Advantages, Limitations, Precautions and Applications of Fluorescence Spectroscopy

Advantages of Fluorescence:

 It’s one of the newer methods and its potentialities are still largely unexplored.

 It also affects precision. Up to 1% can be achieved easily in Flourimetric.

 The method is very sensitive and also possesses specificity because there is a choice of wavelength not only for the radiation emitted, but also for the light which excites it.

Limitations:

 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.

Precautions:

 Fluorescence analysis is especially applicable to trace substances, care must be taken to eliminate contaminations of sample.

 Rubber and cork stoppers contain fluorescent materials and these are extracted if the solvent touches them.

 Filter paper also contains fluorescent material which is extracted by solvents.

 Grease from stop cocks and other sources is a fluorescent contaminant.

 All glasses contain Al, Ca and SiO_2 which may be extracted.

 The most important consideration is the concentration of the reagent. Concentration must be expressed in micro molecules so that the ratio of the reagent to metal may be estimated easily.

 Large temperature change between unknown and standard should be avoided.

 It is also not desirable to expose the solution to ultra violet radiation for longer periods.

Applications:

1 Applications in inorganic chemistry

A) Determination of ruthenium.

B) Determination of boron in steel.It is determined by means of complex formed with benzoin. The boron present in the acid solution of the sample is first converted in to boric acid, which is then separated from the other constituents by co-distillation with methyl alcohol. The resulting distillate containing boric acid is neutralised by NaOH and methyl alcohol is evaporated off.

c)Determination of aluminium in alloys.

D)Determination of chromium and manganese in steel.

E) Determination of uranium salts

F) Estimation of rare earth elements.

G) Estimation of bismuth

H) Estimation of 3,4 benzo pyrene. This compound is carcinogenic found in tobacco plants can be evaluated by fluorescence.

I) Determination of zinc

J) Determination of cadmium

2) Application in Organic Chemistry:

A)Assay of thiamine: Thiamine is a vitamin found in food. It amount can be detected by fluorescence.

B)Fluorescence is also used for analysis of tablets.

3) Investigation of chemical structures and processes

Fluorometric methods have successfully been applied in the investigation of hydrogen bonding, cis trans isomerism polymerisation, tautomerism and rates of reactions etc.

 The free radicles can best be detected with a spectrograph so that the whole spectrum of a short lived component may be photographed at the same time.

 Steric hindrance in diphenyl can be studied

4) Chemical analysis

This type analysis may be quantitative as well as qualitative

 Detection of impurity – solvents used for spectrofluorometry must be free of absorbing impurities. Thus , cyclohexane must be purified until it shows no trace of benzene bands at 2600 A0.

 If absorbing impurities are present in the vitamin, they can be removed either by chemical method or utilizing the change of shape of absorbing band in the presence of an impurity.

 Estimation of fluorescent intensity – intensity of pure fluorescing component in sufficiently low concentration is proportional to concentration, but this condition is not always easy to get. It is therefore desirable to measure the intensity from a specimen.

This method however, suffers some **difficulties**. They are;

 If some of the exciting radiation is absorbed by the impurities, the amount of radiation left to actually

irradiate the specimen is reduced by unknown amount. This is called quenching.

 Impurities present may also deactivate the excited molecules by collision before they have time to radiate the fluorescence. This is called quenching.

 If a major part of the exciting radiation is absorbed by a specimen, further increase in concentration will cause very little increase in fluorescent intensity.

5) Laser induced fluorescence spectroscopy of human tissues for cancer diagnosis

Cancer is one of the most dreaded disease. Early tumours often arise from tissue which have a rapid turnover of cells and are active in repair like transformed mucosa on the surface of hollow organs (oral cavity, gastrointestinal tract, female reproductive organs etc.). Laser spectroscopic techniques use the non-ionizing radiation ensures that the diagnosis can be made repeatedly without any adverse side effects. Laser Induced Fluorescence (LIF) has been used for diagnosing cancer by following method

One approach involves Systemic administration of a drug like hematoporphyrin derivative which is selectively retained by the tumour. When photo excited with light of appropriate wavelength the drug localized in the tumour fluoresces. This fluorescence is used for detection and imaging of the tumour.

6) Study of marine petroleum pollutants

Fluorescence spectroscopy is one of the good techniques to detection of oil slicks on the water surface, determination of petroleum contaminants in seawater and determination of particular petroleum derivative compounds as well as identification of pollution sources. Main components of any oil are hydrocarbons. The other components are primarily derivatives of hydrocarbons containing single atoms of sulfur, oxygen or nitrogen. Only few of hydrocarbons fluoresce, while the major of them show no ability to luminescence. The content of compounds able to fluorescence1 rarely exceeds 10% of the oil mass. At the same time the petroleum strongly absorbs radiation, especially the ultraviolet and blue light.

In spite of this petroleum is a luminescent medium and fluorescence is a phenomenon which allows testing oils. Fluorescence of oils has wavelength over then 260 nm and covers a spectral area of ultraviolet and visible light. The phenomenon is most significant in the 270–400 nm range.

7) Accurate determination of glucose

Glucose is considered as a major component of animal and plant carbohydrates in biological systems. Furthermore, blood glucose levels are also an indicator of human health conditions. The abnormal amount of glucose provides significant information of many diseases such as diabetes or hypoglycaemia. Fluoro photometry was used widely owing to its operational simplicity and high sensitivity.

Fluorescence spectroscopy is a rapid, sensitive method for characterizing molecular environments and events. Fluorescence output is linear to sample concentration over a very broad range.