

FLUORIMETRY AND PHOSPHORIMETRY

III Pharm.D

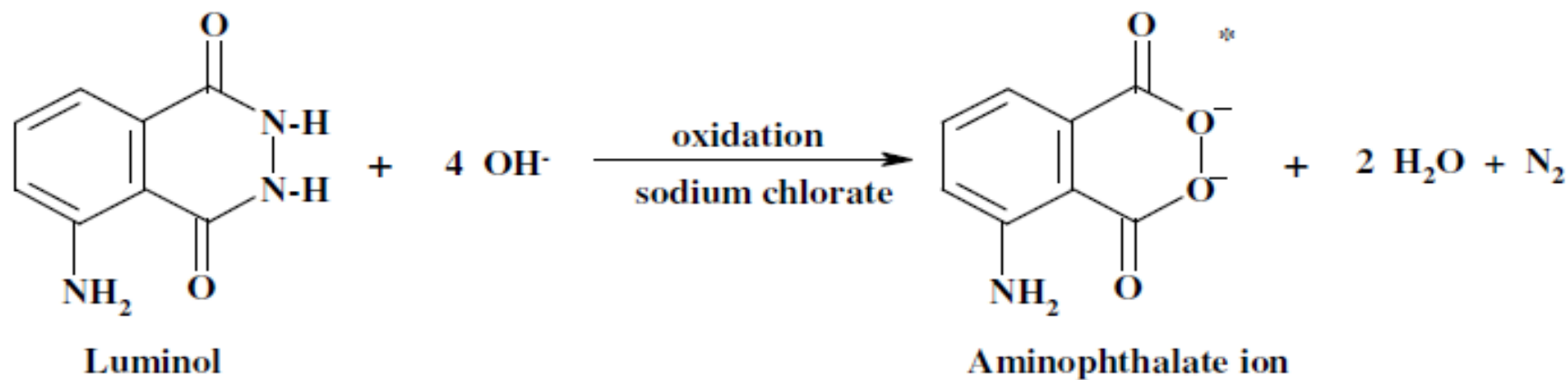
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INTRODUCTION

- Fluorescence is the emission of visible light by a substance that has absorbed light of a different wavelength. The emitted photon has a longer wavelength.
- Phosphorescence is related to fluorescence in emitting a photon, however, a phosphorescent material does not immediately re-emit the radiation it absorbs.
- As the excitation of the molecule is due to the absorption of a photon (light), these types of luminescence are called **photoluminescence**.

Chemiluminescence

- **Chemiluminescence is another phenomenon that falls in the category of luminescence.**
This refers to the emission of radiation during a chemical reaction.
- However, in such cases the excited state is not a result of absorption of electromagnetic radiation. The oxidation of luminol (3-aminophthalhydrazide) in an alkaline solution is an example of chemiluminescence



Jablonski Diagram

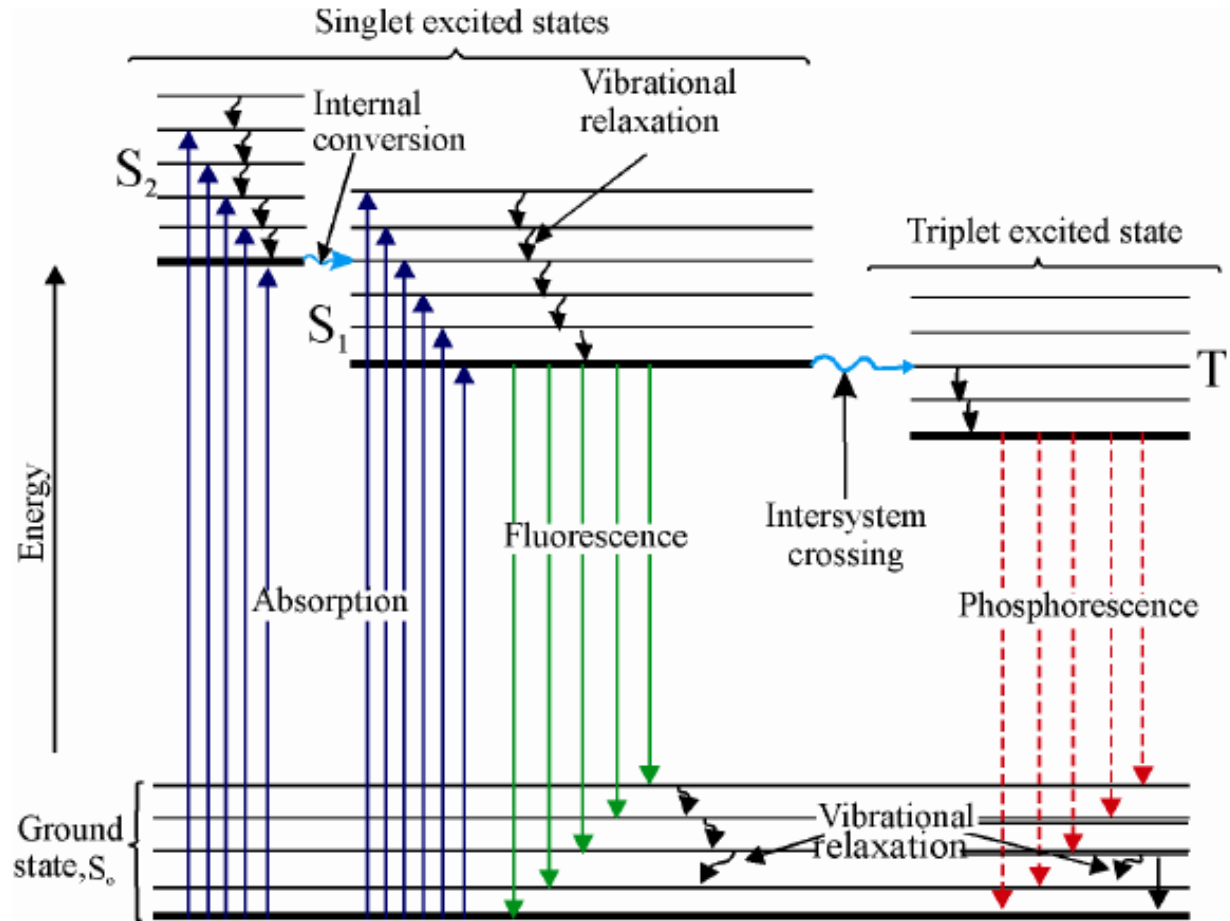


Fig. 5.1: The Jablonski diagram showing the phenomena of fluorescence and phosphorescence

- At the ground state , the molecular orbitals are occupied by two electrons. the spins of the two electrons in the same orbital must be antiparallel. This implies that the total spin, **S , of the molecule in the ground state is zero $[\frac{1}{2} + (\frac{1}{2})]$.**
- This energy state is called “**singlet state**” and is labeled as **S_0** .
- **The electron spins in the excited state achieved by absorption of radiation may either be parallel or antiparallel. Accordingly, this may be a triplet (parallel) or a singlet (antiparallel) state.**

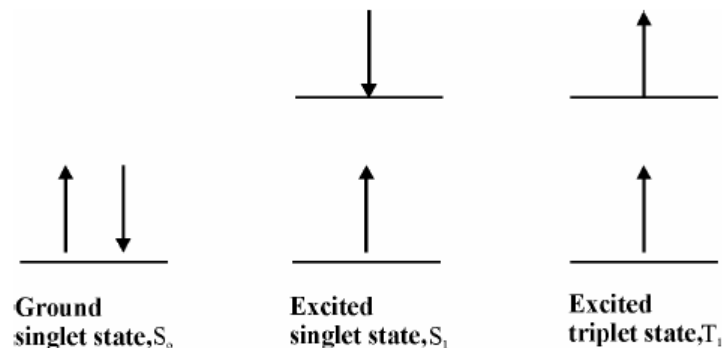


Fig. 5.2: Possible electronic spin states of the molecules

Activation and Deactivation

- The absorption of a photon of suitable energy causes the molecule to get excited from the ground state to one of the excited states. This process is called as **excitation or activation** and is governed by **Franck-Condon principle**.
- According to this principle, the electronic transition takes place so fast ($\sim 10^{-15}$ s) that the molecule does not get an opportunity to execute a vibration,
 - i.e., when the electrons are excited the internuclear distance does not change.
- The basis for the principle is that the nuclei are very massive as compared to the electrons and therefore move very slowly.

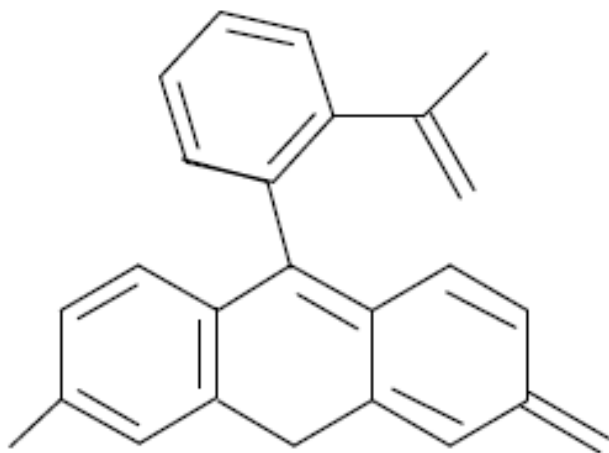
- The deactivation processes can be broadly categorised into two groups given below.
 - Nonradiative deactivation
 - Radiative deactivation

Photoluminescence and Structure

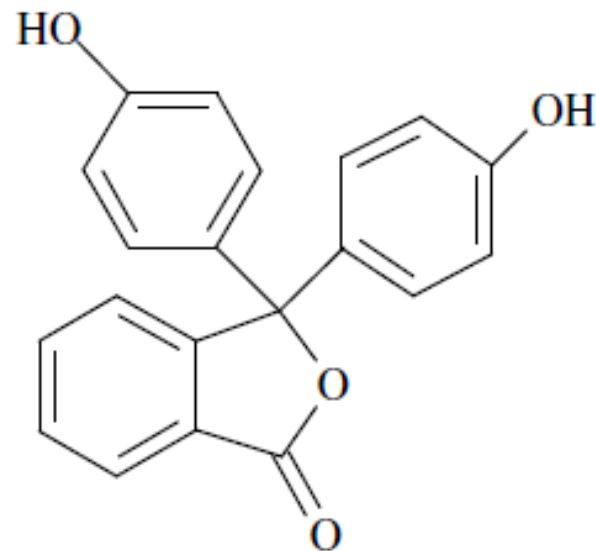
- The presence of the benzene ring and the nature of substituents on it seem to favour the fluorescent behaviour of the molecule.
- The halogen substituents tend to decrease the fluorescence and shift the fluorescence bands to longer wavelengths; the effects increase with increase in the atomic mass of the substituted halogen.

Photoluminescence and Structure

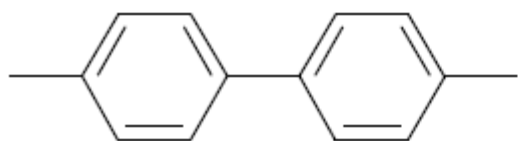
- Compounds with fused ring are found to be especially fluorescent, and the extent of fluorescence is found to be directly proportional to the number of rings in the molecule
- The structural rigidity in a molecule favours fluorescence



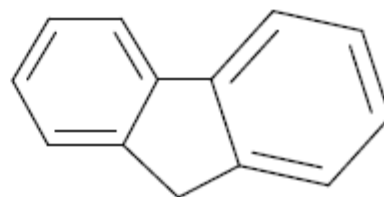
Fluorescein



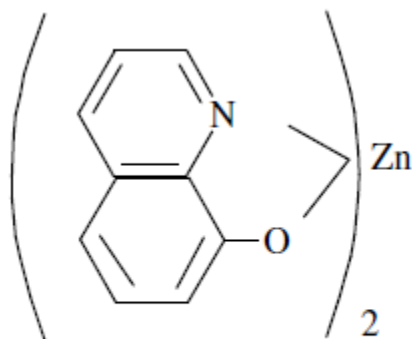
Phenolphthalein



Biphenyl

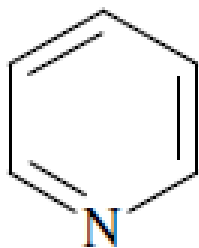


Fluorene

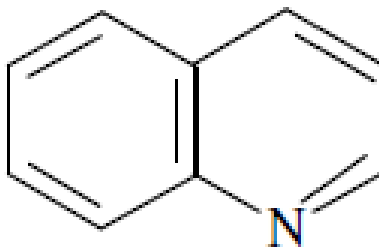


8-Hydroxyquinoline complexed with zinc ions

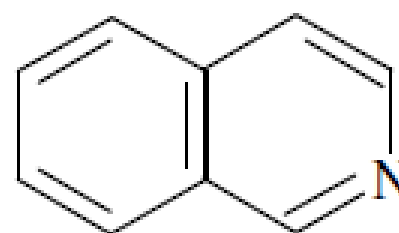
- The fluorescence observed with rigid cyclic molecules with pi-bonds is found to be enhanced by electron donating groups e.g., -NH_2 , OR, -OH and OCH_3 ,
- The electron withdrawing groups such as COOH , NO_2 , N=N and Br, I and CH_2COOH tend to reduce it.
- On the other hand the nonrigid molecules do not fluoresce much, as these rapidly lose the absorbed energy through nonradiative means like, vibrational relaxation or even degradation.



Pyridine



Quinoline



Isoquinoline

- Aliphatic and alicyclic carbonyl compounds or highly conjugated double bond structures also show fluorescence.

- As regards phosphorescence, it has been observed that the introduction of certain paramagnetic metal ions such as copper and nickel give rise to phosphorescence. These ions do not induce fluorescence, on the contrary Mg and Zn compounds show strong fluorescence.
- Phosphorescence is affected by the molecular structure such as unsubstituted cyclic and polycyclic hydrocarbons and those containing $-\text{CH}_3$, $-\text{NH}_2$, $-\text{OH}_2$, $-\text{COOH}$, $-\text{OCH}_3$ substituents which have lifetimes in the range of 5–10 seconds for benzene derivatives and 1–4 seconds for naphthalene derivatives.
- The introduction of a nitro group (NO_2) in a structure diminishes the intensity of phosphorescence, as does the introduction of aldehyde and ketonic carbonyl groups.
- The emission life time (t) is in seconds in rigid media and is 10² –10¹⁰ seconds in fluid media.

FACTORS AFFECTING FLUORESCENCE AND PHOSPHORESCENCE

The common factors affecting the fluorescence are as follows.

- Temperature
- pH
- Dissolved oxygen
- Solvent

Temperature

- A rise in temperature is almost always accompanied by a decrease in fluorescence.
- The change in temperature causes the viscosity of the medium to change which in turn changes the number of collisions of the molecules of the **fluorophore** with solvent molecules.
- The increase in the number of collisions between molecules in turn increases the probability for deactivation by internal conversion and vibrational relaxation.

pH

- Relatively small changes in pH can sometimes cause substantial changes in the fluorescence intensity and spectral characteristics of fluorescence.
 - For example, serotonin shows a shift in fluorescence emission maximum from 330 nm at neutral pH to 550 nm in strong acid without any change in the absorption spectrum.
- In the molecules containing acidic or basic functional groups, the changes in pH of the medium change the degree of ionisation of the functional groups. This in turn may affect the extent of conjugation or the aromaticity of the molecule which affects its fluorescence.
 - For example, aniline shows fluorescence while in acid solution it does not show fluorescence due to the formation of anilinium ion.
- Therefore, pH control is essential while working with such molecules and suitable buffers should be employed for the purpose.

Dissolved oxygen

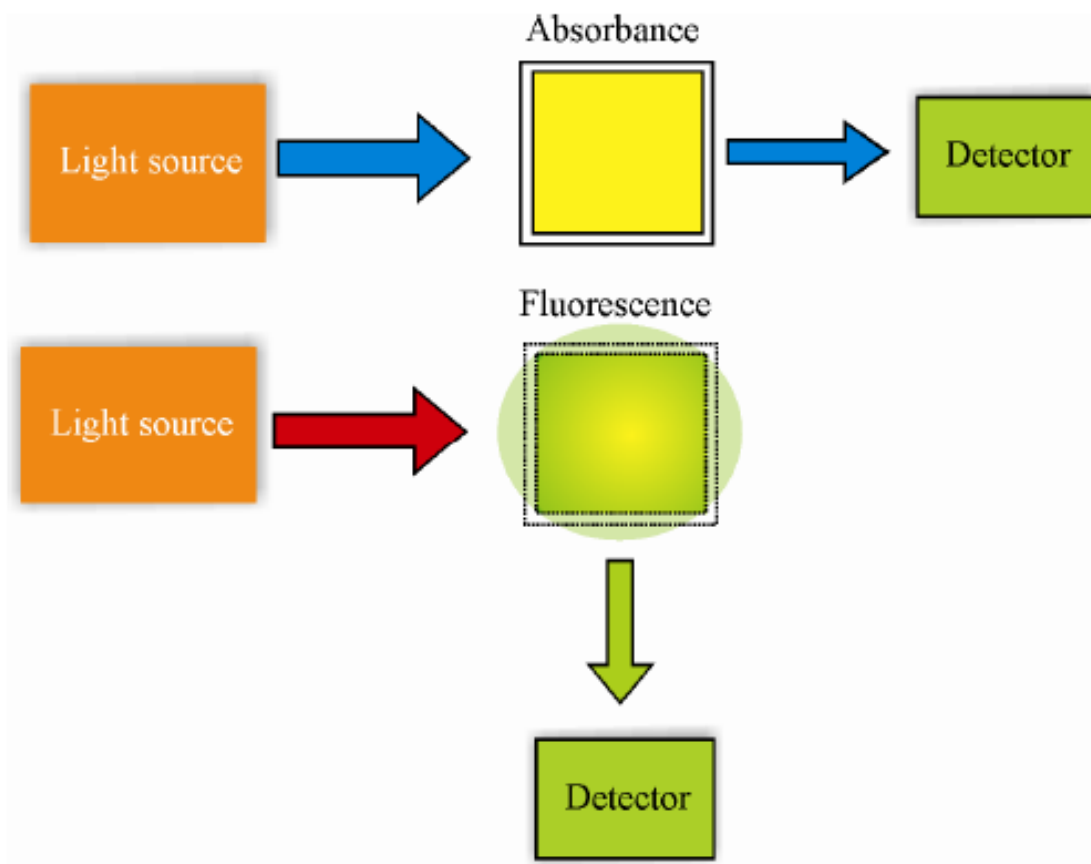
- The paramagnetic substances like dissolved oxygen and many transition metals with unpaired electrons dramatically decrease fluorescence and cause interference in fluorimetric determinations.
- The paramagnetic nature of molecular oxygen promotes intersystem crossing from singlet to triplet states in other molecules.
- The longer lifetimes of the triplet states increases the opportunity for radiationless deactivation to occur.

- Presence of dissolved oxygen influences phosphorescence too and causes a large decrease in the phosphorescence intensity.
- It is due to the fact that oxygen which is in triplet state at the ground state gets the energy from an electron in the triplet state and gets excited.
- This is actually the oxygen emission and not the phosphorescence. Therefore, it is advisable to make phosphorescence measurement in the absence of dissolved oxygen.

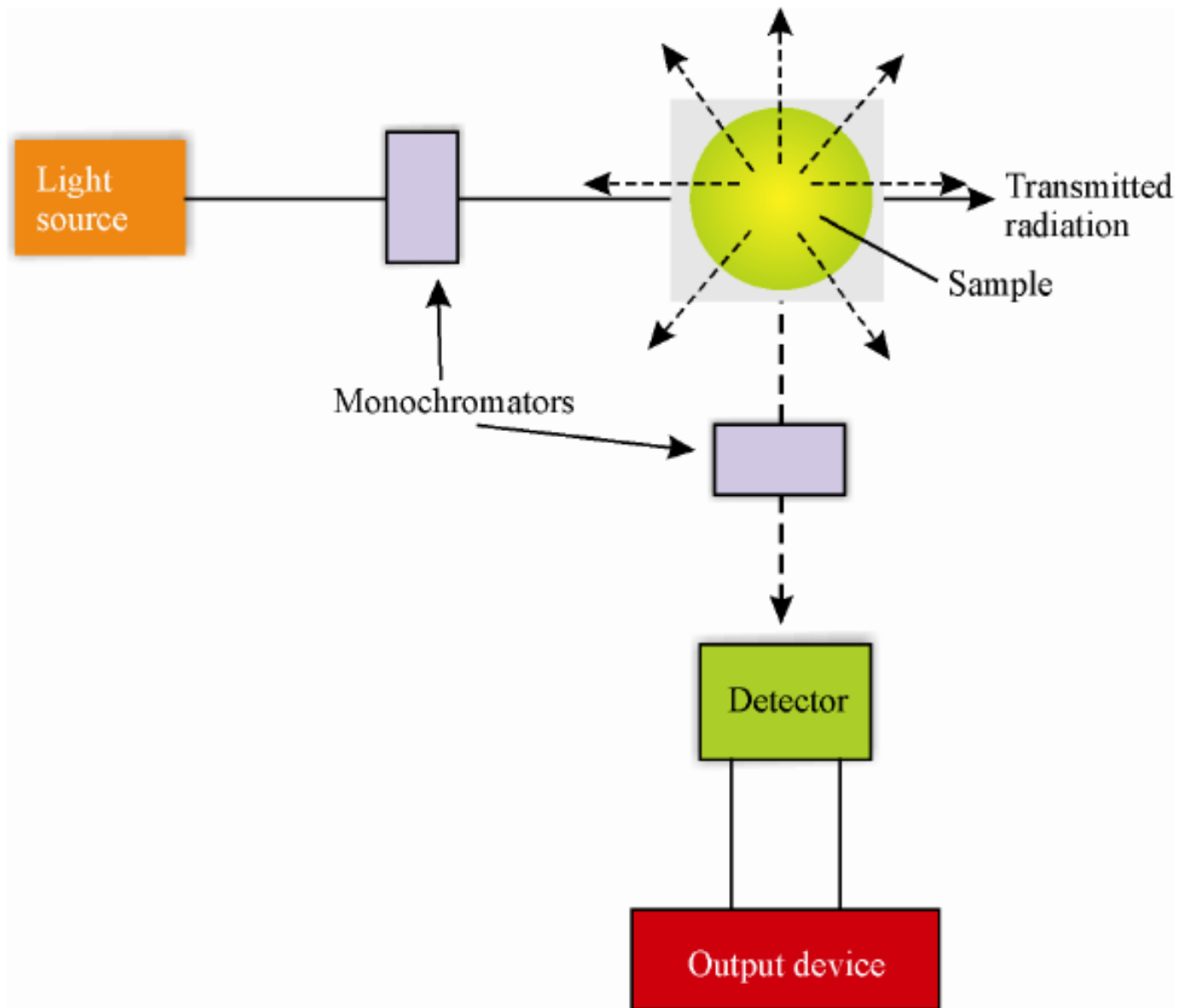
Solvent

- The changes in the “polarity” or hydrogen bonding ability of the solvent may also significantly affect the fluorescent behaviour of the analyte.
- The difference in the effect of solvent on the fluorescence is attributed to the difference in their ability to stabilise the ground and excited states of the fluorescent molecule.
- Besides solvent polarity, solvent viscosity and solvents with heavy atoms also affect fluorescence and phosphorescence.
- Increased viscosity increases fluorescence as the deactivation due to collisions is lowered.
- A higher fluorescence is observed when the solvents do not contain heavy atoms while phosphorescence increases due to the presence of heavy atoms in the solvent.

INSTRUMENTATION FOR FLUORESCENCE MEASUREMENT



- The essential components of an instrument used to measure fluorescence of the sample are:
 - Excitation light sources
 - Filters or Monochromators
 - Sample holder
 - Detector
 - Readout device



INSTRUMENTATION FOR PHOSPHORESCENCE MEASUREMENT

- You know that the basic difference between fluorescence and phosphorescence is that the phosphorescence emission occurs at a different time frame and can be measured only if the sample is solid or is at liquid nitrogen temperatures.
- The basic instrumentation for phosphorescence is similar to that of fluorescence; however, two aspects of the measurement need to be modified. The first is the sampling technique and second being the recording procedure.

Sampling

- Since most of the measurements in phosphorescence are carried out in rigid media at cryogenic temperatures of liquid nitrogen we need to use solvents that have certain special characteristics.
- It is the most important requirement are
 - good solubility of the analyte.
 - The solvent must form a clear rigid glass at 77 K i.e., the temperature of measurement.
 - In addition, it should be highly pure so that there is practically nil background phosphorescence

- Ethanol is an excellent solvent for polar molecules though it may require addition of small quantities of acid or base to produce a clear solid.
- On the other hand a mixture of diethyl ether, isopentane and ethanol in the ratio of 5: 5: 2 respectively, commonly called **EPA** is an **excellent choice for non-polar** compounds.

Phosphorimetry

- Spectrophosphorimeter is similar to a Spectrofluorimeter except that the former instrument must be fitted with
 - 1) A Rotating-shutter device commonly called a phosphoroscope and
 - 2) a sample system which is maintained at liquid nitrogen temperature.

Phosphoroscope

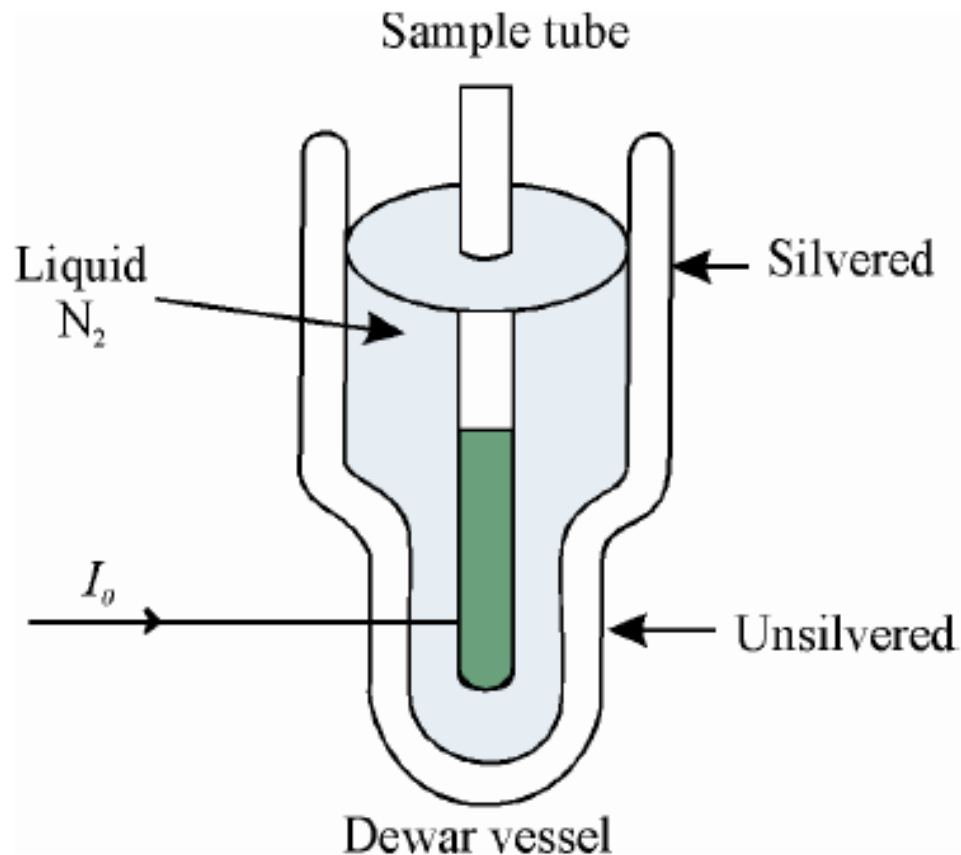


Fig. 5.8: Sample assembly for low temperature phosphorescence measurement

Phosphoroscope

1) The Rotating-Can Phosphoroscope:

- it consists of hollow cylinder having one or more slit which are equally spaced in the circumference.
- This is rotated by a variable-speed motor.
- when the rotating-can is rotated by a motor the sample is first illuminated and then darkened.
- Whenever, there is a dark, phosphorescence radiation passes to the monochromator and be measured

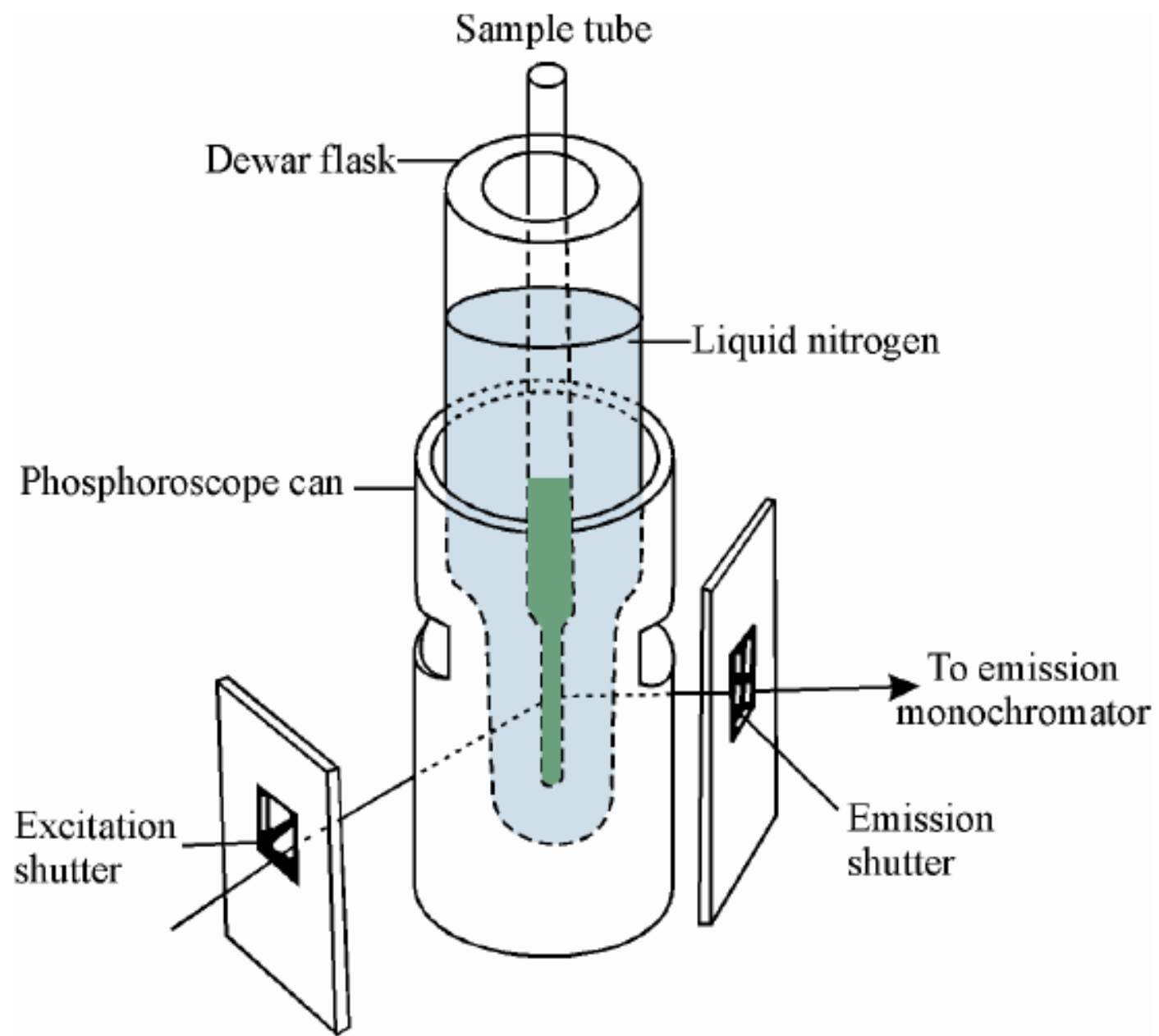
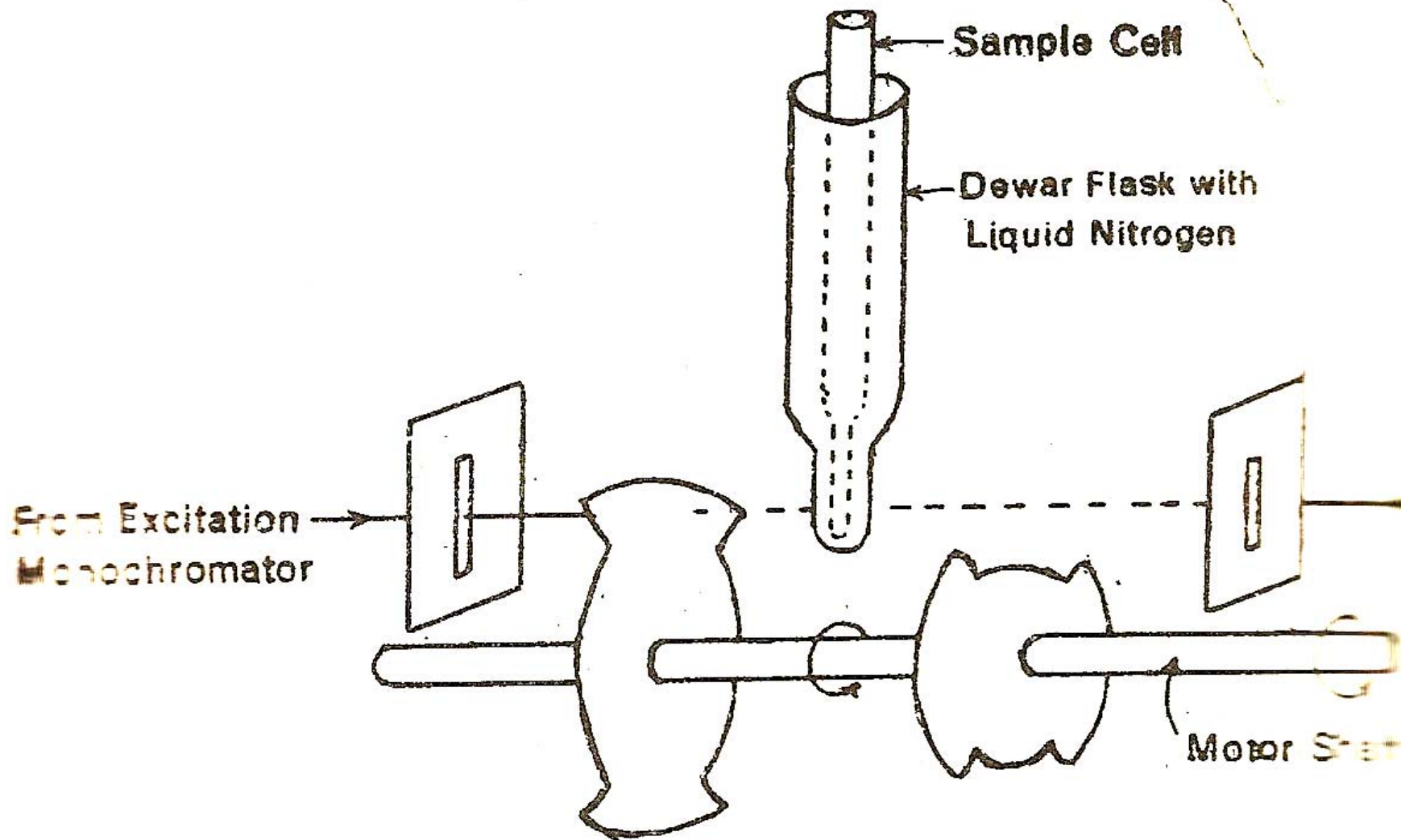


Fig. 5.9: Revolving can shutter system for recording phosphorescence and fluorescence from the same sample

2)The Becquerel or rotating disc phosphoroscope:

- It has two discs which are mounted on a common axis turn by a variable speed motor.
- Both the discs are having openings equally spaced in their circumference.
- On moving becqueral disc the sample is first illuminated and then darkened.



Thanks