Topic 2

Chemicals of Life

Unit covered

2.1 Water

2.2 Carbohydrates, Proteins, Lipids and Nucleic Acids

Introduction

All biological systems are based on the same organic molecules dissolved or suspended in water medium, but the details of those molecules differ among organisms. In this topic, we are going to learn about the structure and properties of water and some of the organic molecules found in biological systems.

Unit 2.1

Water

LEARNING OBJECTIVES

By the end of this unit, learners should be able to:

- state the constituent elements of water.
- describe the chemical structure of water.
- relate the properties of water to its uses in living organisms and as a habitat for living organisms.

Constituent Elements of Water

Water is the most abundant and one of the most important biochemical molecules found in living organism. In most cells, water constitutes about 75% of the total fresh mass and the cells will die if their water content falls much below this. Water consists of two elements; hydrogen (H) and oxygen (O).

Chemical Structure of Water

An individual water molecule is made up of three atoms, two hydrogen atoms, and one oxygen atom. In an individual H_2O molecule, the central oxygen atom forms one covalent bond with each of the two hydrogen atoms. The oxygen–hydrogen bond is called a **polar bond** because there is an unequal distribution of electrons between the two atoms covalently bonded. The bonded electrons are closer to the oxygen nucleus than the hydrogen nucleus. This makes the oxygen end of a water molecule to have a slight negative charge (∂–) and the hydrogen ends to have slight positive charges (∂ +). A molecule where there is an uneven distribution of charge (one with polar bonds) is called a polar molecule. Overall, the

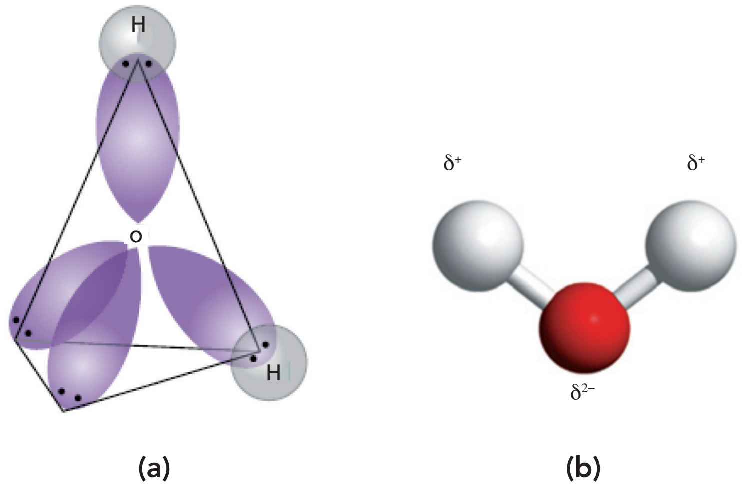


Fig 2.1 Water molecule.

water molecules are neutral because the slight negative charges are equal to the slight positive charges so they cancel each other.

(a) Electronic structure of the water molecule. Four electron orbitals, in an approximately tetrahedral arrangement, surround the central oxygen. Two orbitals participate in bonding to hydrogen (gray), and two contain unshared electron pairs.

(b) The shape of a water molecule and its polarity makes hydrogen bonding possible. A **hydrogen bond** is the attraction of slightly positive hydrogen to a slightly negative atom in the vicinity. Hydrogen bonding is the single most outstanding and important characteristic of water responsible for its anomalous and unique properties. Neighbouring water molecules tend to orient themselves so that each partially positive hydrogen atom is aligned with a partially negative oxygen atom. Hydrogen bonds are stronger than all other intermolecular forces of attraction between molecules. However, hydrogen bonds are not as strong as ionic or covalent bond.

In liquid water, each molecule can potentially form hydrogen bonds with up to four other water molecules, but each bond has a short lifetime. This means that the bonds are constantly being formed, broken and formed in water. As a result, water can form a large fluid network where water molecules are interconnected. Although each hydrogen bond is weak, their collective effort is responsible for the many physical properties of water.

Water is highly cohesive because of its ability to form hydrogen bond. Water's cohesion is responsible for its being a liquid, not a gas, at moderate temperatures. The cohesion of liquid water is also responsible for its **surface tension**. Surface tension is as a result of strong attraction of water molecules to each other than between water molecules and air. As a result, water molecules cling together at a surface exposed to air. Water's high surface tension makes it possible for certain small insects such as the water strider to walk on water without breaking the surface, because at the air–water interface, all the surface water molecules are hydrogen-bonded to molecules below them.

The polarity of water causes it to be attracted to other polar molecules as well. This attraction for other polar substances is called **adhesion**. The attraction of water to substances that have electrical charges on their surface is responsible for capillary action. If a glass tube with a narrow diameter is lowered into a beaker of water, the water will rise in the tube above the level of the water in the beaker, because the adhesion of water to the glass surface, drawing it upward, is stronger than the force of gravity, pulling it downward. The narrower the tube, the greater the electrostatic forces between the water and the glass, and the higher the water rises.

Cohesion and adhesion contribute to the transport system of multicellular organisms that contain internal vessels. In plants, for example, roots are anchored in the soil, where they absorb water, but the leaves are uplifted and exposed to the sun. Water evaporating from the leaves is immediately replaced with water molecules from the xylem vessels that extend from the roots to the leaves. However, because water molecules are cohesive, a tension is created that pulls the water column up from the roots. Adhesion of water to the walls of the xylem vessels also helps prevent the water column from breaking apart.

Most solids are denser than their liquids because in the solid state, the molecules are packed closer and occupy smaller volume. However, solid ice is less dense than liquid water. The hydrogen bonds in ice space the water molecules relatively far apart and as a result ice occupy a larger volume and therefore become less dense.

Important Properties of Water

Water is the biological medium on Earth. The abundance of water is a major reason earth is habitable. The properties of water make it an effective medium to support life. All living organisms require water more than any other substance. Water is transparent which allows light to penetrate through it. Water has a high specific heat capacity which makes it an ideal temperature moderator in living organisms.

Water has high heat of vaporization facilitates cooling in living organisms. Water acts as a solvent for polar molecules and exerts an organizing effect on non-polar molecules. Water is also less dense in its solid form, ice, than as a liquid makes it an ideal habitat for aquatic organisms in almost any place on Earth. All these water properties except for being transparent result from its polar nature.

Uses of Water in Living Organisms

Transparency of Water

The colour of water varies with the ambient conditions in which that water is present. Dissolved or suspended impurities may also affect the colour of water. However, generally speaking, pure water is colourless and therefore transparent. This allows sunlight to penetrate through water bodies and reach aquatic plants. Sunlight is important for photosynthesis in plants. Sunlight is also important for vision in aquatic animals. The sunlight enables animals to see food and potential predators in water. Sunlight is also important for production of vitamin D in aquatic animals.

Fig 2.2 Water transparency is important for aquatic organisms' survival.

Water as a Solvent

The polarity of water molecules and its ability to form hydrogen bonds makes water an excellent **solvent**, which means many other substances (solutes) easily dissolve in it. Substances that dissolve in water are hydrophilic. These include polar molecules such as sugars, some amino acids and proteins, and ionic salts such as sodium chloride.

Polar molecules such as sugars readily dissolve in water, because they contain groups that can form hydrogen bonds with the solvent water molecules. Ionic substances dissolve in water because of its polarity; the slightly positive charge on each hydrogen atom and slightly negative charge on the oxygen atom. When an ionic compound such as sodium chloride ($NaCl$) is added to water, the polar water molecules surround the ions, by aligning their partial charges with the oppositely charged ions. The slightly positive charged hydrogen atoms are attracted to the negatively charged chloride ions and the slightly negative charged oxygen atoms are attracted to the positively charged sodium ions.

Once a chemical substance is in solution, its ions or molecules are free to move about and react with other chemicals inside cells. Most processes in living organisms take place in solution in this way.

Water is also important as a mass flow transport medium in living organisms. In animals, transport systems such as the circulatory system and digestive systems transport many dissolved substances such as metabolites, antibodies, hormones, and waste substances such as carbon dioxide and urea. In plants translocation system also transport many dissolved substances such as mineral salts, sugars and plant hormones.

Water's High Specific Heat Capacity

The temperature of any substance is a measure of how rapidly its individual molecules are moving. In the case of water, a large input of heat energy is required to break the many hydrogen bonds that keep individual water molecules from moving about. Therefore, water is said to have a high **specific heat,** which is defined as the amount of heat energy required to raise or decrease the temperature of 1 kilogram of a substance by 1 °C. Specific heat measures the extent to which a substance resists changing its temperature when it absorbs or loses heat. Water heats up more slowly than almost any other compound and holds its temperature longer, because of its high specific heat.

Water's high specific heat capacity allows them to maintain a relatively constant internal temperature. Enzymes in cells can only function efficiently over a small range of temperature. The heat generated by the chemical reactions inside cells would denature most of the proteins and enzymes inside the cells, effectively stopping or slowing down important biochemical reactions. This can result in cell death. Such damages are prevented by absorption of this heat by the water within cells.

High heat capacity of water also means that water bodies are slow to change temperature as environmental temperature changes. This is important because water provides a stable habitat for aquatic organisms. Organisms are better able to maintain their normal internal temperatures and are protected from rapid temperature changes.

Water's High Latent Heat of Vaporization

The **latent heat of vaporization** is defined as the amount of energy required to change 1 kilogram of a substance from a liquid to a vapour. A considerable amount of heat energy is required to accomplish this change in water. As water changes from a liquid to a gas it requires a lot of heat energy to break its many hydrogen bonds.

The energy transferred to water molecules during vaporisation results in a corresponding loss of energy from their surroundings. This heat energy transfer causes cooling to take place. This is biologically important because it means that living organisms can use vaporisation as a cooling mechanism, as in sweating in humans or panting in mammals. A large amount of heat energy can be lost with minimum loss of water, reducing the risk of dehydration. It can also be important in cooling leaves during transpiration.

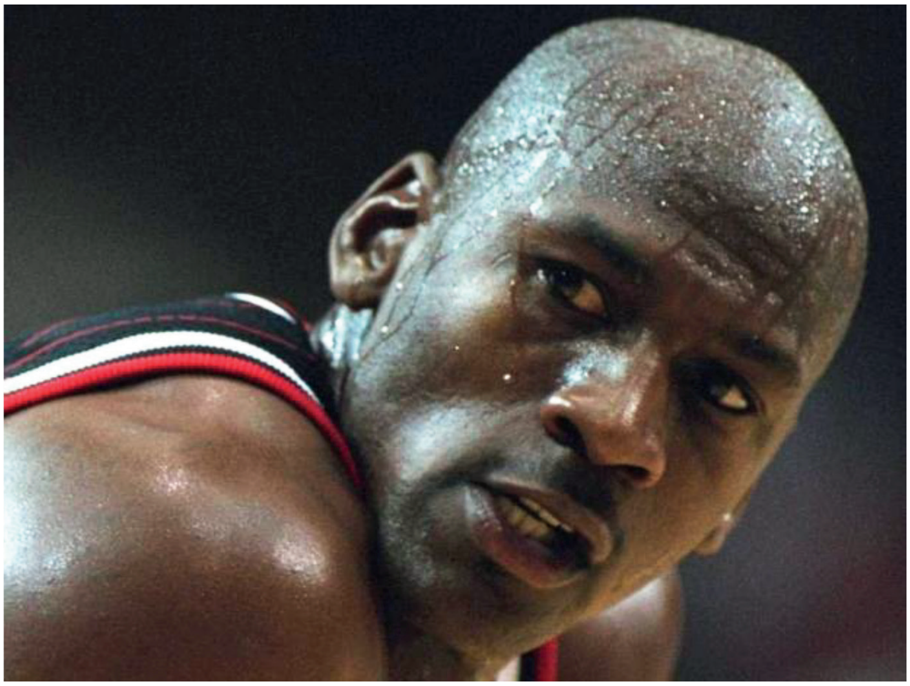


Fig 2.3 An athlete sweating after strenuous exercise results in a cooling effect.

Water as a Habitat

Water is an ideal habitat for many aquatic organisms because of its unique properties. The floating of ice as temperature drops has great biological importance to aquatic organisms. If ice did not float on water, it would sink, and ponds, lakes, and perhaps even the ocean would freeze solid, making life impossible in the water and also on land. Instead, bodies of water always freeze from the top down reducing the tendency for large bodies of water to freeze completely, and increase the chances of life surviving in cold conditions. Furthermore, the sheets of ice that form on the surface of water bodies can insulate the water under them from subfreezing air temperatures. Such 'ice blankets' protect aquatic organisms during cold winters.

Water also draws heat from the environment, helping to prevent a sudden change in temperature that might be harmful to life. This is because of its high heat of fusion which is a measure of the heat energy required to melt ice into liquid water. As ice melts in the spring, it draws heat from the environment, helping to prevent a sudden change in temperature that might be harmful to aquatic life.

Moreover, because of water's high heat of vaporization and ability to hold onto its heat, large water bodies have constant temperatures which makes it an ideal habitat for aquatic organisms which require stable temperatures. During the summer, the ocean absorbs and stores solar heat, and during the winter, the ocean releases it slowly.

The high surface tension of water and cohesion of water makes the surface of liquid water behaves a bit like a sheet of elastic. This allows for certain small organisms, such as water striders, to exploit the surface of water as a habitat, allowing them to settle on or walk over its surface. Cohesion work inside organisms too. Water can move in long, unbroken columns through the vascular tissue in plants.

Fig 2.4 A water strider supported by the surface tension of water

Water as a Reagent

Water takes part as a reagent in some chemical reactions inside cells. For instance, water is essential for all hydrolysis reactions. Water is also important as a reagent in special reactions such as photosynthesis where it is needed as a reagent.

Research

When scientists are exploring if life exists in other planets, the first substance they look for is water. Make a research on planets where water has been detected and explain whether those planets can sustain life or not.

End of chapter questions

1 (a) State the two elements that make up water. [2]

(b) Describe the chemical structure of water. [4]

2 (a) List any three properties of water. [3]

(b) Relate the properties of water you have listed in (a) to the use of water in living organisms. [3]

Unit 2.2 Carbohydrates, Proteins, Lipids and Nucleic Acids

LEARNING OBJECTIVES

By the end of this unit, learners should be able to:

- State the constituent elements of carbohydrates, proteins, lipids and nucleic acids
- Perform tests to identify reducing sugars, non-reducing sugars, proteins and lipids.

Biological Macromolecules

Biological macromolecules are traditionally grouped into carbohydrates, nucleic acids, proteins, and lipids. The major constituent elements that make up biological molecules are carbon (C), hydrogen (H), oxygen (O), nitrogen (N) sulphur (S) and phosphorous (P). In many cases, these macromolecules are polymers. A polymer is a giant molecule made from many similar repeating chemical subunits called **monomers** joined together in a chain.

Carbohydrates

Carbohydrates are molecules that consist primarily of carbon, hydrogen, and oxygen atoms in a 1:2:1 ratio. These may be simple soluble sugars like glucose, fructose, and sucrose, or complex materials that are usually insoluble like starch, cellulose and glycogen. Carbohydrates are divided into three main groups, namely **monosaccharides**, **disaccharides** and **polysaccharides**. The word 'saccharide' refers to a sugar or sweet substance.

Monosaccharides are carbohydrate molecules that consist of a single sugar unit. Glucose, fructose, and galactose are examples of monosaccharides. Glucose a commonly occurring monosaccharide has a chemical formula $C_6H_{12}O_6$ (**Fig 2.6**). Monosaccharides such as glucose are an immediate source of energy for living organisms as they are directly used as a metabolite in cellular respiration. Some monosaccharides are used for structural purposes, for example, ribose and deoxyribose are important components of nucleic acids RNA and DNA respectively.

A disaccharide is a carbohydrate whose molecules contain two sugar units. It is formed when two monosaccharides combine in a **condensation reaction** (**Fig 2.7**). The most common disaccharides in nature are maltose, sucrose, and lactose. Monosaccharides and disaccharides are readily soluble in water. Disaccharides can easily be broken down to monosaccharides in a hydrolysis reaction (see **Fig 2.7**).

When many monosaccharide molecules are joined together, they form a polysaccharide. Common examples of polysaccharides are starch, cellulose, and glycogen. Starch, cellulose and glycogen are made up of many glucose molecules joined together. All the polysaccharides are not readily soluble in water. Polysaccharides differ in which type or types of sugars they are made up of, how the sugar

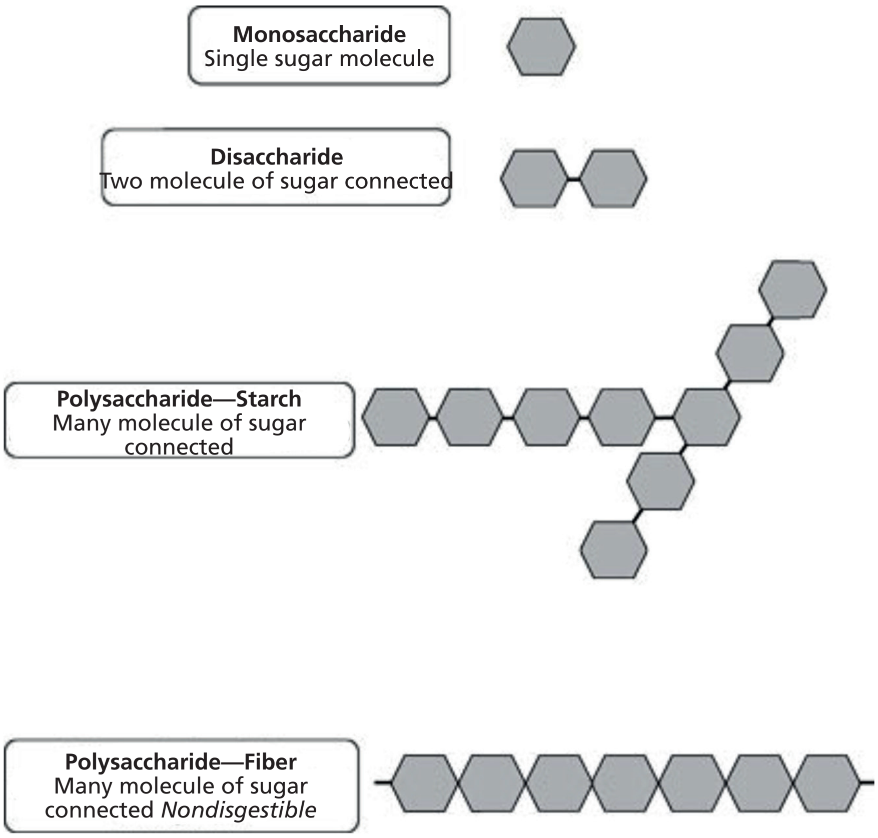


Fig 2.5 Structure of carbohydrates.

monomers are joined, and whether or not they are branched. These differences directly affect their shape as well as function. Each polysaccharide has a specific function in living organisms.

Starch and **glycogen** are polysaccharide made up of hundreds of glucose molecules joined together to form long chains. Glycogen is more branched than starch. **Cellulose** is a straight chain polymer made up of glucose

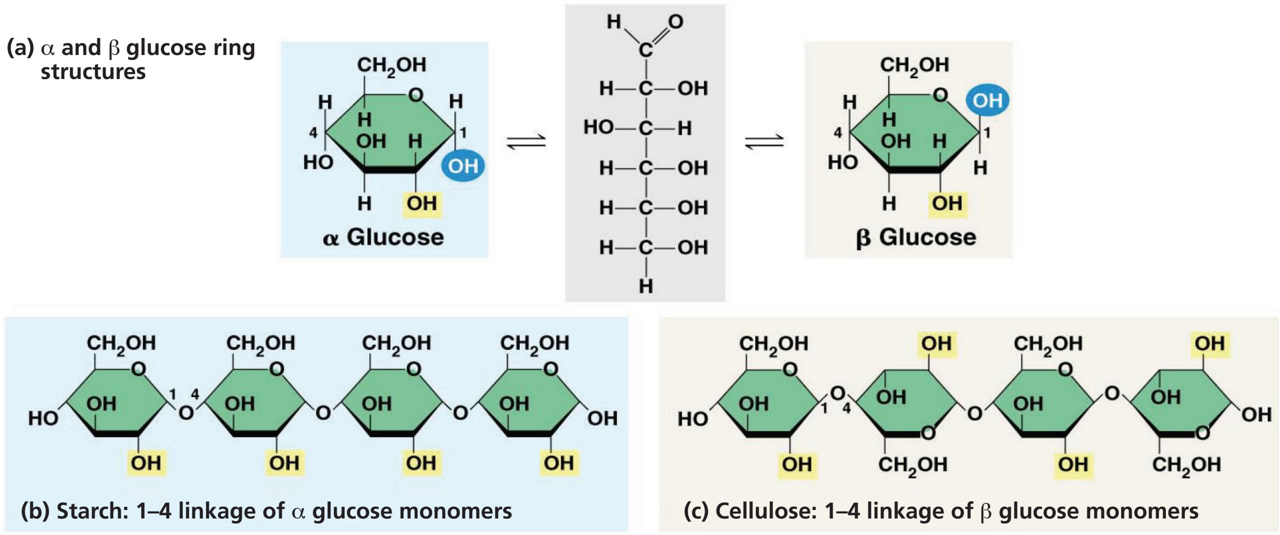


Fig 2.6 Carbohydrates. (a) Glucose structure (b) starch structure (c) cellulose structure. Credit: © 2011 Pearson Education, Inc, publishing as Pearson Benjamin Cummings.

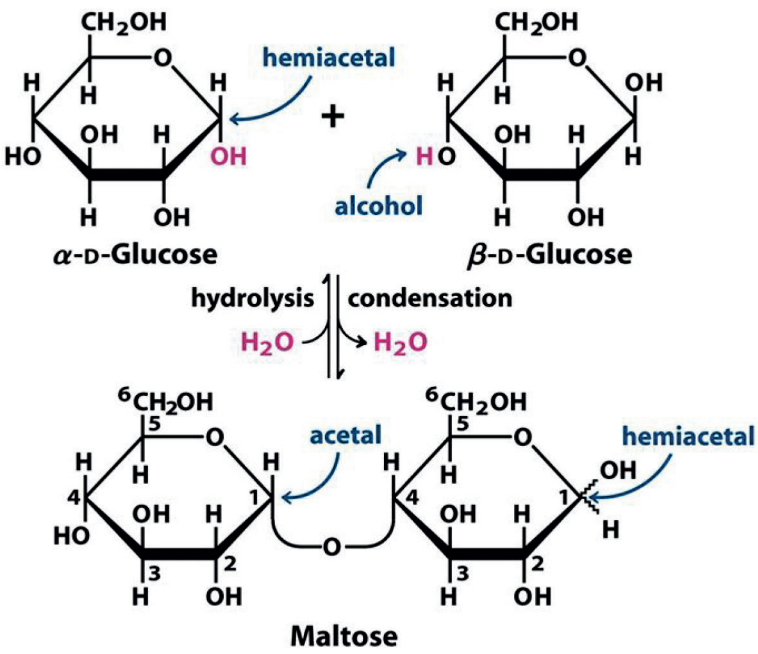


Fig 2.7 Condensation and hydrolysis of maltose. Maltose is formed by condensation of two glucose molecule. Maltose can be hydrolysed into two glucose molecules.

molecules. The chain molecules are grouped together to form microscopic fibres, which are laid down in layers to form the cell wall in plant cells.

Lipids

Lipids are a mixed group of compounds that are insoluble in water but soluble in organic solvents. These compounds are composed of the elements carbon (C), hydrogen (H), and oxygen (O). Lipids are formed by condensing fatty acids combining with an alcohol. The most common lipids are triglycerides which consist of three fatty acids joined to a glycerol molecule. Lipids that are solids at room temperature are known as fats while those that are liquid at room temperature are known as oils.

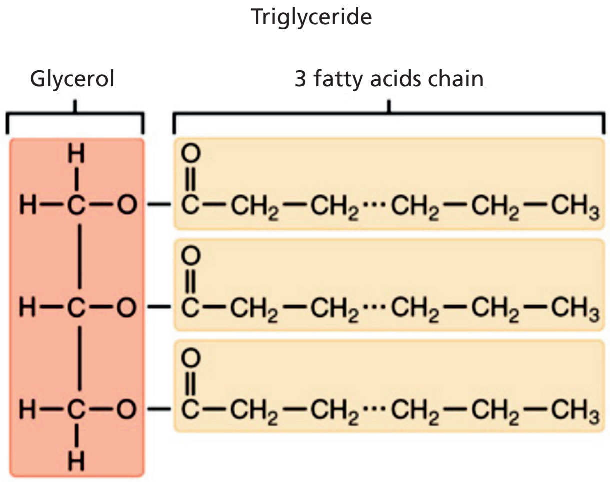


Fig 2.8 Structure of lipids. Triglyceride is formed by the condensation of glycerol and three fatty acid molecules.

Proteins

A protein is an organic compound which consists of one or more chains of amino acids. Amino acids which are the sub-units of proteins are made up of four elements, namely carbon (C), hydrogen (H), oxygen (O) and nitrogen (N). In some amino acids, but not all, sulphur and phosphorous might be present.

Amino acid molecules join up to form long chains called polypeptides. One, two, or more polypeptide chains aggregate to form functional proteins. Proteins fold into precise shapes. The sequence of amino acids in a protein determines the way that it folds and hence determines its three-dimensional shape and function. There is great diversity of proteins in living organisms, so is their functions.

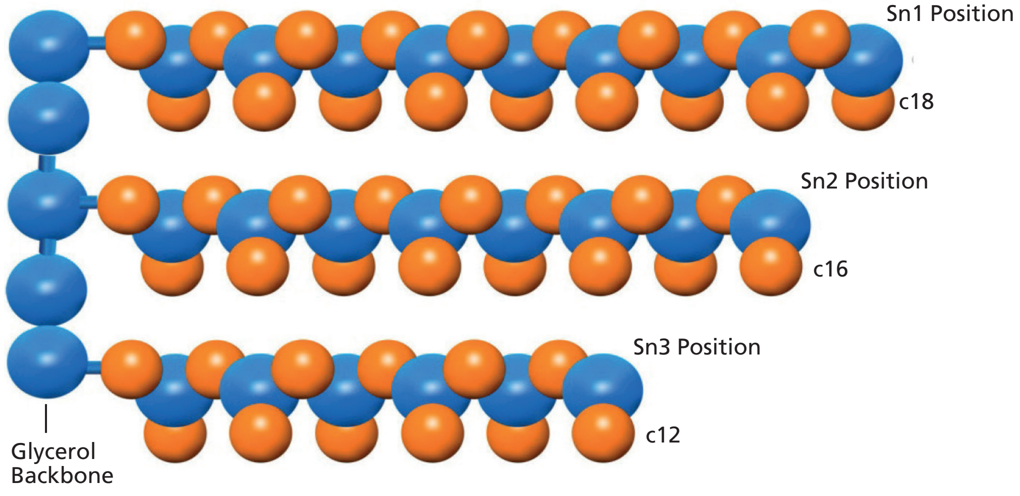


Fig 2.9 Triglyceride space filling model

Nucleic Acids

Nucleic acids are polymers of **nucleotides**. They form the genetic material of all living organisms. Nucleotides are made up of three smaller components, a pentose sugar, a nitrogenous base and a phosphate group. The elements that make up nucleic acids are carbon (C), hydrogen (H), oxygen (O), nitrogen (N) and phosphorous (P).

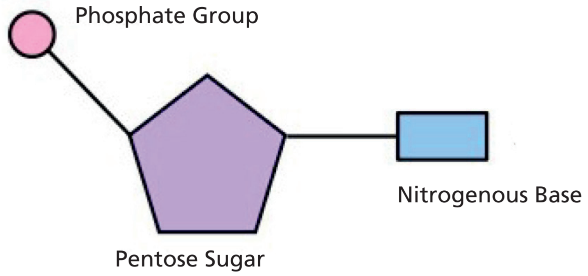


Fig 2.10 A nucleotide made up of a pentose sugar, a nitrogenous base and a phosphate group

Experiment 2.1

BENEDICT'S TEST FOR REDUCING SUGARS

AIM: To test for reducing sugar i.e. glucose.

MATERIALS: reducing sugar solution i.e. glucose solution, distilled water, Benedict's solution, safety goggles, Bunsen burner or spirit burner, beaker and test tubes.

METHOD

1. Add a 2 cm^3 volume of a solution of the reducing sugar to a test tube.
2. Add an equal volume of Benedict's reagent to the solution you are testing.
3. Shake and heat the mixture in a water bath. The heating is done by placing the test-tube in a beaker of boiling water or warming it gently over a blue Bunsen flame (**Fig 2.11**). However, if this second technique is used, the test-tube should be moved constantly in and out of the Bunsen flame or spirit burner to prevent the liquid boiling and shooting out of the tube.
4. Repeat steps 1 to 3 using 2 cm^3 using distilled water in a test tube.

EXPECTED OBSERVATIONS:

If a reducing sugar is present, the solution will gradually turn through green, yellow and orange to red-brown. If the reducing sugar is absent, the solution will remain blue.

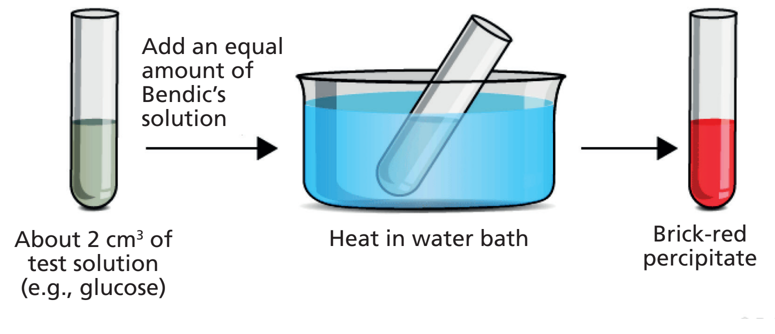


Fig 2.11 Procedure to test for reducing sugar. Credit: BYJU'S

ADDITIONAL INFORMATION

The test is semi quantitative, that is to say a rough estimation of the amount of reducing sugar present will be possible. The intensity of the red colour is related to the concentration of the reducing sugar. An estimate of the concentration of the reducing sugar in the sugar sample used can be conducted by comparing the test colour intensity against colours obtained in tests done with reducing sugar solutions of known concentration.

Experiment 2.2

IODINE TEST FOR STARCH

AIM: To test for starch.

MATERIALS: starch solution, iodine solution, distilled water, test tubes and droppers.

METHOD

1. Add 2 cm^3 of starch solution to a test tube.
2. Add a few drops of iodine in potassium iodide solution (commonly referred to as iodine solution) (**Fig 2.21**).
3. Repeat step 1 and 2 using 2 cm^3 using distilled water in a test tube.

EXPECTED OBSERVATIONS

A blue-black colouration indicates the presence of starch. The solutions will remain brown if starch is absent.

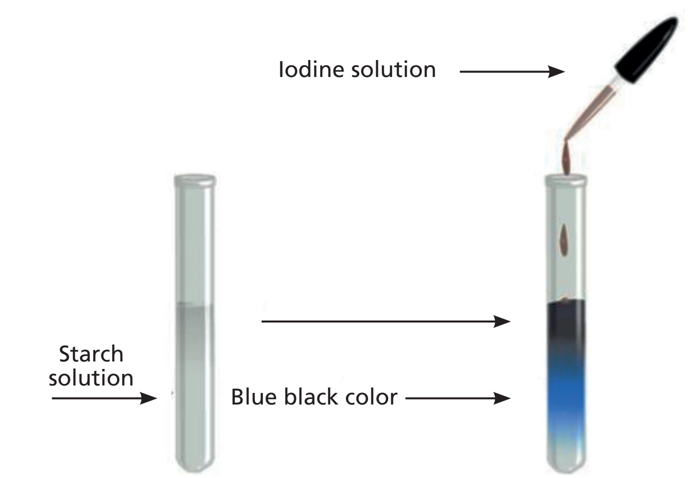


Fig 2.12 Set up to test for starch. Credit: EduRev

Experiment 2.3

EMULSION TEST FOR LIPIDS

AIM: To test for lipids.

MATERIALS: Margarine or cooking oil, ethanol, test tubes and distilled water.

METHOD

1. Add 2 cm^3 of fat or oil substance to a test tube.
2. Add 2 cm^3 of absolute ethanol to the test tube with the sample of the substance to be tested.
3. Dissolve the lipid in the substance by shaking the mixture vigorously.
4. Add 2 cm^3 of distilled water (**Fig 2.13**).
5. Repeat steps 1 to 4 using 2 cm^3 using distilled water in a test tube.

EXPECTED OBSERVATIONS

If a lipid is present, a cloudy white suspension is formed. If there is no lipid present, the ethanol just mixes into the water and the mixture looks completely transparent.

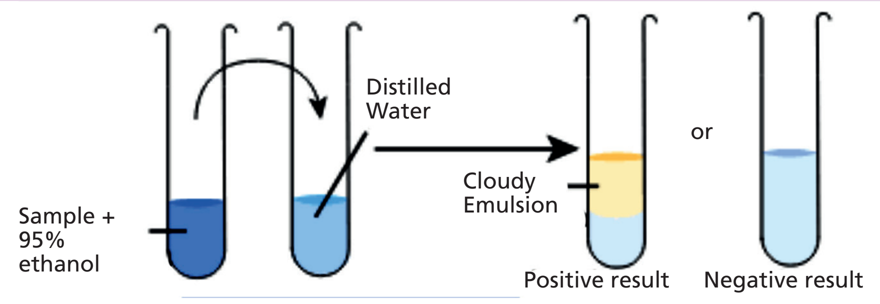


Fig 2.13 Emulsion test. Credit: Socratic

ADDITIONAL INFORMATION

An alternative to this experiment is to take a piece of translucent paper or blotting paper and add a few drops of oil or rub fat on one end of the paper. On the other end of the paper add a few drops of water. Allow the spots to dry and make observations.

Experiment 2.4

BIURET TEST FOR PROTEINS

AIM: To test for proteins.

MATERIALS: 1% solution of albumen (the protein of egg-white), 1% glucose solution, sodium hydroxide, 1% copper sulphate solution test tubes and droppers.

METHOD

1. Add 1% solution of albumen to a test tube.
2. Add 5 cm^3 dilute sodium hydroxide to the test tube with albumen.
3. Add 5 cm^3 1% copper sulphate solution to the test tube (**Fig 2.14**).
4. Repeat steps 1 to 3 using 1% glucose solution.

EXPECTED OBSERVATIONS

A mauve or purple colour that develops gradually indicates that a protein is present. In the absence of protein, the solution remain blue.

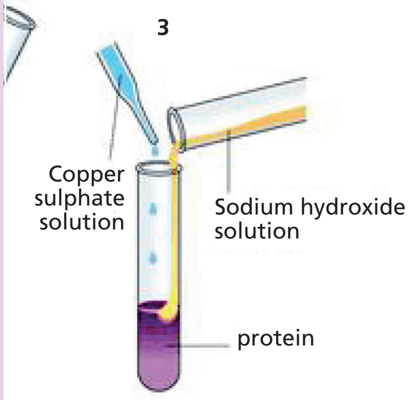


Fig 2.14 Biuret test. Credit: Edmodo Spotlight.

Activity 2.1

Uses the internet to visit sites such as YouTube were you can view videos on identification tests. Watch and share the videos with your classmates.

End of chapter questions

1 List the constituent elements in

(a) Carbohydrate. [3]

(b) Lipids. [3]

(c) Proteins [4]

(d) Nucleic acids [4]

2 Iodine, Benedict's reagent, and Biuret reagent were used in an investigation to identify certain biochemical substances.

(a) (i) Describe the colour change which indicates a positive result when using Biuret reagent. Your answer should state the initial and the final colour. [1]

(ii) Which of the reagents used in the investigation (and listed above) needs to be heated when carrying out the test? [1]

(b) The following statements indicate which tests gave positive results for four different substances.

- Substance A gave a positive result with Biuret reagent.
- Substance B gave a positive result with both Benedict's reagent and with Biuret reagent.
- Substance C initially gave a negative result with both Benedict's reagent and iodine. However, after hydrolysis with hydrochloric acid it gave a positive result with both Benedict's reagent and Biuret reagent.
- Substance D gave a positive result with Benedict's reagent but a negative result with Biuret reagent.

Identify each of the substances:

Substance A

Substance B

Substance C

Substance D [4]

Summary

- Water is a simple covalent molecule made up of two elements hydrogen (H) and oxygen (O). It is a polar molecule because it has an uneven distribution of charge.
- The shape of a water molecule and its polarity makes hydrogen bonding possible.
- Hydrogen bonding is the single most outstanding and important characteristic of water responsible for its anomalous and unique properties such as its solvent properties, high specific heat capacity, high latent heat capacity and its low its low density when it freezes. These properties make water an important substance for living organisms because it is a good solvent, cooling agent, an important reagent for some reaction, and a habitat for many living organisms.
- Carbohydrates are made from monosaccharide units, often glucose. Carbohydrates are used as an energy source. Animals store carbohydrates as glycogen and plants store carbohydrates as starch. Plants also make cellulose, a structural carbohydrate that gives plant cell walls their strength.
- Lipids include fats, fatty acids, and oils. Fats are made up from fatty acids and glycerol.
- Proteins are made up from amino acids joined together.
- Nucleotides are made up of three smaller components, a pentose sugar, a nitrogenous base, and a phosphate group.
- Identification tests can be carried out to identify biological molecules in a sample.
- Brown iodine solutions turns blue black in the presence of starch; blue Benedict's solution turns orange or brick red when heated with a reducing sugar; ethanol turns milky white when mixed with lipids; Biuret's reagent turns mauve or purple in the presence of proteins.

End of chapter questions

1. The tendency of water to resist changes in temperature is due to which of the following properties?
   A. Ability to dissolve solutes
   B. Low density
   C. Specific heat capacity
   D. Transparency
2. What causes water to have a relatively high boiling point?
   A. Hydrogen bonds between water molecules
   B. Hydrogen bonds between hydrogen and oxygen within water molecules
   C. Cohesion between water molecules and the container in which the water is boiled
   D. Covalent bonds between hydrogen and oxygen within water molecules

3. Which nutrient produces a purple colour when mixed with biuret solution?
   A. Fat
   B. Protein
   C. Reducing sugar
   D. Starch

4. Which element is found in proteins but not carbohydrates?
   A. Carbon
   B. Hydrogen
   C. Nitrogen
   D. Oxygen

5. When a food substance is tested with iodine solution, which colour shows the presence of starch?
   A. Blue-black
   B. Brown
   C. Orange
   D. Purple

6. Small molecules are used as the basic units in the synthesis of large food molecules. Which statement is correct?
   A. Amino acids are basic units of carbohydrates.
   B. Fatty acids are basic units of glycogen.
   C. Glycerol is a basic unit of oils.
   D. Simple sugar is a basic unit of protein.

7. **Table 2.1** shows the results of some food tests.

   Which row shows a food containing both protein and starch?

Table 2.1

	Benedict's solution	**biuret test**	**ethanol**	**iodine solution**
A	blue	blue	clear	blue-black
B	blue	purple	clear	blue-black
C	red	blue	cloudy	brown
D	red	purple	cloudy	brown

[5]

8. Proteins are an important part of the diet. These proteins are used in the body to make enzymes and other cell structures.

You are going to estimate the concentration of protein in two solutions, **A** and **B**, using the results from a set of standard protein solutions.

Known concentrations of protein solution were made. Using biuret reagent, the colour intensity score was determined at each concentration.

Step 1: Seven test-tubes were labelled **1**, **2**, **3**, **4**, **5**, **6** and **7**.

Step 2: Solutions containing different concentrations of protein were made using the volumes of 1% protein solution and distilled water shown in **Table 2.3**

Table 2.3

	test tube number						
Test	**1**	**2**	**3**	**4**	**5**	**6**	**7**
volume of 1% protein solution / cm^3	0.00	0.25	0.50	1.00	2.00	3.00	4.00
volume of distilled water / cm^3	5.00	4.75	4.50	4.00	3.00	2.00	1.00
percentage concentration of protein solution	0.00	0.05		0.20	0.40	0.60	

(a) (i) Copy and complete **Table 2.3** by calculating the percentage concentration of the protein solutions in test-tubes **3** and **7**. Write your answers in **Table 2.3**.

Show your working. [2]

(ii) State the colour that shows the presence of protein when tested with biuret reagent. [1]

Step 3: 2 cm^3 of biuret reagent was added to each of the solutions in the test-tubes, **1** to **7**. Each test-tube was shaken gently to mix the contents.

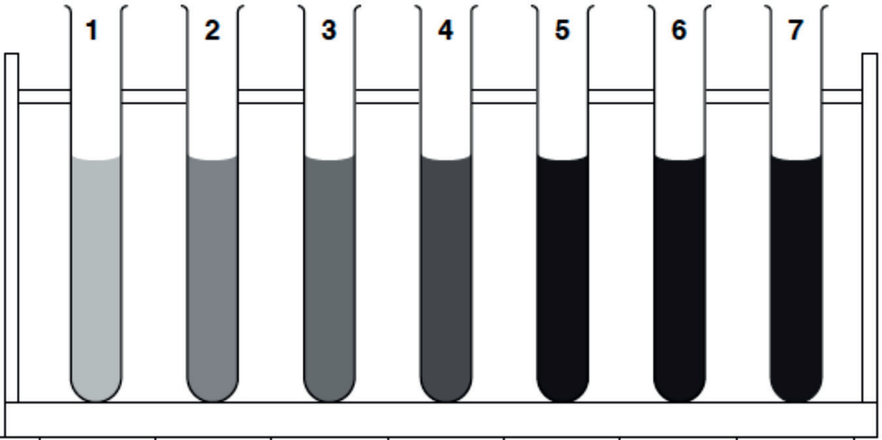
Step 4: The test-tubes were placed in a test-tube rack in order of concentration, from the least concentrated to most concentrated.

Step 5: 2 cm^3 of biuret reagent was added to the protein solution in the test-tube labelled **A** and shaken gently to mix the contents.

Step 6: Step **5** was repeated for the protein solution in test-tube **B**.

Step 7: Test-tubes **1** to **7** were held against a white background so that the colour of the solutions were clearly visible. These test-tubes are shown in **Fig 2.15**.

Step 8: The colour intensity of the solution in test-tube **7** was given a score using **Table 2.4**.



colour intensity score							++++

Fig 2.15

Table 2.4

colour intensity	palest → darkest			
score	+	++	+++	++++

(iii) Use **Table 2.4** to complete **Fig 2.14** by writing in the score for test-tubes **1** to **6**.

Test-tubes that have the same colour intensity should be given the same score. [1]

(iv) Prepare a table to record the results for test-tubes **1** to **7**.

Your table should show:

- the concentration of the protein solutions
- the colour intensity score given to each of the solutions.

The results for tubes **A** and **B** are shown in **Fig 2.16**.

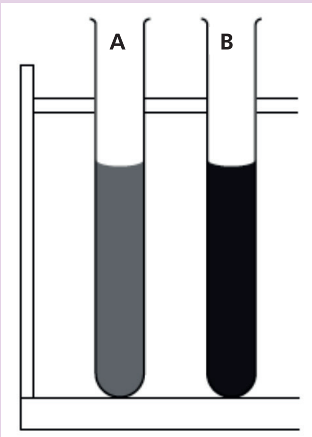


Fig 2.16

(v) Use **Fig 2.15** to determine the colour intensity scores for test-tubes **A** and **B** in **Fig 2.16**.

colour intensity score for test-tube **A**_________________________________. [1]

colour intensity score for test-tube **B**______________________________. [1]

(vi) Use your table from **(a)(iv)** to estimate the percentage concentration of the protein solutions in test-tubes **A** and **B**:

Concentration of the protein solution in test-tube **A** ____________________ %

Concentration of the protein solution in test-tube **B**_____________________ [2]

(b) **(i)** Identify the control for this experiment **and** explain why it is used. [2]

(ii) Explain why the method used to find the concentration of the protein solutions in Test-tubes **A** and **B** can only be an estimate. [2]

[Total: 14]