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Experiment 11 – Paper Chromatography

Discussion

In addition to recrystallization and distillation, chromatography can also be used to separate the components of a homogeneous mixture. Initially, chromatography was used to separate colored substances, hence the name (Greek *chroma*, color). The colors of the components of the mixture are observed as they separate. This technique can also be used with colorless substances if they fluoresce when exposed to ultraviolet light or if they react with a second reagent to produce colored products.

Separation of substances by chromatography depends on the differences between the adsorptive characteristics of the substances with respect to a stationary phase material such as paper. The components of the mixture are adsorbed onto the stationary phase. Continued passage of a solvent in the stationary phase dissolves the adsorbed components of the mixture and moves them along the paper, known as the mobile phase. Each component moves at its own rate; after a given time interval, each component has moved a different distance across the stationary phase.

In this experiment, amino acids will be placed on a sheet of paper and the solvent allowed to travel along the paper by capillary action for a given period of time. The paper serves as the stationary phase, and the amino acids will move along the paper at rates that depend on their structures. Students will determine the distance traveled by certain amino acids; ultimately, an unknown mixture will be analyzed for amino acid(s) present. The ratio of the distance traveled by an amino acid relative to that traveled by the solvent is the R_f value for the amino acid.

$$R_f = \frac{\text{distance traveled by compound}}{\text{distance traveled by solvent}}$$

Since amino acids are colorless, identification of their positions at the end of the experiment is necessary. Ninhydrin will be used to develop a spot of color at the point to which each amino acid has moved.

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Procedure

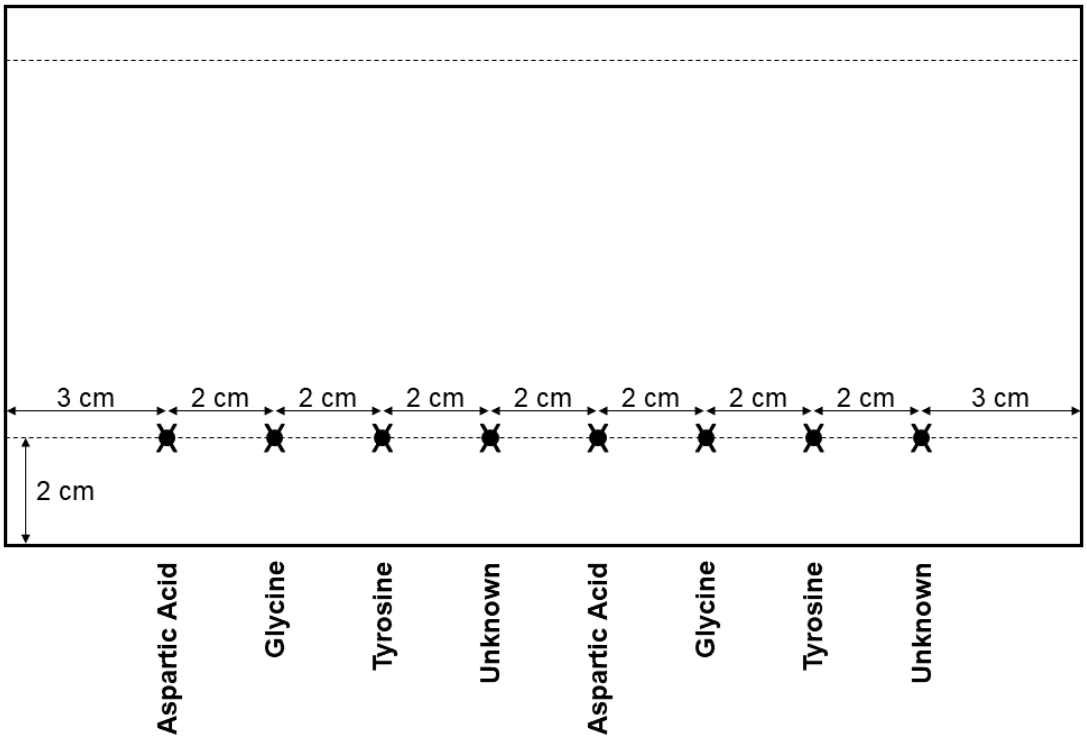
CAUTION! 2% ninhydrin in ethanol will strongly stain the amino acids on your skin. Avoid spilling the ninhydrin solution on yourself.

Prepare a solution of 10 mL 2% aqueous ammonia in 20 mL 2-propanol. The solution should be placed in a 600 mL beaker and covered with aluminum foil. This is your solvent that will be used to flush the known and unknown mixtures along the stationary phase.

Obtain (3) 5-mL beakers. Fill the first beaker with a few drops (no more than 2 mL) of 0.05 M aspartic acid solution in 1.5% hydrochloric acid. Now fill the second beaker with a few drops (no more than 2 mL) of 0.05 M glycine solution in 1.5% hydrochloric acid, and the third beaker with a few drops (no more than 2 mL) of 0.05 M tyrosine solution in 1.5% hydrochloric acid. Also obtain a labeled vial which contains an unknown mixture from your instructor. Make certain to record your unknown mixture code.

You will be provided with a sheet of Whatman No. 1 filter paper, already cut to size 10 x 20 cm. Using a pencil, lightly draw a solid line along the long axis 2 cm from the bottom edge (see Figure One below). Along this line, place eight 'x' marks at equal intervals 2 cm apart, beginning with the first mark 3 cm from the short left edge of the paper. The prescribed distance of 2 cm between marks will allow proper placement of all the 'x' marks. Notice the order of the various amino acids followed by the unknown mixture; you will repeat the order of the amino acids and the unknown for confirmation a second time along the paper.

Figure One. Preparation of Chromatographic Sheet



Using separate capillary tubes for each mixture, place a small drop of each solution at the two respective positions along the line on the filter paper as shown in Figure One. Make sure each spot of solution on the paper is not larger than 2 mm by quickly withdrawing the capillary from the paper when time you touch it. After spotting all samples on the paper, allow the paper to dry in the air for 10 minutes.

Roll the paper into a cylinder such that the line is on the bottom outside of the cylinder. Staple the ends together to hold the cylinder in shape. Place the staples about 4 cm from each edge of the cylinder. Do not allow the edges of the paper to touch when the staples are put in. A small gap in the cylinder is necessary.

Placed the stapled chromatogram paper into the 600 mL beaker, base line down, along the solvent surface BUT NOT COVERED BY IT. Avoid splashing the solvent on the paper. Make sure the paper does not touch the sides of the beaker. Allow the solvent front to migrate up to 1 cm below the edge of the paper (top) for at least 90 minutes. Afterwards, remove the paper from the cylinder, mark the edge of the wet part of the paper, and allow it to air dry on the lab bench top. Once the cylinder is essentially dry, remove the staples and hang it in the hood. Your instructor will spray the paper with a solution of 2% ninhydrin in ethanol. After the spray dries, place the paper in an oven at 100 $^{\circ}$C for 10-15 minutes. Note the spots and circle each one. Measure the distances from the 'x' to each spot and the distance traveled by the solvent. The distance traveled by the solvent is the distance from the liquid level in the beaker to the edge of the wet portion of the paper. Calculate the R_f values and determine the identity of your unknown.

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Data and Calculations for Experiment 11

UNKNOWN CODE: _____

Solution	**Distance Traveled by Amino Acid (cm)**	**Distance Traveled by Solvent (cm)**	**R_f value**
Aspartic Acid			
Glycine			
Tyrosine			
Unknown			

Post-lab Questions

1. How might it be possible to quantitatively determine the composition of an amino acid mixture? You may need to research this question a bit!

2. If two amino acids have the same R_f values in 2-propanol, how might they be separated?

3. Identify the component(s) of your mixture, then draw the structure(s) of the amino acid(s) present. Refer to your Biochemistry notes from lecture for the structures of the various amino acids.