

CHEM 4401 Biochemistry Laboratory I Chromatographic Methods of Amino Acid Separation

Chromatography involves the fractionation of compounds on the basis of their solubility in two different solvents or phases (one typically being polar and the other nonpolar). These phases do not have to be liquid, though typically one of them is. One of the phases is often referred to as the *mobile* phase. The mobile phase moves through some type of inert support (TLC plate, column, capillary tubing) that also allows contact with the *stationary phase*. Solutes move in the direction of solvent flow, but the rate of movement is governed by their relative solubility in the mobile phase. If they are highly soluble, they will move through the support at a higher rate. If they are not very soluble in the mobile phase, they will tend to remain in the stationary phase. Compounds with intermediate solubility will show intermediate movement. This gradient of movement is what allows the high resolution that makes chromatography such a useful technique.

Today, we will be using an inert support (TLC plate) and liquid *mobile phase* consisting of a nonpolar (butanol) fraction and an aqueous, polar fraction (acetic acid:H₂O). We will be using the technique to analyze the polarity of a number of amino acids. Amino acids contain functional groups that contribute to their overall solubility in polar or nonpolar solvents (carboxyl, amino, phenyl, sulfhydryl groups, etc.) Our task today will be to perform an elementary chromatographic separation and analyze how various functional groups contribute to solubility.

Corrections to lab manual

page 24. Definition of R_f should read: $R_f = \text{movement of solute band} / \text{movement of solvent front}$

Procedure Modifications

1. make up 25 ml of chromatography solvent (4 butanol:1 acetic acid: 5 H₂O) = (10 ml butanol: 2.5 ml acetic acid: 12.5 ml dH₂O). Mix well.
2. Prepare a TLC plate (not chromatography paper) with dimensions of 2.5 x 3 inches.
3. Draw a line (pencil) across the plate 1.5 cm from the bottom of the long (3 in.) edge. Mark 6 points (1-6) across the line (left to right, evenly spaced) for spotting the 5 amino acid samples plus the unknown.
4. Use a P-20 pipet to spot 3 ul of each of the 1% amino acid solutions in the following order (L to R): (1) Phe (2) Glu (3) Cys (4) Lys (5) Leu (6) Unknown. Ignore the part in the manual about suspending the plate over the table top and the part about proline but do allow the spots to dry for approximately 5 min.
5. Ignore step 5. We will place the TLC plates into the chromatography jars without bending, stapling, etc.

6. Place your plate in your chromatography jar. Make sure the long edge containing the amino acid spots is in contact with the solvent. Cap the jar and allow the solvent to flow up the plate for 30-45 minutes, or until solvent front is approximately 0.5 cm from the top edge of your TLC plate.
7. Perform the remaining steps as in the lab manual. Remember, ninhydrin is toxic in large amounts, but is nothing to worry about if a little gets on your skin.

Presentation of Data/Lab Report (17 pt. total)

Title page information (1 pt)

Introduction (2 pt)

materials and methods description (2 pt).

Sketch (to scale) a drawing of your TLC plate and results, noting amino acids and unknown, as well as the relative positions of the spots. DO NOT include your actual TLC plate as part of your report (throw away). (2 pt)

Example Rf value calculation (1 pt)

Rf values for each amino acid & unknowns (0.5 pt each, 3 total)

Unknown ID (1 pt)

Discussion (**use question 1 as a basis for discussion** - 2 pt)

Grammar, spelling, coherent sentence structure (1 pt)

Laboratory Performance (2 pt)