Chem 131A: Folin-Ciocalteau Analysis for Protein

This method is quite sensitive. Samples containing as little as 5 ug of protein can be readily analyzed.

The principle depends on the reaction of the phenolic (R) group of tyrosine with the colorless phosphomolybdotungstate ions in the presence of Cu^{2+} ions. The phenol is oxidzed and the complex phosphomolybdotungstate is reduced to a blue chromogen that can be estimated spectrophotometrically.

Important!! Steps 1 and 2 must be done at the same time.

1) Standard Tyrosine Curve: To obtain data for the curve, set up the following tubes:

Tube	1	2	3	4	5	6	UNK	UNK
Standard tyrosine (mL)	0	0.10	0.20	0.30	0.40	0.50	0.30	0.50
Water (mL)	1.20	1.10	1.00	0.90	0.80	0.70	0.90	0.70
Absorbance								

Standard tyrosine solution: 100 μg tyrosine/mL

2) Tyrosine Unknown.

You will receive an unknown containing tyrosine plus an inert compound. The percentage of tyrosine varies from 10 to 90 %. Weigh out an exact amount (calculate) of unknown in a beaker on an analytical balance (~1mg). Dissolve, then dilute to volume in a 10 mL volumetric flask. Set up two tubes as in the table above. Accurately determine the % tyrosine (3 sig. places) in the unknown.

When all 8 tubes have been prepared (this can be shared between two people):

1) Prepare 100 ml of fresh alkaline copper reagent by mixing together in the following order: 1 mL of 1% $CuSO_4 \cdot 5H_2O$, 1 mL of 2% sodium tartrate and 98 mL of 2% Na_2CO_3 in 0.1 M NaOH.

2) Add and mix immediately 6 mL of fresh alkaline copper solution to each tube. Allow all the tubes to stand for 10 minutes at room temperature. Add and mix immediately 0.3 mL of Folin-Ciocalteau reagent to each tube. Allow 30 minutes for full color development. Measure absorbance at 500 nm. (Adapted from Clark and Switzer, EXPERIMENTAL BIOCHEMISTRY, 2nd edition, p.12, 1977, Freeman.)

3) Plot a standard curve: absorbance vs μg of tyrosine. Use the standard curve to determine μg of tyrosine in your unknown.

Important!! Steps 3 and 4 must be done at the same time.

3) Standard protein curve. To obtain data for this curve, set up the following tubes:

Tube	1	2	3	4	5	6	UNK	UNK
Standard Egg Albumin (mL)	0	0.10	0.20	0.30	0.40	0.50	0.30	0.50
Water (mL)	1.20	1.10	1.00	0.90	0.80	0.70	0.90	0.70
Absorbance								

Standard Egg Albumin Solution: 400 μg protein/mL

4) You will be given a solution containing egg albumin at a concentration ranging from 70 to 380 μg/mL. Set up two tubes as indicated in Table 2. Determine and report the concentration of the unknown protein.

When all 8 tubes have been prepared (this can be shared by two people):

1) Add and mix in the same reagents to each of these tubes as described for tyrosine determination (Items 1 and 2 under A).

2) Plot a standard curve: absorbance vs μg of protein. Use the standard curve to determine μg of protein in your unknown.