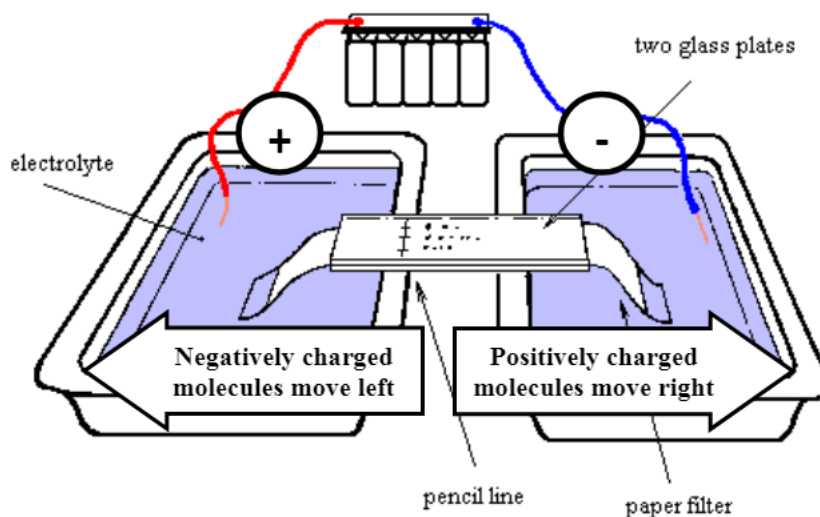


PAPER ELECTROPHORESIS

Introduction

Electrophoresis is a technique that uses an electrical field to separate mixtures of large molecules (macromolecules) such as proteins, polypeptides and DNA. A sample is prepared and carefully placed onto a fixed substrate – normally a gel made of agarose or polyacrylamide – and a voltage of around 50 to 100 volts is applied, which separates a single spot containing a mixture of different species into a number of spots each containing only a single species.

The large molecules contain varying numbers of ionic groups such as amines ($-NH_2$) and carboxylic acids ($-CO_2H$), which under the right conditions will ionise (to $-NH_3^+$ and $-CO_2^-$) to give the large molecules an overall charge. This means the molecules will move towards the positive or negative electrode depending on whether they have a negative or positive overall charge; and since the amount of ionisation is different for each type of molecule, the distance they move will also be different and they will separate.



The molecules are normally dissolved in a buffer solution, the pH of which determines the extent of ionisation of the side-chains, and so plays a big part in determining the separation; buffers of different pH are used to separate different types of proteins.

The 'electro' part of the electrophoresis name comes from the electric field used to separate the molecules, and the 'phoresis' part refers to tiny holes or pores in the gel that allow the the molecules to move through it. Varying either the strength of the electric field or the size of the holes will affect the separation.

Since many macromolecules are colourless, a stain such as bromothymol blue or ninhydrin is often used to show where the spots are.

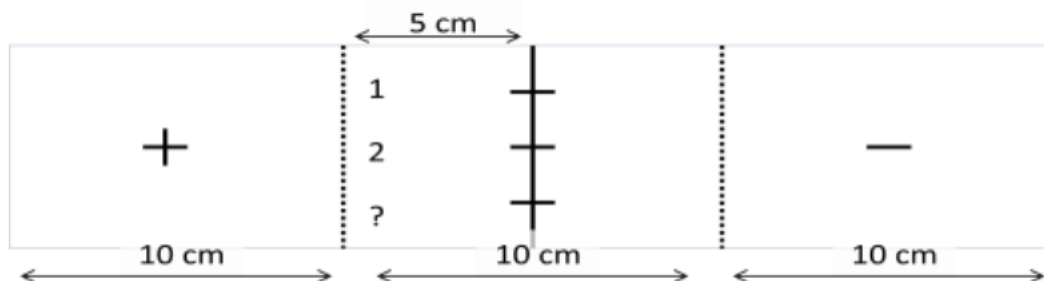
In this experiment, rather than using a gel we will use paper as our substrate and will use it to observe the separation of some known samples of amino acids in order to learn the identity of an unknown one.

Apparatus and Materials

- Standard laboratory glassware
- 45V power supply (5 x 9V batteries in series will do)
- Chromatography paper
- Ruler
- Buffer compartments – ideally two small, trays, but beakers will do
- Pulled capillary tubes
- Two 5 x 10 cm glass or transparent plastic support strips
- Hot plate or drying oven at approx 70°C
- Ethanol
- pH 6 phosphate buffer
- 0.1 M solutions of 2 amino acids, and a further solution of 1 of them labelled 'unknown'
- Ninhydrin spray

Procedure

1. Before you begin, put on some rubber gloves and clean the ruler, your glass/plastic support strips and your workspace with ethanol to remove any contaminating proteins.
2. Obtain a strip of a chromatography paper that is approximately 5 x 30 cm long, you should still be wearing gloves.
3. Using a pencil (not pen!) and ruler mark-up the paper as shown below (the line should be half way).



4. Crease the plate along the dotted lines, so that it will hang nicely into the buffer solutions.
5. Take one of the known amino acid solutions and dip a pulled capillary tube into it to suck up some of the solution. Repeatedly and briefly dab the capillary tube on the paper at the cross-mark in line with the number 1; the idea is to build up a small but concentrated spot of the amino acid.
6. Repeat with the other two solutions, dabbing on the remaining two cross-marks.
7. Fill each of two beakers with buffer solution.
8. Place the paper onto one of the glass/plastic supports and place it (like a bridge) between your two buffer compartments, the ends of the paper should be immersed in the buffer.
9. Use a Pasteur pipette to wet the paper with buffer working in from the edges and alternating sides such that advancing line of buffer meets in the middle at the same time.
10. Carefully place the second glass/plastic support on top of the plate. **Do not slide it around as this will smudge your spots.**
11. Set the power supply to 45V, run wire from the positive terminal into buffer compartment marked '+' and from the negative terminal into the buffer compartment marked '-' and switch it on.
12. After 45 minutes (or as long as time allows) switch off the power supply and carefully remove the top plate.
13. Very carefully, keeping the plate fully horizontal, lift the paper and support out of the buffer solution and place to dry on the hotplate or drying oven.
14. When dry, and wearing gloves, transfer the paper to a fumehood and spray with ninhydrin spray. With luck, this should show up three spots corresponding to each of your amino acid solutions that have moved some distance from the centre line.

Analysis

- a. Measure the distance moved by each of your spots.
- b. Use this to identify your unknown sample.
- c. Calculate the rate of movement by dividing the distance travelled by the run time.
- d. Research (check IB data booklet) and draw the structure of each of the amino acids present in your sample and use this to explain the direction of movement and the difference between the spots.