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BIOLOGY

AQA A-level
Year 1 and AS
Student Book

Mary Jones
Lesley Higginbottom

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Authored by Mary Jones and Lesley Higginbottom
Commissioned by Emily Pither
Project managed by 4science
Edited by Mark Gadd and Gina Walker
Proofread by Alison Walters, Laura Booth and Amanda Harman
Typeset by Jouve
Cover design by We are Laura
Illustrations by Geoff Jones

Printed by Grafica Veneta S.p.A.

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Please note that when teaching the AS and A-level Biology course, you must refer to AQA's specification as your definitive source of information. While this book has been written to match the specification, it cannot provide complete coverage of every aspect of the course.

A wide range of other useful resources can be found on the relevant subject pages of our website: www.aqa.org.uk

CONTENTS

To the student	v		
Practical work in biology	1		
1 – Water and carbohydrates	4	7 – The immune system	120
1.1 Biological molecules	5	7.1 Cell-surface antigens	121
1.2 Water	6	7.2 Phagocytosis	122
1.3 Carbohydrates	10	7.3 The immune response	123
		7.4 HIV/AIDS	133
2 – Lipids and proteins	20	7.5 Monoclonal antibodies	136
2.1 Lipids	21	8 – Exchange with the environment	142
2.2 Proteins	26	8.1 Surface area : volume ratio	143
3 – Enzymes	33	8.2 Gas exchange	145
3.1 Biological catalysts	34	8.3 The human gas exchange system	150
3.2 Enzymes and chemical reactions	34	8.4 Digestion and absorption	157
3.3 How enzymes work	35	9 – Mass transport	165
3.4 Factors affecting enzyme activity	37	9.1 Mass flow	166
4 – Nucleotides	53	9.2 Oxygen transport in mammals	166
4.1 Structure of DNA and RNA	54	9.3 The heart and circulatory system	170
4.2 DNA replication	59	9.4 Blood vessels	176
4.3 ATP	61	9.5 Water transport in plants	185
5 – Cells	67	9.6 Transport of organic substances in plants	191
5.1 Cells and living organisms	68	10 – DNA and protein synthesis	199
5.2 Structure of eukaryotic cells	68	10.1 Genes and chromosomes	200
5.3 Prokaryotic cells and viruses	77	10.2 The genetic code	203
5.4 Methods of studying cells	79	10.3 Protein synthesis	205
5.5 Making new cells	85	11 – Genetic diversity	215
6 – Cell membranes	98	11.1 Mutation	216
6.1 Structure of cell membranes	99	11.2 Meiosis	219
6.2 Diffusion and facilitated diffusion	101	11.3 Natural selection	223
6.3 Osmosis	105		
6.4 Active transport	113		

CONTENTS

12 – Taxonomy and biodiversity	239	13.3 Analysing and interpreting data	264
12.1 The species concept	240	13.4 Statistics	266
12.2 Biodiversity	244	Answers	270
12.3 Investigating diversity	248	Glossary	282
13 – Maths techniques in biology	260	Index	288
13.1 Handling numbers	260	Acknowledgements	302
13.2 Recording and displaying data	262		

TO THE STUDENT

The aim of this book is to help make your study of advanced biology interesting and successful. It includes examples of modern issues, developments and applications that reflect the continual evolution of scientific knowledge and understanding. We hope it will encourage you to study science further when you complete your course.

USING THIS BOOK

Biology is a fascinating, but complex subject – underpinned by some demanding ideas and concepts, and by a great deal of experimental data ('facts'). This mass of information can sometimes make its study daunting. So don't try to achieve too much in one reading session and always try to keep the bigger picture in sight.

There are a number of features in the book to help with this:

- Each chapter starts with a brief example of how the biology you will learn has been applied somewhere in the world, followed by a short outline of what you should have learned previously and what you will learn through the chapter.
- Important words and phrases are given in bold when used for the first time, with their meaning explained. There is also a glossary at the back of the book. If you are still uncertain, ask your teacher or tutor because it is important that you understand these words before proceeding.
- Throughout each chapter there are many questions, with the answers at the back of the book. These questions enable you to make a quick check on your progress through the chapter.
- Similarly, throughout each chapter there are checklists of key ideas that summarise the main points you need to learn from what you have just read.
- Where appropriate, worked examples are included to show how calculations are done.
- There are many assignments throughout the book. These are tasks relating to pieces of text and data that show how biological ideas have been developed or applied. They are not required knowledge for an A-level examination. Rather, they provide opportunities to apply the science you have learned to new contexts, practise your maths skills and practise answering questions about scientific methods and data analysis.
- Some chapters have information about the 'required practical' activities that you need to carry out during your course. These sections provide the necessary background information about the apparatus, equipment and techniques that you need to be prepared to carry out the required practical work. There are questions that give you practice in answering questions about equipment, techniques, attaining accuracy, and data analysis.
- At the end of each chapter are practice questions. These are examination-style questions which cover all aspects of the chapter.

This book covers the requirements of AS Biology and the first year of A-level Biology. There are a number of sections, questions, assignments and practice questions that have been labelled 'Stretch and challenge', which you should try to tackle if you are studying for A-level. In places these go beyond what is required for the specification but they will help you build upon the skills and knowledge you acquire and better prepare you for further study beyond advanced level.

Good luck and enjoy your studies. We hope this book will encourage you to study biology further when you complete your course.

PRACTICAL WORK IN BIOLOGY

Practical work is a vital part of biology. Biologists apply their practical skills in a wide variety of contexts – from conservation to food production; from tracking invasive species to controlling disease. In your AS or A-level Biology course you need to learn, practise and demonstrate that you have acquired these skills.



WRITTEN EXAMINATIONS

Your practical skills will be assessed in the written examinations at the end of the course. Questions on practical skills will account for about 15% of your marks at AS and 15% at A-level. The practical skills assessed in the written examinations are:

Independent thinking

- › solve problems set in practical contexts
- › apply scientific knowledge to practical contexts

Use and application of scientific methods and practices

- › comment on experimental design and evaluate scientific methods
- › present data in appropriate ways
- › evaluate results and draw conclusions with reference to measurement uncertainties and errors
- › identify variables including those that must be controlled

Numeracy and the application of mathematical concepts in a practical context

- › plot and interpret graphs
- › process and analyse data using appropriate mathematical skills
- › consider margins of error, accuracy and precision of data

Instruments and equipment

- › know and understand how to use a wide range of experimental and practical instruments, equipment and techniques appropriate to the knowledge and understanding included in the specification

Throughout this book there are questions and longer assignments that will give you the opportunity to develop and practise these skills. The contexts of some of the exam questions will be based on the 'required practical activities'.



Figure 1 Biologists often use techniques and apparatus in the field as well as in the laboratory.

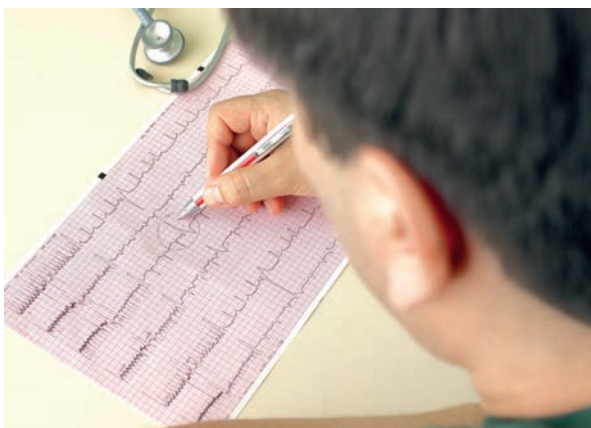


Figure 2 It is important to be able to interpret and analyse data – this doctor is analysing an electrocardiogram; understanding anomalies is crucial when making a diagnosis.



Figure 3 You will need to use a variety of equipment correctly and safely.

ASSESSMENT OF PRACTICAL SKILLS

Some practical skills can only be practised when you are doing experiments. For A-level, these **practical competencies** will be assessed by your teacher:

- › follow written procedures
- › apply investigative approaches and methods when using instruments and equipment
- › safely use a range of practical equipment and materials
- › make and record observations
- › research, reference and report findings

You must show your teacher that you consistently and routinely demonstrate the competencies listed above during your course. The assessment will not contribute to your A-level grade, but will appear as a 'pass' alongside your grade on the A-level certificate.

These practical competencies must be demonstrated by using a specific range of **apparatus and techniques**:

- › use appropriate apparatus to record a range of quantitative measurements (to include mass, time, volume, temperature, length and pH)
- › use appropriate instrumentation to record quantitative measurements, such as a colorimeter or potometer
- › use laboratory glassware apparatus for a variety of experimental techniques to include serial dilutions
- › use a light microscope at high power and low power, including use of a graticule
- › produce scientific drawing from observation with annotations
- › use qualitative reagents to identify biological molecules
- › separate biological compounds using thin layer/paper chromatography or electrophoresis
- › safely and ethically use organisms to measure plant or animal responses, and physiological functions
- › use microbiological aseptic techniques, including the use of agar plates and broth
- › safely use instruments for dissection of an animal organ, or plant organ
- › use sampling techniques in fieldwork
- › use ICT such as computer modelling or data logger to collect data, or use software to process data

For AS, the above will not be assessed but you will be expected to use these skills and these types of apparatus to develop your manipulative skills and your understanding of the processes of scientific investigation.

REQUIRED PRACTICAL ACTIVITIES

During the A-level course you will need to carry out 12 **required practical** activities. These are the main sources of evidence that your teacher will use to award you a pass for your competency skills. If you are doing the AS, you will need to carry out the first six in this list.



Figure 4 Aseptic techniques must be used when handling agar plates and broth.



Figure 5 Dissection tools – such as this scalpel being used to cut a sheep kidney – must be handled with care; they are often surgically sharp, but not always surgically clean!

1. Investigation into the effect of a named variable on the rate of an enzyme-controlled reaction
2. Preparation of stained squashes of cells from plant-root tips; set-up and use of an optical microscope to identify the stages of mitosis in these stained squashes and calculation of a mitotic index
3. Production of a dilution series of a solute to produce a calibration curve with which to identify the water potential of plant tissue
4. Investigation into the effect of a named variable on the permeability of cell-surface membranes
5. Dissection of animal or plant gas exchange or mass transport system or of organ within such a system
6. Use of aseptic techniques to investigate the effect of antimicrobial substances on microbial growth
7. Use of chromatography to investigate the pigments isolated from leaves of different plants, for example leaves from shade-intolerant and shade-intolerant plants, or leaves of different colours
8. Investigation into the effect of a named factor on the rate of dehydrogenase activity in extracts of chloroplasts
9. Investigation into the effect of a named variable on the rate of respiration of cultures of single-celled organisms
10. Investigation into the effect of an environmental variable on the movement of an animal using either a choice chamber or a maze
11. Production of a dilution series of a glucose solution and use of colorimetric techniques to produce a calibration curve with which to identify the concentration of glucose in an unknown 'urine' sample
12. Investigation into the effect of a named environmental factor on the distribution of a given species

Information about the apparatus, techniques and analysis of required practicals 1 to 6 are found in the relevant chapters of this book, and 7 to 12 in Book 2.

You will be asked some questions in your written examinations about skills developed as result of carrying out these required practicals.

Practical skills are really important. Take time and care to learn, practise and use them.

1 WATER AND CARBOHYDRATES

PRIOR KNOWLEDGE

You have probably learned that many substances are made up of molecules, which in turn are formed by groups of atoms bonded together. You may also know that each atom consists of a central nucleus carrying a positive charge, surrounded by a cloud of electrons each with a negative charge. In a neutral atom, the number of negative and positive charges is equal, but an atom that has gained extra electrons, or lost some, has a negative or positive charge, and is called an ion. You may also remember the kinetic theory of matter, which helps us to understand the properties of different states of matter (solids, liquids and gases) by describing how their particles move around and interact with one another.

LEARNING OBJECTIVES

In this chapter, we will use these concepts to explain the structure and roles of some of the most important types of molecules that make up the bodies of all living organisms. You will also learn why water is essential to all life.

(Specification 3.1.1, 3.1.7, 3.1.2)

Many scientists believe there is a strong possibility that there is life elsewhere in the universe, not just here on Earth. Perhaps there are other planets similar

to ours out there somewhere, circling one of the innumerable stars in our galaxy or in other galaxies far away. Nearer to home, could our neighbouring planet, Mars, harbour life?

Biologists searching for life on distant planets tend to begin by looking for water. Although we have no reason to think that life on other planets would look anything at all like the living organisms on Earth, it is very difficult to predict how any life form could exist without water. Water is a unique substance that seems to be essential for the existence of life.

There is now no doubt that there is at least some water on Mars, and that in the past there was much more. NASA's Martian rovers, Curiosity and Opportunity, have found that Mars had a warmer and wetter climate long ago. Lakes used to exist on Mars, and there may even be liquid water present now. There used to be hot springs and flowing streams.

The next question is to find out if there has ever been life there, and the rovers will continue to search for traces of past or present living organisms. They will look for the presence of carbon-containing compounds that are normally produced only by living things. Even if there is no life on the surface now – where conditions seem to be too inhospitable for even microorganisms to live – there is an outside possibility that something may have survived deep underground. Perhaps by the time you read this, a new discovery will have been made.

1.1 BIOLOGICAL MOLECULES

What are living organisms made of? All living organisms, including yourself, plants, bacteria and every other kind of living thing, are made of **molecules**. The study of these molecules is called molecular biology, and the way in which the molecules behave is biochemistry. All life on Earth has similar biochemistry.

Molecules are unimaginably small particles, which themselves are made up of even smaller particles, called **atoms**. In a molecule, atoms are joined by bonds between them, which hold them tightly together (see Figure 1).

Joining together different atoms in different quantities, or even the same atoms in different arrangements, produces molecules that behave in very different ways.

There are more than 100 different kinds of atoms, and an infinite number of different kinds of molecule that can exist. In living things, however, most of the atoms are of just six different kinds – hydrogen, oxygen, carbon, nitrogen, sulfur and phosphorus. Other kinds of atoms are less common, but they are nevertheless vitally important. Atoms of calcium, iron, zinc, magnesium, sodium, chlorine and potassium are all important ingredients in the body of every living organism.

Some of these atoms are important not only when they are part of molecules, but also on their own, particularly when they exist in their charged form as an ion. Whereas uncharged atoms have equal numbers of positively charged protons and

negatively charged electrons, ions have lost or gained electrons, leaving them with a positive or negative charge. Iron ions are an important component of the oxygen-transporting substance haemoglobin, and phosphate ions are found in DNA and other molecules. You will come across these and other examples as you work through your biology course.

In this chapter and the next, we will look at some of the most important molecules that make up living organisms.

Overview of biological molecules

Water (see Figure 1) is a major component of the body of every living organism; in humans, for example, about 70% of our body weight is made up of water.

Water is so important to life that, when searching for life on planets other than Earth, astrobiologists tend to look first for water – it is difficult to imagine any life form that could exist without it. You will find out why this is so when you read the next section in this chapter.

Water has very small molecules, but most molecules found in organisms are much larger. One important class of molecules in living organisms is carbohydrates. The smallest carbohydrate molecules are called sugars, and they are made of several carbon atoms, several hydrogen atoms and several oxygen atoms all joined together. There are many different kinds of sugars, each with molecules in which different numbers of these atoms are linked in slightly different ways. Sugar molecules can join together in long chains, producing huge molecules such as starch, glycogen and cellulose. Sugars, starch, glycogen and cellulose are all **carbohydrates**.

Protein molecules are also made up of long chains of smaller molecules. These smaller molecules are called **amino acids**. Like the molecules that make up carbohydrates, amino acid molecules contain atoms of carbon, hydrogen and oxygen, but they also contain atoms of nitrogen. You will find out more about proteins in Chapter 2.

A third type of molecule found in every living organism is **nucleotides**. These include DNA and RNA. The structures and functions of nucleotides are described in Chapter 4.

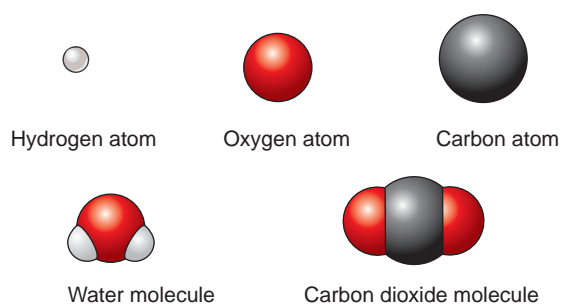


Figure 1 Water and carbon dioxide molecules: molecules are made of atoms held firmly together.

Lipids (also known as fats) often get a bad press, but they are an essential component of all living things. Like carbohydrates, their molecules contain carbon, hydrogen and oxygen atoms, but they are arranged in a very different way. You will learn more about lipids in Chapter 2.

Monomers and polymers

The largest molecules in living organisms are made by joining together long chains of smaller molecules. For example, starch is made by joining together very long chains of glucose molecules (see Figure 2). The glucose molecules are **monomers**, and the long chain of them joined together forms a **polymer**.

Protein molecules are also polymers. Here, the monomers are amino acids.

Nucleotides also form polymers, joining together into long chains to produce polynucleotides.

QUESTIONS

1. Arrange these in order of size, smallest first: protein molecule; carbon atom; amino acid molecule; animal cell.

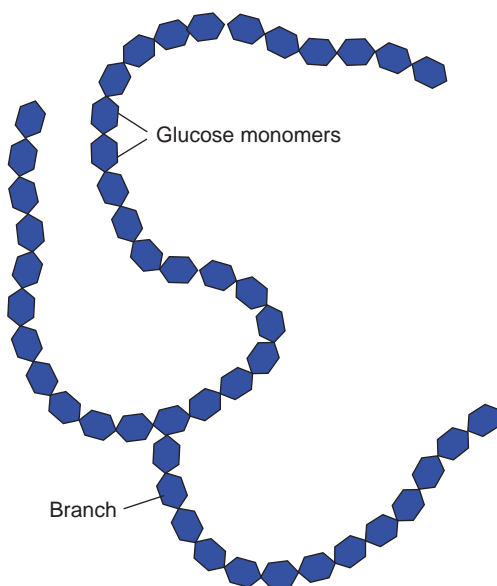


Figure 2 Part of a starch molecule. Starch is a polymer of glucose. Unlike most polymers, starch molecules have branches along their chains of monomers.

KEY IDEAS

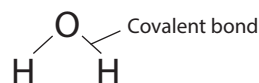
- ▶ Living organisms are made up of many different kinds of molecules, most of which contain atoms of carbon, hydrogen, oxygen and nitrogen.
- ▶ Many biological molecules are polymers, made up of long chains of smaller molecules – called monomers – joined together.

1.2 WATER

Water is an amazing substance. It has a set of properties that are not found in any other substance. Without water, there would be no life on Earth.

The structure of water molecules

To understand why water behaves in the way that it does, we need to understand the structure of its molecules (Figure 3). Here, the symbols of the atoms are used instead of drawing them as balls, as in Figure 1. Lines between the symbols represent bonds that hold them together. A water molecule is made up of two hydrogen atoms joined by **covalent bonds** to a single oxygen atom, so its formula is H_2O . This is a very small molecule compared with most molecules found in living organisms.



Water molecule H_2O

Figure 3 The atoms in water molecules are held tightly together by strong covalent bonds.

Covalent bonds are very strong bonds, formed when two atoms share electrons with each other. It is very difficult to split the hydrogen and oxygen atoms apart – a lot of energy is required to make this happen. As you will see if you continue your studies to A-level, plants have evolved a way to split water molecules using energy from light, which they do in photosynthesis.

Hydrogen bonds

Atoms contain electrons, and an electron has a small negative charge. In a water molecule, the electrons in the chemical bond are not shared equally between

the oxygen and the hydrogen atoms. This results in the oxygen atom having a tiny negative charge, and each hydrogen atom having a tiny positive charge. The water molecule is said to have a **dipole**. Although its overall charge is zero, it has a small positive charge in some places, and a small negative charge in another. These small charges are written δ^- and δ^+ . The symbol δ is the Greek letter delta, so you can say 'delta minus' and 'delta plus'.

Negative charges and positive charges are attracted to one another. This means that each water molecule is attracted to its neighbours. The negative charge on one water molecule is attracted to the positive charge on another water molecule. These attractions are called **hydrogen bonds** (see Figure 4). Though they are important, hydrogen bonds are much weaker than the strong covalent bonds that hold the oxygen and hydrogen atoms firmly together.

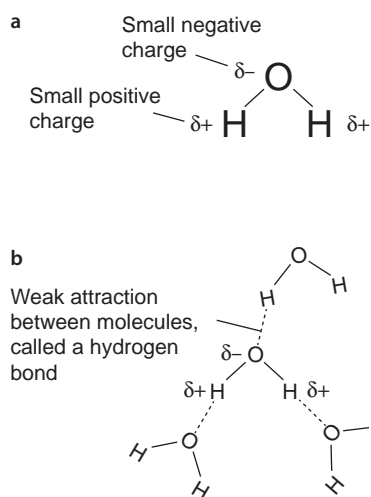


Figure 4 Water molecules are weakly attracted to each other because of their dipoles.

Water helps keep temperatures stable

Molecules are in constant motion. In a solid, they stay in the same place and just vibrate on the spot. In a liquid, they move around more freely, but remain in contact with one another. In a gas, they move completely freely, only rarely making contact.

Temperature is a measure of the kinetic energy of molecules. Kinetic energy is movement energy. A substance in which the molecules are moving very fast has a higher temperature than one where the molecules are moving very slowly.

We can make molecules move around faster by adding heat (thermal) energy to them. For example, you

could put some water into a beaker and heat it over a Bunsen flame. As the heat energy from the flame is transferred to the water, the water molecules move faster and faster. The temperature of the water rises.

However, we find that the temperature of water does not rise as much as it would if a different liquid was heated, such as hexane (C_6H_{14}). This is because of the hydrogen bonds between the water molecules. Hydrogen bonds are weak bonds, and they are easily broken, but it still takes energy to break them. And, although each hydrogen bond between the water molecules in a beaker is very weak, there are billions of them. A lot of the heat energy from the Bunsen flame is transferred in breaking these hydrogen bonds, separating the water molecules from each other. Only the remaining heat energy is transferred to kinetic energy, making the water molecules move around faster and increasing the temperature.

So, to increase the temperature of a beaker of water by $1^\circ C$, we have to put in much more heat energy than to increase the temperature of a beaker of hexane by $1^\circ C$. We say that water has a relatively high **heat capacity**. A lot of heat energy has to be transferred to the water to raise its temperature significantly.

This is very useful to living organisms. Because our bodies are mostly made of water, we have a relatively high heat capacity. The water in and around our cells absorbs a lot of heat energy without its temperature rising very much. The water 'buffers' (tones down) heat changes.

This is also very helpful to aquatic organisms. Large bodies of water, such as the sea or a lake, or even a pond, do not change temperature as quickly or as greatly as the air (Figure 5). As air temperature rises during the daytime, water temperature rises only a little. Similarly, as air temperature drops at night, water



Figure 5 The water in the sea is much cooler than the air on a hot, sunny day.

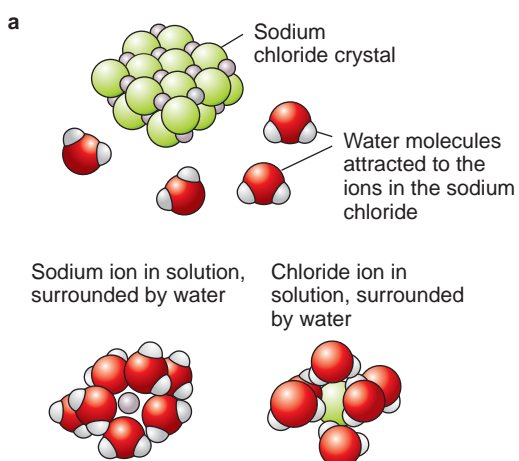
temperature drops by much less. So, aquatic organisms live in an environment where temperature changes are much smaller, and happen much more gradually, than the environment of terrestrial (land-living) organisms.

Water helps keep organisms cool

In a body of water, not all of the water molecules are moving at the same speed. Some have more kinetic energy than others. Some will have enough kinetic energy to escape from the water and shoot off into the air. They have become a gas. This is called **evaporation**. (Do not confuse evaporation with boiling, which happens only when the temperature of a liquid rises to its boiling point. Boiling also happens throughout the liquid, while evaporation happens only at the surface.)

As these fast-moving molecules are lost from the water, their energy goes with them. As more and more of them leave, the average kinetic energy of the molecules that are left behind in the water gradually decreases. The water cools down.

The energy that is lost from the liquid water as it evaporates is called **latent heat of vaporisation**. Water has a relatively high latent heat of vaporisation, so the evaporation of quite small quantities of water has a large cooling effect. We make use of this when we sweat. Sweat, which is mostly water, lies on the skin surface and evaporates. As it evaporates, the skin surface is cooled. Plants, too, are cooled down when water evaporates from their leaves in transpiration.



QUESTIONS

2. Fish are not able to regulate their body temperature in the way that mammals do. Explain how hydrogen bonding in water helps fish to have a relatively constant body temperature.

Stretch and challenge

3. When climbing big mountains, mountaineers often set up a base camp before attempting the final climb to the summit.
 - a. Why do mountaineers find that, when cooking at altitude, their water boils more quickly than when they are at base camp, but it takes longer to cook their food?
 - b. When sterilising water, why do the mountaineers need to boil it for longer at high altitudes?

Water as a solvent

Water is an excellent solvent. A **solvent** is a liquid in which other substances – called **solutes** – can dissolve. Many different substances are able to dissolve in water, and this is all down to its hydrogen bonds.

Figure 6 shows how salt (sodium chloride) dissolves in water. Sodium chloride is made up of positively charged sodium ions, Na^+ , and negatively charged

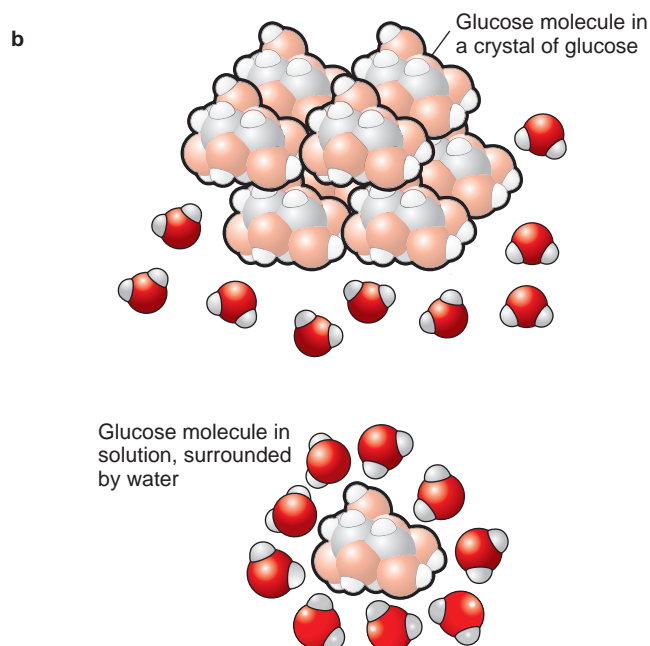


Figure 6 (a) How sodium chloride dissolves in water; (b) how glucose dissolves in water

chloride ions, Cl^- . These are attracted to the δ^- and δ^+ charges on the water molecules. The sodium ions and chloride ions are therefore separated from each other, and spread out in between the water molecules. This is why salt seems to 'disappear' when you stir it into water.

Water can even dissolve quite large molecules, such as sugars. As you will see later in this chapter, sugar molecules have tiny positive and negative charges on parts of their molecules, and these are attracted to the negative and positive charges on the water molecules. When you stir sugar into a cup of tea, the sugar molecules spread out in between the water molecules.

When substances are dissolved in water, their molecules or ions are free to move around and to react with other molecules or ions that are also in solution. The metabolic reactions that take place in your body, and in the bodies of every other living organism, only take place because the reactants are dissolved in water.

Water's solvent properties are also important in transporting substances around the body. Blood plasma is mostly water, and it carries a huge range of different substances – such as glucose, vitamins, urea and carbon dioxide – in solution. Plants transport mineral ions and sugars dissolved in water in their xylem and phloem vessels. We also use water to dissolve substances to be excreted from the body, especially urea. Urine is a solution of urea and other solutes in water.

Water molecules stay together

We have seen that water molecules are attracted to each other. These attractions help to hold water molecules together, so that they can flow as a continuous stream. You see this happening when you watch water flowing from a tap, or along a river. This movement of a whole mass of water together is an example of **mass flow**.

In plants, water moves up xylem vessels as a continuous stream, by mass flow. Water can move like this all the way to the top of the tallest trees. The tall, continuous columns of water inside the tree's trunk and branches can only hold together because of the attraction between the water molecules. The way in which the water molecules tend to 'stick' together is called **cohesion**. You can read more about this in Chapter 9.

Normally, a water molecule is attracted to other water molecules on all of its sides. But at the surface of a body of water, the top layer of water molecules do not have any water molecules above them, so the net attraction is downwards. This produces **surface tension**, making the water behave as though there is a thin 'skin' where it meets air. Surface tension allows small animals to walk on the water surface (Figure 7).

Water as a metabolite

So far, all of the properties of water described in this chapter are physical properties. This means that they involve whole water molecules, which do not break up. The water may change state, but it is still water.

However, water molecules can – and do – break up when they react with other substances. In these chemical reactions, the covalent bonds between the hydrogen and oxygen molecules are broken, and the atoms that made up the water molecule form new bonds with other atoms.

There are many metabolic reactions in living organisms that include water as one of the reactants. For example, digestion – reactions in which large molecules such as starch are broken down into smaller ones such as glucose – requires water as a reactant. These reactions are called **hydrolysis** reactions. Water is also produced when small molecules are joined together to produce larger ones, such as glucose molecules joining together to form starch. These are **condensation** reactions. There are several examples of hydrolysis and condensation reactions discussed later in this chapter and also in Chapter 2.

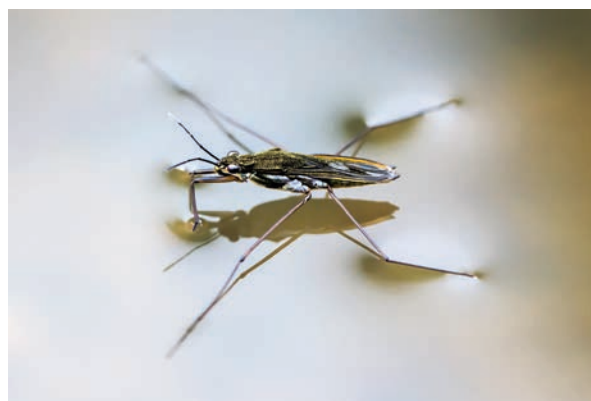


Figure 7 The water's surface tension makes it possible for this pond skater to walk on the pond.

KEY IDEAS

- ▶ Water molecules have dipoles – that is, they have a small negative charge on some parts of the molecule and a small positive charge on others.
- ▶ Water molecules are attracted to each other because of these charges. The weak attractions are called hydrogen bonds.
- ▶ Hydrogen bonding causes water to have a high heat capacity, buffering temperature changes.
- ▶ Water has a high latent heat of vaporisation, which provides a cooling effect when it evaporates.
- ▶ Water is an excellent solvent, in which metabolic reactions can take place and substances can be transported.
- ▶ Cohesion between water molecules enables tall columns of water to be supported in the xylem vessels of plants.
- ▶ Water is a metabolite in many metabolic reactions.

1.3 CARBOHYDRATES

All carbohydrates contain the elements carbon, hydrogen and oxygen. The hydrogen and oxygen atoms are generally in the ratio 2:1, giving carbohydrates the general formula CH_2O .

There are three basic types of carbohydrate molecule: **monosaccharides**, **disaccharides** and **polysaccharides**.

Monosaccharides

Monosaccharides are simple sugars – small molecules that dissolve in water and taste sweet.

These are the monomers from which all larger carbohydrates are made.

Glucose is a monosaccharide. Its structural formula is shown in Figure 8. Five of the carbon atoms (numbered 1–5 in the diagram) form a ring. Glucose can exist in two slightly different forms, depending on the orientation of the hydrogen and hydroxyl ($-\text{OH}$) groups on carbon atom 1. These are known as **α -glucose** (alpha glucose) and **β -glucose** (beta glucose). They are isomers of one another – that is, their molecules are made of the same numbers of carbon, hydrogen and oxygen atoms, but these atoms are arranged differently.

Fructose, another monosaccharide, is shown in Figure 9a. A third commonly occurring monosaccharide is **galactose**, which is shown in Figure 9b. All of these monosaccharides are isomers. They all have the formula $\text{C}_6\text{H}_{12}\text{O}_6$ but have slightly different properties from one another.

All monosaccharides are soluble in water.

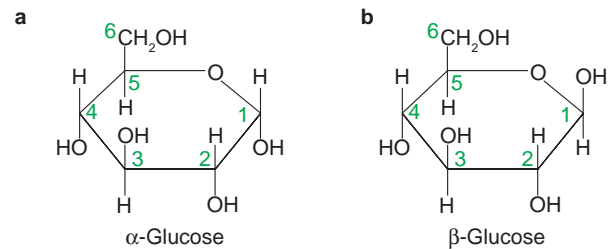


Figure 8 The molecular structures of α -glucose and β -glucose

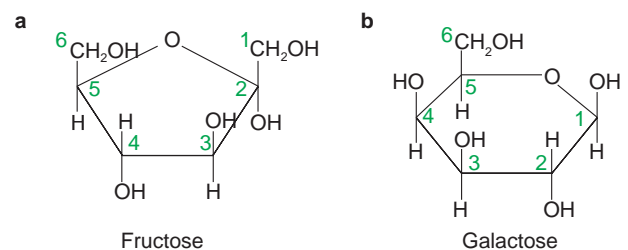


Figure 9 The molecular structures of fructose and galactose

ASSIGNMENT 1: USING CALIBRATION CURVES

(MS 1.3, MS 1.11, MS 3.1, MS 3.2, PS 3.1, PS 3.2, PS 3.3)

A calibration curve can be used to determine the concentration of an unknown solution. Calibration curves can be produced for any substance. In this assignment, we will look at the use of a calibration curve to determine the concentration of glucose in an unknown solution.

If a patient is suspected of having diabetes, a sample of blood is taken after the patient has fasted and the concentration of glucose in the blood is measured. In a person without diabetes, this would be in the range of 80–120 mg of glucose per 100 cm³ of blood. The blood glucose concentration in a diabetic person would be much higher.

Many people like to know how sweet the wine they are buying is, and a glucose calibration curve can be used to measure the amount of glucose in wine. Medium sweet wines have a sugar content between 18 g dm⁻³ and 45 g dm⁻³, while those with a concentration greater than 45 g dm⁻³ are classified as sweet.

Manufacturers of sugar-free and low sugar foods and drinks will regularly test their products to ensure they fall within a narrow range of acceptable values.

The glucose concentration of a solution can be measured using potassium permanganate solution, which is pink. Glucose easily donates electrons to permanganate ions in a redox reaction. The rate of this reaction depends on the concentration of glucose in solution. When potassium permanganate is reduced by accepting electrons, it becomes colourless, and by measuring the time taken for different known concentrations of glucose to turn the solution colourless you can generate the data to produce a graph of *glucose concentration* against *time*.

To produce a glucose calibration curve, you first need to make up a range of glucose solutions of known concentration. For example, to prepare a 1% solution, you dissolve 1 g of glucose in 100 cm³ of water. If you make up a range of different concentrations, and time how long

it takes for a standard volume of standard concentration potassium permanganate solution to be decolourised, you can then plot a calibration curve.

Glucose concentration/ mg 100 cm ⁻³	Time taken for potassium permanganate to go colourless/seconds
0	5
25	12
50	18
75	24
100	30
125	36
150	42
175	48
200	54

Table A1 A set of data for a calibration curve

After generating a calibration curve using a standard glucose solution and potassium permanganate, you can then determine the concentration of glucose in an unknown solution.

Questions

- A1.** Plot the data in Table A1 to produce a calibration curve.
- A2.** Use your curve to determine the glucose concentration of a sample which took:
 - a.** 32 seconds to go colourless
 - b.** 10 seconds to go colourless.
- A3.** How long would it take a glucose solution with a concentration of 160 mg 100 cm⁻³ to go colourless?
- A4.** Suggest the main source of error in determining the data shown in Table A1.
- A5.** Suggest why using a glucose calibration curve to estimate the concentration of sugar in wine might give an underestimate.

Disaccharides

Disaccharides have molecules made of two monosaccharides joined together. Like monosaccharides, all disaccharides taste sweet and are soluble in water.

Examples include **maltose** (made from two glucose molecules), **sucrose** (made from a glucose molecule and a fructose molecule) and **lactose** (made from a glucose molecule and a galactose molecule).

Maltose, or malt sugar, is found in germinating seeds. Sucrose is the sugar we use to add to drinks, or in cooking. Lactose is found in milk.

Figure 10 shows how two α -glucose molecules can join together to form maltose. Figure 11 shows how an α -glucose molecule and a fructose molecule join together to form sucrose.

Both of these reactions are **condensation reactions**, and you can see that a water molecule

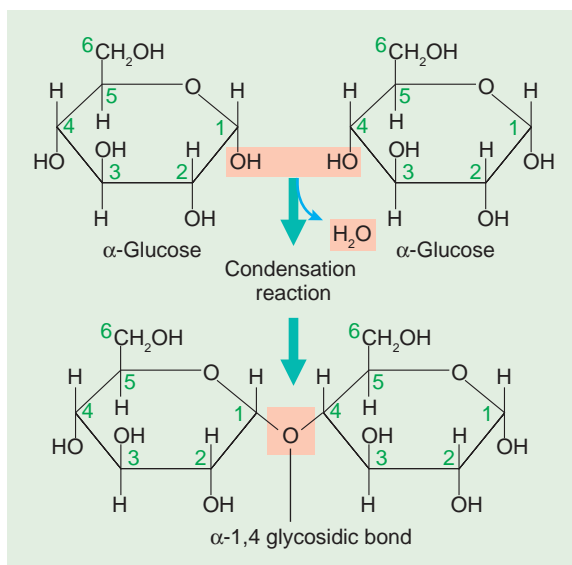


Figure 10 The formation of maltose

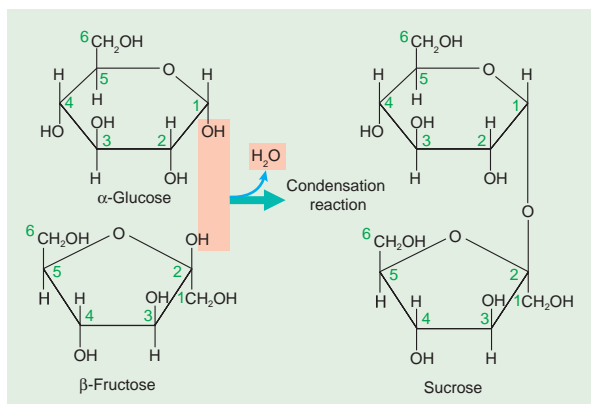


Figure 11 The formation of sucrose

is formed. The bond that is formed between the two sugar molecules is called a **glycosidic bond**.

Disaccharides can be broken down into their component monosaccharides. The reactions shown in Figures 10 and 11 happen in reverse. For this to happen, you can see that a water molecule must be used. For this reason, this kind of reaction is called a **hydrolysis** reaction. 'Hydro' means 'water' and 'lysis' means 'splitting apart'.

QUESTIONS

- A lactose molecule is made from a β -glucose molecule, joined by a glycosidic bond from its carbon-1 to the carbon-4 of a galactose molecule. Use this information, and Figure 9, to draw a diagram of a lactose molecule.
- Draw a diagram to show the hydrolysis of the disaccharide, maltose. Refer to the diagrams of condensation in Figures 10 and 11 to help you.

Polysaccharides

Polysaccharides are giant molecules made up from many monosaccharide molecules joined together by condensation reactions. Starch is a polysaccharide. Part of a starch molecule is shown in Figure 2 and Figure 12. Starch molecules are compact, coiled and branched, making them ideal 'energy' stores. Polysaccharides are insoluble, because their molecules are so large and cannot spread out in between water molecules as smaller molecules do.

We have seen that starch is a polymer – a molecule made up of repeating units, rather like links in a chain. The

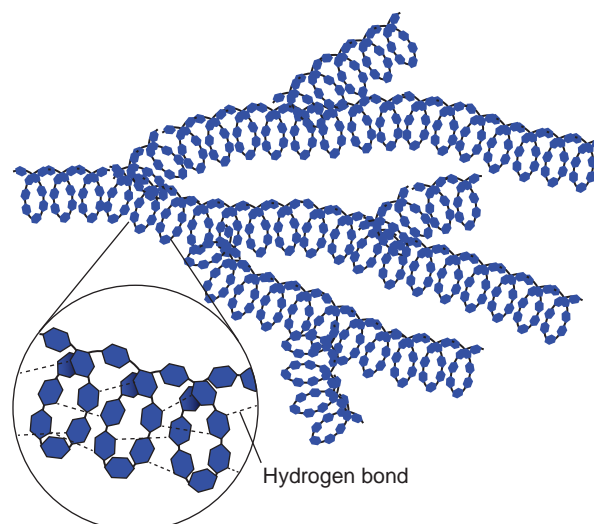


Figure 12 The structure of starch

individual units are called monomers, which in this case are α -glucose molecules. These bonds produce twisted chains of monomers. The coiled and branched chains of starch molecules give them a compact shape which, together with their insolubility in water, makes them ideal 'energy' storage compounds. When required, they can easily be hydrolysed to individual glucose molecules by enzymes that break the α -1,4 glycosidic bonds. The glucose can then be used in respiration to release energy.

Another advantage of using starch as a storage compound is that its large molecules cannot cross membranes. Because of its insolubility, starch does not have any osmotic effects on the cell, as glucose would (it does not affect water potentials). Storing glucose would tend to cause water to move into the cell by osmosis.

Starch is the major storage carbohydrate in plants and thus the major carbohydrate in our diet.

Animals use a very similar substance as their storage carbohydrate, called **glycogen**. (Starch is never found in animals, and glycogen is never found in plants.) Glycogen molecules are very similar to starch molecules – they are made up of α -glucose molecules joined by 1,4 and 1,6 glycosidic bonds – but they have more branches than starch.

Glucose monomers are joined in a very different way to form **cellulose** (Figure 13). Cellulose is the most common polysaccharide in the world, because it is the

major substance from which plant cell walls are made. Cellulose molecules are made up of long chains of β -glucose molecules, all joined through 1-4 glycosidic bonds. There are no branches. This produces long, straight molecules that can lie side by side. Cellulose molecules form huge numbers of hydrogen bonds with all the other cellulose molecules lying alongside them, forming groups of parallel molecules called microfibrils. Although an individual hydrogen bond is very weak, the large numbers of them mean that the microfibrils have great strength. They are also difficult to digest; relatively few organisms have enzymes (cellulase) that can break β -4 glycosidic bonds. So cellulose is an excellent structural substance, rather than an energy storage substance like starch and glycogen.

QUESTIONS

- Construct a table to compare the structure and properties of starch and cellulose. You could include the sugar units from which they are made, the type of glycosidic bonds that join them together, whether or not they branch, the overall shape of the molecules, whether or not the molecules form hydrogen bonds with one another, their solubility, the ease with which they can be digested, where they are found, and their functions.

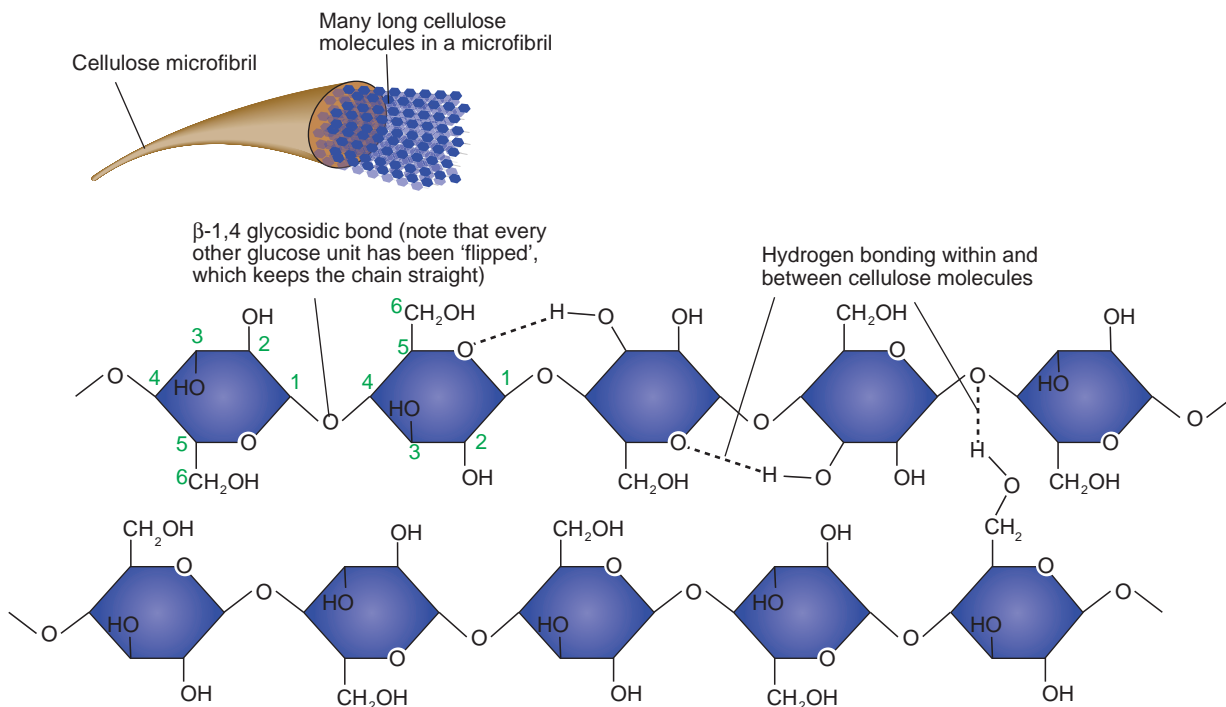


Figure 13 The structure of cellulose

ASSIGNMENT 2: UNDERSTANDING LACTOSE INTOLERANCE

(PS 1.1, PS 1.2)

Lactose is found in milk and dairy products. It is also present in many things you perhaps would not expect – such as crisps, biscuits, crackers, fruit bars, and even some coatings on tablets.

All humans produce lactase in their small intestines when they are babies; this is necessary to digest the lactose present in breast milk or formula milk. However, in most people, lactase production stops as they become adults. There are some exceptions – for example, most people of European descent continue to make lactase throughout their lives.

The lactase in the small intestine hydrolyses lactose into glucose and galactose, which can then be

absorbed into the bloodstream. In people who do not produce lactase, the undigested lactose passes into the colon where bacteria ferment it, producing fatty acids and gases such as carbon dioxide, hydrogen and methane. It is these acids and gases that cause the symptoms of lactose intolerance such as wind, pain and bloating. Some of the gases eventually dissolve in the blood and are breathed out from the lungs.

Questions

- A1.** Use your knowledge of osmosis to explain why water enters the small intestine of a person with lactose intolerance.
- A2.** What is the result of more water in the large intestine?

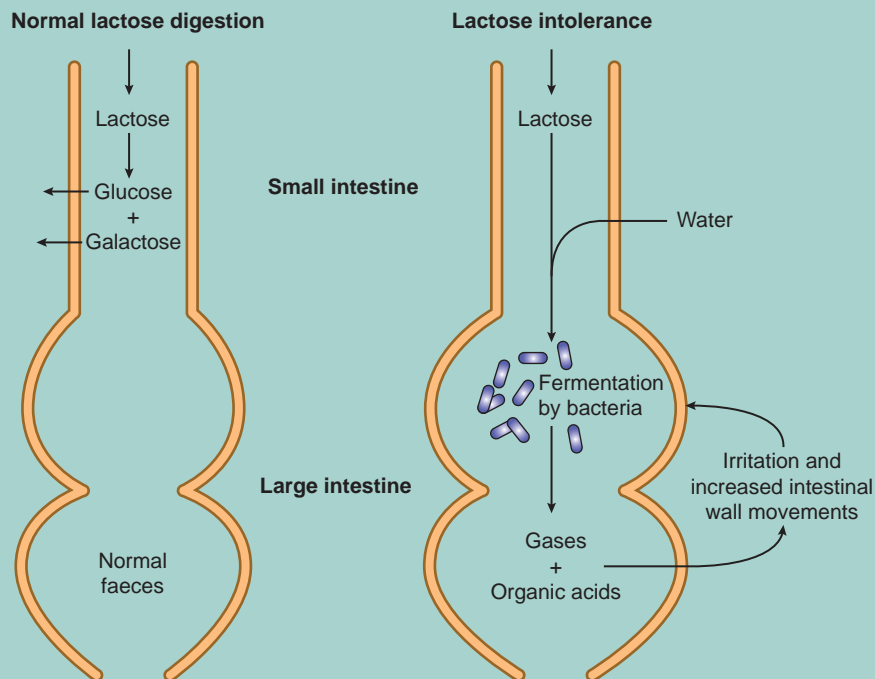


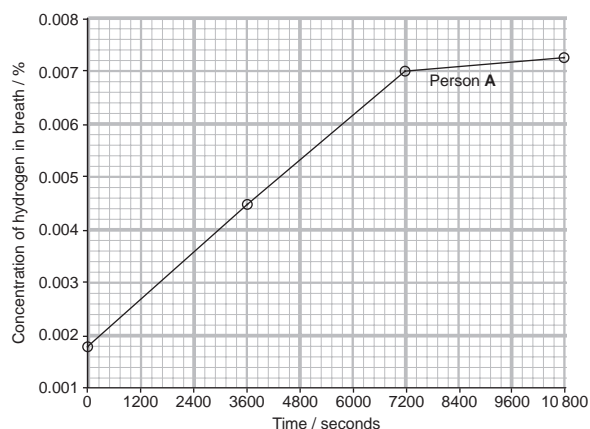
Figure A1 Lack of the enzyme lactase enables bacteria to ferment the sugar producing acids and gases.

Worked maths example: The hydrogen breath test**(MS 0.1, MS 1.1, MS 1.11, MS 2.2, MS 2.3, MS 3.3, MS 3.5, PS 3.1, PS 3.2, PS 3.3)**

Lactose intolerance can be diagnosed in a variety of ways, but the most reliable is the hydrogen breath test. The subject fasts for about 10 hours before having the test to determine how much hydrogen is present in their breath (in parts per million, ppm). This is their baseline. The subject then drinks 250 cm³ of a 10% lactose solution and is tested again each hour for three hours. Lactose intolerance is diagnosed if more than 20 ppm of hydrogen is detected, compared with baseline, after three hours.

Use this graph with results of a hydrogen breath test to work out:

- if Person A is lactose intolerant
- the rate of change in the concentration of hydrogen, in ppm min⁻¹, in the breath of Person A during the first two hours after they drank the lactose solution, to two significant figures
- the percentage error of part b. to three significant figures, assuming the breath testing equipment can read to 0.1 ppm.

**Part a**

We need to know the concentration of hydrogen after three hours.

Note that the tester plotted the graph using per cent (y -axis) and seconds (x -axis), rather than ppm and minutes, so we need to ensure we convert the units.

Firstly, we need to convert three hours into seconds; there are 60 minutes in an hour, and 60 seconds in a minute.

So, **3 hours** = 3×60 minutes = **180 minutes** = 180×60 seconds = **10 800 seconds**.

Next, we must convert per cent to ppm. It may help to think of 'per cent' as 'parts per 100':

$$1\% = \frac{1}{100}$$

$$1 \text{ ppm} = \frac{1}{1000000}$$

There are four zeros between these figures, that's a 10 000 (10^4) fold difference. So, to convert percentage to ppm you must multiply by 10 000, and to convert ppm to percentage you divide by 10 000.

$$1 \text{ ppm} = 0.0001\%$$

Therefore, $10 \text{ ppm} = 0.001\%$

The increments on the y -axis are in 0.001% intervals, so we know that $0.002\% = 20 \text{ ppm}$, $0.003\% = 30 \text{ ppm}$, and so on.

In the first three hours (between 0 and 10 800 seconds), the concentration of hydrogen rose from 18 ppm (baseline) to 72 ppm (0.0018% to 0.0072%), a difference of **54 ppm** (0.0054%).

The hydrogen concentration after three hours is more than *20 ppm greater* than baseline, so it's likely that Person A is lactose intolerant.

Part b

During the first two hours (120 minutes; 7200 seconds), the line is straight, so the relationship is **linear**. We can use the equation $y = mx + c$ to calculate the rate of change in y compared to x :

$$y = mx + c$$

The equation needs to be re-arranged to find the gradient of the line, m . The gradient is the rate of change (see Chapter 13):

$$m = \frac{y - c}{x}$$

Remember the answer needs to be in ppm, so first convert the y and c values:

$$0.007\% = 70 \text{ ppm}$$

$$0.0018\% = 18 \text{ ppm}$$

Then put the values into the equation:

$$\begin{aligned} \text{rate of change in the concentration of hydrogen} \\ &= \frac{(70 - 18)}{120} = 0.4333333 \text{ ppm min}^{-1} \end{aligned}$$

$$= \mathbf{0.43 \text{ ppm min}^{-1}} \text{ to two significant figures}$$

Part c

The equipment measures to 0.1 ppm, so the uncertainty in measurement is 0.05 ppm. In other words, the equipment is accurate to 0.1 ppm \pm 0.05 ppm.

$$\text{percentage error} = \frac{\text{uncertainty}}{\text{value being measured}} \times 100$$

So, in this case:

$$\text{percentage error} = \frac{0.05}{0.43} \times 100 = 11.6279\%$$

= **11.6%** to three significant figures

Biochemical test for starch

To test for starch, add **iodine in potassium iodide solution** to the substance. A blue-black colour indicates the presence of starch.

Biochemical tests for sugars

Tests for sugars can distinguish two groups of sugars: the **reducing sugars** and the **non-reducing sugars**.

A redox reaction is one in which one substance is oxidised while another is reduced:

- oxidation is the loss of electrons or hydrogen, or the gain of oxygen
- reduction involves the gain of electrons or hydrogen, or the loss of oxygen.

Reducing sugars

A reducing sugar readily loses electrons to another substance – the reducing sugar is therefore oxidised, while the other substance is reduced. The monosaccharides glucose and fructose are reducing sugars. The disaccharide maltose is also a reducing sugar.

To test for a reducing sugar, we add Benedict's solution to the substance, then heat the mixture to 80°C in a water bath. An orange-red precipitate indicates the presence of a reducing sugar.

Non-reducing sugars

Some sugars, though, are not readily oxidised and so do not reduce other substances. These are non-reducing sugars. The disaccharide sucrose is a non-reducing sugar.

A non-reducing sugar will not give an orange-red precipitate when heated with Benedict's solution. To test whether there is a non-reducing sugar present, we boil the substance with dilute acid, and then neutralise by adding hydrogen carbonate. Then we test again with Benedict's solution, and if a non-reducing sugar was present then a precipitate will now form.

Figure 14 shows what happens when the reducing sugar glucose loses electrons in a redox reaction. Carbon-1 forms an exposed –CHO group, which can then accept oxygen so that the glucose is oxidised (to gluconic acid).

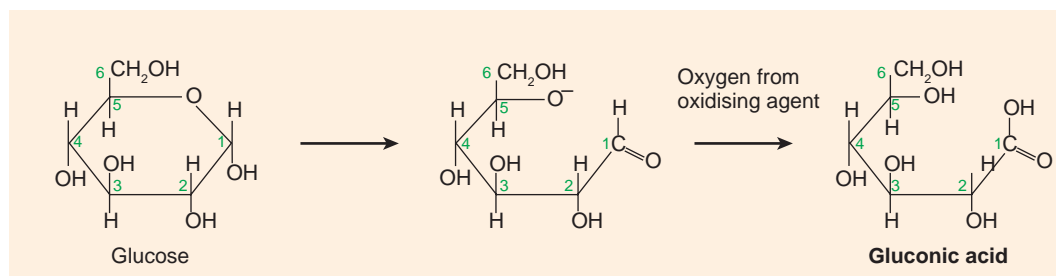


Figure 14 A redox reaction

QUESTIONS

7. Explain why Benedict's test works with maltose but not with sucrose.
8. Boiling a disaccharide with acid hydrolyses it into monosaccharides. Explain why Benedict's test gives a positive result after sucrose has been treated with acid.
9. For low concentrations of glucose, a positive Benedict's test ranges from green through yellow to orange and brick red. By comparing the colour of the sample solution with the colour of a standard solution, the glucose concentration of the sample can be estimated. Explain how you would prepare standard solutions for comparison.

ASSIGNMENT 3: IDENTIFYING CARBOHYDRATES FROM BIOCHEMICAL TESTS**(PS 1.2, PS 4.1)**

Biochemical tests are very important in both nutritional analysis and clinical diagnosis. Samples of blood, urine and other body fluids can be tested for a range of substances to help in the diagnosis of some disorders, such as diabetes. Different microorganisms are able to ferment different sugars and biochemical tests for carbohydrates can be used on bacterial cultures to determine which carbohydrates they are using and so aid in their identification. This is very important if the sample being tested comes from someone who is ill.

Nutritionists and food scientists use biochemical tests to analyse foodstuffs. It is important, for example, that someone who is lactose intolerant is informed when foodstuffs contain lactose.

Most monosaccharides and disaccharides can be fermented by yeast. The products of fermentation are alcohol and carbon dioxide; the formation of bubbles of carbon dioxide is used to confirm that fermentation is occurring. Although yeast has enzymes for the hydrolysis of most disaccharides, it does not have the enzyme necessary for hydrolysing lactose. So, both

lactose and galactose give negative results with this fermentation test. This test can be used to determine whether a substance contains lactose.

Six foodstuffs were tested in a laboratory to determine which carbohydrates were present, to see if they were suitable for lactose-intolerant persons or diabetics. However, the technician had not labelled the test substances, and was relying on the results of biochemical tests. The results of these tests are shown in the table.

Questions

- A1.** Describe how you would test for:
- starch
 - reducing sugar
 - non-reducing sugar.
- A2.**
- Which sample contained no carbohydrates? Explain your reasoning.
 - Which samples were likely to contain sucrose? Explain your reasoning.
 - Which sample(s) would be suitable for someone with lactose intolerance? Explain your reasoning.

Sample	Test for starch	Test for reducing sugar	Test for non-reducing sugar	Fermentation test
A	positive	positive	positive	positive
B	positive	negative	positive	positive
C	negative	negative	negative	negative
D	negative	positive	positive	positive
E	positive	negative	negative	negative
F	negative	negative	positive	positive

KEY IDEAS

- ▶ Carbohydrates are made up of carbon, hydrogen and oxygen.
- ▶ Monosaccharides are single-unit sugars. Glucose, fructose and galactose are monosaccharides. Monosaccharides, such as glucose, can exist in two different forms (for example, α -glucose and β -glucose). These are isomers.
- ▶ Disaccharides are made of two monosaccharide molecules joined by a glycosidic bond. Maltose, sucrose and lactose are disaccharides.
- ▶ The reaction in which two monosaccharides join together is an example of a condensation reaction. The reaction that breaks them apart is a hydrolysis reaction.
- ▶ Polysaccharides are polymers of monosaccharides. Starch, glycogen and cellulose are polysaccharides.
- ▶ Starch and glycogen have compact, coiled and branched molecules made of long chains of α -glucose monomers. They are used as energy stores in plants and animals respectively.
- ▶ Cellulose has straight, unbranched molecules made of long chains of β -glucose monomers. Cellulose molecules associate with each other to form microfibrils. They form cell walls around plant cells.
- ▶ Benedict's solution can be used to test for reducing sugars. Non-reducing sugars do not give a positive result with Benedict's solution.
- ▶ Iodine in potassium iodide solution is used to test for starch.

PRACTICE QUESTIONS

1. Figure Q1 shows a glucose molecule and a fructose molecule.

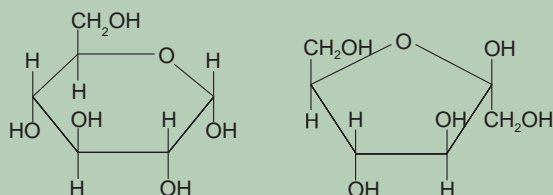


Figure Q1

- a. Is the glucose molecule in the alpha or beta form? Explain your answer.
 - b. Write the molecular formula for fructose.
 - c. Draw a diagram to show how glucose can join to fructose to form a sucrose molecule.
 - d. Name the type of reaction involved in the formation of sucrose.
2. A student was provided with a glucose solution of unknown concentration. He was also given some pure glucose. He had access to Benedict's solution.
- The colour produced when glucose is heated with Benedict's solution is dependent on the concentration of glucose.
- a. Describe how to test a solution for the presence of glucose, and explain what causes the colour change.
 - b. Describe how you could make up 1 dm³ of a 10% solution of glucose.
 - c. Describe how you could use this 10% solution to make a range of less concentrated solutions, each of known concentration.
 - d. Describe how you would use the Benedict's test to produce a set of colour standards using these solutions.
 - e. Explain how you could use these colour standards to find the concentration of the unknown glucose solution.
3. a. Describe the structure of a starch molecule.
b. Explain how this structure is related to the function of starch molecules in plant cells.
4. Plants lose water from their leaves because liquid water evaporates from the surfaces of the mesophyll cells in the leaf, and then diffuses as water vapour into the air, through the stomata.

(Continued)

Figure Q2 shows the water loss from plants kept for five days at either 22 °C or 28 °C.

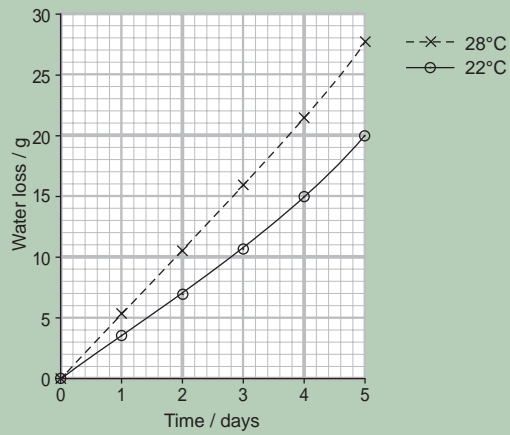


Figure Q2

a. Calculate the mean rates of water loss per day at:

i. 22 °C

ii. 28 °C

b. With reference to the properties of water, explain the reason for the difference in the rates that you have calculated.

c. Explain how the loss of water from the leaves can help to cool the plant.