A Guided Inquiry Approach to Understanding Fluorescence Spectroscopy

This experiment is designed with four parts where each section builds on the previous part. The experiment starts off with exploring the instrument and the data collection parameters. You will explore how data collection parameters play off of each other to generate a spectrum.

The second part actually starts fluorescence data collection. You will determine the optimum wavelength for excitation by looking at the absorbance spectrum, manipulating the data collection parameters, and manipulating the LED excitation with a known sample. The goal of this is purely to get a strong, clear spectrum while manipulating the parameters you just learned about, plus a few more.

In part three, you prepare serial dilutions from a stock solution to investigate the most vital, and perhaps trickiest part of fluorescence spectroscopy, the effect of concentration on fluorescence emission. Finally, in part four, you start to learn about the properties of molecules through fluorescence spectroscopy.

Materials

Part I Operating a Spectrometer: Exploring the Instrument

Chromebook, computer, **or** mobile device

Vernier Spectral Analysis app

Fluorescence/UV-VIS Spectrophotometer[[1]](#footnote-1)

Part II Obtaining an Fluorescence Spectrum

materials from Part I

fluorescence quartz cuvette

375 nm, 450 nm, and 525 nm LEDs

quinine solution (10 mg/L in 0.1 M H2SO4)

H2SO4 (0.1 M)

Part III Conducting a Fluorescence Experiment: Concentration

materials from Part II

quinine solution (100 mg/L in 0.1 M H2SO4)

H2SO4 (0.1 M)

Part IV Learning about Molecules with Fluorescence Spectroscopy

your instructor will provide additional materials as applicable

INVESTIGATION

Part I Operating a Spectrometer: Exploring the Instrument

1. Connect the AC power supply to the Fluorescence/UV-VIS Spectrophotometer. Turn the power switch to the ON position. Allow the spectrophotometer to warm up for a minimum of 10 minutes.
2. After the 10-minute warm up period, connect the spectrophotometer to your computer or Chromebook. Launch Spectral Analysis app and then click or tap Advanced Full Spectrum. **Note**: If using Go Direct Fluorescence/UV-VIS Spectrophotometer, you can also connect to a mobile device.
3. The Spectrometer Settings should be open by default. Select Raw Data mode and observe the data collection parameters. This mode simply reports the raw signal from the lamp onto the detector.
4. Note the Integration Time, Wavelength Smoothing, and Temporal Averaging parameters.
5. Start data collection. Once a spectrum appears, end data collection.
6. Return to the Spectrometer settings, https://lh6.googleusercontent.com/PI4bMEDESSdsUtpLbmn141UypnmyNTRXNQaG9lC_c02Z0CRNzxqPH1C_7VkYbgDwKq_IQsC7lQdWytKuqa6iqu7xXB8UHpEWDrM2_geM6wSV9AJkQ94_YIXv-53DyHerA-JtfSMV, if necessary. Modify the Integration Time, Wavelength Smoothing, and Temporal Averaging parameters systematically to determine what, if any, impact the values have on the lamp output.
7. Stop and Start data collection as appropriate. Note: You will not need these spectra for the remainder of the experiment.

Part II Obtaining a Fluorescence Spectrum

1. Obtain a sample of 10 mg/L quinine in 0.1 M H2SO4 from your instructor. **WARNING**: *Sulfuric acid solution,* H2SO4: *Causes skin and serious eye irritation*.
2. Set the spectrophotometer to Absorbance mode and calibrate it. If necessary, choose Calibrate from Spectrometer Settings and follow the instructions.
3. Prepare a blank by filling the empty quartz cuvette 3/4 full with the solution used to prepare the sample.
4. Place the cuvette in the spectrophotometer.
5. Select Finish Calibration. Select OK.
6. Collect absorbance *vs.* wavelength data.
7. Remove the blank cuvette from the spectrophotometer. Empty the cuvette.
8. Fill a cuvette with ~3 mL of the known sample.
9. Place the sample in the spectrophotometer.
10. Start data collection. Once the absorbance spectrum is displayed, stop data collection. The data are automatically stored. **Note**: Click on the y-axis label to display or hide data sets.
11. Examine the plot of absorbance *vs.* wavelength. Make sure the absorbance values are between 0.1 and 1.0 absorbance units. Any values outside this range may introduce an error. If your sample is outside this range, dilute or concentrate it and repeat Step 6.
12. Use the Statistics feature to determine the wavelength of maximum absorbance.
13. Based on your absorbance spectrum, determine the best LED to excite your samples for the fluorescence spectrum. Insert this LED into the LED slot on the UV-VIS/Fluorescence Spectrophotometer.
14. Switch to Fluorescence mode and set the LED.
15. Set the Integration Time to 200 ms. The Temporal Averaging should be set to 6 and the wavelength smoothing to 1.
16. Set the LED to 100.
17. Collect fluorescence *vs.* wavelength data.
18. Fill a cuvette with ~3 mL of the known sample.
19. Place the sample in the spectrophotometer.
20. Start data collection. Fluorescence is automatically plotted on the right y-axis.
21. Examine the plot of fluorescence *vs.* wavelength. Make sure the fluorescence values are above 0.1 relative units. Any values below this may introduce an error. If your sample is outside this range, adjust the integration time, temporal averaging, or LED intensity based on your investigations in Part I. **Note**: If you adjust these values too much, your baseline will no longer be close to zero. If it floats too far from the zero line, you should stop data collection and recalibrate with the new settings.
22. Once the fluorescence spectrum is displayed, stop data collection. Record the integration time, temporal averaging, wavelength smoothing, and LED intensity values that resulted in the cleanest fluorescence spectrum.
23. Measure the fluorescence emission of your sample with different excitation wavelengths by using the available LEDs.
24. Remove the solution from the spectrophotometer and discard as directed. Rinse the cuvette with 0.1 M H2SO4.

Part III Conducting a Fluorescence Experiment: Concentration

1. Place the correct LED in the spectrometer.
2. Obtain the 100 mg/L stock solution of quinine sulfate in 0.1 M H2SO4. Accurately prepare serial dilutions of this standard to the following concentrations: 75 mg/L, 50 mg/L, 20 mg/L, 15 mg/L, 10 mg/L, 8 mg/L, 5 mg/L, 3 mg/L. Dilute with 0.1 M H2SO4.
3. Collect fluorescence *vs*. concentration data.
4. To set this mode in Spectral Analysis, select New Experiment from the File menu.
5. Fill the cuvette with the most concentrated sample first and place it in the spectrophotometer.
6. Select the Fluorescence vs Concentration experiment.
7. Follow the instructions for selecting the measurement wavelength. Recalibrate with your blank solution.
8. Fill the cuvette with the most concentrated sample first and place it in the spectrometer. Adjust your spectrometer settings to the values that gave you the spectrum in Part II. If necessary, recalibrate with your blank solution.
9. Start data collection. After the fluorescence reading stabilizes, select Keep. Enter the concentration of the solution and select OK. Collect data for all the solutions. **Note**: Do not adjust spectrometer settings during data collection; if you wish to adjust them, stop data collection and start again.
10. Examine the plot of fluorescence *vs.* concentration. Note deviations from expected behavior, if any. Perform a linear regression analysis on all or part of the data.

Part IV Learning about Molecules with Fluorescence Spectroscopy

* Using the skills you have learned from Parts I through III, measure the pH dependence of quinine fluorescence. Adjust the pH of five samples between pH 2 and pH 6. The concentration of quinine should be the same in each solution.
* Using the skills you have learned from Parts I through III, measure the halide quenching of quinine fluorescence. Prepare five solutions of 10 mg/L quinine in 0.1 M H2SO4 with the following concentrations of KBr: 0 mM, 2.5 mM, 5.0 mM, 7.5 mM, and 10.0 mM. Measure the fluorescence of the five solutions. The concentration of quinine should be the same in each solution.

ANALYZING RESULTS

Part I Operating a Spectrometer: Exploring the Instrument

1. Discuss the changes in your spectral output that resulted from adjusting the Integration Time.
2. Discuss the changes in your spectral output that resulted from adjusting the Temporal Averaging.
3. Discuss the changes in your spectral output that resulted from adjusting the Wavelength Smoothing.

Part II Obtaining an Fluorescence Spectrum

1. How did you determine the excitation wavelength for your fluorescence spectrum?
2. Discuss why a graph showing absorbance and fluorescence on a double-y-axis is useful.
3. Did other excitation wavelengths produce an accurate fluorescence spectrum? Why or why not?
4. Discuss the changes in fluorescence emission of your sample when the excitation intensity is increased.

Part III Conducting a Fluorescence Experiment: Concentration

1. Discuss deviations in linearity in your fluorescence intensity *vs.* concentration data.
2. If you were to perform a fluorescence experiment using quinine sulfate, what concentration would you use and why?

Part IV Learning about Molecules with Fluorescence Spectroscopy

1. Discuss the changes in fluorescence emission of your sample as a function of pH.
2. Discuss the changes in fluorescence emission of your sample as a function of halide concentration.

1. The procedure is written for fluorescence/UV-VIS spectrophotometers from Vernier including the Go Direct Fluorescence/UV-VIS Spectrophotometer (order code: GDX-SPEC-FUV) and the Vernier Fluorescence/UV-VIS Spectrophotometer (VSP-FUV). [↑](#footnote-ref-1)