

## AMINO ACIDS AND PROTEINS

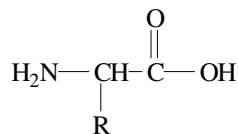
Adapted from S. L. Saeger and M. R. Slabaugh, *Safety-scale Laboratory Experiments for General, Organic, and Biochemistry for Today*, 4<sup>th</sup> Ed., p. 387.

**Materials Needed**

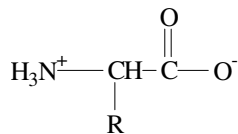
0.12% solutions of aspartic acid, phenylalanine, serine, and aspartame	gloves
Diet Coca-Cola, aged and fresh	capillary tubes
1 mL 3M HCl	heat gun
0.2% ninhydrin spray	drying oven
10 mL 12:3:5 butanol:acetic acid:water solution	400-mL beaker
Equal	250 mL Erlenmeyer
Ruler	100 mL fat-free milk
two 9 x 6 cm TLC plates	5 mL glacial acetic acid

**Introduction**

Alpha amino acids are the monomers, or building blocks, in proteins. These compounds are all similar in structure because each has two characteristic functional groups: the carboxylic acid group (-COOH) and the amino group (-NH<sub>2</sub>). They also contain a characteristic side chain that differentiates each amino acid from others. The general structure for an amino acid is shown. The R represents the side chain of the amino acid.



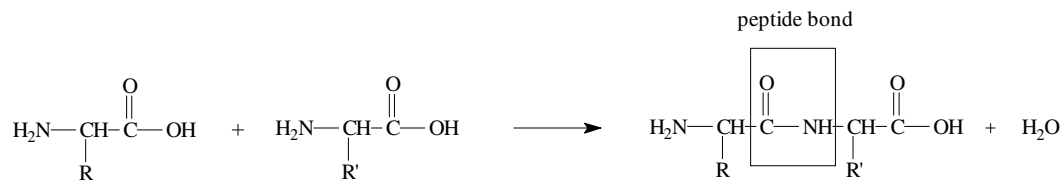
Although the structure is simplified, it is important to note that the amino acid probably does not exist as shown. Because both an acidic and a basic functional group are present, the acid will generally exist in *zwitterionic* form. That is, the acidic proton of the carboxylic acid functional group will protonate the nitrogen on the amino group, leaving an ionic form that is soluble under aqueous conditions.



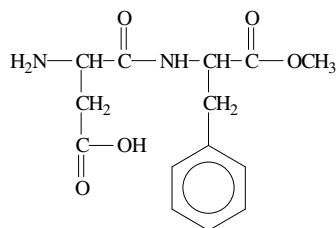
## ZWITTERION

In fact, different pH conditions can lead to different forms of the acid, also. Under acidic conditions, both the carboxy group and the amino group will be protonated. Under basic conditions, the *carboxylate* will be formed and the amino group will remain neutral. The ability for an amino acid to form several different types of ions explains its versatility in many cellular functions. In addition, the various R groups can contribute to its affinity for different solvents. Some R groups contain carbon and hydrogen atoms only which makes the amino acids nonpolar and hydrophobic. Others contain OH or SH groups that makes the amino acids hydrophilic. We can use the properties of the R groups to characterize the amino acids. The various solubilities provided by the side chains can be utilized to identify the different amino acids.

Peptide linkages hold amino acids together. Peptide linkages occur through *condensation* of two separate amino acid groups. The formation of each peptide bond requires the loss of one molecule of H<sub>2</sub>O. Likewise, peptide bonds can be broken through hydrolysis to form individual amino acids.



In this experiment, we will determine the percentage of the protein, **casein**, in non-fat milk. This will be done by precipitating the protein with acetic acid and weighing the amount of protein obtained. We will also use *thin-layer chromatography* to identify **aspartame**, an artificial sweetener found in diet pop, and its hydrolysis products.

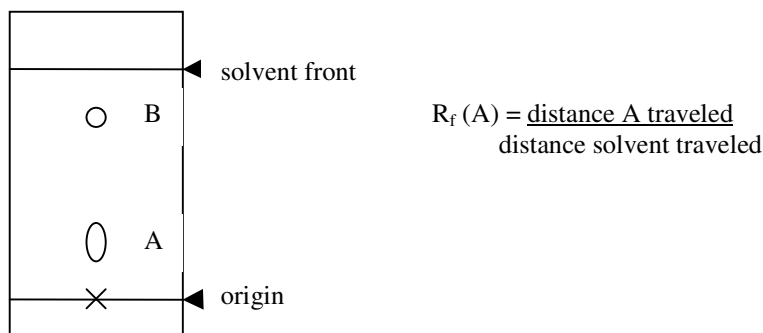


ASPARTAME

If you look closely at the structure of aspartame, you will notice that the terminal acid group is not a carboxylic acid, but rather the methyl ester of carboxylic acid. This type of variation in protein structure is very common throughout all types of amino acids. Therefore, upon hydrolysis, aspartame will yield aspartic acid, phenylalanine, and methyl alcohol.

Thin-layer chromatography is a useful analytical technique that allows various components of a mixture to be separated based on their polarity. Generally, a thin sheet of plastic or glass is used that has been coated with silica gel. Silica gel is a polar substance that will attract other polar substances. As a result, nonpolar substances will not have a strong affinity for the gel. A small drop of the mixture to be separated is applied near one end of the plate. The spotted end of the plate is then dipped into a developing solvent, called the *mobile phase*, which flows up the plate by capillary action. As the developing solvent flows up the plate, it can carry along the components of the mixture. The more soluble the component is in the solvent, the faster it travels up the plate. If it is not very soluble in the solvent, it will remain adsorbed on the surface of the silica gel, or *stationary phase*, and travel at a much slower rate.

The rates of flow are measured in terms of  $R_f$  values. An  $R_f$  is the relative distance that a sample component has moved relative to the distance moved by the developing solvent. The following illustration will demonstrate how the  $R_f$  is calculated.  $R_f$  is measured by dividing the distance the component traveled by the distance the solvent traveled. Therefore, an  $R_f$  value can never be greater than 1.



The  $R_f$  value for a particular component is characteristic for that component in that particular solvent. Therefore, it will always be the same and it can be identified in other mixtures. In this experiment you will first be determining the  $R_f$  value of pure substances and then comparing the data with the experimental data observed from the hydrolysis products of aspartame.

## PROCEDURE

### Aspartame analysis

1. Prepare a boiling water bath in a 250 mL beaker.
2. Obtain a TLC plate. Handle it carefully by the edges and do not bend. Using a lead pencil (not a pen), lightly draw a line across the plate about 1 cm from the bottom. Lightly mark 5 positions where your samples will be spotted. You may want to number the spots to keep them straight. Repeat the procedure for a second TLC plate.
3. Pour 10 mL of the butanol:acetic acid: water solvent mixture into a large (400 mL) beaker. Place a piece of filter paper in the mixture to get it wet and prop the filter paper up along the side. Cover the beaker with a watch glass.
4. Dissolve about 10 mg of Equal in 1 mL of 3 M HCl in a small test tube. Heat the tube in the boiling water bath for 5 min. Make sure the liquid does not completely evaporate! Cool the test tube and label it as hydrolyzed aspartame.
5. In the hood there are samples of each of the following substances: phenylalanine, serine, aspartame, aspartic acid, fresh Diet Coca-Cola and aged Diet Coca-Cola. You will use these samples for spotting your plates.
6. On one of the plates, spot samples of phenylalanine, aspartic acid, serine, aspartame, and hydrolyzed aspartame. Apply the sample to the plate until it spreads about 1 mm in diameter. Be sure to keep a log of which spot contains which sample! Allow the spots to dry.
7. Place the spotted TLC plate in the chamber you prepared in step 3. Make sure that the spots applied to the plate are above the surface of the eluting solvent.
8. On the second plate, spot samples of fresh Diet Coca-Cola on lane 1, aspartic acid on lane 2, aspartame on lane 3, phenylalanine on lane 4, and aged Diet Coca-Cola on lane 5. Multiple spotting will be required for the Diet Coca-Cola samples (about 12-15 times.) Allow the spot to dry each time before reapplying in order to prevent the spotting area from getting too large. Make sure you keep track of which spot is which and allow the spots to dry.
9. This TLC plate should be placed in the same chamber as the first one. The developing process will take a long time. During this time, continue on with the isolation of casein. Watch the plates carefully. When the solvent is about 1 cm from the top, they can be removed from the chamber. When finished, the drying process can be accelerated by using an air gun.
10. Using gloves, spray the plates with ninhydrin solution holding the solution about 6 inches away from the plate. Place the sprayed plates in a drying oven for 2-3 min.
11. Remove the plates from the oven and calculate your  $R_f$  values. Be sure to record your data.

### Isolation of casein

1. Place 100 mL (100 g) of fat-free milk in a 250-mL Erlenmeyer flask.
2. Heat the milk to 50° C in a hot tap water bath.
3. Add glacial acetic acid drop wise. The acid will cause the protein to precipitate out of solution, forming a clump and a clear solution. Add acid until no further precipitation occurs.  
**Caution:** Glacial acetic acid is extremely corrosive! Wear gloves and be careful not to spill any. If you get some on your skin/clothes, rinse off immediately with plenty of water.
4. Allow the sample to cool. Then decant the liquid down the drain, flushing with water from the faucet. Dry the solid with paper towels. Allow the protein to dry for a few minutes.
5. Weigh and record the mass of casein from the milk sample.

## PROCEDURE AND REPORT SHEET

### EXPERIMENT 6 - AMINO ACIDS AND PROTEINS

Name \_\_\_\_\_ Date \_\_\_\_\_

Partners \_\_\_\_\_

Plate I	Distance traveled (mm)		Solvent front (mm)	R <sub>f</sub>	R <sub>f</sub>
Phenylalanine					
Aspartic acid					
Serine					
Aspartame					
Hydrolyzed aspartame					

Plate II	Distance traveled (mm)		Solvent front (mm)	R <sub>f</sub>	R <sub>f</sub>
Aspartame					
Phenylalanine					
Aspartic acid					
Fresh Diet Coca-Cola					
Aged Diet Coca-Cola					

- Calculate the percentage of casein in nonfat milk. ( $\% \text{ casein} = \frac{\text{mass of casein}}{100 \text{ g milk}} \times 100$ )
  
- Name the amino acids you found in the hydrolysate of the sweetener Equal.
  
- Which of the aspartame samples, hydrolyzed or non-hydrolyzed, does the aged Diet Coca-cola best represent?

4. The shelf life of Diet Coca-Cola is ~3 mo. Why do you think this is?

5. Why does the milk protein precipitate when acetic acid is added? (See Chap 18.12 in McMurry)