Fluorescence Determination of Quinine in Tonic Water
Experiment 2

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I. General Information

Date experiment started: February 14, 2013
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Unknown Identification: Mixed® Tonic Water

II. Objective

The purpose of this introductory experiment is to become familiar with a technique often used in quantifying the concentration of unknown substances: fluorescence determination. More specifically, the analysis of quinine concentration in tonic water which makes famous night club drinks fluoresce blue.

III. Instrumentation/Introduction

Course of Action. Spectrofluorometry measures the emission of visible light when electrons excited by ultraviolet (UV) light return to their ground states. Notice the two grating monochromators (which measure wavelength intensity) in Fig. 1.

![Fig. 1. Spectrofluorometer schematic.](image)
In order to measure the fluorescence of a sample, radiation is emitted from the xenon lamp and split after coming into contact with the excitation monochromator. One beam travels to a reference photomultiplier tube (responsible for detecting photons) and the other passes through the sample. Once this second beam passes through the sample, it is bounced to the emission monochromator and the dispersion of radiation demonstrated by the Jablonski diagram in Fig. 2 below is detected by the second photomultiplier tube.

![Jablonski Diagram](image)

**Corrections.** In a spectrofluorometer, instrument readings will vary greatly from day-to-day if left uncorrected because of variances among the instrument such as source intensity, transducer sensitivity, and the synchronization of monochromators. In order to negate these variances, three methods are used in order to provide corrections. The first is to establish a reproducible sensitivity level by testing a set of standards. The second involves manually setting parameters for the emission spectra. As shown in Fig. 3, array-detector spectrofluorometers are able to use CCD detectors to measure the intensity of a sample for the entire range of visible wavelengths by measuring the entire emission spectra at a single excitation wavelength. This determines the calibration factors, and a resulting correction factor is stored on the computer to correct the subsequent spectra automatically. The third involves finding the limit of
detection, which is basically “blanking the blank.” It involves taking many trials of the blank in order to find the lowest value the instrument can read.

**Quenching.** The quenching trend is separate from the objective of this experiment, though it heavily affects the process of fluorescence determination in general. The term “quenching” refers to the process of decreasing the intensity of an analyte’s signal, though the concentrations remain the same. An example of a compound whose addition will “quench” the signal of the analyte is NaCl. This effect increases with increasing concentration of the quenching addition.

### IV. Experimental Procedure

*Preparing Stock Solutions.* The first step for this experiment involved preparing three stock solutions. The calibration standards were prepared by diluting the stock solution of quinine with a diluted H₂SO₄. The H₂SO₄ was diluted to 100 mL of 1 M solution according to the following sample calculation:

\[
SC \ 1 \ \ m_1v_1 = m_2v_2 \\
(17.6 \text{ M}) \times (x \text{ mL}) = (1\text{ M}) \times (100 \text{ mL})
\]

\[
x = 5.68 \text{ mL}
\]
From this, a 0.05 M solution of H$_2$SO$_4$ was prepared according to the following calculation:

\[
SC 2 \quad m_1v_1 = m_2v_2 \text{ (to get a 0.05M H}_2\text{SO}_4 \text{ solution)}
\]

\[
(1 \text{ M}) \times (x \text{ mL}) = (0.05 \text{ M}) \times (500 \text{ mL})
\]

\[
x = 25 \text{ mL}
\]

The quinine stock solution was prepared according to the following cocktail: 10 mg quinine, 5 mL of 1 M H$_2$SO$_4$, and filled to the mark with deionized water.

*Preparing the Fluorometer.* The fluorometer had to be appropriately set in order to progress with the quinine standards’ readings. In Simple Reads mode, the excitation wavelength was set to 350 nm. In Scan mode, the emission wavelength was manually identified as “corrected” at 455 nm by the maximum wavelength identified as the peak of the resulting bell-shaped curve, and set accordingly. Detection limit was also determined by reading the same blank 20 times.

*Gathering Data: Standards.* After preparing a set of quinine standards with concentrations of 0.5, 1.0, 2.0, 3.0, and 4.0 ppm, each solution was poured in a cuvette and placed inside the fluorometer for a rapid reading. Initially, the readings were done with a detector at medium voltage. Based on the extremely high readings, the detector was exchanged for one that could analyze the sample within a reasonable fluorescence percentage.

*Gathering Data: Quinine.* The tonic water sample was then examined at various dilutions (10x, 20x, 50x, and 100x) in order to find which dilution would be within the range of standards in order to have this unknown value fit within a calibration plot.

*Fluorescence Quenching.* In order to investigate a property of fluorescence not associated with the results being manipulated called “quenching,” we ran quinine samples (5 mL) with various amounts of NaCl (0, 0.1, 1.0, 3.0, and 5.0 mL) added to them.
V. Data and Results

Quinine Standards. The quinine calibration standards’ data are presented in Table 1 (medium voltage) and Table 2 (low voltage). A calibration plot is presented in Graph 1 based on the more reasonable readings from the low voltage detector. This plot has a slope of 8.4624 and a regression coefficient ($R^2$) of 0.9997.

Detection Limit. Table 3 shows the data from the 20 readings of the “blank” trial, as well as the pertinent information involved in solving for the limit of detection. Quenching trends are shown in Table 4. These values were solved according to the following calculations:

SC 3 Average of “blank” trials ($X_{bl}$) = (0.064)/20 = 0.003

Conf. Int. (95%): $X_{avg} \pm (ts)/\sqrt{n} = 0.003 \pm (1.729*0.094)/\sqrt{20} = 0.003 \pm 0.036$

Standard deviation of “blank” trials ($s_{bl}$) = 0.094, based on Excel

Minimum detectable signal ($S_M$) = $X_{bl} + 3s_{bl} = 0.285$

Slope of calibration plot (m) = 8.4624

Limit of Detection (LD) = ($S_M - X_{bl}$) / m = (0.285-0.003) / 8.4624 = 0.033

After determining these statistics, we found the concentration of quinine in the tonic water using the best-fit line equation using the 20x dilution.

SC 4 $y = 8.4624x - 0.2022$

$22.641 = 8.4624x - 0.2022$

$x = 2.699$

Concentration of quinine = 20x = 53.99 ppm or mg/L
VI. Discussion and Analysis of Results

**Quinine Concentration.** Unfortunately, the amount of quinine is not listed on the product’s nutrition facts.\(^3\) However, it’s interesting to note that the FDA limits the amount of quinine in tonic water to 83 ppm or 83 mg/L.\(^4\) The experimental value we found of 54 ppm is within this range.

**Quenching Trends.** The experimental values for our quenching portion of this study (Table 4) show the same trend as expected. With increasing concentrations of NaCl—despite constant concentrations of quinine—the intensity of the readings decrease dramatically. In fact, once 5 mL of the NaCl solution was added to 5 mL of the quinine solution, the % fluorescence decreased by half.

**General Assessment.** Overall, this data met the objectives for this experiment and were of good quality. The regression coefficient reading (0.9997) is very high and shows that the best-fit line is of good quality and represents the data well. This is important because we used the slope of this best fit line in later analysis, so this lends to the quality of the calculations as a whole. In addition to manually correcting for emission wavelength setting, a set of quinine standards was used in order to determine an appropriate sensitivity level. These QC methods assisted in the accuracy and precision of our data. Additional ways to improve would be to perform more trials when taking data for the quinine calibration plot (Table 2, Chart 1).

VII. Additional Questions

1. What instruments could you use to determine the excitation wavelength had it not been provided to you?
Depending on the specifications of the instrument, a “quantum counter” could have been used, which would have tested a sample of high quantum efficiency to be used as a reference signal for future trials.

2. Calculate the limit of detection for quinine for the fluorometer you used.
   
   Calculated in “Discussion and Analysis of Results” and shown in Table 3.

3. What was the concentration of quinine in your tonic water sample?
   
   Shown and calculated in “Data and Results”

4. What changes would you expect in the fluorescence signal if HCl had been used to acidify your samples? Why?

   HCl will also quench fluorescence since fluorescence quenching has been related to the halide concentration by the Stern-Volmer equation. [5]

5. What QC procedures should be included to ensure the accuracy of your analyses?

   Reviewed in “Discussion and Analysis of Results”

6. What other methods of analysis could be used for analysis of quinine in beverages? How do these methods compare to fluorescence analysis? Are they more sensitive or accurate? (Bonus 5 pts.)

   Quinine concentration can also be found using HPLC, although this method is not as sensitive. In fact, fluorescence detectors are the most sensitive HPLC detectors on the market. [6] HPLC, however, should provide a better precision and accuracy. [7]

VIII. Bibliography


