Fluorescence

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Analytical techniques, very much like women's clothing styles, tend to go in and out of vogue. This, however, is where the similarity ends. Whereas women's styles are the result of the whims of the designers, analytical methods remain popular only as long as they can perform a particular measurement better, easier, and/or cheaper than a competitive approach. The ability of a technology to survive is thus dependent on state-of-the-art instrumentation and analytical procedures but is restrained by the inherent limitations of the method.

Fluorescence (and closely related phosphorescence and chemiluminescence) spectroscopy is presently enjoying an increase in popularity. This is due to the versatility and sensitivity of the method and to the fact that lack of specificity, a persistent complaint, has been greatly overcome. Improved instrumentation and refined data processing are two key reasons for the current success of fluorescence. Spectroscopists, optical engineers, and computation specialists have all worked together to strengthen the technology of fluorescence spectroscopy. They have added lasers (dye, mode-locked, pulsed, etc.), fast detectors, advanced optical systems, and computers to the spectrometers to increase the utility of the fluorescence approach. Most recently, fiber optic technology has been integrated into these systems to permit remote *in situ* fluorescence analyses.

Fluorescence is an emission phenomenon. When a quantum of light impinges on a molecule, there is the probability, dependent on the wavelength, that the light will be absorbed. The process is nearly instantaneous and results in the visible and UV absorption spectra that are characteristic of many molecules. Once in the excited state, the molecule typically loses a small fraction of the excitation energy and then returns to the ground state by the emission of radiation. Consequently, the emitted light will generally be of longer wavelength than that of the absorbed light. Depending on the nature of the excited state, the process can be short-lived (fluorescence) or long-lived (phosphorescence—times usually greater than 10^{-4} s).

Fluorescence is usually induced by visible and UV radiation. Monochromatic laser sources are normally used to excite the molecule because they provide convenient sources of high intensity radiation and because their monochromaticity ensures that all of the available energy is fully utilized. This gives maximum sensitivity.

Fluorescence is exhibited by both free atoms and molecules. It can occur in the gaseous, liquid, and solid states although not necessarily in all three phases of the same substance. The fluorescence effect at the atomic level is well understood. Fluorescence in molecules is a more complex phenomenon because the electronic excitation and deexcitation processes may be accompanied by secondary changes in the vibrational and rotational energy of the molecule.

Materials that fluoresce naturally, those that can be converted to fluorescent compounds (fluorophors), and those that extinguish fluorescence can be determined quantitatively. Compounds in a mixture can generally be distinguished from one another because (a) compounds that absorb energy at the same frequency typically emit at different frequencies, (b) compounds that emit at the same frequency often absorb at different frequencies, and (c) transitions of different lifetimes can be readily distinguished by modern light-measuring techniques.

The versatility of fluorescence spectroscopy is, therefore, related to the several types of measurements that can be made. It is much more than just a peak intensity versus peak location type of system. Fluorescence yield, decay, polarization, and spectral distribution are only a few of the techniques available. Energy transfer, quenching mechanisms, spectral shifts, and time resolution also yield important information. These are further supplemented by low temperature techniques, stopped-flow fluorescence, synchronous excitation spectroscopy, matrix-isolation spectroscopy, etc.

The applications of fluorescence spectroscopy are numerous. The medical field is one of the prime movers of this technology. It is impossible to cite a representative selection of uses for this technique in a brief editorial, but listing a few seems essential: (a) general analysis: qualitative and quantitative measurements, chemical diagnostics, and detection of GC and LC effluents; (b) molecular information: measurement of intramolecular distances, determination of binding equilibrium, and study of structural transformations; (c) environmental: indentification and quantification of water and air pollutants; and (d) medical: study of membrane structure and function; determination of conformation of antibodies; study of heterogeneity of biomolecules, evaluation of drug interactions, determination of enzyme activity, reactions, etc.; fluoroimmunoassay; and *in situ* monitoring of body chemistry using fiber optics.