Unit 2: Practical Scientific Procedures and Techniques

Paper Chromatography of Amino Acids

Aim: to identify and separate amino acids in mixtures using chromatographic techniques.

Evaluation:

In the experiment, a chromatography paper was used to draw a 1.5cm line above the solvent level because that is the minimum distance between the solvent and the components from dissolving each other, using a ruler and pencil, not a pen otherwise the ink will move along the solvent and mix with other components, giving an unclear qualitative observation and inadequate quality of results. Further improvement, forceps should be used to hold and manipulate the paper to avoid contaminating the chromatogram with fingers or hands. As any traces of sweat from fingers will contain amino acids which may confuse the results.

Moreover, a selection of colourless amino acids such as leucine, phenylalanine, and serine as well as a mixture solution were labelled to prevent confusion and help identify which amino acids were in the mixture. This small range of amino acids could not present all the substances in the mixture, this leads to uncertainty and inaccuracy of the experiment. A better suggestion would be having a broad range of amino acids such

as histidine, glycine, cysteine, and many others to enhance the repeatability of the experiment.

The spots were placed along the baseline 1cm apart to prevent mixing with 2 drops each of the substances with a separate toothpick, however, this method wasn't highly effective, because whenever the toothpick collected drops from the amino acids, they will always be a smaller number of drops on the baseline than on the soaked toothpick as it didn't gather enough. A better tool to collect samples is using a capillary tube since it works by capillary action to contain more drops and gives a clear indication of the substances present on the baseline while preventing contamination of each amino acid. Once all the amino acids and the mixture were dipped on the paper, a 150ml beaker just big enough for the paper to fit in, was needed to add 5cm3 of acetone as the solvent using a pipette, this allows the solvent to be below the baseline of the paper before dissolving any components. The paper was placed in the beaker, closed the top of the beaker with a lid as acetone is volatile and can easily evaporate into the air, losing the amount of solvent in the beaker causes to move slowly and improperly separating the components. This can be improved by adding a lid, this fully saturates the beaker with the solvent vapour as it rises up the paper.

Once that is observed that the solvent reaches near to the top of the chromatography paper, the paper was removed,

measured the values of the components and solvent front to a recorded table. The value of the solvent front depended on the observer's measure on how close or far away the solvent is from the top of the paper which leads to different values for different observers. To improve accuracy, a line (drawn in pencil) near to the top of the paper should show where the solvent must stop before putting into the beaker.

A ninhydrin test was required to see the colours of the chromatogram under supervision or done by a professional, however this was not satisfying enough as the ninhydrin quickly evaporates once the hairdryer dries the chromatogram. This means that there is a limited time on measuring the Rf values before the components become colourless again. Also requires a fume cupboard to well ventilate the room as ninhydrin can be a harmful skin and eye irritant. A more effective method is to use UV lights to detect the colours of the components for a prolonged period of time.

Risks and Hazards	Safety measures
Acetone is an irritant for skin, lungs, and eyes	Use gloves and safety goggles don't breathe acetone
Amino acids can stain clothing	Wear a lab coat and Gloves
Ninhydrin is toxic and UV lights are harmful	Handled by a supervisor

Acetone is highly flammable	No open flames
Hair dryers can overheat	Unplug the hairdryer when not in use

R_f value = distance travelled by the component/distance travelled by the solvent.

Amino acid	Distance of solute	Distance of solvent	Rf value
Serine	6mm	18mm	0.3
Leucine	8mm	18mm	0.5
Alanine	7mm	18mm	0.38
Mixture	9mm	18mm	0.5

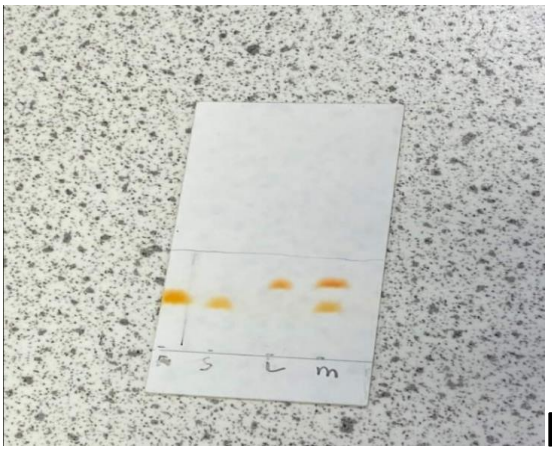

Figure 1.

Conclusion:

As shown in the table, the mixture has been separated and contains alanine, serine and leucine since leucine travelled the furthest and relatively close to the mixture with a high Rf value of 0.5, because leucine interacted less with the stationary phase, and more with the mobile phase. This means leucine is

the most polar out of three due to dipole-dipole interactions and solvent concentration between leucine and acetone causing to move leucine up the paper. In contrast, alanine and serine have the similar polarity but are less polar than leucine and are on the same column as the mixture, and having a lower but similar Rf value of 0.3 and 0.38 than leucine, concluding that serine, alanine and leucine are part of the mixture.

TLC Chromatography of Plant Pigments

Aim: to identify and separate plant pigments in mixtures using chromatographic techniques.

Evaluation:

At the start of the TLC experiment, a penny coin was used to extract the samples of the plant pigment from a green leaf by applying low pressure from the top of the leafs down to the near roots, unnecessary long roots were cutted with scissors and dispose as wasted material. Despite the method collected the sample, they were still small granules which were too large to be placed on to the TLC plate, to increase the effectiveness of this method, a mortar and a pestle can fully grind the small pieces of the leaf to obtain a paste like liquid. This is helpful in collecting samples at an average size as applying too many samples may result in large, diffuse bands of plant pigments moving up the plate, making it difficult to accurately measure the distance the plant pigment has been moved.

The sample was placed along the baseline to the centre of a 5mm line (drawn in pencil), not a pen otherwise the ink will be attracted to the solvent and ruin the results. 5cm3 of the solvent e.g: cyclohexane was added to a 150ml beaker just below the baseline of the TLC plate coated in silica gel to prevent from dissolving the components. Cyclohexane is nonpolar and has a larger molecular size which can lower the Rf values of the plant pigments, making it harder to separate the mixture and see the colours clearly. To improve this, a more polar solvent such as ethanol can separate the components in the mixture. Also, the distance of the solvent wasn't specific to determine how far the solvent is near to the top of the TLC plate, for better observation a solvent front drawn near the top of the TLC plate can enable the observer to know when the solvent should stop.

The silica gel in the TLC plate is used as an adsorbent stationary phase that prevents moisture, as temperatures can affect the separation of components in the TLC plate. If the temperature rises, **the heat transfers further energy to the solvent and component** giving the molecule more energy to escape from the beaker to the vapor phase. This also means less colour from the plant pigment appearing on the TLC plate making the judgement of comparing samples difficult. To improve better results, the experiment can be done in laboratory conditions where the temperature is constant and using a lid on top of the

beaker stops the solvent from evaporating. In addition, once the solvent fully separated the components, no UV light or chemicals needed to observe the colours present which makes the experiment safe.

Risks and Hazards	Safety measures
Ethanol is flammable	No naked flames
Sample can stain clothes and hands	Wear a lab coat and gloves
Plant materials may contain toxins or irritants	Extract the sample carefully
Roots and leaves contain bacteria	Wash the leaves before extracting samples

R_f value = distance travelled by the component/distance travelled by the solvent.

Plant pigment	Present	Colour	Distance travelled by spot	Distance travelled by solvent	Rf value
B- carotene	Yes	Yellow	4.8	4.8	1
Pheophytin	No	No colour	No data	No data	No data
Chlorophyll a	Yes	Light green	3.8	4.8	0.79
Chlorophyll b	Yes	Green	3.5	4.8	0.72

Lutein	Yes	Yellow	3.2	4.8	0.62
Neoxanthin	Yes	Yellow	3.1	4.8	0.31

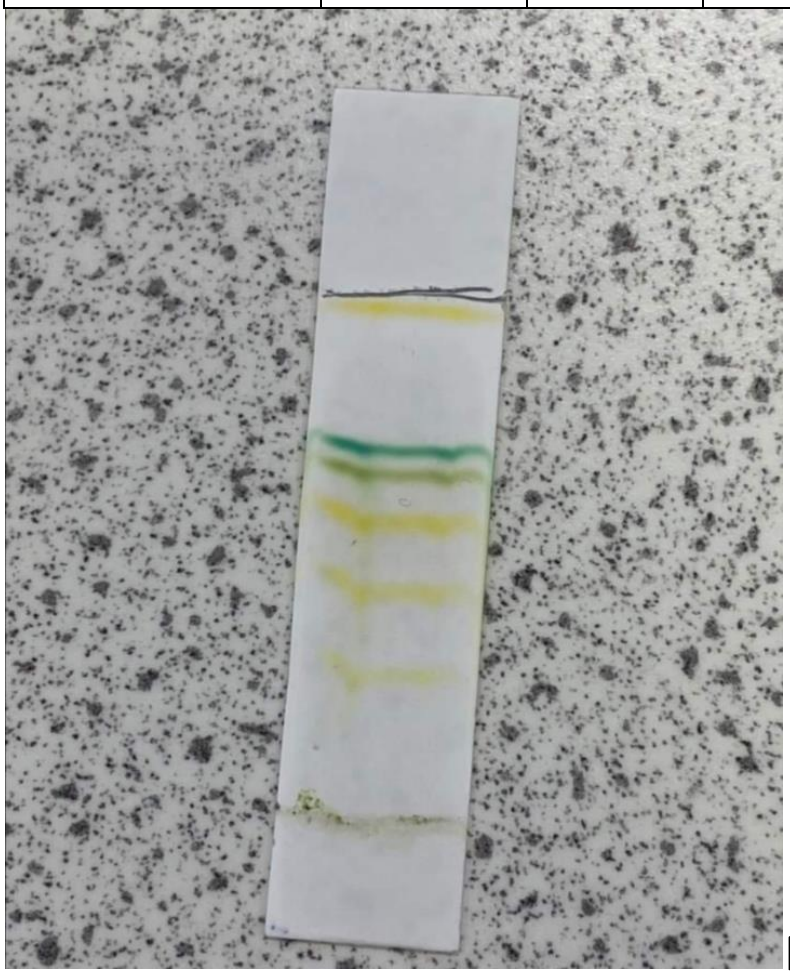
Figure 2.

Conclusion:

From the data in the table and the picture provided above supports that B- Carotene has the highest Rf value of 1.00 since it moved the furthest up the paper, this is because of the fixed amount of solvent below the baseline being able to efficiently separate the components. A bright green colour from Chlorophyll A shows a higher Rf value of 0.79 than a lighter green from Chlorophyll B showing a relatively lower Rf value of 0.72, with a 0.07 difference, giving a clear distinction between

the two chlorophyll pigments. The plant pigments with the lowest Rf value are Lutein and Neoxanthin which all closely match the expected values from the TLC Data Table of Average Rf values, this is because their molecular size is too large to travelled up the TLC plate since it is non polar so it is less attracted to the mobile phase which all further supports the reproducibility of the experiment and confirms the validity of the expriment.