

Lecture Demonstrations of Fluorescence and Phosphorescence

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Abstract: Photoluminescence, the emission of light by molecules after photo excitation, is a standard topic in physical chemistry and analytical chemistry courses. This paper describes two lecture demonstrations of fluorescence of common substances (quinine and chlorophyll) and a demonstration of phosphorescence of naphthalene. A comparison of these two related phenomena provides an insight into electronic transitions and selection rules and the effect of deuterium substitution on the lifetime of phosphorescence.

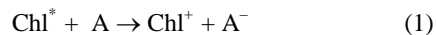
Fluorescence of Quinine Sulfate

Quinine, in the form of its sulfate salt, is a flavor component of tonic water. The bitter taste of anti-malarial quinine tonic led British colonials in India to drink it mixed with gin, thus creating the gin and tonic cocktail. Today, many soft drinks contain a small amount of the substance as a flavoring agent. Upon excitation by UV light, tonic water glows with blue light due to the fluorescence of quinine. Figure 1 shows the well-known Jablonski diagram [1] illustrating the related phenomena of absorption, fluorescence, and phosphorescence. Fluorescence occurs when the first excited singlet state (S_1) returns to the ground singlet state (S_0) by emitting a photon. The emission spectrum has a maximum at about 450 nm, accounting for the bluish color. (Figure 2, left). A very crude upper-limit estimate of the fluorescence lifetime, as defined by the time it takes to decrease the fluorescence intensity to $1/e$ of its original value, can be obtained by quickly blocking the excitation light source and noting the instant disappearance of fluorescence, confirming that the lifetime must be much less than a second. In reality, the fluorescence lifetime of quinine sulfate is about 19 ns [2].

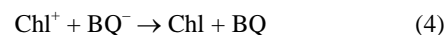
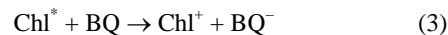
Fluorescence of Chlorophyll

A clear green chlorophyll (Chl) solution can be made by grinding spinach (either from fresh or frozen samples) in a mortar and pestle, adding the broken leaves to a beaker of acetone, and then filtering the solution through cheesecloth or coarse filter paper to remove the solid plant materials. Chlorophyll *a* and chlorophyll *b* molecules, both present in plants, impart the green color to the solution. When irradiated with UV light, these molecules fluoresce at about 650 nm to give a beautiful red solution (Figure 2, right). As with quinine sulfate, the mean fluorescence lifetime for both compounds is very short - about 5 ns [3].

When present in plant cells, instead of fluorescing, an electronically excited chlorophyll molecule (Chl^*) transfers an electron to a nearby acceptor molecule (A) as follows:



The electron is then passed along an electron-transport chain containing a quinone and other molecules to reach the reaction center. Eventually, the excess energy originally captured by the chlorophyll molecules is used to synthesize carbohydrates using atmospheric CO_2 as a carbon source [4, 5]. An interesting extension of this demonstration with chlorophyll is to introduce fluorescence quenching. In solution, we can demonstrate the quenching of chlorophyll fluorescence by subsequently adding 2,5-dimethyl-*p*-benzoquinone (BQ), which is an effective electron acceptor. In the absence of the electron-transport chain present in plants, the ion-pair formation process shown in Equation (1) undergoes rapid spin-allowed electron back transfer to yield ground state reactants:



This process is responsible for quenching the fluorescence.

Phosphorescence of Naphthalene

Like quinine and chlorophyll, the aromatic hydrocarbon naphthalene (C_{10}H_8), which bears a superficial resemblance to the porphyrin ring of chlorophyll, can undergo fluorescence although most of the emitted light is invisible because it is in the UV region. As Figure 1 shows, the first excited singlet state (S_1) of naphthalene can also decay by an alternate radiationless transition to a triplet state (T_1) of lower energy (a phenomenon known as *intersystem crossing*). During this process, some energy is lost as heat to the surrounding molecules. Once a molecule is in the triplet state, it can lose its excess energy and return to the ground state (S_0) either by spontaneously emitting a photon (this process is called phosphorescence), or undergoing another radiationless transition. Because the triplet to singlet transition is spin-forbidden, the lifetime of a triplet state is usually found to persist for seconds, which is much longer than that for fluorescence, as the previous examples demonstrate. To avoid deactivation of the triplet state by collision with other molecules, phosphorescence spectra are usually recorded with samples prepared in a frozen medium.

In this demonstration, we use naphthalene to show the effect of deuteration (that is, C_{10}D_8 versus C_{10}H_8) on the lifetime of

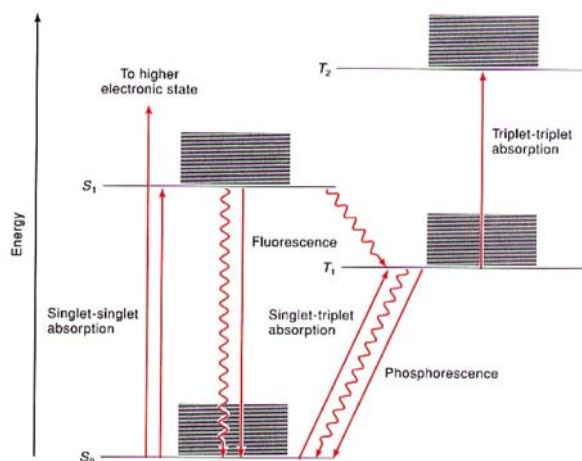


Figure 1. Jablonski diagram showing absorption, fluorescence, and phosphorescence. The wavy lines indicate radiationless transitions; the closely spaced lines represent the vibrational levels.



Figure 2. Left: Fluorescence of tonic water. Right: An acetone solution of chlorophyll under direct UV irradiation.

its phosphorescence. In 1944, Lewis and Kasha postulated that the phosphorescing state of organic molecules must be a triplet, the expected paramagnetism of which was demonstrated by magnetic susceptibility measurements [6]. The definitive proof of the formation of the triplet state, however, was provided by electron paramagnetic resonance (EPR) [7]. The green phosphorescence of naphthalene can be readily observed by dissolving the compound in 2-methyltetrahydrofuran, which forms a clear glass in the frozen state, and irradiating the sample with UV light. The irradiation is carried out with the sample tube immersed in liquid nitrogen, using an unsilvered Dewar designed for low-temperature EPR experiments. With the $C_{10}H_8$ sample, the decay of the resulting phosphorescence, as evidenced by the diminishing green light, persists for a few seconds after the excitation light source is blocked. A dramatic increase in the phosphorescence lifetime is observed when $C_{10}D_8$ is used as the sample (Figure 3, right). In this case, the emitted green light can be observed even after 30 seconds! (The literature values for the phosphorescence

lifetimes are 2.7 s for $C_{10}H_8$ and 22 s for $C_{10}D_8$, respectively [8].)

The reason for the dramatic increase in the phosphorescence lifetime from $C_{10}H_8$ to $C_{10}D_8$ is due to the fact that the relative values of the radiative and radiationless transition probabilities vary considerably between these two molecules. It is found that the complete substitution of deuterium for hydrogen in aromatic hydrocarbons increases both the observed lifetime of the phosphorescence and its quantum yield. However, there is relatively little difference in the energy of these transitions or the nature of the respective excited triplet states, as shown by the similar appearance of the phosphorescence and EPR signals for $C_{10}H_8$ and $C_{10}D_8$. Hence, it has been concluded that deuteration has altered only the relative rate of the radiationless transition. Most theories predict that only high-energy vibrations will be effective in promoting radiationless transitions between the lowest triplet state and the ground state [9]. In aromatic hydrocarbons, the highest-energy vibrations are the C–H stretching motions, which occur at frequencies of about $3,000\text{ cm}^{-1}$. Complete deuteration decreases the frequency of these vibrations by roughly the square root of the ratio of the proton mass to the deuteron mass or to a frequency of about $2,200\text{ cm}^{-1}$. This lower vibrational frequency is believed to be the major cause of the decrease of the rate constant for the radiationless process upon deuteration, and hence the observed longer lifetime of the triplet state.

The above demonstrations are fairly easy to carry out in either a small classroom or a large lecture hall, where a video camera and projection screen may be used to show the light emissions. Although the underlying principles require some knowledge of quantum mechanics, we believe that parts of the demonstrations, namely the fluorescence of quinine and chlorophyll and the quenching of chlorophyll fluorescence, are also suitable for the general chemistry audience.

Experimental

UV irradiation was provided by a short wavelength (254 nm) hand-held UV lamp (model UVG-1), or by a larger UV lamp ("BLAK-RAY" Longwave Ultraviolet Lamp, model B-100A), both from UVP, Inc. Solvents and chemicals [CAS number] (naphthalene [91-20-3], perdeutero-naphthalene [1146-65-2], and 2-methyltetrahydrofuran [96-47-9]) were purchased from Sigma-Aldrich and were used without additional purification. 2,5-Dimethyl-*p*-benzoquinone [137-18-8] was obtained from Fisher Scientific/Acros-Organics. Commercially available tonic water was used directly. Frozen spinach was thawed to room temperature before extraction of chlorophyll. An 8" 5 mm O.D. quartz EPR tube with an attached J. Young valve (Cat. # 701-JY-8) was obtained from Wilmad-Lab Glass, Inc., as was a low temperature EPR Dewar (Cat. # WG-816-B).

A freshly prepared acetone extract of chlorophyll was prepared by crushing ca. 150 g of spinach (after thawing frozen spinach, it should be drained through a metal screen; fresh spinach may also be used) in a medium mortar and pestle. The crushed spinach was transferred to a 250 mL beaker and extracted by swirling with 200 mL of acetone. The acetone extract was then filtered through coarse filter paper (cheesecloth may be substituted) into a 250 mL beaker. A small aliquot (2–3 mL) of this solution is diluted with acetone and the intensity of the resulting red fluorescence upon direct irradiation with the UV lamp is observed. Progressive serial

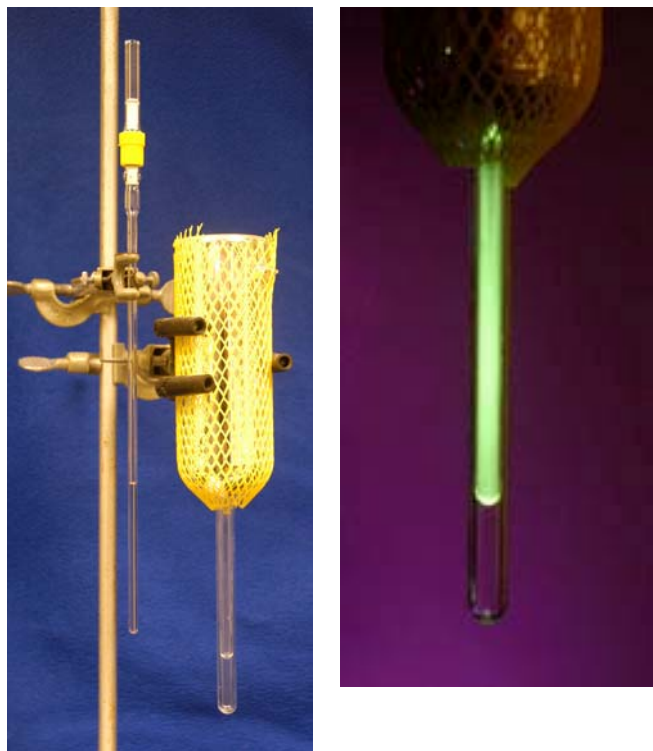


Figure 3. Left: EPR tube and EPR Dewar. Right: Phosphorescence of a frozen solution of $C_{10}D_8$ in 2-methyltetrahydrofuran after brief UV irradiation.

dilution should be carried out until a sample with suitably intense fluorescence is obtained. Use of a Petri dish to display this fluorescence is convenient; the tonic water fluorescence can be easily displayed by direct irradiation of the bottle. A small sample of 2,5-dimethyl-*p*-benzoquinone (ca. 50 mg) can

be gradually added to the acetone solution of chlorophyll under irradiation to demonstrate quenching. Darkening the room makes visualization of the fluorescence easier to observe. Chlorophyll/acetone solutions may be stored in the refrigerator and reused.

A solution of naphthalene, or perdeutero-naphthalene, was prepared by dissolution of 20 mg of the compound in 1 mL of 2-methyltetrahydrofuran in a small test tube. Degassing of solutions was not necessary. The solution was transferred to the EPR tube with a Pasteur pipet and the J. Young valve was sealed. The EPR Dewar was filled with liquid nitrogen and the EPR tube was slowly placed in the Dewar. After ca. 3 minutes, the sample was frozen solid. The sample was then briefly (ca. 15 sec) irradiated in a darkened room with the UV lamp before removing the lamp to allow the resulting phosphorescence to be observed. The sample can be used repeatedly with no noticeable deterioration.

References and Notes

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