**Identification of the amino acids in a protein using paper chromatography STUDENT**

**Introduction**

In this task, you will carry out a practical to explore the techniques associated with paper chromatography and separate and identify the amino acids present in egg white.

**Aim and skill covered**

* To identify the amino acids present in the protein in egg white (albumen) using paper chromatography*.*

**Intended class time**

* 1 hour on one day
* 5 minutes on the next day to remove the chromatogram from the solvent and hang it to dry
* 30 minutes on a third day to develop and interpret the chromatogram

**Equipment**

* Gas jar
* Chromatography paper
* Pencil
* Ruler
* Gloves
* Digested egg white solution
* Solvent
* Capillary tubing
* Ninhydrin spray
* Hair dryer or oven set at 110°C

**Health and Safety**

A fume cupboard is required for the procedure. Ninhydrin spray is harmful. When spraying the chromatograms, the fume cupboard should be used, windows should be open, eye protection and gloves should be worn and no flames should be nearby.

**Procedure**

1. You are provided with a solution of albumen (egg white) that has been incubating with the enzyme trypsin for three days. The result is a solution of amino acids.
2. Wearing eye protection, place your gas jar in the fume cupboard and pour solvent in to a depth of about 3cm. Place the lid on the gas jar and leave it in the fume cupboard so that the atmosphere inside the gas jar becomes saturated with solvent fumes.
3. Wash and dry your hands.
4. Take a piece of chromatography paper - it should be long enough for about 5mm to dip into the solvent and the other end should extend out of the top of the gas jar by about 20mm. Handle the paper as little as possible throughout the experiment.
5. Draw a faint pencil line about 40mm from one end of the chromatography paper.
6. Using the capillary tubing, draw up amino solution and place a tiny dot in the centre of the line.
7. Wait for it to dry.
8. Place another dot of amino acid solution on top of the first one using the capillary tubing and wait for it to dry.
9. Repeat this at least six times letting it dry between applications until you have a very concentrated dot of amino acid solution. Try to keep the dot as small as possible by being patient and letting it dry fully between applications.
10. Carefully lower the paper into the gas jar, making sure it does not touch the sides and allow it to just dip into the solvent. Fold the top of the paper over and replace the lid. Leave for several hours until the solvent has moved to within 2cm of the top of the paper.
11. After several hours and once the solvent has moved to within 2cm of the top of the paper, carefully remove the chromatography paper from the gas jar.
12. Without damaging the paper, draw another pencil line marking the point where the solvent moved to and then hang the paper somewhere warm to dry.



folded over paper

gas jar

pencil line

chromatography paper

amino acid solution

solvent

1. Once the chromatography paper is dry, take it back to the fume cupboard wearing gloves and eye protection.
2. Spray the paper carefully with ninhydrin reagent.
3. Use heat as directed by your teacher to develop the chromatogram. If you continue to apply heat after the paper is dry, the amino acids will start to show up as purple spots which will become denser with more heat.
4. Each purple spot represents one or more amino acid. Outline each spot with pencil and mark the centre of each one.
5. In order to identify them, a measurement called the Rf value is used. Rf stands for “relative front”. It is the ratio of the distance moved by each amino acid to the distance moved by the solvent:

Rf = distance moved by spot

 distance moved by solvent

1. Measure the distance that the solvent moved.
2. Measure the distances that all of the amino acids moved and record in a suitably designed table.
3. Calculate the Rf value for each amino acid and use the table below to try to identify them and record in the table.

|  |  |
| --- | --- |
| **Amino acid** | **Rf value** |
| Lysine | 0.14 |
| Arginine | 0.20 |
| Aspartic acid | 0.24 |
| Glycine | 0.26 |
| Serine | 0.27 |
| Glutamic acid | 0.30 |
| Threonine | 0.35 |
| Alanine | 0.38 |
| Proline | 0.43 |
| Tyrosine | 0.45 |
| Methionine | 0.55 |
| Valine | 0.60 |
| Phenylalanine | 0.68 |
| Isoleucine | 0.72 |
| Leucine | 0.73 |

**Extension Opportunities**

1. What amino acids did you identify as being present in albumen (egg white)?
2. Why might not all of the amino acids known to be present in albumen have appeared on your chromatogram?
3. Why are you advised to handle the chromatography paper as little as possible?

**To submit**

For this piece of work to count towards Practical Activity Group 6 of the Practical Endorsement, you should have evidence of a chromatogram showing separated amino acids. You should have recorded the measurements for the solvent front and the distances moved by the amino acids so that you can calculate the relative front values and then identify the amino acids in the solution. This should all be evidenced in a table. You also need to have considered the above questions as the answers to these questions will aid you in preparation for your written examinations.