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

Chromebook, computer, or mobile device

Vernier Spectral Analysis app

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

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 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 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.

5. Calibrate the spectrophotometer with the blank. Absorbance mode is selected by default. Select Calibrate and follow the prompts to finish calibration.

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. **Note**: The data are automatically stored.

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, record the wavelength of maximum absorbance.

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

10. Prepare the spectrophotometer for measuring the fluorescence of the solution. To do this, click Spectrometer Settings, , and enable Fluorescence. You may want to calibrate with the appropriate solvent to remove any baseline offset.

11. Set an appropriate sampling time for collecting fluorescence data.

1. Set the Integration time to 200 ms.
2. Set Wavelength Smoothing to 0.
3. Set Temporal Averaging to 1.
4. Set the LED to 50.

12. Start fluorescence data collection. A full spectrum graph of the sample will be displayed. Stop data collection.

13. Observe the peak or peaks on the graph. Fluorescence is measured on a relative scale of 0–1, thus the peak fluorescence needs to be less than 1.0. If the peak intensity is greater than 1, repeat Step 24 and decrease the sample time by 10 ms. If the peak is below 0.5, increase the sample time by 10 ms. Collect a new fluorescence spectrum. Increase or decrease the sample time until the peak amplitude is 0.5–1.0. Record the emission wavelength in your data table.

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

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

15. Collect absorbance data for the five standard solutions. Record the absorbance and concentration values in your lab notebook.

16. Switch back to Fluorescence mode. The calibration settings are already stored. The LED should turn on automatically.

17. Collect fluorescence data for the five standard solutions. Record the absorbance and concentration values in the data table.

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

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

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

20. 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.

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)