Amino Acid Identification by Thin Layer Chromatography

Introduction:

Chromatography is a method of separating the components of a mixture based on their differential affinity for two chemicals, one of which is immobilized (the "stationary phase") and the other mobile (the "mobile phase"). As the mobile phase travels across a layer of stationary phase, it will carry with it components of the mixture. Those components that interact well with the mobile phase but poorly with the stationary phase will travel right with the mobile phase; those that interact poorly with the mobile phase but strongly with the stationary phase will not travel as quickly. As the mobile phase moves, then, it carries components of the mixture at different rates. When the mobile phase is stopped, different components will have traveled different distances.

Interactions between the components of the mixture and the stationary and mobile phases may include charge interactions or interactions between polar substances, including hydrogen bonding interactions. For instance, a TLC system can be set up with a very polar stationary phase and a nonpolar solvent. When a mixture containing substances of varying polarity is added to the mobile phase and carried across the stationary phase, the more polar compounds within the mixture will not travel as quickly. The less polar substances in the mixture will, over a limited amount of time, travel farther.

Thin layer chromatography uses a solid stationary phase coated onto a glass or plastic support. Samples are placed on one end of the coated sheet. When the edge of the sheet is placed into a beaker of solvent, the solvent travels up the TLC sheet, picking up and carrying components of the mixture with it. Our TLC plates are coated with silica gel, which is very polar. We will be using a mobile phase composed of ethanol and ethyl acetate in a 90:10 ratio. The mobile phase is more nonpolar than the stationary phase. The system will be used to separate amino acids based on their degree of polarity.

The separation principles are the same in all types of chromatography: gas chromatography, high performance liquid chromatography (HPLC), and column chromatography. These methods are often used not only to separate mixture components but also to identify them (based on their separation behavior under controlled circumstances): finding drugs in blood or urine samples, finding poisons, detecting flame accelerants in arson investigations, etc.. Stationary phase and mobile phase interactions can be of many types, including those based on molecular size or even based on interactions between antigens and antibodies.

Materials:

Amino acid solutions
Ninhydrin solution in spray bottles (use in the hood) (0.1% in acetone)
Solvent for TLC (90:10:: ethanol:acetic acid) or (Butanol:Acetic acid:water:: 5:4:1)
Unknown sample
TLC plates

Special equipment:

Plastic wrap
Capillary pipets for spotting solutions
Rulers
Hot air oven (used in the hood)
Gloves for spraying ninhydrin

Procedure:

1. **Preparation of TLC plates**: There are different types of adsorbent material or stationary phase used to prepare TLC plates some commonly used adsorbent include silica gel, kelsigur and alumina. The choice of adsorbent depends largely on the class of compounds

to be separated but silica gel is usually used for separating a wide variety of substance. Here we are using slical gel for separation of amino acid.

2. Clean the glass plates with detergent and then dry them in oven. Place the glass plate on a plane surface of the bench.

3. **Preparation of slurry**; Silica gel is weighed and is mixed with calcium sulphate Binder in the ration 17:3. (In our case we have slilca gel mixed with binder calcium sulphate). The slurry is prepared by adding 2 parts of distilled water to 1 part of silica adsorbent and 4the resultant mixture is stirred properly. Mix the slurry by stirring it for 2 min. and pour it on plate and spread it uniformly. (Applicator can be used for the purpose)
4. Keep the plate as such at room temp. for 20-30 minutes to dry.
5. Activate the plate by heating them at 100-130^{O}C for 45 min to 2 hours in an oven. These activated plates if not to be used immediately, store in a desiccators.

Sample application

1. Take a graph paper and place glass plates in such a way that it align with the lines on the graph paper. Leave 2.5 cm from the one end of the plate and 2.5 cm from the base of the plate.
2. Apply the sample and the standard by capillary tube. Apply all the sample in such a pattern that first spot must be 2.5 cm, from the base and along side of the plate and subsequent spots are separated by 3 cm each for excellent results.
3. Be careful while applying the samples so that the adsorbent should not flake off.
4. Not the distance from base where you have applied sample and not the spot of standard and sample.
5. Allow the sample to dry at room temperature.
6. Pour the solvent (90:10:: ethanol:acetic acid) or (Butanol:Acetic acid:water:: 5:4:1) in the tank. Allow this solution to stand for 20 to 30 min at room temp to saturate the tank with vapour. This process is technically referred as equilibration.
7. Remove the cover plate and place the plate vertically in the chamber (tank) in such a way that the end of the plate dips into the solvent system.
8. Replace the cover and allow the chromatogram to develop. Do not disturb during run.
9. After 15 cm solvent run (90 min), remove plate from tank.
10. Dry it first at room temp and then 5 min in hot air oven.
11. Spray ninhydrin (0.1% in acetone)
12. After air drying, place the plate in hot air oven till color develops.
13. Calculate Rf values for each amino acid according to this equation:

Rf = distance traveled by spot / distance traveled by solvent

Record Rf values for each amino acid you test as well as the unknown (mixture).