Fluorescence

Fluorescence is a photoluminescence process in which atoms or molecules are excited by absorption of electromagnetic radiation. The excited species then relax to the ground state, giving up their excess energy as photons.

Characteristics of fluorescence:—

(i) molecular fluorescence is very sensitive

ii) For selected species, under controlled conditions, single molecules ~~here~~ may be detected by fluorescence spectroscopy

iii) There is a large linear concentration range of fluorescence method

iv) Disadvantages:

1) Fluoreseeence method is less widely applicable than absorption method because of the relatively limited number of chemical systems that show appreciable fluorescence.

ii) There are many more environmental interference effect than absorption methods.

Theory of molecular fluorescence:—

i) Molecular fluorescence is measured by exciting the sample at absorption wavelength, called the excitation wavelength

ii) Short-lived emission that occurs is called fluorescence and long-lived luminescence is called phosphorescence.

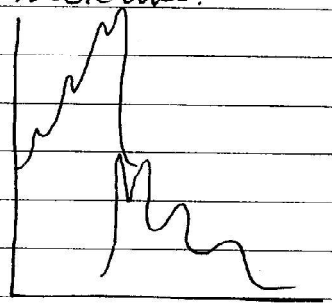
iii) Excited molecule undergo vibrational relaxation and then relaxed molecule undegoes non radiative relaxation and fluorescence emission. Almost always, fluorescence is observed from lowest lying excited electronic state $E_1 \rightarrow E_0$. Also fluorescence ~~usually~~ usually occurs only from the lowest vibrational level of E_1 to many different Vibrational levels of E_0 state

iv) Molecular fluoreseence bands are mostly made up of lines that are longer in wavelength that the band of absorption radiation. This shift of to longer wavelength is called Stokes shift.

Relationship between Excitation spectra and fluorescence spectra:—

i) Energy difference between vibrational states is about the same for both ground and excited states, the excitation spectrum and the fluorescence spectrum for a compound often appear as approximate mirror images of one and another with

overlap occuring near the origin transition. Though there are many exceptions to this mirrore image rule, particularly when the excited and ground states have different geometries or when fluorescence bands originate from different parts of the molecule.



Quantum Yeild :—

Quantum yeild of molecular fluorescence is ratio of the number of molecules that fluoresce to the total number of excited molecules or the ratio of photons emitted to photons absorbed.

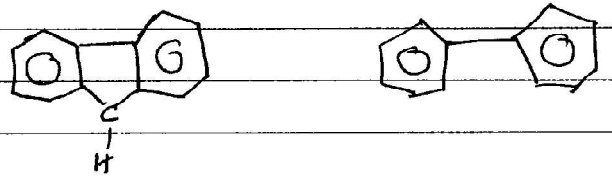
$$\Phi_F = \frac{k_F}{k_F + k_{nr}}$$

k_F = first order rate const for fluorescence relaxation k_{nr} = radiation less relaxation.

Fluorescence & Structure :— Compound containing aromatic rings give the most intense and most useful molecular fluorescence emission certain aliphatic and alicyclic carbonyl compounds as well as highly conjugated double bonded structure also fluoresce.

Most unsaturated aromatic hydrocarbons fluoresce in solution but quantum yeild increases with increasing numbers of rings and their degree of condensation. However hetero atom containing aromatic compounds generally do not fluoresce.

fluorescence is particularly favoured in rigid molecules. for example quantum efficiency of fluorene is ~1.0 but that for biphenyl is ~0.2.



The rigidity lowers the rate of non radiative relaxation.

In addition, enhanced emission frequently results when fluorescing dyes are adsorbed on solid surface.

Temperature and solvent effect :—

For most molecules, quantum efficiency of fluorescence decreases with increasing temp because the increased frequency of collision at elevated temperature increases the probability of non-radiative collisional relaxation.

A decrease in solvent viscosity also leads to the same result.

Effect of Concentration on fluorescence Intensity

The power of fluorescence radiation F is proportional to the radiant power of the excitation beam absorbed by the system :—

$$F = K'(P_0 - P)$$

P_0 = Power of incident beam

P = Power of beam passing through a length of l of the medium.

Now Beer's law may be written as

$$P/P_0 = 10^{-\varepsilon.l.c.}$$

Expansion of the exponential term leads to

$$F = K' P_0 (1 - 10^{-\varepsilon.l.c})$$

$$F = K' P_0 \left[2.3\varepsilon.l.c - \frac{(2.3\varepsilon.l.c)^2}{2} \ldots \right]$$

When $\varepsilon.l.c < 0.05$, the 1st term is much larger than subsequent terms. and we can write

$$F = K' P_0 \varepsilon.l.c$$

For constant incident power P_0

$$F = KC.$$

Thus plot of fluorescence power of a solution as a function of concentration of the emitting species should be linear at low concentration when C becomes large and consequently absorbance is larger than 0.05, the relationship represented in equation becomes non linear. and F lies below an extrapolation of linear plot.

This effect is called inner filter effects.

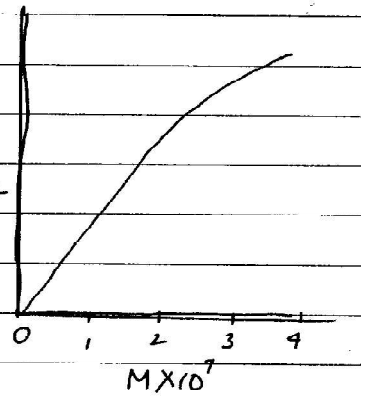
Inner-filter effect correction :

Fluorescence intensity vs concentration plot deviates from linearity due to Primary & secondary absorption. Primary absorption results from strong absorption of the incident beam and fluorescence is no longer proportional to the concentration of fluorescing

materials. Secondary absorption results from the absorption of the emitted radiation by other analyte molecules.

$$F = F' 10^{\varepsilon_m c \frac{l}{2} + \varepsilon_{em} \cdot c \cdot l/2}$$

Where F = corrected fluorescence intensity and F' is observed fluorescence intensity.



Applications of fluorescence:

1) Fluorescence methods are used to study chemical equilibria and kinetics ~~and~~ at lower conc. due to high sensitivity.
2) In many cases where fluorescence monitoring is ordinarily not feasible, fluorescent probes or tags may be attach covalently ~~that enable to study~~ to specific sites in molecule and marking them detectable via fluorescence. This tags can be used to provide information about energy transfer processes, the polarity of the substance molecule, micro environment pH, and distances between reactive sites.
3) Inorganic fluorescence methods are developed by reacting analytes with complexing agent to form fluorescent complex. Some of the fluorescence reagents for cations are 8-hydroxyquinoline (reagent for Be, Al etc) ~~alizg~~ alizarin garnet R (for Al & F^-) flavanol (reagent for Zr & Sn), benzoin (reagent for B, Zn Ge, Si)
Quenching methods may also be used to detect inorganic ions mainly anions.
3) Fluorescence method is also widely used in Biochemistry. The compounds that can be determined by fluorescence ~~is are anions~~ are amino acids, proteins, Co-enzymes vitamins, nucleic acids, alkaloids, ~~ph~~ porphyrins steroids, flavonoids and many more.

REFERENCE: Fundamentals of Analytical Chemistry. Skoog, West, Holler & Crouch, 8th Edition, Cengage Learning publication; page 825-838