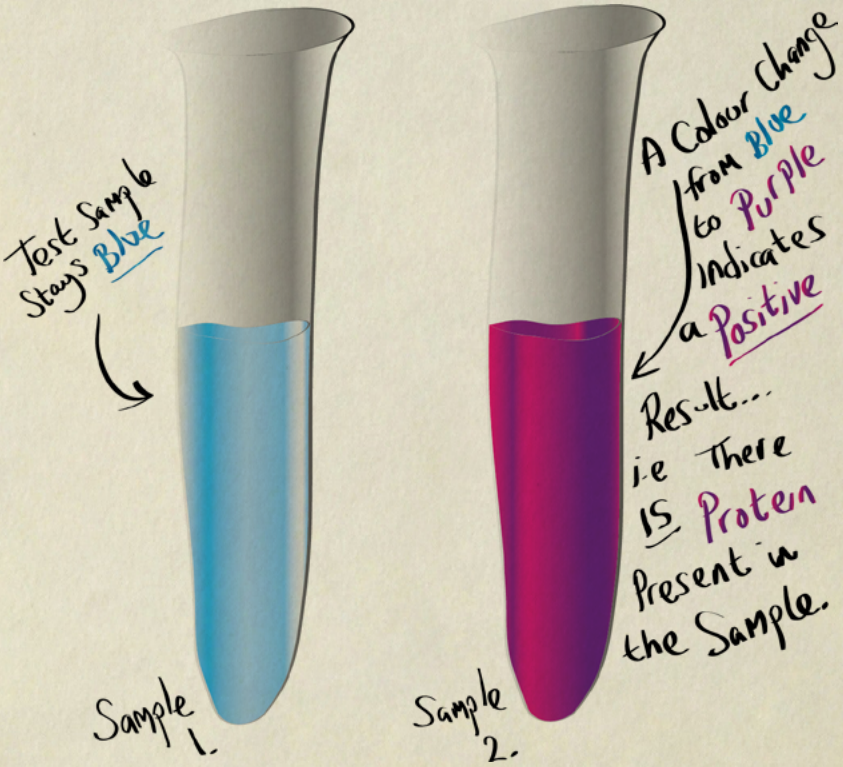
The Biuret Test For Proteins

Revision Notes and Knouledge Check Q & A



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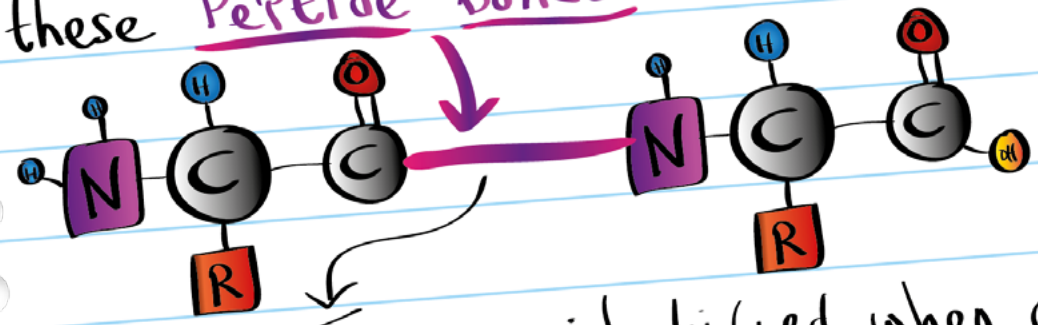
As we have Seen in Previous lessons, Proteins are really Versatile MACROMolecules, which are Essential for life...

We now also KNOW that Proteins are made up from Amino Acids Joined via Condensation reactions, which form Peptide bonds between the Adjacent Amino Acids... In fact, it is these Peptide bonds



That are identified when carrying out the test to find out if a Substance has any Protein Present within it...

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The Biuret Test for Proteins - Revision Notes

The Test is the Biuret Test, which Utilises the Biuret Reagent ... which Contains a 1% Solution of Copper II Sulfate ($CuSO_4$).

It is the Cu^{2+} in the Biuret reagent that forms a COMPLEX with the Peptide bonds found in Proteins ...

∴ Single "free" Amino Acids will NOT produce a Positive Result ...

Since Long chain Poly Peptides (Proteins) have MANY Peptide BONDS the Cu^{2+} will react with these bonds causing a Colour change in the Reagent and ∴ indicating a Positive RESULT!

So, in order to Qualitatively test for the Presence of Protein in a Sample, Simply follow these Steps ...

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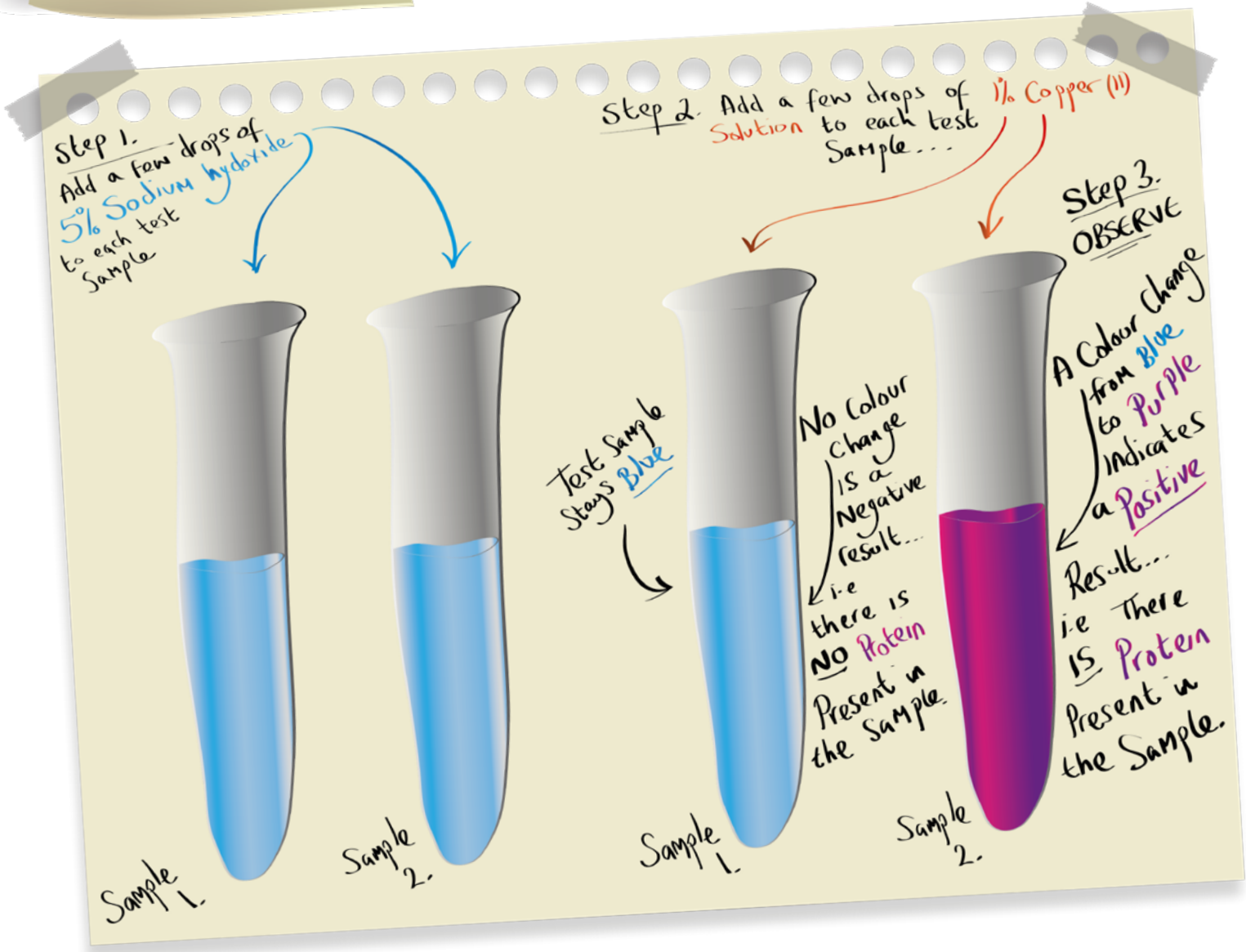
The Biuret Test for Proteins - Revision Notes

Step 1.
To Ensure that the test Sample is ALKALINE add a few drops of :-
5% Sodium Hydroxide Solution
to each Sample being tested...

Step 2.
Now add a few drops of:-
1% Copper II Sulfate Solution
to each Sample being tested...

Step 3. OBSERVE (and record your results)
With a Negative Result (No Protein) The Sample will remain BLUE
However... A Positive Result (PROTEIN Present) The Sample will Change Colour from Blue to PURPLE



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NOTE

If you are using **Biuret Reagent** ensure you state that you **KNOW** it is a prepared reagent of 1% Copper II Sulfate and 5% Sodium Hydroxide...

When the Biuret reagent is added to a test sample, the sample will turn **Purple** if it contains protein.

DON'T forget to show your understanding of why...

i.e.

The 5% Sodium hydroxide Alkalises the sample and the 1% Copper II Sulfate form a **Complex** with the **Peptide bonds** ∴ causing a reaction, which results in a colour change in the sample from **Blue** to **PURPLE**

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After studying the notes and watching the revision video, you should be able to answer the following questions.

Knowledge check Questions.

Q1) Proteins are composed of amino acids. Name the reaction that joins amino acids together.

A1.

Q2) Name the bond that links amino acids together.

A2.

Q3) What must be added to the test sample in step 1? Why is this step required?

A3.

Q4) What must be added to the test sample in step 2?

A4.

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The Biuret Test for Proteins - Knowledge Check

After studying the notes and watching the revision video, you should be able to answer the following questions.

Q5) What colour indicates a positive result for the presence of protein in a sample?

A5.

Q6) Explain why the Biuret Reagent indicates the presence of protein but not amino acids.

A6.

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After studying the notes and watching the revision video, you should be able to answer the following questions.

Q7) Sucrase is an enzyme which hydrolyses the disaccharide sucrose to give a molecule of glucose and a molecule of fructose.

Sucrase + Water $\xrightarrow{\text{Sucrase}}$ Glucose + Fructose

A solution containing the enzyme sucrase was added to a sucrose solution. The mixture was incubated in a test tube at 40°C for 1 hour. Sample A was removed from the tube at the start. Sample B was removed after 1 hour. A biuret test was carried out on sample A. It gave a positive result.

(a) Describe what you would expect to see if the biuret test gave a positive result.

A7 (a).

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After studying the notes and watching the revision video, you should be able to answer the following questions.

Q7 (b) Explain why the biuret test gave a positive result with sample A.

A7 (b).

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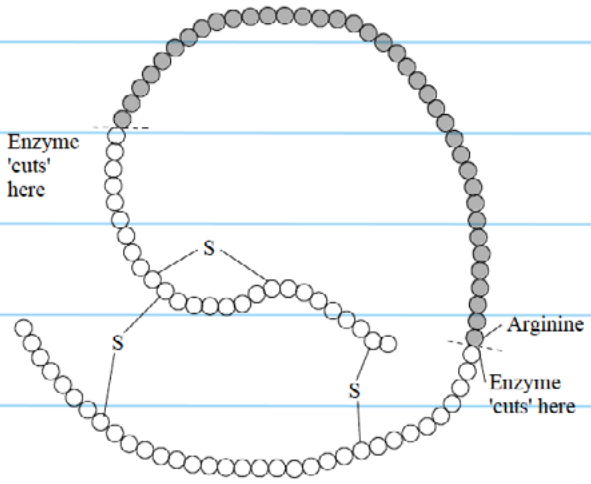
After studying the notes and watching the revision video, you should be able to answer the following questions.

Q8) Insulin is a protein. It is made in the cells of the pancreas from a larger molecule called pro-insulin. An enzyme breaks the pro-insulin into insulin and a short polypeptide.

This is shown in the diagram below.



Explain the result you would expect if the enzyme were tested with biuret reagent. Give reasons for your answer.

A8.

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After studying the notes and watching the revision video, you should be able to answer the following questions.

Q9) The table shows the results of biochemical tests on substances found in food.

Substance	Colour with Benedict's Test	Colour With Iodine Test	Colour with Biuret Test	Colour With Emulsion Test
W	Blue	Orange-Brown	Violet	Clear
X	Blue	Orange-Brown	Blue	Milky
Y	Brick-Red	Orange-Brown	Blue	Clear
Z	Blue	Orange-Brown	Blue	Clear

Identify

W

X

Y

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Lipids. Triglyceride structure and formation.

Answers

Q1. Condensation Reaction
Q2. Peptide bond
Q3. 5% sodium hydroxide solution - To alkalise the sample.
Q4. 1% Copper II sulfate solution.
Q5. Purple / Blue-black / Mauve.
Q6. Proteins are long complex polypeptides with many peptide bonds. The Copper II sulfate solution form a complex with the peptide bonds and causes a colour change. Amino Acids are monomers, since there are no peptide bonds to interact with, a colour change will not take place.

Q7. (a) Sample A contains the enzyme sucrase which is a protein. The Biuret test gave positive result turning sample A purple [blue-black / mauve / lilac].
(b) Sucrase is an enzyme, which is a protein. So Sucrase has many peptide bonds which forms a complex with the Copper II sulfate in the biuret test. The reaction causes the colour change from blue to Purple.

Q8. If the Enzyme were to be tested with the biuret reagent it would give a Positive result showing a Purple [blue-black / lilac / mauve] colour change. The positive result is expected since there is protein present in the form of the Enzyme that "cuts" [hydrolyses the peptide bonds of the protein pro-insulin into insulin]. Enzymes are large complex polypeptides, the Copper II Sulfate in the Biuret Reagent forms a complex with the peptide bonds and causes the Positive result [colour change].

Q9. (a) W = protein.
X = fat /oil / lipid / triglyceride.
Y = reducing sugar.

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