



TLC Analysis

Experiment

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Introduction

Thin Layer Chromatography (TLC) is a technique used to separate mixtures of compounds. This technique can be performed on a sheet of glass, plastic, or aluminum foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminum oxide, or cellulose. This layer of adsorbent is known as the stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved.

Paper chromatography uses paper as the stationary phase and a liquid solvent as the mobile phase. The technique relies on the idea that the solvent and the paper both have an attraction for the components in a mixture. The solvent creeps along the surface of the paper. If a material is placed on one spot on the paper and is soluble in the liquid solvent, the material will be dissolved when the solvent moves over it. The material will move along with the solvent. Each compound in a mixture will have its own characteristic balance of attractions to solvent and to paper, so all will not move at the same speed. Eventually this difference in speed will separate the compounds.

In paper chromatography when the conditions are kept constant, a particular compound always travels a fixed percentage of the distance traveled by the solvent front. The ratio of the distance the compound travels to the distance the solvent travels is called the R_f value. The symbol R_f stands for "retardation factor" or "ratio-to-front". It is expressed as a decimal fraction. When the conditions are duplicated, the same average relative positions will turn up for the solvent and solute; thus the R_f value is a constant for a given compound. The R_f value is a physical property for that compound. The R_f value is useful in identifying compounds, but other properties should be used in combination with the R_f value to confirm compound identification. Since it is difficult for different laboratories to exactly duplicate conditions for a chromatography experiment, R_f values are more useful for comparisons within one lab than for comparisons of data from different labs. In this experiment, you will analyze food dyes using paper chromatography.

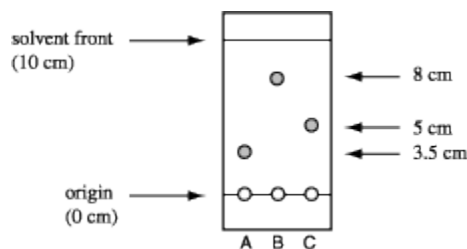
Procedure

1. Obtain an 18.5 x 8 cm square of TLC paper. Using a pencil (not ink from a pen), draw a line ~1 cm from the edge of bottom side of the paper.
2. Make six pencil dots (or however many colors you have) along this line, about 2.5 cm apart. Underneath each dot, label the color of the sample you will test on that spot. You won't have space to write the whole color name. Try B for blue, G for green, or something equally easy.

- Place a few drops of each color on a spot plate. Dip a toothpick into a color and dab the color onto the pencil dot for that color. Use a clean toothpick for each color. Try to keep each dot as small as possible. Allow the filter paper to dry, then go back and add more color to each dot, a total of three times, so you have lots of pigment in each sample.
- When the paper is dry, roll it into a cylinder and staple the ends together and position the chromatography paper into a 250 mL beaker with the spots on the bottom. The beaker should tall enough to extend beyond the paper.
- Fill the beaker with a 1% NaCl solution until the liquid is about 0.5 cm from the bottom of the colored samples. Place a watch glass over the top of the beaker to minimize evaporation of the solvent.
- Capillary action will draw the salt solution up the paper. As it passes through the dots, it will begin to separate the dyes. You will notice some colors may contain more than one dye. The dyes separate because some dyes are more likely to stick to the paper, while other dyes have a higher affinity for the salt water. In paper chromatography, the paper is called the 'stationary phase' and the liquid (salt water) is called the 'mobile phase'.
- When the salt water is about 2.0 cm from the top edge of the paper, remove it from the glass and place it on a clean flat surface to dry. Remove the staples so the paper is flat. Using a pencil, mark the height in which the solvent advanced on the paper.
- When the filter is dry measure the height of the solvent traveled as well as the height that the dyes traveled. For the mixtures, you will have two measurements.

Analysis

Determine which colors were present in each mixture.



Calculate the R_f factor for each colored dye.

$$R_f = \frac{\text{distance dye traveled}}{\text{distance solvent traveled}}$$