Amino acid chromatography

In this experiment paper chromatography was used in order to identify two unknown amino acids using eight known amino ones. The two unknown ones were identified by comparing the distance they travelled up the chromatography paper and their Rf values to the corresponding values of the other eight known amino acids. The unknown amino acids identified were Glycine and Methionine. Introduction Proteins in cells are important in many ways. There are different types of proteins such as contractile proteins, enzymes, hormonal proteins, structural proteins and transport proteins. They are vital to regular cell functioning.

Proteins are made up of amino acids that are joined together by peptide bonds. When fewer than 50 amino acids are joined together, a polypeptide is formed. All proteins have two groups in common. They have a carboxylic group and an amino group. There are 20 types of amino acids that bond together in different combinations to perform different functions. The primary structure of proteins is the order and number of amino acids. Secondary, tertiary and quarternary structures are formed from chains of peptides that are folded into sheets, ribbons and coils so that they form a 3D shape and are more stable.

Different weights of amino acid make them differ in polarity. This characteristic enables the separation of proteins by polarity using chromatography. Paper chromatography is an example of a chromatography technique called absorption chromatography. The paper is the adsorbent, which will bind the components of the mixture. The substance will be “ spotted” onto the chromatography paper and put into a beaker filled with solvent. The solvent will then flow through the paper. The solvent chosen

depends highly on its polarity as this will be the characteristic that will separate the different substances.

Petroleum, ether, hexanes, cyclohexanes and toluene are some examples of solvents with different polarities as well as increasing polarities. In some cases, mixtures of solvents are made to reach a certain polarity. If substances that are needed to be separated are polar, then the solvent must be slightly less polar. Non-polar substances need a polar solvent to be separated. The solvent travels faster than the samples. The Rf value is the ratio of the distance traveled by the sample and the distance travelled by the sample.

Rf = distance travelled by amino acid sample from the origin in mm distance travelled by the solvent from the origin in mm Factors affecting how far the amino acids travel depend on how high the solvent is allowed to rise on the paper, the type of absorbent, the type of concentration of the solvent, temperature and the distance of the origin from the solvent. One type of test to detect proteins is the Ninhydrin test. This test makes the amino acids spots visible. Ninhydrin is a pale yellow solid and it reacts with the amino group in the amino acids and proteins and produces a purple product.

Heat must be used in order to speed up the reaction. Objective The objective of this experiment was to spot various amino acids and an unknown mixture on chromatography paper and run it with a chromatography solvent. The lab period following included treating the samples with Ninhydrin solution and heating it so that the amino acids could be visible. The distance of the samples were then measured in mm from the origin. The measurements were then used to calculate the Rf values for each sample and thus the

unknown sample could be identified. Materials Alanine, 1% Solution Arginine, 1% Solution

Asparagine, 1% Solution Aspartic acid, 1% Solution Glycine, 1% Solution Lysine, 1% Solution Methionine, 1% Solution Tyrosine, 1% Solution Unknown, 1% Solution Chromatography Solvent, 20mL Ninhydrin solution, 2%, 10mL Beaker, 600mL Chromatography paper, 20X10 cm Graduated Cylinder, 25-mL Heat source, drying over or hot plate Microtip pipets, 9 Pencil Ruler Spray bottle Stapler Watch glass or aluminum foil Procedure 1. On a 20cm wide by 10 cm high piece of chromatography paper, a pencil was used to draw a straight line (about 1 cm) from the bottom of the paper from the left to the right side 2.

Nine pencil dots were placed 2cm apart on the line 3. The name of each amino acid was written under each dot in pencil. 20 mL of chromatography solvent was then added to the 600-mL beaker 4. A micropipette was used to obtain a small amount of the first amino acid 5. The tip of the pipette was placed above the chromatography paper directly above the pencil dot and a spot of the amino acid was dropped on the dot 6. Steps 4 and 5 were repeated for the eight amino acid solutions 7. With the sample side facing outwards the chromatography paper was turned into a cylinder and the top and bottom edges of the paper were stapled. .

The paper cylinder was then placed into a beaker with the chromatography solvent. 9. The beaker was then covered with a watch glass 10. The samples were then allowed to run till the solvent level was about 1 cm from the top of the paper. 11. The chromatography paper was then removed from the beaker. The solvent height was then marked with a pencil line and the

staples were removed 12. The chromatography paper was then left to dry During the following lab 13. The chromatography paper was sprayed with a spray bottle containing 10mL of 2 % Ninhydrin solution 14.

The chromatography paper was left to dry for 10-20 minutes 15. The paper was then put in a drying oven or held 10 cm above a hot plate to heat so that the color could develop 16. A dot was placed with a pencil at the centermost point of each amino acid 17. The distance in mm of the solvent traveled from the pencil line till the where the solved stopped traveling was measured. 18. The distance in mm from the origin till where each amino acid traveled was measured 19. The Rf value for each amino acid was calculated Results

Table 1: Distance and Rf values of the amino acids and unknowns Amino Distance(mm)4524272230155742 35/60 Rf Value0. 50. 270. 30. 240. 330. 170. 630. 470. 39/0. 67 The distance traveled by the solvent from the pencil line drawn was 90mm. The unknown samples were found to be Glycine and Methionine by comparing their Rf and distances values to those amino acids with Rf and distance values that were calculated. Discussion Paper Chromatography is used to separate a mixture of compounds into its components.

Pens and markers are not used as their ink will be separated too. Instead, pencils are utilized as they are made from graphite which does not separate. Capillary action is the ability of a liquid to flow in narrow spaces without any help from external forces. This flow is against gravity as well. This happens because of the intermolecular attractive forces between the liquid and the

solid surrounding surfaces. Surface tension and adhesive forces between the liquid and solid also help the liquid rise through the solid.

The Rf value is defined as the ratio of the distance travelled by the amino acid sample from the origin to the distance travelled by the solvent. The ratios, therefore, stay the same regardless of the solvent used. Ninhydrin is used in paper chromatography to identify amino acids. Ninhydrin solution turns the amino acid fingerprints to the color purple, therefore making them visible. For this reason we take care when touching the chromatography paper. The least polar amino acid was alanine as the distance it moved up the paper was the least.