Determination of the solute potential by measuring the degree of incipient plasmolysis

Specification reference: **1.3**

Cell membranes and transport

Introduction

When the water potential of plant tissue and its surroundings are equal, there is no net movement of water in or out of the cell. The cell is neither turgid nor plasmolysed and is at incipient plasmolysis. The cell membrane is withdrawn from the cell wall in places and the cell contents exert no pressure on the cell wall.

In principle, when examined under the microscope, every cell in the tissue would be expected to show its cytoplasm withdrawn in places from the cell wall. Cells, however, show variation in their behaviour. Incipient plasmolysis is the point where plasmolysis just takes place and for practical purposes this is taken to be when half of the cells are plasmolysed and half are not plasmolysed.

The equation describing water potential is $\psi_{cell} = \psi_S + \psi_P$
At incipient plasmolysis, the pressure potential, $\psi_P = 0$
$\therefore \ \psi_{cell} = \psi_S + 0$
$\therefore \ \psi_{cell} = \psi_S$
Thus the water potential of the cells is equal to their solute potential.

The water potential of the cells is also equal to the solute potential of the bathing solution, which is known. Thus, ψ_S of the bathing solution $= \psi_{cell} = \psi_S$.

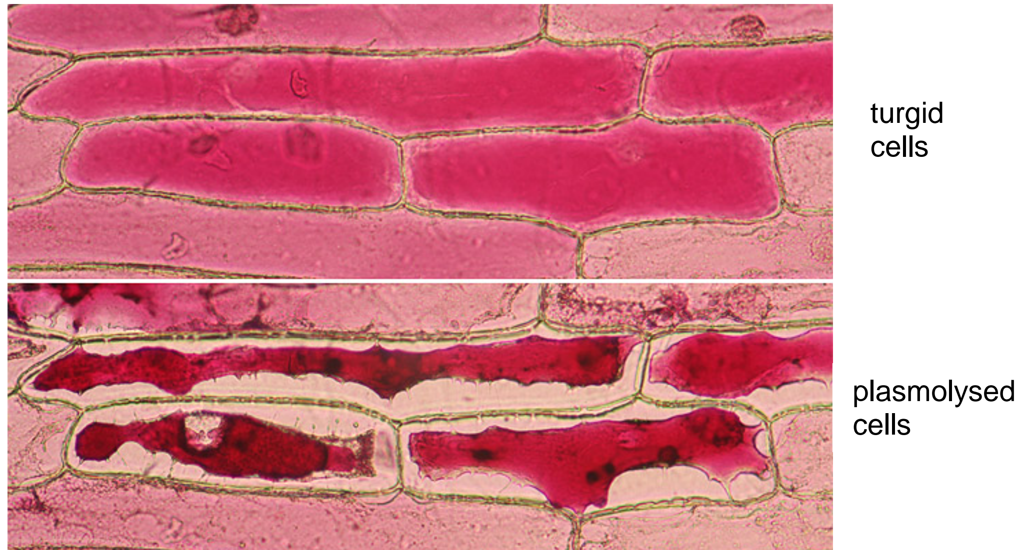
Apparatus

White tile
Fine forceps
Fine scissors
Rhubarb petioles or red onion
5 x 9 cm Petri dishes, 100 cm^3 beakers or watch glasses
Distilled water
sodium chloride solutions 0.2, 0.4, 0.6, 0.8 mol dm^{-3}: instructions for making these solutions is given in the previous experiment.
Stopclock
Microscope slides
Cover slips
Microscope
Dropping pipettes

Method

1. Set up five labelled Petri dishes/ small bottles each containing $10 cm^3$ of one of the following solutions: distilled water, 0.2, 0.4, 0.6, 0.8 $mol\,dm^{-3}$ sodium chloride solution.
2. Insert the fine forceps tip just under the upper epidermis of the onion leaf.
3. Keeping the forceps handles parallel with the epidermis, so as not to penetrate the underlying mesophyll, grip the epidermis and, maintaining the tension in the tissue, pull the epidermis off the mesophyll, away from you and place into distilled water.
4. When several square centimetres of epidermis have been peeled, place one square into each labelled petri dish/small bottle.
5. Leave at room temperature for a minimum of 30 minutes.
6. Carefully spread the tissue out on a microscope slide, so that it is not folded. Take a scalpel and rock the blade backwards and forwards over the tissue in order to cut out a 0.5 x 0.5 cm square.
7. Add two drops of bathing solution and apply a cover slip.
8. If any solution exudes from the cover slip, blot it with filter paper to dry the slide.
9. Using a x10 and then a x40 objective lens, examine all the cells in a field of view and count the number that are turgid and the number plasmolysed.
10. Repeat the counts at all concentrations of bathing solution.
11. Record your results in a table.
12. Plot a graph of % cells plasmolysed against the concentration of the bathing solution.
13. Using the graph, read the concentration of bathing solution that would produce plasmolysis in 50 % of the cells.
14. From the table given in the previous experiment, determine the solute potential of this solution. This is equal to the solute potential of the cells.

The photograph below shows the appearance of turgid and plasmolysed cells.



Risk assessment

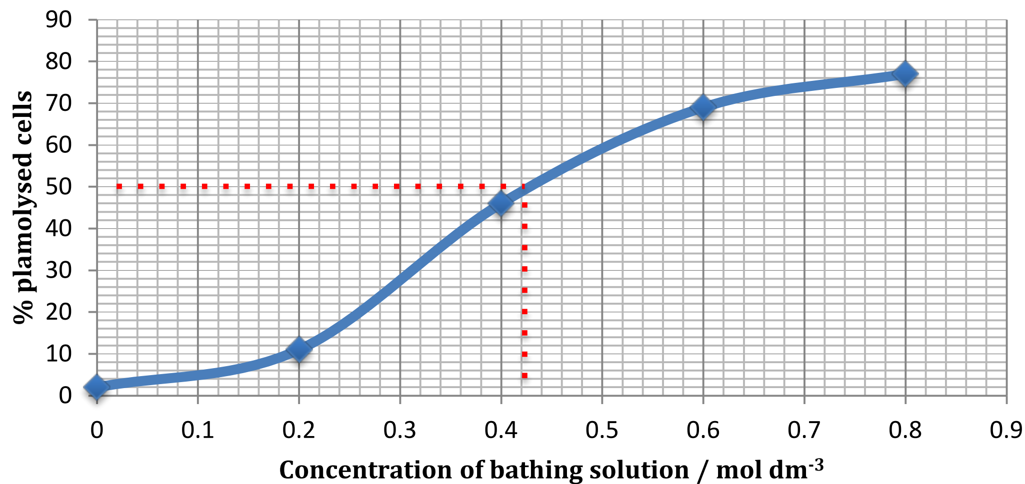
Hazard	Risk	Control measure
Scalpel blades are	may cut skin when cutting cylinders	Cut away from body onto a white tile

Teacher/ Technicians notes

- To ensure that the same cell is not counted more than once, a system of tracking across the specimen may be used. The slide is always moved in the same direction across the field of view and successive fields of view are always separated from the previous one by the width of at least one cell.
- Cells occur on the edge of the field of view and are only partially visible. A rule should be made regarding their scoring, for example, a partially visible cell is never counted or a cell is scored if it is at least half visible.
- In biology, it is normal to join data points to construct a line on a graph. In this situation, a curve of best fit may be used. This takes into account all the data, rather than only the two data points either side of that representing 50% plasmolysis.

Sample results

Concentration of bathing solution / mol dm^{-3}	Number of cells in field of view						Total		
	1		2		3				
	plasmolysed	turgid	plasmolysed	turgid	plasmolysed	turgid	plasmolysed	turgid	% cells plasmolysed
0	1	30	0	32	1	36	2	98	2
0.2	4	28	2	32	5	29	11	89	11
0.4	20	15	10	19	16	12	46	54	46
0.6	20	9	25	11	24	11	69	31	69
0.8	19	7	34	8	24	9	77	23	77



The solute potential of the equivalent concentration may be read from the table given in Experiment 4.

Alternatively, the % plasmolysed cells can be plotted against the solute potential, so that the solute potential of the cells may be read directly from the graph.

Further work

Make a scientific drawing of turgid and plasmolysed cells, labelled to show

- Cell wall
- Cytoplasm
- Position of cell membrane (it is too thin to actually see but the boundary of the cytoplasm marks its position)
- Vacuole
- Nucleus
- Position of bathing solution

Practical techniques

- use appropriate apparatus to record a range of quantitative measurements (to include mass, time, volume, temperature, length and pH)