FLUORESCENCE SPECTROSCOPY

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Paper: MFSN 202 Food Science-II
(Advance analytical techniques and food packaging)

Introduction:

- **Fluorescence spectroscopy** (also known as **fluorimetry** or **spectrofluorometry**) is a type of electromagnetic spectroscopy that analyzes fluorescence from a sample. It involves using a beam of light, usually ultraviolet light, that excites the electrons in molecules of certain compounds and causes them to emit light; typically, but not necessarily, visible light. A complementary technique is absorption spectroscopy. In the special case of single molecule fluorescence spectroscopy, intensity fluctuations from the emitted light are measured from either single fluorophores, or pairs of fluorophores.
- Devices that measure fluorescence are called fluorometers.

Theory

Molecules have various states referred to as energy levels.

•In fluorescence, the species is first excited, by absorbing a photon, from its ground electronic state to one of the various vibrational states in the excited electronic state

- Collisions with other molecules cause the excited molecule to lose vibrational energy until it reaches the lowest vibrational state from the excited electronic state.

•The molecule then drops down to one of the various vibrational levels of the ground electronic state again, emitting a photon in the process have different energies

Therefore, by analysing the different frequencies of light emitted in fluorescent spectroscopy, along with their relative intensities, the structure of the different vibrational levels can be determined

LUMINISCENCE AND THE NATURE OF LIGHT

- A hot body that emits radiation solely because of its high temperature is said to exhibit incandescence. All other forms of light emission are called luminescence.
- When luminescence occurs, the system loses energy and if the emission is to be continuous, some form of energy must be supplied from elsewhere.
- When the external energy supply is by means of the absorption of infrared, visible or ultraviolet light, the emitted light is called photoluminescence and this is the process that takes place in any fluorimetric analysis.

- The energy, E, carried by any one quantum is proportional to its frequency of oscillation,
- Since the amount of energy per einstein is proportional to the frequency of the radiation, it varies enormously over the range of the electro-magnetic spectrum, as shown in the following table:-

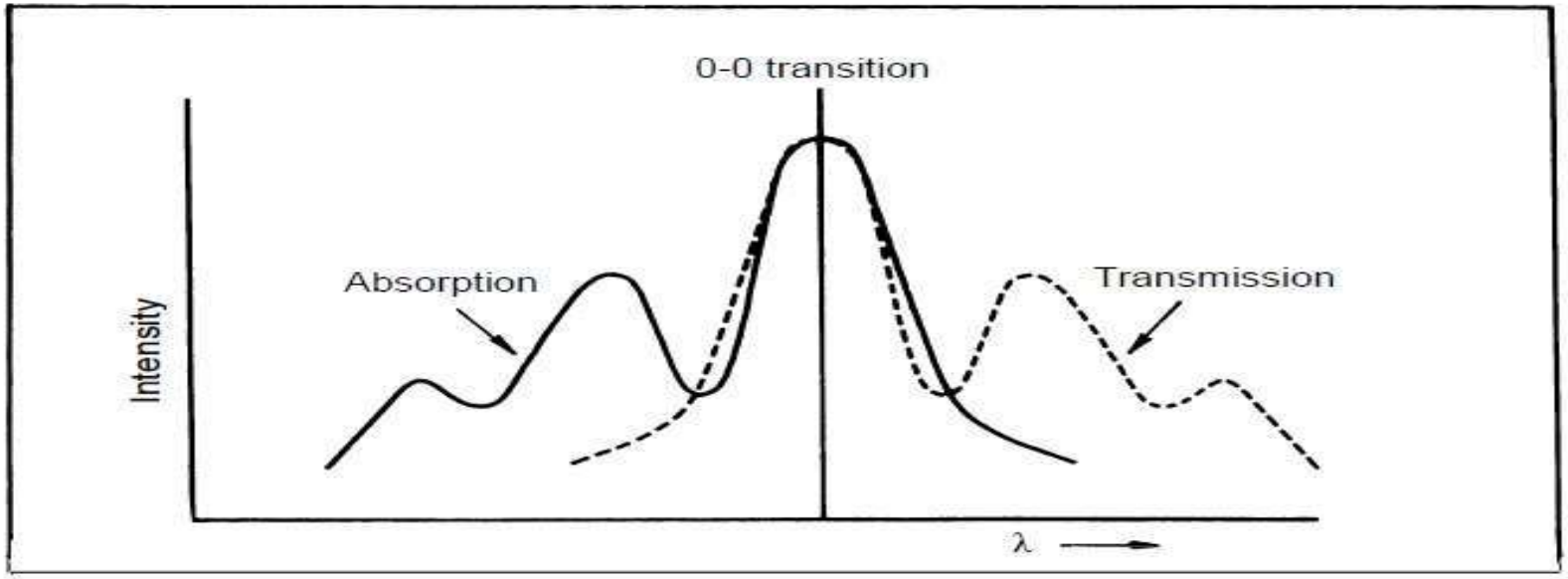
	Approximate sizes of Quanta				
Radiation	**ν(cm) (typical values)**	**Wave-number (μm^{-1})**	**Size of quantum (electron volts)**	**Size of einstein (kilogram calories)**	**Absorption or emission of radiation involves**
Gamma rays	10^{-10}	10^{6}	1.2×10^{6}	2.9×10^{7}	Nuclear reactions
X-rays	10^{-8}	10^{4}	1.2×10^{4}	2.9×10^{5}	Transitions of inner atomic electrons
Ultraviolet Visible	10^{-5} 4×10^{-5} 8×10^{-5}	10^{1} 2.5 1.25	1.2×10^{1} 3.1 1.6	2.9×10^{2} 7.1×10^{1} 3.6×10^{1}	Transitions of outer atomic electrons
Infrared	10^{-3}	10^{-1}	1.2×10^{-1}	2.9	Molecular vibrations
Far infrared	10^{-3}	10^{-2}	1.2×10^{-2}	2.9×10^{-1}	Molecular rotations
Radar Long radio waves	10^{1} 10^{5}	10^{-5} 10^{-9}	1.2×10^{-5} 1.2×10^{-9}	2.9×10^{-4} 2.9×10^{-8}	Oscillation of mobile or free electrons

- The absorption of light results in the formation of excited molecules which can in turn dissipate their energy by decomposition, reaction, or re-emission. The efficiency with which these processes take place is called the quantum efficiency and in the case of photoluminescence can be defined as:

ΦE = einsteins emitted or no. of quanta emitted/ einsteins absorbed or no. of quanta absorbed and never exceeds unity.

FLUORESCENCE

- At room temperature most molecules occupy the lowest vibrational level of the ground electronic state, and on absorption of light they are elevated to produce excited states.

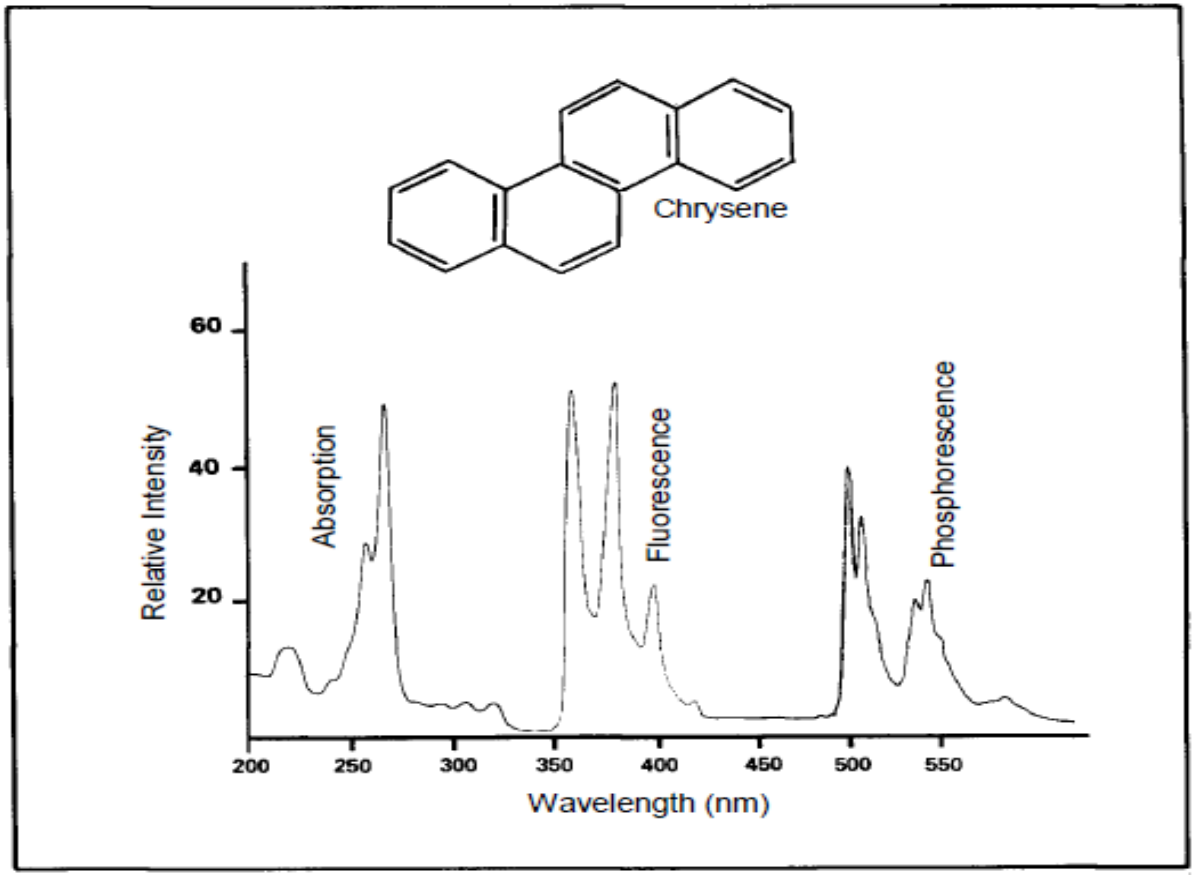


A plot of emission against wavelength for any given excitation wavelength is known as the emission spectrum. If the wavelength of the exciting light is changed and the emission from the sample plotted against the wavelength of exciting light, the result is known as the excitation spectrum. Furthermore, if the intensity of exciting light is kept constant as its wavelength is changed, the plot of emission against exciting wavelength is known as the corrected excitation spectrum. The quantum efficiency of most complex molecules is independent of the wavelength of exciting light and the emission will be directly related to the molecular extinction coefficient of the compound; in other words, the corrected excitation spectrum of a substance will be the same as its absorption spectrum.

PHOSPHORESCENCE

- 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
- Direct transition from the ground state, usually a singlet state, for a molecule with an even number of electrons, to an excited triplet state is theoretically forbidden, which means that the reverse transition from triplet to ground state will be difficult.
- Thus, while the transition from an excited singlet state, for example, S1, to the ground state with the emission of fluorescence can take place easily and within 10-9 - 10-6 seconds, the transition from an excited triplet state to the ground state with the emission of phosphorescence requires at least 10-4 seconds and may take as long as 102 seconds.

- The triplet state of a molecule has a lower energy than its associated singlet state so that transitions back to the ground state are accompanied with the emission of light of lower energy than from the singlet state. Therefore, we would typically expect phosphorescence to occur at longer wavelengths than fluorescence.



LIGHT SCATTERING

- ***Rayleigh-Tyndall scattering***

Incident radiation is not only absorbed or transmitted by the sample but is also scattered in all directions. The scattering takes place either from the molecules themselves (Rayleigh scattering) or from small particles in colloidal suspension (Tyndall scattering).

- ***Raman scattering***

During the Rayleigh scattering process, some of the incident energy can be abstracted and converted into vibrational and rotational energy. The resulting energy scattered is therefore of lower energy and longer wavelength than the incident radiation.

INSTRUMENTATION

- All fluorescence instruments contain three basic items: a source of light, a sample holder and a detector.
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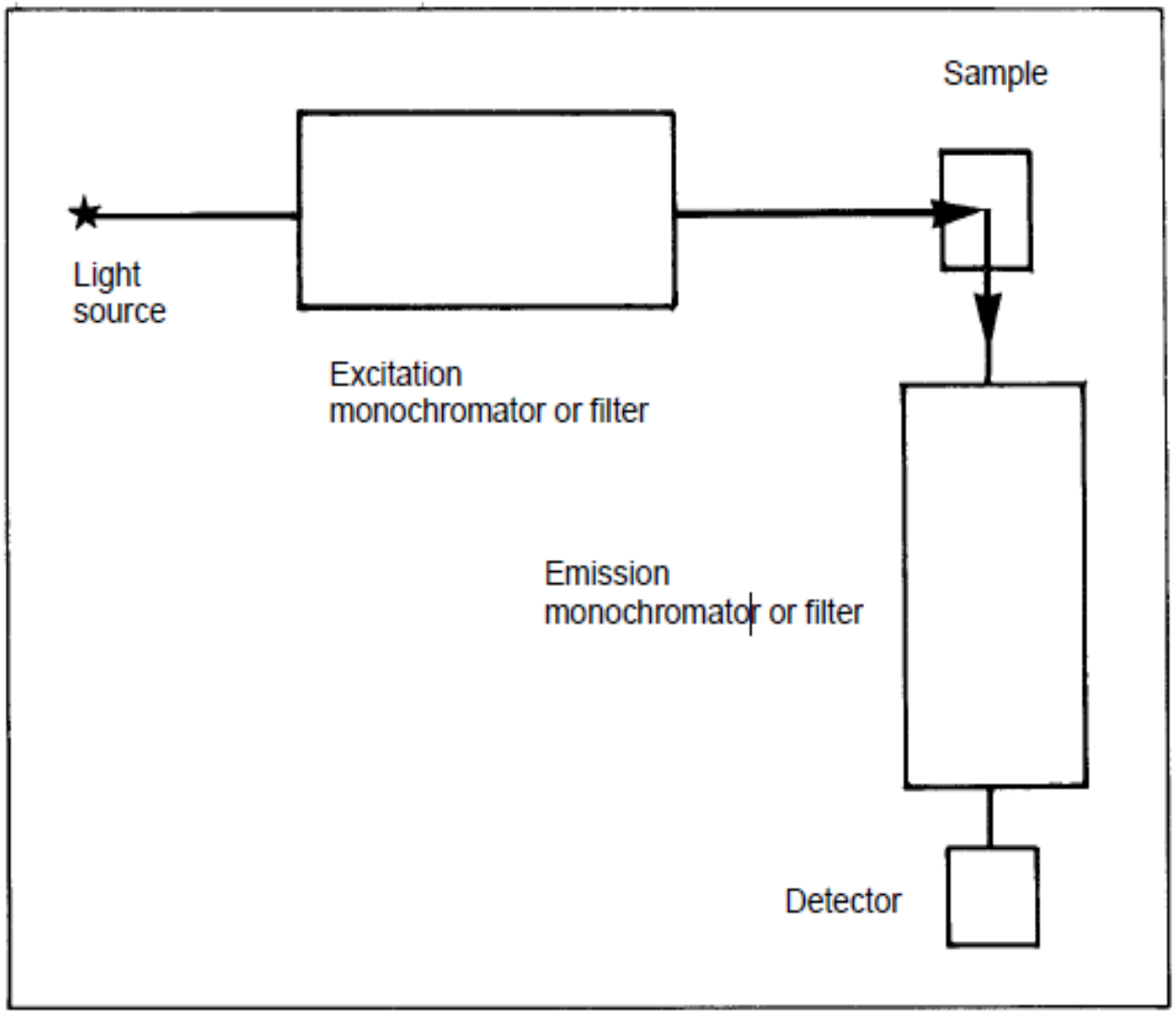
  - Simple and sophisticated filter fluorimeters.
- Simple fluorescence spectrometers have a meanfluorimeters. s of analysing the spectral distribution of the light emitted from the sample, the fluorescence emission spectrum, which may be by means of either a continuously variable interference filter or a monochromator. In more sophisticated instruments, monochromators are provided for both the selection of exciting light and the analysis of sample emission.


Sample
Light
source
Excitation
monochromator or filter
Emission
monochromator or filter
Detector

LIGHT SOURCES

- Commonly employed sources in fluorescence spectrometry have spectral outputs either as a continuum of energy over a wide range or as a series of discrete lines. An example of the first type is the tungsten-halogen lamp and of the latter, a mercury lamp.
- It is advantageous to employ a source whose output is a continuum and the most commonly employed type is the xenon arc.

wAVELENGTH SELECTION

- The simplest filter fluorimeters use fixed filters to isolate both the excited and emitted wavelengths. To isolate one particular wavelength from a source emitting a line spectrum, a pair of cut-off filters are all that is required. These may be either glass filters or solutions in cuvettes.
- Recently, interference filters having high transmission (≈ 40%) of a narrow range (10 – 15 nm) of wavelengths have become available and it is possible to purchase filters with a maximum transmission at any desired wavelength. UV filters of this type, however, are expensive and of limited range.
- However, it is useful to be able to scan the emission from the sample to check for impurities and optimize conditions. A convenient method is to make use of a continuous interference filter so that an emission spectrum can be recorded, at least over the visible region of the spectrum.

- A further refinement would be to use monochromators to select both the excitation and emission wavelengths.
- Such a fluorescence spectrometer is capable of recording both excitation and emission spectra and therefore makes full use of the analytical potential of the technique
- If monochromators are employed, it should be possible to change the slit width of both the excitation and emission monochromators independently.

DETECTORS

- All commercial fluorescence instruments use photomultiplier tubes as detectors and a wide variety of types are available. The material from which the photocathode is made determines the spectral range of the photomultiplier and generally two tubes are required to cover the complete UV-visible range. The S5 type can be used to detect fluorescence out to approximately 650 nm, but if it is necessary to measure emission at longer wavelengths, a special red sensitive, S20, photomultiplier should be employed.

rEAD OUT DEVICES

- The output from the detector is amplified and displayed on a readout device which may be a meter or digital display.
- Microprocessor electronics provide outputs directly compatible with printer systems and computers, eliminating any possibility of operator error in transferring data.

sAMPLE HOLDERS

- The majority of fluorescence assays are carried out in solution, the final measurement being made upon the sample contained in a cuvette or in a flowcell.
- Cuvettes may be circular, square or rectangular (the latter being uncommon), and must be constructed of a material that will transmit both the incident and emitted light.
- Square cuvettes, or cells will be found to be most precise since the parameters of pathlength and parallelism are easier to maintain during manufacture. However, round cuvettes are suitable for many more routine applications and have the advantage of being less expensive.
- The cuvette is placed normal to the incident beam. The resulting fluorescence is given off equally in all directions, and may be collected from either the front surface of the cell, at right angles to the incident beam, or in-line with the incident beam.

Advantages of fluorescence spectroscopy:

Very sensitive
Can be used for quantitation of fluorescent species
Easy and quick to perform analysis

Disadvantages:

Not useful for identification
Not all compounds fluorescence
Contamination can quench the fluorescence and hence give false/no results