Core practical 6: Investigate plant water relations

Objective

- Know how to carry out an investigation to determine the osmotic potential and therefore water potential of plant epidermal cells

Safety

- Take care with glassware, mounting needles and cutting equipment.

Specification links

- Practical techniques 3, 4, 8
- CPAC 1a, 2a, 2b, 4a, 4b

Procedure

Before you start this practical make sure that you understand the terms 'turgid' and 'plasmolysed'. Do some research to see what cells in these conditions might look like under a microscope.

When plant cells are placed in a range of solutions, the concentration that has the same osmotic potential as the cell sap is just concentrated to cause sufficient water loss so that the plasma membrane begins to separate from the cell wall (incipient plasmolysis). It is difficult to measure this because cells within plant tissue plasmolyse at different rates. Instead, we use the osmotic potential of the solution that causes visible plasmolysis in 50% of the cells.

1. Take six thin sections of the available plant tissue. 1 cm^2 is a suitable size to use. The tissue must only be one cell thick to allow the cells to be examined clearly.
2. Label six watch glasses with the different concentrations of salt solution, including 0.0 mol dm^{-3}. Transfer some of each salt solution into the appropriate watch glass with a pipette. Now place one of the five tissue sections into each of the watch glasses and leave for 20 minutes.
3. Remove each tissue section with forceps and place on a slide clearly labelled with the appropriate concentration. Put a drop of the corresponding solution on the slide and float the tissue on to it.
4. Cover each section with a coverslip and observe under the microscope.
5. Observe 25 cells and record how many of them show plasmolysis. Record your findings in a suitable table. Include a column to record the percentage of cells showing plasmolysis.

Notes on procedure

- 1 cm^2 is a suitable size of tissue to use, as it is unlikely to fold. It is important that the tissue has only a single cell layer to allow the cells to be examined clearly under the microscope. Plasmolysed cells should be visible at higher concentrations; if not, slides can be irrigated with iodine solution.
- Pre-prepared salt solutions may be used to save time.
- Clear labelling of the watch glasses and microscope slides with the concentration being used prevents confusion. Floating the tissue on a drop of the solution prevents the tissue folding.
- 25 cells is a suitable number to count and allows students to estimate the osmotic potential from their graphs. However, each cell accounts for 4% of the sample in the final calculations, reducing accuracy. Students should comment on this in their evaluation: a larger sample size would be better. In addition, interpretation of cells as plasmolysed or not is somewhat subjective and there is no clear cut-off. This may reduce accuracy.
- Tables used to display results should have the independent variable (concentration/mol dm^{-3}) in the first column and the number of cells showing plasmolysis in the columns to the right. The percentage of the cells that show plasmolysis should be in the far right column for each concentration. This is a good opportunity to remind students of the correct way to tabulate data.
- Students should be warned of the dangers of damaging microscopes.

Answers to questions

1. The solution closest to 50% plasmolysis will vary according to the tissue used.
2. This will depend on students' results; they should read off the concentration that would give 50% plasmolysis.
3. Water potential is described by the equation $\psi = P + \pi$. At the point of incipient plasmolysis the cell membrane is just beginning to peel away and exerts no pressure on the cell wall, so $P = 0$. Therefore ψ must equal π. There is no *net* movement of water by osmosis at this point.
4. This will depend on students' results. Students will need to estimate between points on the table.

Sample data

Salt concentration/mol dm^{-3}	Number of plasmolysed cells in a sample of 25 cells				Mean percentage plasmolysis (%)
	Repeat 1	Repeat 2	Repeat 3	Mean	
0.0	0	1	0	0	1
0.1	2	3	1	2	8
0.3	8	9	7	8	32
0.5	13	14	14	14	55
0.7	18	17	16	17	68
0.9	22	23	21	22	88

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All the maths you need

- Use ratios, fractions and percentages.
- Find arithmetic means.
- Substitute numerical values into algebraic equations using appropriate units for physical quantities.
- Solve algebraic equations.
- Translate information between graphical, numerical and algebraic forms.
- Plot two variables from experimental or other data.

Equipment

- plant tissue with a single cell layer
- five salt solutions of a suitable range for the tissue to be used, e.g. 0.1 mol dm^{-3}, 0.3 mol dm^{-3}, 0.5 mol dm^{-3}, 0.7 mol dm^{-3}, 0.9 mol dm^{-3} for onion
- distilled water
- six watch glasses
- measuring cylinders
- five 5 cm^3 syringes
- pipettes
- filter paper
- scalpel
- labels or pen for labelling
- microscope, slides and coverslips
- forceps
- iodine solution

Diagram

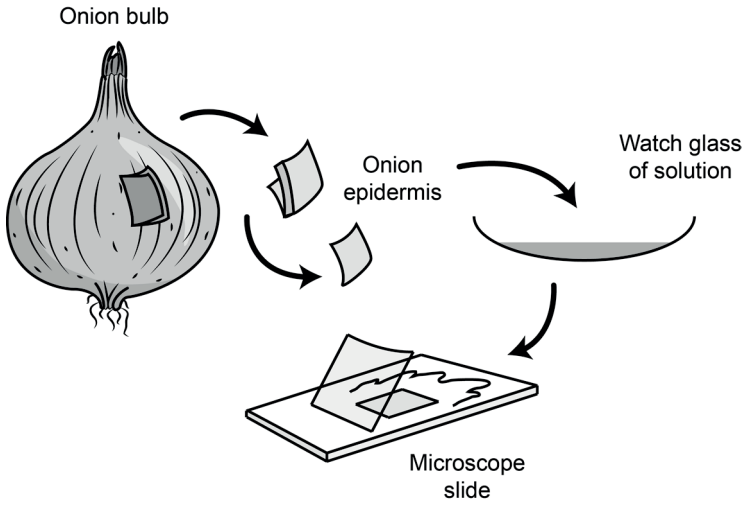

fig A Using the onion epidermis.

Procedure

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Analysis of results

1. Work out the percentage of cells showing plasmolysis at each concentration. If possible, collect results from other students as replicates at each concentration and then calculate a mean.
2. Draw a graph of the percentage of cells plasmolysed against the concentration of the solution.
3. Use your graph to estimate the concentration at which exactly 50% of the cells would be plasmolysed. We can take this point, where half the cells show plasmolysis, to be the concentration at which incipient plasmolysis occurs. At this point, the water potential of the experimental solution is equal to the osmotic potential of the cells.
4. Write a short evaluation of the investigation methods. Were there any areas of inaccuracy or possible errors?

Learning tips

- Only at incipient plasmolysis are water potential and osmotic potential the same.
- Make sure that you understand how other methods can be used to determine water potential, such as changes in mass, density or volume of plant tissue. Note that these techniques allow for estimation of water potential of tissues not cells.

Questions

1. Which of your initial solutions gave results closest to 50% plasmolysis?
2. From your graph, what concentration did you estimate would have exactly the same concentration as the osmotic potential of the cell?
3. The osmotic potential of the cells is equal to the water potential only at the point of incipient plasmolysis. Use the equation $\psi = P + \pi$ to explain why water potential and osmotic potential are equal at this point.
4. Osmotic potential is actually measured in kPa. Use **Table 1** to estimate the approximate osmotic potential of the cells studied.

Table 1 Relationship between molarity and osmotic potential of salt solutions

Salt concentration/mol dm^{-3}	Osmotic potential/kPa
0.1	−270
0.2	−550
0.3	−830
0.4	−1130
0.5	−1460
0.6	−1820
0.7	−2200
0.8	−2610
0.9	−3050
1.0	−3560

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Equipment per student/group	**Notes on equipment**
plant tissue such as onion epidermis or leaves with a single cell layer, e.g. *Elodea* or moss	It is essential that the tissue is only one cell thick so that plasmolysis can be seen clearly. Any tissue of a single cell layer is suitable. Red onion works well, as do leaf epidermal peels.
five salt solutions of a suitable range for the tissue to be used, e.g. 0.1 mol dm^{-3}, 0.3 mol dm^{-3}, 0.5 mol dm^{-3}, 0.7 mol dm^{-3}, 0.9 mol dm^{-3} for onion	Five salt solutions from 0.1–1.0 mol dm^{-3}. It is worthwhile testing the tissue to be used in advance with the lower and upper end of the range to check that full turgor and almost complete plasmolysis are achieved.
distilled water	
watch glasses	six per group
measuring cylinders	Only required if students are to make up their own solutions
5 cm^3 syringes	Only required if students are to make up their own solutions
dropping pipettes	
filter paper	Size is not important, as they are used for soaking up solutions.
scalpel	One per group
labels or pen for labelling	Students need to be able to label the watch glasses. A grease pencil or marker pen is suitable.
microscope, slides and coverslips	One microscope with five slides and coverslips per group
forceps	One pair per group
iodine solution	For use if plasmolysed cells are difficult to distinguish
Notes	