CHAPTER - I

INTRODUCTION

1.0 INTRODUCTION

Photochemistry is concerned with reactions which are initiated by electronically excited molecules. Such molecules are produced by absorption of suitable radiation in the visible and near UV region of the spectrum. Photochemistry is basic to the world we live in. With sun as the central figure, the origin of life itself must have been a photochemical act.

The photochemistry of biological reactions is a rapidly developing subject and helps the understanding of phenomena like photosynthesis, vision and mutagenic effects of light. In doing so, it tries to integrate knowledge of physics, chemistry and biology.

In photochemistry various important photophysical behaviours are observed like fluorescence, phosphorescence etc. which basically belongs to the term luminescence. Luminescence is the emission of light by a substance. It occurs when an electron returns to the electronic ground state from an excited state and loses its excess energy as a photon. Luminescence spectroscopy is a collective name given to three related spectroscopic techniques. They are;

a) Molecular fluorescence spectroscopy

b) Molecular phosphorescence spectroscopy

c) Chemiluminescence spectroscopy.

Fluorescence occur in molecules as a result of and subsequent to a series of physical phenomena, normally beginning with the absorption of light. These phenomena as well as fluorescence and phosphorescence are derived from the electromagnetic nature of light, the details of molecular structure (especially molecular electronic structure) and the nature of the environment of the luminescent molecule. It should be fairly obvious that an appreciation of these phenomena is necessary for the understanding of the relationship between molecular structure and luminescence spectroscopy to chemical and biological problems.

Fluorescence (the name originates from the fluorescent mineral fluorspar) refers to cold light emission (luminescence) by electron transfer in the singlet state when molecules are excited by photons. The electromagnetic

phenomenon, fluorescence is a three-stage process that occur in certain molecules called fluorophores or fluorescent dyes. First, the fluorophore is excited to an electronic singlet state by absorption of an external photon. Second, the excited state undergoes conformational changes and interacts with the molecular environment in a number of different ways, including vibrational relaxation, quenching and energy transfer. Third, a photon is emitted at a longer wavelength, while the fluorophore returns to its ground state. The difference in energy or wavelength between the absorbed and the emitted photon is called the Stokes shift. The fluorescence excitation and emission of light typically occurs within nanoseconds and is independent of temperature. A similar, but slower luminescence phenomenon is phosphorescence in which the photon goes through an intermediate excited triplet state where the afterglow lasts longer than microseconds and which is temperature dependent. The molecular structure and environment is decisive for whether a compound is fluorescent. Fluorescence is often exhibited by organic compounds with rigid molecular skeletons, usually polyaromatic hydrocarbons and heterocycles. The less vibrational and motional freedom in the molecule, the greater the possibility that the difference in energy between the excited singlet state and the ground electronic state is sufficiently large that deactivation by fluorescence will occur. Fluorescence is unique among spectroscopic techniques, because it is inherently multidimensional.

A fluorophore needs a specific level of energy to be excited, and the subsequent emission energy corresponds to the difference between the excited and ground electronic singlet states. Each electronic state has several associated vibrational levels, which implies that excitation does not occur at only one single wavelength, but rather over a distribution of wavelengths corresponding to several vibrational transitions. While the deactivation of the excited state only occurs from the vibrational ground level, emission also occurs at several wavelengths as it may reach different vibrational levels in the electronic ground state. The result is that all fluorophores have independent and specific spectral excitation and emission profiles characterizing their unique fluorescent

properties. These profiles can be measured as excitation and emission spectra or as a complete excitation-emission matrix (EEM), also known as fluorescence landscapes. Besides the high specificity of fluorescence spectroscopy, the Stokes shift is fundamental to the sensitivity of the fluorescence measurements. The Stokes shift, which in contrast to absorption spectroscopy, allows for emission of photons to be detected against a low background, combined with efficient detectors in the visual range makes fluorescence spectroscopy a very sensitive analytical method with possibilities to measure down to parts per billion levels.

Fluorescence spectroscopy is one of the most widely used spectroscopic techniques in the fields of biochemistry and molecular biophysics today. Although fluorescence measurements do not provide detailed structural information, the technique has become quite popular because of its acute sensitivity to changes in the structural and dynamic properties of biomolecules and biomolecular complexes. Like most biophysical techniques, fluorescence spectroscopic studies can be carried out at many levels ranging from simple measurement of steady-state emission intensity to quite sophisticated time resolved studies. The information content increases dramatically as various fluorescence observables are time resolved and combined in global analyses of the phenomena of interest. Nonetheless, quite a good deal of information is available from steady-state measurements for which the requirements in instrumentation are quite modest. Consequently steady-state fluoremeters are routinely used to measure complexation and conformational phenomena of biological molecules.

For more detailed treatment of fluorescence techniques, the four basic rules of fluorescence should be covered. These are as follows:

1. The Franck- Condon principle: The nuclei are stationary during electronic transitions and so excitation occurs to vibrationally excited levels of the excited electronic state.

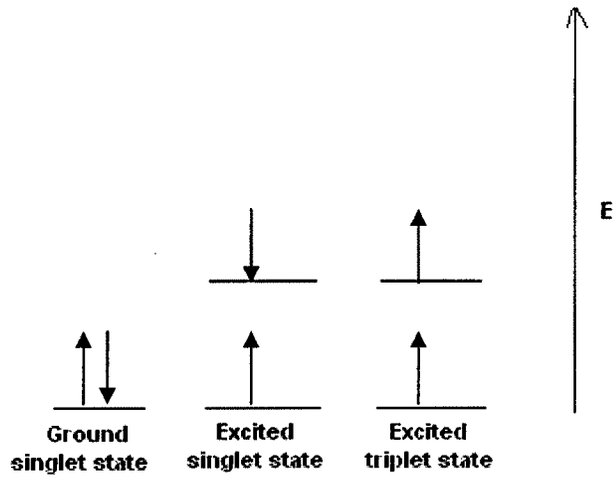
2. Emission occurs from the lowest vibrational level of the lowest excited singlet state because relaxation from the excited vibrational levels is much faster than the emission.

3. The Stokes shift: Emission is always of lower energy than absorption due to nuclear relaxation in the excited state.

4. The mirror image rule: Emission spectra are mirror images of the lowest energy absorption band.

1.1 Fluorescence and Phosphorescence (Photoluminescence)

The electronic states of most organic molecules can be divided into singlet states and triplet states;



Singlet state: All electrons in the molecules are spin paired.

Triplet state: One state of electron spins is unpaired.

1.1.1 Fluorescence:

Absorption of UV radiation by a molecule excites it from a vibrational level in the electronic ground state to one of the many vibrational levels in the electronic excited state. This excited state is usually the first excited singlet state. A molecule in a high vibrational level of the excited state will quickly fall to the lowest vibrational level of this state by losing energy to other molecules through collision. The molecule will also partition the excess energy to other

possible modes of vibration and rotation. Fluorescence occurs when the molecule returns to the electronic ground state, from the excited singlet state, by emission of a photon. If a molecule which absorbs UV radiation does not fluoresce it means that it must have lost its energy some other way. These processes are called radiationless transfer of energy. The emission rates of fluorescence are typically 10^8 S^{-1}, so that a typical fluorescence lifetime is near 10 ns (10×10^{-9} S).

The quantum of energy absorbed or emitted (ΔE) given by Planck's relation,

$$\Delta E = \frac{h.c}{\lambda} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where,

h = Planck's constant

c = Velocity of light

λ = Wavelength

The energy emitted as fluorescence is less than the light energy absorbed and therefore, the wavelength of fluorescence is longer than that of the absorbed light.

Types of fluorescence:

The fluorescence is observed as long as excitation is in process and is known as steady state or prompt fluorescence. When excitation is cut off, fluorescence ceases. However, in some cases even after cutting of source of excitation, the emission of light persists as a glow and is known as delayed fluorescence.

E- type delayed fluorescence:

It is observed when the singlet-triplet energy gap (ΔE) is fairly small as in the case of dye molecules [1]. This type of fluorescence is first observed in eosin. Therefore it is referred as E-type delayed fluorescence. Similar fluorescence was subsequently observed from dyestuffs in fluid solutions [2].

The molecules, initially excited to the singlet energy level, cross over to the triplet level by intersystem crossing mechanism. After vibrational relaxation, if ΔE is small, some of the molecules may be again promoted with the help of thermal energy from the surrounding to the isoenergetic point and cross back to the first excited singlet, subsequently returning to the ground state by radiative process.

P-Type Delayed Fluorescence:

It is so called because it was first observed in pyrene and phenanthrene solutions. In aromatic hydrocarbons singlet-triplet splitting is large and therefore thermal activation to excited singlet state at room temperature is not possible. In such cases lowest excited singlet are formed in triplet-triplet annihilation process. The emission of light occurred from lowest excited singlet during the deactivation in the P- type delayed fluorescence [1,2].

***1.1.2 Phosphorescence*:**

A molecule in the excited triplet state may not always use intersystem crossing to return to the ground state. It could loose energy by emission of photon. Triplet / singlet transition is much less probable than a singlet / singlet transition. The lifetime of the excited triplet state can be up to 10 sec., in comparison with 10^{-5}sec to 10^{-8} sec. average lifetime of an excited singlet state. Emission from triplet / singlet transitions can continue after initial irradiation. Internal conversion and other radiationless transfer of energy compete so successfully with phosphorescence that it is usually seen only at low temperatures or in highly viscous media.

The radiative transition from the lowest triplet to the ground singlet state is longer lived than fluorescence because of the spin forbidden ness (low probability) of the former and is called phosphorescence. Phosphorescence is, like fluorescence, most likely to occur in molecules having restricted vibrational freedom and is thus most often observed in aromatic molecules and their derivatives. However, all aromatic molecules are not phosphorescent because triplet states are so long lived (10^{-5} to several sec.) chemical and

physical processes in solution as well as internal conversion compete effectively with phosphorescence for deactivation of the lowest excited triplet state. Except for the shortest lived phosphorescences, collisional deactivations by solvent molecules. Quenching by paramagnetic species, photochemical reactions and energy transfer processes preclude the observation of phosphorescence in fluid media. Rather, phosphorescence is normally studied in the glassy state at liquid nitrogen temperature, in solution in very viscous liquids where collisional processes can not completely deactivate the triplet state and in low pressure gases.

Phosphorescence usually originates from the lowest vibrational levels of the ground state. Thus the structure of the phosphorescence band represents the vibrational structure of the ground state and the separations between vibronic features in the phosphorescence spectrum can usually be matched with peaks in the infrared or Raman spectra of the phosphorescing molecule, corresponding to normal vibrations of the ground state molecule.

1.1.3 Jablonski Diagram :

The processes that occur between the absorption and emission of light are usually illustrated by Jablonski diagram. Jablonski diagrams are often used as the starting point of discussing light absorption and emission. They are used in variety of forms, to illustrate various molecular processes that can occur in excited states. These diagrams are named after Professor Alexander Jablonski, who is regarded as the father of fluorescence spectroscopy because of his many accomplishments, including descriptions of concentration depolarization and defining the term "anisotropy" to describe the polarized emission from solutions.

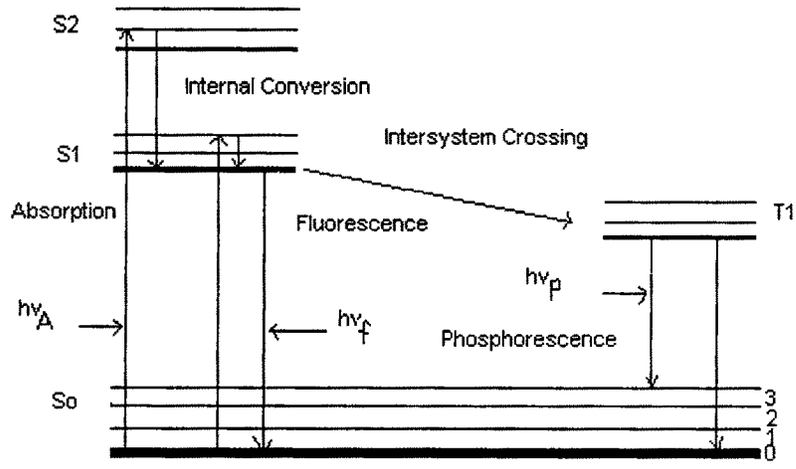


Fig.1.1 Jablonski diagram.

A typical Jablonski diagram is shown in figure 1.1. The singlet ground, first and second electronic states are depicted by S_0, S_1 and S_2 respectively. At each of these electronic energy levels, the fluorophores can exists in a number of vibrational energy levels, depicted by 0,1,2 etc.

In this Jablonski diagram we excluded a number of interactions such as, quenching, energy transfer and solvent interactions. The transitions between states are depicted as vertical lines to illustrate the instantaneous nature of light absorption. Transitions occurs in about 10^{-15} S, a time too short for significant displacement of nuclei. This is the Franck-Condon principle.

At room temperature thermal energy is not adequate to significantly populate the excited vibrational states. Absorption and emission occur mostly from molecules with the lowest vibrational energy. The larger energy difference between the S_0 and S_1 excited states is too for thermal population of S_1. For this reaction, we use light and not heat to induce fluorescence.

Fluorescence is competed by many non-radiative processes, which are illustrated with the help of Jablonski diagram shown in figure. It is important to consider all processes, which occur after photon absorption by a molecule and their relationship to luminescence.

a) Vibrational relaxation (VR):

This process is so efficient that the average lifetime of a vibrationally excited molecule is 10^{-12} sec. or less, a period significantly shorter than the average lifetime of an electronically excited state.

On absorption of energy the molecules are excited to different vibrational levels of excited singlet state. The deactivation of molecule from same vibrational level to which they are excited occurs only in gaseous state. However, in case of solids and solutions the excited molecules do not give emission from the same vibrational level.

But they have to fall into lowest vibrational level of the excited state. This process of loss of energy as a heat is the vibrational relaxation. Thus in this non-radiative process, the molecule fall into the lowest vibrational level of an excited states and then emission occurs [Kasha Rule]. As a result, fluorescence from solution, when it occurs, always involves a transition from the lowest vibrational level of an excited state [2,3]. A consequence of the efficiency of vibrational relaxation is that, the fluorescence band for a given electronic transition is displaced toward lower frequencies or longer wavelengths from the absorption bands (the Stokes shift).

b) Internal conversion (IC) :

The term internal conversion describes intermolecular processes by which a molecule passes to a lower energy electronic state without emission of radiation. Once an excited molecule has relaxed to the lowest vibrational level of the electronically excited state, it can dissipate excitation energy only by going to lower electronic energy level. This can be achieved in three possible ways,

1. If there is a considerable overlap between the lower vibrational levels of the higher electronic state and higher vibrational levels of the lower electronic states then the upper and lower electronic states will be in a transient thermal equilibrium which will allow population of the electronic state. Finally the

molecule crossover from a higher to a lower excited singlet state by this vibrational coupling.

2. If there is no considerable overlap but they separated by a small gap, the molecule in the upper electronic state will convert to the lower electronic state by tunneling mechanism.

3. If the energy separations of the upper and lower electronic states are relatively large, the radiative transition takes place to any one of a number of vibrational levels of the lower electronic state. This radiative transition is nothing but the fluorescence.

This process is very rapid, taking about 10^{-12} sec. The average lifetime of the lowest excited singlet state is the order of 10^{-8} sec. Therefore even if a molecule can not pass efficiently from its lowest excited singlet state to the ground state, it may undergo other processes which may compete with fluorescence [4].

c) External conversion (EC) :

Collisional deactivation:

Deactivation of an excited electronic state may involve interaction and energy transfer between the excited molecule and the solvent or other solutes. These processes are called collectively external conversion or collisional quenching. Evidence for external conversion includes the marked effect upon fluorescence intensity exerted by the solvent, furthermore, those conditions that tend to reduce the number of collisions between particles generally lead to enhanced fluorescence [4].

d) Intersystem crossing (ISC):

The process in which the vibrational coupling between energy states of the different multiplicity population of triplet state by direct absorption from the ground state in many aromatic molecules occurred within the lifetime of an excited singlet state (10^{-8}sec.). This process is spin dependent internal conversion which is referred to intersystem crossing. For the efficient population of triplet state, molecule should have to satisfy the condition as the

energy difference between the lowest singlet state and the triplet state just below it must be small and vibrational coupling should be more between the excited singlet state and triplet state. In aromatic hydrocarbons where singlet-triplet splitting is large, the ISC is less efficient than in certain dye molecules where triplet-triplet splitting is small [5].

Once ISC has occurred, the molecule undergoes the usual IC process and falls to the lowest vibrational level of the first triplet state. Therefore, ISC can compete with fluorescence and thus it decreases the quantum efficiency of fluorescence. The population of triplet state has a significance in producing delayed fluorescence and phosphorescence which is a radiative decay of triplet state molecule to the ground state.

1.2 Factors affecting fluorescence

Several phenomena reduced to the nature of food sample as well as the concentration and the local molecular environment of the inherent fluorophores will influence the fluorescence signal obtainable from biological samples. The effect of quenching, the concentration and the molecular environment of fluorophores plus scatter phenomena will be briefly discussed in the following.

1.2.1 Fluorescence quenching:

The phenonmenon of decrease in intensity of fluorescent compound at its λ_{max} is known as fluorescence quenching. When one compound diminishes the fluorescence of another, it is said to quench the fluorescence. The quenching of one compound by another is often unpredictable [6,4,7,8].

Quenching of fluorescence can occur by,

a) inner filter effect, b) energy degradation, c) chemical change, d) energy transfer, e) electron transfer.

Fluorescence quenching refers to any process which decreases the fluorescence intensity of a sample, a deactivation of the excited molecule by either intra or intermolecular interactions. Quenching is either static or dynamic. Static quenching occurs when formation of the excited state is inhibited due to a ground –state complex formation in which the fluorophore

forms nonfluorescent complexes with a quencher molecule. Dynamic or collision quenching refers to the process when a quencher interferes with (deactivates) the behavior of the excited state after its formation. The excited molecule will be deactivated by contact with other molecules or by intra or intermolecular interactions. Higher temperatures as increase collisional quenching due to the increased velocities of the molecules. Resonance energy transfer can be considered as a kind of dynamic quenching. Resonance energy transfer occurs when the emission spectrum of a fluorophore overlaps with the absorption spectrum of an acceptor molecule. The energy transfer does not involve emission of light, but rather a direct interaction between the donor and acceptor molecule, leading to a full or partial deactivation of the excited fluorophore (donor).

In quenching process, the molecules are not chemically altered. For collisional quenching the decrease in intensity is described by the well known Stern-Volmer equation,

$$\frac{F_0}{F} = 1 + K[Q] = 1 + k_q.\tau_0[Q] \quad \text{.....(1)}$$

In this expression, K is the Stern-Volmer quenching constant, k_q is the bimolecular quenching constant, τ_0 is the unquenched lifetime, and [Q] is the quencher concentration. The Stern-Volmer quenching constant K indicates the sensitivity of the fluorophore to a quencher. A fluorophore buried in a macromolecule is usually inaccessible to water soluble quenchers, so that the value of K is low. Larger values of K are found if the fluorophore is free in solution or on the surface of a biomolecule.

A wide variety of molecules can act as collisional quenchers examples include oxygen, halogens, amines, and electron-defficient molecules like acrylamide. The mechanism of quenching varies with the fluorophore-quencher pair. For instance, quenching of indole by acrylamide is probably due to electron transfer from indole to acrylamide, which does not occur in the ground

state. Quenching by halogen and heavy atoms occurs due to spin-orbit coupling and intersystem crossing to the triplet state.

Aside from collisional quenching, fluorescence quenching can occur by a variety of other processes. Fluorophores can form nonfluorescent complexes with quenchers. This process is referred to as static quenching since it occur in the ground state and does not rely on diffusion or molecular collisions. Quenching can also occur by a variety of trivial i.e. non-molecular mechanisms, such as attenuation of the incident light by the fluorophore itself or other absorbing species.

A wide variety of small molecules or ions can act as quencher of fluorescence, that is, they decrease the intensity of the emission. These substances include iodide (I^-) oxygen and acrylamide. The accessibility of fluorophores to such quenchers can be used to determine the location of probes on macromolecules, or the porosity of proteins and membranes to quenchers.

1.2.2 Resonance energy transfer (RET) :

An important process occurs in the excited state is resonance energy transfer. This occurs whenever the emission spectrum of a fluorophore, called the donor, overlaps with the absorption spectrum of another molecule, called the acceptor [9]. The acceptor does not need to be fluorescent . It is important to understand that RET does not involve emission of light by the donor. RET is not the result of emission from the donor being absorbed by the acceptor.
Such reabsorption processes are dependent on the overall concentration of the acceptor, and on non-molecular factors such as sample size, and thus are of less interest. There is no intermediate photon in RET. The donor and acceptor are coupled by a dipole-dipole interaction for these reasons the term RET is preferred over the term fluorescence resonance energy transfer (FRET), which is also in common use.

FRET is the distance-dependent transfer of energy from a donor molecule to an acceptor molecule. According to Forsters theory, there are many factors that influence resonance energy transfer.

The primary conditions that need to be met in order to occur FRET are relatively few.

a) The donor and acceptor molecules must be in close proximity to one another (typically 10-100 A^0)

b) The absorption or excitation spectrum of the acceptor must overlap with the fluorescence emission spectrum of the donor.

c) The donor and acceptor transition dipole orientations must be approximately parallel. These conditions have been optimized by overlapping of spectra.

The extent of energy transfer is determined by the distance between the donor and acceptor and the extent of spectral overlap. For convenience, the spectral overlap is described in terms of the Forsters distance (R_0).

The rate of energy transfer k_T (r) is given by,

$$k_T(r) = \frac{1}{\tau_D} \cdot \left(\frac{R_0}{r}\right)^6 \qquad \text{.....(2)}$$

where r is the distance between the donor (D) and acceptor (A) and τ_D is the lifetime of the donor in the absence of energy transfer. The efficiency of energy transfer for a single donor-acceptor pair at a fixed distance is,

$$E = \frac{R_0^6}{R_0^6 + r^6} \qquad \text{.....(3)}$$

Here the extent of transfer depends on distance (r).

Where R_0 = Characteristic distance called the Forsters distance or critical distance at which the efficiency of transfer is 50%.

R_0 is expressed as,

$$R_0^6 = 8.8 \times 10^{23} [K^2 . n^{-4} . \phi J.(\lambda)] \text{.....} in A^{0^6} \text{.....(4)}$$

Where, K^2 is the factor expressing the relative orientation of the donor and acceptor molecule, n is the refractive index of the medium, Φ is the quantum yield of the donor in absence of the acceptor, and $J(\lambda)$ is the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor.

The spectral overlap integral J(λ) was calculated by numerical intergration .

$$J = \frac{\int_0^{\infty} F.(\lambda).\in.(\lambda).\lambda^4.\Delta\lambda}{\int_0^{\infty} F.(\lambda).\Delta\lambda} \quad \text{.....(5)}$$

$\lambda^4 = \lambda_{max.}$ of donor, J = overlap area x λ^4

The Forsters distance R_0 has been calculated assuming random orientation of the donor and acceptor molecules.

Fortunately, the Forsters distances are comparable in size to biological macromolecules : 30 to 60 A°. For this reason, energy transfer has been used as a " spectroscopic ruler" for measurements of distance between sites on proteins [10,11]. The value of R_0 for energy transfer should not be confused with the fundamental anisotropies (r_0).

The field of RET is large and complex. The theory is different for donors and acceptors that are covalently linked, free in solution, or contained in the restricted geometries of membranes or DNA. Additionally, depending on donor lifetime, diffusion can increase the extent of energy transfer beyond that predicted by equation 3.

1.2.3 Concentration:

The intensity of fluorescence depends on the concentration, the molar absorptivity and the quantum yield of the fluorophore. The fluorescence signals are ideally additive in mixtures; i.e. the overall fluorescence signal of a given sample can be expressed as the sum of the fluorescence contribution from each of the inherent fluorophores. However, in complex mixtures such as intact food samples, the fluorescence may not be additive due to quenching phenomena and interactions with the molecular environment of the fluorophore.

1.2.4 Molecular environment:

The polarity of the local environment of a fluorophore influences the emission of especially polar fluorophores. In more polar environments, fluorophores in the excited state will relax to a lower vibrational energy state before emission, resulting in emission at lower energies, i.e. longer wavelengths. Shifts in emission spectra of fluorophores can be observed when comparing identical fluorophores in different solvents or as residues in different macromolecules. Also pH and temperature strongly affect the fluorescence signal. With dissociation or protonation caused by pH changes, the rates of nonradiative processes competing with fluorescence can be altered and thereby affect the quantum yield of fluorescence emission. Temperature primarily affects fluorescence through its impact on dynamic quenching.

1.2.5 Scatter:

Scattering and reflection of the incident light has a substantial effect on fluorescence measurements, with respect to both the optical depth of the sampling and the obtained fluorescence signal. The most important parts of the scattered light can be divided into elastic Rayleigh scatter and inelastic Raman scatter. For Rayleigh scatter, the wavelengths of the scattered light are the same as those of the incident light, and in principle this type of scatter should not interfere with fluorescence emission, as these spectral areas can be disregarded when analyzing the fluorescence landscape.

However, when working with instrumental setups with large band widths, Rayleigh scatter can constitute a significant interference to fluorescence emission from fluorophores with small Stokes shift. Raman scatter is related to vibrational states of the bulk substances in the sample and reflects a constant energy loss. Raman scatter can in many cases be neglected because of its weak contribution to the fluorescence signal. Alternatively, corrections of the fluorescence signal can be performed either by substracting the pure solvent / background scatter contribution or by specifically addressing the scatter in the modeling of the fluorescence data.

1.2.6 Effects of solvent:

a) Photophysical processes in molecules in solution:

The arguments developed concerning the spectroscopic behavior of molecules were based upon the intrinsic electronic properties of the molecules themselves. This treatment is complete only for truly isolated and non-interacting molecules, and is realized only in the gas phase at low pressures, and to a fair approximation in dilute solutions in non-polar and non-hydrogen bonding solvents (e.g. hydrogen solvents). However, most electronic spectroscopy is practiced in fluid in rigid solutions involving solvents, many of which are highly polar or capable of hydrogen bonding with the absorbing or emitting molecules. The interactions of solute molecules with polar or hydrogen bonding solvents are capable of profoundly altering the electronic properties of the states from which absorption and emission occur, and any attempt to account for electronic spectral phenomena in solution must take this into account. Fortunately, the effect of solvents on electronic spectra can be treated in terms of the perturbation of the spectra of the isolated molecules by the interaction with the solvent. This will allow us to build upon the theory already developed for the spectra of isolated molecules. Moreover, in activating (polar and hydrogen bonding solvents, interactions between solute molecules (e.g. chemical reactions) which are kinetically or thermodynamically impossible in isolated molecules without solvent assistance often occur in ground and electronically excited states. These interactions usually have profound effects upon the electronic spectra observed and must be taken into account in order to explain spectral behavior in condensed media. We shall consider the effects of solvent-solute and solute-solute interactions upon the properties of $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$ and intramolecular charge transfer transitions and the spectral bands they represent.

Solvent play a major role, both invitro and in vivo in the behavior of molecules in solution. Solvents have their own intrinsic characteristics due to which they can alter the reaction pathways by altering the energies of the reactants and contributing to the final state of a reaction. This hastled to a wide

spread interest to study the effects of solvation and solvation dynamics of molecular systems in different solvents. Solvent molecules can induce stabilization of a probe molecule either through dipolar relaxation between the probe and the solvent molecules or through some specific interactions such as hydrogen bonding.

Aromatic fluorophores bearing constituents on the aromatic ring are known to be particularly sensitive to the chemical and physical properties of the solvents. Redistribution of the electron density in the excited state often leads to a large change in the dipole moment of the molecule. The solvent molecules, being unable to follow this sudden change, undergo a re-orientation around the probe leading to a shift in the fluorescence band of the fluorophore. When the probe molecule does not disturb the solvent structure, the solvatochromic shift of a fluorescence or absorption band is linear with respect to solvent polarity function. However a local enrichment of the solvent molecules around the probe occurs (preferential solvation), a deviation from linearity is obtained. The latter may be due to various interactions viz. electrostatic forces, hydrogen bonding etc. between the probe and the two different solvent components.

b) Solvent effects on electronic spectra:

Solvent interaction with solute molecules are predominantly electrostatic and may be of the induced dipole-induced dipole, dipole-induced dipole or hydrogen bonding types. Additionally, hydrogen bonding usually accompanies dipole-dipole interaction as a mode of solvation. A solvent which has atoms having lone or nonbonding electron pairs is said to be a hydrogen bond acceptor solvent. Qualitatively, a hydrogen bond donor behaves as a very weak Bronsted acid, partially donating a proton to a basic site on the solute molecule. A hydrogen bond acceptor behaves as a very weak Bronsted base, partially accepting a proton from the solute molecule. Because of the involvement of nonbonding and lone pairs in $n \rightarrow \pi^*$ and intramolecular charge transitions, hydrogen bonding solvents have the greatest effect on the positions

of these types of spectra. Because of the large dipole moment changes accompanying electronic reorganization in $\pi \rightarrow \pi^*$and intramolecular charge transfer transitions, these types of spectra are most affected by solvent polarity. A quantitative description of the effects of environment on fluorescence emission spectra is perhaps the most challenging topic in fluorescence spectroscopy. No single theory or type of interactions can be used in all circumstances. A number of factors affect the emission including :

1. General solvent effects due to the interactions of the dipole of the fluorophore with its environment.
2. Specific solvent effects due to fluorophore-solvent interactions.
3. Formation of ICT or TICT (twisted intramolecular charge transfer) states depending on the probe structure and the surrounding solvent.
4. Viscosity and changes in the radiative and nonradiative decay rates.
5. Probe-probe interactions.

Even if only one type of interaction were present, the effects would still be complex and beyond the limits of most models. For instance, the Lippert equation is only an approximation, and ignores higher-order terms. Also, this equation only applies to a spherical dipole in a spherical cavity. More complex expressions are needed for non-spherical molecules, but one cannot generally describe the fluorophore shape in adequate detail. With regard to specific effects, there is no general theory to predict the shift in emission spectra due to hydrogen bond formation. And, finally, for fluorophores, bound to macromolecules or in viscous solvents, spectral relaxation can occur during emission, so that the emission spectrum represents some weighted average of the unrelaxed and relaxed emission.

1.3 Instrumentation

The success of fluorescence experiments requires attention to experimental details and an understanding of the instrumentation. There are also many potential artifacts that can distort the data. Light can be detected with high sensitivity. As a result, the gain or amplification of instruments can

usually be increased to obtain observable signals, even if the sample is nearly nonfluorescent. These signals seen at high amplification may not originate with the fluorophore of interest. Instead, the interference can be due to background fluorescence from the solvents, light leaks in the instrumentation, emission from the optical components, stray light passing through the optics, light scattered by turbid solutions, and Rayleigh and / or Raman scatter, to name a few interference sources.

An additional complication is that there is no ideal spectrofluorometer. The available instruments do not yield true excitation or emission spectra. This is because of the nonuniform spectral output of the light sources and the wavelength-dependent efficiency of the monochromators and detector tubes. The polarization or anisotropy of the emitted light can also affect the measured fluorescence intensities because the efficiency of gratings depends on polarization. It is important to understand and control these numerous factors. The instrumental factors can affect the excitation and emission spectra as well as the measurement of fluorescence lifetimes and anisotropies. Additionally, the optical properties of the samples such as optical density and turbidity can also affect the spectral data.

Instrumentation for the measurement of fluorescence consists essentially of 1) a light source to electronically excite the sample, 2) a monochromator to separate the light of desired energy from the source, 3) a sample compartment, 4) a second monochromator to isolate the samples fluorescence energy from the excitation energy, 5) a photodetector to translate the fluorescent light into an electrical signal, and 6) a readout system such as a galvanometer or a recorder, coupled with an amplifier to determine the intensity of fluorescent light emitted. In addition, sites are usually present on either side of the monochromator to collimate the exciting and fluorescent light and to limit the range of wavelengths (bandpass) of exciting light irradiating the sample and fluorescent light falling on the photodetector. Generally, the fluorescence emission detector is perpendicular to the exciting beam of light. This configuration allows effective separation of exciting light from the

fluorescence emission falling on the photodetector, and in his regard it complements the monochromators. There are two major types of instruments for fluorimetric measurements; filter fluorimeters and fluorescence (scanning) spectrophotometers. The former uses filters as monochromators while the later uses gratings to disperse the exciting light and fluorescent emission into their component wavelengths.

1.3.1 Recording of fluorescence spectra:

The fluorescence and fluorescence excitation spectra of the Biginelli compounds in solution state were recorded on spectrofluorophotometer. The detailed information regarding specifications is listed below-

Instrument	:	PC based spectrofluorophotometer
Make	:	JASCO, Japan
Model	:	FP-750
Light source	:	150W xenon lamp with shielded lamphouse
Monochromator	:	Holographic grating with 1200 lines/mm
Wavelength range	:	220 nm to 730 nm
Spectral bandwidth	:	10, 20 nm on both Ex. and Em monochromator
Wavelength accuracy	:	±3 nm
Wavelength threw speed	:	30,000 nm/min
Wavelength scanning speed	:	60,250,1000,4000 nm/min
Response	:	Fast, Medium, Slow, Auto
Sensitivity	:	Signal to noise ratio of Raman band of water is higher than 300:1
Photometric display	:	-999 to +999
Sample chamber	:	Single cell holder (standard)
Detector	:	Silicon photodiode for Ex. monochromator and Photomultiplier tube for Em. monochromator

During recording of the fluorescence and fluorescence excitation spectra the parameters like spectral bandwidth (10nm) data pitch (1nm) and wavelength scanning speed (250nm/min) were kept constant while other parameters were changed as per requirement of the experiment.

1.3.2 Optical system of FP-750 Spectrofluorometer:

The optical system of the instrument is given in fig.1.2. The light from the source (Xenon lamp) is focused on to the entrance slit of the excitation monochromator by the ellipsoidal mirror Mo. The light from the slit is dispersed by the diffraction grating G_1 and monochromatic light is taken out by the exit slit. A part of the monochromatic light is led to the monitoring silicon photodiode (SP) by the beam splitter chamber by the plane mirror M_2 and ellipsoidal mirror M_3 where it is focused on the center of the sample cell.

The emission from the sample is focused on to the entrance slit of the emission (Em) monochromator by ellipsoidal mirror M_4 and two plane mirrors M_5 and M_6. Monochromator after going through the exit slit form the exit slit and is led to photometric photomultiplier tube PMT by the spherical mirror, M_7.

The schematic diagram for the FP-750 system is shown in Fig.1.3. The light incident on the monitoring detector (Silicon photodiode) and the emission detector (PMT) is converted into an electrical signal and then converted into digital signal by the A/D converter and is introduced to the microcomputer. The signal subjected to arithmetic operation by the microcomputer is outputted to the display unit as digital data or spectrum. Both wavelengths as well as slit drives are controlled by the microcomputer.

The steps involved during recording of the fluorescence emission and fluorescence excitation spectra of luminophors are explained as follows.

1. Visual fluorescence color was observed by exciting the sample at 365nm (Hg line) excitation wavelength.
2. The emission monochromator was set at the approximate wavelength of visually observed color.

3. The excitation monochromator was scanned from 230nm to a wavelength of emission monochromator.
4. The excitation spectrum was recorded and the λ_{ex} was noted.
5. The excitation monochromator was set at λ_{ex} observed in excitation spectrum.
6. The emission monochromator was allowed to scan in the range 300nm to 750nm.
7. The fluorescence emission spectrum was recorded and the λ_{em} was noted.
8. The emission monochromator was then set at the λ_{em} and excitation spectrum monochromator was scanned and thus the excitation spectrum was recorded.
9. Finally the fluorescence spectrum was obtained by setting the excitation monochromator at λ_{ex} obtained in above step. Similarly fluorescence excitation spectrum was obtained by setting λ_{em} observed in the final emission spectrum.

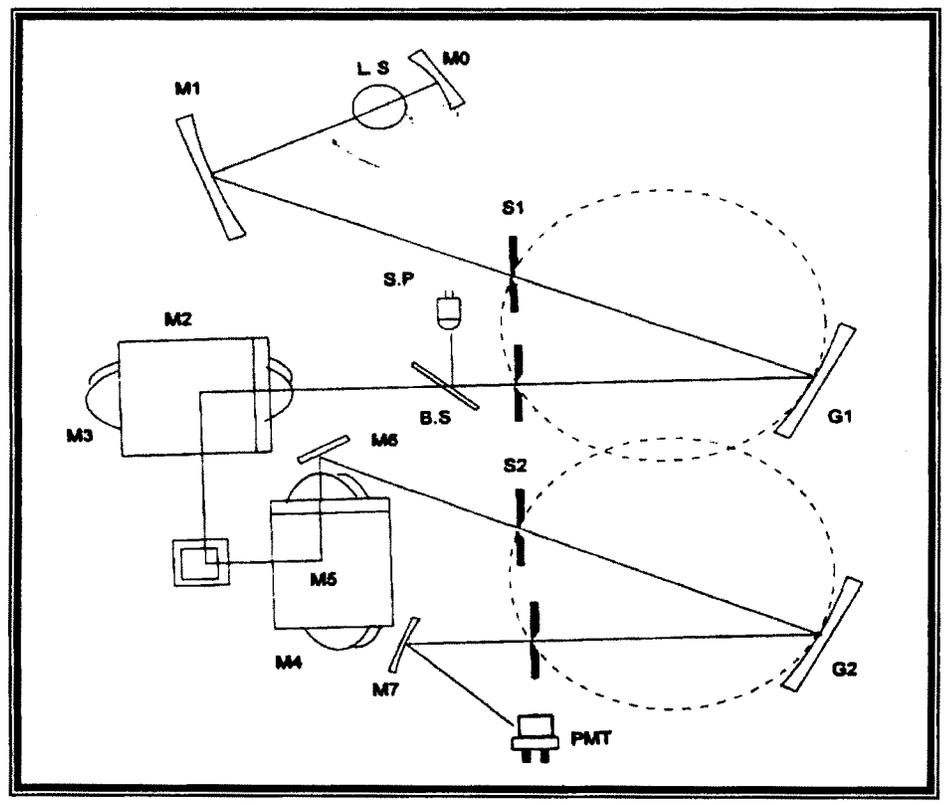


Fig.1.2 Optical system of FP-750

LS: Light source (150W xenon lamp)	M2,M5,M6 : Plane mirror
M1: Ellip soidal mirror	M3,M4 : Ellipsoidal entrance/exit slit
S1: Excitation entrance/exit slit	S2 : Emission entrance/exit slit
G1: Excitation concave diffraction grating (1200lines/mm)	G2 : Emission concave diffraction grating (1200lines/mm)
SP : Silicon photodiode	PMT : Photomultiplier tube
BS : Beam splitter	MO,M7 : Spherical mirror

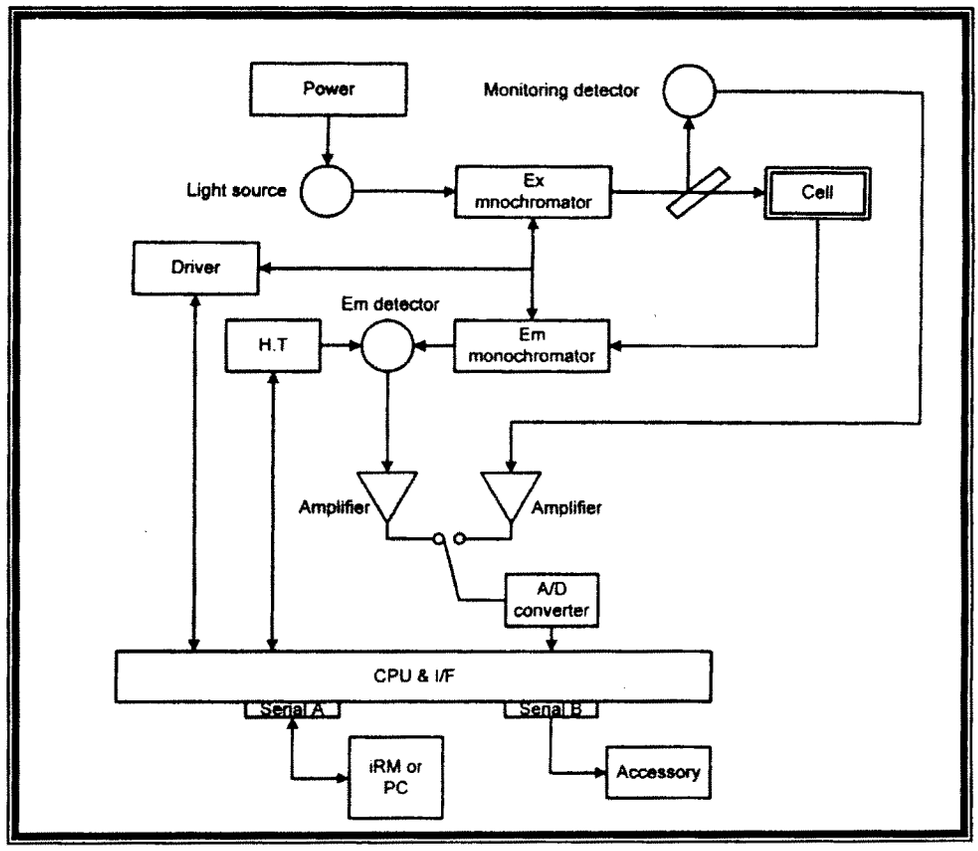


Fig. 1.3 System Diagram.

1.4 Luminescence Measurements

Following experimental observations are used to measure the properties of any luminescent system.

1.4.1 Absorption spectrum:

It shows the dependence of the degree of light absorption by the compound on the wavelength of the light. The quantization condition for the absorption of emission of light by an atom or molecule is given by Einstein relation,

$$h\upsilon = \frac{hc}{\lambda}$$

$$h\upsilon = E_2 - E_1 \qquad \text{----------(1)}$$

Where, E_2 and E_1 are the electronic energy levels,

h = Plancks constant , and

υ = Frequency of the incident photon.

λ = wavelength of the incident photon.

C = Velocity of incident photon.

According to Beer-Lamberts law,

$$\log_{10}(I_o/I) = \epsilon cl \qquad \text{.....(2)}$$

Where, I_O = Intensity of incident light.

I = Intensity of transmitted light.

ϵ = Molar excitation coefficient.

C = Concentration of solution.

l = Path length of the absorbing systems.

$\log_{10}(I_o/I)$ = Optical density / absorbance of the material.

Usually, the absorption spectrum is plotted in terms of molar extinction coefficient (ϵ) against frequency or wavelength.

The probability of the absorption depends upon the degree of overlap of the wave function of the lowest vibrational level at the ground state and the wave function of the vibrational levels of the first excited singlet state. The nature and positions of the absorption peaks are very important for the spectroscopic studies. The monomeric or dimeric state of molecular systems is indicated by the nature of the absorption spectrum. It is significant in the sense to explore whether the ground state dimer or a monomer is excited in the absorption process [12].

Appearance of the broad absorption band in solution state is an indication of dimeric nature of molecules in the ground state while the structural spectrum indicates the existence of monomolecular species [13]. But in solids the absorption spectra are not as structured in solution. The nature of absorption bands also gives an idea about the lattice structures of molecular systems under study and suggests the possibility of formation of dimeric species [4,14].

1.4.2 Emission spectrum :

The relative intensity of radiation emitted at various wavelengths is defined by emission spectrum [4]. The fluorescence emission Spectrum is obtained by irradiating the sample by a wavelength of maximum absorption as indicated by absorption spectrum of the sample. Ground state and excited state associated with the absorption and emission spectra. It is observed that the absorption spectra gives data about the vibrational levels of the excited state and the emission spectra yields data about the vibrational levels of the ground state. The independence of the shape of the fluorescence emission spectrum from the wavelength of the exciting light arises from the fact that the emission always takes place from the lowest excited state. If the shape of the emission spectrum changes with changing wavelengths of the exciting light, then the presence of more than one fluorescent compound should be suspected [4,15].

In most of the organic materials the emission spectrum is the mirror image of the absorption spectrum. This relationship is an indication of the similarity of the respective vibrational wave functions in the excited and ground states.

1.4.3 Excitation spectrum:

It defines as the relative efficiency of different wavelengths of exciting radiation to induce fluorescence. The excitation spectrum is obtained by measuring the fluorescence intensity at a fixed emission wavelength, while the excitation wavelength is scanned. The excitation spectrum is quite stable for most large, complex molecules. It does not depend on the emission wavelength at which it is monitored.

The shape of the excitation spectrum should be identical with the absorption spectrum of the molecule. However, because of the instrumental artifacts, this seldom is the case. Often, the photomultiplier sensitivity changes in spectrofluorometer such that the detector has different responses or sensitivities at different wavelengths. Also in absorption spectrophotometry, the band width of the monochromator, changes at different wavelength in order to keep the energy as constant as possible. In contrast in fluorescence the excitation and emission bandwidth remains constant. If the apparent excitation spectrum was corrected for their instrumental artifacts, one would obtain the true or "corrected" spectrum.

The excitation spectrum will be identical to the absorption spectrum where $\epsilon.c.l << 1$. The measurement of quantum intensity is limited by the sensitivity of the spectrofluorometer and that depends upon the intensity of the excitation source . Parker (1968) estimated that concentrations as low as 10^{-12} M can be detected by excitation spectroscopy compared with a minimum concentration of 10^{-8} M by absorption spectroscopy [2,4,16]. Excitation spectroscopy is also used to determine the quantum efficiency of energy transfer between donor and acceptor molecules.

1.4.4 The Stokes shift:

From the Jablonski diagram, it is obvious that the energy of the emission is less than absorption. Fluorescence typically occurs at lower energies or longer wavelengths. This phenomenon was first observed by Sir. G. G. Stokes in 1852 at the University of Cambridge [17].

Fluorescence radiation always occurs at wavelengths longer than the exciting wavelength by a wavelength interval depending on the energy loss in the excited state due to vibrational relaxation. This separation between the excitation and emission band maxima is known as the Stokes shift. This is the characteristic of all complex molecules. It is usually greater than 10 nm. When the emission band lies within 30 to 50 nm of the excitation wavelength, measurement patterns can arise due to difficulty in separating the Rayleigh scatter of the excitation light from the emission band. The interactions of solute molecules with the solvent usually introduce large spectral red shifts of fluorescence. These shifts are occasionally solvent specific and are also called Stokes shift [4].

Energy losses between excitation and emission are observed universally for fluorescent molecules in solution. One common cause of the Stokes shift is the rapid decay to the lowest vibrational level of S_1. Furthermore, fluorophores generally decay to higher vibrational levels of S_0, resulting in further loss of excitation energy by thermallization of the excess vibrational energy. In addition to these effects, fluorophores can display further Stokes shift due to solvent effects, excited-state reactions, complex formation, and / or energy transfer. When there is large Stokes shift, then it originates from intramolecular charge transfer (ICT) characters of excited state fluorophore.

Stokes shift υ is given by equation,

$$\Delta\nu = \bar{\nu}_{abs} - \bar{\nu}_{em} = \frac{2(\mu_e - \mu_g)^2}{4\pi\epsilon_0 hca^3}\Delta f.(\epsilon, n) + constant$$

$$\Delta f = \frac{(\epsilon - 1)}{(2\epsilon + 1)} - \frac{(n^2 - 1)}{(2n^2 + 1)}$$

Where,

h = Plancks constant = 6.6262×10^{-34} J.

C = 2.99×10^{8} ms^{-1},

ϵ_0 = permittivity of vacuum = 8.8542×10^{-12} $C^2N^{-1}M^2$.

a = Onsager cavity radius.

Onsager cavity radius can be determined by AM_1method using the Gaussian 98 package.

μ_e and μ_g are the dipole moments of the fluorophore in the excited and ground state respectively.

f = solvent polarity, ϵ = dielectric constant of solvent, n = refractive index of solvent.

The Stokes shift ($\upsilon_{abs.}$- $\upsilon_{em.}$) of a solute molecule depends on the dielectric constant (ϵ) and the refractive index (n) of the solvent.

The plot of Stokes shift verses solvent polarity called as Lippert-Mataga plot. If the plot reflect linear relationship and good correlation of Stokes shift with solvent polarity, then it indicates that the dielectric solute-solvent interactions are responsible for the solvatochromic shift.

1.4.5 Fluorescence lifetime and quantum yields:

The fluorescence lifetime and quantum yield are perhaps the most important characteristics of a fluorophore. Quantum yield is the number of emitted photons relative to the number of absorbed photons.

Substances with the largest quantum yields, approaching unity, such as rhodamines, display the brightest emissions. The lifetime is also important, as it determines the time available for the fluorophore to interact with or diffuse in its environment, and hence the information available from its emission. The fluorescence quantum yield is the ratio of the number of photons emitted to the number absorbed. The quantum yield can be close to unity if the radiationless decay rate is much smaller than the rate of radiative decay.

We note that the energy yield of fluorescence is always less than unity because of Stokes losses. The life of the excited state is defined by the average

time the molecule spends in the excited state prior to return to the ground state. Generally, fluorescence lifetimes are near 10 ns. Fluorescence emission is a random process, and few molecules emit their photons at precisely $t = \tau$. The lifetime is an average value of the time spent in the excited state. For a single exponential decay 63% of the molecules have decayed prior to $t = \tau$ and 37% decay at $t > \tau$.

Heavy atoms such as iodine typically result in shorter lifetimes and lower quantum yields. The lifetime of the fluorophore in the absence of non-radiative process is called the intrinsic or natural lifetime. In principle, the natural lifetime τ_n can be calculated from the absorption spectra, extinction coefficient and emission spectra of the fluorophore. The natural lifetime can be calculated from the measured lifetime (τ) and quantum yield.

$$\tau_n = \tau / Q \qquad \text{.....(1)}$$

Many biochemical fluorophores do not behave as predictably as unsubstituted aromatic compounds. Hence there is poor agreement between the value of τ_n calculated from above equation 1 and that calculated from its absorption and emission spectra. These discrepancies occur for a variety of unknown and known reasons, such as a fraction of the fluorophores located next to quenching groups, which sometimes occurs for tryptophan-residues in proteins.

From Stern-Volmer equation,

$$\frac{F_0}{F} = 1 + kq.\tau_0[Q] = 1 + K_{SV}[Q]$$

$$K_q = \frac{K_{sv}}{\tau_0}$$

where,

k_q = quenching constant,

K_{sv} = Stern-Volmer costant,

τ_0 = Fluorescence lifetime of the fluorophore.

The quantum yield and lifetime can be modified by factors that affect the rate constants. For example, a molecule may be nonfluorescent as a result of a large rate of internal conversion or a slow rate of emission. Scintillators are generally chosen for their high quantum yields. These high yields are a result of large rate constant values. Hence, the lifetimes are generally short: near 1 ns. Comparison of the natural lifetime, measured lifetime and quantum yield can be informative.

Fluorescence quantum yields (Φ) in different solvents at room temperature were measured by referencing to some standard solution like quinine sulphate in H_2SO_4 (Φ_f = 0.55). the absorbance at the excitation wavelength was less than 1 to avoid the " inner filter effect".

Sometimes quantum yield of a fluorophore in different solvent varies with solvent polarity. Such a phenomenon of quantum yield variation with solvent polarity indicates 2 mechanisms involved during the course of increasing solvent polarity.

One mechanism is the increase of quantum yield with a suitable enhancement of ICT (intramolecular charge transfer) : the so called " negative solvatokinetic effect". Another mechanism which is usually observed in molecules with ICT character is reduction in quantum yield by strong ICT, the so called, "positive solvatokinetic effect".

In strongly polar solvents the positive solvatokinetic effect plays a predominant role so that the fluorescent quantum yield decreases with increase of solvent polarity due to the strong interaction between the strongly polarized molecule and solvent in the excited state. Moreover, the much lower fluorescence quantum yield in ethanol, butanol like proton solvents can be distributed to the hydrogen bond interaction between the molecule and surrounding solvent, which results in an additional non-radiative decay. Quantum yield in different solvents can be calculated as,

$$\phi = \frac{\phi_{std}.F.A_{std}.n^2}{F_{std}.A.n^2_{std.}}$$

Where,

F & $F_{std.}$ = Peak areas of sample & standard solutions respectively.

A & $A_{std.}$ = Absorbance at excitation wavelengths of sample & standard solutions respectively.

n & n_{std} = Refractive indices of sample and standard solutions respectively.

Φ & $\Phi_{std.}$ = Quantum yields of sample & standard solutions respectively.

1.5 SYNTHESIS AND CHARACTERIZATION OF DHPM

1.5.1 Introduction:

The synthesis of dihydropyrimidones was firstly reported by Pietro Biginelli in 1893. It involves a one-pot condensation of an aldehyde, ethyl acetoacetate and urea in ethanolic medium in presence of strong mineral acid [18]. Though the reaction remained ignored almost for a century, the interest in their synthesis has been greatly increased from last decades, because of the confirmation of diverse and important biological properties of dihydropyrimidones. A latter thing inspired organic chemist to find out more suitable protocol and simple methods for the synthesis of dihydropyrimidones.

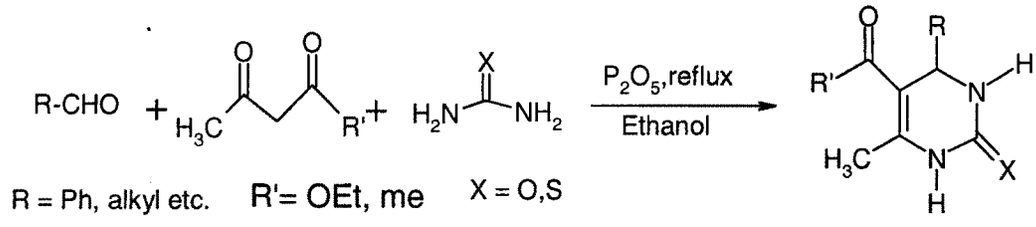
Biginelli compounds show an important biological activities like anti-viral, anti-tumor, anti-bacterial, and anti-inflammatory which have been reported for these compounds [19]. Some compound among these groups was patented as an agent for the protection of wool against moths in 1930 [20], which helps in the development of Nitractin as an antiviral agent [21]. The same compound also show antibacterial activity [22] Some marine alkaloids having dihydropyrimidine-5-carboxylate core have been shown to exhibit interesting biological activities such as potent HIV-gp-120-CD_4.

Because of the strong anti-hypertensive activity, the work on the synthesis of Biginelli compounds has been greatly increased [23].

1.5.2 Synthesis:

The first synthesis of dihydropyrimidones nucleus involves a one-pot condensation of an urea, aldehyde and ethyl acetoacetate in ethanolic medium in the presence of strong mineral acid reported by Scientist Biginelli in 1893. However, this method suffered from lower yields and longer reaction time especially with aliphatic as well as substituted aromatic aldehydes.

There are variety of catalysts but one of the inexpensive catalyst is phosphorus pentoxide and is reported by our colleague [24]. The Biginelli reaction became successful with fulfillment of the entire above requirement in presence of phosphorous pentoxide. This reagent not only acting as an acid but also has tendency to absorb water. In addition, it is water soluble and hence its removal from the resultant reaction product would be much easier. Since, we have decided to use phosphorous pentoxide for the synthesis of Biginelli compounds as per scheme I.



(Scheme-I)

The reaction was extended to a variety of aldehydes, but as reported in the literature, only the optimized quantity of phosphorous pentoxide is used. Aromatic as well as heterocyclic aldehydes afford corresponding dihydropyrimidones in excellent yields.

The versatility of the method was then checked by using thiourea instead of urea to prepare dihydrothiopyrimidones and by replacing ethyl aceto acetate with acetyl acetone, which gave corresponding DHPMs. All these results are summarized in Table-I.

Table I

The synthesis of dihydropyrimidones in presence of phosphorous pentoxide:

Entry	R	R'	X	Time (Hrs)	Yield (%)	MP Obs. (lit) ^{0}C
1	C_6H_5	Me	S	1.3	88	220-223(220-222)[10a]
2	4-(CH_3O)-C_6H_4	OEt	O	1.5	94	201-202(199-201)[10a]
3	4-(NO_2)-C_6H_4	OEt	O	O.5	95	207-209 (207-210)[12c]
4	4-(CH_3O)-C_6H_4	Me	O	1.5	89	165-166(166-168) [10a]
5	C_6H_5	OEt	S	1.0	92	205-209 (208-209) [5c]
6	C_6H_5	Me	O	1.2	91	244 (242-244) [4b]
7	4-(NMe_2)-C_6H_4	OEt	O	1.5	87	251-253(250-252)[9a]
8	2-(Cl)-C_6H_4	OEt	O	1.2	85	216-219(215-218) [4]
9	C_6H_5-CH=CH	OEt	O	1.5	90	230-232 (232) [12b]
10	C_6H_5-CH=CH	OEt	S	1.7	86	222-226(223-225) [8c]
11	4-(Cl)- C_6H_4	OEt	O	0.5	94	215-217(216-217)[8c]
12	2-furyl	OEt	O	2.0	75	208-210 (209-211) [8c]
13	2-(OH)-C_6H_4	OEt	O	2.0	82	199-203(201-203) [10a]
14	4-(CH_3O)-C_6H_4	OEt	S	1.5	92	148-150(150-152) [10a]
15	C_6H_5	OEt	O	1.0	95	204-205 (204) [5c]

All reactions were carried out on 10 mmol scale. All the compounds are reported one and their melting points were well matched with reported value. The two compounds only those showed fluorescence properties were characterized by proton NMR, IR and ^{13}C .The ^{1H} NMR and ^{13}C NMR spectra were recorded by using $CDCl_3$ + DMSO-d6 solvent on Brucker 300 MHz spectrometer with tetra methyl silane as internal standard. The reaction was monitored by TLC (Silica gel 60-F 254 plates).

1.5.3 Spectroscopic data of the representative compound:

5-Ethoxycarbonyl-4-(4-methoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one (fig. 1.5) (entry-2): mp 201-202 °C (Lit mp 199-201 °C)[10];

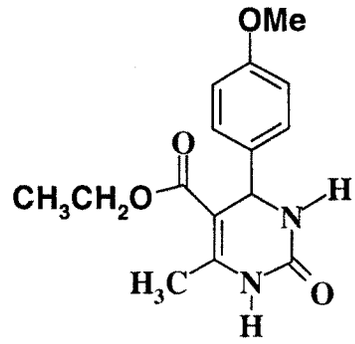


Figure 1.5

The IR spectrum of compound (Entry 2) (Fig. 1.6) showed the peaks at (-NH), (C=O, NH-CO-NH). While the PMR spectrum (Fig. 1.7) showed a triplet at 1.19 and quartet at 4.00 (both with J = 6 Hz) was due to ethyl group. A singlet at 2.32 was due to vinylic methyl; a singlet at 5.34 for a methine proton while two singlets at 5.92 and 8.39 was due to two –NH protons respectively of dihydropyrimidine ring. The doublet of doublet at 6.83 and at 7.21 was due to four aromatic protons ortho coupled to each other. The CMR spectrum of the same showed the signals at 14.02, 17.61, were due to the methyl group of ester function and vinyl methyl protons respectively. The peaks at 53.83 and 59.19 were due the O and carbon-5

attached to N, respectively. This was confirmed through the DEPT scan. A peak at 99.27 was due to vinylic carbon attached to N. The aromatic carbons appeared at 126.22 to 144.78 (six carbons). Lastly three more peaks at 148.19, 152.11 and 165.33 were due to the C_5, C_2 and ester carbonyl carbon respectively.

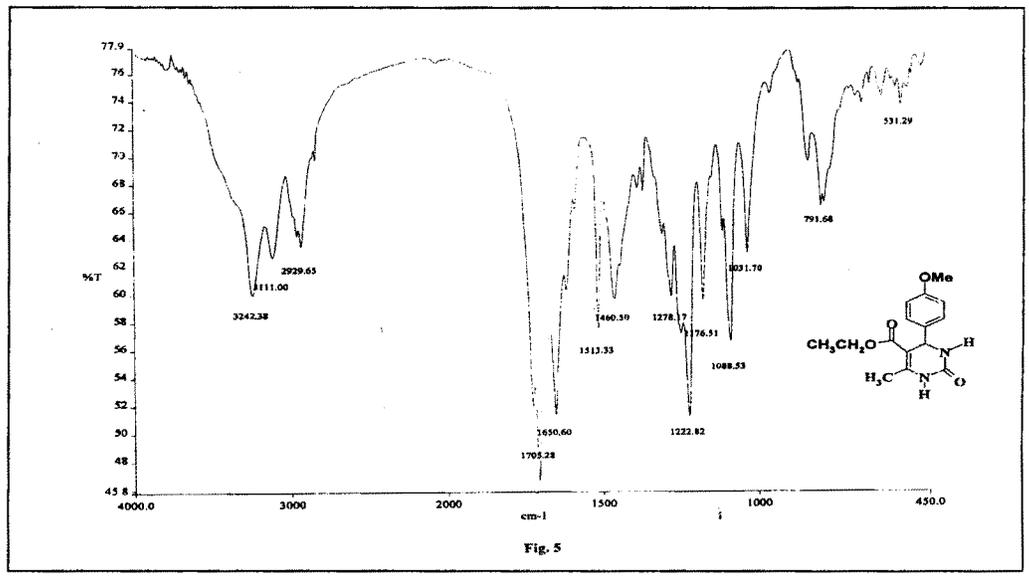


Fig. 5

Fig. 1.6 : IR Spectrum

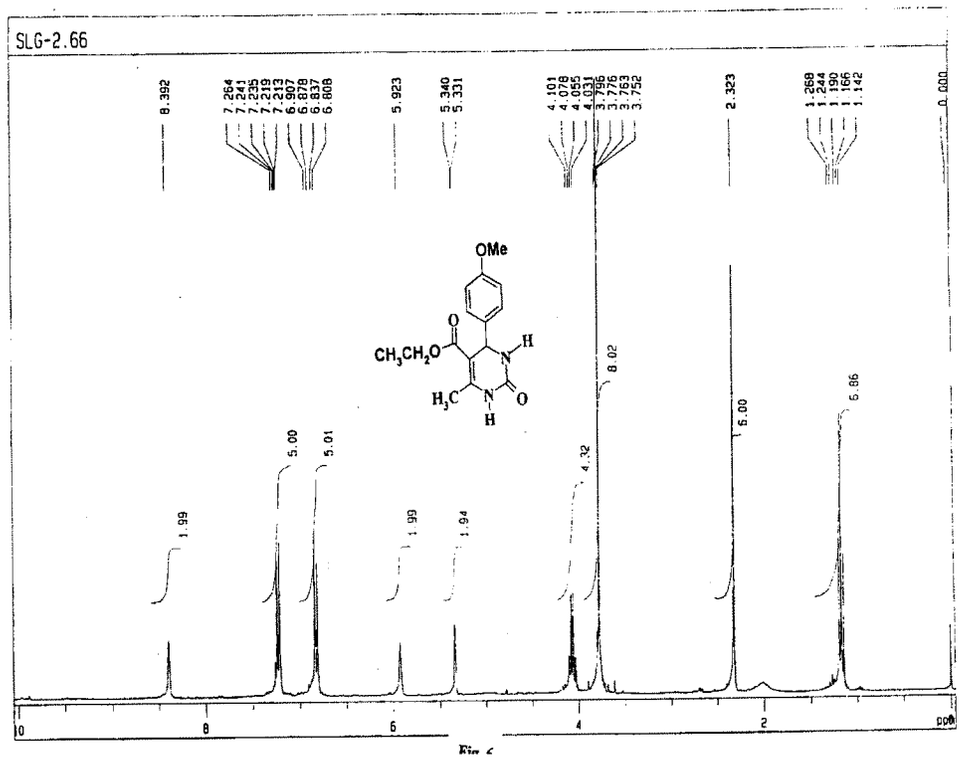


Fig. 1.7 : NMR Spectrum

1.5.4 General procedure:

In a 250 ml round bottom flask, aldehyde (10 mmol), urea / thiourea (30 mmol), ethyl acetoacetate / acetyl acetone (10 mmol) and phosphorous pentoxide 0.5 gms (3.54mmol) were taken. The resulting reaction mixture was refluxed on the water bath till the completion of reaction (TLC). The reaction mixture was poured on the crushed ice after the completion of the reaction. Separation of dihydropyrimidones is carried out with the help of stirring. The solid was filtered, washed with pet-ether, dried and recrystallised using ethanol.

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