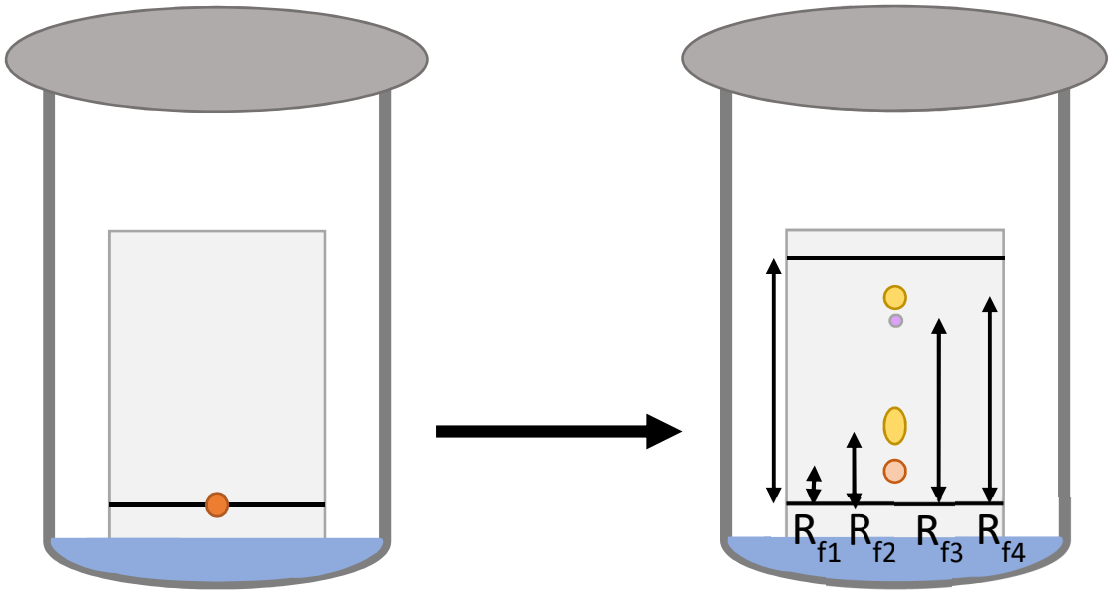
TC3 - Identifying Amino Acids

Because each of the twenty amino acids have different side chains, they will each have slightly different **properties**. For example, the amino acid serine has a hydrophilic OH group, whereas valine has a hydrophobic, hydrocarbon side chain. This means that each molecule will act differently when in a **solvent**, and this allows the two to be **separated** and identified. This can be done with all the amino acids, as they all act slightly differently in solvents. This is the basis for amino acid separation using **chromatography**.

THIN-LAYER CHROMATOGRAPHY

Thin-layer chromatography (**TLC**) is similar to paper chromatography that you may have done in the classroom. It can be used to separate the different amino acids in a mixture, and then identify them.



PREPARING THE TLC PLATE

Although the principles are the same as **paper chromatography**, it uses a **TLC plate** instead. This is a plastic or metal which has been coated in a thin layer of **silica**, which acts as the **stationary phase** through which the **mobile phase** travels. A line is then drawn about 1 or 2 cm from the bottom of the plate using pencil – as pen would dissolve in the solvent and travel up the plate. Then a spot of the solution of the unknown mixture is then added in the centre using a capillary tube. If there are multiple solutions, or mixtures to compare then multiple samples can be added along the bottom of the plate. However, make sure to label each with a number or letter below the spot!

RUNNING THE CHROMATOGRAPHY

Once the TLC plate has been prepared, the process can begin. First, add some of your chosen solvent to the bottom of a suitably sized beaker. Make sure that you don't add too much! You need to make sure that the amount of solvent in the bottom of the beaker will not reach the sample spots on the plate. If the solvent touches this, then the sample will start to dissolve in the solvent. You want to add enough solvent so that the solvent level is just below the level of the sample spots. Then

carefully place the TLC plate in the beaker. Now place a lid over the top of the beaker. This helps to saturate the atmosphere, and helps the solvent to travel through the stationary phase.

THE PROCESS OF CHROMATOGRAPHY

As the solvent starts to travel up the TLC plate, it will reach the sample spot, containing different amino acids. Each of the amino acids will have slightly different **affinities** to the mobile phase (solvent), and the stationary phase (silica), because they each have different side chains which affect their polarity. As the solvent starts to move across the sample, it will interact with the amino acids. Some of the amino acids in the mixture will have a **high affinity** for the mobile phase, and a **low affinity** for the stationary phase, because it interacts strongly with the solvent, and weakly with the silica. This means that when the solvent moves up the TLC plate, it will pull the amino acid along strongly, and the stationary phase won't hinder it much. But if the amino acid has a strong affinity to the mobile and the stationary phases, then although the solvent will be pulling the amino acids up the TLC plate, the silica will be strongly interacting with it too, and hold the amino acid back, so that the amino acid won't travel as far up the plate. This balance of affinities is the key.

INTERPRETING TLC

It is important to take the TLC plate out before the solvent has reached the top of the plate. This is so that you can mark the **solvent front**. The solvent front should be marked on the final TLC by drawing a line across the plate where the solvent has reached. This is to determine the **R_f value**, called the **retention factor**. This is the ratio of the distance that a component of a mixture has travelled relative to the solvent.

$$R_f = \frac{\textbf{Distance travelled by spot}}{\textbf{Distance travelled by solvent}}$$

If you have samples of the amino acids that you think may be in your mixture, then you can run a TLC of these in the same solvent to compare the plates. You can then match up the R_f values and the TLC plates to determine which amino acids are in your sample.

This interpretation is much more obvious when the components of your mixture are all coloured. But what if your components are colourless, such as amino acids?

STAINING AGENTS AND ULTRAVIOLET DETECTION

Not all chemicals are coloured, and this makes it slightly trickier to detect them on a **chromatogram**. There are two key techniques to detect colourless spots on your TLC. The first is to use a **UV lamp**, to show up any spots which are visible in the UV region of the spectrum, then pencil the outline of the spots so that you can calculate the R_f value.

The second is to use a **developing agent** which **stains** the samples. A classic one is **ninhydrin**, which will react with the **ammonium** group of an amino acid, to produce a **purple** compound. Another staining agent commonly used is **iodine**. Once the chromatogram has been produced, it can be placed into another lidded beaker, with a small amount of iodine in the bottom. The iodine will sublimate, and react with the amino acids, staining them. This method must be done in a closed environment such as a lidded beaker so that the atmosphere becomes **saturated**, and to prevent exposure of iodine in the laboratory.