

Biochemical Tests

These five tests identify the main biologically important chemical compounds. For each test take a small amount of the substance to test, and shake it in water in a test tube. If the sample is a piece of food, then grind it with some water in a pestle and mortar to break up the cells and release the cell contents. Many of these compounds are insoluble, but the tests work just as well on a fine suspension.

- **Starch** (iodine test). To approximately 2 cm³ of test solution add two drops of iodine/potassium iodide solution. A blue-black colour indicates the presence of starch as a starch-polyiodide complex is formed. Starch is only slightly soluble in water, but the test works well in a suspension or as a solid.
- **Reducing Sugars** (Benedict's test). All monosaccharides and most disaccharides (except sucrose) will reduce copper (II) sulphate, producing a precipitate of copper (I) oxide on heating, so they are called reducing sugars. Benedict's reagent is an aqueous solution of copper (II) sulphate, sodium carbonate and sodium citrate. To approximately 2 cm³ of test solution add an equal quantity of Benedict's reagent. Shake, and heat for a few minutes at 95°C in a water bath. A precipitate indicates reducing sugar. The colour and density of the precipitate gives an indication of the amount of reducing sugar present, so this test is semi-quantitative. The original pale blue colour means no reducing sugar, a green precipitate means relatively little sugar; a brown or red precipitate means progressively more sugar is present.
- **Non-reducing Sugars** (Benedict's test). Sucrose is called a non-reducing sugar because it does not reduce copper sulphate, so there is no direct test for sucrose. However, if it is first hydrolysed (broken down) to its constituent monosaccharides (glucose and fructose), it will then give a positive Benedict's test. So sucrose is the only sugar that will give a negative Benedict's test before hydrolysis and a positive test afterwards. First test a sample for reducing sugars, to see if there are any present before hydrolysis. Then, using a separate sample, boil the test solution with dilute hydrochloric acid for a few minutes to hydrolyse the glycosidic bond. Neutralise the solution by gently adding small amounts of solid sodium hydrogen carbonate until it stops fizzing, then test as before for reducing sugars.
- **Lipids** (emulsion test). Lipids do not dissolve in water, but do dissolve in ethanol. This characteristic is used in the emulsion test. Do not start by dissolving the sample in water, but instead shake some of the test sample with about 4 cm³ of ethanol. Decant the liquid into a test tube of water, leaving any undissolved substances behind. If there are lipids dissolved in the ethanol, they will precipitate in the water, forming a cloudy white emulsion. The test can be improved by adding the dye Sudan III, which stains lipids red.
- **Protein** (biuret test). To about 2 cm³ of test solution add an equal volume of biuret solution, down the side of the test tube. A blue ring forms at the surface of the solution, which disappears on shaking, and the solution turns lilac-purple, indicating protein. The colour is due to a complex between nitrogen atoms in the peptide chain and Cu²⁺ ions, so this is really a test for peptide bonds.