

Purpose: (4/5)

In this experiment, ion exchange chromatography was performed in order to separate a mixture of glycine, lysine, and aspartic acid. This was achieved by taking advantage of the amino acids' molecular structures and ionic characters. Separation of these amino acids was accomplished by using a gradient of increasing pH that then was eluted in the order of their isoelectric point. A colorimetric assay was then used to test for the presence of the amino acids using Ninhydrin. As well, during this lab an unknown protein was analyzed by using its molecular weight with a technique known as SDS polyacrylamide gel electrophoresis (SDS-PAGE). The unknown protein was then compared with molecular weight markers and the weight of the unknown protein was calculated using a line of regression equation.

Comment [m1]: To determine the amino acid concentration

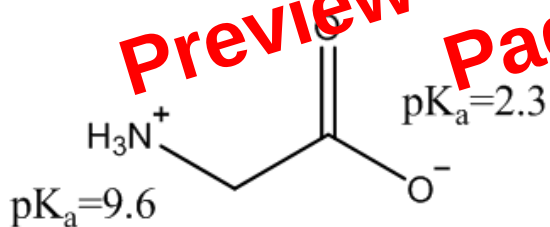
Comment [m2]: separate

Comment [m3]: Molecular weight

Results and Discussion:

R1. (15.5/17)

Glycine



$$\text{pK}_{a1} = 9.60, \text{pK}_{a2} = 2.34$$

$$\text{pI} = \frac{\text{pK}_{a1} + \text{pK}_{a2}}{2}$$

$$\text{pI} = \frac{9.60 + 2.34}{2}$$

$$\text{pI} = 5.97$$

Lysine sample calculations from fraction 13

A570 corrected calculation

$$A_{F13 \text{ corrected}} = A_{F13} - A_{\text{blank}} = 0.373 - 0.054 = 0.319$$

Concentration and moles calculations

Variables: $A_{\text{lys}} = 0.533$, $c_{\text{lys}} = 1 \text{ mM}$, $A_{F13} = 0.319$, $V_{\text{avg}} = 4.6 \text{ mL}$

$$\frac{A_{\text{lys}}}{c_{\text{lys}}} = \frac{A_{F13}}{c_{F13}} \rightarrow A_{\text{lys}}(c_{F13}) = (A_{F13})(c_{\text{lys}})$$

$$c_{F13} = \frac{(A_{F13})(c_{\text{lys}})}{A_{\text{lys}}} = \frac{(0.319)(1 \text{ mM})}{0.533} = 0.598 \text{ mM} = 5.98 \cdot 10^{-4} \text{ M}$$

$$c_{F13} = \frac{n}{V_{\text{avg}}} \rightarrow n = (c_{F13})(V_{\text{avg}}) = (5.98 \cdot 10^{-4} \text{ M})(4.6 \cdot 10^{-3} \text{ L}) = 2.8 \cdot 10^{-6} \text{ mol}$$

The concentrations of aspartic acid, glycine, and lysine for their fractions are $8.8 \times 10^{-5} \text{ M}$, $5 \times 10^{-4} \text{ M}$, and $5.98 \times 10^{-4} \text{ M}$. The μmol for aspartic acid, glycine, and lysine in their fractions are 4×10^{-6} moles, 2.3×10^{-6} moles, and 2.8×10^{-6} moles.

Correct absorbances with blank: 0.9/1.8

Correct formula: 1.8/1.8

Correct calculations: 2.4/2.4

Presence of units: 1.8/1.8

Correct answers: 2.4/2.4

Correct standard used: 1.8/1.8

Comment [m7]: You also have to show calculations for the corrected absorbances of the three standards