Reading the Inside of a Cell

measuring a hidden concentration through osmosis and plasmolysis

Aim: to measure the solute concentration inside onion epidermis cells by finding the external salt concentration at which half the cells are plasmolysed — while practising the core microscope skills: preparing a temporary mount, calibrating an eyepiece graticule, and calculating magnification and actual cell size.

Equipment

- Light microscope
- ×10 eyepiece + graticule
- ×40 objective
- Stage micrometer
- Slides + coverslips
- Forceps, mounted needle
- Red onion
- Distilled water
- NaCl: 0.0, 0.2, 0.3, 0.4, 0.5, 0.7, 1.0 M
- Dropping pipettes
- Filter / paper towel

Safety

Sodium chloride solutions are low hazard. Handle glass slides and coverslips carefully — edges are sharp. Take care with the mounted needle. Wash hands after handling onion and solutions. Report any breakages.

BEFORE YOU START — PREDICT

A cell sits in salt water *stronger* than its contents. Which way will water move, and **why**? Write your prediction before you add any salt.

Part A — temporary mount & the instrument

Peel a thin strip of red epidermis from the inner surface of an onion scale. The red pigment sits in the vacuole, so it makes the living contents easy to see.

STEP 1 · MOUNT Sketch / describe how you place the strip in a drop of distilled water and lower the coverslip.	STEP 2 · COARSE FOCUS Describe finding the cells on low power, then the coarse-focus move.	STEP 3 · FINE FOCUS Describe switching to ×40 and sharpening with fine focus only.

MAGNIFICATION = EYEPIECE × OBJECTIVE

Total magnification = _____ (eyepiece) × _____ (objective) = _____ ×

Always begin on the lowest power to locate the cells, then increase — a wider field is easier to search, and you protect the slide and lens.

Part B — calibrate the graticule, measure a cell

The eyepiece graticule is a ruler with no units until you calibrate it against the stage micrometer (1 mm divided into 100, so each division = 10 µm).

1. **1.** Line the eyepiece scale (eyepiece divisions, **e.p.d.**) against the stage micrometer at ×400.

2. **2.** Count how many stage divisions fit a known number of eyepiece divisions, and complete the calibration below.

> CALIBRATION (DO THIS ONCE AT ×400)
>
> _____ e.p.d. line up with _____ stage divisions = _____ × 10 = _____ µm
>
> So **1 eyepiece division = _____ µm** (your calibration factor at ×400)

Now remove the stage micrometer, return to your onion slide, and measure **ten cells**. Record the length in eyepiece divisions, then convert to micrometres.

CELL	1	2	3	4	5	6	7	8	9	10	MEAN
LENGTH (E.P.D.)											
ACTUAL LENGTH (MM)											

Actual length (µm) = length in e.p.d. × calibration factor. **Mean cell length = _____ µm**

Part C — plasmolysis concentration series

Mount a fresh strip of red epidermis in each salt concentration. Wait two minutes for osmosis to act, then count cells in three separate fields of view. A cell is **plasmolysed** when the red contents have pulled away from the cell wall.

NaCl / mol dm⁻³	Plasmolysed / total cells			Mean % plasmolysed
	Field 1	Field 2	Field 3	
0.0				
0.2				
0.3				
0.4				
0.5				
0.7				
1.0				

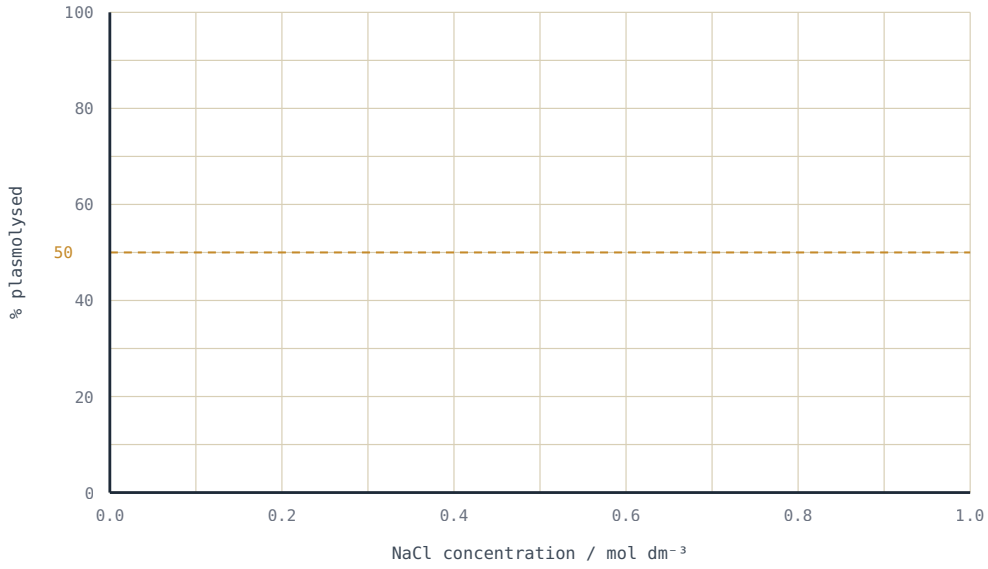
% plasmolysed = (plasmolysed ÷ total) × 100

Qualitative observations

What did the cells actually look like as concentration rose? Note colour, shape, where the membrane sat relative to the wall.

Analysis — find the hidden concentration

Plot mean % plasmolysed (y) against NaCl concentration (x). Draw a smooth best-fit curve, then read off the concentration at **50%**.



[1] **State** the total magnification you used. _____

[2] **Determine** the concentration at which 50% of cells are plasmolysed (from your curve). _____ **mol dm⁻³**

[3] **Explain** what this concentration tells you about the contents of the onion cells.

[4] **Explain**, in terms of water moving down a water-potential gradient, why the cells plasmolyse in strong salt solution. (Avoid saying the salt "pulls" the water.)

Evaluation

[5] Two **limitations** of this method:

[6] One **improvement** for each limitation:

Extension idea for your Internal Assessment: what one variable would you change to investigate further — onion variety, tissue type, temperature, or time in solution?