The use of Fluorophores in Modern Chemistry

Dr. Anubha Sharma
Associate Professor Chemistry
Govt. Autonomous Girls P.G. College of Excellence, Sagar (M.P.)

Abstract -

Fluorophores are the chemical compounds that re-emit light after their excitation with light.They are used in fluorescent labelling,cathode ray tubes,cosmic ray detection and as microbial growth media.Chemically,they may be proteins, small organic compounds, or synthetic polymers.Fluorescent molecules can also be used as tags like in Fluorescence In Situ Hybridization (FISH).

Key words - Fluorescent, cathode ray, microbial, polymer, FISH.

Introduction -

Fluorescence is that property by virtue of which a solid,liquid or gaseous chemical system shows luminescence.It is the emission of visible or invisible radiation from certain substances as a result of incident radiation of a shorter wavelength.This incident radiation may be any radiation of light either from the visible spectrum,ultraviolet or infra-red spectrum.Thus, a fluorophore or fluorochrome is a compound that shows fluorescence. Fluorescent labeling with a synthetic fluorophore was first reported in 1942, when fluorescein isothiocyanate (FITC)-labeled anti-pneumococcal antibodies were obtained1 . Recently, a number of methods have been developed for inserting fluorescent tags into living objects2, for example, genetically encoded chimeras of target cellular proteins (TCPs) with GFP-like fluorescent proteins (FPs)3,4,5. The main advantage of these fluorophores is their small size and the availability of compounds with the desired chemical and photophysical properties.

Mechanism of fluorescence -

Fluorophores usually have multiple energy states including those corresponding

to different electron orbits and vibration/rotation states. The energy states of electron orbits are separated by a large gap compared to the vibration and rotation energy states. Before an excitation, the fluorophore is in the lowest energy, meaning that its electron state is the ground state (S0) and vibration and rotation energy is minimum. A fluorescent molecule can be excited to the excited state (S1) by absorption of a photon. The energy of the absorbed photon (h?) is converted to a change in electron orbit and increases in vibration and rotation of the molecule. The distance between the excitation and emission wavelengths iscalled the Stokes Shift.Stokes Shift helps in the detection of the emitted fluorescence in biological applications. Stokes shift is also a distinct characteristic of each fluorophore.

Types of Fluorophores -

Fluorophores can be broadly divided into two main classes-intrinsic and extrinsic. Intrinsic fluorophores are those that occur naturally. These include the aromatic amino acids, NADH, flavins, derivatives of pyridoxyl, and chlorophyll. Extrinsic fluorophores are added to the sample to provide fluorescence when none exists, or to change the spectral properties of the sample. Extrinsic fluorophores include dansyl, fluorescein, rho-damine, and numerous other substances.

Fluorophores can also be classified into following categories :

- Biological fluorophores;
- Organic dyes;
- Quantum dots

Phycobiliproteins (allophycocyanin), phycocyanin, phycoerythrin, and phycoerythrocyanin) and many other proteins have been designed for use in biological expression systems. Synthetic organic dyes, such as fluorescein, have small sizes. Hence,they can be crosslinked to macromolecules like antibodies, biotin or Avidin, without interfering with proper biological function.

Quantum dots are nanocrystal type of semiconductors that, when excited, emit fluorescence at a wavelength based on the size of the particle; smaller quantum dots emit higher energy than large quantum dots, and therefore the emitted light shifts from blue to red as the size of the nanocrystal increases.

Use of fluorophores -

The molecular fluorophores are used for cellular labeling.Hoechst 33342 is a dye that is used to stain nucleic acids and visualize cell nuclei in the research laboratory. However, more complex challenges, such as those faced in medical diagnostics, require more sophisticated labels.

The Aequorea victoria green fluorescent protein (GFP) undergoes a remarkable posttranslational modification to create a chromophore out of its amino acids (S65, Y66, and G67)6,7,8.The discovery of derivatives of avGFP has advanced our understanding of basic biology.The development of engineered variants of fluorescent proteins has facilitated routine monitoring of gene activation as well as the selective labeling and analysis of single proteins, cellular organelles, and whole cells3,4.Newer fluorophore proteins include blue FP Sirius9 and yellow LSS FP mAmetrine10.

In future,further development of more spectrally distinguishable fluorophores will help in bigger advances in the field of chemistry and spectroscopy.

References -

1. Coons A.H., Creech H.J., Jones R.N., Berliner E., J. Immunol. 1942;45:159-170.
2. Sung M.H., McNally J.G.. Wiley Interdiscip. Rev. Syst. Biol. Med. 2011;3(2):167-182. 3. Chudakov D.M., Matz M.V., Lukyanov S., LukyanovK.A.. Physiol. Rev. 2010;90(3):1103-1163.
4. Day R.N., Davidson M.W.. Chem. Soc. Rev. 2009;38(10):2887-2921.
5. Pakhomov A.A., MartynovV.I.. Chem. Biol. 2008;15(8):755-764.
6. Ormö, M., Cubitt, A. B., Kallio, K., Gross, L. A., Tsien, R. Y. & Remington, S. J. (1996) Science 273, 1392-1395.pmid:8703075.
7. Cubitt, A. B., Heim, R., Adams, S. R., Boyd, A. E., Gross, L. A. &Tsien, R. Y. (1995) Trends Biochem. Sci. 20, 448-455.pmid:8578587.
8. Heim, R., Prasher, D. C. &Tsien, R. Y. (1994) Proc. Natl. Acad. Sci. USA 91, 12501-12504.pmid:7809066.
9. Tomosugi W, Matsuda T, Tani T, Nemoto T, Kotera I, Saito K, Horikawa K, Nagai T. An ultramarine fluorescent protein with increased photostability and pH insensitivity.Nat Methods. 2009;6:351-353.
10. Ai HW, Hazelwood KL, Davidson MW, Campbell RE. Fluorescent protein FRET pairs for ratiometric imaging of dual biosensors. Nat Methods. 2008;5:401-403.