Total protein determination by spectrophotometric biuret method

Biuret method is the most widely used method and the one recommended by the International Federation of Clinical Chemistry (IFCC) expert panel for the determination of total protein.

Principle:

Cupric ions (Cu^{2+}) in the biuret reagent complex with the groups involved in the peptide bond. In the presence of alkaline media i.e. 3% NaOH and at least two peptide bonds a violet colored chelate is formed.

Biuret also contains sodium potassium tartrate which assists in Cu^{2+} complex formation and further prevent their precipitation in an alkaline solution. The absorbance of the coloured chelate is measured at 540nm. The colour intensifies from pink to reddish violet due to the complexity of the peptide bond in the protein.

This method however can't be used to differentiate between various fractions nor sub-fractions of proteins.

Use the reagents and materials provided.

Reagents and Materials

You are provided with the following:

a)- A stock protein standard (150g/L)
b)- A 3% NaOH solution
c)- Normal saline solution
d)- Biuret reagent solution (blue solution)
e)- Distilled water
f)- Control sample Patient sample #1 Patient sample #2

You need to perform the following:

a)- Take **12 plastic tubes**, divide them into two sets **(A)** and **(B),** each with six tubes labeled **1-6**.
b)- Take **set A (1-6)** and prepare protein standards according to **table (1)** below;

Set (A) tubes 1-6	**1A**	**2A**	**3A**	**4A**	**5A**	**6A**
Std concentration g/l	0				120	150
µL of stock	0				800	1000
µl of normal saline	1000				200	0
Total volume (ml)	**1.0**	**1.0**	**1.0**	**1.0**	**1.0**	**1.0**

c)- Cover the tubes in set A with **parafilm** and mix by gentle **inversion 4 to 5 times**.
d)- **Transfer 100µl** of protein standards **from set (A) to set (B),** labeled 1-6 also.
e)- Take **three** more tubes and label them as **Control**, **Patient #1** and **patient #2**.
f)- Now add **100µl** of Control, patient sample #1 and patient sample #2 to appropriate tubes.
g)- To all the **nine tubes** add **4.90 ml of 3% NaOH** solution so that all the tubes have a **total of 5 ml** solution.

h)- Now to all the **nine** tubes add **1 ml of Biuret solution** so that the total volume of solution in each tube is **6ml**.
i)- Cover each tube with **parafilm** and mix by gentle inversion.
j)- **Incubate** at **room temperature** for **15 min.**
k)- Set the spectrophotometer at **540nm** and set the **absorbance at zero** using **distilled water.**
l)- Record the absorbance of each of the standard in **table (2)** provided below
m)- Record the absorbance of Control and Patient samples #1 and #2 in **table (3)** below.
n)- **Draw standard curve** using graph paper provided.

Standard curve: Write the title of the experiment on top and date of the experiment
Write the concentrations of protein standards on x-axis
Write the absorbance of protein standards on y-axis
Clearly label x and y-axis
A linear curve should appear forming a plateau at the last two points.

Table (2)

Protein std (g/L)	**Absorbance**
0	
120	
150	

Table (3)

Samples	Absorbance	Conc g/L	Condition
Control			
Patient #1			
Patient #2			

⇒ You must answer the following questions:

1- Write the condition according to the normal range of total protein, i.e. hypoproteinemia, hyperproteinemia or slight hyperproteinemia or normal.
2- What are possible causes of hyperproteinemia?
3- Write the principle of Biuret method.
4- What other methods may be used for total protein determination.
5- Is determination of total protein alone diagnostic?
6- Which non-pathological conditions may cause hyper or hypo-proteinemia