Review Article

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A REVIEW ON FLOURIMETRY

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Abstract

Fluorescence spectroscopy is a rapid, sensitive method for characterizing molecular environments and events samples. Fluorimetry is chosen for its extraordinary sensitivity, high specificity, simplicity and low cost as compared to other analytical techniques. It is widely accepted and powerful technique that is used for a variety of environmental, industrial, medical diagnostics, DNA sequencing, forensics, genetic analysis and biotechnology applications. It is a valuable analytical tool for both quantitative and qualitative analysis. This article presents a brief overview of the theory of fluorescence spectroscopy, together with examples of applications of this technique in organic and inorganic chemistry, medical diagnosis, medical science etc.

Keywords: Fluorimetry, lamp, fluorescence quench, filters.

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Introduction

Fluorescence is the emission of visible light by a substance that has absorbed light of a different wavelength. Fluorophores play the central role in fluorescence spectroscopy. Fluorophores are the components in molecules that cause them to fluorescence. Majorly fluorophores are the molecule which contains aromatic rings such as Tyrosine, Tryptophan, and Fluorescein etc. It is an analytic method for detecting and measuring fluorescence in compounds that uses ultraviolet light stimulating the compounds, causing them to emit visible light. The energy/light emitted by the substance has a linger wavelength than absorbed. This process of emitting radiation with a longer wavelength than absorbed is known as luminescence (cold light). The mechanism of phosphorescence- As phosphorescing molecules can luminesce for a much longer time than fluorochromes, there must be a difference in the way they store the excitation energy.

The basis for this discrepancy is found in the two forms of excitation levels, the singlet excited state and the triplet excited state, which are based on different spin alignments Fluorescence Fluorescence is a type of luminescence caused by photons exciting a molecule, raising it to an electronic excited state. fluorimetry The slower time scales of the re-emission are associated with "forbidden" energy state transitions in quantum mechanics.As these transitions occur very slowly in certain materials, absorbed radiation may be re-emitted at a lower intensity for up to several hours after the original excitation. The mechanism of fluorescence- Fluorochromes will only fluoresce if they are illuminated with light of the corresponding wavelength.

The wavelength depends on the absorption spectrum of the fluorophore and it has to be ensured that an appropriate quantity of energy is delivered to elevate the electrons to the excited state. After the electrons are excited they can dwell in this high energy state for a very short time only.

Principle

Molecule contains σelectrons,∏ electrons and non bonding (n) electron. The electrons may be present in bonding molecular orbital. It is called as highest occupied molecular orbital (HOMO).it has lest energy and more stable. When the molecules absorbs radiant energy from a light source, the bonding electrons may be promoted bonding molecular orbital (LUMO).

Absorption of UV or visible radiation causes transition of electrons from singlet ground state to the singlet excited state. As this state is not stable, it emits energy in the form of UV or visible radiation and returns to singlet ground state. Fluorescence emission occurs as the fluorophore decay from the singlet electronic excited states to an allowable vibrational level in the electronic ground state. The fluorescence excitation and emission spectra reflect the vibrational level structures in the ground and the excited electronic states respectively. It has more energy

and hence less stable The process of promotion of electrons from HOMO to LUMO with absorption of energy is called as excitation.

Concept of Flourimetry

Singlet state

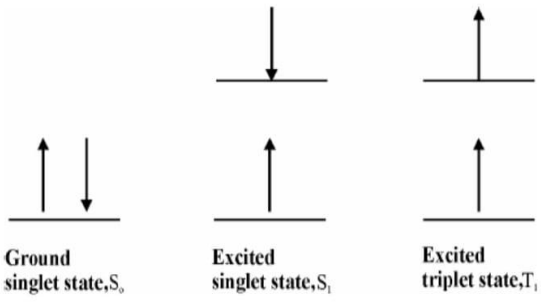
A state in which all the electrons in a molecule are paired ↓↑when all the electron spins are paired in the molecular electronic state and the electronic energy levels do not split when the molecule is exposed to UV radiation. If there is n number of unpaired electrons, it means that (n+1) fold degeneracy (equal energy state) will be associated with the electron spin, regardless of the molecular orbital occupied. Thus if no unpaired electrons are present (n=0), According to the formula: n+1, 0+1 = 1 spin state (singlet state)

Doublet state

A state in which un paired electrons is present ↓ or ↑.A doublet state occurs when there is an unpaired electron that gives two possible orientations when exposed to UV radiation and imparts different energy to the system.

Triplet state

A state in which unpaired electrons of same spin present↑↑A singlet or a triplet can form when one electron is excited to a higher energy level. In an excited singlet state, the electron is promoted in the same spin orientation as it was in the ground state (paired). In a triplet, excited stated, the electron that is promoted as the same spin orientation (parallel) to the other unpaired electron. Singlet, doublet, and triplet is derived using the equation for multiplicity, 2S+1, Where - S is the total spin angular momentum (sum of all the electron spins). Individual spins are denoted as spin up (s = +1/2) or spin down (s = -1/2).



Singlet excited state

A state in which electrons are unpaired but of opposite spin like↑↓ (un paired and opposite spin) When light of appropriate wavelength is absorbed by a molecule the electrons are promoted from singlet ground state to singlet excited state. once the molecule is in this excited state relaxation can occur via several process. For ex by emission of radiation . The process can be the following

1) Collisional deactivation
2) Fluorescence
3)Phosphorescence

Collisional de activation

In which entire energy lost due to collision de activation and no radiation emitted.

Fluorescence

excited singlet state is highly unstable. Relaxation of electrons from excited singlet to singlet ground state with emission of light.

Phosphorescence

At favorable condition like low temperature and absence of oxygen there is transition from excited singlet state to triplet state which is called as inner system crossing.

The emission of radiation when electrons undergo transition from triplet state to singlet ground state is called as phosphorescence.

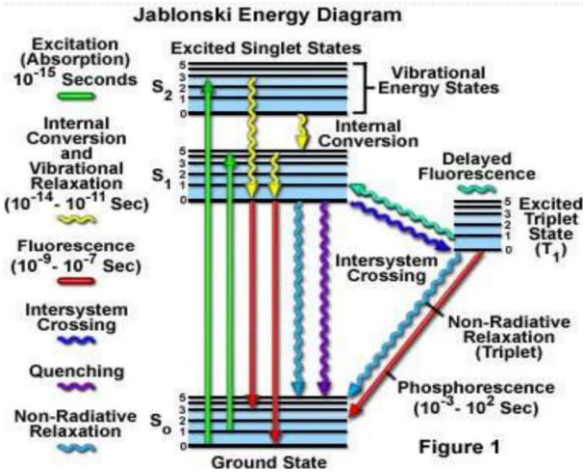


Figure 1

Factors Effecting Fluorescence Intensity

1. Concentration:

Fluorescence intensity is praportnal to concentration of substance only when the absorbance is less than 0.02

A=log Io\It or A= abc

Io=intensity of incident light

a= absorptivity of constant

b= Pathlength

c= concentration

2. Quantum Yield of Fluorescence

(∅)=number of photons emitted\number of photons absorbeds

It Is Always Less Than 1.0 Since Some Energy Is Lost By Radiation less Pathways (Collisional, Intersystem Crossing, Vibrational Relaxation.when light is absorbed ,only a fraction of it is emitted via fluorescence ;the rest of the excited molecules decay via other process.

3. Intensity of Incident Light:

increase In The Intensity Of Incident Light On The Sample Fluorescence Intensity Also Increases.The intensity of light depends on 1.light emitted from the lamp.2.excitation monochromators.3.excitation slit width.higher the intensity of light higher will be the fluorescence.if the intensity is too high too effects can happen:spectral over lap,photodecomposition:the molecule osf solution changes its composition due to photochemical effect thereby affecting our results.

4.Adsorption:

Adsorption Of Sample Solution In The Container May Leads To A Serious Problem. extreme sensitiveness of the method requires very dilute solutions. adsorption of fluroscent substances on the container wall create serious problems. hence strong solutions must be diluted.

5. Oxygen

Oxidation of fluorescent species to a non fluorescent species, quenches fluorescent substance. Prescence of dissolved oxygen often reduces the intensity of fluorescence in a solution. quenches as a result of the paramagnetic properties of moleculer oxygen,which promote intersyetem crossing and conversion of excited molecules to the triplet state. oxygen can also oxidises fluorescing species.

Presence of oxygen may interfere in 2 ways:by direct oxidation of fluorescent substances to non fluoroscents,by quenching of non fluorescence [para-magnetism].

6.PH

Alteration of ph of a solution will have significant effect on fluorescence. With increasing ph, the fluorescence intensity of the j- band increased in proportion to that observed with peak absorption.ph of solution is very important. Fluoroscence aromatic compound with acidic ring substituents is usually dependent. For ex Aniline in alkali medium gives visible fluorescence but in acidic condition gives fluorescence in visible region. Relatively small changes in pH will sometimes radically affect the intensity and spectral characteristics of fluorescence.

7. Temperature and Viscosity

Temperature Increases Can Increase the collisional de activation, and reduce fluorescent intensity. If viscosity of solution is more the frequency of collisions are reduced and increase in fluorescent intensity.increase in temperature leads to increase in collisions of molecules and decrease in fluorescence intensity whilr decrese in temperature leads to decrease in collisions of molecules and increased fluorescence intensity. Changes in temperature affect the viscosity of the medium and hence the number of collisions of the molecules of the fluorophore with solvent molecules. Fluorescence intensity is sensitive to such changes and the fluorescence of many certain fluorophores shows temperature dependence.

8. Photochemical Decomposition

Absorption of intense radiation leads to photochemical decomposition of a fluorescent substance to less fluorescent or non fluorescent substance. Excitation of fluorescencing or dilute solutions with intense light sources will cause photochemical decomposition of the analyte. This is minimized by: use of the longest feasible wave lengths for excitation that does not introduce light – scattering effects. Measure the fluorescence immediately after excitation to decrease the duration of excitation. protect unstable solutions from ambient light by storing them in dark bottles. Remove dissolved oxygen from the solution.decomposition ontroduces non liner response curves and loss of majority of the sample fluorescence.

9.Quenchers

Quenching is the reduction of fluorescence intensity by the presence of substance in the sample other than the fluorescent analyte. Quenching is following types:

Inner Fluorescent Effect

Absorption of Incident (uv) Light Or Emitted (fluorescent) Light By Primary And Secondary Filters Leads To Decrease In Fluorescence intensity.

Self Quenching

At Low Concentration Linearity Is Observed, At High Concentration Of The Same Substance Increase In Fluorescent Intensity Is Observed. This phenomena is called self quenching.

Collisonal Quenching

Collisions between the fluorescent substance and halide ions leads to reduction in fluorescence intensity.

Decrease of fluorescence intensity by interaction of the excited state of the fluorophore with its surroundings is known as quenching and is fortunately relatively rare. Quenching is not random. Each example is indicative of a specific chemical interaction, and the common instances are well known. Quinine fluorescence is quenched by the presence of halide ion despite the fact that the absorption spectrum and extinction coefficient of quinine is identical in 0.5M H_2SO_4 and 0.5M HCl.

Static Quenching

This occurs because of complex formation between the fluorescent molecule and other molecules. Ex: caffeine reduces fluorescence of riboflavin.

10.SCATTER

Scatter is mainly due to colloidal particles in solution. Scatter is mainly due to colloidal particles in solutions scattering of incident light after passing through the sample leads to decrease in fluorescence intensity.a higher fluorescence is observed when the solvents do not contain heavy atoms while phosphorescence increases due to presence of heavy atoms in the solvents. Scattering of incident light after passing through the sample leads to decrease in fluorescence intensity.

11. Conjugation

Conjugation Molecule must have unsaturation i.e. it must have π electrons so that UV/vis radiation can be absorbed. If there is no absorption of radiation, there will not be fluorescence.

12. Rigidity of Structure

Rigid structures will produce more fluorescence, while flexible structure will produce less fluorescence.

Instrumentation

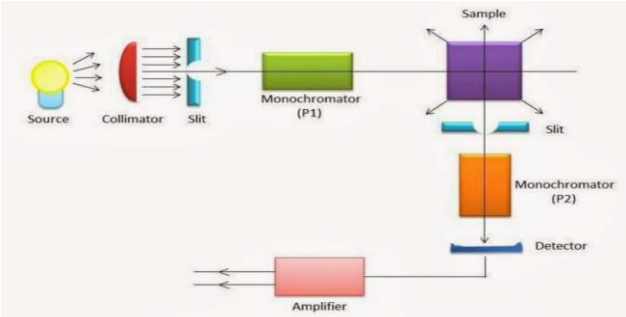
All fluorescence instruments use essentially the same components as are used in absorption spectrophotometers. However, the geometric arrangement of the components is somewhat different. This is due to the reason that any transmitted radiation is not measured along with the fluorescence. You know that the absorption and transmission of radiant energy occur

only along the direction of the incident light whereas the fluorescence radiation emanates in all directions.

The detection of transmitted radiation is avoided by placing the detector at a right angle to the transmitted beam. The essential components of an instrument used to measure fluorescence of the sample are:

- Excitation light sources
- Filters or monochromators
- Sample holder
- Detectors
- Read out device

In addition to the optical components, most fluorimeters and spectrofluorometers have dedicated computers. These are required to control the instrumental operating parameters like, excitation and emission wavelengths, scan rates, monochromator slit widths, detector parameters, etc. These help in the acquisition and post processing of the spectral data. Currently, two broad categories of commercial instruments are being manufactured. The low cost instruments needed for routine measurements are based on filters as wavelength selectors whereas the more sophisticated spectrofluorometers use monochromators. Let us learn about the essential components of a fluorimeter in the following.



Representative Diagram of a Spectrofluorimeter for 90° Illumination

1) Sources Of Light

Sources As mentioned earlier, the fluorescence power or intensity, IF, is directly proportional to the source power, P_0. An increase in P_0 will produce a larger signal for a given concentration and thereby improve sensitivity. Therefore, the source must be more intense than that required for UV-VIS absorption spectroscopy.

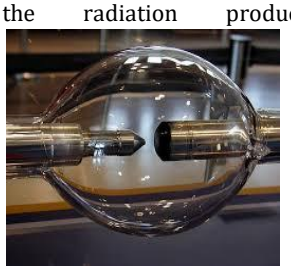
tungsten lamp and deuterium lamp The tungsten filament and deuterium lamps used in absorption spectrophotometers are generally not suitable for fluorescence instruments as they lack the desired intensity.If excitation has to be done in visible region this can be used.It is used in low cost instruments. As molecular absorption spectra usually are broad, a highly monochromatic source is generally not required; an intense continuum source that emits throughout the UV, visible, and near infrared regions is adequate.However, in fluorescence measurements a source with intense emission lines at certain frequencies are desirable.The xenon lamps are usually placed in specially designed housings because of the danger of explosion due to gas in xenon lamps under high pressure (~10 atm.)

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. The emission filter must be chosen so that the Rayleigh-Tyndall scattered light is obscured and the light emitted by the sample transmitted. To avoid high blanks it may also be necessary to filter out any Raman scatter.

mercury vapour lamp Filter fluorimeters often employ a low-pressure mercury vapour lamp. Mercury vapour at high pressure gives intense lines on continuous background above 350nm.Low ressure mercury vapour gives an additional line at 254nm.It is used in filter flourimeter. This source produces intense lines at certain wavelengths. One of these lines will usually be suitable for excitation of a fluorescent sample.

xenon arc lamp

Spectrofluorometers, on the other hand, need a continuous radiation source, are often equipped with a 75-450 W high-pressure **xenon arc lamp**. These produce an intense continuum between about 250 and 600 nm.It gives **more intense** radiation than **mercury vapour lamp.** It is used in spectro flourimetry. As the xenon arc lamp produces lot of heat, the lamp assembly needs to be cooled therefore, these instruments cannot be used for routine work. For certain applications, it is preferable to use a laser excitation source. A tuneable dye laser, using a pulsed nitrogen laser as the primary source can produce monochromatic radiation between 360 and 650 nm. Since the radiation produced is monochromatic,



Wave length selectors

The low-cost instruments designed for routine determinations are simple filter fluorimeters Such instruments are used when it is sufficient to measure fluorescence intensity at a single excitation and emission wavelength. These employ fixed filters to isolate both the excitation and emission wavelengths. In order to isolate one particular wavelength from a source what we need is just a pair of cut-off filters. Absorption filters are comprised of a suitably absorbing substance or substances dispersed in gelatin, glass or plastic. The filter fluorimeters are used primarily in environmental field screening, hospital or clinical settings and other applications in which low cost and small size are crucial.

2) Filters Andmonochromators

Filters

these are nothing but optical filters works on the principle of absorption of unwanted light and transmitting the required wavelength of light. In inexpensive instruments fluorimeter primary filter and secondary filter are present. Filter is a device used to get selected wavelength. It allows the light pass through it but absorbed the light of different wavelength may partially and fully. A specific filter is used to obtain the desired wavelength for special analysis like Primary filter and Secondary filter.

Primary filter

absorbs visible radiation ' and transmit UV radiation.

Secondary filter

absorbs UV radiation and transmit visible radiation.

Monochromators

They convert polychromatic light into monochromatic light. They can isolate a specific range of wavelength or a particular wavelength of radiation from the source. they convert polychromatic light into monochromatic light. They can isolate a specific range of wavelength or a particular wavelength of radiation from a source. monochromators can use either the phenomenon of optical dispersion in a prism or that of diffraction grating ,to spatially separate the colour of light. It usually has a mechanism of directing the selected colour to an exit slit.usually the grating or the prism is uded in a reflective mode. A monochromator provides a selection in moicroplate readers, spectrophotometers and other instruments. It enables the spectral isolation of a wave length from the beam of a light source.

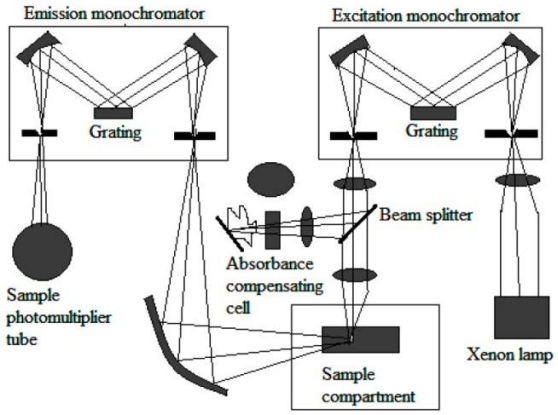


Figure 1.1 Spectrofluorometer

3) Sampling

The majority of fluorescence measurements of the analyte are carried out in solution. For this the sample is taken in a **cuvette** or in a **flow cell.** The cuvettes generally are circular, or square shaped. These are constructed of a material that will transmit both the incident as well as the emitted light. Glass and quartz both qualify this criterion. However, the quartz cuvettes are generally used The cuvette or cell is placed normal to the incident beam. Though resulting fluorescence is given off equally in all directions, it is collected from the right angles to the incident beam. As fluorimetry is a very sensitive technique, the following precautions should be followed without exception while handling the cuvettes.The samples should be transferred to the cuvettes with the help of a dropper or a pipette.The cuvettes should be rinsed with the analyte solution before filling and the overfilling should be avoided.

Cuvettes

One of the advantages of fluorescence procedures compared to equivalent absorption techniques is that routine measurements may usually be carried out in inexpensive test-tubes rather than precision cuvettes without appreciable loss in precision. This benefit is derived from the geometrical layout of simple fluorimeters where only a small central area of the cuvette is actually viewed by the detector, so that the overall dimensions of the cell are less important. However, this statement needs careful qualification, since the use of laboratory grade test-tubes will result in deviation arising from other sources.

4) Detectors

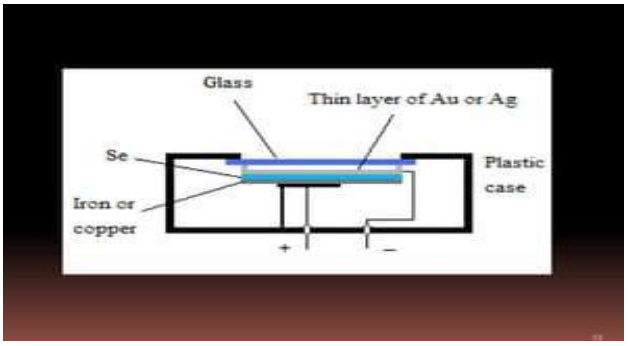
All commercial fluorescence instruments use photomultiplier tubes as detectors and a wide variety of types are available. The material from which the photocathode is made determines the spectral range of the photomultiplier and generally two tubes are required to cover the complete UV-visible range. 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. The limit of sensitivity of a photomultiplier is normally governed by the level of dark current (which is the signal derived from the tube with no light falling on it).The spectral response of all photomultipliers varies with wavelength, but it is sometimes necessary to determine the actual quantum intensity of the incident radiation and a detector insensitive to changes in wavelength is required. A suitable quantum counter can be made from a concentrated solution of Rhodamine 101 in ethylene glycol which has the property of emitting the same number of quanta of light as it absorbs, but over a very wide wavelength range. Some commonly used detectors are as follows

1.Barrier layer cell/Photovoltaic cell
2.Phototube/photo emission tube
3.photomultiplier tube

1. Barrier Layer Cell

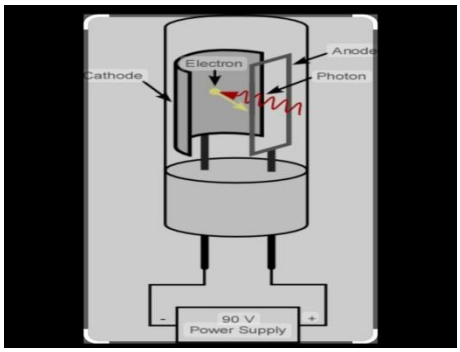
It is employed in inexpensive instruments. For ex: Filter Fluorimeter. ′ It consists of a copper plate coated with a thin layer of cuprous oxide (Cu2o). A semi transparent film of silver is laid on this plate to provide good contact. ′ When external light falls on the oxide layer, the electrons emitted from the oxide layer move into the copper plate. Then oxide layer becomes positive and copper plate becomes negative. ′ Hence an emf develops between the oxide layer and copper plate and behaves like a voltaic cell. So it is called photovoltaic cell. A galvanometer is connected externally between silver film and copper plate and the deflection in the galvanometer shows the current flow through it. The amount of current is found to be proportional to the intensity of incident lightIt is consists of an evacuated glass tube with a photocathode and a collector anode.

The surface of photocathode is coated with a layer of elements like cesium, silver oxide and its mixtures When radiant energy falls on photosensitive cathode, electrons are emitted which are attracted to anode causing flow of current.



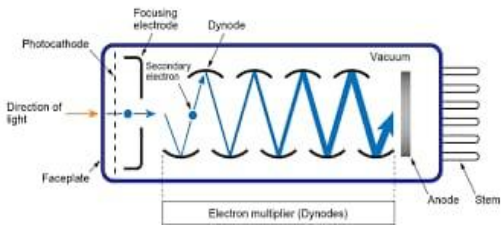
2. Photo Tube/Photo Emission Tube

It is more sensitive than barrier layer cell. These are incorporated in expensive instruments like spectrofluorimeter. Its sensitivity is high due to measuring weak intensity of light. The principle employed in this detector is that, multiplication of photoelectrons by secondary emission of electrons. This is achieved by using a photo cathode and a series of anodes (Dyanodes). Up to 10 dyanodes are used. Each dyanode is maintained at 75100Vhigher than the preceding one. At each stage, the electron emission is multiplied by a factor of 4 to 5 due to secondary emission of electrons and hence an overall factor of 106 is achieved. PMT can detect very weak signals, even 200 times weaker than that could be done using photovoltaic cell. Hence it is useful in fluorescence measurements. ′ PMT should be shielded from stray light in order to have accurate results.



3.Photomultiplier Tube

It is multiply the photoelectrons by secondary emission of electrons.A primary photo-cathode is fixed in a vacuum tube which receives radiation from the sample. Some 08 to 10 dynodes are fixed eachwith increasing potential of 75-100V higher than preceding one.Near the last dynode an electron collector electrode is fixed.It is extremely sensitive to light and detect weaker or low radiation,



CCD Detectors

Charge-coupled devices (CCDs) are very good in sensitivity and have linear dynamic range therefore are very widely used in fluorescence spectroscopy. CCDs typically contain 106 or more pixels where each pixel acts as an accumulating detector where charge accumulates in proportion to total light exposure.

A two-dimensional image can be obtained by reading out the charge at each pixel when desired. Small spectrofluorometers using CCDs are commercially available, have good sensitivity and by using a fiber-optic cable the signal is brought to the device.

They are conveniently interfaced via a USB cable and can have no moving parts, when combined with an LED light source it can be turned to a solid-state device.

5) Read Out Devices

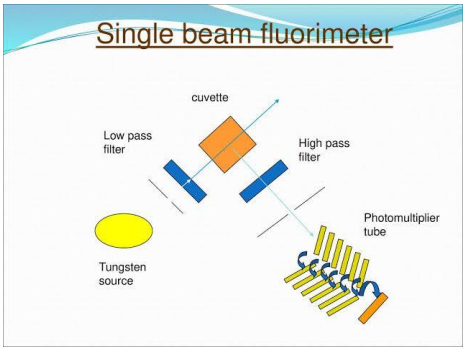
The output from the detector is suitably amplified and displayed on a read out device like a meter or digital display. The sensitivity of the amplifier can be changed so as to be able to analyse samples of varying concentrations. In some instruments the display can be adjusted to directly give the output in terms of the concentration. Nowadays the instruments have microprocessor controlled electronics that provides outputs compatible with the printers and computers whereby minimising the possibility of operator error in transferring data.

The most common types instruments are:-

Single beam fluorimeter
Double beam fluorimeter
spectro fluorimeter

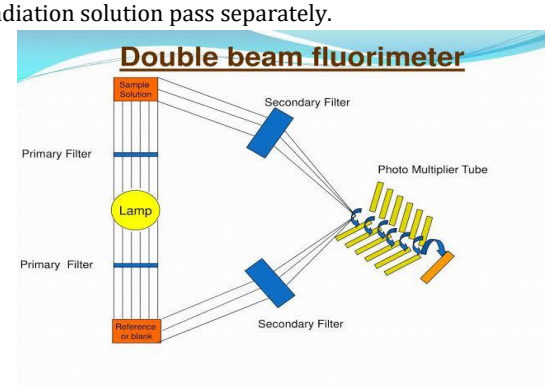
Single Beam Filter Fluorimeter

It contains tungsten lamp as a source of light and has an optical system consists of primary filter. The emitted radiations are measured at 900 by using a secondary filter and detector. Primary filter absorbs visible radiation and transmit uv radiation which excites the molecule present in sample cell. Instead of 90 if we use 180 geometry as in colorimetry secondary filter has to be highly efficient otherwise both the unabsorbed uv radiation and fluorescent radiation will produce detector response and give false result. A sinle beam spectrophotometer is a device that measures a substances light absorption one light beam to determine its concentration, purity and chemical characteristics. single beam covers the uv wavelength to visible wavelength range and offer highly reliable measurements. in a single beam all the light rays pass through the biological sample.



Double Beam Fluorimeter

It is similar to single beam except that the two incident beams from a single light source pass through primary filters separately and fall on the another reference solution. Then the emitted radiations from the sample or reference sample pass separately through secondary filter and produce responsembinly on a detector. a double beam fluorescence spectrophotometetr has been designed for the analytical control laboratory and for some research applications. it is similar to single beam, except the two incidientbeam from single light source pass through primary filter separately used full in circle. Sample or reference solution. The critical radiation from sample or radiation solution pass separately.



In spectrofluorimeter

In this primary filter in double beam fluorimeter is replaced by excitation monochromator and the secondary filter is replaced by monochromator. Incident beam is split into sample and reference beam by using beam spliter.

Applications

- Determination of aluminum in alloys
- Determination of ruthenium
- Determination of chromium and manganese in steel
- Determination of uranium salts
- Estimation of rare earth terbium
- Estimation of bismuth
- Determination of beryllium in silicates
- Determination of cadmium
- Estimation of quinine sulphate
- Estimation of 3,4 benzpyrene

Advantages and Disadvantages:

- Wide ranges of compounds are used.
- Simple in construction.

- Easy to use.
- Economical.
- Sample and reference solution can be analysed simultaneously.
- Very specific.
- Very sensitive.
- Wide concentration range.
- Simplicity and speed.
- Low cost.
- The tests based on fluorimetry are higly sensitive.
- Qualitative and quatitative analysis of organic aromatic compounds present in cigarette smoke, air pollutanta,automobile exhausts etc.
- Nuclear research.
- Fluotimetric techniques have high degree of specificity.

Disadvantages

- ✓ Change in ph effect fluroscence.
- ✓ Oxygen may decrease fluorescence.
- ✓ Heavy metal presence are also decrease flurosecence.
- ✓ When uv radiatin is passed out some time it is reacted with sample.
- ✓ It is not possible to use reference solution and sample solution is at a time .
- ✓ Rapid sacanning to obtain excitation and emission spectrum of the compound is not possible.
- ✓ Rapid scanning is not possible due to use of filters.
- ✓ The susceptibility to environmental conditions and the vitual impossibility of predicting whether a compound will fluroscence.
- ✓ The other major problem is quenching, where by the energy,transferred to the othger molecules.
- ✓ Contamination can quench the fluroscence and hence gives false/no result.

Limitations

- Careful buffering is necessary as fluorescence intensity may be strongly dependent.
- Ultraviolet light used for excitation may cause photochemical changes or destruction of the fluorescent molecule.
- The presence of dissolved oxygen may cause increased photochemical destruction.
- Traces of iodide and nitrogen oxides are efficient quenchers and therefore interfere.
- The method is not suited for determination of major constituents of a sample, because the accuracy is very less for large amounts.
- The extent of applicability of this technique is limited, because of the fact that all elements and compounds are unable to exhibit fluorescence.

Conclusion

Fluorescence is the most sensitive analytical techniques. Detection studies will increase the development of fluorescence field. Fluorimetric methods are not useful in qualitative analysis ,and much used in quantitative analysis. Fluorescence spectroscopy is a sensitive optical emission technique in which sample molecules are excited with a photon source. Those molecules that relax by radiant emission can be subsequently detected by measuring the intensity of that emission. Fluorimetry is generally used if there is no colourimetric method sufficiently sensitive or selective for the substance to be determined. The important applications for determination of organic and inorganic compounds including immunoassays and chemistry of bioluminescence are reviewed. Special fluorimetric applications are also included in this study. At first glance it seems easy to perform fluorescence experiments. However, there are numerous factors that can compromise the data and invalidate the results. One needs to be constantly aware of the possibility of sample contamination, or contamination of the signal from scattered or stray light. Collection of emission spectra, and examination of blank samples, is essential for all experiment.

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