3.5 Testing for Biochemical Substances

Practical biochemistry: testing for the presence of sugars, proteins and lipids

Below is a list of chemical tests for different substances covered throughout chapters 3.2 to 3.4. This builds the *How Science Works* section of the course, and it is important that you know the test for each of them.

Testing for Starch

(See 3.2 Carbohydrates) Starch is an important carbohydrate which is used for energy storage in animals. The test for starch is very simple: prepare the sample you are testing, and add **iodine solution**. If starch is present in the sample, it will turn from a yellow-orange colour to a blue-black colour

Testing for Reducing Sugars

(See 3.2 Carbohydrates) All *monosaccharide* and *disaccharide* sugars are called **reducing sugars**: meaning that a molecule of this sugar can react with other molecules by giving electrons to them – **reduction**

When a reducing sugar is heated with *alkaline copper sulphate* (**Benedict's solution**) the solution will change colour from blue to an orange-red. This is called **Benedict's test**

Benedict's test is a test used frequently in this series of practicals. The result of using Benedict's test is either the same as it was before, or there might be a change. In the test for a *reducing sugar* described above, if it changes to a red-orange colour is it called a **precipitate** because it comes out of the solution and forms solid particles dispersed around the water. Below is a reduction scale used to describe the amount of reducing sugar in a sample:

(nothing) blue → green → yellow → orange → red (lots)

Testing for Non-Reducing Sugars

(See 3.2 Carbohydrates) If the reducing sugar test comes out as negative (no colour change), the **non-reducing sugar** test can be done

If a substance does not react with Benedict's solution, this test is used:

- Boil the sample with hydrochloric acid – this hydrolyses any sucrose present, splitting sucrose molecules to give glucose and fructose (see below)
- Cool the solution and neutralise it by adding sodium carbonate solution (an alkali solution)
- Carry out the reducing sugar test (Benedict's test) again: if there were non-reducing sugars present in the original sample, the test will now come out as positive (as they have been broken down into reducing sugars glucose and fructose)

The sugar *sucrose* is a non-reducing sugar. It is formed in a condensation reaction making a *glycosidic bond* between a glucose molecule and a fructose molecule. Fructose and glucose are both monosaccharides, and sucrose a disaccharide. The glycosidic bond formed in sucrose is different from that one found in, for example, maltose (maltose being a reducing sugar). It is this difference which prevents the sucrose from reacting with the Benedict's solution.

The non-reducing sugar test works because if there is any sucrose present (which is a non-reducing sugar, that we are testing for), it is broken down into those monosaccharides, which can be tested for using the ordinary reducing sugar test. A positive result therefore means non-reducing sugars are present on the original sample. The same scale applies:

(nothing) blue → green → yellow → orange → red (lots)

The table below shows some of the expected results for the two tests for glucose and sucrose:

	Reducing sugar test	Non-reducing sugar test
glucose	green	green
sucrose	blue	green
mixture	green	orange

If you test a sample for both reducing and non-reducing sugars, and the colours produced are the same, the conclusion is that there are *no non-reducing sugars*. If the colour in the non-reducing sugar test is more towards the *red* end of the spectrum, then *non-reducing sugars are present*.

(nothing) blue → green → yellow → orange → red (lots)

We call the tests for reducing and non-reducing sugars **semi-qualitative tests** because they produce results which indicate what *type* of molecule is present, not a specific amount, although the colour spectrum above does give a rough idea of comparison of how much is present. But it is also important that we can do **quantitative tests** to identify exact amounts of *how much* of a molecule is present.

USING BENEDICT'S SOLUTION

- Using Benedict's test will reveal the presence of reducing sugars
- It results in an orange-red precipitate
- The more reducing sugar there is present, the more precipitate will be formed, and the more Benedict's solution (copper sulphate) will be used up
- The precipitate is filtered out then the concentration of the remaining solution can be measured
- This will tell you how much Benedict's solution has been used up allowing you to estimate the concentration of reducing sugar in the original sample

A device called a **colorimeter** can be used to make more accurate measurements. This device shines a beam of light through a prepared sample, and a reading is measured of percentage light transmission.

USING A COLORIMETER

- The solution is places in a clear plastic cuboid called a **cuvette** which then goes into a small chamber in the colorimeter
- The colorimeter shines a beam of light through the sample
- A **photoelectric cell** picks up the light that is passed through the sample (on the other side) and will provide you with a reading of the amount of light that was passed through – transmitted

The more copper sulphate that has been used in the Benedict's test, the less light will be blocked out in the sample, and more transmitted. Therefore the reading gives a measure of the amount reducing sugar based on the Benedict's reaction

Whilst using a colorimeter alone will provide a measure, it doesn't specify an exact amount: in order to **quantify** the amount, a **calibration curve** must be made…

CALIBRATION PLOTTING

- Take a range of known concentrations of reducing sugar, carry out a Benedict's test on each one, then filter out the solution; use a colorimeter to give readings of the amount of light passing through the solutions
- Plot the readings in a graph to show the amount of light getting through (transmission) versus reducing sugar concentration
- Then you can take the reading of an unknown concentration – use the graph to make a precise measurement

TESTING FOR LIPIDS

(See 3.3 Lipids) Testing for the presence of a lipid uses the **ethanol emulsion test**:

- Mix the sample with *ethanol*: this dissolves any lipid present, because they are soluble in alcohols
- Pour the mixture into water contained in another test tube
- If there is lipid present, a cloudy white *emulsion* will form near the top of the water

TESTING FOR PROTEINS

(See 3.4 Amino Acids and Proteins) A protein test uses the **biuret test**. Biuret reagent, which contains sodium hydroxide and copper sulphate, and is pale blue in colour, is added to the sample. These chemicals react with the *peptide bonds* found in proteins, which results in a colour change to lilac

There is a summary of all of these semi-qualitative tests (also known as **food tests**) in the below table:

Test for…	**Description**	**Result (colour changes)**
Starch	Add a few drops of iodine solution	Orange to blue-black
Reducing sugars	Add Benedict's solution, heat to 80^{o}C in a water bath	Blue to orange-red
Non-reducing sugars	(If reducing sugar test is negative) boil with hydrochloric acid, cool and neutralise with sodium carbonate solution, repeat Benedict's test	Initially no change, repeated Benedict's test will turn blue to orange-red
Lipids	Add ethanol, pour mixture into water in another test tube	White emulsion forms in water
Proteins	Add biuret reagent	Pale blue to lilac