Monitoring Reactions by TLC

The fastest and most commonly used method to follow the course of an organic reaction is by thin layer chromatography (TLC). If performed properly, one can use this simple technique to
(1) determine the presence of starting material in the reaction
(2) detect and monitor the (qualitative) rate of formation of product or products
(3) infer a product's identity from its mobility and/or visualization properties

Needed equipment and supplies:

(1) a TLC chamber equipped with a lid

(2) silica gel plates (6 cm x 2.5 cm) impregnated with a phosphorescent dye (Merck silica gel 60 F-254 or JT-Baker Si250F, 0.25 mm thickness).

(3) TLC solvents - A binary mixture of miscible solvents usually works best. TLC solvents are conveniently made up in the TLC chamber just before analysis from solvents kept in dropping bottles. Common TLC solvent combinations are:
    • For neutral organic molecules: hexanes (bp 68-70 °C) as the non-polar component and ethyl acetate (EtOAc, bp 77-78 °C) as the polar component. The choice of solvents with similar boiling points assures that their ratio will not change dramatically over short periods of time due to differential evaporation.
    • For carboxylic acids that tend to "tail": Diethyl ether (2 mL) + acetic acid (2 drops). Because diethyl ether boils at 35 °C, this solvent system should be made fresh each time.
    • For amines and aminoacids: 4:1:1 or 2:1:1 n-butanol-water-acetic acid.

(4) a 10 mL graduated cylinder

(5) supply of disposable hand-drawn glass capillaries. These can be conveniently made by heating a disposable pipette over a Fischer burner then until it is red then quickly drawing out both ends.

(6) a hand-held 254 nm UV lamp

(7) an iodine chamber (iodine/silica gel)

(8) a variety of charring solutions:
    • For general use: Verghn's reagent (12.5 g ammonium molybdate + 0.5 g ceric sulfate dissolved in 250 mL of aqueous sulfuric acid)
    • For general use: 5% (w/v) p-anisaldehyde in 95:5:1 ethanol-acetic acid-sulfuric acid
    • For general use: 2% (w/v) vanillin in 98:2 ethanol-sulfuric acid
    • For 1° and 2 ° amines: 0.3% (w/v) ninhydrin in 97:3 ethanol-acetic acid

Procedure:

Suppose you obtain a chemical sample and want to determine (1) how many components are in the sample and (2) what the identity of the component(s) is (are). Figure 1 shows an idealized depiction of how TLC can be used to address such a problem. In this example, a small quantity of the sample was "spotted" at the origin (lower dotted line) of lanes 1, 3, and 5. The origin can be marked with pencil (not ink). The sample solution can be made by dissolving <1 mg of sample in an appropriate solvent (usually ethyl acetate) in a clean, labeled glass vial. (It is a good idea to spot the sample in 3 lanes to be sure that the solvent front has advanced uniformly otherwise you R_f data will be unreliable.) Authentic sample B was spotted at the origins of lanes 2, 3 (co-spot), and 4. The TLC plate was developed in an appropriate solvent system and the solvent front marked in pencil and the spots visualized (UV, iodine, and/or char). From this TLC, we can conclude that there are 3 compounds in the mixture. Compound B is shown to be identical (by TLC) to an authentic sample so its structure can be assigned.
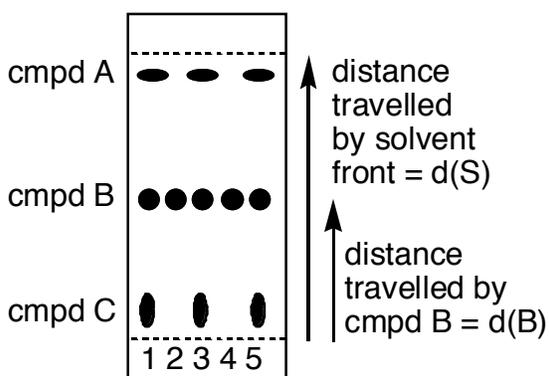
lanes 1, 3, & 5: mixture of cmpds A, B, and C
lanes 2, 3, & 4: authentic sample of cmpd B

Figure 1. Idealized analysis of a mixture by TLC.