Revised Fall 2010

Biuret Protein Assay

Purpose and goals:

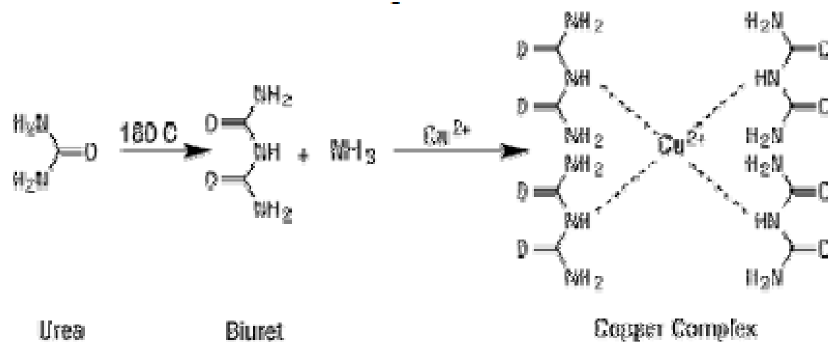
- to pipet accurately
- to prepare a standard curve
- to learn the use of the spectrophotometer
- to assay protein samples of unknown concentration
- to analyze data from standard curve and unknowns

Reagents and equipment needed:

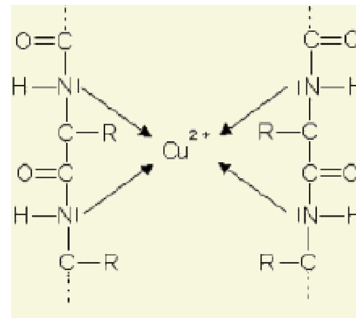
- Stock solution of Bovine Serum Albumin (BSA): 10mg/ml
- Deioninzed H_2O
- 20 test tubes
- P-1000 and P-200 pipetmen with tips
- Biuret reagent
- 0.5% SDS

Background:

There are a number of very good and very sensitive protein assay methods available to scientists. One of the simplest and most common is the Biuret Protein Assay. The name of this assay is somewhat confusing because assaying for proteins using this method does not actually use biuret. Biuret is a small compound that forms when urea is heated which causes two urea molecules to join. Urea molecules fused in this manner produce amide groups (-NH) at the center of the molecule which bind to copper ions at a basic pH. The copper complexes that result from this interaction produce a strong blue color that can be measured with a spectrophotometer.



Proteins also contain amide groups. When an amino group and a carboxyl group join to form a peptide bond, the amino group ($-NH_2$) becomes an amide group (-NH). Therefore, proteins will also complex with copper ions at a basic pH. Because this reaction was first observed with biuret, it is called the biuret reaction, and when this reaction is used to measure protein concentrations, it is called the Biuret Protein Assay. Thus, in this assay you will combine protein samples with Biuret Reagent which contains copper ions in a basic solution. The copper ions will complex with the amide groups in the



proteins to create a blue color that will be measured using a spectrophotometer. The amount of blue color that forms is directly proportional to the quantity of protein in your samples.

Procedure:

In addition to the standard curve, you will be assaying one of seven different protein samples of unknown concentration. Choose one of the unknowns (chicken, beef, pork, fish, egg white, tofu, milk) from the instructor's bench. Weigh out 0.3-0.4 g of the sample into a 50 ml blue cap conical tube. To the sample, add 10-15 colored glass beads and 10 ml of 0.5% SDS. SDS is a detergent that will help to disrupt cellular membranes and release proteins into solution. For milk and egg white samples, vortex the mixture for 10 seconds, then let the samples sit at room temperature for 10 minutes. For the chicken, beef, pork, fish, and tofu samples, vortex the mixture for 1 minute, then centrifuge these mixtures at 2500 rpm for 8 minutes in a swinging bucket centrifuge. When removing the tubes from the centrifuge and carrying them to your table, take care not to shake the sample.

While the sample is spinning, prepare the standard curve as follows. Label 2 sets of test tubes with the numbers 1 through 6. Pipet the amount of water indicated in the chart below into each tube, then pipet the appropriate amount of BSA stock solution (10 mg/ml). Do not add the biuret reagent until your unknown sample is ready.

Tube #	BSA Conc. (mg/ml)	H20 (ml)	BSA stock (ml)	Biuret Reagent (ml)	ABS	ABS	ABS average
1	0	1.0	0	2			
2	1	0.9	0.1	2			
3	2	0.8	0.2	2			
4	3	0.7	0.3	2			
5	4	0.6	0.4	2			
6	5	0.5	0.5	2			

Carefully pipet 1 ml of the liquid portion of your unknown sample into clean, duplicate test tubes (DO NOT add the unknown to the tubes you made for the standard curve). Now add 2 ml biuret reagent to every tube: the 12 tubes for the standard curve and the 2 tubes for your unknown. Cover the tubes with parafilm and briefly vortex to ensure that the sample and the biuret are thoroughly mixed. Allow all of the tubes to stand at room temperature for 15 minutes. While you are waiting, turn on the spectrophotometer and allow it to warm up; this may take 1-2 minutes. Do not push any buttons until the machine is ready (when the ABS reading at 542 nm appears on the screen). Adjust the wavelength to 550 nm.

Before each tube is read in the spectrophotometer, gently wipe the tube with a paper towel to remove fingerprints and dust. After 15 minutes, place tube 1 into the spec

and set the absorbance to zero. This tube will serve as the blank. Record the value for this tube in the chart. Measure the absorbance of the other tube 1 as well. The absorbance should be very close to zero. DO NOT zero/blank the instrument again. Continue measuring the absorbance of the other standards and record them in the chart. Finally, record the absorbance of your unknown. Determine the average ABS for all tubes.

Unknown	Amount sol'n (ml)	Biuret Reagent (ml)	ABS	ABS	ABS average	Conc. (mg/ml)	Conc. (mg/g)

In order to determine the actual concentration of protein in the unknown samples it is necessary to graph the standard curve (concentration on the x-axis and on the absorbance y-axis) and interpolate the absorbance values of the unknowns. A more accurate method is to perform a linear regression analysis on the standard curve which will yield the equation for a straight line: $y = mx+b$. With this equation you can calculate the concentration by entering the absorbance value of each unknown into the equation as the y value and then solve for x.

Instructions for creating a graph (chart) and obtaining the equation for a line in Excel 2007.

Data Input (You are only entering data from the standard curve samples, NOT your unknown!)
-Input x data (protein concentration) in a column.
-Input y data (absorbance) in a column (next to the first).

Create a Graph
-Highlight the data using your mouse.
-Under the "Insert" tab, select "Scatter Chart" and choose the first option (without lines connecting data points). The graph should appear with your data.

Titles
-Under the "Chart Tools" tab, select "Layout".
-Select "Chart Title" and choose the option for "Above Graph". A text box will appear – click in this box and enter an appropriate title (e.g. Biuret Standard Curve).
-Select "Axis Titles" and "Primary Horizontal Title". Select the option for "Title Below Axis". A text box will appear below the x axis. Click on the text box and type in "Protein Concentration (mg/ml)".
-Select "Axis Title" and "Primary Vertical Title". Select the option for "Rotated Title". In the text box that appears on the y axis, type in "Absorbance".
-Select "Legend" and click on "None" to remove the legend from the graph.

Calculating Linear Regression

-Under the "Chart Tools" tab, select "Layout".

-Select "Trendline" and choose the "Linear Trendline" option. A line will appear on the graph.

-Select "Trendline" once more and choose the "More Trendline Options" at the bottom. A separate window will pop up. Click on the box next to "Display equation on chart". A check should appear in the box. Click "Close" to exit the window.

-The regression equation should now be displayed on the graph. You can click on it and move it around to a different spot if you wish.

Save your graph and print.

Use the regression equation to calculate the protein concentration of your unknown sample. (Plug in the unknown absorbance value for y and solve for x).