5. Fluorescence Spectroscopy

5.1. Principles

We have seen in the last two chapters that the absorption of electromagnetic radiation in the ultraviolet and visible region leads to an electronically excited state of a molecule. We shall now discuss the processes that take place when a molecule makes the transition back to its electronic ground state.

All processes that involve the emission of electromagnetic radiation are called luminescence.
If the excited state was generated by absorption of electromagnetic radiation two different luminescences are distinguished. Phosphorescence and Fluorescence. In addition, the energy of the excited state may be dissipated by processes that are radiation less.
Fluorescence and phosphorescence are often observed when aromatic molecules are excited by ultraviolet or visible radiation.

In the electronic ground state of a molecule the orbitals of lowest energy are usually occupied by two electrons. According to Pauli's principle, the spins of the two electrons that occupy the same orbital must be antiparallel. The total Spin S of the molecule in the ground state is zero and the corresponding state of the energy of the molecule is termed "singlet state". In the excited state, π^*, there are unpaired electrons in two molecular orbitals, π and π^* in case of a $\pi \rightarrow \pi^*$ excitation or n and π^* in case of an $n \rightarrow \pi^*$ transition. After excitation, the electron spins may now be either oriented parallel (triplet State, S=1) or antiparallel (singlet state, S=0). The corresponding energies of these two possible states are different, because of the different interactions between electrons of parallel and anti parallel spin. The energy of the triplet state is usually lower than the energy of the singlet state. The possible transitions are depicted in Figure 6.1 (Jablonski diagram see below).

The diagram shows the excitation of an electron from the electronic-vibrational ground state S_0 to excited states S_1, S_2 which are characterized by the different electronic energies and by different vibrational states of the molecule. In other words: The absorption of a photon of suitable energy usually takes place between the state of lowest vibrational energy of an electronic ground state to an excited electronic state according to the Franck-Condon principle.

The absorption to a triplet state is forbidden as a consequence of quantum theory, because it would require a reversal of the electron spin. The probability of such a reversal is very low and corresponding absorption bands are therefore very weak.

The excited electron can revert to the ground state in several ways:

Deactivation processes

Internal conversion refers to the dissipation of vibrational energy to different vibrational modes in the molecule or to the dissipation of vibrational energy to vibrational or translational energy of other molecules, for example in interactions (collisions) with the solvent in which the excited molecule is diluted. This is an exchange by which the molecule returns to thermodynamical equilibrium with its environment. The molecule reverts to the vibrational ground state of the of the corresponding electronically excited state.

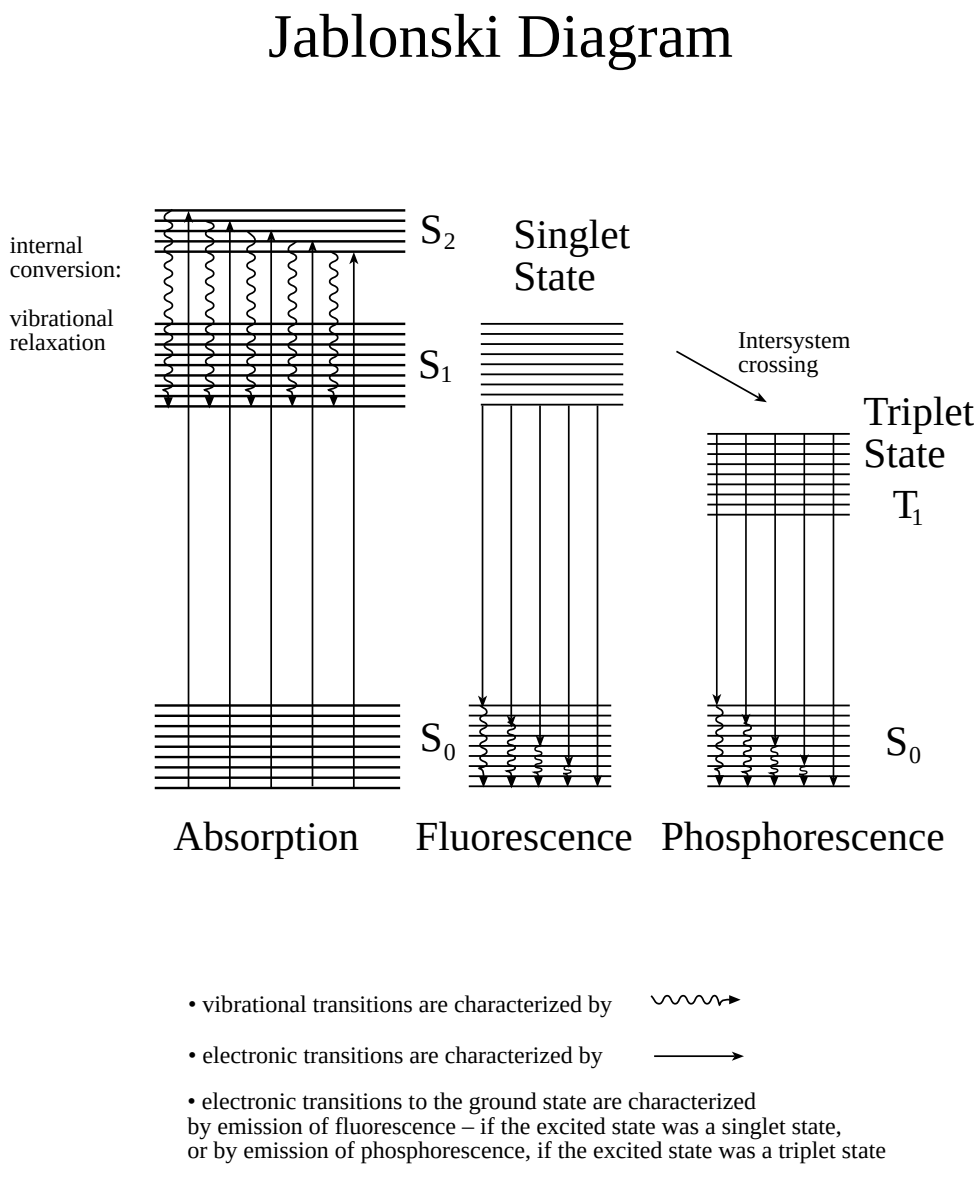


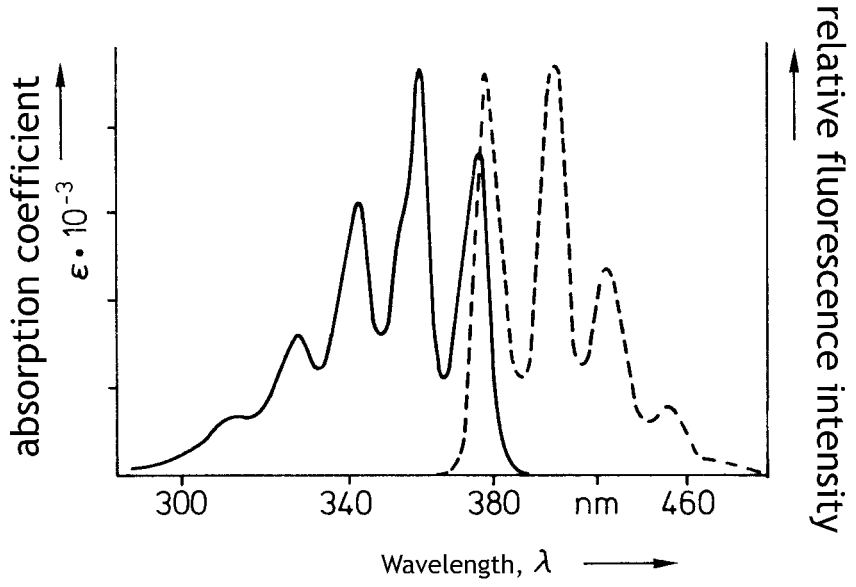
Figure 5.1 Jablonski diagram of transitions between different electronic energy levels.

Nonradiative deactivation. By this mechanism the electronic energy is converted into vibrational energy which is then dissipated to the environment. Similarly to vibrational relaxation, all electronic energy is dissipated in form of vibrational and translational energy to the environment of the molecule. The same process is also responsible for the relaxation of electronic states like S_2 or higher to the S_1 state (an exception is azulene, which shows fluorescence from the S_2 state). One could imagine that nonradiative deactivation also leads to the relaxation of the S_1 state to the electronic ground state S_0. However, the energy difference between the electronic ground state and the S_1 state is higher than the energy difference between S_1 and electronic states of even higher energy. The nonradiative transition between the excited S_1 state and the ground state S_0 therefore requires the concurrent excitation of many vibrational modes which is more unlikely to take place. Therefore, the nonradiative transition from the S_1 to the S_0 state is much slower and Fluorescence can successfully compete with the nonradiative process.

Intersystem crossing. Intersystem crossing is a spin-exchange mechanism by which singlet states can be converted into triplet states and triplet states can be converted into singlet states. This transition is only rarely observed.

In ***fluorescence quenching*** the excitation energy is transferred to special quencher molecules.

Fluorescence is the observed radiation when an electron makes the transition from the lowest vibrational mode of the electronically excited state S_1 to a vibrational mode of the electronic



Absorption and fluorescence spectra of anthracene in cyclohexane. The fluorescence spectrum is almost a mirror image of the absorption spectrum.

Figure 5.2 Fluorescence emission and the absorption spectrum of cyclohexane

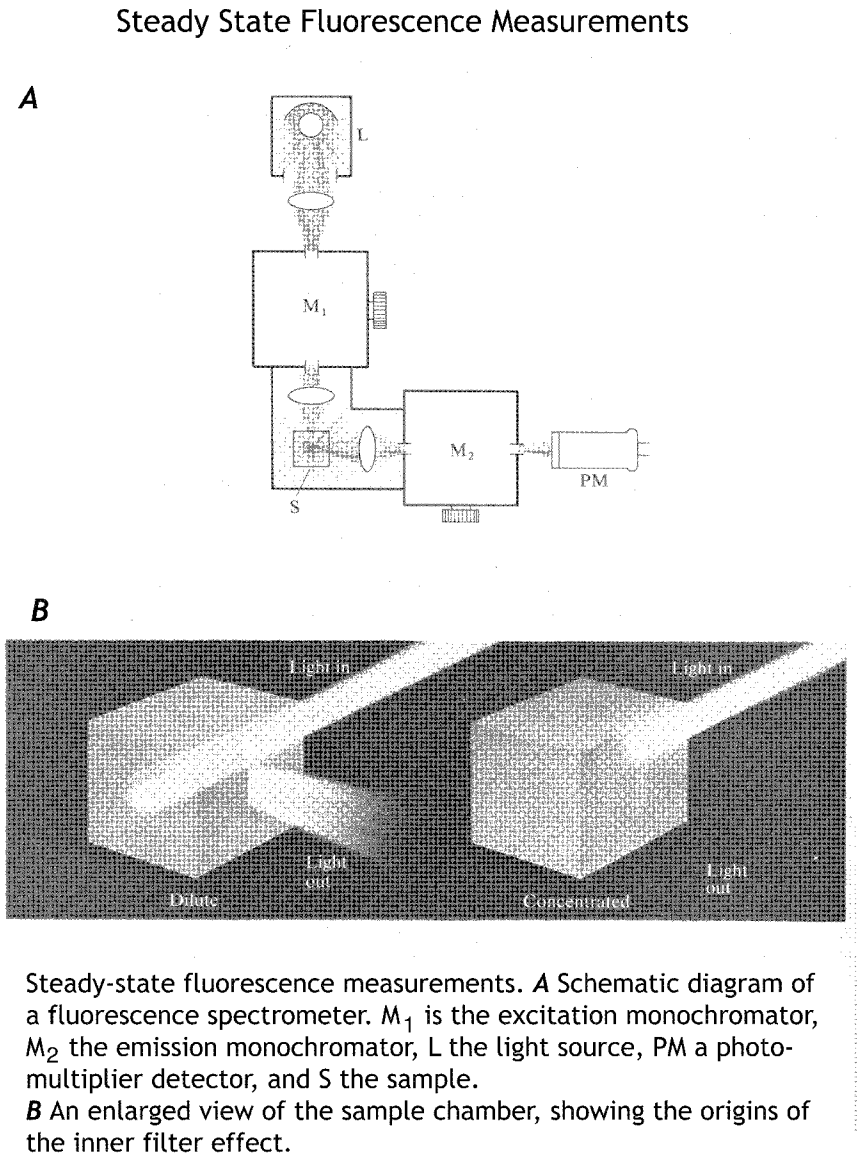
ground state S_0. The rate of such a transition is in the region of 10^7 to 10^8 s^{-1}.

Phosphorescence is the observed radiation when the electron makes the transition from the lowest vibrational mode of a triplet state, T_1, into a vibrational mode of the singlet state S_0. The rate of this transition can be 10^4 s^{-1} to value < 1 s^{-1}. The different possible transitions are depicted in Figure 5.1. The Fluorescence that shall be discussed in this chapter has the following properties:

A. the spectrum of the emitted radiation is independent of the wavelength of excitation.

B. the spectrum is shifted towards longer wavelength (corresponding to smaller frequencies and therefore to smaller energy ($c=\nu\cdot\lambda$ and $E=h\nu$).

C. while the fine structure of the absorption spectrum is characterized by the vibrational modes of the excited state (S_1), the fine structure of the fluorescence spectrum is characterized by the vibrational modes of the electronic ground state (S_0). If the vibrational modes of the



Steady-state fluorescence measurements. *A* Schematic diagram of a fluorescence spectrometer. M_1 is the excitation monochromator, M_2 the emission monochromator, L the light source, PM a photo-multiplier detector, and S the sample.
B An enlarged view of the sample chamber, showing the origins of the inner filter effect.

Figure 5.3 Scheme of a steady state spectrofluorometer.

ground state S_0 and the excited S_1 state are comparable, the absorption an the fluorescence spectra relate to each other like an image to its mirror (Figure 5.2).

5.1. Quantum Yield.

The contribution of fluorescence to the various deactivation processes of the excite state is termed the “quantum yield”. The quantum yield is defined by the quotient of the number of photons that are emitted and the number of photons that are absorbed:

$$\Phi = \frac{\text{number of emitted photons}}{\text{number of absorbed photons}} \leq 1$$

With the transition rates kf for the fluorescence, kic and kisc for the internal conversion and for the intersystem crossing, and with the fluorescence quenching by quencher molecules, the quantum yield can be expressed as

$$\Phi = \frac{k_f}{k_f + k_{ic} + k_{isc} + k_Q}$$

5.2. Fluorescence Lifetime

The fluorescence lifetime is the time the molecule remains on average in the excited state, before emission takes place. If there are N molecules in the excited state, then the decrease dN of molecules that revert to the ground state in a given time interval dt is expressed by the relation

$$-\frac{dN}{dt} = k_f \cdot N$$

The number of excited molecules therefore decreases exponentially with time:

$$N(t) = N_0 \exp\left(-k_f \cdot t\right)$$

The time after which the number of excited molecules decreases from N_0 to N_0 / e is called the fluorescence lifetime. Therefore the fluorescence lifetime is given by the reciprocal fluorescence transition rate:

$$\tau_F = \frac{1}{k_f}$$

In contrast to this radiation lifetime, the real lifetime of the excited state has to include the non-radiative deactivation processes.

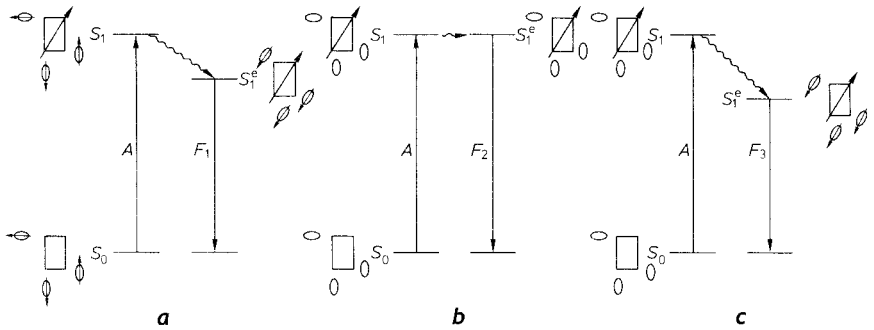
$$\tau = \frac{1}{k_f + k_{ic} + k_{isc} + k_q}$$

The lifetime τ that can be experimentally determined, can be used to calculate the fluorescence lifetime:

$$\tau = \Phi \cdot \tau_F$$

5.3. Solvent effects.

Similarly to absorption spectroscopy, there are solvent effects on the spectral properties of fluorescent molecules. Changes in the fluorescence emission of a molecule can be caused by solvents of different polarity, of different dielectric constant or of different polarizability. In Figure 5.4 , the appearance of possible red and blue shifts of the fluorescent light is explained by dipolar interactions of the fluorophore and the solvent. For reasons of simplicity, we assume that the molecule does not have a dipole moment in the ground state but only in the excited state. The energy difference between the two electronic states is given by the length of the arrows that connect the two states.



Solvent effect on the fluorescence emission. A=Absorption, F=Fluorescence, S = energy of the excited molecule directly after absorption (excited Franck-Condon state), S = vibrational equilibrium state, ***a*** polar solvent, ***b*** apolar solvent with small polarizability, ***c*** apolar solvent with hight polarizability.

Figure 5.4 Red and blue shifts of the fluorescence of a molecule by different solvents depend on solvent polarity and on dipole interactions between the fluorophore and the solvent.

First we shall consider the fluorescent molecule in a polar solvent. The excited Franck-Condon State S_1 has a higher energy than the equilibrium state of the excited molecule S_1E, which is reached after reorientation of the polar solvent molecules.
If the molecule is in an apolar solvent (part b of Figure 5.4) there is no reorientation of the molecules in the apolar solvent, the energies of the states S_1 and S_1^E (vibrational equilibrium state) are very similar to one another. The energy difference between the excited state from which the molecule fluoresces and the ground state is therefore bigger in a apolar solvent than it is in a polar solvent. Thus the emitted light is of higher frequency and smaller wavelength, i.e. blue-shifted.
If, in contrast, the polarizability of an apolar solvent is sufficiently high, than the excited fluorophore can induce dipole moments in the solvent and the energy of the equilibrium state of the fluorophore, S_1^E , is reduced compared to the excited Franck-Condon state S_1. In this case, the energy level of the equilibrium state of the excited molecule in an apolar solvent of high polarizability is often lower than that in a polar solvent. The frequency of the emitted light is then smaller and its wavelength longer, i.e. the fluorescence maximum of the emission spectrum is red-shifted.

5.4. Excitation spectra.

The fluorescence emission is characterized by the transition from the lowest vibrational mode of the electronically excited state to the ground state. Therefore, the shape of the emission spectrum is always the same and independent of the wavelength of the exciting radiation. The recorded fluorescence intensity as a function of the emission wavelength at a fixed excitation wavelength is termed fluorescence emission spectrum. By contrast, if the wavelength of the exciting light is changed and fluorescence emission is monitored at a constant emission wavelength, the fluorescence excitation spectrum is recorded. If the excitation energy is constant the fluorescence excitation spectrum corresponds to the absorption spectrum of the substance.

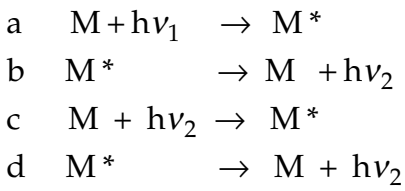
5.5. Concentration dependence of fluorescence.

The fluorescence of concentrated solutions of fluorophores often shows a decrease in the fluorescence intensity, i.e. a decrease in quantum yield. This decrease is often coupled to changes in the spectral lineshape and it has different reasons.

5.5.1 Reabsorption of emitted fluorescence radiation.

The higher the fluorescence the higher the probability that the emitted radiation of a molecule

is reabsorbed by another molecule. The condition for such reabsorption is that the emission and the absorption spectra partially overlap with each other. Reabsorption is therefore possible for the high frequency fraction of the emitted radiation.

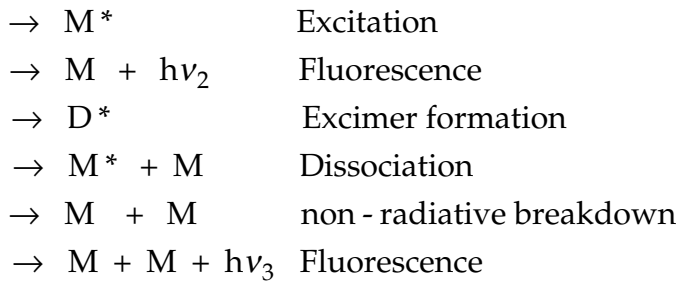


a $M + h\nu_1 \rightarrow M^*$
b $M^* \rightarrow M + h\nu_2$
c $M + h\nu_2 \rightarrow M^*$
d $M^* \rightarrow M + h\nu_2$

The quantum yield for n reabsorption processes is Φ^n. With $\Phi < 1$, the quantum yield approaches 0 with increasing n, and the fluorescence emission disappears in the high frequency region. The shape of the fluorescence spectrum will change significantly, which can be reason for false interpretation of a fluorescence spectrum.

Dimer formation.
If dimers can be formed by monomers in the ground state the absorption spectrum will change if the dimers absorb radiation differently than monomers. The fluorescence spectrum of the monomer is often unaffected since the dimers only are only rarely fluorescent. The appearance of dimers results in a reduced fluorescence intensity of the monomers.

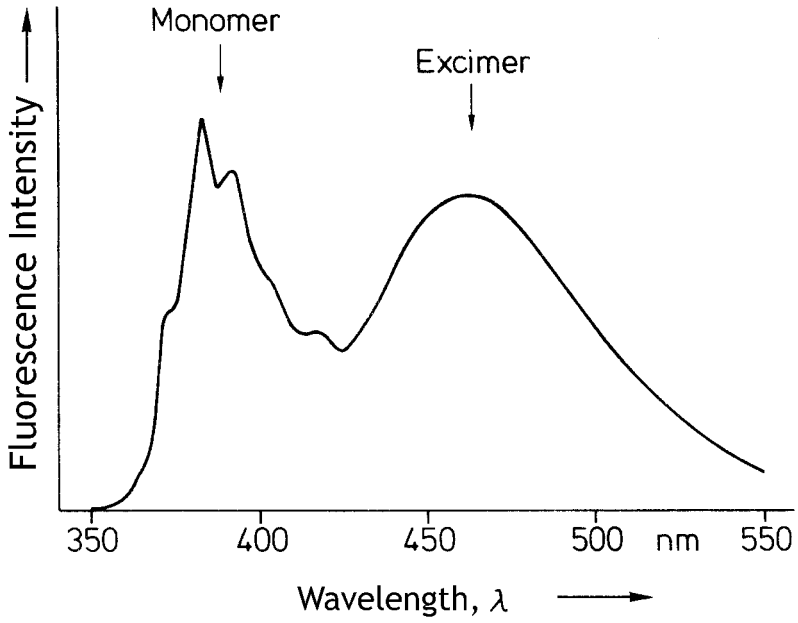
5.5.2 Excited Dimers.
Another concentration dependent mechanism that affects the fluorescence spectrum is the formation of excited dimers (excimers):



a	$M + h\nu \rightarrow M^*$	Excitation
b	$M^* \rightarrow M + h\nu_2$	Fluorescence
c	$M^* + M \rightarrow D^*$	Excimer formation
d	$D^* \rightarrow M^* + M$	Dissociation
e	$D^* \rightarrow M + M$	non - radiative breakdown
f	$D^* \rightarrow M + M + h\nu_3$	Fluorescence

Excited dimers only exist in the exited state. The formation of excimers takes place only after excitation of one monomer that reacts with another monomer in the ground state. The absorption spectrum of the fluorophore remains unaffected by the formation of excited dimers. However, in the fluorescence emission spectrum, an additional band appears (Figure 5.5). At low

concentrations of the fluorophore the processes a and b (excitation and fluorescence of the monomer) are predominant. However, at higher concentrations the probability of excimer formation increases (and more D* is formed). The fluorescence of the excited D* appears at a different, usually lower frequency (higher wavelength).



Fluorescence spectrum of pyrene in n-hexane (0,667 mM)

Figure 5.5 Fluorescence spectrum of pyrene in n-hexane

The formation of excimers can be viewed as a dynamic fluorescence quenching of the monomers and it can be described with a Stern-Volmer dependency.

$$\frac{\Phi^{M}_{\max}}{\Phi^{M}} - 1 = k_a \cdot c \cdot \tau_0$$

were $\Phi^{M}_{\max}$ is the maximum quantum yield of the monomers at infinite dilution, $k_a \cdot c$ the rate of excimer formation and t_0 the life time of the excited monomer.
For the excimer component there is an analogous expression:

$$\frac{\Phi^{E}_{\max}}{\Phi^{E}} - 1 = \left(k_a \cdot c \cdot \tau_0\right)^{-1}$$

The maximum quantum yield of the excimers can be determined at a high concentration of the fluorophore.

5.6. Fluorescence Quenching.

The fluorescence of a substance can be strongly affected by its environment. An example is the fluorescence quenching by so called quencher molecules. These quencher molecules reduce the fluorescence quantum yield. The absorption process remains unaffected but the energy of the excited state is dissipated to the quencher molecules.

There are two kinds of quenching processes

a. dynamic quenching

b. static quenching

The efficiency of the two processes is dependent on the concentration of the quencher molecules.

5.6.1 Dynamic fluorescence quenching.

For the deactivation of the excited state of a fluorophore we must consider

a) fluorescence emission
b) internal conversion and non-radiative transitions
c) collisional quenching

The quantum yield in presence of fluorescence quenchers is given by

$$\Phi = \frac{k_f}{k_f + k_i + k_q}$$

The quantum yield in absence of quencher molecules is given by

$$\Phi_0 = \frac{k_f}{k_f + k_i}$$

and the fluorescence lifetime is given by

$$\tau_0 = \frac{1}{k_f + k_i}$$

The ratio of the quantum yields with and without quencher is thus

$$\frac{\Phi_0}{\Phi} = \frac{k_f + k_i + k_q}{k_f + k_i} = 1 + \frac{k_Q}{k_f + k_i}$$

or

$$\frac{\Phi_0}{\Phi} = 1 + k_Q \tau_0$$

At a constant concentration of the fluorophores the transition rate kQ is proportional with the concentration of the quencher molecules

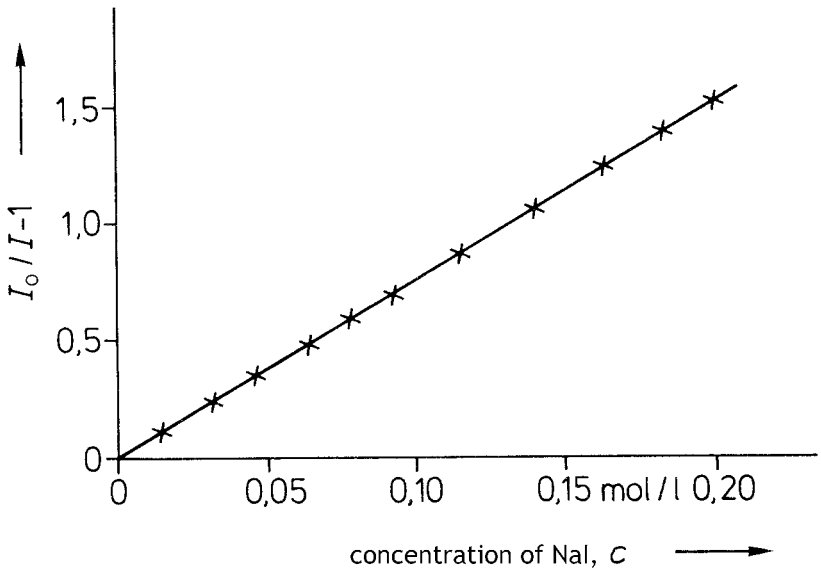
$$k_Q = K \cdot c_Q$$

The proportionality constant is termed quenching constant or Stern-Volmer constant. Since the intensity of the fluorescence signal is proportional to the quantum yield one can write:

$$\frac{I_0}{I} - 1 = K \cdot c_Q \cdot \tau_0$$

This equation is termed “Stern-Volmer” equation.

The quenching constant K can be estimated by a Stern Volmer plot of I_0/I -1 as a function of the quencher concentration (Figure 5.6).



Stern-Volmer plot of the fluorescence quenching of 0.1 mM tryptophan at different concentrations of sodium iodide (NaI).

Figure 5.6 Stern-Volmer Plot

5.6.2 Static Fluorescence quenching.

The fluorescence quenching by complex formation between the fluorophore M and the Quencher molecule Q can be described by the following mechanism:

a	$M + Q \rightarrow MQ$	Complex formation
b	$M + h\nu_1 \rightarrow M^* \rightarrow M + h\nu_2$	Fluorescence
c	$MQ + h\nu_1 \rightarrow (MQ^*) \rightarrow MQ + \text{heat}$	Quenching by the complex

If we assume that the quantum yield of the fluorescence in absence of quenchers is 1:

$$\Phi_0 = \frac{[M^*]}{M} = 1$$

then the quantum yield in presence of the quenchers is given by

$$\Phi = \frac{[M^*]}{[M^*] + [MQ^*]}$$

and therefore

$$\frac{\Phi_0}{\Phi} = \frac{[M^*] + [MQ^*]}{M^*} = 1 + \frac{[MQ^*]}{[M^*]}$$

If the probability of excitation is the same for the fluorescent molecule and the complex of the fluorescent molecule with the quencher, we can write:

$$\frac{[MQ]}{[M]} = \frac{[MQ^*]}{M^*} = k_a[Q]$$

with

$$k_a = \frac{[MQ]}{[M][Q]}$$

the constant of complex formation.

Therefore the ratio of the quantum yields can be expressed as

$$\frac{\Phi_0}{\Phi} = 1 + k_q[Q]$$

If we replace the quantum yields with the corresponding intensities we get:

$$\frac{I_0}{I} = 1 + k_q[Q]$$

Static fluorescence quenching gives a linear dependence of the intensity ratio I_0/I on the concentration of the quencher molecules. In contrast to static fluorescence quenching, the quenching of fluorescence by collision shows an additional dependence on the lifetime of the excited state of the fluorophore, which allows the distinction of two processes. This difference is immediately understood because the probability for the collision between the excited fluorophore and the quencher is the higher the higher the lifetime of the fluorophore.
On the other hand, the formation of a complex will just reduce the concentration of the free fluorophore M and will not affect the lifetime of the excited molecules.

Finally, we shall mention that the distinction that we made between collisional and static quenching after all is just a question of the lifetime of the formed complex. In dynamic or collisional fluorescence quenching, the lifetime of the complex is much shorter than the lifetime of the excited fluorophore. In static quenching, the lifetime of the complex is significantly longer than the lifetime of the excited fluorophore. The two processes are therefore only extreme cases.

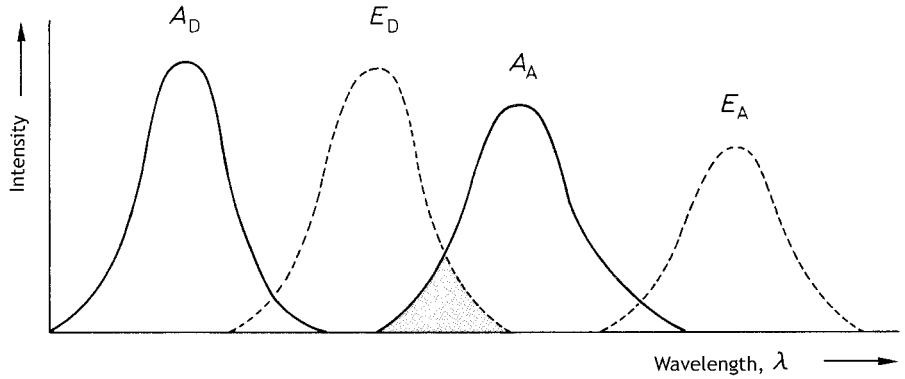
5.7. Energy transfer (Förster transfer).

We have just seen on the example of the collisional quenching, that fluorescence can be quenched by interaction between molecules. In contrast to fluorescence quenching by collision or by complex formation, for which a coupling between electronic orbitals is necessary, energy transfer can also take place over larger distances up to 100 Å. The participating molecules are named donor (D) and acceptor (A). Is the acceptor molecule also a fluorophore, then the energy transfer can be determined from the fluorescence of the acceptor. The emitted radiation from the acceptor is then termed sensitized fluorescence, because the radiation is observed without direct excitation of the acceptor.

The energy transfer can be described by the following processes:

a.	$D + h\nu_1$	$\rightarrow$	D^*				absorption
b.	$D^* + A$	$\rightarrow$	A^*	+	D		energy transfer
c.	A^*	$\rightarrow$	A	+	$h\nu_2$		emission

The mechanism of energy transfer was described by Förster as a resonance phenomenon. The electron system of the excited donor and that of the acceptor may be seen in analogy to two coupled pendulums where the energy of the first pendulum is transferred to the second pendulum.



Absorption and emission spectra of a donor / acceptor pair. The spectral overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor is the necessary condition for Förster energy transfer.

Figure 5.7 Energy Transfer between donor and acceptor requires an overlap between the fluorescence spectrum of the donor and the absorption spectrum of the acceptor.

The transfer of the excitation energy of the donor to the acceptor molecule is only possible if the electronic transition of the donor from the excited state to the ground state corresponds to the absorption frequency of the acceptor. The necessary condition for energy transfer therefore is an overlap of the emission spectrum of the donor and the absorption spectrum of the acceptor molecule.

For energy transfer the following conditions must be met:

a. the donor molecule must be a fluorophore which has a sufficiently long fluorescence lifetime.
b. the emission spectrum of the donor and the absorption spectrum of the acceptor must overlap partially.
c. the transition dipole moments must have a suitable orientation to each other.

d. the distance between donor and acceptor must be within a limiting range (usually smaller than 10 nm).

These conditions are comparable to those found for the reabsorption of emitted radiation by a suitable acceptor molecule. The mechanisms of both processes are completely different. Energy transfer is observed without the concurrent fluorescence of the donor and possible only within a certain distance between donor and acceptor molecules. The table below summarizes the features of the different mechanisms of fluorescence changes.

Characteristic properties of the mechanisms that affect fluorescence in solutions

	Non-trivial transition	*Reabsorption*	*Complex formation*	*Collision mechanism*
Dependence on volume	none	increase	none	none
Dependence on viscosity	none	none	none	decrease
Excitation time of the photosensitizer	minimal	unchanged	unchanged	smaller
Fluorescence spectrum of the photosensitizer	unchanged	changed	unchanged	unchanged
Absorption spectra of photosensitizer and acceptor	unchanged	unchanged	changed	unchanged

Table 5.1 Properties of the mechanisms by which fluorescence is reduced in solutions.

If the rate of energy transfer between donor and acceptor is k_T, then the transfer efficiency E_T is

$$E_T = \frac{k_T}{k_T + k_f^D + k_{ic}^D + k_{isc}^D}$$

with the index D indicating the transition rates of the donor molecule. For an experimental test of the resonance energy transfer, the emission maxima of the donor and the acceptor must be sufficiently separated from each other. It is important that the selected wavelength for the excitation of the donor does not excite the acceptor directly. The efficiency of energy transfer can then be calculated from the ratio of the fluorescence quantum yields Φ_{D-A} and Φ_D of the donor in presence and in absence of acceptor molecules:

$$\frac{\Phi_{D-A}}{\Phi} = \frac{\dfrac{k_f^D}{k_f^D + k_{ic}^D + k_{isc}^D + k_T}}{\dfrac{k_f^D}{k_f^D + k_{ic}^D + k_{isc}^D}} = 1 - E_T$$

In the experiment the donor is excited with radiation of a suitable frequency and the fluorescence emission of the donor is then measured at a selected wavelength in absence and in presence of acceptor molecules.
Alternatively, E_T can be determined by measuring the sensitized acceptor fluorescence and also the lifetime of the excited donor molecules.

The fluorescence lifetime τ_{D-A} of the donor in absence and in presence of the acceptor is given by:

$$\tau_{D-A} = \frac{1}{k_f^D + k_{ic}^D + k_{isc}^D + k_T}$$

and without acceptor

$$\tau_D = \frac{1}{k_f^D + k_{ic}^D + k_{isc}^D}$$

With the equation

$$E_T = \frac{k_T}{k_T + k_f^D + k_{ic}^D + k_{isc}^D}$$

we finally get:

$$\frac{\tau_{D-A}}{\tau_D} = 1 - E_T$$

The estimation of the transfer efficiency E_T by determining the lifetime has the advantage that the there is are no artifacts that could arise from possible reabsorption. The fluorescence lifetime of the donor is only reduced by resonance energy transfer, because the energy

transfer deactivates the excited state of the donor molecule. The reabsorption of radiation does not lead to a change in the lifetime τ_D.

The characteristic dependence of the transfer efficiency on the distance between donor and acceptor can be utilized to determine the structural organization of chromophores in macro molecules. The transfer rate k_T can be written as

$$k_T = \frac{1}{\tau_D}\left(\frac{R}{R_0}\right)^{-6}$$

In this equation, R is the distance between donor and acceptor and R_0 is the critical distance between a donor - acceptor pair, for which the probability of resonance energy transfer and the deactivation of the donor by radiative and non-radiative processes is the same. For any system, R_0 is a constant with typical values between 1 and 5 nm.

The energy of interaction between donor and acceptor corresponds to the interaction of two dipoles and therefore decreases with the third power of the intermolecular distance. The probability for an energy transfer is then proportional to the square of the energy of interaction, which leads to the R^6 dependency.

The transfer efficiency can therefore also be expressed as

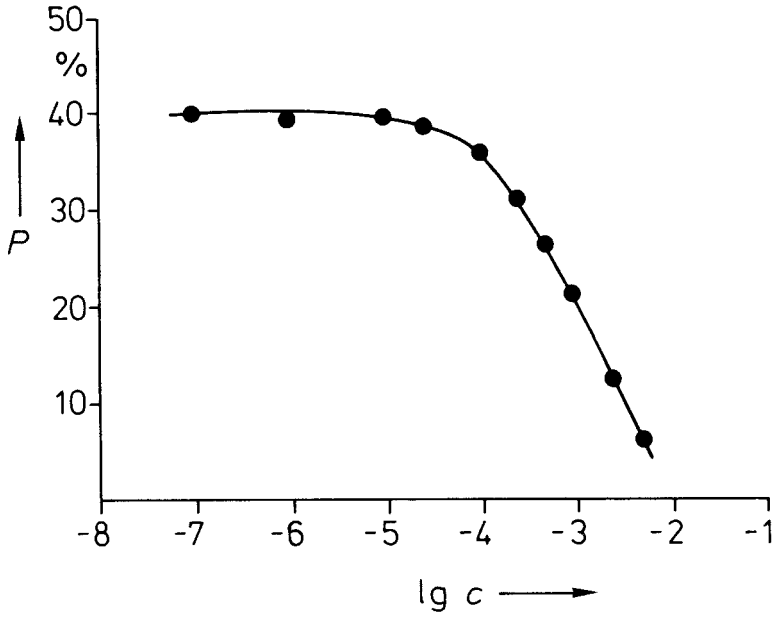
$$E_T = \frac{k_T}{k_T + \frac{1}{\tau_D}}$$

and with the previous equation on the distance dependence of the rate of energy transfer we can write:

$$E_T = \frac{R_0^6}{R^R + R_0^6}$$

A measurement of the energy transfer efficiency therefore allows the determination of the intermolecular distance between donor and acceptor if the distance is close to R_0.
The resonance energy transfer of excitation energy is not limited to the transfer between different molecules but is also possible between similar molecules, because the absorption and emission spectra overlap. This can be demonstrated by fluorescence polarization experiments.
An experiment in which polarized light is passed through a sample of fluorescein in glycerol

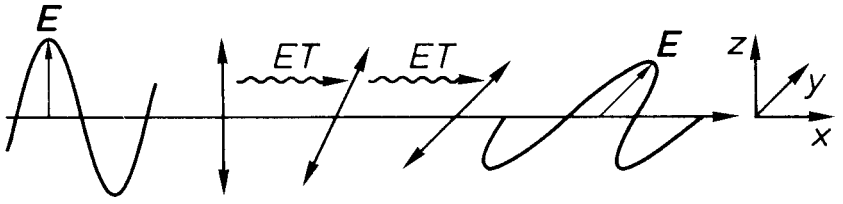
(see Figure 6.8 below) demonstrates that the extend of polarization depends on the concentration of the fluorophore. Excluding the possible reabsorption of radiation, one can observe a decay in the degree of polarization of fluorescence emission if the concentration of the fluorophore is increased. This effect is termed concentration depolarization.
The effect is caused by resonance energy transfer between fluorophores. Since the transition



Concentration depolarization of fluorescein in glycerol (after Pheofilov, P.P., Shveshnikov, B., 1940 J. Phys. USSR 3, 493)

Figure 5.8 Concentration depolarization of fluorescein

dipole moments of the participating molecules have an angle towards each other, the emitted fluorescence radiation will have a different direction of polarization after several energy transfer events. Therefore the degree of polarization will depend on the concentration of the fluorophore.



Rotation of the polarization direction of linearly polarized light by multiple energy transfer events.

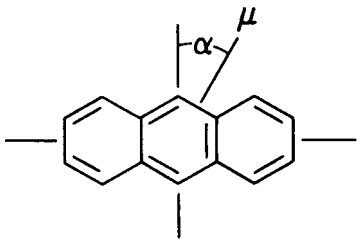
Figure 5.9. Rotation of the direction of polarization by fluorescence energy transfer.

5.8. Fluorescence Polarization.

Fluorescence radiation of a molecule is characterized by a wavelength and, in addition by a direction of polarization, i. e. by a defined direction of the electrical field vector. In analogy to the description of the absorption of electromagnetic radiation by a transition dipole moment of absorption, the emission of electromagnetic radiation can be described by a transition dipole moment of emission.

5.8.1 Qualitative description.

Absorption and emission transition dipole moments have a defined orientation in a molecular coordinate system. Such an orientation is given on the example of anthracene. The emission transition dipole moment of most fluorophores include an angle of 10 to 40 °. The polarization of the radiation in a fluorescent sample is dependent on the orientation of the emission transition dipole moment and therefore on the molecular motions (rotation and flexibility of segments within the molecule).



Direction of the transition dipole moment of absorption in anthracene. (After Yguerabide, J. (1978), in: Cell Membranes and Viral Envelopes, Tiffany, J. M., Blough, H. A., (eds.) Academic Press.)

Figure 5.10 Transition dipole moment in anthracene

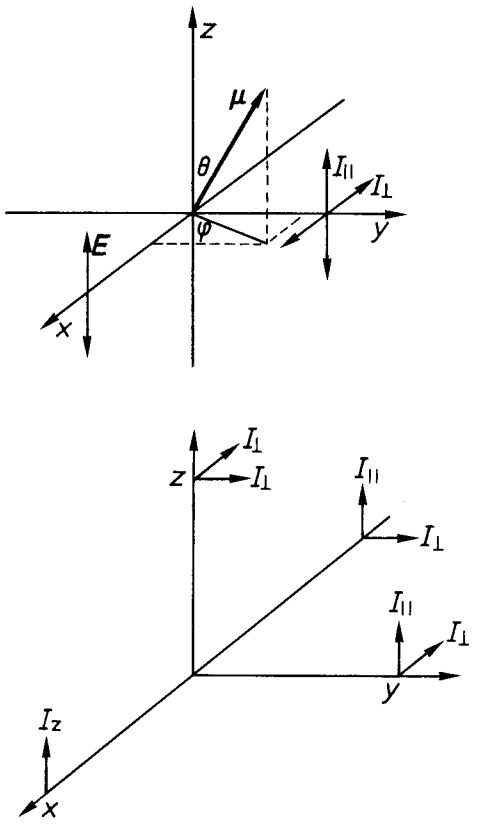
To demonstrate that the direction of polarization of radiation that is emitted by a molecule can be used to obtain information on the rotational mobility, the orientation and the viscosity of the environment of the molecules, we assume that the sample is irradiated by linearly polarized light in the direction of the x-axis. The direction of polarization is the x-y plane. The fluorescent sample is indicated by the vector of the transition dipole moment, $\vec{\mu}_A$. For simplicity, it is assumed that this vector may have the same direction for emission and absorption. The emitted fluorescence is detected perpendicular to the direction of the excitation beam along the y-axis. $I_{||}$ and $I_{\perp}$ are the components of the fluorescence intensity parallel and perpendicular to the excitation beam.
The degree of polarization is defined as

$$P = \frac{I_{||} - I_{\perp}}{I_{||} + I_{\perp}}.$$

Another quantity that is commonly used to express the extent of polarization is the anisotropy

$$A = \frac{I_{||} - I_{\perp}}{I_{||} + 2I_{\perp}}$$

In the literature, the polarization P is more often used to express fluorescence polarization, However, the physically more important parameter is the anisotropy A, since in contains the contributions of all directions of polarization. The fluorescence emission is polarized perpendicular to the excitation beam and can be expressed by a component that is polarized in the x-direction and by a component that is polarized in the y-direction. Both components have the same average intensity $I_{\perp}$, since the distribution of the molecule is independent from the angle ϕ. The total fluorescence intensity is given by $I_{||} + 2I_{\perp}$. If P is close to zero, the radiation is not polarized. If P approaches 1 the radiation is completely polarized, i. e. the emitted radiation does not have a vertical component. If P is less then 1 the radiation is partially polarized.



Schematic description of the experimental determination of fluorescence polarization.

Figure 5.11 Determination of fluorescence polarization