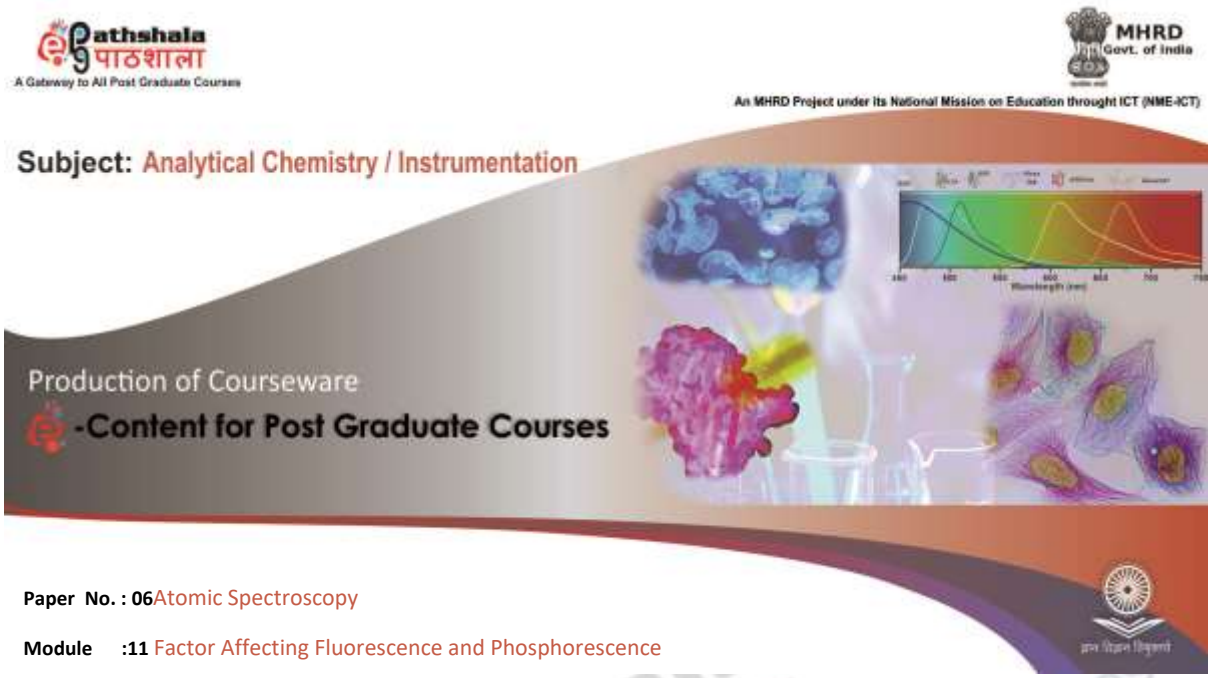

Paper No. : 06 Atomic Spectroscopy

Module :11 Factor Affecting Fluorescence and Phosphorescence

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Description of Module	
Subject Name	Analytical Chemistry / Instrumentation
Paper Name	Atomic Spectroscopy
Module Name/Title	Factor Affecting Fluorescence and Phosphorescence
Module Id	11
Pre-requisites	
Objectives	Brushing the concept of F&P Factors that affect the F&P The source of light and concentration of the analyte can affect the intensity of fluorescence The importance of transition type, structure and its rigidity The importance of solvent, pH of the solution and temperature with respect to F&P
Keywords	Fluorescence, Structure rigidity, triplet state, dissolved oxygen, resonance fluorescence

1. INTRODUCTION

If a molecule is excited to give a species whose emission spectra provides information for qualitative and quantitative analysis. The method is collectively called molecular luminescence procedures. Conveniently luminescence can be defined as the photons emitted by an atom or a molecule from its excited state after absorbing the energy. Luminescence can be classified into two main categories, fluorescence and phosphorescence. The two phenomena fluorescence and phosphorescence are often referred as photoluminescence because the excitation is brought in similar fashion which is by absorption of photons.

Fluorescence and phosphorescence differs from each other in that the electronic energy transition involved in fluorescence is singlet excited state without change in spin in contrast the transition in phosphorescence occur from triplet state with a change in electron spin. As a result the life time of fluorescence is short with luminescence ceasing almost immediately (10^{-5}s), whereas the change in electron spin in phosphorescence causes radiations to endure for an easily detectable time after termination of source of energy. Photoluminescence is always longer in wavelength than the source radiation used for its excitation because of number of reasons involved and the same will be discussed in the topic later.

2. **Theory**: Molecular orbital and atomic orbital are the places where the probability of finding an electron is high therefore these orbital determine the energy of the molecular as well as atomic in a molecule or atom respectively.

The absorption of photons/light results in transition of electrons from ground state of an atom or molecule to excited state. In atoms an electron from an outer shell/orbital is promoted to an empty orbital of higher energy and in case of molecules, promotion of an electron from the HOMO (highest occupied molecular orbital) to the LOMO (lowest unoccupied molecular orbital) takes place. Promotion of a valence electron due to the absorption of a light/photon from its ground state to an excited state with conservation of the electron's spin results in singlet excited state. For example, a pair of electron in an orbital must have opposite spins according to Pauli's exclusion principle, occupying the same electronic ground state (Figure 1) and are said to be in a singlet spin state. After absorbing a photon one of the electrons is excited state (Figure 1b) and goes to next level, a phenomenon called as "excitation" and the state is called excited singlet state as the spin is still paired. Since the excited states are not stable and therefore will not stay indefinitely. The electron will return to ground state by releasing the excess of energy absorbed. This release of energy to return back to normal is a process called decay, deactivation or relaxation. When under some special conditions, this

excess energy absorbed is released during the relaxation in the form of a photons, such type of relaxation is called emission. The phenomenon of release of excess energy or emission of a photon from a singlet excited state to a singlet ground state, or between any two energy levels with the same spin, is known as ***fluorescence***. Since the average lifetime of an electron in an excited state is only 10^{-5}–10^{-8} s the probability of a fluorescent transition is very high and due to this reason fluorescence decays immediately after the removal of excitation source.

Phosphorescence: Phosphorescence is also a radiative process. When the spin of the electron in excited state gets changes it is called triplet excited state (T_1) i.e. the spin of the excited electron is no longer paired with that of the ground state electron (Figure 1c). Due to this reason the lifetime of the electron in excited state increases and when such an electron release that excess energy in the form of light to come to ground state the phenomenon is called phosphorescence.

So phosphorescence describes the radiative process originating from T1 to So. The required spin inversion of one of the electrons when it moves from the LUMO to the half filled HOMO is a forbidden process. When observed it occurs on a timescale much longer than the fluorescence process. In most cases it can only be observed in frozen glass solutions at low temperature (77 K). As a result the average lifetime for phosphorescence ranges from 10^{-4} to 10^4 s, and phosphorescence may continue even after removing the source of excitation.

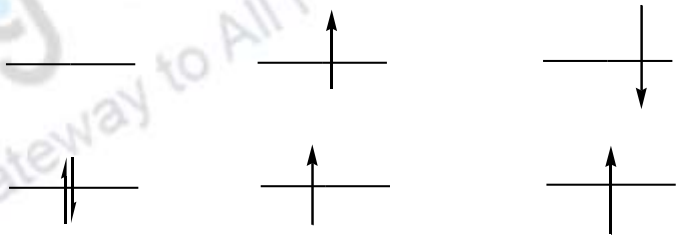


Figure 1 a) Singlet ground state b) Singlet excited state c) Triplet excited state

Fluorescence –can be seen in simple as well as in complex gaseous, liquid and solid chemical system. Vaporized sodium atom is simplest example where 3s electrons are excited to 3p orbit by absorption of λ5896 Å and 5890 Å. After 10^{-5}-10^{-8}sec. Electrons return to ground state emitting radiation of same two wavelengths. Such a phenomenon is known as resonance fluorescence.

The ratio of the number of molecules that luminesces to the total number of excited molecule is fluorescence and phosphorescence. For highly fluorescent molecule the ratio approaches unity for example fluorescein.

3. FACTORS AFFECTING THE FLUORESCENCE

Fluorescence is affected by number of factors. The factors are broadly divided as those, which are related to intrinsic structure of the molecule, called as **intrinsic factors** and the environmental of the molecule called as **extrinsic factors**

3.1. INTRINSIC FACTORS

3.1.1. Transition type in Fluorescence:

Fluorescence produced mainly arises due to transition of electrons from the first excited electronic state to one of the ground vibrational state. The radiation is produced by a transition involving π-π* excited state rather n- π* because for π-π* transition the molar absorptivity is 100 - 1000 folds greater than for an n- π* transition. The inherent life time associate with π-π* is shorter 10^{-7} to 10^{-9} sec as compared to the n-π* (10^{-5} to 10^{-7}) and fluorescence is larger because less time for deactivation is available.

3.1.2. Quantum efficiency or yield:

Quantum yield is the ratio of molecule that fluoresces to the total number of molecules that are excited. For highly fluorescent molecule the quantum efficiency approach unity under the same condition e.g. fluorene. Molecules which do not show fluorescence have quantum efficiency that approach to zero. It is denoted by φ and is determined by rate constant for the processes by which lowest excited singlet state is deactivated e.g. fluorescent (K_f), internal conversion (K_{ic}), external conversion (K_{ec}), intersystem crossing (K_{isc}), predissociation (K_{pd}) and dissociation (K_d)

$$\Phi = \frac{K_f}{K_f + K_{ic} + K_{ec} + K_{isc} + K_{pd} + K_d}$$

The equation permits the quantitative interpretation of structural and environmental factor which effect the fluorescence e.g. K_f, K_{pd}, K_d are mainly dependent on the structure of the molecule, whereas the other on the environment.

Relative fluorescence quantum yields can be determined experimentally, by measuring fluorescence of a fluorophore of known quantum yield with the same experimental parameters (excitation wavelength, slit width, photomultiplier, voltage etc.) as the substance under test.

$$\phi = \phi_R \times \frac{Int}{Int_R} \cdot \frac{1 - 10^{-AR}}{1 - 10^{-A}} \cdot \frac{n^2}{n_R^2}$$

where Φ is the quantum yield, Int is the area under the emission peak (on a wavelength scale), A is absorbance (also called "optical density") at the excitation wavelength, and '*n*' is the refractive index of the solvent. The subscript R denotes the respective values of the reference substance

3.1.3. Structure

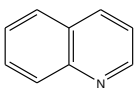
Aromatic compounds or compounds having aromatic functional groups with low energy transition level (π-π*) show the most intense and most useful fluorescent behavior. Aliphatic, alicyclic or highly conjugate systems may also show fluorescence. Most of the unsubstituted aromatic compounds fluoresce is solution. The quantum yield/efficiency increases with increase in number of rings and their degree of condensation. Heterocyclic compounds have lowest energy electronic transition level of n-π* that rapidly converts to the triplet state and thus prevents flourescence e.g. pyridines, furans, thiophene etc. but enhances phosphorescence.

Pyrimidine

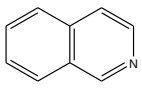
Furan

Thiophene

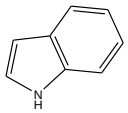
Fused benzene rings also show fluorescence e.g.



Quinoline



Isoquinoline



Indole

Fluorescence decrease with halogen substitution because with increase in atom number of halogens cause (heavy atom effect which enhances inter system crossing). Substitution by carboxylic or carbonyl group also decreases fluorescence due to n-π* transition.

The nature of groups attached to the fluorophore also affects the intensity of fluorescence for example electron donating groups- fluorescence intensity increases**.** That is because the substituents delocalize the pi-electrons and they tend to increase the transition probability between the lowest excited singlet state and the ground state:

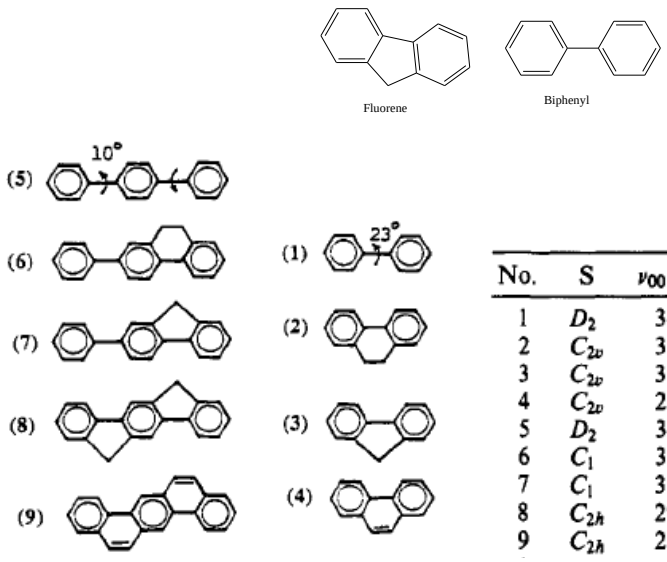
–OH, –NH_2, –OEt, –OMe,

- $-NO_2$, –NHR, $-NR_2$, –CN,
- Electron Withdrawing Groups- fluorescence intensity **Decreases**
- –COOH, –COR, –CHO,
- –COOR,–F, –Cl, –Br, –I, –SH,
- Groups having no effect on fluorescence
- $-NH_4^+$, $-SO_3H$,–alkyl group

3.1.4. Structure rigidity:

Fluorescence from a molecule has been observed to increases with increase in structure rigidity. The shape and wavelength position of the fluorescence spectra and fluorescence parameters of aromatic molecules are largely determined by the molecular structure of the fluorescence species. For example structure less absorption and fluorescence spectra are seen with non-planar molecules where in case of rigid molecules of the high-symmetry group show well resolved absorption and fluorescence spectra with well-resolved vibrational bands are observed. Sometimes the absorption and fluorescence spectra of a planar and rigid compound show a similar structural pattern and display mirror symmetry.

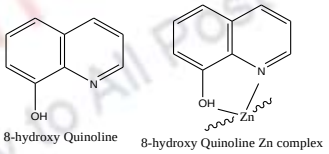
Very often, transition from a nonplanar molecule to a similar but more planar and rigid molecule is accompanied by an increase in quantum yield of fluorescence. For instance, biphenyl in solution is nonplanar and has a very wide structureless absorption band and a structural fluorescence spectrum. They do not show any mirror symmetry between them, but when phenyl rings of biphenyl are forced into a planar position by bridging with the introduction of a methylene group (fluorene), both the absorption and fluorescence spectra become very sharp. They also display mirror similarity, and the quantum yield values increase from 0.18 to 0.80. Earlier it was believed that the ability of some molecules to emit fluorescence radiation was totally attributable to molecular rigidity. Berlman, (Berlman, I. B. J. Phys. Chem. IWO, 74, 3085) however, showed later that rigidity in the SO state was not as important a factor as rigidity in the first excited S^1 excited state, that is, in maintaining a planar or near planar configuration. Currently there is no doubt that the planarity and rigidity of a molecule play important roles in determining the fluorescence parameters of a compound.



No.	S	ν_{00} (cm^{-1})	$\Delta\nu_{st}$ (cm^{-1})	γ
1	D_2	34 600	9260	0.17
2	C_{2v}	32 760	3480	0.50
3	C_{2v}	33 140	2740	0.72
4	C_{2v}	28 900	3260	0.13
5	D_2	31 960	7860	0.84
6	C_1	30 780	6640	0.80
7	C_1	31 080	5940	0.83
8	C_{2h}	29 860	2900	0.86
9	C_{2h}	25 300	2600	0.18

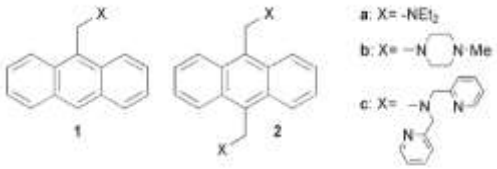
Figure 2: Influence of planarity and rigidity on the absorption and fluorescence paramenters and intersystem crossing rate constant in aromatic molecules (N I Nijegorodov and W S Downey J. Phys. Chem. 1994, 98, 5639-43)

Complexation also results in increase in fluorescence e.g. 8-hydroxy quinolone has less fluorescence than its Zn complex.



8-hydroxy Quinoline 8-hydroxy Quinoline Zn complex

Enhanced fluorescence has been observed on complexation of diethylamine (**1a, 2a**) and N-methyl-piperazine derivatives (**1b, 2b**) with Ni^{2+}, Cu^{2+} and Zn^{2+}. Where as the fluorescence intensities of N,N-bis(2-picolyl)amine derivatives (**1c, 2c**) are enhanced by the presence of Zn^{2+} and vice versa by the presence of Ni^{2+} and Cu^{2+}.



Scheme 1

(Kanji Kubo and Akira Mori, PET fluoroionophores for Zn^{2+} and Cu^{2+}: complexation and fluorescence behavior of anthracene derivatives having diethylamine, N-methylpiperazine and N,N-bis(2-picolyl)amine units. Journal of Materials Chemistry, 2005, 15, 2902–2907)

3.2. Extrinsic factors

3.2.1. The light source

When a sample solution is excited to excited singlet state, the fluorescence that emanates from the sample propagates in all directions. The fluorescence photons that pass through the emission monochromator (EmM, Figure 19) are only a small fraction of the total fluorescence intensity. It is necessary therefore to use a light source that has a relatively high intensity light output. In very simple fluorescence spectrometers used for routine dedicated analyses, a Mercury arc lamp may be satisfactory, but it only produces light at a limited number of wavelengths in the ultraviolet and visible spectral range (e.g. 253.7, 302.2, 313.2, 435.8, 546.1 nm). The continuum lamps used in absorption spectrometry, deuterium and quartz/ iodine sources have too low an intensity and hence the fluorescence intensity is also very low. Most fluorescence spectrometers use a high pressure xenon arc lamp as an excitation source since it produces a useful high intensity continuum light spectrum from 200 nm to 1000 nm (Figure 20). These lamps are designated according to the electrical power consumed, i.e. 150 W, 450 W, 1000 W. In instruments recently made available, a 75 W xenon arc is used, but it is pulsed, so that the peak intensity of each pulse is high. Both molecular and chemical environment determine whether substances will or will not fluoresce

3.2.2. **Concentration:**

The power of fluorescence radiation (F) is proportional to the radiant power of the excitation beam that is absorbed by the system.

$$F = Kc$$

The above equation shows F is directly related to concentration.i.e. with increase in concentration of the fluorescence species the fluorescence will increase linearly but this holds true for dilute solutions only. The graphs below reveal the effect of concentration onfluorescence.

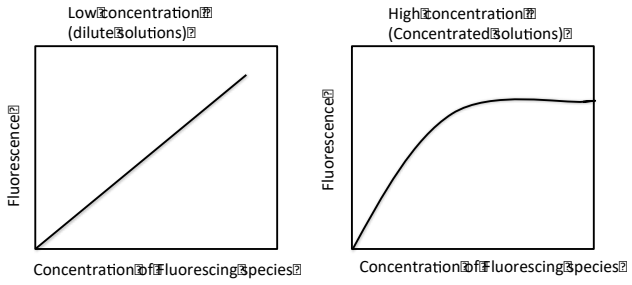


Figure 3: Deviation at higher concentration from the normal may be due to self quenching or self absorption

3.2.3. Temperature

Quantum efficiency decreases with increase in temperature, as frequency of collision increase which increases probability for deactivation by external conversion. Decrease in viscosity of solvent also leads to external conversion and it has same effect as with increase in temperature.

This can be explained by the example of study of fluorescence of a xanthene dye Rhodamine B studies in water using cuvettes at different temperatures.

The Rhodamine, has been extensively used for both fluorescence standard. The temperature dependent emission spectra ofRhodamine-B in water are displayed in Figure 1 from 16 different temperatures between 5˚C to 80˚C. The emission intensity increases as temperature decreases. As the absorbance stays constant over this temperature range we conclude that this is consistent with a change in the fluorescence quantum yield. Temperature dependent changes of Rhodamine-B in a different solvent is different. The temperature also affects the radiative lifetime as shown in Figure 2 for the sametemperature range.

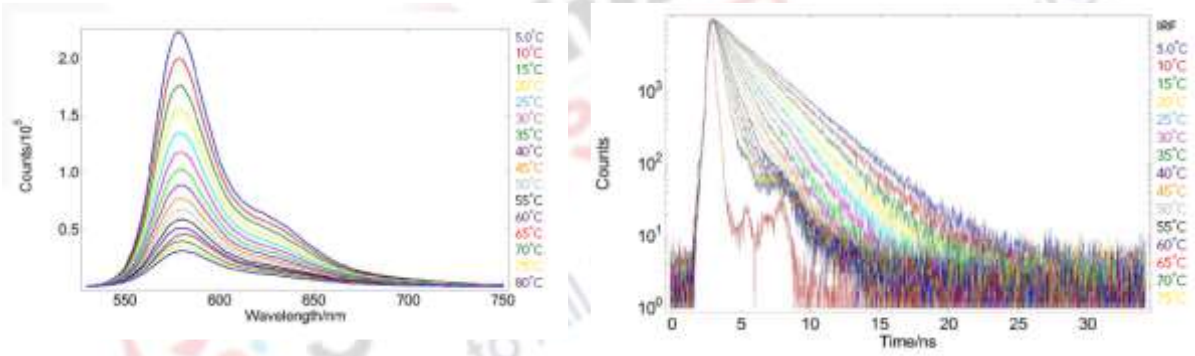


Figure 4: Emission spectra of Rhodamine-B in H_2O for temperatures 5 ˚C – 80˚C and Radiative decays of Rhodamine-B in H_2O from 5 ˚C – 80˚C

Technical note, Quenching of fluorescence with temperature by Edinburgh Instruments limited. www.edinst.com
Source: Georgios Arnaoutakis, Dirk Näther , Quenching of fluorescence with temperature TN_P27 v.2 15 Feb. 16

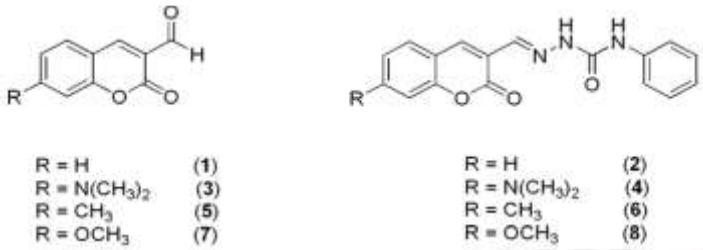
Solvent effect

A change of solvent is accompanied by a change in polarity, dielectric constant and change in polarizability of the surrounding medium. Thus the change of solvent affects the ground state and the excited state differently. The absorption as well as emission spectra are affected by the properties of the solvent. With the increasing solvent polarity, both absorption and emission bands undergo a bathchromic shift, the latter being more pronounced than the former. Polarity of the solvents causes increase in n-π* transition while π-π* suffers opposite. For example coumarin laser dyes C307 and C522B shows a bathchromic shift in solvent with high the polarity indicating $\pi \rightarrow \pi^*$ transition.

Figure 5: Molecular structure of (a) C307 and (b) C522B

Fluorescence decrease by solvent containing heavy atom like carbon tetrabromide, ethyl iodide etc

The effect of solvent on fluorescence of Coumarin compounds is discussed by following example



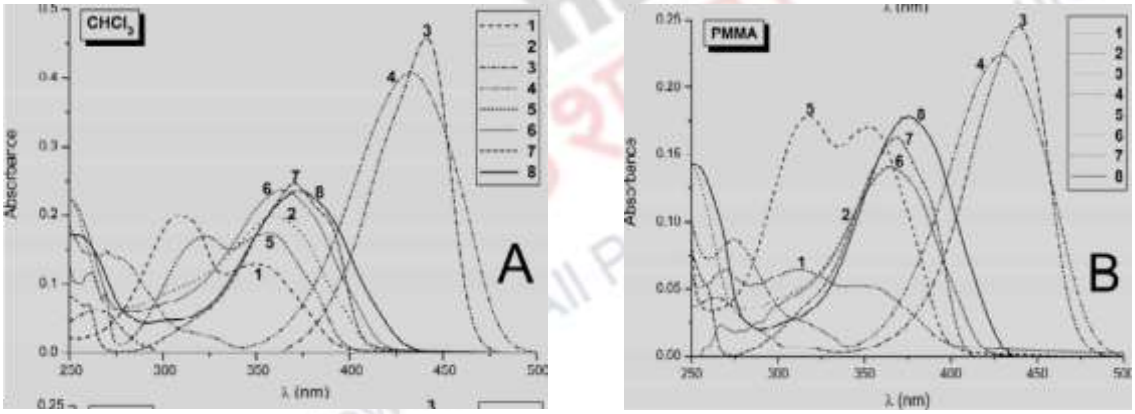
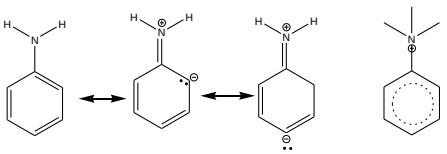


Figure 6. (A) Absorption spectra of coumarins 1–8 in chloroform at 10^{-5} mol·dm^{-3}; (B) absorption spectra of coumarins 1–8 in PMMA at 0.002 mol·kg^{-1} (Source: Molecules 2012, 17, 3259-3276)

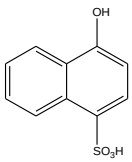
3.2.4. Effect of pH on fluorescence

The fluorescence of the acidic and basic compound is usually pH dependent. Both wave length and intensity are different for the ionized and unionized forms of the compound

e.g aniline and anilinium ion



Aniline in resonance structures and anilinium ion



1- napthol-4-sulphonic acid

The change in the emission results from the differencing number of resonance species that are associated with the acidic and basjjic forms of the molecule. The additional resonance forms leads to a more stable first excited state fluorescence. Fluorescence of phenolic form of 1-napthol-4-sulphonic acid is not detectable by eye but when it is converted to phenolate ion by addition of base. The emission shifts to visible wave length.

7-Hydroxycoumarin exhibits an intense blue or blue-green fluorescence band at λ 460 nm at the pH below 2.2. The change in pH also results in change in emission spectra

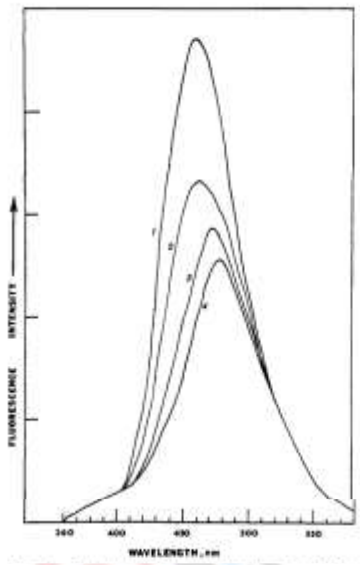


Figure 7: Umbelliferone fluorescence spectrum as a function of pH: 7-Hydroxycoumarin concentration = 8 X 10~7M; excitation wavelength: 330 nm; Spectrum 1) pH 2.2; 2) pH 1.8; 3) pH 1 .5; 4) pH 1.2

Fluorescence emission spectra of indolcarboxylic acids also shows changes in aqueous solution of different pH at 298K

Table 1: The effect of pH on electronic absorption spectral data for the indolecarboxylic acids (pH 1.2, 7.0, 12.5, and in 5 N NaOH in aqueous solutions) (Source: J. J.AARON et al. Journal of Luminescence 33 (1985) 33—51 33)

pH →	1.2	7.0	12.5	5 N NaOH
Acid	λ_{em}(nm)	λ_{em}(nm)	λ_{em}(nm)	λ_{em}(nm)
3-Indoleacetic	369	378	379	433
3-Indolepropionic	377	381	381.5	436
3-Indolebutyric	380.5	381.5	383	440.5
2-Indolecarboxylic	422	372.5	375.5	410.5
5-Indolecarboxylic	454.5	404.5	412	474.5
3-Indolelactic	371	375	377	437
3-Indolepyruvic	405.5	379.5	384.5	429
3-Indoleacrylic	394	372.5, 413	374, 436	452.5

* All concentrations approximately 5×10^{-4} M. The spectra have not been corrected for the instrument reponse. The wavelengths of the main peaks are underlined; the wavelengths of shoulders are given in parentheses. Peak wavelength error: ±1 nm.

In the case of 2-indolecarboxylic acid, 5-indolecarboxylic acid, 3-indoleacetic acid, 3-indolepropionic acid, 3-indolebutyric acid, and 3-indolelactic acid, the UV absorption band maxima wavelengths do not change significantly with pH and they possess a vibrational structure which is independent of pH. For the four latter compounds, the first band contains three maxima characteristic of the indole ring. Even in a strongly alkaline medium (5 N NaOH), the vibrational structure of the absorption bands is not altered, which indicates the absence of any ground-state deprotonation reaction of the N—H group of the indole ring, as it was expected from the large PKa values previously reported for the equilibrium between indole and the indole anion

Effect of dissolved oxygen:

Dissolved oxygen causes decrease in emission intensity of fluorescent solution. It may be due to photochemical oxidation of the fluorescence species. The paramagnetic properties of molecular oxygen are responsible for loss of fluorescence. It also promotes intersystem crossing and conversion of excited singlet state molecule to the triplet state molecule.

Summary:

The topic discuss about the various factors that affect the fluorescence of a molecule. It explains how intrinsic and extrinsic factors increase or decrease the fluorescence intensity of a molecule. The intrinsic properties of a molecule that may affect its fluorescence includes transition type, structure and its rigidity whereas the extrinsic factors include, the light source, concentration, temperature, solvent effect, effect of ph on fluorescence, effect of dissolved oxygen