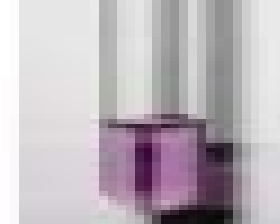# Investigating Protein Concentration with a
Quantitative Biuret Test

Aim: To investigate the protein concentration of an unknown solution.

Essential Information:

To test for proteins the biuret test is used. The presence of protein is indicated by a purple colour when the test reagents are added. The purple colour shows the presence of the protein, but not clearly how much protein.

A **quantitative test** is one that also reveals the concentration of the molecule of interest in the solution, not just whether the molecule is present, but how much. The intensity of the color formed depends on the concentration of protein in the solution. The greater the protein concentration, the deeper the purple.

The intensity of the purple colour can be measured using a colorimeter. A colorimeter will measure the absorbance of the solution, and also the % transmission of the solution. The darker the colour, the greater the absorbance and the lower the % transmission.

The absorbancy or transmission recorded for an unknown protein solution can be compared with a 'standard Curve' of absorbance or transmission produced from known concentrations of protein solutions.

The graph can be used to determine the protein concentration of the unknown solution.

Procedure

1. Label 5 test tubes: 0.1g, 0.2g, 0.3g, 0.4g, Egg. Place in a test tube rack.

2. Pipette 2ml of protein solution 0.1g/100ml into the test tube labeled 0.1g.

3. Add 5ml of Biuret reagent. Then, CAREFULLY swirl the tube to ensure that the materials are uniformly mixed. The color-producing reaction of the reagents with the protein won't occur properly unless the solutions in the test tubes are thoroughly mixed. Do not invert the test tubes, just carefully swirl it to mix the contents.

4. Repeat the above for the other solutions 0.2, 0.3, 0.4 and Egg. Ensure that you use a clean pipette for each solution. Place all of the test tubes in a water bath set at 37°C.

5. Leave the tubes for 15-20minutes (use a stop clock). This will allow full colour development.

During this period, prepare a "blank" solution made up of **2ml Biuret reagent and 2ml water.** Place this solution into the colorimeter and calibrate the dials until the reading shows 100% light transmission. This will ensure that when you measure the transmission and absorbance of the solutions, the results are due to the colour developed because of the presence of protein (and not due to the light absorbed by the glass of the cuvette or the blue colour of the biuret test).

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