

Paper Chromatography- Solutions

Solvent

N Butyl alcohol	250ml
Distilled water	250ml
Glacial acetic acid	60ml

Mix N Butyl alcohol and Distilled water .To this mixture, add Glacial acetic acid and shake well. Keep for 15 minutes for the separation of layers. Carefully draw the upper layer (without mixing the lower layer) and use it as solvent.

Ninhydrin solution

Ninhydrin powder	0.3gm
Acetone	90ml
Distilled water	10ml

Mix 90ml Acetone and 10ml Distilled water. To this add 0.3gm Ninhydrin powder and shake well.

Caution: Ninhydrin is toxic. So handle carefully and wash hands with soap or hand wash after using Ninhydrin.

Preparation of Amino acid samples

Prepare 10% Isopropyl alcohol using distilled water. To 10ml of this solution, add 1mg amino acid powder and mix well. Spot amino acids on the paper (small spot) using fine end toothpick and dry immediately using the hair dryer.

Rf Value

Rf value is the **Retention factor** used to identify the unknown amino acids. It is the rate at which the amino acids move along with the solvent. Each amino acid has a particular rate of movement. So by calculating the Rf value, it is easy to identify the amino acid from the standard Rf value chart.

Rf value = Rate of solute movement / Rate of solvent movement.

That is, measure the distance from the base line to the upper end of solvent moved and the amino acid spot in cm and calculate the Rf value.