Comparison of Analytical Methods: Direct Emission versus First-Derivative Fluorometric Methods for Quinine Determination in Tonic Waters

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Fluorescence spectroscopy is an extremely versatile, sensitive experimental technique used in identification and quantification of many environmentally important compounds such as polycyclic aromatic hydrocarbons, polycyclic aromatic nitrogen heterocycles, and polycyclic aromatic sulfur heterocycles. Through judicious selection of excitation and emission wavelengths, a single desired fluorophore can often be analyzed in complex unknown mixtures containing several absorbing and fluorescing species.

Many laboratory experiments appearing in this *Journal* (*1–9*) and standard laboratory manuals (e.g., ref *10*) have involved determination of analyte concentrations by fluorometric methods. Published methods assume that the observed emission intensity, F, is

$$F = K'C \qquad (1)$$

directly proportional to the molar concentration of the analyte. The proportionality constant, K', depends upon the quantum efficiency (quantum yield) of the fluorescence process, the response of the photodetector at the emission wavelength, and the molar extinction coefficient, which remain constant during any given chemical analysis at fixed excitation and emission wavelengths. Analyte concentrations are determined from a working-curve plot of the measured fluorescence intensity versus the known molar concentrations of the standard solutions.

The aforementioned experimental methods introduce students to fluorescence instrumentation. However, the data analysis will appear rather trivial if UV–vis spectrophotometric, flame emission, or AA analysis has already been performed. Most instrumental analysis textbooks (*11–14*) discuss absorption spectroscopy and applications of the Beer–Lambert law one or two chapters before presenting fluorescence and phosphorescence.

We have found it possible to modernize our existing fluorometric laboratory experiment involving the determination of quinine in tonic waters by statistically comparing values determined from direct emission and first-derivative fluorometric methods. Recent review articles (*15–20*), written in several different languages, have cited numerous examples of the application of derivative spectroscopy to the analysis of food, clinical, pharmaceutical, biomedical, and environmental samples. For the most part, published applications utilize either the first or second derivative. Third and higher-order derivatives have been successfully used in select occasions. The first-derivative spectrofluorometric method is relatively straightforward and will be discussed in terms of an unknown tonic water sample containing quinine. The measured emission intensity is given by eq 1. Differentiation of the solution fluorescence emission with respect to the emission wavelength, λ_{em}, yields the following mathematical expression:

$$dF/d\,\lambda_{em} = (dK'/d\,\lambda_{em})C_{quinine} \qquad (2)$$

For solutions that contain only a single fluorophore, the first derivative corresponds to the gradient $dF/d\,\lambda_{em}$ of the fluorescence emission envelope and for each well-resolved band features only a maximum and trough. The vertical distance is the amplitude, which is directly proportional to the analyte concentration at each wavelength provided that eq 1 is obeyed. The proportionality constant, $dK'/d\,\lambda_{em}$, is obtained from linear least-squares analysis of the first-derivative spectrofluorometric data for standard solutions of known quinine concentration.

The first-derivative method is identical in concept to the more conventional fluorescence method based upon eq 1, except that first-derivative spectra are used in the data treatment. Many scanning spectrofluorometers have built-in software for displaying derivative spectra. We have found that it requires very little additional laboratory time to record first-derivative spectra as part of the experimental laboratory measurements. Students are instructed to compare their quinine concentrations calculated from the direct emission intensities to values obtained from the first-derivative spectra (both positive and negative slopes) to ascertain if there is a significant difference in the analytical methods. Values from the entire class are pooled to increase the number of data points for the statistical treatment. The statistical treatment is discussed in most standard analytical textbooks (*21–23*). Rarely are undergraduate students afforded the opportunity to actually apply the treatment to their experimental data. Such analysis leads into a discussion of factors that are considered in analytical method selection. The selection of an appropriate analytical method is a decision that practicing analytical chemists encounter daily.

Experimental Measurements

The experimental work can be completed easily in a 3-hour laboratory period. We suggest that students work in groups of two to reduce the time needed to prepare solutions. Each group is given 50 mL of tonic water for analysis and told that the sample must be diluted with 0.05 M H_2SO_4 in order to have the measured emission intensity fall in the linear region of the working curve. A 20-fold dilution (5 mL aliquot

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into a 100-mL volumetric flask) is suggested for the initial try.

Students are instructed to use a buret to transfer 20.0, 12.5, 7.5, 2.5, and 1.0 mL of a 10 ppm quinine stock solution into each of five 25-mL volumetric flasks. Each flask is then filled to the mark with 0.05 M H_2SO_4. Fluorescence emission spectra of the five standard solutions, the 10 ppm stock solution, and the diluted tonic water sample are scanned from 400 to 550 nm, using an excitation wavelength of 350 nm. The corresponding first-derivative spectra are obtained from the recorded emission spectra using the spectrofluorometer's built-in software program. Students record the fluorescence emission intensity of all seven solutions at 450 nm, as well as the values of $dF/d\,\lambda_{em}$ at 422 nm (positive slope) and 477 nm (negative slope).

Discussion of Results

Typical student results, measured on a Shimadzu RF-5000U spectrofluorometer, are listed in Table 1 for the determination of quinine by means of the direct emission and first-derivative spectrofluorometric methods. Numerical values for both the positive and negative $dF/d\,\lambda_{em}$ slopes are given. The first six solutions pertain to the calibration curves needed to determine the proportionality constants K' and $dK'/d\,\lambda_{em}$, which govern the change in the measured fluorescence intensity with concentration. Linear least squares analysis of the experimental data yields the following mathematical expressions:

$$F \text{ (at 450 nm)} = 19.598\, C_{quinine} + 4.354 \qquad (r^2 = .9983) \quad (3)$$

$$dF/d\,\lambda_{em} \text{ (at 422 nm)} = 0.186\, C_{quinine} + 0.037 \qquad (r^2 = .9988) \quad (4)$$

$$dF/d\,\lambda_{em} \text{ (at 477 nm)} = -0.127\, C_{quinine} - 0.034 \qquad (r^2 = .9976) \quad (5)$$

The nonzero intercepts likely result from a small background fluorescence caused by an impurity in the 0.05 M H_2SO_4 used to make the dilutions. Near-unity squared correlation coefficients indicate that the measured fluorescence signal does increase linearly with quinine concentration. By substituting the unknown's measured emission intensity into eq 3, one obtains the numerical value of $C_{quinine}$ = 2.165 ppm for the concentration of quinine in the diluted tonic water sample. Similarly, values of $C_{quinine}$ = 2.167 ppm (positive slope) and $C_{quinine}$ = 2.175 ppm (negative slope) are computed from the first-derivative measurements.

Students are reminded during the brief prelaboratory lecture that analytical chemists always report the concentrations in the original samples, and that one must always take into account any dilutions that were been made during the course of the chemical analysis. In the present case, students had to make a 20-fold dilution of the original tonic water sample so that the measured fluorescence emission intensity would fall on the working curve. The concentration of quinine in the tonic water is then found by multiplying the calculated values of $C_{quinine}$ by 20. Experimental results for the entire class are summarized in Table 2. For informational purposes, each group of students was given the same tonic water sample to analyze.

As part of the laboratory experiment, students are asked to determine if there is a difference between the various analytical methods at the 95% confidence level. The statistical treatment employs the *t*-test. The value of *t* is calculated using

$$t = |(x_{method\ 1} - x_{method\ 2})/s_{pooled}|[n_1 n_2/(n_1 + n_2)]^{0.5} \quad (6)$$

where

$$s_{pooled} = \{[s^2_{method\ 1}(n_1 - 1) + s^2_{method\ 2}(n_2 - 1)]/(n_1 + n_2 - 2)\}^{0.5} \quad (7)$$

the average quinine concentration ($x_{method\ 1}$ and $x_{method\ 2}$) for the two methods being compared, as well as the calculated standard deviations ($s_{method\ 1}$ and $s_{method\ 2}$) and number of data points (n_1 and n_2). If the calculated value of *t* is greater than the tabulated *t* at the 95% confidence level for $n_1 + n_2 - 2$ degrees of freedom, then the results determined by the two methods are considered to be different.

For the direct emission versus first-derivative method (positive slope), the calculated value of *t* is *t* = 0.34, which is significantly less than the tabulated value of $t \approx 2.1$ for 18 degrees of freedom. Statistically, there is no difference between the two analytical methods. Had students been given different tonic water samples, then the statistical treatment would be based upon comparing individual differences. Most standard analytical textbooks (*21–23*) discuss the statistical treatments in great detail.

We have found that incorporation of the first-derivative method into our existing fluorescence analysis method for quinine in tonic water greatly enriches the experiment's educational value. Very little additional laboratory time is required for the spectrofluorometer to display the first-derivative spectra. Undergraduate students are exposed to the

Table 1. Representative Experimental Data for the Determination of Quinine in Tonic Water by Direct Emission and First-Derivative Spectrofluorometry

[Quinine]/ ppm	*F* (450 nm)	$dF/d\,\lambda_{em}$ (422 nm)	$dF/d\,\lambda_{em}$ (477 nm)
0.4	8.96	0.08	−0.06
1	22.72	0.22	−0.15
3	65.98	0.62	−0.44
5	106.68	1.00	−0.70
8	161.47	1.52	−1.05
10	197.31	1.88	−1.28
Unknown	46.78	0.44	−0.31

NOTE: Values in columns 2–4 were determined on a Shimadzu RF-5000U spectrofluorometer.

Table 2. Quinine Concentrations in Tonic Water Determined by Direct Emission and First-Derivative Spectrofluorometry

Group No.	Direct Emission		Positive Slope		Negative Slope	
	r^2	ppm	r^2	ppm	r^2	ppm
1	.9985	43.72	.9984	43.43	.9967	42.86
2	.9983	43.30	.9988	43.33	.9976	43.50
3	.9983	43.20	.9980	42.25	.9967	44.42
4	.9983	43.25	.9988	43.57	.9971	43.23
5	.9965	42.67	.9961	42.22	.9969	43.09
6	.9967	44.70	.9946	44.42	.9971	43.85
7	.9982	43.50	.9975	44.17	.9980	41.82
8	.9973	46.96	.9981	45.45	.9976	43.26
9	.9977	44.02	.9975	43.55	.9987	50.29
10	.9979	44.48	.9984	45.45	.9945	45.08
Av (±s)		*43.98 (±1.22)*		*43.80 (±1.12)*		*44.19 (±2.34)*

general method of derivative spectroscopy, which is an important, often-used analytical technique (*15–20*) for eliminating sample matrix and background fluorescence effects and for treating overlapped spectral bands. Moreover, students have the opportunity to actually perform the statistical analysis involved with comparing different analytical methods. Method selection and validation are important items routinely encountered by practicing analytical chemists.

As an informational note, we elected to introduce derivative spectroscopy as part of an existing fluorescence experiment. Students were already performing UV–vis absorbance experiments involving both a bilinear regressional analysis (*24*) and an H-point standard addition method (*25*). A third absorption experiment was not needed. If one does wish an absorption experiment, Stolberg published a derivative spectrometric method for determining saccharin in cola in this *Journal* several years ago (*26*). The five review articles cited earlier (*15–20*) provide specific examples of mixtures that could only be analyzed by derivative spectroscopy. Direct absorption or emission methods would not be applicable because of severe overlap of spectral bands. Many of the cited examples could be modified into a suitable laboratory experiment for students to perform in the instrumental analysis. This was not our intent, however, as we wanted students to use statistical analysis to compare two analytical methods. A statistical comparison would be impossible if only one of the two methods yielded numerical values.

Derivative spectroscopy can also be used in the physical chemistry laboratory to study chemical bonding of direct relevance to quantum theory. For example, Cartwright (*27*) showed that the second-derivative visible spectrum of gaseous iodine facilitated the assignment of band heads and permitted data collection beyond that normally obtained from the undifferentiated spectrum. More recently, Ramachandran and Halpern (*28*) utilized second-derivative analysis in determining the positions of vibronic features and vibrational spacings in the vapor phase absorption spectrum of trimethylamine.

Acknowledgments

Carmen Hernández thanks the University of North Texas and the U. S. Department of Education for support provided under the Ronald E. McNair Postbaccalaureate Achievement Program. Gaddum Reddy thanks the UNT Texas Academy of Math and Science (TAMS) for an undergraduate summer research fellowship. Geo Martinez, Autumn Jackson, and Guenevere Brown thank the U.S. Department of Education for support provided to them under the Upward Bound Math and Science Program.

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