

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 measured 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.

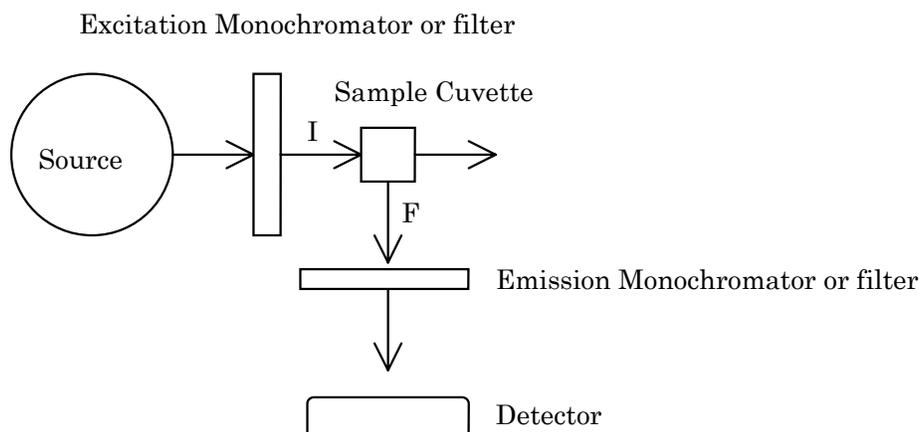


Figure 1. Diagram of the major parts of a fluorometer. The arrows indicate the paths of the EMR. I, incident radiation; F, fluorescent radiation.

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.

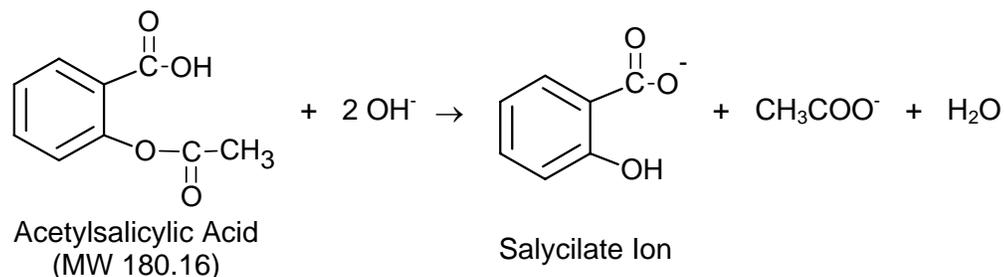
In dilute solutions the intensity of fluorescent radiation is directly proportional to the concentration of the fluorescing substance. Since relative fluorescent intensities rather than absolute fluorescent intensities are usually measured, an equation, such as Beer's law for absorbance measurements, normally is not used to directly calculate the concentration of a

fluorescent species from its fluorescent intensity. Either the working curve method or the standard addition technique generally is used to determine the concentration of a sample solution from its relative fluorescent intensity.

After having chosen the proper wavelengths or filters for the exciting and fluorescent radiation, a blank solution is placed in a cuvette and the cuvette is placed in the instrument. The instrument is adjusted to yield a relative fluorescent reading of 0. With the solution which contains the highest concentration of the fluorescing species in the fluorometer, the instrument is adjusted to yield a relative fluorescent intensity of 100. Without alteration of the adjustments, the relative fluorescent intensity of the remaining solutions is measured and recorded.

Analysis of Acetylsalicylic Acid in an Aspirin Tablet

Acetylsalicylic acid is the analgesic (pain reliever) which is found in aspirin tablets. In addition to acetylsalicylic acid, some aspirin tablets contain other ingredients such as binders and buffering agents. In the experiment, a portion of an aspirin tablet is dissolved in water and converted 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 are prepared; the fluorescence of the standards and the samples are measured; and the working curve method is used to determine the concentration of salicylate ion in the sample solutions. The concentration is used to calculate the percentage of acetylsalicylic acid in the aspirin.

Prelaboratory Assignment:

A 0.1019-g portion of an aspirin tablet was dissolved in sufficient water to prepare 1.000 liter of solution. A pipet was used to transfer 10 mL of the 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, by the working curve method, to correspond to a salicylate ion concentration of 4.80×10^{-5} M. Calculate the percent acetylsalicylic acid in the aspirin tablet.

Apparatus:

fluorometer	100-mL graduated cylinder	2-mL pipet
cuvettes	filter paper (medium porosity)	4-mL pipet
100-mL beaker	glass funnel	6-mL pipet
2-liter beaker	hot plate or Bunsen burner	8-mL pipet
2, 1-liter volumetric flasks	mortar and pestle	10-mL pipet
9, 100-mL volumetric flasks	wash bottle	

Chemicals:

aspirin tablet
salicylic acid (reagent grade)
sodium hydroxide solution (4 M)

Procedure:

1. 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.
2. 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.
3. Place about 1 liter of distilled or deionized water in a 2-liter beaker. Heat the water to just below the boiling point.
4. Fold a piece of filter paper and place it in a glass funnel. Place the funnel in the top of a 1-liter volumetric flask. Use a spray of distilled or deionized water from a wash bottle to rinse the powder in the 100-mL beaker into the funnel. Allow the solution which flows through the funnel to drain into the volumetric flask.
5. Slowly pour the hot water which is in the 2-liter beaker 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 which 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. Dilute the solution to the mark with room-temperature water. Pour at least 500-mL of the hot water through the funnel before concluding that the solid will not dissolve further.
6. Weigh 0.077 g of acetylsalicylic acid to the nearest 0.1 mg. Place the weighed 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 of salicylic acid.
7. Respectively label nine, 100-mL volumetric flasks with B, U1, U2, U3, 1, 2, 3, 4, and 5. Use a pipet to deliver 2 mL of 4 M sodium hydroxide solution to each of the nine flasks. Use pipets to add 2, 4, 6, 8, and 10 mL, respectively, of the salicylic acid stock solution to the 100-mL volumetric flasks which are labeled 1, 2, 3, 4, and 5. Use a pipet to place 10 mL of the 1-liter solution of the tablet into each of the flasks which are labeled U1, U2, and U3. Fill each flask to the mark with distilled or deionized water.
8. If a spectrofluorometer is to be used for the fluorimetric measurements, adjust the monochromator which controls the excitation wavelength to 310 nm and adjust the monochromator which controls the emission wavelength to 400 nm. If a filter fluorometer is to be used, insert a primary filter with a transmission band which is below about 360 nm (Corning 7-60 or the equivalent) and a secondary filter with a transmission band which is below about 460 nm (Wratten 3 or the equivalent). The primary filter is dark violet and the secondary filter is light yellow.
9. Fill a cuvette with the well-stirred solution in one of the nine, 100-mL volumetric flasks. Place the cuvette in the fluorometer. Measure and record the relative fluorescence of the solution. The instructor will provide the operating instructions for the fluorometer.

10. Similarly measure and record the relative fluorescence of each of the remaining eight solutions. Measure the blank solution at least three separate times so that you may calculate a standard deviation for the blank.

Calculations:

1. Use the mass of the salicylic acid (MW 138.13) to calculate the concentration of salicylic acid which is in the 1-liter stock solution.
2. Use the volumes of the stock solutions which were 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 working curve by plotting the relative fluorescence of the solutions which are in flasks B, 1, 2, 3, 4, and 5 (y axis) as a function of the concentration of salicylate ion in each flask.
4. From the working curve determine the concentration of salicylate ion which is in flasks U1, U2, and U3.
5. Use the dilution factor (10 mL diluted to 100 mL) to calculate three values for the concentration of acetylsalicylic acid in the 1-liter solution.
6. Use the three acetylsalicylic acid concentrations and the mass of the tablet which was used to prepare the solution to calculate three values of the percentage of acetylsalicylic acid in the tablet.
7. Determine the mean and standard deviation of the results.
8. Calculate the lower limit of detection for this measurement.