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Fluorescence Experiments with Quinine

Molecular fluorescence is an important analytical method characterized, perhaps most importantly, by the extreme analytical sensitivities possible in many cases. Yet there are few reports of experiments that are really suitable for an undergraduate quantitative analysis or instrumental analysis laboratory *(1–3)*. None of these seem to be easily adaptable to the quantitative analysis of "real" samples, a trend that is developing in many analytical chemistry courses. There are several more physically *(4–7)* or more biochemically *(5, 8)* oriented experiments that have been described.

It is the purpose of this article to present a series of experiments to illustrate the analytical capabilities of fluorescence, and to outline two straightforward quantitative analyses involving real samples. These experiments are employed routinely in our undergraduate instrumental analysis course. Students are required to do certain experiments (recording of spectra on a scanning fluorimeter and preparation of a calibration curve), and then have been permitted to select among the remaining ones.

Although the experiments described herein were performed with an Aminco-Bowman Spectrophotofluorimeter, they can be done with a relatively inexpensive filter fluorimeter and some adjustment of experimental details—concentrations, filters used, etc. One decided advantage in the use of quinine is that most commercial fluorimeter manufacturers provide some discussion of quinine fluorescence and their instrument's sensitivity for quinine fluorescence in the instruction manuals accompanying the instrument. Thus, adapting these fluorescence experiments to the particular experimental apparatus available should be relatively straightforward. Because of the fact that many different types of fluorimeters are available, the procedures described are given in fairly general terms.

Experimental

In 0.05 *M* H_2SO_4, quinine has two analytically useful excitation wavelengths, 250 and 350 nm. Regardless of which excitation wavelength is used, the wavelength of maximum fluorescence is 450 nm. With a scanning fluorimeter, we have used a 350 nm excitation wavelength and 450 nm emission wavelength throughout. The general purpose primary and secondary filters normally supplied with Turner filter fluorimeters by the manufacturer are suitable for these experiments; primary filter B-1 and secondary filter PC-1 are recommended by the manufacturer for quinine assay on the Coleman filter fluorimeter.

The only solution the student really need prepare is a 100.0 ppm quinine stock solution, and various dilutions of this. The stock solution is prepared by accurately weighing 120.7 mg of quinine sulfate dihydrate 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. Quinine solutions must be prepared daily and should be protected from light. The other required solutions can be prepared in large quantities and made available to the student.

Calibration Curve for Quinine. The Sensitivity of Quinine Analysis

A series of quinine standards is prepared by accurate sequential 10× dilutions of quinine solutions with 0.05 *M* H_2SO_4, beginning with the 100-ppm stock solution. The fluorescence intensity is recorded, and the process of dilution and measurement is continued until the fluorescence intensity is approximately that of the blank (0.05 *M* H_2SO_4).

The log of the relative fluorescence intensity is plotted versus log concentration; the same is done for log (relative intensity − blank) if the fluorimeter is the type that can produce a separate reading for the apparent fluorescence of the blank, that is, an instrument that does not have to be zeroed with the blank. Any deviation from linearity in these plots should be discussed.

The student has now prepared a calibration curve for quinine fluorescence that can be used for analysis of quinine in synthetic "unknowns," tonic water, or urine. In the concentration range used, there is departure from linearity in the calibration curve at high quinine concentrations (10 and 100 ppm) due to concentration quenching, and at very low concentrations due to significant contribution from the blank.

In more advanced classes, it may be desirable to have the students formally determine the analytical "sensitivity" for quinine determination. For example, in various places in the manufacturer's literature accompanying the Aminco-Bowman fluorimeter used, at least three different "sensitivities" for quinine were stated—depending on the definition of sensitivity used. Perhaps the most commonly accepted definition of the detection limit of spectral analysis methods is that concentration which results in a signal-to-noise ratio of two. In this case, the student can plot the ratio of the signal from the analyte solution to the rms noise level of the blank tracing as a function of concentration for several solutions near the detection limit, report the concentration where this ratio is two, and compare this to the manufacturer's stated value.

Parsons *(9)* and Ingle *(10)* have presented basic discussions of the definition of detection limits, and Winefordner and coworkers *(3, 11, 12)* have presented discussions of the signal-to-noise ratio in fluorescence techniques which can be consulted for further details.

Fluorescence Analysis of Quinine in Tonic Water

According to the Federal Food, Drug, and Cosmetic Act *(13)*, quinine, as the hydrochloride or sulfuric acid salt, may be added to carbonated beverages as a flavoring up to a maximum level of 83 ppm quinine. The quinine content of commercial tonic water usually runs between about 25 and 60 ppm. The determination of quinine in commercial tonic water is quite straightforward *(14, 15)*. Tonic water is a "real" sample, and thus quite interesting and relevant to students; yet this analysis does not suffer from one of the serious drawbacks inherent in the analysis of most real samples—excessively long laboratory time for sample preparation.

The only normal interference in the fluorometric assay for quinine in tonic water is chloride ion, which quenches quinine fluorescence. Strache *(14)* indicates that this interference is negligible as long as the chloride ion concentration in the tonic water is below 0.4 m*M*, as it almost invariably is. For example, with this chloride level and a 1:10 dilution, an analytical error of only −0.4% is obtained.

Procedure. Pipet 5.00 ml of tonic water into a 250-ml volumetric flask, dilute to volume with 0.05 *M* H_2SO_4, and mix thoroughly; pipet 5.00 ml of the resultant solution into a 25-ml volumetric flask and dilute to volume with 0.05 *M* H_2SO_4. Record the fluorescence intensity of the final solution and determine the quinine concentration from a calibration curve. This total 250× dilution results in a final quinine concentration of about 0.2 ppm, which is normally in the middle of the linear portion of the quinine calibration curve.

If it is desired to include "accountability" into the laboratory work, and have some objective standard whereby student performance can be evaluated, it is sufficiently easy to give each student a synthetically prepared quinine unknown to analyze. Since quinine solutions do decompose with time, it is imperative that the unknown stock solution be prepared fresh daily. And, since commercial quinine and quinine sulfate samples may have variable waters of hydration *(16)*, it is best to prepare the stock unknown solution from the same bottle of quinine the student will use in preparing his stock solution.

Fluorimetric Assay of Quinine in Urine

A small (7 oz.) bottle of commercial tonic water normally contains about 5–15 mg of quinine. Since quinine is excreted directly and rather rapidly in the urine, ingestion of one gin and tonic or a few ounces of tonic water, surprisingly enough, produces easily measurable levels of quinine in urine within a few hours. For example, Mulé and Hushin *(17)* report that quinine was detected in the urine of each of three human volunteers for a period of 10–11 days after ingestion of 325 mg of quinine sulfate. Screening of urine for quinine is a fairly common method for effective surveillance of heroin abuse within a narcotic control, treatment, or aftercare program, since quinine is a common diluent of illegal heroin samples.

Since this seemed like an intriguing experiment on a "real" unknown, students were permitted to perform this on an optional basis; they seemed to be quite interested in the results. Student ingestion of tonic water was done outside the laboratory; standard medical specimen cups were provided for urine collection. The extraction and assay procedure, which is reasonably rapid and straightforward, is essentially adapted from the report of Mulé and Hushin *(17)*. This extraction procedure is much simpler than others normally employed *(18)*, and seems to work quite well.

Procedure. Two milliliters of urine is transferred (pipet) to a 15-ml centrifuge tube and the *p*H adjusted to 9–10 with 3.7 *N* NH_4OH. 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.

The same operations are performed on a blank consisting of 2.00 ml of distilled water, and on a standard consisting of 2.00 μg of quinine in 2.00 ml of 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. 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.

From the emission intensity of the extracted samples and the calibration curve, the quinine level of the original urine sample is obtainable. The standard serves as a check on the method.

Mulé and Hushin *(17)* indicate that the minimal concentration reliably detected for quinine was 0.10 μg/ml of urine; the recovery of quinine from spiked urine samples was 95 ± 3%. The final value for the quinine level of urine should be corrected for this incomplete extraction.

Quenching of Quinine Fluorescence by Chloride Ion[a]

NaCl Concentration (ppm)	Relative Fluorescent Intensity I	I/I_0[b]
0	0.63	1.00
50	0.58	0.91
100	0.52	0.82
300	0.37	0.59
1000	0.18	0.28
2000	0.10	0.16

[a] Solutions 1 ppm in quinine sulfate and 0.05 *M* in H_2SO_4; excitation wavelength: 350 nm, fluorescence wavelength: 450 nm.
[b] I_0 is the relative fluorescent intensity of the solution with no NaCl present.

Students reported the total mg of quinine ingested, the time after quinine ingestion that the urine sample was taken, the quinine level in the urine in ppm, and the μg of quinine taken and recovered for the standard sample. Our results show that with ingestion of from 3–7 oz of tonic water (4–15 mg quinine), quinine levels in urine ranged from about 0.6–3 μg/ml for urine samples taken from 3–24 hr after ingestion. Our results indicate a quinine recovery of 103 ± 5%, so that it may not be necessary to correct for incomplete quinine recovery.

Again, this analysis is certainly interesting and "relevant," but there is really no "correct" analytical result; so it may be desirable to have students also analyze a synthetic quinine unknown as part of the experiment.

Quenching of Quinine Fluorescence by Chloride Ion

Fluorescence quenching is an important consideration which must be investigated in the development of any analytical fluorometric method. As was mentioned previously, chloride ion is the only major interferent in the analysis of quinine in tonic water *(14)*.

Procedure. Prepare six solutions containing 1 ppm quinine and 0.05 *M* H_2SO_4 each and also containing 0, 50, 100, 300, 1000, and 2000 ppm NaCl, and measure the fluorescent intensity of each solution.

The table illustrates some typical student data for the chloride quenching experiment. Actually, any quinine concentration between about 0.1 and 10 ppm is suitable, depending on the characteristics and response of the instrument used, as long as the fluorescent intensities are on a linear portion of the calibration curve. At 0.1 or 10 ppm quinine, the relative fluorescent intensities differ from those for 1 ppm by a factor of 10, but the ratios of the quenched to the non-quenched fluorescent intensities (I/I_0) are identical to those in the table, within experimental error.

The experiment as described is designed to illustrate the analytical effects of quenching. It shows, quite dramatically, that the selection of an appropriate standard compound and the other reagents used in solutions for the preparation of an analytical calibration curve is a matter of some concern. Quinine dihydrochloride, another common commercial form of quinine, for example, would exhibit some quenching effects at higher concentrations due to added chloride; and hydrochloric acid cannot be used instead of sulfuric to acidify quinine solutions without serious consequences in fluorescence measurements.

It may be more appropriate, in certain courses, to stress the more theoretical aspects of quenching by conversion of all concentration units to a molar basis and treating the data in terms of quenching constants, similar to the approach of Eisenbrand and Raisch *(19)*, or apply Stern-Volmer theory to the data to determine quenching rate constants. The quenching experiments can be extended to include bromide and iodide ions, which give similar results, except they are approximately 1.5 and 2 times as effective as chloride ion on a molar basis as quenchers.

Conclusion

These experiments have been designed to illustrate primarily the practical aspects involved in the use of fluorescence. It is intended that the instructor choose those experiments most appropriate to the educational requirements of his students, and to the laboratory time available. It is estimated that the total laboratory time necessary for a reasonably proficient pair of students to perform all the experiments described herein is approximately 6–7 hr. The tonic water analysis experiment by itself, including preparation of a stock solution and a calibration curve, takes less than about 3 hr lab time; the urine analysis experiment would take approximately an hour longer due to increased sample preparation time.

In general, the students were quite pleased with the use of real samples in the laboratory; it is a simple enough matter to keep the lab sufficiently quantitative in nature by the inclusion of a synthetic quinine unknown for analysis.

Literature Cited

(1) Willard, H. H., Merritt, L. L., Jr., and Dean, J. A., "Instrumental Methods of Analysis," 4th Ed., D. Van Nostrand Company, New York, **1965**, pp. 390–392.
(2) Meloan, C. E., and Kiser, R. W., "Problems and Experiments in Instrumental Analysis," C. E. Merrill, Columbus, O., **1963**, p. 22.
(3) Cetorelli, J. J., McCarthy, W. J., and Winefordner, J. D., J. CHEM. EDUC., **45**, 98 (1968).
(4) Duchin, K. L., Lee, Y. S., and Mills, J. W., J. CHEM. EDUC., **50**, 858 (1973).
(5) Russo, S. F., J. CHEM. EDUC., **46**, 374 (1969).
(6) Ellis, D. W., J. CHEM. EDUC., **43**, 259 (1966).
(7) Steinfeld, J. I., J. CHEM. EDUC., **42**, 85 (1965).
(8) Carper, M. A., and Carper, W. R., J. CHEM. EDUC., **45**, 662 (1968).
(9) Parsons, M. L., J. CHEM. EDUC., **46**, 290 (1969).
(10) Ingle, J. D., Jr., J. CHEM. EDUC., **51**, 100 (1974).
(11) Winefordner, J. D., and O'Haver, T. C., *Fluorescence News*, **5**, 1 (1971).
(12) O'Haver, T. C., and Winefordner, J. D., *Fluorescence News*, **6**, 1 (1972).
(13) *Federal Register*, **27**, 2456 (March 15, 1962).
(14) Strache, F., *Deut. Lebensm. Rundschau*, **55**, 153 (1959).
(15) Hadorn, H., and Zürcher, K., *Mitt. Geb. Lebensmittelunters. Hyg.*, **55**, 194 (1964).
(16) "The Merck Index," 7th Ed., Merck & Co., Inc., Rahway, N.J., **1960**, pp. 888–892.
(17) Mulé, S. J., and Hushin, P. L., *Anal. Chem.*, **43**, 708 (1971).
(18) Udenfriend, S., "Fluorescence Assay in Biology and Medicine," Volume I, Academic Press, New York, **1962**, pp. 401–403.
(19) Eisenbrand, J., and Raisch, M., *Z. Anal. Chem.*, **179**, 352 (1961); p. 406.