

Methods of fluorescence determination :

Three methods

1)Direct method

2)Indirect method

3)Quenching method

1) Direct method

In this method, material is naturally fluorescence and this is directly measure
i.e sample should be naturally fluorescence. So directly study it

2) Indirect method

Sample is non fluorescent, it is converted in to fluorescent by following ways

By forming complex

Sometimes compound is non fluorescent but its derivative is fluorescent. So by
derivatization we can make it fluorescence.

By labelling it with fluorescent dye and then study it

3) Quenching method:

It is process in which decrease in fluorescence intensity takes place. For example our compound is fluorescent, we use it for the determination of iron in water. Iron can form complex with dye and decrease fluorescence. There should be linear relationship. Suppose fluorescent dye intensity is 1.

When it add in one ppm solution of water, its fluorescence decrease to 0.9

In 2 ppm solution, it fluorescence decrease to 0.8

In 3ppm solution, it decrease to 0.7.

It means intensity of fluorescence decrease and quenching increase.

By making graphs we determine the iron.

Natural Fluorophores

Natural existing compounds that show fluorescence are called natural fluorophores.

These are **1) Vitamin A and E**

2) Carotenes

3) Quinine

4) Steroids

5) Aromatic amino acids

6) Co-enzymes

7) Nucleotides

8) fluorescent proteins etc.

Quenching

It is reduction in the intensity of fluorescence due to the specific effect of constituents of compounds is called quenching. Fluorescent compound quench the light radiations.

Those substance which are responsible of quenching is called **Quenchers**. For example oxygen, iodide ion, cesium ion, acrylamide.

Four possibilities of quenching process or deactivation process

1)Heat

2)Relaxation by photochemical reaction

3)Reaction between excited species and other species

4)Dissipation of emission of photon.

1)Heat:

The atom may re-emit its extra energy in the form of heat and may come to the ground state.



This process give **different names** like

a)Non- radioactive deactivation

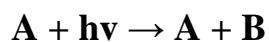
b)Collisional deactivation

c)Vibrational Relaxation

d)Thermal Relaxation

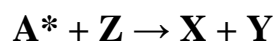
2)Relaxation by photochemical Relaxation:

This may happen through decomposition of excited atom



3)Reaction between excited species and other species:

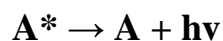
The molecule A^* may react with other species present in surrounding. They can give us different products. For example



It is **rare process**

4)Dissipation by emission of photon:

The extra energy of excited molecule is dissipated by emission of photons



This is also known as photo deactivation process. It is also **rare process**.

Types of quenching:

Three types

1)Concentration Quenching

2)Self- Quenching

3)Chemical Quenching

Concentration quenching:

It is the type of quenching which occur when the emitted radiations are absorbed solution. The radiations emitted from the excited state atoms are absorbed by the ground state atoms being present at high concentration. So the fluorescent

intensity decreases. This is called concentration quenching. It is also called **inner filter effect**.

Self-Quenching:

It is the type of quenching which occur when the emitted radiation are absorbed by sample present in solution.

Chemical Quenching:

Decrease in intensity of fluorescence because of actual changes in chemical nature of fluorescent substance is called chemical quenching.