SLE111 Practical 2: Behaviour of the cell membrane

Learning outcome of the practical activity

Students who successfully complete this practical will be able to demonstrate that they can: Focus a light microscope at X40, X100 and X400 magnification.

Explain how plant and animal cells behave differently in hypo-, iso-& hyper-tonic solutions and describe the structural features responsible for those differences

Draw conclusions about membrane permeability to various solutes based on experimental observations.

K – Knowledge and S - Skills in C - Context for 'real-world' relevance

K - The knowledge that you will be developing during this practical relates to the microanatomy of Eukaryotic cells, the structure of the cell membrane, how and why that structure allows certain solutes to cross the membrane while others cannot.

S - The skills that you will be developing (reinforcing) are basic light microscopy and simple sample preparation for light microscopy.

C – This type of knowledge and these skills are needed for research and diagnostic laboratories for optimal handling of biological specimens.

Associated assessment

The pre-practical quiz on Moodle will account for 1 of the 5 marks.

The worksheet will account for 4 of the 5 marks.

Complete all drawings, tables and questions on your own worksheet and submit at completion of the class.

WARNING: 0.05M NaOH (sodium hydroxide), corrosive liquid—wear safety clothing including safety glasses.
RISK: Low.

Relationship to ULOs and GLOs and CLO's

This task is intentionally aligned to the development of:

ULO 1 & 4: ULO 1 & 4: because it allows you to practically test the theoretical principles of osmosis and membrane permeability discussed in classes and record and report on those observations

GLO 1, 6: because it consolidates foundational biological knowledge covered in classes (GLO1). This is a self-paced exercise and you are responsible for ensuring you finish all the activities in the allotted time, you are also required to work safely and responsibly in a laboratory environment (GLO6).

Pre-lab activities

- Revise the lecture on biological membranes
- Read/revise Campbell Chapter 7 and Chapter 3 Concept 3.3 (pH).
- Complete the pre prac quiz on the SLE111 page on Moodle. This quiz will test your knowledge of practical 2 and ensure you have prepared yourself for the class. You have 2 attempts to answer the quiz. After your first attempt you will be informed which questions you answered incorrectly. Your highest score for the quiz will stand. Your result from this quiz will contribute 1% of your overall SLE111 mark.

Practical 2: The behaviour of the cell membrane

Introduction

The cell membrane (or plasma membrane) forms a fine boundary between a living cell and its surroundings and controls the movement of molecules into and out of the cell. The cell membrane is selectively permeable allowing some substances to cross more easily than others while blocking the passage of some substances altogether. The cell membrane takes up substances the cell needs and disposes of the cell's wastes.

For a structure that separates life from non-life, the cell membrane is surprisingly thin and not visible under the light microscope. However, it shows up clearly in an electron micrograph where the membrane can be seen to have three bands: two dark bands with a lighter band in the middle (see figure 6.6, p. 101 in the textbook).

Part 1: Osmotic effects on plant and animal cells

Procedure

You can work in groups of 3 or 4 for this section with each group member using a different salt solution

Osmosis in plant cells

Take three clean slides and label them A, B and C.

Remove thin strips of red onion tissue with fine forceps and place one piece on each slide. Then treat each slide as follows:

Slide A: add two drops of distilled water.

Slide B: add two drops of dilute salt solution (0.17 M sodium chloride).

Slide C: add two drops of strong salt solution (2 M sodium chloride).

Place a coverslip on each slide and observe the cells over a period of time.

The red onion cells have large vacuoles that normally take up most of the interior of the cell.

Exercise 1 to be completed on worksheet

1. **Draw and compare the cells from each treatment.**

   Briefly describe what has occurred to the red onion cells in the three solutions.

Carefully remove the coverslip from the red onion cells in slide C (strong salt solution). Rinse the tissue and place in two drops of distilled water and add a clean coverslip. Note any change in the appearance of the cells.

2. **To what extent do the red onion cells recover after washing in distilled water?**

Osmosis in animal cells

Observe the three slides/photomicrographs labelled A, B and C, demonstrating the reaction of horse blood cells when placed in three different solutions:

A drop of distilled water.

A drop of dilute salt solution (a solution that is isotonic to blood).

A drop of strong salt solution.

3. **Compare the cells on each slide/photomicrograph. Can you suggest which solution (A, B, or C) each blood cell preparation has been exposed to? What has happened to the cells in each solution? Explain the water movement in each case. Remember osmosis is the diffusion of water across semi-permeable membranes, and the direction of net movement is from dilute solution to a more concentrated solution.**

4. **How do plant and animal cells differ in behaviour when placed in distilled water? What structural difference between plant and animals cells accounts for this observation?**

Part 2: Examination of the behaviour of the cell membrane

Background information

In this section of the practical we will use dialysis tubing to represent the cell membrane or, at least, the phospholipid bilayer component of the cell membrane to determine which substances can cross easily. Dialysis tubing acts as a semipermeable membrane. It is made from cellulose acetate and behaves like a molecular sieve separating particles by size. Smaller particles can pass through easily whilst larger substances are too big to pass through the pores in the tubing. Dialysis tubing does not tend to inhibit or allow passage based on any chemical properties. We are going to examine whether the following substances can pass through

Starch

Starch is a carbohydrate polymer made by joining together hundreds of glucose monomers. It is a storage form of carbohydrate in plants.

Iodine

Exists as a solid as I_2. When it dissolved in water it forms I^- or I_3^-. When starch and iodine react together they form a complex that is blue in colour.

NaOH

When NaOH is dissolved in water it separates into Na^+ and OH^-. The OH^- makes the solution more basic (alkaline)

Neutral red

Is a largish molecule (but it is not a polymer) Neutral Red is a dye that changes colour according to the pH of its environment. The colour changes are due to changes in its structure. The following diagram summarises the changes but does not represent what the molecules actually look like.

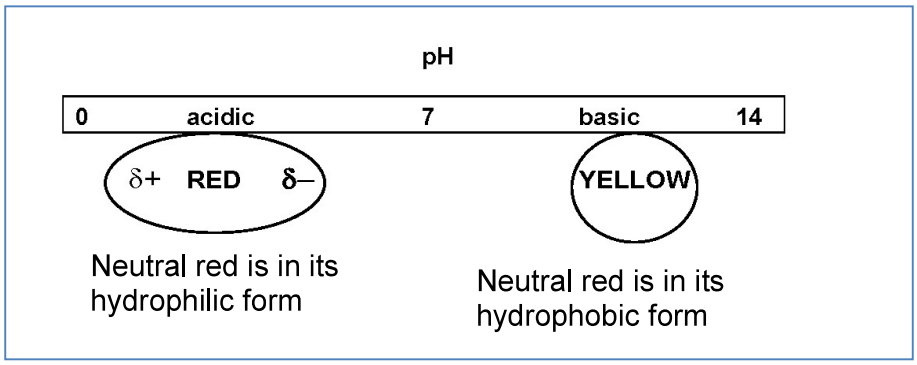


Figure 1: The colour and hydrophobicity of Neutral Red at differing pH levels.

These changes in the colour of Neutral Red (NR) at different pH levels enable it to be used as an indicator of pH (similar to Litmus paper). That is we can tell if a solution is acidic if we add NR and the NR in solution becomes red whereas if the NR turns orange/yellow we know that solution is basic. In addition to changing colour when encountering different pH solutions NR changes also occur to NR chemical structure. As figure 1 indicates when in an acidic solution NR is hydrophilic and when placed in a basic solution NR is hydrophobic

Procedure

You may work in pair for this exercise.

Materials

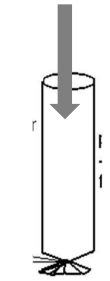
- 4 pieces of dialysis tubing
- Marker
- 5 beakers
- Iodine solution
- 0.5M NaOH (**WARNING**: 0.05M NaOH (sodium hydroxide), corrosive liquid—wear safety clothing including safety glasses. **RISK**: Low.)
- 0.5% Starch solution
- 0.005M Neutral Red
- 0.05M Sodium Hydroxide
- Pipette

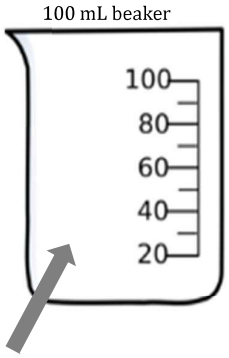
Procedure 1 - Iodine & Starch

1.Half fill a 250 mL beaker with tap water and dip a piece of dialysis tubing (pre-cut to size), into the water to soften. You may need to dip the tubing multiple times but DO NOT leave it to soak. Once the tubing is pliable tie a double knot in one end. Be sure to pull it tight. It is essential the tubing is tightly sealed to prevent leaks. Leave it in the water until needed (but try not to leave it soaking for a long period of time). Set-up the dialysis tubing and a 100 mL beaker as per diagram A. Then prepare a second piece of dialysis tubing (as above) and new 100 mL and set up experiment B

A

5 mL water + 1 ml iodine solution

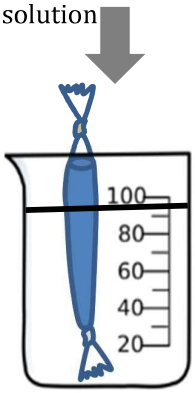




60 mL 0.5 % starch solution

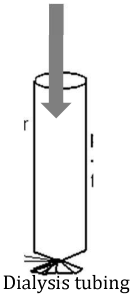
2. Observe the colour of solutions and record it on your worksheet.

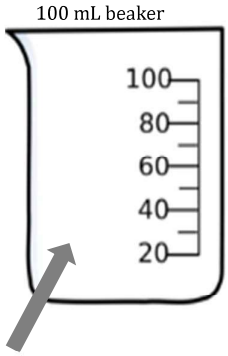
3. Tie the opposite end of the dialysis bag securely in a double knot, **RINSE the outside of the bag my dipping it in the beaker of WATER** and place the bag into the beaker of starch solution. Keep the top knot above the starch solution



B

5 mL 0.5 % starch solution

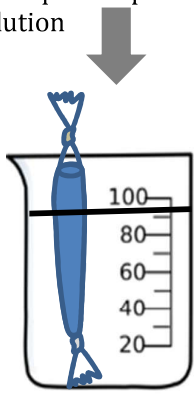




50 mL water + 10 ml % iodine solution

2. Observe the colour of solutions and record it on your worksheet.

3. Tie the opposite end of the dialysis bag securely in a double knot, **RINSE the outside of the bag my dipping it in the beaker of WATER** and place the bag into the beaker of starch solution. Keep the top knot above the starch solution



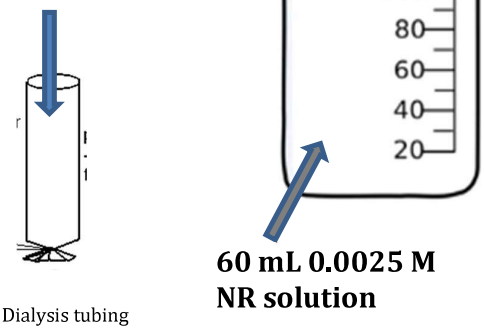
4. Observe the colour of solutions after 10 minutes and record the observation in your worksheet

Procedure 2 - Neutral Red & Sodium hydroxide

1. Half fill a 250 mL beaker with tap water and dip a piece of dialysis tubing (pre-cut to size), into the water to soften. You may need to dip the tubing multiple times but DO NOT leave it to soak. Once the tubing is pliable tie a double knot in one end. Be sure to pull it tight. It is essential the tubing is tightly sealed to prevent leaks. Leave it in the water until needed (but try not to leave it soaking for a long period of time). Set-up the dialysis tubing and a 100 mL beaker as per diagram A. Then prepare a second piece of dialysis tubing (as above) and new 100 mL and set up experiment B

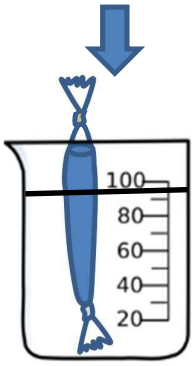
A

5 mL 0.5 M NaOH solution



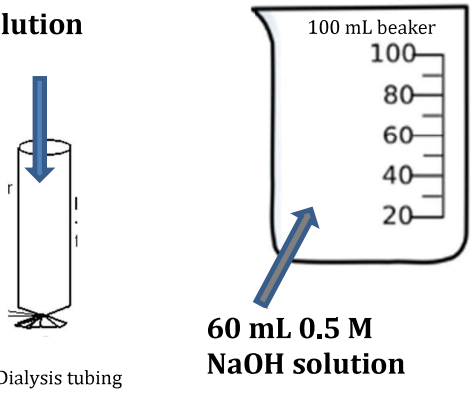
2. Observe the colour of solutions and record it on your worksheet.

3. Tie the opposite end of the dialysis bag securely in a double not and place the bag into the beaker of starch solution. Keep the top knot above the starch solution



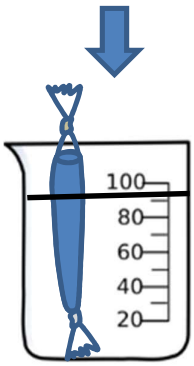
B

5 mL 0.0025 M NR solution



2. Observe the colour of solutions and record it on your worksheet.

3. Tie the opposite end of the dialysis bag securely in a double not and place the bag into the beaker of starch solution. Keep the top knot above the starch solution



4. Observe the colour of solutions after 10 minutes and record the observation in your worksheet. Answer any related questions on your worksheet.

When you have finished making your observations make sure you clean your workplace and put everything away as directed.