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Fluorescence Spectroscopy as a Basic Tool of Analytical Chemists: A Review

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I. Introduction

What an irony fate of the present civilisation, scientifically and technologically so advanced! On the one hand, industrialisation is indispensible for any healthy, wealthy and civilised nation; on the other hand, it is largely responsible for chemical hazards of environmental pollution now hanging over us like Damocles' sword threatening the very existence of our entire eco-system. At this critical juncture, analytical chemists of high manipulative skills have great responsibilities and in fact, they can accept the challenge of environmental pollution problem. Significant roles of inorganic chemicals particularly heavy metals at extremely low concentrations are being recognised gradually which are proved to be micronutrient and/or highly toxic to humans. Ultra-trace analysis techniques are not only required for controlling the environmental pollution but also for controlling the quality of industrial products. Some industries (e.g. semiconductor) demand highest purity of the material which must not contain foreign impurities above parts per trillion levels.

Among various modern trace analysis techniques employed in solutions, Molecular Photo-fluorescence Spectrometry or Fluorimetry or Fluorescence Spectroscopy has been rated to be one of the most powerful and successful tools recognised to-day for this purpose at the hands of the analytical chemists. In some cases, it is the only technique suitable. Fluorimetry is extremely sensitive, so much so that sometimes pictogram (10^{-12}g) level or less can be determined. It encompasses practically all the fields of chemical science and is so broad that it can be rated as a versatile technique. The present status of fluorimetry is too great to be evaluated. Only a brief account of the salient features will be discussed here in a much simplified way readable by a layman.

II. Historical Account

The phenomenon of luminescence was first recognised by Spanish botanist and physician, Nicolas Monardes in 1565. He observed a blue shimmer in the water contained in a cup made from a specific wood, Ligirium Nephiticiem. In the next century, the celebrated chemist Robert Boyle also encountered the same phenomenon. In 1833, Sir David Brewster found a red emission from a solution of green leaves. Although he explained the phenomenon differently in his own way, to-day we know that the red emission is fluorescence from chlorophyll. The first fluorescence emission spectrum was recorded by Herschel in 1845 from quinine. But it was George Stokes in 1852 who proposed the use of fluorescence as an analytical tool. The term ***fluorescence*** was also coined by him. The first fluorescence based chemical analysis was performed by Goppelsrode in 1867. He developed a method for trace determination of non-luminescent aluminium by forming a strong fluorescent Morin-aluminium complex. Further in 1877, Adolf Baeyer demonstrated the link between the two rivers Rhine and Danube by means of fluorescence. He threw 10 kg of a strongly fluorescence substance called fluorescein into the river Rhine and three days later the green fluorescence of fluorescein was detected in the other river Danube indicating the link between the two rivers.[1]

III. Theoretical Aspects

To the common man **fluorescence** is the beautiful bright light having a specific colour emitted when a substance is brought in the light [2]. There is another very closely related phenomenon called **Phosphorescence.** They differ in their time of glow. Fluorescence is very short-lived with time of glow 10^{-9} to 10^{-7} s and the glow stops immediately as the light source is removed. But Phosphorescence has longer time span of 10^{-3} to 10 s and the glow continues even after the removal of the light source. Both fluorescence and phosphorescence are called luminescence.

An electromagnetic spectrum is a continuum of electromagnetic radiations from cosmic ray with wavelength range of 10^{-14} m to radio wave having wavelength range 10^{2} m [3]. Our human eye can detect only a very small part of this spectrum. This part is called visible region extending from wavelength 400 nm (red) to 800 nm (violet). Ordinary light is a part of this visible region. These radiations such as ordinary light are given the name electromagnetic radiation because of their association with electric and magnetic forces when they propagate as wave. All the electromagnetic radiations are described by various parameters such as wavelength, frequency, wave number, and energy. But they have different penetrating powers and thus they require different

handling techniques. Thus when they interact with matter the consequent results are different and hence the information deduced are also different. Accordingly, employing different electromagnetic radiations with suitable handling techniques, various analytical spectroscopic techniques have been developed. These are, to mention a few, IR spectroscopy, UV-Visible spectroscopy, Nuclear Magnetic Resonance (NMR) spectroscopy, Electron Spin Resonance (ESR) spectroscopy, Raman spectroscopy, Mossbauer spectroscopy etc. Dr. C.V. Raman, an Indian born scientist, won Nobel Prize in Physics in 1930 for his discovery of Raman Scattering employing IR radiation which became the foundation of Raman Spectroscopy.

3.1. Mechanism of fluorescence [1]: The electrons in a molecule have definite amount of energy at a particular temperature. This is called electronic state of the molecule. Within this electronic state there are vibrational energy states and within each vibrational state there are still rotational energy states. Under standard temperature, more than 99% of the molecular species around us are in their lowest electronic state or ground state. The following figure depicts mechanism of fluorescence and related phenomena.

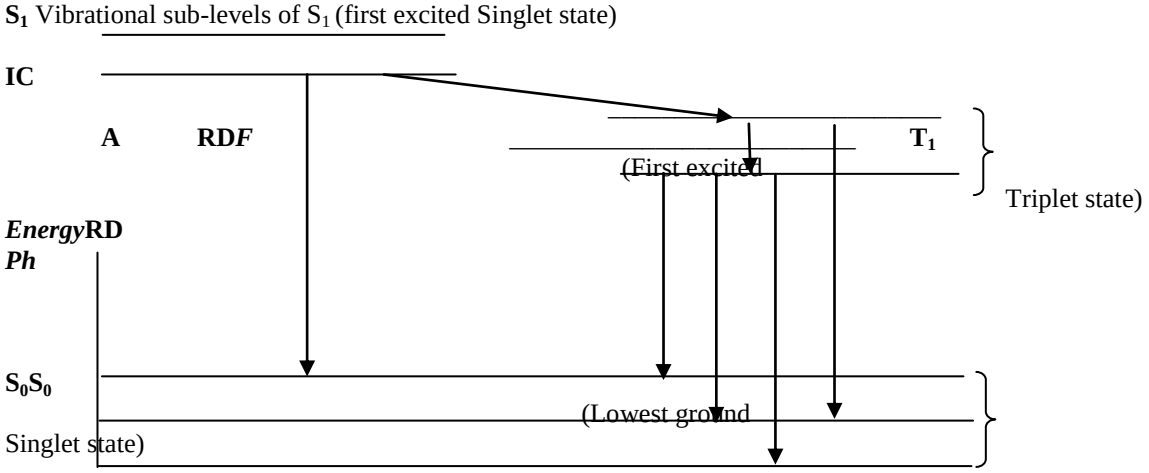

Fig. Mechanisms of absorption, fluorescence and phosphorescence, **A**-Absorption, **RD**-Radiation less Deactivation,
F-Fluorescence, ***Ph.***-Phosphorescence, IC-Intersystem crossing.

Most of the molecules in their ground states are also in the **Singlet state**. Singlet state refers to a molecular state having spin-paired electrons. When a molecule in the ground state, S_0 is irradiated with UV-Visible light, the electrons in the molecule are promoted to a higher energy level, S_1 due to absorption of energy from the electromagnetic radiation. The amount of energy absorbed is equal to the difference between the two energy states.

Thus, $\Delta E = S_1 - S_0$, Where ΔE = Amount of energy absorbed, S_0 = Energy of the ground electronic state,
S_1 = Energy of the first excited state

In the S_1 electronic level, the excited molecule may lose the excess energy in different routes. A very common way is the loss of energy by intermolecular collisions[4]. In this process, the excess energy is utilised as kinetic energy and converted into heat. After losing the energy as heat, the excited molecule returns to its ground state. Such relaxation is called Radiation less de-activation. However in a very rare case, certain molecules adopt another route for losing the excess energy. Here, the excited molecule in S_1 rapidly comes down to its lowest vibrational sub level by a radiation less process and from here the molecule loses the excess energy in the form of a radiation of visible light and by doing so the molecule returns to its ground state. This form of energy transition from excited singlet state to ground singlet state is called Fluorescence. The time between absorptionof energy and emission of light to return to the ground state is 10^{-9} s to 10^{-7} s. Further in more rarer cases, before radiation of light, the excited molecule in the lowest vibrational sub-level of S_1may undergo a process called Intersystem crossing to transform to Triplet State. A triplet state refers to a molecular state having electrons of unpaired spins. Thus intersystem crossing involves only flipping of one of the electronic spins so that the electronic spins become unpaired. The excess energy from the excited molecule in this triplet state T_1may then be lost in the form of radiation to return to the ground state. This mode of energy transition from excited triplet state to ground singlet state is what we call Phosphorescence. The time between the initial absorption of energy and return to the ground state in the case of phosphorescence is 10^{-3} s to 10 s.

3.2. **Sensitivity of fluorescence:** The first and foremost criterion of any ultra-trace analysis technique is its ultra-sensitivity. The limitless sensitivity of fluorimetry owes very much to its mathematical working equation as under [5]:

$$F = KLIck \quad \textit{or} \quad F \alpha c$$

Where,

F is Fluorescence intensity.
K is Instrumental constant.
L is the product of log conversion factor (2.303) and fluorescence quantum yield.
I is intensity of incident radiant power.
c is concentration of fluorescent solute.
k is chemical and experimental constant of the solution.

From the above equation, it is evident that any decrease in 'c' (low concentration) can well be compensated by increasing 'I' (intensity of incident light) or amplification of photo detector system accounted in instrumental constant 'K' or by both. Also direct proportionality of fluorescence intensity (F) to the intensity of incident radiation (I) confers virtually limitless sensitivity of fluorimetry.

IV. Applications

Fluorescence spectroscopy is an extremely powerful tool in the hands of analytical chemists. All molecules absorb electromagnetic radiation but very few of them fluoresce. This makes fluorescence spectroscopy an attractive technique in resolution of fluorescent components in complex mixtures. The application of fluorescence spectroscopy has now covered almost all areas of science ranging from biology to physics. It has been successfully applied in the determination of DNA, RNA, proteins, enzymes and other bio-molecules [1]. Toxic metals have been determined in sub-Nano gram levels. In this process toxic metals such as mercury, arsenic, chromium, nickel, cobalt, vanadium etc. are first converted into fluorescent agents by complexing with suitable reagents [6]. Since fluorescence intensity is directly proportional to concentration, the amount of the metal in the complex can be determined. Fluorescence quenching is also another fast developing technique in fluorescence based chemical analyses. In this case, the native fluorescence of a reagent is quenched by the addition of a metallic salt solution. From the amount of fluorescence quenching, the amount of the metal is determined [7]. The vast applications of fluorescence spectroscopy may be summarised as[5].

C	-	Clinical	/**C**ytochemistry
L	-	Life science	/**L**ipid studies
E	-	Environmental	/**E**nzyme analysis
O	-	Oceanography	/**O**ccupational chemical hazards
P	-	Pharmacology	/**P**ublic health
A	-	Abused drugs	/**A**gro-chemical analysis
T	-	Toxicology	/**T**race-element analysis
R	-	Radio-dosimetry	/**R**are earth analysis
A	-	Antigent, antibody	/**A**mino acid assay

V. Future

Fluorescence spectroscopy has a quite promising future. With the fast development of instrumentation, computerisation and better understanding of bio-analytical processes, it will become a must-have technique in the scientific laboratories. With its simplicity in handling, rapidity in analysis and sensitivity in application it will find an important role in all areas ranging from biology to physics.

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