Proteins & Amino Acids

Notes For Use

A01557 Proteins and Amino Acids

Contents

Notes For Use

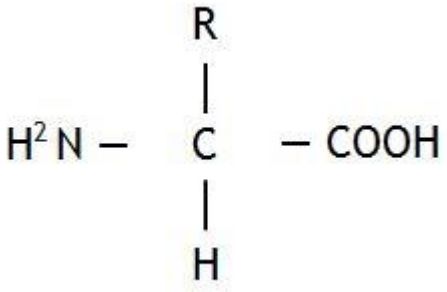
Introduction:

Proteins are fundamental to the structure and function of all cells, where they represent 50% or more of the dry matter of cell contents. The structures of cells — walls, membranes, organelles — all contain protein molecules. Enzymes, nature's biocatalysts, are largely protein in nature. A number of hormones are polypeptides, such as insulin and glucagon and some proteins perform a variety of transport functions, such as albumin and haemoglobin in the blood.

Other proteins are involved in a variety of storage functions — ferritin is a combination of iron and the protein apoferritin. In animals, protection against infectious agents depends partly on the immunoglobulins, the anti-bodies, which are a group of interrelated proteins.

Thus, many different proteins are known, having a wide variety of functions. They are all polymeric molecules built up from simpler building blocks, or sub-units. These sub-units are the amino acids, of which about twenty different kinds have been found in proteins. Formula 1 is a general formula for an amino acid showing the amino group substituted on the C atom in the position ∝ to the carboxyl group. The group R represents the variable parts, or the side chains of amino acids.

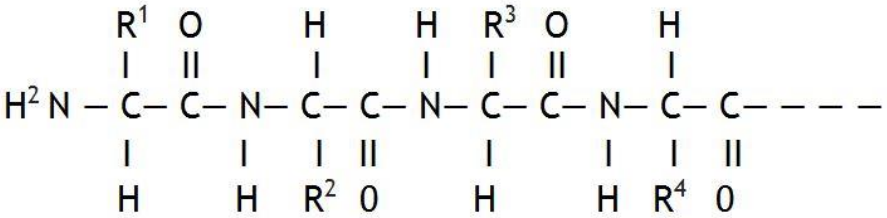
Formula 1:



When R is H — the compound is glycine; when R is CH^{3-} the compound is alanine, and so on. The combination of amino acids to form proteins is through the amino and carboxyl groups, with the formation of amide, or
peptide, bonds.

Formula 2 illustrates a section of a protein.

Formula 2:



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It is clear that the nature and sequence of the R groups can be altered in a large variety of permutations and combinations. Some R groups are non-polar, as in valine and leucine.

Some are polar, as in lysine and arginine, which have basic R groups, or as in aspartic acid and glutamic acid, which have acidic groups. In solution a protein behaves in a manner dependent on a number of factors, including the amino acid content of the protein, the properties of the solvent and on possible interactions with other solutes. Experiments illustrating these are outlined below.

A. Chemical reactions of proteins and amino acids:

Proteins and amino acids undergo a wide variety of reactions. Some of these are of importance in quantitative estimations, while others are of importance in the determination of the amino acid sequences of proteins. The former group will be illustrated; the latter will be mentioned here, but only briefly.

FDNB (fluorodinitrobenzene), dansyl chloride (5-dimethylamino-naphthalene-l-sulfonyl chloride) and phenylisothiocyanate (Edman reagent) all react with amino acids and proteins. Free amino groups in proteins are confined to N-terminal residues and to lysine. Therefore a polypeptide may be labelled
with one of the reagents above, and then hydrolysed and analysed. In this way the labelled amino acids can readily be identified. It is especially useful that the release of a residue labelled with Edman reagent can be achieved without the hydrolysis of the remainder of the polypeptide. Thus the shortened polypeptide can be recovered, and the new N-terminal residue labelled, removed and identified. With sequential labelling, hydrolysis, and identification the sequence of amino acids in a polypeptide can be determined. In addition to the above, some reactions will be described which are specific for one amino acid or group of amino acids. These no longer find widespread use in research or routine analysis. However, they do illustrate the differences between amino acids, and they can be used to set up exercises in deduction based on chemistry.

General safety note:

When heating substances or handling corrosive liquids always wear safety goggles. When using ninhydrin wear gloves and work in a fume cupboard.

Colour reactions — ninhydrin and biuret tests:

Notes on reagent preparations:

Proteins:

For these tests protein solutions should be approximately 1% (w/v).

Albumin, gelatin, haemoglobin, peptone:

Dissolve in distilled water. Do not heat the albumin solution. The others may be heated gently. Make to the required volume and filter. If the gelatin solution turns cloudy before use heat it gently.

Casein:

Dissolve in a minimum of 2N NaOH. Heat gently for 10 minutes to assist solution; filter, neutralise (check with indicator paper or pH meter) and make to required volume with distilled water.

Fibrin:

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Suspend the powder in 2N NaOH. Boil and stir for 10 minutes. Cool and filter.

Amino acids:

These should be about 0.1% (w/v).

Glycine, methionine, arginine, lysine, glutamic acid, etc:

Dissolve in distilled water.

Tyrosine, cystine:

These are insoluble in water: directions for their use are given below.

Volumes required:

In the ninhydrin reaction 0.1 cm^3 of each test solution used. To allow for repeats and spillages, prepare 0.5 cm^3 (minimum) of each test solution for each individual or group of individuals performing the ninhydrin tests.

In the biuret reaction 1.0 cm^3 of each test solution is used. To allow for replicates prepare 5 cm^3 of each test solution per individual or group performing the tests.

Ninhydrin reagent:

Dissolve 2.1g citric acid in 50 cm^3 distilled water. Adjust to pH 5.0 with 2N NaOH. Make the final volume to 100 cm^3. Dissolve 4g ninhydrin in ethanol (absolute or 95%) 100 cm^3 . Combine the ninhydrin and citrate buffer solutions.

If the reagent is not to be prepared fresh daily:

1. Add 160mg $SnCl^2$ (reagent grade) to 100 cm^3 citrate buffer before adding to ninhydrin solution.
2. Exclude light and air while preparing the ninhydrin solution.

Volume required:

Two cm^3 of this reagent is required per test. Allow 6 cm^3 for replicate tests on each single compound to be tested. Multiply this by the number of compounds to be tested and further multiply by the number of students or groups performing the tests independently e.g. for 10 students each testing
10 compounds prepare 600 cm^3 ninhydrin reagent.

Biuret reagent **(<u>danger: corrosive and harmful</u>):**

Volume required:

To carry out the qualitative tests the volume of biuret reagent needed is the same as that of the ninhydrin reagent — 600 cm^3. For the quantitative section 40 cm^3 per test is needed. Allow for replicates and the number of groups or single students using the reagent e.g. 10 groups — 800 cm^3 reagent.

The ninhydrin reaction:

Principle:

Notes For Use

This reaction may be used qualitatively or quantitatively. Ninhydrin is a powerful oxidizing agent and brings about the oxidative deamation of the ∝-amino group of amino acids, liberating the following: ammonia, carbon dioxide, the aldehyde corresponding to the original amino acid and reduced ninhydrin. The ammonia formed in the reaction then reacts with one mole of oxidized ninhydrin and one mole of reduced ninhydrin to yield a violet coloured complex that absorbs maximally at 570 μm. This absorption is directly proportional to the amount of amino groups originally present and so provides a convenient colourimetric assay for amino acids and proteins. The amino acids proline and hydroxyproline react with ninhydrin to give a yellow complex that absorbs maximally at 440 μm. Although the ninhydrin reaction is a general one for amines, the evolution of carbon dioxide in the reaction is reasonably diagnostic of ∝—amino acids.

A major use for ninhydrin is in the automatic analysis of amino acids in hydrolysates of pure protein or of foodstuffs. The amino acids are separated chromatographically and identified by reference to standards. In that way the lack of specificity of ninhydrin is unimportant.

Ninhydrin test procedure:

Prepare a number of clean test-tubes, one for each compound to be tested. To each tube add 2.0 cm^3 of ninhydrin reagent. Add 0.1 cm^3 of a test solution to a test tube, mix, and boil for 2 minutes in a fume cupboard. A violet colour is the positive reaction. This will be difficult to observe in the case of
haemoglobin: spectrophotometric analysis would confirm the reaction.

The biuret reaction:

Principle:

Biuret is a compound obtained when urea is heated to 180°C. It reacts with copper sulphate in alkaline solution to give a violet colour with an absorption maximum at 550nm. This compound has given its name to the colour reaction which was also found to occur with other compounds — those having two or more amide groups or peptide bonds joined directly together, or through a single atom of carbon or nitrogen. An inspection of structures will show that tripeptides and polypeptides satisfy that requirement, and therefore give a positive biuret test. Amino acids and dipeptides give a negative reaction.

Procedure:

To 1 cm^3 of each compound to be tested add 1 cm^3 biuret reagent. Mix and observe any colour change over the next ten minutes. A change from the blue of copper sulphate to violet constitutes a positive reaction.

The biuret reaction is used in a quantitative manner and a number of variations of the copper sulphate reaction have been devised to make the method more sensitive. The principle of the method may be demonstrated as follows: prepare five test tubes of similar thickness and diameter: to these add 0, 0.4, 0.8, 1.2 and 1.6 cm of casein solution (1.0% w/v) prepared as described previously. Add sufficient water to adjust the volume in each tube to 2.0 cm^3. Add 8cm of biuret reagent to each tube and mix thoroughly.

Colour will develop, the intensity of which is proportional to the protein concentration. A colorimeter or spectrophotometer may be used to measure the intensity of the colour at 550 μm. The absorbancy of the solution in the control, or blank tube, that to which no protein was added, should be subtracted from the values obtained for the other tubes. Alternatively, the instrument may be zeroed against this blank or control. For teaching purposes the first procedure may be better in that it will illustrate the importance of taking account of control or blank values in solutions.

Note:

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If the quantitative demonstration is to be omitted then the biuret test may be performed in the following way:-

To 1 cm^3 of each test add 0.1 cm^3 of 40% NaOH solution and 0.1 cm^3 of copper sulphate solution (5% in water). Mix and observe the colour change.

Specific reactions of amino acids:

Lead sulphide test for cysteine and cysteine:

Principle:

The sulphur group of cysteine and cystine is liberated by heating in strong alkali. If lead ions are present lead sulphide is formed as a dark precipitate. This reaction distinguishes between these two amino acids and methionine: treatment with alkali does not liberate S from methionine.

Reagents:

NaOH 40% in H^2O:	100 cm^3 needed for 10 test groups, allowing for replicates.
Cystine:	Suspend 25mg cystine in 50 cm^3 40% NaOH - sufficient for 10 groups.
Lead acetate 2% in H^2O:	10 cm^3 per 10 groups or individuals.

Procedure:

To 2 cm^3 of each test solution (cystine and any other amino acid to be used as cross reference) add 0.1 cm lead acetate solution. Heat for five minutes on a boiling water bath. A darkening of the solution - black or brown - is indicative of cystine or cysteine. Perform the test also using 2 cm^3 H^2O in place of an amino acid.

Millon's test for tyrosine:

Principle:

This test is specific for phenolic compounds which have no ortho substituents. A red colour develops when nitrous acid reacts with a mercuric derivative of such a compound.

Reagents:

Mercuric sulphate (Millon's Solution A):	10% $HgSO^4$ in 10% $H2SO^4$ (<u>danger – toxic and corrosive</u>). Mercuric nitrate may replace the $HgSO^4$: do not use mercuric chloride.

Volume required:

2cm^3 per test compound per test group or individual.

$NaNO^2$ 1% in H^2O: 0.2 cm^3 required per test compound per group or individual.

Procedure:

To 1 cm^3 of water in a test tube add a small quantity of tyrosine (about 2mg is enough). Add 1 cm^3 of acidic mercuric sulphate solution. Boil gently for about 1 minute. Cool, add 1 drop of 1% $NaNO^2$ solution. Warm gently: a red colour indicates tyrosine.

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Xanthropeteic test for tyrosine and tryptophan:

Principle:

Nitration of aromatic rings with concentrated HNO^3 produces compounds of yellow colour (greek, xanthos = yellow). Tyrosine and tryptophan give positive reactions, but phenylalanine does not.

Reagents:

Conc. nitric acid, Sodium hydroxide: 5%
Amino acids: Use as solids, putting about 5 mg in each test tube.

Procedure:

To each test tube containing about 5mg of amino acid add 1 cm^3 conc. nitric acid. HANDLE WITH CARE.
Heat very carefully to boiling in a fume cupboard. Cool the solutions carefully, then add 5% NaOH slowly until the solutions are alkaline. CARE!!

Prior to the addition of alkali a yellow colour or precipitate may develop, indicating a positive reaction. Following the addition of alkali an intense orange colour is a positive reaction.

Sakaguchi's test for arginine:

Principle:

Ammonia and ammonium ions give positive reactions in this test. For this and other reasons reagents always give some colour in the reaction. Therefore a blank or control containing reagents should be prepared for comparison.

Reagents:

4% NaOH in H^2O: 2 cm^3 per compound to be tested per individual or group.

Naphthalen-1-ol 1%
in ethanol: 0.5 cm^3 per compound to be tested per testing individual or group.

Sodium hypochlorite 10%
in H^2O: 2 cm^3 per compound to be tested per testing individual or group.

Amino acids 0.1% in H^2O:
4 cm^3 of each compound to be tested per testing group or individual.

Procedure:

To 2 cm^3 of water and of each solution to be tested add 1 cm^3 of 4% NaOH and then 2 drops of Naphthalene-1-ol (1% in ethanol). Add 1 cm^3 of 10% sodium hypochlorite to each solution and mix. Development of a bright red colour on standing is indicative of arginine. The reaction occurs with proteins which contain arginine as well as with the free amino acid.

Test for methionine:

Reagents:

Notes For Use

20% HCl in H^2O: 10 cm^3 per compound to be tested per testing group.
40% NaOH in H^2O: 1 cm^3 per compound to be tested per testing group.
1% glycine in 1 H^2O: 2 cm^3 per compound per group.
Sodium nitroprusside (TOXIC): 10%, freshly prepared, in H^2O: 0.6 cm^3 per compound per group.
Methionine: 0.1% in H^2O: 12 cm^3 per group.

Procedure:

To 5 cm^3 methionine solution add the reagents as follows:

(a) 0.5 cm^3 40% NaOH. Mix well.
(b) 1.0 cm^3 1% glycine. Mix well.
(c) 0.3 cm^3 sodium nitroprusside. Mix well.

Heat the mixture gently (35 — 40°C) for 5 - 10 minutes. Cool in an ice bath for 10 minutes. Add 20% HCl, slowly, with stirring, keeping the mixture on ice: it is essential that the solution be kept cold. A red colour indicates methionine. Any test giving a green colour may be ignored.

Summary of reactions:

The following table, in conjunction with the tests described above, shows how an unknown amino acid from a specified group may be identified.

Amino acid	Tests				
	Solubility in water	Lead sulphide	Millon's	Sakaguchi's	Xanthoprotei c
Glycine	+	-	-	-	-
Tyrosine	-	-	+	-	+
Cystine	-	+	-	-	-
Arginine	+	-	-	+	-
Tryptophan	+	-	-	-	+

B. Physical properties of proteins:

Solubility of proteins:

Amino acids are dipolar ions. In solution their net charge, therefore, depends on the pH of the solution. Proteins are also charged molecules, their charges arising from free terminal —NH^2 and —COOH groups, and from the side chains of certain residues such as lysine, arginine, aspartic acid and glutamic acid. At a particular pH the net charge on an amino acid or protein may be zero: this pH, at which the negative and positive charges are balanced, is called the isoelectric point. At its isoelectric point a protein often has decreased solubility and it may precipitate. When milk is soured the lactic acid, responsible for the sour taste and smell, also lowers the pH and the casein precipitates when its isoelectric point is reached.

Alteration of the dielectric constant of a solvent or solvent mixture may also affect protein solubility. Thus addition of organic solvents to protein solutions often causes precipitation.

Proteins remain in aqueous solution by virtue of their interaction with water. If that interaction is altered, and particularly if protein — protein interaction is promoted, then insoluble aggregates may be formed. This is the basis of the "salting-out" effect and of precipitation following heat denaturation. In the latter case the application of heat disrupts hydrogen bonds which are partially
responsible for maintaining the three dimensional structure of the protein: the protein chains unwind and interact with each other excluding water and forming large poorly solvated masses which precipitate.

Notes For Use

Isoelectric precipitation of casein:

Reagents:

Casein solution: Prepared as described before (p. 3). 10 cm^3 required per testing group or individual.
Acetic Acid: 20% in H^2O 10 cm^3 per testing group.

Procedure:

To 2 cm^3 casein solution in a test tube add 20% acetic acid dropwise. Mix well after each addition. Note the quantity of acetic acid added when precipitation occurs. Repeat the procedure but add less acetic acid — say 0.2 cm^3 less.

Repeat again with a further decrease in addition. In this way prepare a series of tubes with different pH values. Observe the tubes for 30 minutes and measure the pH of each solution using pH indicator papers. It should be noted that precipitation is quite marked in the region of pH 4.7. Failure to mix
thoroughly following addition of acid can cause precipitation at apparently higher pH values: this is because localised regions of lower pH exists before mixing.

"Salting - in" and "Salting - out":

Principle:

Neutral salts have marked effects on protein solubility. At low concentrations they increase the solubility of most proteins, a phenomenon known as "salting-in". That is, increasing concentrations of salt stabilize the charged groups on the protein thereby increasing its solubility. "Salting-out", observed at higher ionic strengths, is due to competition between the protein and the salt ions for the available water molecules for the purpose of solvation. When sufficient water for full solvation of the protein is unavailable, protein—protein interactions become more important than protein-water interaction and precipitation of protein aggregates takes place.

Salting-out is the basis for the widely used technique of ammonium sulphate fractionation and purification of proteins. Ammonium sulphate is the salt routinely used because it has a high solubility and is relatively cheap.

Reagents:

$(NH^4)^2SO^4$: Saturated solution in H^2O. 30 cm^3 per student or group.

Albumin and gelatin: 1% solutions prepared as described previously (p. 3).

Procedure:

To test tube No. 1 add 2 cm^3 gelatin solution and 2 cm^3 H^2O.
To test tube No.2 add 2 cm^3 albumin solution and 2 cm^3 H^2O.
To test tube No. 3 add 2 cm^3 gelatin and 2 cm^3 albumin solution.

To each test tube add saturated $(NH^4)2SO^4$ solution slowly with constant stirring. Note that gelatin precipitates when 3 – 4 cm of salt solution has been added. At that point leave all tubes to equilibrate for 10 minutes and allow fuller precipitation. Then centrifuge the contents of test tube No. 3 and decant the supernatant solution into a clean test tube. To that solution and to

the gelatin solution in test tube No. 2 add further saturated $(NH^4)2SO^4$ and note the increased quantity of salt required to precipitate the protein.

Organic solvents:

Principle:

The solubility of proteins is a function of the dielectric constant of the medium. Thus, solvents such as water or dimethylsulphoxide that have high dielectric constants favour protein solubility, whereas proteins are much less soluble in solvents with low dielectric constants e.g. acetone, methanol.

Addition of organic solvents, such as acetone, to protein solutions in aqueous media effectively lowers the concentration of water necessary for full solvation of the protein. Protein—protein interaction thereby increases and aggregation and precipitation take place. Since proteins vary in their solubilities in organic solvents, judicious choice of solvent and concentration of solvent allows fractionation of mixtures of proteins. Normally such work is carried out below 0°C to avoid protein denaturation. However the principle may easily be demonstrated at room temperature.

Reagents:

Propanone (Acetone)

Albumin and gelatin solutions (p. 3): 6 cm^3 each per testing group.

Procedure:

Prepare two test tubes, one contaning 3 cm^3 albumin solution and the other containing 3 cm^3 gelatin solution. From a burette, or safety pipette, add propanone to these solutions, slowly, drop by drop. The solutions should be mixed constantly in the course of these additions. Note the number of drops,
or volume, required to bring about precipitation of each protein.

Heat denaturation:

Principle:

Application of heat to a protein solution disrupts H bonds, particularly above 40°C. Since H bonds play an important part in maintaining the structure of each protein molecule heat clearly disrupts the equilibrium of these molecules. New conformations are adopted and frequently these promote protein—protein interaction, with the formation of insoluble aggregates.

Reagent:

Albumin (p. 3): 4 cm^3 per group.

Procedure:

Heat a solution of albumin and note the temperature at which precipitation, or coagulation, occurs.

Salts of heavy materials:

Principle:

Notes For Use

Salts of heavy metals, such as lead acetate, precipitate proteins because the metal ions form ionic bridges between protein molecules, building up large aggregates and decreasing protein—water interaction.

Reagent:

Albumin solution (p. 3): 6 cm^3 per testing group.

Procedure:

Add lead acetate solution (2% w/v in water) to albumin solution (3 cm^3 of albumin) drop by drop, with constant mixing. Note the effect of the lead acetate and the volume needed to precipitate the protein. Anomalous results may be obtained with gelatin and casein solutions depending on their source and purity.

Separation of 3 amino acids by paper chromatography and identification of an unknown amino acid:

Paper chromatography:

One of the most important separating techniques in chemistry and bio-chemistry is a process known as chromatography. In this experiment paper chromatography may be used to separate three amino acids and identify an unknown amino acid.

When filter paper (which consists of cellulose and about 20% absorbed water) is inserted into a chromatography tank containing an organic solvent, the solvent ascends the paper by capillary action without disturbing the absorbed water. If the paper is first spotted with amino acid solutions then the amino acids undergo innumerable partitions between the solvent (the moving phase) and the water (the stationary phase). If a number of different amino acids have been applied to the paper they will move at different rates; some will be largely retained by the absorbed water and move very little.

The amino acids are themselves colourless but when the paper is sprayed with a solution of ninhydrin a reaction occurs and the locations of the amino acids are easily discernible from the purple spots which appear. The centre of each spot is measured and its distance from the point of application of the amino acid is measured. The distance from the point of application to the solvent front is also measured. The relative rate of movement R^f value, is a characteristic of a given compound under fixed conditions (solvent, stationary phase, temperature etc.).

The R^f of a compound is defined as:

$$\frac{\text{distance a compound moves}}{\text{distance the solvent moves}}$$

The R^f of a spot may be used as a criterion of identity, particularly if known materials are used on the same paper as unknowns. The intensity of a spot is a measure of the amount of material present.

Materials:

Equipment:

- Large glass jars (with lids) at least 20cm high and with a cross-section not less than 8cm (e.g. large Kilner jars or Shandon chromatography jars)
- Whatman filter paper, 20 x 20cm
- Finely drawn glass capillary tubes
- Spray bottle or can

Notes For Use

Reagents:

2% ammonia solution:	10 cm^3 per chromatography jar.
Propan-2-ol (Isopropyl alcohol):	20 cm^3 per chromatography jar.
Glycine, leucine and aspartic acid:	0.1% w/v in H^2O.
Ninhydrin reagent:	Dissolve 1g ninhydrin in 100 cm^3 ethanol (absolute or 95%). Prepare daily.

Procedure:

i. Add 2% ammonia solution (10 cm^3) and propan—2—ol (Isopropyl alcohol) (20 cm^3) to a clean, dry chromatography tank. Cover the tank with a piece of glass or lid.

ii. Make a light pencil mark parallel to the bottom of a piece of 20cm x 20cm Whatman filter paper (Figure 1 overleaf). Along this line place eight light X's, two for each known and two for the unknown. Under each X place identifying marks to label the spots.

iii. Using capillary tubes place a small amount of each appropriate solution on its two positions along the line on the filter paper. Avoid getting the spot on the paper larger than about 2mm in diameter. Although your paper gives you two chances to make a proper addition it is advisable first to practise transferring solution to an ordinary piece of filter paper. Let the paper dry for a few minutes in air. Add a second portion of the unknown to one of its positions, to make certain that sufficient quantities of each component of the unknown will be present for good visual observation when the paper is developed.

iv. Roll the paper into a cylindrical form, and staple the ends together about a third of the way in from the edge (Figure 2 overleaf). Staple the paper in such a fashion that the ends of the paper do not touch one another — otherwise the solvent will flow more rapidly at that point and form an uneven front. When the spots on the cylinder of paper are dry (it may be necessary to place the paper in an oven for a short time; or a hair dryer may be used) place it carefully in the tank and cover with a piece of glass or lid. Make certain that the paper does not touch the sides of the tank and use care in keeping solvent from splashing onto the paper.
Let the solvent rise up the paper for at least 1 1/2 hours. If the time is shorter the components may not be sufficiently separated for easy identification. Remove the paper, mark the "solvent front" with a pencil, and place it upside-down on the bench to dry. When most of the solvent has evaporated open the cylinder by tearing it apart where it was stapled and hang it in a fume cupboard.

v. Spray the paper lightly but completely with a solution of ninhydrin and leave the paper in the fume cupboard until the spray solution is dry. Place the paper in an oven at 100 — 110°C for about 10 minutes — or until all the spots have developed.

vi. Circle each spot with a pencil and measure the distance that each spot has travelled (use the centre of the spot for measurement) from the starting line.

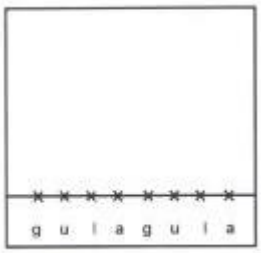

Fig.1 Preparation of paper for chromatography

Notes For Use

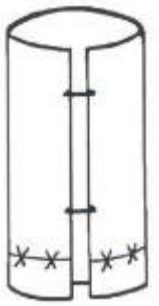

Fig.2 Stapled paper for chromatography

Results:

Calculate R^f values for each of the amino acids that you have used.

Determine the composition of the unknown by visual comparison of spot colours and by the relationship of the R^f values. Write a short account of the experiment.

Amino Acid Set:

Chemicals and Reagents:

Ninhydrin aerosol	
Ninhydrin 5g	
Biuret reagent	500 cm^3
Copper sulphate	500g
Sodium hydroxide	500g
Sodium hydroxide 2.0M	2.5 litres
Lead acetate	500g
Mil|on's Reagent	250 cm^3
Sodium Nitrite	250g
Sodium Hypochlorite 10%	2.5 litres
Ammonium Sulphate	500g
Propanone	1 litre
Propan-2-ol	500 cm^3
Tin (11) chloride	100g
Citric Acid	500g

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