Characterization of Quinine and Its Determination

Adapted From: Donald T. Sawyer**,** William R. Heineman, Janice M. Beebe**,** Chemistry Experiments for Instrumental Methods, Experiment 10-1, 271-273, 1984.
and http://webpages.acs.ttu.edu/dpappas/index.html

Purpose:
Quinine is a strongly fluorescent compound in dilute acid solution with two excitation wavelengths (250 and 350 nm) and a fluorescence emission at 450 nm. The factors which affect the quantitative determination of quinine (such as concentration quenching and chemical quenching) will be studied as well as the interpretation of the emission spectra.

Chemicals:
- Quinine stock solution: (100.0 µg/mL), 120.7 mg quinine sulfate dihydrate or 100.0 mg quinine, transfer to a 1-L volumetric flask, add 50 mL 1 M sulfuric acid (H_2SO_4), and dilute to mark with water (**prepared by the stockroom and protected from light**).
- Unknown: Quinine solutions (in 0.05 M H_2SO_4)
- 0.05 M H_2SO_4 for dilutions
- 10.0 µg/mL Quinine stock solution, prepared from the 100.0-µg/mL solution
- 0.05 µg/mL sodium bromide (**prepared by the stockroom**)
- Buffers (pH 1, 2, 3, 4, 5, 6)
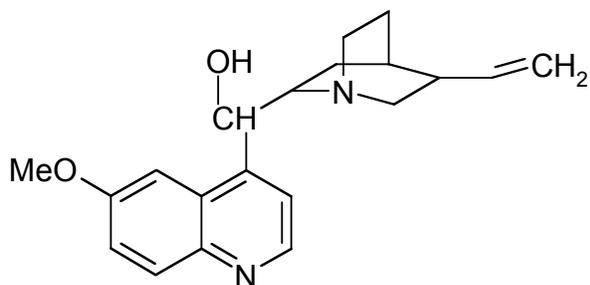
QUININE

Theory:
For low concentrations, the fluorescence intensity is directly proportional to the concentration as well as to the intensity of the incident radiation. The equation
$F = P_0(2.3\varepsilon bc)Q_f k$ holds generally for concentrations up to a few micrograms per milliliter. Where
- F = intensity of fluorescence
- ε = molar absorptivity
- b = path length
- c = concentration
- P_0 = power of incident radiation
- Q_f = quantum efficiency = photons emitted/photons absorbed
- k = photons measured/photons emitted

Reduction in intensity of fluorescence can be due to specific effects of constituents of the solution itself. The term quenching is used to describe any such reduction in intensity. Types of

quenching include *concentration quenching* (a decrease in the fluorescence-per-unit-concentration as the concentration is increased), also referred to as an inner filter effect, and *chemical quenching*. Concentration quenching results from excessive absorption of either primary or fluorescent radiation by the solution. Collisional quenching may be caused by nonradiative loss of energy from the excited molecules, and the quenching agent (such as oxygen) may facilitate conversion of the molecules from the excited singlet to a triplet level. Chemical quenching is due to actual changes in the chemical nature of the fluorescent substance (conversion of a weak acid to its anion with increasing pH). Aniline is an example. It fluoresces when the molecule is between pH 5 and pH 13. Below pH 5 it exists as the anilinium cation, and above pH 13 it exists as the anion: neither fluoresce.

Excitation and Emission Spectra

The fluorescence (emission) spectrum of an organic molecule is roughly a mirror image of its excitation (absorption) spectrum. This is due to the fact that the vibrational levels in the ground and excited states have nearly the same spacing (see "Additional Reading, Figure 10-1"), and the molecular and orbital symmetries do not change. Assuming that all of the molecules are in the ground state before excitation, the least energy absorbed in the excitation process (i.e., of the longest wavelength) equals the greatest energy transition in the fluorescence process (i.e., of the shortest wavelength). (See "Skoog: 402-410" for the absorption and emission spectra of quinine.) If a significant number of molecules are already in a vibrationally excited state before excitation, some overlap occurs in the absorption and emission spectra because less energy is needed to excite these molecules to the S_1 level.

The types of transitions associated with fluorescence include π*-to-π and π*-to-n; σ*-to-σ transitions are seldom observed because fluorescence spectra from absorptions at wavelengths shorter than 250 nm are not likely to occur – the high energy causes deactivation of excited states by predissociation or dissociation. At 200 nm (equal to 140 kcal/mol) bond ruptures often take place. The molar absorptivity for a π-to-π* transition (excitation) is 100 to 1000 times that for a n-to-π* transition. The time involved for the former is of the order of 10^{-7} to 10^{-9} s; that for the latter is 10^{-5} to 10^{-7} s.

Under usual conditions and at a concentration of about 2 μg/mL quinine, the two excitation peaks (250 and 350 nm) and one emission peak (450 nm) will be seen. However, for carefully controlled conditions other peaks appear due to grating monochromator peculiarities and Rayleigh, Tyndall, and Raman scattering. Rayleigh scattering refers to radiation scattered in all directions by elastic collisions (impinging and dispersed radiation are the same wavelength and are radiated in a random manner). In Raman scattering, the collisions are nonelastic, due to the mixing of the electromagnetic energy with the rotational and vibrational energy of the colliding molecule, and the emerging radiation will be at a different wavelength.

Procedures
A. Preparation of a Calibration Curve: Determination of Quinine in Unknowns and Limit of Detection

Prepare a series of quinine standards from the 100-μg/mL stock standard. Make sequential tenfold dilutions with 0.05 *M* H_2SO_4. This should give concentrations of 10, 1, 0.1, 0.01, 0.001, 0.0001 μg/mL, etc. Collect replicate scans of the blank. Make dilutions until the most dilute solution gives a fluorescence intensity approximately that of the blank (0.05 *M* H_2SO_4).

Read carefully the instructions for the use of the particular fluorometer to be used. If a spectrofluorometer is available, record the excitation and emission spectra for the 1-µg/mL solution, using 0.05 M H_2SO_4 as a blank. The emission wavelength is the same (450 nm) for either of the excitation wavelengths (250 and 350 nm). Also measure the fluorescence of a sample of quinine tonic water after the following dilutions: pipet 5.00 mL of tonic water into a 250-mL volumetric flask, dilute to the mark with 0.05 M H_2SO_4; pipet 5.00 mL of this solution into a 25-mL volumetric flask and dilute to volume with 0.05 M H_2SO_4.

Collect a UV-Visible absorption spectrum of the 1 µg/ml quinine solution a UV-Visible Spectrophotometer.

Treatment of Data:
Plot the relative fluorescence intensity versus the quinine concentration. **Discuss any deviation from linearity in the plots.** Account for deviations if present and delete these points from your calibration curve. Determine the concentration of the unknown quinine sample and of the tonic water using a least squares spreadsheet and report the appropriate uncertainties. Remember to calculate and report the concentration of quinine in the **orginal** tonic water.

In your discussion *compare and contrast* the excitation spectrum collected with fluorometer with the UV-Visible absorption spectrum. How do the sensitivities compare?

Determine the limit of detection, c_m, using the standard deviation of the blank intensity, s_{bl}, and the instrumental sensitivity, m.

B. pH Dependence of Quinine:
Pipet 2.0 mL of the 10-µg/mL standard quinine solution into a 25-ml volumetric flask and dilute to the mark with a pH 1 buffer solution. Measure the exact pH of the resulting solution with a pH meter. Repeat the above procedure with five other buffer solutions between pH 2 and pH 6. The concentration of quinine will be the same in each solution. Measure the fluorescence intensity of these six solutions.

Treatment of Data
Plot the fluorescence intensity versus the pH for the six solutions. On the basis of these results, what conclusions can be drawn about the pH dependence of quinine?

C. Halide Quenching of Quinine Fluorescence:
Pipet 2.0 mL of the 10-µL standard quinine sulfate solution into a 25-mL volumetric flask, add 1.0 mL 0.05 M NaBr., and fill to the mark with 0.05 M H_2SO_4. Prepare four more solutions with 2.0 mL of 10-µg/mL quinine in each and add 2.0-, 4.0-, 8.0-, and 16.0-mL portions of NaBr. Measure the fluorescence of the five solutions.

Treatment of Data
Plot fluorescence intensity versus **concentration** (not volume) of bromide ion. Explain the results. Could hydrochloric acid be used to dilute the standard quinine solutions in place of 0.05 M H_2SO_4? Why?

References:

1 A.J. Pesce, C.G. Rosen, and T.L. Pasby (eds.), "Fluorescence Spectroscopy: An Introduction for Biology and Medicine," Dekker, New York, 1971.

2 H.H. Bauer, G.D. Christian, and J.E. O'Reilly (eds.), "Instrumental Analysis," Allyn & Bacon, Boston, 1978, chap. 9.

3 H. H. Willard, L.L. Merritt, Jr., J.A. Dean, and F.A. Settle, Jr., "Instrumental Methods of Analysis," 6th ed., Van Nostrand, New York, 1981, Chap. 4.

4 G. W. Ewing, "Instrumental Methods of Chemical Analysis," 4th ed., McGraw-Hill, New York, 1975, chap. 4.

5 J.E. O'Reilly, *J. Chem. Ed., 53,* 191 (1976).

6 J.E. O'Reilly, *J. Chem. Ed., 52,* 610 (1975).

7 M.W. Legenza and C. J. Marzzacco, *J. Chem. Ed., 54,* 183 (1977).

8 J.D. Winefordner, S.G. Schulman, and T.C. O'Haver, "Luminescence Spectrometry in Analytical Chemistry," Wiley, New York, 1972, p. 293.

9 S. Udenfriend. "Fluorescence Assay in Biology and Medicine," Academic Press, New York, vol. I, 1962; vol II, 1969.

10 G.G. Guilbault, "Practical Fluorescence: Theory, Methods, Techniques," Dekker, New York, 1973.

11 C. E. White and R.F. Argauer, "Fluorescence Analysis. A Practical Approach," Dekker, New York, 1970.

12 E.D. Olsen, "Modern Optical Methods of Analysis," McGraw-Hill, New York, 1975, chap. 8.

13 W. R Seitz in "Treatise on Analytical Chemistry," P.J. Elving, E.J. Meehan, and I.M. Kolthoff (eds.), 2nd ed., part 1, vol. 7, Interscience, New York, 1981, chap. 4.