be suitable for optimum yield. These were then adopted throughout the synthetic work.

In the presence of Mn (II), Fe (III), Co (II), Co (III), Ni (II) and Zn (II) the products obtained with the valine Schiff base are in good yields and are all distinctly coloured, stable to air and sunlight. The solubility of the complexes was tested in common organic solvents. The complexes were found to be soluble in solvents of high dielectric strength, like DMSO and

DMF but only slightly soluble in solvents like methanol, acetone, chloroform, etc. The complexes have high decomposition temperatures (Table I).

Products isolated in the presence of all the first row transition metals with cysteine Schiff base and in the presence of some of the metalsi.e. VO(II), Cr(III) and Cu(II) with valine Schiff base were Ruhemann`s purple. This was confirmed by the similarity of physical properties, molar conductance, analytical and spectroscopic analysis. The respective metal salts were also recovered by concentrating from the filtrate and after usual work up.

Table I. Physical Properties of Metal Complexes of IDIDP and Other Related Products.

| | | Colour and Appearance | Yield (%) | Melting/Decomposition temp (°C) |
|---|---|---|---|---|
| Complex of IDIDP | Mn(II) | Red brown, Powder | 50.6 | >360 |
| | Fe(II) | Brown, Powder | 55.0 | >320 |
| | Co(II) | Light green, Crystalline | 60.6 | >360 |
| | Co(III) | Greenish brown, Powder | 56.7 | >320 |
| | Ni(II) | Dark green, Powder | 56.7 | >360 |
| | Zn(II) | Brown, Crystalline | 56.2 | >360 |
| Complex of Tyrosine Schiff base | Zn(II) | Green, Powder | 54% | >360 |
| Complex of Cysteine Schiff base | Cd(II) | Greyish green, Powder | 52.1% | >200 |
| Solid isolated with valine Schiff base in the presence of: | VO(II) | Red brown, Fluffy, Crystalline | 24.5 | 275 |
| | Cr(III) | Red brown, Fluffy, Crystalline | 16.9 | 270 |
| | Cu(II) | Brown, Fluffy, Crystalline | 22.5 | 270-272 |
| Ruhemann's purple | | Red brown, Fluffy, Crystalline | - | 270 |
| Solid isolated with cysteine Schiff base in the presence of: | Mn(II) | Dark brown, Fluffy, Crystalline | 29 | 272 |
| | Fe(III) | Brown, Fluffy, Crystalline | 23 | 270 |
| | Co(III) | Red brown, Fluffy, Crystalline | 30 | 270 |
| | Co(II) | Red brown, Fluffy, Crystalline | 21.2 | 270 |
| | Ni(II) | Dark brown, Fluffy, Crystalline | 13.0 | 270 |
| | Zn(II) | Dark brown, Fluffy, Crystalline | 19 | 270-274 |
| | VO(II) | Dark brown, Fluffy, Crystalline | 26 | 270 |
| | Cr(III) | Brown, Fluffy, Crystalline | 18 | 270-272 |
| | Cu(II) | Brown, Fluffy, Crystalline | 20 | 272 |

Room temperature experiments to synthesize Co(II), Ni(II), Cu(II) and Zn(II) complexes of serine Schiff base (IDIHP) gave products that were the same as the corresponding products in the synthetic procedure with refluxing [50]. That is, exactly same physical characteristics and identical IR and electronic spectra, etc. However, the products in the cold had a much smaller yield (<35 %) than with refluxing (> 50 %) and need an extended time, usually not less than three days. This indicates that refluxing facilitates the reaction [49].

Ruhemann`s purple complex of Zn(II) and Co(II) were found to be different from that of the Schiff base complexes. And this was revealed by physical properties of the products and spectroscopic studies (Table II). Cu(II) did not form a complex with Ruhemann`s purple and the product is exactly the same as that of the product of VO(II), Cr(III), Cu(II) of valine Schiff base and that of cysteine. This may lead to a conclusion that Cu(II) forms a complex neither with Ruhemann`s purple nor with the Schiff base, and the isolated products must have been Ruhemann`s purple. Literature reveals that Cu(II) – Ruhemann`s purple complexes are unstable salt in aqueous ethanol solutions [30,31].

Table II Data of products obtained with Ruhemann`s purple complex and the general procedure (valine)

| | Ruhemann's Purple complex | | | Schiff base complex | | |
|---|---|---|---|---|---|---|
| | Co (II) | Zn (II) | Cu(II) | Co(II) | Zn(II) | Cu(II) |
| Colour | Brownish green | Orange | Red brown | Light green | Brown | Red brown |
| Appearance | Powder | Powder | Fluffy, Crystalline | Powder | Crystal | Fluffy, Crystalline |
| m.pt/dec. temp. ($^0$C) | > 320 | > 360 | 278 | > 360 | >360 | 272-274 |
| $\lambda_{max}$ (nm) | 420, 580 | 408, 572 | 365, 491 | 496 | 206 | 365,492 |

## 4.2 Analytical Studies

### 4.2.1 Elemental Analysis

The analytical data of the compounds are given in Table III. The data show a metal to ligand ratio of 1:2 in Co(II), Ni(II) and Zn(II) complex of the valine Schiff bases (IDIDP). Co(III) and Fe(III) complexes of the valine Schiff base (IDIDP) show a 1:1 metal to ligand ratio with additional water and chlorides. The results of the analysis agree well with the proposed stoichiometry. The data found with cysteine Schiff base indicates that it did not form metal complex with any of them. The same results were observed with VO(II), Cr(III) and Cu(II) for the valine Schiff base (IDIDP), and this implies that they did not form any kind of complex with the Schiff base and Ruhemann's purple was isolated as the final product.

### 4.2.2 Conductivity Data of Metal Complexes

The conductivity measurements were carried out in 1 mM DMSO solutions. The molar conductivity values are given in Table (III). The low molar conductivity values are indicative of non- electrolytic nature of the complex, except the Fe(III) complex, which is 1:1 electrolyte [51].

From the elemental analysis and conductivities of Fe(III) complex, it can be concluded that out of the two chlorides, one is in the ionisation sphere, while the other in the coordination sphere. In the case of Co(III) complex, the two chlorides are in the coordination sphere.

The molar conductance values of the products obtained with cysteine Schiff base and IDIDP with VO(II), Cr(III) and Cu(II) were very small (< 10 $\Omega^{-1}$ $cm^{-1}$ $mol^{-1}$) and almost all the values are equal. Their non- electrolytic nature, along with the absence of a metal as shown from metal analysis, may suggest a large organic molecule, which is a Ruhemann's purple.

Table III Analytical and Conductance Data for Metal Complexes of IDIDP and Other Related products.

| | | % Metal Calc. (found) | % Chloride calc (found) | % Hydrogen Calc (found) | % Carbon calc (found) | % Nitrogen Calc (found) | $\Lambda_m$ ($\Omega^{-1}$ $cm^{-1}$ $mol^{-1}$) DMSO |
|---|---|---|---|---|---|---|---|
| IDIDP Complex | Mn(II) | 9.60(9.20) | - | 4.20( ) | 58.85( ) | 4.90( ) | 23.00 |
| | Fe(III) | 13.80(13.20) | 17.83(17.69) | 3.79( ) | 39.82( ) | 3.32( ) | 63.00 |
| | Co(II) | 10.24(9.98) | - | 4.17( ) | 58.44( ) | 4.87( ) | 33.60 |
| | Co(III) | 14.50(14.40) | 17.69(17.59) | 3.76( ) | 39.54( ) | 3.29( ) | 8.60 |
| | Ni(II) | 10.21(10.24) | | 4.17( ) | 58.46( ) | 4.87( ) | 36.90 |
| | Zn(II) | 11.24(11.08) | | 4.13( ) | 57.79( ) | 4.81( ) | 30.40 |
| Tyr. Schiff base | Zn(II) | 9.2(8.96) | - | | | | 23.21 |
| Cys. Schiff base | Cd(II) | 17.61(17.56) | | | | | 19.06 |
| Prod. With VO(II), Cr(III), Cu(II) and cysyeine Schiff base with all the metals | | > 9 (<0.5) | - | - | - | - | 2-10 |

C, H, N Experimental data to be acquired very soon

In DMSO $\Lambda_m$; 0-40 ➔ non -electrolyte

40-90 ➔ 1:1 electrolyte [51].

The presence of M(II) ions in the system most probably favours the condensation of ninhydrin and valine by enhancing the polarisation of carbonyl groups, there by promoting the nucleophilic attack resulting in the production of indane-1, 3-dione-2-imine-N- (3,3 dimethyl)-

propaionic acid (IDIDPA), which in turn binds the metal ion after deprotonation. The neighbouring - COOH with a replaceable hydrogen increases the basicity of the azomethine group in IDIDP thus favouring the formation of a stable metal chelate[45].

## 4.3 Infrared Spectral Studies

The infrared spectral results (Table IV and V) of the complexes gave strong evidence for the formation of the proposed ligand framework. The data clearly show two groups of products in the valine Schiff base. In addition, the data found from the cysteine Schiff base is identical with the second group of the valine Schiff base. Group I contains metal complexes obtained with Fe(III), Mn(II), Co(III), Co(II), Ni(II) and Zn(II), and group II contains products obtained in the presence of VO(II), Cr(III) and Cu(II) and the products obtained with cysteine Schiff base.

The infrared spectra show characteristic bands assignable to $\nu_{C=O}$, $\nu_{C=N}$ and $\nu_{C-O}$ which are indications of specific functional groups.

The absence of absorption peaks in the complexes in the range of 3200-3000 $cm^{-1}$ indicates the derivatization of the amino acid NH group and the OH group of the ninhydrin[52]. The infrared spectra of the complexes of IDIDP show the isopropyl group of the valine amino acid, C-H bending at 1368 and 1380$cm^{-1}$ (doublet). However, the products isolated from cysteine Schiff base do not show the S-H stretch, which should have appeared at 2500(w).

In the spectra of Fe(III) and Co(III) complexes the broad strong bands with distinct structure in the region of 3400-3200 $cm^{-1}$ can be attributed to OH stretching of vibration of coordinated water. The presence of $H_2O$ molecules in Fe(III) and Co(III) complexes were proved with

further evidence from the non-ligand bands assigned to rocking and wagging modes at 1630 - 1600 $cm^{-1}$ (HOH bending vibration) and 600 –200 $cm^{-1}$ [18,23].

Ninhydrin shows three bands in the C=O stretching region; 1768 $cm^{-1}$, 1754 $cm^{-1}$ and 1720 $cm^{-1}$ [53]. The 1754 and 1720 $cm^{-1}$ bands are characteristic of its 1, 3 - dicarbonyl functional group [56] and the 1768 $cm^{-1}$ band is characteristic of the intermediate carbonyl in the tricarbonyl species, which is in equilibrium with the dihydroxy species. The infrared frequencies for the carbonyl groups in ninhydrin are shifted from 1754 $cm^{-1}$ and 1720 $cm^{-1}$ to 1730 - 1710 and 1712 - 1702 $cm^{-1}$ in the first group, IDIDP complexes. This indicates the involvement of one of the two carbonyl groups of ninhydrin in coordination with metal ion [18,53]. In this group, the complexes show a band in the range of 1650 - 1610 $cm^{-1}$, which is assigned to $\nu_{COO(ass)}$. A positive shift of 10-50 $cm^{-1}$ is observed in the anti symmetric stretch frequencies of carboxylate group of the ligand while symmetric frequencies decrease due to co-ordination with the metal ion [57]. The usual frequency at which $COO^-$ antisymetrical stretch appears is in the range 1600-1590(w) and $COO^-$ sym. stretch is in the range 1400-1350 $cm^{-1}$ (s)[52]. The separation between the antisymmetric and symmetric frequencies varies with the metal ion involved and the pattern observed is consistent with the literature [57,58].

The infrared frequency corresponding to the N-H of valine and -OH of ninhydrin are absent in the spectra of the complexes. Instead, a new strong and sharp peak, characteristic of $\nu_{C=N}$, has been observed at 1510 - 1500 $cm^{-1}$ suggesting the formation of an azomethine group due to condensation of valine and ninhydrin. The usual frequency at which $\nu_{C=N}$ appears is about 1670 - 1550 $cm^{-1}$. Its negative shift by about 100 $cm^{-1}$ is a clear indication of the participation of

azomethine nitrogen in coordination. This fact is further confirmed by the absence of such a peak in the individual spectrum of either valine or ninhydrin.

The non-ligand bands appearing in 750-600 $cm^{-1}$ region of spectra of the complexes may be assigned to $\nu_{M-N}$ and $\nu_{M-O}$ modes. The ligands behave as a tridentate ONO donor involving a carbonyl oxygen and an azomethine nitrogen in coordination.

The second group shows carbonyl stretching in the range 1730-1740 $cm^{-1}$ which is close to that of Ruhemann's purple carbonyl stretching, 1738 $cm^{-1}$. In the group, the 1768 $cm^{-1}$ band of ninhydrin is lost showing its condensation and formation of azomethine group. The $\nu_{C=N}$ do not show any negative shift, it is observed in its usual range 1670-1550 $cm^{-1}$, indicating the azomethine group is not involved in any coordination.

The observations from the infrared spectrum are: the first group which are the complex formed with IDIDP have formed a metal complex and the ligand behave as a tridentate ONO donor. However, the second group which include the products found from the cysteine Schiff base and from the metal (VO(II), Cr(III), Mn(II), Fe(III), Co(II), Co(III), Ni(II), Cu(II), Zn(II)) including the products found from the valine Schiff base and the metals (VO(II), Cr(II), Cu(II)) did not form a metal complex and the end products are Ruhemann's purple.

Table IV Infrared Spectral Data of the Metal Complexes of IDIDP and Starting Materials (KBr pellets) [ $cm^{-1}$]

| Compound | $\nu_{N-H}$ | $\nu_{OH}$ | $\nu_{C=O}$ | $\nu_{assCOO^-}$ | $\nu_{C=C}$ | $\nu_{C=N}$ | $\delta_{CH3}$ | $\nu_{C-H}$ | $\nu_{sCOO^-}$ | $\nu_{M-O-C}$ | $\nu$(M-N, M-O, M-Cl...) $\rho_r H_2O$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Ninhydrin[53] | - | 3200-2920(b,s) | 1768(s) 1754(s), 1720(s) | - | 1590(s) | - | - | - | - | - | |
| Valine[54] | 3200-2700 (vb) | | | 1590(s) | | | 1420 | 1368 1382d | | | |
| Mn(II) Complex | | | 1731(s) 1700(m) | 1643(vs) | 1604(s) | 1517(vs) | 1420(s) | 1368(d) | 1331(s) | 1202(s) | 700(m), 665(m),605, 522(s) |
| Fe(III) Complex | | 3381-2715 | 1738(s) 1700(m) | 1650(s) | 1639(s) | 1511(vs) | 1420(s) | 1378(d) | 1331(s) | 1220(s) | 700(m), 665(m),605, 545(s) |
| Co(III) Complex | | 3421-2955 | 1731(s) 1700(m) | 1630(s) | 1604(s) | 1530(vs) | 1458(s) | 1370(d) | 1338(s) | 1210(s) | 691(m), 612(m), 520(s) |
| Co(II) Complex | | | 1725(s) 1700(m) | 1644(vs) | 1590(s) | 1530(vs) | 1445(m) | 1390(d) | 1321(s) | 1220(s) | 690(m), 665(m),601, 520(s) |
| Ni(II) Complex | | | 1731(s) 1700(m) | 1643(vs) | 1604(s) | 1517(vs) | 1420(s) | 1368(d) | 1331(s) | 1210(vs) | 700(m), 665(m),605, 500(w), 405(w) |
| Zn(II) Complex | | | 1737(s) 1700(m) | 1650(vs) | 1611(s) | 1511(s) | 1458(s) | 1370(d) | 1331(s) | 1206(vs) | 700(m), 675(m),599, 530(s) |
| Zn(II) tyr schiff. | | 3600-3320 | 1738 1700(m) | 1650(s) | 1640 | 1511 | | | 1321 | 1220 | 700(m),650 600(m) |
| Cd(II) cys shiff. | | | 1731(s) 1700 | 1643(vs) | 1604 | 1517(vs) | | **-SH 2500(w)** | 1331 | 1210 | 700(m),665, 605(m) |

- Intensities in parenthesis - b - broad, vs- very strong , s- strong, m - medium, w-weak a - asymmetric, s- symmetric, arom - aromatic.
- Assignments based on K. Nakamoto[57], $\nu$-designate stretching vibration , d- designate doublet.

Table V Infrared Data of Rhemann's Purple and the Products Isolated in the Presence of Some Metals (KBr Pellets)

| Compound | | | $\nu_{C=0}$ | $\nu_{C=C}$ | $\nu_{C=N}$ | $\nu_{C=C}$ (enol) | $\nu_{C-0}$ | $\delta_{C-H}$ (arom) | $\nu_{S-H}$ | $\nu_{C-H}$ Dimethyl |
|---|---|---|---|---|---|---|---|---|---|---|
| Products isolated with | Valine | VO(II) | 1736(s) | 1600 | 1637 | 1557 | 1100 | 721(s) | - | - |
| | | Cr(III) | 1738(s) | 1590 | 1633 | 1556 | 1120 | 721(s) | - | - |
| | | Cu(II) | 1738(s) | 1600 | 1638 | 1557 | 1121 | 720(s) | - | - |
| | Cysteine | VO(II) | 1737(s) | 1590 | 1638 | 1557 | 1100 | 721(s) | - | - |
| | | Cr(III) | 1735(s) | 1595 | 1637 | 1557 | 1100 | 720(s) | - | - |
| | | Mn(II) | 1732(s) | 1592 | 1632 | 1557 | 1120 | 720(s) | - | - |
| | | Fe(III) | 1738(s) | 1591 | 1638 | 1558 | 1121 | 720(s) | - | - |
| | | Co(III) | 1737(s) | 1591 | 1637 | 1557 | 1111 | 721(s) | - | - |
| | | Co(II) | 1737(s) | 1600 | 1634 | 1554 | 1101 | 722(s) | - | - |
| | | Ni(II) | 1736(s) | 1591 | 1638 | 1558 | 1110 | 721(s) | - | - |
| | | Cu(II) | 1738(s) | 1596 | 1637 | 1558 | 1120 | 720(s) | - | - |
| | | Zn(II) | 1737(s) | 1601 | 1638 | 1557 | 1121 | 721(s) | - | - |
| Ruhemann's Purple | | | 1738(s) | 1617 | 1664 | 1560 | 1119 | 721(s) | | |

- Intensities in parenthesis - b - broad, vs- very strong , s- strong, m - medium, w-weak, a - asymmetric, s- symmetric, arom - aromatic.
- Assignments based on K. Nakamoto[57], ν-designate stretching vibration , d- designate doublet.

## 4.4 Electronic Spectra

The electronic spectral data of the metal complexes of IDIDP and the products isolated from the cysteine Schiff base are given in Table VI. As it has been observed from the infrared spectral data here also there are two groups observed. The first group include metal complexes of IDIDP, Mn(II), Fe(III), Co(III), Co(II), Ni(II) and Zn(II) complexes. The second group include the products isolated in the presence of VO(II), Cr(III) and Cu(II) of the valine Schiff base and the products isolated in the presence of all the selected metals VO(II), Cr(III), Mn(II), Fe(III), Co(III), Co(II), Ni(II), Cu(II) and Zn(II) of the cysteine Schiff base.

The electronic spectrum of the metal complexes shows number of bands in the range of 41494 - 14286 cm $^{-1}$. The band positions are observed at around 41494, 35714, 27027, 20161 cm $^{-1}$. The ligands show a high intensity band centred at 493 nm (20289 cm $^{-1}$) which may be assigned to a combination of $\pi$-$\pi^*$ and n-$\pi^*$ transitions which are characteristic of an azomethine chromophore. The electronic spectra of the metal complexes show bathochromic shifts of $\nu_{c=N}$ chromophore recorded in the range of 400 nm to 430 nm in the free ligand to the extent of 5 to 20 nm, which may be interpreted as a consequence of the involvement of this group in metal binding process [59]. Besides this clear indication, there are a number of non-ligand electronic spectral bands observed in the spectra of metal complexes, which can be assigned to various d-d transitions which are characteristics of the electronic configurations and geometries around the metal ions.

The bands 35714 - 27248 cm $^{-1}$ are assigned for a n-$\pi^*$ transitions of carbonyl (C=O). Bands at 38610-41494 cm $^{-1}$ may be attributed to $\pi$-$\pi^*$ transition of benzene ring (C=C). The positions of most of these bands at relative higher wavelength region could be attributed to the extended conjugation.

The electronic spectrum of Co(II) complex of IDIDP shows two bands located at 15873 cm$^{-1}$ which may be considered as $^4T_1 \rightarrow {}^4A_2(P)$ and at 20161 cm$^{-1}$ assigned to $^4T_{1g} \rightarrow {}^4T_{2g}(P)$. The band which was expected at around 7000 cm$^{-1}$ $^4T_1 \rightarrow {}^4T_{2g}(F)$ is not observed [25]. The electronic spectrum of Co(II) complex of IDIDP suggests an octahedral geometry. The electronic spectra of Co(III) complex of IDIDP also suggest an octahedral geometry.

The spectrum of Ni(II) complex shows absorption bands located in the range of 8200 - 27780 cm$^{-1}$, the band present at 8264 cm$^{-1}$ is assigned to $^3A_2 \rightarrow {}^3T_2$ while those at 17544, 27778 cm$^{-1}$ are assigned to $^3A_2 \rightarrow {}^3T_1$ (F), $^3A_2 \rightarrow {}^3T_1$ (P) transitions for octahedral geometry, respectively [18,25].

In the electronic spectrum of Fe(III) and Mn(II) complexes of IDIDP a number of low intensity bands in the region 17826 cm$^{-1}$- 22200 cm$^{-1}$ that may be attributed to the doubly forbidden transitions from $^6A_1$ ground state to various high energy quartet states are seen. These observations support octahedral geometry. For these complexes and the Zn(II) complex octahedral geometry is suggested based on similarity of their properties to each other, elemental, IR and conductance studies.

The other group which did not form metal complex show bands in the range 22489 – 23600 $cm^{-1}$ which is characteristic peak of Ruhemann's purple. This group and Ruhemann's purple show bands at around 40000 and 33000 $cm^{-1}$, which can be attributed to $\pi$ - $\pi^*$ and n-$\pi^*$ of benzene. Moreover, other band around 27280 $cm^{-1}$ assigned to n-$\pi^*$ of C=O. Besides there is a band from the range 22489-23600 $cm^{-1}$ which is $\pi$ -$\pi^*$ and n-$\pi^*$ of Ruhemann's purple chromophore.

Table VI Electronic Spectral Data of Metal Complexes of IDIDP and their Assignments.

| Complex | Non ligand electronic spectral bands | $\varepsilon_{max}$ ($Lmol^{-1}$ $cm^{-1}$) in DMSO | Assignments |
|---|---|---|---|
| Mn(II) | 18181 (very broad) | 19.02 | Spin forbidden $^6A_1 \rightarrow {}^4F$, $^4D$ or $^4G$ |
| Fe(III) | 20784 (very broad) | 26.11 | Spin Forbidden $^6A_1 \rightarrow {}^4A_1$, $^6A_1 \rightarrow {}^4E(G)$ $^6A_1 \rightarrow {}^4T_1(D)$ and $^6A_1 \rightarrow {}^4E(D)$ |
| Co(III) | 16273<br>20284<br>26174 | 31.00<br>54.06<br>62.68 | $^1A_1 \rightarrow {}^3T_1$<br>$^1A_1 \rightarrow {}^1T_1$<br>$^1A_1 \rightarrow {}^1T_2$ |
| Co(II) | -<br>16873<br>20161 | <br>14.04<br>9.12 | $^4T_1 \rightarrow {}^4T_2(F)$<br>$^4T_1 \rightarrow {}^4A_2(P)$ |
| Ni(II) | 14286<br>16000<br>24699 | 32.5<br>96.36<br>144.62 | $^3A_2 \rightarrow {}^3T_2$<br>$^3A_2 \rightarrow {}^3T_1$<br>$^3A_2 \rightarrow {}^3T_1(P)$ |
| Zn (II) | - | - | - |

## 4.5 Anti-microbial Studies

The diameter of the inhibition zones around each sample; controls and solvent were measured. These data were then converted in to a conventional way of expressing bacterial activity (+ and -) by taking some approximate ranges compared to the activity of the starting material ninhydrin.

Ninhydrin was found to be strongly active against the three bacteria studied. The activities of the metal complexes, ninhydrin and Ruhemann`s purple are given in Table VII. Many of the complexes of both Schiff bases are active against one gram-negative bacterium *Proteus* mirabilis and the gram-positive bacterium *Staphylococcus aureus*.

Co(III) and Mn(II) complexes of the IDIDP showed a greatly enhanced activity than the starting material, ninhydrin. However, they exhibited a highly reduced activity against the gram – negative bacteria *Escherichia coli* compared to ninhydrin activity. The corresponding metal salts and the solvent have not shown any anti microbial activity. The observed enhancement of activity of those complexes found to be due to a synergistic or combination effect of the derivatization, complexation and the side group of the amino acid. This may also be associated with electronic distribution and stereochemistry of the ligand due to complexation. Compared to the inactivity of the metal complexes of glycine Schiff base, IDIA, against these three bacteria [45], the activity of the current new complexes may be mainly due to the side groups

and/or structural variations. Ruhemann`s purple also show some activity, though reduced, against *Proteus mirabilis* and *Staphylococcus aureus* at the concentration studied.

Table VII Results of Anti-microbial Studies on Ninhydrin, the Metal Complexes of IDIDP and Related Products.

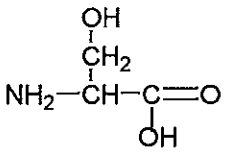
| Compound | | Test organism | | |
|---|---|---|---|---|
| | | Escherichia | Proteus mirabilis | Staphylococcus |
| Ninhydrin | | ++ | ++ | ++ |
| Ruhemann's Purple | | -- | + | + |
| Metal Complexes of IDIDP | Mn (II) | -- | ++++ | ++++ |
| | Fe (III) | -- | + | + |
| | Co (III) | ++ | ++++ | ++++ |
| | Co (II) | + | ++ | ++ |
| | Ni(II) | -- | ++ | ++ |
| | Zn(II) | -- | - | ++ |
| Products isolated from cysteine Schiff base and in the presence of | Mn (II) | -- | - | + |
| | VO (II) | -- | + | + |
| | Cr (II) | -- | - | + |
| | Fe (II) | -- | + | -- |
| | Co (III) | -- | -- | -- |
| | Co (II) | -- | -- | -- |
| | Ni(II) | -- | -- | - |
| | Cu(II) | -- | -- | -- |
| | Zn (II) | -- | -- | -- |
| IDIDP in the presence of | VO (II) | -- | + | + |
| | Cr (III) | -- | + | -- |
| | Cu (II) | -- | -- | -- |

* ++++ = Very high activity; +++= High activity, ++ =Moderate activity, + = Some activity; --= No activity.

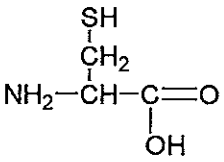
Table VIII Data Available on the Studies of Metal Complexes Derived from Different Amino acids and Ninhydrin.

| Complex | | Color | % Metal Calc(found) | Melting/Decomp.pt. °C |
|---|---|---|---|---|
| Glycine | Co(II) | Dark green | 12.08(12.02) | >320 |
| | Ni(II) | Green | 11.74(11.96) | >360 |
| | Zn(II) | Brown | 12.82(13.15) | >320 |
| Alanin | Co(II) | Bright green | 11.34(11.03) | >350 |
| | Ni(II) | Greenish brown | 11.30(10.70) | >360 |
| | Zn(II) | Dark brown | 12.49(11.77) | >340 |
| Valin | Co(II) | Light green | 10.24(9.98) | >360 |
| | Ni(II) | Dark green | 10.21(10.24) | >360 |
| | Zn(II) | Brown | 11.24(11.08) | >360 |
| Serine | Co(II) | Red | 14.94(14.75) | >330 |
| | Ni(II) | Bright green | 10.65(9.95) | >350 |
| | Zn(II) | Dark brown | 11.73(11.55) | >330 |
| Tyrosine | Zn(II) | Green | 9.2(8.96) | >320 |
| Cysteine | Co(II) | Red brown | 10.10(0.24) | 270 |
| | Ni(II) | Dark brown | 10.07(0.17) | 270 |
| | Zn(II) | Dark brown | 11.09(0.28) | 270-274 |

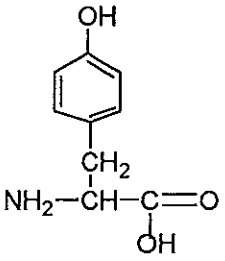
From the data available i.e., percentage metal, it is obvious that the cysteine amino acid did not form a metal complex with any of the Schiff bases. In addition, it was possible to isolate the metal salts from the filtrate.



Serine



Cysteine



Tyrosine

A plausible explanation why cysteine does not form metal complexes lies on the side group, -SH. Hence, with this assumption, the first case considered was the acidity behaviour of the side chain. Since the –SH group of cysteine is acidic unlike –OH group of serine, acidity might have an effect for the cysteine not forming metal complex. Thus another acidic amino acid (tyrosine) was taken for comparison purpose. Tyrosine was chosen, since it has an acidic side chain due to the presence of the phenyl group. Interestingly, a 1:2 metal to ligand ratio complex was formed, comparable to the complexes formed with the glycine, alanine, valine and serine. This shows that acidity is not a reason for cysteine not forming metal complex.

The other situation to be considered is, that cysteine has a soft sulphur side group which combines with soft acceptors. However, the metals used for the synthesis are all hard as well as intermedediate in their properties. Literature reveals that amino acids which contain sulphide ion ($S^-$) have a property of hindering the approach of metal ion, even though that group does not involve in the eventual bonding/coordination [60].

Cadmium (divalent) a soft acid, on the other hand has formed a complex with a 1:2 metal ligand ratio complex as the other amino acids. This shows that the soft sulphur group is the reason for the cysteine not forming metal complexes with the first row transition metals.

# CONCLUSION

With the support of all the available data, two groups were found from the isolated products. The first group consists of Mn(II), Co(II), Co(III), Ni(II) and Zn(II) complexes of IDIDP. The second group is the products isolated with cysteine Schiff base in the presence of the first row transition metals metals and from valine Schiff base in the presence of VO(II), Cr(III), and Cu(II). From the data acquired, the second group do not form metal complexes, rather Ruhemann's purple.

For the first group the ligand (Schiff base) behaves as a monobasic tridentate ONO donor and an octahedral geometry is assumed for all the complexes in the group. Based on the available data the structures proposed are the following (Fig. 5, 6,7).

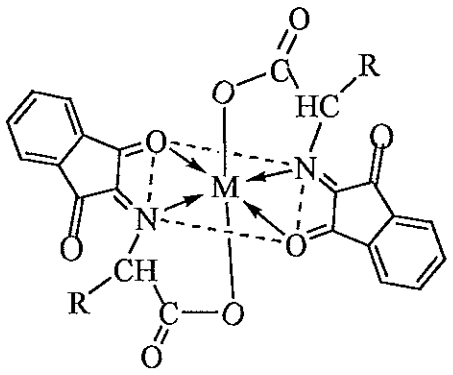



**Fig.5** Metal complexes of IDIDP (R= Isopropyl)

$[ML_2]$, M= Mn (II), Co (II), Ni (II) and Zn (II)

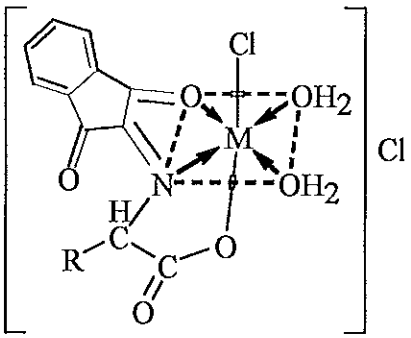



**Fig.6** Metal complex of IDIDP (R= Isopropyl)

$[ML(H_2O)_2Cl]^+Cl^-$, [M=Fe(III)

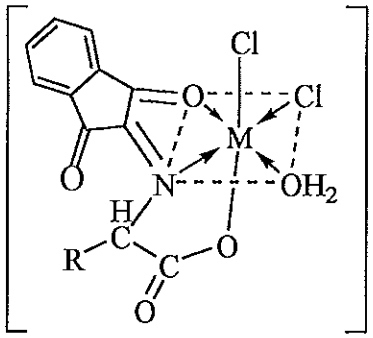



**Fig.7** Metal complexes of IDIDP (R= Isopropyl)

$ML(H_2O)Cl_2$ [M= Co(III)]

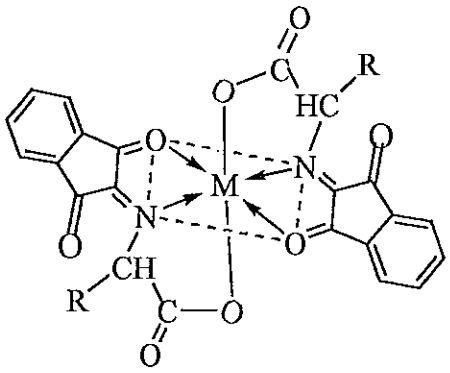


**Fig.8** Zn(II) complex of tyrosine Schiff base (Ketimine)

(R= Phenol) $ML_2$

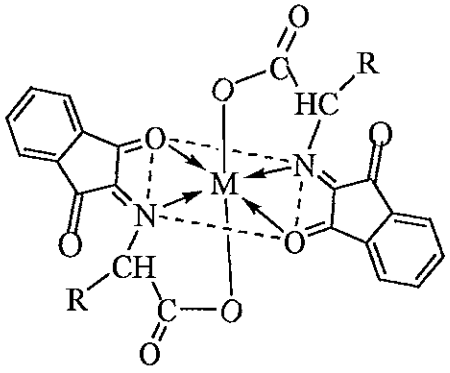


**Fig.9** Cd(II) complex of cysteine Schiff base (R= -SH)

$[ML_2]$, M= Cd(II)

The project focused on the studies of the metal complexes formed in the reaction of α - amino acid with ninhydrin in the presence of selected transition metal. The results found from the current studies and from literature revealed that there is a positive implication towards a possible method to identify a specific amino acid. The data from current studies show that Co(II) may be used to specifically identify and quantify serine from glycine, alanine and valine, Fe(III) may be used to discriminate alanine from the others, Mn(II) may be used to discriminate valine from the others and Zn(II) may be used to discriminate tyrosine from the others.

The studies should continue with different amino acids and based on all possible data found, it may be possible to attain the goal of identifying specific amino acids using transition metal.

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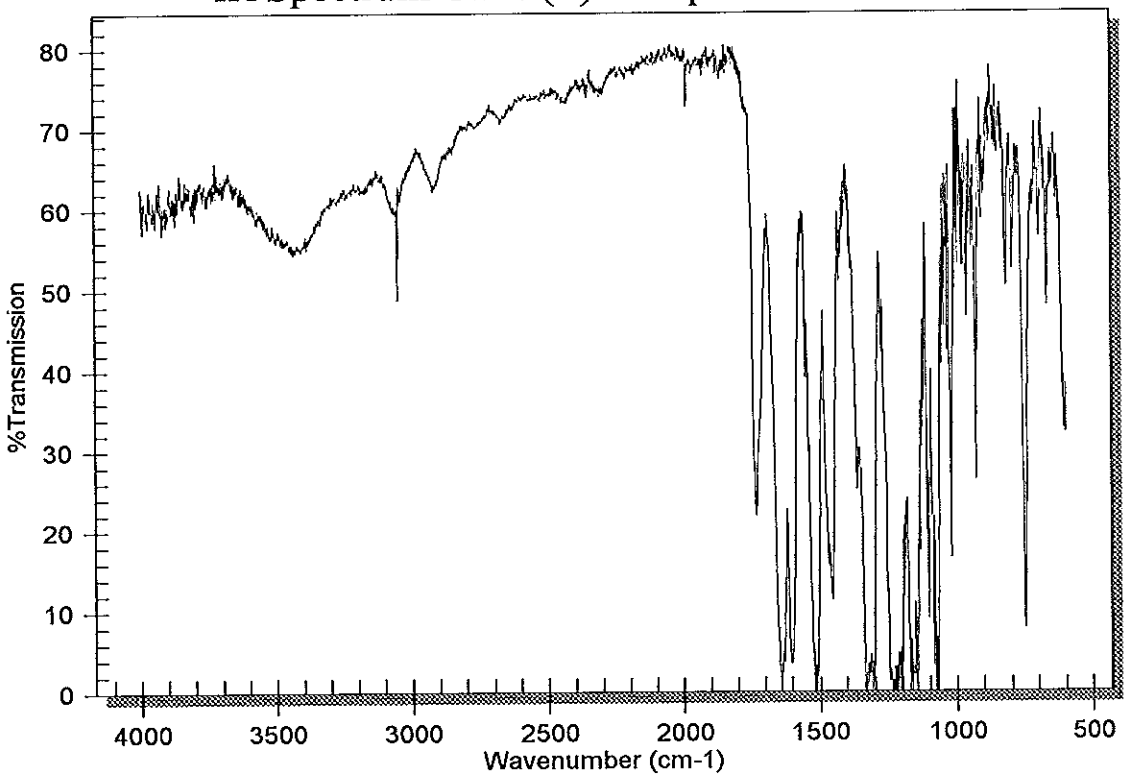
IR Spectrum Of Ni(II) Complex Of IDIDP

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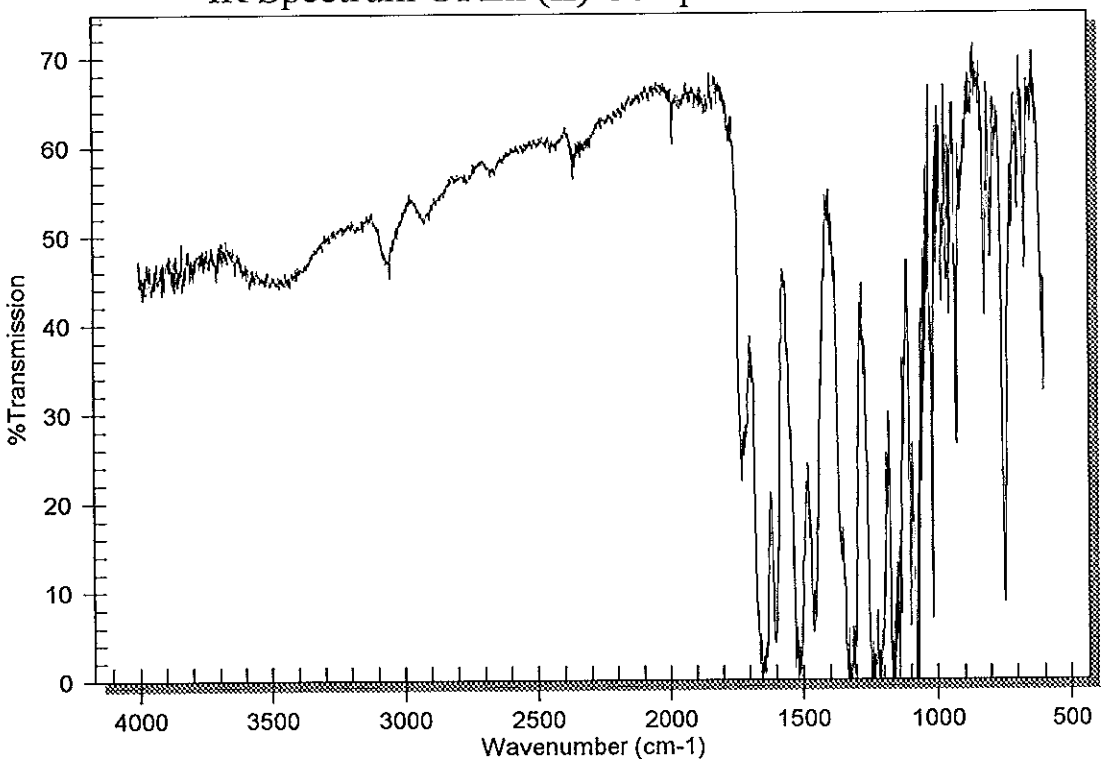

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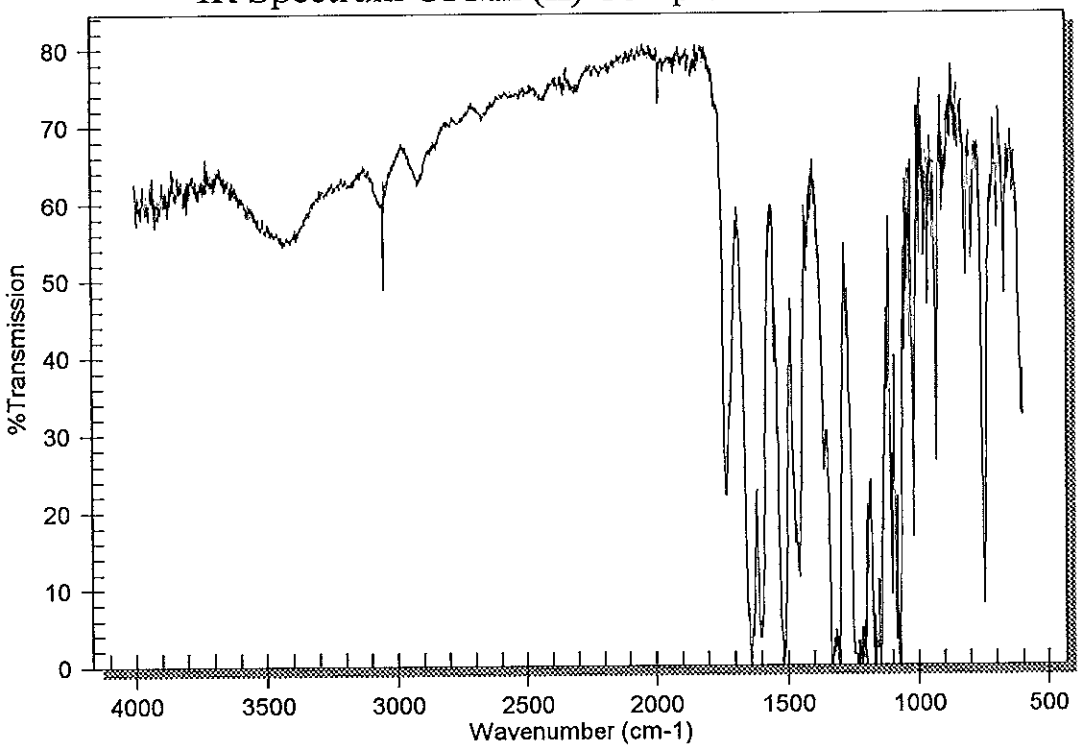
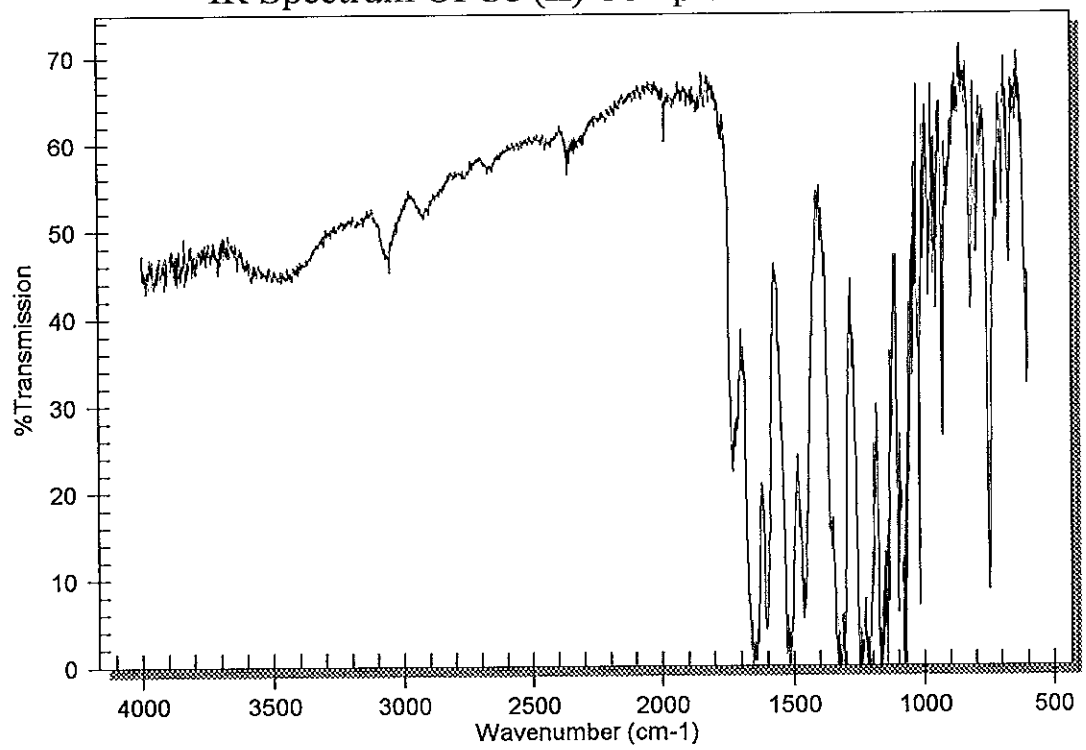
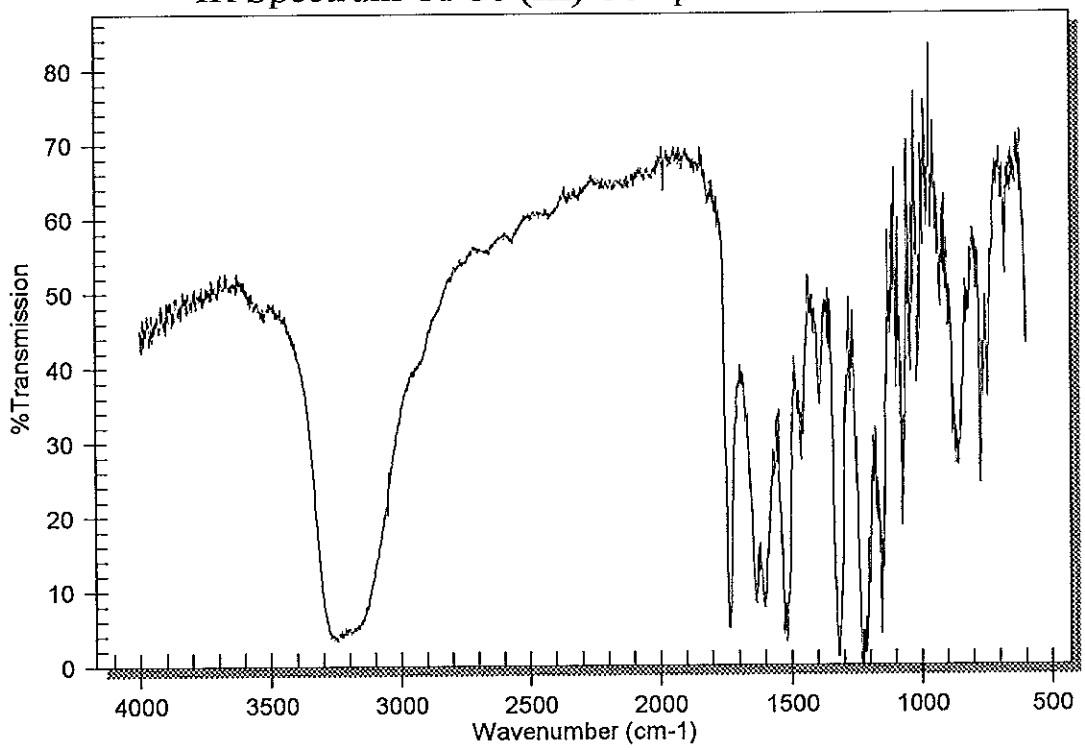


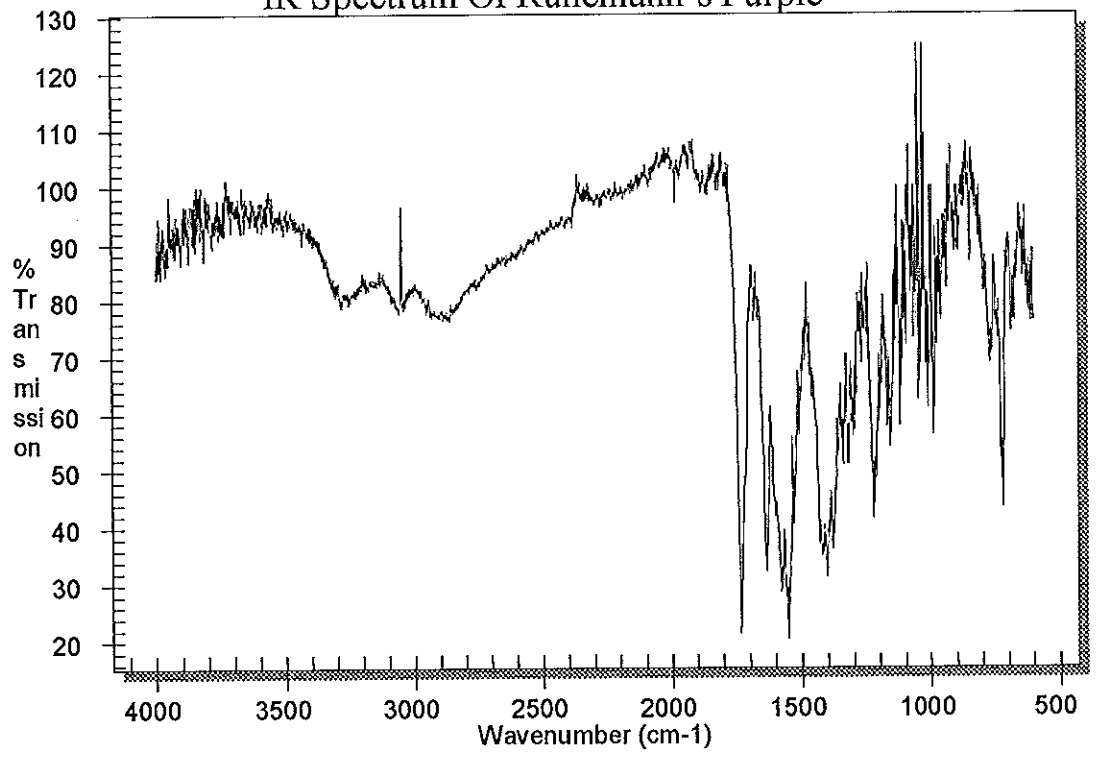

IR Spectrum Of Co (II) Complex Of IDIDP



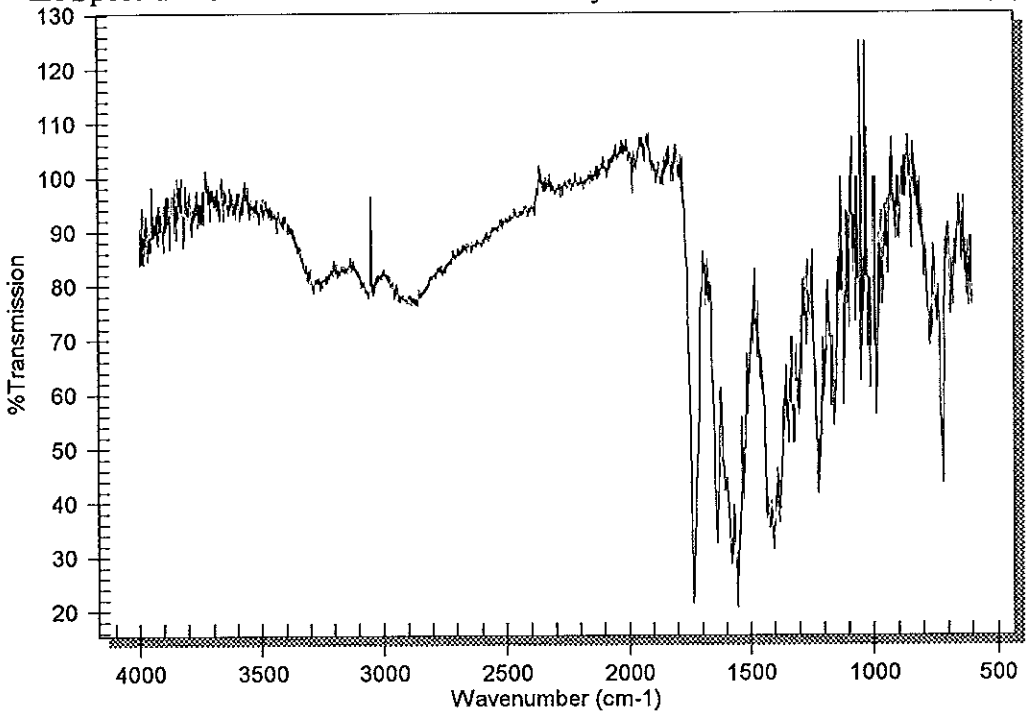
IR Spectrum Of Co (III) Complex OF IDIDP



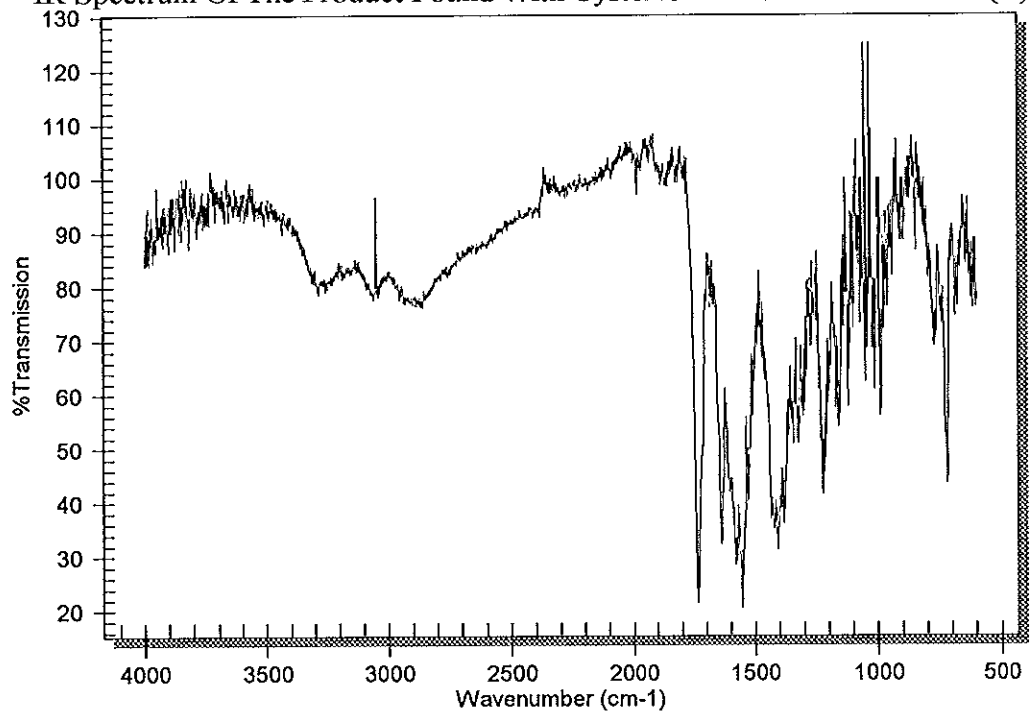

IR Spectrum Of Ruhemann`s Purple



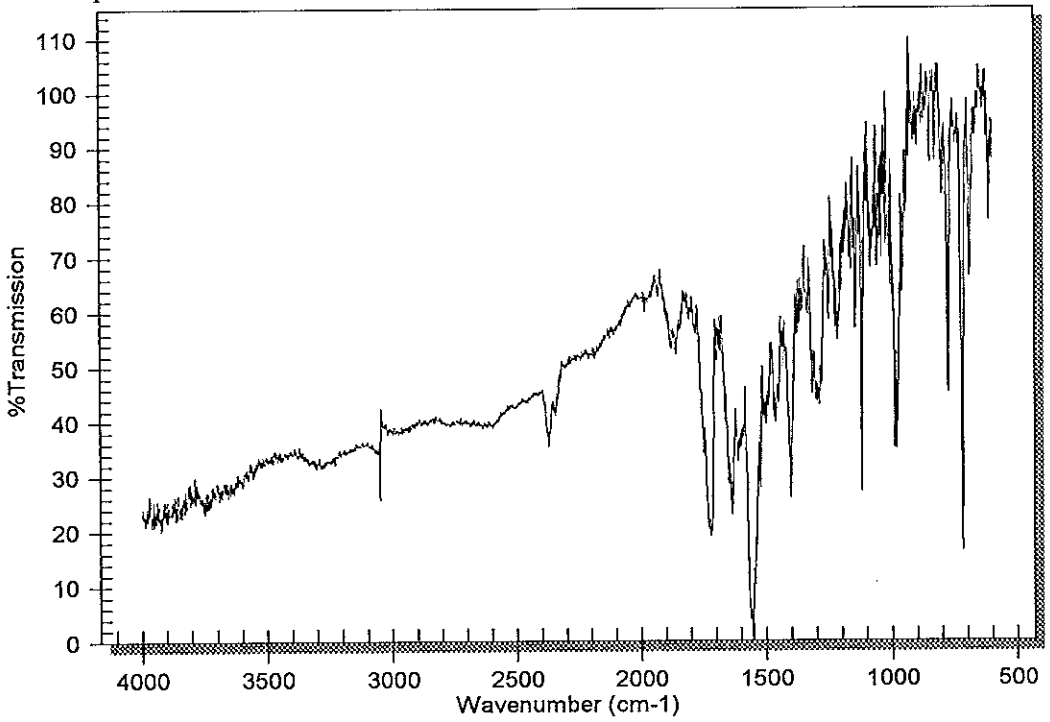
IR Spectrum Of The Product Found With Cysteine In The Presence Of Ni (II)



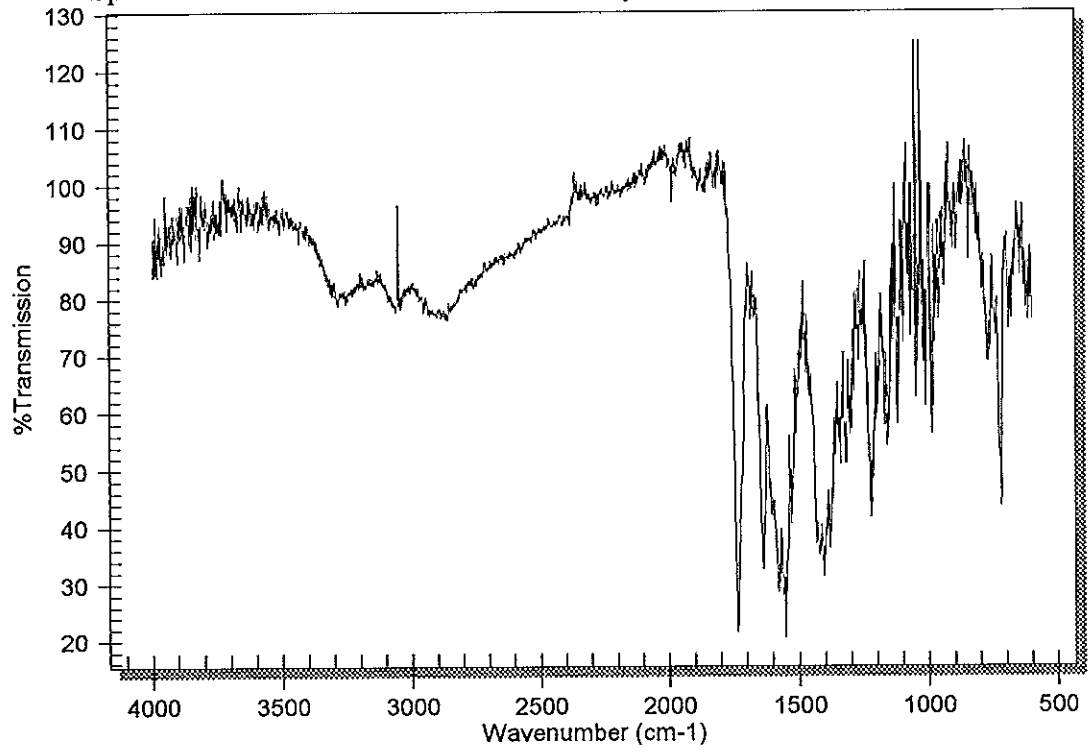

IR Spectrum Of The Product Found With Cysteine In The Presence Of Zn (II)



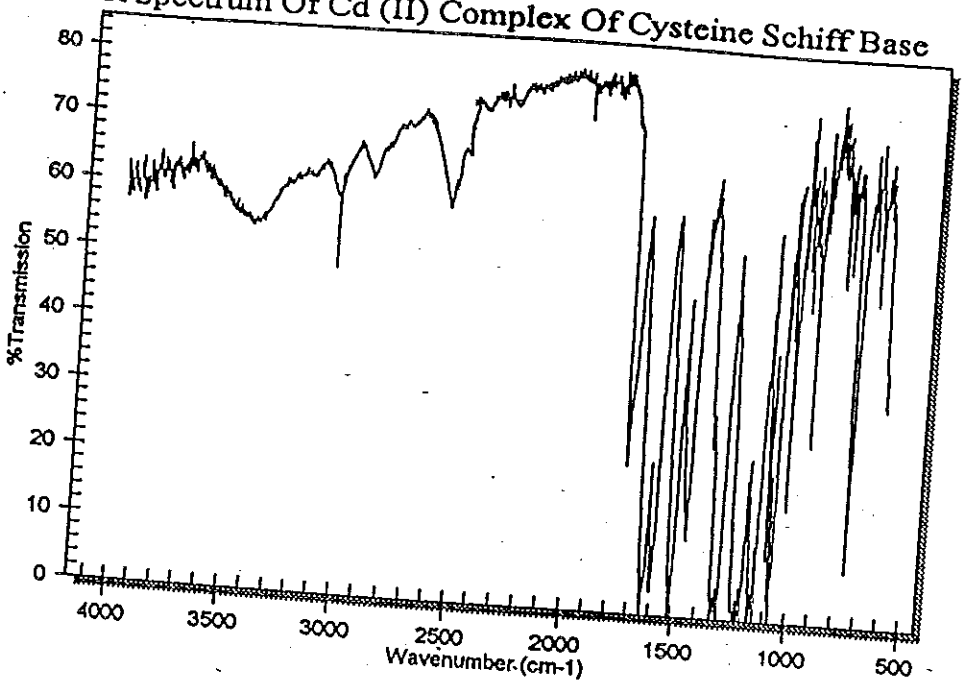
IR Spectrum Of The Product Found With Cysteine In The Presence Of Mn (II)



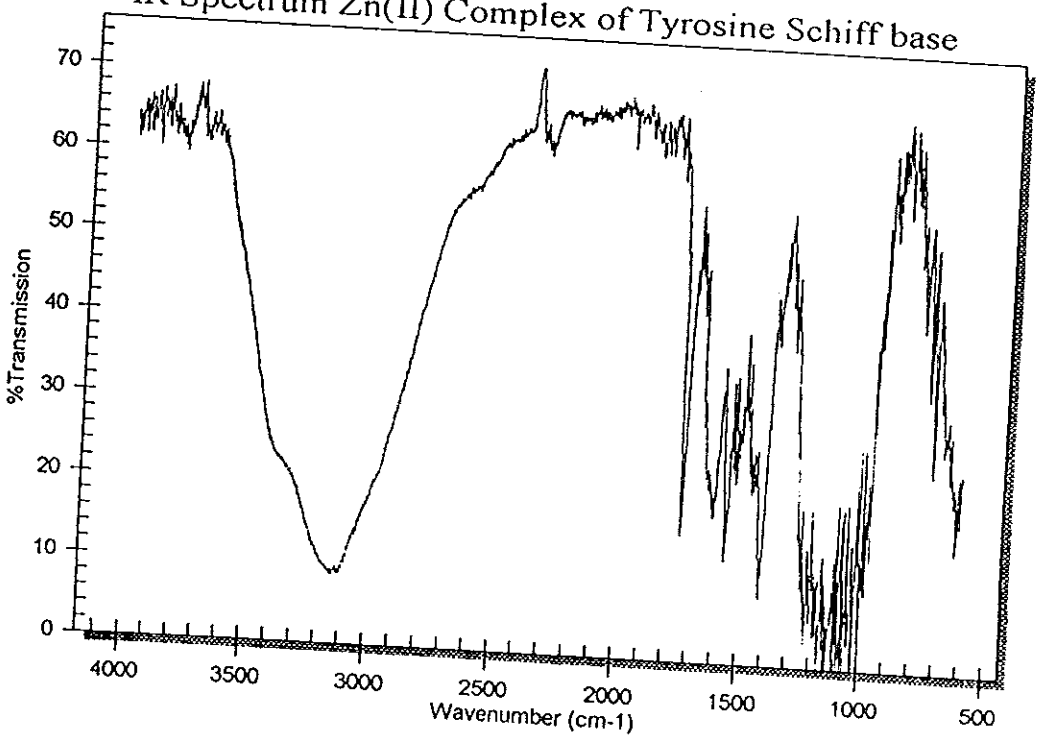

IR Spectrum Of The Product Found With Cysteine In The Presence Of Co (II)



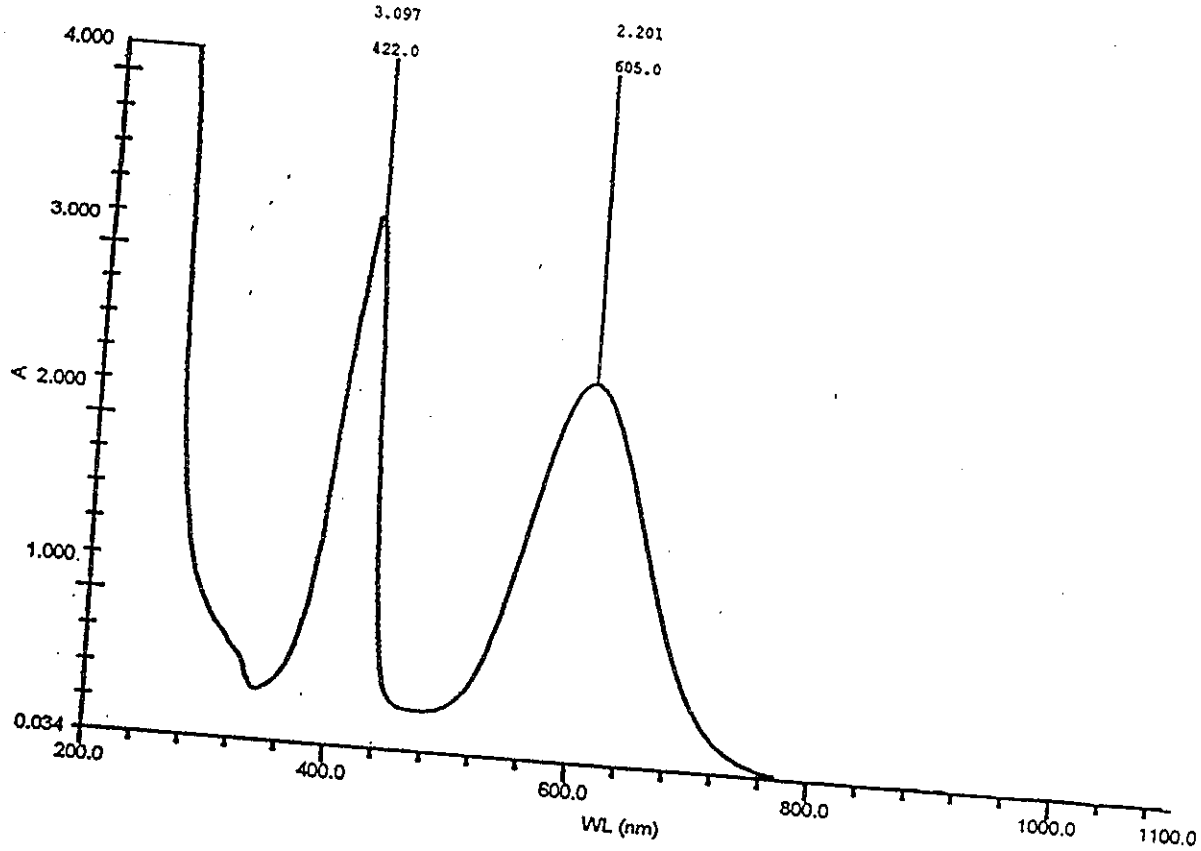
IR Spectrum Of Cd (II) Complex Of Cysteine Schiff Base



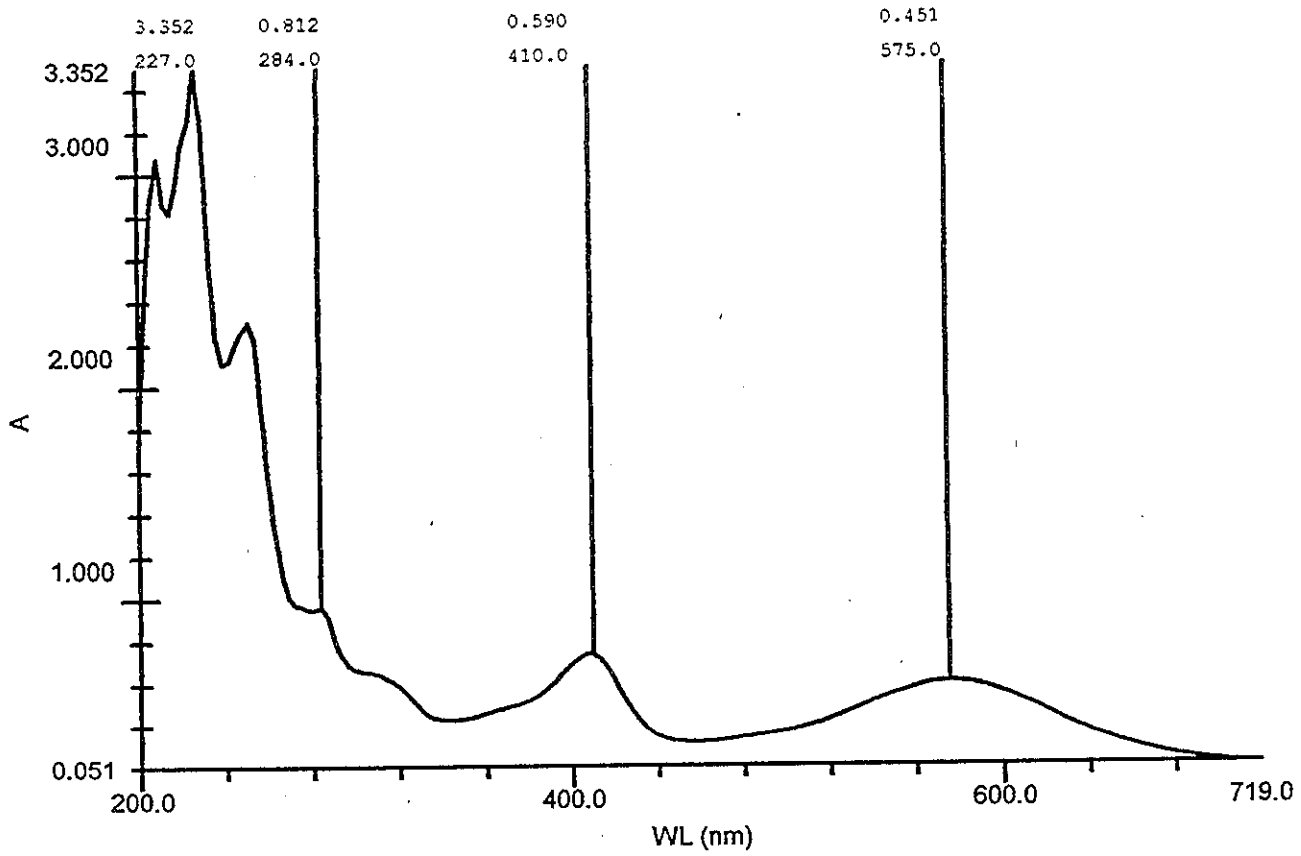

IR Spectrum Zn(II) Complex of Tyrosine Schiff base



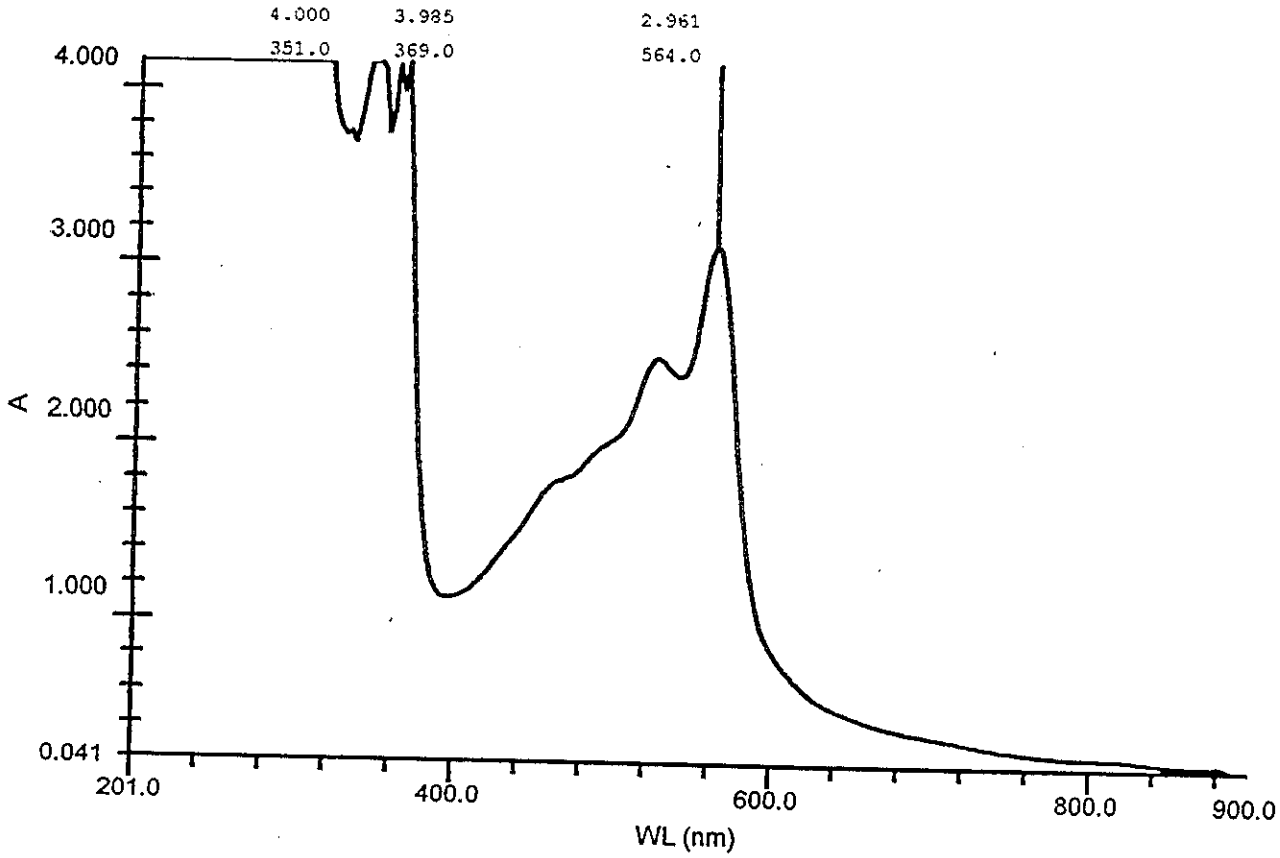
UV Spectrum Of Co(III) Complex Of IDIDP



IR Spectrum Of Ruhemann`s Purple



UV Spectrum Of The Product Isolated With Cysteine In The Presence Of Co(II)



UV Spectrum Of Cd(II) Complex Of Cysteine Schiff Base