The discovery and characterization of Luminescence

Nicolás Monardes (1506) was the first to describe the bluish opalescence of the water infusion from the wood of a small Mexican tree.

Galileo (1612) described the emission of light (phosphorescence) from the famous Bolognian stone, discovered by Vincenzo Casciarolo, a Bolognian shoemaker. Galileo wrote: "It must be explained how it happens that the light is conceived into the stone, and is given back after some time, as in childbirth."

Sir John Herschel (1845) made the first observation of fluorescence from quinine sulfate

Sir George Stokes (1852) coined the term "**Fluorescence"**

Stokes used sunlight to illuminate a quinine solution and observed the emission through a stained glass filter

He showed that the fluorescence emission occurred at a higher wavelength (lower energy) than the excitation light. This displacement is now called the **Stokes Shift**

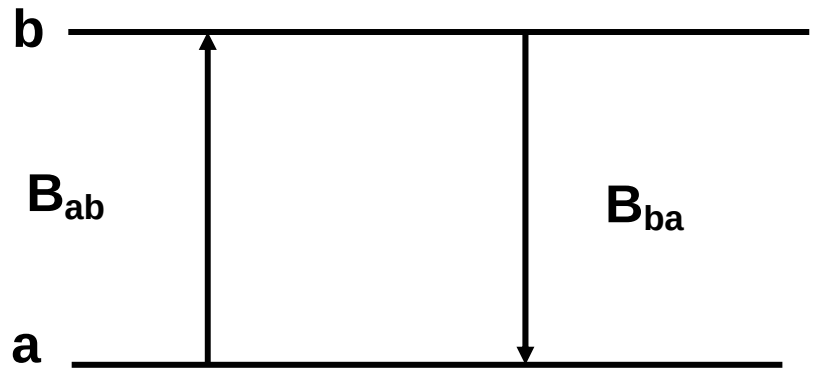
Adolph Von Baeyer (1871), a German chemist, synthesized the fluorescent dye, fluorescein.

Edmund Bequerel (1880) Showed that certain metal ion complexes emit radiation with a very long decay time.

Fluorescence spectroscopy: Part I

Consider a molecule with two energy levels

S_a and S_b



Define the rate of transition B_{ab} induced by light of frequency ν

$$B_{ab} = (3\pi/2h^2) \left| \langle \psi_b \left| \mu \right| \psi_a \rangle \right|^2$$

We have seen also that the **radiation induced** process B_{ba} occurs at the same rate

$B_{ab} = B_{ba}$ Einstein coefficients

There is a different way for a molecule to pass from, S_b to S_a: **SPONTANEOUS EMISSION**

Designate by A_{ba} the rate of spontaneous emission. A_{ba} must be independent of the radiation density $I(\nu)$.

At equilibrium the rate of conversion $S_a \leftrightarrow S_b$ must be the same.

$$\frac{n_a}{n_b} = \frac{B_{ba}I(\nu) + A_{ba}}{B_{ab}I(\nu)} = 1 + \frac{A_{ba}}{B_{ab}I(\nu)} \qquad (1)$$

n_a are molecules in state A

n_b are molecules in state B

But at equilibrium, from a statistical mechanics point of view

$$\frac{n_a}{n_b} = e^{-\frac{(E_a - E_b)}{kT}} = e^{\frac{h\nu}{kT}} \qquad (2)$$

Equation 1 and 2 must be equal.

$$e^{\frac{h\nu}{kT}} = 1 + \frac{A_{ba}}{B_{ab} I(\nu)} \qquad (3)$$

At equilibrium I(ν) is the radiation density of a black body at temperature T

$$I(\nu) = \frac{8\pi h\nu^3}{c^3} \frac{1}{e^{\frac{h\nu}{kT}} - 1}$$

Using this equation in 3

$$e^{\frac{h\nu}{kT}} = 1 + \frac{A_{ba}}{B_{ab}} \frac{e^{\frac{h\nu}{kT}} - 1}{8\pi h\nu^3 c^{-3}}$$

Solving for A_{ba}

$$A_{ba} = \frac{B_{ab}}{c^3} \frac{8\pi h\nu^3}{c^3}$$

The rate of spontaneous to stimulated emission increases as ν^3

B_{ab} can be measured from the area under the absorption band and is proportional to the extinction coefficient ε. In principle the rate of spontaneous emission can be measured from the absorption spectrum.

Because A_{ba} is the rate of deactivation of state S_b, the corresponding **radiative lifetime** will be

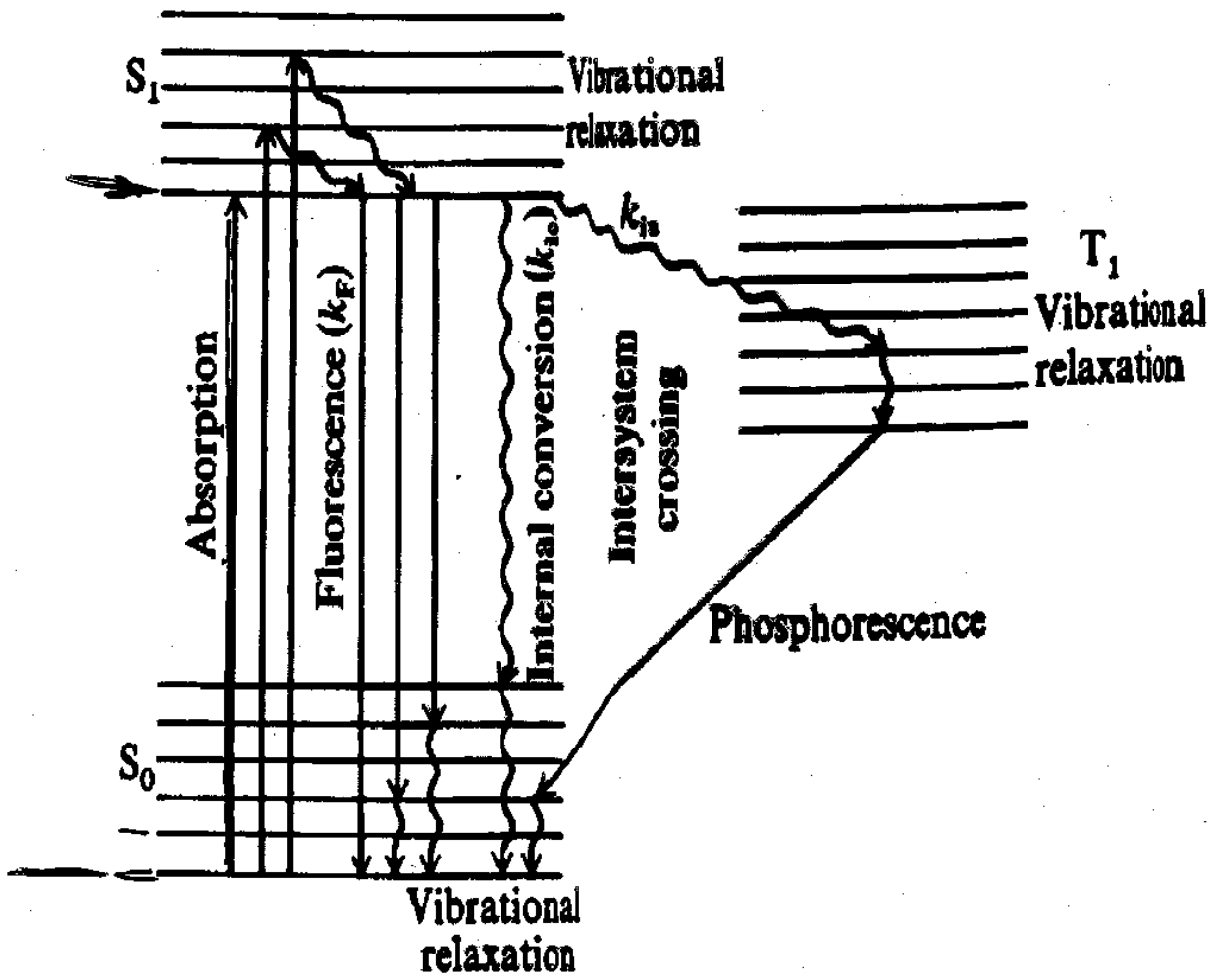
$\tau_r = 1/A_{ba}$

This equation shows the general principle that the stronger the absorption, the shorter the radiative lifetime.

The radiative relationship is valid in the absence of radiation or any other kind of perturbation. This relationship is also valid if the same state that absorbs the light is the one from which the light is emitted.

τ_r is called the **radiative unperturbed lifetime**. In reality there are many different ways an excited state can be perturbed. The de-excitation process gives valuable information about the structure and dynamics of biological molecules.

The general scheme for de-excitation



1. Absorption
2. Vibrational relaxation
3. Fluorescence
4. Internal conversion
5. Intersystem crossing
6. Phosphorescence

Vibrational de-excitation (internal conversion)

This process is caused by collisions with solvent molecules and with phonon modes in the molecule.

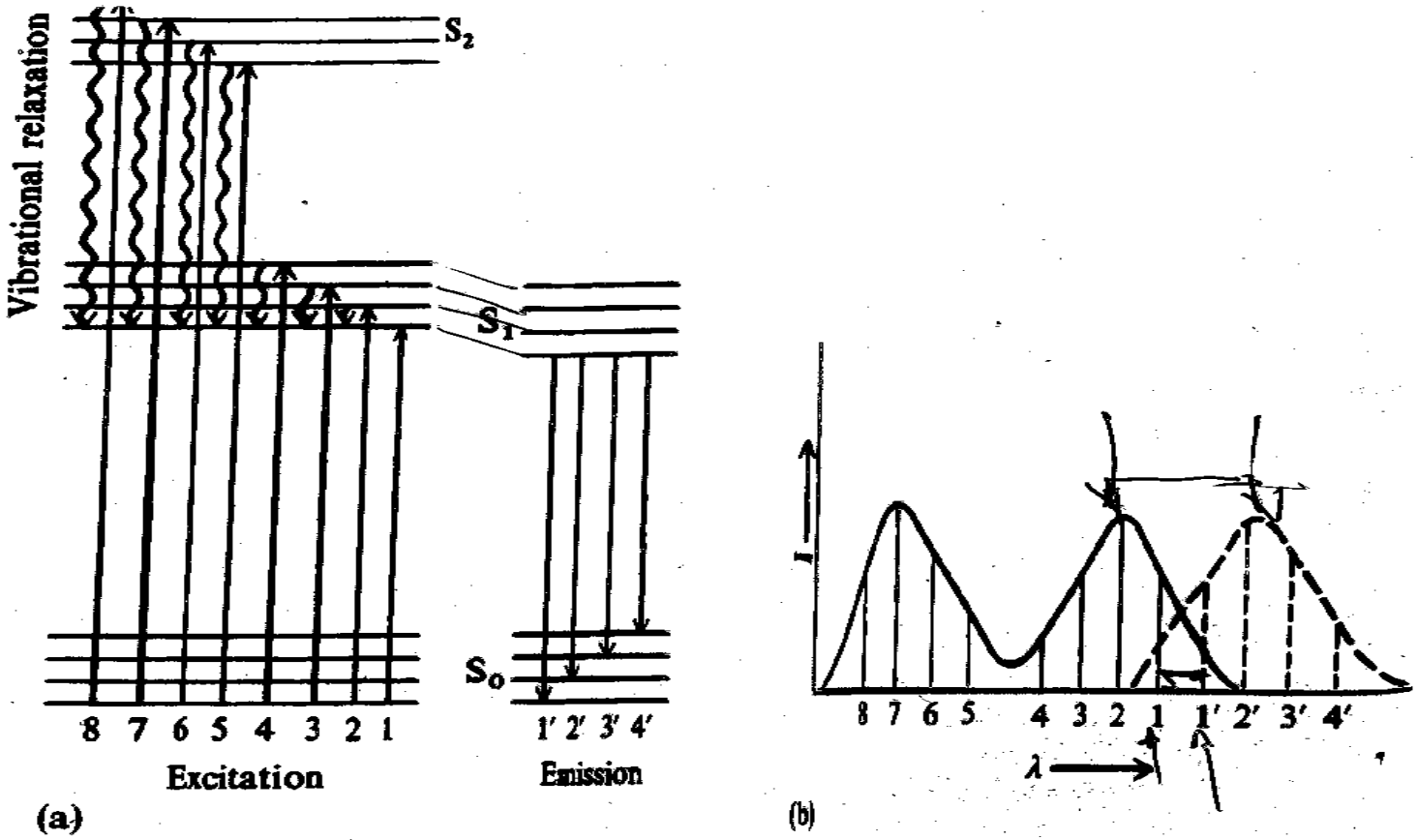
Generally vibrational relaxation is much faster than photon emission. The consequence is that only the ground vibrational level of the excited state is populated at the emission time. This observation has several important consequences

- The spectrum of the emitted light is independent of excitation wavelength

- The shape of the emission band is the mirror image of the absorption band provided that they have similar vibronic structure.

- The transition from S_a ground to S_b lower vibrational level will be at the same energy as S_b lower level to S_a ground state, lower level.

Usually this is not the case. During excitation the environment can be considered fixed. If after excitation the solvent has time to relax before emission, the energy level of S_b will change. This effect leads to a shift in the lower level of the excited sate to ground level transition, called **Stokes-shift.**



Stokes shifts on the order of 20 to 50 nm are usually observed in the range of 300 nm absorption (about 5 to 20 % of the absorption energy).

These shifts correspond to energies of about 5000 cm^{-1} or 15Kcal/mole.

- If a second electronic state exists and it is excited, the conversion from the second level to the first one can be very rapid.

Decrease of the fluorescence intensity

1. Internal conversion

In this process, energy is lost by collision with the solvent molecules or by dissipation in internal vibrational modes.

Collisions and phonons will increase as the temperature is increased. This will cause a decrease of fluorescence intensity when the temperature increases.

If the rate of spontaneous emission is

$$k_f = A_{ba} = 1/\tau_r$$

We define **QUANTUM YIELD** of the fluorescence the ratio

$$\Phi_F = k_f / (k_f + k_{nr})$$

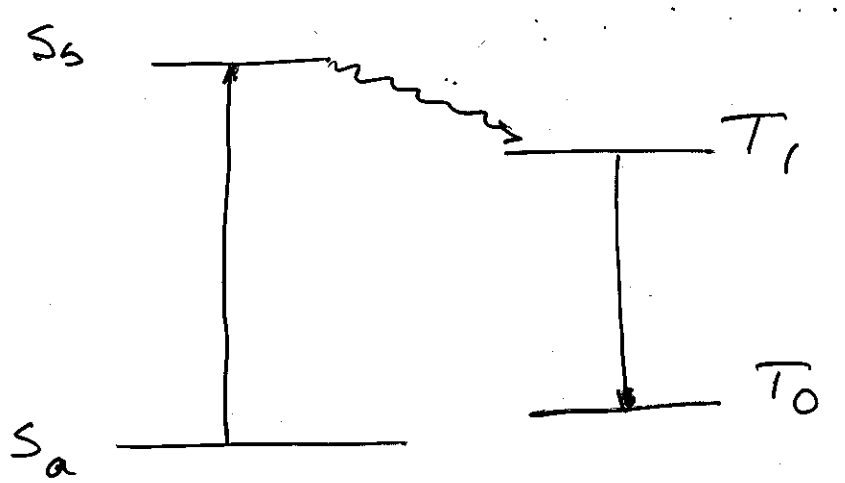
where k_{nr} indicates the non-radiative decay rate of the excited state. The maximum value of the quantum yield is 1. There are substances with quantum yield close to one (fluorescein, rhodamine).

2. Intersystem crossing

In this process a formally forbidden transition occurs.

A singlet state is converted into a triplet state. The triplet state can in turn be converted to a ground level by a thermally induced decay or by emission of a photon. This later process is called **phosphorescence**.

The triplet state is generally lower in energy than the singlet state.



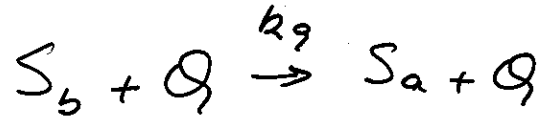
Phosphorescence occurs at longer wavelengths with respect to fluorescence. Because the triplet to singlet transition is forbidden, the lifetime of the triplet state is very long (milliseconds to seconds)

Collisions and internal conversion compete strongly with phosphorescence. To observe phosphorescence the sample must be cooled and the movement of the solvent must be avoided (protein phosphorescence).

3. Quenchers

These molecules are ground state triplets.

O_2, I_2^- and NO are typical quenchers. This process is in principle a bimolecular reaction.



Upon collision the excited state S_b is converted to a triplet state and the triplet state is then deactivated by thermal processes.

Because of all mechanisms mentioned

- Internal conversion
- intersystem crossing
- quenching

the effective lifetime of the excited state is **shorter** than the radiative lifetime.

If all deactivation processes operate independently, the concentration of S_b will decay by

$$-dS_b/dt=(k_f+k_{ic}+k_{is}+k_q[Q])S_b$$

where

- k_f is the radiative rate
- k_{is} is the intersystem crossing rate
- k_{ic} is the internal conversion rate
- k_q is the quenching rate

The solution of the above equation is an **exponential**

$$S_b(t)=S_b(0)\exp(-t/\tau_F)$$

$\tau_F = 1/(k_f + k_{ic} + k_{is} + k_q[Q])$

quantum yield $\Phi_F = \tau_F / \tau_r$

The quantum yield gives an idea of how competitive other deactivation processes are with respect to fluorescence. Generally the fluorescence lifetime is on the order of 10^{-10} to 10^{-6} s.

Tab. 6.1. Standards for the determination of fluorescence quantum yields

Range	*Compound*	*Temp. (°C)*	*Solvent*	Φ_F	*Ref.*
270–300 nm	Benzene	20	Cyclohexane	0.05 ± 0.02	1
300–380 nm	Tryptophan	25	H_2O (pH 7.2)	0.14 ± 0.02	2
300–400 nm	Naphthalene	20	Cyclohexane	0.23 ± 0.02	3
315–480 nm	2-Aminopyridine	20	0.1 mol L^{-1} H_2SO_4	0.60 ± 0.05	4
360–480 nm	Anthracene	20	Ethanol	0.27 ± 0.03	1, 5
400–500 nm	9,10-diphenylanthracene	20	Cyclohexane	0.90 ± 0.02	6, 7
400–600 nm	Quinine sulfate dihydrate	20	0.5 mol L^{-1} H_2SO_4	0.546	5, 7
600–650 nm	Rhodamine 101	20	Ethanol	1.0 ± 0.02	8
				0.92 ± 0.02	9
600–650 nm	Cresyl violet	20	Methanol	0.54 ± 0.03	10

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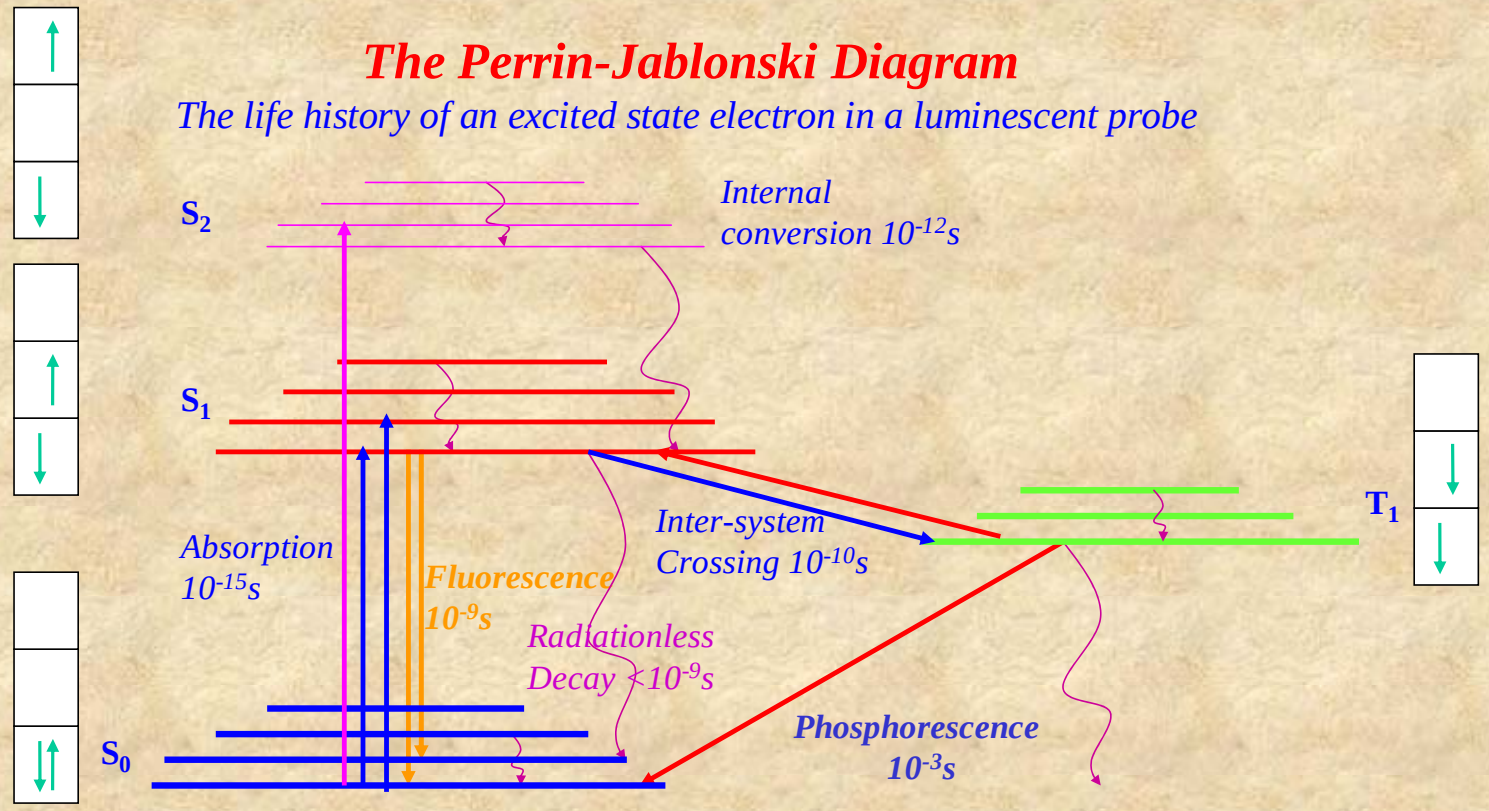
The Perrin-Jablonski Diagram

The life history of an excited state electron in a luminescent probe



Key points:

- ✓***Excitation spectra are mirror images of the emission spectra***
- ✓***Emission has lower energy compared to absorption***
- ✓***Triplet emission is lower in energy compared to singlet emission***
- ✓***Most emission/quenching/FRET/chemical reactions occur from the lowest vibrational level of $[S]_1$***

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Virtually 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

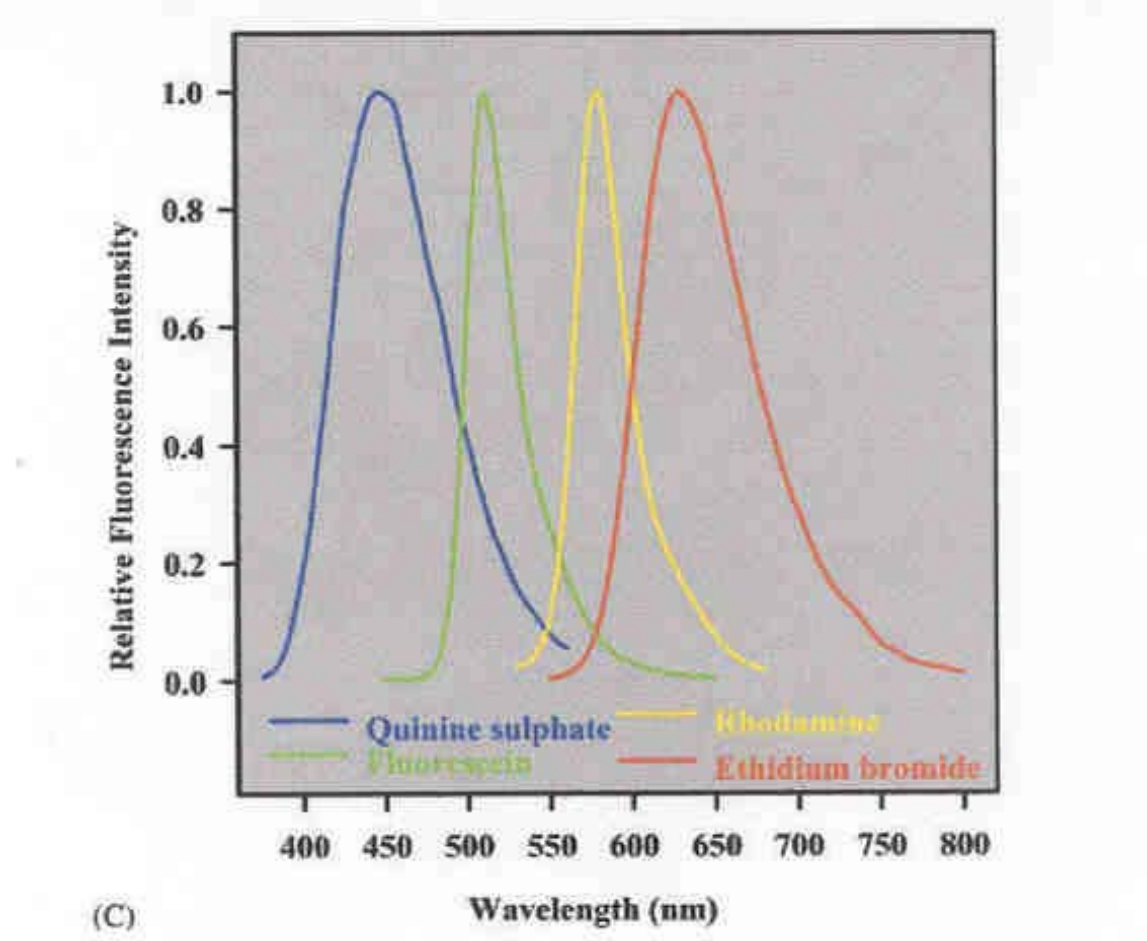
4. The polarization (anisotropy) of the emission

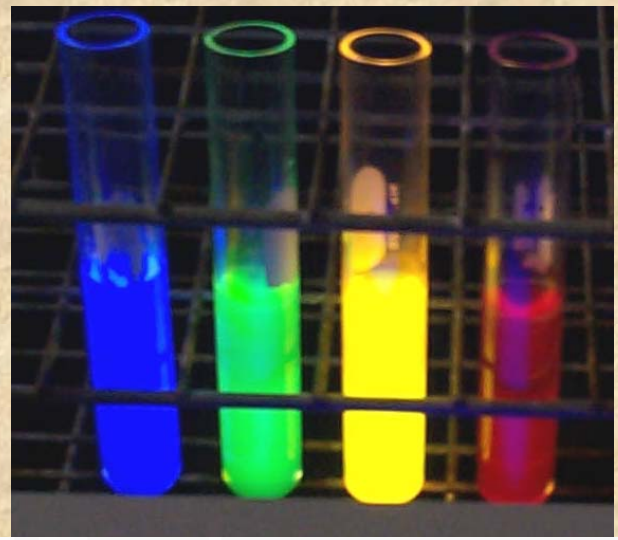
5. The fluorescence lifetime

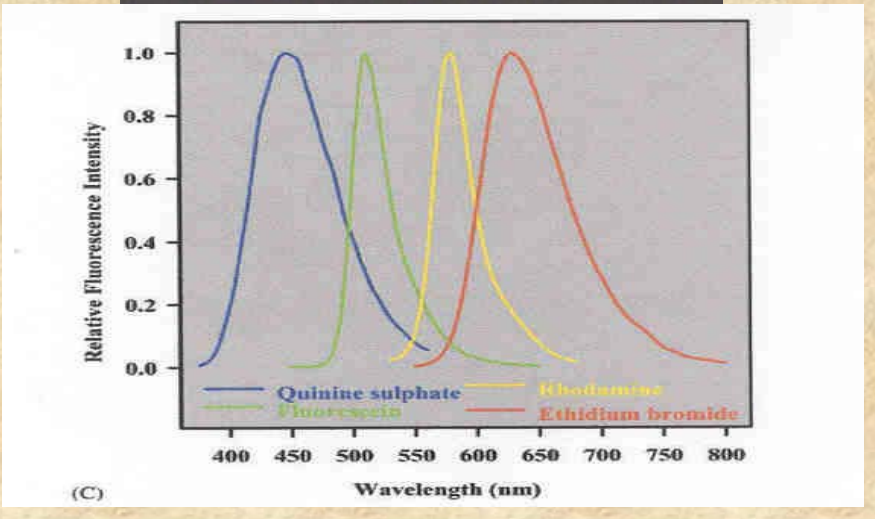
We briefly examine each of these categories and discuss historical developments, underlying concepts and practical considerations

The fluorescence emission spectrum

In a typical emission spectrum, the excitation wavelength is fixed and the fluorescence intensity versus wavelength is obtained






Relative Fluorescence Intensity
1.0
0.8
0.6
0.4
0.2
0.0
Quinine sulphate
Fluorescein
Rhodamine
Ethidium bromide
400
450
500
550
600
650
700
750
800
Wavelength (nm)
(C)

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

2) The fluorescence spectrum lies at longer wavelengths than the absorption

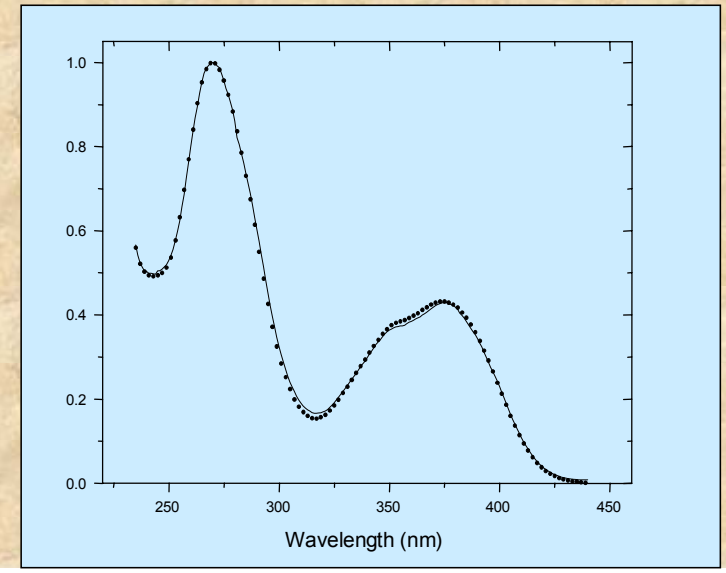
3) The fluorescence spectrum is, to a good approximation, a mirror image of the absorption band of least frequency

These general observations follow from consideration of the Perrin-Jabłoński diagram shown earlier

The fluorescence excitation spectrum

The relative efficiencies of different wavelengths of incident light to excite fluorophores is determined as the excitation spectrum. In this case, the excitation monochromator is varied while the emission wavelength is kept constant if a monochromator is utilized - or the emitted light can be observed through a filter.

If the system is “well-behaved”, i.e., if the three general rules outlined above hold, one would expect that the excitation spectrum will match the absorption spectrum. In this case, however, as in the case of the emission spectrum, corrections for instrumentation factors are required.



Overlay of Absorption Spectrum and Corrected Excitation Spectrum for ANS in ethanol