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Quinine Assay with Home-Built UV-LED Fluorometer: Quantitative Analysis, Photo-Bleaching, Fluorescence Quenching, and Urine Analysis

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ABSTRACT. Quinine quantitative analysis, photo-bleaching, fluorescence quenching, and urine analysis have been performed by means of a UV-LED fluorometer, which can be easily built and used in a high-school laboratory. The quinine detection range is estimated to be 0.05-80 ppm, enough for many classroom luminescence experiments. The quinine content in commercial tonic water is determined from the calibration curve, and UV photo-bleaching of this anti-malarial drug is demonstrated with clear wavelength dependence. Halide quenching of quinine fluorescence is also observed and the increase in quenching efficiency in the order of Cl^-, Br^-, and I^- is evident. Urine analyses for the student volunteers have been carried out and the results clearly reveal excretion of the ingested quinine. The student participants are exuberant throughout the course of this study and sense the practices resourceful.

Key words: Quinine, Fluorescence, UV-LED, Fluorescence quenching, Urine analysis

INTRODUCTION

Fluorescence is easy to observe, instructive, and applicable to many chemical systems, from acid-base titration to coordination of biological molecules.[1,2] The molecular luminescence also provides high sensitivity adaptable to quantitative analyses of real samples and provides students opportunities to garner invaluable skills in the experimental trials of analytical and biochemical subjects.[3-5] However, sophisticated instrumentation or lasers for its practices are not always available. Here we propose simple student experiments based on a home-built fluorometer using a UV-LED and a fiber-optic spectrometer. Working with electronic circuits and instrumentation not only add fun, but also are educational for the participants to understand the operational principles of analytical instruments.

Quinine, a well-known anti-malarial drug and muscle relaxant with a bitter taste,[6] absorbs near ultraviolet light (UV, λ_{max}=250 and 350 nm) and releases strong fluorescence centered at 450 nm.[7-9] Due to its relatively constant and well-known fluorescence quantum yield, this chemical has been considered as a standard for molecular luminescence. Unlike many other luminescent molecules, its emission is not quenched by oxygen. It is, however, quite susceptible to halide quenching.[8] This conjugated compound, a combination of aromatic quinolone and bicyclic quinuclidine systems, is first synthesized by R. B. Woodward and W. E. Doering in 1944.[10] The bitter taste of anti-malarial quinine tonic led British colonial in India to mix with gin, thus creating the popular cocktail "gin and tonic". Like vitamin B's, quinine is excreted directly and rather rapidly in the urine, allowing easy detection of its intake.[7,11]

In this study, we report a fluorimetric assay of quinine using a home-built fluorometer with a UV-LED,[12] which is easy to assemble, operate, and maintain. The experiments reported here are all carried out by 1st and 2nd year high school students. The quinine content in commercial tonic water is measured, photo-bleaching monitored, fluorescence quenching by halide anions observed, and amount in urine measured.

EXPERIMENTAL

UV-LED Fluorometer

A fluorometer for student experiments has been built using a UV-LED as a light source and a fiber-optic spectrometer with a 3648-pixel linear CCD array (USB 4000, Ocean Optics) (*Fig.* 1). A UV LED (λ_{max}=361 nm, luminous intensity=750 μW, LED Supply part # L5-0-UTH15-1), which is glued in a center hole of a plastic round plate, is fixed in a mirror mount. This light source is appropriate to the quinine absorption characteristic (λ_{max}=250 and 350 nm), but it is readily exchangeable with other LED's covering other wavelength regions. The UV LED is run by a

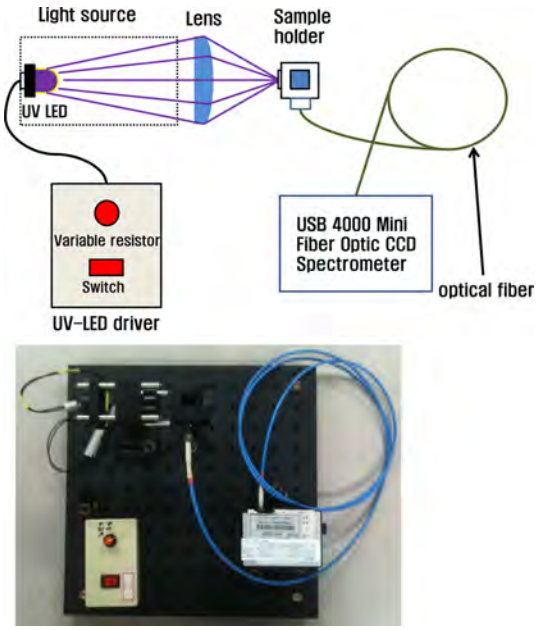

***Fig.* 1.** Schematic diagram of home-built fluorometer used in this study. The fluorometer consists of a UV-LED glued on a circular plastic plate and its driver shown in *Fig.* 2, a lens (focal length = 25 mm), and a miniature fiber-optic CCD spectrometer (USB 4000, Ocean Optics) connected to a sample holder via optical fiber. A photo of the home-built spectrometer is also shown.

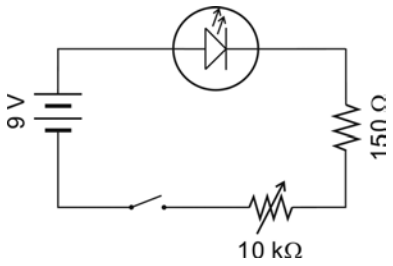

***Fig.* 2.** Circuit diagram for running UV-LED. A simple driver circuit is employed to run the UV-LED used in this study.

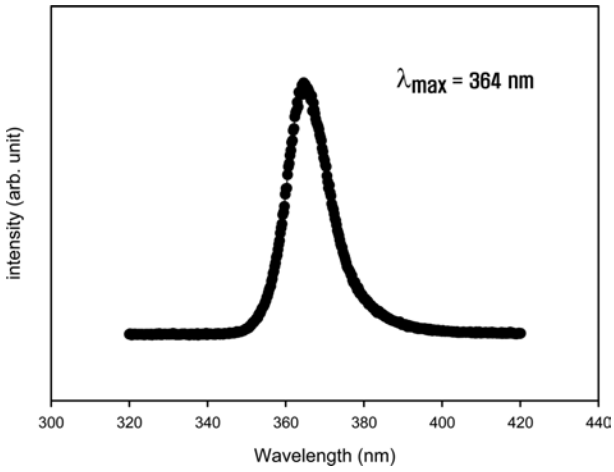

***Fig.* 3.** Emission spectrum of UV-LED recorded with a home-built fluorometer used in this study. λ_{max} is measured to be 364 nm, slightly different from the product specification of the UV-LED.

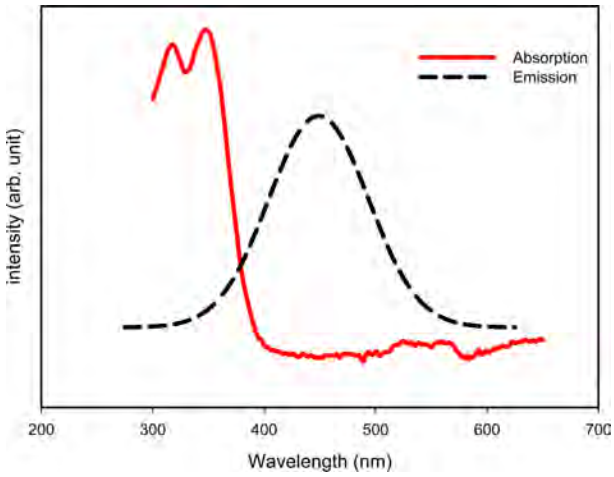

***Fig.* 4.** Absorption (solid red line) and emission (broken black line) spectra of quinine. Quinine releases characteristic 450-nm blue fluorescence.

simple circuit (*Fig.* 2), and its emission profile recorded with USB 4000 is shown in *Fig.* 3. The UV light is focused by a lens (focal length=25 mm) at the center of the sample in a quartz (or PMMA) cuvette with a pathlength of 1 cm, and its fluorescence emission from the sample is collected at 90° and conveyed with an optical fiber to a miniature fiber-optic CCD spectrometer as shown in *Fig.* 1. The fluorescence intensity from quinine is observed at 450 nm (*Fig.* 4), λ_{max} of quinine fluorescence, which reportedly does not change with the excitation wavelength.

Quinine Stock Solution

100.0 ppm quinine stock solution is prepared by weighing 120.7 mg of quinine sulfate dehydrate or 100.0 mg of quinine, transferring to a 1 L volumetric flask, adding 50 mL of 1 M H_2SO_4, and diluting to volume with distilled water.[7] Other quinine solutions are provided by various dilution of the stock solution with 0.05 M H_2SO_4. Quinine solutions must be made daily and protected from light to avoid photo-bleaching. Under our experimental condition, the quinine concentration decreases ~20% a day without proper UV protection.

RESULTS AND DISCUSSION

Calibration Curve for Quinine and the Detection Range of Quinine

A series of quinine standards is prepared by sequential

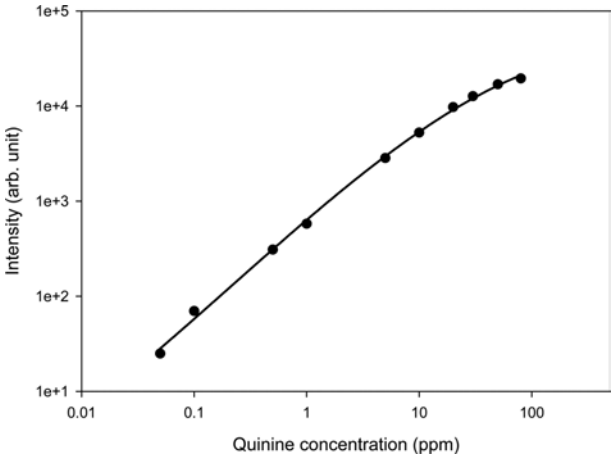

***Fig.* 5.** Calibration curve of quinine. Due to degradation of quinine, the quinine stock solution was prepared daily, and so was the calibration curve. The operation range of the home-built spectrometer for quinine is estimated to be 0.05-80 ppm.

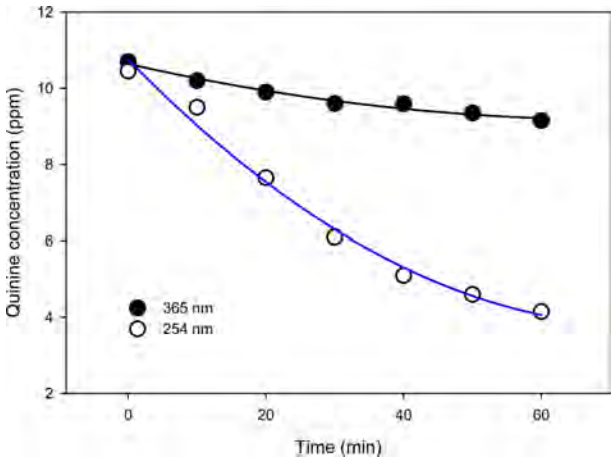

***Fig.* 6.** Photo-bleaching of quinine. Quinine is evidently very sensitive to UV. Notice that shorter wavelength UV (254 nm, hollow circle) is far more destructive than longer wavelength UV (365 nm, solid circle).

dilution of quinine solutions with 0.05 M H_2SO_4, beginning with the 100 ppm stock solution. The process of dilution is continued until fluorescence intensity-to-noise ratio reaches 2:1, and the blank solution is used to set the zero intensity. A calibration curve is shown in *Fig*. 5. The quinine detection range with the home-built UV-LED fluorometer is estimated to be 0.05-80 ppm. The signal-to-noise ratio drops below 2:1 with quinine concentration lower than 0.05 ppm, and the increase in signal intensity as a function of concentration is not consistent above 80 ppm.

Quinine in Tonic Water

10.0 mL of commercially available tonic water (Jinro Mixer) is transferred to 100 mL volumetric flask and diluted to volume with 0.05 M H_2SO_4. The possible interference in the fluorometric assay for quinine is chloride ion, which quenches quinine fluorescence. However, the previous studies have shown that this effect is negligible as long as the chloride ion concentration is below 0.4 mM,[13] which is the case for tonic water.[7] The quinine concentration is determined from the fluorescence intensity and calibration curve. In order to confirm the result, the trial is repeated with 2 X dilution of the tonic water sample. We estimate the quinine concentration in tonic water to be 18.1 ppm. Unfortunately our result is not validated. The quinine content is not specified on the product used in this study, and the producer, the sole supplier of tonic water in Korea, does not provide the information upon our request.

Photo-Bleaching of Quinine

10 ppm quinine solution in a quartz cuvette is irradiated (at a distance of ~5 cm) with a UV lamp (black light, λ=254 or 365 nm switchable, UVP Model UVGL-58) while the quinine fluorescence intensity is measured every 10 min for an hour. The quinine concentration at each point is determined from the calibration curve. This procedure has been done for both 254 and 365 nm, and the exponential-type decays are illustrated in *Fig.* 6. Clearly the photo-bleaching at the shorter wavelength is much higher; ~65% of quinine dissociates in an hour with λ=254 nm under our experimental condition, and the bleaching rate is ~7 times higher than that at 365 nm, a good example of faster photo-reaction by higher photon energy. The half-life of quinine with λ=254 nm is ~35 min in this experiment.

Quenching of Quinine Fluorescence by Halide Anion

Quenching of quinine fluorescence has been an interesting topic of chemical kinetics.[8] The experiment enhances the student's understanding and experience in the technique of fluorimetry and how the halide ion (X^-) in a solution influences the rate of equation. A fluorescent molecule (D) absorbs a photon (1) and later releases a longer wavelength photon (2). Addition of X^- leads to a decrease in luminescence intensity (quenching) by depleting the excited species (3).

$$D + h\nu \rightarrow D^* \quad (1)$$

$$D^* \xrightarrow{k_1} D + h\nu \text{ or } \Delta \quad (2)$$

$$D^* + Q \xrightarrow{k_2} D + Q + \Delta \quad (3)$$

D and D^* are the fluorescent species in its ground and

excited states, k_1 the first-order decay rate constant of the excited state, k_2 the bimolecular rate constant for depleting the excited state by quencher (Q), and Δ the excess energy released to the surroundings in the form of heat. These three equations yield the Stern-Volmer relation (4)

$$\frac{I_0}{I} = \frac{k_1 + k_2[Q]}{k_1} = 1 + K_{SV}[Q] \quad (4)$$

$$K_{SV} = \frac{k_2}{k_1} \quad (5)$$

where I_0 and I are the luminescence intensities before and after adding the quencher (X^-), [Q] the molarity of quencher, and K_{SV} the Stern-Volmer quenching constant.[9] Therefore, if $I_0/I - 1$ is plotted against [Q], the data ought to be linear with a slope equal to K_{SV}, which is the ratio between the first-order decay constant (k_1) and the second-order quenching constant (k_2) of the excited state by the quencher.

A 0.01 M stock solution of the halide ion is prepared by weighing potassium halide (0.7455, 1.190, and 1.660 g of KCl, KBr, and KI), transferring to 1 L volumetric flask, and diluting to volume with distilled water. 0, 1, 3, 5, 10, and 20 mL of the KCl stock solution are pipetted to 100 mL volumetric flasks, and 1 mL of 100 ppm quinine solution added to each flask and diluted to volume. The quinine fluorescence intensities of the KCl solutions are measured, and the intensity (I) and $I_0/I - 1$ are plotted against KCl concentration. The procedure is repeated with KBr and KI.

Fig. 7 shows the intensity variation with halide anion concentration. The quenching effect of halide anion becomes clear at high concentration of halide anion; a higher concentration of halide anion clearly leads to a lower intensity of quinine fluorescence. In a separate trial, near complete quenching of the quinine fluorescence is also demonstrated in a higher concentration range of X^- ($1–20\times10^{-3}$ M). These results also confirm the increase in quenching efficiency of halide anion in the order of Cl^-, Br^-, and I^-. The heavier anion yields a higher K_{SV}; the Stern-Volmer constants for quinine fluorescence quenching with Cl^-, Br^-, and I^- are measured to be 130, 200, and 310 M^{-1}, respectively, which are comparable to the previous results.[8]

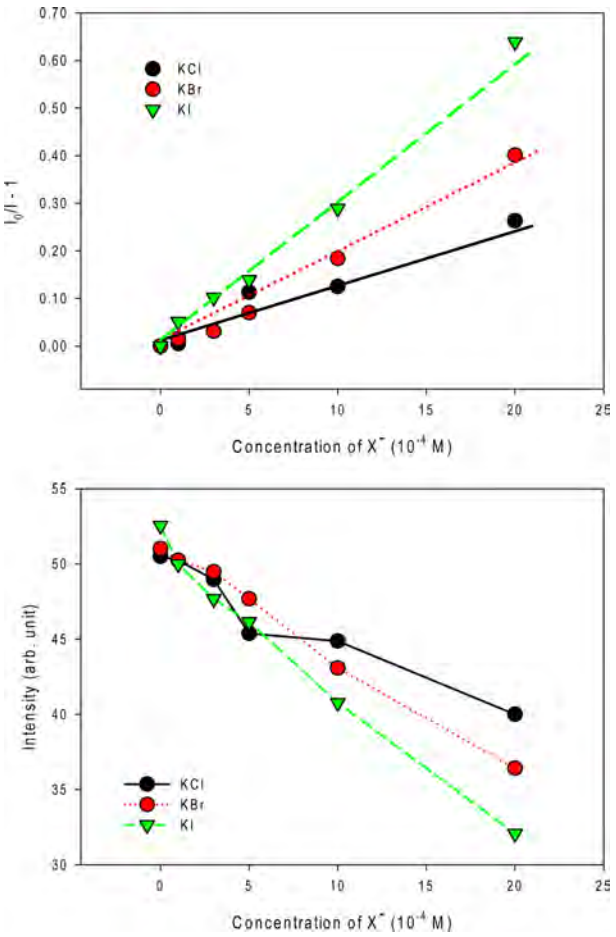

***Fig.* 7.** Quenching of quinine fluorescence with X^-. While oxygen is not a quencher for quinine fluorescence unlike many other fluorescing materials, the halide anion effectively depletes the emission. The quenching efficiency increases in the order of Cl^-, Br^-, and I^-.

Fluorimetric Assay of Quinine in Urine

Since quinine is excreted directly and fairly rapidly in the urine (half-life in human body=~18 hours[14]), ingestion of one gin and tonic or a few hundred mL of tonic water provides easily measurable levels of quinine in urine within a few hours. Screening of urine for quinine is a common method for surveillance of heroin abuse because quinine is a common diluent of illegal heroin samples.[7] We estimate from the measured quinine concentration that a 300 mL bottle of tonic water (Jinro Mixer) contains 5.43 mg of quinine. Each student volunteer consumed two bottles of Jinro tonic water (600 mL, 10.9 mg of quinine) and collected a urine sample every three hours in the following 20-26 hours.

The urine sample was prepared basically following the previously reported method.[7] Two milliliters of urine is transferred to a 15 mL centrifuge tube and the pH adjusted to 9-10 with 3.7 N NH_4OH (normally 2 drops). Four milliliters of chloroform-isopropanol (3:1 v/v) is added to each tube and the samples are shaken by hand for 1 min. The layers are allowed to separate; centrifuge if necessary. Two milliliters of the lower organic phase is transferred to a clean, dry centrifuge tube. Two milliliters of 0.05 M H_2SO_4 is

added, and the tube is shaken for 1 min. The layers are allowed to separate and the upper aqueous phase is transferred to a fluorescence cuvette for measuring the fluorescence intensity. The quinine concentration drops to half the original value in this sample preparation.

The same operations are performed to check the method on a blank consisting of 2.00 mL of distilled water and on a standard 2 ppm quinine solution in distilled water. In both of these cases it is actually preferable to use quinine-free urine instead of distilled water for the blank and the standard, as there is an unknown fluorescent interferent in urine which is also extracted by this procedure. From the emission intensity of the extracted samples and the calibration curve, the quinine level of the original urine sample is determined. The wavelength of maximum fluorescence of this impurity is 425 nm, and overlaps the quinine fluorescence at 450 nm; on the average, this interference produces a fluorescent intensity equivalent to about 0.4 ppm quinine in the original urine sample.[7,15]

The presence of quinine in urine is easily noticeable with the characteristic blue (λ_{max}=450 nm) fluorescent emission (*Fig*. 8). During the procedure, students were able to immediately differentiate a quinine-containing sample from a quinine-free one with the color of the sample under black light. The accumulated amount of quinine excreted from our three volunteers is shown as a function of time in *Fig*. 9. The present results indicate that ~20% of the ingested quinine is defecated during the 20 hours after intake, which is lower than expected. The half-life of quinine in human body is known to be ~18 hours,[14] and a previous study shows a quinine recovery of 100% 24 hours after intake.[7] While the reason for the lower recovery of quinine in our trials is not clear at the moment, a possible reason is the high salt content in Korean diet. The chloride

***Fig.* 8.** Fluorescence from a quinine-containing urine sample. One can easily distinguish with its characteristic fluorescence a quinine-containing urine sample from quinine-free samples.

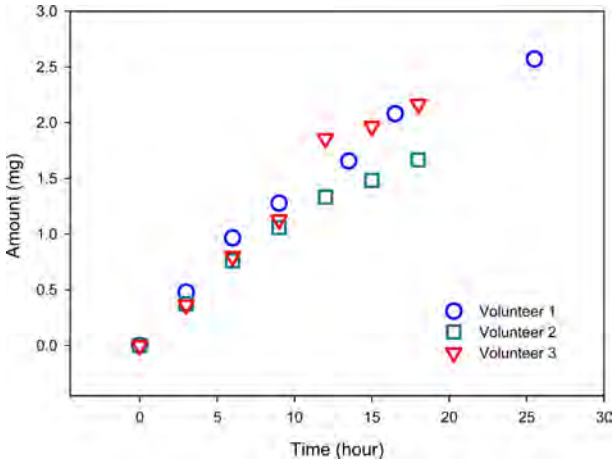

***Fig.* 9.** Amount of quinine excreted in urine. The urine samples are collected from three student volunteers every three hours after drinking two bottles of tonic water, and the accumulated amount of defecated quinine from each student is measured. These results show a quinine recovery of about 20% within 20 hours, which is in fact lower than expected (see text).

anion is a well-known quinine fluorescence quencher.[8] Non-negligible amounts of Cl^- might be transferred to the fluorimetric urine samples, leading to mistakenly low quinine concentrations. Further future study is needed to examine this effect.

CONCLUSIONS

The fluorescent emission of quinine is observed with a home-built fluorometer with a UV-LED and a CCD spectrometer. The spectrometer not only can be easily assembled in a high school chemistry laboratory, but also is simple, educational, and easy to use. The quinine detection range is estimated to be 0.05-80 ppm. The quinine content in tonic water is measured from its fluorescence intensity and a calibration curve generated with standard solutions of known concentrations. Photo-bleaching of quinine is monitored as a function of time, determining the decay rate.

Quenching of quinine fluorescence by halide anions is also investigated, confirming the increase in quenching efficiency in the order of Cl^-, Br^-, and I^-, due to stronger binding of the heavier halide anion with the fluorescent compound. Fluorimetric quinine assay has also been carried out for the urine samples of student volunteers. The quinine-containing urine samples are easily distinguishable by its characteristic fluorescence from the quinine-free samples. The quinine recovery is measured with time and found to be lower than those reported previously. All the

trials are done by high school students, and they are pleased and motivated throughout this study.

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