

Chemistry 365 Biochemistry

Laboratory Unit #2

Spectrophotometric Determination of Amino Acids with Ninhydrin

References:

Lee, Y.P.; Takahashi, T. *Anal. Biochem.* **1966**, *14*, pp 71-77.

Robyt, J.F.; White, B.J. *Biochemical Techniques Theory and Practice*; Waveland: Prospect Heights, IL, 1990.

Solutions:

- 1) ninhydrin reagent - 63 % glycerol (volume), 37% 0.5 M citrate buffer, pH 6.5 containing 7.1 g ninhydrin per liter.
- 2) amino acid solutions - 0.002 M solutions of phenylalanine, glycine, and aspartic acid

Laboratory:

- 1) Prepare a blank by mixing 3.8 mL ninhydrin reagent and 0.2 mL distilled water.
- 2) For each amino acid, prepare a range of four concentrations by mixing 3.8 mL ninhydrin reagent and varying amounts of amino acid stock solution and water that add to 0.2 mL.
- 3) Mix each solution well and heat in a boiling water bath for 45 minutes.
- 4) Cool the solutions and read the absorbances versus the blank at 570 nm.

Report:

- 1) Write a brief summary of the experiment and spectrophotometric analysis in your notebook.
- 2) Include the structures of ninhydrin and each of the three amino acids in your notebook.
- 3) Prepare a Table, using a spreadsheet, for each of the 2 amino acids you used that includes a title with complete explanation, the **actual** amino acid concentrations in the final mixtures, the corresponding absorbances, and the regression results with best fit line.
- 4) Prepare a Figure for each showing the graph of absorbance (570 nm) versus concentration (M) for each of the 2 amino acids that includes a title with complete explanation, the experimental data points and the best fit line.
- 5) Write an abstract to report the results of your study that contains:
 - a) a statement of the purpose of the experiment (hypothesis)
 - b) a statement of the experimental plan used (refer to authors' names and year of publication)
 - c) a statement of the principal results (the molar absorptivities of the colored products, with uncertainty; proper units and significant figures)
 - d) a statement of your conclusion (did your results support or disprove the hypothesis)
- 6) Hand in your abstract and the 2 tables and the 2 figures.