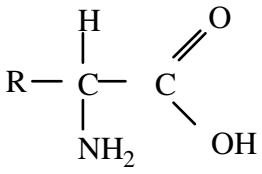
SEPARATION OF AMINO ACIDS BY PAPER CHROMATOGRAPHY

Chromatography is a convenient and useful method for the separation of mixtures and for the identification of substances. The method has been especially valuable for the separation of closely related compounds. There are many different types of chromatography, but in this experiment we will illustrate the method with the separation of amino acids by paper chromatography.

We will be using four common amino acids: arginine, glutamic acid, leucine and valine. Each has a structure, which can be represented by



where R represents the parts of the structure which are different with each amino acid. The two groups common to all amino acids are the -NH_2 group (the amino group) and the -COOH group (the carboxylic acid group).

The basic procedure in this experiment consists of applying a small drop of the solution containing the substances to be separated near one end of a strip of absorbent paper. This end of the paper is then placed into a developing solvent, which flows upward along the paper by capillary action. The degree of solubility of the components of the mixture in the solvent, as well as the degree of attraction of these components to the wet cellulose molecules in the paper fibers, will determine the distance that the solvent will carry each substance along the paper during a given time interval. Those components that are quite soluble in the developing solvent, or that have a low affinity for cellulose, will be carried the greatest distance from the origin. The finished paper, with its spots, is called **a chromatogram.**

In this experiment, distances traveled by spots of various compounds will be compared on a single sheet of paper. It is a common procedure to measure the ratio of the distance the spot moves to the total distance the solvent front moves (beyond the original location of the spot) under the conditions of the experiment. This ratio is called the R_f value and is defined as:

$$R_f = \frac{\text{distance the spot travels}}{\text{distance the solvent travels}}$$

Tables of R_f values are published for many compounds under various conditions. Distances are measured to the center of the spots. When spots of unknown compounds are placed on the same chromatogram as spots of known materials it is often sufficient, under good experimental conditions, to compare distances from the starting line for knowns and unknowns. In simple cases, this is all that is required in order to identify the unknown.

The positions of colored substances on a chromatogram are easily detected by the human eye, while a colorless substance may be made visible by treatment with a reagent that converts it to a colored compound. For example, amino acids, which are all colorless by themselves, give a blue or purple color when they react with ninhydrin. Therefore, amino acids may be detected on a chromatogram by treatment with ninhydrin reagent. Other methods of detecting colorless materials on a chromatogram include the use of ultraviolet light to detect fluorescent compounds, or the use of a Geiger counter to detect radioactive samples.

In this experiment you will carry out paper chromatography in order to understand the basic principles of this separation technique. You will calculate the R_f values for known and unknown components of your amino acid solutions and use this information to identify the components of your unknown amino acid solution.

Safety Precautions

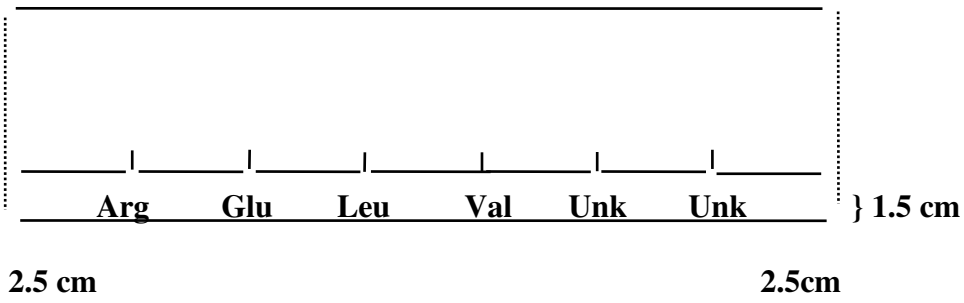
- **Wear gloves when using ninhydrin solvent**
- **Use different toothpicks for each amino acid and unknowns so that cross contamination can be avoided.**
- **Dispose of any left over developing solvent (butanol/acetic acid/water) in reclaim container provided. No solvent should be allowed to go down the drain.**
- **Butanol is a flammable liquid. Make sure there are no open flames and/or heat near the developing solvent.**

EXPERIMENTAL PROCEDURE

1. PREPARATION OF THE CHROMATOGRAPHIC PAPER

CAUTION: Wearing gloves, handle the paper (Whatman no. 1) only along the narrow edges. Use paper towels to protect the paper from the desk surface **and from your** hands. Remember that your skin contains amino acids and therefore you must avoid any contact with the part of the paper to be used in the analysis. Take a rectangle of the paper that is cut (typically about 11 cm, x 20 cm) so that the width of the paper is just a little less than the depth of an 800 mL beaker and its length is enough to permit the formation of a cylinder that will fit inside of the beaker without touching its walls.

Still wearing your gloves, make a light pencil line 1.5 cm from one long edge. Place six equally spaced pencil marks along this line, keeping the end markings about 2.5 cm from the ends. Label the first four marks with the abbreviations of the four amino acid knowns, and the remaining two spots with the unknown numbers.



Use a toothpick to put a small drop of the solution onto the paper at the appropriate mark along the line. When the solvent has evaporated, transfer another drop to the paper. Repeat this 4 or 5 times, allowing the spot to dry each time. The goal is to keep the spot under 4 mm in diameter and to make repeat applications to build up the concentration of the material without enlarging the spot appreciably. Be sure that you use toothpicks only for their designate solutions and do not mix applicators among solutions. Allow the spots to dry again.

Be sure to record in your notebook any observations you may make about the physical properties of the known and unknown amino acid solutions.

2. SETTING UP THE DEVELOPING SOLVENT

Use a clean dry 800 mL beaker and the developing solvent (butanol/acetic acid/water) from the supply in the HOOD, taking only what you need (ONLY 30 mL). Record your observations on the properties of the solvent. Note also the location of the "reclaim bottle" in the HOOD that will be used for any unused or extra solvent at the end of the experiment. NO SOLVENT SHOULD BE ALLOWED TO GO DOWN THE DRAIN. Use a glass rod to add enough solvent to the beaker to cover the bottom to about 0.5 cm in depth. Do this without splashing the sides of the beaker with the solvent.

3. RUNNING THE CHROMATOGRAPHIC SEPARATION

Coil the spotted paper into a cylinder and fasten the two short ends together with stapler. Do not overlap the ends of the paper. A gap of one or two millimeters space between paper edges is helpful. Set the paper cylinder, spotted edge down, into the beaker containing the solvent solution. The pencil line with the spots must be above the liquid level. The paper should not touch the sides of the beaker. Cover the beaker with plastic wrap and leave the paper cylinder in the beaker until the solvent has nearly reached the top edge of the paper. **NOTE: Once you set your chromatogram inside the beaker with the solvent, do not move or agitate the beaker while the solvent is flowing upward. Any slight movement of the beaker will cause the components (the spots) to shift positions.** Keep an eye on the level of the solvent in the beaker so that the

chromatogram does not go dry during the process. Record the total time for the chromatographic process.

4. ANALYSIS OF THE CHROMATOGRAM

Remove the paper from the beaker, lay it flat on a clean paper towel and immediately mark the solvent front with a pencil. Allow the paper to dry. Take the paper to the HOOD and spray it with ninhydrin solution. **(Caution: remember that this material will form colored compounds with all amino acids, including those in your skin. Wear latex gloves)** Spray the chromatogram with a fine, even layer of the ninhydrin, then dry the chromatogram under a heat lamp. While they are still visible, circle each spot. The color may fade with time.

Return the unused or extra solvent to the reclaim bottle provided in the HOOD. Do not put solvent down the drain. Discard the latex gloves in the trash bin.

ANALYSIS

1. Calculate the R_f value for each known and unknown spot.

   Remember that more than one spot may develop for each of the knowns due to the presence of isomers, and that you are expecting more than 1 spot in your unknown, which is a mixture.

2. Use the R_f values and the general appearance of the spots to identify the amino acids in your unknown.

POST LAB QUESTIONS

In addition to your pre-lab, data and observations, answer the following questions in your lab notebook.

1. What are amino acids used for in the body? Where do they come from?

2. Why don't all of the amino acids travel up the paper at the same rate?

3. Look up structural formulas for the four amino acids, which we used in this experiment and include them in your report. What is the relationship between structure and Rf value?

4. What significance is it that some side chains are polar while others are nonpolar, or that some side chains are charged while others are not?