Separating amino acids by paper chromatography

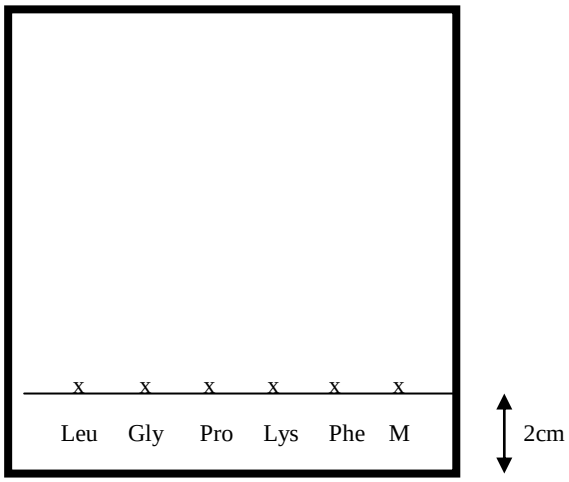
The aim of the experiment:

The aim of the experiment is to determine the composition of an unknown amino acid mixture.

Experimental protocol

Spotting the chromatogram with samples

On your filter paper draw a line across one end of the paper about 2 cm from the end (use a pencil only). Mark 6 points on the baseline in even distances.



Dip one end of the micro-pipette into the solution of an amino acid. It will cause a small amount of the sample to rise into the tube by capillary forces. Then touch the loaded pipette lightly onto the filter paper at a point marked on a baseline. This will cause some of the liquid in the pipette to be drawn onto the adsorbent, forming a visible ring. Using a hair dryer dry the spot and repeat the procedure, trying to keep the spot as small as possible. Then proceed the same way with the remaining amino acids and a mixture obtained from the teacher.

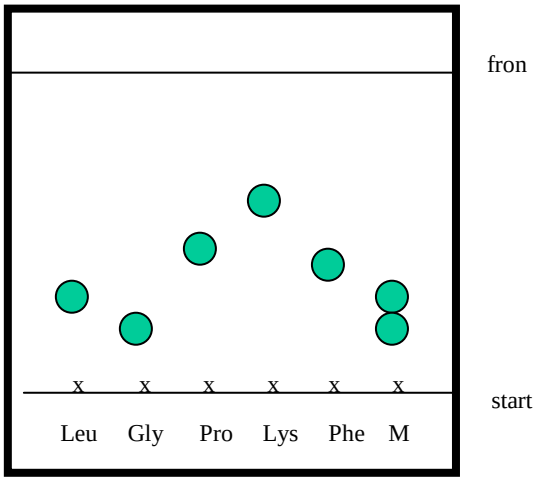
Developing a chromatogram

Once the spots have been applied, place the chromatogram in the tank for development. The chromatogram must be placed in the tank carefully, so that it doesn't touch the walls of the tank. The solvent level on the bottom of the tank must not be above the baseline, otherwise amino acids will be washed to the solvent instead of undergoing chromatography. Once the chromatogram has been placed correctly, replace the cover on the tank and wait for the solvent to advance up by capillary action (2-3 h).

As the solvent rises, the filter paper becomes visibly moist. When the solvent has advanced 80-90% of the length of the chromatogram, it should be removed and the position of the solvent front should be marked *immediately* by scoring the paper along the solvent line with a pencil. Then the chromatogram must be dried.

Visualizing the developed chromatogram

The visualization reagent used in case of amino acids is ninhydrine. Ninhydrine reacts with amino acids to form complexes that are purple or yellow (in case of proline which is a secondary amine). Under the hood spray the whole chromatogram with ninhydrine and dry it using a hair-dryer. Color spots will appear.



Calculating the R_f value

Under the established conditions, such as solvent system and temperature, a given compound always travels a fixed distance relative to the distance the solvent front travels. The ratio of the distance the compound travels to the distance the solvent travels is called R_f value. The symbol R_f stands for "retention factor" and it is expressed as a decimal fraction:

$$R_f = b/a$$

where: a = distance traveled by solvent front

b = distance traveled by substance (measured to the center of the migrated spot).

Calculate R_f values for all standard amino acids and make a table as below:

Amino acid	**A [cm]**	**B [cm]**	**color**	**R_f**
Phe				
Leu				
Met				
Pro				
Lys				

Calculate R_f values for the unknown amino acids in the mixture and compare with the standards. Name the amino acids present in the mixture.

The protocol should contain:

1. The title of the experiment
2. Table with R_f values
3. Chromatogram