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Current Achievement and Future Potential of Fluorescence Spectroscopy

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1. Introduction

Spectrofluorometric methods of analysis are the most commonly analytical techniques and continue to enjoy wide popularity. The wide availability of the instrumentation, the simplicity of procedure, sensitivity, selectivity, precision, accuracy, and speed of the technique still make the spectrofluorometric methods attractive. These features make fluorescence spectroscopy an attractive technique as compared to other forms of optical spectroscopy or other analytical techniques such as chromatography and electrophoresis. Fluorescence spectroscopy has been used widely as a tool for quantitative analysis, characterization, and quality control in the pharmaceutical, environmental, agricultural, nanotechnology and biomedical fields.

The emission of light from an excited electronic state of a molecular species is called luminescence. The discovery and characterization of luminescence begun from the 15th century. In 1506 Nicolas Monardes was the first to describe the bluish opalescence of the water infusion from the wood of a small Mexican tree. In 1612 Galileo described the emission of light (phosphorescence) from the famous Bolognian stone, which discovered by Vincenzo Casciarolo, a Bolognian shoemaker. Galileo wrote: "It must be explained how it happens that light is conceived into the stone, and is given back after some time, as is childbirth". Even though, some of the first scientific reports of luminescence appeared in the middle of the 18th century. In 1845 Sir J.F.W. Herschel reports on an experiment he did twenty years earlier. Herschel made the first observation of fluorescence from quinine sulfate (quinine: (*R*)-(6-methoxyquinolin-4-yl)-((2*S*, 4S, 8R)- 8-vinylquinuclidin-2-yl)methanol, $C_{20}H_{24}N_2O_2$, quinine absorbs in the UV region), he observed that an otherwise colorless solution of quinine in water emitted a blue color under certain circumstances. Herschel concludes that a species in the solution, "exert its peculiar power on the incident light" and disperses the blue light. The experiment can be repeated simply by observing glass of tonic water exposed to sunlight. Often a blue glow is visible at the surface (Rendell, 1987).

The phenomenon of fluorescence was known by the middle of the nineteenth century. British scientist Sir George G. Stokes first made the observation that the mineral fluorspar exhibits fluorescence when illuminated with ultraviolet light, and he coined the word "fluorescence". In 1852, Sir G.G. Stokes studied the same compound (quinine) that has been used by Herschel and found that the fluorescing (*emitted*) light has longer wavelengths than the excitation (*absorbed*) light, a phenomenon that has become to be known as the Stokes

shift. Stokes' paper demonstrated the fundamental property of fluorescence, which simply can be summarized as a photon of ultraviolet radiation collides with an electron in a simple atom, exciting and elevating the electron to a higher energy level. Subsequently, the excited electron relaxes to a lower level and emits light in the form of a lower-energy photon (*higher wavelength*) in the visible light region. In 1871 Adolph Von Baeyer, a German chemist, synthesized the fluorescent dye, fluorescein. In 1880 Edmund Bequerel showed that certain metal ion complexes emit radiation with a very long decay time (Rendell, 1987).

Jabłoński and others developed a modern theoretical understanding of Stokes observation some 70 years later. In the 1920s and 1930s Jabłoński investigated polarized light and fluorescence and was able to show that the transition moments in absorption and emission are two different things. Thus the foundation for the concept of anisotropy was laid. For that and other accomplishments Jabłoński has been referred to as the father of fluorescence and his work has had a major impact on the theoretical understanding of photophysics (Rendell, 1987; Lakowicz, 2006).

How and why do certain molecules known as fluorophores or fluorescent molecules (such as: dyes, polyaromatic hydrocarbon, or heterocyclic,…etc.) emit different colors of light?. Briefly the answer for this question is that some molecules are capable of being excited, via absorption of light energy, to a higher energy state, also called an excited state. The energy of the excited state, which cannot be sustained for long, "decays" or decreases, resulting in the emission of light energy. This process is called *fluorescence*. To "fluoresce" means to emit light via this process. A fluorophore is a molecule that is capable of fluorescing due to the presence of certain chromophores within a molecule. In its ground state, the fluorophore molecule is in a relatively low-energy, stable configuration, and it does not fluoresce. When light from an external source hits a fluorophore molecule, the molecule can absorb the light energy. If the energy absorbed is sufficient, there are multiple excited states or energy levels that the fluorophore can attain, depending on the wavelength and energy of the external light source. Since the fluorophore is unstable at high-energy configurations, it eventually adopts the lowest-energy excited state, which is semi-stable. The length of time that the fluorophore is in excited states is called the *excited lifetime*, and it lasts for a very short time, ranging from 10^{-15} to 10^{-9} seconds. Next, the fluorophore rearranges from the semi-stable excited state back to the ground state, and the excess energy is released and emitted as light. The emitted light is of lower energy, and thus longer wavelength, than the absorbed light. This means that the color of the light that is emitted is different from the color of the light that has been absorbed. Emission of light returns the fluorophore to its ground state. The fluorophore can absorb light energy again and go through the entire process repeatedly (Lakowicz, 2006).

The cyclical fluorescence process, shown in Figure 1, can be summarized as: 1. Excitation of a fluorophore through the absorption of light energy, the excitation wavelength is usually the same as the absorption wavelength of the fluorophore; 2. A transient excited lifetime with some loss of energy, during this period, the fluorophore undergoes conformational changes and is also subject to possible interactions with its molecular environment, with two important consequences: first, the energy of higher excited state is partially dissipated as a heat, yielding a relaxed lowest singlet excited state from which fluorescence emission originates, second, not all the molecules initially excited by absorption (stage 1) return to the ground state by fluorescence emission, as other processes such as collisional quenching,

fluorescence energy transfer, and intersystem crossing may also depopulate the first excited state ; and 3. Return of the fluorophore to its ground state, accompanied by the emission of light. The light energy emitted is always of a longer wavelength (*lower energy*) than the light energy absorbed, due to the energy dissipation during the transient excited lifetime, as shown in Step 2. Consequently, the ratio of the number of fluorescence photons emitted (stage 3) to the number of photons absorbed (stage 1) represents the *fluorescence quantum yield* (Lakowicz, 2006).

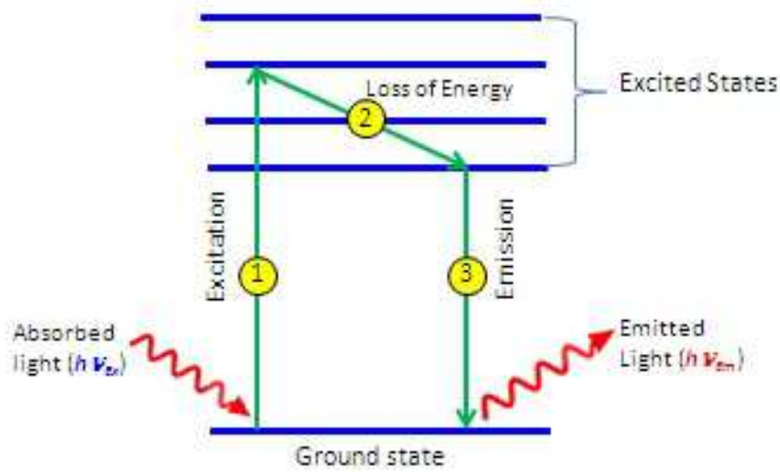

Fig. 1. The Jablonski diagram illustrates the three stages involved in the creation of an excited electronic singlet state by optical absorption and subsequent emission of fluorescence

A fluorophore can repeatedly undergo the fluorescence process—in theory, indefinitely. This is extremely useful, because it means that one fluorophore molecule can generate a signal multiple times. This property makes fluorescence a very sensitive technique for visualizing microscopic samples—even a small amount of the stain can be detected. In reality, however, the fluorophore's structural instability during the excited lifetime makes it susceptible to degradation. High-intensity illumination can cause the fluorophore to change its structure so that it can no longer fluoresce—this is called photobleaching. When a fluorescent sample, such as a slide with mounted tissue, is photobleached, the fluorophores are no longer promoted to an excited state, even when the required light energy is supplied (Lakowicz, 2006).

Now that we've introduced the general process of fluorescence, let's take a look at the basic properties of the light spectrum and its importance in fluorescence. The visible spectrum (Figure 2) is composed of light with wavelengths ranging from approximately 380 nanometers to 750 nanometers.

Light waves with shorter wavelengths have higher frequency and higher energy. Light waves with longer wavelengths have lower frequency and lower energy. As we stated before, an excited fluorophore emits lower-energy light than the light it absorbed. Therefore, there is always a shift along the spectrum between the color of the light absorbed by the fluorophore during excitation, and the color emitted.

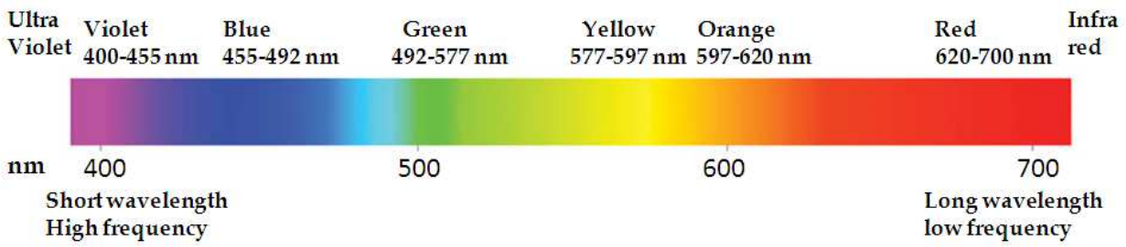

Fig. 2. Visible light spectrum

For example, let's say that we have a tube that contains a particular fluorescent dye. If we shine 480 nanometer light at the dye solution, some of the fluorophore molecules will become excited. However, the majority of the molecules are not excited by this wavelength of light. As we increase the excitation wavelength, say to 520 nanometers, more molecules are excited. However, this is still not the wavelength at which the proportion of excited molecules is maximal. For this particular dye, 550 nanometers is the wavelength that excites more fluorophores than any other wavelength of light. At wavelengths longer than 550 nanometers, the fluorophore molecules still absorb energy and fluoresce, but again in smaller proportions. The range of excitation wavelengths can be represented in the form of a fluorescence excitation spectrum, which looks like the spectrum shown in Figure 3.

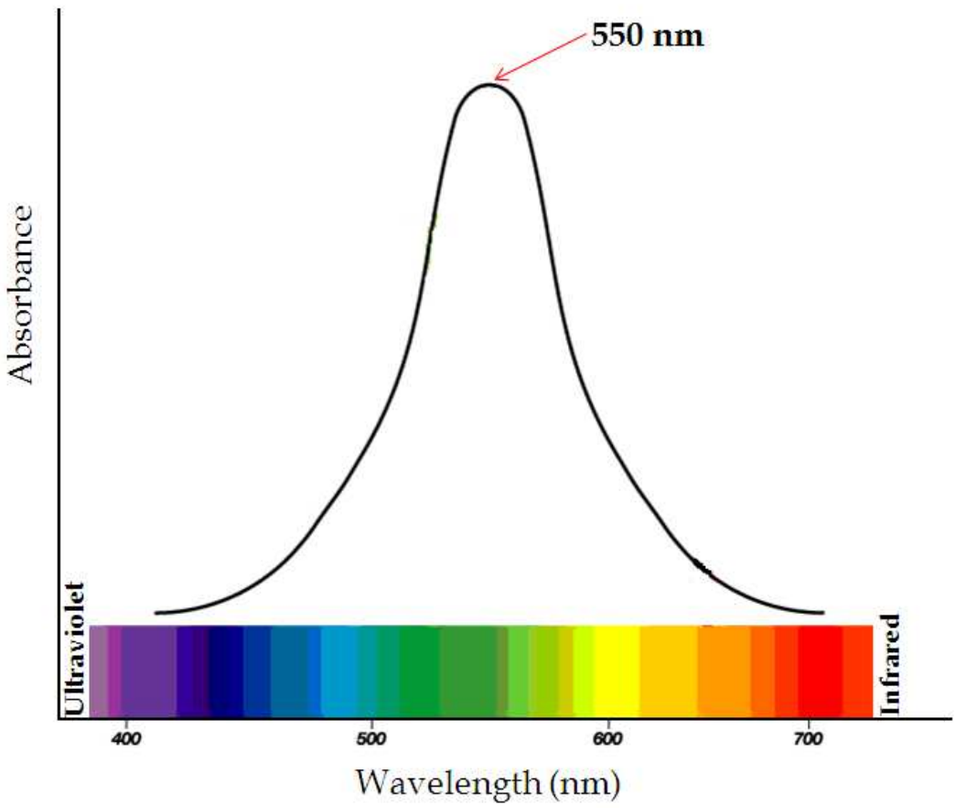

Fig. 3. Excitation spectrum of dye solution recorded at λ_{em} = 570 nm, the excitation wavelength maximum at 550 nm.

A fluorescent molecule absorbs light over a range of wavelengths—and every chemical molecule has a characteristic excitation range. However, some wavelengths within that range are more effective for excitation than other wavelengths. This range of wavelengths reflects the range of possible excited states that the fluorophore can achieve. Thus for each fluorescent molecule, there is a specific wavelength—the excitation maximum—that most effectively induces fluorescence. Now let's look at the light that is emitted by the fluorophore molecules when they are excited at the optimal excitation wavelength. Just as fluorophore molecules absorb a range of wavelengths, they also emit a range of wavelengths. There is a spectrum of energy changes associated with these emission events. When we excite the previously described dye solution at its excitation maximum, 550 nanometers, light is emitted over a range of wavelengths. A molecule may emit at a different wavelength with each excitation event because of changes that can occur during the excited lifetime, but each emission will be within the range. Although fluorophore molecules emit same intensity of light, the wavelengths and therefore the colors of the emitted light are not homogeneous. However, a larger population of molecules has most intensely fluorescence at 570 nanometers (Lakowicz, 2006).

Based on this distribution of emission wavelengths, we say that the emission maximum of this fluorophore is 570 nanometers. The range of wavelengths is represented by the Fluorescence Emission Spectrum, Figure 4.

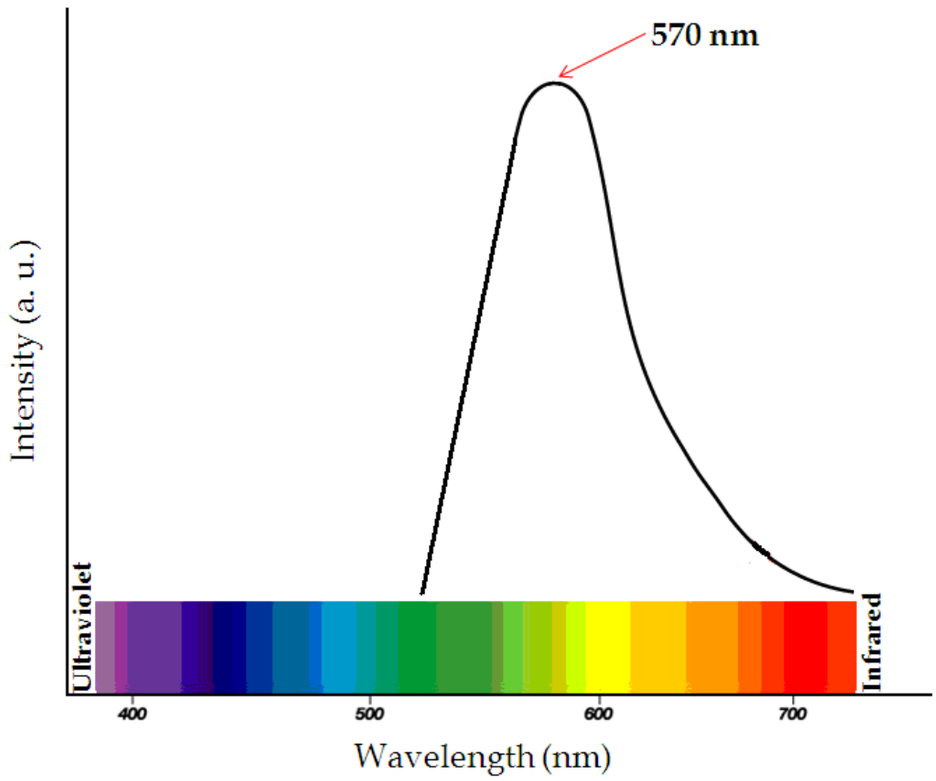

Fig. 4. Fluorescence Emission Spectrum of dye solution monitored at λ_{ex} = 550 nm

The emission intensity is proportional to the amplitude of the fluorescence excitation spectrum at the excitation wavelength. Fluorescence emission intensity depends on the same

parameters as absorbance—defined by the Beer-Lambert law as the product of the molar extinction coefficient, optical path-length, and concentration—as well as on the fluorescence quantum yield, the intensity of the excitation source, and the efficiency of the instrument and, in dilute solutions, is linearly proportional to these parameters.

The summary points of this introduction to fluorescence are: 1. Fluorophores are molecules that, upon absorbing light energy, can reach an excited state, and then emit light energy. 2. The three-stage process of excitation, excited lifetime, and emission is called fluorescence. 3. Fluorophores absorb a range of wavelengths of light energy, and also emit a range of wavelengths. Within these ranges are the excitation maximum and the emission maximum. Because the excitation and emission wavelengths are different, the absorbed and emitted lights are detectable as different colors or areas on the visible spectrum.

The purpose of this chapter is to review the articles on the interior cited aspects published since 2000 about various aspects of application of fluorescence spectrophotometry in chemical analysis.

2. Theoretical and instrumental aspects

2.1 Basic theory of fluorescence

This section provides a basic tutorial on specific topic in luminescence, namely fluorescence, and fluorescent instrumentations. To be able to understand the basic theoretical principles of luminescence spectroscopy, which include the electronic transitions, one should have a basic background on quantum mechanics and atomic orbitals, which was developed by Schrödinger in 1926. A tutorial review of Schrödinger's wave equation is out of the scope of this chapter, but briefly the most fruitful outcome of solving Schrödinger's wave equation is a set of wave functions (*called orbitals*), and their corresponding energies. An orbital is described by a set of three quantum numbers (Principal (n), Angular momentum (*l*), and magnetic (m_l) quantum numbers). Later a fourth quantum number, that describes the magnetic field generated due to the spinning of an electron on its own axis, was discovered and named as spin magnetic quantum number (m_s). The spin quantum number has only two allowed values: +1/2 and −1/2. According to Pauli Exclusion Principle, no two electrons in the same atom can have identical sets of four quantum numbers, *n, l,* m_l*, and* m_s. Thus any two electrons in same orbital (*n, l,* m_l) must have different spins either m_s=+1/2 or m_s= -1/2. The total spin is defined by $S = \Sigma m_s$ and the Multiplicity (M) is defined as M = 2S+1(M=1, 2, 3… Singlet, Doublet, and Triplet, respectively,..) as shown in Figure 5.

The photophysical processes that occur from absorption to emission are often shown in a so-called Jabłoński diagram. Of course all possible energy routes cannot be encompassed in single figure, and different forms of the diagram can be found in different contexts. Figure 7 is a simple version of Jabłoński diagram, where absorption ($S_0 \rightarrow S_1$ or $S_0 \rightarrow S_2$), fluorescence (emission not involving spin change, $S_1 \rightarrow S_0$), phosphorescence (emission involving spin change, $T_1 \rightarrow S_0$), intersystem crossing (non-radiative transition with spin change, as an example from singlet to triplet states; $S_1 \rightarrow T_1$), internal conversion (non-radiative transition either to lower state when vibrational energy levels "match" or to lower state by collisional deactivation, $S_2 \rightarrow S_1$)), vibration relaxation (within the vibrational levels in any excited electronic state) as well as intermolecular processes (radiationless relaxations) are shown.

Other intermolecular processes (e.g. quenching, energy transfer, solvent interaction etc.) are omitted (Rendell, 1987; Lakowicz, 2006).

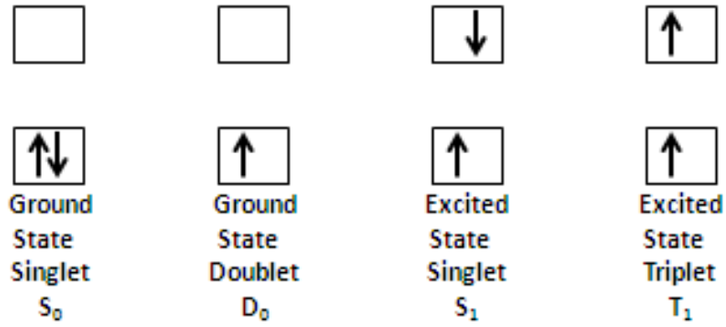

Fig. 5. Possible energy states according to their spin multiplicity

Once a molecule is excited by absorption of light it can return to the ground state with emission of fluorescence, but many other pathways for de-excitation are also possible, these are summarized in Figure 6.

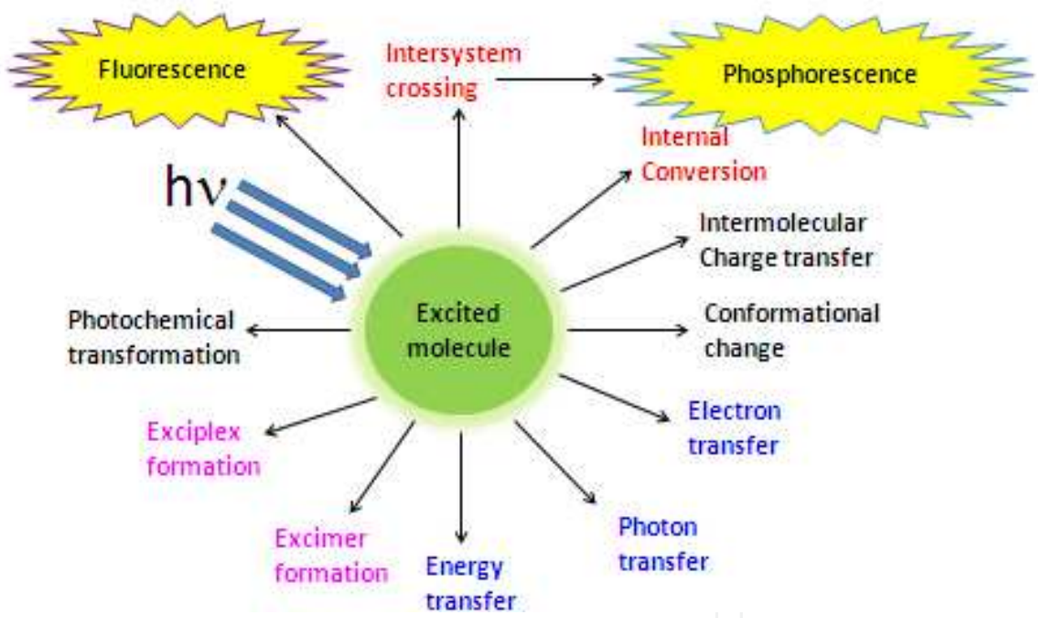

Fig. 6. All possible pathways for de-excitation processes

Jablonski diagram (Fig. 7) explains the mechanism of light emission in most organic and inorganic luminophores. Upon absorption of the light by a molecule, the electron promoted from ground electronic state (S_0) to an excited state that should possess the same spin multiplicity (such as, S_1, S_2,….) this process usually occurs within ~10^{-15} s. This excludes the triplet excited state as the final state of electronic absorption because transitions between states of different multiplicities are improbable "*forbidden*" (e.g. T→S or S→T). According to the quantum mechanical selection rules for electronic transitions, spin state should be maintained upon excitation because it is harder for an electron to go from a singlet state to

triplet state since the spin has to be flipped (i.e. *change in spin during the electronic transitions is not allowed*). Therefore, to go from a singlet to a triplet state ($\Delta M = 1$) is so-called forbidden transition and occurs with a small rate constant and typically too weak to be of much importance. At room temperature the higher vibrational energy levels are in general not populated (less than 1% according to Boltzmann statistics). The magnitude of the absorbed energy decides which vibrational level of S_1 (or S_2) becomes populated. This process is very fast and happens within 10^{-15} s. In the next 10^{-12} s the molecule relaxes to the lowest vibrational level of S_1, a process called internal conversion. Since emission typically occurs after 10^{-9} s the molecule is fully relaxed at the time of emission, hence, as a rule, emission occurs from the lowest vibrational level of S_1 (Kasha.s rule) and the fluorescence spectrum is generally independent of the excitation wavelength. After emission the molecule returns to the ground state, possibly after vibrational relaxation. This completes the simplest case of fluorescence: excitation, internal conversion, emission and relaxation. The energy lost to the surroundings, due to vibrational relaxation and internal conversion is the reason why a Stokes' shift (*defined as the energy difference between the emission and absorption peak maxima for the same electronic transition*) is observed (Figure 8). The Stokes shift is due to the fact that some of the energy of the excited fluorophore is lost through molecular vibrations that occur during the brief lifetime of the molecule's excited state. This energy is dissipated as heat to surrounding solvent molecules as they collide with the excited fluorophore. The magnitude of the Stokes shift is determined by the electronic structure of the fluorophore, and is a characteristic of the fluorophore molecule (Rendell, 1987; Lakowicz, 2006).

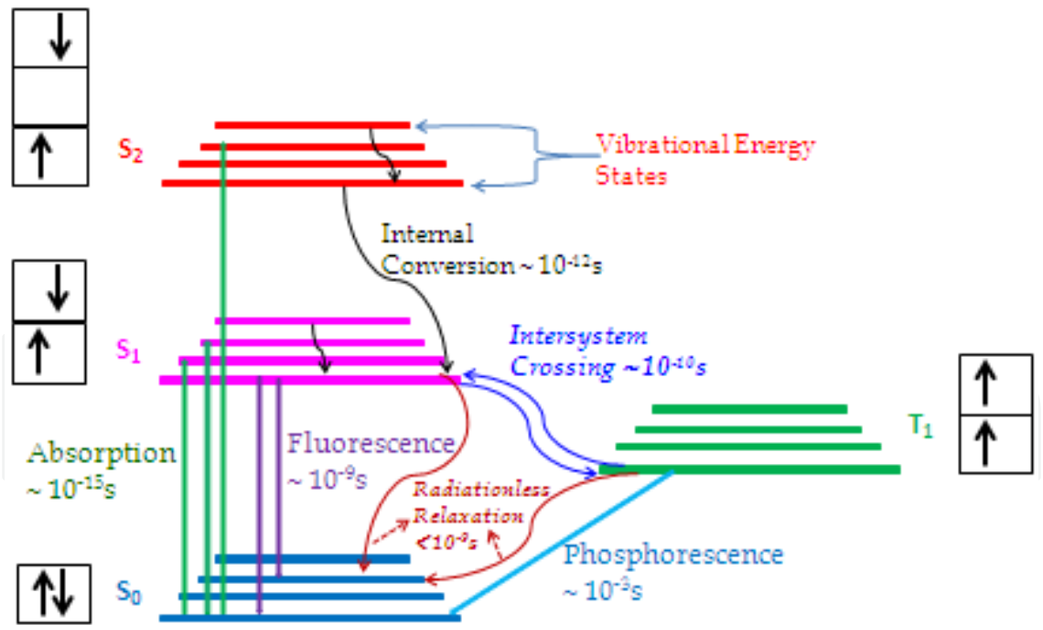

Fig. 7. The Jablonski diagram. Four electronic levels are depicted along with four vibrational energy levels.

Since the energy spacing between the vibrational levels in S_0 or S_1 is of the same size, there often exist mirror image symmetry between the emission spectrum and the $S_0 \rightarrow S_1$ absorption spectrum (not the $S_0 \rightarrow S_2$ absorption) (Fig. 8), needless to say there are plenty of

exceptions to the rule. The transitions that will predominate can be justified by Franck-Condon principle. The principle states that since the electronic absorption of light occurs in extremely short time (~10^{-15}s), thus during the time scale of absorption the nuclei are assumed to be frozen, that is that the transitions between various electronic levels are vertical (Rendell, 1987; Lakowicz, 2006).

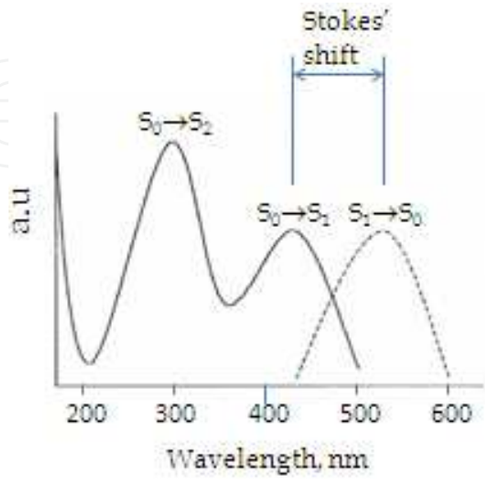

Fig. 8. Typical absorption and emission spectra.

The typical molecular photoluminescence relaxation processes that illustrated by Jablonski diagram (Fig. 7) can be classified to two main type of transition, these are radiative and nonradiative transitions. The non-radiative relaxation processes are vibrational relaxation (a rapid relaxation of excited molecules from higher to lowest vibrational level, occur within ~10^{-14}-10^{-12} s), internal conversion (a rapid relaxation of excited molecules to the lowest energy singlet excited state (S_1) from higher energy excited singlet state (such as S_2 in Fig. 7), occur within a time scale of 10^{-12} s), and intersystem crossing (relaxation between excited states of different spin multiplicity, such as $S_1 \rightarrow T_1$ in Fig. 7, occur within a time scale of 10^{-8} s). Intersystem crossing occurs more slowly than internal conversion since it's a less probable process than internal conversion due to spin multiplicity is not conserved.

The radiative processes are fluorescence and phosphorescence (Figure 9) (Lakowicz, 2006). The fluorescence refers to the emission of light associated with a radiative transition from an excited electronic state that has the same spin multiplicity as the ground electronic state ($S_1 \rightarrow S_0$, Fig. 7). Fluorescence transitions are spin allowed, thus they occur very rapidly and the average lifetimes of the excited states responsible for fluorescence are typically 10^{-9}-10^{-5} s. Phosphorescence refers to the emission of light associated with a radiative transition from an excited electronic state that has a different spin multiplicity from that of the ground electronic state ($T_1 \rightarrow S_0$, Fig. 7). Phosphorescence transitions are spin forbidden, thus they are less probable than spin allowed transitions and the average lifetimes of the excited states responsible for phosphorescence are typically 10^{-3} s. However, spin forbidden transitions become more probable when a significant interaction between the spin angular moment and the orbital angular momentum is observed (spin-orbit coupling increases), this can be observed in the presence of heavy atoms. Furthermore, in solutions, the presences of paramagnetic species such as molecular oxygen increase the probability of intersystem crossing and consequently make the spin forbidden transitions more probable.

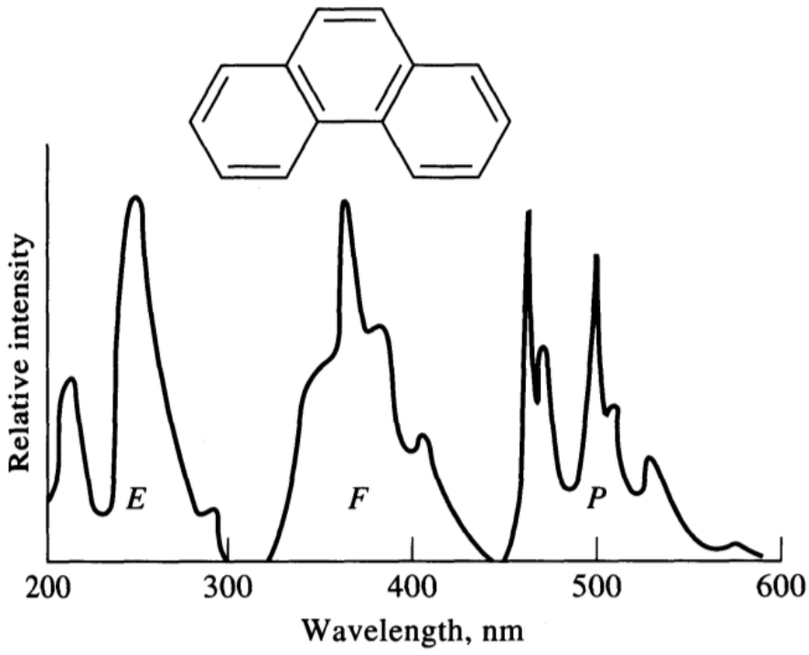

Fig. 9. Typical excitation (E), fluorescence (F), and phosphorescence (P) spectra of phenanthrene (Lakowicz, 2006).

The relative intensity of fluorescence peak is controlled by the Frank-Condon principle, but also the total fluorescence peak intensity (I) is related to the fluorescence quantum yield (Φ_F), which defined as the ratio of number of photons emitted to number of photons absorbed. Furthermore, the fluorescence quantum yield (Φ_F) can be expressed as the rate of photons emitted divided by the total rate of depopulation of the excited state (Equation 1) (Rendell, 1987; Lakowicz, 2006).

$$\Phi_F = \frac{k_F}{k_F + \Sigma k_{nr}} \tag{1}$$

Where k_F and k_{nr} are the rate constant of fluorescence and non-radiative processes, respectively. The fluorescence quantum yield (Φ_F) value in the range of 0.0 to 1.0. If the non-radiative relaxation is fast compared to fluorescence ($k_{nr} > k_r$), Φ will be small, that is the compound will fluoresce very little or not at all. Often different non-radiative events are limited in the solid phase, and long-lived luminescence (e.g. phosphorescence) is often studied in frozen solution or other solid phases. Quenchers make non-radiative relaxation routes more favorable and often there is a simple relation between Φ and the quencher concentration. The best-known quencher is probably O_2, which quench almost all fluorophores; other quenchers only quench a limited range of fluorophores. If a molecule is subject to intramolecular quenching, Φ may yield information about the structure.

The factors that affect the fluorescence quantum yield (Φ_F) are: (1) the excitation wavelength (λ_{ex}), the short wavelengths' break bonds and increase the rate constant of dissociation processes, the most common excitation wavelength are involving the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$

transitions; (2) lifetime of the excited state, the transition probability measured by the molar absorbitivity (ε), large ε implies short lifetime, thus largest fluorescence are observed from short lifetime and high ε state, as an example $\pi^* \rightarrow \pi > \pi^* \rightarrow n$ (10^{-9}-10^{-7} s > 10^{-7}-10^{-5} s); (3) structure of the molecule, emission of light is favored in aromatic molecules (conjugated systems) involving $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions, and fluorescence increased by number of fused rings and substitution on or in the ring, such as pyridine, pyrrole, quinoline and indole; (4) rigidity of the structure, fluorescence quantum yield increases with increasing the rigidity of the molecules specially with chelation, such as fluorine and biphenyl; and (5) the fluorescence quantum yield is highly dependence on the temperature, pH and solvent (Rendell, 1987; Lakowicz, 2006).

The total fluorescence intensity (I) is given by Equation 2:

$$I_F = 2.303 I_o \Phi \varepsilon c b \tag{2}$$

Where I_o is the incident radiant power, the term εcb is deduced from the well known Beers' law expression for the absorption (ε is the molar absorptivity, c is the molar concentration of the fluorescent substance, and b is the path length of the cell). The Beers' law is valid only for diluted solutions (εbc< 0.05) (Guilbault, 1990)

When an emission spectrum is obtained, data are typically collected for more than 0.1 sec. at each wavelength increment (typically 1nm), but since fluorescence lifetimes typically is measured in nanoseconds, it follows that the obtained spectrum is a time-average of a many events. The time averaging loses much information, and time-resolved experiments are often the more interesting when a system is investigated. The fluorescent lifetime of the excited state, τ_F, is the average time a molecule stays in the excited state before returning to ground state. Thus τ_F can be expressed as the inverse of the total depopulation rate as in Equation 3 (Rendell, 1987; Lakowicz, 2006).

$$\tau_F = \frac{1}{k_F + k_{nr}} \tag{3}$$

Where k_F and k_{nr} are the rate constant of fluorescence and non-radiative processes, respectively.

Typically fluorescence lifetime values are in the 5-15 ns range. The expression in Eq. 3 is related to the expression for Φ_F, in that way that they have a common denominator. Actually an approximation of τ_F can be obtained by measuring Φ_F in aired and degassed solutions.

In the absence of non-radiative relaxation (k_{nr}= 0), the lifetime becomes the inverse of k_r and is often called the natural lifetime, denoted τ_N. For many compounds the natural lifetime can be calculated from the measured lifetime τ and the measured quantum yield Φ_F, Equation (4) (Rendell, 1987; Lakowicz, 2006).

$$\tau_N = \frac{\tau_F}{\Phi_F} \tag{4}$$

It is important to notice that the fluorescent lifetime is what is experimentally obtained, and the natural lifetime can be calculated.

The fluorescence lifetime, τ_F, is determined by observing the decay in fluorescence intensity (decay profile) of a fluorophore after excitation. Immediately after a molecule is excited the fluorescence intensity will be at a maximum and then decrease exponentially according to Equation 5 (Rendell, 1987; Lakowicz, 2006).

$$I(t) = I_o e^{-t/\tau_F} \tag{5}$$

Thus after a period of τ_F the intensity has dropped to 37% of I_0, that is 63% of the molecules return to the ground state before τ_F. In many cases the above expression needs to be modified into more complex expressions. First of all it is assumed that the instrument yields an infinite (or very) short light pulse at time zero. In cases where τ_F is small I_0 must be replaced by a function, which describes the lamp profile of the instrument. Also, more than one lifetime parameter is often needed to describe the decay profile, which is I(t) must be expressed as a sum of exponentials. Finally the concept of anisotropy should be mentioned. Anisotropy is based on selectively exciting molecules with their absorption transition moments aligned parallel to the electric vector of polarized light. By looking at the polarization of the emission the orientation of the fluorophore can be measured. The anisotropy of the system is defined as (Equation 6) (Rendell, 1987; Lakowicz, 2006):

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}} = \frac{3\left\langle \cos^2(\theta) \right\rangle - 1}{2} \tag{6}$$

The oval in Figure 10 symbolized the absorption transition moment. Vertical polarized excitation light enters along the x-axis and $I_\perp$ and $I_\parallel$ are measured along the y-axis, setting the emission polarizer perpendicular and parallel to the excitation polarizer respectively. Θ is the angle of the emission to the z-axis (see Figure 10), the squared brackets indicates that it is the average value. If all absorption transition moments are aligned along the z-axis then $I_\perp$ = 0 and θ = 0, leading to r = 1, the maximum anisotropy.

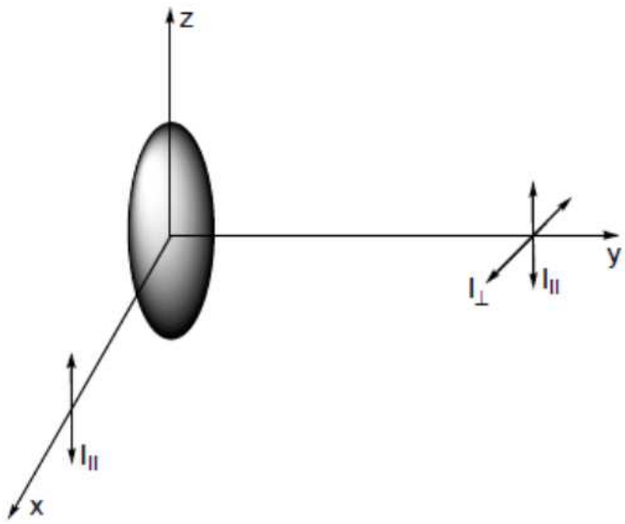

Fig. 10. The absorption dipole is aligned along the z-axis. The excitation light is vertically aligned and follows the x-axis. Emission is measured along the y-axis.

By combining anisotropy with time-resolved measurements it is possible to measure the mobility of a fluorophore. Immediately after excitation all excited molecules will be oriented along a common axis. In the solid phase the system will retain its anisotropy until emission. However, if the fluorophores are free to move, the anisotropy of the system will decrease before emission.

2.2 Instrumentation

The principal sketch of a typical fluorescence spectrophotometer is shown in Figure 11. It consists of a light source, an excitation and emission monochromator (*grooves/mm*), polarizers (*prisms*), sample chamber and a detector (*such as photomultiplier tube*). For steady state measurements the light source usually consists of a 450W xenon arc lamp, and for time resolved measurements it is equipped with nanosecond flash lamp. Most simple spectrometers have a similar geometry, but often extra detectors and/or light sources are fitted resulting in a T- or X-geometry.

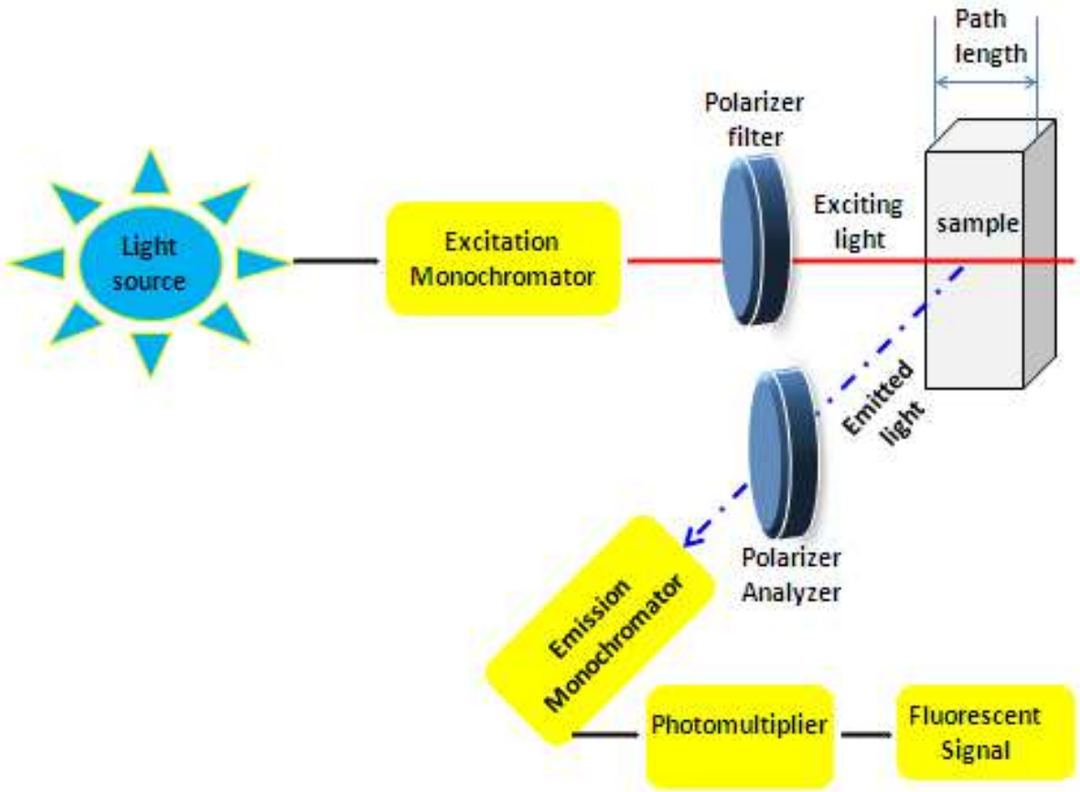

Fig. 11. Schematic representation of a fluorescence spectrophotometer.

The light source produces light photons over a broad energy spectrum, typically ranging from 200 to 900 nm. Photons impinge on the excitation monochromator, which selectively transmits light in a narrow range centered about the specified excitation wavelength. The transmitted light passes through adjustable slits that control magnitude and resolution by further limiting the range of transmitted light. The filtered light passes into the sample cell causing fluorescent emission by fluorphors within the sample. Emitted light enters the

emission monochromator, which is positioned at a 90^{o} angle from the excitation light path to eliminate background signal and minimize noise due to stray light. Again, emitted light is transmitted in a narrow range centered about the specified emission wavelength and exits through adjustable slits, finally entering the photomultiplier tube (PMT). The signal is amplified and creates a voltage that is proportional to the measured emitted intensity. Noise in the counting process arises primarily in the PMT. Therefore, spectral resolution and signal to noise is directly related to the selected slit widths (Guilbault, 1990; Rendell, 1987; Lakowicz, 2006).

Not all fluorimeters are configured as described above. Some instruments employ sets of fixed band pass filters rather than variable monochromators. Each filter can transmit only a select range of wavelengths. Units are usually limited to 5 to 8 filters and are therefore less flexible. Fiber optics are also employed for "surface readers", to transmit light from the excitation monochrometers to the sample surface and then transport emitted light to the emission monochrometers. This setup has the advantage of speed, but has the disadvantages of increased signal to noise, due to the inline geometry, and smaller path length which increase the probability of quenching.

Fluorescence requires a source of excitation energy. There are several main types of light sources that are used to excite fluorescent dyes. This section introduces the types of commonly used excitation sources and presents some of the ways that filters can be used to optimize your experimental result. The most popular sources used for exciting fluorescent dyes are broadband sources such as the mercury-arc and tungsten-halogen lamps. These produce white light that has peaks of varying intensity across the spectrum. In contrast, laser excitation sources, which will be described later, offer one or a few well-defined peaks, allowing more selective illumination of your sample. More recently, high-output light emitting diodes, or LEDs, have gained popularity due to their selective wavelengths, low cost and energy consumption, and long lifetime.

When using broadband white light sources it is necessary to filter the desired wavelengths needed for excitation; this is most often done using optical filters. Optical filters can range from simple colored glass to highly engineered interference filters that selectively allow light of certain wavelengths to pass while blocking out undesirable wavelengths. For selective excitation, a filter that transmits a narrow range of wavelengths is typically used. Such a filter is called a band pass excitation filter.

The high intensities and selective wavelengths of lasers make them convenient excitation sources for many dyes. The best performance is achieved when the dye's peak excitation wavelength is close to the wavelength of the laser. Compact violet 405 nm lasers are replacing expensive UV lasers for most biological work. The most commonly used lasers are the 488 nm blue-green argon laser, 543 nm helium-neon green laser and 633 nm helium-neon red laser. Mixed-gas lasers such as the krypton-argon laser can output multiple laser lines and therefore may still require optical filters to achieve selective excitation. While a given dye's excitation maximum may not exactly match the laser's peak wavelength, the high power of the laser can still produce significant fluorescence from the dye when exciting at a suboptimal wavelength. Filters are important for selecting excitation wavelengths. They are also important for isolating the fluorescence emission emanating from the dye of interest. Detecting the fluorescence emission of a sample is complicated by the presence of

stray light arising from sources other than the emitting fluorophore—for example, from the excitation source. This stray light must be kept from reaching the light-sensitive detector in order to insure that what the instrument "sees" is due only to the fluorescence of the sample itself. When a single dye is used, a filter that blocks out the excitation light to reduce background noise, but transmits everything else is often a good choice to maximize the signal collected. Such a filter is called a long pass emission filter (Guilbault, 1990; Rendell, 1987; Lakowicz, 2006).

If multiple dyes are used in the sample, a band pass emission filter can be used to isolate the emission from each dye. Careful filter selection helps to ensure that the detector registers only the light you are interested in—the fluorescence emitted from the sample.

LEDs are relatively new light sources for fluorescence excitation. Single-color LEDs are ideal for low-cost instrumentation, where they can be combined with simple long pass filters that block the LED excitation and allow the transmission of the dye signal. However, the range of wavelengths emitted from each LED is still relatively broad. Currently their application may also require the use of a filter to narrow the bandwidth.

There are many options for light sources for fluorescence. Selecting the appropriate light source, and filters for both excitation and emission, can increase the sensitivity of signal detection to an astounding degree. Making fluorescence labeling one of the most sensitive detection technologies available.

With recent advances in sensitive array detectors, fiber optic wave guides, high speed electronics and powerful software, many new generations of spectrofluorometers have been developed. These new spectrofluorometers use charged couple devices (CCDs) or photodiode arrays to replace the photomultipliers and avalanche photodiodes used in conventional spectrometers. They offer excellent performance for a wide range of spectroscopic applications from the UV to the near IR. Because of their unique combination of outstanding sensitivity, high speed, low noise, compactness, instantaneous capture of full spectra, low cost and robustness, these detectors have revolutionized spectroscopic detection. A quick glance at today's instrumentation market indicates the popularity of the CCD as the detector of choice. The overwhelming benefits of array detectors are simultaneous and multi-wavelength data acquisition. On the other hand, the use of fiber optics as light guidance allows a great modularity and flexibility in setting up an optical measurement system. Recent applications and a critical comparison between simple luminescence detectors using a light-emitting diode or a Xe lamp, optical fiber and charge-coupled device, or photomultiplier for determining proteins in capillary electrophoresis are presented by Casado-Terrones et al. (Casado-Terrones, 2007).

In summary, from the preceding discussion, four essential elements of fluorescence detection systems can be identified: (1) an excitation source, (2) a fluorophore, (3) wavelength filters to isolate emission photons from excitation photons and (4) a detector that registers emission photons and produces a recordable output, usually as an electrical signal or a photographic image. Regardless of the application, compatibility of these four elements is essential for optimizing fluorescence detection.

For the sample holders, the majority of fluorescence assays are carried out in solution, the final measurement being made upon the sample contained in a cuvette or in a flowcell.

Cuvettes may be circular, square or rectangular (the latter being uncommon), and must be constructed of a material that will transmit both the incident and emitted light. Square cuvettes or cells will be found to be most precise since the parameters of path length and parallelism are easier to maintain during manufacture. However, round cuvettes are suitable for many more routine applications and have the advantage of being less expensive. The cuvette is placed normal to the incident beam. The resulting fluorescence is given off equally in all directions, and may be collected from either the front surface of the cell, at right angles to the incident beam, or in line with the incident beam. Some instruments will provide the option of choosing which collecting method should be employed, a choice based upon the characteristics of the sample. A very dilute solution will produce fluorescence equally from any point along the path taken by the incident beam through the sample. Under these conditions, the right-angled collection method should be used since it has the benefit of minimizing the effect of light scattering by the solution and cell. This is the usual measuring condition in analytical procedures.

2.3 Sample preparation

Fluorescence is a very sensitive technique. This is the one criterion that makes it a viable replacement to many radioisotope-labeling procedures. However, it is extremely susceptible to interference by contamination of trace levels of organic chemicals. Potential sources of contamination are ubiquitous since any aromatic organic compound can be a possible source of fluorescence signal. For example, the researcher is a possible source of this type of contamination since oils secreted by the skin are fluorescent. Good laboratory procedure is essential in preventing solvents and chemicals from becoming contaminated with high background fluorescence that could hinder low-level measurements. Solvents should be of the highest level purity obtainable commercially. In addition, care must be taken to eliminate all forms of solid interference (suspended particulates such as dust and fibers). These will float in and out of the sampling area of the cuvette via convection currents, and cause false signals due to light scattering while they remain in the instrument's beam.

Fluorescence spectra and quantum yields are generally more dependent on the environment than absorption spectra and extinction coefficients. For example, coupling a single fluorescein label to a protein reduces fluorescein's quantum yields ~60% but only decreases its molar extinction coefficient by ~10%. Interactions either between two adjacent fluorophores or between a fluorophore and other species in the surrounding environment can produce environment-sensitive fluorescence.

Many environmental factors exert influences on fluorescence properties. All fluorophores are subject to intensity variations as a function of temperature, pH of the aqueous medium, and solvents polarity. In general fluorescence intensity decreases with increasing temperature due to increased molecular collisions that occur more frequently at higher temperatures. These collisions bleed energy from the excited state that produces fluorescence. The degree of response of an individual compound to temperature variations is unique to each compound. While many commercially available dyes are selected for their temperature stability, care should be taken to eliminate exposure of samples to drastic temperature changes during measurement. If possible, the temperature of instrument's sample compartment should be regulated via a circulating water bath. At lower assay temperatures, higher fluorescence signal will be generated. It has been found that a 50%

decrease in the fluorescence signal of yellow-green microspheres when exposed to 160°C for 15 minutes (Guilbault, 1990).

Fluorescence variations due to pH changes are caused by the different ionizable chemical species formed by these changes. The results from these pH variations can be quite drastic since new ionization forms of the compound are produced. Fluorescence spectra may be strongly dependent on solvent. This characteristic is most usually observed with fluorophores that have large excited-state dipole moments, resulting in fluorescence spectral shifts to longer wavelengths in polar solvents. As the polarity of environment decreases, the fluorophore shows a shift to longer wavelength with an increase in fluorescence quantum. Also, in polar environments the fluorescence quantum yield decreases with increasing temperature, while in nonpolar environment very little change in the fluorescence quantum yield was observed. Never the less, the environmental sensitivity of a fluorophore can be transformed by structural modifications to achieve desired probe specificity (Guilbault, 1990; Rendell, 1987; Lakowicz, 2006).

In summary, all fluorescence data required for any research project will fall into one of the following categories: (1) the fluorescence emission spectrum, (2) the excitation spectrum of the fluorescence, (3) the quantum yield, and (4) the fluorescence lifetime. In a typical emission spectrum, the excitation wavelength is fixed and the fluorescence intensity versus wavelength is obtained. Early examination of a large number of emission spectra resulted in the formulation of certain general rules: (1) in a pure substance existing in solution in a unique form, the fluorescence spectrum is invariant, remaining the same independent of the excitation wavelength (known as Kasha's rule), (2) the fluorescence spectrum lies at longer wavelengths than the absorption, (3) the fluorescence spectrum is, to a good approximation, a mirror image of the absorption band of least frequency. These general observations follow from consideration of the Jablonski diagram shown earlier. The fluorescence spectrum gives information about processes that happens when the molecules are in the excited stat.

3. Applications

In this section, the applications of fluorescence spectrophotometry as a powerful tool for quantitative analysis, characterization, and quality control in different fields will be reviewed and discussed in details. This section will include the use of fluorescence spectrophotometry as a powerful spectroscopic tool in several fields of science.

3.1 Organic analysis

In the period reviewed so many papers using fluorescence spectrophoometry for analysis, characterization, and as a tool for identification of several compounds are appeared in the literature. In this section, few recent methods will be summarized.

For example, the design and development of artificial molecular systems for sensing anions in biologically relevant conditions is a challenging task in supramolecular chemistry. In particular, sensing fluoride anion has attracted increasing interest in the molecular recognition community because of its pivotal importance in many areas of biological and chemical sciences. In recent years high levels of fluoride in drinking water have caused numerous human diseases, creating a crucial need for artificial sensors to detect fluoride anions in an aqueous environment. Recently, highly sensitive fluorescence "Turn-On"

indicators, triisopropylsilyl-protected coumarin derivatives, for fluoride anion with remarkable selectivity in organic and aqueous media have been developed (Sokkalingam & Lee C-H., 2011). This developed method exhibited new fluorescent sensors systems for fluoride anion detection that proved to be simple, inexpensive, and highly selective and, achieve accurate determination with a low detection limit. In addition, the results of this study showed that the system can detect inorganic fluorides as quickly as organic ones by simply introducing chelating agents such as crown ethers. The easily prepared indicator system synthesized here could be an ideal chemodosimeter for detecting and determining fluoride anion in both organic and aqueous solutions and could lead to development of a convenient and reliable detection method for fluoride anion in practical and commercial applications.

Fluorescence is a powerful tool for structural and functional studies of a diversity of molecules. Among the various fluorophores, pyrene derivatives are attractive fluorescent probes. Therefore, pyrene "click" conjugates of 7-deazapurine and 8-aza-7-deazapurine nucleosides and a basic pyrene compound has been synthesized (Ingale et al., 2011). The influence of the nucleobase on fluorescence quenching was studied on nucleoside and oligonucleotide level. This study showed that the favorable photophysical properties of 8-aza-7-deazapurine pyrene conjugates improve the utility of pyrene fluorescence reporters in oligonucleotide sensing as these nucleoside conjugates are not affected by nucleobase induced quenching. This improves the utility of pyrene fluorescence reporters for detection of oligonucleotides.

Selective recognition of Ag^+ ions and amino acids is an important area of research due to their involvement in chemical, biological, and environmental applications. Silver compounds are used as antimicrobial agents, and the activity is closely related with the interaction of Ag^+ with sulfurdryl (−SH) groups. There are several chemosensors reported in the literature for the recognition of Ag^+ ion in nonaqueous and aqueous systems. However, the molecular receptors which can recognize Ag^+ followed by amino acids are indeed limited in the literature. Recently, a new 1,1′-thiobis(2-naphthoxy)-based receptor molecule (L) containing a benzimidazole moiety has been synthesized (Dessingou et al., 2011). The selectivity of L has been explored in aqueous methanol, resulting in selective (7.5 ± 0.5)-fold *switch-on* fluorescence response toward Ag^+ among 14 different transition, alkali, and alkaline earth metal ions studied.

There is a growing interest in the development of molecular sensors that can detect selectively metal ions even in low concentrations. Among the various techniques used for this purpose fluorescence-based methods have gained in importance because of their sensitivity. These methods depend upon the change of fluorescence intensity and/or a shift in the fluorescence band of the sensor upon interaction with the metal ion. Although such a methodology has been successful for diamagnetic metal ions, its application to paramagnetic metal ions is fraught with difficulties in view of the fact that the latter quench fluorescence either via energy or electron transfer and only in some instances a fluorescence enhancement has been observed. In this regard, development of fluorescence-based sensors for Cu(II) has assumed importance in view of the fact that it is an essential trace element and yet at slightly increased concentrations, it is toxic, being implicated in gastrointestinal, liver, and kidney diseases as well as in neurological diseases such as Alzheimer's or Parkinson's. Therefore, Chandrasekhar et al. have demonstrated a simple approach for the design of

fluorescence-based sensors (Chandrasekhar et al., 2009). This methodology consists of assembly of phosphorus-supported coordinating platforms whose fluorescence properties are modulated by binding with Cu(II) as well as by the number of coordinating arms that the ligand possesses. We believe that this design is quite general and can be applied for selective detection of other type of metal ions also.

In recent decades, colorimetric and fluorometric sensors have been used in various scientific fields. In biology, for instance, such sensors are useful reagents for living cell imaging. It is important to design novel sensors because they have the potential to overcome many technical limitations in experiments. Imidazo[1,2-a]pyrazin- 3(7H)-one (*imidazopyrazinone*) often is used as a bioluminescent substrate, and it is an attractive core structure for useful sensors. In this regard, a new series of imidazopyrazinones [7-benzylimidazo[1,2 a]pyrazin-3(7H)-one derivatives] have been prepared and their fluorescent properties in the presence of various metal ions (M^{n+}) have been studied (Hirano, et al., 2010).

Copper amine oxidases (CAOs) are a large family of copper-containing quinone-dependent amine oxidases that can be found in all living organisms including bacteria, yeast, plants, and mammals. Human CAOs have been implicated in a number of diseases, including atherosclerosis, cardiovascular diseases, diabetes, Alzheimer's disease, and cancer. To study the kinetic behavior of highly potent inhibitors of the CAO bovine plasma amine oxidase (BPAO), it has been sought a sensitive and real time assay to monitor low levels of enzyme activity. The most sensitive assay for CAOs is the fluorometric coupled assay, which monitors generation of hydrogenperoxide during substrate turnover using horseradish peroxidase (HRP) as a secondary detecting enzyme. In this regard, a novel fluorogenic substrate of bovine plasma amine oxidase (BPAO), namely, (2-(6-(aminomethyl)naphthalen-2-yloxy)ethyl)trimethylammonium (ANETA) has been reported, which displays extremely tight binding to BPAO (K_m 183 (14 nM) and yet is metabolized fairly quickly (k_{cat} 0.690 (0.010 s^{-1}), with the aldehyde turnover product (2-(6-formylnaphthalen-2 yloxy)ethyl)trimethyl-ammonium serving as a real time reporting fluorophore of the enzyme activity (Ling et al., 2009). This allowed for the development of a fluorometric noncoupled assay that is two orders of magnitude more sensitive than the spectrophotometric benzylamine assay. ANETA represents the first highly sensitive, selective, and tight binding fluorogenic substrate of a copper amine oxidase that is able to respond *directly* to the enzyme activity *in real time*.

Organic molecules containing a fluorophoric unit combined with site(s) for guest binding purposes have found application in building up florescent signaling systems for biomedical research and chemical logics. Metal ions can act effectively as guests for these molecules because of their ability to enhance, shift or quench luminescent emissions of these organic ligands by coordination. The changes brought about by metal binding are mechanistically of four types, photoinduced electron transfer (PET), photoinduced charge transfer (PCT), formation of monomer/excimer, energy transfer and proton transfer. Transition metal ions with partially filled d-orbitals are known to induce fluorescence quenching by oxidative or reductive PET and energy transfer processes. In this regard, dioxomolybdenum(VI) complexes $[MoO_2(B^2)H_2O]$, $[MoO_2(B^2)EtOH]$, $[MoO_2(B^3)EtOH]$ and $[MoO_2(B^4)EtOH]$ were synthesized using several Schiff base ligands (B^1, B^2, B^3, and B^4) (Gupta et al., 2009). These ligands (B^1, B^2, B^3, and B^4) were prepared by condensation of 1-(2-pyridyl)-5-methyl-3-pyrazole carbohydrazide with salicylaldehyde, o-hydroxy acetophenone, 5-bromo

salicylaldehyde and 5-nitro salicylaldehyde, respectively. Due to the presence of a substituted 1-(2-pyridyl) pyrazole unit, these ligands exhibit fluorescent emissions. As the ligands are capable of using different binding modes, according to the demands of the guest metal ions, their emission properties also change accordingly.

3.2 Inorganic analysis

The development of sensors for metal ions in solution has always been of particular importance for cations with biological and environmental interest. The molecular devices converting metal ions recognition in physical recordable signal are continuously growing. In the last few years great attention has been paid to fluorescent chemosensors and many new systems were synthesized. In particular, an effective fluorescent sensor for metal ions is a system able to interact with the metal ion in solution signaling its presence by changing fluorescence properties, as the wavelength or emission intensity, as well as by the appearance of a new fluorescence band different from those of the free sensor. Recently, paper reviews ligand molecules containing fluorophores synthesized and employed in metal ions sensing in solution in the last few years has been published (Formica et al. 2012). The large number of references reported in the review highlights the synthesis of new fluorescent chemosensors able to sense and signal metal ions in solution. This is a prosperous and still emerging field. In this review the authors concluded that the discovery of newer and more efficient emitting units is desirable. In this case, research is now focused on the synthesis of fluorescent units able to shift the emission in an optical range where the biological noise is reduced in the near infrared region (NIR) thus to increase the sensitivity of the chemosensor for in vivo analysis.

Metal–polypyridine complexes are extensively used in photochemical applications, such as solar energy conversion (Akasheh & AI-Rawashdeh, N.A.F, 1990), due to their peculiar excited-state dynamics. These complexes, of which ruthenium tris-bipyridine ($[Ru(bpy)_3]^{2+}$) is considered the prototype, exhibit a visible absorption band due to the singlet metal-to-ligand charge transfer state. The principle of the dye sensitized solar cells is based on the use of such metal-based molecular systems, of which the RuN3 ($[Ru(dcbpyH_2)_2(NCS)_2]$) dye is the most popular, adsorbed onto a semiconductor substrate (usually TiO_2). In this regards, femtosecond-resolved broadband fluorescence studies are recently reported for $[M(bpy)_3]^{2+}$ (M=Fe, Ru), RuN3 and RuN719 ($(Bu_4N)_2[Ru(dcbpyH)_2(NCS)_2]^{2-}$) complexes in solution (Bram et al. 2011). In this study, the pump wavelength dependence of the fluorescence of aqueous $[Fe(bpy)_3]^{2+}$ and the solvent and ligand dependence of the fluorescence of Ru-complexes excited at 400 nm have been investigated. The RuN3 and RuN719 are asymmetric complexes contrary to $[Ru(bpy)_3]^{2+}$, which allows us to explore the effects of molecular geometry on the ultrafast relaxation dynamics of this class of molecules.

Ru(II) polypyridyl complexes have been widely used as DNA, cation, and anion sensors, because their outstanding photophysical and electrochemical properties are quite sensitive to external stimuli (Schmittel, 2007, as cited in Cheng, 2010). Transfer of protons can be regarded as one of the simplest external stimuli and can induce the switching of properties such as fluorescence and UV–Vis absorption for pH sensors, so some Ru(II) polypyridyl complexes containing imidazole fragment have been synthesized. Imidazole-containing ligands are poor π-acceptors and good π-donors and have the appreciable ability to control orbital energies by proton transfer compared with pyridine-, pyrazine-, and pyrimidine-

containing ligands, but in most cases these complexes with imidazole rings coordinated to the metal center are nonemissive or weakly emissive and only display deprotonating process [Ayers, 2002, as cited in Cheng, 2010). In this regards, tripodal ligands 1,3,5-tris{4-((1,10-phenanthroline-[5,6-d]imidazol-2-yl)phenoxy)methyl}-2,4,6-trimethylbenzene (L1), 1,1,1 tris{4-((1,10-phenanthroline-[5,6-d]imidazol-2-yl)phenoxy)methyl}propane (L2), 2,2',2''-tris{4-((1,10-phenanthroline-[5,6-d]imidazol-2-yl)phenoxy)ethyl}amine (L3), and corresponding Ru(II) complexes $[(bpy)_6L(Ru(II))_3](PF_6)_6$, have been synthesized (Cheng et al., 2010) . This study showed that the fluorescence spectra of these complexes are strongly dependent on the pH of the buffer solution. These complexes act as pH-induced off–on–off fluorescence switch through protonation and deprotonation of the imidazole-containing ligands. For the same purpose, two novel tetrapodal ligands tetrakis{4-((1,10-phenanthroline-[5,6-d]imidazol-2-yl)phenoxy)methyl}methane (L1), tetrakis {3-((1,10-phenanthroline-[5,6-d]imidazol-2-yl)phenoxy)methyl}methane (L2), and corresponding Ru(II) complexes $[(bpy)_8L(Ru(II))_4](PF_6)_8$ have been synthesized (Cheng et al., 2011). The two complexes act as off–on–off fluorescence pH switches with a maximum on–off ratio of 5. This on–off ratio is moderate compared with those reported for imidazole-containing Ru(II) complexes (Cheng et al., 2010). They have potential utility to detect pH variations of external environment due to their interesting photon-dependent photophysical properties.

Crystal engineering of coordination polymers and supramolecules have attracted lot of attention due to their potential applications as functional materials, as well as their fascinating architectures and topologies (Moulton & Zaworotko, 2010, as cited in Leong, 2009). A successful strategy in the construction of such networks is to utilize appropriate multidentate ligands with flexible backbone that are capable of binding metal ions in different modes. In this regards, a series of metal (Cu(II), Ni(II), Mn(II), Zn(II), Mg(II), Ca(II), and Al(III)) complexes containing the 4-methylumbelliferone-8-methyleneiminodiacetic acid (H_3muia, also named as Calcein Blue) has been synthesized (Leong et al., 2009). In this study, the fluorescence spectroscopy has been used to investigate the hydrogen bonding interactions along with π–π stacking in the synthesized complexes solid-state structures. Solid-state fluorescence studies indicate that complexes of muia have the similar emission properties as in the solution state. Transition metal ions quench the fluorescence of muia while alkali earth and post-transition metal complexes of muia exhibit strong blue emission.

A considerable number of papers have focused on the use of anthracene containing compounds as protein photo cleavers (Hasewage et al., 2006, as cited in Oliveria, 2007), organic light-emitting diodes and materials (Jou, et al., 2006, as cited in Oliveria, 2007), crystal engineering , molecular imprinted polymers, sensors and chemosensors (Magri, et al., 2005, as cited in Oliveria, 2007). Anthracene is one of the most employed chromophores due to its ability to induce PET (photoinduced electron transfer) processes. In this regard, a new scorpionate system (L) containing an emissive anthracene pendant arm, derived of O^1,O^7-bis(2-formylphenyl)-1,4,7-trioxaheptane and tren, has been synthesized (Oliveira et al., 2007). The fluorescence spectroscopy studies conducted suggest that this ligand is an effective complexation molecular device for several divalent metal ions of biological importance as well as for Al(III) and Cr(III), both metals of great relevance in medicine and environmental chemistry. The results of this study could be used as a starting point to develop a more efficiently fluorescence chemosensor based on macrocyclic ligands for these metals.

Since some of lanthanide ions, especially Eu^{3+} and Tb^{3+}, posses good luminescence characteristics (high color purity) based on the 4 f electronic transitions, a variety of rare earth compounds activated by Eu^{3+} and Tb^{3+} have been studied for practical applications (Zhang, et al., 1999, as cited in Xu, et al., 2004). Polydimethylsiloxane (PDMS) materials have broad application in a variety of industrial area so their well-established surface modifying properties. In this regard, Eu(III)-containing polymer complex was synthesized, in which polydimethylsiloxane was used as a polymer ligand (Xu, et al., 2004). The result of this study showed that the fluorescence intensity change of Eu(III)-PDMS complex displays typical fluorescent concentration quenching behavior, in which the emission intensity of the complex was enhanced with increasing content of Eu (III) ion and reaches a maximum at 2 wt.%, and then decreased with further increasing content of Eu(III) ion. These results indicate that the complex contains ionic aggregates in which Eu(III) ions are located close together. Eu(III)-PDMS could transform harmful ultraviolet radiation to blue luminescence that is needed by plants effectively. So Eu(III)-PDMS has a great application foreground.

Schiff base ligands have been extensively studied in coordination chemistry mainly due to their facile syntheses, easily tunable steric, electronic properties and good solubility in common solvents. Transition metal complexes with oxygen and nitrogen donor Schiff bases are of particular interest (You et al., 2004) because of their ability to possess unusual configurations, be structurally labile and their sensitivity to molecular environments. In this regard, the preparation and structures of nickel(II) zinc(II) and cadmium(II) complexes with the related Schiff base ligand N-2-pyridylmethylidene-2- hydroxy-phenylamine have been investigated (Majumder et al. 2006). The results of this study showed that the complexes with zinc(II) and cadmium(II) metals can serve as potential photoactive materials as indicated from their characteristic fluorescence properties. Since the complexes with zinc(II) and cadmium(II) metals possess an intense fluorescence property at room temperature, which is not observed for complexes with Ni(II). It is suggested that complexes with Zn(II) and Cd(II) exhibit potential applications as photoactive materials.

3.3 Pharmaceutical analysis

Over the last three decades, wide ranges of thermodynamic data concerning the guest host complexation have been reported. Cyclodextrins (CDs) are cyclic oligosaccharides composed of glucopyranose units linked together via oxygen bridges at the 1 and 4 positions (a-(l,4)-glycoside bonds) (Bender& Komiyama, 1978). This class of organized media possesses a hydrophilic upper and lower rims lined with hydroxyl groups and a hydrophobic cavity due to C^3H, C^5H, and C^6H hydrogen's and O^4 ether oxygen. This structure gives CDs the ability to extract a variety of organic guest molecules of appropriate size and hydrophobicity from the bulk aqueous solution (Szejtli, 1988). Complexation of various guest compounds with CDs generally results in the improvement of some physical properties of the guest molecules, such as stability, bioavailability, membrane permeability, and solubility. In luminescence studies, CDs have been employed to enhance fluorescence emission of different luminophors (Bender & Komiyama, 1978; Szejtli, 1988) and to induce room temperature phosphorescence under appropriate conditions. In this regards, there is a lot of literature reported in several journals of interest, as an example, the inclusion of the anti-inflammatory drug, Nabumetone (NAB), in γ-cyclodextrin (γ-CD) was studied by fluorescence measurements (Al-Rawashdeh. N.A.F., 2005). Nabumetone is poorly soluble in water and exhibits intrinsic fluorescence. The results of this study showed that the emission

fluorescence spectrum, of NAB reveals a maximum whose intensity increases with the different γ-CD's growing concentrations. It is noteworthy mentioning that significant alterations in the physicochemical properties of the included molecule (NAB) are observed upon forming the inclusion complex with γ-cyclodextrin, such as stability and solubility in aqueous media.

Imidazoline-derived drugs are a family of drugs that is structurally distinguished by the existence of the heterocyclic ring of imidazoline that enables these drugs to interact with adrenergic receptors via stimulating presynaptic and postsynaptic a-adrenoceptors (Parini et al., 1996). The majority of the imidazoline-derived drugs are frequently used for their agonist activity, whereas others are used for either antihypertensive, antihistaminic, or agonistic activity (Kaliszan et al., 2006). However, the principle pharmaceutical applicability of these drugs is due to their vascoconstrictive effects. Three typical imidazoline-derived drugs were selected for this study; Naphazoline (NP) (4,5-Dihydro-2-(1-naphthalenylmethyl-1H-imidazole), Antazoline (AN) (4,5-Dihydro-Nphenyl-N-(phenylmethyl)-1H-imidazole-2-methanamine, 2-[(N-benzyl anilino) methyl]-2-imidazole, and Xylometazoline (XM) (2-(4-tert-Butyl-2,6-dimethylbenzyl)-2-imidazoline). While CDs have a wide range of applications, using CDs as additives in various separation and pharmaceutical sciences is still the foremost application of them. Hence, adding the CDs to the separation media can significantly enhance the separation process, whereas employing CDs as additives to drugs formulation can promote the bioavailability through enhancing the stability and solubility of selected drugs. In this regard, the inclusion complexes of selected imidazoline-derived drugs, namely Antazoline (AN), Naphazoline (NP) and Xylometazoline (XM) with β-cyclodextrin (β-CD) were investigated using steady-state fluorescence (Dawoud & Al-Rawashdeh, N. 2008)). Their results confirmed the formation of the inclusion complexes between the three studied drugs and β-cyclodextrin using steady-state fluorescence spectroscopy. Importantly, the results of their study showed that the geometrical size and polarity of various substituents, such as phenyl and naphthyl groups, have dramatically altered the stability and geometrical configuration of the inclusion complexes. These studies present the state-of-the-art of macromolecular binders and provide detailed illustrative examples of recent developments bearing much promise for future pharmaceutical applications.

Propranolol is a beta-adrenergic blocking drug widely prescribed for the treatment of cardiac arrhythmia, sinus tachycardia, angina pectoris and hypertension (Parfitt, 1990). It has also been suggested for use in a number of other conditions including dysfunctional labour and anxiety. When administered over a long period of time it reduces mortality caused by hypertension and lengthens survival in patients with coronary heart disease. It is also used in low activity sports, reducing cardiac frequency, contraction force and coronary flow. Therefore, it has been included in the list of forbidden substances by the International Olympic Committee. The Spanish Olympic Committee has decided that only a qualitative determination of propranolol in urine is necessary. In this regards, a sensitive fluorescence optosensor for the drug propranolol to use in the analysis of pharmaceutical preparations and as a doping test for the qualitative analysis of propranolol in human urine without lengthy preliminary procedures have been developed (Fernandez-Sanchez et al. 2003).The effect of proteins presents in urine samples were evaluated using the developed flow-through fluorescence optosensor. The results of this study showed that the proposed methods for analysis were satisfactorily applied to commercial formulations and urine

samples, which offers excellent analytical parameters, such as sensitivity, selectivity, versatility, and ease of use. The development of optosensing techniques has led to a shorter turnaround analysis time and reduced costs for doping controls. As a large part of the samples prove to be non-doped, rapid analytical methods such as doping tests that provide reliable 'yes/no' responses are of increasing interest. These tests can usually be described as systems that 'filter' samples to select those with analyte content levels 'similar to' or 'higher than' a previously established threshold. These 'probably doped' samples must then be examined with more exact instrumental methods. Doping tests can significantly cut costs and save time.

Anthraquinones are known to be present in many different families such as Polygonaceae, Leguminosae, Rubiaceae, Liliaceae and Rhamnaceae. Recently, a number of pharmacological tests revealed that the anthraquinone derivatives present various biological activities including antifungal (S K. Agarwal, 2000, as cited in He, 2009), antimicrobial (Y. W. Wu, as cited in He, 2009), anticancer (J. Koyamma, as cited in He, 2009) , antioxidant (G. C. Yen, as cited in He, 2009), and antihuman cytomegalovirus activity (D. I. Barnard. As cited in He, 2009). In general, fluorescence detection is sensitive and selective. The five anthraquinones are known to possess natural fluorescence, but it is difficult to analyse and determine their contents by conventional fluorimetry due to their similar molecule structures. Therefore, recently a simple, rapid and sensitive reversed-phase high-performance liquid chromatography (RP-HPLC) method, using fluorescence detection, to simultaneously quantify aloe-emodin, emodin, rhein, chrysophanol and physcion in medicinal plants and their pharmaceutical preparations was developed by He et al. (He et al., 2009). Their method was suitable for use as a tool for routine quality assurance and standardization of the anthraquinone from the raw material and commercially available pharmaceutical preparations containing rhubarb.

Fluorimetry from the early fifties has been among the most frequently used techniques for determining both therapeutically and abuse drugs; probably due to its excellent selectivity and the low detection limits. Also, the fluorimetric technique is the recommended choice for quantifying the purity of active principles. The research on analytical fluorescence applications is in continuous expansion to new automated or semi-automate processes like classic or emergent methodologies on the continuous-flow field. In this way, fluorescence-based methods have found a wide range of analytical applications (Calatayud & Zamora, 1995)). In this regard, a new strategic tool to predict the native fluorescence of organic molecules has been proposed (Albert-Garcia et al. 2009). For this purpose, the molecular connectivity indices of different organic substances (pharmaceuticals and pesticides) were calculated. The work presented in this paper was focused to present a new tool for enhancing the research yield on new analytical applications of fluorescence.

3.4 Biological and biomedical analysis

Cancer has overtaken heart disease as the world's top killer by 2011, part of a trend that should be more than double global cancer cases and deaths by 2030. Cisplatin (cis diamminedichloroplatinum(II)) is one of the most effective anticancer drugs in the treatment of a variety of tumors and it has been clinically used widely. However, its limited usefulness in the development of resistance in tumor cells and the significant side effects have generated new areas of research, which mainly focused on searching for new metal-based

complexes with low toxicity and improved therapeutic properties. In this regards, 3-Carbaldehyde chromone thiosemicarbazone and its transition metal (Cu(II), Zn(II) and Ni(II)) complexes were synthesized and characterized systematically (Li et al., 2010). The results of this study showed that the Zn(II) complex can emit blue fluorescence under UV light in solid state and may be used as an advanced material for blue light emitting dioxide devices. However, almost the solid fluorescence of Cu(II) and Ni(II) complexes could not be observed. Thus, the fluorescence property of these complexes was used as a helpful tool to understand the interaction mechanism of small molecule compounds binding to DNA. It was believed that the information obtained from the present work would be useful to develop new potential antioxidants and therapeutic agents for some diseases.

The quantitative determination of micro-amounts of nucleic acid has attracted a great deal of attention in the fields of medicine and molecular biology. Many methods have been developed, such as direct determination, including ultraviolet absorption and determination of ribose or deoxyribose in nucleic acid, spectrophotometry, chemiluminescence, electrochemical chromatography, including high-performance liquid chromatography and paper chromatography, capillary electrophoresis, and resonance light scattering. However, low sensitivity and easy interruption by protein and other biomolecules existed in these methods in common. However, the fluorometric methods make predominant concern because of their high sensitivity and selectivity. Generally, the fluorescence intensity of DNA must be enhanced by fluorescent probes because it emits weak fluorescence itself. In this regard, method for the determination of DNA based on the fluorescence intensity of the gatifloxacin-europium(III) (GFLX-Eu^{3+}) complex that could be enhanced by DNA was developed (Wang et al. 2011). The GFLX-Eu^{3+} complex showed an up to 6-fold enhancement of luminescence intensity after adding DNA. On the basis of the above findings, the fluorescence enhancement effect of the GFLX-Eu^{3+} complex by DNA was investigated in this study in detail.

The green fluorescent proteins (GFPs) originated from the bioluminescent jellyfish Aequorea victoria, were discovered by Shimomura in the early 1960s (Shimomura et al., 1962). In the last few years, green fluorescent protein (GFP) has become one of the most widely used tools in molecular and cell biology. As a noninvasive fluorescent marker in living cells, GFP allows for numerous applications where it functions as a probe of gene expression, intercellular tracer or as a measure of protein-protein interactions. The green fluorescent protein (GFP) has emerged as a powerful reporter molecule for monitoring gene expression, protein localization, and protein–protein interaction. However, the detection of low concentrations of GFPs is limited by the weakness of the fluorescent signal and the low photostability. Recently, the proximity of single GFPs to metallic silver nanoparticles increases its fluorescence intensity approximately 6-fold and the decrease in decay time has been observed by Fu et al. (Fu et al., 2008). Furthermore, single protein molecules on the silvered surfaces emitted 10-fold more photons as compared to glass prior to photobleaching. The photostability of single GFP has increased to some extent. Accordingly, longer duration time and suppressed blinking were observed. The single-molecule lifetime histograms indicate the relatively heterogeneous distributions of protein mutants inside the structure.

Detection of DNA in solution is an important problem in a large variety of biochemical assays. The most popular agents for DNA quantitation are fluorescent dyes that strongly

interact with nucleic acids and significantly increase their emission intensity in the DNA complex. Fluorescent dyes are used in real-time PCR, DNA-based cell quantitation, gel staining, chromatin and other DNA-based approaches (Glazer and Rye, 1992, Jing et al., 2003, Lakowicz, 2006, Le Pecq and Paoletti, 1967, Lim et al., 1997, Szpechcinski et al., 2008, as cited in Dragan, 2010). In this regard, both a theoretical and experimental analysis of the sensitivity of a DNA quantitation assay using a fluorescent chromophore which non-covalently binds dsDNA were investigated (Dragan et al. 2010). It is well-known that the range of DNA concentrations available for fluorescence quantitation depends on the concentration of the chromophore, its affinity for nucleic acids, the binding site size on DNA and the ratio between the fluorescence intensity of the chromophore when bound to DNA compared to free chromophore in solution. In this study an experimental data obtained for a PicoGreen® (PG)/DNA quantitation assay was presented, which is in complete agreement with the results of our theoretical analysis. Furthermore, it has been shown, both theoretically and experimentally, that DNA assays based on the MEF of PG demonstrate sensitivity to DNA concentration of ≈1 pg/ml, which is several orders of magnitude more sensitive than without the silver nanoparticles, suggesting the broader practical use of this approach (metal-enhanced PicoGreen® fluorescence) for the ultra-sensitive detection of double stranded nucleic acids.

Identification of living organisms (eukaryotes, bacteria, viruses etc.) by means of quantitative analysis of their specific DNA sequences, which represent different genomes, is a challenging aim, which faces many scientists today. It also concerns the search and detection of different microorganism mutations and strains of pathogenic bacteria and causes of severe diseases in humans. In recent short communication, further development of the "Catch and Signal" technology—principles of a 2-color DNA assay for the simultaneous detection/quantification of two genome-specific DNAs in one well was presented (Dragan et al., 2011) In this method a combination of the Metal-Enhanced Fluorescence (MEF) effect and microwave-accelerated DNA hybridization has been utilized. Furthermore, it is shown that fluorescent labels (Alexa 488 and Alexa 594), covalently attached to ssDNA fragments, play the role of biosensor recognition probes, demonstrating strong response upon DNA hybridization, locating fluorophores in close proximity to silver nanoparticles, which is ideal for MEF. The 2-color "Catch and Signal" DNA assay platform can radically expedite quantitative analysis of genome DNA sequences, creating a simple and fast bio-medical platform for nucleic acid analysis. Their results clearly showed that the 2-color DNA assay can effectively be employed as a new "Rapid Catch and Signal" technological platform in the creation of an ultra-sensitive, sequence-specific approach for the fast analysis of genetic material from different organisms, for potential analysis of bacteria and virus pathogens, and search for possible mutations and sequence variations. This technology being fast, ultra-sensitive and inexpensive can effectively compete with the PCR technique, especially for the routine and rapid analysis in Point-of-Care settings and bio-medical laboratories.

3.5 Environmental analysis

For the past 20 years there has been continued growth in the applications of fluorescence spectroscopy in physical and biological sciences. Because of the sensitivity of fluorescence detection, the fluorometeric method has been one of the selected techniques to determine compounds at low concentrations (Lakowicz, 2006). Examples of such compounds are

agrochemicals (pesticides, fungicides, insecticides, etc), which have attracted attention worldwide due to their usage in agriculture. Therefore, the potential increase in fluorescence of a benzimidazole-type fungicide (carbendazim) due to complexation with cucurbit[6]uril was reported (Saleh & Al-Rawashdeh, N.A.F., 2006). The fluorescence enhancement of the fungicide carbendazim by cucurbit[6]uril has been observed in solution due to formation of a host–guest inclusion complex. The enhancement of the carbendazim fluorescence (maximum factor of 10) is accompanied by a significant blue shift in the spectrum (11 nm). In general, Saleh and Al-Rawashdeh work (Saleh & Al-Rawashdeh, N.A.F., 2006) demonstrates the potential usefulness of cucurbit[6]uril in fluorometric analysis of fungicide for agricultural and environmental applications.

Recycling in agriculture of organic wastes produced by various animal breeding, such as pig slurry (PS), is a common practice throughout the world, which has recently raised serious environmental concerns. In particular, Cu(II) and Zn(II) ions, which are used abundantly as pig feed additives, may be introduced in relatively large amounts into PS-amended soils, thus representing an actual risk of phytotoxicity and/or leaching downward the soil with potential endangering groundwater quality (L'Herroux et al., 1997, Saviozzi et al., 1997, Giusquiani et al., 1998, Nicholson et al., 1999, Aldrich et al., 2002, Taboada-Castro et al., 2002, as cited in Hernandez, at al. 2006).

Bioavailability, mobility and transport of metal ions in soils are strongly influenced by binding reactions with soil organic matter, and especially its humified fractions, i.e., humic substances (HS), of which humic acid (HA) is the major component (Aldrich et al., 2002, as cited in Hernandez, at al. 2006). For these reasons, the effects of PS application on the compositional, structural and functional properties of native soil HA, and especially on their Cu(II) and Zn(II) binding behavior, need to be accurately evaluated. Therefore, The effect of the consecutive annual additions of pig slurry on the Cu(II) and Zn(II) binding behavior of soil HAs was investigated in a field experiment by a fluorescence titration method (Hernandez et al. 2006). In Hernandez et al. study (Hernandez et al. 2006), a fluorescence titration method was used for determining metal ion complexing capacities and stability constants of metal ion complexes of humic acid isolated from pig slurry and unamended and amended soils. The results of this study is expected to have a large impact on bioavailability, mobilization, and transport of Cu(II) and Zn(II) ions in pig slurry-amended soils (Hernandez et al. 2006).

Moreover, a luminescent sensor utilizing the substrate 2,6pyridine-dicarbox-aldehydebis-(*o*-hydroxyphenylimine) has been developed for low concentration detection of the environmental mercuric ion pollutants (Kanan et. al 2009). The sensor selectively detects mercury in the presence of several ions that are commonly found in aquatic environments. The sensor was found to be highly selective with strongest binding was observed between the mercuric ions and the substrate at a pH range of 6.5-7.5 which makes the substrate a distinctive fluorescent sensor for detecting mercury under normal environmental conditions. No effect was demonstrated with the addition of any other metal ions commonly found in water, making this compound selective to mercuric ions.

Dissolved organic carbon (DOC) refers to the hundreds of dissolved compounds found in water that derive from organic materials, and is composed of 'organic acids', 'organic bases', and 'neutral groups'. The amount of DOC in the hydrosphere (700 gigatons) is almost the

same as the amount of carbon in the atmosphere (750 gigatons). However, a large proportion of rainwater DOC is still uncharacterized. A novel method for qualitatively characterizing DOC compounds in rainfall is to use fluorescence. A large proportion of DOC is fluorescent and this fraction can be used to characterize DOC compounds (Baker & Spencer, 2004, as cited in Muller, 2008), yet its full potential for analyzing rainwater DOC compounds has yet to be achieved. Fluorescence can be used to fingerprint fluorophores, identify DOC compounds, and detect small concentration levels which may have otherwise gone undetected, with just 0.04 ml samples. Given the importance of better characterization of rainwater DOC, and the presence of fluorescent DOC compounds, using fluorescence spectrophotometry, primarily sought to examine the fluorescent DOC compounds present in precipitation in Birmingham, UK has been investigated (Catherine et al., 2008). Furthermore, how the fluorescence of the identified DOC compounds varies with meteorological parameters, by assessing the variations with stratiform/convective storm types; examining the variations with air mass type and source area using back-trajectory analysis; and investigating variations with wind speed and wind direction have been investigated (). The results of this study demonstrate the utility of fluorescence analysis for identifying and characterizing rainwater DOC compounds. This research has revealed information regarding fluorescent rainwater DOC compounds in Birmingham, UK and provides evidence in support of using fluorescence spectrophotometry as a means of qualitatively characterizing rainfall DOC.

Organic pollution originating from industrial, agricultural and municipal wastewater discharge has become a common environmental problem in many lakes, rivers, estuaries and coastal waters. The commonly-used methods to study the composition of dissolved organic matter (DOM) (nuclear magnetic resonance, gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry, size exclusion chromatography, etc.) need complicated sample pretreatment procedures and are not suitable for on-line or real-time determination. Excitation-emission matrix (EEM) fluorescence spectroscopy has been suggested as a powerful tool to characterize aquatic DOM which could detect the specific fluorescent fractions in organic matter from different sources with much higher sensitivity (Coble et al., 1996, as cited in Guo, 2010). Therefore, wastewater dissolved organic matter (DOM) from different processing stages of a sewage treatment plant in Xiamen was characterized using fluorescence spectroscopy by Gue at al. (Guo et al., 2010). The results of this study showed that excitation emission matrix fluorescence of wastewater DOM from a sewage treatment plant revealed high concentration of degradable protein-like fluorescence and the occurrence of xenobiotic-like component. This technique provides a fast and sensitive way to monitor the qualitative and quantitative variation of DOM during the whole sewage treatment process.

Perylene-3,4:9,10-tetracarboxylic bisimide, so-called perylene bisimide (PBI) is known as red vat dyes (*fluorescent dyes*), and has been applied to pigments such as automotive finishes due to its light-fastness, thermal stability, and chemical inertness . Perylene bisimide (PBI) is a fluorescent dye which has strong emission and high photostability. Although PBI has been widely used for industrial materials, the application of PBI in analytical fields was limited mainly due to its high hydrophobicity. In recent years, however, unique and useful analytical methods based on PBI platform are being successfully developed by utilizing the characteristic features of this compound including its high hydrophobicity. In this regard, the recent trend of environmental and biological analysis using PBI was reviewed by Soh &

Ueda (Soh & Ueda 2011). Furthermore, the analytical methods presented in this study are classified based on the detection mechanisms.

3.6 Food analysis

Fluorescence spectroscopy is an instrumental technique whose theory and methodology has been widely exploited for studies of molecular structure and function. It is therefore applicable to address molecular problems in food. Since many foods exhibit auto fluorescence it is considered highly relevant for characterization purposes and high-throughput screening. The main advantages of molecular fluorescence spectroscopy are its sensitivity and selectivity, in addition to its ease of use, instrumental versatility, speed of analysis and its non-destructive character.

Recently, it has been shown that excitation-emission matrix (EEM) fluorescence spectroscopy and three-way statistical methods, concretely Parallel Factor Analysis (PARAFAC) can be used for distinguishing between wine samples of different appellations, or between wine samples obtained with different ageing procedure (Airado-Rodrıguez et al., 2011). At first, excitation-emission matrices (EEMs) were obtained and examined with the aim of identifying the main families of fluorescent compounds. Then PARAFAC was applied for exploratory analysis and the scores of the four selected components were obtained. The set of sample score values obtained in the PARAFAC decomposition were plotted against each other, to visualize clustering trends of samples belonging to different appellations. The potential of the autofluorescence of wine, through its Excitation-emission matrices (EEMs), combined with the three-way method PARAFAC for the purpose of discrimination of wine according to the appellation of origin, is shown in this paper. Wine samples were monitored with rapid and non-destructive front face fluorescence spectroscopy, yielding information about the wine throughout its appellation and ageing conditions. PARAFAC revealed four groups of fluorophores that were responsible for the main fluorescence of Spanish wines. Two of them were assigned to benzoic-like phenolic acids and phenolic aldehydes, and to monomeric catequins and polymeric proanthocyanidin dimers, respectively. The exploration of the matrix of score values obtained by PARAFAC reveals some distribution trends of the samples according with their appellation.

Rapid measurements of milk properties and discrimination of milk origin are necessary techniques for quality control of milk products. A study was undertaken to evaluate the potential of using front face fluorescence spectroscopy (FFFS) and synchronous fluorescence spectroscopy (SFS) for monitoring the quality of forty-five ewe's milk samples originating from different feeding systems by Hammamia et al. (Hammamia et al., 2010). Whereas, the physicochemical analyses and fluorescence spectra were conducted on samples during lactation periods (the first 11 weeks). Results obtained showed a good discrimination among milk samples with regard to feeding systems given to the ewes throughout the lactation periods. In addition, a better discrimination was observed with front face fluorescence spectroscopy than with synchronous fluorescence spectroscopy.

Fumonisins (FBs) are worldwide distributed and produced by Fusarium verticillioides and Fusarium proliferatum, mainly in corn and corn-based products (Soriano & Dragacci, 2007, as cited in Silva et al., 2009). Although several other fumonisin analogues have been characterized, fumonisin B1 (FB1) remains the most abundant in naturally contaminated

Corn-based foods followed by fumonisin B2 (FB2).The problems and risks associated with fumonisin contamination have resulted in the development of precise, reliable and sensitive methods for its determination in corn and corn-based foods (Magan & Olsen, 2004, as cited in Silva et al., 2009). Therefore, the quality parameters in the analysis of FB1 and FB2 in corn-based products obtained with LC with fluorescence detector have been investigated (Silva et al., 2009). Furthermore, a comparison study between fluorescence detector (FD), mass spectrometry, and tandem mass spectrometry with a triple quadrupole (QqQ) analyzer using an electrospray ionization interface for the determination of fumonisin B1 and B2 in corn-based products has been performed. A comparative study of the three LC detectors, FD, single quadrupole, QqQ for the analysis of fumonisins in corn samples has been performed. The response achieved by the three detectors was sensitive enough to study the maximum contents established by the EU legislation. These LC detectors would be appropriate for quantification purposes but the acquisition of at least two transitions achieved with QqQ provided a univocal identification.

Low-molecular-weight compounds have been used widely as animal drugs, food additives, and pesticides, to achieve maximum productivity and profits directly or indirectly through food products. However, residues of such low-molecular-weight compounds in food products have been proven to be detrimental to human health. Therefore, development of a microsphere-based competitive fluorescence immunoassay for the determination of hazardous low-molecular-weight compounds in food has been described (Zou et al. 2008). In this method, antigens are covalently bound to carboxy-modified microspheres to compete monoclonal antibody with low-molecular- weight compounds in food samples; mouse IgG/fluorescein isothiocyanate conjugate is used as the fluorescent molecular probe. Thus, the hazardous low-molecular-weight compounds are quantified using a multiparameter flow cytometer. This method has been evaluated using clenbuterol as a model compound. It has a sensitivity of 0.01 ng/mL with dynamic range of 0.01–100 ng/ mL, and the concentration of clenbuterol providing 50% inhibition (IC50) is 1.1 ng/mL. The main advantages of this method are its high efficiency, biocompatibility, and selectivity, as well as ultralow trace sample consumption and low cost.

The aspects of fluorescence spectroscopy that may have value for solving problems in food science and technology have been summarized in a review article by Strasburg & Ludescher (Strasburg et al., 1995). In this review article, the techniques described, which depend on the measurement of the intensity, energy and polarization of fluorescence emission, have been illustrated by examples taken from the food science and related literature.

3.7 Optical sensors

Glucose is considered as a major component of animal and plant carbohydrates in biological systems. It acts not only as a source of energy of the living cells but also as metabolic intermediate in the synthesis of other complex molecules. 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 hypoglycemia. Accurate determination of glucose is very important in clinical diagnosing as well as in food analysis. To date, various sensors for glucose analysis have been reported, and among them, fluorophotometry was used widely owing to its operational simplicity and high sensitivity (Shang et al., 2008, Li et al., 2009, Shiang et al., 2009, as cited in Jin et al., 2011). Recent

advances in the noble metal clusters open a promising field toward the development of a satisfying fluorescence probe. In this regard, recently biomolecule-stabilized Au nanoclusters were demonstrated as a novel fluorescence probe for sensitive and selective detection of glucose (Jin et al., 2011). The fluorescence of Au nanoclusters was found to be quenched effectively by the enzymatically generated hydrogen peroxide (H_2O_2). By virtue of the specific response, the present assay allowed for the selective determination of glucose in the range of 1.0×10^{-5} M to 0.5×10^{-3} M with a detection limit of 5.0×10^{-6} M. In addition, it has been demonstrated the application of the present approach in real serum samples, which suggested its great potential for diagnostic purposes. In comparison with previous approaches for glucose detection, this method required no complicated preparation procedure, and used only commercially available materials. It also exhibited environmentally friendly feature and good sensitivity. Furthermore, the present nanosensor possessed red emission and excellent biocompatibility, which presage more opportunities for studying the biological systems in future applications.

Recently, metalloprotein design and semiconductor nanoparticles have been combined to generate a reagent for selective fluorescence imaging of Pb^{2+} ions in the presence red blood cells (Shete et al., 2009). A biosensor system based on semiconductor nanoparticles provides the photonic properties for small molecule measurement in and around red blood cells. Metalloprotein design was used to generate a Pb^{2+} ion selective receptor from a protein that is structurally homologous to a protein used previously in this biosensing system. This designed protein demonstrates a highly sensitive and selective biosensor that can reversibly detect Pb^{2+} ions in aqueous solutions. The modularity of semiconductor nanoparticle-based biosensors has allowed metalloprotein design and different semiconductor materials to be combined to significantly improve the detection of soluble, exchangeable Pb^{2+} ion concentrations to address inefficient Pb^{2+} ion chelation therapy.

The development of artificial receptors for molecular recognition studies of zwitterion amino acids under the physiological conditions is a very important research area since it can help to understand the important roles of free amino acids in biological systems. In this regard, a new fluorescence macrocyclic receptor based on the Zn(II) complex of a C_2-terpyridine and a crown ether has been developed for molecular recognition of zwitterion amino acids in water/DMF solution with remarkable selectivity towards L-aspartate ($K = 4.5x10^4\ M^{-1}$) and L-cysteine ($K = 2.5x10^4\ M^{-1}$) (Kwong et al., 2009).

Copper is an essential trace element, its deficiency is one of the causes of anemia, but it is toxic at higher concentration levels. The uptake of copper by human beings above a certain level is known to cause gastrointestinal catarrh, Wilson's disease, hypoglycemia, and dyslexia. Increases in copper concentration in water and plants have resulted from industrial and domestic waste discharge, refineries, disposal of mining washings, and the use of copper as a base compound for antifouling paints. Therefore, the trace copper content in water and food must be monitored on a daily basis. In this regard, a highly sensitive and selective optical sensor for the determination of trace amounts of Cu^{2+} based on fluorescence quenching has been developed (Aksuner et al., 2009). The sensing membrane was prepared by immobilization of a novel fluorescent Schiff base ligand 4-(1-phenyl-1-methylcyclobutane 3-yl)-2-(2-hydroxy-5-romobenzylidene) aminothiazole, on polyvinlyl chloride. The accuracy of the proposed sensor was confirmed by analyzing standard reference materials of natural water and peach leaves. The sensor was successfully applied for the determination of copper

in tap water and tea samples. This study showed the application of a PCT dye for preparation of a new Cu^{2+} sensitive optical chemical sensor for the first time. The sensor shows a high selectivity and quick response for Cu^{2+} over other common metal ions.

Heavy metal pollution is a global problem and it causes threat to the environment and human beings. Among the different heavy metal ions, mercury has received considerable attention due to its highly toxic and bioaccumulative properties. It is released from coal burning power plants, oceanic and volcanic emissions, gold mining, and solid waste incineration. Mercury vapor lamps, fluorescent lamps, electrical switches, batteries, thermometers and electrodes are the second largest sources of mercury discharge to the environment. In this regard, an ultrasensitive and selective spectrofluorimetric determination of Hg(II) using 2,5-dimercaptothiadiazole (DMT) as a fluorophore was developed (Vasimalai & John, 2011). In this study, the practical application of the present method was demonstrated by determining Hg(II) in tap water, river water and industrial waste water samples. The obtained results have a good agreement with inductively coupled plasma atomic emission spectrometric (ICP-AES) and atomic absorption spectrometric (AAS) methods. According to the literature, this is the first report for the lowest detection with the highest selectivity for Hg(II) in a water medium by the fluorimetric method.

3.8 Nanomaterials

Noble metal nanocrystals exhibit extraordinary plasmonic properties. The excitation of their localized surface Plasmon resonance modes results in the confinement of electromagnetic waves in regions below the diffraction limit near the metal surface. On the other hand, the localized plasmon modes of elongated metal nanocrystals are inherently anisotropic. The optical signal amplification is therefore expected to be strongly dependent on the excitation polarization direction. Recently, the strong polarization dependence of the plasmon-enhanced fluorescence on single gold nanorods has been reported (Ming et al., 2009). In this study, it has been observed that the fluorescence from the organic fluorophores that are embedded in a mesostructured silica shell around individual gold nanorods was enhanced by the longitudinal Plasmon resonance of the nanorods. The polarization dependence is ascribed to the dependence of the averaged electric field intensity enhancement around the individual gold nanorods on the excitation polarization direction. The maximum fluorescence enhancement occurs when the longitudinal Plasmon wavelength of the Au nanorods is nearly equal to the excitation laser wavelength.

Organic dyes, such as fluorescein, rhodamine, and cyanine, and fluorescent proteins, such as green fluorescent protein (GFP) and its variants, are popular probes for cellular organelles and lipid and protein dynamics, due to their small sizes (<5 nm), high specificity, and aqueous solubility. However, because these fluorescent markers rapidly photobleach, have low quantum yield, and exhibit blinking, it is difficult to obtain quantitative spatial and temporal data on the cellular structures they probe (Yao et al., 2005, as cited in Muddana et al., 2009). Recently, the principle photophysical properties of calcium phosphate nanoparticles (CPNPs) using steady-state and time resolved fluorescence spectroscopy, to demonstrate the potential of these particles for biological imaging and drug delivery and to understand the underlying photophysical mechanisms of encapsulation-mediated fluorescence enhancement have been characterized (Muddana et al., 2009). The enhanced

photophysical properties together with excellent biocompatibility make CPNPs ideal for bioimaging applications ranging from single-molecule tracking to in vivo tumor detection while pH-dependent dissolvability of calcium phosphate offers the possibility of timed co-delivery of drugs to control cell function.

Fluorescent nanoparticles have attracted increasing research attention due to their promising applications covering electro-optics to bio-nanotechnology. In this regard, monodispersed water-soluble fluorescent carbon nanoparticles (CNPs) were synthesized directly from glucose by a one-step alkali or acid assisted ultrasonic treatment (Li et al., 2011). The results showed that the particle surfaces were rich in hydroxyl groups, giving them high hydrophilicity. The CNPs could emit bright and colorful photoluminescence covering the entire visible-to-near infrared (NIR) spectral range. In this study they conclude that combining free dispersion in water (without any surface modifications) and attractive photoluminescent properties, CNPs should serve as a promising candidate for a new type fluorescence marker, bio-sensors, biomedical imaging, and drug delivery for applications in bioscience and nanobiotechnology.

Recent advances in ultrasensitive protein biosensors have brought significant impacts to proteomics, biomedical diagnostics, and drug discovery (Zhu et al. 2001, as cited in Huang & Chen, 2008). Advanced nanoscale biosensors based on nanoparticles, nanowires, and other nanomaterials have been developed to detect various proteins with improved sensitivity, specificity, and reliability (Fu et al., 2007, as cited in Huang, 2008). Ultrasensitive fluorescence nanosensors can detect the fluorescence signal from a fluorescence tag bound specifically with a single target molecule, but the plain fluorescence intensity measurement can hardly discriminate against a nonspecifically bound tag. Therefore, an electrically modulated fluorescence protein assay that can detect specific fluorescence from a single molecule assembled on an Au nanowire by manipulating the molecule with an electrical potential applied on the nanowire have been developed (Suxian et al., 2008). In their study, they conclude that the simple electrically modulated fluorescence detection method can be generally applied to various bioassays. The essential requirement of the method is to selectively modulate the specific fluorescence from the target molecules by an external reference field, which can be achieved by electrical, optical, magnetic, mechanical, or biochemical interactions etc.

Inorganic nanomaterials have been widely used in biological and environmental fields such as bio-labeling, imaging, drug delivery, separation processes and optical sensing. In these applications, the size and the shape of nanomaterials have very important effects on their properties. Recently, much attention has been given to one- dimensional nanomaterials for building various sensors, due to its high activity, high surface-to-volume ratio, easy assembly in an array for the device and especial suitability for intracellular detection by inserting it into cell (Zhang et al., 2004; Park et al., 2007, as cited in Xu et al., 2011). Therefore, a fluorescence sensor for selective detection of Cu(II) realized by covalently immobilizing derivatives of rhodamine6G (R6G) on the surface of silicon nanowires (SiNWs) has been designed and fabricated (Xu et al., 2011) The fabricated SiNWs-based chemosensor can be electively used for detection of Cu(II) with Cu(II)-special fluorescence enhancement over other metal ions. The Cu(II) sensor exhibits a good selectivity and sensitivity.

Drug delivery systems (DDS) are intensively investigated in the recent years for their great potential to improve the therapeutic index of small molecular drugs. In this regard, recently, new types of fluorescent nanoparticles (FNPs) were prepared through ionic self-assembly of anthracene derivative and chitosan for applications as drug delivery carriers with real-time monitoring of the process of drug release (Wei et al., 2011). In this study, the potential practical applications as drug delivery carriers for real-time detection of the drug release process were demonstrated using Nicardipine as a model drug. Upon loading the drug, the strong blue fluorescence of FNPs was quenched due to electron transfer and fluorescence resonance energy transfer (FRET). With release of drug in vitro, the fluorescence was recovered again. The relationship between the accumulative drug release of FNPs and the recovered fluorescence intensity has been established. Therefore, they conclude that such FNPs may open up new perspectives for designing a new class of detection system for monitoring drug release.

Finally, the current state-of-the-art of gold nanoparticles in biomedical applications targeting cancer has been revised (Cai et al., 2008). In which, gold nanospheres, nanorods, nanoshells, nanocages, and surface enhanced Raman scattering nanoparticles have been discussed in detail regarding their uses in *in vitro* assays, *ex vivo* and *in vivo* imaging, cancer therapy, and drug delivery.

3.9 In vivo fluorescence spectroscopy

Monitoring phytoplankton classes and their abundance is a routine task in marine scientific research. With the frequent occurrence of red tide (a colloquial term used to refer to one of a variety of natural phenomena known as a harmful algal blooms), there is an urgent need for rapid analytical methods that can provide qualitative and quantitative information. Therefore, in vivo synchronous fluorescence spectra (SFS) of phytoplankton samples for determining the relative abundance of specific classes of phytoplankton (plant-like organisms that can form dense, visible patches near the water's surface) was investigated (Li et al., 2008). This study demonstrates the potential for determining phytoplankton class abundance by in vivo SFS. The database could be expanded to include more phytoplankton species grown under different nutrient, temperature, and photon flux densities. This work brings an in situ method for determining major phytoplankton class abundance by fluorescence measurement of seawater and deserves further studies.

Magnetic nanoparticles have been widely used in biomedical research such as magnetic carriers for bioseparation (Dolye et al., 2002, as cited in Yoo at al., 2007), enzyme and protein immobilization (Cao et al., 2003, as cited in Yoo at al., 2007), and contrast-enhancing media (Wu et al., as cited in Yoo at al., 2007). In this regard, in vivo fluorescence imaging method of novel threadlike tissues (Bonghan ducts) inside the lymphatic vessels of rats with fluorescent magnetic nanoparticles has been investigated (Yoo et al., 2007). The results of this study showed new applications of nanoparticles for in vivo imaging of hardly detectable tissues using fluorescence reflectance imaging and magnetophoretic control.

Chlorophyll fluorescence has been used as an accurate and nondestructive probe of photosynthetic efficiency, which can directly or indirectly reflect the impacts of environmental factors and changes in the physiological state of the plants (Baker, 2008, as cited in Falco et al., 2011). Recently, the first in vivo observation of chlorophyll fluorescence

quenching induced by gold nanoparticles has been observed (Falco et al., 2011). The results showed that laser-induced fluorescence spectroscopy can be used to investigate the alterations in the physiological response of plants induced by gold nanoparticles, and that both excitation wavelengths, 405 nm and 532 nm, were able to detect the presence of the gold nanoparticles inside the plants. Even though, further investigations must be conducted to clarify the processes of penetration, translocation, and accumulation of nanoparticles in plants.

With the increasing nutrient pollution of freshwater ecosystems, lots of reservoirs, including those used as drinking water resources, suffer from extensive cyanobacterial blooms in the summer. Most of cyanobacteria produce a broad range of compounds with various chemical and toxicological properties. Therefore, their occurrence in recreational or drinking water reservoirs represents high health risk for humans and other organisms. In vivo fluorescence methods have been accepted as a quick, simple, and useful tool for quantification of phytoplankton organisms. In this regard, a case study in which fluorescence methods were employed for the selective detection of potentially toxic cyanobacteria in raw water at the drinking water treatment plant has been presented (Gregor et al., 2007). In this study the author demonstrated that presence of cyanobacteria in raw water can be easily monitored by phycocyanin fluorescence. Measured values were in good correlation with the other parameters of cyanobacterial biomass (chlorophyll a, cell counts). However, the blue light-excited fluorescence of eukaryotic algae should be also monitored to avoid false positive signals.

Photodynamic therapy (PDT) is a treatment modality that may in some cases replace invasive, more harmful or more expensive therapies for certain disorders such as cancer (Dougherty et al., 1998; Ochsner et al., 1997, as cited in Fischer at al., 2002). The PDT treatment process uses photosensitizing compounds that are selectively retained in abnormal tissue some hours to days after administration. At an optimal time after the administration, the photosensitizer is activated with visible light, causing the formation of reactive oxygen species that can initiate a destruction process of the tissue in which they are located. In addition to this photosensitization process, however, most photosensitizers used in PDT show characteristic fluorescence properties (Wagnieres at al., 1998, as cited in Fischer at al., 2002). In this regard, in vivo fluorescence imaging using two excitation and/or emission wavelengths for image contrast enhancement has been described (Fischer et al., 2002). In their study, in vivo measurements in mice where the second image, usually the background signal only, contains new unwanted image data were described. This simple method can successfully resolve the desired image, thus demonstrating the versatility of the image processing procedure.

Currently, the use of 5-aminolevulinic acid (5-ALA) (Zaak, et al., 2001, as cited in Bulgakova et al., 2009) offers the most promising outlook for a fluorescence diagnosis method to reveal superficial bladder cancer. In this regard, a methodological approach combining fluorescence imaging with in vivo local fluorescence spectroscopy (LFS) was clinically tested in order to improve the specificity of photodynamic diagnosis of superficial bladder cancer after intravesical instillation of Alasense (Bulgakova et al., 2009). This preliminary study, which included 62 patients, suggests that in vivo LFS in the course of fluorescence cystoscopy examinations could possibly minimize false positive fluorescence cases and reduce the required number of biopsies from 5-aminolevulinic acid (5-ALA)-based agent induced red fluorescence zones.

4. Comparison of fluorescence spectroscopy with other spectroscopic methods as an analytical technique

Compare to other analysis techniques, it must suffice here to add that fluorescence measurements are rapid, accurate and require only very small quantities of sample (nanomole or less). Fluorescence instrumentation is also relatively inexpensive and easy to use. In general, fluorescence experiments are relatively easy to perform; as in many fields, it is the planning of appropriate experiments, the analysis, and accurate interpretation of the data that require more extensive experience.

Fluorescent methods have three significant advantages over absorption spectroscopy and other typical optical spectroscopy. First, two wavelengths are used in fluorimetry, but only one in absorption spectroscopy. Emitted light from each fluorescent color can be easily separated because each color has unique and narrow excitation spectra. This selectivity can be further enhanced by narrowing the slit width of the emission monochromator so that only emitted light within a narrow spectral range is measured. Multiple fluorescent colors within a single sample can be quantified by sequential measurement of emitted intensity using a set of excitation and emission wavelength pairs specific for each color. The second advantage of fluorescence over absorption spectroscopy is low signal to noise, since emitted light is read at right angles to the exciting light. For absorption spectrophotometry, the excitation source, sample and transmitted light are configured in line, so that the absorption signal is the small difference between the exciting light and the transmitted light, both of which are quite intense. The third advantage is that fluorescent methods have a greater range of linearity. Because of these differences, the sensitivity of fluorescence is approximately 1,000 times greater than absorption spectrophotometric methods (Guilbault, 1990). However, a major disadvantage of fluorescence is the sensitivity of fluorescence intensity to fluctuations in pH and temperature. However, pH effects can be eliminated by using nonaqueous solvents, and normal room temperature fluctuations do not significantly affect the fluorescence intensities of commercial dye solutions.

In addition there are other useful fluorescent techniques that have an advantages over the other typical optical spectroscopy, that not have been mentioned in this chapter, these are: (1) polarization (anisotropy), this technique gives information about the rotation of a fluorophore, and allow to infer protein shape, membrane fluidity (order parameters), and binding analysis; (2) quenching fluorescence, these processes can occur during the excited state lifetime –for example collisional quenching, energy transfer, charge transfer reactions or photochemistry –or they may occur due to formation of complexes in the ground state, thus this technique can give a useful kinetic information of the tested system, and give information about accessibility of fluorophoresis obtained allowing to correlate it with changes in protein or membrane structure; (3) Förster resonance energy transfer (FRET), this technique can be considered as a molecular ruler, which allow to determine distance from 10 to 80 Å; (4) fluorescence microscopy for image analysis, this technique gives magnification (in order to see small parts), resolution (in order to distinguish details of the small parts), and contrast (in order to magnify and resolve details, fluorescence emission provides contrast); (5) multiphoton excitation fluorescence microscopy, as an example for two photon excitation a molecule can be excited by using simultaneous photons and get fluorescence as happens with one photon excitation, this techniques has so many advantages such as sectioning effect without pinholes, low photobleaching and photodamage rate, separation of

excitation and emission, no Raman from the solvent, deep penetration in tissues, single excitation wavelength for many dyes, avoid chromatic aberrations, and no expensive UV optics (for UV excited fluorophores) needed. Even though it has some disadvantages such as only is suitable for fluorescence images (reflected light images is not currently available), the technique is not suitable for imaging highly pigmented cells and tissues which absorb near infrared light, and laser source is expensive. It is worth mentioning that all analysis that can be done using fluorescence spectroscopy can be done using a multi-photon excitation fluorescence microscopy. However, multi-photon excitation fluorescence microscopy has a unique analysis applications over the fluorescence spectroscopy such as: analysis of deep tissue imaging (Brain, skin, etc), can prime photochemical reaction within subfemtoliter volumes inside solutions, cells and tissues (photolabile "caged" compounds), can be used for imaging of living specimen for longer period of time, and for live animal imaging (intrinsic fluorophores).

5. Conclusion

It could be concluded at the end of this chapter, that the fluorescence spectroscopy is intensely employed as a powerful spectroscopic tool for quantitative analysis, characterization, and quality control in different fields such as inorganic, organic, pharmaceutical, biological and biomedical, food, and environmental analysis. Furthermore, fluorescence spectroscopy has so many applications in optical sensors, and nanamaterials.

Compare with other analytical techniques, it must suffice here to add that fluorescence measurements are rapid, accurate and require only very small quantities of sample (nanomole or less) with high selectivity. The principal advantage of fluorescence over radioactivity and absorption spectroscopy is the ability to separate compounds on the basis of either their excitation or emission spectra, as opposed to a single spectra. This advantage is further enhanced by commercial fluorescent dyes that have narrow and distinctly separated excitation and emission spectra.

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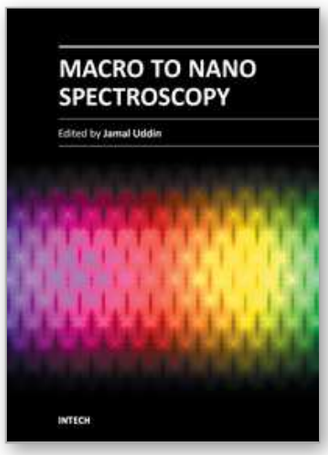

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In the last few decades, Spectroscopy and its application dramatically diverted science in the direction of brand new era. This book reports on recent progress in spectroscopic technologies, theory and applications of advanced spectroscopy. In this book, we (INTECH publisher, editor and authors) have invested a lot of effort to include 20 most advanced spectroscopy chapters. We would like to invite all spectroscopy scientists to read and share the knowledge and contents of this book. The textbook is written by international scientists with expertise in Chemistry, Biochemistry, Physics, Biology and Nanotechnology many of which are active in research. We hope that the textbook will enhance the knowledge of scientists in the complexities of some spectroscopic approaches; it will stimulate both professionals and students to dedicate part of their future research in understanding relevant mechanisms and applications of chemistry, physics and material sciences.

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