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Fluorimetry

17.1 General

In the previous chapters we have learnt that when on atom or a molecule absorbs radiant energy it can produce electronic excitation and ionization in certain cases. If the energy is high enough to allow the electrons to be raised to excited states and then lose the energy by reemission in random directions, the process is called *luminescence*. The luminescent process is of two types. (a) fluorescence and (b) phosphorescence. The two phenomenon can be differentiated from each other in terms of (i) excited electronic state and (ii) the time interval between the absorption and reemission of the radiant energy. The excited and reemitted radiation may normally lie in the visible and U.V. regions of the spectrum.

In order to understand details of fluorimetry and its instrumentation, it is necessary that we clarify our mind on some of the fundamentals such as difference between *electron* orbital and *electronic state* An orbital essentially describes the volume element at a given distance and direction from the nucleus where there is a high probability of finding an electron. Normally, the electrons in a molecule occupy orbitals that are atomic in character. Therefore, we can say that the properties of all electrons in all the orbitals of a molecule are responsible for the electronic state of the molecule i.e. the wave function of an electronic state will be a combination of the wave functions of the electrons in each of the orbitals of a molecule. When an electron is moved from one orbital to another, the state of the molecule is changed, therefore it is necessary to consider the electronic state of the molecule rather than consider only the orbitals involved in such an electronic transition.

Further, we must know that in a molecule three types of electronic states are possible namely ; (i) ground state (ii) transition state and (iii) excited state. The most normal state of the molecule is the ground state i.e. the state of lowest energy. The transition state is considered to be a sort of a vibrationally excited ground state i.e. a state of strained condition. On the contrary, the excited state will be a state much higher than the ground state. It will have a different electron distribution from the ground state. Normally, there can be only one ground state in a molecule where as many different excited states are possible. The exact nature of these excited states depends upon the particular orbitals involved in the molecule.

A majority of organic compounds contain an even number of electrons, hence their electronic states may be either a *singlet state* or a *triplet state*. The ground state of most organic molecules will be a *single state* in which all of the electrons in the molecule will have their spins paired ($\uparrow\downarrow$). If one set of electron

spins in a molecule becomes unpaired ↑↑ or ↓↓ the resulting electronic state is called a *triplet state* .

Since substances which contain delocalized π—electrons are expected to show fluorescence, ethylene molecule may be utilized as a model example for studying such electron excitation. The position with ethylene is that it is in sp^2 hybridized configuration. Each carbon atom forms three sp^2 hybrid orbitals with an angle of 120° to each other and having a p_z orbital perpendicular to the plane of the three sp^2 orbitals. During bond formation each of the carbon atom will utilize two of the sp^2 orbitals to form σ bonds with hydrogen atoms and the third sp^2 orbital can form a low energy *bonding* σ orbital or a higher energy *antibonding* σ* orbital. The two p_z orbitals of the carbon atoms will combine to form *bonding* π orbital and *antibonding* π* molecular orbital. Thus in the ground state, ethylene molecule will have two electron in σ orbital and two electrons in the p orbital while the σ * and π* will remain vacant. This state will be represented by $\sigma\ {}^2\pi^2$. The various conditions for electronic states in ethylene are shown in Fig. 17.1.

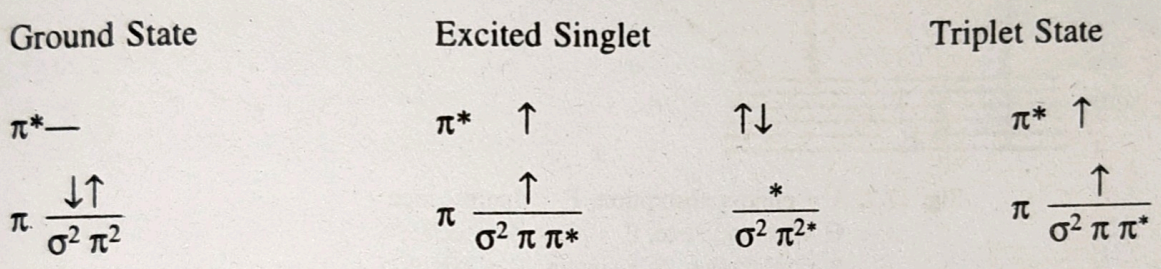

Fig. 17.1. Electronic states in ethylene.

As π and π* orbitals are already at higher energy states than σ and σ* orbitals, the former only will be of any significance for any such electron excitations. Thus the exacitation of π electrons will give rise to two possible excited singlet states in ethylene i.e. $\sigma^2\ \pi\ \pi^*$ and $\sigma^2\ \pi^{*2}$. The $\sigma^2\ \pi\ \pi^{*2}$ involves raising of one electron from π orbital and placing it in π* orbital and $\sigma^2\ \pi^{*2}$ singlet state consists in promoting both π electrons to the π* orbital. A third excited state called *triplet state* is also possible and involves raising of a π orbital electron into a π* orbital but with a change in the spin. The above three excited states of ethylene involve only π electrons and result from $\pi \rightarrow \pi^*$ transitions.

17.2 Fluorescence

When a molecule is exposed to a photon, it will absorb energy and in the process an electron will be raised into an orbital of higher energy. It the source of energy is removed the excited molecule while coming down to the ground state will reemit the absorbed radiation by a radiationless process. This radiationless or luminescent process will be either fluorescence or phosphorescence depending upon the type of excited state from which luminescence occurs and its relationship to the ground state.

If a molecule at the lowest vibrational level of an excited state returns to the ground state by emission of a photon, the process is called *fluorescence* . As the life time of an excited state is approximately 10^{-8} seconds, therefore the life time of fluorescence will be the intrisic life time of the excited state. Due to the vibrational (thermal) relaxation of the molecule after photon absorption and before photon emission the emitted photon in fluorescence will have lower energy (i.e. longer wavelength) than the excited photon absorbed by the molecule. This shift in the fluorescence spectrum from a shorter wavelength to a longer wavelength is known as *stokes shift*. Thus the phenomenon of fluorescences comprises in the absorption of a photon to go to an excited state, relaxation from higher vibrational levels of that excited state to its lowest vibrational level followed by emission of a photon to the vibrationally excited state of the ground state and again relaxation of the molecule to the lowest vibrational level of the ground state.

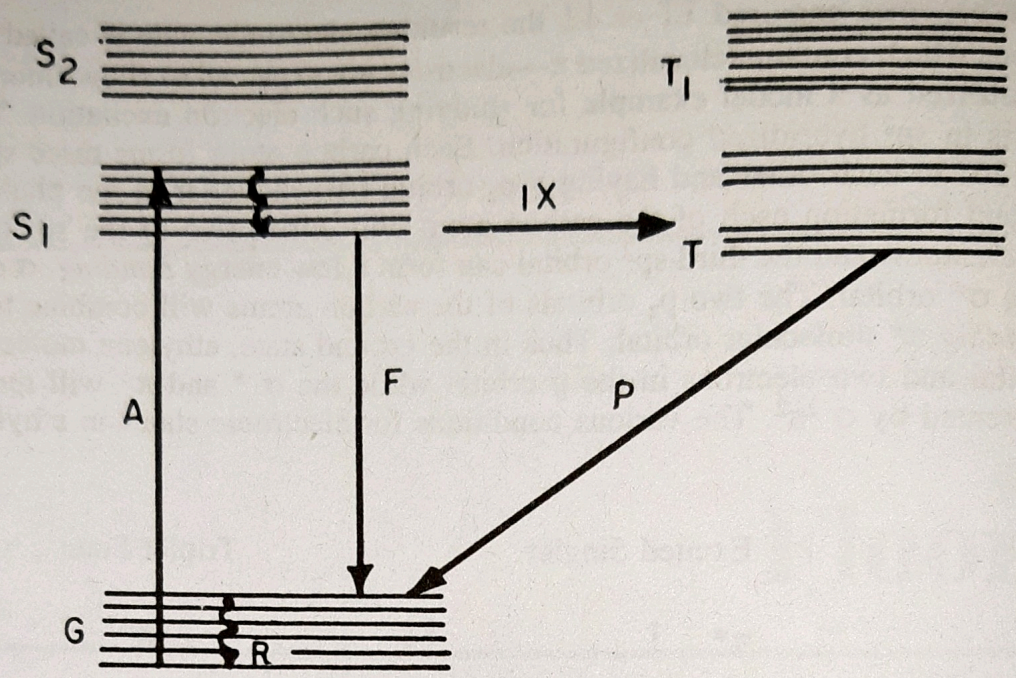

Fig. 17.2. A = photon absorption, F = fluorescence
G = ground state, P = phosphorescence
R = relaxation, S = singlet, T = Triplet
IX = Interstate cross over.

17.3. Phosphorescence

The transition from ground state of a molecule to the lowest triplet state though forbidden is possible and then the emission of light from the lowest triplet state can occur in two different ways. One way is by intersystem crossing of electrons from zeroth vibrational level of the lowest excited state of the molecule by vibrational coupling with the lowest triplet state. This emission is called *phosphorescence*. The life time of the lowest triplet state is 10^{-4} to 10 second which is the life time of phosphorescence. Therefore this phenomenon is characterized by an after glow not observed for fluorescence. A second way of emission of light from the lowest triplet state is by minimizing collisions of electrons in the triplet state by placing the molecule in a rigid medium to remove the vibrational energy in excess of the zeroth vibrational level of the triplet state. Thus phosphorescence involves a triplet — singlet transition.

17.4 Fluorescence and Molecular Structure

Saturated aliphatic compounds do not show fluorescence because they do not strongly absorb in the UV or visible region of the EM spectrum. However, some of the unsubstituted cyclic organic compounds do exhibit fluorescence in these regions. On the otherhand a majority of organic compounds that contain multiple-con-jugated double bonds with a high degree of resonance stability are expected to show fluorescence. It has been observed that the greater the number of rings in an aromatic hydrocarbon the lower is the energy of the emitted photon. Among the aromatic compounds, the nature of the substituent groups greatly affects their degree of fluorescence, e.g. electron donating groups like $-NH_2$, $-OH$ etc increase the degree of fluorescence while electron withdrawing groups like halogens, $-COOH$, NO_2 etc diminish or destroy fluorescence. Thus it may be noted that cyclohexane is non-fluorescent, benzene shows weak fluorescence while compounds like anthracene, riboflavin, thiamine and epinephrine are strongly fluorescent.

Benzene, when substituted by groups like –F, $-NH_2$, –OH and $-OCH_3$ shows more intense fluorescence than the parent, But, when substituted with groups like, I, Br and Cl does not show any fluorescence, instead gives phosphorescence involving intersystem crossing.

Substances like benzoic acid, nitrobenzene, benzenesulphonamide, benzaldehyde do not show any fluorescence, but benzonitrile shows intense fluorescence due to interaction of C ≡ N group with p electron of benzene. It is interesting to note that cis-stilbene shows less intense fluorescence than the trans isomer. This is due to the geometry of the molecule, the cis isomer is non-planer. Hydrocortisone though is a weak fluorescent substance. shows strong fluorescence in concentrated H_2SO_4 and ethyl alcohol.

The fluorescence is also affected by the ionization is some organic molecules in their excited states. For example β— naphthol shows different acidities in its lowest excited singlet states (ionic, fluoresces) and in its ground state (non-ionic does not fluoresce). Thus, fluorescence intensity is a function of pH of the solution in some organic molecules.

The intensity of fluorescence in organic compounds may be diminished or stopped due to competing quenching processes taking place in excited states. The quenching of fluorescence takes place because of the transfer of energy from the excited molecules to a particle with which at collides.

Ring closure is conductive to fluorescence of aromatic compounds e.g. fluorescein, phenolphthalein. Similarly metal chelation promotes fluorescence.

17.5 Fluorescence and Concentration

The degree of total fluorescence of a solution will depend upon the number of fluorescent molecules in the solution. It is improbable that all of the energy absorbed by a fluorescent molecule will be reemitted as fluorescence because some of the energy is lost through competing quenching process during the fluorescence process. Inorder to calculate fluorescence intensity use of quantum efficiency of fluorescence ϕf is made. It is defined as the number of quanta emitted by the fluorescent process to the number of quanta absorbed in unit time.

Beer's law states that in a solution of an absorbing substance the absorbance is directly proportional to the concentration. Thus we can say that fluorescence intensity will be proportional to the concentration of molecules in the excited state and therefore of the intensity of radiation. The light absorbed by the sample and intensity of the exciting light does not remain constant but decreases as it travels through the sample. In a dilute solution significant absorption will not occur and the decrease in the exciting light will not be significant. In a concentrated solution, the intensity of light might be different in regions of the sample being irradiated.

Thus the fluorescence intensity will be proportional to the amount of light absorbed which can be expressed as :

$$F = k \phi_f (I_0 - I)$$

where F = intensity of fluorescence

k = proportionality constant

ϕ_f = quantum efficiency

I_0 = Initial light

I = Transmitted light

As per Beer's Law the fraction of light transmitted is :

$$I = I_0 e^{-\epsilon bc} \text{ or } \frac{I}{I_0} = e^{-\epsilon bc}$$

where ε = molar absorptivity or molar extinction coefficient

b = path length (cm)

c = concentration in moles/litre.

The coressponding fraction of light absorbed in then :

$$1 - \frac{I}{I_o} = (1 - e^{-\varepsilon bc})$$

On rearranging the above equation we have

$$I_o - I = I_o (1 - e^{-\varepsilon bc})$$

But the total fluorescence intensity will be equal to the rate of light absorption (quanta per unit time) multiplied by the quantum efficiency of fluorescence ϕ_f ,

$$F = k \phi_\phi I_o (1 - e^{-\varepsilon bc})$$

The above equation can be expanded according to the series :

$$e^x = 1 + x + \frac{x^2}{2!} + \cdots + \frac{x^n}{n!}$$

$$\text{Thus } F = k \phi I_o \varepsilon bc \left[1 - \frac{\varepsilon bc}{2!} + \frac{(\varepsilon bc)^2}{3!} + \cdots + \frac{(\varepsilon bc)^n}{(n+1)!} \right]$$

It is to be noted that the dectector does not measure total fluorescence intensity but intensity form a segment of the sample.

$$\text{So } S_f = k \phi_f g \Phi I_o \varepsilon bc \left[1 - \frac{\varepsilon bc}{2!} + \frac{(\varepsilon bc)^2}{3!} + \cdots + \frac{(\varepsilon bc)^n}{(n+1)!} \right]$$

where S_f is signal generated by detector.

g = a constant representing sensitivity and amplification of signal

Φ = a constant represent geometry of the system, rather a solid angle of light viewed by detector.

When the concentration is small or the solution is very dilute, only a small fraction of light is absorbed, the term εbc is not greater than 0.05.

$S_f = k \phi_f g \Phi I_0 \varepsilon bc$ then only linear relationship will exist between concentration and fluorescence (Fig. 17.3).

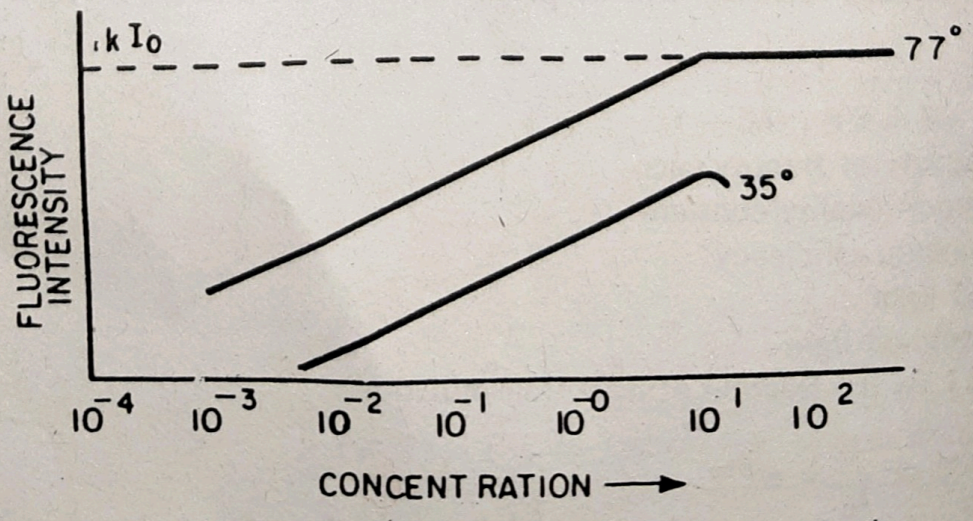

Fig. 17.3. Relation between fluorescence intensity and concentration.

When concentration is large, $e^{-\varepsilon bc}$ will become zero and the equation $S_f = k\ \phi_f\ g\ \Phi\ I_0$ will be independent of concentration.

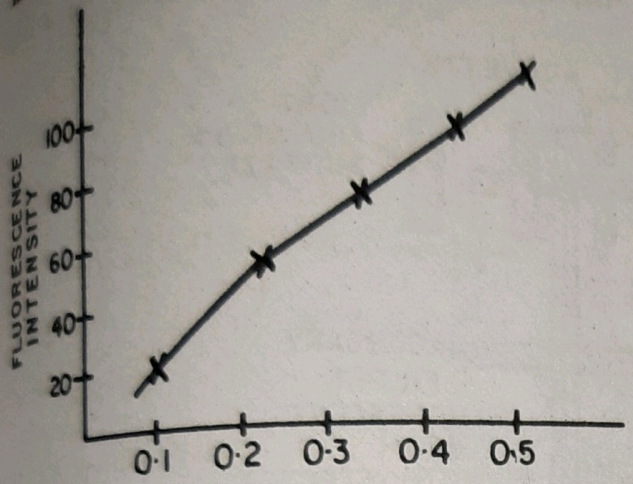

Fig. 17.4. Concentration of Quinine sulphate (mg %)

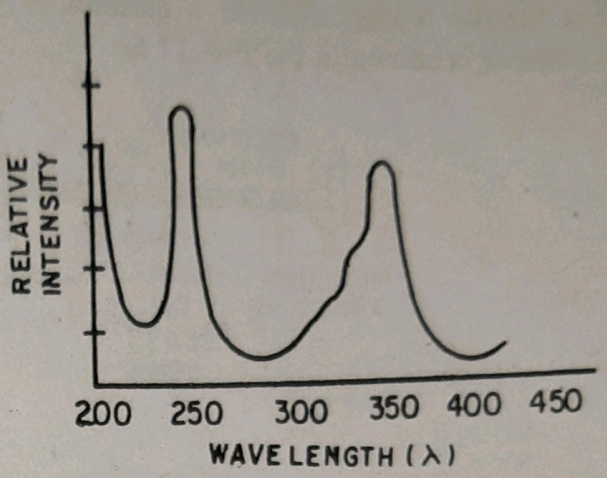

Fig. 17.5. excitation spectra of quinine in 0.05 M H_2SO_4.

17.6 Quenching

As already pointed out fluorescence process is competed by quenching process taking place in the excited states. The quenching can occur in three different ways as explained below.

(i) **Collisional Impurity Quenching :** The loss of fluorescence can occur by formation of excited complex between the excited sample species and the ground state impurity molecule and then subsequent non-radiative energy losses e.g. dissolved O_2 as impurity quencher.

(ii) **Energy Transfer Quenching :** This is another type of impurity quenching and will take place when an impurity present whose first excited singlet state is at an energy below that of excited singlet of the sample species. non-radiative energy transfer can occur between the impurity and the sample species.

(iii) **Concentration Quenching :** This is a kind of self-quenching and take places with increasing concentration. The quenching occurs due to the formation of a collisional complex between one molecule of the sample species that has been excited to singlet state and a second identical unexcited molecule.

The reactions for fluorescence can be represented as shown below :

$$M + hv \longrightarrow M^* \text{ (Absorption)}$$
$$M^* \longrightarrow M + hv \text{ (Fluorescence)}$$
$$M^* + Q \longrightarrow Q^* + M \text{ (Quenching)}$$
$$Q^* \longrightarrow Q + \text{Energy.}$$

where M is a sample in ground state,

M* is the excited sample,

Q is a quenching agent

hv is the photon of radiant energy

Quenching takes place due to the presence of an anion or ions with loosely bound electrons. Iodide, thiocyanate are powerful quenchers than those with tightly bound electrongs like fluoride and perchlorate. The oxidizing ions like nitrate has a good quenching effect. Organic substances like polyhydroxy phenols, amines as well as antioxidants are efficient quenchers.

During quenching there is no permanent reaction between the fluorescent substance and the quencher. There is usually a reversible oxidation-reduction reaction involved between the quencher and the excited molecule and then the regeneration of the original substance.

17.7 Instrumentation Aspects

The main components of a fluorimeter are (i) a source of radiant energy with which to irradiate the sample, (ii) a sample holder and (iii) a detector to measure fluorescence. A schematic diagram of a filter fluoremeter is shown in the Fig. 17.6.

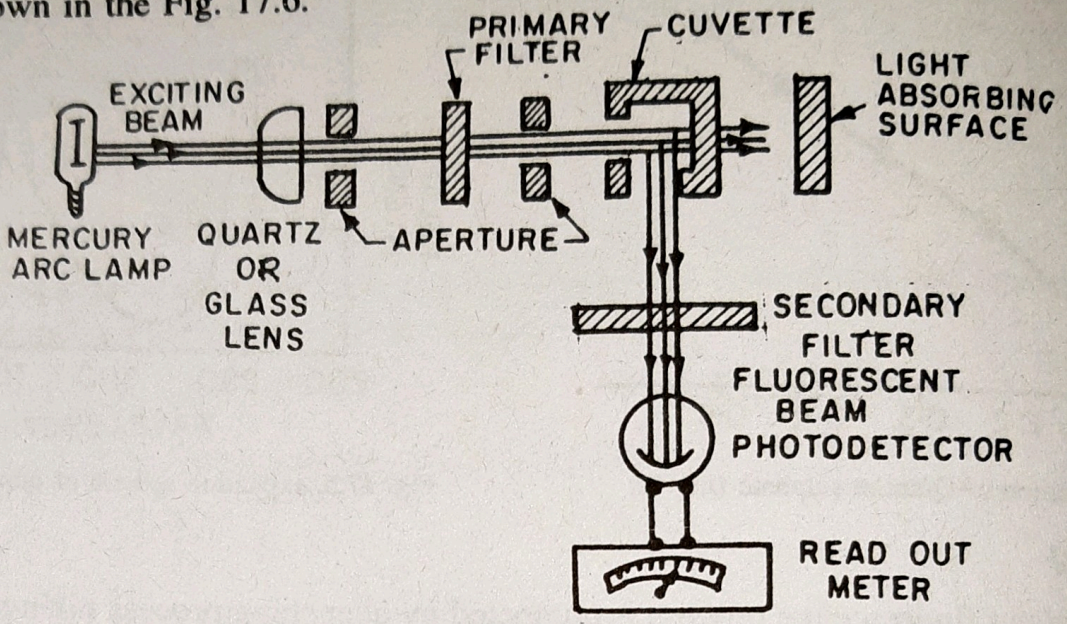

Fig. 17.6 A schematic diagram of a typical fluorimeter.

A mercury vapour lamp with glass or fused silica envelope is a common excitation source from which a suitable activation beam is isolated by means of a filter. The principle lines from mercury discharge lamp are at 366, 405, 436, 546 and 578 nm. The selection of the exciting wavelength is made by inserting a primary filter in the incident beam before the sample. A light source like high-pressure xenon-arc lamp provides a high level of ultraviolet and visible light.

When both excitation and emission spectra are needed two monochromators are necessary, one for the light source and one for fluorescence. For studies in ultraviolet, lenses or cuvettes made of quartz or fused silica are used.

The phototube is placed at right angles to incident beam and with masking slits in position the detector views only the illuminated liquid and not the illuminated cuvette faces. This also minimizes the reflection of any of the intense exciting radiation. A secondary filter is placed in front of the detector to remove the reflected and scattered exciting radiation. The secondary filter should be selected such that they absorb the scattered primary radiation but not the fluorescent light. Although fluorescence is emitted in all directions and can be viewed from any angle. But in practice 90° angle is the most convenient angle selected in designing fluorimeters.

Different models of spectrofluorimeters including those with microprocessor controls are available which can provide both the fluorescence emission spectrum and the excitation spectrum. Their operation consists in that a suitable wavelength in the emission spectrum is selected, and the second monochromator is set at this point. The excitation spectrum is then plotted by scanning the first monochromator. The optical diagram of a spectrofluorimeter is shown is Fig. 17.7 below.

A 150-w xenon lamp is used as the source and the chopped radiation is allowed to pass through a Czerny—Turner monochromator. It is then focused on to a sample cuvette where it is partially absorbed. The fluorescent emission is analysed by the second monochromator. Further, a beam splitter is inserted to cut away a fixed fraction of incident beam from the main path and monitered by means of an auxillary detector. This helps in correcting the output of the main photomultiplier tube for changes in the luminescence of the xenon lamp as the wavelength is scanned.

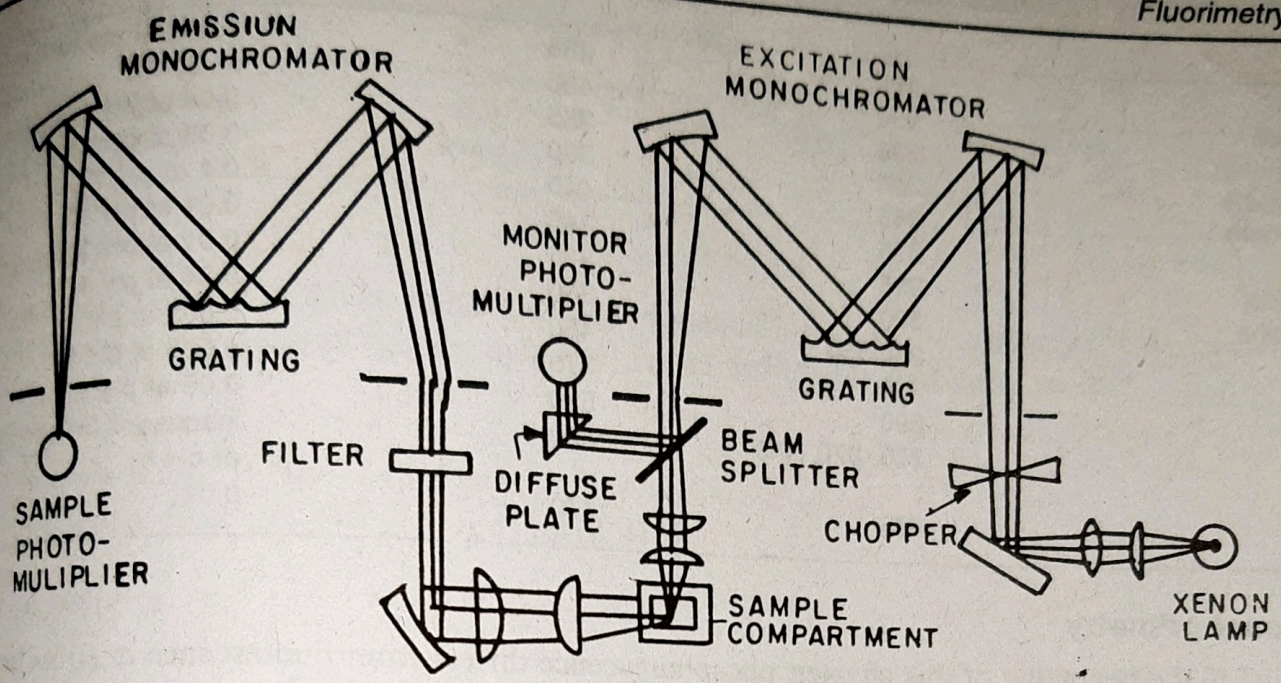

Fig. 17.7 optical diagram of a spectrofluorimeter.

17.8 Applications

Fluorimetry is selective because fewer substance show efficient fluorescence. It is also a very sensitive method because determination in the range of nanogram quantities or below can be measured. A variety of inorganic substances and minerals show fluorescence in the visible region while organic and organometallic compounds absorb in the ultraviolet region. For example selenium is determined by its complexation with 3, 3′— diaminobenzidine. Similarly cadmium is determined by reaction with bis (dimethylaminomethyl) fluorescein after it has been freed from other interfering ions.

A large number of pharmaceutical compounds have been assayed by fluorimetric procedure. Some of these compounds are listed in Table 17.1.

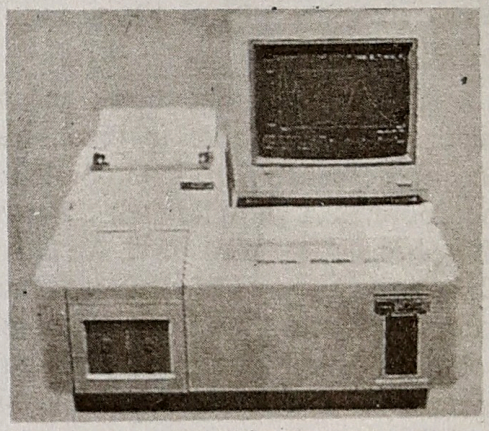

Fig. 17.8 Spectrofluoro-Photometer Model FP-777 JASCO (Courtesy of JASCO)

Table 17.1. Some pharmaceutical Compounds analysed by fluorimetry

Compound Name	Excitation (nm)	Fluorescence (nm)	Concentration required (µg/ml)
Allylmorphine	285	355	0.1 at pH 1
Amytal	265	410	0.1 at pH 14
Aureomycin	355	445	0.02 at pH 14
Chloroquine	335	400	0.05 at pH 11
Chlorpromazine	350	480	0.1 at pH 11
Cinchonidine	315	445	0.01 at pH 1.
Cinchonine	320	420	0.01 at pH 1.
Epinephrine	295	335	0.07 at pH 1.
Folic Acid	365	450	0.01 at pH 1.
Hydrocortisone	460	520	75% v/v in H_2SO_4 in ethanol

(Contd.)

LSD	325	465	0.002 at pH of
Nicotinamide	250	430	in CNCL
Norepinephrine	295	335	0.06 at pH 1.
Oxychloroquine	335	380	0.08 at pH 11.
Pentobarbitone	265	440	0.1 at pH 13.
Procaine	275	345	0.01 at pH 11.
Procainamide	295	385	0.01 at pH 11
Physostigmine	300	360	0.04 at pH 1.
Quinine	250,350	450	0.002 at pH 1.
Reserpine	300	375	0.008 at pH 1.
Terramycin	390	520	0.05 at pH 11.
Riboflavine	270, 370, 445	520	inaqueous buffer at pH 6
Vitamin A	325	470	0.01

17.9 Phosphorimetry

As explained in the beginning of this chapter, phosphorescence differs from fluorescence in (i) its longer decay time i.e. 10^{-1} to 10 seconds (10^{-7} to 10^{-9} seconds for fluorescence) and (ii) by a shift to longer wavelength.

A compound that shows phosphorescence should also possess fluorescence therefore a phosphorimeter should be able to distinguish between the two phenomena. One method of measurement of phosphorescence is to incorporate a rotating shutter called phosphorescope in an instrument. This device incorporates a definite delay between the time intervals during which the sample is irradiated and phosphorescence measured. An alternative method is to use a pulse technique instead of a rotating shutter. The primary source of radiation consists of a flash tube which is triggered by an electronic timing circuit. After a selected time interval, the photomultiphier is activated and phosphorescence measured. Another technique possible in phosphorimetry is time resolution. This technique is useful in the determination of mixtures or multicomponent analysis provided the individual components of the mixture have different decay times.

EXERCISE : 1. To draw the calibration plot for the fluorimetric estimation of quinine sulphate.

Reagents

0.1N Sulphuric Acid : Take about 300 ml distilled water in the 1 litre flask, add 3 ml conc. H_2SO_4 slowly (use 10 ml measuring cylinder). Add distilled water to make the volume upto 1 litre. Mix well.

Quinine Sulphate Solution

Stock Solution A : Weigh 100 mgms quinine sulphate powder. Transfer to a 100 ml volumetric flask, using a small funnel. Add 30-40 ml. 0.1N-H_2SO_4 and dissolve the powder. Make the volume to 100 ml with 0.1N-H_2SO_4. Mix well and label. This contains 1 mgm/ml of quinine sulphate.

Stock Solution B : Pipette 1 ml of stock solution A to another 100 ml volumetric flask. Make the volume to 100 ml using 0.1N-H_2SO_4. Mix well and label. This contains 10 µg/ml of quinine sulphate.

Stock Solution C : Pipette 10 ml of stock solution B to a 100 ml volumetric flask. Make the volume to 100 ml using 0.1N-H_2SO_4. Mix well and label. This contains 1 µg/ml quinine sulphate.

Standard Solution : Using stock solution C, prepare standard solutions of known concentrations, as given below (Use graduated pipettes) :

Test-tube No.	0	1	2	3	4	5
ml. of stock solution C	0.0	1.0	2.0	3.0	4.0	5.0
ml. of 0.1N-H_2SO_4	10.0	9.0	8.0	7.0	6.0	5.0

Procedure

Follow the procedure as given in the instruction manual or as per the instructor. Use standard solution No. 6 (containing 0.5 µg/ml quinine sulphate) for adjusting 100 on the dial scale.

Filters

Primary filter : B Hg - 1 (365 nm)
Secondary filter : N 440 (450 nm) (or B 470)

Observations

S. No.	Concentration (µg/ml)	Fluorescence Intensity (F)
*1	0.0	
2	0.1	
3	0.2	
4	0.3	
5	0.4	
6	0.5	

Plot Fluorescence intensity vs. Concentration of quinine sulphate.
*This is F_o (blank).

Results

1. The calibration plot for quinine sulphate is attached.
2. The plot is linear/non-linear in the concentration range studied (.....µ.g/ml to µg/ml).
3. The concentration of quinine sulphate in the given sample (concn. unknown) is .....µ.g/ ml.

Sample Calculations

Ex. The following data was obtained for fluorimetric estimation of Penicillin as an aminoacridine derivative :

S.No.	Sample Concentration (µg/10 ml)	Fluorescence Intensity (F)	
1.	0.0 (Blank)	9.1	(F_o)
2.	1.5 (Standard)	32.7	(F_s)
3.	Sample	28.8	(F_u)

What is the concentration of penicillin (in µg/ml) in the sample ?

$$\text{Conc. (unknown)} = \text{Conc. (std)} \times \frac{(F_u - F_o)}{(F_s - F_o)}$$

$$= 1.5 \times \frac{(28.8 - 9.1)}{(32.7 - 9.1)} = 1.5 \times \frac{19.7}{23.6}$$

$$= 1.25 \text{ µg/10 ml or } 0.125 \text{ µg/ml}$$

The concentration can also be read directly from the calibration plot.

EXERCISE : 2. To study the pH dependence of the fluorescence of quinine sulphate solutions.

Reagents

Prepare stock solutions of quinine sulphate (stock solution A, B and C) as in experiment No. 1, except that use distilled water instead of 0.1N-H_2SO_4 in all cases.

Prepare 10 ml each of the standard solutions of quinine sulphate as given below :

Test-tube No.	1	2	3	4	5
Volume of stock solution C (in ml.)	1.0	2.0	3.0	4.0	5.0
Distilled water (in ml.)	9.0	8.0	7.0	6.0	5.0

Procedure

Follow the procedure as given in the instruction manual or as per instructor. Use the same settings of control knobs as in Experiment No. 1.

Observations

S. No.	*Concentration (µg/ml)*	*Fluorescence Intensity*
1	0.1	
2	0.2	
3	0.3	
4	0.4	
5	0.5	

Plot Fluorescence intensity vs. Concentration, at the neutral pH.

Result

Compare F vs C plot at neutral pH (as above) with the plot at acidic pH (as in Experiment No. 1)

Record your conclusions regarding the effect of pH on the fluorescence of quinine sulphate solutions.

EXERCISE : 3. To study the "quenching" effect due to the presence of varying concentrations of iodine ion on the florescence of quinine sulphate solution.

Materials

Quinine sulphate solution (Stock solution C,
Potassium iodine
0.1N sulphuric acid
1 volumetric flask (100 ml) for KI solution.
10 ml graduated pipette for KI solution.
20 ml test-tubes.
1 Test-tube stand.

Reagents

1. **Potassium Iodide Solution** : Dissolve 200 mgms. KI solution in 100 ml. of 0.1N-sulphuric acid (contains 2.0 mg/ml KI).
2. **Quinine Sulphate Solution :** Use stock solution C, prepared in Experiment No. 1 (1 µg/ml quinine sulphate). Prepare standard solutions as given below (use graduated pipette) :

Test tube No.	1	2	3	4	5
ml. of stock solution C.	5.0	5.0	5.0	5.0	5.0
ml. of KI solution	0.0	1.0	2.0	3.0	4.0
ml. of 0.1N H_2SO_4	5.0	4.0	3.0	2.0	1.0

Procedure

Follow the procedure as described in the instruction manual or as per the instructor. Use the same settings of control knobs as in Experiment No. 1.

Observations

S. No.	*Concentration KI* (mg/ml)	Fluorescence Intensity
	0.0	
1	0.2	
2	0.4	
3	0.6	
4	0.8	
5		

Plot Fluorescence intensity vs. Concentration of potassium iodine.

Result

Compare F vs. C plots in presence of iodine ion with the plot obtained in its absence (Exercise No. 1). Record your conclusions.