EXPERIMENT-8(b)

AIM

To separate the given mixture of amino acids (glycine and valine) using radial paper chromatography.

Report their R_f values.

Solvent system is butanol: acetic acid: water

The spraying reagent is Ninhydrin reagent.

APPARATUS REQUIRED

Square whatman filter paper, petri dish, cotton, fine capillary

CHEMICALS REQUIRED

Glycine, valine, butanol, acetic acid, distilled water, ninhydrin reagent.

THEORY

Chromatography is a physicochemical method for separation of complex mixtures and was discovered in 1903 by Russian-Italian botanist M. S. Tswett. Chromatography is a modern and sensitive technique used for rapid and efficient analysis and separation of

components of a mixture and separation of components of a mixture and purification of compounds.

The basis of principle of chromatographic technique is based on the differential migration of the individual components of a mixture through a stationary phase under the influence of moving phase.

Stationary phase – the phase that is immobilized on the support particles, or on the inner wall of the column tubing.

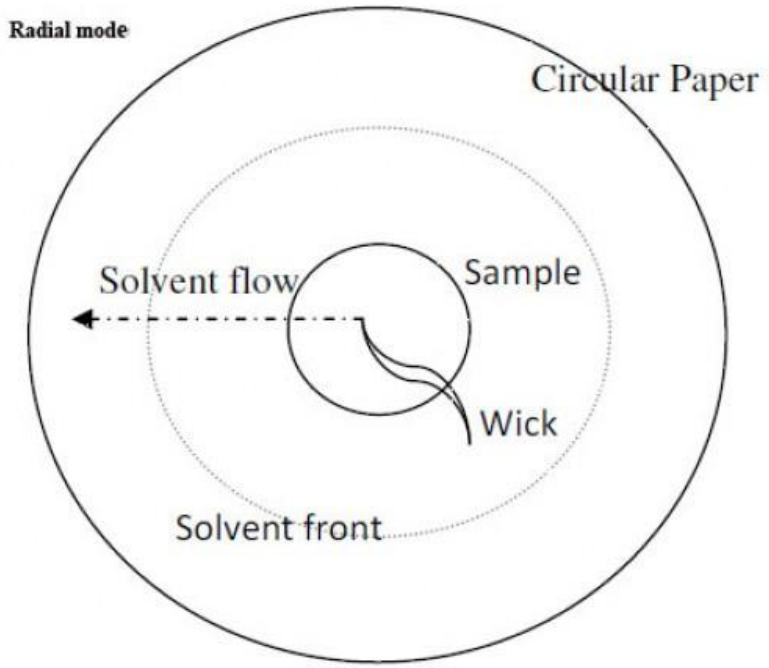
Mobile phase – the phase that moves in a definite direction.

Paper chromatography – This is a type of partition chromatography which involves paper placed in a jar containing a shallow layer of solvent and sealed. A small dot of sample solution is placed onto a strip of chromatography paper. As the solvent rises through the paper by capillary action, it meets the sample mixture which starts to travel up the paper with the solvent. Components of the mixture are carried along with the solvent up the paper to varying degrees, depending on the compound's performance to be adsorbed onto the paper v/s being carried along with the solvent. This paper is made of cellulose to which polar water molecules are adsorbed while the solvent is less polar usually consisting of a mixture of water and an organic liquid. The compounds within the mixture travel farther if they are non-polar. More polar substances bond with the cellulose paper quickly and therefore, do not travel so far.

Radial chromatography – It is also known as horizontal or circular chromatography. The method radially from centre (mid point) towards the periphery of a circular chromatogram with the help of a wick and petri dish. A circular chromatograph of suitable size is taken and two

perpendicular diameters are drawn to locate the centre of the chromatograph. The sample is spotted at the centre of the circular paper and is placed in a peti dish containing the solvent whose size is smaller than the paper. The horizontal development takes through the help of a wick held at the centre of the paper. The components appear as concentric bands rather than ovals or spots. Hence this mode gives a better resolution as compared to ascending or descending chromatography.

FIGURE 1: RADIAL CHROMATOGRAPHY



Identification of metals by radial paper chromatography:-

Radial paper chromatography as discussed earlier is based on the migration of sample from the centre of paper towards the periphery (horizontal or radial development). Two petri dishes, one inverted over

the other may serve as small tank. The filter paper disc with the applied sample is stretched between the two dishes, and the mobile phase is fed in through a cotton wick from the lower dish to the centre of the application of the sample. The solvent climbs the wick, touches the sample, and resolves it into the concentric rings.

In this experiment, dilute solutions of amino acids (glycine and valine) are applied or spotted in the centre of the circular chromatograph. If a comparison is to made, then each solution should be applied on the same place (centre). After the solutions have been applied, the paper is dried and placed in a petri dish which contains a few milliliters of the liquid mobile phase. For separation of amino acids, a mixture of solvents is employed as the mobile phase. As soon as the paper is placed in the mobile phase, the solution (sometimes called the eluting solvent) will begin to rise in the horizontal direction of the paper by the capillary action.

As the mobile phase rises on the paper, it will ultimately develop the amino acids as concentric circles. The outcome of each amino acid in the mixture depends on the affinity for the mobile phase than the stationary phase. If a amino acid has a higher affinity for the mobile phase than the stationary phase, it will tend to travel with the solvent front and be relatively unobstructed by the paper. In contrast, if the amino acid has a higher affinity for the paper than the solvent, it will tend to stick to the paper and travel more slowly than the solvent front. It is these differences in the amino acids affinities that lead to their separation on the chromatograph. The affinities of different amino acids for the mobile phase can be further linked to their solubilities in the mobile phase (developing solvent). This means that a amino acid

which is highly soluble in the mobile phase will have a higher affinity for the mobile phase.

FIGURE 2: STRUCTURE OF GLYCINE

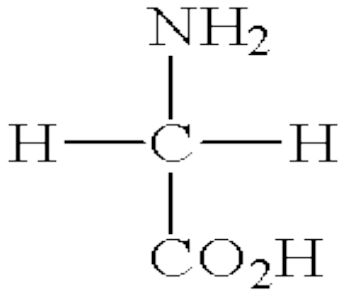
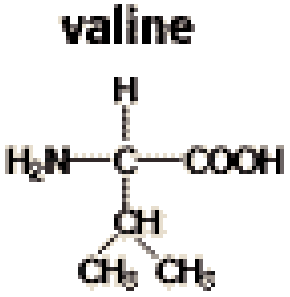


FIGURE 3: STRUCTURE OF VALINE



When the solvent reaches three-fourth of the paper, it is removed and the distance known as solvent front is marked with a pencil. The circular bands corresponding to each amino acid can be visualized by spraying the paper with visualizing agent called ninhydrin which reacts with metals to form colored complex.

The further the circular band from the starting spot, the higher the affinity of the amino acids for the mobile phase and faster is its migration. The relative distances are measured in terms of retention factor (R_f).

$$\mathbf{R_f = \frac{distance\ travelled\ by\ component\ from\ application\ point}{distance\ travelled\ by\ solvent\ from\ application\ point}}$$

DETECTION

If the components are of different color, the position of each component on the paper can be seen.

If the components are colorless there are number of techniques used to detect each component. For example:

a. Reactions with color producing reagent such as alizarin.
b. Place the sample under ultraviolet light.

FIGURE 4: STRUCTURE OF NINHYDRIN

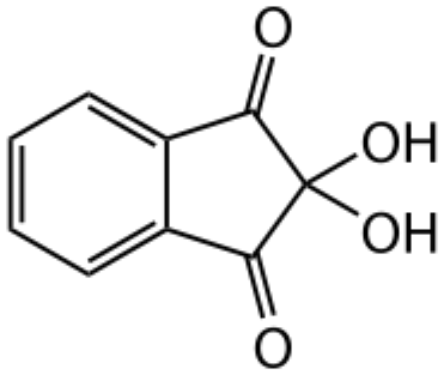
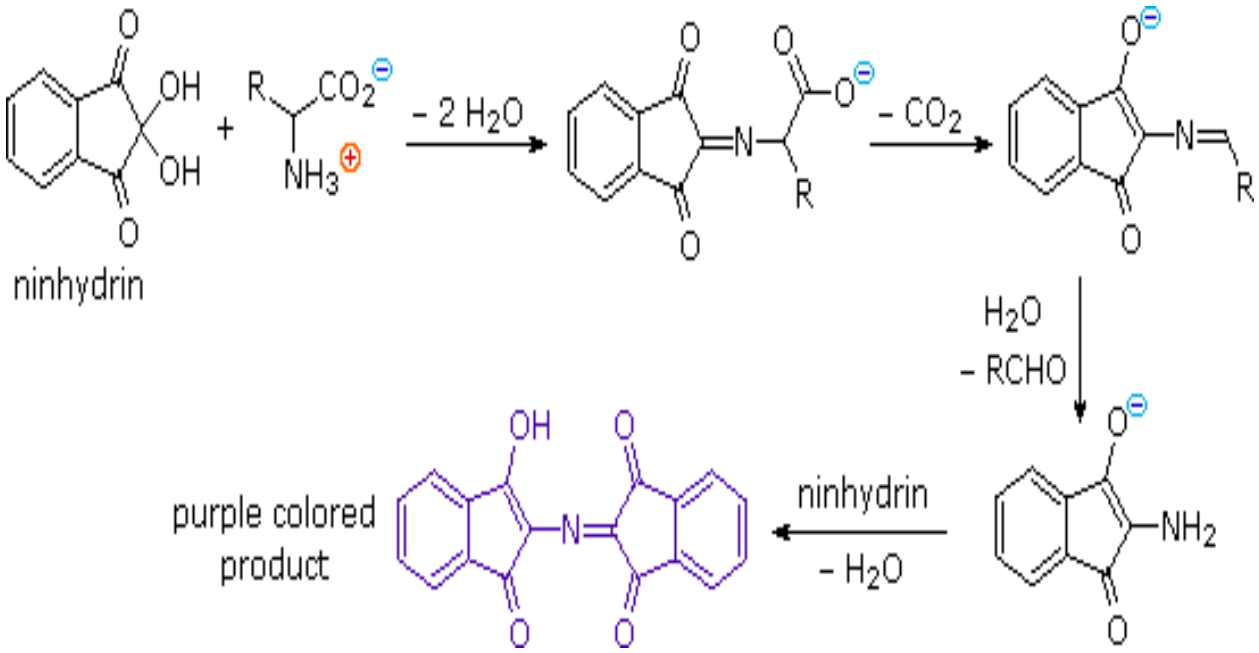


FIGURE 4: REACTION OF AMINO ACIDS WITH NINHYDRIN



PROCEDURE

1. Take a square whatman filter paper and mark the centre by drawing two perpendicular diameters. Make a hole with a pin at this point.
2. Make a wick of cotton and insert it through the hole of the chromatogram.
3. In a petri dish, place the solvent mixture (40-50 mL) and cover it with another petri dish.
4. Remove the wick from the chromatogram and apply the given mixture of amino acids (glycine and valine) using a fine capillary to spot at the centre of centre of the chromatogram.
5. Repeat the spotting two times and insert the wick back when the spot has dried.
6. Introduce the system of chromatogram and wick into the chromatography chamber (containing the solvent system).

7. Ensure that the chromatogram rests on the rim of the petri dish and does not dip into it. The chromatogram should remain in a stretched state and now cover it with another petri dish.
8. Leave the system undisturbed to allow the development of chromatogram.
9. When the solvent front has almost reached three-fourth of the chromatogram, remove it and immediately mark the solvent front with a pencil.
10. Spray the chromatogram with spraying reagent (ninhydrin reagent) to visualize the separated components. Calculate R_f value of each components at four different points.

OBSERVATIONS

S. No.	Component	Distance travelled at different points/cm				Mean distance/cm
		A	B	C	D	
1	Solvent					
2	Glycine					
3	Valine					

CALCULATIONS

A. Calculation of R_f value of glycine:-

$$R_f = \frac{\text{distance travelled by glycine from application point}}{\text{distance travelled by solvent from application point}}$$

B. Calculation of R_f value for valine:-

$$R_f = \frac{\text{distance travelled by valine from application point}}{\text{distance travelled by solvent from application point}}$$

RESULT

1. R_f value of glycine =

2. R_f value of valine =

INTERPRETATION OF RESULT

R_f value of valine is greater than glucose. This is because valine is more soluble in mobile phase than in stationary phase and has more affinity towards the mobile phase and hence travels further. Glycine is less soluble in mobile phase than in stationary phase and has more affinity towards the stationary phase and hence do not travels further.

PRECAUTIONS

1. The chromatogram should remain in the stretched position on the rim of the petri dish.
2. Do not disturb the system while the chromatogram develops.
3. The chromatogram should be dried properly before introducing it in the chromatography chamber.
4. The solvent mark should be marked immediately with a pencil after removing it from the chromatography chamber.