

Experiment 2: Analytical Methods for Amino Acid Separation and Identification

In this experiment, you and your lab partner will be separating certain amino acids via two methods: by charge (using ion exchange chromatography) and by thin layer chromatography (TLC). To make efficient use of your time, one member of your pair should prepare the column in part A while the other member prepares the TLC standards in part B. Modifications to the text protocol are as follows:

Part A.

- The columns (with corresponding stopcock) to be used are already fitted with a porous disk which removes the need for glass wool as described in the first half of the paragraph 1 of the Experimental Procedure. You can pour your column by adding the resin slurry directly and allowing the resin to settle as described in the text. As stated in the text you should NOT allow the buffer to run below the top of the resin layer.
- You will be collecting 2 mL fractions rather than the 1 mL fractions in the text. This will require doubling the total buffer volume in each of the three cases but you will still use 0.5 mL of your sample and follow with 1 mL of the pH 3.0 buffer. Rather than spend time in the measuring of fraction tubes, you can count 40 drops for each fraction.

Part B.

- For the analytical separation, you will be using a 10 X 20 cm sheet of chromatography paper. Mark the long (20 cm) axis as directed in the text using a pencil. (N.B. Pen and marker inks contain components that will cause false results)
- To apply your known samples to the TLC plate, you can use a 10 μ L graduated capillary tube to spot the sample. While multiple spotting will result in more attractive TLC results, you may apply the entire 10 μ L in one application. You should still be able to discern the knowns and unknowns regardless.
- To apply your unknown samples, remember that the solutions have been diluted considerably during the chromatographic separation. The two drops used to test for the presence of amino acid in your fractions is about 100 μ L. Depending on the intensity of the test strip spots, you may wish to spot with more or less sample.
- You will be eluting each plate in a solution of chloroform/methanol/ammonia. Rather than rolling your plate as described in the text, we will be using a TLC rack which allows up to 10 plates to run at once. Once your TLC has finished eluting, you do not have to wait for the plate to dry to spray with ninhydrin but should dry the plates before developing the plates in the oven.

Prelaboratory Exercise

For the chemicals to be used in the laboratory (listed below), refer to the Materials Safety Data Sheets to determine the following:

- What are the immediate hazards from the use of these compounds in laboratory?
 - What preventative measures can be taken to prevent injury/harm from use or misuse of these compounds?
 - What are the requirements for disposal for each compound?
1. citric acid
 2. tris buffer [tris(hydroxymethyl)aminomethane]
 3. ninhydrin
 4. silica
 5. chloroform