FIUORIMETRY

➢ THEORY:-

The emission of light by a substance it occurs when an electron returns to electronic ground state from an exited state and loses it's excess energy as photon is called as Luminescence.

Luminescence is of two types -

- Fluorescence.
- Phosphorescence.

1. FLUORESCENCE:-

If a phenomenon of emission of radiation when molecules are exited By radiation at certain wavelength when a beam of light is incident on certain substance they emit visible light or radiation this phenomenon is known as Fluorescence. **2. PHOSPHORSCENCES:-**

When light radiation is incident of certain substance they emit light continuously even after incident light is cut off this type of delayed fluorescence is known as Phosphorscenes.

➢ CONCEPT OF SINGLE TRIPLET STSTE:-

Singlet State: A molecular electrons state in which all electron spins are paired is called a singlet state and no splitting of electronic energy levels occurs when the molecule is exposed to a magnetic field.

Doublet State: Free radical (due to odd electron). Net spin S is 1/2. Spin Multiplicity 2S + 1 = 2.

Triplet State: Electron Spins in the ground and excited electronic states are not paired.

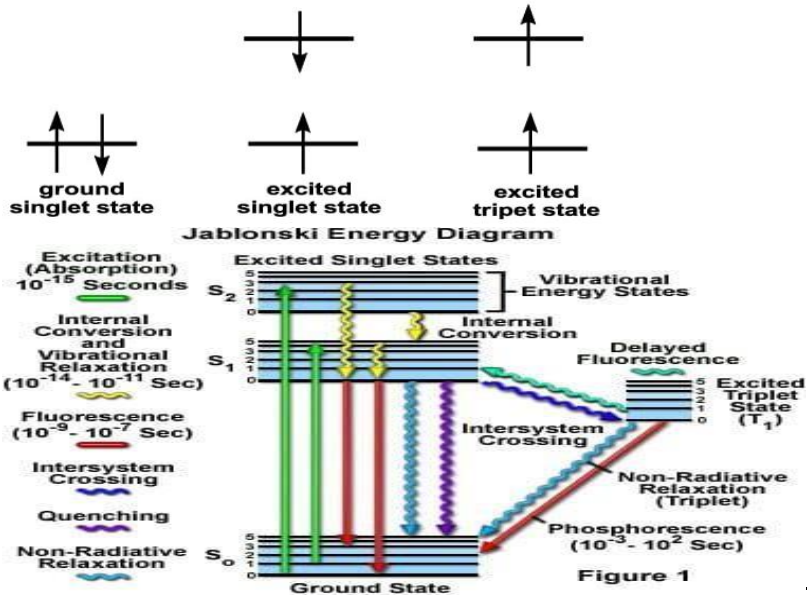


Figure 1

➢ FACTOR AFFETCTING ON FLUORESCENES :-

1) Oxygen:-
2) Photodecomposition:-
3) PH:-
4) Temperature And Viscosity:-
5) 5 Impurities and other substances:-
6) Chemical Quenching:-
7) Inter Filter effect:-

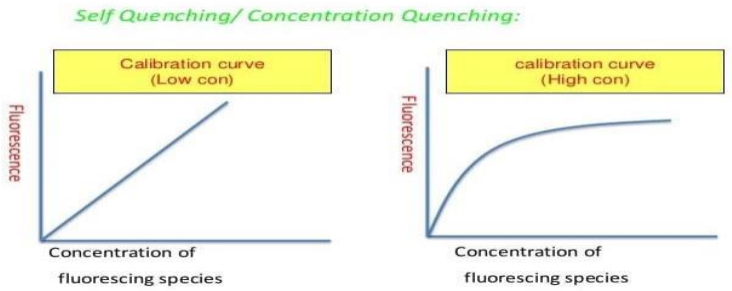
QUENCHING:-

- Decrease in Fluorescence intensity due to specific effects of constituents of the solution.
- Due to concentration ph, pressure of chemical substances , temperature, viscosity, etc

TYPES OF QUENCHING:-

1) Self quenching.
2) Chemical quenching.
3) static quenching.
4) Collosion quenching.

1) SELF QUENCHING:-



Deviation at higher concentration can be attributed to self quenching

2) CHEMICAL QUENCHING:- Here decrease in fluorescence intensity due to the factors live change in ph, presence of oxygen-bolides & heavy metals.

Ph,Halides,Heavy metal

3) STATIC QUENCHING:-

This occure to complex formation. Eg. Caffine reduces the fluorescence of riboflavin by complex formation.

4) COLLISION QUENCHING:-

It reduces fluorescence by collision where no. collision increased hence quenching take place.

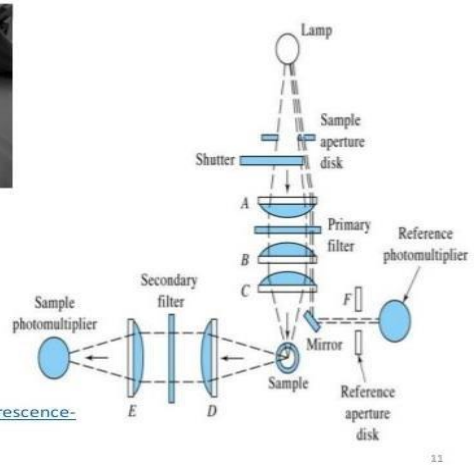
➢ INSTRUMENTATION:-

1) Instrument for fluriometric Analysis:-

The instruments used for the measurement of fluorescence are known 56 fluorescence on Fluorophotometer In these Filters are used to 150late the wavelength of excitation

➢ FLUROMETER:-

A-1 filter fluorometer



https://www.edinst.com/blog/fluorescence-measurements-introduction/

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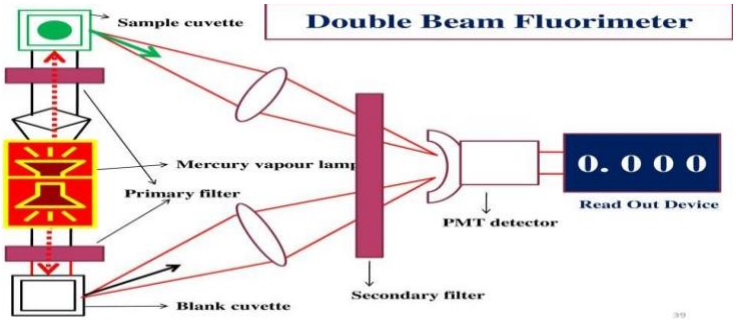
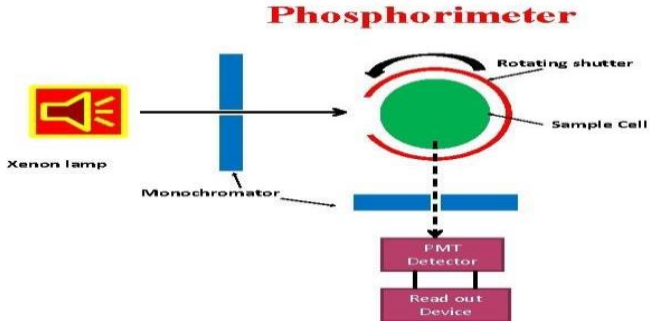
basic arrangement for a single-beam, 90°, filter fluorimeter is shown in Fig. 16.7., a condensing lens, a primary filter, a sample container, a secondary filter and a receiving photocell.



A double-beam filter fluorimeter is shown in Fig. In this instrument, a specially designed mercury vapour lamp is employed.

INSTRUMENT OF PHOSPHOROMETRIC ANALYSIS:-



A spectrophosphorimeter is similar to a spectrofluorimeter except that the former instrument fitted with (1) a rotating shutter device commonly called a phosphoroscope and temperature.

THE ROTATTING-CAN PHOSPHOROSCOPE:-

It consists of a hollow cylinder having one or more slats wh are equally spaced in the circumference.

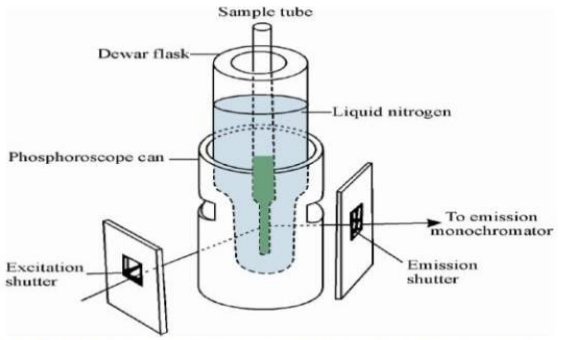
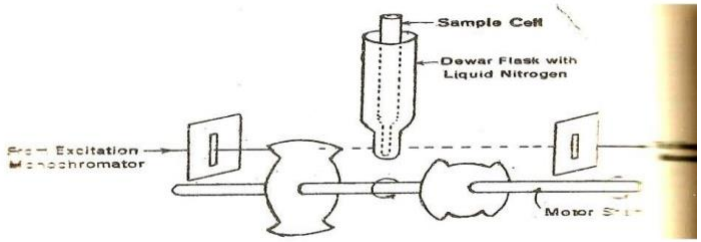


Fig. 5.9: Revolving can shutter system for recording phosphorescence and fluorescence from the same sample



APPLICATION OF FLUORIMETRY:-

1)Determination of inorganic substances:-

2)Nuclear research:-

3)Fluorescent Indicator

4) Fluorometric reagent:-

5) Organic analysis:-

➢ REFERENCE

Pharmaceutical Analysis Dr. A.V. Kastur Dr. S.G. Wadokar Pg. no. 193-194

Instrumental Method Of Analysis Gurudeep r. Chatwal Sham K. Anand Pg no. 2400-2413

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