Experiment 20

Paper Chromatography

Objective

To illustrate the use of paper chromatography for the separation and identification of amino acids.

Results

Amino Acid	Distance travelled /cm		R_f value
	by solvent	by amino acid	
Aspartic acid			
- alone	5.3	1.2	0.2264
- in mixture	5.3	1.2	0.2264
Leucine			
- alone	5.3	3.95	0.7453
- in mixture	5.3	4.3	0.8113
Lysine			
- alone	5.3	3.1	0.5849
- in mixture	5.3	3.3	0.6226

Special Questions

1.> For each amino acid, compare your two R_f values with each other and with the specimen results

Why do you think there is some variation?

Both R_f values are the same for aspartic acid but there is some variation for leusine & lysine. This may due to:

1.> The grease on our fingers contaminated on chromatographic paper will affect greatly on the amino acids that are less polar.

2.> The measurement error of the distance travelled by solvent and/or amino acid.

3.> The shape of chromatographic paper is not exactly square-shaped, so that one side of the paper may be lifted and the speed of the solvent travel in one side is faster.

4.> The different proportion of chemical s (∵measured by measuring cylinder) may be used between the researchers and us, and the temperatures may also different, thus R_f values obtained are different.

2.> Why do R_f values change when a different solvent is used?

Each amino acid has different polarity and each solvent has different polarity, too. So, when a different solvent is used, amino acids will travel at a different speed.

For example, if a more polar solvent is used, the more polar amino acid will travel at a faster speed and has a larger R_f value.

On the contrary, if a less polar solvent is used, the non-polar amino acid will travel faster and the slowest one should be the polar amino acid.

3.> Why is it so important to avoid touching the chromatographic paper with your fingers?

There are some secretion on our fingers - amino acids and grease.

Amino acids on our fingers will result in the unexpected colour zone appears on chromatographic paper after s praying ninhydrin solution. This will make us confused and more difficult to obtain the appropriate R_f values.

Grease on our fingers will affect non-polar amino acids most, since they are more likely to dissolve in grease than more polar developing solvent, they will move at a higher speed. Hence the R_f values are affected.

Moreover, there may have some chemicals on our fingers, e.g. ammonia, water, etc. These will affect the polarity of the developing solvent and thus the R_f values.

Precaution

1.>Small area with concentrated spot of amino acid should be applied to obtain a deeper colour of spots.

2.> The edges of chromatographic paper should not touch the sides of the beakersince he

solution at edges will move faster due to capillary action.

The spots will bend and R_f value is affected.

3.> The beaker should be saturated with the solvent before putting the chromatographic paper in. If the atmosphere is not saturated, there is a

diffusion gradient between chromatographic paper and air, some solvent may vapourize from chromatogram and results in long tail of spots.

4.> The origin line should be marked by pencil, not ball pen, since the ball pen ink can dissolve in the developing solvent and the line fades gradually

or even an additional spot can be observed.

5.> Ninhydrin solution should not spray too much on chromatogram since it will blur and spreads the coloured spots. It's because the solvent that are

used to dissolve ninhydrin can also dissolve amino acids.

Discussion

Paper chromatography is useful for separation and identification of many substances, e.g. amino acids, dyes, etc. Its principle is based on the difference of the relative adherence of a solute between a stationary phase and a mobile phase. Since paper consists of cellulose that contains a large number of —OH groups, a layer of water will be permanently attached to the paper. This layer is the stationary phase. Under capillary action, the developing solvent will move upwards, which is the mobile phase. The solute particles are moved upwards with the solvent and distribute between the stationary phase and mobile phase.

Paper chromatography can be used for analysis of solutes because at a constant temperature, each solute has a particular retention factor (R_f) in a particular set of mobile phase & stationary phase. Base on the R_f values, the solutes can be identified.

$$\text{where } R_f = \frac{\text{distance moved by spot}}{\text{distance moved by solvent front}}$$

The higher the R_f, the faster is the movement of the solute.

If the solute particles is less polar than the stationary phase (i.e. less soluble in stationary phase), it will move up with the mobile phase (less polar). Otherwise, if the solute particles is highly polar, it will only stay in the stationary phase. In other words, the difference in partition coefficients of the solute particles enable the solute particles to move with different speeds. In this experiment, leusine has the highest solubility in mobile phase (least polar) than in stationary phase thus its motion is fastest. Aspartic acid has the lowest solubility in mobile phase (most polar) than in stationary phase thus it moves slowest. After the mobile phase moved a certain distance, the spots (after drying) may be cut out and extracted with an appropriate solvent.

If the solute is colourless such as amino acid, it can be located by spraying the chromatogram by ninhydrin solution to form a coloured spot. The colour of the complex formed between ninhydrin & aspartic acid is deep blue whereas that of leusine and lysine

are purple. Other organic solutes may also be located by ultraviolet light (if it gives fluorescence in UV light) or by iodine vapour (I_2 will dissolve in organic compound which results in a brown spot).

2-way chromatography (i.e. turn the chromatogram for 90° and obtain a second chromatography) may be applied for further chromatography to obtain a more distinct spot.

If the paper medium is replaced with silica gel or alumina coated on a glass plate, it's called thin layer chromatography. In this case the separation is based on the effect of both adsorption and partition.