Experiment 30
Chromatography

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Name:

Date:

Key Objectives

1. Identify polar and nonpolar functional groups and amino acids.
2. Understanding of basic chromatography concepts - solid phase, liquid phase R_f value.
3. Understand which amino acids travel fastest/furthest in polar/non-polar solvents.

Discussion

Proteins are an important class of macro-molecules with a wide variety of chemical, physical and biological properties due to the unique structure of the individual amino acids that make up the primary, secondary, tertiary and quartenary structure of these versatile molecules.

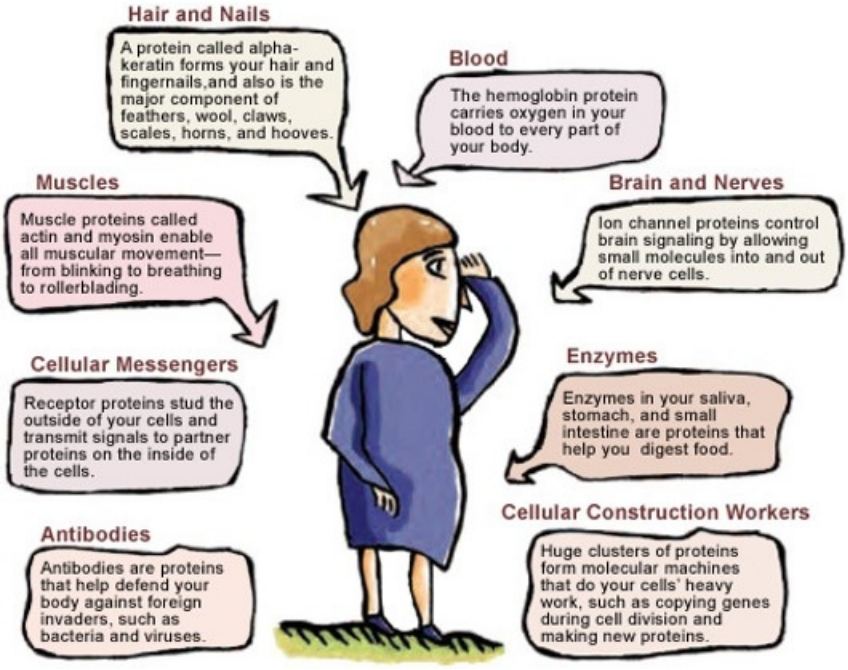


Figure 30.1: Proteins are an important macromolecule with a variety of uses. credit: `http://nnhsbiology.pbworks.com/w/page/109339297/Honors%20Proteins`

Proteins are made up of 20 different amino acids that share a common structure called the conserved region which consists of the central α-carbon to which is attached a H, an amine group, and a carboxylic acid group. The fourth bond is variable and the R-group which is attached varies for each amino acid and is what gives them their unique properties. Of note is that most amino acids are also chiral, though only the L-enantiomer is biologically important, much like for sugars only the D-isomers are used.

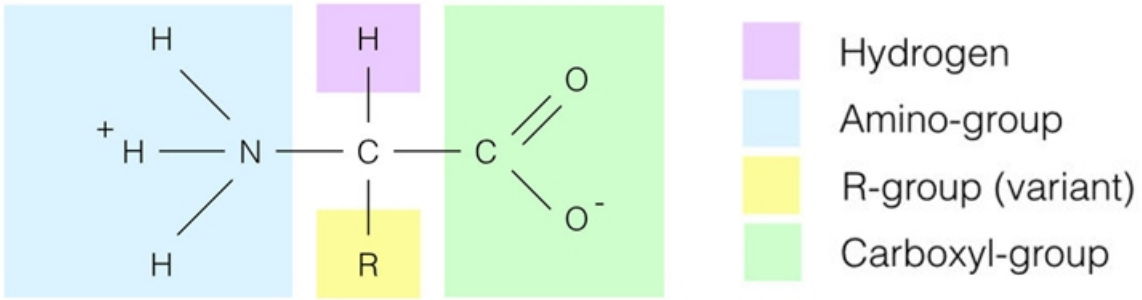


Figure 30.2: General structure of an amino acid illustrating the conserved regions - H, COOH, NH_2 and variable region - R. credit: unknown

The properties of the R-group that is unique to each amino acid is what makes separating them by chromatography relatively easy. In general the R-groups are categorized as acidic (- charge), basic (+ charge), hydrophylic (polar) and hydrophobic (non-polar) as shown in Figure **??**

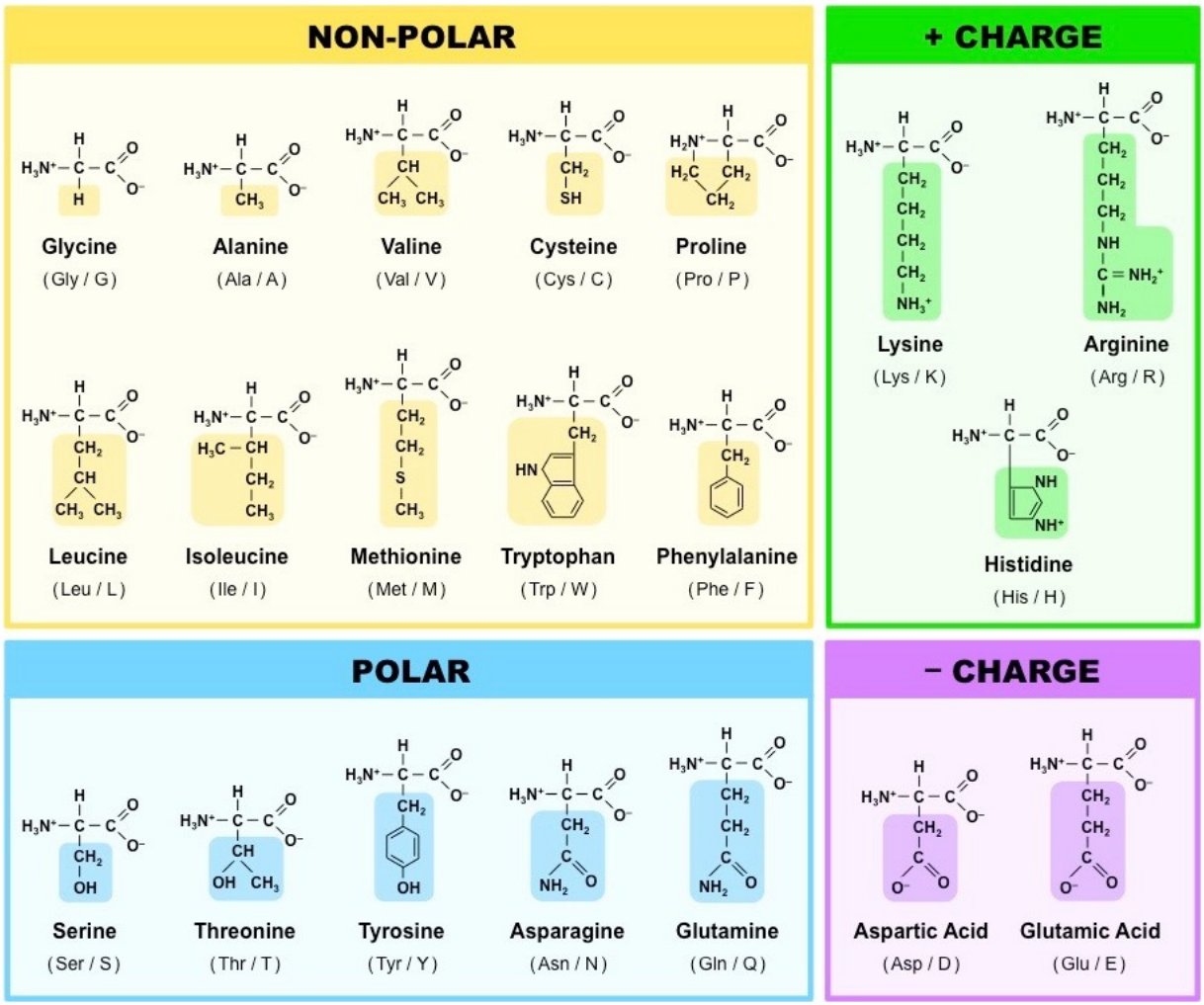


Figure 30.3: The different R-groups on an amino acid result in different properties. The figure shows four different categories amino acids can be divided into. credit: unknown

Chromatography is a standard technique used to separate compounds in a mixture (anything from colored dyes, DNA, RNA or in this lab amino acids) based on the compounds different rate of travel between two phases (often solid/liquid). The key part that is the same about all chromatography experiments is that the separation of the substances is due to their different solubility in each phase.

The stationary phase (usually a solid) and the mobile phase (usually a liquid or gas) interact with the compounds to be separated based on differences in the structure of the molecule and the molecules solubility in the mobile phase. The easiest type of separation is based on whether a molecule is hydrophobic or hydrophyllic which will determine if it is attracted to the solid phase and moves slowly up the paper or is attracted to the liquid phase which will move rapidly up the paper. Depending on the choice of stationary phase (hydrophobic or hydrophyllic) and the liquid phase (usually the opposite so in this case hydrophyllic or hydrophobic) the molecules will travel up the stationary phase at different rates.

The ratio of the distance traveled by a compound and the distance traveled by the mobile/solvent phase is relatively unique for different compounds. Mathematically this is shown in Figure 31.4

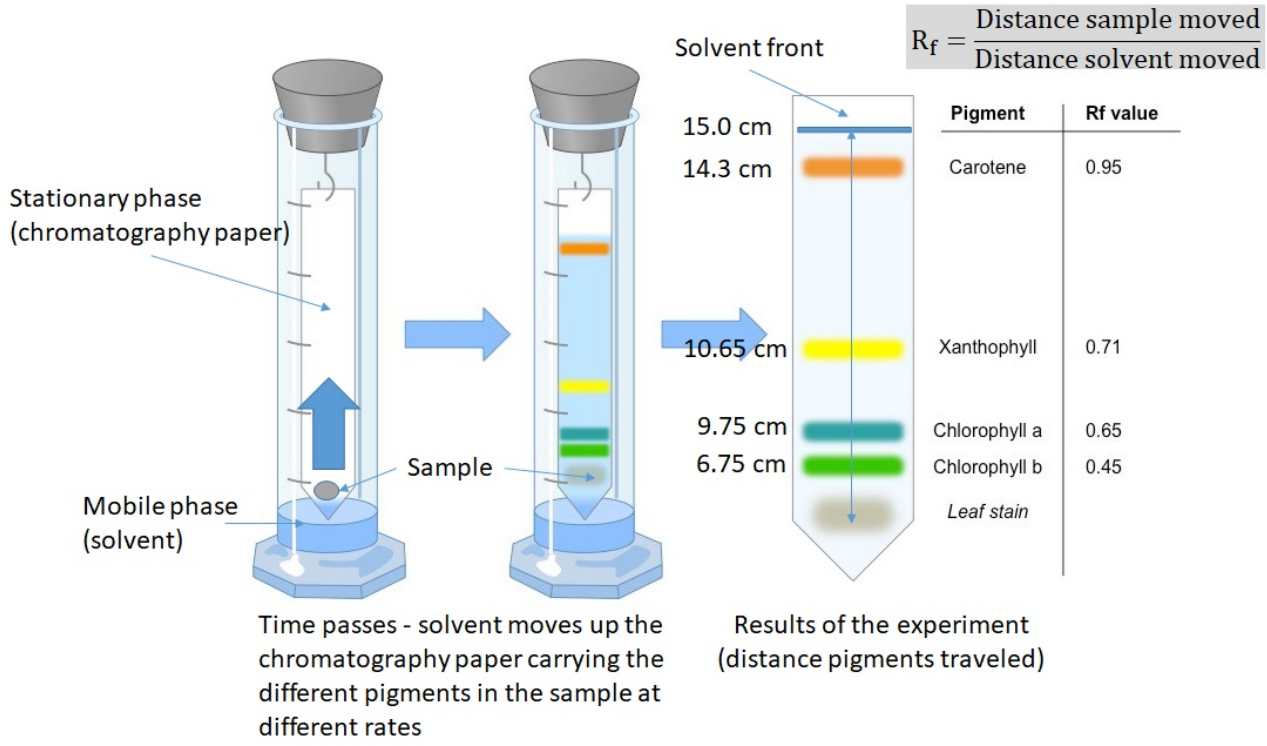


Figure 30.4: Typical chromatography experiment and calculation of R_f values. credit: author modified from `https://microbenotes.com/paper-chromatography/`

The separation of different compounds is effected by several variables and can vary considerably depending on the choice of stationary phase (polar or nonpolar), mobile phase (polar or nonpolar), the concentration of each phase (can be varied to determine the best separation) and temperature. As such R_f values must be determined for a specific set of conditions (stationary phase, mobile phase and temperature) and samples of the pure compound must generally be compared to mixtures to determine the identity of an unknown compound.

Procedure

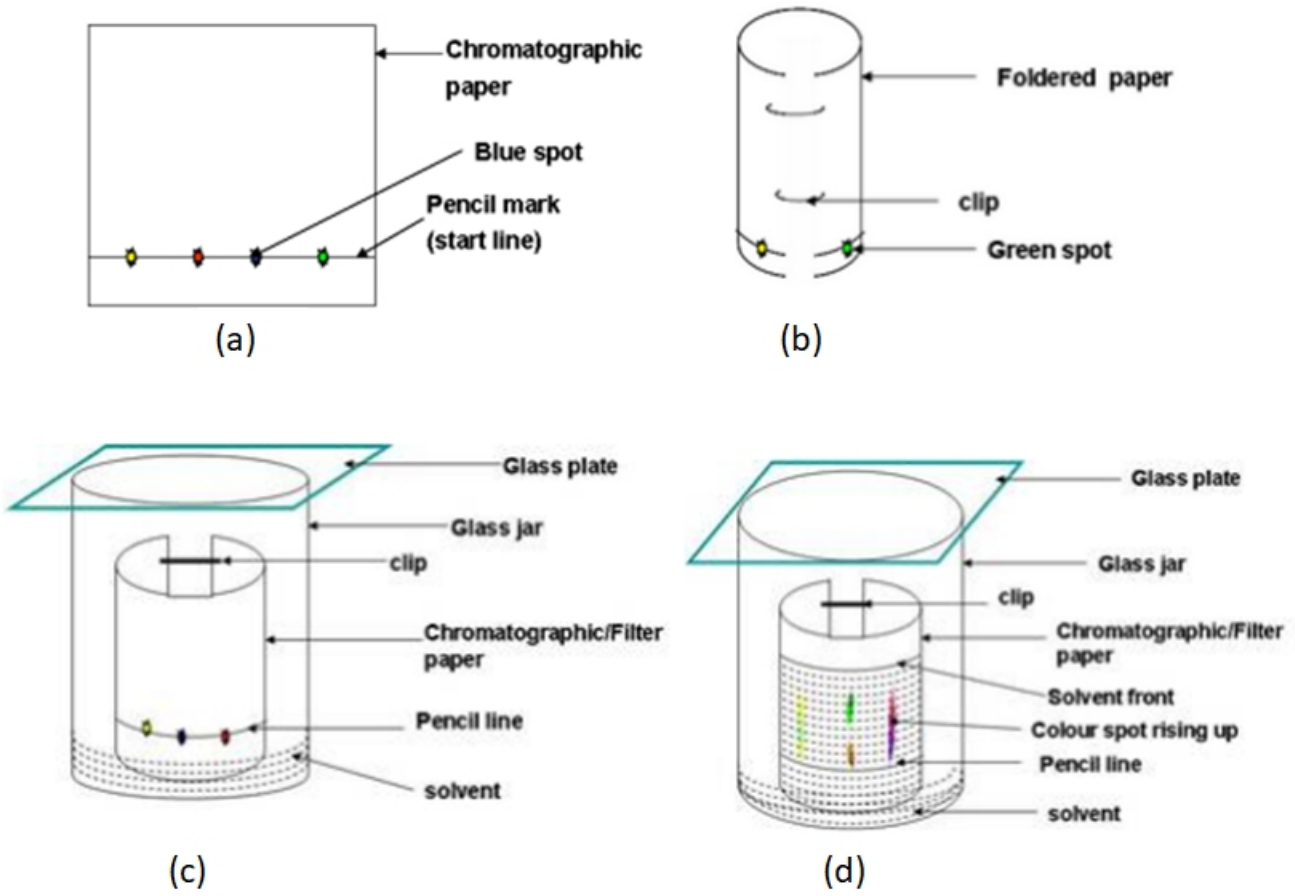


Figure 30.5: (a) preparation of chromatography paper (b) rolled into a self-supporting tube held together by staples, (c) placed in solvent jar (solvent **must** start below the compounds to be tested (d) results after elapsed time. credit: `https://www.open.edu/openlearncreate/pluginfile.php/70646/mod_page/content/1/CHE_Pack_5.pdf`

Day 1 - Calculating R_f for known amino acid samples

1. Cut a piece of filter paper into a 15.0 cm square. Touch the filter paper as little as possible, your fingers are filthy! (and also contain proteins which might effect your results).

2. Using a pencil (not a pen, it will smear) draw a line about 2.0 cm from the bottom and measure out 4 equally spaced dots approximately 3.0, 6.0, 9.0 and 12.0 cm from one end) as shown in Figure 31.5. The line must be above the level of the solvent or the amino acids on the chromatography paper will dissolve in the solvent and not travel upwards over time.

3. Before going on to the next step grab a scrap piece of chromatography paper and a hollow glass tube and practice placing small dots on it using water. The goal is as small a dot as possible so it does not spread as it goes up the chromatography paper. When you feel confident (ask your instructor) move onto the next step.

4. At each dot place a small drop (less than 0.5 cm wide) of each known compound (amino acid) on the paper. Wait 2 minutes and repeat the process for a total of 3 times to increase the concentration of the amount of sample on each spot.

5. Wait 2 minutes and roll the paper into a tube (be sure the bottom is level and the spots are on the **outside**) and place two staples in it to hold it together.

6. Obtain a 1000 mL beaker and pour 50.0 mL of the solvent (acetic acid/1-butanol) in the bottom of the flask.

7. Place the rolled tube into the beaker, be sure that the spots are above the level of the solvent (liquid phase/mobile phase).

8. Place saran wrap or tin foil tightly over the top of the beaker to seal it in place. Be careful not to splash/slosh the solvent onto the paper)

9. Wait for the solvent to move up the chromatography paper until it is about 2-3 cm from the top of the paper (or you run out of time in class). While waiting for solvent to move up the chromatography paper work on post-lab questions or homework.

10. Remove the chromatography paper and mark the solvent line with pencil. Allow the paper to dry for 15 minutes, use a hair dryer if one is available. Dispose of the liquid phase (solvent) as directed by your instructor.

11. In the hood, spray the paper with a fine mist of ninhydrin solution, this should make the amino acid spots appear.

12. Calculate the R_f value for each spot and place it into the appropriate data table.

13. Save your chromatography paper, you may need to refer to or use it later and will need to turn it in with the lab.

Day 2 - Determining the identity of an unknown mixture of amino acids.

1. Obtain or make a known sample containing all four amino acids assigned to you **AND** an unknown sample from your instructor.

2. Run a chromatography experiment on both the known **AND** unknown samples at the same time using the procedure from Day 1. Since there are four spots you can run each twice.

3. Compare your known and unknown mixture to the results from Day 1.

4. Calculate R_f for each sample, average the results of the known together and average the results of the unknown together.

5. Identify the composition of you unknown.

6. Turn in your chromatography paper(s) to your instructor.

7. Dispose of the liquid phase (solvent) as directed by your instructor.

A blank or not so blank page that is the question.

Name: _____ Date: _____ Score: _____/80

Results

Day 1 Results

Assigned Amino Acid	**Distance Traveled (cm)**	**R_f value**
Solvent Front		
AA 1 -		
AA 2 -		
AA 3 -		
AA 4 -		

1. Why did you use pencil instead of pen to label your chromatography paper?

2. Why is it important to not touch the chromatography paper with your fingers?

3. Why is it important to have as small a spot as possible **AND** have it be as concentrated as possible?

4. Why is it important to have the compounds to be tested be above the mobile phase (solvent) in the beaker?

5. What is the purpose of spraying the chromatography paper with ninhydrin? What is the ninhydrin reacting with?

Day 2 Results

Use as many spaces as required in the table below for your known and unknown.

	Distance (cm)								
	Solvent	**Spot 1**	**Spot 2**	**Spot 3**	**Spot 4**	**R_f 1**	**R_f 2**	**R_f 3**	**R_f 4**
Mixture 1									
Mixture 2									
AVERAGE									
Unknown 1									
Unknown 2									
AVERAGE									

Postlab Questions

For each amino acid in your unknown calculate the percent error in the R_f value measured on Day 1 compared to the value measured on Day 2. Show a calculation for Spot 1.

	ID of AA	**R_f Known**	**R_f Unknown**	**Percent Error**
Spot 1				
Spot 2				
Spot 3				
Spot 4				

1. Based on your data from Day 1 and Day 2 what is the identity of the amino acids in your unknown?

2. Why did your known solution contain a mixture of all the amino acids?

3. Can you with 100% confidence state that you know the identity of the amino acids in your unknown sample. Explain.

Nothing to see here, move along

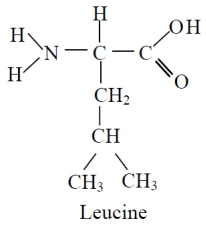
Name: _____ Date: _____ Score: _____/20

Prelab Questions

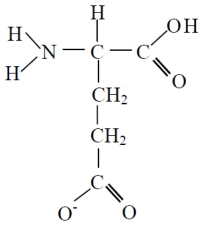
1. What part of an amino acid is always the same (conserved)? What part is always different? Draw a picture of an amino acid to illustrate this.

2. Draw **AND** name **three** functional groups that are hydrophobic/non-polar and **three** functional groups that are hydrophyllic/polar.

3. Given the following two molecules, if the stationary phase is polar and the mobile phase is non-polar, which amino acid will travel the furthest? Explain.

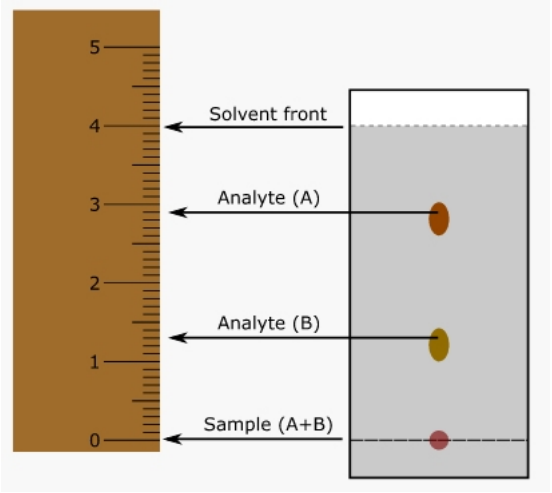


Leucine



Glutamate

4. Given the following chromatography experiment and data, determine the R_f value for each component. Explain.



Hello!