Thin Layer Chromatography

7 Thin Layer Chromatography (TLC) is the second of 3 types of chromatography you will use in this laboratory course:
- Gas Chromatography
- Thin Layer Chromatography
- Column Chromatography

7 Chromatography has been a very important development in improving chemists’ ability to separate and purify organic compounds.
All forms of chromatography work on the same principle. They all have a *stationary phase* (a solid, or a liquid supported on a solid) and a *mobile phase* (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it. If the components interact differently with the stationary phase, separation occurs.

Thin layer chromatography is done exactly as it says - using a thin, uniform layer of a porous solid material coated onto a piece of glass, metal or rigid plastic. In our lab, we use silica gel as the solid phase. The mobile phase is a liquid solvent or mixture of solvents that moves through the porous stationary phase.
Stationary Phase – Silica Gel

7 Silica gel is a form of silicon dioxide (silica). The silicon atoms are joined via oxygen atoms (Si-O-Si bonds; Si is tetrahedral) in a giant covalent structure. However, at the surface of the silica gel, the silicon atoms are attached to -OH groups.

[Diagram of silica structure]

main body of silica structure

Stationary Phase – Silica Gel

7 The surface of the silica gel is very polar because of the -OH groups, and can adsorb compounds onto the surface by forming hydrogen bonds as well as by van der Waals dispersion forces and dipole-dipole attractions.

[Diagram of silica structure]

main body of silica structure
Steps in TLC separations
(See Lab Manual – pages 43 – 47)

1. Adsorb sample onto plate by ‘spotting’. The sample is dissolved in a volatile solvent (~ a 1% solution; 10 mg/mL).

   Dip the microcap into solution - the arrow points to the microcap; it is tiny and hard to see.

   Make sure it is filled - hold it up to the light if necessary.

   Touch the filled microcap to TLC plate to spot it – use multiple touches to keep spot small (1-2 mm).

Steps in TLC separations

2. Place Plate in Developing Chamber. The chamber should contain 5-6 mm of solvent in the bottom and should have been filled and capped prior to spotting the plates to allow evaporation of solvent in the chamber.

   Cap the chamber immediately after placing the plate in the chamber.
Steps in TLC separations

3. Allow solvent to ascend the plate. The solvent will move up the plate by the effect of capillary action – the silica surface attracting the solvent onto it. As the solvent moves past the adsorbed compound, a distribution between plate and solvent will be established.

4. Remove the plate from the chamber and mark the solvent front with a pencil line before solvent evaporates. Allow solvent to evaporate.

5. The next step is to mark the location of the spots on the plate. When the compounds separated are not colored, we have to use a visualization technique.
6. Visualization. To visualize colorless components on a TLC plate you must either use a plate with a fluorescent indicator and observe the plate under ultraviolet illumination, or you can use a second method: Spray Reagents. These reagents are mixtures of chemicals that, when sprayed on the plate and allowed to react, will cause colorless compounds to change to colored compounds.

- We will use UV light and a fluorescent indicator in this experiment since our analgesic components all absorb light in the UV region.

6. Our TLC plates contain a compound added to the silica gel that absorbs light in the UV region and then emits that energy in the visible (green) region. If an organic compound is present on the plate to absorb the UV light, little or no fluorescence is observed and the area will appear dark.

- You need to let all of the eluting solvent evaporate before you use the UV light.
- You have to mark the location of the spots while the plate is under the UV light.
Steps in TLC separations

7. The final step is to calculate $R_f$ (ratio of fronts) values for the spots. This value is the distance traveled by a spot (center of spot) divided by the distance traveled by the solvent front.

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R \text{ (blue)} = \frac{d(2-1)}{d(4-1)}
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R \text{ (red)} = \frac{d(3-1)}{d(4-1)}
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Analgesics

7. Definition – Analgesics are a class of drugs used to relieve pain.

7. The pain relief induced by analgesics occurs either by blocking pain signals going to the brain or by interfering with the brain's interpretation of the signals, \textit{without} producing anesthesia or loss of consciousness.
Analgesics

There are two types of analgesics – narcotics and non-narcotics. All non-prescription analgesic preparations contain only non-narcotic active ingredients. Currently, only five compounds are approved by the FDA for OTC analgesic preparations: aspirin, acetaminophen, ibuprofen, ketoprofen, and naproxen sodium.

Structures of Analgesics

caffeine
1,3,7-trimethylxanthine
The eight preparations studied today are: Advil, Aleve, Anacin, Bayer, Excedrin Extra Strength, Excedrin Tension Headache, Motrin, and Tylenol. Some of these are single ingredient preparations while others have more than one ingredient.

Each of you will analyze one of these preparations. You will cooperate with two other students to determine the best of three different solvents to analyze these preparations. You will then do one more analysis of your sample with the best solvent to determine the components present.
Solvent Selection

7 Since silica gel is quite polar, most organic compounds with polar functional groups are adsorbed relatively tightly.
7 A non-polar solvent will leave polar compounds very near the original spot and will show little separation of components.
7 Very highly polar solvents will move non-polar compounds very near the solvent front and again show little separation between similar compounds.

Procedure Comments

7 You will use commercial microcapillary pipettes as spotters. Do not waste these capillary tubes. Take what you need; keep your unused ones clean and return any unused tubes to the dispenser.
Cleanup and Safety

7 Silica gel dust can damage corneas of eyes. Wash hands after handling plates.
7 Avoid looking directly into a UV lamp.
7 Dispose of chemicals, spotting capillaries and pasteur pipettes in the appropriate containers.
7 Rinse your beaker with analgesic with acetone into the waste bottle.

Cleanup and Safety

7 Although these analgesics are OTC drugs, they are considered toxic and should not be disposed of down the drain.
7 Do not rinse or wash the TLC jar. Dispose of the solvent in the container and recap the container after solvent has evaporated.