Biological Chemistry: Separating Amino Acids

Proteins are macromolecules or polymers composed of many monomer units of amino acids. There are 20 common amino acids, nine of which are considered essential. The essential amino acids cannot be made in the body and must be supplied by the foods we eat. Each amino acid has its own unique characteristics. Some amino acids have polar R-groups, some have non-polar R-groups, others have an acidic R-group and the last group has a basic R-group. Understanding how a protein functions is tied to knowing the protein's composition and how that composition can dictate molecular structure.

The traditional method of separating amino acids uses paper chromatography. Amino acid solutions are spotted onto the chromatography paper. The spots are allowed to dry. The paper is then inserted into a chromatography chamber. The chamber contains a solvent which moves by capillarity up the paper. If the amino acid is soluble in the solvent, the amino acid dissolves in the solvent and is carried up the paper. If the amino acid is not soluble in the solvent it remains at the origin (where the amino acids were spotted on to the paper). The distance an amino acid travels is determined by the solubility of the amino acid and it size. After a period of time the paper is removed and air dried. The paper is then sprayed with an indicator (ninhydrin) and heated. Heating ninhydrin facilitates color development. Blue spots will appear to indicate the locations to which the amino acids migrated. The size of the colored spot is directly proportional to the amount of amino acids in the spot.

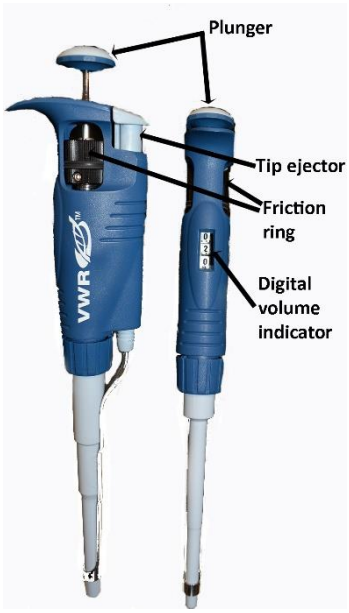
Materials

Chromatography paper
Chromatography solvent
Ruler
Stapler
Pencil
Hood (clothesline and clothespins)

Amino acid solutions (cysteine, alanine, phenylalanine, mixture or unknown amino acid)
Micropipette – 0-20 µL
Pipette tips
Ninhydrin

Procedure

How to use a micropipette



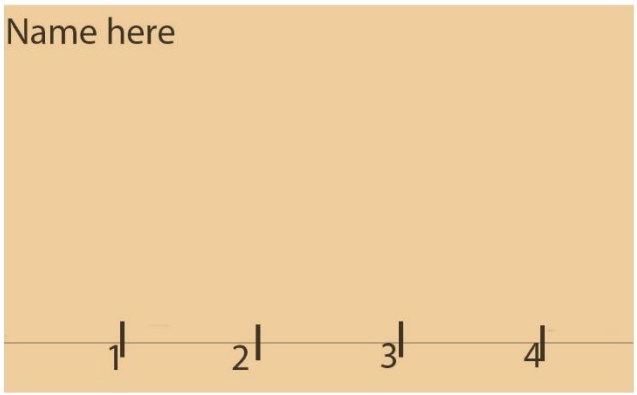
Micropipettes are used to reliably transfer very small quantities of liquid. It is important to handle these tools carefully. They are valuable and fragile. They should never be used without a pipette tip. They should never be laid on the benchtop with an attached pipette tip. Fluids in the pipette tip can run down the bore and damage the pipette

1. Add a pipette tip to the micropipette.
2. Adjust the volume to 5 µL. Volume is adjusted by rotating the black friction rings below the plunger. The volume is shown in a small window called the digital volume indicator. The volume should read 05.0.

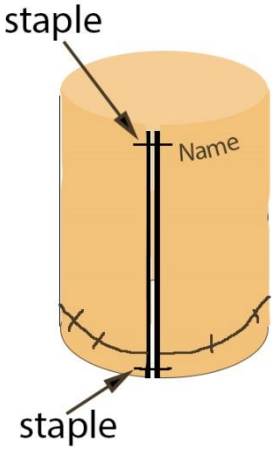
3. The plunger has 2 stop points. Test the plunger by pushing it down with your thumb. The first stop represents the volume indicated on the digital volume indicator. Release the plunger.
4. Push the plunger all the way down. This is sometimes called the blow out stop. Pushing the plunger all the way down will force all of the liquid in the pipette tip out.
5. To use the micropipette, push the plunger down to the first position. Insert the pipette tip into a solution and gently release the plunger. The micropipette has withdrawn the amount of fluid indicated on the digital volume indicator.

Separating Amino Acids

1. Obtain a sheet of chromatography paper from the supply table. Carry the paper by the edges. Skin secretes a number of organic compounds, including amino acids that can interfere with the chromatogram.
2. Place the chromatography sheet on a clean piece of paper on your bench. Use a pencil and a ruler to draw a line across the bottom of the paper approximately 1.5 cm above the bottom edge. Use the pencil to make 4 hatch lines along this line. Label the first hatch line, '1', the second '2', the third '3 and the fourth '4'. These hatch lines should be equally spaced along the line at least 3 cm apart. These are the hatch lines on which you will dispense the amino acid samples.



3. Print your name in pencil along the top of the paper (opposite to the hatch lines).
4. Use the micropipette to add 5 µL of phenylalanine to the first hatch line (hatch line labeled 1). Remove the pipette tip.
5. Add a fresh pipette tip and pipette 5 µL of cysteine to the next hatch line. Remove the pipette tip.
6. Add a fresh pipette tip and pipette 5 µL of alanine to the third hatch line. Remove the pipette tip.
7. Add a fresh pipette tip and pipette 5 µL of the amino acid mixture to the last hatch line. Remove the pipette tip.
8. Allow the paper to dry. Repeat steps 4 through 7.
9. Allow the paper to dry. Repeat steps 4 through 7 and allow the paper to dry.
10. Grasp the edges of the chromatography paper and bring the edges together but not <u>overlapping.</u> Bring the right top corner of the paper next to the left top corner and staple the edges together. Bring the left bottom corner of the paper to the right bottom corner of the paper and staple. You should have a cylinder of paper stapled at the top and bottom. The paper edges should not overlap. If they do, remove the staple and reposition the edges, and staple.



11. Place the chromatography paper in the chromatography jar with the edge marked in pencil down (bottom of the paper). The solvent should not cover the pencil line.
12. Re-cover the chromatography jar. Do not move the jar.
13. Wait until the solvent has moved at least 75% of the way up the paper (~45 minutes).

14. Remove the chromatography paper from the jar. Place the cover back on the jar. Remove the staples from the paper.
15. Hang the chromatography paper in the hood or outside as directed by your instructor.
16. Once dry, your instructor will spray the chromatograms with ninhydrin and place the papers in a drying oven to facilitate color development.
17. Remove the paper after 15-20 minutes and examine the results.

Which amino acid was most mobile (traveled the farthest from the origin)?

Which amino acid was the least mobile (stayed closest to the origin)?

Which amino acid/s was/were in the 'mixture'? How do you know?

What is the basis for the separation of amino acids using chromatography?

Ninhydrin is often shown being used by crime scene investigators on television. Why? Or how would it be useful?

Keeping in mind the separation of amino acids that you observed, was the solvent hydrophilic or hydrophobic? (You may need to research the hydrophilicity of the amino acids to answer this question.)

Draw or attach a photograph of your chromatogram below.