Acetone

LIST OF APPARATUS:

1 100ml graduated cylinder
1 dozen capillary tubes (10µL)
1 1500ml or 1000ml beaker
1 30cm ruler and a pencil
1 50ml graduated cylinder

GENERAL:

2-3 large sheets of chromatography paper (filter paper), 2 cans of ninhydrin spray, aluminum foil, oven (100-110°C), solvent (isopropanol and ammonium hydroxide), unknown samples and standard amino acids.

PROCEDURE:

Solvent phase. Obtain from the laboratory instructor 10ml of 2% ammonium hydroxide and 20ml of isopropyl alcohol, and pour into you're your beaker. Cover tightly with foil and allow solvent vapour to saturate the atmosphere inside the beaker. Label five clean test tubes and initial them G, T, L,A and U. Obtain from the instructor several drops each of glycine, tyrosine, leucine, aspartic acid and an unknown sample. Record the number of the unknown. (each solution is about 0.05M amino acid in a solution of 1.5% hydrochloric acid.)

Stationary phase. Obtain from the instructor a clean sheet of whatman #1 filter paper, about 12cm by22cm, and make a light pencil line parallel to the bottom (fig.1) and about 2.0cm away. Along this line, at intervals of about 2cm, place ten light x's under each x place identifying marks, two for each known and two for the unknown. 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. (practise spotting on a loose piece of paper of paper first and check with T.A for correct procedure.) let the paper dry for a few minutes in air. Add a second portion of the unknown to one of its two 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

Roll the paper into a cylindrical form, and staple the ends together about a third of the way in from the page (fig.2. ) staple the paper in such a fashion that the ends of the paper do not touch each other - otherwise the solvent will flow more rapidly at that point and form an uneven front.

When the spots on the cylindrical paper are dry (it may be necessary to place the paper in an oven at about 100°C for a short time), place it carefully in the beaker of solvent, and cover carefully and tightly with the aluminum foil.

Do not allow the paper to touch the sides of the beaker nor solvent to slash onto the paper.

Let the solvent rise up the paper for at least 1.5 hours, if the time is shorter, the components may not be sufficient separated for easy identification. Remove the paper and mark the solvent front before the solvent dries. Place the chromatogram upside down on the desk top 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 hood (Fig.3).

Spray the paper lightly but completely wig a solution of ninhydrin *, and leave in the hood until the spray solution is dry