INVESTIGATION OF COMPLEXATION BEHAVIOR OF NINHYDRIN AND AMINO ACIDS TOWARDS COBALT(II) IONS USING FLUORESCENCE SPECTROSCOPY

By

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Abstract

The amino acids (glycine and alanine) and ninhydrin derivatives of shiff base (ketimine) and Ruhemann's purple complexes of Co(II) were prepared and studied using fluorescence and UV/VIS absorption spectroscopy. The Ruhemann's purple complex were found to have two emission bands at 420nm ($23810cm^{-1}$) and 490nm ($20410cm^{-1}$). The existence of iso-emission point in the emission spectra of Ruhemann's purple complex shows the existence of two structures of the complex at equilibrium. The fluorescence spectra of glycine ninhydrin shiff base in DMF solution were found to have also an iso-emission point showing possible two structures. The emission band lies in ultra violate region and 350nm ($28570cm^{-1}$) band can be seen resolved. From the spectra of both complexes the octahedral symmetry group were suggested for the two complexes.

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Tesfaye Assefa

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Introduction

The α-amino acids and the ninhydrin reaction is used to estimate the amino acids in very small amount. Ninhydrin reaction with α-amino acids gives derivatives with an intense purple colored compound, known as the Ruhemanns purple. The reaction of ninhydrin with amino acids can be altered in the presence of metal ions with the end products being metal complexes. All complexes were distinctly colored. This physical color distinction can be a better way of determination for individual amino acids. If molecules are irradiated by light of a wave length that corresponds to the energy of the allowed transition from the ground state there will be resonance absorption of photons followed by the release of resonance radiation in decay back to original level. The plot of exciting wave length with florescence emission is called excitation spectra. The plot of fluorescence wave length with fluorescence emission is called emission spectra. The excitation spectrum is in theory, identical with the absorption spectrum. Most transition metal complexes are highly fluorescent. The fluorescence spectroscopy of Co(II) complexes of shiff base derived from ninhydrin and glycine reveals this strong fluorescence emission property. The Ruhemanns purple complexes have also the same emission property.

Chapter 1

Literature review

Proteins have been long known to be the basis of life. Most biological activities are determined and regulated by proteins. Proteins are made from the chains of relatively smaller molecules called amino acids. Complex biological characteristic of a protein profoundly depends on the composition of amino acids that make them. Therefore, our understanding of the properties of amino acids is crucial for determination of different kinds biological behaviors.

The reaction of α- amino acids with ninhydrin $(1,2,3-triketohydrindene)$ has long been used in their identification and for its sensitivity in the formation of a purple colored compound called Ruhemann's purple. It was also, discovered that there are many intermediate products in this reaction. Amino acids can react with transition metal ions and form metal complex. Such complex formation is also discovered to exist between transition metal ions such as iron(II), cobalt(II) and copper(II) ions with the intermediate compounds from the reactions of ninhydrin and α-L amino acids such as glycine and alanine.

Metal complexes of transition metal ions mostly have good emission bands in ultra violate and visible (UV/VIS) spectral ranges. The emission bands are dependent on

many factors, one of the main factors is the structural symmetry group of the metal complex. Emission spectra study on transition metal complex of intermediate products of ninhydrin and $\alpha-$L amino acid, reveals a lot about their alleged biologically active property.

1.1 General structures and properties of amino acids

Amino acids are building blocks of protein. Many of amino acids in living organisms fall in a category called the α-amino acid. In each amino acid there is one central carbon atom ($\alpha-$ carbon) bound to hydrogen atom, the amino group ($-NH_2$), a carbonyl group ($-COOH$) and side chain represented by R [1]. The nature of R group determines the physical and the chemical differences among amino acids [2]. There are two broad classes of amino acids based on whether the R group is hydrophobic or hydrophilic. The hydrophobic amino acids tend to repeal the aqueous environment. This class of amino acids does not ionize nor participate in the formation of H-bonds. The hydrophilic amino acids tend to interact with the aqueous environment, are often involve in the formation of H-bonds. The $\alpha-$ amino acids have a common structure as shown in the figure below.

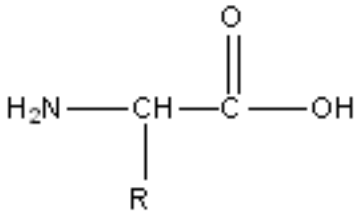

Figure 1.1: *General structure of $\alpha-$ L amino acids*

Crystalline amino acids have relatively high melting and decomposition point, usually above 200^0C. They are much more soluble in water than in less polar solvents. If amino acids are crystalized in non ionic form, they would be stabilized by the much weaker Van Der Waals force and would have low melting point. In water solution amino acids can be found as being acidic, basic or neutral depending on the properties of their R groups. Amino acids exist in aqueous solutions as dipolar ions, known as zwitterion form. High dielectric constant and their large dipole moment are evidences for their form as dipolar ion in aqueous solution[3].

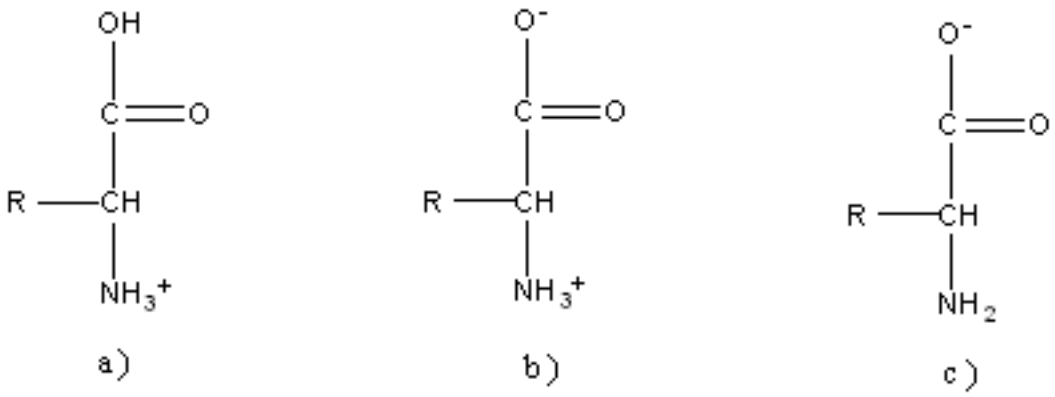

Figure 1.2: *a), b) and c) are Zwitterion structures of* $\alpha-$ *amino acids in water solution*

All $\alpha-$amino acids have good absorption spectra in infrared (IR) region. The theoretical IR spectra of glycine and alanine derived using Hartree-Fork approximation method as in Figure 1.3 show intense absorption in IR region[4]. None of the twenty amino acids found in proteins absorb in visible range. But all amino acids absorb

in far ultra violate region ($< 220nm$)[5]. A tetrahedral carbon atom with 4 distinct constituents is said to be chiral. The one amino acid not exhibiting chirality is glycine since it's R-group is a hydrogen atom. Chirality describes the handedness of a molecule that is observable by the ability of a molecule to rotate the plane of polarized light either to the right (dextrorotatory) or to the left (levorotatory). All of the amino acids in proteins exhibit dextrorotatory property. Therefore, they are all $\alpha-$L amino acids. D-L amino acids are levorotatory and never found in proteins, although they exist in nature. D-L amino acids are often found in polypeptide antibiotics. The aromatic R-groups in amino acids absorb ultraviolet light with an absorbance maximum in the range of 280nm. The two simple amino acids are glycine and alanine. Glycine

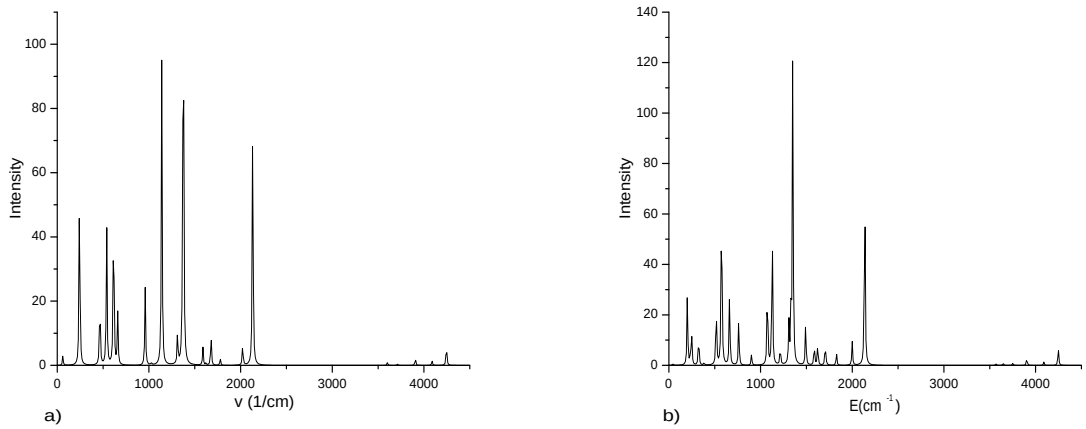

Figure 1.3: *IR spectra of a) Glycine and b) Alanine*

(NH_2CH_2COOH) is the simplest amino acid. Unlike other amino acids, it has no center of chirality. While glycine can exist as a neutral molecule in the gas phase, it exists as a zwitterion in solution and in the solid state. In the solid state, glycine crystallizes in three forms (α, γ and β) which have been studied by x-ray and neutron

diffraction. The α form consists of hydrogen bonded double layers of molecules, which are packed by van der Waals forces. The unstable β form, whose single molecular layers possess an internal arrangement the same as the α form, readily transforms to the α form in air. The γ form crystallizes with a trigonal hemihedral symmetry. While the γ form is stable at room temperature, it is reported to irreversibly convert to α form upon heating above 165^0C. α and γ remain unchanged for a long time under normal conditions, α glycine is commonly available[6]. Due to its dipolar nature, glycine has a high melting point.

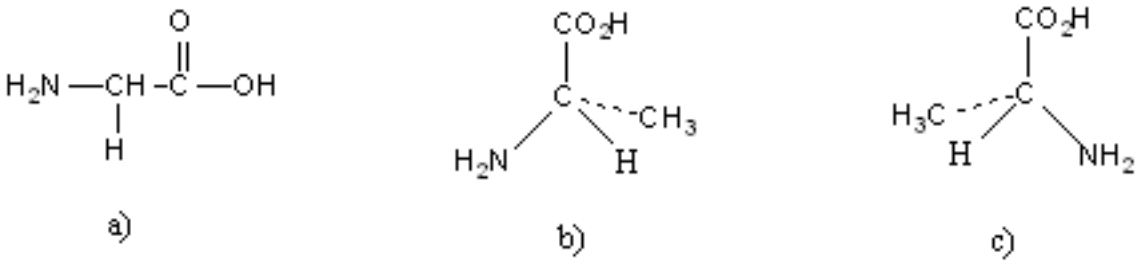

Figure 1.4: *Structures of a)glycine b)α-alanine and c)D-alanine*

A complex or coordination compound is a one with several atoms or groups are directly and less firmly attached to a central atom or ion. Amino acids like glycine and alanine have long been known to form complexes with transition metal ions like copper(II) and cobalt(II). Glycine copper(II) complex has no ionic charge[7]. Copper(II) complexes of glycine and alanine in aqueous solution have similar absorption spectra, in which the characteristic 500nm to 550 nm absorption band was common.

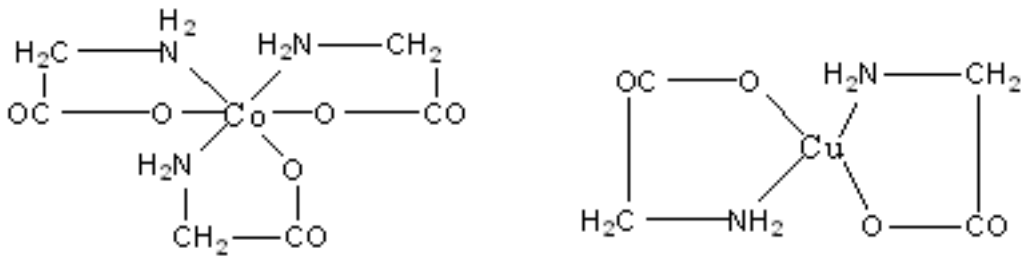

Figure 1.5: *Copper and cobalt complexes of glycine*

1.2 Amino acid reactions with ninhydrin

Ninhydrin ($1,2,3-triketohydrindene$) shows an absorption curve that has several plateaus at $pH = 4.8$, some of which may represent absorption maxima. The main maximum is the one at λ_{max}= 232nm[8]. A reaction of $\alpha-$amino acids with ninhydrin, can be utilized to estimate amino acids in very small amount. Ninhydrin reacts with $\alpha-$amino acids to give a derivative with an intense purple color (maximum absorption at λ_{max}= 570nm) and with few a yellow color. The purple colored compound is generally known as Ruhemann's purple. Oscillation of an electron in Ruhemann's purple between the atoms of the nitrogen and oxygen bridge would be the main resonance in an oxazine-free radical, which would account for the absorption band in the visible region between 520nm to 560 nm. The reaction of ninhydrin with $\alpha-$ amino acids have many intermediate products before reaching its final product, the Ruhemann's purple.

An important compound from the intermediate products of $\alpha-$amino acids with ninhydrin is the ketamine, the ketamine can form stable coordination compounds with transition metals. Such complexes of transition metal ions with the ketimine

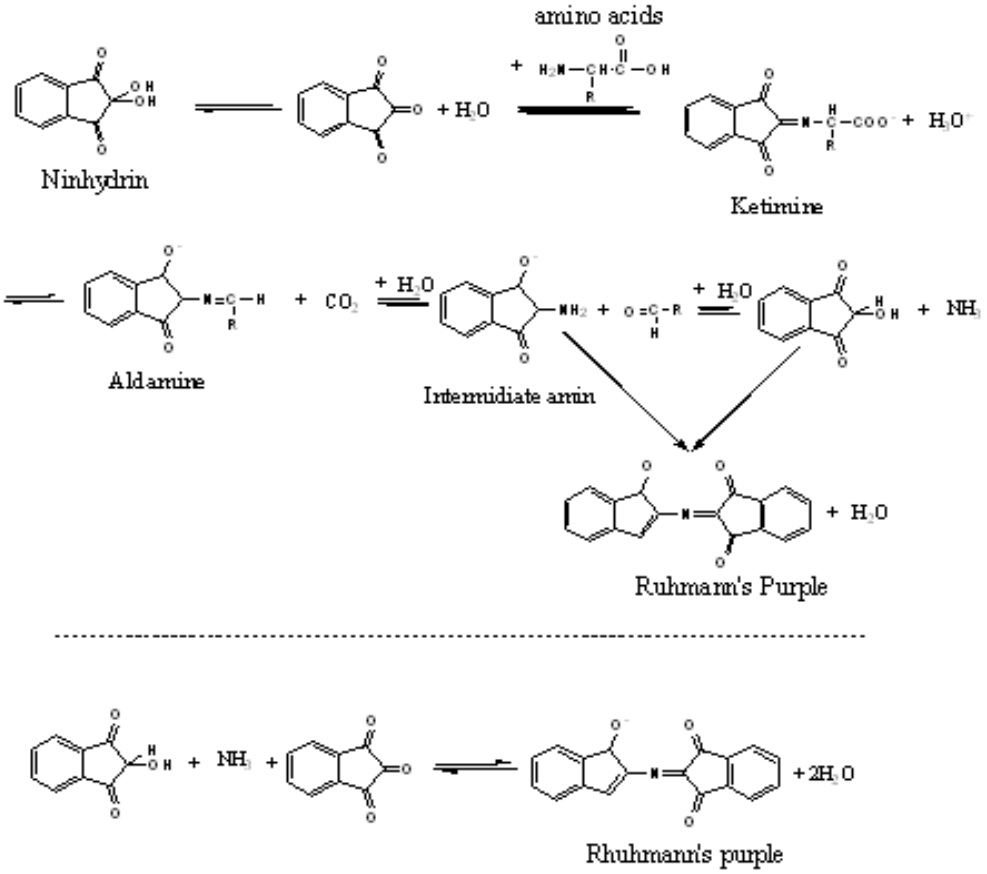

Figure 1.6: *Reaction of $\alpha-$amino acids with ninhydrin*

were successfully synthesized and characterized[9]. The complexes of transition metals with the ketamine were also found to be distinctly colored. The shiff base transition metal complexes were also found to be soluble in solvents of higher polar solvents like DMF and DMSO.

1.3 Transition metal complexes

When a metal ion combines with an electron donor, it is called metal complex or coordination compound. A molecule containing two or more donor groups forming ring with metal is known as metal chelate, the donor compound is called chelating agent. The donor atoms that form complex with metals are restricted to nonmetallic elements of group V and VI (N,O and S are the only common ones). The molecules or ions surrounding the central metal ion are called ligands.

Consider simple transition metal complex $Fe(H_2O)_6^{3+}$. Iron has an electronic configuration given as $1s^2 2s^2 2p^6 3s^2 3p^6 3d^6 4s^2$. When it forms an Fe^{3+} ion it loses the 4s electrons and one of the 3d electrons and it's electronic configuration is $1s^2 2s^2 2p^6 3s^2 3p^6 3d^5$. The single electrons in the 3d level are not involved in the bonding in any way. Instead, the ion uses 6 orbitals from the 4s, 4p and 4d levels to accept lone pairs from the water molecules. Before they are used, the orbitals are re-organized (hybridized) to produce 6 orbitals of equal energy. This orbitals then form a coordination compound with 6 molecules of water. This implies that Fe^{3+} has coordination number of six. The co-ordination number of a complex ion counts the number of co-ordinate bonds being formed by the metal ion at its center. The water

Ion	Cu^{+2}	Co^{+2}	Co^{+3}	Ni^{+2}	Zn^{+2}	Mo^{+4}
Coordination number	4	6	6	6	4	8

Figure 1.7: *Coordination numbers of some transition metal ions*

molecule in $Fe(H_2O)_6^{3+}$ is called unidentate. Unidentate ligands are molecules that form single bond with the central metal ion. Bidentate, tridentate, quadridentate,

etc., denote complexes formed with molecules or ions containing two, three, four,etc., donor groups. The stabilities of complexes of bivalent metal ions follow the order $Pd > Cu > Ni > Co > Zn > Fe > Mn > Mg$ irrespective of the nature of the ligands involved[10].

The color of chelate compounds is generally significant, colors are usually considered as an indication that a chelate may be present. Its apparent that many chelate compounds have characteristic absorption spectra, a large proportions are colorless or do not have absorption spectra that differs significantly from their metals or their chelating agent. Inorganic ions may fluoresce directly or when combined with an organic ligands. Its apparent that many chelate compounds have characteristic to form a highly fluorescent metal chelate. Non-bonding n-electrons may be involved in bonding with a metal ion and create a strongly fluorescent $\pi \rightarrow \pi^*$ transition results for excitation rather than $n \rightarrow \pi^*$ transition of low fluorescence yield.

1.3.1 Structures of cobalt(II) complexes

Cobalt can be found in one of the two most common form, i.e Co^{2+} Or Co^{3+} in its ionic form. The two ions, Co(II) and Co(III), have d^7 and d^6 orbital configuration respectively. Most complexes of cobalt(II) have an octahedral or tetrahedral symmetries[11]. Cobalt(II) can form four, five or six bonds with ligands. The preferred arrangement of four bonds with cobalt(II) ions is a tetrahedral one, which is found in the CoX_4^{2+} ions ($X = Cl, Br, I$). There is a distortion of tetrahedral coordination group if the ligands are bidentate. In case of cobalt(II) with five bonds three groups of structures can be formed. They are trigonal bipyramidal complexes with unidentate ligands, square bipyramidal complexes and square planar formed from dimeric complexes. The six bond arrangement in cobalt(II) is mostly octahedral.

1.4 Structures of Cobalt(II) ion complexes with products of amino acids and ninhydrin reaction

Its acceptable to propose an octahedral symmetry configuration withe the coordination complexes of cobalt(II) ion. In the intermediate compounds and the Ruhmann's purple, there are possible sites for the formation of bond with cobalt(II) ion. In all possible formations of complexes the chelating agents are oxygen and nitrogen forming tridentate ligand to form complexes with the central cobalt(II) ion. From the observation of reactions of ninhydrin with α-amino acids in the presence of cobalt(II) ions it was found out that cobalt(II) can form two complexes, with ketimine and Ruhmann's purple. The possible octahedral structures of the two complexes with glycine and alanine shiff bases and Ruhmann's purple structures in their solution form are shown as follows.

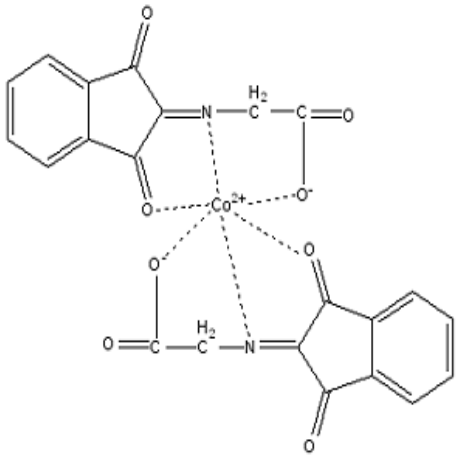

Figure 1.8: *Possible octahedral structures of cobalt(II) complex of glycine ninhydrin derived ketimine*

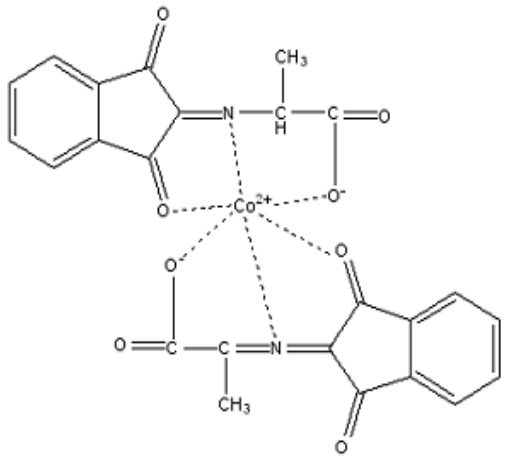

Figure 1.9: *Octahedral structures of cobalt(II) complex of alanine ninhydrin derived ketimine*

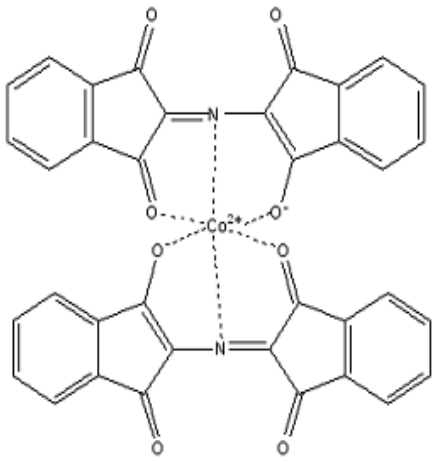

Figure 1.10: *octahedral structure of cobalt(II) Ruhmann's purple complex*

Chapter 2

Theory

Most objects around us are colored not only because they scatter light but also absorb light of certain frequency. Atoms absorb frequencies of light in the ultra violate and visible region. Atomic absorption can be easily seen in a gas phase with very small pressure exposed to white light where the absorption line appears as a dark line in it's spectra. Sodium has a strong absorbtion lines in the yellow region at 589nm and 589.6nm. The electronic and absorption spectra in molecules also lie mostly in ultra violate and visible regions. Molecules have a freedom to vibrate. These molecular vibration frequencies lie in infrared region. Rotation is also a possibility in a molecule and the corresponding spectrum lies in microwave region.

2.1 Theories of interaction of matter with radiation

2.1.1 Classical theory of absorption

A simple description of classical theory of the interaction of electromagnetic field with material medium can be explained by the assumption of Lorentz electron oscillator model[12]. A motion of an electron is described by Newton's second law of

motion for a charged particle in an electromagnetic field propagating in Z direction. The total force is given by a Lorentz force.

$$\vec{F} = e(\vec{E} + \vec{V} \times \vec{B}) \tag{2.1.1}$$

where e is the charge of an electron, $\vec{E}$ is electric field, $\vec{V}$ is velocity of charged particle and $\vec{B}$ is magnetic field. Assuming that the atoms in the molecules are dielectrics, the term with magnetic interaction is ignored. Electrons in matter have a natural vibration about their stable equilibrium point. The equation governing electrons motion in matter is given as

$$\frac{d^2}{dt^2}\vec{x(t)} = \frac{e}{m}\vec{E} - \omega_0^2\vec{x(t)} \tag{2.1.2}$$

where $\omega_0^2 = \sqrt{\frac{k}{m}}$ is the natural frequency of vibration of electrons. In material medium molecules are subjected to collisions with one another, which affects the electrons motion as a drag force. The drag force is given by

$$\vec{F_{drag}} = -b\frac{d}{dt}\vec{x(t)} \tag{2.1.3}$$

The electric field $\vec{E}$ of electromagnetic radiation is given by $\vec{E} = \vec{\varepsilon}E_0 \exp -(\omega t - kz)$, where ω, k and E_0 are frequency, wave vector and amplitudes of propagating wave. The complete equation in an electron oscillator model is expressed as

$$\frac{d^2}{dt^2}\vec{x(t)} + 2\beta\frac{d}{dt}\vec{x(t)} + \omega_0^2\vec{x(t)} = \hat{\varepsilon}\frac{e}{m}E_0 \exp -j(\omega t - kz) \tag{2.1.4}$$

where $\beta = \sqrt{\frac{b}{2m}}$ is dumping constant. The solution of the above equation is

$$\vec{x(t)} = -\hat{\varepsilon}\frac{(\frac{e}{m})E_0}{\omega^2 - \omega_0^2 + 2j\beta\omega} \exp -j(\omega t - kz) \tag{2.1.5}$$

The dipole moment of an atom is

$$\vec{p} = e\vec{x(t)} = -e\hat{\varepsilon}\frac{(\frac{e}{m})E_0}{\omega^2 - \omega_0^2 + 2j\beta\omega} \exp -j(\omega t - kz) \tag{2.1.6}$$

The complex polarizibility α is defined by the ratio of magnitudes of the dipole moment and the Electric field.

$$\alpha(\omega) = \frac{\frac{e^2}{m}}{\omega_0^2 - \omega^2 - 2j\beta\omega} \tag{2.1.7}$$

The complex polarization $\vec{P}$ in the Lorentzian oscillator model is the product of the number of absorbing atoms or molecules per unit volume N and the dipole moment. In terms of the relationship of dipole moment and polarizabilty, the polarization is expressed as

$$\vec{P} = \hat{\varepsilon} N\alpha(\omega)\vec{E}_0 \exp -j(\omega t - kz) \tag{2.1.8}$$

The filed in the material medium satisfies the inhomogeneous wave equation

$$\nabla^2\vec{E} - \frac{1}{c^2}\frac{d^2\vec{E}}{dt^2} = \frac{1}{\epsilon_o c^2}\frac{d^2}{dt^2}\vec{P} \tag{2.1.9}$$

Substituting equation 1.1.8 in equation 1.1.9 and simplifying gives

$$k^2 = \frac{\omega^2}{c^2}\{1 + \frac{N\alpha(\omega)}{\epsilon_o}\} = \frac{\omega^2}{c^2}n(\omega)^2 \tag{2.1.10}$$

where $n(\omega)$ is the complex refractive index of the medium. Lets consider the refractive index is composed of real and complex part $n(\omega) = n(\omega)_r + jn(\omega)_c$, substituting this expression in traveling component of the field

$$E(\vec{z}, t) = \hat{\varepsilon}\vec{E}_0 \exp -n(\omega)_c\frac{\omega z}{c} \exp -j(t - n(\omega)_r\frac{z}{c}) \tag{2.1.11}$$

The result shows that in a material medium the amplitude of the field decreases with the distance traveled in the medium. The decrease in the intensity of light is proportional to the square of the field

$$I(\omega)\alpha|\vec{E}|^2 = |\vec{E}_0|^2 \exp \frac{2n(\omega)_c\omega}{c} \tag{2.1.12}$$

This equation can be summarized as

$$I(\omega) = I_0 \exp -a(\omega)z \tag{2.1.13}$$

where I_0 is the intensity of incident radiation and $a(\omega) = \frac{2n(\omega)_c\omega}{c}$ is the absorption coefficient of the medium. Equation 1.1.13 is called Bear-Lambert's Law. As light passes through a material medium it's intensity decreases exponentially.

2.1.2 Semiclassical theory of absorption and emission

Consider a single molecule in a vacuum interacting with electromagnetic wave moving in Z direction with respect to fixed laboratory axis. Such a wave is represented by a real part of vector potential

$$\vec{A}_R = Re[\vec{A}_0 \exp j2\pi\nu(t - \frac{z}{c})] = \frac{1}{2}[\vec{A}_0 \exp j2\pi\nu(t - \frac{z}{c}) + \vec{A}_0^* \exp j2\pi\nu(t - \frac{z}{c})] \tag{2.1.14}$$

where $\vec{A}_0^*$ is the complex conjugate of the amplitude of vector potential $\vec{A}_0$. The electric field $\vec{E}$ and magnetic field $\vec{B}$ vectors of the wave are obtained from Maxwell Electromagnetic equations as

$$\vec{E} = -\frac{1}{c}\frac{\partial}{\partial t}\vec{A} \quad and \quad \vec{B} = \vec{\nabla} \times \vec{A} \tag{2.1.15}$$

The vector potential satisfies the condition of Coulomb's gauge,

$$\vec{\nabla}.\vec{A} = 0 \tag{2.1.16}$$

The interaction hamiltonian of charges produce a time dependent perturbation. This happens as a result of the interaction of charges with time dependent field of the electromagnetic wave. The total interaction hamiltonian is

$$H' = \sum_j \frac{q_j}{m_j c} Re(\vec{A}_j.\vec{p_j}) \tag{2.1.17}$$

where q_j, m_j and p_j are the charge, mass and linear momentum of the j^{th} particle. Let H_0 be the molecular hamiltonian and the molecule is initially at the stationary state $|\alpha, t = 0>$. $\{|n>\}$ are all possible eigen states of H_0 and $H_0|n>= E_n|n>$. The molecule is initially at the state $|k>$ which is one of the eigen states of H^0. The state ket at $t > 0$ must satisfy the time dependant Schrodinger equation.

$$(H_0 + H')|\alpha, t >_s= i\hbar\frac{\partial}{\partial t}|\alpha, t >_s \tag{2.1.18}$$

The subscript s on the state ket indicates the state ket is in Schrodinger picture, to solve the above equation we rather use an interaction picture[13]. In the interaction picture the state kets, evolution operator and time dependent perturbation potential are related with their corespondents in Schrodinger picture by

$$|\alpha, t_o; t >_I= \exp(\frac{iH_0t}{\hbar})|\alpha, t_o; t >_s \tag{2.1.19}$$

$$U_I(t, t_o) = \exp(\frac{iH_0t}{\hbar})U_s(t, t_o)\exp(\frac{-iH_0t}{\hbar}) \tag{2.1.20}$$

and

$$V_I(t) = \exp(\frac{iH_0t}{\hbar})V_s(t)\exp(\frac{-iH_0t}{\hbar}) \tag{2.1.21}$$

The state ket $|\alpha, t_o; t >_I$ in terms of the eigen states of H_0 can be stated by

$$|\alpha, t_o; t >_I= U_I(t, t_o)|\alpha, t_o >_I= \sum_l |l > C_{kl}(t) \tag{2.1.22}$$

where $C_{kl}(t) =< l|U_I(t, t_o)|\alpha, t_o >$ is the transition amplitude for a system to jump from state $|k>$ to state $|l>$.The above time dependant state ket must satisfy equation of motion of state kets in interaction picture.

$$V_I(t)|\alpha, t_o; t >= i\hbar\frac{\partial}{\partial t}|\alpha, t_o; t > \tag{2.1.23}$$

Substituting equation 1.2.9 in the above equation implies that, the transition amplitude also satisfies the equation stated below.

$$\frac{\partial}{\partial t}U_I(t,t_o) = \frac{1}{i\hbar}V_I(t)U_I(t,t_o) \tag{2.1.24}$$

The solution of the above equation comes in a famous series called Dyson series.

$$U_I(t,t_o) = \{1 + \frac{1}{i\hbar}\int_{t(o)}^{t} dt' V_I(t') + [\frac{1}{i\hbar}]^2 \int_{t(o)}^{t} dt' \int_{t(o)}^{t'} dt'' V_I(t'')V(t') + ...\} \tag{2.1.25}$$

The transition amplitude of the state ket is then expressed as

$$C_{kl}(t) =< l|\{1+\frac{1}{i\hbar}\int_{t(o)}^{t} dt' V_I(t') + [\frac{1}{i\hbar}]^2 \int_{t(o)}^{t} dt' \int_{t(o)}^{t'} dt'' V_I(t'')V(t') + ...\}|k > \tag{2.1.26}$$

The zero order term of the above equation gives the initial state of the system, $C_{kl}(t)^0 = C_{kl}(t_o) = \delta_{kj}$. The first order term of the above equation give the transition between two levels of the molecule,

$$\begin{aligned} C_{kl}(t)^1 &= \frac{\pi}{h}[< l|\sum_j \frac{q_j}{m_j c}\vec{A}_0.p_j \exp(-\frac{2\pi i\nu z_j}{c})|k > \times \frac{1-\exp[2\pi i(\nu_{lk}+\nu)t]}{2\pi(\nu_{lk}+\nu)} \\ &+ \frac{\pi}{h}[< l|\sum_j \frac{q_j}{m_j c}\vec{A_0}^*.p_j \exp(-\frac{2\pi i\nu z_j}{c})|k > \times (\frac{1-\exp[2\pi i(\nu_{lk}-\nu)t]}{2\pi(\nu_{lk}-\nu)})] \end{aligned} \tag{2.1.27}$$

where $\nu_{lk} = \frac{(E_l - E_k)}{h}$. From the equation, there are two terms that can lead to two consequences, absorbtion and emission. Let $P_{k\to l} = |C_{kl}|^2$ be the probability that a transition takes place from state $|k>$ to $|l>$ with absorption of a photon, in this case $\nu_{jk} > 0$ and C_{kl} is only appreciable only when $\nu \approx \nu_{lk}$. So the first term in 1.2.14 can be ignored and the probability of transition with the absorption of photon is

$$P_{k\to l} = \frac{\pi^2}{h^2}|< l|\sum_j \frac{q_j}{m_j c}\vec{A_0}^*.p_j \exp(-\frac{2\pi i\nu z_j}{c})|k > |^2 \times |\frac{1-\exp[2\pi i(\nu_{lk}-\nu)t]}{2\pi(\nu_{lk}-\nu)}|^2 \tag{2.1.28}$$

For a perfectly monochromatic absorbtion of a radiation whose frequency satisfies the condition that $\nu = \nu_{lk}$ the second term approaches to one, transition probability

$$P_{k\to l} = \frac{\pi^2}{h^2}| < l| \sum_j \frac{q_j}{m_j c} \vec{A_0}^* . p_j \exp(-\frac{2\pi i \nu z_j}{c})|k > |^2 \tag{2.1.29}$$

According to quantum theory emission occurs when a molecule jumps from higher energy state to a lower energy state. In an emission of a radiation whose frequency is $\nu_{lk} = \frac{E_l - E_k}{h}$, the transition probability from excited state $|l >$ to the initial state $|k >$ through an emission of photon is

$$P_{k\leftarrow l} = \frac{\pi^2}{h^2}| < l| \sum_j \frac{q_j}{m_j c} \vec{A_0} . p_j \exp(-\frac{2\pi i \nu z_j}{c})|k > |^2 \tag{2.1.30}$$

Generally in quantum mechanics absorption and emission are opposite processes. In absorption a system gains energy and jumps to a higher state, which is unstable and in an emission process a system loses energy and turns to a lower state through radiation. The above description is a semiclassical approach of quantum mechanics towards the interaction of matter with radiation, in full quantum mechanical description the radiation is quantized.

2.1.3 Enstine theory of interaction of matter with radiation

The first quantum theory of interaction of matter with radiation is proposed by Albert Enstine in 1905 for his explanation of photo electric effect. The Enstine's theory is farther developed by Bohr's atomic model that suggest electrons in atoms have a restricted energies. Enstine theory deduces that matter can interact with radiation according to the five ways described in figure 2.1.

- 1. Absorption: It is a process by which an atom or molecule absorbs an energy $\triangle E = E_j - E_i$ and raised from lower energy state $|i >$ to a higher energy state

$|j>$.

- 2. Spontaneous Emission:The atom or molecule emits a photon of energy $h\nu = E_j - E_i$ by deexcitation from an upper state to a lower state. The emission occurs spontaneously without external interaction.

- 3. Stimulated Emission: It is a process by which molecules emit an a photon which is an exact replica of the photon already present in the system.

- 4. Absorption of photon:Molecules absorb a photon of energy $h\nu = E_j - E_i$ and raise themselves from lower state to a higher state.

- 5. Non radiative dexcitation:It is a process by which atoms or molecules return from upper states to the lower ones without photon emission. The energy is converted in to other forms like vibrational energy, rotational energy and translational kinetic energies.

Let's see the relation between absorption, stimulated emission and spontaneous emission. Consider a system with only two possible states, lower level $|i>$ and higher level $|j>$, with number of molecules in state $|i>$ is n_i and state $|j>$ is n_j. The rate at which the number of molecules in the excited state n_j varies is given as follows,

$$\frac{d}{dt}n_j = n_i B_{ij}\rho(\nu) - n_j(B_{ji}\rho(\nu) + A_{ji}) \tag{2.1.31}$$

where B_{ij}, B_{ji} and A_{ji} are known as Enstine coefficients of absorbtion, stimulated and spontaneous emission and $\rho(\nu)$ is the energy density in a given volume. If the system is in equilibrium with radiation at a given temperature T, the net rate of change of the number of molecules in any state is zero. In thermal equilibrium, the

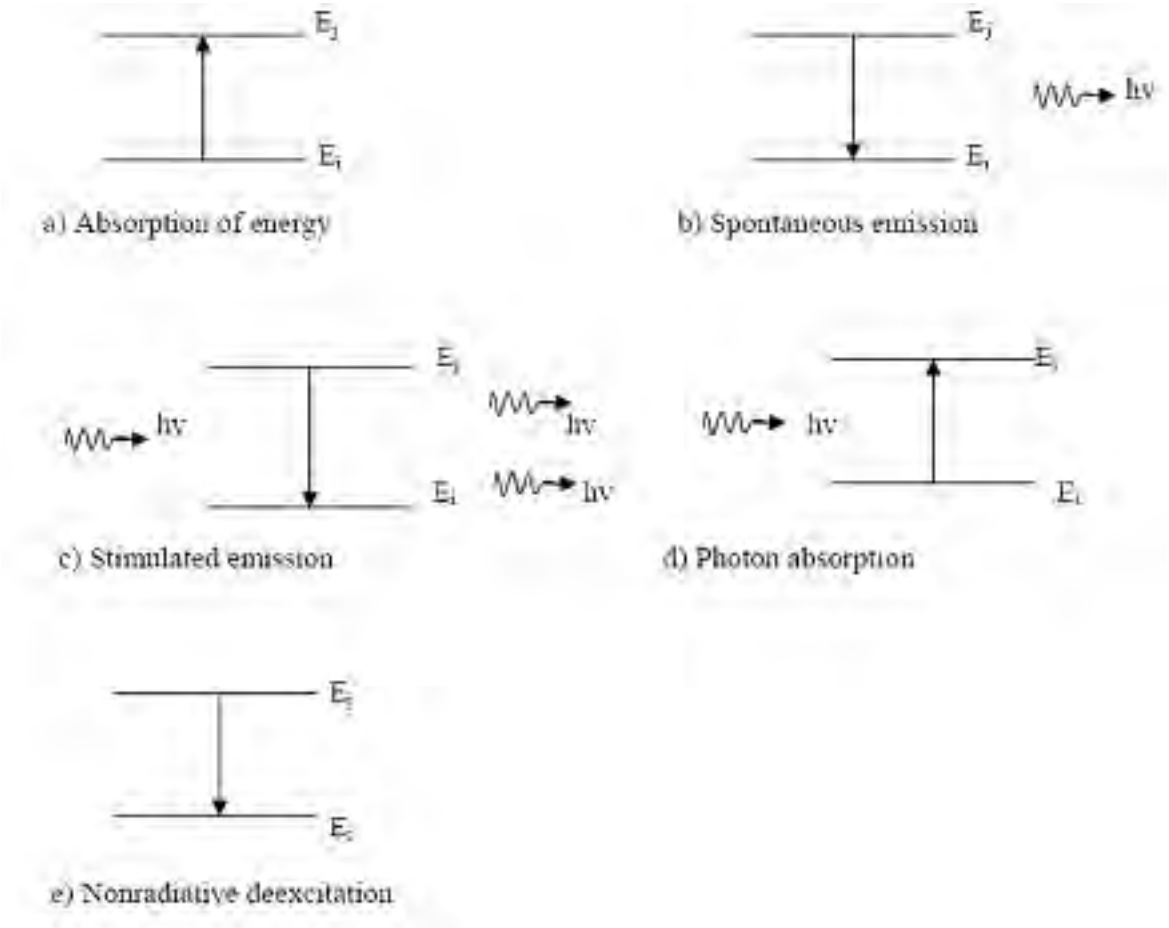

Figure 2.1: *Enstine's theory of interaction of matter with radiation*

numbers of molecules in the higher state and lower state is given by the Boltzman distribution law[14].

$$n_j = n_i \exp(-\frac{E_j - E_i}{\kappa T}) \tag{2.1.32}$$

where κ is Boltzman constant. From Planck's Distribution law, the energy density is stated as

$$\rho(\nu) = \frac{8\pi h\nu^3}{c^3} \frac{1}{\exp(\frac{h\nu}{\kappa T}) - 1} \tag{2.1.33}$$

where $\nu = \frac{(E_j - E_i)}{h}$. The rate of spontaneous emission and stimulated emission is finally related as follows,

$$\frac{A_{ji}}{B_{ji}} = \frac{8\pi h\nu^3}{\rho(\nu)c^3} \tag{2.1.34}$$

from the above result we see that stimulated emission is hardly possible at ordinary conditions. Spontaneous emission is significant only in special conditions, e.g Laser cavities. In atomic and molecular studies of interaction of matter with radiation, the only significant terms are absorption and spontaneous emissions.

2.2 Molecular quantum mechanics

Molecular quantum mechanics is the formalism that is used to describe molecular properties and interaction with applications of the principles of quantum mechanics. Molecular interactions are far more complex from atomic ones in their types and structures, though their fundamental concept begins from our understandings of the behaviors of atoms. The quantum mechanical theory of molecules begins with the formation of molecular hamiltonian and its application on Schrodinger equation. A very interesting part lies on the fact that the exact solution of Schrodinger equation is practically impossible. In molecular quantum mechanics the fundamental problems lie on the development of approximation methods to attain the solution in Schrodinger equation. There are many of such theories that use different kinds of formalisms from quantum mechanics, mathematics, electrodynamics, etc. The fundamental problem in molecular mechanics lies on the determination of the electronic states of the molecules and their interactions with electromagnetic radiation.

2.2.1 Molecular hamiltonian and Born-Oppenhimer approximation

The nonrelativistic molecular hamiltonian can be given by the sum of five terms as follows

$$\hat{H}_T(q,Q) = -\frac{\hbar^2}{2}\sum_{\eta}\frac{1}{M_\eta}\nabla_\eta^2 - \frac{1}{2}\sum_{i}\frac{\hbar^2}{m_i}\nabla_i^2 + \bar{V}_{ee}(q) + \bar{V}_{nn}(Q) + \bar{V}_{en}(q,Q) \quad (2.2.1)$$

where the Q_η are the normal coordinates of the nuclei and q_i are electronic coordinates. The first two terms in the above equation represent the kinetic energy of electrons and the nuclei, the next two terms are electron-electron and nuclei-nuclei electrostatic repulsive potentials and the last term belongs to the electron-nuclei electrostatic attractive potential. The molecular hamiltonian is more compactly written as

$$\hat{H}_T(q,Q) = \bar{T}_e(q) + \bar{T}_n(Q) + \bar{V}_{ee}(q) + \bar{V}_{nn}(Q) + \bar{V}_{en}(q,Q) \quad (2.2.2)$$

Let define the electronic hamiltonian by

$$\hat{H}_{el}(q,Q) = \bar{T}_e(q) + \bar{V}_{ee}(q) + \bar{V}_{nn}(Q) + \bar{V}_{en}(q,Q) \quad (2.2.3)$$

then the total hamiltonian is given by

$$\hat{H}_T(q,Q) = \bar{T}_n(Q) + \hat{H}_{el}(q,Q) \quad (2.2.4)$$

The molecular Schrodinger wave equation

$$\hat{H}_T(q,Q)\Psi_K(q,Q) = E_K\Psi_K(q,Q) \quad (2.2.5)$$

is solved using various approximations. Unfortunately, the term $\bar{V}_{en}(q,Q)$ prevents us from separating $\hat{H}$ into nuclear and electronic parts, which allows to write the

molecular wave function as a product of nuclear and electronic terms. We thus introduce the Born-Oppenheimer approximation, by which we conclude that this nuclear and electronic separation is approximately correct. The term $\bar{V}_{en}(q,Q)$ is large and cannot be neglected; however, we can make the Q dependence parametric, so that the total wave function is given as $\Psi(q,Q) = \Phi(q;Q)\chi(Q)$. The Born-Oppenheimer approximation bases on the fact that the nuclei are much more massive than the electrons, which allows us to say that the nuclei are nearly fixed with respect to electron motion[15]. We can fix Q, the nuclear configuration, at some value Q_0, and solve for the electronic wave function $\Phi(q;Q_0)\chi(Q_0)$, which depends only parametrically on Q. If we do this for a range of Q, we obtain the potential energy curve along which the nuclei move. The electronic Schrodinger equation is

$$\hat{H}_{el}(q,Q)\Phi_K(q,Q) = W_K(Q)\Phi_K(q,Q) \tag{2.2.6}$$

where $\Phi_K(q,Q)$ depends parametrically on Q. In Born-Oppenheimer approximation it is assumed that the solution of equation 2.2.5 takes the simple product form

$$\Psi_K(q,Q) = \Psi_{ki}(q,Q) = \Phi_k(q,Q)\chi_{ki}(Q) \tag{2.2.7}$$

where $\chi_{ki}(Q)$, the vibrational function is only of nuclear coordinates. In the assumption of fixed nuclear coordinates, $\hat{H}_{el}$ commutes with $\chi_{ki}(Q)$ also $\bar{T}_n(Q)$ approximately commutes with $\Phi_k(q,Q)$. Thus

$$\bar{T}_n(Q)\Phi_k(q,Q)\chi_{ki}(Q) \approx \Phi_k(q,Q)\bar{T}_n(Q)\chi_{ki}(Q) \tag{2.2.8}$$

Equating the above equations gives the Born-Oppenheimer nuclear equation

$$[T_n(Q) + W_k(Q)]\chi_{ki}(Q) = E_{ki}\chi_{ki}(Q) \tag{2.2.9}$$

The Born-Oppenhimer approximation requires that the electronic function $\Phi_K(q, Q)$ is a slowly varying function of nuclear coordinates such that equation 2.2.8 is valid. Equation 2.2.9 express an idea that the nuclei can be viewed as moving in a potential (single potential energy surface) $W_K(Q)$, which can be vibrational, rotational vibrational-rotational or multiplicative potential energy operators of the nuclei.
The formulations in Born-Oppenhimer approximation strictly require a single vibronic function (i). The above discussion requires modification for degenerate states and causes break down of Born-Oppenhimer approximation. From here on different techniques of perturbation and mathematical approximations are used to get the solutions of molecular Schrodinger equation.

2.2.2 Molecular transitions and the Franc-Condon principle

Consider an electronic transition between a specific vibrational electronic state A to a specific vibrational electronic state B. The states are given as

$$|A\alpha i\rangle \equiv \Phi_{A\alpha}(q, Q)\chi_i(Q)|B\beta j\rangle \equiv \Phi_{A\beta}(q, Q)\chi_j(Q) \tag{2.2.10}$$

where the α and β represent the components of electronic states A and B, and i and j label vibrational states associated with A and B. The vibronic transition matrix element of μ_γ, where γ indicates the vector component of electric dipole operator, is expressed as

$$\langle A\alpha i|\mu_\gamma|B\beta j\rangle = \langle \Phi_{A\alpha}(q, Q)\chi_i(Q)|\mu_\gamma(q)|\Phi_{A\beta}(q, Q)\chi_j(Q)\rangle \tag{2.2.11}$$

The Franc-Condon principle states that the electronic transition takes so rapidly in comparison with the vibrational motion, that almost no changes occur in the process[16]. Transitions that incorporate ground vibrational states in both ground and

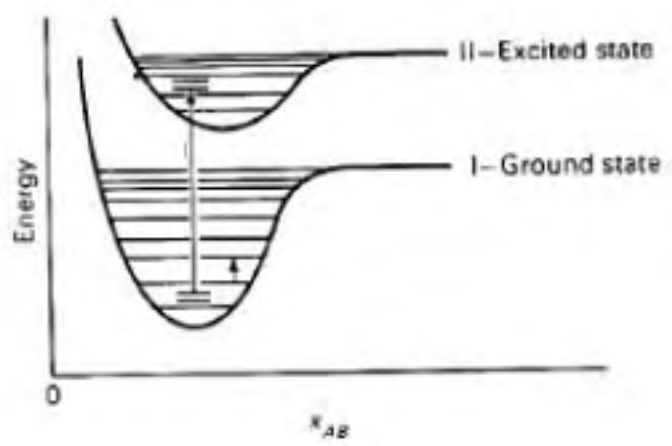

Figure 2.2: *Vibrational transitions in Franc-Condon principle*

excited electronic states are intense and have higher transition dipole moment values, where as transitions between vibronic states of the nuclei in different electronic states have much smaller values, due to motion in excited vibrational states. The transition moment integral can be modified in Franc-Condon principle with an assumption that the electronic functions are slowly varying functions of Q, so that it can be approximated by equilibrium value Q_0 in the ground electronic state.

$$\langle A\alpha i|\mu_\gamma|B\beta j\rangle \approx \langle \Phi_{A\alpha}(q,Q_0)|\mu_\gamma(q)|\Phi_{A\beta}(q,Q_0)\rangle\langle\chi_i(Q)|\chi_j(Q)\rangle \tag{2.2.12}$$

The above equation is called Franc-Condon approximation. Franc-Condon approximation predicts vibrational progression in the electronic dipole transitions. The first term of the left side in equation 2.2.12 is non zero then the transition is known as electric dipole allowed electronic transition. $\langle\chi_i(Q)|\chi_j(Q)\rangle$ is an overlap integral between vibrational wave functions of two electronic states and it governess the band shape in Born-Oppenhimer and Frank-Condon approximation.

2.3 Ligand field theory and d^7 electronic structure

Transition metal atoms are different from other atoms by their partially filled d orbital. Complexsation behaviors in transition metals require a different approach for their explanation. Crystal field theory and ligand field theory are the theories that explain the electronic structures in transition metal complexes. In a metal complex, if perturbation is weak,the ligands are treated as point charges. This kinds of weak interaction of ligands with central metal atoms or ions is studied by crystal field theory. When metal-ligand interactions are strong then the ligands are no more point charges and they are studied by ligand field theory.

Consider weak interaction between ligands and transition metal centers in tetrahedral and octahedral symmetry point groups. Due to the weak interaction the degenerate metal d orbitals (d_{xy}, d_{yz}, d_{zx}, $d_{x^2-y^2}$ and d_{z^2}) will be perturbed and the result is the splitting of the d orbitals. The reason lies on the fact that $d_{x^2-y^2}$ and d_{z^2} orbitals

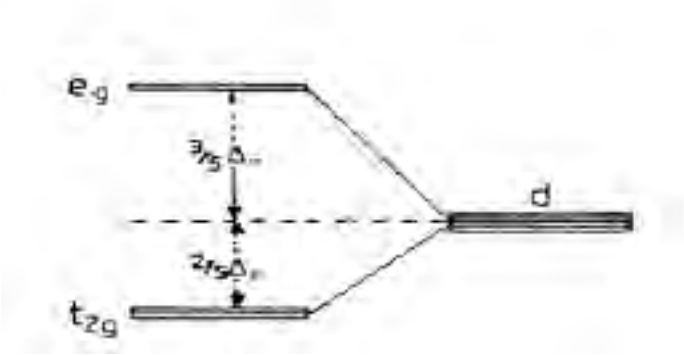

Figure 2.3: *Splitting of d orbitals in octahedral symmetry*

experience much repulsion by ligand electrons rather than d_{xy}, d_{yz} and d_{zx} orbitals[17]. The result is formation of E_g and T_{2g} orbitals with energy difference Δ_o. Δ_o is called crystal field splitting energy. The d orbitals in transition metals can form hybrid

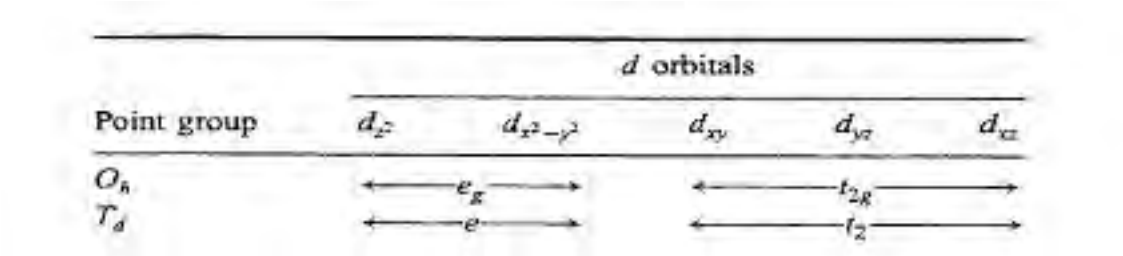

Figure 2.4: *Symmetry species in octahedral and tetrahedral point groups*

orbitals due to strong metal interaction. This phenomena is explained by Russell-Sanders coupling or L-S coupling. L in Russell-Sanders coupling represents the total orbital angular momentum of the electrons and S represents the total spin angular momentum of electrons. As atomic orbitals, the orbitals in Russell-Sanders coupling uses letters S, P, D, F, etc., which represent the corresponding L values of 0, 1, 2, 3, etc,. The Russell-Saunders term symbols for the d^7 configuration in ground and excited states are given by ^4F, ^4P, ^2H, ^2G, ^2F, 2^2D and ^2P. The indices on the orbitals represent the spin multiplicity of the orbitals. d^7 splitting forms a spin doublets and quartets.

For d^7 octahedral and tetrahedral complexes, as in figure 2.5 and 2.6 can be used to interpret the observed electronic absorption spectra[18]. Note that the transitions that occur are dependent on the sizes of Δ and B and the A_{2g} term may be either higher or lower than the $T_{1g}(P)$ term.

The selection rules in transition metals follow $\Delta\zeta = 0$ (Hund's Rule) where $\zeta = 2S+1$ represents spin multiplicity and $\Delta L = +/-1$ the Orbital Rule(Laporte's Rule). The first rule represents that transitions that involve the promotion of electrons from spin multiplate states (singlets ($\zeta = 1$), doublets($\zeta = 2$), triplates($\zeta = 3$), quaretates($\zeta = 4$), etc.,) without a change in their spin multiplicity are allowed transitions. The

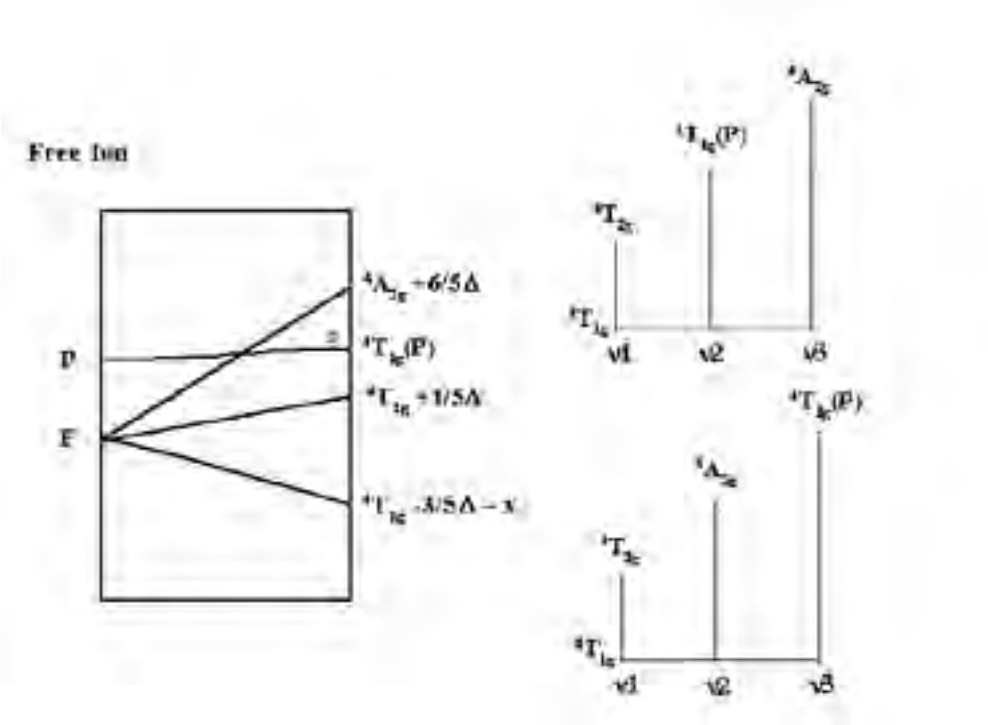

Figure 2.5: *Splitting of ground state* ^4F *and excited state* ^4P *in octahedral* d^7 *ion-complex*

second rule says that transitions within a given set of p or d orbitals are forbidden if the molecule has a center of symmetry. Relaxation of the Rules can occur through spin-orbit coupling, vibronic coupling and π-acceptor and π-donor ligands can mix with the d-orbitals so transitions are no longer purely d-d. Types of transitions in metal complexes are charge transfer from ligand to metal or metal to ligand, which is extremely intense and found in the UV but may have a tail into the visible and $d-d$ transition can occur in both UV and visible, its a weak transition[19]. The hydrated cobalt ion $[Co(H_2O)_6]^{2+}$ has a broad band whose maxima is at $\lambda = 510nm$ (19600 cm^{-1}) in visible region. The $510nm$ band includes two quartet transitions of $^4T_{1g}(P) \leftarrow^4 T_{1g}$ at (19400 cm^{-1}) and $^4A_{2g} \leftarrow^4 T_{1g}$ at (16000 cm^{-1})[20]. The above

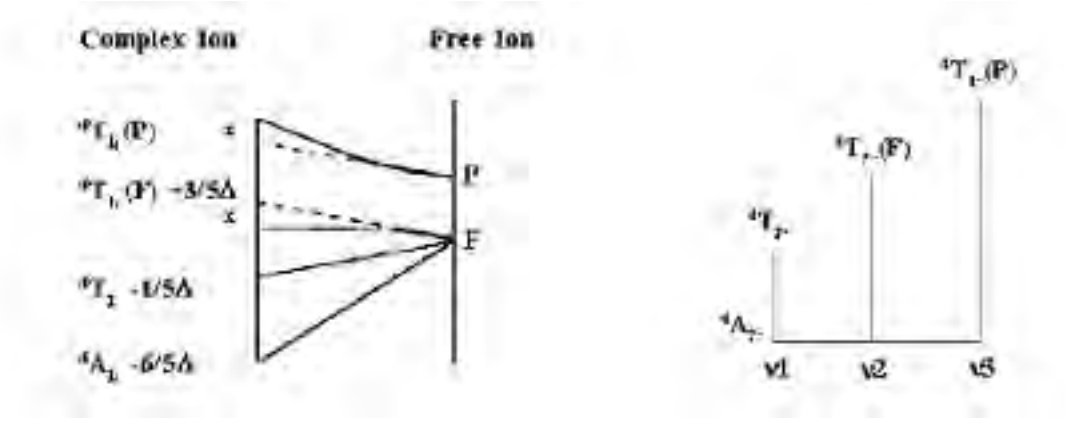

Figure 2.6: *Splitting of ground state* ^4F *and excited state* ^4P *in tetrahedral* d^7 *ion-complex*

transitions signify that $\Delta \approx 9000\ cm^{-1}$ and $B \approx 900\ cm^{-1}$.

2.4 Fluorescence spectroscopy

A property of hot body to emit radiation because of high temperature is known as incandescence. All other forms of light emission are called luminescence. In luminescence energy must have to be supplied to the system. If luminescence occurs due to the absorption of energy in the form of electromagnetic radiation, it is called photoluminescence. The energy in the absorption and emission process of light can only occur for a discreet qantas of energy ($E = h\nu$), where ν represents the frequency of radiation. There are two types of photoluminescence, fluorescence and phosphorescence. In fluorescence emission occurs from singlet electronic excited state to ground singlet state and in phosphorescence indirect process occurs by a conversion from the excited singlet state produced by absorption of energy to a triplet state, which is known as intersystem crossing[21]. Transition from triplet to ground state will be take long time, while the transition from an excited singlet state, for example

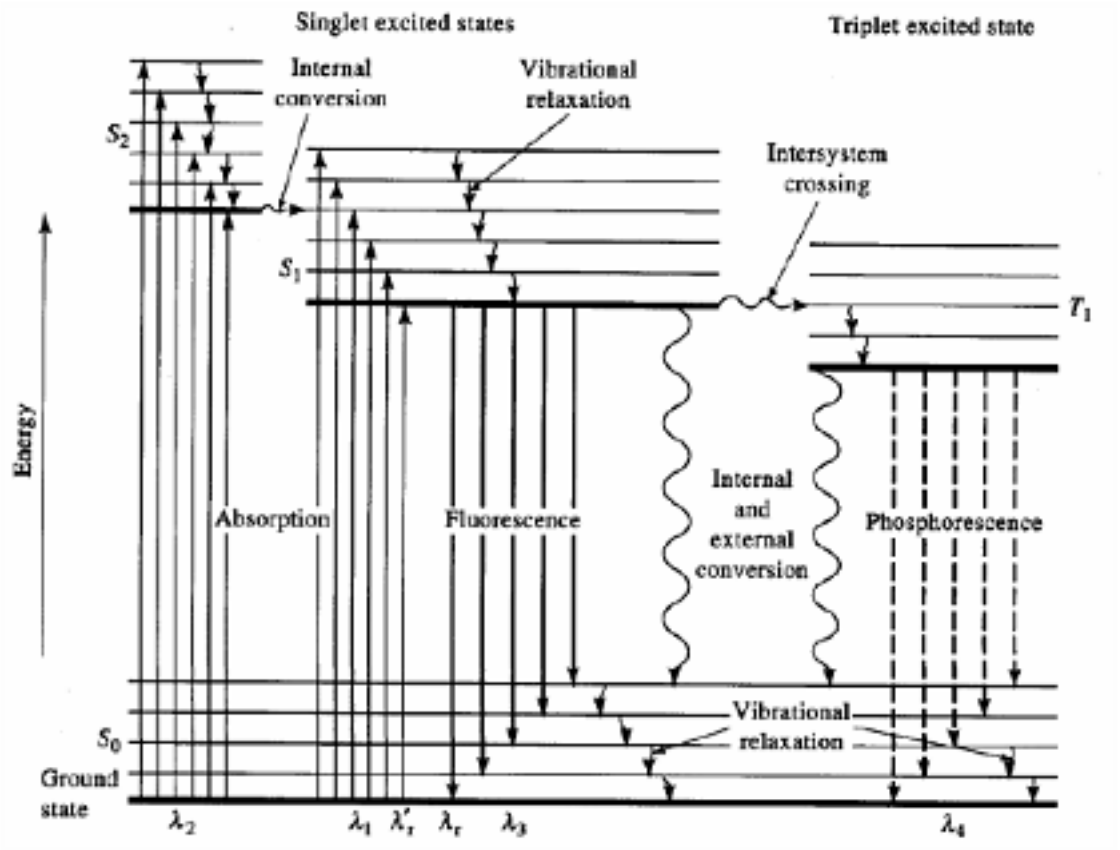

Figure 2.7: *Jablonski diagram showing Fluorescence and Phosphorescence spectral transitions*

S_1 to the ground state S_0 with the emission of fluorescence can take place easily and within $10^{-9} - 10^{-6}$ seconds, the transition from an excited triplet state to the ground state with the emission of phosphorescence requires at least 10^{-4} seconds and may take up to 100 seconds. The fluorescent molecule does not have to relax always by radiative dexcitation but they can also relax through thermal process resulting non-radiative transition. The quality of fluorescent molecule is directly related to the rates of these two decay modes. Let Γ and κ represent the rates of radiative and

non-radiative dexcitations, the life time of the molecule in the excited state S_1 is given by

$$\tau = \frac{1}{\Gamma + \kappa} \tag{2.4.1}$$

In the absence of non-radiative decay only dexcitation through emission of photon is possible. The measured life time is called the intrinsic life time of florescent molecule.

$$\tau_0 = \frac{1}{\Gamma} \tag{2.4.2}$$

The quantum efficiency of florescent molecule is defined by

$$\tau_0 = \frac{\Gamma}{\Gamma + \kappa} \tag{2.4.3}$$

The more the quantum efficiency of the molecule the strong the intensity of fluorescence emission. A transition from the lowest vibrational level in the ground electronic state to the lowest vibrational level in the first excited state, the 0 - 0 transition is common to both the absorption and emission phenomena. Other absorptions require more energy than any transition in the fluorescence emission. We can therefore expect the emission spectrum to overlap the absorption spectrum at the wavelength corresponding to the 0 - 0 transition.

Emission of a molecule for a given excitation wavelength is known as the emission spectrum. The plot of the emission intensity over the excitation wave length at constant emission wave length is called excitation spectrum. The process of absorption does not perturb the distribution of vibrational levels in the excited state, the vibrational levels are similar in both the ground and first excited states. The energy differences between the bands in the emission spectrum will be similar to those in the absorption spectrum and frequently the emission spectrum will be approximate to a mirror image of the absorption spectrum.

The emission of fluorescence always takes place from the lowest vibrational level of the first excited state to the ground state, the shape of the emission spectrum is always the same, despite changing the wavelength of exciting light. The fact that the molecule in the excited state reaches the vibrational ground state long before remission takes place, has the following important consequences[22]. The first one implies that fluorescence spectrum is independent of the exciting wavelengths, it is a characteristic of the molecule. The fluorescence spectrum is red-shifted relative to the absorption spectrum.

In fluorescence excitation or emission spectra three kinds of peaks, the excitation or emission peak, the Rayleigh peak and Raman peak. The incident radiation is scattered in all directions, the scattering takes place either from the molecules themselves (Rayleigh scattering) or from small particles in colloidal suspension (Tyndall scattering). Both types of scattered light are collected together with fluorescence. Rayleigh scattering is strong narrow peak near the excitation wavelength. Some of the incident energy can be abstracted and converted into vibrational and rotational energy. The resulting energy scattered is therefore of lower energy and longer wavelength than the incident radiation. The result is a weak emission, Raman band. Since the amount of energy abstracted is always constant, Raman bands appear separated from the incident radiation by the same frequency difference.

All fluorescence instruments contain three basic items: a source of light, a sample holder and a detector. In addition, to be of analytical use, the wavelength of incident radiation needs to be selectable and the detector signal capable of precise manipulation and presentation. Simple fluorescence spectrometers have a means of analyzing

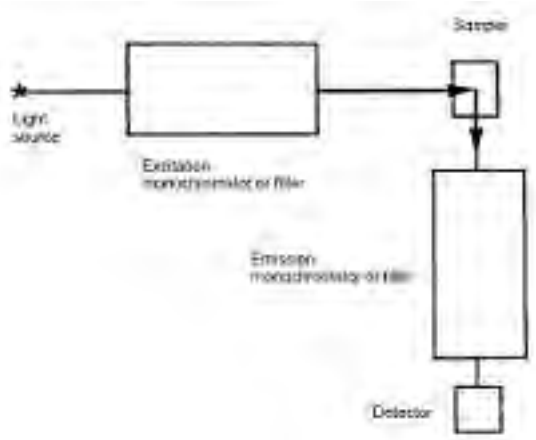

Figure 2.8: *Basic set up of fluorescence spectrometers*

the spectral distribution of the light emitted from the sample, the fluorescence emission spectrum, which may be by means of either a continuously variable interference filter or a monochromator. In more sophisticated instruments, monochromators are provided for both the selection of exciting light and the analysis of sample emission. Such instruments are also capable of measuring the variation of emission intensity with exciting wavelength, the fluorescence excitation spectrum.

Chapter 3

Sample preparation and Experimental procedure

3.1 Sample preparation

Two types of amino acids were used in this project, glycine and D-alanine. The other chemicals used in the experiment are cobalt dichloride hexahydrate and ninhydrin. Cobalt dichloride hexahydrate was checked for its purity using standardization procedure for determination of cobalt using xylenol orange as an indicator[23]. An analytical solution of 0.1 molar each of the two amino acids, ninhydrin and cobalt dichloride hexahydrate were prepared with deionized water. The amino acids formed colorless aqueous solutions, ninhydrin formed yellow solution and cobalt dichloride hexahydrate formed a reddish pink solution.

The following four solutions were prepared as bulk solutions.

- A: 250ml of 0.1 molar cobalt dichloride hexahydrate solution in deionized water.
- B_1: 100ml of 0.1 molar solution of glycine in deionized water.
- B_2: 100ml of 0.1 molar solution of alanine in deionized water.

- C: 200ml of 0.1 molar solution of ninhydrin in deionized water.

The above solution were then mixed as follows.

- D: mixture of 5ml each of solution A with solution C.
- E: mixture of 5ml of each solution A with solutions B_1 and B_2.
- F: mixture of 5ml of each solution A and C with solutions B_1 and B_2.
- G: mixture of 5ml each of solutions B_1 and B_2 with C.

3.2 Measurement and Procedure

Measurement of UV/VIS absorbtion spectra using PerkinElmer UV/VIS/NIR Spectrophotometer Lambda 19 for each solutions were made immediately after preparation, also the UV/VIS spectra of glycine, alanine, ninhydrin and cobalt dichloride hexahydrate were taken. The absorption spectra for each solutions were made after a day or days from preparation. The ninhydrin, glycine and cobalt dichloride hexahydrate solution after days were filtered and the UV/VIS spectra of filtered solution were taken. The precipitation from the filtered solution were dried and its UV/VIS absorption spectra were taken immediately after it was dissolved in DMF. The same procedures were alanine.

The UV/VIS fluorescence spectra of filtered precipitation of ninhydrin, glycine and cobalt dichloride hexahydrate solution in DMF and the filtered solution were measured using Shimadzu spectrofluorophotometer(P/N 206-81601) RF-5301. In both absorption and fluorescence measurements the back ground measurements were run to prevent solvent effects in the corresponding spectral data. The results of the data from the two spectrophotometers will be discussed in the next chapter.

Chapter 4

Results, Analysis and Discussion

Solution F made from mixtures of ninhydrin which is yellow and amino acids (glycine and alanine) which are colorless starts to form a bluish purple color which increases in its depth in time and forms dark purple color over days. Solution E made from the mixtures of amino acids and cobalt dichloride hexahydrate preserves the pink color of cobalt dichloride hexahydrate solution. There is no noticeable change of color in cobalt dichloride hexahydrate and ninhydrin mixed solution D over days of observation. The solution made from the mixtures of solutions of glycine, ninhydrin and cobalt dichloride hexahydrate which is yellow pink forms a faint purple color immediately after preparation, after a day it forms a thin shinny dark green film on the sample holder test tube and the solution attains reddish color both colors will be intensified in depth over days. Alanine also forms a bright green precipitation. Both green precipitations were found to be insoluble in water but are highly soluble in DMF which agrees with literatures.

4.1 Absorption spectra data of different samples

The absorption spectra of glycine and alanine shown in figure 4.1, indicates they have no absorption band in visible region, but they have an intense absorption below 250nm ($40000cm^{-1}$) as sited in [5]. This is found out to be the common properties of simple amino acids. The absorption spectra of ninhydrin shown in figure 4.2.a shows several maxima in ultra violate region and the main maxima is at 230nm ($43200cm^{-1}$) as sited in [8]. The absorption spectra of cobalt dichloride hexahy-

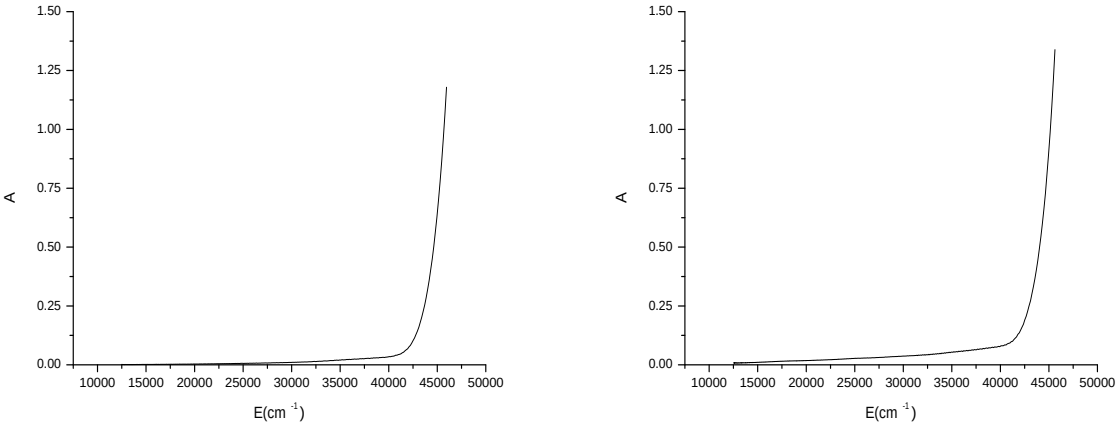

Figure 4.1: *Absorption Spectra of glycine and alanine in water solution*

drate in water shown in figure 4.2.b have broad band emission in visible range whose maxima is at 515nm ($19600cm^{-1}$). The spectra of cobalt dichloride hexahydrate shows three local peaks at $16000cm^{-1}$, $19600cm^{-1}$ and $21000cm^{-1}$ shows corresponding transitions of ${}^4A_{2g} \leftarrow {}^4T_{1g}$, ${}^4T_{1g}(P) \leftarrow {}^4T_{1g}$ and the spin orbit coupling allowed transition[19-21]. Figure 4.3 shows solution D and E made no considerable change. The absorption spectra of solution D of ninhydrin and cobalt shows the $19600cm^{-1}$ and $21000cm^{-1}$ bands of cobalt dichloride hexahydrate and ninhydrin peaks and

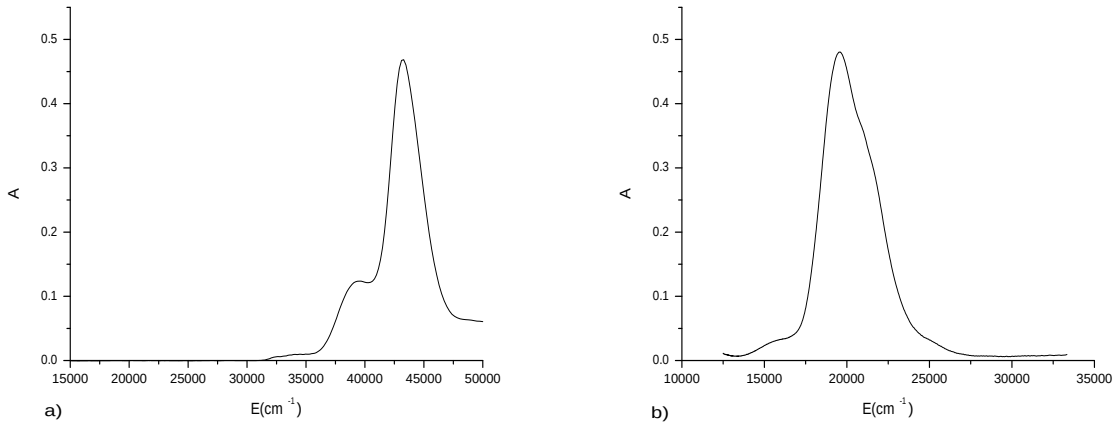

Figure 4.2: *a) Absorption Spectra of ninhydrin and b) Absorption Spectra of cobalt dichloride hexahydrate in water solution*

stretch below 25000cm^{-1}, this implies that there is less interaction between ninhydrin and Co^{2+} ions and they have not formed considerable amount of new compound. Also the spectra has shown no considerable change over days after its preparation. The absorption spectra of solution E of glycine and cobalt dichloride hexahydrate only adds the visible range absorption bands of cobalt dichloride hexahydrate (16000cm^{-1}, 19600cm^{-1} and 21000cm^{-1}) since glycine had no observed maxima in visible range. This shows that there is no considerable chemical reaction has been undergone between glycine and Co^{2+}. The absorption spectra of solution F of glycine, ninhydrin and cobalt dichloride hexahydrate shows a new property. The solution had shown a color change after it was prepared. The absorption spectra immediately after its preparation has two maxima in visible range, at 400nm (25000cm^{-1}) and 570nm (17540cm^{-1}). It also has intense absorption in ultra violate region below 300nm. Its absorption spectra after a day shows two bands in visible range but there are two resolved peaks in visible the first 400nm (25000cm^{-1}) peak dominated by new

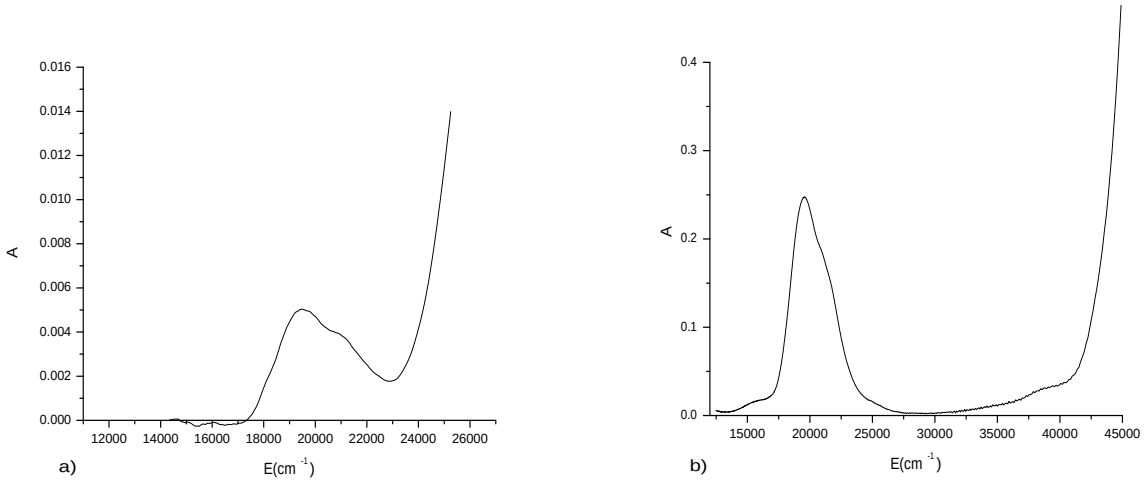

Figure 4.3: *a) Absorption Spectra of cobalt dichloride hexahydrate solution mixture with the solution of ninhydrin (D) and b) Absorption Spectra of cobalt dichloride hexahydrate solution mixture with the solution of glycine (E)*

370nm ($27000cm^{-1}$) band. The comparison of area normalized absorption spectra taken immediately and after a day were the solution were shown in figure 4.4. From the comparison the only thing happened in the spectra is the intensification of 370nm ($27000cm^{-1}$) peak. From the figure it can be seen that the cobalt dichloride hexahydrate peaks ($16000cm^{-1}$, $19600cm^{-1}$ and $21000cm^{-1}$) are completely disappeared. Solution F of glycine, ninhydrin and cobalt dichloride hexahydrate were filtered using standard filter paper after days from its preparation. The filtered green precipitation (shiff base complex) is dissolved in DMF. Filtered red solution (Ruhemann's purple complex) has the same absorption peaks at 400nm and 570nm spectra with solution F of glycine, ninhydrin and cobalt dichloride hexahydrate as one expects because the green precipitation of the solution is water insoluble. The absorption spectra of DMF solution of the shiff base complex has two absorption maxima at 420nm ($23810cm^{-1}$) and 695nm ($14390cm^{-1}$). The absorption spectra of mixed solutions of glycine and

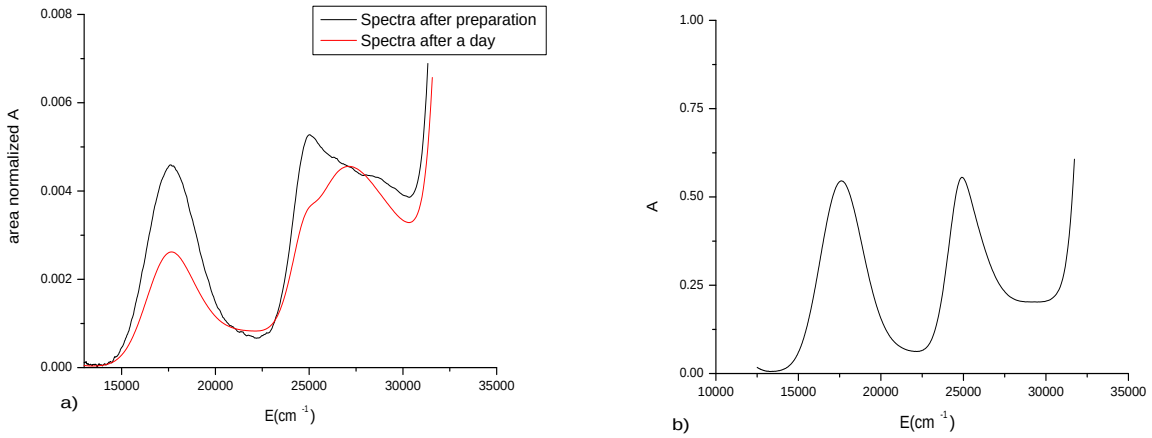

Figure 4.4: *a) Absorption Spectra of the mixture of glycine, ninhydrin and cobalt dichloride hexahydrate solutions (F) and b) Ruhemann's purple (G)*

ninhydrin that formed deep purple color were found to be at 400nm ($25000cm^{-1}$) and 570nm ($17540cm^{-1}$) which is the characteristic absorption of Ruhemann's purple. The peaks at 400nm ($25000cm^{-1}$) and 570nm ($17540cm^{-1}$) in glycine, ninhydrin and cobalt dichloride hexahydrate can be represented as the absorption bands of Ruhemann's purple.

The same measurements for alanine based samples were made and all solutions showed no spectral change compared to their components except the alanine, ninhydrin and cobalt dichloride hexahydrate solution. The solution has an absorption maxima at 335nm ($29850cm^{-1}$), 400nm ($25000cm^{-1}$) and the broad band 560nm ($17860cm^{-1}$). The DMF solution of the green precipitation (alanine derived schiff base Co(II) complex) has absorption maxima of relatively sharp peak at 420nm ($23809cm^{-1}$) and a broad band peak maximum at 600nm ($16666cm^{-1}$).

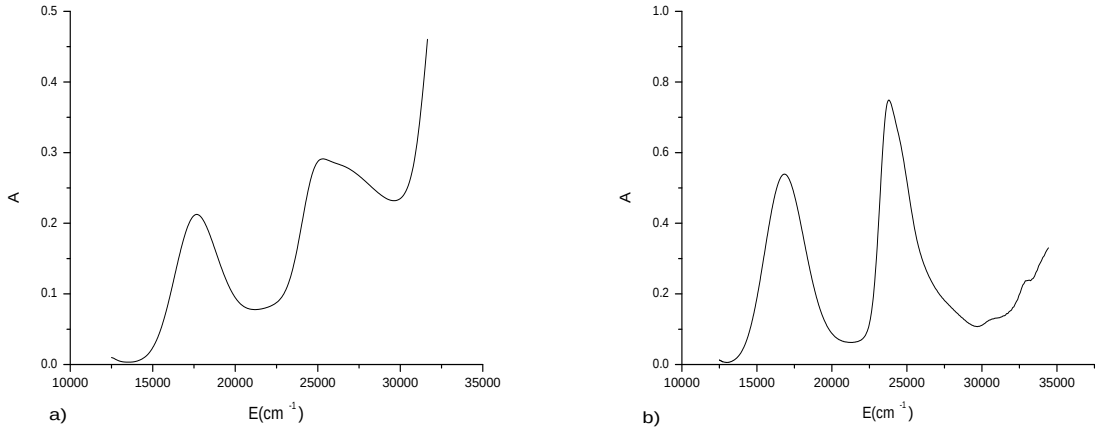

Figure 4.5: *a) Absorption Spectra of the mixture of filtered glycine, ninhydrin and cobalt dichloride hexahydrate solutions (F) and b) Absorption Spectra of green precipitation of (F) in DMF*

4.2 Data of fluorescence spectra of samples

The fluorescence spectra of the filtered red solution of solution F and green precipitation of solution F in DMF solution were taken, in both cases the spectra were found to contain two emission maxima and indicates the existence of an iso-emission point. The existence of iso-emission point implies two structures exist at equilibrium in the solution. The different possible structures of the ligands, shown in figures 5.1 - 5.2, may have affected their combination forming different emission maxima. The Ruhemann's complex shows two fluorescence maxima at points of 420nm ($23810cm^{-1}$) and 490nm ($20410cm^{-1}$). Scanning using excitation wave lengths from 280nm to 310nm shows an increment of 420nm ($23810cm^{-1}$) emission band and a decrease in 490nm ($20410cm^{-1}$) emission band.

In the scanned wavelengths from 320nm to 390nm had shown a decrease in 420nm ($23810cm^{-1}$) emission band and an increase in 490nm ($20410cm^{-1}$) emission band.

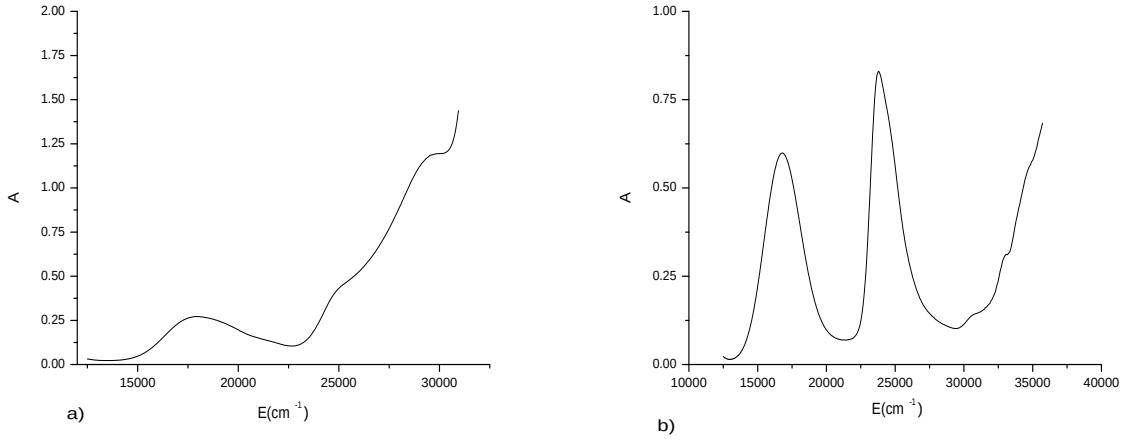

Figure 4.6: *a) Absorption Spectra of filtered mixture of alanine, ninhydrin and cobalt dichloride hexahydrate solution (F) and b) Absorption Spectra of green precipitation of (F) alanine shiff base complex in DMF*

The above observation defies a fact that, if two structures A and B exist at equilibrium ($A \rightleftharpoons B$) there should be a continuous increase and decrease in the emission bands A and B as we scan through the emission bands by varying excitation wavelengths. The possible suggestion can be the fluorescent structures of Ruhmann's purple- Co^{2+} complexes in water solution contain an equilibrium structural transition of ($A \rightleftharpoons B \rightleftharpoons A$). This proposition recognizes the two equilibria structures of Ruhmann's purple-Co^{2+} complexes have a form ($A \rightleftharpoons B \rightleftharpoons A$) and are responsible for the discussed change in the fluoresce emission spectra as proposed in figure 1.12. The proposed fluorescence transition is ${}^4T_{1g}(P) \rightarrow {}^4T_{1g}$ in octahedral structure of cobalt(II) with two tridentate ligands of Ruhmann's purple forming at list to possible structures.

The fluorescence spectra of glycine derived shiff base in DMF solution shows two fluorescence emission bands in the ultra violate region. One of the peaks is not observed much because the instrument uses xenon lamp which is unable to produce ultraviolet

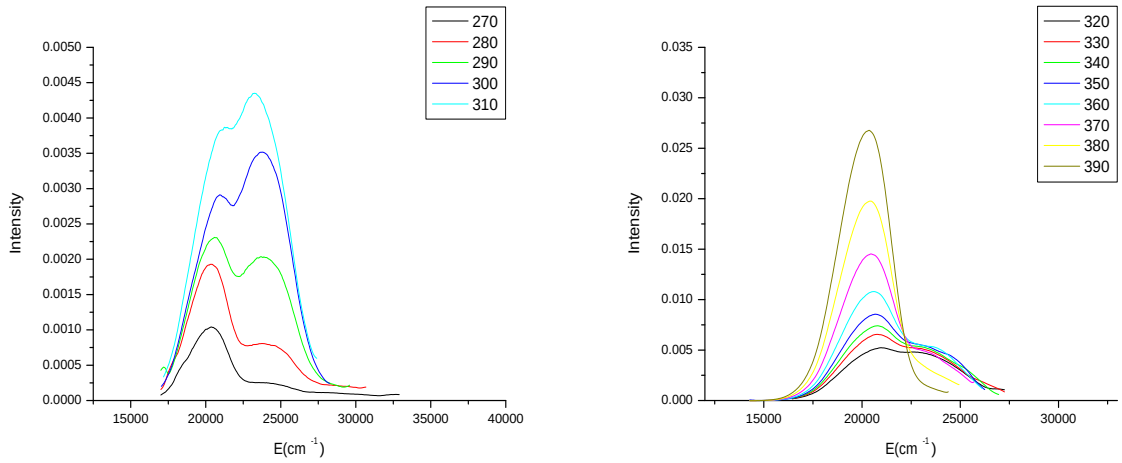

Figure 4.7: *Area normalized fluorescence spectra using excitation wave lengths from 270nm to 310nm and 320nm to 390nm*

light below 260nm[22]. The well resolved fluorescence maxima is observed at 350nm ($28570cm^{-1}$). The spectra also shows isoemission point indicating the existence of equilibrium structures. The suggested possible structures were given in figure 1.11. The fluorescence transitions is far pushed to ultra violate region, so transition from the $^4T_{1g}$ to higher quartette states may have been taken place.

4.3 Comparison of fluorescence and absorption spectra

It is a theoretically correct that absorption and excitation spectra have the similar band shape. The fluorescence emission spectra and the excitation spectra are mirror image to each other. The excitation of Ruhmann's purple Co^{2+} complex has maxima at 355nm ($28170cm^{-1}$) and 420nm ($23810cm^{-1}$), which is expected due to

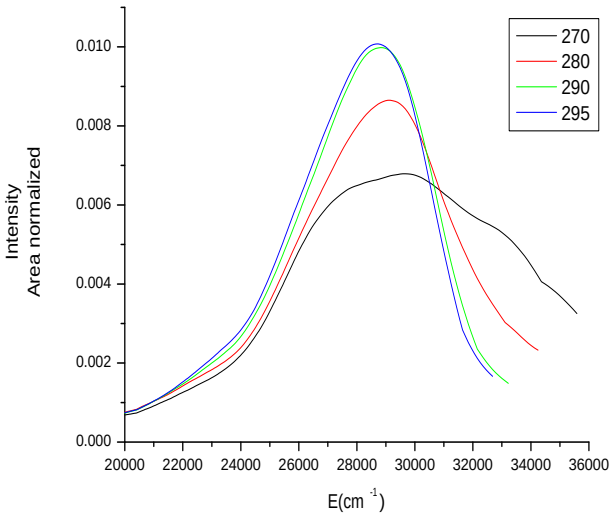

Figure 4.8: *Fluorescence spectra of glycine derived shiff base*

two fluorescence emission bands. The excitation spectra at 355nm ($28170cm^{-1}$) corresponds to 420nm ($23810cm^{-1}$) fluorescence band and the excitation spectra at 420nm ($23810cm^{-1}$) corresponds to 490nm ($20410cm^{-1}$) emission band. It was also observed that the emission bands and excitation bands are approximately mirror images to each other.

The excitation spectra of glycine shiff base Co^{2+} complex as in figure 4.10 shows an excitation maxima at 300nm ($33333cm^{-1}$) taking in account that the other band was out of the detection limit of the instrument.

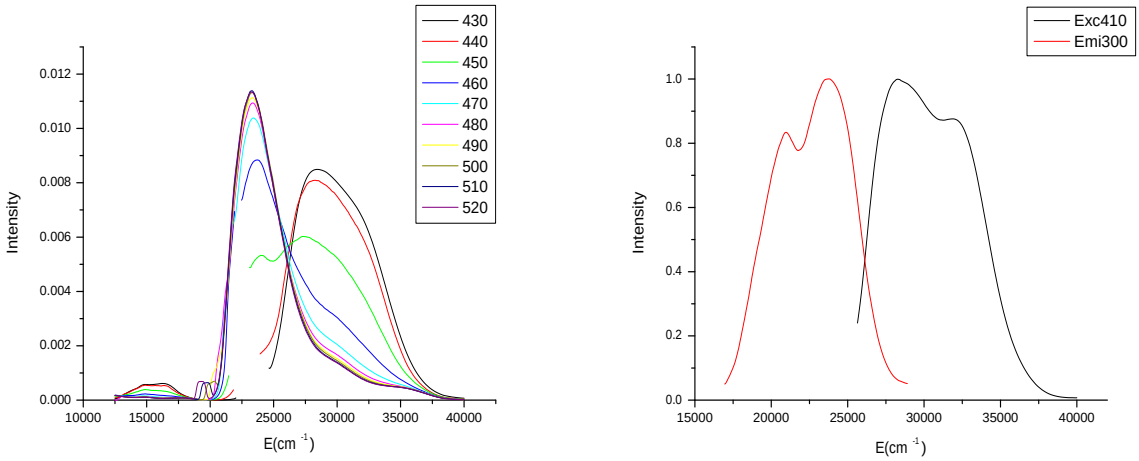

Figure 4.9: *The excitation spectra of Ruhmann purple Co^{2+} complex and the comparison of emission and excitation spectra*

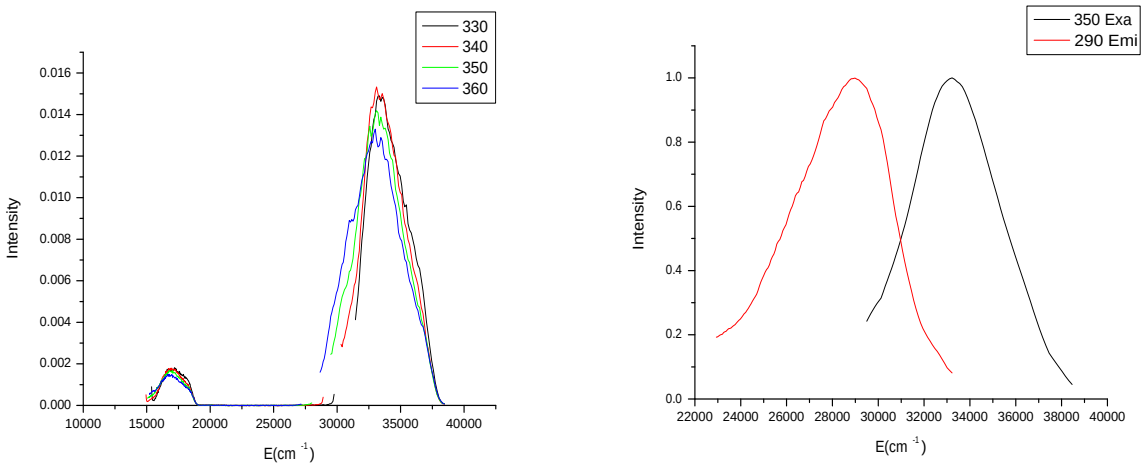

Figure 4.10: *The excitation spectra of glycine derived shiff base Co^{2+} complex and the comparison of emission and excitation spectra*

Chapter 5

Conclusion and Recommendations

5.1 conclusion

Complexes of Co^{2+} were prepared from reaction derivatives of amino acids (glycine and alanine) with ninhydrin. It was also observed that Co^{2+} ion can form two kinds of complexes, with shiff base and Ruhmann's purple. The shiff base complex were found to form a green precipitation insoluble in water for both glycine and alanine. The Ruhmann's complex were found out to be red colored and found out to have dual fluorescence emission peaks at 420nm ($23810cm^{-1}$) and 490nm ($20410cm^{-1}$) were observed, implying two structures of Ruhmann's complex at equilibrium. The shiff base complex has strong fluorescence in ultra violate range at 350nm ($28570cm^{-1}$) and the shiff base also has an isoemission point, certifying the existance of two equilibrium structures. The probable structure of suggested as in figure 5.1 and 5.2. Possible fluorescence emission transition ${}^4T_{1g}(P) \rightarrow {}^4T_{1g}$ were suggested. The shiff base complex of alanine were also prepared and found to have the similar absorption band with that of the shiff base complex of glycine.

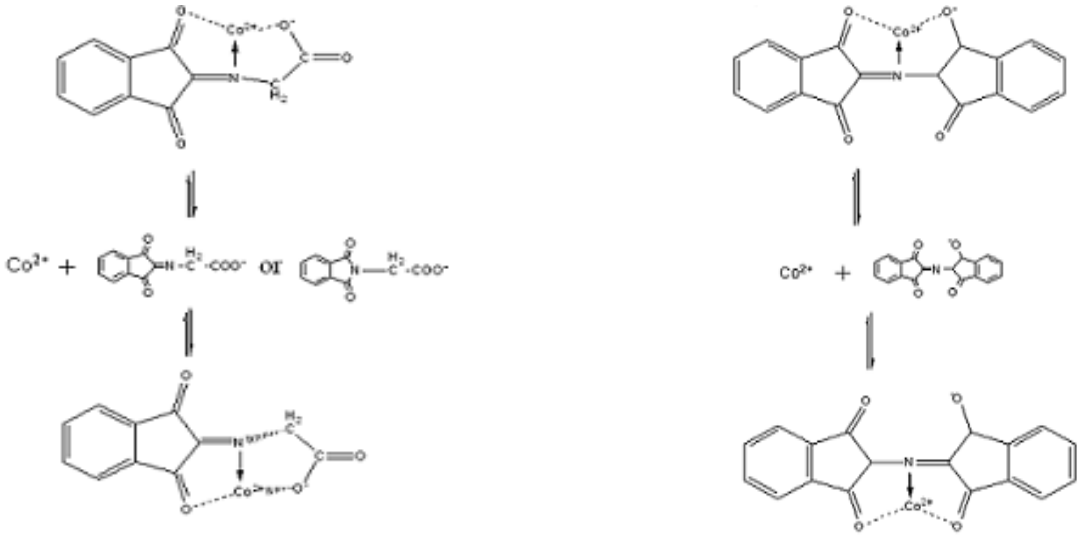

Figure 5.1: *Possible structures of glycine derived shiff base Co(II) complex in solution form and Ruhemann's Co(II) complex in water solutions*

5.2 Recommendations

Fluorescence property of the Co^{2+} complexes of the shiff base and the Ruhmann's purple can be studied using different solvents and pH variations. Additional experiments of appropriate type should be done to obtain the exact equilibrium structures of the complexes which is confirmed by their fluorescence spectra.

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