Determination of Quinine in Tonic Water by Fluorescence (DQF)

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CHEM438 - 021L

I submit this laboratory report as an original document. I assert that all ideas and discussion of data contained herein are my own work, unless otherwise referenced.

ABSTRACT

The fluorescence measurement of quinine was found to have the excitation and emission wavelengths of 340 and 470 nm respectively, which were in agreement with the wavelengths determined from the charts in pre-lab assignment. A stock solution of quinine sulfate was made (33.21 μM) and 5 sample solutions were prepared to obtain a calibration curve. The fluorescence, F is directly proportional to the quinine sulfate concentration, [Quinine Sulfate], by the following equation: $F = (708.3 \pm 29.6)\ \mu M^{-1} \cdot [Quinine\ Sulfate] + (332 \pm 233)$, with an R^2-value of 0.9948, within the concentration region of 0.66 to 13.28 μM. The linearity behaved as expected as it was ensured to be within the concentration region where the overall linear trend of fluorescence versus concentration still holds (peak of fluorescence was approximately around 40 μM). The average fluorescence of an 80x-diluted unknown tonic water after applying the correction from the blank's fluorescence was found to be 8,535. From the calibration line, the concentration of the 80x-diluted tonic water was computed to be 11.3 ± 1.9 μM (95% C.I., n = 5). The original quinine concentration from the tonic water bottle was calculated to be (9.0 ± 1.5) x 10^{-4} M (95% C.I., n = 5). The limit of detection of the fluorescence measurement was determined to be 274.15 in the fluorescence unit, which was equivalent to 0.39 μM in molar concentration units. From this determined value, the fluorescence measurement has a lower detection limit as it is more sensitive than the absorbance measured by UV-visible spectroscopy.

INTRODUCTION [1]

Tonic water contains both quinine and sodium benzoate, which are essential for human consumption. The concentration of quinine in tonic water can be determined by using the fluorometer. Quinine fluoresces strongly, which makes it easier to be detected at even very low concentrations. Fluorescence measurement is very selective in which one or two compounds in a mixture can be detected, saving some time from separating these two compounds if one were to analyze the sample with a method other than fluorescence.

The measurement of emission intensity of the fluorescence is proportional to the incident power applied on the instrument, the molar absorptivity and the concentration of the desired analyte. Similar to Beer's law, there is also a limitation in fluorescence measurement, where it will stop behaving linearly between the fluorescence and the concentration as it reaches a specific maximum concentration.

Fluorescence is also directly proportional to a collection of terms: quantum yield, collection and detection efficiencies. The collection efficiency term is not always constant as it will decrease when the concentration of the analyte observed increases. This is associated with the reabsorption of its own fluorescences emission photons at the specified emission wavelength. However, for simplicity, this effect is neglected or known as inner filter effects, as it is not relatively larger than the effect of the concentration itself. This assumption will no longer hold if the emission and excitation spectra of the fluorescence overlap significantly, in addition to a near-strong absorption region of the emission wavelength.

PROCEDURE [1]

To collect the fluorescence spectrum of samples, the excitation and emission wavelength, λ_{ex} and λ_{em} respectively should be determined first. When collecting the spectrum of excitation wavelengths, the λ_{em} was set to a fixed value according to the pre-lab assignment, as a guess value. The same procedure was repeated by scanning the spectrum of emission wavelengths, by fixing the λ_{ex} at a value obtained from pre-lab assignment. From these spectrums, the values of wavelength at respective peaks were used throughout the collection of spectrum for the rest of the samples.

A stock solution of quinine sulfate was prepared by adding a known mass of salt into a 50-mL volumetric flask and diluted with 0.05 M H_2SO_4. From this stock solution, 5 different samples were prepared, by pipetting 10, 7, 5, 1, and 0.5 mL of the stock solution by using the respective volumetric pipette into a 25-mL volumetric flask per sample and diluted with the same diluent as the stock's. For each sample, the fluorescence data at λ_{em} = 470 nm was collected 3 times and averaged. The fluorescence of the diluent which acted as the blank fluorescence was also collected 3 times, and used to correct the fluorescence of all 5 samples. A calibration curve of fluorescence versus the final concentration of the sample was plotted and ensured to be approximately linear.

A sample of tonic water as unknown was prepared by diluting the provided 40x-diluted tonic water further into 80x-diluted, by pipetting 25 mL of the 40x-diluted unknown into a 50-mL volumetric flask and diluted with the same diluent, to ensure the fluorescence of the unknown fell within the calibration curve.

In order to determine the detection limit (LOD) of the measurement, 2 more blank fluorescence data were collected, resulting in 5 fluorescence data in total. The data was averaged and the standard deviation was calculated to determine the LOD.

RESULTS AND DISCUSSION

The predictions made in pre-lab assignments for λ_{ex} and λ_{em} were 350 and 470 nm, while the experimental values were found to be 340 and 470 nm respectively. The prediction and experimental values were in agreement with each other, with only a 10-nm difference in λ_{em}.

Table 1. Fluorescence data of 5 quinine sulfate sample solutions.

Sample	[a] $V_{stock\ added}$ (mL)	Concentration (µM)	[b] F_1	[b] F_2	[b] F_3	[c] F_{avg}	[d] $F_{avg} - F_{blank}$
1	10.00	13.28	9,800	9,598	9,630	9,800	9,676
2	7.00	9.30	7,336	7,276	7,318	7,336	7,310
3	5.00	6.64	5,501	5,755	5,708	5,501	5,655
4	1.00	1.33	1,381	1,450	1,351	1,381	1,394
5	0.50	0.66	856	905	859	856	873

[a] Volume of stock solution added for each sample ($[Quinine\ Sulfate]_{stock}$ = 33.21 µM)
[b] Fluorescence data at λ_{em} = 470 nm
[c] Average of 3 fluorescence data
[d] Average fluorescence corrected with the blank's fluorescence (F_{blank} = 227.8)

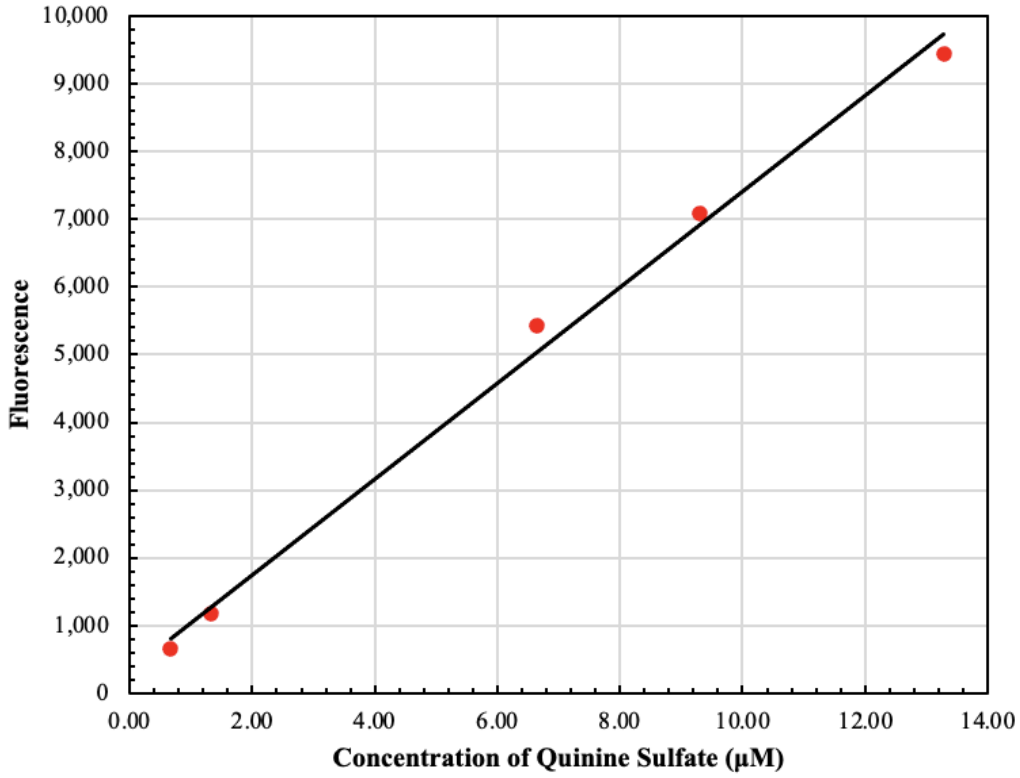


Figure 1. Calibration curve of quinine sulfate sample solution.

All five data points of fluorescence versus the concentration of quinine sulfate in the solution tabulated in **Table 1** were plotted and fitted with a linear trendline as shown in **Figure 1**. The trendline has an equation as provided in **Eq. 1** below.

$$F = (708.3 \pm 29.6)\ \mu M^{-1} \cdot [Quinine\ Sulfate] + (332 \pm 233) \qquad \textbf{Eq. 1}$$

The trendline has an R^2 value of 0.9948. From a statistical point of view, the five data points were fitted almost perfectly with a linear regression since the R^2 value is very close to 1. From a practical and conceptual meaning of the plot, the trendline should start from the origin, where there will be no fluorescence for a zero concentration of quinine sulfate. After fitting the 5 data points to the origin, the resulting R^2-value is 0.9912, which is still reasonably close to what was originally obtained. Either way of fitting the data will not have a significant impact on the overall data analysis.

The linear behavior is as predicted. Fluorescence has an upper limit value where it will stop behaving linearly beyond a certain concentration of the analyte solution. The concentration where the fluorescence will disobey linearity is approximately 4×10^{-5} M, which is 40 μM. The sample solutions were prepared carefully in which the concentrations were ensured to be lower than this maximum concentration. The stock solution was calculated to have a concentration of 33.21 μM, prepared from 0.0013 g of salt. The calibration curve obtained in **Figure 1** was just a small cut-section of the overall curvature of fluorescence throughout all concentrations, where it should result in a linearity.

The unknown tonic water was prepared to be 80x-diluted and the three fluorescence obtained were 8,411, 8,573, and 8,620. The average fluorescence was 8,535, which was then corrected using the blank's fluorescence to be 8,307. From this fluorescence value, substituting into **Eq. 1** will result in quinine concentration of 11.3 μM. With 95% confidence interval, the concentration of quinine if the 80x-diluted solution was 11.3 ± 1.9 μM (95% C.I., n = 5). Taking into account the dilution factor, the original concentration of quinine from the tonic water bottle was $(9.0 \pm 1.5) \times 10^{-4}$ M (95% C.I., n = 5). From an article published by Harvard Health Publishing of Harvard Medical School, tonic water should have no more than 2.56×10^{-4} M of quinine.[2] The calculated and literature data agree in magnitude of concentration, though the calculated concentration is slightly higher. This could be due to the inaccuracy in preparing the sample/unknown solutions, which will result in slightly higher concentration calculated.

Table 2. Detection limit of the fluorescence measurement.

Run	1	2	3	4	5
$\mathbf{F_{blank}}$	213	225	225	254	222

Table 2 showed the blank's fluorescence obtained five times for the determination of the limit of detection. The average fluorescence, $\overline{F}_{blank}$ was 227.8 with a standard deviation, σ_{blank} of 15.4 The limit of detection (LOD) was computed by using **Eq. 2** below, which then can be used to determine LOD in molar concentration units from **Eq. 3**.

$$LOD = \overline{F}_{blank} + 3\sigma_{blank} \qquad \textbf{Eq. 2}$$

$$LOD\ (M) = \frac{LOD\ (fluorescence\ unit)}{m},\ m\ =\ slope\ of\ the\ calibration\ line\ (708.3\ \mu M^{-1}) \qquad \textbf{Eq. 3}$$

The LOD in fluorescence unit of the measurement was 274.15. Meanwhile, the LOD in molar concentration units was found to be 0.39 μM. Fluorescence should have lower detection limits than those obtained from absorbance measurements of UV-Visible spectroscopy because fluorescence is more sensitive.[3] They are measured in different ways, where the fluorescence is measured directly without any reference beam, unlike the absorbance measurement. Fluorescence is measured over a dark as opposed to bright background, in which it is easier to detect lower levels of light.

CONCLUSIONS

Fluorescence measurement technique can be used to determine the concentration of quinine in tonic water, as quinine fluoresces strongly under the specified emission wavelength, making it easier to be detected. Like Beer's law, fluorescence behaves linearly from the lower concentration region up to a maximum concentration, where the fluorescence will start to decrease as the concentration increases. Calibration curve approach is used to determine the concentration of unknown tonic water, and the samples were prepared by making sure the concentrations lie within the region where the linearity between fluorescence and the concentration still holds. Moreover, the detection limit of fluorescence measurement is expected to be lower as it is more sensitive than the absorbance measurement from UV-visible spectroscopy.

REFERENCES

[1] Cruz, F. *CHEM438 Instrumental Methods Laboratory - Determination of Quinine in Tonic Water lab*; University of Delaware: Newark, Delaware, 2018; pp 1-5.

[2] Will tonic water prevent nighttime leg cramps? https://www.health.harvard.edu/newsletter_article/will-tonic-water-prevent-nighttime-leg-cramps (accessed Oct 21, 2021).

[3] Why is fluorescence more sensitive than UV-vis absorption spectroscopy?: AAT Bioquest. https://www.aatbio.com/resources/faq-frequently-asked-questions/Why-is-fluorescence-more-sensitive-than-UV-VIS-absorption-spectroscopy (accessed Oct 21, 2021).

APPENDICES

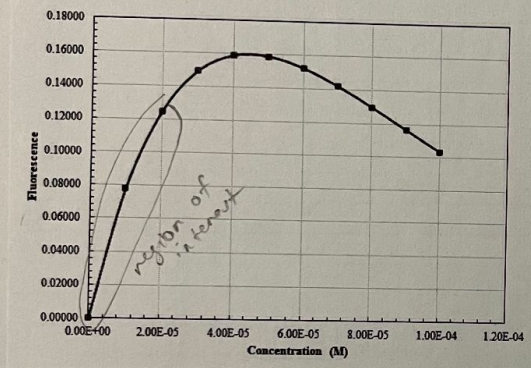
Lab Notebook

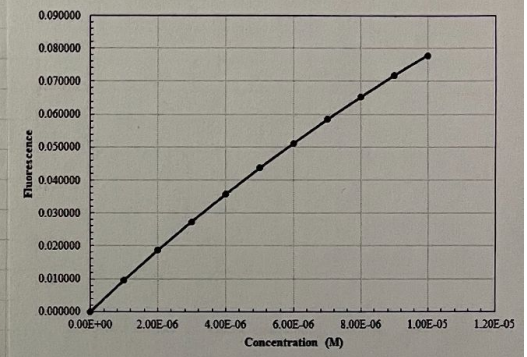
Exp. No. 5	Experiment/Subject CHEM 438 (PCR)	Date 10/11/2021	17
Name Abdul Fayeed	Lab Partner Justin M	Locker/ Desk No.	Course & Section No. 021L

PRE-LAB Questions

1. λ_{ex} = 340nm (peak @ 3400 Å) → no peak for toluene @ this λ.
   λ_{em} = 460nm (peak @ 4600 Å)

2. $\varepsilon = 9750\ M^{-1}cm^{-1}$ (from the graph @ $\lambda_{obs} = 340nm$)
   $\Omega = 2$, $P_o = 0.5$, $b = 1cm$

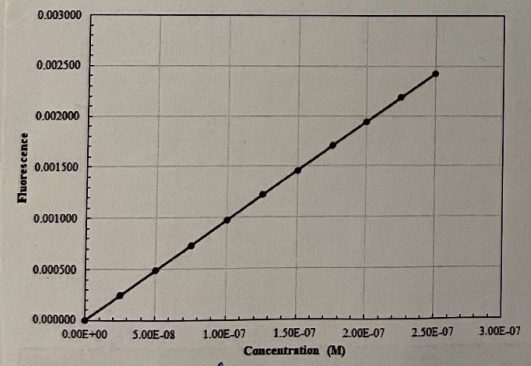




(The specified conc. range) (10-fold dilution range)

⇒ should dilute the quinine sample to get linear range of calib. curve.

3. Tonic water should be diluted. 40-dilution factor ~~can should~~ be used.



4. Take average of blank values then use the standard deviation to calculate the limit of detection.

Witness/TA	Date
	10/11/2021

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Exp. No. 5	Experiment/Subject CHEM 438 (DCF)	Date 10/11/2024	
Name Abdul Fayyad	Lab Partner Justin M	Locker/ Desk No.	Course & Section No. 021L

Objective

Purpose of this lab is to determine the concentration of quinine in tonic water by fluorescence.

Introduction

Fluorescence is ~~effective~~ selective to one or 2 compounds in a mixture. Tonic water has both quinine and benzoate. Appropriate λ_{ex} and λ_{em} have to be picked to ensure that only one compound can be fluorescese at a time. Fluorescence spectrum also has its limit where it reaches a maximum fluorescence then drop down as concentration increases.

Procedure

1. Determine λ_{ex} and λ_{em}.
   λ_{ex} = 340nm, λ_{em} = 460nm
   350nm / 470
2. Set non-scanned (excitation) wavelength to appropriate λ_{ex} value.
3. Measure fluorescence excitation ~~of~~ spectrum of quinine once, using the highest concentration, using λ_{em} to scan the excitation wavelength.
4. Measure the fluorescence emission spectra of quinine by fixing λ_{ex} and scanning over emission wavelength
5. Prepare a set of fluorescence calibration standards to determine the diluted tonic water 'unknown'. Dilute solution w/ 0.05M H_2SO_4.
6. Make measurement to determine the LOD of fluorescence. (to find?)

λ_{ex} = 340nm, λ_{em} = 470nm

mass of quinine sulfate (g) = 0.0013 g

stock concentration = 3.32×10^{-5}M (50mL volume)

Sample	Vol of stock (mL)	(25mL V) conc (M)	F_1	F_2	F_3
1	10mL	1.33×10^{-5}	9800	9598	9630
2	7	9.3×10^{-6}	7336	7276	7318
3	5	6.64×10^{-6}	5501	5755	5708
4	1	1.33×10^{-6}	1389	1450	1357
5	0.5	6.64×10^{-7}	856	905	859
Tonic	80x diluted	25mL into 50mL	8411	8573	8620
Blank	0	0	213	225	225

Conclusions

Quinine can be detected and measured based on its concentration by fluorescense. Fluorescence is selective to only one or 2 compounds, so it can save time to separate the compound from mixture.

Signature	Date 10/11/2024	Witness/TA	Date

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