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CH 362  
Professor Attygalle  
I pledge my honor that I have abided by the Stevens Honor System.

**1) Title of Experiment:**

Determination of Concentration of Quinine in Tonic Water using Spectrofluorometry

Date: October 24, 2020  
Name of Technique: Spectrofluorometry

**2) Technique:**

Spectrofluorometry is a technique that takes advantage of the fluorescent properties of some compounds to determine the concentration of those compounds in unknown solutions. The setup begins with a xenon lamp emitting light into an excitation monochromator, which usually chooses to select a wavelength in the UV region. Then, this beam is shined into a fluorometric cuvette, which excites electrons in the fluorescent material inside. Electrons are excited to a higher state, and as they come back to the ground state, they release light. The emitted light from the compound is passed through a second monochromator, which selects different wavelengths to be transmitted to a photodetector to build a fluorescence spectrum for the compound. This setup is visually shown in Figure 1.

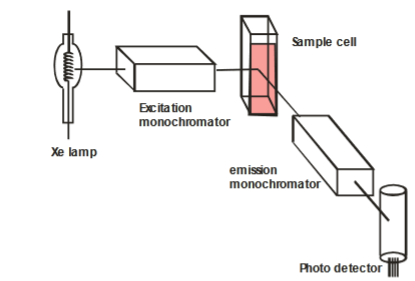


Figure 1: Internal Setup of Spectrofluorometer

Because increased concentrations of fluorescent compound would increase the intensity of the fluorescence, and the intensity on the fluorescence spectrum, a combination of solutions of known and unknown concentrations can be used to determine the concentration of the unknown compound with ordinary linear calibration.

**3) Application of the Technique to my Experiment:**

In this experiment, spectrofluorometry is targeting the compound quinine, which is added to tonic water as a bitter flavoring agent. As a strongly fluorescing compound, comparison of fluorescence spectra between calibration solutions and tonic water will yield the concentration of quinine in the tonic water.

To begin, a stock solution of quinine will be prepared from quinine sulfate and dilute sulfuric acid. From this stock solution, five calibration solutions of 0.1, 0.2, 0.6, 0.8, and 1.0 ppm will be created by diluting aliquots of the stock solution with more dilute sulfuric acid. Next, commercial tonic water will be transferred to a beaker, shaken to reduce effervescence, and identical aliquots of the tonic water will be pipetted to three beakers and diluted with dilute sulfuric acid to create three identical diluted samples of the tonic water.

With each sample prepared, the spectrofluorometer will be calibrated by setting the excitation wavelength to 247 nm, and the fluorescence start and end wavelengths to 350 nm and 600 nm, respectively. Then, a calibration cuvette will be filled with only the dilute sulfuric acid solvent. After calibrating the machine with this sample, each of the calibration and unknown solutions will be measured by the spectrofluorometer and their fluorescence spectrums will be recorded. After finding the maximum fluorescence wavelength of quinine, the absorption readings at those points will be used to set up a calibration curve and the unknown concentration of quinine in the tonic water can be calculated.

**4) Calculations:**

**Preparation of stock solution of quinine**

|  |  |
| --- | --- |
| Mass of ascorbic acid used | 0.010 g |
| Volume of solution | 250.0 mL |
| Concentration of solution (ppm) | 0.010 \* 1000 / 250 / 1000 = 40.00 ppm |

**Preparation of calibration solutions**

**Each mL of stock solution contains 0.010g \* 1/250 = 0.0004 g = 0.04 mg of quinine.**

|  |  |  |  |
| --- | --- | --- | --- |
| Desired concentration (ppm) | Desired Volume (mL) | Mass of quinine needed (mg) | Volume of stock solution needed (mL) |
| 0.1 | 100.00 | 0.01 | 0.25 |
| 0.2 | 100.00 | 0.02 | 0.50 |
| 0.6 | 100.00 | 0.06 | 1.50 |
| 0.8 | 100.00 | 0.08 | 2.00 |
| 1.0 | 100.00 | 0.10 | 2.50 |

**5) References:**

1. Attygalle, A. Instrumental Analysis I Lecture and Laboratory Manual <https://sit.instructure.com/courses/38802/files/6982711?module_item_id=1042514> (accessed Oct 11, 2020).
2. Harris, D. C. *Quantitative Chemical Analysis*, 8th ed.; W.H. Freeman and Co: New York, 2010. Chapter 17.

**6) MSDS:**

**Quinine Sulfate dihydrate:**

CAS No.: 6119-70-6  
Molecular Weight: 782.96  
Chemical Formula: C40H54N4O10S  
Appearance: white solid powder  
Lab Protective Equipment: Lab coat, goggles

**Health effects:**Harmful if swallowed, in contact with skin, or inhaled. Causes skin and serious eye irritation. May cause respiratory irritation.

**First Aid measures:**Eye contact: rinse cautiously with water, for several minutes. Remove contact lenses if present and easy to do. Continue rinsing, and if irritation persists, contact a doctor.  
Skin contact: wash off immediately with plenty of water and soap. Call a poison control center or doctor if you feel unwell. Get medical attention if skin irritation or rash occurs. Remove contaminated clothing.  
Inhalation: Move to fresh air and put in comfortable breathing position. Consult a physician if feeling unwell.  
Ingestion: Call a poison control center or doctor if feeling unwell. Rinse mouth.

**Other hazards:**Fire: not known to be a fire hazard.   
Explosion: not known to be an explosion hazard.

**0.05 M Sulfuric Acid:**

CAS No.: 7664-93-9  
Molecular Weight: 98.079  
Chemical Formula: H₂SO₄  
Appearance: clear, colorless liquid  
Lab Protective Equipment: Lab coat, goggles  
Corrosive to metals.

**Health effects:**Can cause eye and skin irritation. Can cause respiratory and digestive tract irritation if inhaled or swallowed.

**First Aid measures:**Eye contact: rinse immediately with water, especially under eyelids, for >15 minutes. Consult a physician.  
Skin contact: wash off immediately with plenty of water. Consult a physician.  
Inhalation: Move to fresh air. If not breathing, give artificial respiration. Consult a physician.  
Ingestion: Do not induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.  
**Other hazards:**Fire: not known to be a fire hazard.   
Explosion: not known to be an explosion hazard.

7) Pre-lab Questions

1. While cuvettes used for fluorometric and UV determinations are both made of quartz, fluorometric ones must be clear on all sides because fluorescence emits in all directions, while UV determinations follow the beam of the UV energy to the detector. This is because spectrofluorometry is based on the fluorescence that occurs when an electron comes down from an excited state to the ground state after being excited by UV light, while UV spectrometry is based on a compound absorbing the original UV beam. In the spectrofluorometer, the detector is not aligned with the direction of the original UV beam, but at a 90-degree angle, detecting emission in the visible spectrum coming out of the side of the cuvette. This would not work with a typical UV cuvette, as not being clear on all sides means the side of the UV cuvette would absorb some of the visible light emitted from the fluorescence.