AMINO ACID CHROMATOGRAPHY

Solutes can be separated from solvents and identified by various chemical and physical means or a combination of both. A Russian scientist, Tswett, developed a technique to separate compounds from a solution. This technique is called chromatography. Tswett worked with plant pigments.

Chromatography is a physical process in which compounds are separated from a solution. A common feature in any chromatographic method is the use of two phases. One phase is stationary, the other is mobile. The stationary phase can be solid or liquid. The mobile phase can be liquid or gas. The mixture to be separated is distributed between the two phases. In this investigation you will use paper chromatography. **What are the two phases in this lab?**

In paper chromatography, the solution is "spotted" near the edge of a piece of filter paper and allowed to dry. The filter paper is then placed in a container of specific solvent. The spots of dried solution are placed slightly above the solvent level. The filter paper absorbs the solvent and the component parts of the dried solution will "move" upward at a specific rate in relationship to the moving solvent.

Unknown solutes from a solution or its component parts may be identified by computing the R value of the known components in the solvent systems. The Rf value is the ratio of the distance traveled by the solute to the distance traveled by the solvent. By comparing Rf values of unknown components with the Rf values of known components, an unknown substance can be identified.

In General Biology, you learned to identify proteins. In this investigation, you will identify some amino acids which are building blocks of proteins. By comparing the Rf value of **one or two** unknown amino acids with the Rf values of known amino acids, you will identify the unknown amino acids.

Materials

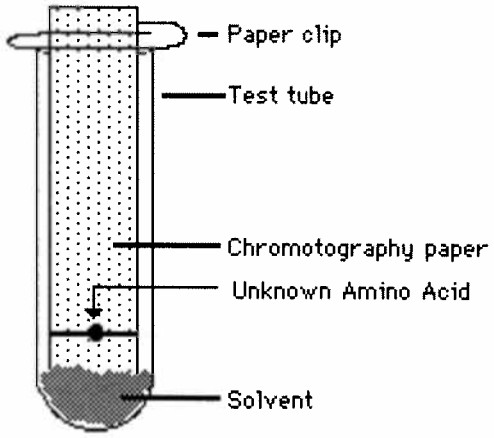
Amino acid samples, methanol **(flammable and poisonous, avoid contact with the skin)**, ninhydrin (**extremely toxic**) disposable gloves, chromatography paper strip (1.5 x 21 cm), large test tube, parafilm, paper clips, Pasteur pipette, unknown amino acid samples and a pencil.

Procedures

1. Using disposable gloves, obtain one filter paper strip chromatogram. CAUTION: Do <u>not</u> touch filter paper with your bare hands. Chemicals in sweat can interfere with your results.
2. With a pencil, draw a line 2.5 cm from the bottom of the chromatogram.
3. Dip the tip of the Pasteur pipette into one unknown amino acid solution, do **<u>not</u>** draw anything up using the bulb, capillary action will do the work.
4. Touch the end of the pipette to the middle of the pencil line drawn on the chromatogram. The amino acid solution will be transferred from the pipette to the chromatogram and will appear as a wet spot about 0.5 to 1 cm in diameter. **Make sure you do not mix up the pipettes.**
5. With a **<u>pencil</u>**, write the **letter** of the sample you used and your **initials** and **period** at the top of the chromatogram.
6. Record the letter of your in your lab notebook.
7. Using figure 1, on the **<u>top</u>** of the next page as a guide (**Do not add solvent until step 11**), using a large paper clip, fold and clip the lettered end of the chromatogram as shown.

*** **Your set-up should look just like figure 1 on the next page. However, there should be <u>no</u> solvent in the test tube!**

Figure 1



8. Place the chromatogram into the **empty** test tube.
9. Adjust the paper height on the paper clip so that the chromatogram just touches the bottom of the test tube when the paper clip supporting the chromatogram is placed across the mouth of the test tube.
10. Remove the chromatogram from the empty test tubes.
11. Pour the solvent (methanol) into each test tube to a depth of 1.5 cm.
12. **CAUTION**: DO NOT breathe fumes from solvent. **These solvents are both poisonous and flammable.**
13. If spillage occurs, rinse with water and call your teacher.
14. Place the chromatogram back into the test tubes and rest the paper clip on the mouth of the test tube. **The solvent should be about 1 cm below pencil line.**
15. Gently seal the top of each test tube with parafilm wrap.
    The seal will help keep the air inside the test tube saturated with solvent.
16. Your finished assembly should look like Figure 1.
17. Place the assembly into a flask in an **upright** position.
18. Allow the jars to sit for at least **15** minutes.
19. At the end of **15** minutes, remove the chromatogram from the jars.
20. Using a pencil, **immediately** draw a line to indicate how far the solvent has moved up the chromatogram. This line is called the leading edge of the solvent.
21. Air dry the chromatogram by moving them back and forth.
22. Using a pump spray bottle, spray the area between the leading edge and the bottom pencil line of each chromatogram with ninhydrin solution.
    CAUTION: Ninhydrin is very toxic. Do not inhale fumes. **Spray the ninhydrin solution under the fume hood.**
23. After air drying, place the chromatograms near a lighted incandescent bulb for 3 to 5 minutes. A colored spot or spots will appear on each chromatogram. The spots represent a specific amino acid.
24. Outline the spots with a pencil.
25. On the chromatogram, measure the distance from the top of the colored spot to the bottom pencil line. Also measure the distance from the leading edge of the solvent to the bottom pencil line (B on Figure 2 on the next page).

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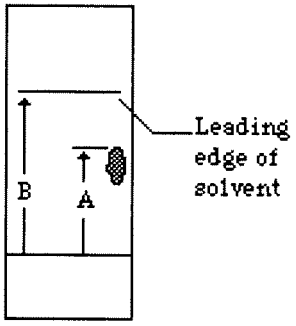
Figure 2



26. Record these distances in your data chart. Using the formula:

$$\frac{\text{Distance traveled by amino acid solution (B}}{\text{Distance traveled by the solvent (A)}} = \text{Rf}$$

Compute the Rf values for your unknown amino acids. Identify the unknowns by comparing your Rf values and the colors of your amino acids to those listed in the table below.

Physical Properties of Amino Acids

Amino Acid	Color	Rf Value
Aspartic Acid	Purple, yellow shadow	0.29
Leucine	Dark Purple	0.86
Lysine	Yellow and purple	0..67
Proline	Yellow	0.55
Serine	Purple	0.46

FORMULATING GENERALIZATIONS

1. Fill the chart below.

Unknown	Rf value	Color	Amino Acids

2. What information helped you to conclude which amino acid was on each chromatogram?
3. What was the purpose of the ninhydrin spray?
4. Suppose you saw 1 purple spot and 1 red spot 2 cm apart on your developed chromatogram. What could you conclude about the 2 spots?
5. Arginine, an amino acid with an Rf value of 0.80, appears as a dark purple spot on a developed chromatogram. If arginine were one of the unknowns used in this investigation, would you be able to identify it? **Why?**
6. Suppose arginine has a Rf value of 0.60. Could it be confused with any other unknown amino acids in this investigation? **Why?**
7. What is the practical value of chromatography to a scientist? (two functions)