Lab 3b: Chromatographic Analysis of Synthesized Acetylsalicylic Acid (Aspirin)

PURPOSE

In this week's lab activity, you will use thin layer chromatography (TLC) to analyze the purity of the acetylsalicylic acid you synthesized in lab. TLC will also be used to analyze other active ingredients found in an over-the-counter (OTC) pain reliever. Also in this lab, we will start to work on quality lab technique!

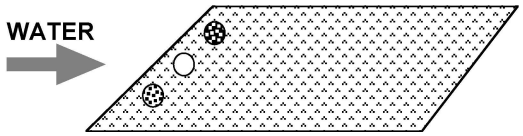
INTRODUCTION

Chromatography is a term used to describe a general method for separating a mixture of compounds. The term literally means, "to write with color" since this process was originally used to separate colored plant pigments. The following is a brief introduction chromatography. In this explanation, we use a model system that uses the commonly known material, Velcro™. Please note, the use of Velcro™ is ***only*** a macroscopic analogy to chromatography and not actually the way it operates on the scale of molecules.

Imagine a tube several inches in diameter and a foot long, as shown below. The inside of the tube (the stationary phase) is covered with Velcro™. There are three balls; one is smooth, like a marble, another is completely covered with Velcro™ and the last ball is partially covered with Velcro™. The three balls are placed at the left side of the tube as below. A strong air stream (the mobile phase) is directed through the tube and is allowed to blow the balls through the tube. Which ball will travel fastest through the tube? Which ball will travel slowest through the tube?

After contemplation, it is not unreasonable to assume that the smooth, marble-like ball would not interact with the Velcro™ and hence would travel through the tube the fastest. Similarly, it is reasonable to assume that the ball completely covered with Velcro™ would interact with the inside of the tube and would travel through the tube the slowest. The ball partially covered with Velcro™ would interact less with the tube than the fully covered ball, but would interact more than the smooth ball, hence it is reasonable to assume that this ball would travel at an intermediate speed through the tube.

The main principle of chromatography is that compounds (the balls) interact with the stationary phase (Velcro™) to different degrees and hence travel at different rates. If, instead of a tube of Velcro™, a flat surface is used, and instead of air, water is used, the results would be the same for the same three balls. The Velcro™ covered ball would travel the slowest, the smooth ball would travel the fastest, and the partially covered ball would move at a speed between the other two.



Now let us consider a more chemically based set of circumstances. First our flat surface will no longer be a Velcro™ covered plate, but rather a piece of chromatography paper; this chromatography paper will be orientated vertically. The Velcro™ covered balls are analogous to chemical compounds. The solvent will move the chemical compounds up the chromatography paper just like the air moved the balls through the tube or the water moved the Velcro™ balls across the plate. The chemicals compounds that interact less with the chromatography paper will move up the paper faster than those chemicals that interact more. A schematic of this system is shown to the right.

The above model systems have been given to illustrate the concepts and vocabulary of chromatography. The chromatography paper is referred to as the ***stationary phase***. The solvent that moves through the chromatography paper is referred to as the ***mobile phase***. The chemical compounds that interact to a different extent with the stationary and mobile phase are referred to as ***solutes***. We can use the ratio of the distance the solvent traveled to the distance the solutes travel to identifying the compound in the mixture. This ratio is known as the ***retention factor*** or R_f and is calculated by the following equation:

$$R_f = \frac{\text{distance to spot}}{\text{distance to solvent front}}$$

The retention factor is always between 0.0 and 1.0. Ideally, the R_f determined under a certain set of chromatography conditions will always be the same for a single compound.

In this experiment you will use a thin layer chromatography plate to separate a mixture of solutes that are found in your synthesized acetylsalicylic acid (aspirin). TLC plates are made of silica adhered to a plastic film. Silica is a relatively ***polar*** stationary phase. We will be using a mobile phase that is a mixture of toluene, diethyl ether, acetic acid and methanol. The TLC

plates that we will be using have a fluorescent dye mixed into the silica that allows us to easily see the solutes on the TLC plate. The plates are "developed" vertically in a beaker with the mobile phase. Because acetylsalicylic acid interacts differently with the stationary phase than salicylic acid, we will be able to determine the relative amounts of these two solutes in your final product. We will determine if your synthesis was complete (one spot on the TLC plate that is acetylsalicylic acid) or not complete (two spots on the TLC plate, suggesting both acetylsalicylic acid and salicylic acid are present). You will also analyze a sample of pure salicylic acid, aspirin, caffeine (which is often found in over-the-counter pain relievers), and one over-the-counter pain reliever.

PROCEDURE

Weigh... watch glass/filter paper/product from last week and record in your lab notebook.

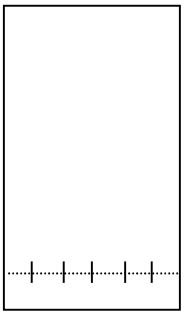
Preparation of samples.

Obtain the following compounds. In a small beaker dissolve ~5 mg (half the size of a match head) of each chemical compound in ~2 mL of methanol. Each compound is placed in a separate small test tube.

1) Salicylic acid
2) Acetylsalicylic acid (aka aspirin)
3) Your synthesized sample
4) Caffeine
5) An over-the counter-pain reliever.

Preparation of TLC plate.

Obtain a 10 x 5 cm TLC plate (touch only on the edges—don't touch the white surface) and *lightly* draw a line with a pencil (do not disturb the silica layer) about 2.0 cm from the end (as shown below). Draw 5 hash marks (starting ~1 cm in from the edge) that are ~1 cm apart. Label the marks 1, 2, 3, 4, 5; these numbers correspond to the compounds listed above. In addition, put your initials on the top edge of the TCL plate.

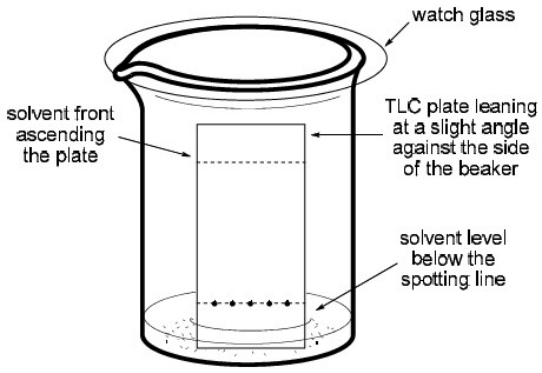


"Spotting" the plate.

"Spotters" have been made for us by the our lab manager…thanks Steve! Take a spotter and break it in half (***safety glasses are optional at this point since the small fragments of glass that may be produced while breaking the spotter are easily removed from your eyes…NOT!***). Dip the tip (small end) into one of your samples and then touch the tip to the correct hash mark on the plate to deposit the sample. Let the solvent evaporate for a few seconds and repeat the procedure until all of the sample has been deposited. Repeat the procedure with the other samples.

"Developing" the TLC plates.

Make a TLC chamber using a 400 mL beaker. Place a small amount (~10 mL) of prepared solvent (120 parts toluene, 60 parts diethyl ether, 20 parts glacial acetic, acid and 1 part methanol) in the bottom of the beaker. Place your TLC plate in the beaker (***make sure the pencil line and spots are above the solvent***) and cover the beaker with a watch glass. The mobile phase will start to move slowly up the plate. When the solvent front gets close (~1cm) to the top of the plate, remove the plate from the beaker, and place in on a paper towel in the hood. Draw a line with a pencil to mark the solvent front and let the TLC plate dry ***in the hood***.



Viewing the TLC plate.

Place the plate under the UV light and turn the light on. ***Caution: UV light is harmful to the eyes and skin: Do not look directly at the light.*** Lightly mark the spots with a pencil and note the color of each of the spots in your lab notebook. Return to your lab bench, sketch a picture of the TLC plate in your lab notebook, and measure the distance to the solvent front and each individual spot moved. Make a table in your lab notebook to contain all of this information. Calculate the R_f value of each compound. Indicate in your lab notebook the compounds that were in each of the individual spots. Comment on the purity of your synthesized aspirin sample.