*Tested Studies for Laboratory Teaching*
*Proceedings of the Association for Biology Laboratory Education*
Vol. 36, Article 29, 2015

# Demonstrating Osmotic Potential: One Factor in the Plant Water Potential Equation

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Water potential in plants depends on osmotic and pressure potentials. Pressure potential is easily understand by monitoring changes in tissue mass when immersed in various sugar solutions. Osmotic potential can be visualized by comparing the cell sap's density with these same sugar solutions in which it will rise, sink, or hover, in which the latter represents the concentration of equal density suggesting an isosmotic condition. Students can use this method to compare the density of various plant tissues (e.g. potato vs apple) or construct a Höfler plot using the Van't Hoft equation to illustrate relationships between osmotic and pressure potential.

**Keywords**: water potential, osmotic potential, plant physiology

## Introduction

In *General Biology*, a typical laboratory about osmosis might use plant tissue or alga placed in various sugar concentrations to observe the response. Changes in the cells are easily visualized since the cells walls allow either pressure to build within the cell (increased turgor pressure) when placed in hypotonic solutions and the cytoplasm to shrink (plasmolysis) with the loss of water in hypertonic solutions. Terms like *hypotonic*, *isotonic*, and *hypertonic* are introduced to explain the results and reflect the differences in the concentrations between the cell and the surrounding solution.

In plant biology, the topic of water movement is more complex. Now, osmosis is incorporated into the more complex concept of water potential that describes the free energy related to water movement. Water potential becomes an equation with symbols for each component in order to predict whether or not water will move within the plant or even from the soil to the plant. The symbol of water potential is Ψ, and the units are reported using the energy term, MPa (megapascals). There are three components of water potential as seen in the equation:

$$\Psi = \Psi s + \Psi p + \Psi m$$

Ψs or sometimes Ψπ (**Osmotic Potential**) describes the influence of solutes

Ψp (**Pressure Potential**) describes the influence of pressure

Ψm (**Matric Potential**) describes the influence of adhesion to nondissolved structures (i.e. cell walls)

While the osmotic potential can be measured directly using an osmometer, the instruments are expensive and do not allow students to visualize the effect of solute concentrations, which can be demonstrated by comparing density of cell sap with known concentrations of sugar. It is important to note that this is an indirect indicator of osmotic potential. The definition of osmotic potential can be explained as the reduction of the ability of water to do work when solutes are added to a solution since they are bound to solutes. The two other factors (pressure potential and matrix potential) must also be considered when predicting water movement from the soil to the plant or within the plant itself where the protoplasts are interconnected and most cells walls are fully hydrated. Water moves towards a more negative water potential—that is, water moves in a downhill direction.

### Osmotic Potential

Osmotic potential (Ψs) describes the effect solutes have on water potential. Pure water (when no solutes are present) has a Ψs = 0 MPa. When solutes are added, the water's free energy is decreased because water molecules interact with the solute molecules and cannot move as easily. Ψs < 0 when solutes are present.

The value of the osmotic potential is related to the number of particles present in the solution so that when more

particles are present, Ψs becomes more negative. The value of the osmotic potential can be determined using the Van't Hoff equation:

$\Psi s = -Ci RT$

where: C is the molar concentration of the solutes (molarity = moles $L^{-1}$),

*i* is the osmotic coefficient (the value of *i* is 1 for molecules that do not dissociate in solution (sucrose) and can be 2 or more for molecules that completely dissociate (NaCl),

R is the gas constant (8.31 J $K^{-1}$ $mol^{-1}$), and

T is the absolute temperature (room temperature in K, i.e. K = °C + 273°).

and: $iRT = 2.48$ MPa $mol^{-1}$ at 25 C for sucrose since $i = 1$.

The pressure potential of an open solution or **the cell extract** is 0 MPa by definition so that:

$\Psi s = -C \times 2.48$ MPa $mol^{-1}$ Note that Ψs is proportional to –C.

# Student Outline

Osmotic potential (Ψs) reflects the number of solute particles present in the solution so that when more particles are present, Ψs becomes more negative. Pure water (when no solutes are present) has a Ψs = 0 MPa. When solutes are added, the water's free energy is decreased because water molecules interact with the solute molecules and cannot move as easily. Ψs < 0 when solutes are present. Its value can be determined using the Van't Hoff equation:

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where: C is the molar concentration of the solutes (molarity = moles $L^{-1}$),
*i* is the osmotic coefficient (the value of *i* is 1 for molecules that do not dissociate in solution (sucrose) and can be 2 or more for molecules that completely dissociate (NaCl),
R is the gas constant (8.31 J $K^{-1}$ $mol^{-1}$), and
T is the absolute temperature (room temperature in K, i.e. K = °C + 273°).
and: $iRT = 2.48$ MPa $mol^{-1}$ at 25 C for sucrose since $i = 1$.

The pressure potential of an open solution or **the cell extract** is 0 MPa by definition so that:
$\Psi s = -C \times 2.48$ MPa $mol^{-1}$. Note that Ψs is proportional to -C.

We will be determining the osmotic potential of the plant cell sap **indirectly** based on the density of the cell sap. If the density of the cell sap is similar to a known solution concentration, then we have some knowledge of its osmotic potential. Because the cell sap also contains water contained in the cell walls, we will correct the value using an approximate number.

**The Experiment**

*Materials*

<u>For preparing sugar dilutions</u>
Sucrose 1M solution
Distilled water
Test tubes and rack
Pipettes and pipetting aids

<u>For preparing cell sap</u>
Potato or apple
Mortar and pestle
Methylene blue powder
Glass Rod
Transfer pipettes
Micropipettor P100
1.5ml Microfuge tubes
Microfuge

*Method*

1. Prepare tubes containing decreasing concentrations of sucrose (0.6 M – 0 M).
   a. Make 5 ml of each sucrose solution into appropriately labeled test tubes (Table 1).
   b. Set the solutions aside until the cell sap is prepared.
2. Prepare cell sap dyed with methylene blue.

   a. Chop about 1 $cm^3$ piece of potato or apple into small pieces with a single-edge razor blade, and then grind it to a pulp using a mortar and pestle. Do not add water.
   For potato: By compressing the pestle again the plant tissue, squeeze out a small volume of sap and place into a microfuge tube using the transfer pipette. This step may need to be done several times to collect enough sap. Centrifuge the plant extract for 2 minutes at about 6,000 rpm in the microcentrifuge. (This step will remove cell debris including starch granules. (You will need about 0.4 ml.)
   For apple: Fill a 1.5 ml microfuge tube with macerated fruit pulp and centrifuge for 10 min at about 6000 rpm.
   b. Remove the supernatant using a transfer pipette and place in a new microfuge tube. (The experiment requires about 0.4 ml of cell sap.)
   c. Dip a dry glass rod or the tip of a dry transfer pipette into the powdered methylene blue and mix this small amount of dye with the potato extract. Have faith that some power has attached to the glass rod. After mixing, the solution should be a dark blue.

**Table 1**. Preparation of sucrose dilutions and calculation of their osmotic potentials.

| Final Concentration (M) | Volume,1 M sucrose (ml) | Volume, Water (ml) | Osmotic potential, MPa * |
|---|---|---|---|
| 0.6 | 3.0 | 2.0 | _____ |
| 0.5 | 2.5 | 2.5 | _____ |
| 0.4 | 2.0 | 3.0 | _____ |
| 0.3 | 1.5 | 3.5 | _____ |
| 0.2 | 1.0 | 4.0 | _____ |
| 0.1 | 0.5 | 4.5 | _____ |
| 0 | 0 | 5.0 | _____ |

* The water potential is calculated using the Van't Hoff equation: $\Psi s = -Ci RT$,
where $iRT = 2.48$ MPa $mol^{-1}$ at 25 C for sucrose since $i = 1$.

3. Estimating the osmotic potential of the cell sap.
   a. Using a P100 micropipettor, remove a small amount (30 µL) of the cell sap dyed with the methylene blue.
   b. Place the colored cell sap into the middle of 0 M solution. Gently release the drop.

**Note whether the dye sinks, disperses, or floats to the surface in this solution. Subjectively estimate whether it does so rapidly or slowly**.

   c. Record your results in Table 2 and repeat this procedure for each of the solutions.
   d. If the isosmotic point seems to between two concentrations (i.e. the drop sinks in 0.3 M and floats in 0.4 M), make a solution that is midway between the two, and then test the cell sap using the same procedure.

4. Analysis
   a. What is the **solute concentration** of the sample based on your observations (Table 2)? _____
   b. What is the **osmotic potential** of the sample? _____
   c. To correct for the fluid in the cell walls, which accounts for about 15% of the total volume of the cell sap and does not contribute to the osmolarity of the cell sap, correct your value for the osmotic potential by multiplying it by 0.85.

**Corrected value of the osmotic potential of the cell sap** _____

**Table 2**. Response of cell sap drops (float, sink, or hover) when placed in various concentrations of sucrose.

| Sucrose Solution (M) | Drop response: float, sink, hover | Approximate rate of response. |
|---|---|---|
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## Notes for the Instructor

This method does not give a true reading of osmotic potential because it is measuring density against a sucrose solution, which is the predominant sugar in plant cells. Potato and apple tissue should have equal density at about 3.5 M. Example data for apple sap is shown below (Fig. 1).

This lab could be extended to include several types of tissues or coupled with an experiment measuring the differences in mass of tissue in these same solutions to prepare a Höfler plot.

## Literature Cited

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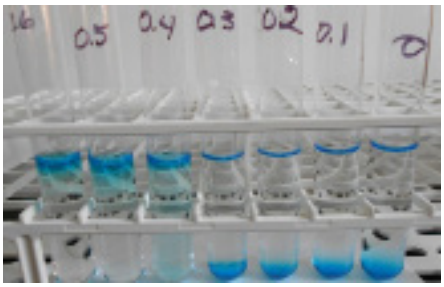


**Figure 1**. Apple sap stained with methylene blue. Sucrose solutions range from 0.6 M to 0 from left to right. Sap is floating in 0.4 – 0.6 M and sinks in 0 – 0.3 M. From this data student would then make a 3.5M solution and test the apple sap.

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## Citing This Article

Ford, R. 2015. Demonstrating Osmotic Potential: One Factor in the Plant Water Potential Equation. Article 29 in *Tested Studies for Laboratory Teaching,* Volume 36 (K. McMahon, Editor). Proceedings of the 36th Conference of the Association for Biology Laboratory Education (ABLE). http://www.ableweb.org/volumes/vol-36/?art=29