Experimental Protocol

Part 1: Prepare the Chromatogram

A. Preparation of the Mobile Phase

1. Carry your chromatography jar over to one of the fume hoods and add 20 ml of the mobile phase into your chromatography jar. The mobile phase for today's experiment is 70% 1-propanol. 1-propanol is a primary alcohol with the empirical formula $\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}$. It is fully miscible in water and all common solvents.

2. Place the jar at a convenient spot on the lab bench surrounding the room. You should place it in a position that will allow you to remove the lid and work with the jar without moving the jar. Once you have positioned the jar, cover it with the over-sized petri dish cover.

B. Preparation of the Stationary Phase

1. Obtain one sheet of chromatography paper from the instructor's bench. The paper measures 15 cm (h) x 20 cm (w). With a pencil, write your initials in the upper right hand corner of the paper.

2. Using a plastic ruler, draw a light pencil line across the paper 15 mm from the bottom edge, then place 9 small pencil dots at 2 cm intervals along this line (see figure).
C. Collect Samples of 3 Amino Acids.

Each lab group is assigned 3 amino acids to analyze (see appendix following this protocol).

1. Using a wax pencil, label depressions of a spot plate A - C.

2. Using the pasteur pipets that come with each amino acid, half-fill each of the labeled depressions with the amino acids your are assigned.

3. Label a fourth depression D and half-fill depression D with water.

D. Apply samples of each Amino Acid to the Chromatography Paper.

1. Using a capillary pipet calibrated for 2 μl, apply 2 μl samples of your first amino acid (amino acid A) to each of the first three pencil dots. Clean the pipette by pulling distilled water from depression D into the pipet several times. Expel the rinse water onto a piece of filter paper.

2. With your cleaned pipette, apply 2 μl samples of your second amino acid (amino acid B) to each of the second three pencil dots, then clean the pipette with water as you did above.

3. Now apply 2 μl samples of your third amino acid (amino acid C) to each of the last three pencil dots. Clean the pipette a final time, then put it away.

4. Allow the samples to dry.

Part 2: Run the Chromatogram

1. Gently roll the paper into a cylinder (sample side out), then loosely staple the ends together.

2. With the spots towards the bottom and without moving your chromatography jar, gently and in one smooth motion place the chromatogram cylinder into the middle of your chromatography jar containing the mobile phase. Cover immediately and do not disturb.

3. Ideally, we would like to give the nonpolar solvent time to rise to within about 10 mm (½”) from the top of the paper. However, we only have about 60 - 70 minutes to allow the chromatogram to develop. This will be enough time for the mobile phase to migrate to within about 7 – 7.5 cm from the top of the paper. This is the best we could do.
4. When the solvent front has migrated to within about 7 – 7.5 cm from the top of the paper, carefully remove the paper from the jar **without moving the jar**, immediately remove the staples and accurately trace the solvent front lightly with a pencil. (Once the solvent dries, your trace will be the only way of knowing exactly where the solvent front was.)

**Part 3. Dry and Stain the Chromatogram**

1. Hang the chromatogram under the hood and let the chromatogram dry for 10 minutes.

2. Using a large pair of forceps, grasp the dried chromatogram at its edge and dip it into the ninhydrin staining solution, remove it quickly, let it drip back into the staining solution, then re-hang the chromatogram under the hood.

3. Allow the chromatogram to dry under the hood for 2 minutes, then place it in a 105 degree oven. As the chromatogram heats in the oven, the amino acid spots will begin to appear. When the stain is fully developed, remove it from the oven.

**Part 4. Collect the Raw Data**

**A. The Microsoft Excel® Worksheet**

Before you begin, open the Microsoft Excel® Worksheet that goes with this experiment and make sure you have it saved on your computer. Read the instructions carefully. Go to the tab labeled **My Raw Data**. Table #1 contains cells into which you will enter your raw data. There are also cells reserved for a variety of calculations, such as the Rf of each sample and the Mean Rf and standard deviation for each amino acid. The table is NOT programmed for you. You will need to program these cells and calculate these numbers before you leave lab today. Your instructor will provide you with instructions on how you can program the cells to make these necessary calculations.

Tips:

- to calculate Mean: =AVERAGE()
- to calculate Standard Deviation: =STDEV.S() or =STDEV()

When you are ready, proceed to collect and record your raw data.

**B. Collect and Record the Raw Data**

1. Accurately trace the outer edge of each amino acid spot with a pencil line. Then, estimate the center of each spot and mark it with a pencil point.
2. For each amino acid sample applied to the chromatography paper, collect two pieces of raw data and record the data in Table 1:

a) Measure the distance the sample migrated from the origin in mm \(D_{sample}\). To do this, measure from the origin to the center of the spot.

b) Measure the distance the mobile solvent migrated from the origin at that point in mm \(D_{solvent}\). Make a separate measurement of \(D_{solvent}\) for each amino acid.

As you enter the data, the Rfs, Means and standard deviations will be calculated for you.

C. Compiling the Class Data

Now that you have collected, recorded and saved your data, your instructor will instruct you to transfer your mean Rf for each of your 3 amino acids in the pooled class data table at the instructor’s computer. Your instructor will then compile the data from all groups in your section to create a hydrophobicity scale of 18 amino acids that will be shared with you. Once the data are pooled, return to your Excel worksheet and click on the tab labelled Section Hydrophobicity Scale. Follow the instructions on the worksheet to record your class hydrophobicity scale and compare it to the scales reported by others.

D. Save the Excel Worksheet

When all of the data have been collected & recorded, save the Microsoft Excel© worksheet to your computer. When you save the file, it is recommended you change the filename to YourName.CellBiologyOLM_Lab02_2011.xlsx

E. Homework Assignment

Your instructor will likely follow today’s work with a homework assignment. Make sure you understand the assignment before you leave lab today.

F. Clean Up

Once you saved the Excel table to you computer and entered your data in the instructor’s computer, today’s experiment is complete. Before you shut down, you should make sure the Excel file is saved to your computer. You can logout of the lab manual. Before you leave you must clean up your place so it looks the way it did when you walked into lab today.