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Chapter 18

Measurement of Water Potential: Comparative Analysis of Three Distinct Methods in Biological Systems

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1. Introduction:

In thermodynamics, each component within a system contains a certain amount of free energy that can perform work under constant temperature conditions (Taiz et al., 2015; Tripathi et al., 2013). The process of osmosis involves water movement driven by differences in free energy across a semi-permeable membrane (Salisbury and Ross,1992). For non-electrolytes, this free energy per mole is called chemical potential (Ψ). Specifically, for water, it is known as water potential (Ψw). Osmotic pressure (OP) in a solution arises from the presence of solutes, which lowers the water potential. Thus, osmotic pressure quantifies the reduction in water potential, termed osmotic potential (Ψs) (Westgate and Boyer 1985; Tripathi et al., 2028; Yadav et al., 2023). Though OP and Ψs are numerically equal, Ψs is negative (-). The pressure generated by a fluid is called pressure potential (Ψp).
Therefore, Ψw = Ψs + Ψp If Ψp is disregarded, then Ψw = Ψs Given OP and Ψs are numerically similar but oppositely signed, Ψs = OP

Water potential (Ψw) can be determined using the plasmolytic method by calculating the

osmotic pressure with the formula OP = CRT, where:

OP = Osmotic pressure

C = Concentration of cell sap

R = Gas constant

T = Temperature (in Kelvin)

In the experiment, solutions of varying molar concentrations are used. The cell sap has a specific molar concentration, and the cell membrane acts as the semi-permeable membrane. By observing the number of plasmolyzed cells in solutions of different

concentrations, the cell sap concentration can be determined. Plasmolysis happens when the protoplasm shrinks away from the cell wall in solutions with higher concentrations than the cell sap. In a hypertonic solution, most plant cells plasmolyze. At the isotonic point, where the solution concentration equals the cell sap concentration, incipient plasmolysis occurs, marking the beginning of protoplasm

withdrawal from the cell wall.

At this point, since the external solution concentration matches the cell sap concentration, the cell sap concentration can be found, allowing the determination of osmotic pressure.

$\Psi w = \Psi s + \Psi p$ DPD = DP – TP (WP) Thus, Ψw equals DPD. The DPD value can be indirectly measured from the OP value at the concentration where no absorption or desorption occurs, expressed as DPD = OP = CRT.
C = Molar concentration
R = Universal gas constant
T = Absolute temperature

2. By Plasmolytic Method:

2.1. Materials Required:

1. Leaves of Rhoeo discolor (*Tradescantia spathacea*).
2. Sucrose.
3. Chemical balance,
4. Weight box,
5. Compound microscope
6. Glassware/Plastic ware
7. 12 Pairs of petri dishes,
8. Beakers
9. Measuring cylinder,
10. Slides
11. Forceps,
12. Needle
13. Blade
14. Cover slip
15. Distilled water

2.2. Preparation of Reagents:

A molar solution contains 1 mole (=molecular weight) of solute dissolved in 1 litre (1000 cc) of distilled water. For this experiment, a 1 M sucrose solution is used as the stock solution. Sucrose has a molecular weight of 342.3 g, and 342.3 g needs to be

dissolved in 1000 cc of distilled water to create a 1 molar solution. Table1: Preparation of sucrose stock solution.

Sr. No.	1M Stock Solution (cc)	Distilled Water (cc)	Total Volume (cc)	Working Strength (M)
1	1	9	10	0.1
2	2	8	10	0.2
3	3	7	10	0.3
4	4	6	10	04
5	5	5	10	0.5
6	6	4	10	0.6
7	7	3	10	0.7
8	8	2	10	0.8
9	9	1	10	0.9
10	10	0	10	1.0

Table1: Preparation of sucrose stock solution.

2.3. Preparation of Varying Concentrations of Molar Solution:

Different molar solution concentrations for this experiment need to be made using the prepared stock solution.

2.3.1 Procedure:

1. Carefully detach a small segment from the lower surface of the leaf either by tearing the leaf obliquely with a single jerk or scraping it with a safety blade.
2. Place the peel in a drop of water on a slide and cover it with a coverslip. Observe it under a microscope.
3. Prepare sugar solutions of different molar concentrations ranging from 0.1 M to 1 M.
4. Take another peel and mount it similarly on a slide, but this time in a drop of sugar solution with varying concentrations on different slides.
5. After 30 minutes, observe each preparation under the microscope.

2.4 Observations:

Table 2: Percentage of plasmolyzed and non-plasmolyzed cells with an increase in molar concentration.

S.No	Molar Concentration (M)	Number of Cells Considered	Number of Plasmolysed Cells	Number of Non-Plasmolysed Cells	Percentage of Plasmolysed Cells (%)	Percentage of Non-Plasmolysed Cells (%)
1						
2						

Record the number of cells considered, plasmolyzed cells, non-plasmolyzed cells, and calculates the percentage of each. Plot a graph correlating the percentage of plasmolyzed cells against sucrose concentrations. Identify the concentration at which 50% of cells are plasmolyzed, indicating the stage of incipient plasmolysis. From the graph, determine the isotonic point, where 50% of the cells are plasmolyzed. Using the formula OP = CRT, calculate the concentration at this point.

3. Weight Method:

3.1. Materials Required:

1. Fresh potato tuber.
2. Sucrose ($C_{12}H_{22}O_{11}$).
3. Weighing balance.
4. Measuring cylinder,
5. Test tube, beaker,
6. Petri dishes
7. Distilled water,
8. Cork borer
9. Blotting paper

3.2. Procedure:

1. Use a cork borer to obtain uniformly bored pieces of potato tuber.
2. Weigh each piece accurately (initial weight) and place them into petri dishes containing sucrose solutions of known concentrations.
3. Allow the setup to sit undisturbed for an hour, then remove the tuber pieces and blot them dry. Weigh each piece again (final weight).
4. Repeat the process for all tuber pieces, noting the initial and final weights of each.

3.3. Observations:

Observe that the tuber pieces gain weight with increasing concentration of the molar solution up to a certain point, beyond which a corresponding decrease in weight is observed. Record these observations and plot them on a graph.

Table 3: Percentage weight of potato tuber with the increase in molar concentration.

S. No.	Molar concentration (M)	Initial weight (W1) (mg)	Final weight (W2) (mg)	Difference in weight (W2 -W1) (mg)	Percentage of difference in weight	Remark
1.						
2.						
3.						

Calculate the percentage difference in weight for each concentration and note any significant observations.

3.4. Result:

Utilize the relationship DPD = OP – TP, where TP = O (Turgor Pressure is negligible). Calculate the water potential (Ψw) directly from the graph by determining the molar concentration where there is no turgor pressure.

3.5. Precautions:

1. Use distilled water for all solutions.
2. Ensure thorough drying with blotting paper before weighing the samples.

4. Density Method:

4.1. Materials Required:

1. Peeled potato tuber
2. Sucrose solutions (0.1 M, 0.2 M, 0.3 M, 0.4 M, 0.5 M),
3. Methylene Blue
4. Weighing balance
5. Test Tubes, Pipette, Dropper
6. Forceps,
7. Cork borer

8. Test Tube Stand

4.2. Procedure:

1. Dissolve 34.2 g of sugar in 100 cc of distilled water to make a 1 M stock solution.
2. Prepare duplicate sets of test tubes with different molar concentrations of sucrose solution (0.1 M, 0.2 M, 0.3 M, 0.4 M, 0.5 M).
2. Cut small cylindrical pieces of potato, dry them between folds of filter paper, and place them in one set of test tubes.
3. Add methylene blue to the other set of test tubes until the solution turns sufficiently blue.
4. Allow the test tubes to sit undisturbed for about 20 minutes.
5. Transfer a drop of colored solution from the blue set into the corresponding molar concentration test tube of the potato set.
6. Observe the movement of the drop in the sugar solution.

4.3. Observations:

Record whether the drop rises, falls, or diffuses in each test tube, indicating changes in density.

4.4. Result:

Calculate the water potential by observing the movement of the drop using the formula OP = CRT, where the concentration of the molar solution is where the drop disperses.

4.5. Precautions:

1. Make sure that only distilled water is used to prepare the solutions.
2. Keep the Petri plate covers on during the experiment.
3. Make sure that cells at every concentration are rigorously exposed for the same amount of time.
4. The samples must be dried with blotting paper before being weighed.

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