

Unit 4: Cells and molecules

Unit 4 is externally assessed. The assessment consists of an external examination (50 %) and the preparation of a plan for an investigation (50 %). The nature of the investigation will be specified by OCR and presented to you six weeks before the external examination.

It is supported by two types of resource material:

- supporting activities which develop and provide opportunities to cover the practical aspects of the unit (enclosed in this pack); and
- a revision guide which covers and tests students' understanding of the unit content (available separately).

In Unit 4 students learn about:

- planning an investigation
- the structure of the cell
- some molecules found within the cell
- investigating cells and cell types, measuring them and counting cell numbers
- investigating the work of molecular biologists in cellular research.

The activities

The following supporting activities give students experience of and an opportunity to demonstrate their ability to:

- produce a slide of cellular tissue and describe the structures observed within the cell using a light microscope
- describe the additional structures observed using an electron microscope
- explain how and why scientists in biomedical research and pathology laboratories study cells, cell counts and manifestations of cell changes
- find out how cervical smear tests are analysed in a hospital laboratory for positive and negative results
- describe the process of osmosis and explain how cells maintain their correct water balance
- carry out tests for reducing sugar, non reducing sugar and starch
- carry out tests for lipids
- carry out tests for proteins
- use an eyepiece graticule to determine the relative sizes of cells or tissue structures
- use a stage micrometer to determine the actual dimensions of cells
- use a haemocytometer to determine the number of cells in a specific volume of liquid
- explain how and why the brewing industry and pathology laboratories use Coulter counters
- explain how and why scientists in biomedical research and pathology laboratories study cells, cell counts.

The activity summaries below provide more opportunities than students will probably have the time for and so suitable ones will need to be selected.

Note: The activity summaries refer to those aspects that link directly to the specification. All are set in context and have other parts to them.

The activities have been devised to develop knowledge, understanding and skills in a logical and progressive way. It's suggested that they are used in the given order.

A What's in a cell?

Students:

- obtain a mouth swab from the inside of their cheek
- produce a stained, bubble free, mounted slide of the cells from the swab
- set up a light microscope to view the cells
- record their observations
- compare what they saw with a light microscope with images obtained using electron microscopy
- find out about primary prevention using health checks and screening
- investigate some of the aspects of cervical screening, where microscopy is used to examine cells from the cervix.

B Water balance and osmosis

Students

- investigate the movement of water in and out of plant cells by osmosis
- investigate the effect of metabolic poison on the ability of the cell to absorb water by osmosis.

C Biological Molecules

Students

- use qualitative tests to find out which type of food is contained in a number of unlabelled food samples
- use a quantitative colorimetric technique to determine how much reducing sugar there is in a particular sample
- devise a qualitative method to determine the presence and distribution of food types in plant tissue samples
- devise a quantitative method to determine which food sample contains the most reducing sugars
- apply knowledge of the above tests to a glucose case study.

D Counting and measuring

Students

- learn how to use an eyepiece graticule
- learn how to use a stage micrometer
- calibrate an eyepiece graticule
- use a calibrated graticule to determine the actual size of the yeast cells
- use a haemocytometer to determine the number of yeast cells in a given volume of liquid
- use data to determine if three blood samples are from a patient suffering from anaemia.

OCR
AS GCE APPLIED SCIENCE
UNIT 4: CELLS AND MOLECULES

WATER BALANCE AND OSMOSIS

Specification links

This investigation relates to the following parts of Unit 4: Cells and Molecules

You need to:

- describe the process of osmosis and explain how cells maintain their correct water balance.

THE INVESTIGATION

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THE INVESTIGATION

WATER BALANCE AND OSMOSIS

Osmosis is the diffusion of water from a region of low solute concentration to a region of high solute concentration. It's a vital process.

But sometimes it can be harmful: if you put a saltwater fish into fresh water, it would die. It's because the liquid in its body is less salty than the water it swims in. So, the water inside the fish moves out through the fish's skin to the saltwater. Saltwater fish drink enormous amounts of water to keep from drying out. If it was put into freshwater, the osmosis process would reverse - but the fish, doing what it's designed to do, would keep drinking. It would die, quickly.

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The science at work

Causes of disease

Sickle cell anaemia is a genetic disease that causes some of the red blood cells to change from their normal round shape to a sickle cell shape. This extremely painful condition can block small blood vessels and prevent oxygen reaching the tissues of the body.

The change in shape of the red blood cells causes water to enter or leave the red blood cells. It does so by a process called osmosis. Using what scientists know about osmosis in red blood cells may help in the development of new drugs to treat sickle cell anaemia.

Sand dunes and salt marshes

Sand dunes and salt water marshes are important habitats around the shores of the UK. But they are not just homes for wildlife. They also act as a barrier against the sea by preventing, or at least reducing, coastal erosion. This is an increasing problem as sea levels begin to rise. This extract comes from <http://news.bbc.co.uk/1/hi/sci/tech/4651876.stm>:

'Global sea levels could rise by about 30 cm during this century if current trends continue, a study warns. Australian researchers found that sea levels rose by 19.5 cm between 1870 and 2004, with accelerated rates in the final 50 years of that period.'

The dunes and marshes are bound together by plants that grow in them. These plants must withstand extreme changes in their environment, from fresh rain water to salt water. By studying how plants cope with changes caused by osmosis, scientists are better able to help to protect these important habitats.

Your brief

In this investigation you will find out about osmosis and water potential. Then you will work in pairs to investigate:

- the movement of water in and out of plant cells by osmosis;
- how a metabolic poison affects the ability of the cell to absorb water by osmosis.

For all analytical work record your observations and interpretations, and answer the questions that come with each task.

Remember risk assessments must be performed and checked with your teacher for all practical work.

The investigation

Finding out about osmosis

Before you carry out the practical investigations, do some research to find out about osmosis. You can look in biology textbooks or on the internet. One very good site is the BBC's AS Guru Biology. Here are the specific pages for osmosis and water potential:

<http://www.bbc.co.uk/education/asguru/biology/01cellbiology/05pathways/10osmosis/index.shtml>

http://www.bbc.co.uk/education/asguru/biology/01cellbiology/05pathways/10osmosis/03osmosis_b/index.shtml

You may want to look at other topics available at this site.

Following your research, write definitions and/or explanations for:

- osmosis;
- water potential;
- solute and solvent;
- partially permeable membrane;
- isotonic, hypotonic and hypertonic solutions.

You might find it useful to draw some labelled diagrams to go along with these definitions and/or explanations.

Osmosis in plant cells: making standard solutions

Prepare a range of standard solution of sucrose using *Making up solutions*. You will use these in the investigation, so label them and store them somewhere safe.

Questions

- 1 What is meant by the term **standard solution**?
- 2 Explain how you made up your 1 mol dm^{-3} of sucrose solution.
- 3 Why is distilled water used rather than tap water to make the solutions?
- 4 Why are the solutions made up from the stock solution rather than by weighing out sucrose?

Osmosis in plant cells: water potential of potato tissue

You will be given a fresh potato tuber. Use *Determining the water potential of potato tissue: the weighing method*.

Questions

- 1 What value did you get for the water potential of potato cells? Compare it with the results of others in your group.
- 2 Explain why you should repeat the procedure and obtain mean values for your results.
- 3 Identify any sources of error and explain how you could modify the procedure to make it more reliable.
- 4 Suggest an alternative method that you could use for this procedure.

Osmosis in plant cells: water potential of onion epidermal cells

You will be given a fleshy scale leaf from a fresh onion. Use *Determining the water potential of onion epidermal cells*. The method involves using a microscope, so make sure you know how to set up and use one before starting.

Questions

- 1 What value did you get for the water potential of onion epidermal cells? Compare it the results of others in your group.
- 2 Make fully labelled drawings of your observations made with the microscope. How many types of plasmolysis did you observe?
- 3 Compare the values you obtained for the water potential of potato cells and onion epidermal cells. What do they tell you about the two different cells?
- 4 In the winter, grass often dies near roads that have been salted to remove ice. What causes this to happen?

Osmosis in plant cells: the effect of a poison

Toxic substances change cell structures. These changes result in different osmotic behaviour. Toxicity can be studied by comparing osmosis in untreated cells with that in cells that have been treated with the toxic substance. Use *The effect of lead nitrate on onion epidermal cells* to investigate the toxicity of lead nitrate.

Questions

- 1 Describe your observations and explain what they tell you about the effect of lead nitrate.
- 2 Suggest what structural damage lead nitrate does to onion epidermal cells.
- 3 A solution of lead nitrate in water contains lead ions, Pb^{2+} , and nitrate ions, NO_3^- . How could you decide whether toxicity is due to lead ions or nitrate ions? If you have time, design and carry out an investigation to find the answer to this question.

Your findings

Write a report of your investigation into osmosis in potato and onion epidermal tissue. Make sure you:

- accurately record all your analytical data and observations using sucrose and lead nitrate solutions;
- compare your results with other members of the class;
- produce a conclusion about the effect of lead nitrate on the cells.

Don't forget to ...

- set out your report clearly and logically - use headings and sub headings;
- begin by explaining why it is useful to understand osmosis and cells;
- keep a glossary of key terms in your own words;
- keep your notes, calculations and the record of your practical work to aid your revision.

Useful resources

<http://www.bbc.co.uk/education/asguru/biology/01cellbiology/05pathways/10osmosis/index.shtml>

http://www.bbc.co.uk/education/asguru/biology/01cellbiology/05pathways/10osmosis/03osmosis_b/index.shtml

<http://www.chemsoc.org/networks/learnnet/cfb/cells.htm>

<http://www.biotopics.co.uk/life/osmdia.html>

Making up solutions

Equipment and materials

- 50 g of sucrose
- access to a balance that can measure 0.0 to 10.0 g to an accuracy of 0.1 g
- 100 cm³ volumetric flask
- 2 x 50 cm³ burettes
- distilled water
- 5 x small conical flasks (or similar containers)

Procedure: stock solution

The stock solution should be 1 mol dm⁻³ of sucrose solution. That is 340 g of sucrose in 1 dm³ of solution (NOT in 1 dm³ of water).

1. Calculate the mass of sucrose needed to make 100 cm³ of 1 mol dm⁻³ sucrose stock solution.
2. Using a 100 cm³ volumetric flask, prepare 100 cm³ of 1 mol dm⁻³ sucrose stock solution. Pour the solution into a suitably labelled container.

Procedure: standard solutions by dilution

1. Set up two burettes, one containing distilled water and the other containing 1 mol dm⁻³ sucrose solution.
2. Label 6 conical flasks:
 - A 0.2 mol dm⁻³ sucrose solution
 - B 0.4 mol dm⁻³ sucrose solution
 - C 0.6 mol dm⁻³ sucrose solution
 - D 0.8 mol dm⁻³ sucrose solution
3. Using the burettes, add the following to the labelled flasks:

	Volume distilled water / cm ³	Volume 1.0 mol dm ⁻³ sucrose solution / cm ³
A	16	4
B	12	8
C	8	12
D	4	16

You should now have a range of different concentration sucrose solutions, from 1 mol dm⁻³ (the original stock solution) to 0.2 mol dm⁻³.

Determining the water potential of potato tissue: the weighing method

Background

When a plant cell is immersed in a solution of the same water potential, its mass and volume remain the same, because water enters and leaves it at the same rate.

If samples of a tissue are immersed in a range of solutions of different concentrations, the cells will gain water, and mass, in solutions of higher water potential and lose water, and mass, in solutions of lower water potential. The water potential of the tissue is equal to that of the solution in which it neither gains nor loses mass. The purpose of this practical is to estimate the water potential of potato tuber cells.

Equipment and materials

- sucrose solutions (0.2, 0.4, 0.6, 0.8, 1.0 mol dm⁻³), prepared previously
- 5 x boiling tubes with stoppers
- boiling tube rack
- wax pencil
- cork borer about 10 mm diameter
- sharp knife or scalpel [CARE]
- filter papers
- forceps
- access to a balance that can measure 0.0 to 10.0 g to an accuracy of 0.1 g
- distilled water
- large potato tuber

Procedure

1. Consult a risk assessment for this procedure and consider whether it needs to be adapted to suit the particular conditions under which you are working. Implement the control measures identified, modifying them as necessary, but first ask your teacher to check your risk assessment.
2. Label six boiling tubes: DW (distilled water), 0.2, 0.4, 0.6, 0.8, 1.0 mol dm⁻³. Place about half a tube-full of distilled water in the first tube and the appropriate sucrose solution in each of the other tubes. Firmly stopper each tube.
3. Label in pencil six pieces of filter paper 0, 0.2, 0.4, 0.6, 0.8, 1.0.
4. Using a cork borer and sharp knife, prepare six potato cylinders, each about 10 mm in diameter and 50 mm long. Place each potato cylinder on the appropriate piece of filter paper.
5. For each cylinder, record its mass on the filter paper and transfer it to one of the boiling tubes with forceps. Stopper the tube.
6. Re-weigh and record the mass of the filter paper on its own.
7. Once all the cylinders have been transferred to the boiling tube. Note the time.
8. Calculate the initial mass of each cylinder.
9. After at least 25 minutes remove the cylinders from the tubes in the same order that you inserted them.
10. Remove any surplus fluid quickly and gently with filter paper. Do not squeeze the cylinders. Then reweigh each cylinder and record its mass.

Calculations

- Work out the percentage change in mass of each cylinder using the formula:

$$\% \text{ change in mass} = \frac{\text{change in mass}}{\text{original mass}} \times 100$$

- Plot a graph of *percentage change in mass* against *molarity of sucrose solution*.
Your vertical axis needs to take into account the fact that you will have both positive and negative numbers. Join adjacent points with straight lines.
- Calculate the water potential of the potato cells:
 - find where your line crosses the place on the vertical axis corresponding to no change in mass;
 - read off the horizontal axis the molarity of sucrose at this point;
 - from the table below find the water potential of a sucrose solution of that molarity. This is the water potential of your sample of potato cells;
 - express your result in kilopascals (kPa).

Table to show the relationship between molarity and solute potential of sucrose solutions

Molarity / mol dm ⁻³	Solute potential / kPa
0.05	-130
0.1	-260
0.15	-410
0.2	-540
0.25	-680
0.3	-860
0.35	-970
0.4	-1120
0.45	-1280
0.5	-1450
0.55	-1620
0.6	-1800
0.65	-1980
0.70	-2180
0.75	-2370
0.8	-2580
0.85	-2790
0.9	-3000
0.95	-3250
1.0	-3500

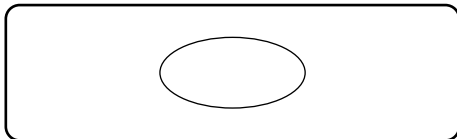
Determining the water potential of onion epidermal cells

What is plasmolysis?

Plasmolysis occurs when a plant cell membrane shrinks away from the cell wall. It happens when the cell loses water by osmosis from an area of high water potential in the cell to an area of low water potential outside the cell, through the cell membrane. The cell loses turgor and the plant becomes wilted.

Cell to cell communication can occur through small pores in the cell wall called plasmadesma. The cytoplasm of one cell is continuous with the cytoplasm of an adjacent cell through these small pores. As the cytoplasm shrinks away from the cell walls during plasmolysis, the last point of attachment is the plasmadesma. For this reason different types of plasmolysis may be visible as each cell becomes plasmolysed.

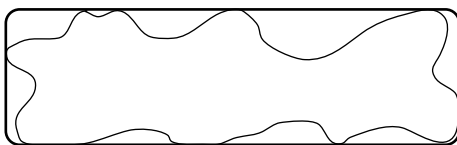
Different types of plasmolysis



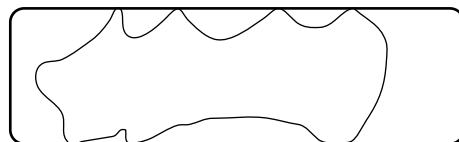
complete plasmolysis



cap plasmolysis



plasmodesmata plasmolysis



plasmodesmata and cap

Look for each of these four types when carrying out this procedure.

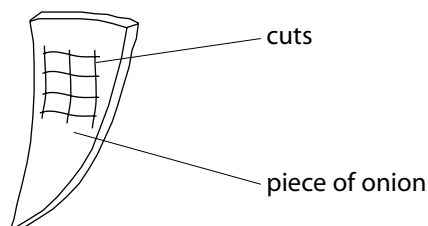
WATER BALANCE AND OSMOSIS

Equipment and materials

- 1.0, 0.8, 0.6, 0.4, 0.2, and 0 mol dm⁻³ solutions of sucrose
- one piece of fleshy scale leaf from a fresh onion submerged in distilled water
- dropper pipette and means of washing it
- sharp knife or scalpel [CARE]
- white tile or cutting board
- paper towels
- 5 x microscope slides with cover slips
- glass marker pen
- stopwatch or sight of a clock
- microscope with:
 - low power objective lens, e.g. x10
 - high power objective lens, e.g. x40

Procedure

1. Consult a risk assessment for this procedure and consider whether it needs to be adapted to suit the particular conditions under which you are working. Implement the control measures identified, modifying them as necessary, but first ask your teacher to check your risk assessment.
2. Remove the piece of the onion from the distilled water.
3. Make small cuts on the inner concave surface of the onion scale to give at least six squares of about 5 mm by 5 mm as shown.



4. Use the tip of the scalpel blade to carefully peel off the first 5 mm x 5 mm square of thin epidermal tissue and mount it on a microscope slide in 0.0 mol dm⁻³ (distilled water) sucrose solution. Cover with a glass cover slip.
5. Label the slide appropriately with a marker pen.
6. Repeat the procedure with the other five squares of epidermal tissue using a different concentration of sucrose solution with each slide.
7. Examine each slide under the microscope.
8. Produce a table to record your observations.
9. Estimate the solution of sucrose that has a concentration most similar to that of the epidermal cell contents. Then calculate the water potential.
10. Make a large labelled drawing to compare the cells in both the 1 mol dm⁻³ and 0.0 mol dm⁻³ solutions.

Report

The report should include:

- how you performed your observations;
- your drawings of the different cells;
- your conclusion;
- how you could make the procedure more accurate.

The effect of lead nitrate on osmosis in onion epidermal cells

Background

The procedure can be used to test any substance for toxic effects on cells by observing the effect of the toxin on cell structure and its subsequent effect on the ability of the cell to carry out osmosis.

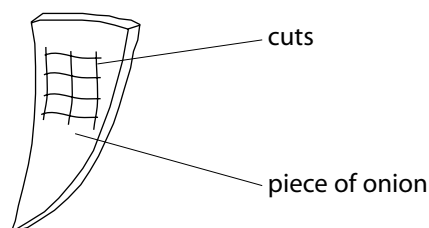
The effect of 1 mol dm^{-3} sucrose solution and 1 mol dm^{-3} sucrose solution containing lead nitrate is investigated on onion epidermal cells. The reversibility of the effects of the toxin is then investigated.

Equipment and materials

- 5 cm^3 of 1 mol dm^{-3} solution of sucrose
- 5 cm^3 of 1 mol dm^{-3} lead nitrate solution [TOXIC]
- test-tube with rack
- one piece of fleshy scale leaf from a fresh onion submerged in distilled water
- dropper pipette and means of washing it
- sharp knife or scalpel [CARE]
- white tile or cutting board
- paper towels
- 3 x microscope slides with cover slips
- stopwatch or sight of a clock
- microscope with:
 - low power objective lens, e.g. x10
 - high power objective lens, e.g. x40

Procedure

1. Consult a risk assessment for this procedure and consider whether it needs to be adapted to suit the particular conditions under which you are working. Implement the control measures identified, modifying them as necessary, but first ask your teacher to check your risk assessment.
2. Mix equal volumes of 5 cm^3 of 1 mol dm^{-3} sucrose solution and 5 cm^3 of 1 mol dm^{-3} lead nitrate solution in the test-tube.
3. Remove the piece of the onion from the distilled water.
4. Make small cuts on the inner concave surface of the onion scale to give at least two squares of about 5 mm by 5 mm as shown.



5. Use the tip of the scalpel blade to carefully peel off the first 5 mm x 5 mm square of thin epidermal tissue and mount it on a microscope slide in 1 mol dm^{-3} sucrose solution. Cover with a glass cover slip.
6. Label the slide appropriately with a marker pen.
7. Repeat the procedure with the other square of epidermal tissue but this time mount the tissue in the solution containing both sucrose and lead nitrate.

WATER BALANCE AND OSMOSIS

8. Examine each slide using your microscope.
9. Produce a table to record your observations.
10. Make a large labelled drawing to compare the cells in both the 1 mol dm^{-3} sucrose and 1 mol dm^{-3} sucrose with lead nitrate solutions.
12. Carefully remove the cover slips from both slides and irrigate the slide with distilled water. Try to wash away any traces of the previous solutions. But take care as you do not want to damage the tissue.
12. Re-mount both pieces of tissue using distilled water.
13. View both of the epidermal tissue samples using your microscope and record your observations in your table.
14. Make a large labelled drawing to compare both sets of tissue with your original observations.

TEACHING GUIDANCE

WATER BALANCE AND OSMOSIS

Outline

Students work in pairs to investigate the movement of water in and out of plant cells by osmosis. In addition they look at the effect of a metabolic poison on this process.

Finding out about osmosis

Before carrying out any practical work, students should use the Internet and textbooks to carry out some research into osmosis.

Osmosis in plant cells: making standard solutions

Students are provided with 10 g of sucrose from which they make up a molar solution. They then use this solution to make up a series of other concentrations of sucrose solution. Students should practice this skill prior to the investigation as any error in the concentrations of solution could affect their overall results for the rest of this investigation.

Osmosis in plant cells: water potential of potato tissue

Students use the sucrose solutions that they have made to discover the effect of each concentration of the size of a piece of potato tissue. They should discover that chips of potato tissue increase in size in distilled water but decrease in size in 1 mol dm^{-3} sucrose solution. By plotting a graph of the results for different concentrations of sucrose solution, they can then interpolate from their graph what the concentration of the potato cell sap actually is. Results from different groups could then be used to determine the mean result for the whole class.

In practice, none of the experimental solutions is likely to have exactly the same water potential as the cells, but the solution in which there would have been no gain or loss in mass can be estimated from a graph.

In principle this technique can be applied to any plant tissue.

Osmosis in plant cells: water potential of onion epidermal cells

Students could work in pairs for the whole of this activity, particularly if there is a shortage of microscopes. The previous activity is now extended to include onion epidermal cells. This activity required much more skill as the onion epidermal cells are examined for plasmolysis using a light microscope. The student uses different strength solutions to determine the point of insipient plasmolysis for the onion cells. The results are then compared with those for potato cells. Students could also compare the strength and weaknesses of each procedure.

Osmosis in plant cells: the effect of a poison

Students now compare the effect of a 1 mol dm^{-3} sucrose solution on onion epidermal cells with a 1 mol dm^{-3} sucrose solution containing lead nitrate. Students examine the effect of lead nitrate on the structure of the cell and discover that one of the effects of lead nitrate is the destruction of the cell, in particular the cell membrane. When students place cells from the two solutions into distilled water they should notice that whilst turgor is regained in those cells placed only in sucrose solution, turgor is not regained in those cells placed in sucrose and lead nitrate solution. This effect is due to the destruction of the cell membrane through which osmosis normally takes place.

Health and safety information

Lead nitrate is a TOXIC substance. It should be handled with care and disposed of after the investigation using suitable precautions and following the proper procedure.

TECHNICAL INFORMATION

WATER BALANCE AND OSMOSIS

Equipment and materials

Making up solutions

- 50 g of sucrose
- access to a balance that can measure 0.0 to 10.0 g to an accuracy of 0.1 g
- 100 cm³ volumetric flask
- 2 x 50 cm³ burettes
- distilled water
- 5 x small conical flasks (or similar containers)

Determining the water potential of potato tissue: the weighing method

- sucrose solutions (0.2, 0.4, 0.6, 0.8, 1.0 mol dm⁻³), prepared previously
- 5 x boiling tubes with stoppers
- boiling tube rack
- wax pencil
- cork borer about 10 mm diameter
- sharp knife or scalpel [CARE]
- filter papers
- forceps
- access to a balance that can measure 0.0 to 10.0 g to an accuracy of 0.1 g
- distilled water
- large potato tuber

Determining the water potential of onion epidermal cells

- 1.0, 0.8, 0.6, 0.4, 0.2 and 0 mol dm⁻³ solutions of sucrose
- one piece of fleshy scale leaf from a fresh onion

submerged in distilled water

- dropper pipette and means of washing it
- sharp knife or scalpel [CARE]
- white tile or cutting board
- paper towels
- 5 x microscope slides with cover slips
- glass marker pen
- stopwatch or sight of a clock
- microscope with:
 - low power objective lens, e.g. x10
 - high power objective lens, e.g. x40

The effect of lead nitrate on onion epidermal cells

- 5 cm³ of 1 mol dm⁻³ solution of sucrose
- 5 cm³ of 1 mol dm⁻³ lead nitrate solution [TOXIC]
- test-tube with rack
- one piece of fleshy scale leaf from a fresh onion submerged in distilled water
- dropper pipette and means of washing it
- sharp knife or scalpel [CARE]
- white tile or cutting board
- paper towels
- 3 x microscope slides with cover slips
- stopwatch or sight of a clock
- microscope with:
 - low power objective lens, e.g. x10
 - high power objective lens, e.g. x40

