**Investigation 3: Chromatography**

**Focus Questions:** What factors affect the retention times of a compound in gas-solid adsorption chromatography? How are intermolecular forces related to relative retention in a chromatography experiment? Design a method for separating amino acids: What do you expect to happen and how can you measure it?

**Pre-lab required reading**

*Chemistry; an Atoms-Focused Approach*: Section 6.1-6.2

**Technical Primers:**
- Keeping a Laboratory Notebook
- Gas Chromatography
- Thin Layer Chromatography

**Safety and Waste Disposal**

- Use caution when handling syringes. They are very fragile.
- Strong acids are used in the mobile solvent phase of TLC, THIS MUST BE CONDUCTED IN THE HOOD. Goggles and gloves should be worn when handling.
- Ninhydrin will discolor bare hands (which contain amino acids) for several days – use gloves when using.
- Used solvents should be placed in an appropriate waste container.

**Background**

Almost all substances we come into contact with on a daily basis are impure; that is, they are mixtures. Similarly, compounds synthesized in the chemical laboratory are rarely produced in a pure state. They are almost always produced with impurities including reaction byproducts and leftover reactants. As a result, a major focus of research in chemistry is designing methods of separating and identifying the various components of mixtures.

Many of these separation methods rely on **physical** differences between the components of a mixture. Undoubtedly, you are already familiar with several means chemists use to effect separations based on physical differences. These techniques include: *Filtration*, in which separation may be effected because substances are present in different states (solid vs. liquid); *Centrifugation*, where separation is effected by differences in density; and *Distillation*, in which separation is effected by taking advantage of differences in boiling temperatures of the various components. In this laboratory exercise, we will effect a separation of a mixture compounds using gas chromatography.

All chromatography techniques have three important components: the **analyte** or mixture of species being separated, a **mobile phase**, and a **stationary phase**. The mobile phase is a flowing liquid or gas used to push the analyte over or through a stationary porous material (the stationary phase). Because of physical interactions between the analyte and the stationary phase, the analyte moves through or over the stationary phase more slowly than the mobile phase does. Furthermore, because physical interactions between the analyte and the stationary phase can be different for each component of the mixture, the different components transit the stationary phase at different speeds. Those that strongly interact with the stationary phase lag behind those that interact only weakly. As a result, the components of the mixture may be separated.

By comparing the retention times of a series of known compounds having different physical properties, the factors affecting retention time can be determined.

**Procedure**

**Part one:**

Draw the Lewis structures for the following compounds:

- hexane, CH₃CH₂CH₂CH₂CH₃
- acetic acid, CH₃COOH
- butan-2-one, CH₃CH₂C(O)CH₃
- butan-1-amine, CH₃CH₂CH₂CH₂NH₂
- butan-1-ol, CH₃CH₂CH₂CH₂OH
Use Valence Shell Electron Pair Repulsion Theory to predict the shape around each of the atoms (except hydrogen) in the compounds and label the bond angles. Determine whether each molecule is polar or nonpolar.

Analyze one or more samples (as directed by your instructor) using gas chromatography.

**Carefully follow your instructors’ directions when using the gas chromatograph.**

1. Make sure the syringe is well conditioned with the sample to be injected. This conditioning can be accomplished by drawing solution into the barrel of the syringe and then expelling it into a container for waste. Repeat at least twice.

2. Draw between 0.5 and 1 μL of sample into the syringe.

3. Insert the syringe into the injection port. There will be resistance as the needle enters the port (as though you were trying to pierce a rubber ball) but it should not feel as though the needle is hitting a hard surface. If there is excessive resistance as the syringe enters the port, remove the syringe and try to insert it again.

4. Once the needle is completely inserted into the port, depress the plunger to inject the sample and simultaneously start the program run (this is done by pressing either the space bar or the ‘+’ key depending on instrument).

5. Five of the samples to be analyzed contain two compounds (hexane plus one of the substances modeled in Part I above) and therefore will result in two peaks in the chromatograph. The other sample contains only hexane and will display only one peak. Once the compound or compounds have eluted and the line on the chromatogram has returned to the base, end the run (this is done by pressing either the ‘END’ key or the ‘-’ key based on the instrument).

6. Record the retention time observed for the components analyzed.

**Part two: Background**

Proteins are biological molecules that play vital roles in the body. Proteins are responsible for a variety of functions, from making muscles contract to replicating DNA to carrying oxygen to cells. Proteins are made in the body when many amino acids – small organic molecules found either in food or produced by the body – are bonded together to make a very large molecule in much the same way that beads are strung together to make a necklace. The identity of the protein depends on the number and arrangement of these amino acids.

Amino acids are the building blocks of proteins. These building blocks contain two common functional groups that make up the backbone of proteins: 1) a carboxylic acid group and 2) a basic amino group. Figure 2 illustrates the architecture of an amino acid with a R group representing the side chains that are different for each amino acid. Protein folding and catalysis depend on the properties of the R groups. The chemical properties provided by these R groups are also utilized to characterize and identify each amino acid. Thus, understanding their chemical properties as well as how these groups can form intermolecular interactions is essential in biochemistry.

![Amino Acid Structure](image)

**Figure 2** Architecture of an amino acid.
Depending on the chemical structure of their side chains, amino acids can be classified roughly as “polar” or “nonpolar”. The intermolecular forces between these amino acids determine the three-dimensional structure of the protein. The protein’s three-dimensional structure, in turn, determines the biological function of the molecule.

The different side chains (R groups), and the solubilities provided by the side chains, affect their migration in thin-layer chromatography (TLC). In thin-layer chromatography, the stationary phase is a thin layer of adsorbent silica particles attached to a plastic plate. A small amount of sample is applied (spotted) near the bottom of the plate and the plate is placed in a solvent mobile phase. This solvent is drawn up by capillary action. Separation occurs as each component being different in chemical and physical composition, interacts with the stationary and mobile phases to a different degree creating the individual bands on the plate. TLC can be utilized to identify the different amino acids. Amino acids are stained with ninhydrin to aid in visualizing them.

**Procedure**
Your goal is to determine a simple procedure to separate mixtures of the following amino acids in order to determine which amino acids are present in an unknown mixture:

Valine (R=\text{CH}(\text{CH}_3)_2), lysine (R=\text{CH}_2\text{CH}_3\text{CH}_2\text{CH}_2\text{NH}_2), asparagine (R=\text{CH}_3\text{C(O)NH}_2), and leucine (R=\text{CH}_2\text{CH}(\text{CH}_3)_2).

First, draw Lewis structures for the amino acids listed above.

Since the amino acids are significantly larger than the compounds used in the first week, gas chromatography is not the best method to use to separate them. You will be given a mixture of three of the four amino acids. Design a set of experiments using Thin Layer Chromatography to separate the mixture of the three of amino acids and identify them. A mobile phase which contains butanol: acetic acid: water (5:3:2, v/v/v) will be used on polar silica plates. The plates should be dried under a hood with a hair dryer and then stained with ninhydrin (Note that ninhydrin will discolor bare hands (which contain amino acids) for several days.) Place sprayed plates in a drying oven set at 105-110°C for 3 minutes. Remove the plates from the oven, mark the center of the spots and calculate the $R_f$ values for each spot.

**References**