

# BCM 101

## BIOCHEMISTRY

### Week 5 Practical

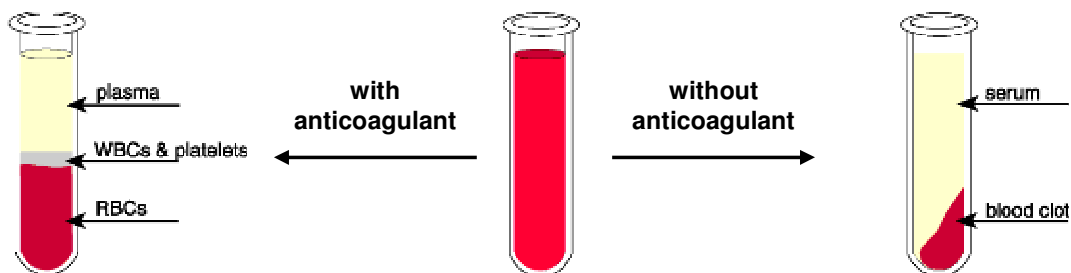
## “Colorimetric estimation of plasma proteins”

Blood, consists of 45% formed elements (cells) and 55% plasma. Plasma (the liquid portion of blood) consists mainly of water (about 90-92%), proteins, salts, oxygen, carbon dioxide, nutrients and waste.



“**Plasma proteins**” are of 3 major types: albumin, globulins and fibrinogen. They are all synthesized in the **liver**, with the exception of the gamma globulins which are produced by B-lymphocytes.

**N.B. Plasma** is obtained from blood in which an anticoagulant is added, while **serum** is obtained when no anticoagulant is added.



### Functions of plasma proteins:

1. Plasma proteins maintain blood osmotic pressure, pH and volume.
2. Albumin transports many substances in blood including hormones and some drugs (plasma protein-bound drugs).
3. Gamma globulins (antibodies) fight infection.
4. Fibrinogen is necessary for blood clotting.

### Normal value of plasma proteins:

The normal value of plasma proteins in humans is **6 - 8 g/dl** (dl= 100 ml).

### Clinical significance:

In some cases, the value of plasma proteins is lower than normal (below 6 g/dl); this is known as **hypoproteinemia** and can be caused by malnutrition, liver disease or severe burns. In other cases, the value of plasma proteins is higher than normal (above 8 g/dl); this is known as **hyperproteinemia** and can be caused by dehydration due to severe vomiting or diarrhea.

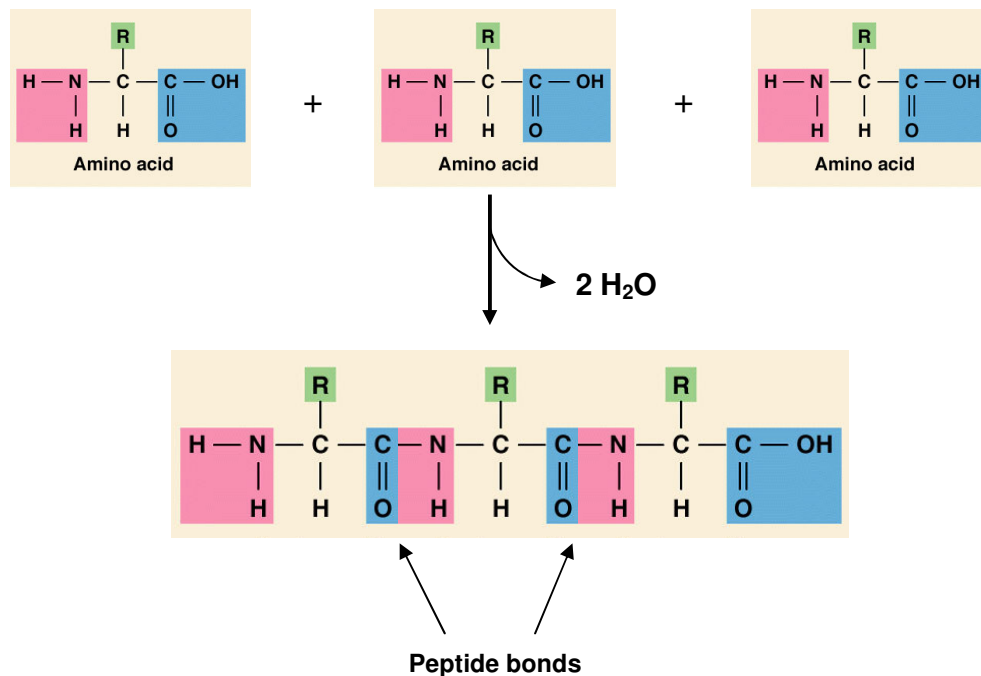
The **aim** of this practical session is to:

1. Estimate the concentration of proteins in a plasma sample using a colorimetric method, the “**Biuret method**”.
2. Comment on the provided case.

## Biuret method for colorimetric estimation of plasma proteins

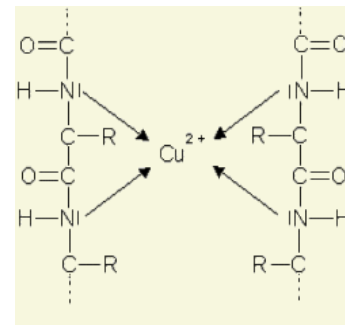
### Chemical structure of proteins:

Proteins are polymeric compounds composed of “**amino acids**” joined together by “**peptide bonds**”.



### Principle of the biuret method:

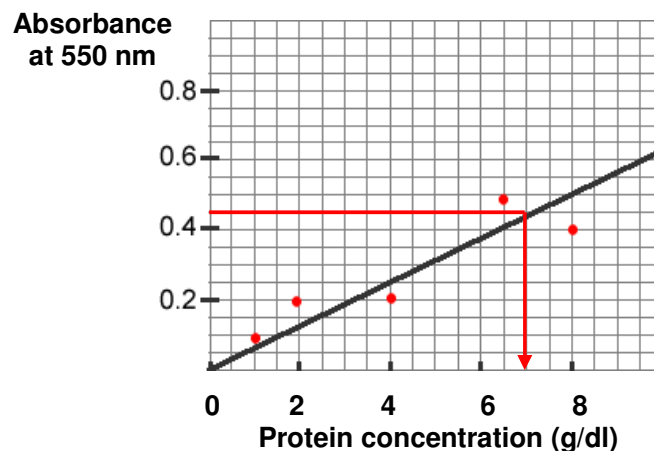
- The biuret reaction is a method that can be used to determine the amount of protein in a solution.
- The biuret reagent (copper sulfate in a strong base) reacts with peptide bonds in plasma proteins to form a violet complex known as the “**biuret complex**”.
- **N.B.** Two peptide bonds at least are required for the formation of this complex.
- A **colorimeter** can be then used to measure the intensity of the color produced; the more protein present the darker the color.
- To estimate the concentration of plasma proteins, one of the two following methods can be used:
  1. Performing the biuret reaction on a “**standard**” protein solution (i.e. of known concentration) and then applying the following equation:



**Biuret complex**

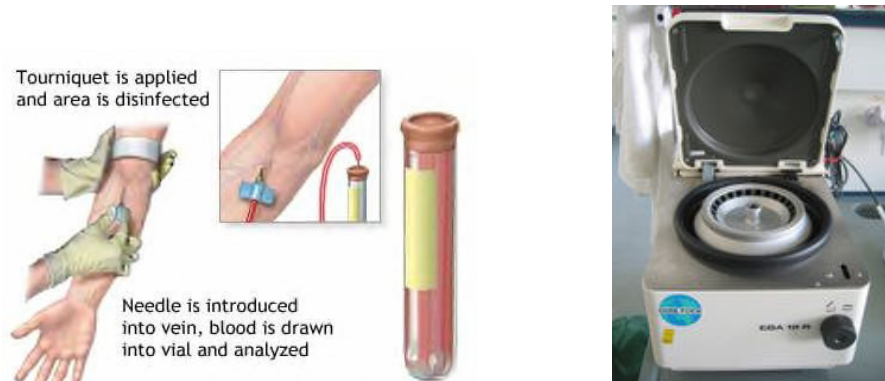
$$C_{\text{test}} = C_{\text{std}} \times A_{\text{test}} / A_{\text{std}}$$

2. Performing the biuret reaction on a series of standard protein solutions and then constructing a “**standard curve**” by plotting the absorbance on the y-axis and the concentration on the x-axis. From this curve, the absorbance reading of any sample can be converted into its corresponding concentration.



## Practical:

1. Blood is drawn from a vein and transferred into a centrifuge tube containing an anticoagulant. In this case, blood will not clot and blood cells will settle to the bottom of the tube leaving plasma on the top.
2. Plasma is obtained by centrifugation of blood for 10 minutes.



3. Determine the protein concentration in the provided plasma sample of patient **1**, **2** or **3** using the **biuret method** as follows:

- In a clean dry test tube, add 0.5 ml of distilled water (blank) or plasma sample (test), then add 2 ml of biuret reagent.

	<b>Blank</b>	<b>Test</b>
<b>Distilled water</b>	0.5 ml	—
<b>Test</b>	—	0.5 ml
<b>Biuret reagent</b>	2 ml	2 ml



blue



violet

- Mix the content of each tube.
- Allow to stand for 15 minutes.
- Read the absorbance at 550 nm.

- Construct a “**standard curve**” for plasma proteins using the values in the table below showing the absorbance reading of protein solutions of different concentrations.

<b>Concentration</b> (g/dl)	<b>Absorbance</b> (at 550 nm)
<b>0</b>	<b>0.00</b>
<b>1</b>	<b>0.07</b>
<b>2</b>	<b>0.14</b>
<b>4</b>	<b>0.28</b>
<b>7</b>	<b>0.49</b>
<b>10</b>	<b>0.70</b>
<b>12</b>	<b>0.84</b>

- Determine the concentration of plasma proteins (g/dl) in the provided sample.
- **Comment** on the provided case.

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Student Name: ..... Student number: .....

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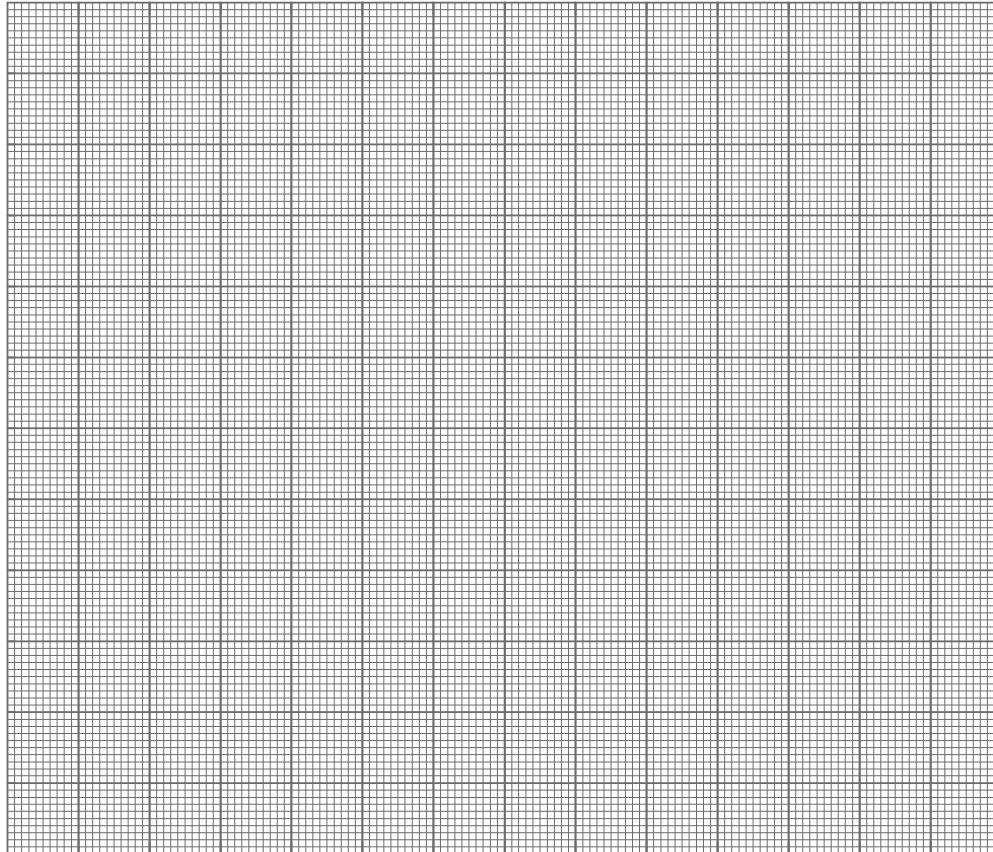
**Laboratory exercise:**

1. Determine the concentration of proteins in the provided plasma sample using the biuret method.

**Results:** Patient number .....

$A_{\text{test}}$  = .....

$C_{\text{test}}$  = .....



2. Write your **comment** on the case:

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