

The Fluorometric Determination of Acetylsalicylic Acid in an Aspirin Tablet

Introduction:

Fluorescence is the emission of radiation from an atom or polyatomic species after the substance has been exposed to electromagnetic radiation. The incident radiation results in excitation of the substance. Fluorescent radiation is emitted during de-excitation of the substance

A fluorometer is a device which measures fluorescence. Radiation from a source passes through either a filter (the primary filter) or a monochromator (the excitation monochromator) which limits the width of the wavelength band. The radiation subsequently travels through the cuvette which contains the sample solution and causes excitation of the sample. Although the resulting fluorescent radiation is emitted in all directions from the cuvette, customarily only that portion of the radiation which is emitted perpendicularly to the incident radiation is measured. By measuring the fluorescent radiation at 90° from the incident radiation, interference from the incident radiation is minimized.

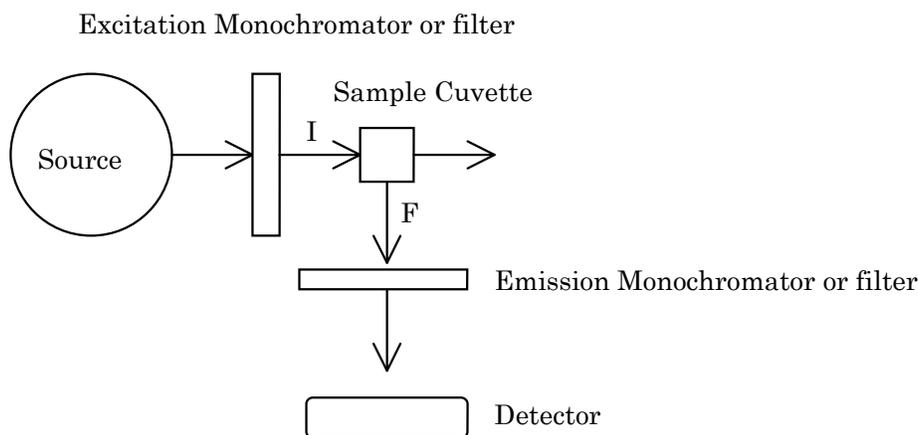


Figure 1. Diagram of the major parts of a fluorometer. The arrows indicate the paths of the EMR. I, incident radiation; F, fluorescent radiation.

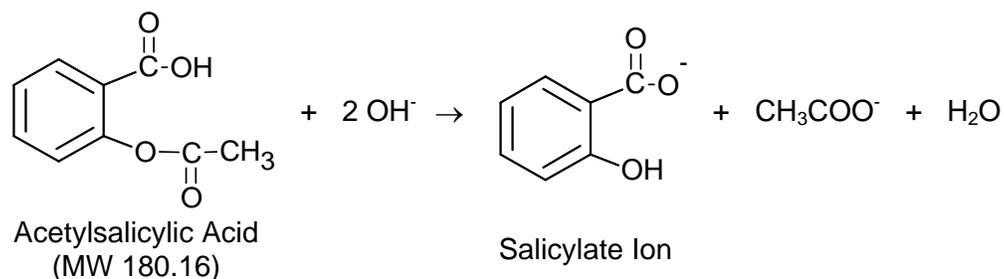
The fluorescent radiation passes through a second filter (the secondary filter) or monochromator (the emission monochromator) and strikes a detector. The filter or monochromator serves to allow the fluorescent radiation to strike the detector while preventing scattered exciting radiation from reaching the detector. The detector is a device which measures the intensity of the fluorescent radiation. If the fluorometer contains filters it is called a filter fluorometer. If it contains monochromators, it is called a spectrofluorometer.

Spectrofluorometers can be used to obtain fluorescent spectra, whereas filter fluorometers usually cannot be used to obtain spectra. A fluorescent spectrum which is obtained by holding the emission wavelength constant while varying the excitation wavelength is an excitation spectrum. A spectrum which is obtained by holding excitation wavelength constant while varying the emission wavelength is an emission spectrum.

In dilute solutions the intensity of fluorescent radiation is directly proportional to the concentration of the fluorescing substance. Once a wavelength of maximum emission is chosen, you will make a calibration curve using the intensity of emission for each standard at this wavelength.

Analysis of Acetylsalicylic Acid

Acetylsalicylic acid (ASA) is the analgesic (pain reliever) which is found in aspirin tablets. In addition to ASA, some aspirin tablets contain other ingredients such as binders and buffering agents. In order to maximize the detection of ASA in an aspirin tablet, you will first convert it to salicylate ions by the addition of sodium hydroxide.



The salicylate ion strongly fluoresces at about 400 nm after it has been excited at about 310 nm. A series of standard solutions of the salicylate ion will be prepared; the fluorescence of the standards and the samples will be measured; and the concentration of ASA in the aspirin tablet will be determined. In addition, a numbered unknown solution of salicylate ion will be measured using a similar technique.

Prelaboratory Assignment:

A 0.1019 g portion of an aspirin tablet was dissolved in sufficient water to prepare 1.000 liter of solution. A 1-mL portion was transferred to a 100-mL volumetric flask and diluted to the mark. A pipet was used to transfer 2 mL of the second solution to a 100-mL volumetric flask. A 2-mL portion of 4 M sodium hydroxide solution was added to the flask, and the resulting solution was diluted to the mark with water. The fluorescence of the diluted sample solution was found to correspond to a salicylate ion concentration of 1.0×10^{-7} M. Calculate the percent acetylsalicylic acid in the aspirin tablet.

Chemicals:

- aspirin tablet
- salicylic acid (reagent grade)
- sodium hydroxide solution (4 M)
- numbered unknown salicylic acid solution (in vial)

Note: This experiment will be performed in pairs or groups. Please read the entire procedure before beginning.

Procedure:

A. Preparation of calibration standards for salicylic acid

1. Accurately weigh approximately 0.077 g of salicylic acid to the nearest 0.1 mg. Place the acid in a labeled, 1-liter volumetric flask. Add about 500 mL of distilled or deionized water and shake the flask until the solid has dissolved. Dilute the solution to the mark with water. The result is a stock solution (A) of salicylic acid with a concentration of approximately 5.6×10^{-4} M.

- Using a volumetric pipet, transfer 1 mL of your stock solution A to a 100 mL volumetric flask. Dilute to the mark with water and mix well. This stock solution (B) will be used to make the calibration standards in the table below.
- Label six 100 mL volumetric flasks 0-5. Using a graduated cylinder or pipet, dispense 2 mL of 4 M NaOH into each flask.
- Using volumetric pipets, dispense each volume of your stock SA solution B in the table below into the appropriate flask. (Flask "0" is the blank which should contain NaOH and water but no SA.)

Flask	Volume stock B (mL)
0	none
1	1.00
2	2.00
3	3.00
4	4.00
5	5.00

- Dilute all flasks to the mark and mix well. Calculate the concentration of SA in each of the flasks.

B. Preparation of salicylic acid unknown

Your unknown for this experiment is a solution. When you obtain your unknown, you need to quantitatively transfer it to a 100 mL volumetric flask and dilute it to the mark, resulting in the "prepared" unknown solution. You are to report the results of this "prepared" unknown.

- Using a volumetric pipet, transfer 5 mL of your prepared unknown (i.e. the solution in the volumetric flask, not the solution in the original vial) into a 100 mL volumetric flask. Dilute to the mark with water and mix well.
- Label three 100-mL volumetric flasks U1, U2 and U3. To each of these flasks, transfer 2 mL of 4 M NaOH as above.
- Using a volumetric pipet, transfer 5 mL of your diluted unknown (made in step B-1 above) to each flask.
- Dilute each flask to the mark and mix well.

C. Preparation of aspirin tablet

Note: In order to complete the experiment in a timely fashion, the aspirin sample should be prepared and analyzed at the same time as your standards and SA unknown.

- Obtain an aspirin tablet from the instructor. If the tablet has a sample number, record it. Record the brand name or manufacturer of the tablet if it is available.
- Before grinding the tablet, weigh it to the nearest 0.1 mg on an analytical balance.
- Place the tablet in a clean, dry mortar. Use a clean pestle to grind the tablet into a powder. Weigh 0.1 g of the powder to the nearest 0.1 mg into a 100-mL beaker.
- Place about 1 liter of DDI water in a 2-liter beaker or flask. Heat the water to just below the boiling point.
- Fold a piece of filter paper into a cone and place it in a funnel. Place the funnel in the top of a 1-liter volumetric flask. Use a spray of DDI water from a wash bottle to rinse the powder in the 100-mL beaker into the funnel. Allow the solution that flows through the funnel to drain into the volumetric flask.
- Slowly pour the hot water from the 2-liter beaker or flask over the solid and through the funnel. The acetylsalicylic acid, which is in the powder, slowly dissolves in the water and drains into the funnel. Some tablets contain binders that will not dissolve. The insoluble

binders are separated from the acetylsalicylic acid during this step. After the solid has completely dissolved, or after no further solid appears to dissolve, allow the solution in the flask to cool to room temperature. Pour at least 500-mL of the hot water through the funnel before concluding that the solid will not dissolve further. Dilute the solution to the mark with room-temperature water. This is your “prepared aspirin.”

7. Using a volumetric pipet, transfer 1 mL of your prepared aspirin solution to a 100 mL volumetric flask. Dilute to the mark with water and mix well. This is your dilute aspirin solution.
8. Label three 100-mL volumetric flasks with A1, A2 and A3. To each of these flasks, transfer 2 mL of 4 M NaOH as above.
9. Use a volumetric pipet to transfer 2 mL of the diluted aspirin solution (made in step C-7 above) into each of the flasks.
10. Dilute each flask to the mark with DDI water and mix well.

D. Fluorimetric Analysis

Consult your instructor for directions for the proper use of the spectrofluorometer.

1. Use your most concentrated standard (5) to collect an excitation spectrum from 200 to 400 nm while monitoring emission at 450 nm. Adjust the slit widths to maintain a maximum emission of approximately 1×10^5 counts. (Consult your instructor for tips on how to do this and how to inspect your spectrum for evidence of self-absorption.)
2. Use the highest excitation determined in the previous step to choose an excitation wavelength for your sample. Collect an emission spectrum from 300 to 600 nm using this wavelength for excitation. Again, adjust the slit widths to maintain a maximum emission of approximately 1×10^5 counts. Determine the wavelength of maximum emission.
3. Collect emission spectra of each of your standards (1-5) as in the previous step. Create a calibration curve using the emission intensity for each standard at the maximum emission wavelength determined in the previous step (you should use the same wavelength for each standard).
4. Using the same conditions as before, collect emission spectra of all of your unknowns (Flask A1, A2, A3, U1, U2 and U3) and your blank (Flask 0). Use the emission intensity at the wavelength determined above to find the concentration of SA in these samples. Measure the blank at least three times in order to determine a limit of detection for the instrument.

Calculations:

1. Use the mass of the salicylic acid (MW 138.13) to calculate the concentration of salicylic acid in the 1-liter stock solution (A) and diluted stock solution (B).
2. Use the volumes of stock solution B added to the 100-mL volumetric flasks to calculate the concentrations of salicylate ion which are in flasks 1, 2, 3, 4, and 5.
3. Prepare a calibration curve by plotting the fluorescence of the solutions in flasks 1, 2, 3, 4, and 5 (y axis) as a function of the concentration of salicylate ion in each flask.
4. From the calibration curve determine the concentration of salicylate ion in flasks U1, U2, U3, A1, A2 and A3.
5. Determine the concentration of SA in the prepared unknown (in % w/v) using the concentration determined for each sample.

6. Determine the % w/w of ASA in the original aspirin sample using the mass of the tablet you analyzed. Using the mass of the original tablet, compare this result to the manufacturer's claimed value for the medication.
7. Determine the mean, standard deviation and 95 % confidence interval of all results.
8. Using the blank, calculate the lower limit of detection for this measurement.