

Experiment-8: Paper Chromatography for separation of Amino acids

Aim: To separate Amino acids by paper chromatography.

Principle:

Amino acids are the building blocks of peptides and proteins. They possess two functional groups: the carboxylic acid group gives the acidic character, and the amino group provides the basic character.

The R-represents the side chain that is different for each of the amino acids that are commonly found in proteins. However, all 20 amino acids have a free carboxylic acid group and a free amino (primary amine) group except proline, which has a cyclic side chain and a secondary amino group.

We use the properties provided by these groups to characterize the amino acids. The common carboxylic acid and amino groups provide the acid–base nature of the amino acids. The different side chains, and the solubilities provided by these side chains, can be utilized to identify the different amino acids by their rate of migration in paper chromatography.

In this experiment, we use paper chromatography to identify aspartame, an artificial sweetener, and its hydrolysis products from certain foods.

Materials:

1. 0.1% solutions of aspartic acid, phenylalanine, and leucine
2. 0.5% solution of aspartame (Equal)
3. Diet Coca-Cola
4. 3 M HCl
5. 0.2% ninhydrin spray
6. Butanol: acetic acid: water–solvent mixture
7. Equal sweetener
8. Aluminum foil
9. Whatman no. 1 chromatographic paper: 15x 8 cm; 12x 4 cm
10. Ruler
11. Polyethylene gloves
12. Capillary tubes open on both ends
13. Heat lamp or hair dryer
14. Drying oven, 110°C
15. Wide-mouth jar
16. Iodine crystals
17. Test tubes (100x13 mm)
18. Test tubes (75x10 mm)

Procedure:

1. Dissolve 10 mg of the sweetener Equal in 1 mL of 3 M HCl in a test tube (100 x 13 mm). Heat the solution with a Bunsen burner, using a small flame (or with a micro burner) to a boil for 30 sec. Do not heat the bottom of the test tube, but heat slightly above the surface level of the solution. As the solution boils, do not let the liquid evaporate completely. Set the solution aside to cool; this is the hydrolyzed aspartame.
2. Label six small test tubes (75x10 mm) as follows: (1) phenylalanine, (2) aspartic acid, (3) leucine, (4) aspartame, (5) hydrolyzed aspartame, (6) Diet Coca-Cola. Place about 0.5 mL samples in each test tube.
3. Use plastic gloves throughout in order not to contaminate the paper chromatogram. Take a strip of Whatman No. 1 chromatographic paper, 8x15 cm and 0.016 cm thick. With a pencil, lightly draw a line parallel to the 8-cm edge 1 cm from the edge. Mark the positions of 6 spots, placed equally, where you will spot your samples

Spotting: For each sample, use a separate capillary tube. Then apply a drop of sample to the paper until it spreads to a spot of 1 mm diameter. Further, dry the spot. (If a heat lamp is available, use it for drying.) Do the spotting in the following order: a) Phenylalanine 1 drop; b) Aspartic acid 1 drop; c) Leucine 1 drop; d) Aspartame (in Equal) 1 drop; e) Hydrolyzed aspartame 6 drops; f) Diet Coca-Cola 10 drops.

4. When you do multiple drops of nos. 5 and 6, allow the paper to dry after each drop before applying the next drop (Avoid putting a hole in the paper!). Do not allow the spots to spread to larger than 1 mm in diameter.

5. Pour about 15 mL of solvent mixture (Butanol: acetic acid: water) into a large (1L) beaker. Place a glass rod over the beaker. Using clear tape, affix the chromatographic paper to the rod so that when you lower the rod over the beaker, the paper will dip into the solvent but the pencil marks and the spots will be above the solvent surface.

Cover the rod and beaker with aluminum foil. Place the beaker on a hot plate. Turn to a low setting (e.g., no. 2 out of 10 on the dial) and heat the beaker to about 35°C. Allow the solvent front to advance at least 6 cm (it will take 40–50 min.), but do not allow it to get closer than 1 cm from the top edge. Watch the temperature and make sure it does not rise above 35°C.

While you are waiting for the solvent front to complete its rise, you can do the short chromatography experiment in Part B. Your instructor may choose to make this optional.

6. When the solvent front has advanced at least 6 cm, remove the rod and the paper from the beaker. You must not allow the solvent front to advance up to or beyond the top edge of the paper. Mark immediately with a pencil the position of the solvent front. Under a hood, dry the paper with the aid of a heat lamp or hair dryer. With polyethylene gloves on your hands, spray the dry paper with ninhydrin solution. Be careful not to spray ninhydrin on your hands and do not touch the sprayed areas with bare hands. If the ninhydrin spray touches your skin (which contains amino acids), your fingers will be discolored for a few days. Place the sprayed paper into a drying oven set at 105–110°C for 2–3 min.

7. Remove the paper from the oven. Mark the center of the spots and calculate the Rf values of each spot. Record your observations on the Report Sheet.

8. If the spots on the chromatogram are faded, you can visualize them by exposing the chromatogram to iodine vapor. Place your chromatogram into a wide-mouth jar (or a large beaker, 1-L size) containing a few iodine crystals. Cap the jar (or cover the beaker with aluminum foil) and warm it slightly on a hot plate to enhance the sublimation of iodine. The iodine vapor will interact with the faded pigment spots and make them visible. After a few minutes' exposure to the iodine vapor, remove the chromatogram and mark the spots immediately with a pencil. The spots will fade again with exposure to air. Measure the distance of the center of the spots from the origin and calculate the Rf values.

Calculation

$R_f = \text{Distance traveled by the amino acid} / \text{Distance traveled by the solvent front}$

Table 6.1: Table of Aminoacids and Rf values

S No	Band Colour	Amino acid	Distance (cm)	Rf values
1				
2				
3				
4				

Result:

Reference:

- 1) Sadasivam S and Balasubramanian T (1985). Practical Manual (Undergraduate), Tamil Nadu Agriculture University, Coimbatore, p.2.
- 2) Sadasivam S, Manickam A (2008). Biochemical Methods, New Age International Publishers, 3rd Edition, ISBN: 978-81-224-2140-8.