

SEPARATION OF AMINO ACIDS BY PAPER CHROMATOGRAPHY

AIM- The aim of the experiment is to perform Ascending paper chromatography for the separation of amino acids present in the given sample.

BACKGROUND-

Chromatography is used to separate mixtures of substances into their individual components. All forms of chromatography work on the same principle. They all have basic requirements of *stationary phase* (a solid or a liquid supported on a solid) and a *mobile phase* (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it. Different components travel at different rates based on their affinities toward stationary phase and mobile phase.

In paper chromatography, the stationary phase is a very uniform adsorbent paper. The mobile phase is a suitable liquid solvent or mixture of solvents.

Retention (or) retardation factor (R_f)-

Retention factor is defined the ratio of the distance travelled by the solute to the distance travelled by solvent. The distance travelled relative to the solvent is called the R_f value.

$$R_f = \frac{\text{distance moved by the substance from origin}}{\text{distance moved by solvent from origin}}$$

The value of retardation factor should lie between 0-1.it has no units.

The sequence of steps involved during the reaction between ninhydrin and amino acids to produce purple (or) yellow-orange colour.²

REQUIREMENTS-

Apparatus: Glass beakers

Whatmann filter paper

Petridishes

Measuring cylinder

Developing chamber and capillary tubes etc.

Chemicals: n-butanol

Glacial acetic acid

Distilled water (4:1:5)

Amino acids (Tryptophan and threonine)

Ninhydrin reagents.

Solvents system and its preparation methods

1. n-butanol and water are taken in 4:5 ratios in a conical flask and allow

it to saturate for 24 hours. By using separating funnel separate nbutanol and water.

The saturated n-butanol and Glacial acetic acid are taken in the ratio of 4:1 which can be used as a solvent system (or) mobile phase.

Ascending paper chromatography

The procedure for ascending paper chromatography method is quite simple as compared to other methods of chromatography.

The chromatography paper is cut into rectangular strips and marks a line on the paper with pencil at about 2 cm from the bottom. With the help of capillary tube, the samples are applied at different points on the starting line

Now, place the chromatography paper in the developing chamber, which contains the mobile phase. While placing the paper, it is important that the solvent level should not reach the starting line or the sample spots and paper shouldn't touch the walls of the developing chamber. After sometime the solvent rises up the paper or the stationary phase by capillary action and dissolves the sample. The components of the sample move along with the solvent in upward direction.

Check if the solvent has reached near the top level of chromatography paper. Then the paper is removed when it reaches the top and marked the level with pencil. This level (or) height is called the "solvent front". By using UV light, ninhydrin or iodine vapors examined the different spots of varied colors. Each spot represents a specific component of the sample.

CONCLUSION-

For ascending paper chromatography

The distance moved by tryptophan and threonine is cm and cm respectively, and the solvent iscm

Rf value of tryptophan is

Rf value of threonine is

Rf value of unknown mixture is &

By performing the Ascending paper chromatography, the distance moved by the sample and unknown mixture is noted and by substituting these values in the given Rf formula, the Rf values of tryptophan, threonine and unknown samples are known.

By performing the Ascending paper chromatography Rf values of both tryptophan and threonine are found to be & respectively, and the unknown samples are found to be tryptophan and threonine.

By performing the radial paper chromatography Rf values of both tryptophan and threonine was found to be & respectively, and the unknown samples were found to be tryptophan and threonine.

REFERENCES-

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