EXPERIMENT 8

THIN LAYER CHROMATOGRAPHY AND MELTING POINT DETERMINATION: DETECTION OF CAFFEINE IN VARIOUS SAMPLES

Additional Resources

http://orgchem.colorado.edu/hndbksupport/TLC/TLC.html
http://coffeefaq.com/caffaq.html

Materials Needed

TLC equipment: 1 5-cm x 8-cm TLC plate, 2 capillary micropipettes, TLC tank and lid, ruler, UV lamp
Extraction Equipment: spatula, 2 small test tubes, Pasteur pipet and bulb
Chemicals: caffeine, ethanol, dichloromethane, TLC solvent (5% acetic acid in ethyl acetate)
Meltemp apparatus and capillary tube
Three of the following samples for analysis: diet cola, diet lemon-lime soda, analgesic pill, instant coffee, instant iced tea

Purpose

In this experiment, thin-layer chromatography (TLC), a standard technique for the qualitative analysis of mixtures, will be introduced. First, a sample of caffeine will be authenticated by measuring its melting point. The authentic caffeine will then be used as a standard for the TLC analysis one of the following items: a diet soda, tealeaves, instant coffee, cocoa powder, or an analgesic pill. The caffeine present in these substances must first be separated from dissimilar materials in the substance using a process called extraction. Extraction simply involves treating the substance with an organic solvent that will dissolve out the caffeine and other soluble organic materials but leave insoluble matter (such as cellulose in leaves and inorganic binders in a pill) behind.

Background

Caffeine. Caffeine is a heterocyclic (i.e., has atoms other than carbon in a ring) amine found in many plant materials including tealeaves, coffee beans, kola nuts, cocoa beans, and guarana seeds. Compounds found in nature are referred to as "natural products" and amine natural products are called alkaloids. Hence, caffeine is an alkaloid. Many alkaloids, including caffeine, have pharmacological effects. Some other examples are morphine, codeine, cocaine, and nicotine.

Caffeine-containing beverages, mainly tea and coffee, have been used for their stimulant effect for over a thousand years. It was only recently that caffeine became available to consumers in essentially pure form (No-Doz). Pure caffeine was first isolated from coffee beans in 1821 by Pierre Robiquet.

Caffeine is also a diuretic and a vasoconstrictor (constricts blood vessels). Recognition of the vasoconstriction effect suggested caffeine's use as a treatment for headaches especially migraines. (Although it has recently been argued by some that it is caffeine withdrawal that is the root cause of the great majority of migraines.) In fact, it is often added to headache remedies, including Excedrin and other OTC analgesics and is also a key ingredient in the new prescription drug, Fioret.

Caffeine's side effects and addictive nature lead many people to use decaffeinated coffee, cola, etc. The caffeine can be removed from coffee beans by extracting the beans with an organic solvent (the old method) or with supercritical carbon dioxide. The caffeine so removed can then be isolated and purified and used in other products. In fact, decaffeination of coffee beans is the primary source of pure caffeine used in stimulant pills (No-Doz), headache medications, and non-cola caffeinated soft drinks such as Mountain Dew.

Thin-Layer Chromatography. In a TLC experiment, a small spot of the sample to be analyzed is made at one end of a glass or plastic plate that has been coated with a thin layer of silica gel ($SiO_2(s)$). In a process known as "**development**" the plate is then immersed spot-end-down in a pool of solvent (the exact solvent used depends on the sample and is determined by experimentation). The solvent is allowed to move up the plate by capillary action (the silica gel "soaks it up"). Compounds present in the sample are carried up the plate by the solvent. However, different compounds generally move at different rates. Therefore, if the sample is a mixture of compounds it will separate into a series of spots at varying distances up the plate (Figure 1). If the sample is pure (i.e., only a single compound is present) then only one spot will result.

If the compounds in the sample are colorless then the spots will be hard, if not impossible, to see against the white background of the silica gel and a process for "**visualizing**" them must be used. A UV light source can be used for this purpose if the silica gel contains a small amount of a fluorescent substance. (The commercial TLC plates used in this experiment have silica gel to which the compound, fluorescein, has been added). Under UV light the spots will show up as dark spots against a bright background.

How far a particular compound moves from the original spot depends on the rate at which the solvent moves it. The fastest the solvent can move the compound is the same rate at which the solvent moves. In this case the compound forms a spot at the same distance from the original spot as the distance the solvent was allowed to move (the spot's R_f value = 1.0, see below). At the other extreme, a compound may be moved so slowly by the solvent that it's spot remains where the original spot was placed ($R_f = 0.0$, see below). By measuring the distance its spot moves and the distance the solvent moves we can quantify the rate of migration of any compound using the ratio referred to as the R_f value. The R_f

$$R_f = \frac{distance\ spot\ moves}{distance\ solvent\ moves}$$

value of a particular compound is an identifying characteristic of the compound just like its boiling point and melting points are. Therefore, spots with identical R_f values in two different samples can reasonably be concluded to indicate the presence of the same compound in both samples. For example, if TLC analysis of an unknown sample gave two spots with R_f values of 0.22, 0.57, and 0.77 (as in Figure 1.b) then the sample can be concluded to consist of a mixture of three compounds. Further, if TLC analysis of a known sample of pure caffeine resulted in a spot with a R_f value of 0.23, then one may reasonably conclude that one of the compounds present in the unknown sample is caffeine. (The small difference between the two values, 0.22 and 0.23, is ascribed to unavoidable random error in the measurement of R_f values, which at best are accurate to no more than ±0.02.)

Laboratory Procedures

Melting Point Procedure. Use the capillary melting point technique learned in Experiment #2 to obtain the mp range of pure caffeine. Looking up the literature mp of caffeine prior to measuring the mp will give you an idea of the approximate temperature to be expected. Carefully note your observations on what the sample looks like as it heats up and then melts.

Each group should extract and analyze one instant beverage sample, one analgesic tablet, and one liquid pop sample.

Extraction Procedures

1   **Solid samples – instant beverages and analgesic pills.** Use 500 mg of the sample. Make sure to record the actual mass used. If a solid sample is not a fine powder to begin with, then you will need to grind it to a powder, preferably by using a mortar and pestle. Put each powder into a small, labeled test tube. Add 2.0 mL ethanol to each test tube and shake to mix thoroughly. Heat the tubes **gently** on a hot water bath for 2-3 minutes and then allow the contents to cool and settle. Not all of the powder will dissolve; the inorganic buffering agent (if present) and binder are insoluble in ethanol. However, any organic compounds present will dissolve. Remove the ethanol solution from undissolved solids using a pipet. Use this solution to spot the TLC plate.

2.   **Liquid samples.** Measure 4 mL of the liquid into a large test tube. Wear gloves during the rest of this step. Add 5 mL of CH_2Cl_2 to the tube. Cap the tube with a tight fitting rubber sleeve stopper or cork and shake gently at first, frequently removing the stopper to allow venting of any pressure build up. Shake well then allow the layers to separate. Caffeine is much more soluble in CH_2Cl_2 than in H_2O so any caffeine present goes into the CH_2Cl_2 layer. Use a Pasteur pipet to

transfer most of the lower CH_2Cl_2 layer to a small test tube. Add a few $CaCl_2$ pellets to this tube and let stand for 5-10 minutes – the solution should clear up as the $CaCl_2$ absorbs any remaining water ("dries the solution"). Use this solution to spot the TLC plate.

TLC Procedures

3. Put approximately 25 mg of caffeine in a small, labeled test tube. Add 4.0 mL of ethanol mix, and heat gently to dissolve the caffeine.

4. Obtain a TLC plate. The plate should be handled using forceps so as to avoid contamination. A light pencil line should be drawn using a straight edge about 1 cm from one end of the plate.

5. The instructor will demonstrate the spotting technique. Use a capillary micropipette to make a small spot of the pure caffeine solution (made in step 3) on the plate. Place the spot 1 cm from the left edge along the pencil line you drew. Also spot the TLC plate with each of the solutions prepared in steps 1 and 2. Make these thre spots at 1-cm intervals to the right of the caffeine spot. In order to avoid confusion make sure to label the spots lightly in pencil bellow the line.

6. Develop the TLC plate by placing it in a beaker that has been filled with developing solvent (5% acetic acid in ethyl acetate) to a level of **less than 1 cm** high (the spot on the TLC plate should be **above** the level of the solvent). Cover the beaker with aluminum foil immediately after the TLC plate is immersed. Allow the solvent to migrate up the TLC plate until it is about one centimeter from the top. **Do not allow the solvent line to reach the top of the plate.**

7. Remove the TLC plate and mark the level to which the solvent rose with a pencil. Allow the solvent to evaporate off of the plate in the hood and then visualize the plate under UV light. Outline all spots with a pencil.

8. Measure the distance the solvent moved as well as the distances of all spots. Carefully sketch the TLC plate in the space provided on the report sheet.

Post-Lab Calculations

Calculate the R_f of all of the spots. (Remember the R_f is simply the distance the spot moved divided by the distance the solvent moved (see Fig 1).

Figure 1. Calculating the R_f of Spots in TLC (a) before development (b) after development.
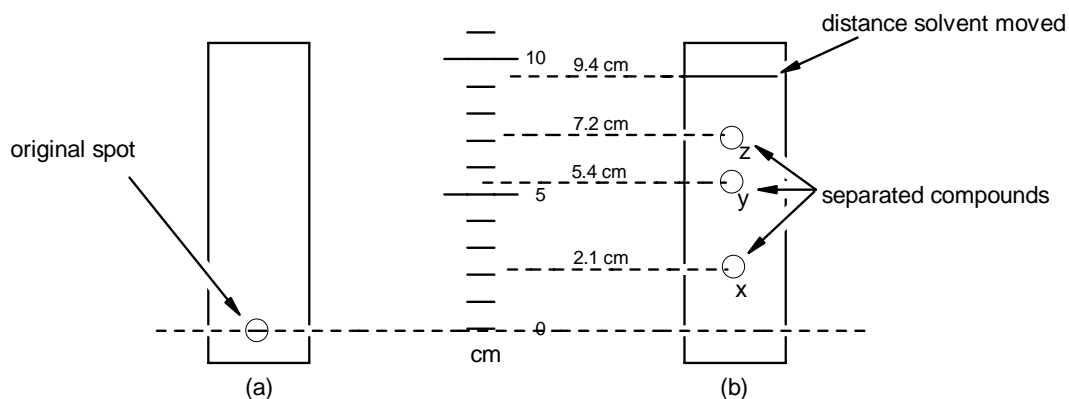
R_f of compound x = 2.1/9.4 = 0.22

R_f of compound y = 5.4/9.4 = 0.57

R_f of compound y = 7.2/9.4 = 0.77

EXPERIMENT 8

THIN LAYER CHROMATOGRAPHY AND MELTING POINT DETERMINATION: DETECTION OF CAFFEINE IN VARIOUS SAMPLES

PRE-LABORATORY QUESTIONS

Name _____ **Section** _____ **Date**_____

1. Give the structure of caffeine in the space below. Explain why it is considered an amine.

2. Give the structure of the other organic compounds being used as solvents in this experiment: acetic acid, ethyl acetate, ethanol, and dichloromethane. Identify the functional group family to which each of these belongs.

EXPERIMENT 8. TLC AND MELTING POINT DETERMINATION: DETECTION OF CAFFEINE IN VARIOUS SAMPLES

IN-LAB OBSERVATIONS/DATA

Name _____ **Section** _____ **Date** _____

Team Name/Partners _____

Pure Caffeine

General Observations _____

Melting point range _____Literature mp _____ Reference_____

Observations on melting process_____

_____

Samples analyzed data and observations

Sample #1(instant beverage) _____

Sample #2 (liquid soda) _____

Sample #3 (analgesic pill) _____

Amounts taken : Caffeine _____ Sample 1 _____ Sample 2 _____ Sample 3 _____

Observations on solutions used to spot TLC plate:

Caffeine _____

Sample #1 _____

Sample# 2_____

Sample# 3_____

Observations on TLC _____

_____

_____

_____

_____

_____

TLC Results

Distances moved:
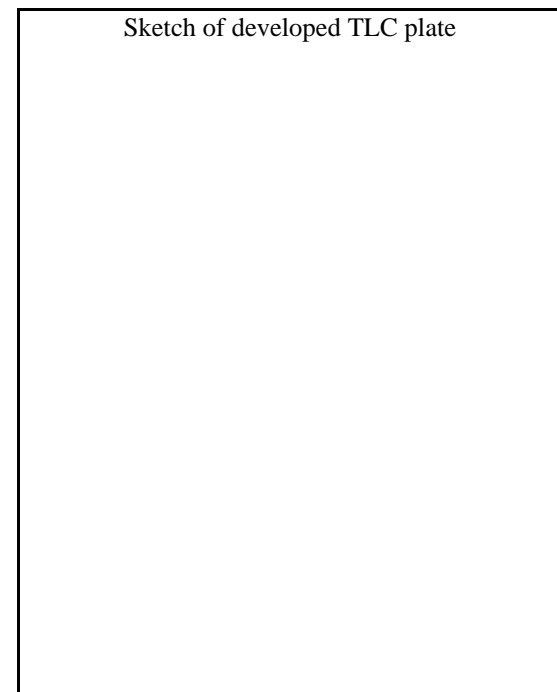
solvent   _____

caffeine   _____

spots in sample#1   _____

                                       _____

                                       _____

spots in sample#2   _____

                                       _____

                                       _____

spots in sample#3   _____

                                       _____

                                       _____

Sketch of developed TLC plate

The sketch should be as realistic as possible. It should be done to scale and not only show the positions of the spot but their sizes and relative darkness as well.

Other Notes:

EXPERIMENT 8. DETECTION OF CAFFEINE IN VARIOUS SAMPLES
REPORT SHEET

I. Melting Point of Caffeine

Mp (˚C) _____ Literature mp (˚C) _____ Reference_____

II. TLC Results Table

Solution	R_f value(s)	Approximate Relative Darkness and Size of Spot (compared to caffeine standard)
Caffeine		
Sample 1: _____	1. 2. 3.	
Sample 2: _____	1. 2 3.	
Sample 3: _____	1. 2 3.	

Questions (Type or neatly write in pen on a separate sheet and attach to this report.)

    (1) How well does the measured melting point of caffeine agree with the literature value? Discuss the accuracy of the measurement and identify some sources of experimental error in it. Does the measured mp prove that the sample was truly caffeine, (Be careful…"prove" is a strong word!)

    (2) For which of the samples was caffeine detected? Explain. How sure can you be that these samples in actual fact do really contain caffeine?

    (3) For each sample estimate the amount of caffeine present. (Compare the darkness of the spot to the darkness of the caffeine spot. You know how much caffeine was in the pure caffeine sample so you can use that amount as a basis for the comparison.)

    (4) For the samples in which caffeine was not detected can you state categorically that absolutely no caffeine was present? Explain.

    (5) If one of your samples contained additional spots with R_fs not corresponding to caffeine's R_f value then do some research and come up with some possible identities for these spots.