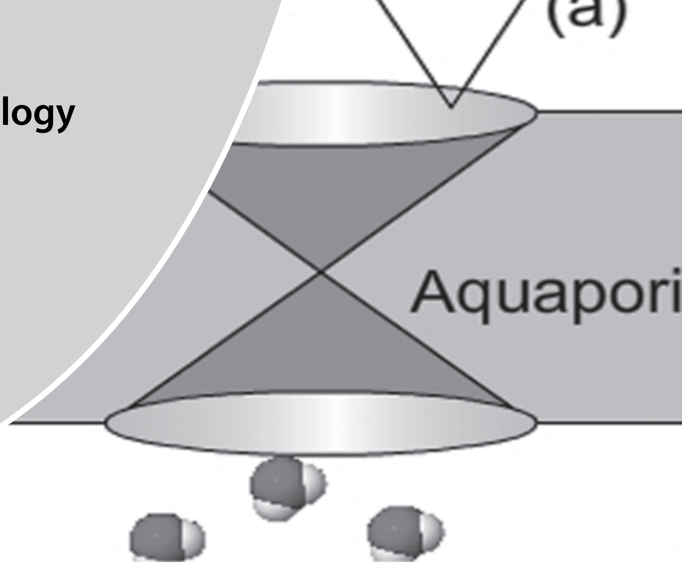
Teaching Osmosis to Biology Students



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Abstract

Osmosis is a fundamental concept of great importance to understanding natural biological, physical, and chemical processes. We provide an instructional guide to assist instructors of advanced high school biology and college biology students in defining questions that are central to deriving a highly developed understanding of osmosis. We present teaching activities that focus on advancing multiple hypotheses about the cause of osmosis, presenting a tentative explanation and model of osmosis, and drawing scientifically accepted conclusions about osmotic processes.

Key Words: *critical thinking; inquiry instruction; osmosis.*

○ Introduction

Osmosis is a fundamental concept of great importance to understanding natural biological, physical, and chemical processes. Water occupies more than two-thirds of a cell's volume, and is related to the colligative properties of solutions and the thermodynamic energy of Brownian motion. Osmosis occurs when a solvent and a solution are separated by a selectively permeable membrane, resulting in spontaneous movement of the solvent through the membrane. Research on the association of osmotic pressure with concentration of solute particles is comprehensive and easily observed with aqueous solutions. Osmotic pressure refers to the pressure needed to prevent the flow of a solvent across a selectively permeable membrane. Despite the specificity of these concepts, scientists do not always agree on explanations of the phenomena and contend that the causes of osmosis remain unclear, obscure (Kiil, 1989), and incomplete (Hammel, 1999). The phenomenon of osmosis offers a challenge to intuition, and incorrect conceptions have been uncovered even in college freshman biology and chemistry textbooks (Kramer & Myers, 2013).

Osmosis is a fundamental concept of great importance to understanding natural biological, physical, and chemical processes.

We provide an instructional guide to define essential questions regarding osmosis and give advanced high school biology teachers and college biology instructors tools to help students think critically about osmosis. We begin with a brief description of historical events regarding osmosis and provide a definition of knowledge and question types. We then advance a theoretical framework that delimits declarative and procedural knowledge, and apply this to pose descriptive and causal questions. Subsequently, we advance multiple hypotheses about the cause of osmosis and present a tentative explanation and model of the phenomenon.

○ Historical Events Regarding Osmosis

In 1837, Dutrochet measured the osmotic pressures of dilute solutions by placing an animal bladder over the end of a thistle tube containing sugar solution then immersing the membrane end in water. He referred to his results as the attraction of water by the solute (Hammel, 1999). The term "osmosis" was first offered by British chemist Thomas Graham in 1854, and was first meticulously studied by a German plant physiologist, Wilhelm Pfeffer, in 1877. Jacobus van 't Hoff won the first Nobel prize in Chemistry in 1901 for his discoveries in chemical equilibrium and osmotic pressure (Nobel, 2017). He provided the first thermodynamic derivation describing osmotic pressure as being generated across a semipermeable membrane (Kiil, 1989), and determined that osmotic pressure had the same colligative properties as vapor pressure lowering, where the solution depends on the mole fraction of the nonvolatile solute and not the nature of the solute itself.

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In 1903, George Hulett found that when external pressure and temperature are applied to solute dissolved in water, the water in solution and pure liquid water change in an equivalent manner. He characterized osmosis as the internal tension in the force bonding the water (Hammel, 1999). Harmon Morse received the Avogadro Medal in 1916 for improving van 't Hoff's work (Remsen, 1923). The Morse equation (Equation 1)—where i is the van 't Hoff factor representing the activity coefficient, M is the mobile solute concentration, R is the gas constant = 8.3 J/mol · K, and T is the absolute temperature—predicts osmotic pressure.

$$\pi = iMRT \qquad \text{(Eq.1)}$$

Molecular kinetic theory of osmosis evolved between 1900 and 1950 and was first published in 1951 (Moos & Freeman, 1951). Solvent tension theory peaked in the 1970s (Kramer & Myers, 2013). Peter Agre won the 2003 Nobel Prize in Chemistry (Nobel, 2017) for identifying aquaporin water protein channels. This provided a molecular basis for water transport in living organisms. Agre's pioneering research brought about discoveries of evolutionary relationships among aquaporins from bacteria, yeast, plants, and mammals (Gomes et al., 2009).

○ Theoretical Framework: Knowledge types

Students' knowledge of osmosis manifests itself as declarative and procedural. Declarative knowledge refers to "knowing the answer to" and procedural refers to "knowing how to" (Gagne, 1985). Each knowledge type has corresponding question types that best elicit that knowledge. Declarative knowledge questions ask for recall of specific information. Procedural knowledge is associated with two question types: descriptive questions, which can be used to identify or describe interesting phenomena, and causal questions, which can used to ask why a phenomenon occurs. Causal questions ask students to construct multiple hypotheses to provide tentative explanations of the process of osmosis. Hypothesizing is an integral part of the nature of learning and science, requiring logical reasoning that facilitates the connection evidence and assumptions with scientific conclusions (AAAS, 1990). Learning is more complete when procedural knowledge is used to construct declarative knowledge (Lawson, 2001).

○ Methods of Science

There is not just one "scientific method." There are many methods of science. Science involves questioning, experimentation, observation, description, and other processes, but does not include fixed processes. Ecologists may not do classical experimentation because they cannot control the environment, just as a geologist cannot control the earth's mantle. On the other hand, biochemists can change the concentration of a solution and observe the effects on cells lending itself to experimentation (Crowther et al., 2005).

In each case, questions and investigations lead to conclusions, but the pathway may be different. Below is a pathway we selected to help advanced high school biology teachers and college biology instructors facilitate examination of osmosis. The questions, observations, conclusions, and hypotheses are subject to change as evidence and information about osmosis is accumulated, reflecting the nature of science and the many modes of knowing.

○ Declarative Knowledge Questions

Traditionally, declarative knowledge has been taught and assessed with end-of-unit exams and standardized tests. Success on such tests depends on recall of specific information. Of course, a declarative question may be interesting, yet many things remain to be discovered. Questions such as "What is a solution?" or "What are solvents and solutes?" may represent important knowledge but do not invoke critical thinking in students. Good research questions require thinking skills and knowledge construction based on both indirect and direct data, logic, analogy, controlled experiments, and many other process skills (Lawson, 2001). Conversely, declarative knowledge questions simply require recall of established ideas.

An example of a declarative question might be "What is an aquaporin?" Answers to such questions can be found in books, electronic resources, periodicals, and prior experience. Thinking skills are not required to answer the question—either you know the answer or you don't. Usually the answer is clear and agreed upon by scientists—declarative knowledge of well-established hypotheses, theories, laws, and facts.

○ Using Procedural Knowledge to Answer Descriptive Questions

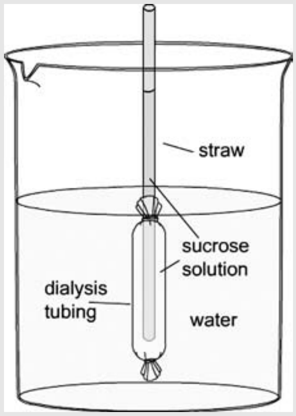
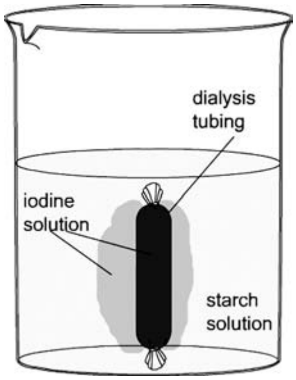
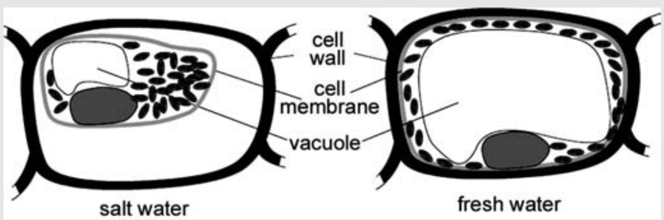
Many descriptive questions call for information and data collection beyond what traditional textbooks offer, and require procedural knowledge to design experiments, control variables, collect and analyze data, draw conclusions, and present findings.

Descriptive questions are excellent for guiding identification and discovery of important scientific phenomena, and make good initial research questions. They ask for systematic observations resulting in a data-supported conclusion. What happens when dialysis tubing, which is tied off at one end with a straw sticking out the other end, containing sugar (sucrose), is placed in tap water? Of course, the question could be answered with a textbook, but the question can also be used to guide systematic observation and quantification to reveal interesting phenomena. Suppose during initial observations, water rose into the straw. It would be unwise to draw conclusions without first conducting a controlled experiment based on a descriptive question.

Table 1 provides three descriptive questions about osmosis with possible observations. Consideration of these questions may be necessary to begin construction of a model about osmosis.

- Question(s) 1: (a) What is the effect of sucrose concentration on the height that water rises in the straw? Or, (b) What is the correlation between sucrose concentration and the height that water rises in the straw? Or, (c) What happens to the height of the water when sucrose concentration increases? Answering these question(s) will require a carefully designed experiment in which variables are identified and controlled. For these questions, the independent variable is the sucrose concentration, and the dependent variable is the height of water rise. Constants include use of the same type of dialysis

Table 1. Descriptive questions about osmosis.

Question (Q)	Laboratory set-up	Observation (O)
Q1. What is the effect of sucrose concentration on the height of water rise in the straw?		O1. Solutions of different concentrations separated by dialysis tubing produces pressure, as evidenced by the solution rise in the straw.
Q2. What happens when iodine solution, placed in dialysis tubing with each end tied off, is put in starch solution?		O2. Dialysis tubing is selectively permeable to some molecules, as evidenced by the starch indicator of iodine.
Q3. What happens to cells placed in salt water?		O3. Salt water increases and fresh water reduces osmotic pressure, as evidenced by the change in the vacuole.

tubing, the same placement of the tubing in the water (depth of water), and the same sucrose solution temperature. A control is a standard for comparison—students could use tap water in the dialysis tubing as a control. The procedure for collecting data is developed after variables are defined and data tables are constructed.

- Possible observations 1: The higher the sucrose concentration, the higher the water rises.
- Possible conclusions 1: Dialysis tubing containing solution, tied off at one end and placed in water, results in pressure, causing the solution to rise in the straw.

- Question 2: What happens when iodine solution, placed in dialysis tubing with each end tied off, is put in starch solution?
  - Possible observations 2: Dialysis tubing containing iodine, tied off at each end and placed in starch solution, produces a purple-black color in the starch solution. Dialysis tubing containing starch solution placed in iodine provides evidence that only iodine is moving through the tubing because the starch inside the dialysis tubing turns a purple-black color.
  - Possible conclusions 2: Dialysis tubing is selectively permeable to some molecules.

- Question 3: What happens to cells placed in salt water?
  - Possible observations 3: *Elodea* leaf cells can be observed under a light microscope to shrivel in salt water and enlarge in tap water.
  - Possible conclusions 3: The salt water increases and tap water reduces pressure across the *Elodea* leaf cell's membrane.

Descriptive questions may require statistical analyses, which could range from visual inspection of central tendency and comparison groups to hypothesis testing in more advanced students. Graphing, data organization, and presentation, as well as basic technology skills (using spreadsheets and statistical software) can be facilitated as students generate and investigate descriptive and correlative questions.

Constructing a Scientific Model of Osmosis

Scientific modeling is an important tool for helping students understand, define, quantify, visualize, or simulate scientific phenomena based on evidence and reasoning (Treagust et al., 2002), and has received great emphasis in the NGSS Scientific Practices and Crosscutting Concepts (NGSS Lead States, 2013). Data collection, pattern recognition, and proposing, revising, or defending explanatory models can occur after or concurrently with data collection and analysis. Models may include drawings, diagrams, computers, mathematical derivations, and prose.

The scientific evidence summarized in Table 1 supports the following simple model (Figure 1):

- Dialysis tubing is selectively permeable to some molecules.
- Dialysis tubing containing solution and placed in tap water produces pressure.
- A solution of water (e.g., with sugar or salt) increases pressure, whereas plain water reduces pressure.
- In Figure 1a, water will move into the dialysis tubing, and water will move out of the dialysis tubing in Figure 1b.

Using Procedural Knowledge to Answer Causal Questions

If interesting phenomena are revealed, the next logical step is to formulate a causal question and tentative hypotheses. For example, after careful observations and data analysis, a student may conclude that the higher the solution concentration, the higher the pressure (water rises in straw). The resulting causal question may be: Why does the water rise higher with increasingly higher sucrose concentration?

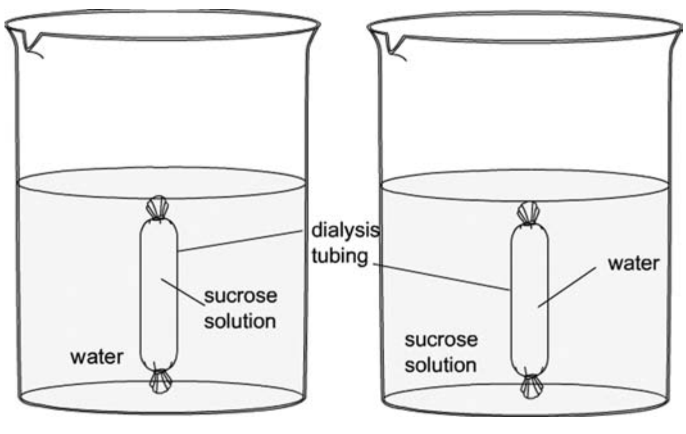


Figure 1. Model of osmosis.

Unlike descriptive questions, causal questions ask why a phenomenon occurs. Like descriptive questions, causal questions require procedural knowledge of experimental design, including controlling variables, collecting and analyzing data, drawing conclusions, and presenting findings. Experimental design is not limited to laboratory conditions. Some scientists use thought experiments based on scientific laws and embedded theories. Thought experiments play important role in scientific inquiry (Gendler, 1998), and have been used to explain osmosis (Kramer & Myers, 2013; Hammel & Schlegel, 2005). According to Kiil (1989, p. R806), Arrhenius conducted a thought experiment with "analogy by showing that the same expression for osmotic potential is obtained if a membrane separates a permeant from an impermeant gas."

Formulating hypotheses is perhaps the most important activity associated with exploration of causal phenomena. Providing plausible explanations of phenomena is a high point of critical thinking and problem solving, and should be a major goal of science instruction. However, this can be challenging. Exploring descriptive questions first may facilitate formulation of a causal question. Multiple hypotheses are constructed to explain the phenomenon, experiments are designed and conducted to test students' hypotheses, expected outcomes determined, and conclusions drawn. It should be emphasized to students that hypotheses are not best guesses but plausible explanations based on prior knowledge, logic, and observations.

○ Writing Multiple Hypotheses: What Causes Osmosis?

What causes osmosis, which students observe as water rising in the straw? The next step is to brainstorm multiple hypotheses. Does water simply diffuse down its diffusion gradient from high to low concentration? Or do solute particles block the water from moving back through the membrane? Perhaps intermolecular forces are stronger in solutes, or solutions have greater entropic forces than pure water. Other experience-derived knowledge that is necessary to understand osmosis includes pressure, characteristics of solvents, solutes and solutions (colligative properties), intermolecular forces, and enthalpy and entropy.

Table 2 offers a standard way to write a hypothesis with the "If, because, and, and then" model. "If" describes the phenomena, "because" the hypothesis, "and" the experiment, and "then" the expected outcome if the hypothesis is supported (Lawson, 2003). This model provides structure for organization by defining the boundaries of an experiment. Hypothesizing is not guessing but providing a possible explanation supported by logic and prior experience. If the "if/then" method for writing a hypothesis is too basic for college biology students, then we recommend providing the hypotheses and asking students to support each hypothesis with background knowledge and/or design experiments to test each hypothesis.

Hypothesis 1

If water diffuses down its diffusion gradient from high to low concentration because the concentration of water is higher in pure water than in a solution of nonvolatile solute particles, then there should be no water movement (osmosis) when 0.2 molal sucrose

Table 2. Writing formal hypotheses.

"If" describes the phenomena	"If" water diffuses down its diffusion gradient from high to low concentration
"because" the hypothesis	"because" the concentration of water is higher in pure water than a solution of nonvolatile solute particles
"and" the experiment	"and" we separate 0.2 molal sucrose and 0.2 molal sodium chloride by a membrane permeable to only water
"then" the expected outcome if the hypothesis is supported	"then" there should be no water movement.

solution and 0.2 molal sodium chloride solution are separated by a membrane permeable to only water.

Possible observations and conclusions: Adding the same amount of solute can result in different concentrations of water. In an experiment, if 50 ml of water is added to 50 ml of water, the final volume is 100 ml. But if 50 ml of ethanol is added to 50 ml of water, the volume of the mixture is less than 100 ml because the ethanol molecules are smaller and fit between the molecules of water. Similarly in a thought experiment, we could consider the case where a 0.2 molal sucrose solution has a water concentration of 958 g/L, and 0.2 molal NaCl solution has a water concentration of 995 g/L. If diffusion of water were responsible for osmosis, then NaCl would have a greater osmotic pressure than sucrose solution. Kosinski and Morlok (2009) found that a potato core takes up water from sucrose solutions and loses water in NaCl solutions. If water concentration causes osmosis, then sucrose should gain water from NaCl solution when separated by a selectively permeable membrane.

Hypothesis 2

If water diffuses down its diffusion gradient from high to low concentration, then osmosis should occur at the same rate as diffusion of water.

Possible observations and conclusions: In a thought experiment, we define diffusion flux as the rate of movement of molecules across a unit of area. The coefficient for diffusion of water in water is approximately $10^{-5} cm^2/s$. Osmotic flux of water is two to six times larger than flux driven by a water concentration gradient, as determined by water diffusing in red blood cells, as compared to water flux resulting from osmosis (Kiil, 1989; Kramer & Myers, 2013).

Hypothesis 3

If water moves from high to low concentration because nonvolatile solute particles block the water from moving through the membrane, then large biomolecules (e.g., hemoglobin) should have a greater osmotic effect than small molecules (e.g., NaCl).

Possible observations and conclusions: Masterson, Slowinski, and Stanitski (1981) noted that water has a tendency to move from high vapor pressure to low vapor pressure, which is responsible for osmosis. A lab experiment using a lower vapor pressure as a corollary could be conducted by placing a few corks on the surface of water. Blocking the surface does not reduce the rate of evaporation (Peckham, 1998). Another thought experiment: hemoglobin molecules have a partial molar volume over 700 times the size of water, yet the number of solute particles, not the size of the solute particles, determines osmotic pressure according to the Morse equation.

Hypothesis 4

If water moves from high to low concentration because the intermolecular forces are stronger in solutes, then enthalpy should be greater for a solution than for pure water.

And similarly,

Hypothesis 5

If water moves because of entropic force, then solutions should have greater entropy than pure water.

Possible observations and conclusions: Consider a thought experiment. According to Masterson et al. (1981), only if all of the reactants and products are gases can a change in enthalpy be obtained from bond energies, making osmotic pressure a result of entropy effects (Peckham, 1998). Kiil (1989) stated intermolecular forces cancel each other out and do not effect osmotic potential. Kramer and Myers (2013) held that water flow relies on Brownian motion to carry solute molecules away from the aquaporin aperture of a membrane pore, causing an imbalance of forces resulting from entropy (Lambert, 2002).

○ A Tentative Explanation and Model of Osmosis

Conclusions drawn about newly formed hypotheses should always be considered tentative. Students should be encouraged to consider variables that have not been controlled, potential confounding variables, and potential errors that may have occurred during the experiment, including sampling errors. Additional research may be necessary to support or refute tentative hypotheses—the process of scientific inquiry is never-ending and the terms are never final. Although full consensus about the process of osmosis has yet to be reached across the scientific community, we believe that teachers can assist students in developing a model of osmosis as a physical process that consists of molecular interactions. We describe this learning target as follows.

Osmosis is not caused by diffusion of water or from particles blocking the membrane pores. Nor does the size of the solute molecules or the intermolecular forces cause osmosis. Osmosis occurs because solutions have greater entropy than pure water. Leff (1996) characterized entropy as the degree of energy spreading and sharing. Entropic spreading and sharing of energy is maximal at thermodynamic equilibrium, and Sachs and Sivaselvan (2015) indicated that osmotic equilibrium is reached when the entropic energy $T\Delta S$ for water movement is equal to $P\Delta V$, where P is the hydrostatic pressure of water in the free volume, ΔV.

Kramer and Myers (2013) described osmosis as bulk flow resulting from membrane-driven fluid flow by rectification of Brownian motion of water through the hydrophilic channel of an aquaporin.

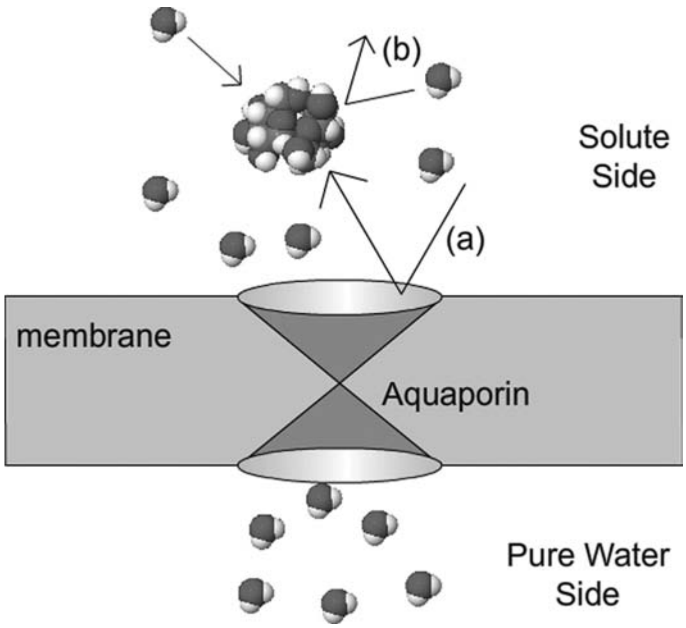


Figure 2. Hydrophilic channel with an aquaporin present.

The challenge to our intuition is the assumption that concentration gradients themselves somehow cause pressure gradients. The Morse equation does not refer to actual pressure but to the pressure needed to stop osmotic flow. Pressure requires a physical force, meaning osmotic pressure can only arise if there is a physical object, the semi-permeable membrane, present to apply force to the solute particles. Only when solute molecules have had a chance to diffuse to the membrane will the latter begin to rectify their Brownian motion and so transmit force to them, and the fluid. The force and fluid displacement requires work. The source of energy for the work of rectification is from a decrease in order on the solute side (Nelson & Doniach, 2004)—in other words, increased energy spreading and sharing. Consequently the second law of thermodynamics is not violated. Figure 2 provides an illustration of the force exchanged when pure water is separated from a solution of water with a hydrophilic channel of an aquaporin present. Only water is allowed to travel through the pore in single file (Agre, 2006). Momentum is exchanged by random collisions between the membrane and solute (Figure 2a), and neighboring solvent and solute molecules (Figure 2b).

Due to Brownian motion, particles are randomly moving on both sides of the membrane. When a solute particle comes in contact with the membrane, energy is exchanged, shifting the direction of momentum. The momentum is spread and shared (entropy) with water molecules, directing water away from the membrane. The membrane exerts a force on the solution as a whole, increasing the hydrostatic pressure, which results in bulk flow of water through the aquaporin.

○ Conclusions

Osmosis is an excellent topic to guide systematic observation, experimentation, reasoning, and hypothesizing. Historical events regarding research about osmosis demonstrate the tentative nature of science and explaining scientific phenomena. Descriptive questions are necessary to describe osmosis. Descriptive questions provide structure for model construction and writing causal questions. Scientific modeling is an important communication tool, helping students visualize or simulate scientific phenomena based on evidence and reasoning.

Writing multiple hypotheses and providing tentative explanations is a pinnacle of critical thinking and problem solving, and should be a major goal of instruction. Formulating questions and guiding experimentation about osmosis will be challenging, but it can be used as a model for "teaching fewer concepts with greater depth," resulting in better understanding than covering osmosis more superficially. "Students will gain the skills of inquiry and scientific attitudes desired by the standards, and gain greater knowledge of how scientific research is actually conducted" (NSTA, 2003, pp. 18–19).

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