Anion Analysis 1 - Simultaneous Determination of Chloride and Bromide by a Fluorescence Quenching Method

Introduction

Fluorescence spectrophotometry in its pure form is inherently a very sensitive analytical method. Single molecules have been observed in fluorescence microscopy due to the very low signal in the absence of fluorescent analytes (often referred to as zero background) and the possibility of producing many photons from a single molecule using a very bright excitation source. There are two types of fluorometric methods used in the determination of inorganic species. Direct methods involve the formation of a fluorescent chelate and the measurement of its fluorescence. Indirect methods are based on the quenching of a fluorescent indicator by the species being determined. The latter technique has been most widely used for anion analysis. Note that the indirect method (used here) is not a zero background method as regards the analyte.

The quenching, a decrease in fluorescence intensity, of a fluorescent indicator (usually an aromatic organic dye) can be described by the Stern - Volmer equation:

$$F_0 / F = 1 + K [Q]$$
 (1)

where F_0 and F are the fluorescence intensity in the absence and presence of a quencher (Q), K is the quenching constant, and [Q] is the molar concentration of the quencher. If the value of K is known or has been determined, the concentration of the quencher [Q] can be determined by measuring F_0 and F.

If two quenchers (Q_A and Q_B) are present and operate independently of one another, the Stern - Volmer equation takes the form:

$$F_0 / F = 1 + K_A [Q_A] + K_B [Q_B]$$
 (2)

To determine the concentrations of two quenchers (*e.g.*, chloride and bromide), it is necessary to carry out two series of fluorescence measurements with two fluorescent indicators (1 and 2).

$$(F_0 / F)_1 = 1 + {}^{1}K_{CI} [CI^{-}] + {}^{1}K_{Br} [Br^{-}]$$
(3)
$$(F_0 / F)_2 = 1 + {}^{2}K_{CI} [CI^{-}] + {}^{2}K_{Br} [Br^{-}]$$
(4)

The molar concentrations of chloride and bromide in an unknown mixture can be calculated by solving the above two simultaneous linear equations, if the quenching constants (K) are all determined in separate calibration experiments. In this experiment, you will use the fluorescence quenching method to analyze a solid unknown challenge sample to determine the weight percentages of NaCl and KBr. The fluorescent indicators **quinine** and **acridine** are used. Be sure to provide molecular structures of quinine and acridine in the Introduction section of your report and discuss briefly why they are good fluorescers.

Apparatus

fluorescence cuvettes (3) 25 mL volumetric flasks (12) 100 mL volumetric flasks (3) 1 mL pipet (2)

Instrumentation (See Appendix for Operating Instructions):

PTI Spectrofluorometer with FELIX data system

Solutions available

- (1) 1.25 M H_2SO_4 : pour out less than 5 mL, only 1 mL is needed.
- (2) 1.0×10^{-4} M quinine prepared in 1.25 M H₂SO₄: measure out about 15 mL in a small beaker.
- (3) 1.0×10^{-4} M acridine prepared in 1.25 M H₂SO₄: measure out about 15 mL in a small beaker.

Solutions to be prepared

- (1) <u> 3.0×10^{-2} M NaCl solution</u>: Weigh out about 0.175 g (to 0.001 g) of NaCl (FW = 58.442) and quantitatively transfer to a 100 mL volumetric flask, dissolve with deionized water, fill to the mark, and mix thoroughly.
- (2) <u> 3.0×10^{-2} M KBr solution</u>: Weigh out about 0.357 g (to 0.001 g) of KBr (FW = 119.002) and quantitatively transfer to a 100 mL volumetric flask, dissolve with deionized water, fill to the mark, and mix thoroughly.
- (3) <u>Solution of solid sample:</u> Weigh out about 0.15 g (to 0.001 g) of solid NaCl and 0.35 g (to 0.001 g) KBr and quantitatively transfer both to a 100 mL volumetric flask, dissolve with deionized water, fill to the mark, and mix thoroughly.

Calculate and record the analytical concentrations for all three solutions.

Procedure

Note: In this experiment, two fluorescent indicators are used to allow the measurement of two anionic analytes. The fluorescence excitation and emission spectra for the two indicators are different, so you will first obtain these spectra and select appropriate excitation and emission wavelengths for each dye. During the quenching measurements, significant errors may be introduced if the wavelengths are not set reproducibly. To avoid changing the wavelengths and introducing the associated errors, you should try to complete all of the measurements with one indicator before switching to the other one. The text below describes the set of operations for the first dye quinine but you will perform an identical (except for the excitation and emission wavelengths) procedure for acridine.

(1) <u>Preparation of solutions:</u>

Using <u>twelve</u> 25 mL volumetric flasks, prepare the samples for fluorescence measurement by adding the following:

#1: 1 mL of 1.25 M H₂SO₄ only (the solvent blank)

#2: 1 mL quinine only (the unquenched indicator)

#3,4,5,6: 1, 2, 3, and 4 mL of NaCl standard + 1 mL of quinine (the NaCl standards)#7,8,9,10: 1, 2, 3, and 4 mL of KBr standard + 1 mL of quinine (the KBr standards)

#11: 1 mL of the solution of the solid sample + 1 mL of quinine (low unknown)#12: 2 mL of the solution of the solid sample + 1 mL of quinine (high unknown)

Fill all of the flasks to the mark with deionized water and mix thoroughly. Label the flasks carefully to avoid mixing them up (they all look like clear liquids).

(2) Recording the fluorescence excitation and emission spectra

Three cuvettes should be used for each set of measurements: one for the <u>solvent blank</u>, one for the <u>unquenched indicator</u>, and one for the <u>standards</u> or <u>samples</u>. Record the fluorescence <u>excitation spectrum</u> for quinine as explained in the Appendix. First you will record a blank using the sulfuric acid solution in flask #1 and the software will store the blank value. Next you will set the emission wavelength to 450 nm and the excitation wavelength scan to go from 300 nm to 425 nm and you will record the spectrum of the unquenched dye in flask #2. Then with the same solution in the cuvette, you will switch to the <u>emission spectrum</u> mode and set the excitation wavelength to the maximum from the excitation spectrum and the emission scan to run from 375 nm to 650 nm. Record the spectrum and note the maximum value to use for the emission wavelength.

(3) <u>Measurement of fluorescence quenching</u>

Set the excitation and emission wavelengths to the maxima that you determined from the two spectra recorded above. Measure the fluorescence intensity of the solutions described above (in flasks 2 -12) using the <u>time-based measurement</u>. *Hint: The instrument used in this experiment may or may not produce stable fluorescence readings, because changes in the light source and detector can both change the results. If the results appear irreproducible, you could try the following optional procedure:*

1) use the solvent blank cuvette to autozero the reading, 2) measure the unquenched and quenched samples in the order $F_0 F F_0$ to determine the stability of the measurements; if there is a significant difference between the first and second unquenched readings, you may need to probe the variability by 3) doing the

measurement sequence in five steps, alternatively measuring the fluorescence of the unquenched indicator (F_0), and the fluorescence of the standard or sample (F) in the order $F_0 F F_0 F F_0$. 4) If you do it this way, you can use the standard deviation of the three F_0 values for a given dye as a measure of the precision in placing the cuvette in the instrument.

Whether you use the procedure above or not, each set of F_0 and F measurements can be used to determine the short term precision in the measurement by calculating the standard deviation of each 30 second data set. You should comment in your Discussion section on which of these measures of uncertainty is the most applicable in determining the overall uncertainty of the measurement used here.

Acridine spectra and fluorescence quenching measurments

Repeat the above procedure for the measurements with acridine. For the excitation spectrum, start with an emission wavelength of 488 nm.

<u>Report</u>: In preparing the Partial Report describing this experiment, you should consider/complete/discuss the following:

(1) Fluorescence spectra:

Include two figures showing the excitation and emission spectra (combined on one plot) for the two fluorescent dyes. Identify the maxima that you selected for the quenching measurements on the graphs.

(2) <u>Quenching constants:</u>

Tabulate all data and plot the quenching ratio (F_0 /F) *vs.* concentration of chloride and bromide for quinine and acridine (four calibration sets). You can usually put two plots on a graph, if they are distinct (*i.e.*, if the points and lines dont overlap). Derive the linear equations and calculate the four quenching constants and their uncertainties. Be sure to briefly explain how you obtained the values and uncertainties in the Discussion section of your report and to state the quenching constants you obtain in both the Results and Abstract sections of your report.

(3) Chloride and bromide concentrations in the challenge samples:

Calculate the concentrations of chloride and bromide in the unknown challenge solutions using equations 3 and 4. Comment on whether the concentrations for the low unknown and high unknown samples make sense, based on how they were made. The proper way to obtain an uncertainty for the unknown concentrations combines the uncertainties in the quenching constants with the uncertainty in the F_0/F ratio as determined from the standard deviations of the F_0 measurements. Be sure to state these values in the Results section of your report and explain clearly but briefly how you obtained the uncertainty in your Discussion section.

(4) Chloride and bromide in the solid sample:

Calculate the weight percentages of NaCl and KBr in your solid sample using the concentrations determined above. Since the solid sample contains only NaCl and KBr, we have an additional equation: mass of sample = mass of NaCl + mass of KBr. You can thus comment on the accuracy of the technique in determining the total anion concentration and the specific recoveries/accuracies for chloride and bromide (although these are clearly inter-related). A summary statement should be included in the Abstract.

Revised 2012-1-11 - DBA