Biology and Biochemistry Department

BIOC311

Biochemistry Lab

Experiment #5

Title: The Separation of Amino Acids by Ion Exchange Chromatography.

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Objective:

The objective of this experiment is to observe the rate of elution of different amino acids and the properties that they have that affect their rate of elution.

Introduction:

Ion chromatography, which includes ion exchange, ion-partition/interaction, and ion-exclusion chromatography, is an important analytical technique for the separation and identification of ionic compounds. Ion chromatography uses ionic interactions between ionic and polar analytes, ions in the eluent, and ionic functional groups connected to the chromatographic support to separate them. Separation occurs in ion chromatography through two distinct processes: ion exchange due to competitive ionic binding and ion exclusion due to repulsion between similarly charged analyte ions and the ions fixed on the chromatographic support. (1)

Peptides, proteins, nucleic acids, and related biopolymers are separated using this chromatography, which is one of the most extensively used adsorption methods for separating charged molecules of various sizes and kinds. Ionic bonds are formed between the charged groups of the biomolecules and the oppositely charged ion-exchange gel/support to achieve separation. Because of their different charge properties, biomolecules interact with charged chromatographic mediums in varying degrees. (2)

Materials:

- Chromatography column (20 cm x1.5 cm)
- Dowex 50 (100-200 mesh) suspended in 0.04 M Na citrate pH 2.8
- Sand
- 0.04 M Na citrate pH 2.8
- 0.08 M Na citrate pH 3.4
- 0.1 M Na citrate, pH 4.8
- 0.4 M Na citrate, pH 7.0
- Glass wool
- Amino acid mixture: 2 mg/ml each of L-aspartic acid, L-tyrosine, and
- L-arginine in 0.04 M sodium citrate buffer, pH 2.8 (sample 1)
- Unknown amino acid (sample 2)
- Buffered Ninhydrin reagent: 2 g of ninhydrin and 0.3 g of hydrindantin in 75
- ml of 2-methoxy ethanol (methyl cellosolve) followed by dilution with 25 ml
  of 4 N sodium acetate (pH 5.5). Store in a dark bottle.
- Boiling water bath.

Methods:

1. a 1x15 cm column was used for this purpose. The stopcock was closed.

2. 6 ml of water was poured and the level was marked to which filled
the column.

3. the water was poured out slowly and enough Dowex 50 was added to fill the column to the 6ml mark. The resin is in a slurry of about 50% solids. the mixture was swirled to suspend the beads.

4. The suspension was pipetted into the column. the resin was allowed to settle, and the step was repeated until the resin level is at ~6-ml.

5. about 1-2 ml of sand was slowly poured on top of the resin in the column. The sand prevented the resin from being disturbed by the elution buffers.

6. The stopcock was opened and was allowed 15 ml of 0.04M sodium citrate buffer, pH 2.8 to pass through to equilibrate the resin.

Application of Sample

1. The liquid was allowed above the resin to drain through the column until the level reached the top of the column and the stopcock was shut.

2. exactly 4 ml of the sample 1 solution was Carefully added to the top of the column, being careful not to disturb the top of the resin.

3. The sample was allowed to pass into the resin and the first 4 ml of breakthrough effluent was collected.

4. The stopcock was shut and the wall of the column was washed with 2 ml of the pH 2.8 citrate buffer.

5. The effluent was collected for a total of 6 ml of breakthrough effluent and will be designated as "Fraction 1”.

Elution of Amino Acids

1. The following buffers will be used sequentially to elute the amino acids from the column, one at a time.

2. They will be used in the correct order or the experiment will fail.

1) 0.08 M Na Citrate, pH 3.4 (48 ml)

2) 0.1 M Na Citrate, pH 4.8 (60 ml)

3) 0.4 M Na Citrate, pH 7.0 (60 ml)

3. When the amino acid solution and wash have passed completely into the column, the stopcock will be closed and 3 ml of the first buffer (pH 3.4) will be carefully added to the top of the column.

4. The buffer will be added slowly, against the sides of the column walls so that the sand will not be disturbed so much as to affect the resin underneath.

Data and results:

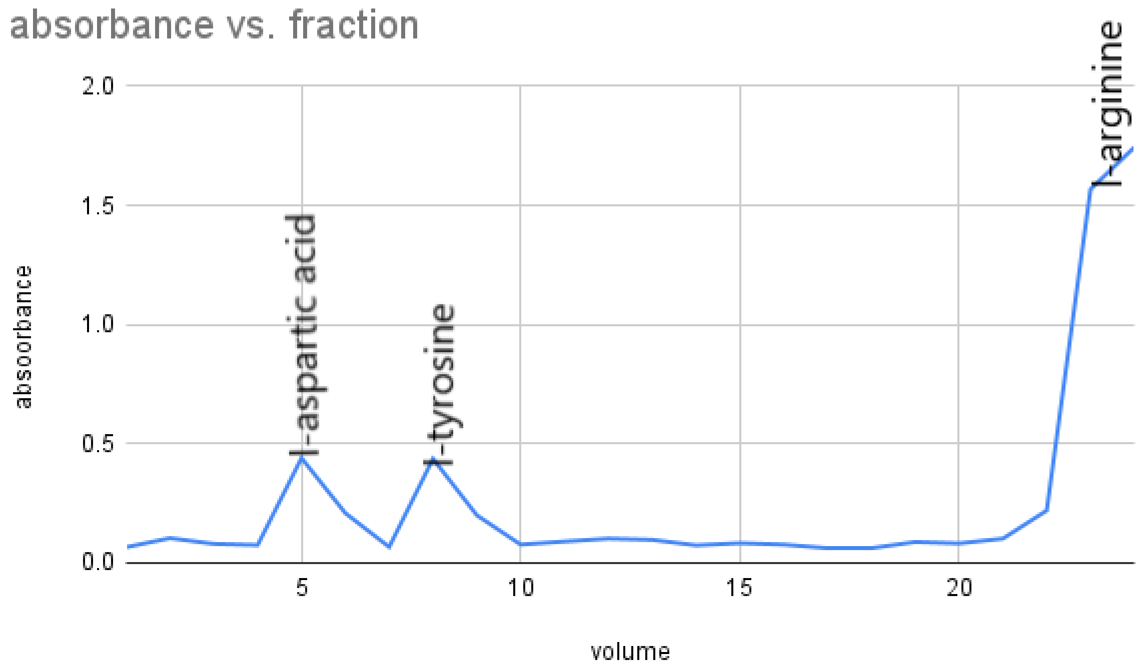

Chart 1: absorbance of a mixture of amino acids (l-aspartic acid, l-tyrosine, and l-arginine at 570nm)

Discussion:

Ion exchange chromatography is an efficient way to evaluate amino acids. This is due to the fact that each amino acid has a specific charge at a specific pH which allows for ion exchange. In chart 1, 3 different peaks occur, proving the existence of three different amino acids. The first amino acid is assumed to be aspartic acid. This is due to the fact that aspartic acid has the closest pI with the column itself (around 3.9). Also, this gives aspartic acid a positive charge, making it elute first.

The second peak is that of tyrosine, which has a slightly higher pH than aspartic acid (around 5). Tyrosines peak showed up when the pH was increased with Na Citrate. This made tyrosine have a slightly more positive charge and leave its typical zwitterion state.

Lastly, arginine was the third and final peak. This is because arginine is highly basic, which makes it the most anionic. Arginine typically has a pH of about 12.5, but since we used l-arginine its pH was around 7. The point is, arginine performed a cationic exchange with the addition of Na citrate pH7. This had arginine leave its zwitterion phase and elute.

Conclusion:

In conclusion, each amino acid has a charge depending on its pH. A lower pH results in a cationic amino acid, and a higher pH results in an anionic amino acid. Depending on whether the amino acid is cationic or anionic, there will be an anionic/cationic exchange with the chromatography tube.

References:

1. ***Haddad, P. R., & Jackson, P. E. (1990). Ion chromatography. Elsevier.***
2. ***Grodzki, A. C., & Berenstein, E. (2010). Antibody purification: Ion-exchange chromatography. In Immunocytochemical Methods and Protocols (pp. 27-32). Humana Press.***

Appendix:

***** the following data is from group 3 sec 2 lab.***