Investigation 3: Chromatography and Molecular Modeling

Focus Questions: *What do molecules look like and how does that effect how they interact with each other? How can we model molecules and determine dipole moments? How are intermolecular forces related to relative retention in a chromatography experiment? How can we use a similar method for separating amino acids?*

Pre-lab required reading

Chemistry; an Atoms-Focused Approach: Section 6.1-6.2
Technical Primers:
Keeping a Laboratory Notebook
Gas Chromotography
Thin Layer Chromotography
Video:
How to set up a TLC plate

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.

Part I: Separating hydrocarbons

Procedure

Molecular Modeling – Visualizing Molecules

1. Draw the Lewis structures for the following compounds*:*

   hexane, $CH_3CH_2CH_2CH_2CH_2CH_3$ acetic acid, CH_3COOH

   butan-2-one, $CH_3CH_2C(O)CH_3$ butan-1-amine, $CH_3CH_2CH_2CH_2NH_2$

   butan-1-ol, $CH_3CH_2CH_2CH_2OH$

2. Use Valence Shell Electron Pair Repulsion Theory to predict the shape around ***each*** of the atoms (except hydrogen) in the four compounds that you drew Lewis structures for in part one and label the bond angles. Determine whether each molecule is polar or nonpolar. Include all drawings in your lab notebook.

3. From the Chemistry Department web site access the WebMO Program. Use the login and password provided by your instructor. From the WebMO Job Manager, Create New Job from the pull down menu.

   Build one of the five compounds. You can add double bonds by dragging an extra line between two atoms. Once the balls (representing carbons or other elements) and sticks (representing bonds) of the basic structure have been drawn, the molecule will need to be "cleaned-up" before any calculations are done. Go to the "Clean-Up" pull down menu. First add hydrogens, then go to the "Clean-Up" menu again and click on hybridization. Go to the "Clean-Up" menu again and click on geometry. Go to the "Clean-Up" menu one last time and click on mechanics optimize.

   At this point the molecule is ready to run the job, so click on the arrow on the bottom right of the window. A new window will appear with a space to name the job and several options. The appropriate choices for this exercise are:

   Name: actual name of compound **plus group identifier**
   Calculation: Geometry Optimization
   Theory: RM1
   Charge: 0 (unless your compound is charged – indicate charge here)
   Multiplicity: Singlet.

   A click on the arrow on the bottom right of this window will send the job to the server and return you to the Job Manager window where you may monitor the progress. It will take no more than a few seconds to calculate the result. When the job is finished, it will be indicated in the Job Manager. You may then access the data by clicking on the magnifying glass icon under the heading "Actions". Click here to access the results of the calculation. The structure should appear in the "molecule viewer" window as a three-dimensional ball and stick representation with each atom in the structure numbered.

   To freely rotate the model (the ball and stick representation), select the curvy arrow located on the top of the tool bar. Use this tool to rotate the molecule and view it from different directions. Compare this ball-and-stick representation to the structure you predicted with VSEPR theory.

   Scroll down the page to see the calculated quantities. In the first box under Overview, you will find the calculated value for the dipole moment. **Record this value.** A greater dipole moment means the molecule is *more polar*.
   Clicking on this magnifying glass icon will display the dipole moment on the molecule in the molecule viewer window. This shows you the direction of polarity in the molecule. **Take a screenshot of your molecule with the dipole moment arrow superimposed.** Note: it may be absent for hexane.

   Repeat the modeling process for the other molecules.

4. Determine whether each molecule is polar or nonpolar.

Gas Chromatography

Analyze the following compounds using gas chromatography*:*

hexane, $CH_3CH_2CH_2CH_2CH_2CH_3$ acetic acid, CH_3COOH

butan-2-one, $CH_3CH_2C(O)CH_3$ butan-1-amine, $CH_3CH_2CH_2CH_2NH_2$

butan-1-ol, $CH_3CH_2CH_2CH_2OH$

Carefully follow your instructors' directions when using the gas chromatograph.

1. Make sure the syringe is well conditioned with the sample to be injected. Condition 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(s) observed for the components analyzed.

7. Wait for the temperature to cool back down to starting temperature and then repeat with each mixture.

Part II: Identifying amino acids by separation

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 1 illustrates the architecture of an amino acid with an 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.

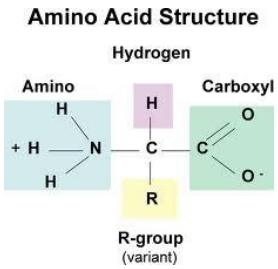


Figure 1 Architecture of an amino acid.

Depending on the chemical structure of their side chains (R group), amino acids can be classified roughly as "polar" or "nonpolar". 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 (polar) attached to a plastic plate (Figure 2). 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.

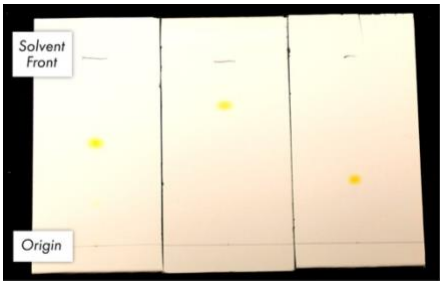


Figure 2 Example of a TLC experiment

Procedure

Thin Layer Chromatography

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 ($R=CH(CH_3)_2$, lysine ($R=CH_2CH_2CH_2CH_2NH_2$), asparagine ($R=CH_2C(O)NH_2$), and leucine($R= CH_2CH(CH_3)_2$) .

1. Draw Lewis structures for the amino acids listed above in your lab notebook. Based on your structures and the results from your WebMO analysis of the molecules in Part I, rank the four amino acids by expected polarity.

2. Review the Thin Layer Chromatography primer. 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 with standards of each available.

   Design an experiment using Thin Layer Chromotography 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.

   TLC plates should be set up by first marking the spotting location for each sample in a straight line approximately 5 mm from one end of the plate with a pencil. Spot a tiny amount of each sample onto the appropriate pencil mark as accurately as possible.

Once the plates have been run, mark the distance to the solvent front with a pencil. The plates should then 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 105-110ºC for 3 minutes.

Remove the plates from the oven, and mark the center of the spots with a pencil. Optional, but recommended: take a photograph of your plates. Calculate the R_f values for each spot. The R_f value is defined as the distance traveled from the origin divided by the distance traveled by the mobile phase, which is the solvent front you marked earlier.

References

Atkins, P.; Jones, L. "Chemical Principles: The Quest for Insight", 6th ed.; Freeman: New York. **2013**.
Csizmar, C.M.; Force, D. A.; Warner, D. L. *J. Chem. Educ*. **2012**, *89,* 379-382.

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