Modern Laboratory Experiment for Instrumental Analysis

Analytical Method for Simultaneous Determination of Chloride and Bromide Ions Based Upon Fluorescence-Quenching Methods

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Fluorescence spectroscopy is an extremely versatile, sensitive experimental technique used in identification and quantification of many environmentally important compounds: 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 can often be analyzed in complex unknown mixtures containing several absorbing and fluorescing species.

Over the past few years many laboratory experiments have appeared in *this Journal* (*1–7*) and standard laboratory manuals (e.g., ref *8*) involving determination of analyte concentrations based upon spectrofluorometric methods. Published methods assume that the observed emission intensity, F, is directly proportional to the molar concentration of the analyte fluorophore.

$$F = K'C \tag{1}$$

where the proportionality constant, K', depends upon the quantum efficiency (quantum yield) of the fluorescent process, response of the photodetector at the emission wavelength, and solute molar extinction coefficient, which should remain constant during any given chemical analysis. Analyte concentrations are determined from a working-curve plot of the measured fluorescence intensity versus the known molar concentration of 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 carried out. Most instrumental analysis textbooks (*9–12*) discuss absorption spectroscopy and applications of the Beer–Lambert law one or two chapters before presenting fluorescence and phosphorescence. We found it possible to incorporate all of these fundamental principles into a relatively simple fluorescence-quenching experiment involving the simultaneous determination of chloride and bromide in unknown mixtures while also introducing students to the basic chemical kinetics associated with competing reaction processes in solution.

The experimental method involves an unusual application of fluorescence: Emission-quenching is monitored as the analytical procedure. The experiment provides a very convenient analytical method for determining chloride and bromide anion concentrations. There are very few standard analytical methods in the chemical literature for halide anions.

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Effect of Two Quenching Agents Upon Fluorescence Emission

Quenching agents decrease fluorescence emission through collisional deactivation involving the excited fluorophore molecule (dynamic quenching) or by formation of nonfluorescent quencher–fluorophore ground-state complexes (static quenching). Both processes give a similar mathematical expression. However, we will consider only the case of collisional deactivation by two quenching agents, quenchers 1 and 2.

Dynamic Quenching

$$\text{fluorophore}^* + \text{quencher 1} \xrightarrow{k_{Q1}} \text{fluorophore} + \text{quencher 1}$$

$$\text{fluorophore}^* + \text{quencher 2} \xrightarrow{k_{Q2}} \text{fluorophore} + \text{quencher 2}$$

where k_{Q1} and k_{Q2} refer to the second-order rate constants for quenching.

In the absence and presence of the two quenching agents, the change in the molar concentration of the excited fluorophore species with time is given by

$$\frac{d[\text{fluor}^*]}{dt} = k_{abs}[\text{fluor}] - k_{fluor}[\text{fluor}^*] - k_{int\ con}[\text{fluor}^*] \tag{2}$$

$$\frac{d[\text{fluor}^*]}{dt} = k_{abs}[\text{fluor}] - k_{fluor}[\text{fluor}^*] - k_{int\ con}[\text{fluor}^*] - k_{Q1}[\text{quencher 1}][\text{fluor}^*] - k_{Q2}[\text{quencher 2}][\text{fluor}^*] \tag{3}$$

Under constant illumination or, if one prefers, under steady-state conditions,

$$\frac{d[\text{fluor}^*]}{dt} = 0$$

eqs 2 and 3 are solved for the molar concentration of the excited fluorophore. This must be directly proportional to the emission signal F because the fluorescence process begins with absorption of excitation radiation.

Through suitable mathematical manipulation of eqs 1–3 (with $d[\text{fluor}^*]/dt = 0$), a relatively simple expression is derived for relating the measured fluorescence emission intensity to both quencher concentrations.

$$[\text{fluor}^*] = k_{abs}[\text{fluor}](k_{fluor} + k_{int\ con})^{-1} \times \left(1 + k_{Q1}(k_{fluor} + k_{int\ con})^{-1}[\text{quencher 1}] + k_{Q2}(k_{fluor} + k_{int\ con})^{-1}[\text{quencher 2}]\right)^{-1} \tag{4}$$

$$\frac{F_o}{F} - 1 = k_{Q1}(k_{fluor} + k_{int\ con})^{-1}[\text{quencher 1}] + k_{Q2}(k_{fluor} + k_{int\ con})^{-1}[\text{quencher 2}] \tag{5}$$

$$\frac{F_o}{F} - 1 = K_{Q1}[\text{quencher 1}] + K_{Q2}[\text{quencher 2}] \quad (6)$$

where F_o refers to the measured fluorescence intensity in the absence of quenching agents.

Monitoring Fluorescence Emission

Numerical values for the two K_{Q1} and K_{Q2} coefficients are determined by preparing two sets of standard solutions having known quencher concentrations. This is like experimentally determining the molar absorptivity coefficient in the Beer–Lambert equation ($A = \epsilon bC$), except that students are now monitoring fluorescence emission instead of the absorbance of the solution.

Inherent in the above treatment is the assumption that the stoichiometric concentration of the fluorophore is constant for all of the solutions, and that the quenching process results from collisions between the excited fluorophore and quenching reagents. The mathematical treatments for cases involving formation of two nonnradiative fluorophore–quencher ground-state complexes (*13*) and a mixed dynamic-static quenching mechanism (*14*, *15*) are published elsewhere.

Experimental Procedure

The laboratory portion may take one of two different pathways, depending upon the number of students enrolled and the time devoted to spectrofluorometry. If time is available, we recommend Method 2 because the experimental procedure is slightly more sophisticated, and the method is applicable to both aqueous solutions and solid samples containing inert "filler" materials. Irrespective of the path selected, two equations must be generated in order to uniquely determine the concentrations of both quenching agents.

Method 1

Students are given an unknown solid sample containing only a mixture of sodium chloride (NaCl) and potassium bromide (KBr). Both anions are known to quench the fluorescence emission of quinine (*16*). Separate stock solutions of 4.0-ppm quinine dissolved in 0.5 M H_2SO_4, 1.8×10^{-2} M NaCl, and 1.8×10^{-2} M KBr are prepared ahead of time by the instructor or laboratory assistant for use in determining the K_{Q1} and K_{Q2} coefficients. Students are then instructed to prepare a series of 0.32-ppm quinine solutions containing 3.60×10^{-4}, 7.20×10^{-4}, 1.44×10^{-3}, and 2.16×10^{-3} M NaCl and 3.60×10^{-4}, 7.20×10^{-4}, 1.44×10^{-3}, and 2.16×10^{-3} M KBr by pipetting appropriate quantities of stock solutions into 25-mL volumetric flasks and diluting to the mark with 0.5 M H_2SO_4. Fluorescence emission intensity of these eight solutions is measured at about 450 nm, with excitation at about 350 nm. If a scanning spectrofluorometer is available, students should record the excitation and emission spectra in order to verify these wavelengths. From the measured intensities, average values of K_{Q1} and K_{Q2} are computed (see eq 6).

Students dissolve about 100 mg of their unknown sample in a 100-mL volumetric flask and dilute to the mark with deionized water. Two milliliters of the unknown solution are transferred with a pipet to a 25-mL volumetric flask containing 2.00 mL of the 4-ppm quinine stock solution, and the emission intensity is measured after dilution to the mark with deionized water.

The concentrations mentioned above give reasonable fluorescence signals on our Shimadzu RF-5000U spectrofluorometer with the detector set at high sensitivity. Excitation and emission slit widths were 15 nm and 3 nm, and all measurements were carried out using standard 1-cm^2 disposal methacrylate cuvettes. The fluorophore and quencher concentrations may have to be adjusted for other instruments.

Calculations

The percentage of NaCl and KBr in the unknown sample is computed by solving the following two equations simultaneously.

$$\text{mass of NaCl} + \text{mass of KBr} = \text{mass of sample taken} \quad (7)$$

$$\frac{F_o}{F_{unk}} - 1 = K_{Cl}\left(\frac{2}{25}\right)\left(\frac{\text{mass of NaCl}}{FW_{NaCl}}\right) + K_{Br}\left(\frac{2}{25}\right)\left(\frac{\text{mass of KBr}}{FW_{KBr}}\right) \quad (8)$$

where FW_{NaCl} and FW_{KBr} refer to the molar masses of sodium chloride and potassium bromide.

The first mathematical constraint (eq 7) arises because the sample is a mixture of only sodium chloride and potassium bromide. Numerical values of the two quenching constants of $K_{Cl} \approx 203$ and $K_{Br} \approx 200$ are based upon typical results obtained by undergraduate students enrolled in our instrumental analysis course. Student analyses were reproducible, and the calculated weight percentages differed from the so-called true values by 3% (relative error) or less.

Method 2

Students are given an unknown solution containing sodium chloride and potassium bromide (approximately 0.005–0.040 M in each anion). To determine both anion concentrations, it is necessary to carry out a second series of fluorescence measurements because the mathematical constraint imposed by eq 7 has been removed. Possible fluorophores include acridine, harman, calcein, calcein blue, harmine, harmol, and *N*-methylacridone (*16*). Acridine serves as a good second fluorophore, and it is commercially available in reasonably pure form (Aldrich, 99+%).

The experimental portion again involves preparing the eight quinine solutions listed under Method 1 plus an additional set of eight solutions containing 2.00-mL aliquots of 10^{-5} M acridine (dissolved in 0.5 M H_2SO_4) in place of the stock quinine solution. Acridine solutions are excited at about 360 nm, with the fluorescence emission measured at about 472 nm. The unknown solution is prepared by transferring 2.00-mL aliquots from the sodium chloride and potassium bromide sample into two separate 25-mL volumetric flasks that contain either 2.00 mL of the quinine or 2.00 mL of the acridine stock solution. The fluorescence emission intensities of acridine and quinine solutions are recorded after dilution to the mark with 0.5 M H_2SO_4.

Fluorescence-Quenching Equations

The molar concentration of bromide and chloride ions in the unknown mixture are computed by solving the following two fluorescence-quenching equations simultaneously.

For quinine

$$\frac{F_o}{F_{unk}} - 1 = K_{Cl}\left(\frac{2}{25}\right)[Cl^-] + K_{Br}\left(\frac{2}{25}\right)[Br^-] \quad (9)$$

For acridine

$$\frac{F_o}{F_{unk}} - 1 = K_{Cl}\left(\frac{2}{25}\right)[Cl^-] + K_{Br}\left(\frac{2}{25}\right)[Br^-] \quad (10)$$

Representative student results gave $K_{Cl} \approx 73$ and $K_{Br} \approx 380$ for the chloride and bromide quenching constants for acridine. Again, the method yields molarities that are within 2–3% (relative error) of the "true" values based upon typical student results. Wolfbeis and Urbano (*16*) showed that iodide concentrations can also be determined in this fashion.

Inner-Filtering Artifacts

The quenching study presented here can serve as a "stand alone" experiment. Alternatively, if one prefers, the laboratory portion can be expanded to include a brief examination of primary and secondary inner-filtering artifacts. One possible inner-filtering study would be the effect of $K_2Cr_2O_7$ on the fluorescence emission intensities of quinine sulfate as recently published in *this Journal* (*17*).

Conclusion

To our knowledge there has been only one fluorescence-quenching experiment published in *this Journal* during the past 15 years. Unlike this work, which is designed as an analytical chemistry laboratory experiment, Fraiji et al. (*14*) treat the quenching process from a physical chemistry point-of-view. The authors calculate equilibrium and rate constants and enthalpies and entropies of complex formation, as opposed to this work, which determines percentages and molar concentrations of unknown samples.

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