Biochemistry I Laboratory
CHEM 4401
Chromatographic Methods of Lipid Separation

References:

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Moran, L.A. and K.G. Scrimgeour. 1994. Biochemistry resource book. Prentice-Hall, Inc., Upper Saddle River, NJ. 287 pp.

Plummer, D.T. 1971. An introduction to practical biochemistry. McGraw-Hill Book Co. (UK), Ltd., Maidenhead, Berkshire, UK. 369 pp.

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Background

As we have seen, Thin Layer Chromatography (TLC) is a very simple but effective procedure for the separation and identification of biochemical compounds. In addition to amino acids (e.g. "Separation of Amino Acids Using Thin Layer Chromatography" experiment), TLC can be used to separate and identify carbohydrates, proteins, peptides, sterols, steroid hormones, fatty acids, and other organic acids.

As we have seen, perhaps one of the most significant properties affecting the separation and subsequent migration of biochemical compounds via TLC is the polarity of the solvent used relative to the polarity of the solute. A high velocity of migration in the direction of capillary flow indicates greater attraction of the solute for the solvent, whereas low migration velocity indicates the opposite. By varying the polarity of the solvent(s) used in TLC, a wide variety of separation regimes may be explored.

Factors other than polarity are also important for determining the rate of solute migration. These include the molecular weight of the solute, the type of chromatography substrate used (paper vs. silica-coated plates), the size of the sampling chamber, the relative miscibility of the solvents used, ambient temperature, and so forth. The migration rate of the solute in the direction of the solvent flow is characterized by the term R_f, which is the distance the solute migrates from the starting point divided by the distance traveled by the solvent front:

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent band}}$$

Using Thin Layer Chromatography, it is possible to separate the major components of lipid fractions (hydrocarbons; tri-, di-, and mono-glycerides; sterols; phospholipids; etc.) from one another. Using samples of known hydrocarbons, sterols, phospholipids, and organic acids, these

may be easily compared to those fractions separated from natural lipid mixtures, such as vegetable, olive, or sunflower oil. Further characterization of these lipid fractions may be performed by utilizing solvents of varying polarity.

For example, cooking oils (vegetable, canola, corn, olive, sunflower, etc.) contain a complex mixture of triacylglycerol, fatty acids and other lipids. To identify these components, a variety of known samples will also be chromatographed to establish comparative Rf values.

α-tocopherol

Phosphatidylcholine

Cholesterol

Oleic Acid
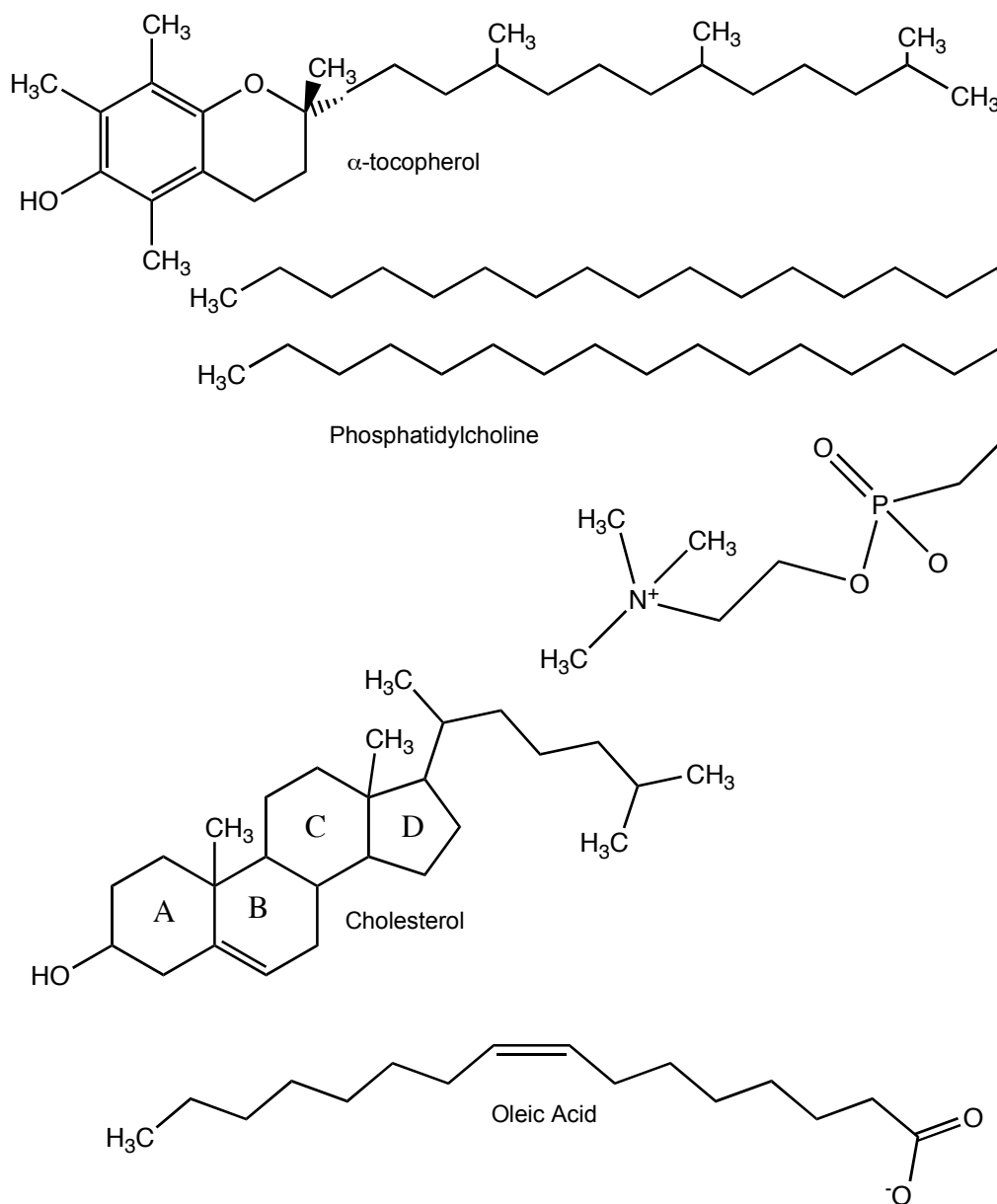
Lipid Fraction Separation via TLC:

Procedure:

1) Prepare one, 6.5 x 15 cm TLC plate.

2) Using a pencil, draw a line 1 cm from the bottom of the short side of the TLC plate. Equally space 6 marks across this line for spotting of lipid samples
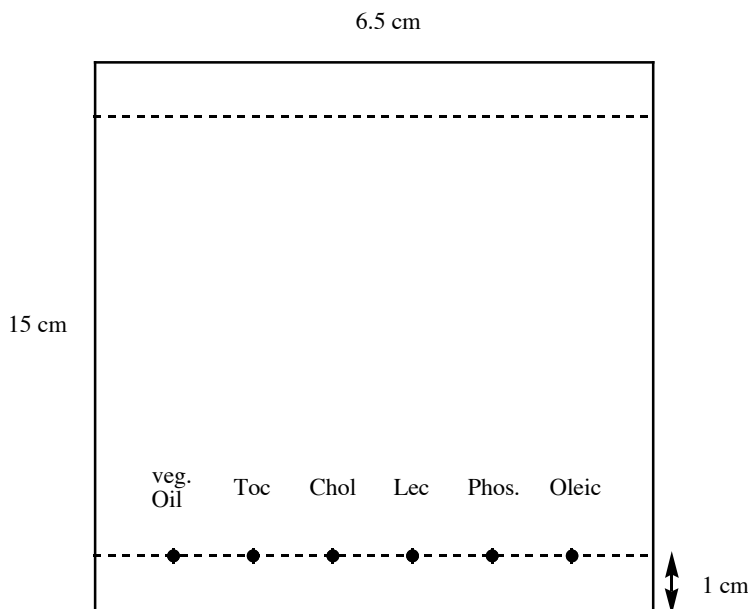
3) Using a capillary tube (**NOT a pipettor**), spot **3 drops** of the <u>unknown</u> (vegetable oil) on the first mark of your TLC plate. Keep the spots small (2-3 mm) in diameter. Your instructor will demonstrate the proper technique.

4) Spot <u>known</u> samples on the remaining marks: 2) tocopherol, 3) cholesterol, 4) lecithin, 5) phosphatidylcholine, 6) oleic acid.

5) Prepare 25 ml of chromatography solvent (Hexane/Ethyl Ether/Glacial Acetic Acid 80:20:1). 25 ml is enough for **two student groups** (the two groups will share a single, large chromatography jar). Transfer chromatography solvent to jar.

6) Place TLC plate (spotted edge down) in solvent-containing chromatography jar. Seal lid and allow separation to occur until solvent front is approximately 1 cm from top of TLC plate. Chromatographic separation typically takes 30-40 minutes.

7) Remove TLC plate and mark solvent front with a pencil. Allow plate to dry completely (5 min.) on your bench

8) Spray dry plate with 10% phosphomolybdic acid solution in chemical fume hood.

9) Dry plate using the oven in the Organic Chemistry Laboratory. Drying time is 5-10 minutes.

10) Calculate Rf values for all major spots on plate.

Results

1) Prepare a *sketch* of your TLC plate (to scale) (figure1).

    a. Figure numbered with appropriate legend (1 pt)
    b. Samples identified at starting line (1 pt)
    c. Solvent front identified (1 pt)
    d. Spots identified (2 pt)

2) Table (1) containing R_f values for each of the <u>known</u> samples (use largest spot if more than one is present).

    a. Table is numbered and contains an appropriate description (1 pt).
    b. R_f values for <u>known</u> 5 samples (2.5 pt)
    c. Example calculation for <u>one</u> sample is shown below table (1 pt).

3) Table (2) of R_f values for each of spot from the <u>unknown</u> sample (vegetable oil).

    a. Table is numbered and contains an appropriate description (1 pt).
    b. R_f values for each spot (2.5 pt)
    c. Table contains a column with tentative identification of spots, where possible (2 pt).

***Bonus* (2 pt).** The vegetable oil sample will likely contain some spots that are not readily identifiable. Do some some research on vegetable oil content, especially in terms of saturated and unsaturated fatty acid content. What are the identities of the major lipids contained in vegetable oils? What types of R_f values might you expect these lipids to have (low?, medium?, high?) if they were chromatographed in this solvent? Justify your reasoning.

Laboratory performance (2 pt)