Fluorescence Spectroscopy

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Abstract. Fluorescence spectroscopy analyzes interactions between light and matter by observing the emission of light as excited electrons return to their ground state. This emitted light can be used to analyze various properties of the sample. In this study, various dyes were diluted in solvents that best exhibited their spectroscopic properties. A number of procedures were then run on each sample using a spectrofluorometer. Experimental data was then compared to commonly accepted values found in literature.

INTRODUCTION

Fluorescence spectroscopy is an analytical tool that examines the encounter between light and matter by monitoring the light given off as falling electrons return to their ground state. In so doing, an electron absorbs energy and migrates to an excited singlet level, where spin orientation is reverse to that at the ground level. The electron returns to the ground state with no spin orientation change, and in so doing, emits light. The light emitted is characteristic of the material and can be utilized to analyze various properties of the sample.

One of the main instruments used for fluorescence spectroscopy is the spectrofluorometer, which utilizes monochromators to measure absorption, emission, and excitation profiles. A monochromator is a device that filters out specific wavelengths of light from a wide spectrum. A monochromator consists of several components: an input slit, a collimator mirror to produce a parallel light beam, a diffraction grating to disperse the light, a focusing mirror to steer the selected wavelength on to the exit slit, and the exit slit. The diffraction grating is tasked by diffracting the incoming light at angles dependent on the light wavelength and groove spacing of the grating grooves. The monochromator can select the wavelength to be analyzed by tilting the diffraction grating orientation. Monochromators are applied in fluorescence spectroscopy to select desired wavelengths to measure excitation and emission. When a measurement of the absorption spectrum is made, an interval of wavelengths is passed onto the sample and transmitted light intensity measured for each one. The level of absorption at each wavelength is determined by subtracting the transmitted intensity from the incident intensity and finding the wavelengths on which the sample absorbs light

most strongly. That data is used to establish the excitation wavelength for later measurements.

To produce an emission spectrum, the monochromator disperses the previously measured absorption wavelength to excite the sample. The light emitted by the sample is then collected, and its intensity is measured over a range of wavelengths. This emission spectrum is the probability distribution of photons emitted at various energies, showing the wavelengths at which the sample re-emits absorbed energy most effectively. An excitation spectrum is achieved by keeping the emission wavelength fixed and scanning over a series of excitation wavelengths. Surprisingly, the excitation spectrum will be similar to the absorption spectrum, as both are a measure of the sample's ability to absorb light at various wavelengths. Despite their similarity, the emission spectrum is always seen to be at higher wavelengths than the excitation spectrum. This is because these materials are photon down converters. This means that when the molecules drop from their excited state to their ground state, they emit photons of higher wavelengths, which have less energy than the incident excitation photons. To examine the emission characteristics more closely, a 3-D emission spectrum may be obtained. In this procedure, the sample is excited at multiple excitation wavelengths and the associated emission spectra are measured for each excitation wavelength. The wavelengths of emission are plotted as a function of the respective excitation wavelengths using a color map indicating emission intensity. The 3-D presentation provides an extensive map of fluorescence behavior of the sample describing how emission varies with changing excitation energies.

METHODS

This lab occurs in two segments. First, the samples must be prepared. The samples used in this lab were Coumarin 440, Coumarin 460, DCM, and fluorescein. The DCM was dissolved in DMSO, while the other three were dissolved in methanol. [1] [2] The samples were prepared in small vials. First, approximately 5 mL of the solvent was added, then a very small amount of each solute, about 1-3 mg, was added to the solvents. This noticeably changed the color of most of the samples, with the exception of Coumarin 440. The amounts of solvent and solute were not measured in exact amounts, so care needed to be taken not to saturate the solutions too much. If this happens, the light from the spectrofluorometer is not able to pass through the sample well enough, which can make measurement difficult. This was the case for DCM, so it was diluted before measurement.

The second part of the experiment involved measuring the optical properties of the samples with the use of a spectrofluorometer. In this case, an FP-8550 spectrofluorometer from Jasco, Inc. was used. The original idea for the experiment was to measure the absorbance spectra for the samples first, then use that peak value obtained to measure the emission and use the emission peak value to measure the excitation spectra. Unfortunately, this did not work out because issues were encountered while taking the absorbance spectra. The issues were not able to be addressed within the timeframe of the lab, so it was determined that literature values of the absorbance peak values would be used instead. Once these values were determined, they were used as the fixed excitation value for measuring the emission spectra of the samples. From there, the emission peak wavelengths were found using the software provided with the spectrofluorometer and were used as the fixed emission values while measuring the excitation spectra.

RESULTS

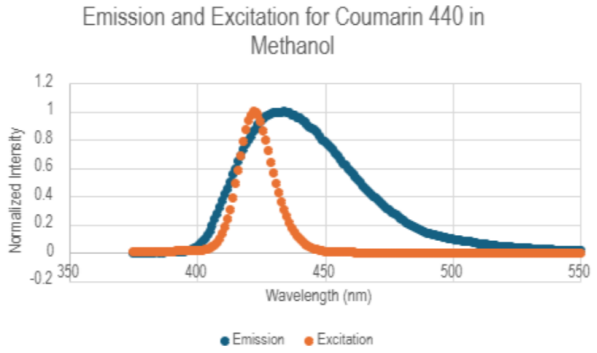

FIGURE 1. Emission and Excitation Spectra for Coumarin 440 in Methanol.

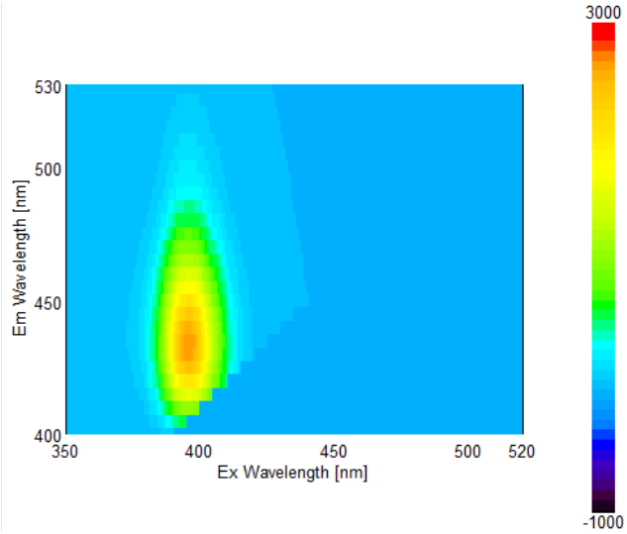

FIGURE 2. 3-D Emission vs. Excitation Profile for Coumarin 440.

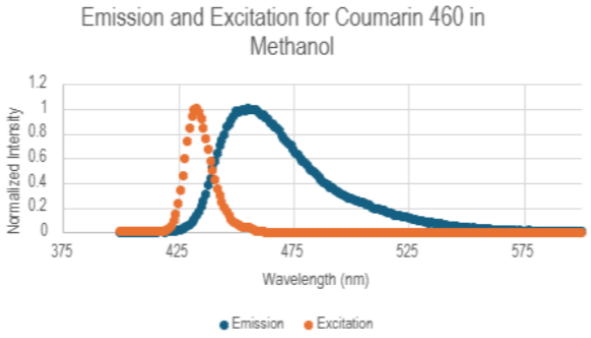

FIGURE 3. Emission and Excitation Spectra for Coumarin 460 in Methanol.

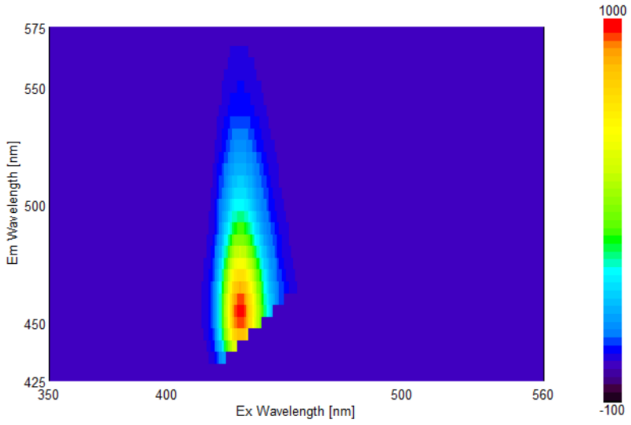

FIGURE 4. 3-D Emission vs. Excitation Profile for Coumarin 460.

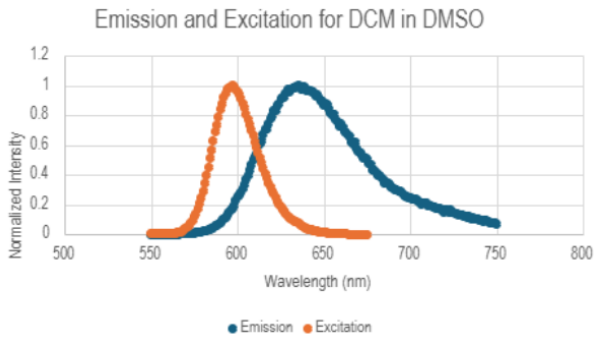

FIGURE 5. Emission and Excitation Spectra for DCM in DMSO.

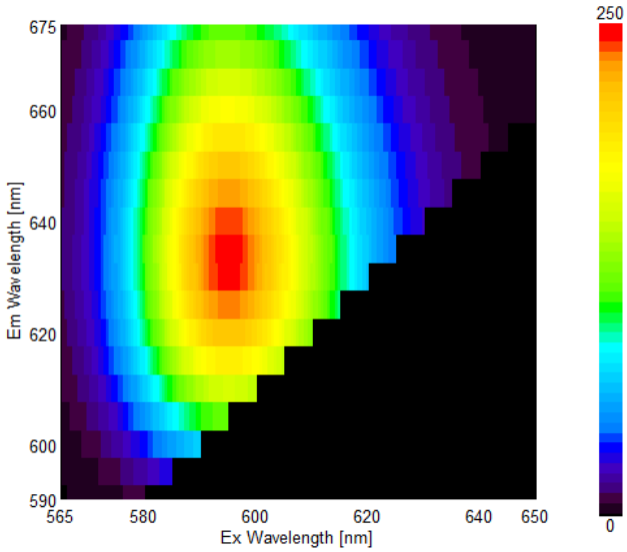

FIGURE 6. 3-D Emission vs. Excitation Profile for DCM.

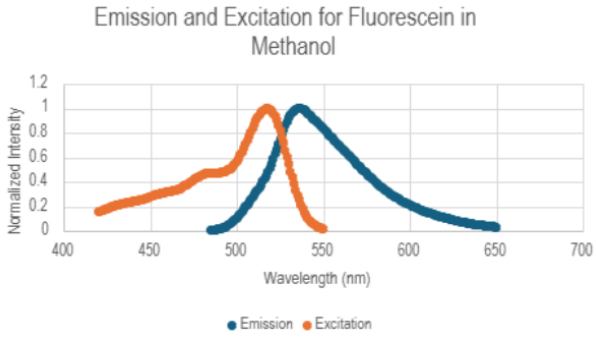

FIGURE 7. Emission and Excitation Spectra for Fluorescein in Methanol.

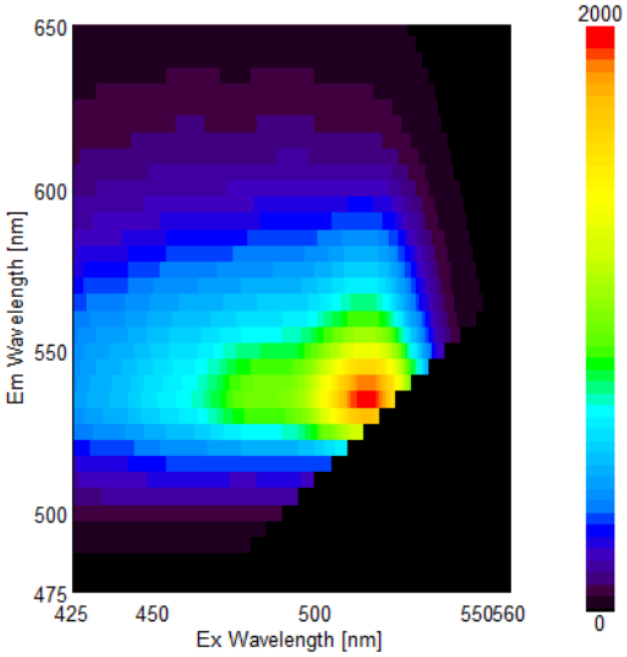

FIGURE 8. 3-D Emission vs. Excitation Profile for Fluorescein.

TABLE 1. Spectroscopic Data of Various Compounds

Compound	Solvent	Absorbance Peak (nm)	Emission Peak (nm)	Excitation Peak (nm)
Coumarin 440	Methanol	352.0	433.6	397.2
Coumarin 460	Methanol	374.0	455.4	432.8
DCM	DMSO	480.0	634.6	597.0
Fluorescein	Methanol	470.0	538.2	517.4

DISCUSSION

In order to find the emission spectra for our chosen dyes, an absorbance or excitation value is needed as a starting point. In this case, external sources were used to find the literature values of the absorbance peak for most of the dyes. [1] This included Coumarin 440, Coumarin 460, and DCM. The final dye tested, which was fluorescein, was not included in the packet and its peak needed to be found online. [2] The authors of the paper chose to excite their fluorescein dye at 470 nm, so that protocol was used in this lab as well. All of this data, along with the following

emission and excitation peak wavelengths, can be seen in Table 1.

Once the starting values had been found, the emission spectra could be measured. As detailed in the methods, the samples were put in a 1 cm cuvette, loaded into the spectrofluorometer, then the excitation value was set, as previously determined, before running the test. After determining the peak emission values, they were used to measure the excitation spectra. The peak wavelengths for the emission and excitation of each sample can also be seen in Table 1.

For Coumarin 440, an emission peak of 433.6 nm and an excitation peak of 397.2 nm were measured. The full spectra are shown in Figure 1. The individual spectra can also be compared to the 3D spectrum taken, shown in Figure 2. It can be seen that the emission peak is much wider than the excitation peak, which is confirmed by the 3D spectrum, showing a much taller peak than wide. Comparing the emission to the accepted value, which is 441 nm, a small discrepancy can be seen. [3] This difference is reasonable and can be attributed to differences in equipment and sample concentration. The emission can also be compared to the literature, which does not give an explicit peak value, however, using the spectrum, the peak value is estimated to be 430 nm, agreeing with the value found in the lab. [3]

Coumarin 460 showed an emission peak of 455.4 nm and an excitation peak of 432.8 nm. It has very similar properties to the Coumarin 440, with a narrow excitation peak and a wider excitation peak, however at a slightly higher wavelength. This can be seen in Figures 3 and 4. Similarly, emission peak comparison with other sources, which gave values of 462 nm and approximately 455 nm, respectively, yields agreement with the measured value. [1] [4]

DCM gave an emission peak of 634.6 nm and an excitation peak of 537.0 nm. Like the Coumarins, it also had a narrow excitation peak and wider emission peak, but both were a little wider than the Coumarins. Figures 5 and 6 show this. The wavelengths are also much higher than the Coumarins. Like before, the emission is compared to literature values. The peak given is 661 nm, which shows a noticeable discrepancy. [1] This may be due to the equipment used. Comparing to the 630 nm found in literature, a much smaller difference is found. [5] Due to the somewhat wide emission peak, the discrepancies are not a major concern.

Lastly, fluorescein showed an emission peak of 538.2 nm and an excitation peak of 517.4 nm. These values are both higher than the Coumarins and lower than DCM, putting fluorescein in the middle of the dyes tested. It also shows very different emission and excitation spectra behavior from the previous dyes. In Figure 7, both peaks are wide and tapering, slowly climbing to the peak

before quickly falling off, excitation cutting off toward higher wavelengths, and emission toward lower wavelengths. The 3D spectrum in Figure 8 also shows this behavior, also showing excitation dropping slower toward lower wavelengths compared to the emission. The emission, compared to the accepted value of 515 nm, shows close agreement. [2]

In general, excitation spectra for the dyes tested, dissolved in the solvents used could not be found. This is why the excitation peak wavelengths were not compared to literature values.

CONCLUSION

The primary objective of this lab was to characterize the fluorescence properties of various dyes. These include DCM, Coumarin 440, Coumarin 460, Fluorescein, and Nile Blue in their respective solvents using fluorescence spectroscopy. DCM was dissolved in DMSO, and Coumarin 440, Coumarin 460, Fluorescein, and Nile Blue were dissolved in Methanol. Using an FP-8050 spectrofluorometer, excitation and emission spectra for these dyes were recorded. As our spectrofluorometer did not allow direct absorbance measurements, publicly available fluorescence data was used. The experimental values found closely agreed with accepted values found from reputable sources. This study not only introduced us to the field of spectroscopy, but provided specfific insights into fluorescence spectroscopy and its unique properties and applications.

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