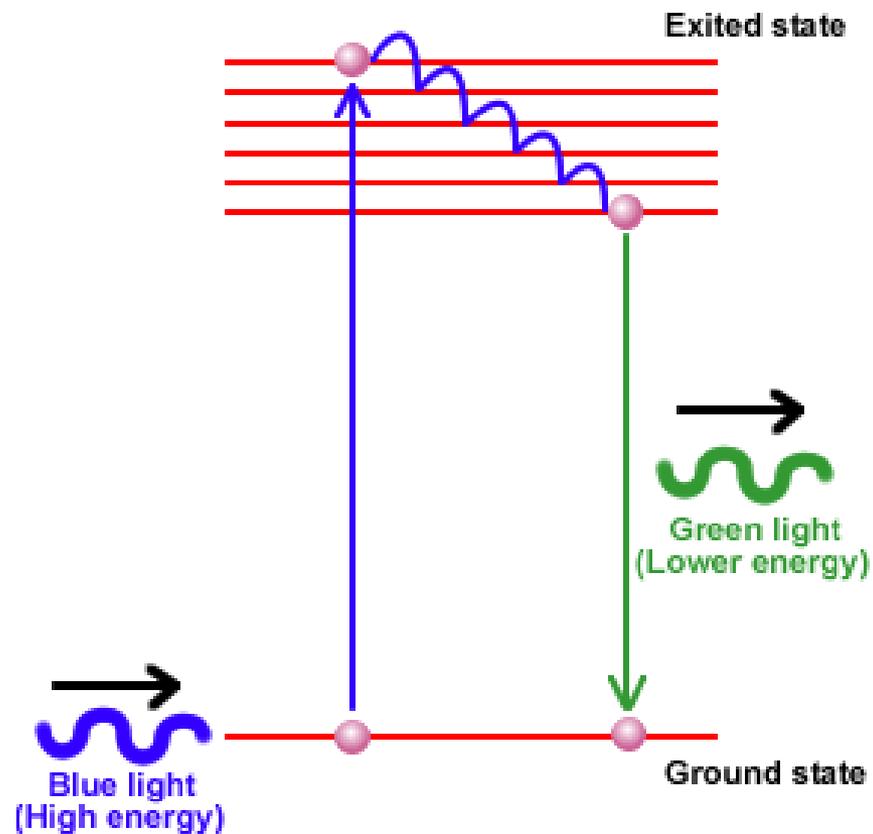


FLOURIMETRY



By
K. RAKESH GUPTA

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- ❑ INTRODUCTION
 - ❑ DEFINITION
 - ❑ THEORY
 - ❑ FACTORS AFFECTING FLOURESCENCE
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 - ❑ CONCLUSION
 - ❑ REFERENCES
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INTRODUCTION

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 is of 3 types.

- **Fluorescence spectroscopy.**
- **Phosphorescence spectroscopy.**
- **Chemiluminescence spectroscopy**

FLUORESCENCE

When a beam of light is incident on certain substances they emit visible light or radiations. This is known as **fluorescence**.

Fluorescence starts immediately after the absorption of light and stops as soon as the incident light is cut off.

The substances showing this phenomenon are known as **fluorescent substances**.



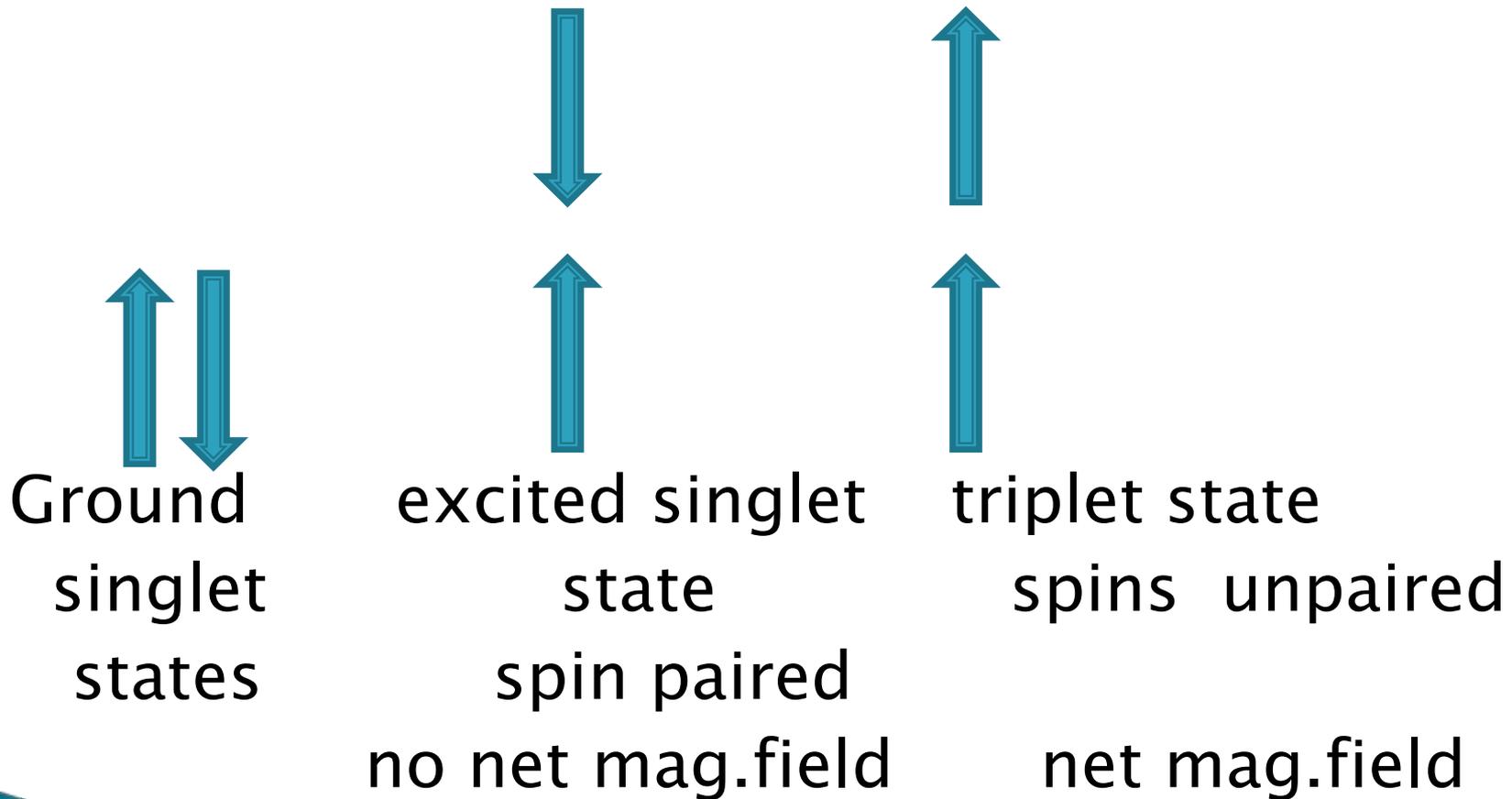
PHOSPHORESCENCE

- ▶ **When light radiation is incident on certain substances they emit light continuously even after the incident light is cut off.**
 - ▶ **This type of delayed fluorescence is called phosphorescence.**
 - ▶ **Substances showing phosphorescence are phosphorescent substances.**
- 

THEORY OF FLOURESCENCE AND PHOSPHORESCENCE

- ❑ A molecular electronic state in which all 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**.
- 

SINGLET/TRIPLET STATE

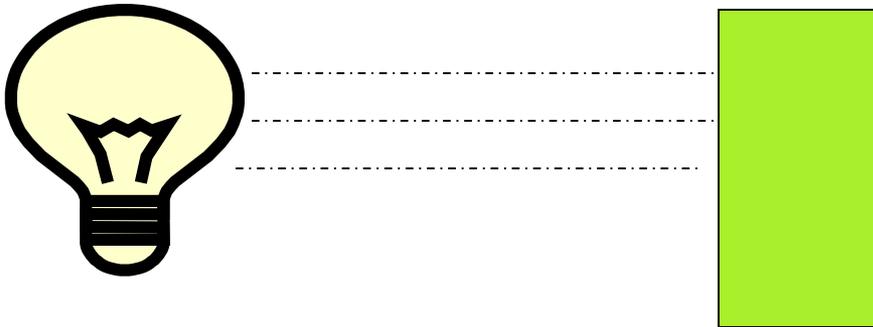


From the excited singlet state one of the following phenomenon occurs

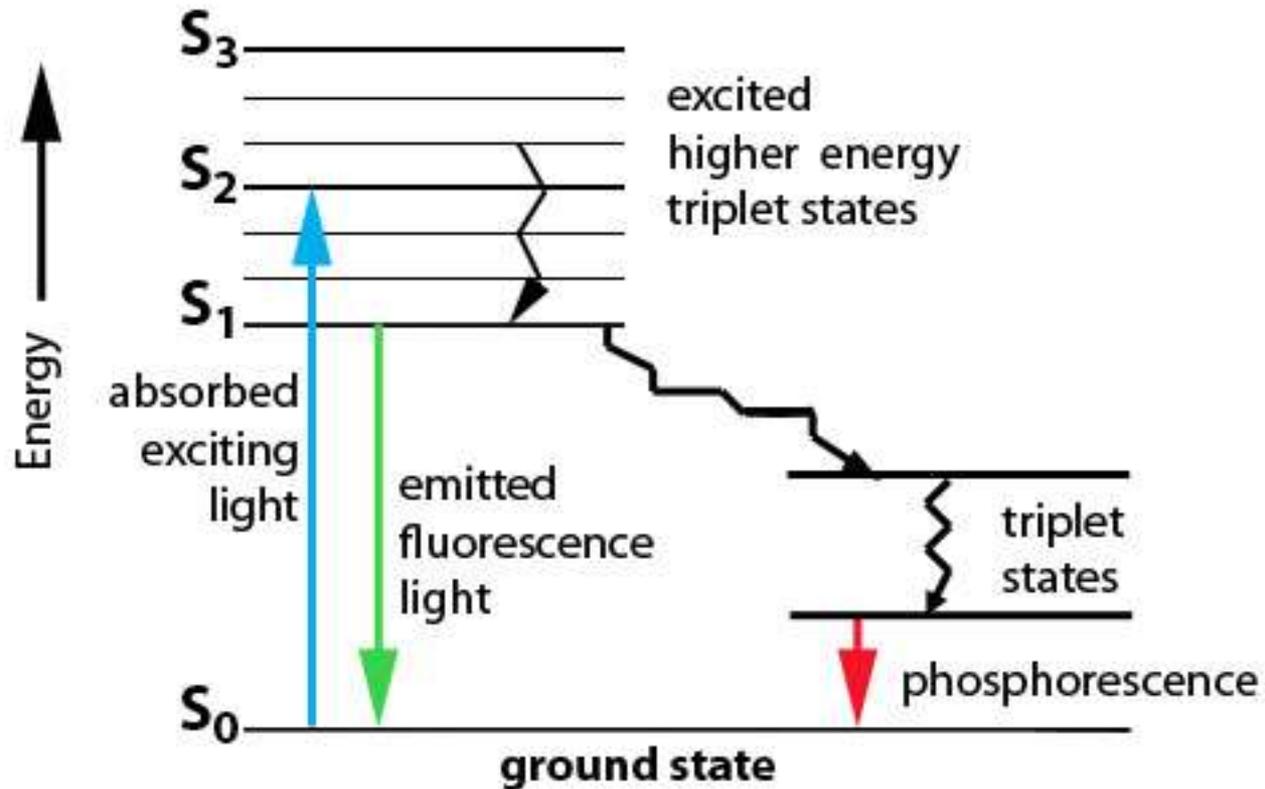
- ❖ **Fluorescence**
- ❖ **Phosphorescence**
- ❖ **Radiation less processes**
 - ❖ **Vibration relaxation**
 - ❖ **Internal conversion**
 - ❖ **External conversion**
 - ❖ **Intersystem crossing**

FLUORESCENCE

LIGHT EMITTING AT ONCE SOURCE STARTS &
STOPS WHEN SOURCE STOPS

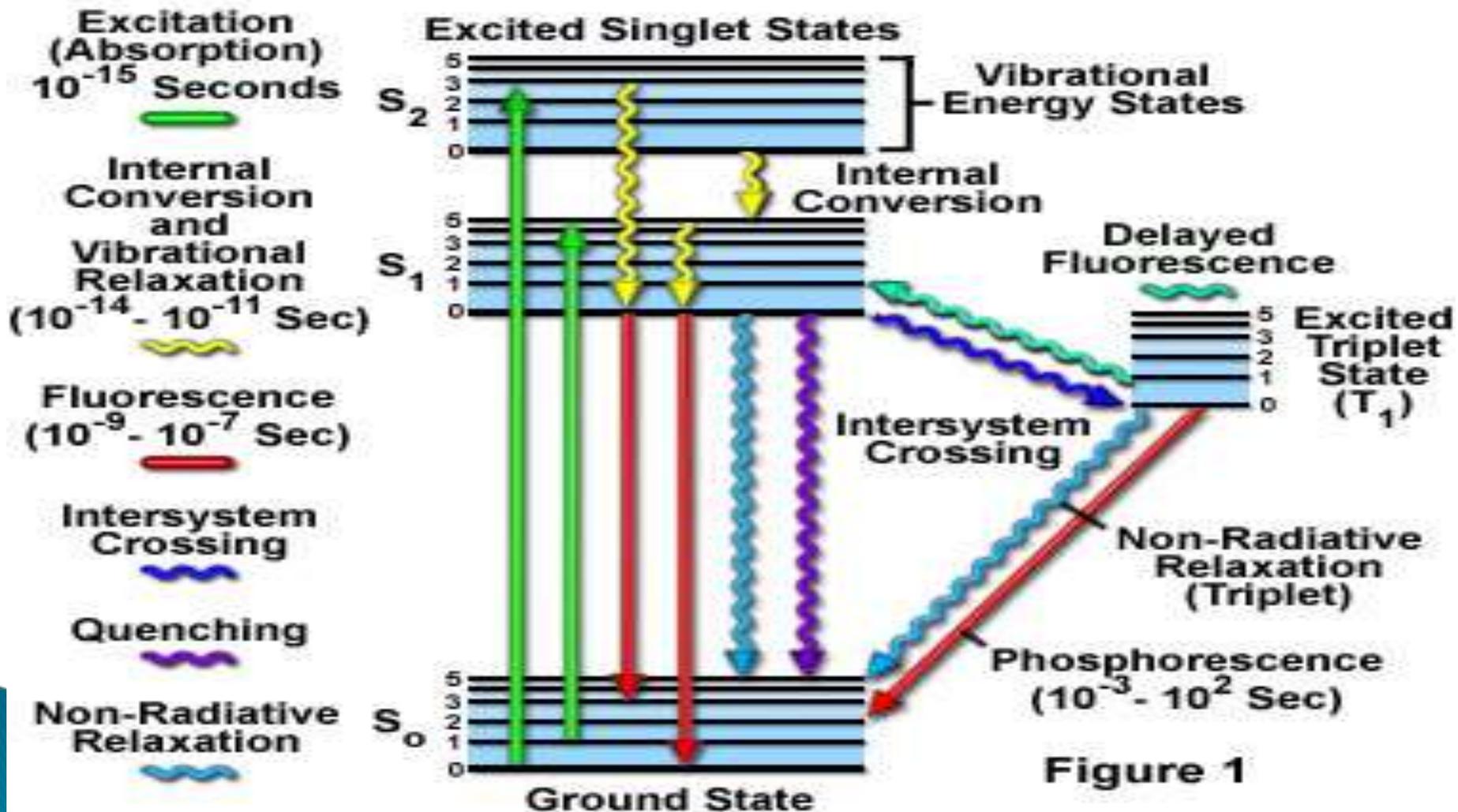


FLOURESCENCE & PHOSPHORESCENCE



JABLONSKI ENERGY DIAGRAM

Jablonski Energy Diagram



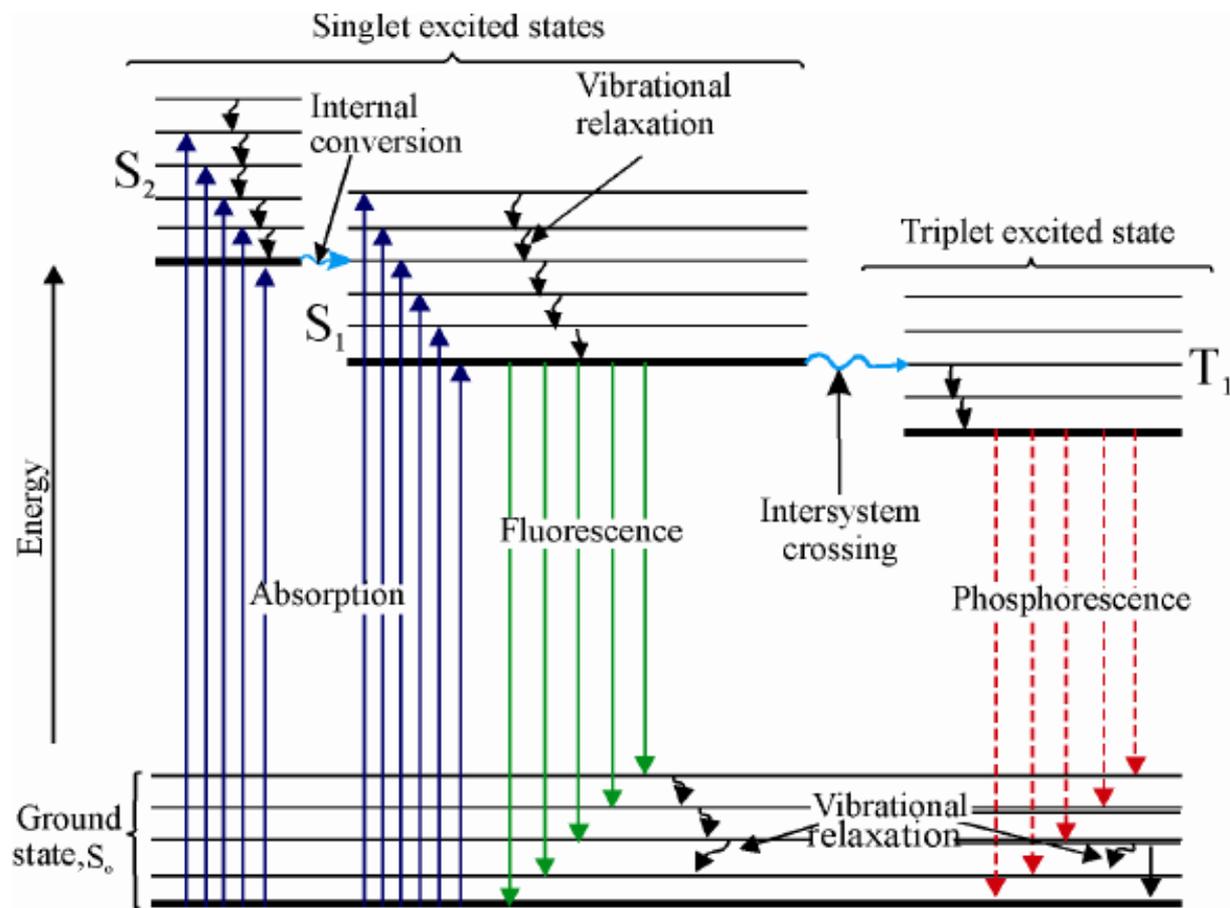
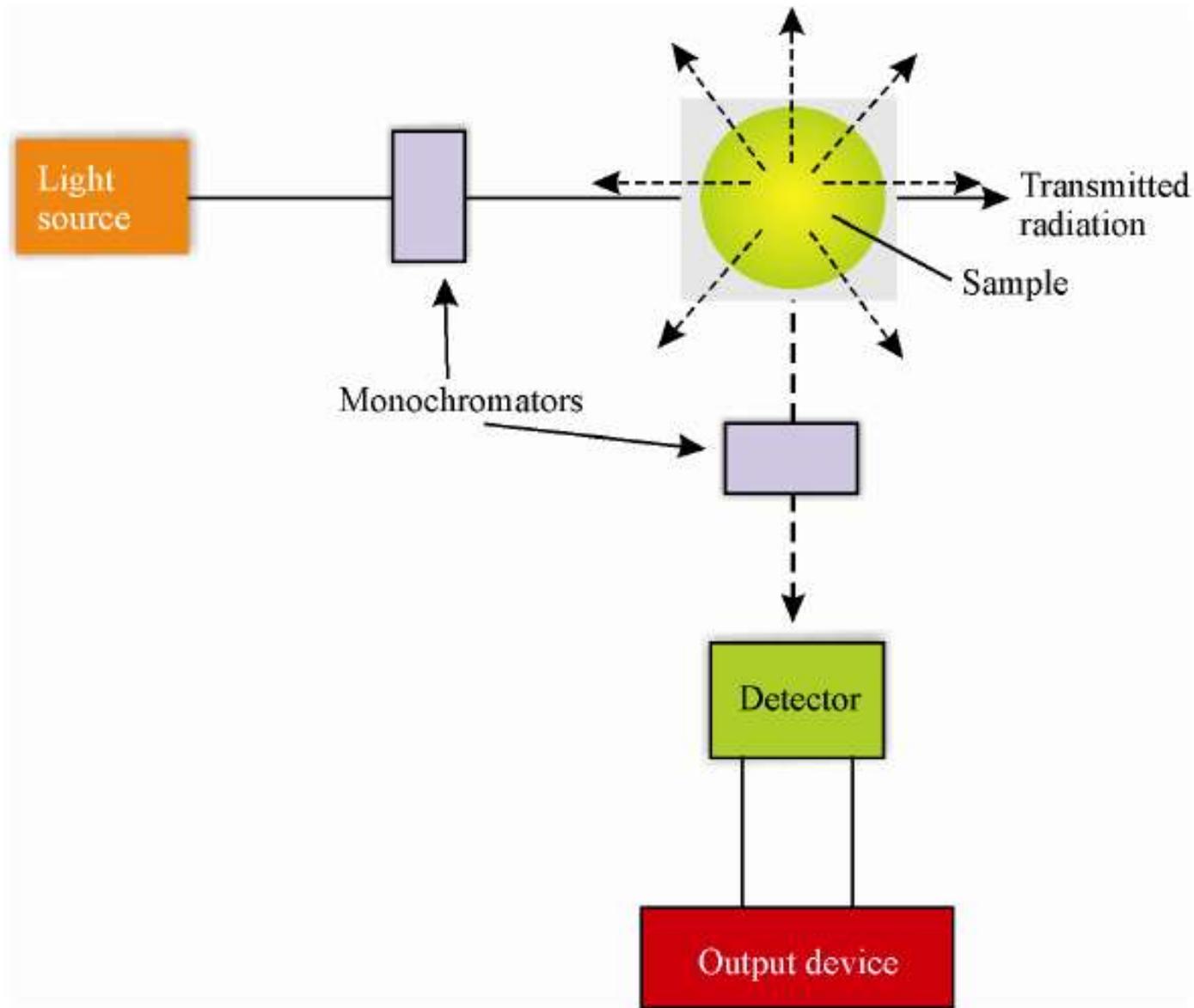


Fig. 5.1: The Jablonski diagram showing the phenomena of fluorescence and phosphorescence

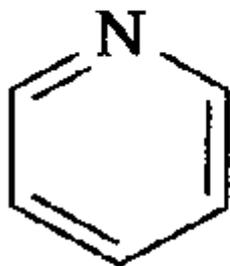


FLUORESCENCE AND CHEMICAL STRUCTURE

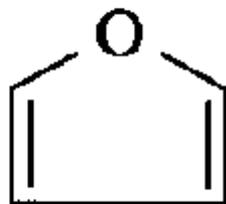
- ❖ Fluorescence is most commonly observed in compounds containing aromatic functional groups with **low energy**.
- ❖ Most unsubstituted aromatic hydrocarbons show fluorescence - quantum efficiency increases with the **no: of rings and degree of condensation**.

CONTD...

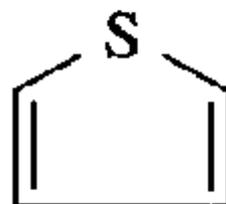
- ❖ Simple **heterocyclic** do not exhibit fluorescence.
- ❖ The $n - \pi^*$ singlet quickly converts to the $n - \pi^*$ triplet and prevents fluorescence.



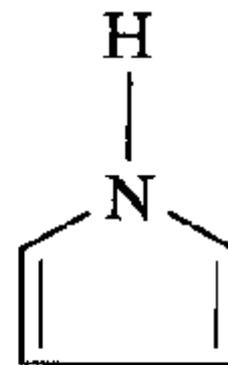
pyridine



furan



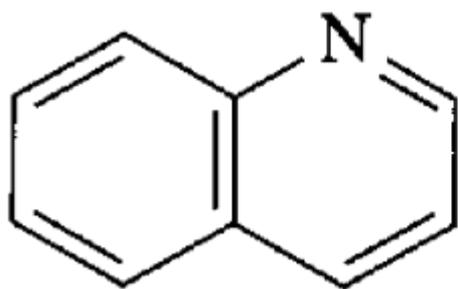
thiophene



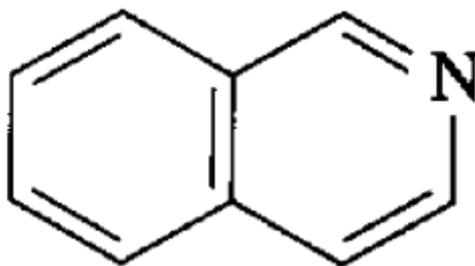
pyrrole

CONTD..

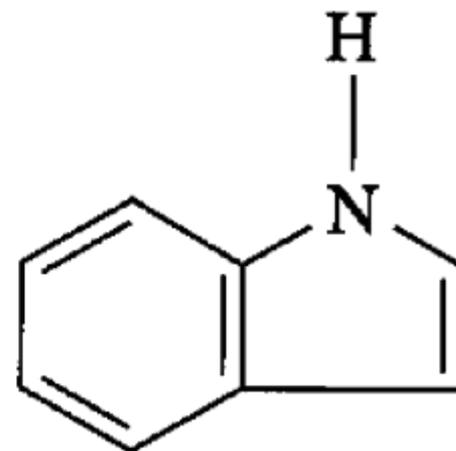
- ❖ Fusion of heterocyclic nucleus to benzene ring increases fluorescence.



quinoline



isoquinoline

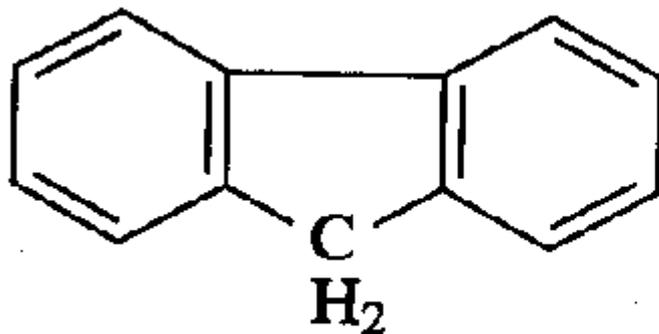


indole

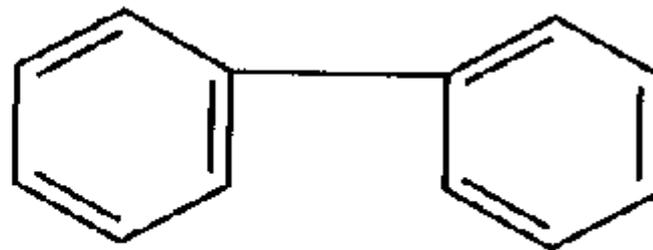
CONTD..

- ❖ **Substitution on the benzene ring shifts wavelength of absorbance maxima and corresponding changes in fluorescence peaks**
 - ❖ **Fluorescence decreases with increasing atomic no: of the halogen.**
 - ❖ **Substitution of carboxylic acid or carboxylic group on aromatic ring inhibits fluorescence.**

STRUCTURAL RIGIDITY



fluorene



biphenyl

- ❖ **Fluorescence is favored in molecules with structural rigidity.**
- ❖ **organic chelating agents complexed with metal ion increases fluorescence.**

FACTORS AFFECTING FLUORESCENCE INTENSITY

- Nature of molecule
- Nature of substituent
- Effect of concentration
- Adsorption, Light
- Oxygen, ph
- Photodecomposition
- Temp . & viscosity
- Quantum yield
- Intensity of incident light
- Path length

nature of molecules

- **All the molecules cannot show the phenomenon of fluorescence.**
 - **Only the molecules absorbs uv/visible radiation can show this phenomenon.**
 - **Greater the absorbency of the molecule the more intense its fluorescence.**
- 

nature of substituent

- ❖ **Electron donating group enhances fluorescence – e.g.: NH_2 , OH etc.**
- ❖ **Electron withdrawing groups decrease or destroy fluorescence.
e.g.: COOH , NO_2 , $\text{N}=\text{N}$ etc.**
- ❖ **High atomic no: atom introduced into π electron system decreases fluorescence.**

EFFECT OF CONCENTRATION

- Fluorescence is directly proportional to concentration.

Contd...

$$FI = Q \times I_a$$

i.e, $F = QI_{oact}$

Q = Constant for a particular substance

I_o = Constant for an instrument

a = Molecular extinction coefficient

t = Path length

C = Concentration of the substance

F = KC Where K represents all constants

$FI \propto \text{Concentration.}$

ADSORPTION

- ❑ **Extreme sensitiveness of the method requires very dilute solution.**
 - ❑ **Adsorption of the fluorescent substances on the container wall create serious problems.**
 - ❑ **Hence strong solutions must be diluted.**
- 

LIGHT

- Monochromatic light is essential for the excitation of fluorescence because the intensity will vary with wavelength.

OXYGEN

The presence of oxygen may interfere in 2 ways.

- 1] by direct oxidation of the fluorescent substances to non fluorescent.
- 2] by quenching of fluorescence.

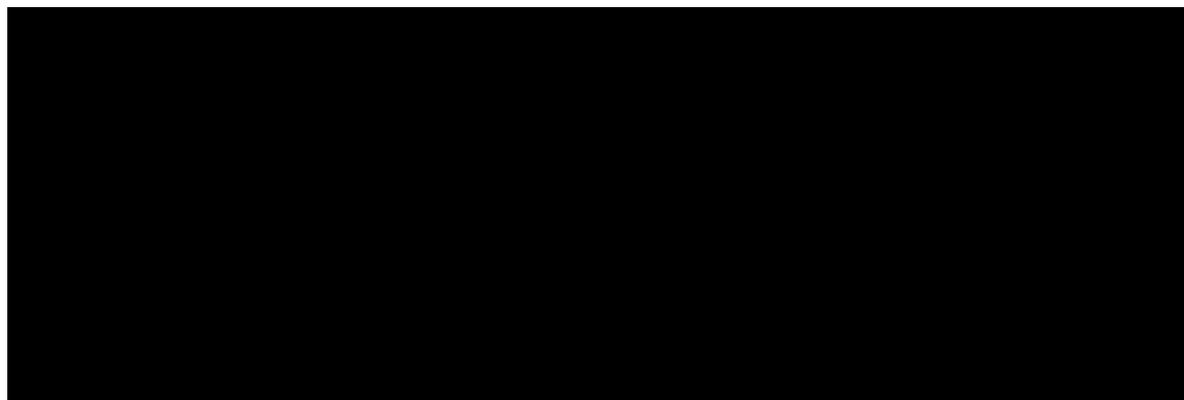
PH

- ❑ Alteration of the ph of the solution will have significant effect on fluorescence.
- ❑ Fluorescent spectrum is different for ionized and un-ionized species.

TEMPERATURE & VISCOSITY

- ❑ Increase in temperature/decrease in viscosity will decrease fluorescence.

fluorescence quantum yield:



K_f = fluorescence

k_{ec} = external conversion

k_{ic} = internal conversion

k_{isc} = intersystem crossing

k_{pd} = pre dissociation

K_d = dissociation

INTENSITY OF INCIDENT LIGHT

- ▶ **Increase** in intensity of light incident on sample **increases** fluorescence intensity.
- ▶ The intensity of light depends upon
 - 1)light emitted from the lamp.
 - 2)Excitation monochromaters
 - 3)Excitation slit width

PATH LENGTH

- ▶ **The effective path length depends on both the excitation and emission slit width.**
- ▶ **Use of microcuvette does not reduce the fluorescence.**
- ▶ **Use of microcell may reduce interferences and increases the measured fluorescence**

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

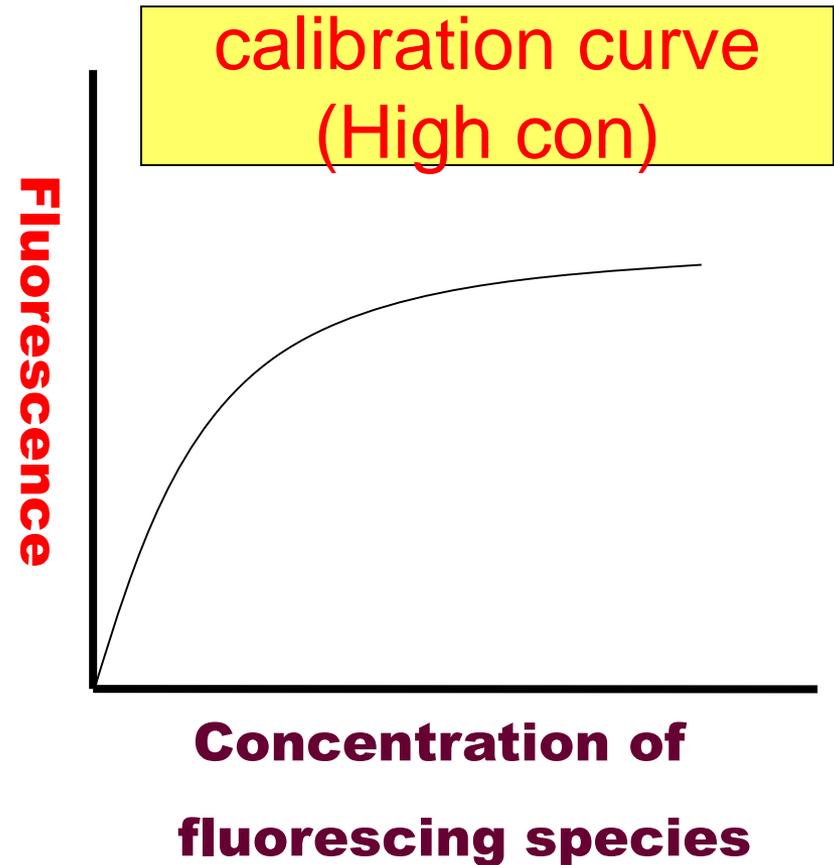
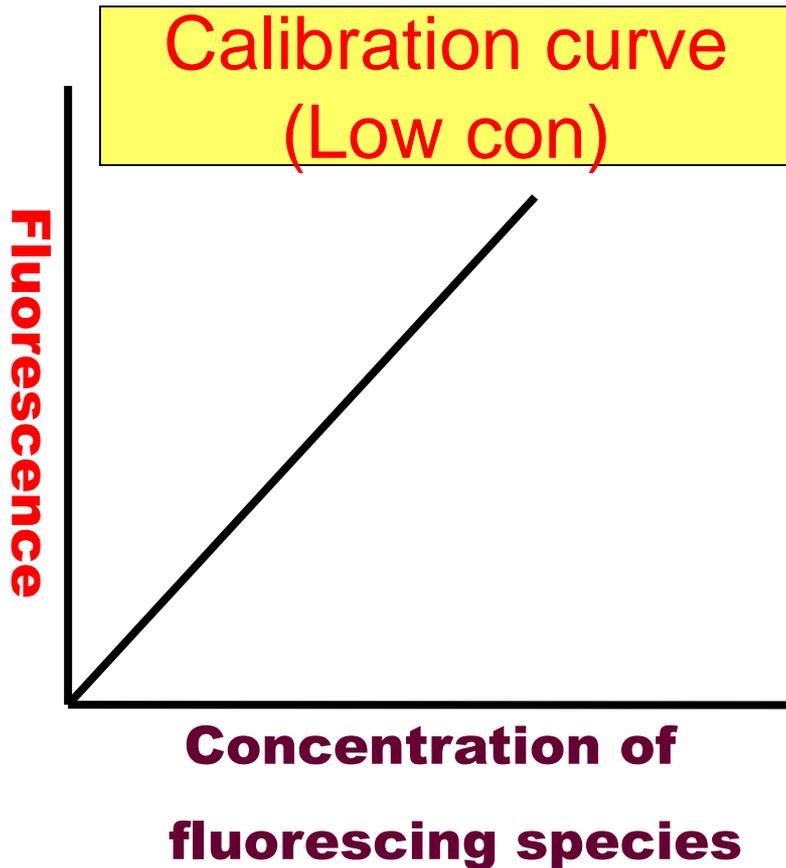
Self quenching

Chemical quenching

Static quenching

Collision quenching

SELF QUENCHING/CONC. QUENCHING



Deviations at higher concentrations can be attributed to self-quenching or self-absorption.

CHEMICAL QUENCHING

- ❑ Here **decrease** in fluorescence intensity due to the factors like change in pH, presence of oxygen, halides & heavy metals.
- ❑ **pH** – aniline at pH 5–13 gives fluorescence but at pH <5 & >13 it does not exhibit fluorescence.
- ❑ **halides** like chloride, bromide, iodide & electron withdrawing groups like NO_2 , 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.
e.g., caffeine reduces the fluorescence of riboflavin by complex formation.

COLLISIONAL QUENCHING

- ❑ It reduces fluorescence by collision. where no. of collisions increased hence quenching takes place.

INSTRUMENTATION

COMPONENTS OF FLUORIMETERS AND SPECTROFLUORIMETERS

- ❖ SOURCE OF LIGHT
 - ❖ FILTERS AND MONOCHROMATORS
 - ❖ SAMPLE CELLS
 - ❖ DETECTORS
- 

SOURCE OF LIGHT

- ❑ MERCURY ARC LAMP.
 - ❑ XENON ARC LAMP.
 - ❑ TUNGSTEN LAMP.
 - ❑ TUNABLE DYE LASERS.
- 

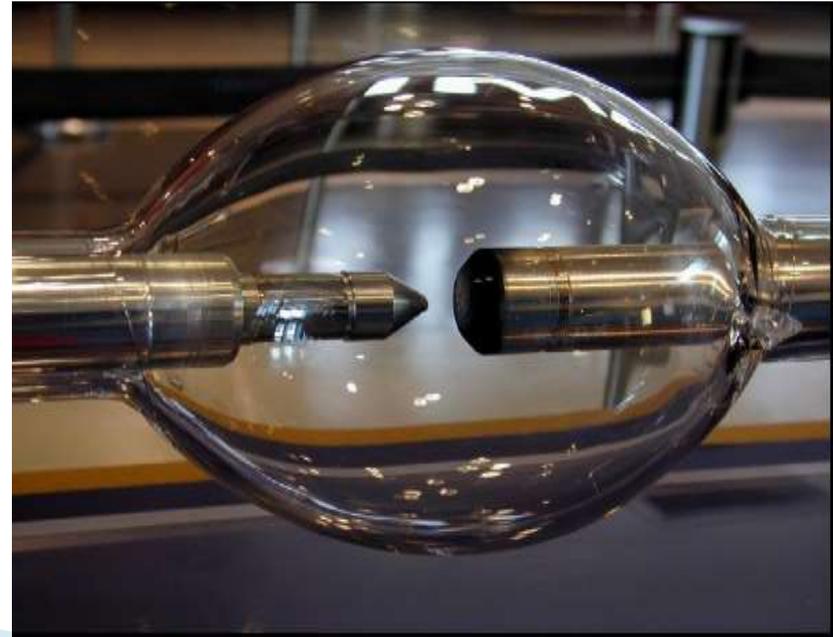
MERCURY ARC LAMP

- ❖ Produce intense line spectrum above 350nm.
- ❖ High pressure lamps give 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 470nm.



TUNGSTEN LAMP

- ❖ **Intensity of the 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.**
- 

FILTERS & MONOCHROMATORS

FILTERS

Primary filter—absorbs visible light & transmits uv light.

Secondary filter—absorbs uv radiations & transmits visible light.

MONOCHROMATORS

Excitation monochromators—isolates only the radiation which is absorbed by the molecule.

Emission monochromators—isolates only the radiation emitted by the molecule.



SAMPLE AND SAMPLE HOLDER

- ❖ **The majority of fluorescence assays are carried out in solution.**
- ❖ **Cylindrical or 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 fluorimetry.**



DETECTORS

❖ **PHOTOVOLTAIC CELL**

❖ **PHOTO TUBE**

❖ **PHOTOMULTIPLIER TUBES – Best
and accurate.**

PHOTOMULTIPLIER TUBE

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

DETECTOR – PHOTOMULTIPLIER TUBE

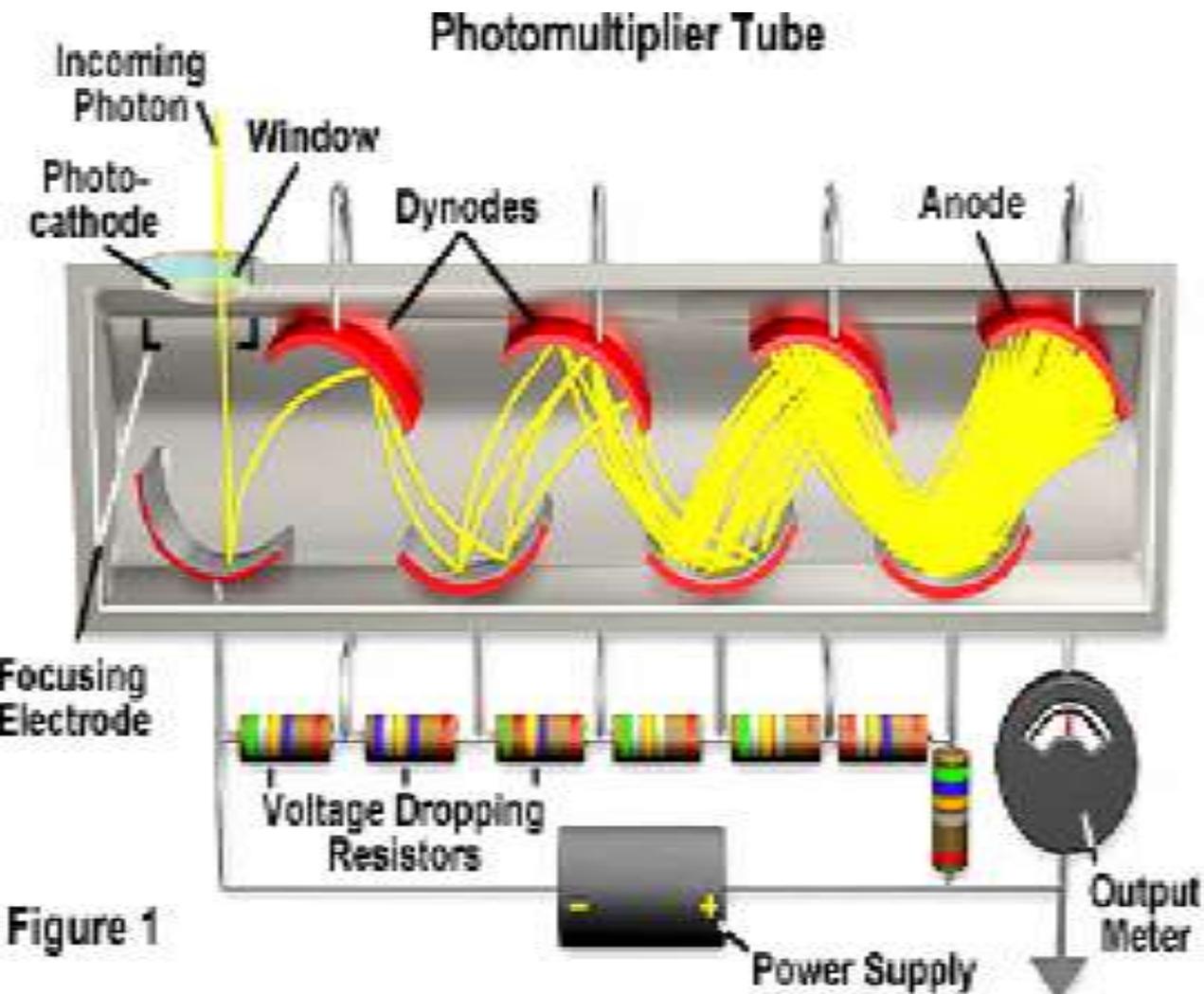
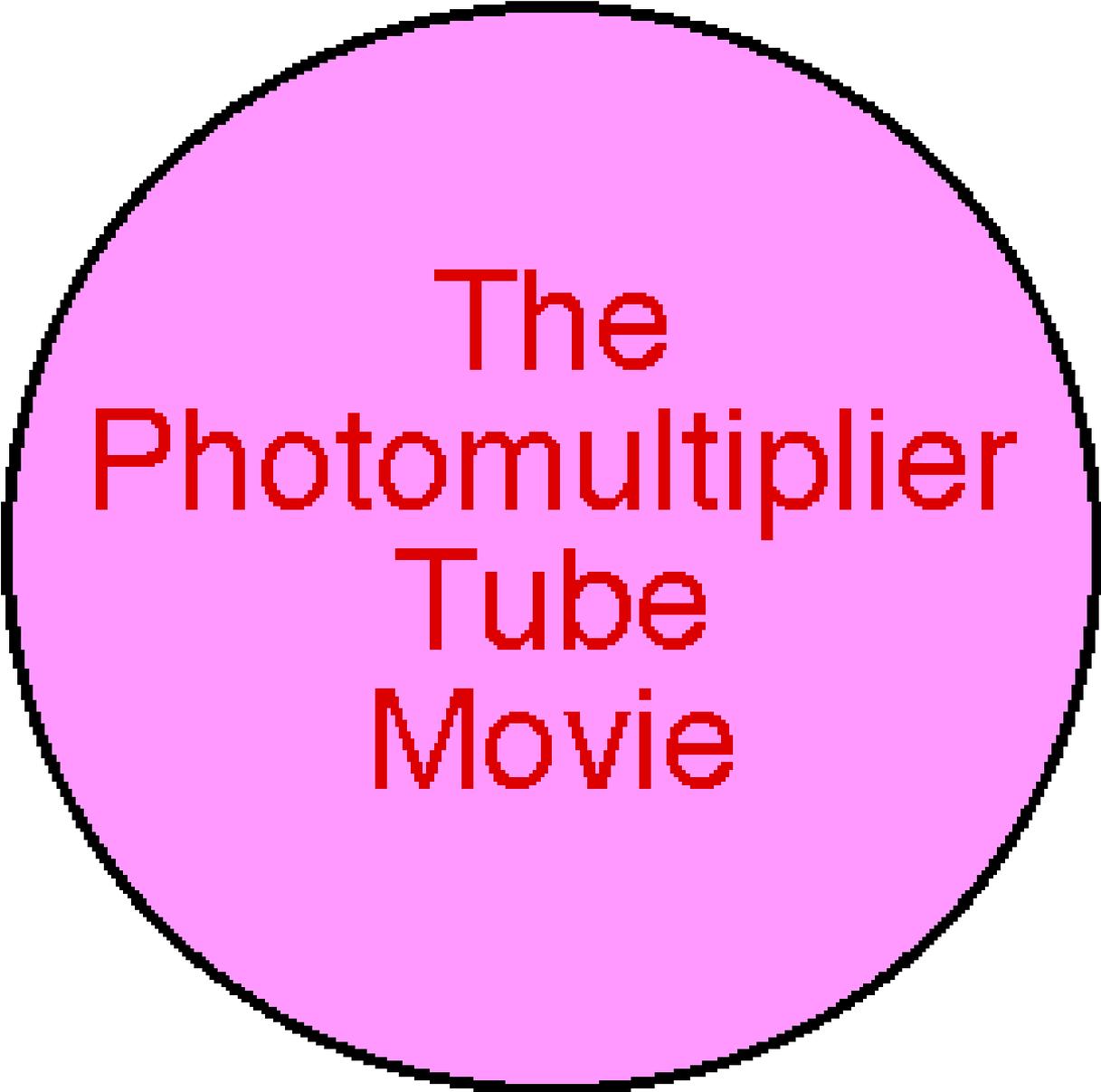
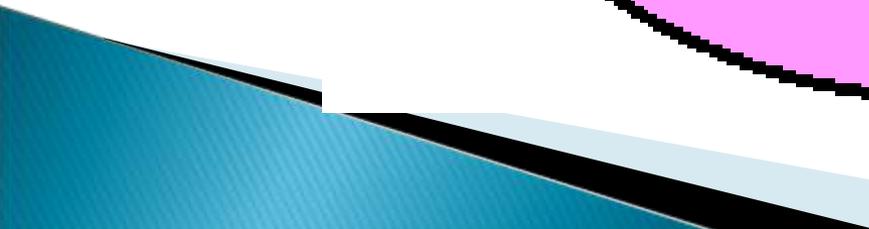


Figure 1





The
Photomultiplier
Tube
Movie



INSTRUMENT DESIGNS



SINGLE BEAM FLUORIMETER

- ❖ **Tungsten lamp as source of light.**
 - ❖ **The primary filter absorbs visible radiation and transmits uv radiation.**
 - ❖ **Emitted radiation measured at 90° by secondary filter.**
 - ❖ **Secondary filter absorbs uv radiation and transmits visible radiation.**
- 

Advantages

- ▶ Simple in construction
- ▶ Easy to use.
- ▶ Economical

disadvantages

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

DOUBLE BEAM FLUORIMETER

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



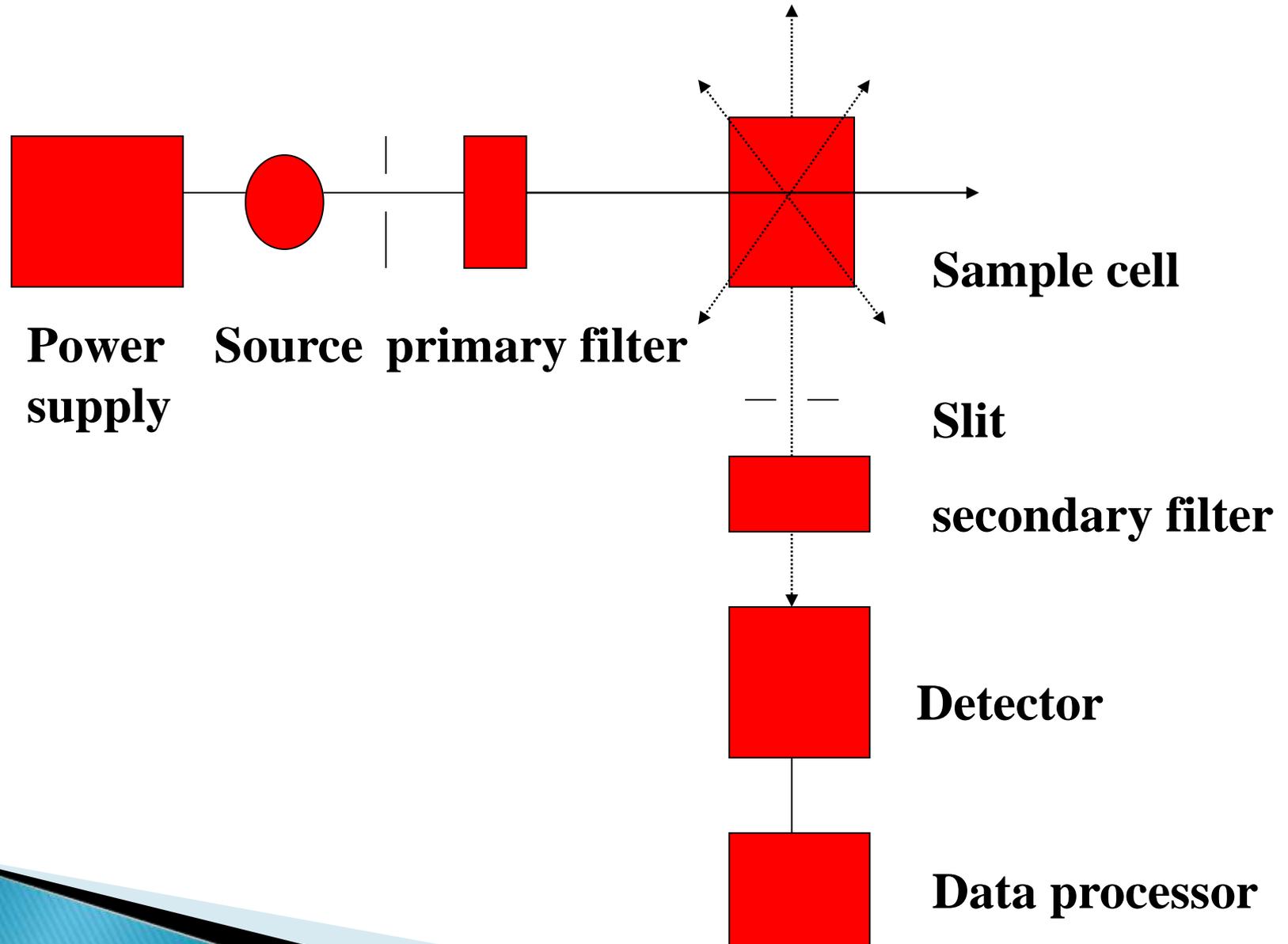
Advantages

- ❑ Sample & reference solution can be analyzed simultaneously.

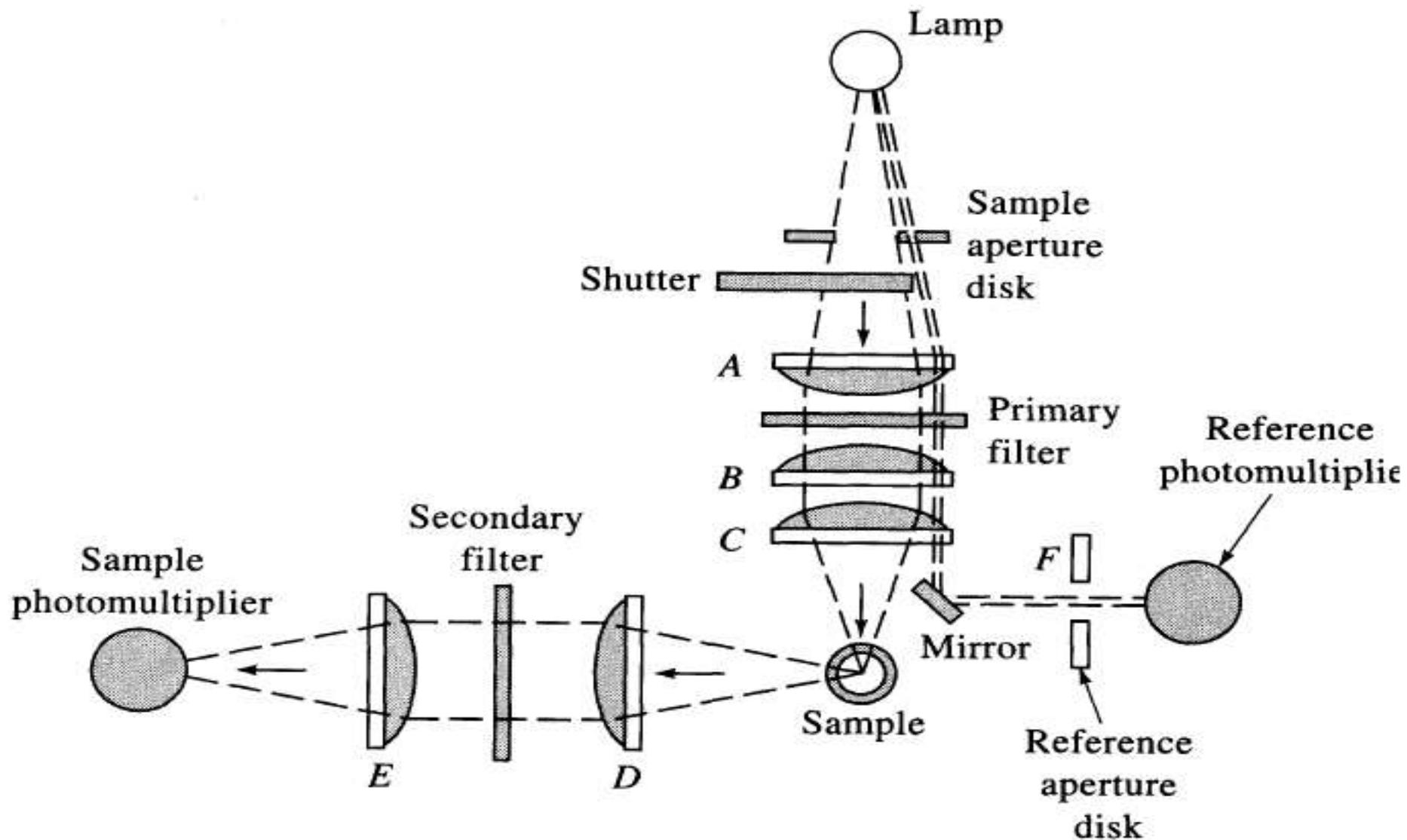
disadvantage

- ❑ Rapid scanning is not possible due to use of filters.

GENERAL LAYOUT OF FLUORIMETER



SCHEMATIC DIAGRAM OF FLUORIMETER



APPLICATIONS OF FLUORIMETRY

1] Determination of inorganic substances

- ❖ Determination of ruthenium ions in presence of other platinum metals.
- ❖ Determination of aluminum (III) in alloys.
- ❖ Determination of boron in steel by complex formed with benzoic acid.
- ❖ Estimation of cadmium with 2-(2 hydroxyphenyl) benzoxazole in presence of tartaric acid.

2] Nuclear research

- ▶ Field determination of uranium salts.

3] fluorescent indicators

Mainly used in acid–base titration.

e.g.:

eosin– colorless–green.

Fluorescein: colourless–green.

Quinine sulphate: blue–violet.

Acridine: green–violet

4] Fluorometric reagent

❖ Aromatic structure with two or more donor functional groups

Reagent	Ion	Fluorescence wavelength	Sensitivity
Alizarin garnet B	Al^{3+}	500	0.007
Flavanol	Sn^{4+}	470	0.1
8-Hydroxy quinoline	Li^{2+}	580	0.2

5] organic analysis

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

6] pharmaceutical analysis

compound	reagent	excitation wavelength	fluorescence
hydrocortisone	75%v/v H ₂ SO ₄ in ethanol	460	520
nicotinamide	cyanogen chloride	250	430

7] Liquid chromatography

❖ Fluorescence is an imp method of determining compounds as they appear at the end of chromatogram or capillary electrophoresis column.

8]determination of vitamin B1 &B2.



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THANK YOU

