CHEM 333: Lab Experiment 6: INTRODUCTION TO CHROMATOGRAPHY;

Thin Layer and High Performance Liquid Chromatography:

Pre-lab Reading Chapters 7, 8 and 9

> "*This lab can actually be kind of fun (in a geeky sort of way). The techniques you will use this week are "analytical"and you will be able to make actual determinations of caffeine levels in beverages. Next week you will learn to perform chromatography on a preparative (though still small) scale."*

Liquid Chromatography

Liquid Chromatography is a powerful technique to separate and purify organic compounds. This week's laboratory deals with Thin Layer Chromatography (TLC) and High Pressure Liquid Chromatography (HPLC), which are used as analytical tools to analyze samples. In next week's laboratory you will use column chromatography. Column chromatography is a preparative method used to isolate or purify larger amounts of material (from milligrams to kilograms).

> After reading the introductory information in the text, it is very important that you understand chromatography's relationship to the polarity of organic compounds. Be able to recognize polar functional groups, especially those that can form hydrogen bonds with the solid phase (*i.e.* 1° and 2° amines, alcohols, and especially carboxylic acids). Make note of Table 7.1 for its ordering of solvent polarities.

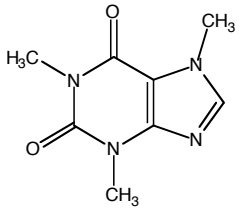
High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (or High Pressure Liquid Chromatography) is a very sensitive analytical technique. It has the capacity to be employed as both an analytical (milligrams) and preparative (hundreds of milligrams) tool for separating mixtures of closely eluting compounds. The chromatogram of materials separated can also be integrated to give quantitative data on the amounts present in the original mixture (given proper calibration). Two types of detectors are widely used for monitoring separation. An absorbance detector measures the absorbance of a particular wavelength of ultraviolet light, usually tuned for aromatic compounds. The alternative, is a detector that monitors changes in the refractive

index of the solvent being eluted. This allows for detection of compounds that may be transparent to UV radiation.

A variation of standard chromatography frequently appears with HPLC: the use of "reverse-phase" columns. The solid support in a reverse-phase column is coated with a greasy, non-polar substance (usually a long-chain hydrocarbon mix tethered to the support through a silicon linkage). In this way, the polarity rules are more or less reversed: non-polar compounds get bound to the column and move slowly, while polar compounds move more quickly with the solvent. More polar solvent mixtures (often aqueous) are usually employed.

Chromatography Part I. The purpose of this experiment is to isolate caffeine from tea



Caffeine

There are three steps: 1. Extraction, 2. Sample Preparation, 3. analysis by HPLC.

Procedure:

1) You will be provided with tea (40 mL). Add 15g of sodium chloride and 0.5g of calcium hydroxide to precipitate tannins
2) Use suction filtration to filter the latter solution through Celite (enough to cover the Büchner funnel) and filter paper
3) Transfer the filtrate from step 6) to a separatory funnel and extract 3 times with 10 mL portions of 1-propanol. Perform the extractions carefully inside the hood.
4) Combine the propanol extracts and transfer it into a round bottom flask. Rotovap the solution to dryness (set the bath temperature at ~60-70°C). After evaporating all the solvent off you should get a yellowish solid.
5) Rinse the solid off of the round bottom flask with two 5 mL portions of acetone to extract the pure caffeine. (You should get a heterogeneous solution at this point)
6) Filter the solution from step 5 using a Büchner funnel and transfer the solution into a small beaker.

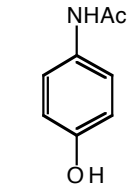
7) Rotovap the acetone from the latter solution until you see a white solid (which would be the caffeine)
8) Analyze your caffeine using thin layer chromatography (TLC) and compare it to a standard caffeine solution that will be provided to you. For the TLC eluent, use BuOH: ethyl acetate: ether in a 2:9:9 ratio. (Your TA should explain you how to run a TLC). Draw your TLC plate in your lab-notebook and record the Rfs for the standard and your caffeine product
9) Analyze your product by HPLC (Your TA should assist you for doing this part of the lab)

Chromatography Part II. Thin Layer Chromatography (TLC) analysis of analgesics

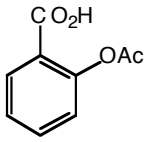
Your text has a good discussion of thin layer chromatography. Note the definition of the **R_f value**. Note that R_f is unit-less, unlike the retention *times* used for GC or HPLC. The most common uses of TLC are monitoring reactions, *i.e.* the disappearance of starting material and appearance of product(s), and as a companion to column chromatography (where fractions eluted from a column are monitored for the presence of compounds. TLC will be employed for both these purposes in future labs!

Procedure:

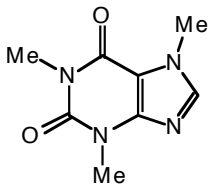
You will be given plastic TLC plates with silica adsorbent (containing a fluorescent dye). Your T.A. will show how to prepare your spotters (the most difficult part of TLC for novices). Note that if you are careful to rinse your spotter between samples, it is not necessary to have a *new* one each time: two or three good spotters should be enough. The solvent system we will be using is butanol–ethyl acetate–ether (2:9:9). You will be given an unknown sample, a chloroform solution containing two of the three analgesic compounds shown below. This sample will be analyzed by TLC.



Acetaminophen



Aspirin



Caffeine

For these known compounds, share the materials with your bench mate. Place approximately 10 mg of each of the references in the wells of the depression plates provided, and dissolve the

samples in a few drops of chloroform. Also, make up a small amount of a mixture of all three compounds. Be sure to keep your samples labeled!

Spot your first TLC plate as shown in Fig. 8.2 on page 74. These plates are easy to mark with a pencil. A light mark at either side about 1 cm from the bottom of the plate should do to mark the origin; if you wish you can make light scratches at the bottom to mark your "lanes." Fill your spotter with the desired solution, then carefully touch it to the TLC plate at the origin as shown. (You may wish to make a few practice spots on your plate: simply use chloroform solvent, and spot at the top of the plate where your marks will not interfere with the elution). Spot carefully to keep the spots as small as possible. Do <u>not</u> put down the entire contents of your spotter at once. Rather, spot, allow it to dry, and spot again at the same place. Repeat this several times. You may need to refill your spotter and spot again in the same place to ensure that you have sufficient sample on the plate. To prepare your spotter for the next solution, rinse the spotter with 2 or 3 dips in chloroform (spot on a paper towel to clear). Then continue with the next sample. If your spotter clogs, break off the end and try again, or use a new one.

Elute your plate in the jars provided. Put enough eluting solvent in the jar to cover the bottom to a depth of 5–6 mm. Make certain that it is not so deep as to cover the origin on your plate! To keep elution even, place a filter paper around the inside of the jar, dipped in the solvent, to help saturate the jar with solvent vapors. The plate will take a few minutes to develop. When the solvent is within 1 cm from the top, remove the plate and mark lightly to indicate the solvent boundary. Examine the chromatogram under a UV lamp and sketch the appearance of the plate in your notebook, indicating the location and approximate size of the spots and any distinctive colors. After this examination, place the plate in an iodine developing chamber for about 30 seconds, then remove and again record the appearance. Identify the spots in the chromatogram, including the spots in the lanes of your unknowns and measure the R_f of the major component in each sample.