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Chemistry Practical, Instruction Sheet
Lab Manual

Objective:

Find out the RF value of the given amino acid by thin layer chromatography (TLC) and identify the amino acid present in a given mixture by TLC.

Theory:

Chromatography is a separation technique which depends on the differential distribution of the components of a mixture between a mobile (bulk) phase and an essentially thin film stationary phase. The stationary phase may be either in the form of a packed Column (Column Chromatography) through which a mobile phase is allowed to flow, or in the form of a thin layer adhering to a suitable form of backing material (Thin Layer Chromatography) over which the mobile phase is allowed to ascend by capillary action.

In the Thin Layer Chromatography it is usual to employ glass plates coated with layers of solid stationary phase which adhere to the plates generally by virtue of a binding agent such as Calcium Sulphate which is incorporated. The prepared Thin Layer Chromatography is often called a Chromaplate. The most commonly used stationary phases which are available include Silica gel, alumina, and Kieselguhr and Cellulose powder.

RF Value:

The movement of any substance relative to the solvent front in a given chromatography system is constant and characteristic of substance. The constant is the RF value and is defined as

$$\text{RF value} = \frac{\text{Distance moved by substance}}{\text{Distance moved by the solvent front}}$$

GENERAL PROCEDURE:

1. Preparation of Plates:

Before glass plates are coated with adsorbent they must be carefully cleaned with detergent and rinsed thoroughly with water and dried in oven.

A slurry is prepared by the slow addition with shaking of 30 g of adsorbent (mostly silica gel) to 100 ml of CCl_4 contained in a wide necked bottle. A pair of microscopic slides are held together and dipped into slurry. Slowly withdrawn allowed to drain momentarily whilst held over the bottle. The slides are parted of carefully and placed horizontally in a rack and dried for 10 minutes.

2. Loading of plates:

Wipe excess adsorbent from the back and edges of the plates. The sample is applied by means of a sample application (capillary tube) at a distance nearly 5mm from the bottom edge.

Plates are then placed to the jar containing developing solvent so that bottom of the adsorbent layer is submersed. The solvent level should not however as far as the spots. Cover the jar and allow the solvent to ascend by capillary action to the finishing line which has been scored across the plate. After removal, the plate is dried suitably depending upon the volatility of the solvent.

3. Location of spot :

The position of the colored components can usually be seen without any difficulty. Most of the colorless organic compounds can be detected with the help of iodine vapours. The dried plates are allowed to stand in a closed tank containing a good supply of iodine crystals scattered over the tank bottom. Usually the spots are revealed as brown strain.

Another general locating procedure involve spraying of the plate with conc. H_2SO_4 in methanol and then heat the plates in oven to about 200°C until the organic materials are revealed as dark charred spots.

Chemical methods for the detection of colorless compounds by the use of a suitable chromogenic spray reagent are widely used. Many of these are for a particular functional group or groups and may be extremely sensitive e.g. ninhydrin reagent for the detection of amino acids.