# Fate of Excited-State Molecules

After a molecule absorbs a photon, what happens next? One of the possibilities is the emission of a photon – *photoluminescence* – and this process is the basis of a number of analytical techniques, notably those based on fluorescence. For that reason, we are concerned with the processes that occur once the molecule is promoted into an excited state. Many of the possible fates are summarized in the following figure.
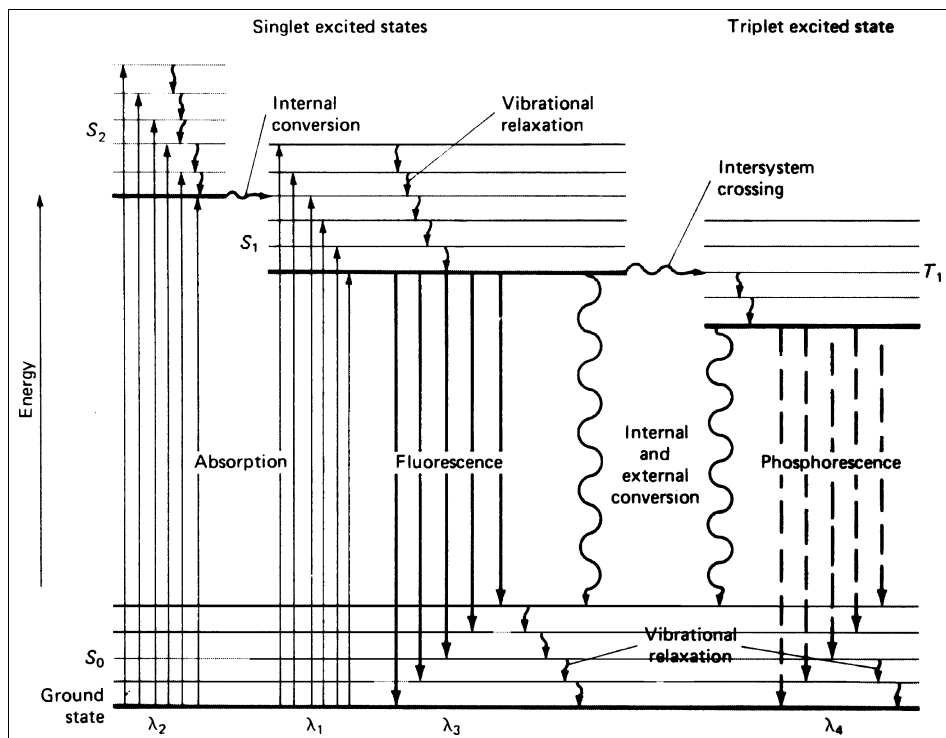
Glossary of Terms

The above figure has a lot of information; the terms used in the figure are defined and described in the following paragraphs.

w*singlet* and *triplet* electronic states. The *singlet* electronic state describes the situation when all of the electrons in a molecule (or atom, for that matter) are "paired," so that there is no net electronic spin. Many molecules have a singlet ground electronic state, although there are a number of exceptions; the dioxygen molecule, O_2, has a triplet ground state, for instance. The *triplet* state has two unpaired electrons.

win this figure, ***photon absorption*** (duration: 10^{-15} sec) promotes the molecule from the ground singlet electronic state, S_0, into an excited electronic singlet state, S_1 or S_2. Note that some of the photon's energy may also cause *vibrational excitation* of the molecule.

w*radiationless deactivation* is the general term used to describe the processes by which a molecule's excess electronic and vibrational energy is lost without the emission of a photon. In the figure, the radiationless transitions are represented by the wavy lines. On the other hand, *radiative deactivation* is the loss of excess energy through the emission of a photon; the radiative transitions (absorption, fluorescence and phosphorescence) are represented by the straight arrows.

wif the molecule is in solution, then any excess vibrational energy will be almost immediately (10^{-10} sec) lost to the surroundings as heat. This process is called *vibrational relaxation*; as a result, the molecule will quickly relax into the ground vibrational level of the excited electronic state. Fluorescence, if it occurs at all, will originate from the ground vibrational level of an excited state.

wthe term *internal conversion* (10^{-9} sec) is a catch-all phrase to describe the relaxation of a molecule from a higher to a lower electronic state without emitting a photon or interacting with another species. The process by which this occurs is not well understood, but it is especially efficient when there is an overlap between vibrational levels of the two electronic states, as there is between S_2 and S_1 in the figure. In solution, internal conversion will be quickly followed by vibrational relaxation to the ground vibrational level.
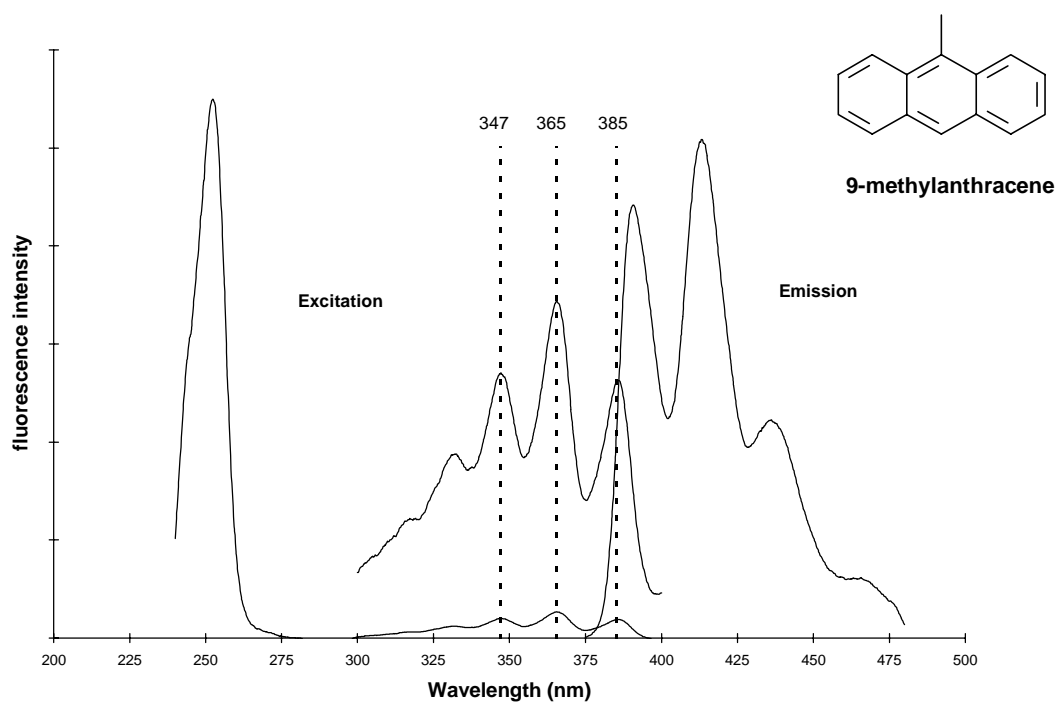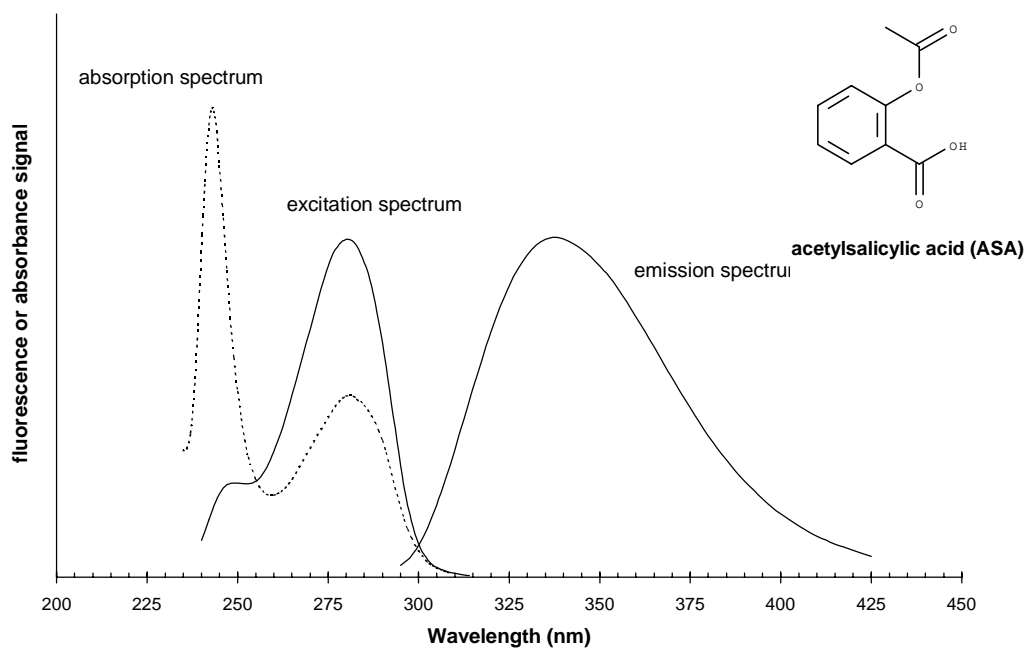
Rapid internal conversion from higher-lying electronic states into the LUMO S_1 is common enough that fluorescence, if it occurs, will usually originate in the LUMO. This general behavior is known as *Kasha's Rule*. There are, however, exceptions to this rule.

wthe excess energy of an excited valence electron may be lost through the spontaneous emission of a photon; if the electron does not change its spin during the emission (e.g., $S_1 \rightarrow S_0$), then the process is *fluorescence* (10^{-9} – 10^{-6} sec). On the other hand, if the spin of the electon changes during the photon emission, the process is known as *phosphorescence* (10^{-3} – 10^3 sec). Phosphorescence is much less common than fluorescence.

w*intersystem crossing* is a radiationless transition between electonic states of different multilicity, such as the singlet S_1 and triplet T_1 states in the figure. Since triplet states have lower energy than the corresponding singlet state, the excess energy causes an increase in vibrational energy, which in solution is quickly lost to the solvent through vibrational relaxation.

w*external conversion*, or *quenching*, is the radiationless deactivation of an excited state through interaction with another molecule, such as the solvent or another solute. Collisional deactivation is an example of quenching.

Molecular Fluorescence

absorption spectrum

excitation spectrum

emission spectrum

acetylsalicylic acid (ASA)

fluorescence or absorbance signal

Wavelength (nm)

347   365   385

Excitation

Emission

9-methylanthracene
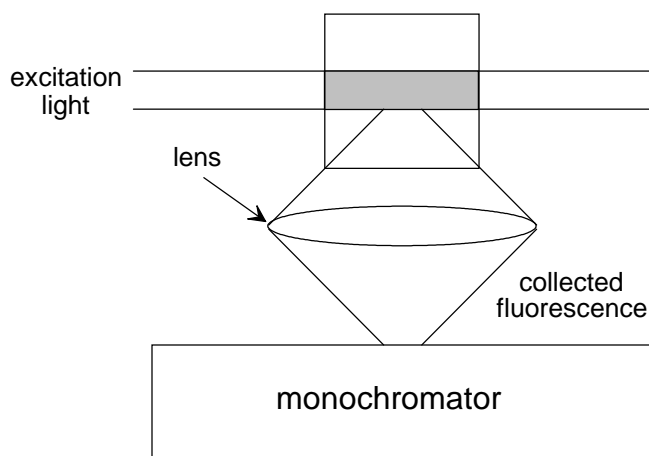
fluorescence intensity

Wavelength (nm)

Quantitative Relationships in Fluorometry

In fluorometry, excitation light that is incident to the sample solution is absorbed by the analyte, which then emits fluorescent light in all directions, as shown in the following figure.
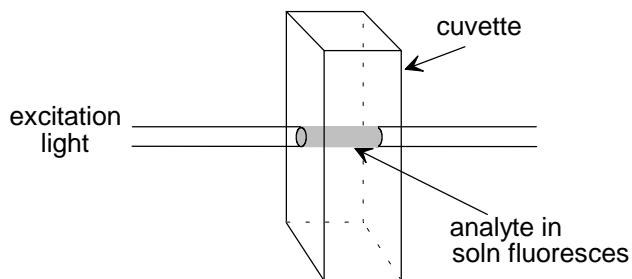
A portion of the emitted fluorescence is collected, most commonly with a right-angle geometry, as shown in the next figure.


With these issues in mind, we can begin to explore the relationship between the intensity of fluorescence emitted by the analyte and the concentration. First, we need to examine the optical geometry of the cuvette in a little more detail. The next figure shows a top view of the cuvette.

TOP VIEW
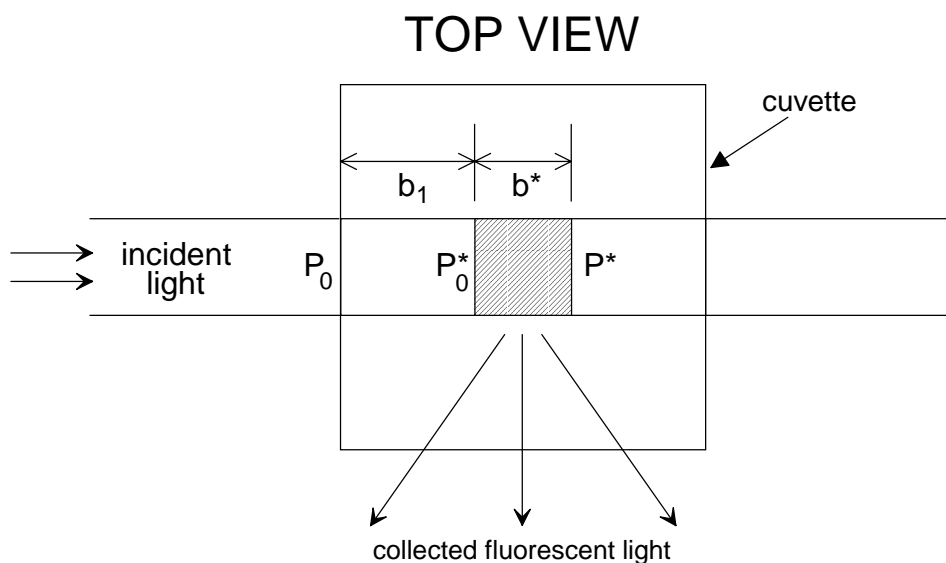
The excitation light defines a narrow "ribbon" in the solution; all along this volume, the analyte will emit fluorescent light. This ribbon will be imaged onto the entrance slit of the emission monochromator; however, only the central portion (the cross-hatched portion in the figure) will actually enter into the monochromator. Thus, the volume of solution that can give a detected fluorescence signal is a fraction of the total volume of solution that fluoresces.

The excitation light has a radiant power of P_0 upon entering the analyte solution. According to Beer's Law, the intensity of the light will decrease exponentially as it travels through the solution. The radiant power of the excitation light has fallen to a value of P_0^* when it enters the detected volume. Of course, the light intensity will continue to fall through the detection region; upon exiting, the radiant power is P^*. The following figure shows the decrease in excitation light intensity as it travels through the sample:
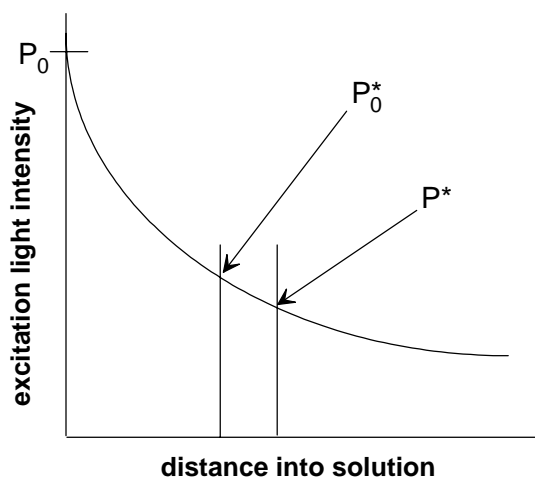
The fluorescence, P_F^*, emitted by analyte molecules in the detection volume will be directly proportional to the light absorbed, P_A^*, by the analyte in this region. The ratio of the number of photons absorbed to the number of fluorescence photons emitted is given by the fluorescence quantum efficiency, ϕ_F, of the analyte. Thus, we may write,

$$P_F^* = \phi_F P_A^* = \phi_F(P_0^* - P^*)$$

According to Beer's law,

$$P^* = P_0^* \cdot 10^{-ab^*C}$$

where a is the absorptivity of the analyte, b^* is the pathlength of the light through the detection region, and C is the analyte concentration of the solution. Substituting,

$$P_F^* = \phi_F P_0^*(1 - 10^{-ab^*C})$$

Now we must solve for P_0^* in terms of the incident light intensity, P_0. Again using Beer's Law,

$$P_0^* = P_0 \cdot 10^{-ab_1 C}$$

The difference $P_0 - P_0^*$ is light that has been absorbed *before* entering the detection volume. The excitation light is said to be *prefiltered* before entering the detection zone. We may substitue in the above equation to obtain an expression for the radiant power of the fluorescence emitted by analyte molecules in the detection volume:

$$\boxed{P_F^* = \phi_F P_0 10^{-ab_1 C}(1 - 10^{-ab^*C})}$$

[1]

Thus, we see that *the fluorescence calibration function is not at all linear* with analyte concentration. In the figure on the next page, $\dfrac{P_F^*}{P_0}$ is plotted as a function of analyte concentration (in M). The following values where used, all of which are reasonable for a strong fluorophore: $a = 10^4$ M^{-1}cm^{-1}; $b_1 = 0.45$ cm; $b^* = 0.1$ cm; $\phi_F = 0.25$. The pathlength values reflect the situation with a 1 cm cuvette and emission monochromator slit widths of 1 mm (and 1:1 imaging onto the slit).

Fluorescence Calibration Curve
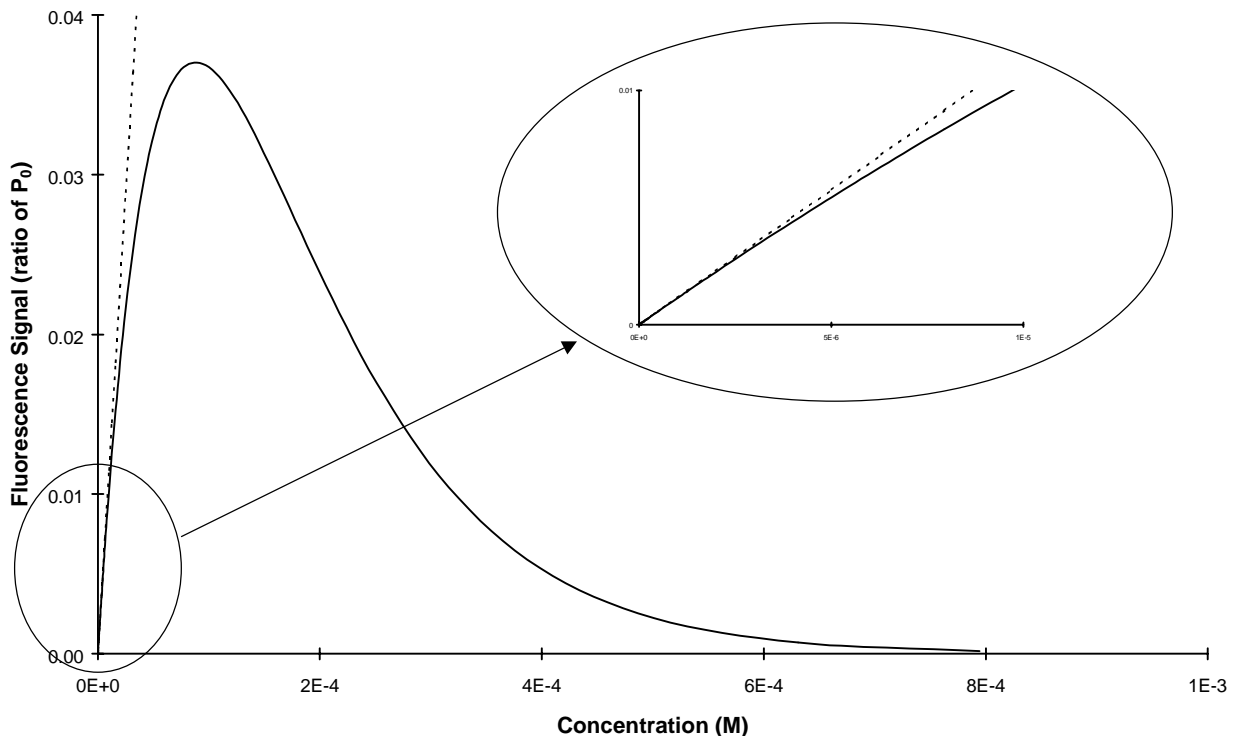
As shown in the figure, at low enough analyte concentrations, the calibration function is approximately linear. At low analyte concentrations, we can make the following two assumptions:

1. There is no prefilter effect, so that $P_0^* = P_0$ (i.e., the first exponential term disappears);

2. $10^{-ab^*C} \approx 2.303(1 - ab^*C)$. This expression comes from a Taylor expansion of the exponential.

Both of these assumptions are reasonably valid if the analyte concentration is small enough. With these assumptions, equation 1 is simplified to

linear calibration region
$$\boxed{P_F^* \approx 2.303\phi_F P_0 abC = kC}$$

This is the linear region of the calibration curve. As before, the sensitivity is directly proportional to the quantum efficiency and the incident light intensity. This equation is shown as the dashed line in the previous figure.

Note: the fluorescence calibration curve is reasonably linear so long as the absorbance, $A = abC$, over the entire 1 cm cuvette is less than about $A = 0.05$. What is interesting about this fact is that this absorbance is close to the lower limit of values that can be measured reliably by photometry. The fluorometric and photometric methods compliment each other in this respect: low concentrations can be measured with the more sensitive fluorometric method, while higher concentrations can be analyzed photometrically.