Absorbance and Fluorescence Characterization of Quinine

Quinine was first isolated in 1820 from the bark of the cinchona tree. In the past, it has been a common treatment for malaria. The typical medicinal form is quinine sulfate dihydrate, (C20H24N2O2)2•H2SO4•2H2O. Its molecular weight is 782.98 g/mol.

Quinine is a strongly fluorescing compound, particularly in dilute acidic solutions. This makes it ideal for investigation using a fluorescence spectrophotometer. In this experiment, you will measure the absorbance spectrum of quinine to determine the best wavelength for fluorescence excitation. You will then prepare a calibration curve by measuring the fluorescence intensity of quinine standards of known concentration. After measuring a sample of tonic water with published quinine concentration, you will compare absorbance and fluorescence spectroscopic techniques.

Materials

LabQuest or computer

LabQuest App or Logger *Pro*

Vernier Fluorescence/UV-VIS Spectrophotometer

fluorescence quartz cuvette

375 nm LED

0.05 M H2SO4 (aq)

quinine stock solution (100 mg/L)

prepared tonic water (with quinine) sample in 0.05 M H2SO4

HAZARD ALERTS

The chemical safety signal words used in this experiment (DANGER, WARNING, and N/A) are part of the Globally Harmonized System of Classification and labeling of Chemicals (GHS). Refer to the Safety Data Sheet (SDS) that came with the chemical for proper handling, storage, and disposal information. The SDS can also be found online from the manufacturer.

Sulfuric acid, 0.05 M, H2SO4: **WARNING**: *Sulfuric acid solution,* H2SO4: *Causes skin and serious eye irritation*.

Procedure

Part I Prepare a Series of Quinine Standards

1. Obtain and wear goggles and gloves.

2. Accurately prepare a series of quinine standards from the 100 mg/L stock solution. Make five dilutions with 0.05 M H2SO4. This should result in five diluted samples with concentrations of 20 mg/L, 10 mg/L, 8 mg/L, 5 mg/L, and 2.5 mg/L.

Part II Measure an Absorbance Spectrum and Fluorescence Spectrum for a Quinine Sample

3. Connect the AC power supply to the Vernier Fluorescence/UV-VIS Spectrophotometer. Turn the power switch to the ON position. Allow the spectrophotometer to warm up for a minimum of 10 minutes.

4. After the 10-minute warm up period, connect the spectrophotometer to the USB port of LabQuest or a computer. Start the data-collection program, and then choose New from the File menu.

5. Calibrate the spectrophotometer in Absorbance mode.

1. Absorbance mode is selected by default. To calibrate the spectrophotometer, choose Calibrate ► Spectrometer from the Experiment menu of Logger *Pro* or the Sensors menu of LabQuest App.
2. Prepare a blank by filling the empty quartz cuvette 3/4 full with the 0.05 M H2SO4 used to prepare your sample.
3. Place the cuvette in the spectrophotometer.
4. Select Finish Calibration. Select OK.

6. Collect absorbance *vs.* wavelength data.

1. Remove the blank cuvette from the spectrophotometer. Empty the cuvette.
2. Fill a cuvette with ~3 mL of the 20 mg/L quinine sample.
3. Place the sample in the spectrophotometer.
4. Start data collection. Once the absorbance spectrum is displayed, stop data collection.

7. 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.

8. Using the Statistics feature in Logger *Pro* or LabQuest App, record the wavelength of maximum absorbance.

9. Store the run. In Logger *Pro*, do this by choosing Store Latest Run from the Experiment menu. In LabQuest App, you can store a run by tapping the file cabinet icon.

10. Obtain the 375 nm LED and insert it into the LED slot on the Fluorescence/UV-VIS Spectrophotometer.

11. Switch to Fluorescence mode and set the LED.

1. To switch to fluorescence mode, choose Change Units ► Spectrometer from the Experiment menu in Logger *Pro* or the Sensors menu in LabQuest App, and choose Fluorescence.
2. Set the Sample Time to 200 ms.
3. Set the LED to 50. Select Apply.

12. Calibrate the spectrophotometer for Fluorescence mode.

1. Choose Calibrate ► Spectrometer from the Experiment menu of Logger Pro or the Sensors menu of LabQuest.
2. Prepare a blank by filling the empty quartz cuvette 3/4 full with the 0.05 M H2SO4 used to prepare your sample.
3. Place the cuvette in the spectrophotometer.
4. Select Finish Calibration. Select OK.

13. Collect fluorescence *vs.* wavelength data.

1. Remove the blank cuvette from the spectrophotometer. Empty the cuvette.
2. Fill a cuvette with ~3 mL of the 20 mg/L quinine sample.
3. Place the sample in the spectrophotometer.
4. Start data collection.
5. Examine the plot of fluorescence vs. wavelength. Make sure the fluorescence values are between 0.1 and 1.0 relative units. Any values outside this range may introduce an error. If your sample is outside this range, adjust the sample time or LED intensity as in Step 11. **Note**: If you adjust these values too much, you may need to stop data collection and recalibrate with the new settings.
6. Once the spectrum is acceptable, stop data collection.

14. Using the Statistics feature in Logger *Pro* or LabQuest App, record the wavelength of maximum absorbance.

Part III Measure Absorbance *vs*. Concentration data and Fluorescence *vs*. Concentration data for the Quinine Standards

15. Switch back to Absorbance mode. The absorbance calibration settings are already stored. The LED should turn off automatically.

16. Collect absorbance *vs.* concentration data.

Logger *Pro* (If you are using LabQuest App, see below)

1. Click Configure Spectrometer Data Collection, . Select Absorbance *vs.* Concentration as the Collection Mode. Change the units to mg/L.
2. Make sure the wavelength of maximum absorbance (λ max) is properly selected. Change the center wavelength value by clicking the peak absorbance on graph or by choosing a wavelength from the list.
3. Select OK. A message will appear warning you to either save or discard the full spectrum run. Make your choice and proceed with data collection. Storing the full spectrum run will show the full spectrum as the top graph with your absorbance *vs*. concentration data on the bottom graph.
4. Proceed to Step 17.

LabQuest App

1. On the Meter screen, tap Mode and change the data-collection mode to Events with Entry.
2. Enter the Name (e.g., Concentration) and Units (e.g., mg/L). Select OK. A message will appear warning you to either save or discard the full spectrum run. Make your choice and proceed with data collection.
3. Confirm the wavelength that you wish to measure on the Meter screen. To change the wavelength, tap the meter itself, and select Change Wavelength. Enter the wavelength of your choice and select OK. If the wavelength you type in is not measured by the spectrometer, the LabQuest will automatically choose the wavelength closest to your choice.
4. Proceed to Step 17.

17. The first solution should still be in the spectrophotometer. Start data collection. After the absorbance reading stabilizes, select Keep. Enter the concentration of the solution and select OK. Collect data for the five solutions.

18. When all the sample data are collected, end data collection.

19. Store the run. In Logger *Pro*, do this by choosing Store Latest Run from the Experiment menu. In LabQuest App, you can store a run by tapping the file cabinet icon.

Part IV Measure an Absorbance Spectrum and Fluorescence Spectrum for Tonic Water

20. Measure the absorbance of the tonic water sample. Record the absorbance value.

21. Switch to Fluorescence mode and set the LED.

1. To switch to fluorescence mode, choose Change Units ► Spectrometer from the Experiment menu in Logger *Pro* or the Sensors menu in LabQuest App and choose Fluorescence.
2. Set the Sample Time to 200 ms.
3. Set the LED to 50. Select OK.

22. Collect fluorescence intensity *vs.* concentration data.

Logger *Pro* (If you are using LabQuest App, see below)

1. Insert the first quinine standard sample in the spectrometer.
2. Click Configure Spectrometer Data Collection, . Fluorescence *vs.* Concentration should be selected.
3. Change the center wavelength value by clicking the peak fluorescence on graph or by choosing a wavelength from the list.
4. Select OK. A message will appear warning you to either save or discard the full spectrum run. Make your choice and proceed with data collection. Storing the full spectrum run will show the full spectrum as the top graph with your fluorescence *vs.* concentration experiment on the bottom graph.
5. Proceed to Step 23.

LabQuest App

1. Insert the first quinine standard sample in the spectrometer.
2. Confirm the wavelength that you wish to measure on the Meter screen. To change the wavelength, tap the meter itself, and select Change Wavelength. Enter the wavelength of your choice and select OK. If the wavelength you type in is not measured by the spectrometer, the LabQuest will automatically choose the wavelength closest to your choice.
3. Proceed to Step 23.

23. The first solution should still be in the spectrophotometer. Start data collection. After the fluorescence reading stabilizes, select Keep. Enter the concentration of the solution and select OK. Collect data for the five solutions.

24. When all the sample data are collected, end data collection.

25. Measure the fluorescence of the tonic water sample. Record the fluorescence value.

26. Perform a linear regression analysis for the absorbance and fluorescence runs.

27. When your data collection is complete, turn the spectrophotometer power switch to the OFF position.

Discussion

* What was the concentration of quinine in tonic water?
* What is the Stokes shift for quinine?
* Compare the absorbance analysis of the tonic water sample with the fluorescence analysis. Which technique is more sensitive?
* Discuss deviations in linearity in your fluorescence intensity vs. concentration data.

EXTENSIONS

* Measure the excitation intensity dependence of quinine. Choose one sample and measure its fluorescence as a function of LED intensity. Discuss the changes in fluorescence emission of quinine when the excitation intensity is adjusted.
* Measure the pH dependence of quinine fluorescence. Adjust the pH of five quinine samples between pH 2 and pH 6. The concentration of quinine should be the same in each solution. Discuss the changes in fluorescence emission of quinine as a function of pH.
* Measure the fluorescence emission of quinine with different excitation wavelengths. Collect fluorescence spectra of quinine with other excitation LEDs available. Discuss the dependence of fluorescence measurements on excitation wavelength.
* Measure the halide quenching of quinine fluorescence. Prepare five solutions of 20 mg/L quinine in 0.05 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. Discuss the changes in fluorescence emission of quinine as a function of halide concentration.