

APPLICATIONS OF SPECTROSCOPY

MODULE:02

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PREREQUISITE: Student should know principles of spectroscopy, various types of spectroscopy and the techniques involved.

COURSE OUTCOME: Illustrate the concept of emission spectroscopy and describe the phenomena of fluorescence and phosphorescence in relation to it.

INTRODUCTION : The emission spectrum of a chemical element or chemical compound is the spectrum of frequencies of electromagnetic radiation emitted due to an atom or molecule making a transition from a high energy state to a lower energy state. Each element's emission spectrum is unique. Therefore, spectroscopy can be used to identify the elements in matter of unknown composition. Similarly, the emission spectra of molecules can be used in chemical analysis of substances.

The course provides principle, instrumentation and applications of Emission spectroscopy-Flame Photometry, introduction to fluorescence and phosphorescence, Jablonski diagram, applications of fluorescence in only medicinal field.

1. Explain briefly about emission spectroscopy.

- A. i. The basis of emission spectroscopy is the emission of radiation from the excited state to the ground state.
- ii. It deals with atom and the sample must be present initially in solid state.
- iii. It comprises of the excitation of a sample using thermal or electrical energy and the emitted radiations are analysed.
- iv. When a sample is excited thermally or electrically, its valence electrons get excited from lower energy level to higher energy level, and they rapidly emit a photon of energy and return to the ground state.
- v. The liquid solutions are evaporated and then analysed.
- vi. The metals and metalloids can be identified in very low concentration, [1ppm]
- vii. A metal can be analysed in a very short time of about one minute.

2. Explain the principle of Flame emission spectroscopy or flame photometry.

- A.i. Flame photometry is based on the measurement of the emitted light intensity when a metal is introduced into the flame.
- ii. The wavelength of the colour gives the information about the element and the colour of the flame gives information about the amount of the element present in the sample.
- iii. The flame is a low energy source and is useful for only alkali[Group 1] and alkaline earth metals,[Group 2] and not for metalloids and transition metals.
- iv. Flame photometry is used in Inorganic chemical analysis to determine the concentration of certain metal ions like sodium, potassium, lithium, calcium, caesium etc.
- vi. Alkali and alkaline earth metals are sensitive to flame photometry, since their energies required for exciting is low.
- vii. The compounds of group 1 and 2 metals dissociate into atoms when introduced into flame.

viii. Some of the dissociated atoms get excited to higher energy levels, but these atoms are not stable at higher energy level, so they return back to the ground state by emitting radiation which lie in the visible region of the spectrum.

ix. Each of the alkali and alkaline earth metal has a specific emitted wavelength and a specific colour in visible region as given below:

Element	Emitted wavelength	Flame color
Sodium	589 nm	Yellow
Potassium	766 nm	Violet
Barium	554 nm	Lime green
Calcium	622 nm	Orange
Lithium	670 nm	Red

3. What are the events or processes occurring in the flame photometry?

A. Following five processes take place in flame photometry:-

i. Desolvation: It involves drying a sample in a solution. The metal particles in the solvent are dehydrated by the flame and thus the solvent is evaporated.

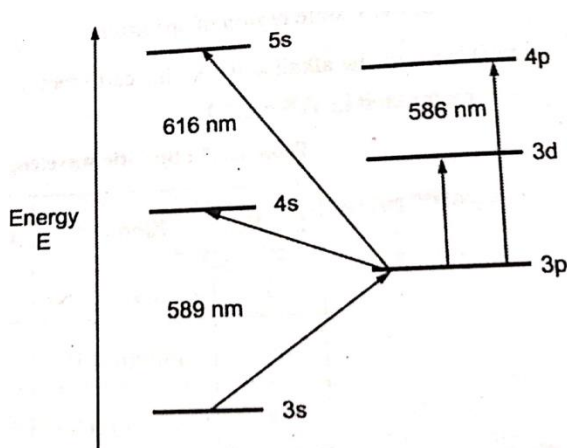
ii. Vapourisation: The metal particles in the solvent are dehydrated by the flame and thus the solvent is evaporated.

iii. Atomisation: Atomisation is the separation of all the atoms in a chemical substance. The metal ions in the sample are reduced to metal atoms by the flame.

iv. Excitation: The electrostatic force of attraction between the electron and the nucleus of the atom helps them to absorb a particular amount of energy due to which the atoms jump from higher energy excited state.

v. Emission: Since the higher energy excited state is unstable, the atom jumps back to the lower energy ground state and becomes stable. The jumping of atoms from excited state to the ground state emits a radiation of characteristic wavelength which is measured by a photo-detector.

For eg: In the energy level diagram of sodium atom, the intensity of the light emitted can be described by the Scheibe-Lomakin equation as follows:



$I = K \times C^n$ where,

I = intensity of emitted light

K = proportionality constant

C = concentration of the element.

In the linear part of the calibration curve, $n = 1$,

Therefore, $I = K \times C$

Thus, the intensity of the emitted light is directly proportional to concentration of the element.

4. Explain the instrumentation or different parts of Flame photometry.

A. A simple flame photometer consists of the following basic components/ parts:

i. **Source of flame:** A Burner in the flame photometer is the source of flame. It can be maintained at a constant temperature. The temperature of the flame is one of the critical factors in flame photometry. It depends on the type of burner used and the ratio of the type of fuel and oxidant. Commonly used fuel-oxidant mixtures and the corresponding temperatures of the flame are listed below:

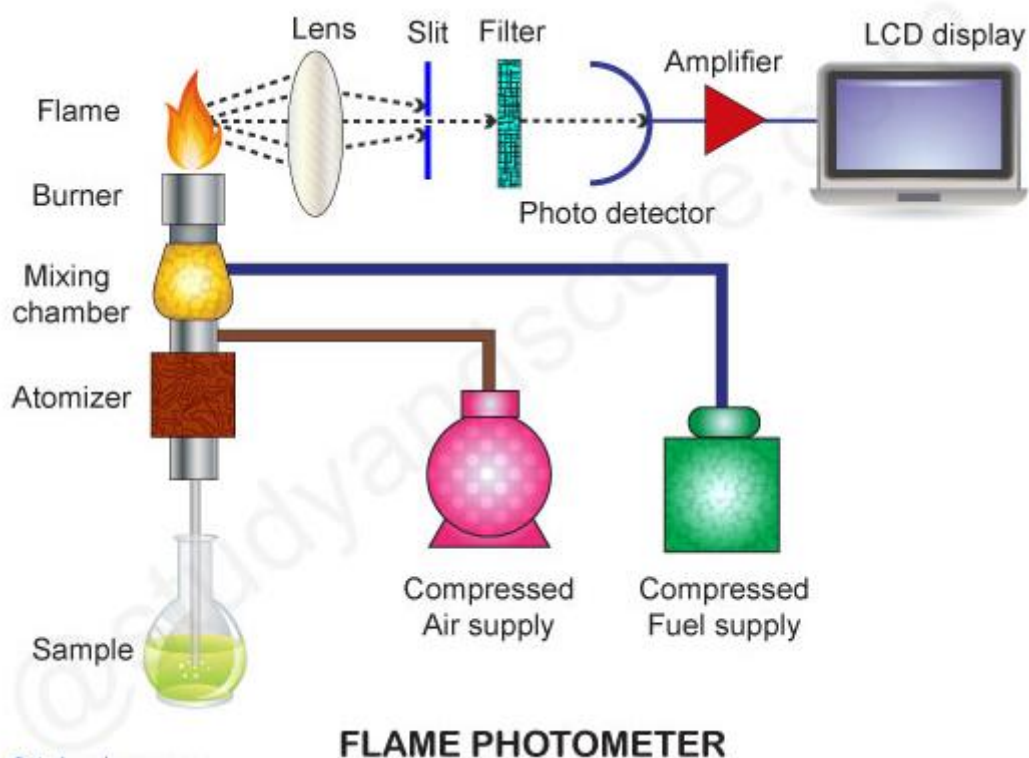
Fuel-Oxidant mixture	Temperature (°C)
Natural gas-Air	1700
Propane-Air	1800
Hydrogen-Air	2000
Hydrogen-Oxygen	2650
Acetylene-Air	2300
Acetylene-Oxygen	3200
Acetylene-Nitrous oxide	2700
Cyanogen-Oxygen	4800

ii. **Nebuliser:** Nebuliser is used to send homogeneous solution into the flame at a balanced rate.

iii. **Optical System :** The optical system consists of convex mirror and convex lens. The convex mirror transmits the light emitted from the atoms. Convex mirror also helps to focus the emissions to the lens. The lens helps to focus the light on a point or slit.

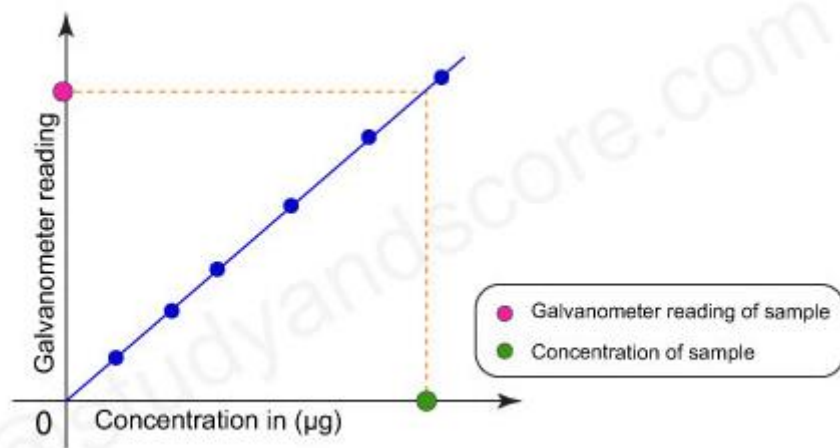
iv. **Simple colour filters:** The reflections from the mirror pass through the slit and reach the filters. Filters will isolate the wavelength to be measured from that of irrelevant emissions. Thus the filters act as interference type of colour filters.

v. **Photo-detector:** The intensity of radiation emitted by the flame is measured by photo detector. Here the emitted radiation is converted to an electrical signal with the help of photo detector. These electrical signals are directly proportional to the intensity of light.



5. Explain the working of flame photometer.

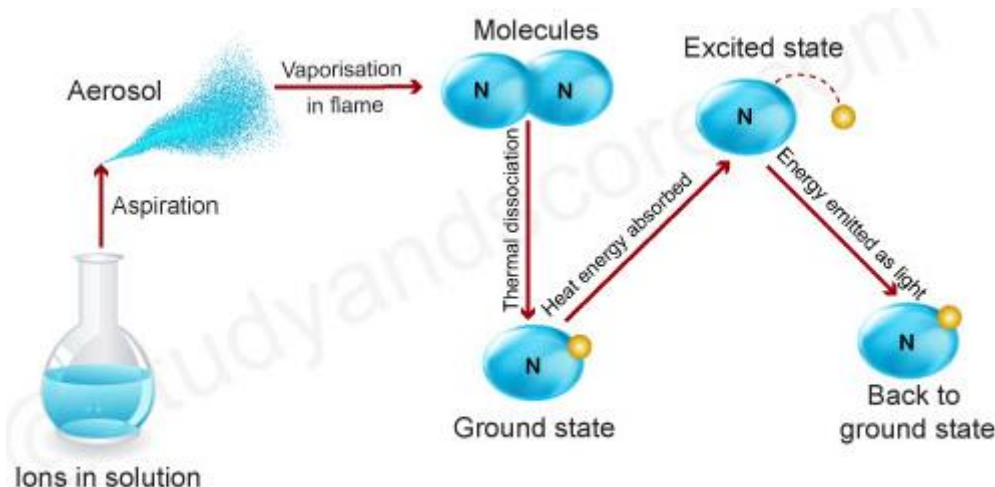
- A.i. The standard stock solution and sample solution are prepared in fresh distilled water.
- ii. The flame of the photometer is calibrated by adjusting the air and gas. Then the flame is allowed to stabilize for about 5 min.
- iii. The instrument is switched on and the lids of the filter chamber are opened to insert appropriate colour filters.
- iv. The readings of the galvanometer are adjusted to zero by spraying distilled water into the flame.
- v. The sensitivity is adjusted by spraying the most concentrated standard working solution into the flame and the full scale deflection of the galvanometer is recorded.
- vi. Again distilled water is sprayed into the flame to attain constant readings of galvanometer. Then the galvanometer is readjusted to zero.
- vii. Each of the standard working solutions is sprayed into the flame for three times and the readings of galvanometer are recorded. After each spray, the apparatus must be thoroughly washed.
- viii. Finally sample solution is sprayed into the flame for three times and the readings of galvanometer are recorded. After each spray, the apparatus must be thoroughly washed.
- ix. Calculate the mean of the galvanometer reading.
- x. Plot the graph of concentration against the galvanometer readings to find out the concentration of the element in the sample. The graph is as shown below:



FLAME PHOTOMETER: GRAPH

6. What are the processes [overview] taking place in flame photometer?

- A.i. The solvent is first aspirated to obtain fine solid particles.
- ii. These molecules in the solid particles are moved towards the flame to produce gaseous atoms and ions.
- iii. These ions absorb the energy from the flame get excited to high energy levels from the ground state.
- iv. But as these ions are unstable, they return back to ground state. While returning they emit characteristic radiation.
- v. The intensity of emitted light is proportional to the concentration of the element.



OVERVIEW OF FLAME PHOTOMETRY

7. Give the advantages and disadvantages of flame photometry.

A. Advantages of Flame photometry are as follows:

- i. The method of analysis is very simple and economical.
- ii. It is quick, convenient, selective and sensitive analysis.
- iii. It is both qualitative and quantitative in nature.
- iv. Even very low concentrations (parts per million/ppm to parts per billion/ppb range) of metals in the sample can be determined.

- v. This method compensates for any unexpected interfering material present in the sample solution.
- vi. This method can be used to estimate elements which are rarely analysed.

Disadvantages of Flame photometry are as follows:

- i. The accurate concentration of the metal ion in the solution cannot be measured.
- ii. It cannot directly detect and determine the presence of inert gases.
- iii. Though this technique measures the total metal content present in the sample, it does not provide the information about the molecular structure of the metal present in the sample.
- iv. Only liquid samples may be used. Also sample preparation becomes lengthy in some cases.
- v. Flame photometry cannot be used for the direct determination of each and every metal atom. A number of metal atoms cannot be analysed by this method. The elements such as carbon, hydrogen and halides cannot be detected due to their non-radiating nature.

8. Give the applications of flame photometry.

A. The applications of flame photometry are as follows:

- i. Flame photometer can be applied both for quantitative and qualitative analysis of elements. The radiations emitted by the flame photometer are characteristic to particular metal. Hence with the help of Flame photometer we can detect the presence of any specific element in the given sample.
- ii. The presence of some group II elements is critical for soil health. We can determine the presence of various alkali and alkaline earth metals in soil sample by conducting flame test and then the soil can be supplied with specific fertiliser.
- iii. The concentrations of Na^+ and K^+ ions are very important in the human body for conducting various metabolic functions. Their concentrations can be determined by diluting and aspirating blood serum sample into the flame.
- iv. Soft drinks, fruit juices and alcoholic beverages can also be analysed by using flame photometry to determine the concentrations of various metals and elements.

9. Define luminescence.

A. Luminescence is any emission of light (electromagnetic waves) from a substance which does not arise from heating. In this process, light is produced at lower temperature, eg, light from kerosene lamp. Therefore the light produced by luminescence process is considered as the cold light or light without heat.

10. Explain the process of Fluorescence.

A.i. Fluorescence process involves the emission of light by a molecule, called fluorophore, that gets excited by a shorter wavelength than the emitted one.

ii. Fluorescence intensity detection gives the measurement of this emitted light.

iii. The most striking example of fluorescence occurs when the absorbed radiation is in the ultraviolet region of the spectrum, and thus invisible to the human eye, while the emitted light is in the visible region, which gives the fluorescent substance a distinct color that can be seen only when exposed to UV light.

- iv. When an atom or a molecule absorbs a photon of light, it gets excited, and in a very short duration of the order of nanoseconds after excitation, it emits a photon of light of longer wavelength, called as fluorescence.
- v. The device used to measure the parameters of visible spectrum fluorescence is called 'Fluorimeter'.
- vi. It measures the intensity and wavelength distribution of emission spectrum after excitation by a certain wavelength of light.
- vii. These parameters are used to identify the presence and the amount of specific molecules in a medium.
- viii. The technique is called 'Fluorimetry' or 'Fluorescence Spectroscopy'.
- ix. It analyses fluorescence properties of a sample by determining the concentration of an analyte in a sample.
- x. It is a very sensitive technique, fast, simple and inexpensive method.
- xi. Some of the examples of fluorescence substances are calcium fluoride, sodium, iodine and mercury vapours.

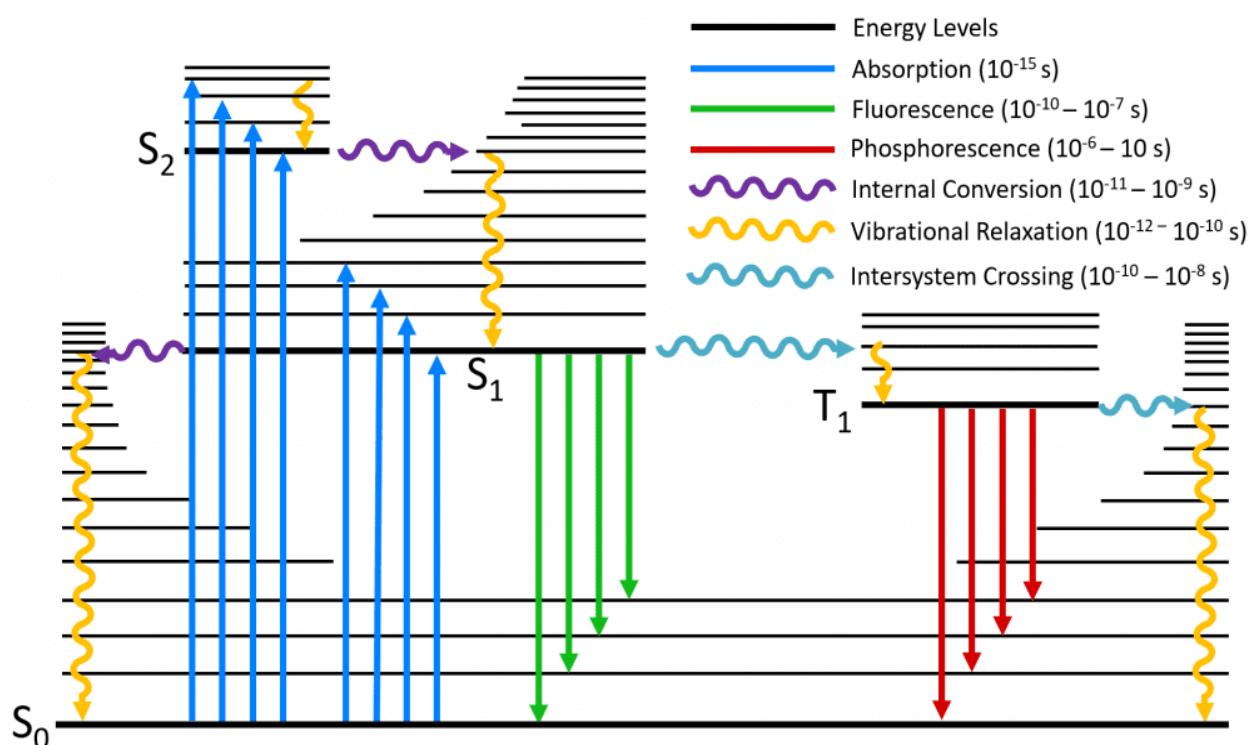
11. Explain the process of Phosphorescence.

- A.i. Phosphorescence is the process of emission of light by a molecule called phosphorophore, which is excited by light with a longer wavelength of the order of milli-seconds after excitation so that the emission continues after the excitation ceases.
- ii. Phosphorescence material does not immediately re-emit the radiation it absorbs.
- iii. The absorbed transitions may be re-emitted at a lower intensity for upto several hours after the original excitation.
- iv. For eg: Paints, clock dials, toys glowing in the dark show applications of phosphorescence.
- v. Phosphorimetry is the technique of chemical analysis based on the nature and intensity of the phosphorescent light emitted by an excited molecule.
- vi. Phosphorescence is mainly caused by ultraviolet and visible light.
- vii. Some of the examples of phosphorescence substances are zinc sulphide, calcium sulphide, barium sulphide and strontium sulphide.

12. What is Jablonski diagram? Explain in detail a typical Jablonski diagram.

- A. Aleksander Jablonski in 1935 suggested the diagram to explain both the kinetics and spectra of fluorescence and phosphorescence.
- i. It is widely used in Fluorescence spectroscopy to illustrate the excited electronic states of the molecule and the radiative and non-radiative transitions occurring between them.
- ii. The Jablonski diagram is the powerful means for visualising the possible transitions that can occur after a molecule has been photoexcited.
- iii. A Jablonski diagram is basically an energy diagram, arranged with energy on a vertical axis. These energy levels can be quantitatively denoted, but most of these diagrams use energy levels schematically.

iv. A typical Jablonski diagram is shown below:



The terms involved and the different possible transitions shown in the diagram is explained as follows:

I. Energy levels:

- i. The energy levels of a molecule are shown by the horizontal bold lines, with the energy increasing along the vertical axis of the diagram.
- ii. The bold lines represent the lowest vibrational level of each electronic state, with the higher vibrational levels represented by thinner lines.
- iii. The vibrational levels become more closely spaced as the energy increases and eventually forms a continuum. Only a subset of these vibrational levels are represented on the diagram.
- iv. The naming of the electronic states is based on the spin angular momentum configuration of each state.
- v. Singlet states, a total spin angular momentum of zero, are denoted by 'S' and the triplet states, a total spin angular momentum of one, are denoted by 'T'.
- vi. for e.g. 'S₀' is the singlet ground state of the molecule. 'S₁' is the first excited singlet state, 'S₂' is the second excited singlet state, 'S₃' is the third excited singlet state and 'S_n' is the nth excited singlet state. Similarly 'T₁' is the first excited triplet state 'T₂' is the second excited triplet state and 'T_n' is the nth excited triplet state.

II. Radiative transitions: These are the transitions between two molecular states where the energy difference is emitted or absorbed by photons. They are represented by straight arrows in Jablonski diagram. Absorption, fluorescence, Phosphorescence and delayed fluorescence are all radiative transitions.

1. Absorption:

- i. A molecule is promoted from its ground state to a higher state by absorption of a photon which is represented by the arrow upwards.

- ii. It is the fastest transition in the Jablonski diagram, occurring on a timescale of order 10^{-15} seconds.
- iii. Absorption of a photon promotes the molecule from the S_0 to one of the vibrational levels of the singlet excited states S_1, S_2, \dots

2. Fluorescence:

- i. This is a radiative transition between two electronic states of the same spin multiplicity. It occurs on a timescale of 10^{-10} to 10^{-7} seconds.
- ii. Molecules occupying the lower vibrational level of the ground electronic energy state absorb light and get excited to higher energy state [S_1 or S_2].
- iii. The excited molecule rapidly loses its excess of vibrational energy by collision and falls to the lower vibrational level of the excited state.
- iv. Almost all the molecules in the highly excited electronic states undergo internal conversion and pass from the lowest vibrational level of the upper state to a higher vibrational level of a lower excited state having same energy.
- v. From there, the molecules again lose energy until the lowest vibrational level of the first excited state is reached.
- vi. From this level, the molecule can return to any of the vibrational levels of the ground state, emitting its energy in the form of fluorescence.

3. Phosphorescence:

- i. This is a radiative transition between two electronic states of the different spin multiplicity. It occurs on a timescale of 10^{-6} to 10 seconds.
- ii. In the process of phosphorescence, when a molecule in the T_1 state returns to the ground state S_0 , emission of radiation occurs at a slow rate.
- iii. Such transitions are spectroscopically forbidden, and hence, its life time is much longer (10^{-3} seconds or more). It can occur only when the intersystem crossing T_1 to S_0 does not happen rapidly.
- iv. They are represented by straight arrows in Jablonski diagram.

4. Delayed Fluorescence:

- i. Delayed fluorescence occurs when a molecule in the T_1 state transits to the S_1 state followed by a radiative transition to the S_0 state, which results in an emission identical in wavelength to standard fluorescence but occurring on a longer timescale.
- ii. Delayed fluorescence occurs through two distinct mechanisms namely, Thermally activated delayed fluorescence [E-type delayed fluorescence] and Triplet-Triplet Annihilation [P-type delayed fluorescence].

III. Non-radiative transitions: These transitions are from the higher excited states S_3, S_2, T_3, T_2 to the first excited state S_1 or T_1 which doesn't involve emission of any radiation. They are represented by undulating arrows (zig-zag arrows) in Jablonski diagram. Vibrational relaxation, Internal Conversion, Intersystem crossing are all non-radiative transitions.

1. Vibrational Relaxation:

- i. In vibrational relaxation, the transition occurs very quickly ($< 10^{-12}$ s). It is enhanced by physical contact of an excited molecule with other particles, with which energy in the form of vibrations and rotations can be transferred through collisions.
- ii. The excess vibrational energy is lost to vibrational modes within either the same molecule (intramolecular) or to surrounding molecules (intermolecular) until the lowest vibrational level of the electronic state is reached.

2. Internal Conversion:

- i. Internal conversion is immediately followed by vibrational relaxation to the lowest vibrational level of the electronic state.
- ii. The rate of internal conversion is inversely proportional to the energy gap between the two electronic states. Internal conversion of the closely spaced higher lying singlet excited states [$S_3 \rightarrow S_2$, $S_2 \rightarrow S_1$, etc] will proceed rapidly on a timescale of 10^{-11} to 10^{-9} seconds.
- iii. In contrast, the energy gap between the S_1 and the S_0 is much wider and internal conversion between these states occur on a slower timescale.

3. Intersystem Crossing :

- i. In Intersystem crossing, the energy of activated molecule is dissipated through transitions between states of different spins. For eg S_2 to T_2 or S_1 to T_1 .
- ii. Such transitions are spectroscopically forbidden due to conservation of spin angular momentum and the orbital angular momentum that makes it weakly allowed.
- iii. It occurs on a timescale of 10^{-10} to 10^{-6} seconds.
- iv. Unlike fluorescence and internal conversion, intersystem crossing is too slow to be relevant for the majority of purely organic molecules.
- v. After intersystem crossing, the molecule will immediately undergo vibrational relaxation to the ground vibrational level of T_1 .

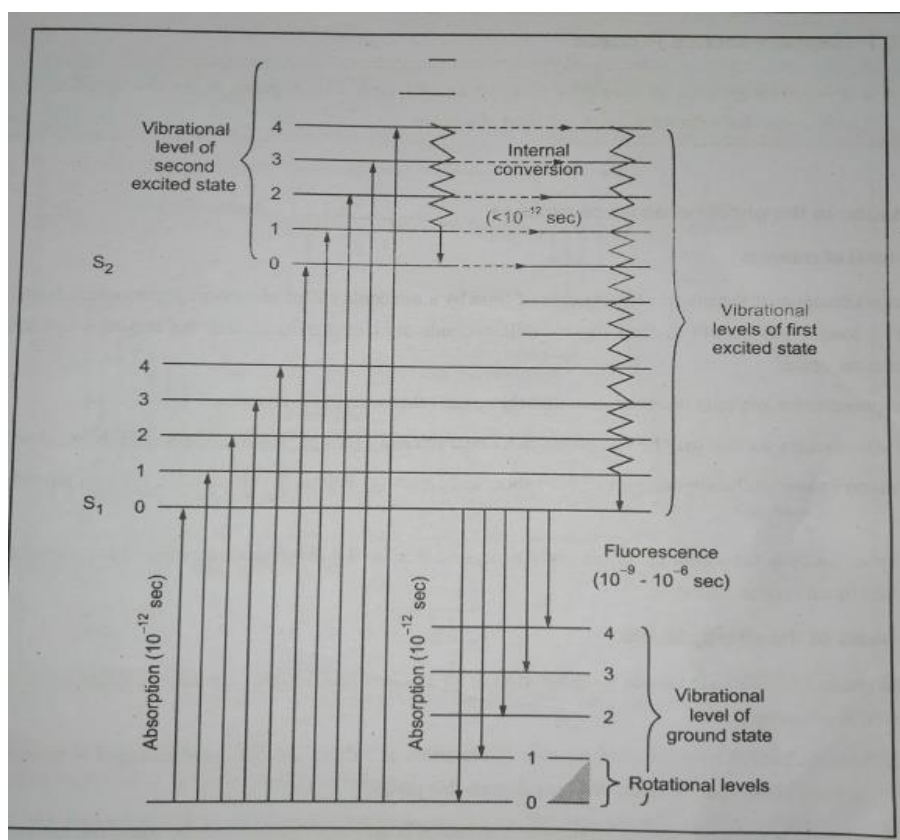
13. Explain the phenomena of fluorescence and phosphorescence on the basis of energy levels.

A. Fluorescence :-

- i. Most molecules at room temperature occupy the lower vibrational level of the ground electronic energy state. On absorption of light in the form of energy as discrete quanta, they get excited to higher energy state [S_1 OR S_2].
- ii. After absorbing energy and reaching one of the higher vibrational levels of an excited state, the molecule rapidly loses its excess of vibrational energy by collision and falls to the lower vibrational level of the excited state. However, the rotational levels associated with each vibrational level are neglected.
- iii. Almost all the molecules in the highly excited electronic states undergo internal conversion and pass from the lowest vibrational level of the upper state to a higher vibrational level of a lower excited state having same energy.
- iv. From there, the molecules again lose energy until the lowest vibrational level of the first excited state is reached.

v. From this level, the molecule can return to any of the vibrational levels of the ground state, emitting its energy in the form of fluorescence. It occurs on a timescale of 10^{-10} to 10^{-7} seconds.

vi. The energy level diagram in the fluorescence is shown below:



Phosphorescence :-

i. Excited states of molecules can have unpaired electrons. States with two unpaired electrons are called triplet states [T].

ii. These excited triplet states are normally more stable than the corresponding singlet excited states because, by Hund's rule less inter electronic repulsion is expected with unpaired electrons rather than paired electrons.

iii. The excited triplet state T_1 lies between the S_0 and S_1 state.

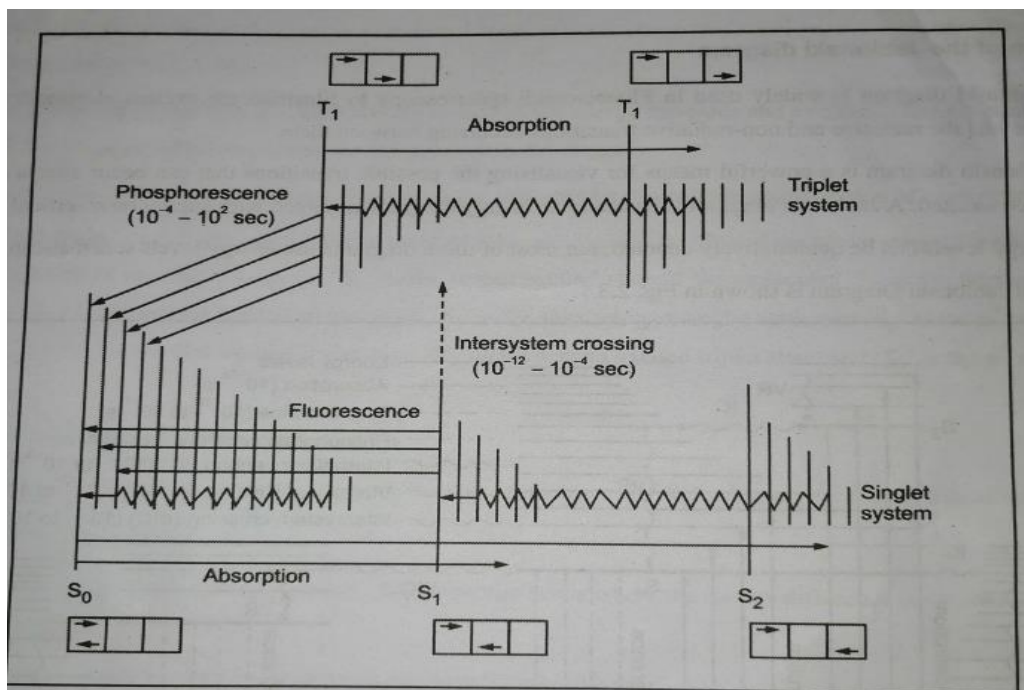
iv. The indirect process of conversion from the excited state produced by absorption of energy, the singlet state to a triplet state, is known as intersystem crossing..

v. It can occur in many substances when the lowest vibrational level of the excited state S_1 , has the same energy level as an upper vibrational level of the triplet state as shown in the diagram.

vi. Direct transition from the ground state S_0 for a molecule with an even number of electrons to an excited state, is theoretically forbidden.

vii. Therefore the reverse transition from an excited triplet state to the ground state becomes difficult and requires atleast 10^{-4} seconds to as long as 10^2 seconds and is accompanied by the emission of phosphorescence.

viii. The energy level diagram for phosphorescence is shown below:



14. Explain with diagram two mechanisms of delayed fluorescence.

A. i. Delayed fluorescence occurs when a molecule in the T_1 state transits to the S_1 state followed by a radiative transition to the S_0 state, which results in an emission identical in wavelength to standard fluorescence but occurring on a longer timescale.

ii. Delayed fluorescence occurs through two distinct mechanisms namely, Thermally activated delayed fluorescence [E-type delayed fluorescence] and Triplet-Triplet Annihilation [P-type delayed fluorescence].

I. Thermally Activated Delayed Fluorescence [E-type delayed fluorescence]:

i. In TADF, the molecule transits from the T_1 state back into the S_1 state through reverse intersystem crossing [rISC] which is thermally activated so that the molecule possess enough thermal energy to overcome the energy gap between the S_1 and the T_1 states.

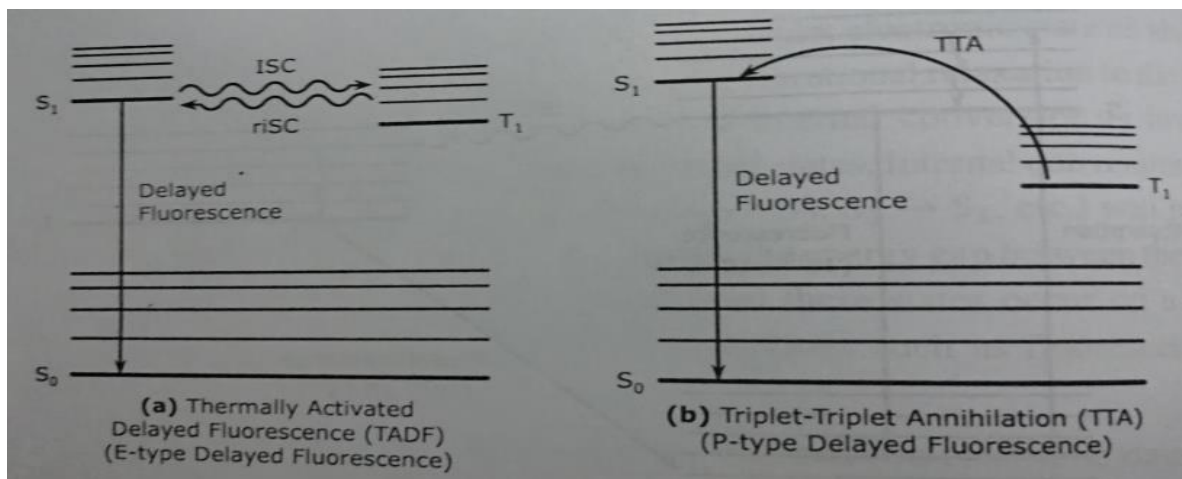
ii. For efficient rISC to occur, the energy gap should be comparable to the thermal energy, kT , which is approximately 25 meV at room temperature.

iii. The mechanism is also known by the name E-type delayed fluorescence as it was first observed in Eosin

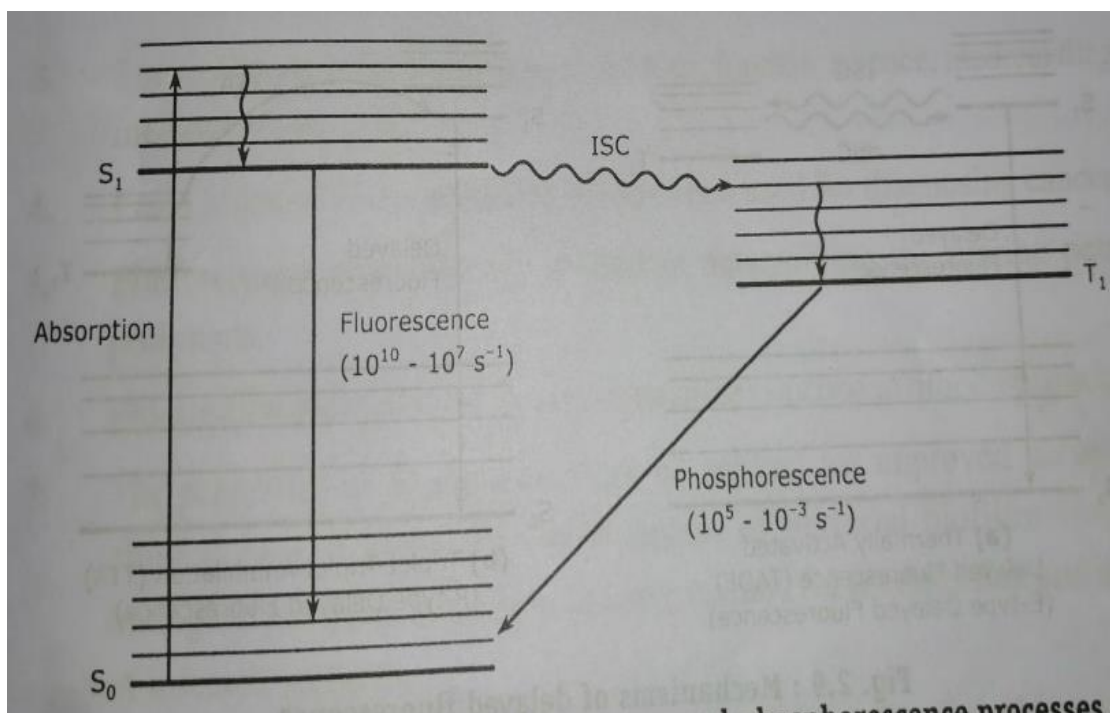
II. Triplet-Triplet Annihilation [P-type delayed fluorescence].

i. In this type of delayed fluorescence, two molecules in the T_1 state undergo energy transfer which promotes one molecule to the S_1 state while the other returns to the S_0 state.

ii. It is also known as P-type delayed fluorescence as it was first observed in Pyrene.



15. Draw Jablonski diagram of fluorescence and phosphorescence processes.



16. Give the applications of fluorescence in medicine.

- A. i. Fluorophotometry can be used to determine glucose in blood very accurately.
- ii. It is an excellent diagnostic as well as excellent research tool in medical microbiology field with high sensitivity and specificity.
- iii. Fluorescent dyes are used to light up specific targets, fluorescent –activated cell sorters can be used to separate white blood cells from other cells found in blood.
- iv. Fluorescence spectroscopy has been extensively used in drugs, glass, petroleum products, and biological samples.
- v. The principles of fluorescence can be applied for improved diagnosis and clinical care in detection and diagnosis of bacterial biofilms from target biological samples. It is non-invasive method of detection and diagnosis of infected surfaces.
- vi. The techniques is also used in food analysis, forensic science, medical diagnosis, medical science etc. Laser induced fluorescence(LW) has been used for diagnosing cancer.

vii. Fluorescence spectroscopy is promising diagnostic technique with high sensitivity and specificity for micro-organisms associated with infections, disease such as bacteria, fungi, viruses and parasites.

viii. Fluorescence spectroscopy is used in determining marine petroleum pollutants.

17. Give the difference between fluorescence and phosphorescence.

A.

Pt. No	Fluorescence	Phosphorescence
1	It is the absorption of energy by atoms or molecules followed by immediate emission of light or electromagnetic radiation.	It is the absorption of energy by atoms or molecules followed by delayed emission of electromagnetic radiation.
2.	The emission of radiation or light suddenly stops on removal of source of excitation.	The emission of radiation remains for sometime even after the removal of source of excitation.
3.	The excited atom has comparatively short lifetime before its transition to low energy state.	The excited atom has comparatively long lifetime before its transition to low energy state.
4.	The emitted photon has lower energy than the absorbed photon and emission occurs at longer wavelength than incident light.	The emitted photon has lower energy than the absorbed photon and emission occurs at longer wavelength than the fluorescence.
5.	Fluorescent materials give an immediate flash or afterglow on excitation	Phosphorescent material appear to glow in the dark because of slow emission of light overtime.
6.	It is the radiation emitted in a transition between states of same multiplicity.	It is the radiation emitted in a transition between states of different multiplicity.
7.	For eg: calcium fluoride, sodium, iodine and mercury vapours.	Foreg: zinc sulphide, calcium sulphide, barium sulphide and strontium sulphide.

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