Biochemistry and Immunology Lab (BBT209)

Experiment 3 a

Estimation of total protein in unknown sample by biuret method using standard curve

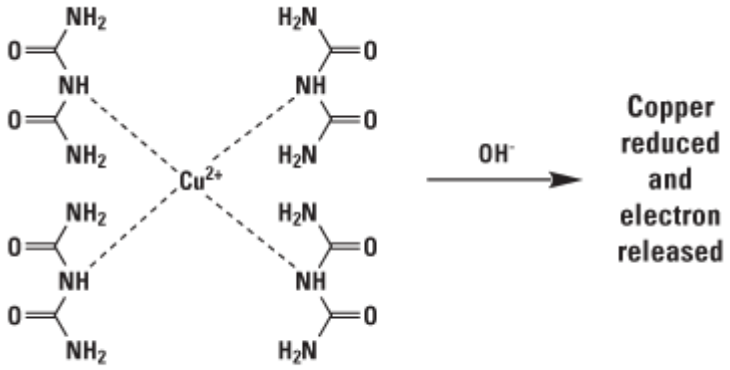
Background:

Plasma proteins are synthesized predominantly in the liver plasma cells, lymph nodes, spleen and in bone marrow. In the course of disease the total protein concentration and also the percentage represented by individual fractions can significantly deviate from the normal values. Hypoproteinemia can be caused by diseases and disorders such as loss of blood, nephritic syndrome, severe burns, salt retention syndrome and kwashiorkor (acute protein deficiency). Hyperproteinemia can be observed in case of severe dehydration and illness such as multiple myeloma changes in the relative percentages of one plasma protein fraction. Often in such cases the amount of total protein does not change. The A/G is commonly used as in index of the distribution of albumin and globulin fractions. Marked changes in this ratio can be observed in cirrhosis of the liver, glomerulonephritis, nephrotic syndrome, acute hepatitis and lupus erythrometosus as well as in certain acute and chronic inflammations. The total protein measurements are used in the diagnosis and treatment of a variety of diseases involving the liver, kidney or bone marrow as well as other metabolic or nutritional disorders.

Principle:

Biuret methods are based on the formation of coloured complexes between peptide bonds and cupric ions, where divalent copper reacts with the peptide bonds of protein under alkaline conditions to form an intensive violet-blue complex. Iodine is included as an antioxidant.

$$\text{Protein} + \text{Cu} \xrightarrow{\text{Alkaline pH}} \text{Cu-protein complex}$$



Biuret Reaction

Reagents:

Protein standard	Bovine serum albumin (BSA)	5 g/dl
R Biuret	Sodium iodide	100 mmol/l
	Potassium iodide	5 mmol/l
	Copper (II) sulphate	19 mmol/l

Procedure:

Specimen: Bovine Serum Albumin (BSA)

Standard preparation: Using the given BSA solution, prepare 5 standards of different concentrations (200, 400, 600, 800 and 1000 mg/dl) using dilution from a stock solution of 5 g/dl

	Desired Concentration (mg/dl)	**Dilution**	**Volume of BSA solution (µl)**	**dH_2O (µl)**	**Total volume (ml)**
5.	1000	1:25			1.0
4.	800	1:25			1.0
3.	600	1:25			1.0
2.	400	1:25			1.0
1.	200	1:25			1.0
Blank	0	1:25			1.0

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Standards: BSA solution in different concentration 200mg/dl, 400mg/dl, 600mg/dl, 800mg/dl & 1000mg/dl.

Assay preparation:

Take 7 test tubes and level them as B (Blank), Std1, Std2, Std3, Std4, Std5, and Unknown Solution.
Prepare the test tubes as shown in the table below:

Tube	**B**	**1**	**2**	**3**	**4**	**5**	**SA**	**SP**
Reagents(R)	1ml	1ml	1ml	1ml	1ml	1ml	1ml	1ml
Standards	50µl of dH_2O	50 µl of 200mg/dl	50µl of 400mg/dl	50µl of 600mg/dl	50µl of 800mg/dl	50µl of 1000mg/dl	50µl of unknown Serum solution	50µl of unknown Protein solution
Absorbance								

Mix well and incubate for 5 minutes at 37ºC or 10 minutes at room temperature.
Measure the absorbance of the blank and standards at 545 nm.
Colour is stable for at least 30 minutes.

Graph preparation: Plot an abs vs. concentration graph using the values,
Y-axis concentration of standards
X-axis absorbance of the standards

Experiment 3 b

Estimation of total protein in serum by enzymatic method using semi-automated Biochemistry Analyzer

Reagents:

Protein standard	Bovine albumin	5 g/dl
R Biuret	Sodium iodide	100 mmol/l
	Potassium iodide	5 mmol/l
	Copper (II) sulphate	19 mmol/l

Procedure:

Sample: Serum

Sample collection: Sample must be collected from any healthy individual. Specimen may be stored at 2-8ºC prior to analysis.

Sample preparation: Blood cells are removed by centrifugation. After that the top clear layer of serum is collected by pipetting carefully.

Prepare the tube as shown below:

	Blank	**Standard**	**Sample**
R	1.0 ml	1.0 ml	1.0 ml
Standard	-	10 µl	-
Sample	-	-	10 µl
dH_2O	10 µl	-	-
Absorbance			

Mix well and incubate for 5 minutes at 37ºC or 10 minutes at room temperature.
Measure the absorbance of sample at 545 nm. Colour is stable for at least 30 minutes.

Calculation: Calculate the total Protein concentration by using the following formula:

Total Protein concentration = (Absorbance of sample/Absorbance of standard) x [Standard]

(Unit conversion: mg/dl x 1.45 = ___ mmol/L)

Expected value:

Adults 6.3-8.3 g/dL

Children

> 1 year	6.0-8.0 g/dL
< 1 year	4.6-7.6 g/dL