Assessed practical BI308:

The aim of this practical test is to assess your accuracy and manual skills. The task is to determine the concentration of a protein solution using the Biuret-method.

1. You are provided with a solution of bovine serum albumin (BSA) of concentration 10mg/ml.

    For your standard curve, set up 8 test tubes:

Test tube no	1	2	3	4	5	6	7	8
BSA (ml)	0	0.1	0.2	0.3	0.4	0.5	0.7	1
H$_2$O (ml)	1	0.9	0.8	0.7	0.6	0.5	0.3	0
Total volume (ml)	1	1	1	1	1	1	1	1
BSA (mg/ml)								

    Calculate the concentration of BSA (mg/ml) in each tube, and fill in the table above.

2. You are provided with a protein solution of unknown concentration (A, B, C, D, E, or F). Set up 3 tubes from one (!) of the solutions with

'unknown' (ml)	0.2
H$_2$O (ml)	0.8
Total volume (ml)	1

3. To each of the tubes above, add 7ml of the biuret reagent 1.5g CuSO$_4$.5H$_2$O, 6g NaK tartrate, 1g KI and 30g NaOH per litre), mix well and leave for 30 min. at room temperature.

4. Pour the blank solution (i.e. tube 1, no BSA) into one of the cuvettes and 'zero' the instrument at <u>550 nm</u>.

6. Replace by each of the other samples in turn and read the extinction at 550 nm. (Check the stability of the instrument by re-reading the blank from time to time).

7. Plot a graph of extinction against the amount (concentration in mg/ml) of BSA present. From this graph, determine the concentration of the unknown sample. Give an average of your measurements.

    COSHH Regulations 1988. This instruction sheet has been subjected to a formal risk assessment. **<u>Safety specs and gloves must be worn at all times</u>**.

    Signed: Date: