ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

SYNTHESIS AND CHARACTERIZATION OF SOME METAL COMPLEXES OF SCHIFF BASES DERIVED FROM NINHYDRIN AND EACH OF a, L- SERINE AND a, L- ALANINE

MEHABAW GETAHUN DEREBE

JUNE 2001

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MEHABAW

By

Mehabavv £etahun Derebe

Department of Chemistry

Science Faculty

Approved by the Examining Board:

Prof. Neeussie Retta _____
Advisor

Dr. Yilma Guitneh _____
Examiner

Dr. Negussie Meeersa _____
Examiner

Dr. Yonas Chebude _____
Examiner

To:

– *My mother Demekech Dametie and my brother Abere Getahun, who left so untimely*

&

– *Asegedech, Yoni and Natl*

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Table of Contents

List of Tables

List of Figures

List of Abbreviations and Symbols

DMSO – Dimethyl sulfoxide

DMF – Dimethyl formamide

mM – Millimolar

M – Molar

M. pt. – Melting point

Dec. temp. – Decomposition temperature

IR – Infra Red

UV-Vis – Ultraviolet-Visible

NMR – Nuclear Magnetic Resonance

nm – Nanometer

cm – Centimeter

λ_{max} – Wavelength of maximum absorbance

ε_{max} – Molar absorptivity at λ_{max}

Λ_m – Molar conductance

Ct - Ohm

v - Stretching vibration

8 - Bending or deformation vibration

IDIHP – Indane-1,3-dione-2-imine-N-2-(3-hydroxy)-propionate, Schiff base derived from serine and ninhydrin

IDIP – Indane-1,3-dione-2-imine-N-2-propionate, Schiff base derived from alanine and ninhydrin

ABSTRACT

SYNTHESIS AND CHARACTERIZATION OF SOME METAL COMPLEXES OF SCHIFF BASES DERIVED FROM NINHYDRIN AND EACH OF a, L- SERINE AND a, L- ALANINE

BY

MEHABAW GETAHUN DEREBE

ADVISOR: Prof. NEGUSSIE RETTA

Complexes of Mn(II), Fe(III), Co(III), Co(II), Ni(II) and Zn(II) with two different intermediate Sohiff bases, one derived from ninhydrin and serine (Indane-1,3-dione-2-imine-N-2-(3-hydroxy) propionate, IDIHP) and another derived from ninhydrin and alanine (Indane-1,3-dione-2-imine-N-2-propionate, IDIP) were successfully synthesized. All complexes were distinctly colored and were characterized by elemental analysis, molar conductance, infrared and electronic spectral studies. The ligand (Schiff base) was shown to behave as a monobasic tridentate ONO donor. The Mn(II), Fe(III) complexes of both Schiff bases and Co(II) complex of IDIHP were found to contain only one ligand molecule plus water and chloride(s) per metal ion, while all the others contain two ligand molecules per metal ion. An octahedral geometry is proposed for the metal complexes.

In light of the anti-microbial activity of ninhydrin, comparative anti-microbial study of the complexes was undertaken against two gram-negative bacteria Escherichia coli and Proteus mirabilis and one gram- positive bacteria Staphylococcus aureus. It was revealed that most of the complexes of both Schiff bases and particularly Mn(Il), Fe(III) and Co(II) complexes, have a much enhanced activity than ninhydrin against P. mirabilis and S. aureus. All complexes except Co(II) complex of IDIHP showed a reduced activity against E. Coli.

INTRODUCTION

Identification and quantification of constituent amino acids in a mixture is required in the biochemical investigations of proteins and peptides. The most extensively used method is the ninhydrin reaction, in which ninhydrin (1,2,3-triketohydrindene hydrate or 2,2-dihydroxy-1,3-indandione **I** & **II – Scheme 1)** reacts with amino acids to give a characteristically blue/purple colored compound, except some that give yellow, popularly known as Ruhemann's Purple **VIII** [1, 2]. It was by chance that Sigfried Ruhemann first observed this color reaction in 1911 [3, 4]. The blue compound **VIII** maximally absorbs at 570 mil [1, 5, 6], and this forms the basis for the spectrophotometric quantitative determination of amino acids that can detect as little as one microgram quantity [1, 7]. Several workers introduced modifications to the method [1], the most noticeable ones being those by Moore, Stein et al. [8 - 12] and Doi et al. [13]. The effect of metals and various other substances on this color reaction has also been studied by several workers [14 – 19].

The chemistry of the reaction of ninhydrin with amino acids is extensively studied by several workers [6, 20, 21]. The mechanism, however, was not well understood until very recently and this gave rise to a series of theories as reviewed by McCaldin [20]. A simplified form of the mechanism proposed by Filippovich and McCaldin is shown in Scheme 1 [20 – 22]. It has a condensation step that leads to a Schiff base formation followed by decarboxylation, hydrolysis and finally further condensation with another ninhydrin molecule to give the final product, Ruhemann's purple [20 – 22]. The kinetic aspects are well studied by Friedman and Sigel [5].

Schiff bases are good ligands to metal ions [23, 24]. The ketimine IV is, therefore, a potential ligand to metal ions, that can act as a tridentate ligand forming two stable five membered rings on complexation. If the side group of the amino acid is not considered for the present discussion, the Schiff base can have metal binding sites as shown in Fig. 1.

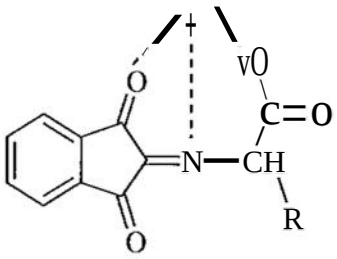
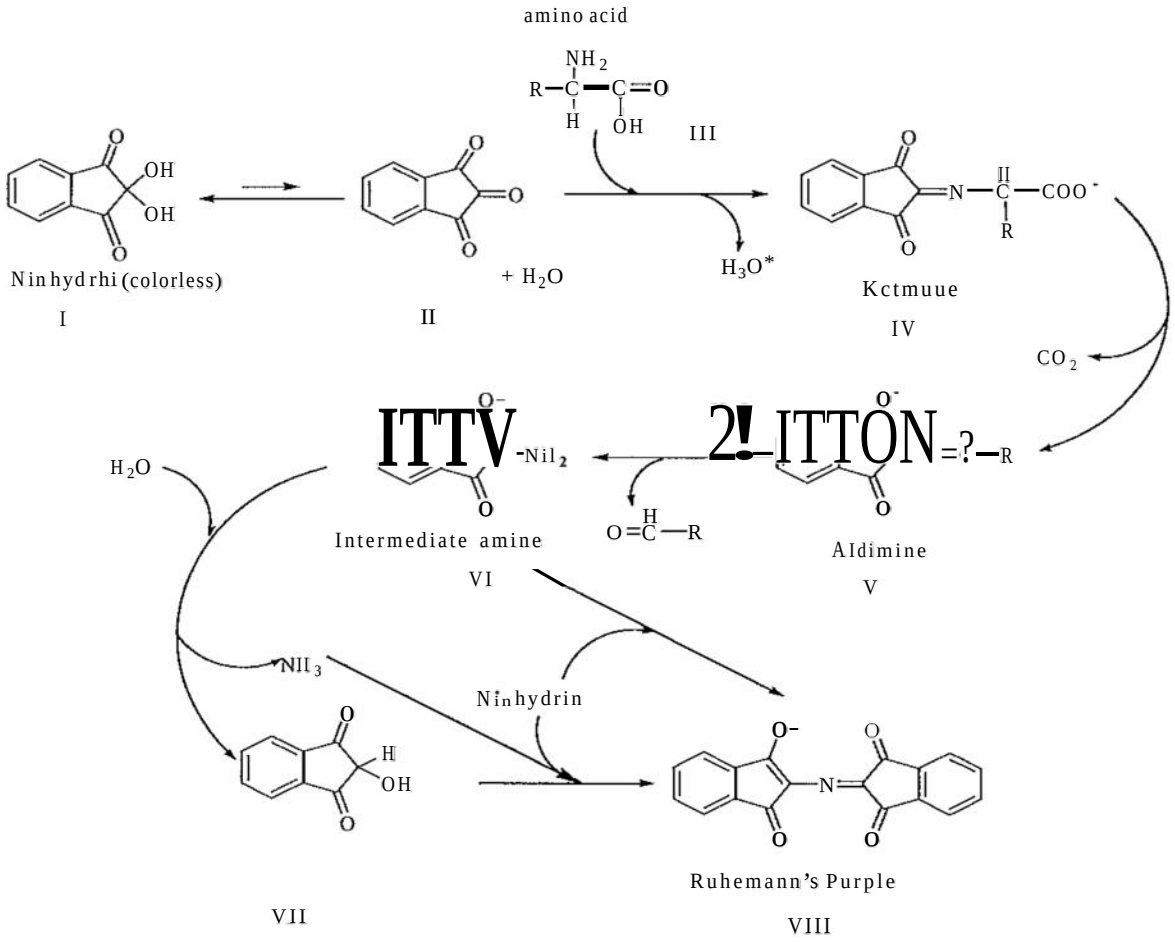


Fig. 1 Possible metal binding centers of a Schiff base derived from ninhydrin and an a-amino acid.

In support of this, a fairly recent study on the coordination of the Schiff base derived from ninhydrin and glycine to some metal ions showed that if a metal ion is present before the reaction commences, the reaction does not proceed to the final product, but stops at the first step, the metal ion forming a highly stable complex with the Schiff base [25]. The metal complexes were characteristically colored, 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-color can thus be developed based on the results and this can form a basis for amino acid identification and determination.

Ninhydrin has been shown to exhibit strong antibacterial, antiviral, etc activities [26]. It has also been shown that physiological activity of organic compounds is much enhanced

on coordination to metals [25, 27], particularly, Schiff bases and their metal complexes have been shown to exhibit enhanced activities [25].



Scheme 1. The reaction of ninhydrin with a-amino acids [22].

The present investigation is, therefore, to synthesize metal complexes of Schiff bases derived from ninhydrin and serine (Indane-1,3-dione-2-imine-N-2-(3-hydroxy)-propionate, IDIHP) and alanine (Indane-1,3-dione-2-imine-N-2-propionate, IDIP). The complexes were characterized by analytical methods and anti-microbial studies against Escherichia coli, Proteus mirabilis and Staphylococcus aureus were also undertaken.

CHAPTER 1 THEORETICAL BACKGROUND

1.1 The Chemistry of Amino Acids [28]

1.1.1 General Structure, Properties and Classification [2, 28 – 30]

Amino acids are low molecular weight (~100 – 200) organic molecules and are monomers of peptides and proteins. Any amino acid is characterized by a unique side group, plus at least one amino and one carboxyl groups. As long as there is no specific requirement as to the chain length, position of the amino group, etc, many amino acids can, by principle, exist. However, until very recently about 173 amino acids have been identified to exist in nature [29], only 25 of which are found in proteins of mammalian tissues [28], of which only 20 are ubiquitous. These 20 are all a-amino acids (except proline and hydroxyproline that are a-imino acids), i.e., the amino group at an a- carbon to the carboxyl group. The greater proportion of these naturally occurring a-amino acids exist in the L- configuration. However, in recent years a constantly increasing number of observations have shown the widespread natural occurrence of amino acids in the D-configuration, chiefly in the metabolic products of lower organisms.

The a-amino acids have the following general structure (Fig. 2).

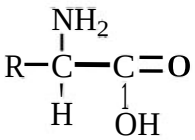


Fig. 2 General structure of a-amino acids

Amino acids may be classified based on the nature or polarity of the side group, the ability of the body to synthesize the amino acid as essential and nonessential, or any other suitable system. Based on the nature of the side group they can be classified as [2, 30] (i) amino acids with uncharged polar side group (e.g. glycine, serine); (ii) amino acids with non polar side group (e.g. alanine); (iii) amino acids with polar side groups with an additional carboxyl group (e.g. aspartic acid) and (iv) amino acids with polar side groups with additional ionizable nitrogen containing groups (e.g. histidine).

1.1.2 Physicochemical Aspects

As the name implies amino acids are at the same time substituted bases and substituted acids [28]. This capacity to act as amphoteric electrolytes conferred on them many remarkable electrochemical properties not shared by any other product of natural origin, and hence, are called ampholytes [2, 29]. In view of this and that acidic ionization is produced in alkaline solution and basic ionization in acid solutions, the following equilibrium was implicated, representing the amino acid as $NH_2RCHCOOH$.

$$^{+}NH_3RCHCOOH + OH^{-} \leftrightarrows NH_2RCHCOOH \leftrightarrows NH_2RCHCOO^{-} + H^{+}$$

The acid dissociation constant K_a is given by:

$$K_a = [NH_2RCHCOO^{-}][H^{+}]/[NH2RCHCOOH]$$

and the base dissociation constant given by:

$$K_b = [^{+}NH_3RCHCOOH][OH^{-}]/[NH2RCHCOOH]$$

$$= K_w[^{+}NH_3RCHCOOH]/[H^{+}][NH2RCHCOOH]$$

$\Rightarrow$ $K_w/K_b = K_b' = [H^+][NH2RCHCOOH]/ [^+NH_3RCHCOOH]$

But, K_b' was found to be several million times as great as K_a [28, 31] and K_b' is the measure of the ionization of the carboxyl group, implying that the amino acid will be almost exclusively the 'inner salt', $^+NH_3RCHCOO^-$, with less than one parts per million of the true amino acid, $NH_2RCHCOOH$. The equilibria involved in aqueous solvent must then be the following:

$$^+NH_3RCHCOOH \leftarrow {}^+NH_3RCHCOO^- \rightarrow NH_2RCHCOO^- + H^+$$

The amino acid is then said to exist in the dipolar form, called zwitterion, an internally neutralized ion. This dipolar form has been verified by various experiments [28]. The following conclusions can be drawn based on the dipolar form of the amino acids: (i) an increase in the solubility in water of other electrolytes, including other amino acids, (ii) a decrease in solubility in media of low dielectric constant such as alcohols and (iii) a high melting point when in the solid state. All these consequences seemingly derive from the strong intramolecular electrical forces characteristic of the dipolar ion form. This 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 no electrophoretic mobility. This point is called the 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 in solution with cations and anions other than H^+ and OH^-. In view of these, another point, called the isoionic point, was defined as the condition in which the ampholyte does not combine with ions other than H^+ and OH^-.

1.1.3 Chemical Reactions of Amino Acids

Because of the bifunctional nature of the amino acids, reactions as both acids and bases are expected. The reaction products of amino acids are in most cases colored or fluorescent compounds, which can be used in qualitative and quantitative analysis [2]. Some of the reactions which are analytically useful as tests for amino acids include [1]: the ninhydrin test, the ninhydrin and ninhydrin-collidine test, the Isatin-zinc acetate-pyridine test, the Pauly reagent for tyrosine and histidine, the flourescamine test [7], the dansyl chloride test, etc. An important group of reaction is the condensation of the amino group with many carbonyl-bearing compounds. Condensation reactions of this type produce ketimine or aldimine Schiff bases depending on the type of the carbonyl compound. These Schiff bases have been very important as ligands to metals and due to their biochemical importance [23].

The Ninhydrin Reaction

Identification and quantitative determination of amino acids in the past, including the more automated amino acid analyzers used in recent years, has been mainly based on methods with ninhydrin. The usual procedure of analyzing proteins is to hydrolyze with acid, separate the amino acids by chromatography or electrophoresis and identify the individual amino acids using a locating reagent, which in most cases is ninhydrin. Ninhydrin (1,2,3-triketohydrindene) oxidatively decarboxylates α-amino acids to CO_2, NH_3 and an aldehyde with one less carbon atom than the parent amino acid, under fairly acidic conditions (pH 3-6) [2]. The reduced ninhydrin then reacts with the liberated ammonia and another ninhydrin to yield a blue complex (some yellow), called

Besides this, free amino acids at the pH where the species $NH_2RCHCOO^-$ is predominant are good ligands and form a stable five membered chelate ring, involving the amino and carboxylate groups, of the general structure shown below (Fig. 3) with a metal ion.

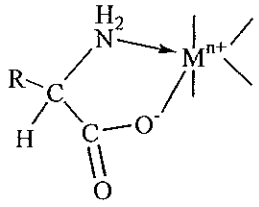


Fig. 3 General structure of a five membered chelate ring formed between a metal ion and α-amino acids.

Under other pH conditions this may not be the case; thus at lower pHs the amino acid may coordinate as a neutral ligand. When the carboxylate group is not part of such a chelate ring, then often four membered rings are formed in which both oxygen atoms are bound to the metal. Alternatively, the carboxyl group may bridge two metal centers. Such amino acid- metal chelates have not only been of theoretical significance but also of practical importance.

A very well known amino acid- metal complex is the copper complex of glycine, which has been characterized to have a square planar geometry with two glycine molecules per metal ion, each glycine as a bidentate ligand with the structure shown (Fig. 4) [28].

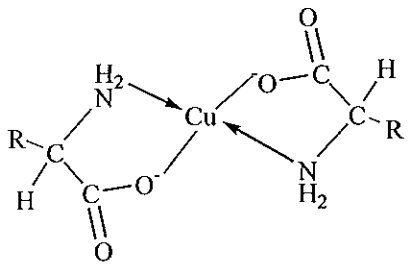


Fig. 4 Cu(II) – glycinate complex [28]

Studies on the stability and ease of formation of metal complexes [28] revealed that the stability diminishes as the side chain lengthens and as the distance between amino and the carboxyl groups increased. Thus with α-amino acids, highly stable five-memebered rings are formed, with β-amino acids six-memebered rings with diminished stability would be expected to form, whereas with γ-, δ- and ε- amino acids still higher memebered 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: $Hg^{2+}>Cu^{2+}>Ni^{2+}>Zn^{2+}> Co^{2+}>Cd^{2+}>Fe^{2+}>Mn^{2+}>Mg^{2+}> Fe^{3+}$ [28, 37, 38]. Stability was also found to be a function of pH [28, 39].

Schiff bases, named after Hugo Schiff who first reported them in 1864 [24], are usually formed by the condensation of a primary amine with an active carbonyl compound. They owe themselves to an extensive study as ligands to transition and non-transition metal ions due to synthetic flexibility of their formation, formation of stable metal complexes, possibility to synthesize wide variety differing in denticity, nature of donor atoms and in electronic properties, etc [23, 24]. Salicylaldehyde was the carbonyl precursor first used by Pfeiffer and Tsumaki [23, 24]. Such condensation products

(Schiff bases) of amino acids with ketones or aldehydes have also been interesting ligands for metals. Several metal complexes were synthesized and characterized with Schiff base formed from salicylaldehyde and amino acids or their corresponding hydrochloride salts [23, 28, 40]. Others involved ketones, like pyridoxal, and metal complexes of its condensation derivatives with amino acids have been successfully synthesized [28, 41] with a decarboxylation step. A racemization/tautomerism step is also involved in both types. These complexation reactions have been of special interest because they are related to some biochemical systems [28].

1.2 Chemistry of the Metal Ions [42, 43]

The coordination chemistry of the transition metal ions considered in the investigation will be discussed particularly from the stereochemistry point of view. The metal ions used are VO(II), Cr(II), 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

VO(II) 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±50 cm^{-1} [42, 43]. Coordination of a ligand to the sixth coordination position, i.e., the position trans to the V=O band brings a drop of ~50 cm^{-1} in the V=O stretching frequency of the parent complex.

VO(II) and Cu(II) ions resemble each other magnetically in having one unpaired electron. The ligand field picture of VO(II) complexes was first formulated by Ballhausen and Gray [44]. The electronic spectra for octahedral VO(II) complexes are characterized by three spin-allowed d-d transitions; $^2B_{2g} \rightarrow {}^2E_g$, $^2B_{2g} \rightarrow {}^2B_{1g}$ and $^2B_{2g} \rightarrow {}^2A_{1g}$ that are generally observed in the region 10000 - 15000 cm^{-1}, 15000 - 19000 cm^{-1} and 19000 - 26000 cm^{-1}, respectively [42 - 44].

Chromium (III) Complexes

Chromium (III), a d^3 system, forms numerous complexes and in most cases rivals Co(III) in its coordination chemistry since many analogous Co(III) and Cr(III) complexes are known [43]. Many Cr(III) complexes with polydentate ligands exist, most of which assuming an octahedral geometry. Octahedral Cr(III) complexes with $^4A_{2g}$ ground state are expected to have three spin-allowed transitions, $^4A_{2g} \rightarrow {}^4T_{2g}$, $^4A_{2g} \rightarrow {}^4T_{1g}$ (F) and $^4A_{2g} \rightarrow {}^4T_{1g}$ (P). These three transitions for $Cr(H_2O)_6^{3+}$ appear at 17400 cm^{-1}, 24500 cm^{-1} and 38000 cm^{-1}, respectively while for $Cr(NH_3)_6^{3+}$ appear at 15300 cm^{-1}, 21500 cm^{-1} and 28500 cm^{-1}, [44] respectively. In addition, spin-forbidden transitions $^4A_{2g} \rightarrow {}^2T_{2g}$ and $^4A_{2g} \rightarrow {}^2E_g$ are also sometimes observed [42 - 44].

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

Manganese (II) Complexes

This 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. The crystal field spectra of these complexes are therefore expected to be very weak. d-d transitions are both Laporte and spin forbidden. Thus, the d-d electronic spectra are of very low intensity and are of little use in determining metal stereochemistry. However, 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 $^6A_{1g} \rightarrow {}^4A_{1g}$ [42, 43].

There are relatively few low spin Mn(II) complexes and they are only usually found in such complexes as $Mn(CN)_6^{4-}$ and $Mn(CNR)_6^{2+}$ [43].

There are few four-coordinated (tetrahedral and square planar), five-, seven- and eight-coordinated complexes known.

Iron (III) Complexes

Iron(III), which is isoelectronic with Mn(II), has the spin free ion electronic configuration of d^5 in the ground state. Chelates of Fe(III) with many bidentate, tridentate, tetradentate and polydentate Schiff bases have been reported [42]. It mostly forms octahedral complexes and comparatively less number of tetrahedral and square planar complexes.

In high spin octahedral Fe(III) complexes, all electronic transitions are both spin and Laporte forbidden and hence, the ligand field bands in the spectra of these complexes are very weak. The spectra have not been adequately characterized and are frequently

not well resolved and complicated by the presence of charge transfer bands, which obscure the higher energy spin forbidden bands. Some of the transitions reported, though not certain, are $^6A_{1g} \rightarrow {}^4T_{1g}$ (G) (10700 – 14200 cm^{-1}), $^6A_{1g} \rightarrow {}^4T_{2g}$ (G) (12700 – 19700 cm^{-1}), $^6A_{1g} \rightarrow {}^4E_g$ (G) and $^6A_{1g} \rightarrow {}^4A_{1g}$ (13200 –25400 cm^{-1}), $^6A_{1g} \rightarrow {}^4T_{2g}$ (D) (14100 – 28800 cm^{-1}) and $^6A_{1g} \rightarrow {}^4E_g$ (D) (14100 – 30200 cm^{-1}) [42, 43].

Low spin octahedral complexes have been found with strong field ligands, like CN^-, Phenanthroline, bipyridine, etc.

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. Trigonal bipyramidal Fe(III) exhibits spin forbidden bands between 5000-25000 cm^{-1} and a charge transfer absorption between 27000-33000 cm^{-1} [42].

Cobalt (III) Complexes

Complexes of Co(III) played a very important role in the development of coordination chemistry. It was in the last decade of the nineteenth century that Alfred Werner set our present-day formulations and postulates of the cobaltammines, which laid the foundation for modern complexation theory and inorganic stereochemistry. Large numbers of Co(III) complexes are known, virtually all of these with the octahedral structure [43].

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 Co(III) complexes are low spin (t_{2g}^6 configuration) with the $^1A_{1g}$ ground state. For such low spin Co(III)

complexes two spin allowed transitions from $^1A_{1g} \rightarrow {}^1T_{1g}$ (15700 – 22000 cm^{-1}) and 1A1g $\rightarrow {}^1T_{2g}$ (22800 – 25800 cm^{-1}) are expected. Other two spin forbidden bands corresponding to $^1A_{1g} \rightarrow {}^3T_{1g}$ (8000 – 13700 cm^{-1}) and $^1A_{1g} \rightarrow {}^3T_{2g}$ (12500 – 17500 cm^{-1}) may also be observed but often not found [42 - 44].

Only with fluoride ligands are there high spin complexes with a $^5T_{2g}$ ground state.

Very few complexes of other geometries, like tetrahedral, trigonal bipyramidal, etc, have been observed.

Cobalt (II) Complexes

Most complexes of this ion are basically tetrahedral or octahedral. It forms more tetrahedral complexes than any other transition metal ion. The relatively low difference in crystal field stabilization energies between octahedral and tetrahedral Co(II) (d^7) complexes, compared to other d^n configurations, is at least partly responsible for this.

The electronic spectrum of high spin octahedral complexes usually shows three spin-allowed transitions corresponding to $^4T_{1g} \rightarrow {}^4T_{2g}$ (F) (6200 – 11300 cm^{-1}), $^4T_{1g} \rightarrow {}^4T_{2g}$ (P) (a multiple band near 20000 cm^{-1}) and $^4T_{1g} \rightarrow {}^4A_{2g}$ (P) (12000 – 16000 cm^{-1}), frequently not observed [42, 43].

Tetrahedral Co(II) complexes are high spin with the $^4A_2$ ground state and three spin – allowed transitions with a much greater intensities than octahedral case are expected corresponding to $^4A_2 \rightarrow {}^4T_1$ (F) (4600 – 7780 cm^{-1}), $^4A_2 \rightarrow {}^4T_1$ (P) (13250 – 16300 cm^{-1}) and $^4A_2 \rightarrow {}^4T_2$ [42].

region with a much greater intensity than the octahedral ones [42]. 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. In square planar Ni(II) complexes, 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. Majority of the square planar Ni(II) complexes exhibit strong absorptions in 15000-25000 cm^{-1} and 23000-30000 cm^{-1} regions [42].

The square planar Ni(II) complexes do not have any absorption band below 10000 cm^{-1}, due to large crystal field splitting. Hence, they can be clearly distinguished from octahedral and tetrahedral complexes [42].

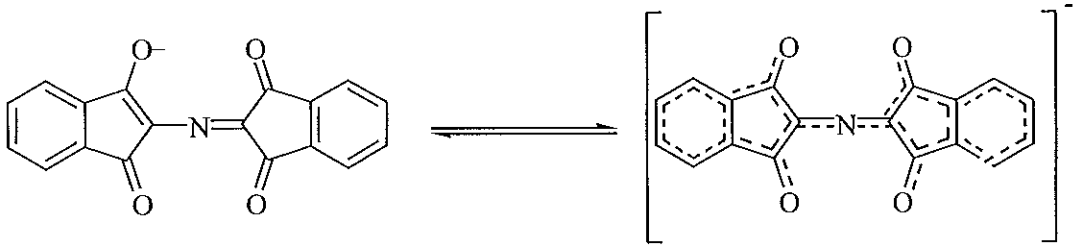
Copper (II) Complexes

Copper is one of the most extensively studied transition metal from the point of view of complex formation. The usual coordination numbers adopted by Cu(II) are four, five and most commonly, six [42, 43]. The Cu(II) ion with its d^9 configuration in octahedral and tetrahedral environment is highly susceptible to Jahn-Teller distortion, and hence, regular octahedral coordination is very rare.

Octahedral complexes without any distortion are expected to have only one d-d absorption band corresponding to $^2E_g \rightarrow {}^2T_{2g}$ transition. For distorted octahedral complexes, several weak absorption bands are observed around 1600 cm^{-1} and often a broad band in the near IR region. In the axially elongated tetragonal distortion three

CHAPTER 2 LITERATURE SURVEY

It was in 1911 [4, 45] that Sigfried Ruhemann discovered the ninhydrin reaction by chance, in an attempt to study the enolization of cyclic dicarbonyl compounds [4]. He first detected its reaction with ammonia and extended to α-amino acids, peptides and proteins [45]. Since then, he studied several systems and reported lots of results [3, 45–48] that come now known to be ninhydrin chemistry, even though the word ninhydrin was not coined by Ruhemann but later in 1913 by Abderhalden and Schmidt [49, 50]. S. Ruhemann proposed structure **VII** [47] for the purple colored reaction product of ninhydrin with α-amino acids, ammonia or related primary amine compounds, which was later modified to **IX**.



VII **IX**

Fig. 5 Structure of Ruhemann's purple

Several investigators followed Ruhemann to develop suitable analytical methods that can be used to analyze amino acids and proteins qualitatively and quantitatively. Most credit goes to S. Moore and W. H. Stein, who developed a colorimetric method of estimation of amino acids with a series of improvements [8 – 12]. These improvements

are usually to increase the color yield, render the product more stable so that it stays longer, prevent the offensive odor during analysis, etc.

C. J. Lennard, et al. [33] studied the application of the ninhydrin reaction in the analysis of fingerprints. Its application in the quantitative determination of nitrogen of a sample was studied by H. Riffart [32]. Both of these analyses are actually based on the presence of primary nitrogen similar to α-amino acids.

The effect of metals and other substances on the ninhydrin reaction has also been studied, usually post reaction studies. J. V. Singh, et al [17] found that Mn^{2+}, Fe^{2+} and Mo^{2+}, if within a specified concentration limit (5 – 10 μg/2mL), increase the intensity of the color developed while Cd^{2+}, Zn^{2+} and Al^{3+} depress the color formation.

Hanan Meyer and Emanuel Riklis [18] found that very small amounts of the cations Al^{3+}, Hg^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, Sn^{2+}, Ag^{+} and Cu^{2+} entirely abolished color formation while Ca^{2+}, Ba^{2+} and Cd^{2+} caused formation of red colors.

Krysciak Jan [16] has shown that Al^{3+}, Hg^{2+} and Cu^{2+} inhibited color reaction while the yield of the color produced decrease in the presence of Co^{2+}, Ni^{2+}, Cr^{3+}, Fe^{2+} and Ti^{3+}. Non-typical colors are produced in the presence of Cd^{2+} and Zn^{2+} and precipitate is formed in the presence of Co^{2+}, Ni^{2+}, Fe^{2+}, Fe^{3+}, Ti^{3+}, Al^{3+}, Hg^{2+} and Cu^{2+}.

A. D'Aniello et al. [19] on studying the effect of salts, acids, alkali and buffer on the color development of the Cd-ninhydrin-amino acid reaction revealed that of many salt solutions studied only Cu^{2+} and Fe^{3+} interfered with the color yield.

K. V. Giri, et al. [51] developed a method of quantitative determination of amino acids separated by paper chromatography based on ninhydrin-Cupric nitrate (NCN). A similar result was also found by E. D. Moffat and R. I. Lytle [52] and also by A. L. Levy and D. Chung [53].

Mechanistic studies on the reaction were done by several workers. Filippovich, et al. [21] suggested a five step mechanism involving condensation that leads to Schiff base formation, reamination, decarboxylation, self-forming (ninhydrinolysis) and a protonation step to give the final purple product, Ruhemann's purple. They proposed that the first step is due to the high mobility of the carbonyl oxygen of the middle oxo group. Several other workers suggested other mechanisms, which gave rise to a series of theories, as very well reviewed by McCaldin [20].

Friedman and Sigel [5] studied the kinetics of the reaction at 100°C and 30°C and reported that both polar and steric factors influence rates of the ninhydrin reaction. They also gave polar and steric substituent constants for the rates of the reaction.

In an attempt to conserve amino acid chromatograms, Kawerau and Wieland [54] studied the complexation of the blue pigment, Ruhemann's purple, with several metal ions. They revealed that complexation with a metal ion renders a great deal of stability. The metal complexes in most metal ions were found to have a reddish coloration. They suggested this red complex salt to be formed from two molecules of the blue pigment with one atom of the metal being held between the two nitrogen atoms.

L. J. Nunez and G. L. Eichhorn studied the rate of formation of Ni(II) and Cu(II) complexes of salicylaldehyde-glycine Schiff base by varying the order of mixing of the

reactants [40]. The effect of a methyl group was also studied by taking sarcosine instead of glycine. It was found that the order in which the reactants are mixed determine the nature of participation of the metal ions in Schiff base formation.

Similarly, in accordance with the proposal that the Schiff base (the ketimine) derived from ninhydrin and α-amino acids [Scheme 1] is a good chelating system, Rao and Reddy [25] synthesized and characterized Co(II), Ni(II) and Zn(II) complexes of a Schiff base derived from ninhydrin and glycine (Indane-1,3-dione-2-imine-N-acetate, IDIA). It was found that the metal complexes were characteristically colored, non-electrolytes and decompose at high temperatures. The Schiff base ligand, IDIA, was speculated to behave as a monobasic tridentate ONO donor. They suggested an octahedral geometry for the complexes (Fig. 6) and proposed a mechanism for its formation (Scheme 2).

They also performed comparative anti-microbial study of the complexes against four bacteria (Escherichia coli, Proteus mirabilis, Streptococcus faecalis and Staphylococcus aureus). The complexes were found to be inactive. Tiffany, et al. [25, 26] reported that ninhydrin exhibits strong bacteriostatic, virucidal, fungistatic, antigen-antibody reactive, inhibitive and diabetogenic properties. Chrisey, et al. [25, 27] has shown that physiological activity of organic compounds undergoes a significant enhancement on addition of metals. Particularly, Schiff bases and their metal complexes have attracted a great deal of attention in biochemistry due to their vast applications as anticancer, antitubercular, anticonvulsant, insecticidal, antibacterial, etc agents.

CHAPTER 3 EXPERIMENTAL

3.1 Chemicals and Materials

Most chemicals used in the investigation were of AnalaR grade. Metal salts used were $CoCl_2.6H_2O$ and $CrCl_3.6H_2O$ (Riedel-de Haën), $Ni(NO_3)_2.6H_2O$, $NiCl_2.6H_2O$, $FeCl_3$, $VOSO_4$, $ZnCl_2$ and $MnCl_2$ (BDH), $CuCl_2.2H_2O$ (Merck), and $[Co(NH_3)_5Cl]Cl_2$ (synthesized in the laboratory). Other chemicals used include ninhydrin (Pharmacos), serine, alanine (Aldrich), formic acid, HNO_3, $HClO_4$, HCl, sodium and solvents like methanol, ethanol, DMSO, DMF, acetonitrile, ethyl acetate, cyclohexane, petroleum ether, acetone, chloroform, benzene and distilled water. Ethanol (Fluka) was used as a solvent throughout the investigation. 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 were done in the range of 200-1100 nm using SPECTRONIC GENESYS 2PC with a 1cm cell at a concentration of $1.5\times10^{-3} - 10^{-4}$ M in DMSO and chloroform at room temperature.

Melting or decomposition temperatures were determined with 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.

Electrical conductivities of the complexes were studied at room temperature with freshly prepared 1mM solutions in DMSO using a Jenway 4330 Digital Conductivity and pH meter and a Philip Harris Conductivity meter. The cell constants were first determined using KCl solutions of known concentrations and conductivities. The conductivity of the solvent was also measured.

The complexes were analyzed for metal using a Varian SpectrAA–20 Plus Atomic Absorption Spectrometer at the Ethiopian Geological Survey Laboratory. Chloride from some samples was determined as AgCl by the sodium fusion method using standard methods [55, 56].

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.

The purity of the complexes was tested by thin layer chromatography.

3.3 Synthesis

3.3.1 Synthesis of Metal Complexes of Schiff Base Derived from Ninhydrin and Serine (Indane-1,3-Dione-2-Imine-N-2-(3-Hydroxy)-Propionate, IDIHP)

The complexes of Mn(II), Fe(III), Co(III), Co(II), Ni(II) and Zn(II) were synthesized using a general procedure. The ligand (Schiff base) was prepared in-situ, i.e., within the reaction mixture in the presence of the metal ion. 0.01 mole (1.78g) of ninhydrin was dissolved in the minimum possible amount of ethanol (~150 mL) and 0.01 mole of a

metal salt was added to the resulting solution, which was shaken and/or heated until all of it dissolved. This mixture was refluxed for about half an hour, followed by addition of 0.01 mole (1.05g) serine to the hot mixture and refluxed for further two hours. The resulting colored precipitate was then filtered off in hot conditions through a Whatman #50 filter paper by suction, washed with enough distilled water, ethanol and finally with a 50:50 (v/v) 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 Alanine and Ninhydrin (Indane-1,3-Dione-2-Imine-N-2-Propionate, IDIP)

Exactly the same procedure as in 3.3.1 above was followed to synthesize metal complexes of the Schiff base derived from ninhydrin and alanine, the only changes made being the use of $NiCl_2.6H_2O$ instead of $Ni(NO_3)_2.6H_2O$ and 0.01 mole (0.89g) alanine instead of serine.

3.4 Studies on the Coordination Property of Ruhemann's Purple

In order that no confusion occurs, the coordination of Ruhemann's purple with the metal ions Co(II), Zn(II) and Cu(II) was studied. For this purpose, a 1:2 mixture of serine and ninhydrin (0.01 and 0.02 moles) were dissolved in the minimum possible amount of ethanol and left overnight. A metal salt (0.01 mole) was then added and refluxed for more than two hours. The resulting solid was filtered, washed, purified, dried and characterized as for the metal complexes of the Schiff bases.

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

3.5 Anti-microbial Studies

These were done by the 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. 10 μL (250 mg) and 20 μL (500 mg) of each sample, controls and solvent were absorbed on paper discs (Antibiotic – Assay Disc, 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

Ninhydrin and serine (or alanine) form a deep purple colored compound known as Ruhemann's purple, which maximally absorbs at 570 nm and 416 nm within 20 minutes of mixing [5]. A preliminary survey with several different mixtures of metal ions, ninhydrin and amino acid showed that in the presence of some metal ions Ruhemann's purple is not formed, even after standing for several days. This was revealed by color comparison and spectroscopic studies (Table I). A follow up experiment for the formation of Ruhemann's purple was also done for about four hours at an interval of five minutes and it has shown that its characteristic band (570 nm) appeared after some 15 minutes and the Schiff base did not show any new band.

Several preliminary experiments were done for a single metal ion, 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 a published work in a similar study [25] and by being common solvents for all the reactants. Molar ratios of metal ion: amino acid: ninhydrin tried 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. Ethanol and 1:1:1 ratio of ninhydrin, metal ion and amino acid were found to be suitable solvent and molar ratio respectively for optimum yield. These were then adopted throughout the synthetic work.

Table I. Colors developed right after mixing and upon standing for different mixtures (serine: metal ion: ninhydrin: 1:1:1) and their absorption maxima

Metal ion	*Color right after mixing*	*Color after standing for several days*	λ_{max} *(nm) (Solvent - C_2H_5OH)*
Cr(III)	Light green	Light purplish green	573
Mn(II)	Colorless	Deep blue	387, 612
Fe(III)	Yellow	Yellow	—
Co(II)	Very faint blue	Blue	510
Ni(II)	Colorless	Reddish pink	483
Cu(II)	Light blue	Light blue	—
Zn(II)	Colorless	Reddish pink	486

The complexes obtained in good yields (50 – 62%) from the reactions of Mn(II), Fe(III), Co(III), Co(II), Ni(II) and Zn(II) with both Schiff bases were distinctly colored, stable to air and sunlight (Tables II and III). They decompose at high temperatures (>330^{0}C) and are highly soluble in solvents of high dielectric strength, DMSO and DMF, but only partially soluble in methanol and chloroform.

The same procedure was followed to synthesize the complexes of VO(II), Cr(III) and Cu(II) with the Schiff bases but found Ruhemann's purple as the end product. This was revealed by physical property (color, appearance and melting point), molar conductance, analytical and spectroscopic analyses. The respective metal salts were also recovered by concentrating from the filtrate and after usual work-up.

Room temperature experiments to synthesize Co(II), Ni(II), Cu(II) and Zn(II) complexes of serine's Schiff base (IDIHP) gave products that are exactly the same as the corresponding products in the synthetic procedure with refluxing. That is, both have exactly the same color, very nearly equal melting/decomposition temperatures, identical IR and electronic spectra. However, the products in the cold had a much smaller yield (<35%) than with refluxing (>50%) and need an extended time to isolate the solid products, usually not less than three days. This shows the necessity of refluxing to facilitate the reaction, and is in agreement with literature [5, 9, 57, 58].

Ruhemann's purple synthesized by mixing amino acid and ninhydrin coordinate with Co(II) and Zn(II). Their comparison with the Schiff base complexes derived from serine is given in Table IV. These data agree with literature data [54], and show that the metal complexes of Co(II), Zn(II) and other cations of the group synthesized using the general procedure are not that of Ruhemann's purple. However, for Cu(II) and its group mates (VO(II) and Cr(III)) the products isolated with Ruhemann's purple and the general procedure do not show any difference. Products with Cu(II) following both procedures are very alike. This may lead to the conclusion that Cu(II) forms a complex neither with Ruhemann's purple nor the Schiff bases, and the isolated products must have been Ruhemann's purple. This observation is consistent with the report by Kawerau and Wieland [54] that the copper – Ruhemann's purple complex is a very unstable salt in aqueous or ethanol solutions.

Table II Physical properties of the metal complexes of IDIHP and other related products

Complex		*Color and Appearance*	*Yield (%)*	*Melting/decomposition temp (°C)*
Mn(II)		Dark red, Crystalline, Shiny	56.3	>330
Fe(III)		Brown, Crystalline	54.4	>330
Co(III)		Bluish brown, Powder	52.2	>340
Co(II)		Red, Crystalline, Shiny	55.2	>330
Ni(II)		Bright green, Powder	61.02	>350
Zn(II)		Dark brown, Powder	58.13	>330
Solids isolated with	VO(II)	Red brown, Fluffy, Crystalline	4.3	~280
	Cr(III)	Red brown, Fluffy, Crystalline	15.3	275 - 280
	Cu(II)	Brown, Fluffy, Crystalline	19.5	270 - 275
Ruhemann's purple		Red brown, Fluffy, Crystalline	—	~270

Table III Physical properties of the metal complexes of IDIP and other related products

Complex		*Color and Appearance*	*Yield (%)*	*Melting/decomposition temp (°C)*
Mn(II)		Bright red, Crystalline, Shiny	52.5	>350
Fe(III)		Light pink, Crystalline, Shiny	53.7	>350
Co(III)		Dark brown, Powder	51.7	>350
Co(II)		Bright green, Crystalline, Shiny	51.64	>350
Ni(II)		Greenish brown, Powder	58.3	>360
Zn(II)		Dark brown, Shiny, Powder	60.3	>340
Solids isolated with	VO(II)	Red brown, Fluffy, Crystalline	21.6	275 – 280
	Cr(III)	Light brown, Fluffy, Crystalline	22.0	~ 280
	Cu(II)	Red brown, Fluffy, Crystalline	19.5	~ 270
Ruhemann's purple		Red brown, Fluffy, Crystalline	—	~270

Table IV Data of products obtained with Ruhemann's purple and the general procedure (serine)

	Ruhemann's purple			*General procedure*		
	Co(II)	Zn(II)	Cu(II)	Co(II)	Zn(II)	Cu(II)
Color	Brownish Green	Red Yellow	Red Brown	Bright Red	Dark Brown	Red Brown
Appearance	Powder	Powder	Fluffy, crystalline	Crystalline	Powder	Fluffy, crystalline
M.pt/dec. temp.(°C)	>360	>360	~275	>330	>330	~275
Char. UV-Vis maxima λ_{max} (nm)	423, 582	408, 570	362, 488	492	485	362, 492

4.2 Analytical Studies

4.2.1 Elemental Analysis

The analytical data of the compounds are given in Tables V and VI. The data shows a metal to ligand ratio of 1:2 in Co(III), Ni(II), Zn(II) complexes of both Schiff bases and Co(II) complex of IDIP. Fe(III), Mn(II) complexes of both Schiff bases and Co(II) complex of IDIHP show a 1:1 metal to ligand ratio with additional water and chloride(s). The analytical data fits the formulation with two moles in the Fe(III) complexes and three moles in the Co(II) complex of IDIHP and Mn(II) complexes. The data is consistent with the proposed formulation. VO(II), Cr(III) and Cu(II) have not been detected (using AAS) in the products, 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 Conductance Studies

The molar conductance values of metal complexes of the Schiff bases IDIHP and IDIP and other related products are given in Tables V and VI.

The molar conductance (Λ_m) values were calculated from conductivity measurements of the solvent (DMSO), metal complex solutions in DMSO and determination of cell constant using the following relation.

$\Lambda_m\ (\Omega^{-1}cm^2mol^{-1}) = 1000\kappa\ /C$

Where: κ - the specific conductivity; $\kappa(\Omega^{-1}cm^{-1}) = (1/R - 1/R_{DMSO})b$

C - concentration in $molL^{-}$

b - the cell constant; b (cm^{-1}) = l/A; l-length and A-area

1/R - the measured conductivity

The data shows that Fe(III) and Mn(II) complexes of both Schiff bases and Co(II) complex of serine's Schiff base are 1:1 electrolytes while all the others are non electrolytes [59]. This also shows the presence of one counter ion, chloride, in the 1:1 electrolytes, which is also confirmed by chloride analysis. The other chloride determined in the Fe(III) complexes of both Schiff bases must then be in the inner coordination sphere.

The molar conductance values of the products obtained with VO(II), Cr(III) and Cu(II) are very small (in the range of 2 – 10 $\Omega^{-1}cm^2mol^{-1}$) and very nearly equal. This shows their non-electrolytic nature and in the absence of a metal as shown from metal analysis, this may suggest the products to be Ruhemann's purple.

Table V Analytical and conductance data for metal complexes of IDIHP and other related products[£]

Complex	*%Metal calc (found)*	*%Chloride calc (found)*	$\Lambda_m(\Omega^{-1} cm^2 mol^{-1})$ *DMSO*
Mn(II)	14.07 (14.40)	9.09 (8.92)	46.8
Fe(III)	14.29 (13.46)	17.73 (17.61)	45.1
Co(III)	10.70 (10.48)	_	29.6
Co(II)	14.94 (14.75)	9.72 (9.58)	50.1
Ni(II)	10.65 (9.95)	_	31.7
Zn(II)	11.73 (11.55)	_	30.1
Prod. with VO(II), Cr(III) & Cu(II)	>9(<0.5)	_	2 – 10

[£] In DMSO Λ_m between 0 – 40 → non-electrolytes
between 40 – 90 → 1:1 electrolytes [59]

Table VI Analytical and conductance data for metal complexes of IDIP and other related products[£]

Complex	*%Metal calc (found)*	*%Chloride calc (found)*	$\Lambda_m(\Omega^{-1} cm^2 mol^{-1})$ *DMSO*
Mn(II)	14.67 (14.08)	9.51 (9.70)	50.8
Fe(III)	14.90 (14.12)	18.07 (17.84)	50.1
Co(III)	11.34 (10.95)	6.40 (7.34)	31.4
Co(II)	11.34 (11.03)	_	30.1
Ni(II)	11.30 (10.70)	_	26.7
Zn(II)	12.49 (11.77)	_	30.1
Prod. with VO(II), Cr(III & Cu(II)	>9(<0.5)	_	2 – 10

[£] In DMSO Λ_m between 0 – 40 → non-electrolytes
between 40 – 90 → 1:1 electrolytes [59]

4.3 Infrared Spectral Studies

The Infrared Spectral data are given in Tables VII, VIII and IX. The data clearly show two groups of products in both amino acids: group I containing metal complexes obtained with Fe(III), Mn(II), Co(III), Co(II), Ni(II) and Zn(II) (Tables VII and VIII), while group II containing products obtained in the presence of VO(II), Cr(III) and Cu(II) (Table IX).

In both groups 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, indicating the derivatization of these groups. The nitrogen may have been converted into a tertiary nitrogen that does not show any peak in the region.

The first group of complexes of IDIHP show a broad strong band in the range 3700 – 2500 cm^{-1}. This is assigned to OH stretching vibration of the free, uncoordinated or unionized OH group of the derivatized serine, i.e., the Schiff base IDIHP, plus, in Fe(III), Mn(II) and Co(II) complexes, coordinated water. The Fe(III) and Mn(II) complexes of alanine's Schiff base IDIP also show this band assigned to OH stretching vibration of coordinated water, since the Schiff base derived from alanine has no any OH group. The presence of coordinated water in these complexes is supported by other water bands in the regions 1630 – 1600 cm^{-1} (HOH bending vibration) and 600 – 200 cm^{-1}. The second group does not show this band indicating that serine has been derivatized and they do not contain any coordinated water.

Ninhydrin shows three bands in the C=O stretching region; 1768 cm^{-1}, 1754 cm^{-1} and 1720 cm^{-1} [60]. The 1754 and 1720 bands are characteristic of its 1, 3- dicarbonyl

functional group [61] and the 1768 band is characteristic of the intermediate carbonyl in the triketo species, which is in equilibrium with the dihydroxy species. In the products there are only one or two carbonyl stretching peaks at decreased wave numbers, which shows the derivatization of the higher frequency (1768 cm^{-1}) carbonyl group of ninhydrin. The first group of complexes of both IDIHP and IDIP show two strong bands in the carbonyl-stretching region, which is characteristic of the 1, 3- dicarbonyl functionality. There is, however, a decrease of their positions (15 – 50 cm^{-1}), which indicates the involvement of one of the carbonyls in coordination [61]. The 1768 cm^{-1} C=O stretching band of ninhydrin is lost, and along with the loss of the NH stretching band of the amino acids shows the condensation reaction of these functional groups and this is consistent with the formation of the Schiff base. The second groups show carbonyl stretching in the range 1735 – 1738 cm^{-1}, which is very nearly the same to that of Ruhemann's purple (1738 cm^{-1}). On the one hand, there is no significant decrease, indicating the absence of coordination to metal ions of the group, and on the other hand, the 1768 cm^{-1} band of ninhydrin is lost showing its condensation.

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} [62 - 64]. In the first group of complexes of both Schiff bases, the anti symmetric stretching mode appears between 1665 – 1630 cm^{-1}, while the symmetric stretching mode appears in the range 1330 – 1302 cm^{-1}. The observed positive shift of the anti symmetric stretching peak and the negative shift of the symmetric stretching peak is a strong evidence of coordination through an ionized carboxyl group via one of the oxygens [25, 62]. The separation between the two frequencies varies with the metal ion involved and the pattern observed

with both Schiff bases is consistent with literature [25, 34, 62]. The second group, however, does not show these bands.

A new strong and sharp band is observed in the spectra of the products, which is absent in the starting materials, assignable to the azomethine (C=N) group. Azomethine group absorbs between 1640 – 1620 cm^{-1} in aromatic ketimines [24, 61, 63, 64]. In the first group of complexes of both Schiff bases the C=N group shows a very strong, sharp peak between 1520 – 1500 cm^{-1}. This negative shift, by as much 100 cm^{-1}, is a strong support for the coordination of the azomethine nitrogen [25]. The second group actually shows this band but at/near 1635 cm^{-1}, which clearly shows the absence of coordination of the azomethine nitrogen to metal ions.

The first group of complexes shows M-N and M-O bands in the region 750 – 200 cm^{-1}. M-Cl band is also observed for Fe(III) complexes of both Schiff bases near 250 cm^{-1}. Other supporting bands in the first group of complexes include M-O-C stretching vibration that appears between 1245 – 1200 cm^{-1} (s), CH bending vibration of CH_2 and CH_3 groups that occur in the range 1480 – 1350 cm^{-1}, aromatic C=C stretch near 1590 cm^{-1} and a strong band near 750 cm^{-1} showing ortho disubstitution.

In conclusion, the IR data is consistent with both amino acids and clearly showed that in the first group of complexes the Schiff bases (IDIHP and IDIP) behave as tridentate monobasic ONO donor ligands involving a carbonyl, azomethine nitrogen and carboxyl oxygen. In the second group, the spectra indicate that of Ruhemann's purple. This shows that VO(II), Cr(III) and Cu(II) do not form complexes with the Schiff bases (IDIHP and IDIP) or Ruhemann's purple and the latter is isolated as the final product.

Table VII Infrared spectral data of the metal complexes of IDIHP and starting materials* (KBr pellets)

Compound	*νOH*	*νC=O*	*$\nu_a COO^-$*	*νC=C (arom)*	*νC=N*	*δCH_2*	*$\nu_s COO^-$*	*νM-O-C*	*Other non ligand Bands ν(M-N, M-O,M-Cl,...)ρ,H_2O,*
Ninhydrin [60]	3200 – 2920(b,s)	1768(s), 1754(s), 1720(s)	—	1590(s)	—	—	—	—	—
Serine [65] **νN-H** 3500(s)	3300 – 2400(b,s)	—	1600(s)	—	—	1460(s)	1410(m)	—	—
Mn(II) Complex	3610 - 2700 (b,s)	1715(s), 1685(s)	1665(vs)	1595(s)	1515(vs)	1455(m)	1330(vs)	1215(vs)	700(m), 685(s), 640(s), 595(s), 400(m)
Fe(III) Complex	3660 – 3000(b,s)	1710(s), 1680(m)	1637(vs)	1585(m)	1503(vs)	1440(m)	1317(vs)	1220(vs)	700(w), 625(w), 600(w), 540(w), 250(w)
Co(III) Complex	3620 – 2500(b,s)	1730(m), 1690(s)	1642(vs)	1580(s)	1505(s)	1450(m)	1325(vs)	1223(vs)	748(m), 620(w), 530(s)
Co(II) Complex	3600 – 3000(b,s)	1719(m), 1688(m)	1651(vs)	1591(s)	1520(vs)	1455(m)	1330(vs)	1225(vs)	668(w), 603(w), 539(m), 370(w)
Ni(II) Complex	3700 – 3000(b,s)	1729(s), 1697(m)	1630(vs)	1590(s)	1512(vs)	1448(m)	1320(vs)	1210(vs)	665(w), 600(s), 550(s), 535(s)
Zn(II) Complex	3700 – 3000(b,s)	1723(m), 1705(m)	1632(vs)	1591(s)	1503(vs)	1445(s)	1319(vs)	1209(vs)	660(m), 600(s), 548(s), 527(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 [62], ν - designates stretching vibration, δ - bending or deformation

Table VIII Infrared spectral data of the metal complexes of IDIP and starting materials* (KBr pellets)

Compound	*νOH*	*νC=O*	*$\nu_a COO^-$*	*νC=C (arom)*	*νC=N*	*δCH_2, δCH_3*	*$\nu_s COO^-$*	*νM-O-C*	*Other non ligand Bands ν(M-N, M-O,M-Cl,...)$\rho_r H_2O$,...*
Ninhydrin [60]	3200 – 2920(b,s)	1768(s), 1754(s), 1720(s)	—	1590(s)	—	—	—	—	—
Alanine [66] **νN-H** 3200(s)	3300 – 2550(b,s)	—	1595(s)	—	—	1458(s), 1355(s)	1410(m)	—	—
Mn(II) Complex	3620 – 2640(b,s)	1715(m), 1690(m)	1661(vs)	1588(s)	1505(vs)	1445(m), 1350(s)	1314(vs)	1232(vs)	715(s), 680(m), 590(m), 519(m), 470(m)
Fe(III) Complex	3600 – 2700(b,s)	1710(s), 1680(m)	1630(vs)	1592(s)	1503(vs)	1 468(m), 1320(s)	1318(vs)	1210(vs)	705(m), 660(m), 540(m), 519(m) 255(s)
Co(III) Complex	—	1729(s), 1687(s)	1630(vs)	1590(s)	1501(vs)	1450(m), 1356(s)	1324(vs)	1225(vs)	605(m), 530(m), 430(m)
Co(II) Complex	—	1730(s), 1710(m)	1635(vs)	1598(s)	1508(vs)	1449(m), 1350(m)	1302(vs)	1200(vs)	660(m), 595(m), 530(m), 492(m)
Ni(II) Complex	—	1735(s), 1714(s)	1631(vs)	1592(s)	1516(vs)	1447(m), 1350(s)	1327(vs)	1245(vs)	698(m), 672(m), 600(m), 540(s)
Zn(II) Complex	—	1733(m), 1710(m)	1652(vs)	1595(s)	1508(vs)	1450(m), 1350(m)	1320(vs)	1232(vs)	687(w), 670(w), 594(m), 545(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 [62], ν - stretching vibration, δ - bending or deformation

Table IX Infrared spectral data of Ruhemann's purple and the products isolated in the presence of VO(II), Cr(III) and Cu(II) with both amino acids* (KBr pellets)

Compound			νC=O	ν C=C	νC=N	νC=C (enol)	νC-O	δC-H (arom)
Products isolated with	Serine	VO(II)	1737(m)	1590(m)	1638(s)	1557(s)	1120(s)	721(s)
		Cr(III)	1737(s))	1580(m)	1638(s)	1560(s)	1122(s)	720(s)
		Cu(II)	1737(m)	1595(m)	1635(s)	1555(s)	1125(s)	722(s)
	Alanine	VO(II)	1737(s)	1613(m)	1635(m)	1556(s)	1120(s)	721(s)
		Cr(III)	1737(s)	1610(m)	1635(s)	1557(s)	1121(s)	720(s)
		Cu(II)	1735(s)	1615(s)	1635(s)	1556(s)	1120(s)	721(s)
Ruhemann's Purple			1739(s)	1581(m)	1637(s)	1555(s)	1119(s)	723(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 [62],
ν - stretching vibration,
δ - bending or deformation

4.4 Electronic Spectral Studies

The electronic spectral data of the metal complexes of both Schiff bases and other related products are given in Tables X, XI and XII. This data again shows two groups of products, one group contains products obtained with Mn(II), Fe(III), Co(III), Co(II), Ni(II) and Zn(II) and the second group contains products isolated with VO(II), Cr(III) and Cu(II).

The first group of complexes with both Schiff bases show some common bands characteristic of the ligands. The bands near 42,000 cm^{-1} and 40,000 cm^{-1} are assigned to $\pi \rightarrow \pi^*$ transition of the benzene moiety [40, 67] and that near 35,500 cm^{-1} is assigned to $n \rightarrow \pi^*$ transition of the carbonyl group [40, 67]. The complexes in this group also show a very strong band near 20,000 cm^{-1} ($\varepsilon_{max} > 10,000$) assigned to a combination of $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of the azomethine chromophore [24, 42], which in the free state absorbs between 25,000 – 23,256 cm^{-1}. The observed bathochromic shift is a strong support for the coordination of the azomethine nitrogen [24, 42]. This band, because it is very strong, is thought to obscure some d-d bands of some complexes of the group in the region, which could otherwise be useful for structure elucidation.

The electronic spectrum of the Co(II) complex of IDIHP shows two d-d bands at 16051 cm^{-1} and 9901 cm^{-1} assignable to $^4T_{1g} \rightarrow {}^4A_{2g}$ (P) and $^4T_{1g} \rightarrow {}^4T_{2g}$ (F) respectively in octahedral geometry [24, 42 – 44, and 68 – 71]. The third d-d band expected near 20000 cm^{-1} ($^4T_{1g} \rightarrow {}^4T_{2g}$ (P)) must have been obscured by the very strong band of the azomethine group at 20120 cm^{-1}. The electronic spectrum of Co(II) complex of IDIP also show a similar pattern suggesting an octahedral geometry.

The electronic spectrum of the Ni(II) complex of IDIHP show three d-d bands at 28571 cm^{-1}, 15974 cm^{-1} and 10460 cm^{-1} assignable to $^3A_{2g} \rightarrow {}^3T_{1g}$ (P), $^3A_{2g} \rightarrow {}^3T_{1g}$ (F) and $^3A_{2g} \rightarrow {}^3T_{2g}$ respectively in octahedral geometry [24, 42 – 44, 68 – 71]. The electronic spectrum of the Ni(II) complex of IDIP also show a very similar pattern suggesting octahedral geometry.

The electronic spectra of Co(III) complexes of both Schiff bases also suggest an octahedral geometry as the number of spectral bands and their positions show.

The Mn(II) and Fe(III) complexes of both Schiff bases showed very few d-d bands, which could not be enough to elucidate the structure, the others most likely being obscured by the strong azomethine band. So, these and the Zn(II) complexes are assumed to have an octahedral geometry based on close resemblance of their properties to the others in the group, elemental, IR, conductance and other data.

The products obtained with VO(II), Cr(III) and Cu(II) with both amino acids show electronic spectra much similar to the isolated Ruhemann's purple. Along with other data (elemental, IR and conductance), these metal ions do not form any complex with the Schiff base or Ruhemann's purple and Ruhemann's purple is isolated as the final product.

Table X Electronic Spectral data of Metal Complexes of IDIHP and their assignments

Complex	*Non-ligand Electronic Spectral Bands (cm^{-1})*	*ε_{max} ($Lmol^{-1}cm^{-1}$)*	*Assignment*
Mn(II)	23,365 (very broad)	55.70	Mixture of $^6A_{1g} \rightarrow {}^4T_{2g}(G)$, $^6A_{1g} \rightarrow {}^4E_g(G)$, $^6A_{1g} \rightarrow {}^4A_{1g}(G)$
Fe(III)	28,571(very broad)	32.20	Mixture of $^6A_{1g} \rightarrow {}^4A_{1g}$, $^6A_{1g} \rightarrow {}^4E_g(G)$
	27,174	37.20	$^6A_{1g} \rightarrow {}^4T_{1g}$ (D) & $^6A_{1g} \rightarrow {}^4E_g$ (D)
	12,690	17.90	$^6A_{1g} \rightarrow {}^4T_{1g}(G)$
Co(III)	27,174	255.00	$^1A_{1g} \rightarrow {}^1T_{2g}$
	16,051	58.00	$^1A_{1g} \rightarrow {}^1T_{1g}$
	10,460	12.00	$^1A_{1g} \rightarrow {}^3T_{1g}$
Co(II)	16,051	35.80	$^4T_{1g} \rightarrow {}^4A_{2g}$ (P)
	9,901	13.60	$^4T_{1g} \rightarrow {}^4T_{2g}(F)$
Ni(II)	28,571	744.00	$^3A_{2g} \rightarrow {}^3T_{1g}$ (P)
	15,974	110.00	$^3A_{2g} \rightarrow {}^3T_{1g}(F)$
	10,460	100.00	$^3A_{2g} \rightarrow {}^3T_{2g}$
Zn(II)	--	--	--

Table XI Electronic Spectral data of Metal Complexes of IDIP and their assignments

Complex	*Non-ligand Electronic Spectral Bands (cm^{-1})*	*ε_{max} ($Lmol^{-1}cm^{-1}$*	*Assignment*
Mn(II)	31,250	22.90	Mixture of $^6A_{1g} \rightarrow {}^4E_g(D)$ & $^6A1_g \rightarrow {}^4T_{1g}(P$
	27,397 (very broad)	15.11	Mixture of $^6A_{1g} \rightarrow {}^4T_{2g}(D)$ & $^6A_{1g} \rightarrow {}^4A_{1g}(G$
Fe(III)	28,571	28.02	Mixture of $^6A_{1g} \rightarrow {}^4E_g(G)$, $^6A_{1g} \rightarrow {}^4A_{1g}$,
	27,397	32.06	$^6A_{1g} \rightarrow {}^4E_g(D)$, $^6A_{1g} \rightarrow {}^4T_{1g}(D)$ & $^6A_{1g} \rightarrow {}^4T_{2g}($
	12,739	14.47	$^6A_{1g} \rightarrow {}^4T_{1g}(G)$
Co(III)	31,250	457.00	$^1A_{1g} \rightarrow {}^1T_{2g}$
	14,456	50.00	$^1A_{1g} \rightarrow {}^1T_{1g}$
	12,407	39.00	$^1A_{1g} \rightarrow {}^3T_{1g}$
Co(II)	14,837	17.00	$^4T_{1g} \rightarrow {}^4A_{2g}(P)$
	12690 - 12547	11.00	$^4T_{1g} \rightarrow {}^4T_{2g}$
Ni(II)	27,174	235.00	$^3A_{2g} \rightarrow {}^3T_{1g}(P)$
	15,385	120.00	$^3A_{2g} \rightarrow {}^3T_{1g}(F)$
	10,460	30	$^3A_{2g} \rightarrow {}^3T_{2g}$
Zn(II)	--	--	--

Table XII Electronic Spectral data of Ruhemann's purple, products isolated with VO(II), Cr(III) and Cu(II) and their assignments

Compound	*Electronic Spectral Bands (cm^{-1})*	*Assignment*
Ruhemann's purple and products isolated with VO(II, Cr(III Cu(II)	~41,000 and ~34,000	$\pi \rightarrow \pi^*$ of benzene
	~28,000	$n \rightarrow \pi^*$ of C=O
	27,397 and 20,000	Mixture of $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ of C=N
	17,544	$\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ of the Ruhemann's purple chromophore

4.5 Anti-microbial Studies

The diameter of the inhibition zones around each sample, controls and solvent were measured. These data were then converted into 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 Ruhemann's purple and Zn(II) complex of Ruhemann's purple against the three test organisms are given in Tables XIII. Many of the complexes of both Schiff bases are active against one gram-negative bacterium, Proteus mirabilis and the gram-positive bacterium, Staphylococcus aureus. Some of them, particularly the Co(II), Fe(III) and Mn(II) complexes of both Schiff bases showed a greatly enhanced activity than the starting material, ninhydrin. However, they exhibited a highly reduced activity against the gram-negative bacterium, Escherichia coli compared to ninhydrin's activity. The corresponding metal salts and the solvent have not shown any anti microbial activity. So, the observed enhancement of activity of those complexes found to be more active than ninhydrin must be due to a synergistic or combination effect of the derivatization, complexation and the side group of the amino acids. 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's Schiff base, IDIA, against these three bacteria [25], the activity of the current new complexes may be mainly due to the side groups and/or structural variations. It is also interesting to note that Ruhemann's purple and its Zn(II) complex show some activity, though reduced, against Proteus mirabilis and Staphylococcus aureus at the concentration studied.

Table XIII Results of anti microbial studies on ninhydrin, the metal complexes of IDIHP and IDIP and related compounds.[#]

Compound / Complex			*Test Organism*		
			Escherichia Coli	*Proteus mirabilis*	*Staphylococcus aureus*
Ninhydrin			++	++	++
Ruhemann's Purple			--	+	+
Zn(II) complex of Ruhemann's purple			--	+	+
Metal Complexes of IDIHP		Mn(II)	+	++++	++++
		Fe(III)	+	++++	++++
		Co(III)	--	--	+++
		Co(II)	+++	++++	++++
		Ni(II)	--	++	++
		Zn(II)	--	+	+
Metal Complexes of IDIP		Mn(II)	+	++++	+++
		Fe(III)	--	++++	+++
		Co(III)	+	++	++
		Co(II)	+	++++	++++
		Ni(II)	--	++++	++
		Zn(II)	--	--	--
Other products isolated in the presence of	Serine	VO(II)	--	--	--
		Cr(III)	--	+	+
		Cu(II)	--	--	--
	Alanine	VO(II)	--	+	+
		Cr(III)	--	+	+
		Cu(II)	--	+	+

[#] - ++++ = very high activity; +++ = high activity; ++ = moderate activity; + = some activity; -- = no activity;

CONCLUSION

All data with both amino acids showed two groups of products. The first group including those isolated with Mn(II), Fe(III), Co(III), Co(II), Ni(II) and Zn(II) confirms with the proposed structure of the metal complexes of the Schiff bases. The other group, including the solids isolated in the presence of VO(II), Cr(III) and Cu(II) are not metal complexes but 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. So, based on all available data the following structures and composition are proposed (Figs. 7 – 9).

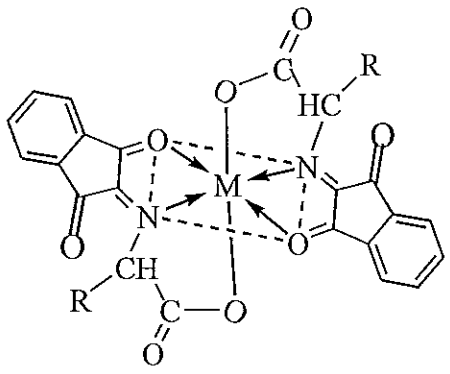


Fig. 7 Proposed structure of Metal complexes of IDIHP or IDIP (-R = -CH_2OH in IDIHP and -CH_3 in IDIP) [$\mathbf{ML_2}$], [M = Co(III), Co(II) (IDIP complex only), Ni(II) and Zn(II)]

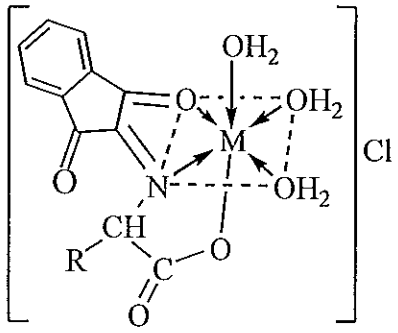


Fig. 8 Proposed structure of Metal complexes of IDIHP or IDIP (-R = $-CH_2OH$ in IDIHP and $-CH_3$ in IDIP) $\mathbf{[ML(H_2O)_3]^+Cl^-}$, [M = Mn(II) and Co(II) (IDIHP complex only)]

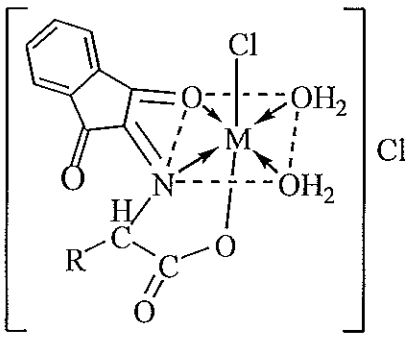


Fig. 9 Proposed structure of Metal complexes of IDIHP or IDIP (-R = $-CH_2OH$ in IDIHP and $-CH_3$ in IDIP) $\mathbf{[ML((H_2O)_2Cl]^+Cl^-}$, [M = Fe(III)]

From the anti-microbial studies most of the metal complexes of both Schiff bases, especially those of Mn(II), Fe(III) and Co(II) show a highly enhanced anti-bacterial activity than the starting material ninhydrin against Staphylococcus aureus and Proteus

mirabilis. In contrast, almost all complexes showed a much-reduced activity against the gram-negative bacteria E. coli.

With these and other similar data analytical procedure may be devised to analyze a sample for its constituent amino acids based on the colors of the metal complexes of the Schiff bases derived from ninhydrin and the amino acid. Four amino acids, glycine [25], serine, alanine (current work) and valine (unpublished similar work) have been studied to date. The data shows Co(II) may be used to specifically identify and quantify serine from the other three, Fe(III) may be used to discriminate alanine from the others and Zn(II) or Mn(II) may be used to discriminate valine from the others. However, to reach at an overall analytical method all possible amino acid – metal ion combinations must be systematically studied and the method carefully designed.

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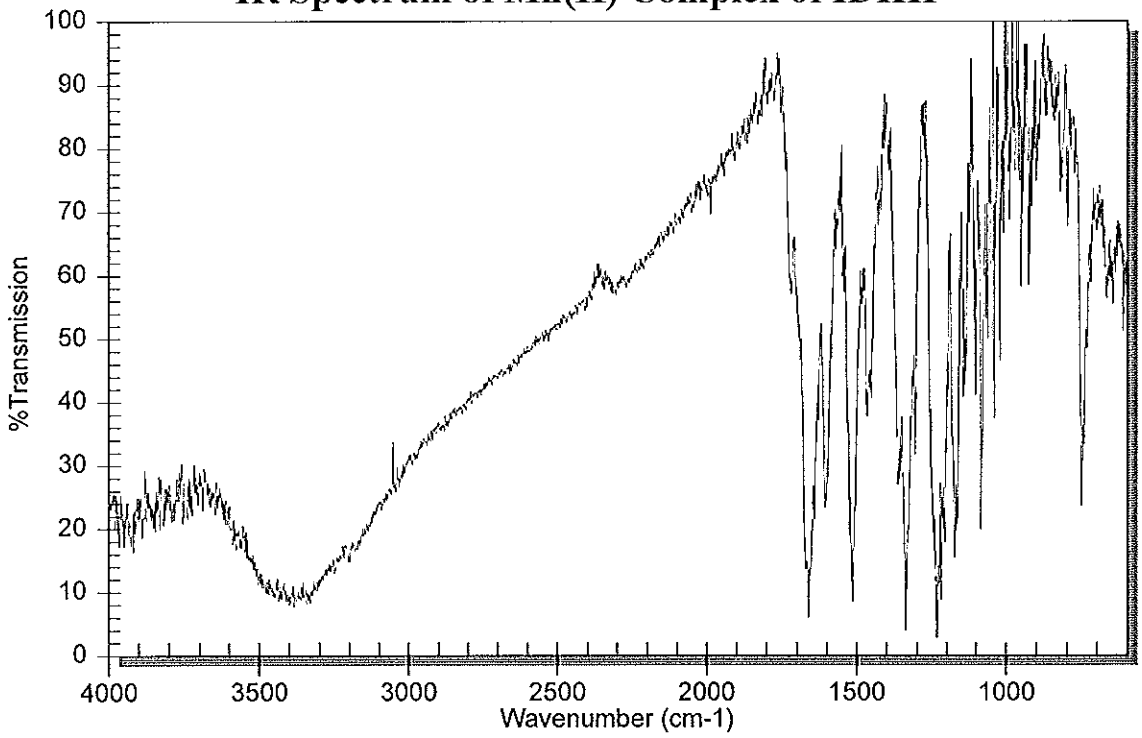
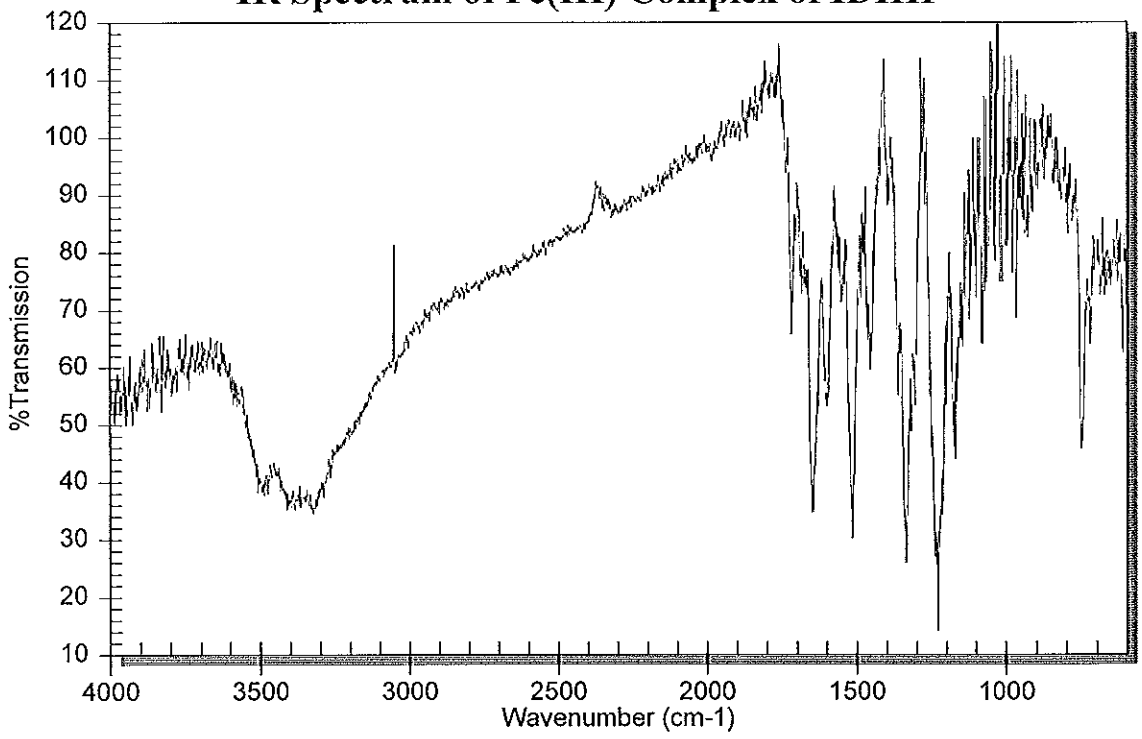
APPENDICES

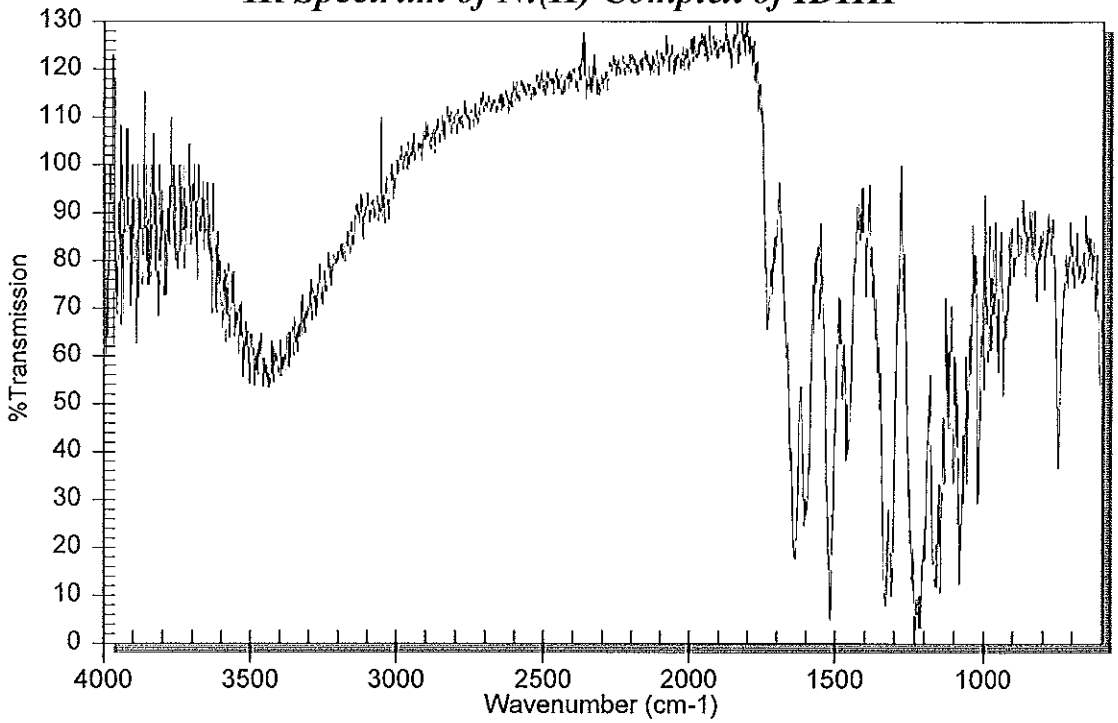
IR Spectrum of Mn(II) Complex of IDIHP



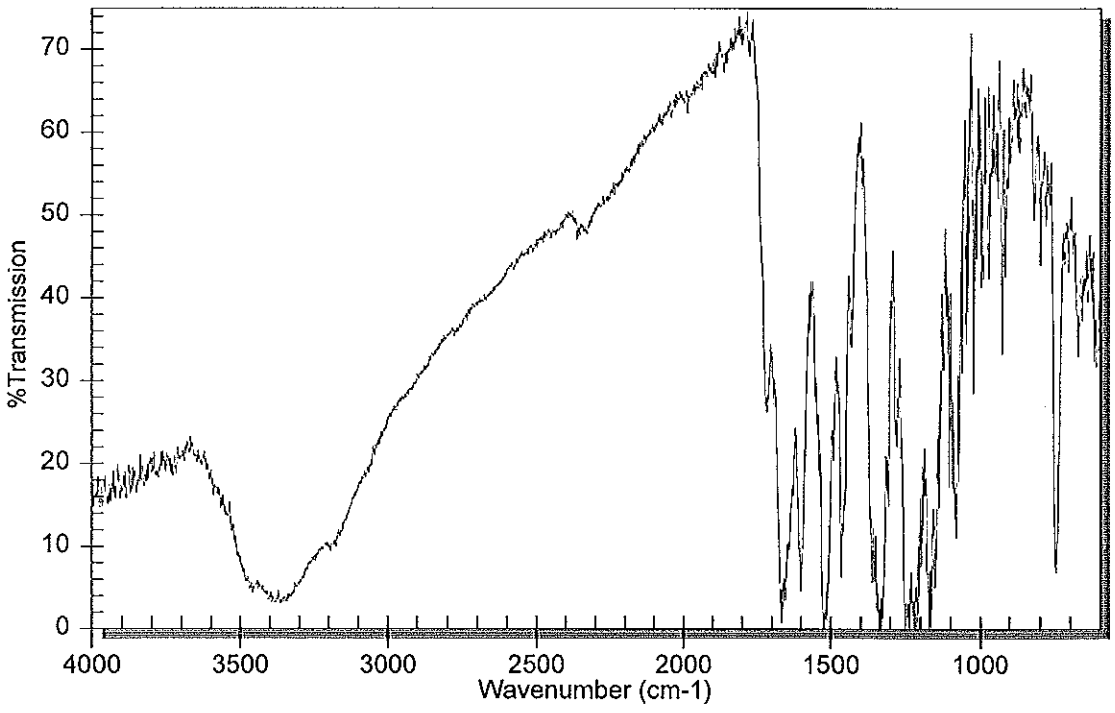
IR Spectrum of Fe(III) Complex of IDIHP



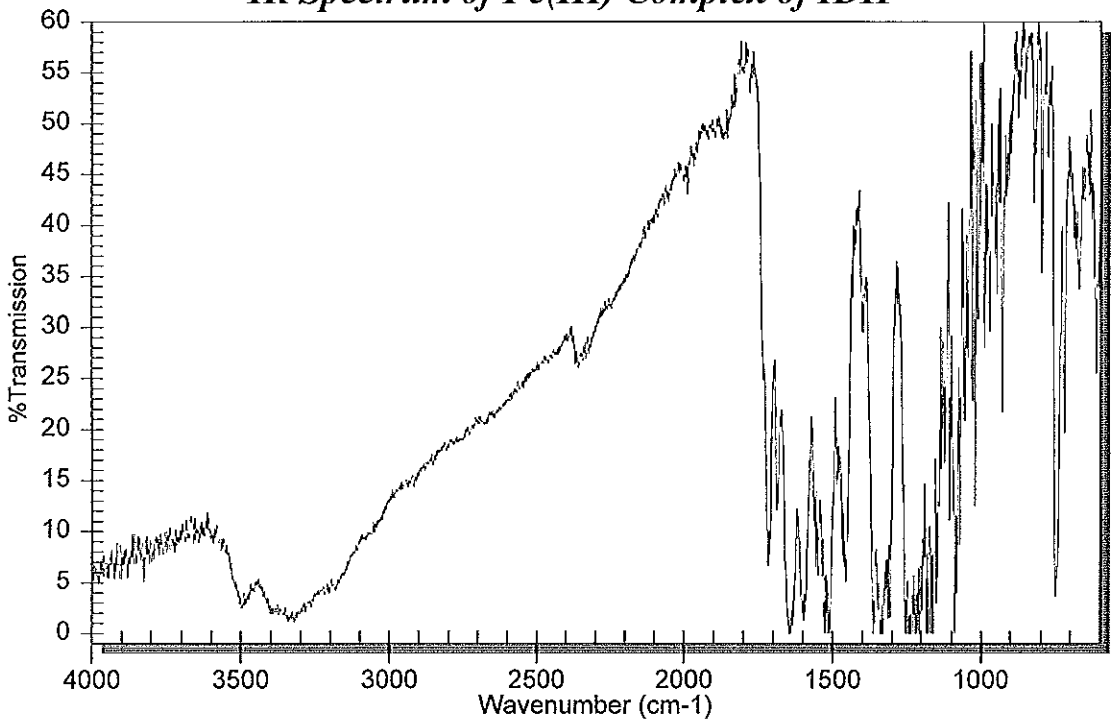
IR Spectrum of Ni(II) Complex of IDIHP



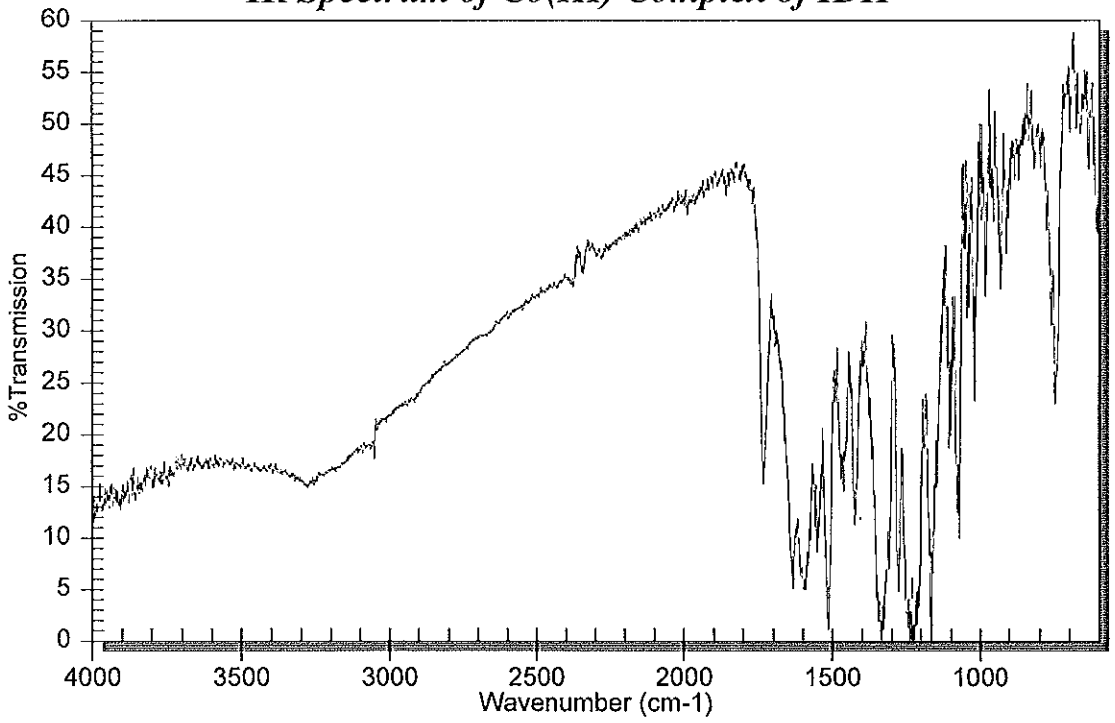
IR Spectrum of Mn(II) Complex of IDIP



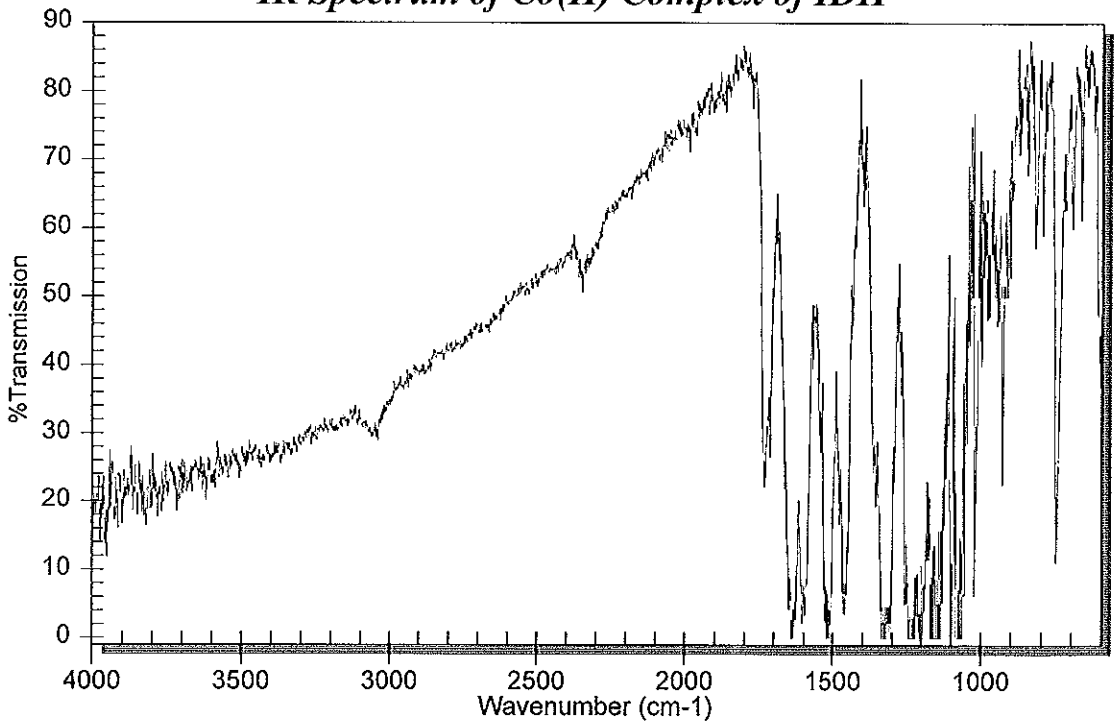
IR Spectrum of Fe(III) Complex of IDIP



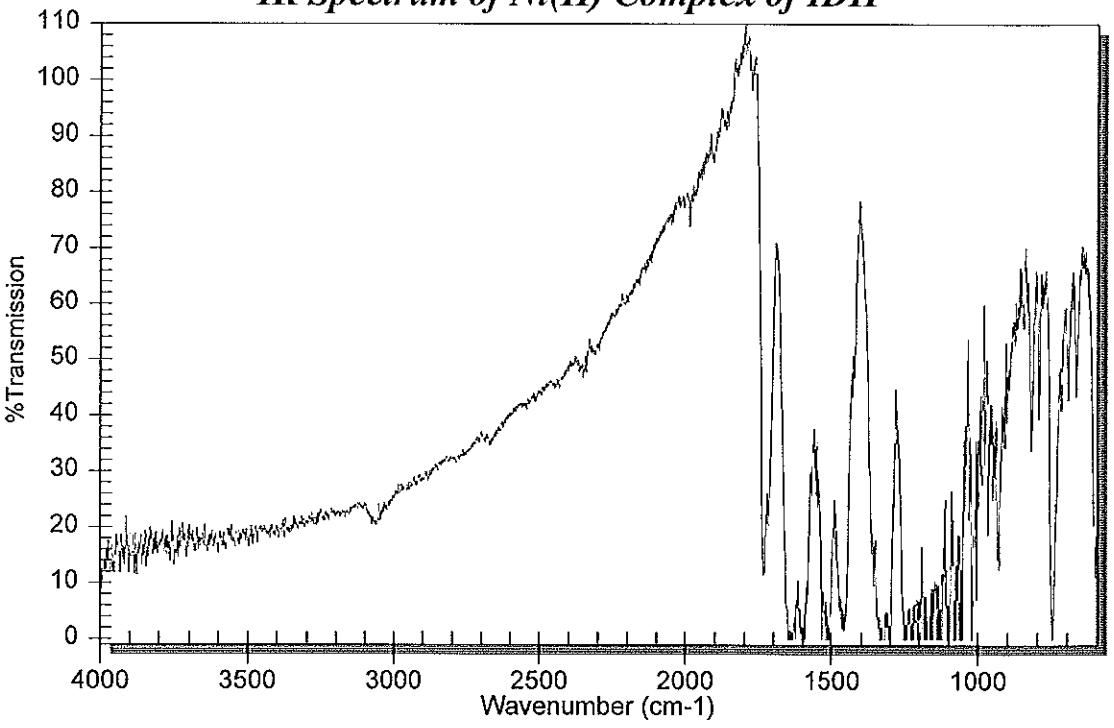
IR Spectrum of Co(III) Complex of IDIP



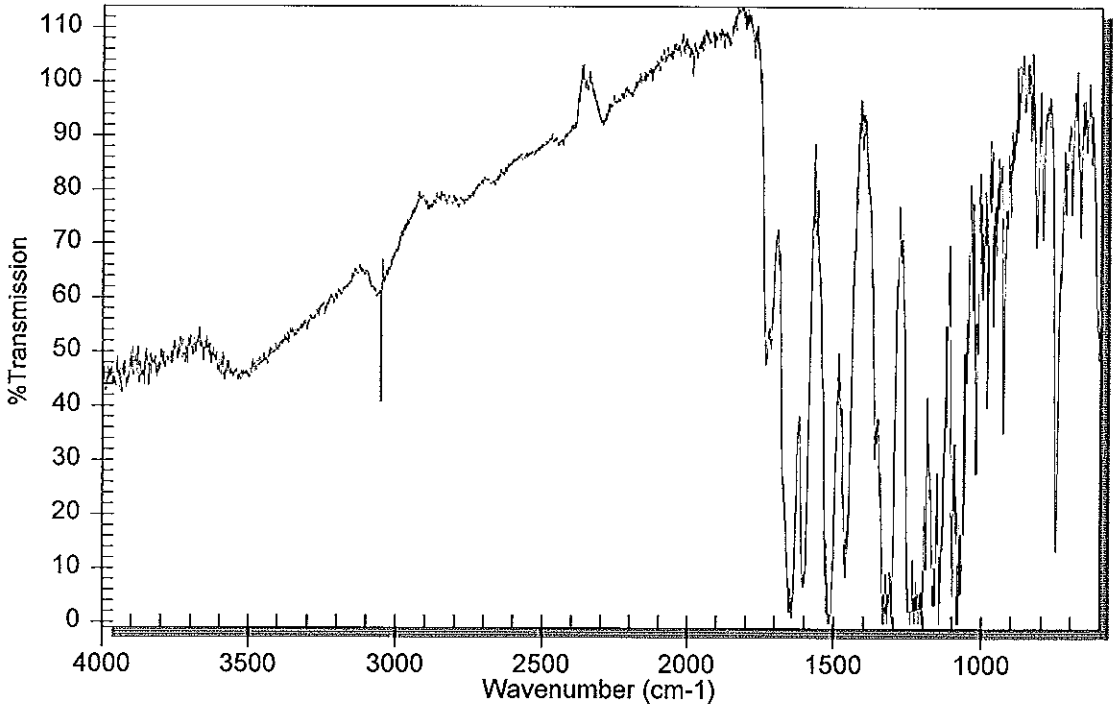
IR Spectrum of Co(II) Complex of IDIP



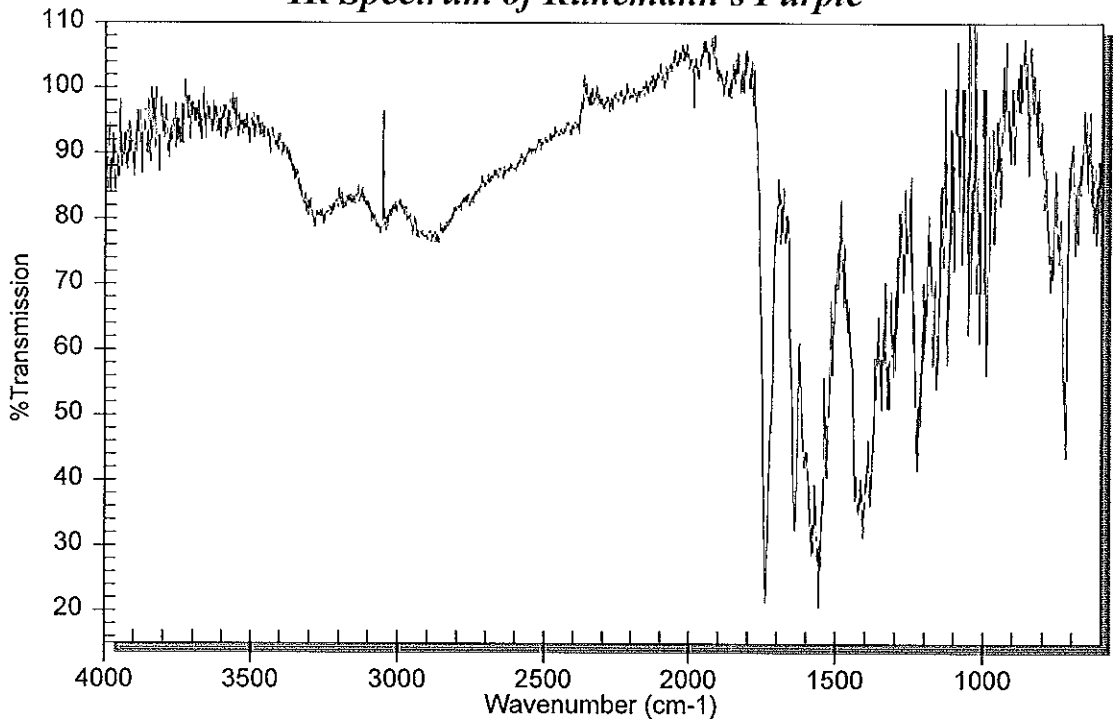
IR Spectrum of Ni(II) Complex of IDIP



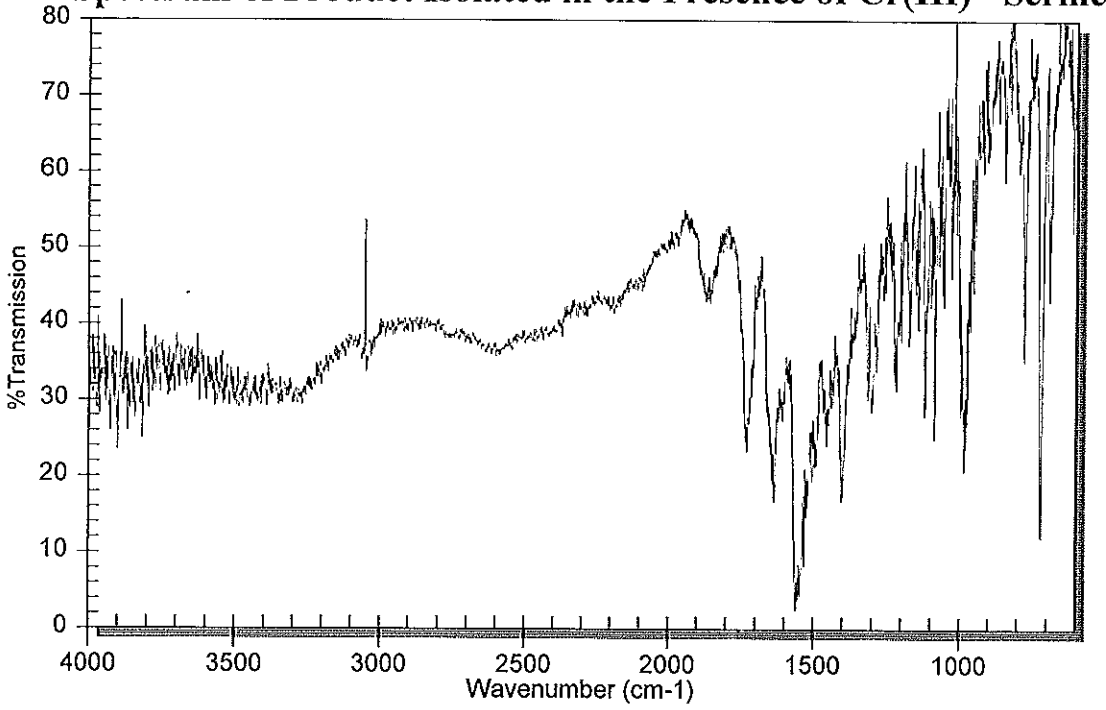
IR Spectrum of Zn(II) Complex of IDIP



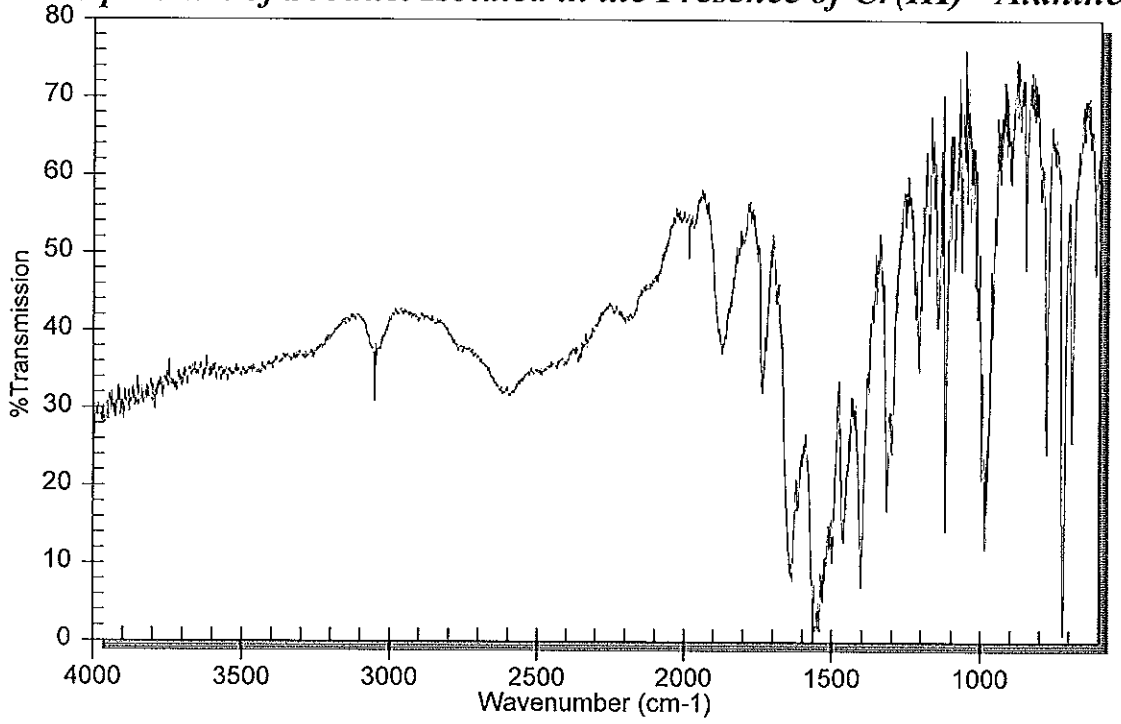
IR Spectrum of Ruhemann's Purple



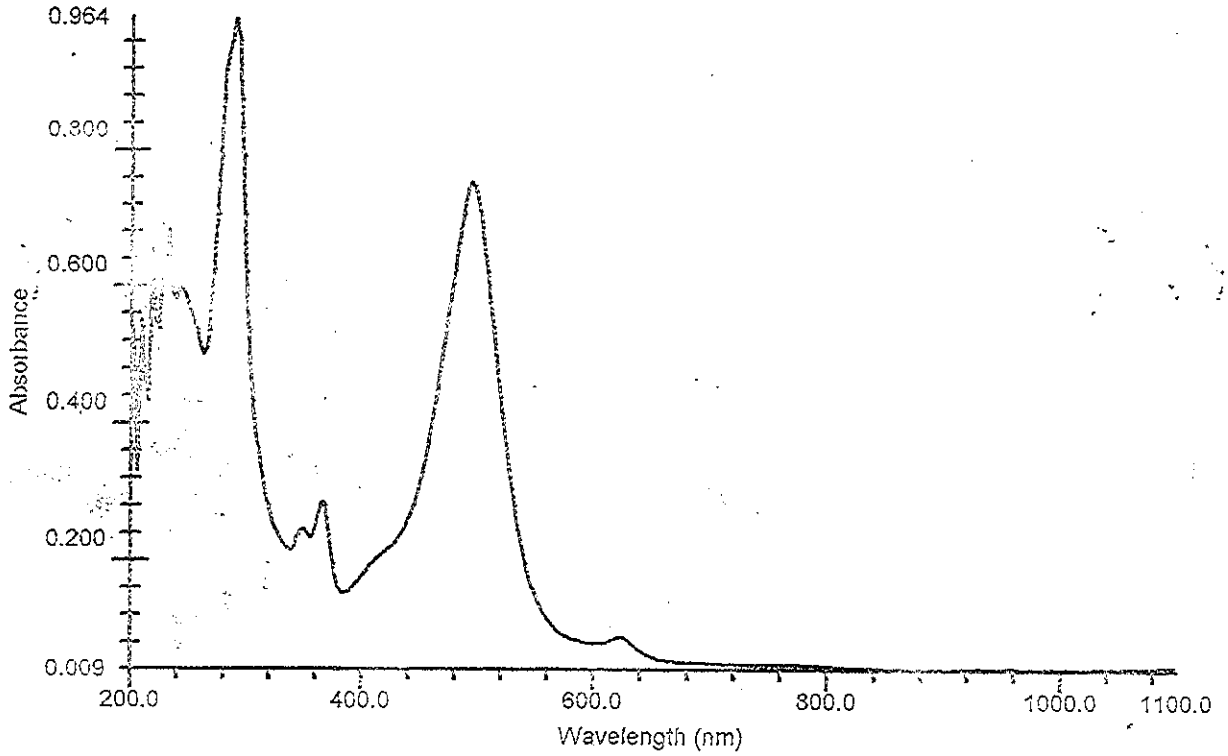
IR Spectrum of Product Isolated in the Presence of Cr(III) - Serine



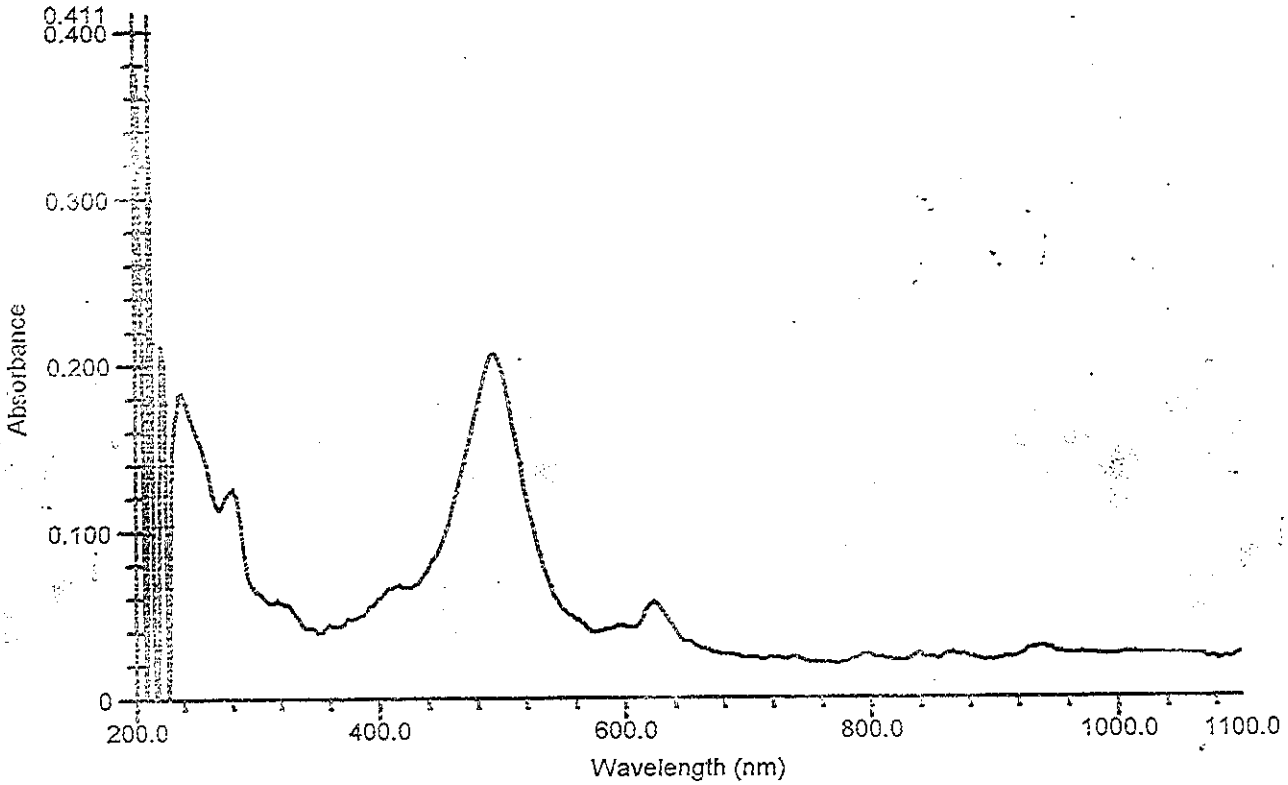
IR Spectrum of Product Isolated in the Presence of Cr(III) - Alanine



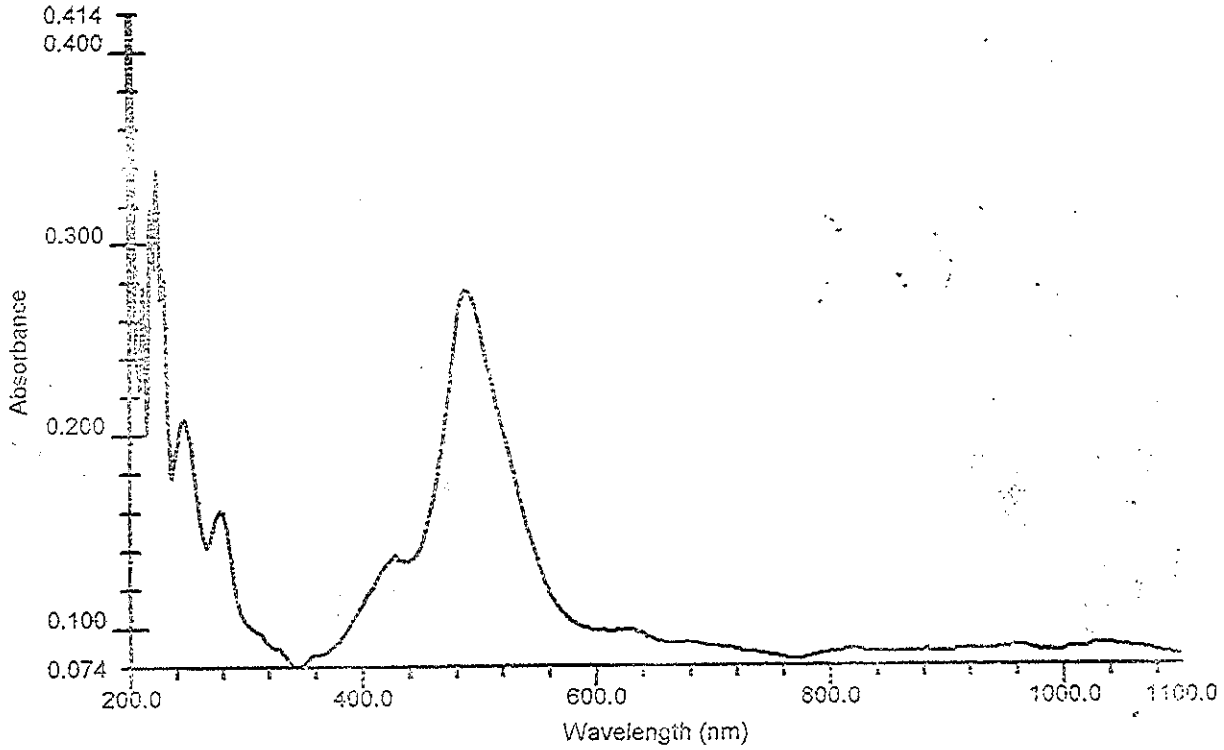
Electronic Spectrum of Co(II) Complex of IDI

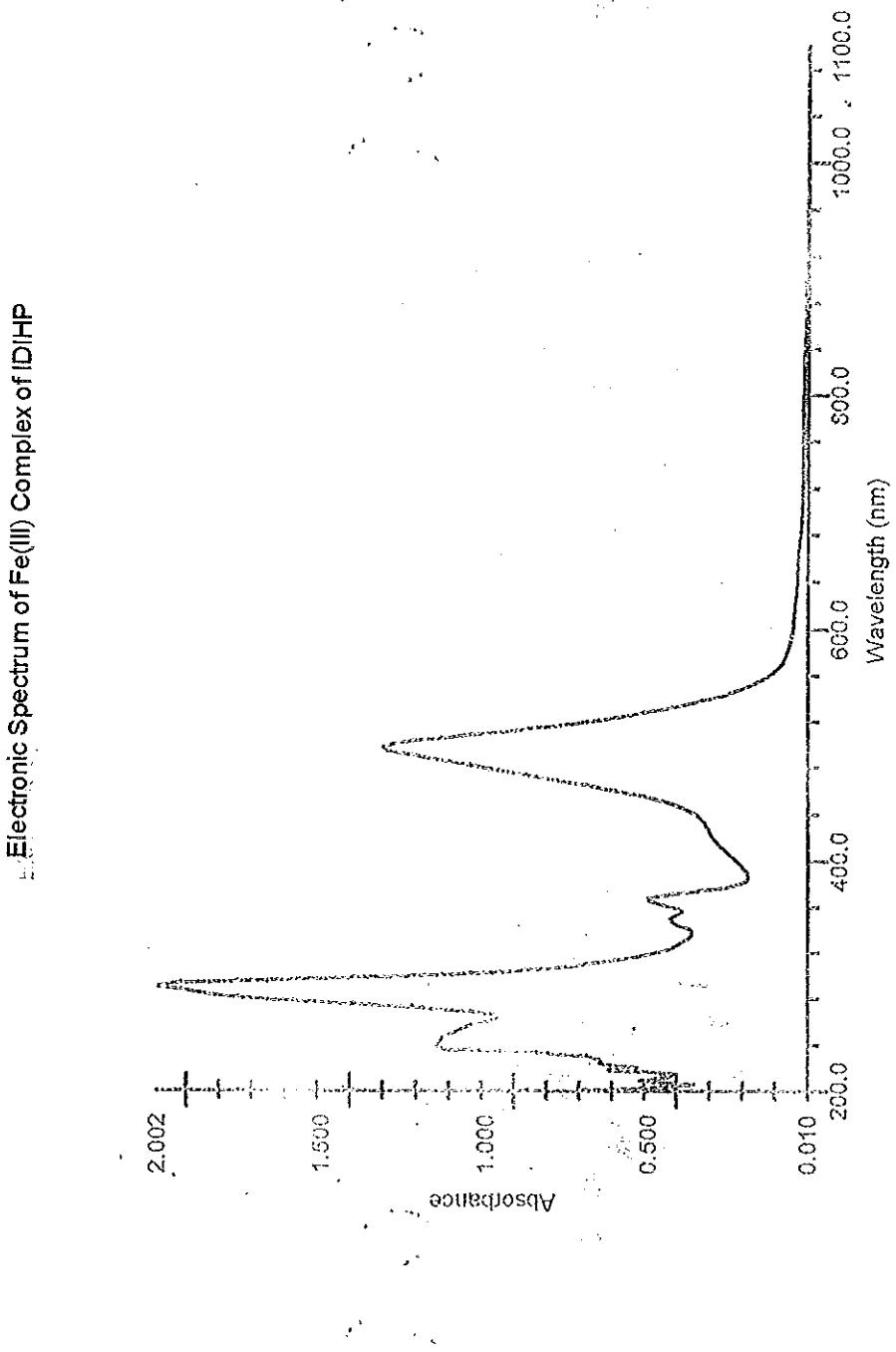


Electronic Spectrum of Co(II) Complex of IDIHP

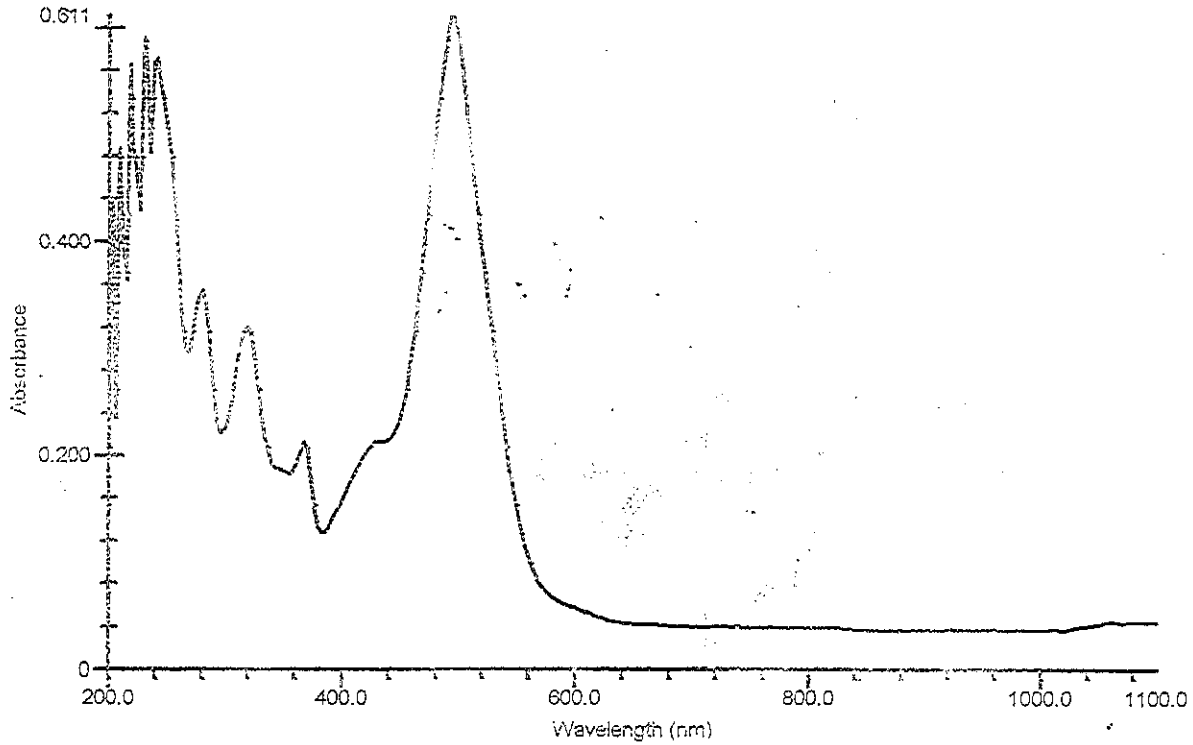


Electronic Spectrum of Mn(II) Complex of IDIHP




Electronic Spectrum of Fe(III) Complex of IDHP
Absorbance
2.002
1.500
1.000
0.500
0.010
200.0
400.0
600.0
800.0
1000.0
1100.0
Wavelength (nm)

Electronic Spectrum of Mn(II) Complex of IDIP



Electronic Spectrum of Fe(III) Complex of IDIP