Regulation of Water in Plant Cells

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Abstract: Cell water relationships are important topics to be included in cell biology courses. Differences exist in the control of water relationships in plant cells relative to control in animal cells. One important reason for these differences is that turgor pressure is a consideration in plant cells. Diffusion and osmosis are the underlying factors involved in the control of water in plant cells; however, additional attention must be given to osmotic pressure, osmotic potential, and water potential. This discussion shows how these parameters relate to each other, and how they are explained by thermodynamics and the universal gas laws. Detailed laboratory exercises are described that demonstrate these principles. The laboratory exercises include data collection, graphing of data, statistical analysis of data, and calculations of osmotic potential and diffusion pressure deficit from the data.

Keywords: Turgor, osmotic pressure, osmotic potential, water potential, diffusion pressure deficit

Introduction

Cell water relationships are deemed by most cell biologists to be an important topic for inclusion in cell biology courses. Still, some cell biology textbooks do not thoroughly cover the topic. In most textbooks, the topic is at least discussed with an explanation of osmosis; however, some of these discussions are without any reference to the thermodynamics involved. In other cases, there is a complete omission of the concept of turgidity. The control of water in plant cells is considerably different from the control in animal cells because of cell walls and turgor, a topic not always covered in detail in some cases.

Almost all of the textbooks reviewed have general explanations of osmosis. Pollard and Earnshaw (2002), however, did not include any discussion on the subject. In addition to osmosis, a number of textbooks also included some discussion on water relationships in plants by providing descriptions of plant cell walls, vacuoles, turgor, and plasmolysis. The concept of thermodynamics, however, was not related to water movement in these textbooks (Bolsover *et al.,* 2004; Lewin *et al.*, 2007; Karp, 2008; Alberts *et al.*, 2010). Cooper and Hausman (2007) included an excellent discussion of the contrast of water relationships between plant and animal cells. Becker *et al*. (2009) additionally discusses the 2nd Law of Thermodynamics, free energy, and entropy. The most quantitative descriptions of water movement were found in outdated textbook editions that are probably out of print (Wolfe, 1993; Tobin and Morel, 1997).

Background Information

Osmosis, in a very technical sense, is the movement of water across a membrane whereby the net movement occurs from a lesser negative water potential to a more negative water potential. The following discussion explains the basis of that definition. Osmosis is an event of diffusion, and it occurs because of the random movement of molecules due to kinetic energy. Diffusion is the tendency of molecules to undergo a net movement in the direction of lesser concentration. The rate of diffusion is proportional to one over the square root of the molecular weight of the molecule involved. Temperature, the distance to travel, and the area of diffusion are other factors involved in the rate of diffusion. A diffusion gradient, therefore, will result in a substance undergoing a net movement from a region of greater concentration (C_1) towards a region of lesser concentration (C_2). The diffusion of solute particles is similar to the diffusion of gases, except that the particles are in a solvent. Hence, gas laws can be applied in order to analyze the movement of solutes.

Osmosis generally refers to the net movement of water across a membrane. Osmosis occurs, for example, when an aqueous solution is separated from pure water by a semi-permeable membrane. One way to demonstrate osmosis is a simple set-up using a thistle tube. For example,

consider the thistle tube containing a sucrose solution, covered with a semi-permeable membrane, and placed into a beaker of pure water (Figure 1). Such a device, called an osmometer, is a simple way to measure osmotic pressure in addition to demonstrating osmosis. The aqueous solution in the thistle tube will rise because of the entrance of water from the beaker due to osmosis. The amount of rise of water in the tube, designated *h* for height, is a crude but valid way to measure osmotic pressure. A more sophisticated osmometer is a device equipped with a moveable piston in the tube connected to a pressure gauge. Such a system would provide a more quantitative measure of the amount of pressure necessary to prevent water from rising in the system's tube due to osmosis.

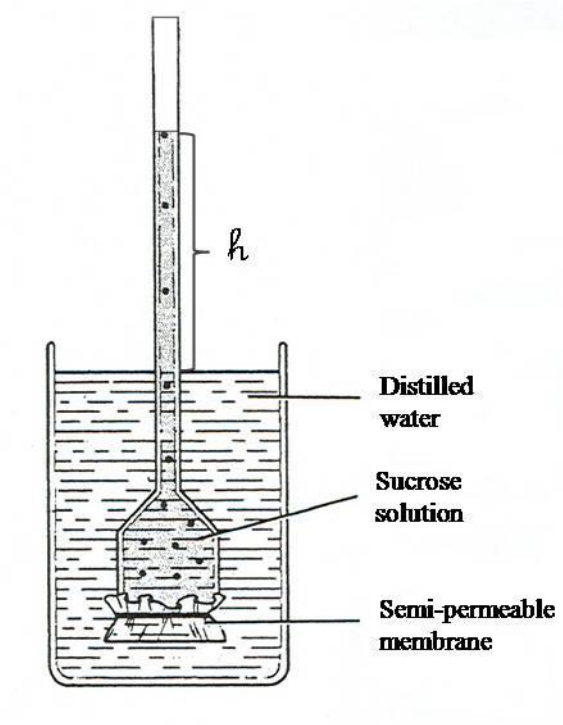

Figure 1. A typical demonstration of osmosis. The rise of water (*h*) represents osmotic pressure.

Unconfined aqueous solutions not in an osmometer also have an osmotic pressure. However, such solutions are described as having an osmotic potential (OP) rather than an osmotic pressure. All aqueous solutions have an osmotic potential. A beaker of tap water, a cup of coffee, a mud puddle, etc. all have an osmotic potential if solute particles are dissolved in the water forming the solvent of the solution. The magnitude of the osmotic potential is based on the colligative property of the solution; that is, OP is directly proportional to the solute concentration. The colligative property is determined solely by the number of solute molecules per unit of the solvent volume and not by the size of the molecules. For example, no difference in the colligative property exists between soluble large protein molecules and an equal number of soluble miniscule ions. Osmotic potential is referred to as Ψs (psi solutes).

Additional terms and their acronyms are often applied to water relationships in both animal and plant cells. The concept of thermodynamics (second law) and free energy can be incorporated into the understanding of these relationships. Water potential given as Ψw (psi water) is a good starting point. Water potential is an expression of the free energy of water, and it is equal to the difference between the free energy of water in an aqueous solution at any point in a biological system and that of the free energy of pure water under standard conditions. Water potential reflects the ability of water to diffuse. Pure water has the highest Ψw, and it is given the arbitrary value of 0. Therefore, all water potentials of solutions are less than 0 meaning they have a negative value. Water potential is actually equal to osmotic potential when turgor is not involved.

The departure of water potential of a solution from the water potential of pure water (0 value) can be expressed using a thermodynamic equation:

$$\Psi w = R\ T\ \ln (e/e^{o})$$

where R = ideal gas constant (1.987), T = absolute temperature (degrees K), ln = the natural log with base 2.718, e^0 = vapor pressure of pure water, and e = vapor pressure of water in the system. Calculations are usually made in bars or atmospheres. When e/e^o is less than 1, Ψw will be calculated as a negative number, the usual situation for an aqueous solution. The more negative the water potential of a system, the greater the tendency for water to diffuse into it. In other words, the more solute particles, the more negative the value assigned to the water potential.

In animal cells, water potential (Ψw) is equal to osmotic potential (Ψs). Animal cells regulate the movement of water by controlling the movement of ions; that is, they cope with osmotic pressure by balancing ionic concentrations on the two sides of the cell membrane. Also, animal cells have membranes containing aquaporins or water channels. In this way, animal cells can, to some extent, prevent

severe swelling or shriveling. In this way animal cells have selective water transport mechanisms.

With plant cells, turgor (Ψtp) must be considered. Plant cells are highly vacuolated and thus they act like an osmometer. Vacuoles are selectively permeable and can make up 75% of the volume of a plant cell, accumulating high concentrations of soluble molecules. Turgor pressure occurs due to osmotic pressure that causes the cell to take in water and, in turn, causes the cell contents to push against the cell wall. An equal and opposite pressure to turgor pressure is wall pressure (Ψwp). The cell walls of plants are relatively inelastic, and this rigidity is sufficient to prevent plant cells from bursting under osmotic pressure. In plant cells, therefore, overall water potential is equal to osmotic potential plus turgor pressure,

$$\Psi w = \Psi s + \Psi tp$$

Matrix (Ψm) forces, which are water-binding forces, can also be involved in water uptake by plant cells, but these forces are generally small and therefore ignored. As previously mentioned, Ψs is negative but Ψtp is a positive pressure, and the two pressures work in opposition to each other. Ψw is made less negative by turgor. When a cell is fully turgid, water flows equally in both directions across the membrane, and no net water flow occurs.

It is evident then, that water relationships of plant cells can extend beyond the usual definition of "water movement occurs from greater concentration to lesser concentration." Nonetheless, solutions in the external environments of plant cells that have greater solute concentrations than the cellular contents will draw water out of plant cells, causing wilting. Plants residing along the edges of sidewalks are often killed because of salt application to the walkways. This same situation can become problematic for farmers who apply too much fertilizer on their crops (called burning). On the other hand, these water relationships also explain why vegetable sections of grocery stores are periodically sprayed with tap water. The maneuver keeps the plant tissues turgid and more appealing to consumers. Turgor pressure also contributes to the movement of water into roots, and to some extent to the movement of water through the cellular tissue in stems. Root cells contain protein molecules in solution to cause water to flow into the plant.

Laboratory Exercises

Maintenance of the physiologically active state in cells and whole organisms depends upon the relative constancy of a number of conditions, one of which is a favorable water balance. Plant cells are ideal for demonstrating this concept. The purpose of this exercise is to learn about the physical principles governing net water fluxes in osmotic systems and to become familiar with simple methods for measuring water parameters in plant cells. These exercises, however, require meticulous attention to measurement techniques. The data collected are conducive to statistical analyses.

PART 1. Determination of the osmotic potential (OP) of plant cells

Use clean watch glasses (or comparably small glass vessels) to set up the following series of sucrose solutions made with distilled water. Volumes of 10 mL can be made for each vessel by mixing 1.0 M sucrose with distilled water in appropriate proportions. For example, combining 5.5 mL of 1.0 M sucrose solution with 4.5 mL of distilled water makes the 0.55 M sucrose solution. Concentrations other than those listed sometimes have to be used, depending on the osmotic potential of the tissue.

0.55 M	0.35 M	0.15M
0.50 M	0.30 M	0.10 M
0.45 M	0.25 M	0.05 M
0.40 M	0.20 M	0.00 M

A red onion is one type of tissue that can be used in this exercise. Carefully obtain thin layers of the membrane-like layer of tissue from the outer purplish-red side of concave pieces of the bulb. This layer is partly made up of pigmented cells, making microscopic examination of the protoplasm (cellular contents) relatively easy. Use small strips of the tissue (3 to 10 mm long) to avoid folding of the tissue in the sucrose solutions. Tissue samples are obtained by bending a layered piece of the onion bulb towards the purple side until it breaks and then gently pulling it apart. Use a forceps to obtain small sections of the thin tissue, carefully choosing tissue that shows considerable color. This tissue is usually only one cell layer thick. Students who react to onion odor may want to wear goggles and gloves. Place a strip of tissue into each of the sucrose preparations at approximately 2-minute time intervals. Make sure that the tissue stays submerged. A small paper clip can be placed on the tissue to prevent it from floating. At about 45 minutes after submergence, mount the tissue on a blank slide with a small drop of the same solution in which it was submerged and apply a cover

glass. Examine the dark red cells for plasmolysis using the 10X objective lens of the microscope. Ignore those cells devoid of color, which in some cases could be a considerable number. Plasmolysis is the shrinkage of the protoplasm and thus its separation from the cell wall due to loss of water. Score 40 to 60 cells from each solution as plasmolyzed or not plasmolyzed. Any amount of protoplasm shrinkage should be scored as a plasmolyzed cell (Figures 2 and 3).

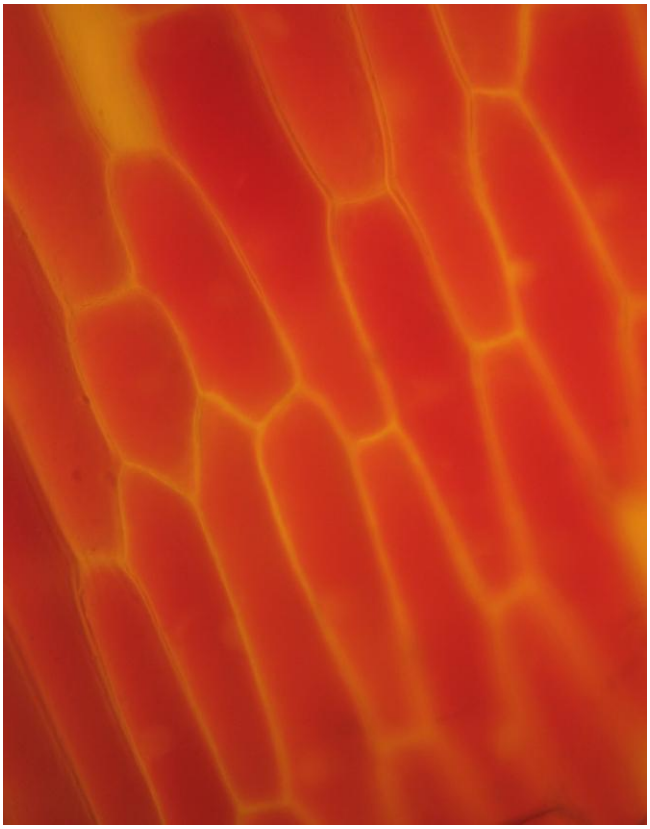

Figure 2. Red onion cells in distilled water showing all cells fully filled with protoplasm. Cells completely without color (upper left) are not scored.

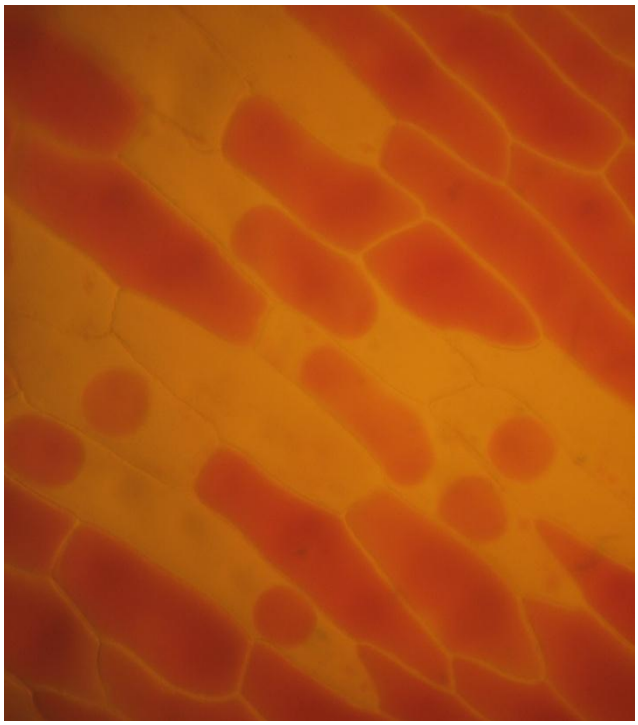

Figure 3. Red onion cells in a sucrose solution. Note cells undergoing plasmolysis among other cells without plasmolysis activity. Again, several cells are void of any color and are not scored.

Other tissues such as certain plant leaves can be used to determine OP, especially if they contain anthocyanin. The lower epidermis of leaves from *Rhoeo discolor* is a good source. However, an occasional student may be allergic to handling these leaves.

Record the number of cells plasmolyzed and not plasmolyzed from the series of solutions (See Table 1 for sample data). Then plot the percentage of cells plasmolyzed against the molarities using regression analysis (Figure 4). Vernier Graphical Analysis was used in this case. More reliable results are obtained if class data are combined. The concentration in which 50% of the cells are plasmolyzed is arbitrarily defined as incipient plasmolysis. Incipient plasmolysis is considered to be the same molarity as the osmotic potential of the cells. Always use the point of 50% plasmolysis obtained by the regression equation since that calculation is based on 10 to 12 different data points.

Table 1. Sample data plotting percent plasmolyzed cells of onion tissue over a range of different sucrose concentrations

Sucrose (M)	Plasmolyzed cells	Total cells	Percent Plasmolyzed cells
0.55	50	50	100
0.50	46	50	92.0
0.45	50	64	78.1
0.40	44	64	68.8
0.35	25	50	50.0
0.30	12	62	19.4
0.25	5	48	10.4
0.20	3	55	5.5
0.15	2	57	3.5
0.10	1	41	2.4
0.05	0	40	0.0

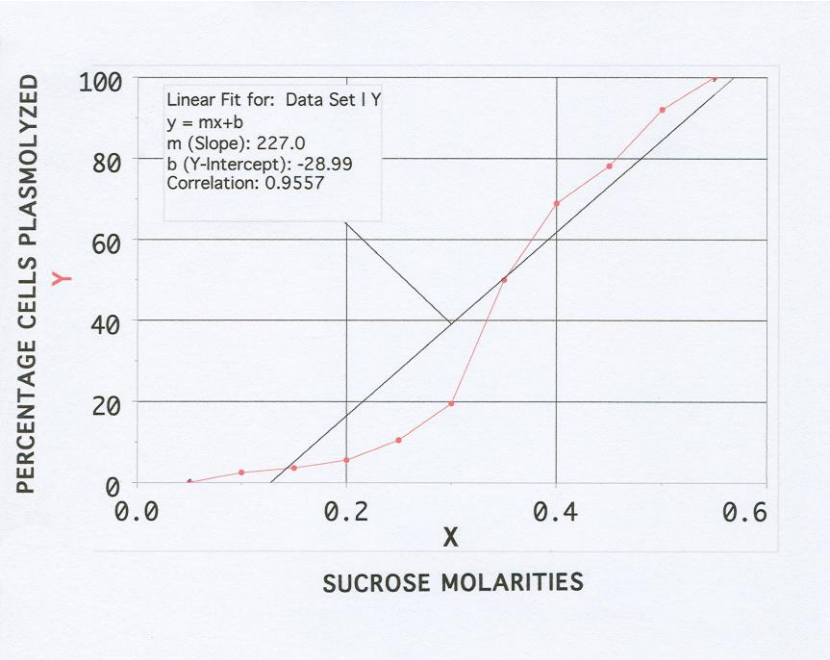

Figure 4. Regression analysis and linear fit of sample data generated by plotting percent cells undergoing plasmolysis on the Y-axis versus sucrose molarities on the X-axis. Incipient plasmolysis is arbitrarily assigned as 50% plasmolysis and used to determine OP.

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The osmotic potential of the cells can be calculated with the following equation. The units are atmospheres, a familiar unit of measurement for pressure.

OP = (22.4) x (M) x (T)/273

Where: OP is osmotic potential
M is the molarity of the external solution at incipient plasmolysis
T is the absolute temperature calculated as room temperature in degrees Celcius plus 273

PART 2. Determination of the diffusion pressure deficit (DPD) of cells

Diffusion pressure deficit is older terminology, but still useful for analysis purposes. DPD is the difference in diffusion pressures between an aqueous solution and pure water. In the case of plant cells, DPD = OP + TP where OP is negative and TP is positive. DPD is an actual deficit and therefore it too is regarded as an absolute quantity (positive).

Prepare the sucrose solutions of the following molarities by appropriately diluting a 1.0 M sucrose solution. Make volumes of 100 mL in clean beakers for this series. For example, the 0.55 M solution would be made by combining 55 mL of 1.0 M sucrose solution with 45 mL of distilled water. Again, other concentrations can be used depending on the DPD of the tissue.

0.55 M	0.35 M	0.15 M
0.50 M	0.30 M	0.10 M
0.45 M	0.25 M	0.05 M
0.40 M	0.20 M	0.00 M

The following steps should be carried out as quickly as possible. Use a standard cork borer with about an 8 mm diameter to push out a cylinder of tissue from a potato, beet, or similar plant tissue (beet is used for demonstration in this exercise). Discard the hard outer covering at the ends of the cylinder and cut about 5 cm of the cylinder into disks, each disk approximately 3 mm in thickness. If the tissue is hard and rigid, it may be necessary to remove the cork borer with the help of pliers. Also a metal rod may be necessary to force the cylinder of tissue out of the borer. If using beets, wear gloves. Weigh the complete group of tissue disks from one cylinder and immediately place the group of disks into one of the solutions. Repeat this procedure for each of the sucrose solutions. Use a separate weigh paper each time.

After about 75 minutes, remove the tissue by pouring the solution with the tissue into an ordinary strainer. When dripping has stopped, very briefly blot the tissue in paper toweling to remove excess wetting due to the solution and weigh. Repeat this procedure for all samples, again using separate weigh papers each time. Keep the technique as uniform as possible from one sample to another. Using the equation below, calculate the percent change in weight in each case. These solutions can result in either positive or negative percent weight changes (Table 2).

Percent change in weight = final weight – original weight/ original weight X 100
Plot the percent changes in weight against the molarities using regression analysis (Figure 5). Again, results are more reliable when class data are combined. Use the equation below to calculate the diffusion pressure deficit (DPD) of the tissue. In this case, M is the sucrose concentration in which no change in weight occurred. The units are in atmospheres.

DPD = (22.4) x (M) x (T)/273

Analyses

Sample osmotic potential (OP) data are shown in Table 1, and these data when graphed are illustrated in Figure 4. The osmotic potential calculated from these sample data by regression analysis is shown below in which Y is set at 50 because osmotic potential is assumed to occur at the concentration in which 50% of the cells underwent plasmolysis:

y = (m) (x) + b
m (slope) = 227.0 and b (intercept) = -28.99
50 = (227.0) (x) + (-28.99)
Solving for x at y = 50 is .348
The correlation of these data is 0.96

This molarity (.348) in which 50% of the cells were plasmolyzed at 23 degrees C (room temperature) is placed into the following equation:

OP = (22.4) (.348) (296/273) = 8.45 atmospheres

Table 2. Sample data plotting percent change in weight of beet tissue over a range of different sucrose concentrations

Sucrose (M)	Original weight in g	Final weight in g	Change in weight in percent
0.55	2.865	2.460	- 14.14
0.50	2.732	2.407	- 11.90
0.45	2.807	2.666	-5.02
0.40	2.474	2.422	-2.10
0.35	3.101	3.152	+1.64
0.30	3.060	3.118	+1.90
0.25	2.549	2.642	+3.65
0.20	2.801	2.899	+3.50
0.15	2.357	2.428	+3.01
0.10	2.675	2.754	+2.95
0.05	2.413	2.528	+4.77
0.00	2.880	3.060	+6.25

Sample diffusion pressure deficit (DPD) data are shown in Table 2, and these data when graphed are illustrated in Figure 5. The diffusion pressure deficit calculated by regression analysis is shown below in which (x) is set at 0 because this is the point in which no loss or gain in weight occurred in the tissue:

$y = (m)(x) + b$

m (slope) = -0.024 and b (intercept) = 0.264

$y = (-0.024)(0) + 0.264$

Solving for y at x = 0 is 0.264

The correlation of these data is - 0.88

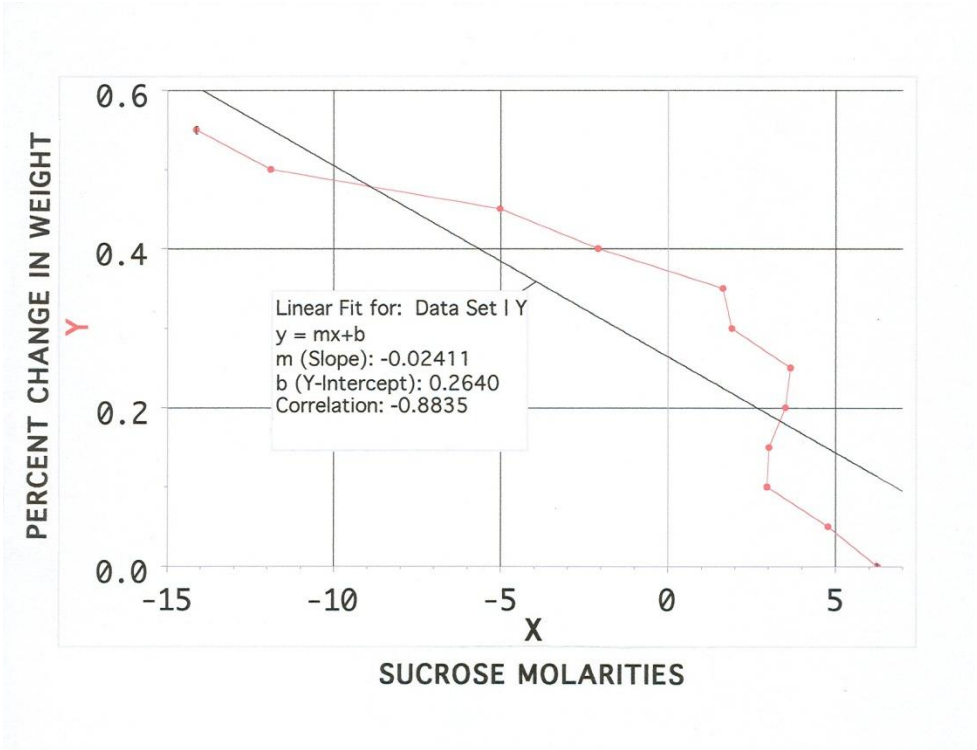

Figure 5. Regression analysis and linear fit of sample data generated by plotting percent change in weight on the X-axis versus sucrose molarities on the Y-axis. The percent change of weight equal to 0 is used to determine DPD.

This molarity (0.264) in which 0% change in weight occurred at 23 degrees C (room temperature) is placed into the following equation:
DPD = (22.4) (.264) (296/273) = 6.41 atmospheres

Additional Explanations

Turgor pressure (TP) is equal to –OP + DPD, and this parameter could be calculated if the OP and DPD determinations were made from the same type of tissue. However, TP is always a positive quantity.

Osmotic relationships can be likened to the gas laws. The ideal gas law equation is given as follows:

P V = n R T

Where: P = pressure
V = volume
n = number of molecules
R = universal gas constant
T = temperature Kelvin

Therefore, P = nRT/V

Mathematically, this is the same as n/V R T
And n/V is equal to M
Hence, P = M R T

One mole of gas occupies a volume of 22.4 liters at 273 degrees K.
Thus, pressure = (22.4) (M) (Temperature K/273), and the units of measurements are in atmospheres.

Osmotic potential is determined at the point of incipient plasmolysis. All turgor pressure has been removed at this point. Therefore, the osmotic potential in the cell is equal to the osmotic potential of the particular sucrose solution causing incipient plasmolysis without turgor pressure interfering with the measurement. Diffusion pressure deficit, on the other hand, is determined as the point in which no loss or gain in weight occurs in the tissue. Consequently, the measurement at this point takes into account both the osmotic potential and the turgor pressure of the cell, resulting in the diffusion pressure deficit.

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