EXPERIMENT 6

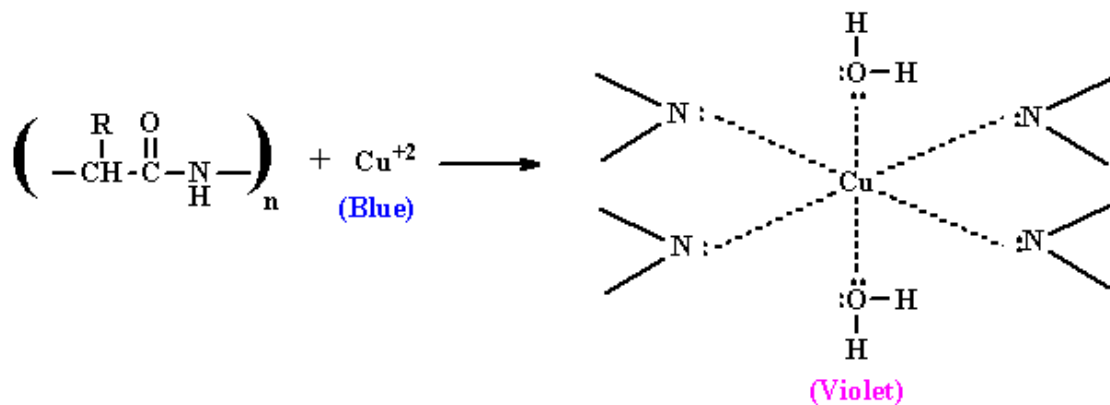**PROTEIN EXTRACTION AND PROTEIN ANALYSIS USING BIURET METHOD**

Figure 1: Biuret assay is based on the reaction of protein with copper (III) sulfate and positive result is the formation of a violet colored complex. (Source: http://people.uwplatt.edu/~sundin/351/351h-pro.htm)
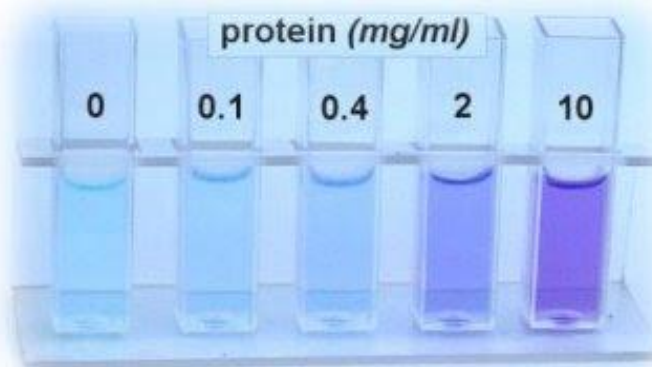

Figure 2: Color changes based on protein concentration. (Source: http://igbiologyy.blogspot.my/2012/12/33-biuret-test-for-proteins.html)

OBJECTIVES:

Student should be able to

1. To explain and conduct protein extraction

2. To explain and conduct protein analysis using biuret method.

MATERIAL

- Culture sample (3 samples x 5 mL each)
- Eppendorf or centrifuge tube
- Sterile distilled water
- Universal bottles
- Glass pipettes
- Pipettor
- Biuret reagent
- 0.2 M NaOH
- Bovine serum albumin, BSA (5 mg/mL)

METHODOLOGY

1) Standard curve for protein concentration determination using Biuret Reagent
   i) Standard curve is prepared by diluting BSA stock solution to five different concentrations with distilled water.
   ii) Take 1.0 mL from each sample into a new bottle and mix with 2 mL Biuret Reagent. Mix or vortex thoroughly.
   iii) Let the mixture stands in room temperature for 15 mins.
   iv) Then, analyze the absorbance of each sample mixture at 550 nm.
   v) Plot an appropriate graph to establish a standard curve for protein concentration determination.

2) Sample preparation

   i) Centrifuge the culture samples given and separate the upper part (supernatant) and transfer it to an empty bottle. DO NOT THROW IT AWAY.
   ii) As for the cell pellet, add in 0.5 mL of 0.2 M NaOH and mix thoroughly.
   iii) Incubate the tubes for 5 minutes in 90 C waterbath.
   iv) Centrifuge to discard any cell debris and collect the supernatant.
   v) Mix the supernatant with 0.5 mL of distilled water.
   vi) Then, proceed with the biuret assay, together with the supernatant that you kept previously in the first step.
   vii) Estimate the protein concentration of each sample using the standard curve you plotted previously.

3)

QUESTIONS

1. Give an example of other methods to estimate protein concentration.
2. Estimation with color differentiation could give false results for some proteins. Give three reasons that may contribute to this problems.