

Fluorescence Spectroscopy

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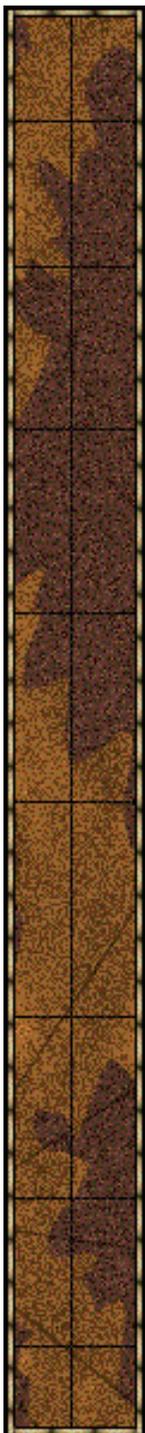
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Introduction

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- A large numbers of substances are known which can absorb UV or Visible light radiation. But these substances lose excess energy as heat through collisions with neighbouring atoms or molecules.
 - However, a large numbers of important substances are also known which lose only part of this excess energy as heat and emit the remaining energy as electromagnetic radiation of a wavelength longer than that absorbed.
 - This process of emitting radiation is collectively known as luminescence.



- 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.
- In luminescence, light is produced at low temperature; therefore the light produced by this process is regarded as “light without heat” or “cold light”.
- Luminescence spectroscopy is a collective name given to *three* related spectroscopic techniques. They are:
 - Molecular fluorescence spectroscopy
 - Molecular phosphorescence spectroscopy
 - Chemiluminescence spectroscopy

Principle of fluorescence and phosphorescence (photoluminescence):

The electronic states of most organic molecules can be divided into *singlet* states and *triplet* states:



Singlet state: All electrons in the molecule are spin-paired

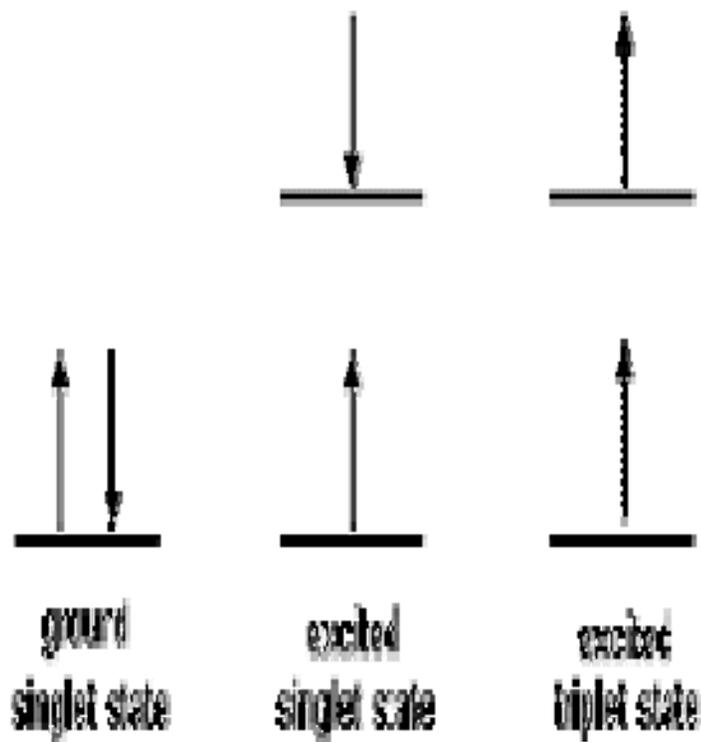
Symbol: $\uparrow\downarrow$

Singlet excited state: Unpaired electrons of opposite spin present

Symbol: $\uparrow\downarrow$

Triplet state: Unpaired electrons of same spin present

Symbol: $\uparrow\uparrow$





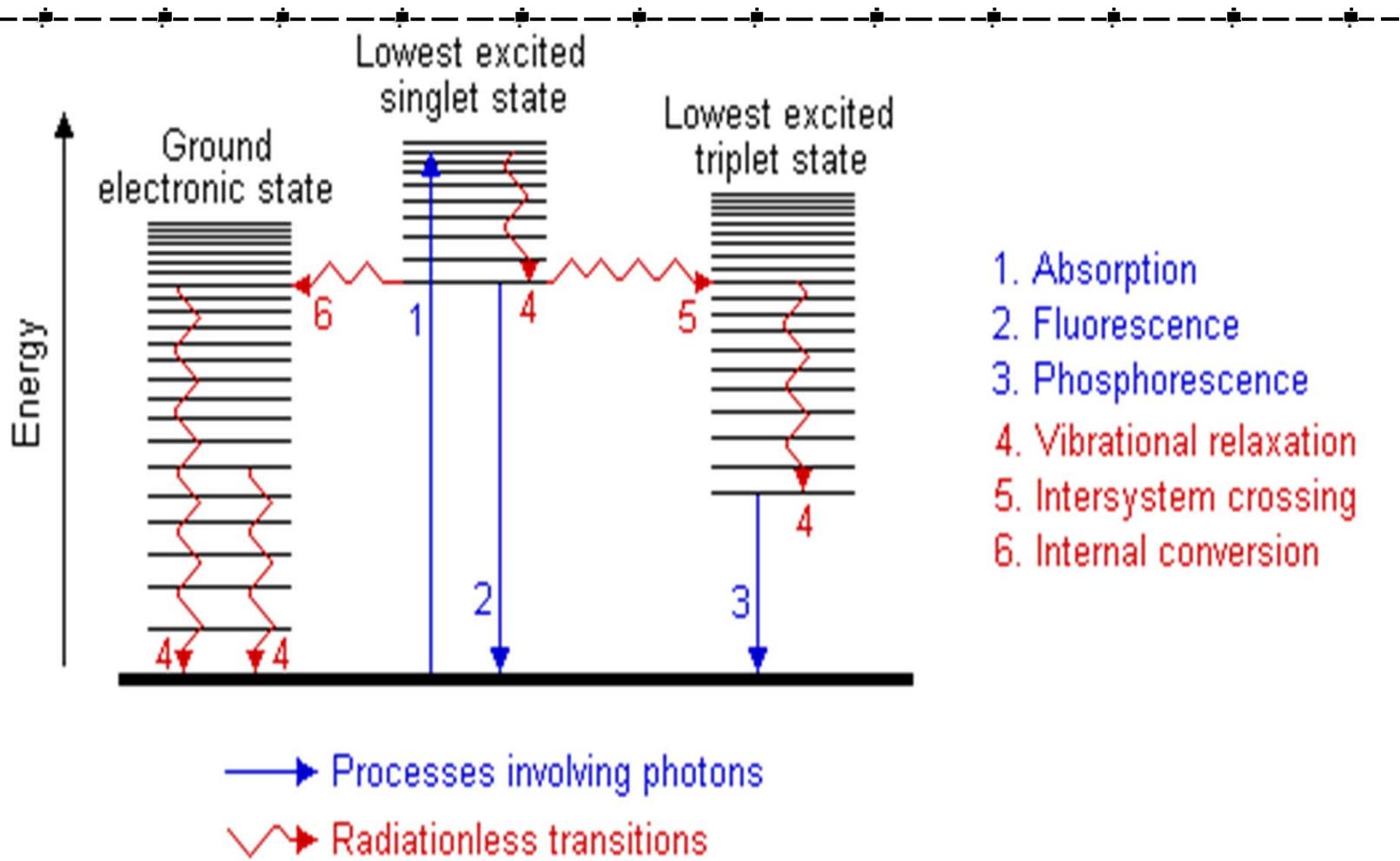
Fluorescence:

Fluorescence is the phenomenon of emission of radiation when electrons undergo transition from singlet excited state to singlet ground state.

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- Absorption of UV/Visible radiation by a molecule excites it from a vibrational level in the electronic ground state to one of the many vibrational levels in the electronic excited state.
 - This excited state is usually the first excited *singlet* state and is not stable.
 - A molecule in a high vibrational level of the excited state will quickly fall to the lowest vibrational level of this state by losing energy to other molecules through collision.
 - Fluorescence occurs when the molecule returns to the *electronic* ground state, from the excited singlet state, by emission of a photon or radiation of longer wavelength than the incident or absorbed radiation.



- This is because the energy of emitted radiation is less than that of incident or absorbed radiation because a part of energy is lost due to vibrational or collisional processes. Hence the emitted radiation has longer wavelength (less energy) than the absorbed radiation.
- The wavelength of absorbed radiation is called excitation wavelength (λ_{ex}) and that of emission radiation is called as emission wavelength (λ_{em}).
- These two wavelengths are specific or characteristic for a given substance under ideal conditions.
- If a molecule, which absorbs UV/Visible radiation, but does not fluoresce it means that it must have lost its energy as some other way. These processes are called radiation less transfer of energy.

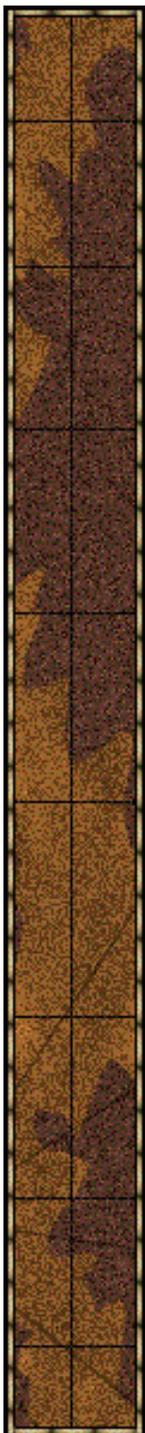




Phosphorescence:

Phosphorescence is the phenomenon of emission of radiation when electrons undergo transition from triplet state to singlet ground state.

- The spin of an excited electron can be reversed, leaving the molecule in an excited *triplet* state, this is called *intersystem crossing*.
- The triplet state is of a lower electronic energy than the excited singlet state.
- A molecule in the excited triplet state may not always use intersystem crossing to return to the ground state. It could lose energy by emission of a photon.



- A triplet/singlet transition is much less probable than a singlet/singlet transition.
- The lifetime of the excited triplet state can be up to 10 seconds, in comparison with 10^{-5} s to 10^{-8} s average lifetime of an excited singlet state.
- Emission from triplet/singlet transitions can continue after initial irradiation. Internal conversion and other radiation less transfers of energy compete so successfully with phosphorescence that it is usually seen only at low temperatures or in highly viscous media.

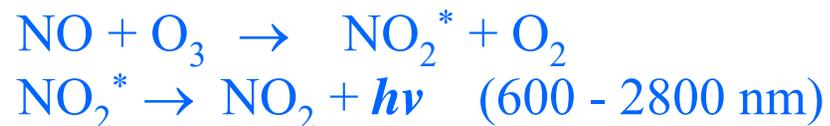


Chemiluminescence:

Chemiluminescence occurs when a chemical reaction produces an electronically excited species, which emits a photon in order to reach the ground state.

The number of chemical reactions, which produce chemiluminescence, is small. However, some of the compounds, which do react to produce this phenomenon, are environmentally significant.

A good example of chemiluminescence is the determination of nitric oxide:





Difference between fluorimetry and absorptiometry:



1. Sensitivity
2. Specificity
3. Selectivity
4. Effect of temperature
5. Standard value of absorptivity ($A_{1\% 1\text{ cm}}$), ϵ and λ_{max}



Types of fluorescence:



A) Based upon the wavelength of emitted radiation when compared to absorbed radiation

- 1) **Stoke's fluorescence:** eg. Conventional fluorimetric experiments
- 2) **Anti-stock's fluorescence:** eg. Thermally assisted fluorescence
- 3) **Resonance fluorescence:** eg. Mercury vapour at 254 nm



B) Based upon the phenomenon

- 1) **Sensitized fluorescence:** eg. Elements like thallium, zinc, cadmium or an alkali metals are added to mercury vapour
- 2) **Direct line fluorescence:** Even after the emission of radiation, the molecules remain in metastable state and finally comes to ground state after loss of energy by vibrational processes
- 3) **Stepwise fluorescence:** eg. Conventional fluorimetry
- 4) **Thermally assisted fluorescence:** The excitation is partly by electromagnetic radiation and partly by thermal energy



Factors influencing fluorescence intensity:



1. Nature of molecule (conjugation)

5. Temperature

2. Nature of substituent group

6. Viscosity

3. Rigidity of structure

7. Oxygen

4. Adsorption

8. pH

9. Photochemical decomposition

10. Concentration (Concentration reversal or Self quenching or Concentration quenching)



Quenching and Types:



- Quenching is the decrease in fluorescence intensity.
- These effects may be due to various factors like concentration, pH, presence of specific chemical substances, temperature, viscosity etc.

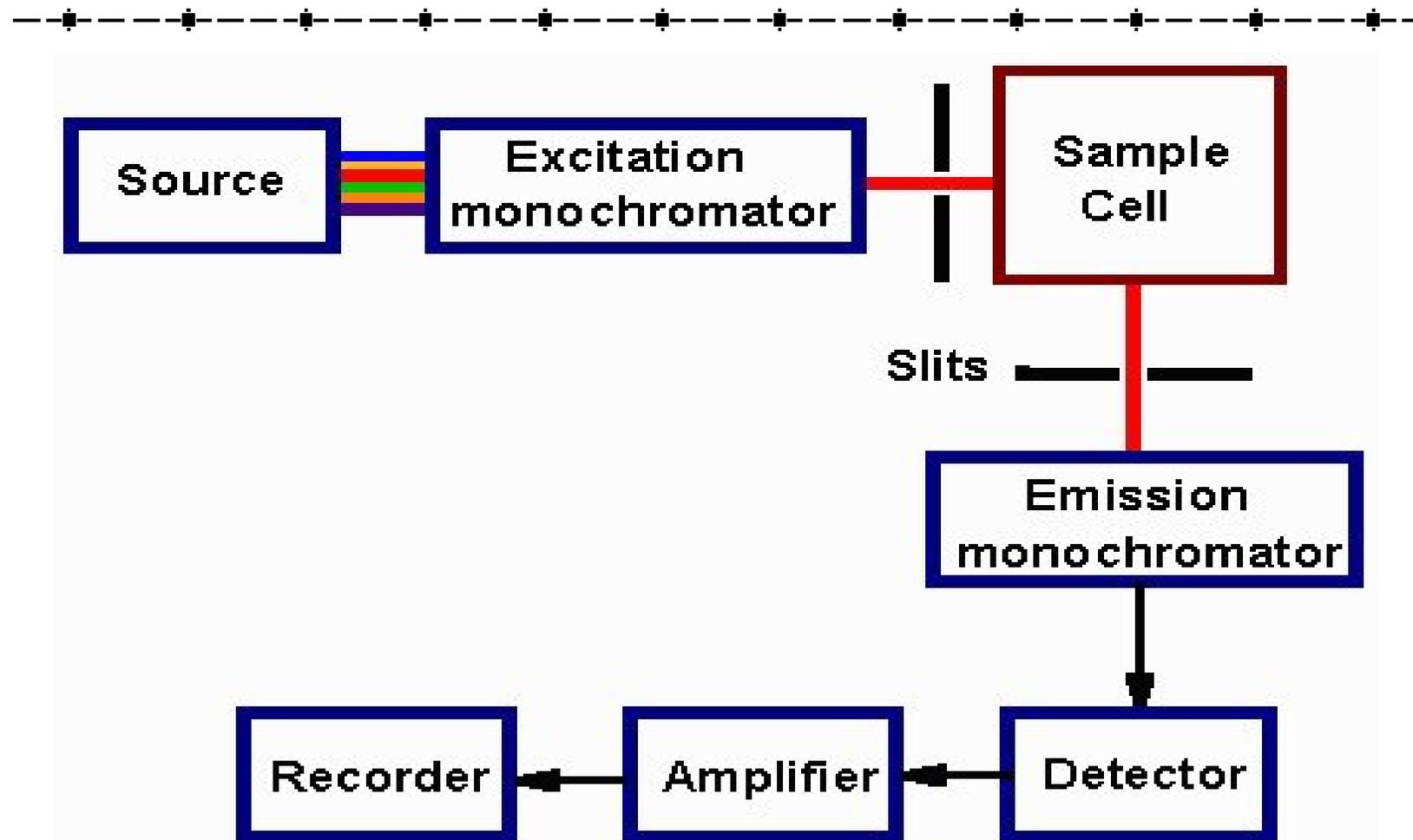
1. **Self quenching or concentration quenching or concentration reversal**

2. **Chemical quenching:** eg. Change in pH, presence of oxygen, halides or heavy metals

3. **Collisional quenching:** eg. Halides, heavy metals etc.

4. **Static quenching:** eg. Because of complex formation between quenchers and fluorescent compound

Instrumentation





A generalized luminescence instrument consist of:

- A source of light
- A primary filter or excitation monochromator
- A sample cell
- A secondary filter or emission monochromator
- A fluorescence detector and
- A data read out device (recorder)



- Molecules in solution are usually excited by UV light and the excitation source is usually a deuterium or xenon lamp.
- The primary or excitation monochromator select specific band or wavelength of radiation from the light sources and direct them through the sample in the sample cell.
- The secondary filter or emission monochromator isolate the resultant fluorescence and directed to the photo detector (PMT), which measure the intensity of fluorescent radiation.
- Simple instruments sometimes use only a band pass filter to select the excitation wavelength.



Applications of fluorescence spectroscopy:



- 1. Determination of uranium salts and this is used extensively in the field of nuclear research.**
- 2. Determination of inorganic ions. These ions form fluorescent chelates with non-fluorescent organic molecules.**
 - Determination of ruthenium ion in the presence of other platinum metals
 - Determination of aluminium (III) in alloys
 - Estimation of traces of boron in steel
 - Estimation of cadmium
 - Determination of calcium



3. Fluorescent indicators:

Eosin

Fluorescein

Quinine sulphate

Acridine

2-napthaquinone

2-hydroxy cinnamic acid

4. Fluorimetric reagents (Determination of inorganic substances)

8-hydroxy quinoline - Lithium

Benzoin - Zinc

Alizarin garnet-B - Aluminnium

Flavanol - Tin

Napthoic acid – Berylium



5. Determination of organic substances

- Fluorimetry has been used to carry out qualitative as well as quantitative analysis for a great many aromatic compounds present in a cigarette smoke, air-pollutants, concentrates and automobile exhausts.

eg. Determination of benzopyrene in the nanogram range

- Aromatic polycyclic hydrocarbons, indoles, naphthols, proteins, plant pigments, steroids etc. can be determined at low conc. by fluorimetry.

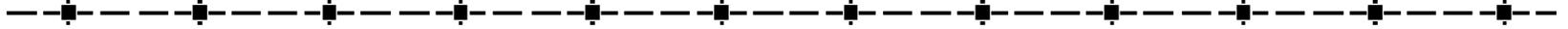


6. Pharmaceuticals applications

- **Compounds which are fluorescent**
- **Compounds readily converted to fluorescent products by chemical reactions**

7. Determination of vitamin B₁ (Thiamine or Aneurine hydrochloride)

8. Determination of vitamin B₂ (Riboflavin)



Thank you