Thin layer chromatography (TLC)

Objectives:

1. Separate and identify a mixture of different amino acids by TLC.
2. Detect the presence of amino acids in urine.

Introduction:

In TLC the stationary phase is a thin layer of silica gel spread uniformly on a glass plate. The mobile phase is a solvent that consists of a mixture of water, acetic acid and methanol.

The separation of compounds by chromatography depends on several factors:

1. Partition of a solute between a moving solvent phase and a stationary aqueous phase. The solute moves in the direction of a solvent flow at a rate determined by the solubility of the solute in the moving phase. Thus a compound with high mobility is more attracted to the moving organic phase than to the stationary phase.
2. Ion exchange effect: any ionized impurities in the support medium will tend to bind or attract oppositely charged ions (solutes) and will therefore reduce the mobility of these solutes.
3. Temperature: Since temperature can effect the solubility of the solute in a given solvent temperature is also an important factor.
4. The molecular weight of a solute can also affects the solubility and hence chromatographic performance.
5. Adsorption of compound (solute) onto support medium: Although the support medium (silica gel) is theoretically inert, this isn’t always the case. If a solute tends to bend to the support medium this will slow down its mobility in the solvent system.
6. The composition of the solvent: since some compounds are more soluble in one solvent than in the other, the mixture of the solvents used will affect the separation of compounds.

Expression of the results in chromatography

The term "Rf" (relative flow) is used to express the performance of a solute in a given solvent system / support medium. The term Rf value may be defined as the ration of the distance moved by the solvent. Rf value is constant for a particular compound, solvent system and insoluble matrix.

Rf = Distance of migration of solute

Distance moved by solvent

Material and method:

As in lab sheet