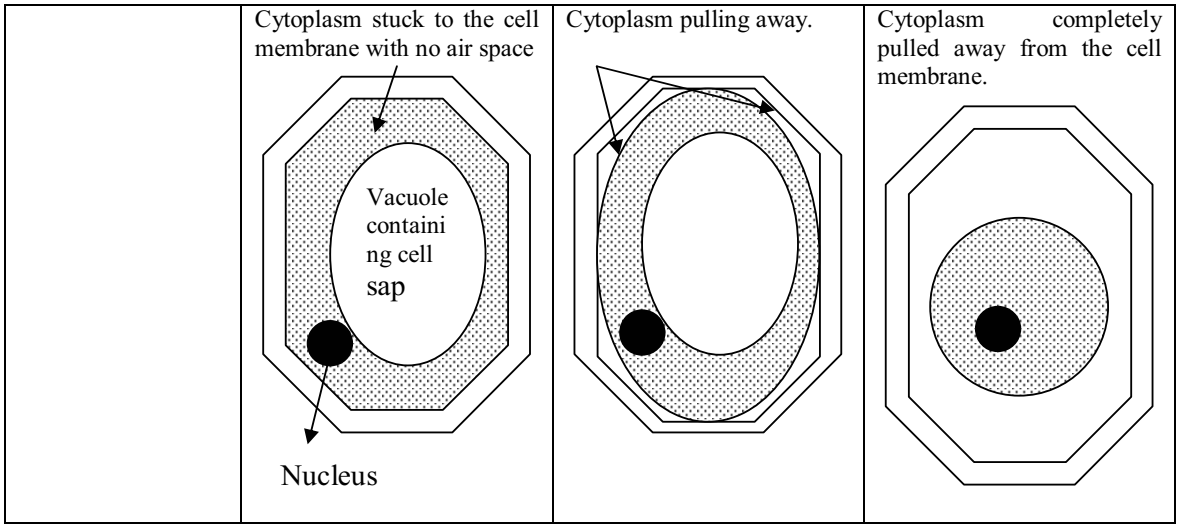
Planning (a)

Aim: To determine the osmotic pressure of sucrose solution at 50% plasmolysis.

Theory: Osmosis is the movement of water molecules from a region of high water potential to a region of lower water potential through a semi-permeable membrane. If a solution is separated by its pure solvent by a membrane, the pressure which must be applied to stop water entering the solution, and so prevent osmosis is called the osmotic pressure. The more concentrated a solution is the greater is its positive osmotic pressure as the concentration gradient is higher.
The vacuole of plant cells contains cell sap which consists of nutrients and salts dissolved in water. If the water potential of the vacuole of a plant cell, and therefore the cell, is less than the water potential of the solution in which the cell is placed then endosmosis will take place and water will diffuse into the cell through osmosis and even though the cell swells up due to the water entering, it doesn't burst as the cell wall is able to withstand the pressure and the cell instead becomes turgid.
However if a plant cell is placed in a concentrated solution where the water potential of the surrounding of the cell is lower than the water potential of the cell sap inside the cell, then exosmosis takes place and water moves out of the cell across the cell membrane and the cytoplasm of the cell starts to shrink. When the cytoplasm no longer presses on the cell wall, it is said to have reached incipient plasmolysis. Further loss of water from the cell causes the cytoplasm to shrink and pull away from the cell wall. This condition is called plasmolysis and the cell is said to be flaccid.

The table below summarizes the affect of different concentration gradients on the direction of movement of water and on the condition of the cell:

Water Potential of surroundings as compared to that of the cell	Higher	Equal	Lower
Net movement of water	Enters Cell	Neither enters nor leaves	Leaves Cell
Cytoplasm	Swells	No Change	Shrinks
Condition of Cell	Turgid	Incipient Plasmolysis	Plasmolysed (flaccid)



Hypothesis: If the solute concentration of the sucrose solution is very high, most of the cells will be plasmolysed due to lower external water potential. However if the sucrose solution is rather dilute with low solute concentration, then very little or no cells will undergo plasmolysis, but will instead turn turgid as water will enter these cells instead of leave them. Therefore the percentage of plasmolysed cells will increase with the concentration of the sucrose.

Variables: The independent variable for this investigation is the sucrose concentration of the solution in which the lower surface epidermis of the leaf is kept as this can be varied to study the affect of different sucrose concentration solutions on the plant cells. The dependent variable is the plasmolysis percentage as it depends directly on the sucrose concentration. The time period – 30 minutes - for which the epidermis is kept in each of the different sucrose solutions is the constant variable as it is kept unvarying.

Planning (b)

Apparatus & Material:

- Rhoea discolor leaf
- 0.5 M sucrose solution
- Distilled water
- 5 Petri dishes
- 5 microscope slides
- Cover slips
- Syringe / 10 cc measuring cylinder
- Microscope
- Scissors
- Brush
- Forceps

Procedure:

1. The room temperature at which the experiment was performed was recorded.
2. First 10ml of 0.4M, 0.3M, 0.2M and 0.1M sucrose solution each was prepared from 0.5M solution by adding distilled water to the solution in appropriate proportions as shown below.

Concentration /M	Sucrose solution/ml	Water/ ml
0.5	10	0
0.4	8	2
0.3	6	4
0.2	4	6
0.1	2	8

3. Then 10 ml of each solution was taken separately using a syringe and put in the different Petri dishes which were labeled with the concentration of the solution they were containing.
4. A long strip of epidermis from the purplish lower surface of the Rhoea discolor leaf was peeled and this strip was cut into 5 small pieces.
5. 2 pieces of epidermis were immersed in each solution in the different Petri dishes which were then covered and left for 30 minutes.
6. The epidermis pieces from the Petri dishes were them mounted onto different slides with one drop of the same solution the piece was contained in, and then covered with a cover slip. These slides were examined under a high power microscope.
7. The number of total cells (unplasmolysed + plasmolysed) and the number of plasmolysed cells was counted in three different fields for each strip.
8. The percentage of plasmolysed cells in each piece of epidermis was then calculated.
9. All the observations and calculations were recorded and then a graph of percentage of plasmolysed cells against sucrose concentration was plotted.
10. The exact concentration of sucrose for which 50% plasmolysis of the plant cells was caused was determined by reading off the graph.
11. The osmotic pressure of that concentration of sucrose was calculated by using the formula:

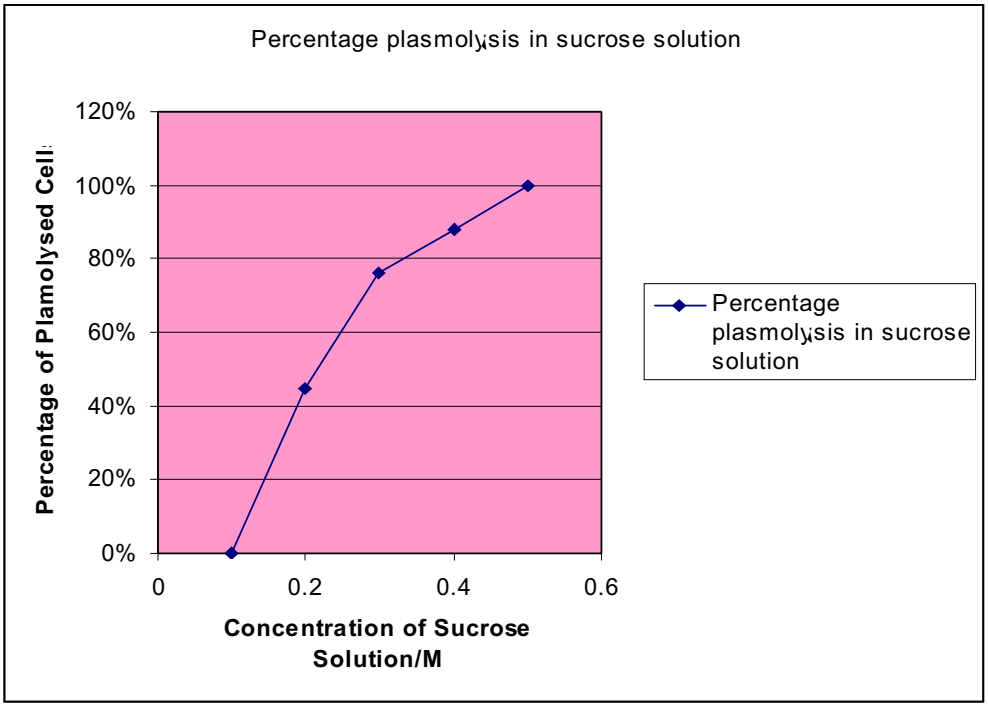
$$\text{Osmotic pressure} = \frac{22.4 \times (t + 273)}{273} \text{ atmospheres}$$

Where:
22.4 = pressure (in atmospheres) of 1.0m sucrose solution,
x = molar concentration of sucrose which gives 50% plasmolysis,
t = room temperature
273 = absolute temperature.

Data Collection:

	Total Number of cells			Number of Plasmolysed Cells			Plasmolysis %
Concentration	1	2	Avg.	1	2	Avg.	
0.1	22	24	23	0	0	0	0%
0.2	25	22	23.5	10	11	10.5	45%
0.3	23	22	22.5	16	18	17	76%
0.4	21	21	21	19	18	18.5	88%
0.5	23	21	22	23	21	22	100%

Data Analysis:



Osmotic pressure= $\frac{22.4 \times 0.19 \times (34 + 273)}{273}$ = 4.78 atmospheres

Evaluation

The procedure of the investigation provides a reliable method for deducing the osmotic pressure at which plant cells are 50% plasmolysed in sucrose solution. The hypothesis that we had formulated has been verified through this experiment. The least number of cells were plasmolysed when the concentration was 0.1M and maximum when the concentration was 0.5M. The third hypothesis was also proved right as 50% plasmolysis occurred when the concentration was 0.19M that is close to 0.2M thus proving over entire hypothesis right. This hypothesis has been proved right because when the concentration of the solution was 0.1M there was more water potential was greater outside this there was no flow of water molecules from the inside of the cell to the outside and this is the reason why the number of plasmolysed cells was zero. As the water potential in the solution outside was decreasing there was concentration gradient developing from inside the cell to the outside and this is the reason why the number of cells increased with the increase in concentration.

Limitations

Certain limitations in the practical procedures may have contributed to the inaccuracy of the experiment and provided scope for error in the results. These limitations are:

- The concentration of the sucrose solutions of different concentrations may not be totally accurate due to imprecision in the volume of solution and water mixed to produce the solutions of desired concentration. Therefore there may be errors in the percentage plasmolysis observed at each concentration and therefore in the final result.
- The molar concentration of sucrose solution which gives 50% plasmolysis is not determined directly, but only through indirect means. The estimated value of sucrose concentration at which 50% of the plant cells undergo plasmolysis is determined only through the graph which is plotted on the base of other observations, and no direct procedure is used to determine this value. Therefore this estimation leads to inaccuracy in the results.
- Human error in the manual process of counting the number of plasmolysed cells and subjective judgment in the classifying a cell as a plasmolysed or not could variably lead to inaccuracy in the results.

Precautions

Several precautions which must be taken to minimize the error in this investigation are:

- The same unwashed syringe must not be used to measure the required volumes of distilled water and sucrose solutions of different solutions
- When measuring the volume of solutions into the Petri dishes using a measuring cylinder, ensure that the eye is in level with the meniscus.
- Be careful when handling the solutions, as they can easily be contaminated which will alter the concentration and disturb the experiment.
- Also when preparing the slide with the leaf pieces to be viewed under the microscope, be careful with all the instruments specially the forceps. Forceps have a sharp point; hence scratch on the body from them should be avoided.

Modifications

- First, the experiment can be repeated several times to increase the accuracy of the results obtained. Not only this but during the experiment, several observers can be used to count the number of cells and then the mean value can be found. This will help to avoid any error from incorrect human observations.
- Also, the leaf tissue used in the experiment could be collected from several areas on the leaf.

Conclusion

Through this experiment we have managed to find out the osmotic pressure of that leaf and since our hypothesis was proved right the osmotic pressure must also be close to the literature value. So the conclusion is that the vacuole must exert a pressure of 4.78 atmospheres after 50% of plasmolysis to prevent more water from exiting the cell.