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A Review on Flourimetry

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ABSTRACT
Absorption of UV/Visible radiation cause transition of electrons from singlet ground state to singlet excited state. As this state is not stable, it emits the energy in the form of UV/Visible radiation and returns to singlet ground state. This study or measurement of this emitted radiation is the principle in fluorimetry. Phosphorescence is also a related phenomenon which is the study of emitted radiation when electrons undergo transition from triplet state to singlet ground state.This review summarizes the scientific trends associated with the technique principle instrumentation factors affecting fluorimetry applications of fluorimetry in the field of pharmaceutical industry

KEYWORDS-
flourescence,phosphorescence,Jablonsky energy diagram, quenching,stoke'sflourescence

I. INTRODUCTION OF FLOURIMETRY

Luminescence is the emission of light by a substance. It occurs when an electron returns to the electronic ground state from an excited state and loses its excess energy as a photon.It's of 3types

- Flourescence spectroscopy
- Phosphorescence spectroscopy
- Chemiluminescence spectroscopy

1.1 .Flourescence

- When a beam of light is incident on certain substances they emit visible light or radiations. This is known as Flourescence.
- Flourescence starts immediately after the absorption of light and stops as soon as the incident light is cut off.
- The substance showing this phenomenon are known as florescent substance

1.2.Phosphorescence

When light radiation is incident on certain substances they emits light continuously even after the incident light is cut off This type of delayed Flourescenceis called Phosphorescence Substances showing Phosphorescence are phosphorescent substances[1]

II. PRINCIPLE OF FLOURIMETRY

Spectroflourimetry is defined as the measurement and interpretation of emission of the radiation after absorption. This emission of the radiation is generally called as the photoluminescence. This photoluminescence is divided into two types based on the time taken for the emission of the radiation. It is also known as atomic emission spectroscopy Before understanding it is important to understand some electric States.

1. **Singlet ground state :**A state in which all the electrons in a molecule are paired
2. **Doublet state :**A state in which an unpaired electron is present.Eg:Free radical
3. **Triplet state :**A state in which unpaired electrons of same spin present.
4. **Singlet excited state:**A state in which electrons are unpaired but of opposite spin[2]

Types of Flourescence The florescence is classified based onthe emitted radiation wave length and based on the phenomenon. They are asfollows:

1.Based upon the emitted radiation:
There are mainly three types:

a) **Stoke's fluorescence**:The emitted radiation wave length is longer than the absorbed radiation wave length. Example:Conventional Flourimetry
b) **AntiStoke'sFlourescence:**The emitted radiation wave length is shorter than the absorbed radiation. Example:Thermal Flourimetry.

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c) **Resonance Fluorescence:** The emitted radiation wave length is equal to the absorbed radiation. Example:Mercury vapour at 254nm[3]

2. Based upon the phenomenon:

There are three types:

a) **Sensitised Fluorescence**:When the elements such as thallium, zinc, cadmium, are added to the mercury vapour, it is sensitized and produces the fluorescence.

b) **Directline Flourescence:** Afterthe emission of the radiation, the molecules remain in the metastable state and finally come to the ground state.

c) **Step Wise Flourescence:** The part of energy is lost by vibrational transition before the emission of the fluorescent radiation.

When a beam of light is incident on certain substances they emit visible light radiations. This known as fluorescence Fluorescence starts immediately after the absorption of light and stop as soon as the incident light is cut off. The substances showing this phenomenon are known as flourescent substances (Light emitted within 10106). Phosphorescence When light radiation is incident on certain substances they emit light continuously even after the incident light is cut of. This type of delayed fluorescence is called phosphorescence Substances showing phosphorescence are phosphorescent substances. The time delay of the emission of the radiation is within 10-8s[4]

Theory Of FlourescenceAnd Phosphorescence

- A molecular electronic state in which are of the electrons are paired are called singlet state.
- In a singlet state molecules are diamagnetic.
- Most of the molecules in their ground state are paired
- When such a molecule absorbs UV/Visible radiation, one or more of the paired electron raised to an excited Singlet state /excited triplet state.

From the excited Singlet state one of the following phenomenon occurs

❖Flourescence
❖Phosphorescence
❖Radiation less processes
❖Vibration relaxation
❖Internal conversion
❖External conversion
❖Inter system crossing

Flourescence and chemical structure

- Flourescence is most commonly observed in compounds containing aromatic functional groups with low energy.
- Most unsubstituted aromatic hydrocarbons show fluorescence quantum efficiency increases with the number of rings and degree of condensation.
- Simple heterocyclic do not exhibit fluorescence
- Substitution on the benzene ring shifts wavelength of absorbance maxima and corresponding changes in flourescence peaks
- Flourescence decreases with increasing atomic no:of the halogen.
- Substitution of carboxylic group on aromatic ring inhibits flourescence.

Rigidity

- Flourescenceisfavoured in molecules with structural rigidity
- Organic chelating agent complexed with metal ion increases fluorescence[5]

Factors Affecting Flourescence Intensity

- Nature of molecule
- Nature of substituent
- Effect of concentration
- Adsorption, Light
- Oxygen, pH
- Photodecomposition
- Temperature Viscosity
- Quantum yield
- Intensity of incident light
- Path length
- Scatter
- Solvent [6]

2.1Quenching

- Decrease in flourescence intensity due to specific effects of constituents of the solution
- Due to concentration, ph, pressure of chemical Substances, temperature, viscosity

Types of quenching

- Self quenching

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- Chemical quenching
- Static quenching
- Collision quenching

Self quenching

- At low concentration linearity is observed, at high concentration of the same substance increase in fluorescent intensity is observed. This phenomenon is called self quenching
- It is also called as concentration quenching.

Chemical quenching

- Here decrease in flourescence intensity due to the factors like change in ph,presence of oxygen, halides and heavy metals.
- Ph-aniline at ph 5-13 it does not exhibit flourescence.
- Halides like chloride, bromide, iodide and electron withdrawing groups like NO2,COOH etc. leads to quenching .
- Heavy metals leads to quenching because of collisions of triplet ground state.

Static quenching

- This occurs due to complex formation. Eg:Caffeine reduces the flourescence of riboflavin by complex formation

Collision quenching

- It reduces flourescence by collision. Where no. of collisions increased hence quenching takes place[7].

III. INSTRUMENTATION OF FLUORIMETRY

Components of fluorimeters and spectroflourimeter

- Sours of light
- Filters and monochromaters
- Sample cells
- Detectors

Source of light

1. Mercury arc lamp
2. Xenon arc lamp
3. Tungsten lamp
4. Tunable dye lasers

Mercury Arc Lamp

- Produce intense line spectrum above 350nm
- High pressure lamp gives lines at 366,405,436,546,577,691,734nm
- Low pressure lamps give additional radiation at 254nm

Xenon Arc Lamp

- Intense radiation by passage of current through an atmosphere of xenon.
- Spectrum is continuous over the range between over 250-600nm, peak intensity about 740nm

Tungsten lamp

- Intensity of lamp is low
- If excitation is done in the visible region this lamp is used
- It does not offer UV radiation

Tunable Dye Lasers

- Pulsed nitrogen laser as the primary source.
- Radiation in the range between 360 and 650 nm is produced[8].

3.1.Filters& Monochromators

Filters

Primary filters -absorbs visible light &transmits UV light.

Secondary filter -absorbs UV radiation &transmits visible light.

Monochromators

Excitation monochromaters -isolates only the radiation which is absorbed by the molecule. Emission

Emission monochromaters -isolates only the radiation emitted by the molecule.

Sample And Sample Holder

- The majority of Flourescence assays are carried out in solution.
- Cylindrical /rectangular cells fabricated of silica or glass used.
- Path length is usually 10mm or 1cm.
- All the surfaces of the sample holder are polished in flourimetry[9].

3.2.DETECTORS

- Photovoltaic cell
- Photo Tube
- Photomultiplier Tubes -Best and accurate

Photomultiplier Tube

- Multiplication of photo electrons by secondary emission of radiation s
- A photo cathode and series of dynodes are used.
- Each cathode is maintained at 75-100v higher than the preceding one used.
- Over all amplification of 10^6 is obtained.

Photomultiplier Tube Movie

Single Beam Flourimeter

- Tungsten lamp as source of light.
- The primary filter absorbs Visible radiation and transmits UV radiation.
- Emitted radiation measured at 90 degree by secondary filter

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- Secondary filter absorbs UV radiation and transmits visible radiation

Advantage

- Simple in construction
- Easy to use
- Economical

Disadvantage

- It is not possible to use reference solution &sample solution at a time.
- Rapid scanning is to obtain Excitation and emission spectrum of the compound is not possible

Double beam Flourimeter

- Similar to single beam instrument
- Two incident beams from light source pass through primary filters separately and fall on either sample or reference pass separately through secondary filter.

Advantage

1)Sample and reference solution can be analysed simultaneously

Disadvantage

2)Rapid scanning is not possible due to use of fil[10].

3.3.APPLICATIONS OF FLOURIMETRY

1)Determination of inorganic compound

- Determination of ruthenium ions in presence of other platinum metals.
- Determination of aluminum in alloys
- Determination of boron in steel by complex formed with benzoin
- Estimation of calcium with 2-(2hydroxy phenyl)benzoxazole in presence of tartarate.

2)Nuclear research

- Field determination of Uranium salts

3)Fluorescent indicators

- Mainly used in acid -base titration
- Eg:

Eosin-colourless-green
Flourescein:colourless -green
Quinine sulphate :blue -violet

4)Flourometric reagent

- Aromatic structure with two or more donor Functional groups

5) organic analysis

Qualitative and quantitative analysis of organic aromatic compounds present in cigarette smoke, airPollutants, automobile exhausts etc.

6)pharmaceutical analysis

7)Liquid Chromatography

Flourescence is an important method of determining compounds as they appear at the end of chromatogram or capillary electrophoresis column[11].

8)Determination of vitamin B1&

IV. CONCLUSION

Fluorescence is a phenomenon of emission of radiation when the molecules are excited by radiation al certain wavelength Flourimetry is measurement of fluorescence intensity at a particular wavelength with the heelpost a Filter fluorimeter or a spectroflourimeter. There is various factors which effect flourescence intensify like concentration, temperature viscosity, etc. oxygen, adsorption. The Flourimetry methods are not useful in qualitative analysis, and much used in quantitative analysis Flourescence is the most common sensitive analytical techniques detected studies will increase the development of Flourescence field

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