PAPER CHROMATOGRAPHY OF AMINO ACIDS LAB



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1: PAPER CHROMATOGRAPHY OF AMINO ACIDS

1.1: Paper Chromatography of Amino Acids Lab Procedure

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1.3: Paper Chromatography of Amino Acids (Report)

Adapted from

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1.1: Paper Chromatography of Amino Acids Lab Procedure

Learning Objectives

- To use paper chromatography to identify amino acids.
- To identify components of unknown amino acid sample by calculating Rf values

Background

Chromatography is a collective term for a set of analytical techniques used to separate mixtures. Chroma means color and graph means to write or draw. Paper chromatography is an analytical technique used to separate mixtures of chemicals (sometimes colored pigments) using a partitioning method. The paper in this method is called the stationary phase because it does not move and serves as a substrate or surface for the separation. Analytes (substances being analyzed) are separated from each other based on a differential affinity to a solvent. The solvent dissolves and carries the analytes along the matrix of the stationary phase. Since the solvent moves through a wicking action, it is called the mobile phase.

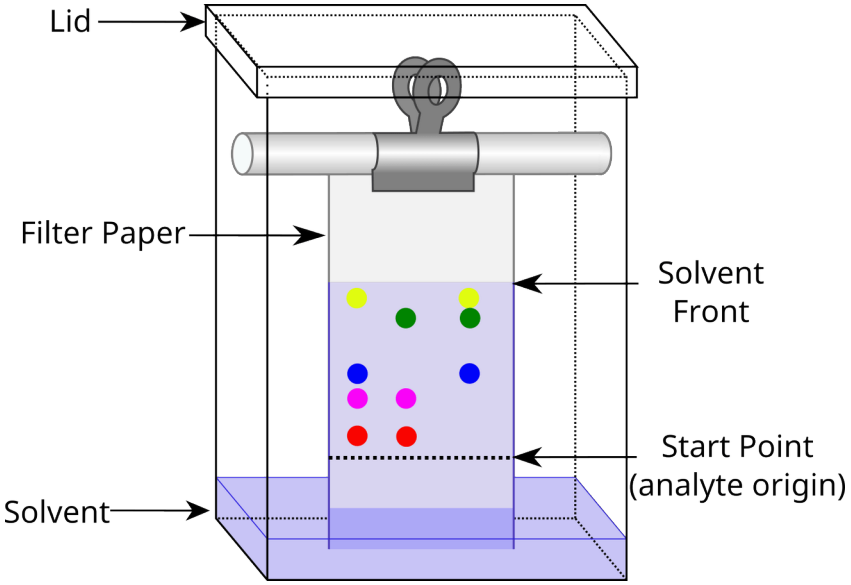


Figure 1.1.1: Credit: Theresa Knott [CC BY-SA 3.0 or GFDL]

The figure above shows a chamber containing mobile phase and a prepared paper stationary phase. A line drawn at the bottom edge of the paper is the starting point/line. The starting line and sample dots must be above the level of the mobile phase when the paper is placed inside the chamber. If the starting line is below the liquid level, the samples will wash out into the mobile phase rather than elute up the stationary phase. Another line is drawn about 10 cm above the bottom edge of the paper. This is the finish line. Its location was chosen for this experiment because when the eluting solution reaches that line, any inks that are mixtures should be clearly separated.

Note

When the solvent front reaches the finish line, the paper should be removed immediately from contact with the mobile phase.

The figure below shows a typical paper chromatogram of the separation of pen inks. There are a few difficulties commonly encountered in the elution process. One problem is that spots tend the spread out as they elute, and can bleed into each other as they proceed up the paper. This can be confusing when interpreting the chromatogram. To avoid this problem, space the spots

of sample far apart and make repeated, tiny applications of sample to prevent spreading. Another problem is an uneven solvent front. This can happen if the beaker is nudged – if the mobile phase sloshes inside, the elution trails may travel diagonally, which makes interpretation very difficult. This can also happen if the two edges of the chromatogram are allowed to touch when they are stapled or taped together to form a cylinder.

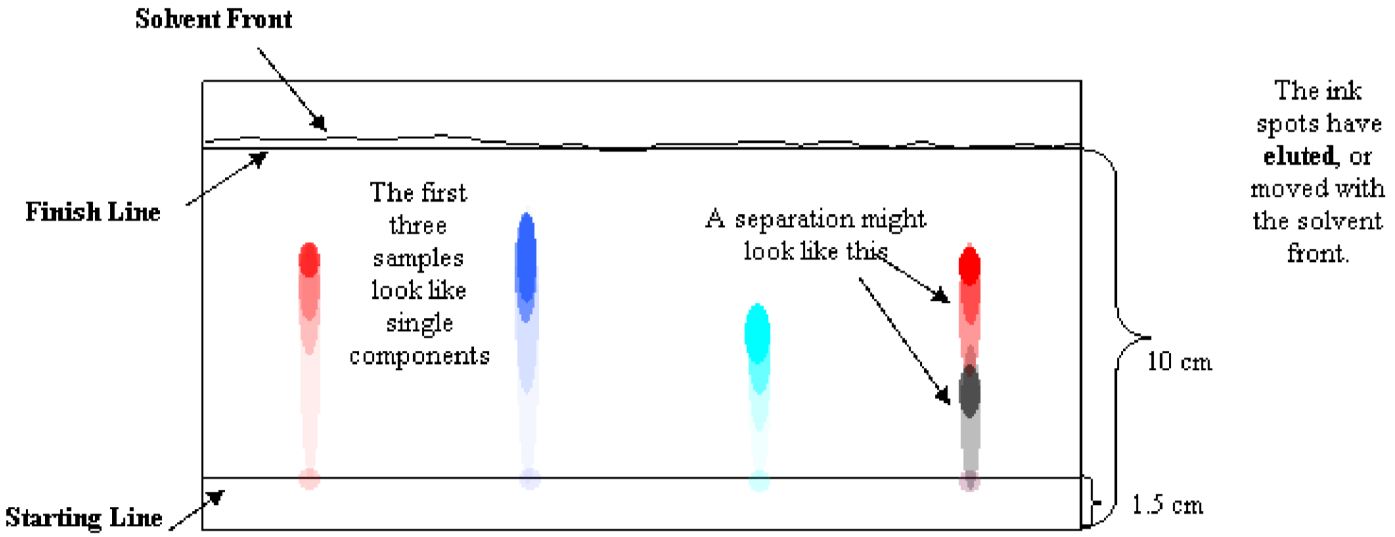


Figure 1.1.2:

A component with a given solubility travels along with the mobile phase at one rate, regardless of what other components are present in the sample. If the red part of purple ink travels at the same rate as pure red ink, and both stop in the same place, the two should be the same red ink. The two red spots should have the same Retention Factor, Rf. The Rf is the distance, D, traveled by the spot divided by the distance traveled by the eluting solution, or Solvent Front, F.

$$R_f = D/F \tag{1.1.1}$$

Comparing the Rf values allows the confirmation of a component in multiple samples because unique components have unique Rf values.

Experimental Procedure

Materials and Equipment

Materials: chromatography paper, amino acids: 6 known amino acids (list will be provided in lab), unknown, eluting solution A, eluting solution B, 0.2% ninhydrin spray.

Equipment: 600-mL beaker, pencil, ruler, plastic wrap, toothpicks, hair dryer, stapler, gloves, regular printer paper, and paper towels.

Safety

Wear safety goggles at all times. Use eluting solution **only in the hood**. Do not breathe fumes from the eluting solution. Be sure to handle only the dry part of the chromatogram when removing it from the beaker. Wash hands thoroughly if the eluting solution touches your skin. Gloves are recommended when handling the chromatogram, but use good glove practices. If your gloves get contaminated - change them. Recommendation: 1 pair of gloves for preparing your chromatography paper and chamber and developing your chromatogram, then a second pair for removing from the chromatography chamber and analyzing your chromatogram.

Part A: Preparation of Chromatography Chamber

1. You will do this experiment with a partner. One of you will test one eluting solution, while one tests the other eluting solution provided.
2. Label your 600-mL beaker with your name and pour about 30 mL of your eluting solution into the beaker. Cover and seal well with plastic wrap. (Your partner will do the same with their eluting solution).
3. Allow to equilibrate in the hood.

Part B: Preparation of Chromatography Paper

1. Wash your hands thoroughly to remove excess oils from your skin. You may want to wear gloves. Obtain a ruler and a piece of chromatography paper from the fume hood. Handle the paper only on the edges to avoid leaving fingerprints, as these may hinder the elution process.
2. Place the chromatography paper on a sheet of clean notebook paper or paper towel to avoid picking up dirt or contaminants from the bench top. Orient the paper into a “landscape” position and write your name on the top edge of the paper in one corner. Using a pencil and ruler to measure accurately, draw a straight line across the paper, about 1.5 cm above the bottom edge. This is the starting line. At the top of the paper draw a straight line across the paper about 2 - 3 cm from the top, this is the finish line.
3. On the starting line, measure in from one side about 2.5 cm and lightly draw a small line centered on the starting line. Mark 8 total spots evenly along the starting line. Number them so you don't get confused and make a key in your notes.
4. In the center of each mark, make a small spot of amino acid sample in the order: the 6 known amino acids, your unknown and your partner's unknown. Each spot should be a very small dot - don't let it spread out. When the spot dries, you won't be able to see it but it will be there.
5. The same should be done for the chromatography paper your partner is setting up. Coordinate so you both get all your data for both solvents.
6. When you have finished, you should have something that looks like the chromatography paper below, except with 8 spots instead of 7.
7. Gently curl the paper into a cylinder, with the spots on the outside. Staple the ends together near the top and bottom, taking care that the two edges of the paper do not touch. If they do touch, the eluent will creep on a diagonal, and the spots will run together, or not in straight lines.

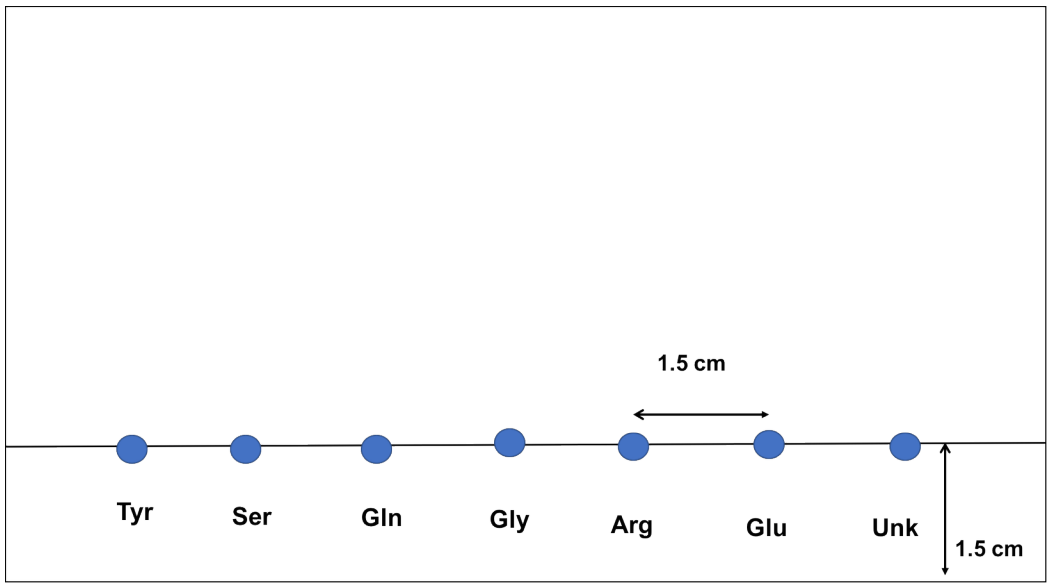


Figure 1.1.3: Prepared chromatography paper.



Figure 1.1.4: Chromatogram in solvent chamber

Part C: Acquisition of Chromatogram

1. Gently place the paper cylinder into the beaker and cover the top with the plastic wrap as shown in the picture above. Remember that the spots must be above the liquid level for the experiment to work. Watch the eluent creep up the paper until it is about 2-3 cm from the top. It will take at least 40 minutes for the solvent front to reach the finish line. Since you have a partner, you can trade off on watching the chromatogram.
2. When the solvent front reaches the finish line, remove the paper from the beaker, being careful to touch only the top (or wear gloves). Let excess eluent drip into the beaker. Gently remove the staple and lay the chromatogram on a piece of paper towel. Use a hair dryer to dry the chromatogram completely.
3. If you are working in a hood without a waste container, cover the beaker with the plastic wrap to take it across the room to the correct hood. Pour the eluting solution in the organic waste container in the fume hood.
4. Check with your instructor or authorized individual for help with spraying the chromatogram lightly with the ninhydrin solution. Dry the sprayed chromatogram with a hair dryer, distinct colored spots will appear as a result of the ninhydrin reacting with the amino acids.
5. CAUTION: Your instructor will use the ninhydrin inside the fume hood. Do not breathe the fumes or get spray on your skin.

Part D: Interpretation of Chromatogram

1. Circle around each color spot. Make sure to record any distinguishing features of each spot: color, how the amino acid spreads, etc.
2. Use a ruler and draw a line through the center of each spot (see description in lab). Measure the distance from the starting line to the middle of the spot. Record this distance (D) for each spot on your lab report. These are the D values, in cm.
3. Measure the distance between the starting line and the finish line or, the farthest up that the solvent front reached. Record this distance. This is the solvent front (F) value, in cm.
4. Make sure to do this for both chromatograms. Take a picture of both chromatograms to submit with your report.
5. Calculate the retention factor (Rf) for each spot and record the values in your lab report.
6. You and your lab partner will each complete your own report.

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1.2: Paper Chromatography of Amino Acids Pre-lab Assignment

Pre-Lab

1. Write the pre-lab as per usual, based on the instructions.
2. Make sure to include a clearly labeled table for the 6 known amino acids and the 2 unknown amino acids (yours and your partners). You will need 2 of these tables - one for each eluting solution. Leave space to label each table, so you know which eluting solution is which!
3. Each person needs their own pre-lab.
4. You will select partners at the start of lab.

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1.3: Paper Chromatography of Amino Acids (Report)

After Lab

Look up the structure of each of the amino acids we used in lab. You will need this to be able to determine its relative polarity.

Calculations

Show calculation of Rf for all spots with proper significant figures and units. Organize your calculated values into a clearly labeled table for each chromatogram, so it is easy to interpret the results.

Conclusions

Make sure your conclusion includes all information below. DO NOT write your conclusion as bullet points, but as sentences and paragraphs addressing these questions, based on your results:

1. Make sure to clearly identify your unknown amino acid, along with the unknown number or other provided identifier.
2. Justify your identification of your unknown, using the data gathered in lab.
3. Are any of the Rf values the same (or similar, within 0.1)? What would the same or similar Rf values indicate? How can you tell between the substances?
4. Which amino acid had the highest Rf value? What does this indicate about that component's attraction to the mobile phase? To the stationary phase?
5. Which of the amino acids tested had the lowest Rf value? What does this indicate about that component's attraction to the mobile phase? To the stationary phase?
6. Based on your results, clearly rank the amino acids tested in order of polarity (which one is least polar? which is most polar?). Support your ranking with your results from **both** chromatograms.
7. Does the polarity order you observed agree with the known structures of these amino acids?

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