**Chromatography of amino acids**

Amino acids have no colour. Therefore all of these procedures need to be carried out "blind", and the results will only be seen after drying when a revealing agent (ninhydrin) from the solvent acts on the resulting chromatogram.

You are provided with some solutions of amino acids, and solution X (a mixture of 2 amino acids). Do not use the same pipette for more than one liquid.

Only the very top of the thin layer chromatography (TLC) plates may be touched with the hands.

Use plastic/rubber gloves and work on a clean surface (e.g. a clean sheet of blotting paper).

Take a TLC plate (slightly longer than the frame in the glass chromatography chamber) and carefully mark it with a thin **pencil** line about 2cm from the bottom.

NB The plate’s coating will crumble if undue pressure is used which will affect the results.

Using a pencil, put 5 small crosses evenly spaced on the line, the outer ones labelled with (3 letter codes for) the amino acids you are going to use, and X in the middle. Initial your plate at the top.

Using 5 different micropipettes, place a drop of each amino acid, and the mixture X, at the appropriate positions on the line. Repeat the spotting process (on exactly the same positions) to raise the concentration of the amino acids, but ensure that the paper is completely dry before re-spotting to prevent the solution ‘spreading’.

**In a fume cupboard**:
There is a pre-prepared glass chamber containing chromatography solvent (butanol/ethanoic acid) (to about 1 cm depth). The lid should be left on as much as possible to allow the atmosphere to become saturated with vapour and avoid the solvent evaporating from the face of the plates during the separation process. Leave the chamber in the fume cupboard in its final position, so that it does not splash up; bring the TLC plate to it. A metal frame is placed in the chamber from which to suspend the TLC plates.

Line up the plate with the frame and clip it onto a wire so that the solvent touches the lower part of the paper but does not cover the drops on the line.

Replace the lid as soon as possible. The TLC plates must not touch the sides of the chamber.

The solvent will gradually rise up the plate, passing the line and heading upwards.

After about 1 hour, the solvent should have risen about three-quarters of the height of the plate. If the solvent nears the top of the paper, proceed immediately to the next stage.

Remove the plate from the apparatus, and immediately use a pencil to mark the highest position of the solvent (the solvent front). Up to 5 plates can be placed across a clean A4 sheet of paper, stapled at the top of the chromatograms.

Place the chromatograms into an oven/incubator at about 45⁰C to dry.
Whilst in the oven colour will develop as the ninhydrin present in the chromatography solvent takes effect. Spots should become visible as purplish smears on the paper.

The image of the spots may be enhanced if the sheet is photocopied on a darker than normal setting.

**Calculation of Rf values**

Measure the distance from the start line to the solvent front and to the front of each spot.

For each spot, calculate the Rf value (Rf means *relative to front*):

       distance moved by spot
distance moved by solvent front

Compare the values you obtain with reference Rf values below.

Different solvents and different types or makes of chromatogaphy papers will give slightly different results.

One or both of the spots from solution X may be at the same level as another (known) amino acid alongside it. This should assist in identification.

|  |  |  |  |
| --- | --- | --- | --- |
| **Amino acid** | **Rf value** | **Amino acid** | **Rf value** |
| Alanine (ALA) | 0.38 | Leucine (LEU) | 0.73 |
| Arginine (ARG) | 0.20 | Lysine (LYS) | 0.14 |
| Asparagine (ASN) | 0.5 | Methionine (MET) | 0.55 |
| aspartic acid (ASP) | 0.24 | Phenylalanine (PHE) | 0.68 |
| Cysteine (CYS) | 0.4 | Proline\* (PRO) | 0.43 |
| Glutamine (GLN) | 0.13 | Serine (SER) | 0.27 |
| glutamic acid (GLU) | 0.30 | Threonine (THR) | 0.35 |
| Glycine (GLY) | 0.26 | Tryptophan (TRP) | 0.66 |
| Histidine (HIS) | 0.11 | Tyrosine (TYR) | 0.45 |
| Isoleucine (ILE) | 0.72 | Valine (VAL) | 0.61 |

 \* not a *true* amino acid - shows up as yellow

Show your all measurements *and* processed data, including an identification of the amino acids in the unknown mixture using a suitable table.