CHROMATOGRAPHY EXPERIMENT: SEPARATION OF AMINO ACIDS

separation of a mixture using stationary and mobile phases.

Objective: *To demonstrate the use of paper chromatography in separating and identifying amino acids in a mixture.*

Introduction and Theory: In order to determine the identity of the components of a mixture, it is necessary to separate the components and then apply tests or reagents that will identify them. There are many methods of separation available. Chromatography methods include column, paper, thin layer, and gas chromatography.

Chromatography is a method of analysis in which a solvent (mobile phase) promotes the separation of a mixture by differential migration through an adsorbing medium (stationary phase). In this experiment, a separation of amino acids (the building blocks of life) is made using filter paper as the stationary phase and isopropyl alcohol in ammonia solution as the mobile phase. The solvent migrates up the paper causing a separation of the amino acids. The amino acid that is least strongly adsorbed to the paper moves most rapidly and the one that is more strongly adsorbed moves very slowly.

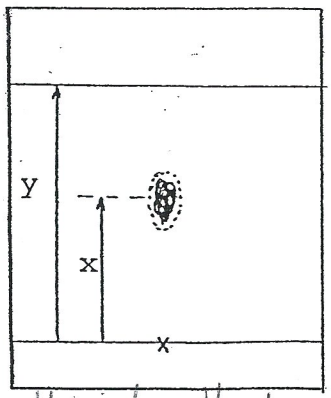
To analyze the separation of the amino acids on the paper chromatograph, R_f values are calculated for each amino acid. The definition of R_f is given by:

$$R_f = \frac{\text{distance compound has moved from origin}}{\text{distance of solvent front from origin}} = \frac{x}{y}$$

• component separated due to different in preference for mobile + stationary phases

* stationary phase: filterpaper
* mobile phase: 1:2 mixture of NH_4O and isopropyl

* to separate amino acids



SF = Solvent Front

S = Substance

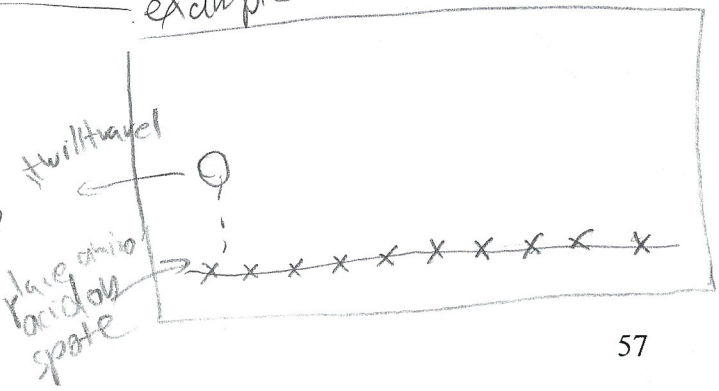
O = Origin

• distance of migration of components is dictated by the amino acids solubility in mobile phase vs. the amino acids attraction (intermolecular forces) the stationary phase

V traveled further than L that means ← example

V has a larger R_f value than L since V traveled further than

Fig 1: Paper Chromatograph



V is more soluble in the mobile phase than L → because the ligand absobed time the V traveled with it

* L has stronger attraction to its stationary phase than V → because it didn't travel like V.

The substance was originally present at the origin O and has moved a distance x during the time in which the solvent front has moved a distance y. The solvent front (SF) is the furthest distance reached by the advancing solvent on the paper. Visualization of the amino acids is accomplished by spraying with a ninhydrin solution. Ninhydrin reacts with amino acids to yield highly colored products.

The R_f values for a series of amino acids depend on various properties such as the molecular weight (molar mass), solubility, and how strongly the amino acid is adsorbed on the surface of the stationary phase medium. Amino acids have the general formula given below where the R group can vary.

Procedure: Follow the steps given below:

1. Solvent: Obtain a 1000 mL beaker containing a solution of 10 mL of 2% ammonium hydroxide in 20 mL of isopropyl alcohol covered tightly with a piece of plastic wrap. Do not disturb this beaker and allow it to reach equilibrium.

2. Capillary tubes. Heat a piece of capillary tubing in the center with continuous and even rotation in the hot part of a Bunsen burner flame. When the glass softens, quickly draw it out to form a capillary of about 1/2 the original diameter. After cooling, cut the capillary in the middle of the drawn out portion. This will form two fine capillaries for transferring known and unknown solutions to the chromatographic paper later in this experiment. You will need at least 5 fine capillary tubes.

3. Knowns and unknown. Prepare five labels with the following identification:

   Glycine (G) Aspartic acid (D) Valine (V) Leucine (L) Unknown (U)

   Place the labels on five clean, 75 mm test tubes and place one capillary into each tube. From the stock solutions on the shelves (containing about 0.05M solutions of the amino acids in 1.5% hydrochloric acid), transfer 2 drops of the appropriate material to the 5 test tubes. Obtain the unknown number from the instructor. (The unknown will contain from one to four of these same amino acids.)

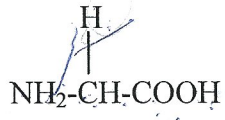
4. Stationary phase. Obtain a sheet of filter paper, 11cm x 22cm. Handle this paper at all times with Kimwipes or small pieces of paper. Do not touch the paper with your fingers as amino acids will be transferred from your fingers to the paper. Make a light pencil line about 1.5 cm from the bottom of the paper (see Fig. 2). Along this line starting 2 cm from the edge, at intervals of about 2 cm, place ten light penciled x's. Under each x place identifying letters, two for each of the knowns and unknowns.

Disposal: The amino acid solutions can be poured down the drain and the test tubes rinsed with water.

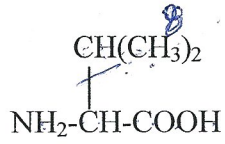
Note: Ninhydrin should be kept off the skin because it reacts with proteins in the body to form a rather long-lasting purple discoloration. The spray reagent is prepared as a 2% solution of ninhydrin in ethyl alcohol. Ninhydrin has the formula $C_9H_6O_4$.

Formulas of the amino acids used:

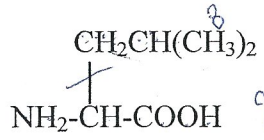
glycine



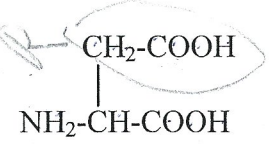
valine



leucine



aspartic acid



Data: Draw a sketch of the completed paper chromatograph in your lab notebook.

Calculations and Results:

1. Trace around each spot with a pencil.
2. Measure the distance each spot traveled (use the center of the spot for measurement).
3. Measure the distance the solvent traveled at each position.
4. Mark these distances on the chromatograph.
5. Calculate the R_f values for each amino acid.
6. Determine the composition of the unknown by visual comparison of spot colors and by the relationships of R_f values. If the unknown contains more than one spot, then calculate an R_f value for each spot.

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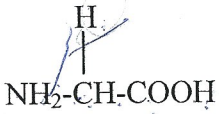
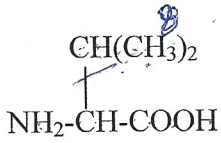
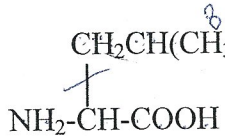


valine



leucine



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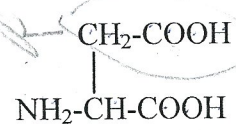

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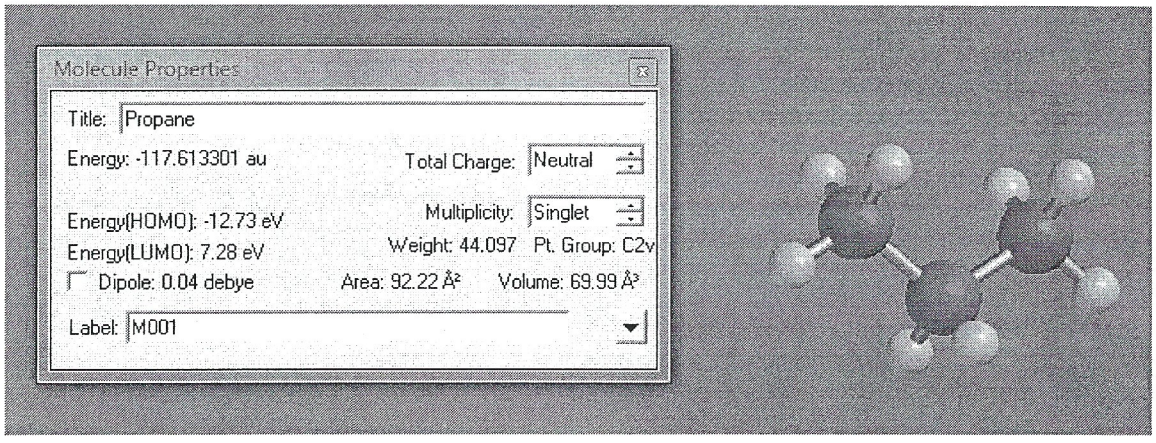
CHROMATOGRAPHY EXPERIMENT: MOLECULAR MODELING COMPONENT

Objective: *To use molecular modeling to study physical properties of amino acids in order to determine what factor(s) contribute most to their separation using chromatographic methods.*

Introduction and Theory: As seen in last week's experiment, molecular modeling is a powerful tool used by chemists to learn about the chemical and physical properties of molecules. In our previous experiment, we used the modeling program just to look at bond lengths and bond angles (which *determine* the overall molecular geometry of the molecule), as well as polarity (which is largely *determined* by the overall geometry of the molecule.) However, there is far more information that can be gained from molecular modeling studies. The screenshot below shows a representative output displaying molecular properties for the compound propane (C_3H_8). While the bond length and bond angle information is not provided in this screenshot, plenty of other useful information is seen there.



The goal of modeling the amino acids is to determine which molecular property (or properties) directly relates to the compounds' R_f values that you will determine using paper chromatography. Remember that the R_f value is a measure of how far the compound moves relative to how far the solvent travels during the same time. Thus, if a compound moved 80% of the distance that the solvent traveled, the compound would have an R_f value of 0.80. There are a number of factors that might determine how far a compound will travel during a chromatography experiment. Most of those factors are present in the screenshot shown above. Using the Spartan Molecular Modeling program, you and your lab partner must calculate and record the various molecular property values for each of the amino acids studied in this experiment. Then, for your lab report, you will need to determine which of the property(ies) is/are directly related to your measured R_f values. For example, consider the following data:

Compound	Measured R_f	Molecular Property "X"	Molecular Property "Y"
A	0.84	23.0	192.9
B	0.72	25.0	211.0
C	0.46	18.0	218.3
D	0.32	30.0	225.3

In this example, the measured R_f values decreases in the order A>B>C>D. When this trend is compared to molecular property X, it is clear that there is no pattern to the data. Thus, you could

conclude that molecular property X does not determine the R_f values for compounds A-D. By contrast, notice that molecular property Y increases in the order A<B<C<D. Thus, you could conclude that as molecular property Y *increases*, the measured R_f value for the compound *decreases*. In other words, there appears to be an inverse relationship between property Y and the compounds' R_f values.

The molecular properties that we are interested in for this experiment are described below. In each case, not only is the property described, but a reasonable hypothesis is provided as to WHY that particular property might be most influential in determining the R_f value for the compound. Note that some of the properties seen in the screenshot have been left out.

Energy – This value represents the total energy of the molecule. It can be thought of as how much energy it would take to break all of the bonds in the molecule and is reported in "atomic units" (1 atomic unit = 2625 kJ/mol)

Hypothesis – The most energetic molecule should have the highest R_f value because the more energy it contains the further it should be able to travel in a given amount of time.

Energy (HOMO) – HOMO stands for Highest Occupied Molecular Orbital. This measured value tells you what the energy of the highest occupied orbital in the molecule is. (Occupied means it contains one or two electrons.) To relate this to what you have learned in the course, the highest occupied orbital for a Ne atom would be a 2p orbital.

Hypothesis – Since the highest occupied orbital in an atom involves the VALENCE electrons, and since the valence electrons are considered to be the most important electrons when determining atomic properties, it can be assumed that the highest occupied orbital in a molecule would be the most important when determining molecular properties. Thus, the compound with the highest HOMO energy should have the highest R_f value because the more energy those electrons contain the further the molecule should travel in a given amount of time.

Energy (LUMO) – LUMO stands for Lowest Unoccupied Molecular Orbital. This measured value tells you what the energy of the lowest unoccupied orbital in the molecule is. To relate this to what you have learned in the course, the lowest unoccupied orbital for a Ne atom would be a 3s orbital.

Hypothesis – The compound with the lowest LUMO will likely also be the compound that contains the lowest energy electrons (in other words if the empty orbitals are low in energy then it can be assumed that the filled orbitals will also be very low in energy). Thus, the compound with the lowest LUMO should have the lowest R_f value because that molecule will be least energetic.

Dipole Moment – As seen in last week's experiment, this value tells you the relative polarity of the molecule. The larger this value is, the more polar the compound is.

Hypothesis – A very polar compound is more likely to "stick" to the paper than is a nonpolar compound (or vice versa). The stickier the compound is, the lower its R_f value should be.

Molecular Weight - Self-explanatory

Hypothesis – A lighter molecule will be able to travel further in a specific period of time than a heavier molecule.

Area – This value represents the surface area of the molecule.

Hypothesis – The larger the surface area of the molecule, the more it will stick to the paper, and the lower its R_f value should be.

Volume – This value represents the 3-dimensional volume occupied by the molecule.

Hypothesis – The larger the volume of the molecule, the more it will stick to the paper, and the lower its R_f value should be.