Dipeptide bonds
Aspartame

Peptides
- Linking the α- amino group of one amino acid to the carboxyl group of a second amino acid produces a peptide.
- A peptide has two ends, the α- amino group is called the N-terminal amino acid residue and the free carboxylic end is called the C-terminal residue.

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\begin{align*}
\text{Aspartic acid residue} & \quad \text{Phenylalanine residue} \\
\text{H-NH-C(CH}_2\text{CO}_2\text{H})\text{C-NH-C(CH}_2\text{C}_6\text{H}_5\text{)}\text{-C-O-CH}_3 &
\end{align*}
\]

- Peptides - Amino Acids joined by Amide Bonds
- Peptides are named from the N-terminal amino acid to the C-terminal terminal amino acid. Aspartame is aspartylphenylalanine methyl ester (Asp-phe).

Some Biological Functions of peptides:
- Act as hormones (oligopeptides) with physiological functions such as pain relief and control blood pressure.
- Oligopeptides are produced and released in small amounts. They are rapidly metabolized because their physiological functions are necessary for only a short time.
- Examples: Somastatin (14-peptides) inhibits the release of other hormones such as insulin, glucagons and secretin. It has a half life of less than 4 minutes. Oxytocin, a peptide hormone produced in the pituitary cause contraction of the smooth muscles and is used to induce labor.
Aspartame

- Aspartame is the most common artificial sweetener.
- Aspartame is broken down to aspartic acid, phenylalanine and methanol.
- Conflicting reports in the effect of the aspartame’s breakdown products. Pro aspartame literature indicates that both aspartic acid and phenylalanine are naturally occurring amino acids and that although methanol is a toxic by-product, it is not produced in significant amounts to cause harm.

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\text{Aspartame breaks down into its respective Amino acids}
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- A number of literatures have indicated the toxic effect of aspartame. Proteins in food are broken down during digestion and released in the blood stream. The various amino acids compete for attachment sites on the enzymes and cell structures. This competition restricts any one type of amino acid from becoming too dominating causing an imbalance in the normal ratio.
• The amino acid isolates from aspartame is absorbed more quickly into the body because it does not require much to break it down as with protein.

• Ingestion of artificial sweetener such as aspartame spikes the plasma phenylalanine/large neutral amino acid ratio (LNAA ratio) leading to increase uptake of phenylalanine into the brain and a decreased uptake of other LNAA.

• It is believed by some researchers that excessive spiking of plasma phenylalanine levels can affect the levels of serotonin in the brain and possibly lead to neurological problems such as depression (Pardridge 1986).

• Synergistic damage also results form the absorption of methanol which can further oxidize to formaldehyde and then formic acid.

• Some symptoms attributed to aspartame are seizures, memory loss, depression and vision deterioration or loss.

Experiment

• Determine the presence of aspartame isolates and methanol in hydrolyzed aspartame and diet drink.

• The presence of aspartame breakdown products, aspartic acid and phenylalanine will be determined using paper chromatography.

• Determine if methanol is present in the hydrolyzed aspartame solution and in the hydrolyzed diet drinks using the KMnO₄ and ceric nitrate tests.

Paper Chromatography

1. Saturation of Chamber. The solvent is placed in the chamber and covered for several minutes. This creates equilibrium between the vapor and the liquid to develop in the chamber allowing the spots and the solvent to move up the TLC plate without evaporation, speeding the chromatographic run.
2. Draw the **line of origin** with a pencil 1cm from the bottom of the paper. Wear gloves when handling the paper.

3. All solutions on the Table 1 are prepared and dispensed in microfuge tubes. Read your labels very carefully.

4. Since you are not using stretch pipets, # of spots has been reduced. Table 2 change all # spots 2-3 to 1-2 and all 6 spots to 3. Spot over the original spot but LET DRY BETWEEN SPOTTING.

5. Running the Chromatogram. Make sure that the line of origin is **above** the solvent line and let it run until the solvent line is within 1-2 cm from the top of the plate. Mark the solvent line.

6. Separation of components. The components of the sample is separated based on their affinity to the solid phase (cellulose) and mobile phase (solvent; n-butanol, acetic acid and water). Remember, like dissolves in likes. The components that are non-polar will be held by the non-polar stationary phase and the more polar components will be carried by the polar solvent. Spots will be visualized by spraying the TLC plate with ninhydrin and developed in the oven.

7. Identification of components. Pure compound or standards (aspartame, phenylalanine, and aspartic acid) are run along side of the solutions to be tested. Rf values are determined by measuring the distance the individual spots and solvent traveled. A ratio is calculated

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R_f = \frac{\text{Distance a spot traveled (middle of the spot)}}{\text{Distance the solvent traveled}}
\]

- An Rf value needs to be calculated for each spot.

The Rf of the solutions are compared to Rf of the standards.
- What would affect the difference in Rf values of the same compounds?
- What compound’s presence are you confirming?
Test for methanol
1. Test freshly prepared aspartame, hydrolyzed aspartame, hydrolyzed beverage, regular beverage, methanol and distilled water for methanol using the KMnO$_4$ Test. The methanol is oxidized to acid and KMnO$_4$ is reduced to MnO$_2(s)$. Manganese dioxide is brown and potassium permanganate is purple.

2. Ceric Nitrate test. Positive test for alcohol is indicated by change in color from yellow to red/brown.

Data Analysis
- Determine the Rf values of the standards and samples.
- Did the hydrolyzed aspartame break down to aspartic acid and phenylalanine? Is heat needed to break down aspartame? Justify.
- Did the hydrolyzed beverage break down to aspartic acid and phenylalanine?
- Were aspartic acid and phenylalanine present in the aged beverage?
- What can you conclude from your data?