A SIMPLE STUDENT LABORATORY ON OSMOTIC FLOW, OSMOTIC PRESSURE, AND THE REFLECTION COEFFICIENT

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Osmosis is usually taught from the point of view of the osmotic pressure developed when solutions of different concentrations of solute are separated by an ideal semipermeable membrane. The osmotic pressure is defined at equilibrium when there is no net flow, and it takes some time to reach this equilibrium. Although the osmotic pressure is certainly important, teaching only this point of view implicitly diminishes the importance of osmotic flow, which begins almost instantaneously across a membrane. A device was constructed with which students could measure the flow across a model membrane (dialysis tubing) as a function of concentration for solutes of different sizes. The device produced flows that were linearly proportional to the concentration, providing a confirmation of van’t Hoff’s law. Separate student groups repeated these experiments using both different solutes and different dialysis membranes. The combined results of four student groups showed that the flow across these nonideal membranes depends on the solute and membrane as well as the concentration of solute. Given a value for area times filtration coefficient \(A \times L_p\) for the membranes (determined beforehand by their instructor), the students could calculate the reflection coefficient \(\sigma\) for three solutes and two membranes. The results showed that large solutes had large \(\sigma\) and that less porous membranes had larger \(\sigma\). A concurrent demonstration using this device and membranes showed that the osmotic flow can generate large pressures. These experiments and demonstration provide a balanced view of osmotic flow and pressure.

Key words: artificial membranes; dialysis; sucrose; polyethylene glycol

The primary observations of osmosis were originally reported by Pfeffer in 1877 (5). Pfeffer made an artificial membrane in the walls of an unglazed porcelain cup by reacting copper salts with potassium ferrocyanide. He placed a sucrose solution inside the cup and water outside, and he found that water moved from the water side to the sucrose side. He then made three important observations. First, he found that the rate of flow was proportional to the sucrose concentration. Second, he observed that a pressure applied inside the cup produced a filtration flow proportional to the pressure. Finally, he found that a closed cup containing a sucrose solution would develop a pressure proportional to the sucrose concentration. He defined the osmotic pressure as the pressure applied to the solution side that is necessary to stop the osmotic flow. This definition is restricted to osmosis across an ideal, semipermeable membrane that allows water to pass but is impermeant to the solute. The pressure defined in this way holds only at equilibrium, that is, when there is no net fluid movement. The magni-
tude of the pressure is then given by van't Hoff's law

$$\pi = R T C$$

where \( \pi \) is the osmotic pressure, \( R \) is the gas constant, \( T \) is the absolute temperature, and \( C \) is the concentration of impermeant solute.

Pfeffer's result, that the flow is linearly related to the concentration, allows us to write the equation

$$Q_v = -AL_p \Delta \pi$$

where \( Q_v \) is the flow of volume across the membrane (in units of \( \text{cm}^3/\text{min} \)), \( A \) is the area of the membrane (in \( \text{cm}^2 \)), and \( L_p \) is variously called the filtration coefficient, hydraulic conductivity, or hydraulic permeability. The minus sign in this equation indicates that the water flow is from the region of low osmotic pressure (water, \( \pi = 0 \)) to the region of high osmotic pressure (sucrose, \( \pi = R T C \)). In the absence of solute, Pfeffer found that the filtration flow is proportional to the hydrostatic pressure (\( \Delta P \))

$$Q_v = A L_p \Delta P$$

Results of experiments (4) and theoretical results from irreversible thermodynamics show that the \( L_p \) in Eq. 2 is identical to the \( L_p \) in Eq. 3. The net flow in the presence of both a pressure and osmotic gradient is

$$Q_v = A L_p (\Delta P - \Delta \pi)$$

In the case of nonideal membranes, which are permeable to solute, the observed osmotic pressure is reduced because of the ability of the solute to penetrate the membrane, albeit less effectively than water. This has led to the notion of the reflection coefficient (\( \sigma \)), originally defined as the ratio of the observed osmotic pressure to the pressure calculated from the van't Hoff law for an ideal membrane. In the case of the nonideal membrane, the flow equation is

$$Q_v = A L_p (\Delta P - \sigma \Delta \pi)$$

From the foregoing discussion, it is clear that the osmotic pressure is an important concept that describes only one of the forces driving fluid flow across membranes. It is the flow that is physiologically important and results in cell swelling or shrinking. The swelling or shrinking requires fluid movement, which occurs at some quantitative rate. These concepts are not easy to convey and are often omitted from the usual laboratory exercises. Attempts to teach osmosis range from using animated films (2) to computer simulations (7) and weighing potato cubes with time (1). A recent laboratory manual describes a simple "osmometer," consisting of a one-hole stopper inserted into dialysis tubing and secured with string (11). The open end of the tubing is also tied, and then a 1-ml pipette is inserted through the hole in the stopper. The dialysis tubing thus forms a compartment that is open to the air only through the 1-ml pipette. The dialysis tubing is then placed in a 1-liter beaker of water, and the dialysis tubing is filled with either 0, 15, or 30% sucrose solutions, and the position of fluid rise in the pipette is recorded with time. The students then plot the volume of fluid movement with time and obtain the flow rates. The results should confirm that the flow is twice as great with 30% sucrose as with 15% sucrose, in agreement with van't Hoff's law.

A variant of this laboratory exercise was the starting point for the development of the exercise described here. We found that it was difficult to seal the dialysis tubing successfully, the results were not reproducible from group to group, and flow was often nonlinear because of the compliance of the dialysis tubing. Because our goal was to offer an exercise investigating osmotic flow, osmotic pressure, and the reflection coefficient, we concluded that it was necessary to prevent the hydrostatic pressure developed by a vertical column of fluid (thereby eliminating the problem of compliance of the dialysis tubing compartment) and to accurately control the surface area of the exposed dialysis membrane. Thus the laboratory exercise described herein was developed to give students a hands-on appreciation for the origin of osmotic pressure and flow, and an understanding of the importance of the interaction of the solute and membrane in producing these quantities. In addition, the exercise was meant to encourage a simple quantitative analysis of laboratory data that could be used to obtain meaningful parameters describing osmosis, including the
hydraulic conductivity and the reflection coefficient for particular solute-membrane pairs.

THE FLOW APPARATUS

The main problems in accurately measuring flow across membranes are to maintain a steady hydrostatic pressure, expose a reproducible membrane area to the solutions, and prevent leaks in the membrane. The device (Figs. 1–3) surmounted these difficulties. Here a known area of dialysis tubing separates a solution inside the tube from pure water outside. The membrane could be sealed by simply tying it off, but then it would be difficult to produce a known area of membrane. Therefore the membrane was sealed at both the top and bottom by an O ring and clamp, where the O rings were seated in a machined groove. To save time in the laboratory, the membranes were installed beforehand by the instructor. Spectrapor membranes, 29 mm in diameter and of 3,500 and 1,000 mol wt cutoff (MWCO), were used in all of the exercises. A hollow, truncated Delrin cone was used to assist in installing the membranes and O rings (Fig. 2), and excess membrane was cut off. To test for leaks, the inner chamber was filled with water, and then a 60-ml

Apparatus for measuring osmotic flow. The horizontal flow tube was calibrated in centimeters and was supported in a level position by the lip of the beaker and a clamp on a ring stand. The membranes used were Spectrapor 1,000 or 3,500 mol wt cutoff (MWCO). The membranes were sealed using an O ring and a metal circular clamp at the top and bottom of the tube. The O rings were seated in grooves machined in both the clamp and the plastic supports, thereby providing a well-defined surface area for the membrane. Leaks were tested (A) by applying a pressure using a 60-ml syringe. When no leaks were detected, the syringe was removed and replaced with the horizontal flow tube. The water solution in the inner compartment was removed and replaced with test solution (B). After filling, the filling syringe was removed, and the position of the solution in the horizontal tube was recorded at regular time intervals (C).
Dimensioned drawings of the body, ring clamps, and assembler. The ends of the body were fabricated from methyl methacrylate plastic. A, inside the 3/8”-diameter hole was an O ring, PRP-568-012, 3/8” ID x 1/2” OD x 1/16” cross section. B, semicircular groove for O ring was 1/64” deep and 1/16” wide; O ring was PRP-568-018, 3/4” ID x 7/8” OD x 1/16” cross section. C, 30° relief angle. D, rods connecting the ends were 1/8” stainless steel. Ring clamps were made from aluminum. O ring shown with the ring clamp is the PRP-568-018 O ring as described in B. The groove in the ring clamp to receive the O ring was 0.095” wide and 0.055” deep. All edges on the ring clamp were rounded to avoid cutting the dialysis tubing. Screws for ring clamp assembly were 4-40 socket head cap, 1/2” long. The assembler was constructed from acetal plastic (Delrin). The 30° angle and dimensions of the assembler were designed to fit over either end of the body to aid in placing the dialysis tubing over the body.

Luer lock plastic syringe was fitted to the opening for the flow tube (Fig. 1A). This opening was sealed with an O ring and fit the 60-ml syringe snugly. Leaks were tested by adding a large pressure via the syringe. About one in four membranes leaked and were replaced. In our experience, the leaks were due not to unsuccessful seals around the O rings but to scratching the membrane and producing a hole during assembly. After testing for leaks, the horizontal flow tube assembly was attached (Fig. 1A and Fig. 3). The water in the inner compartment was removed and replaced with test solution, as shown in Fig. 1B. The test solution was added until it began to flow down the horizontal tube; the fill syringe was then removed and flow measurements were begun. During measurement of flow, the inlet aperture was left open to the atmosphere. Thus the pressure on the fluid in the horizontal calibrated tube was maintained at atmospheric pressure while fluid flowed down the tube. The tube was narrow enough so that the moving water column had a vertical meniscus that could easily be read on the scale.
Students were instructed to fill the dialysis sac until there was some fluid already in the tube, and to avoid air bubbles anywhere in the flow path because these would interfere with the flow measurements. Although flow began almost instantly, the students were instructed to wait for up to 5 min before beginning their measurements.

THE SOLUTIONS

The solutes used in these experiments were urea, sucrose, and polyethylene glycol (PEG). Both urea and sucrose are readily available and solutions are straightforward, but a note of caution is needed for the PEG. PEG, with an average molecular weight of 3,350, was obtained from Sigma Chemical (catalogue no. P-3640). However, this is a weight-average molecular weight. Osmotic experiments depend on the number-average molecular weight, which is smaller than the weight-averaged molecular weight for a polydisperse material. A number-average molecular weight of 2,400 was used for this material. It is probably unnecessary to explain this complication to students, but a discussion of the issue could be raised if desired (8). Stock solutions of 250 ml were made, and ~ 55 ml filled the devices.

DETERMINING FLOW RATES

Students were assigned to one of four groups, each of which had a corresponding set of test solutions and a single test membrane. Each group was instructed to measure the position of the meniscus at 5-min intervals and to record the result in tables provided in their handout. The students also wrote their observations on the chalk board as they progressed so that the data would be immediately available to all students. Students plotted the distance the meniscus traveled against the elapsed time. The results of duplicate laboratory sessions performed in 1993 and 1994 for the 1,000-MWCO membrane are shown in Fig. 4. The flow was routinely linear with time and was reproducible from group to group and from year to year. Although average numbers are used here, the data are sufficiently reliable that single runs will provide similarly quantitative and identically qualitative re-
I N N O V A T I O N S  A N D  I D E A S

FIG. 4.
Position of meniscus in the flow tube related to elapsed time. Position at time 0 was subtracted so that all flows would begin on the origin. Flow is shown for 0.75 M sucrose (○), 0.50 M sucrose (●), 0.25 M sucrose (□), 0.075 M PEG (△), 0.0375 M PEG (□), and 1 M urea (•). The membrane was Spectrapor 1,000 MWCO. Values are means ± SE of 4 separate determinations.

The data in Fig. 4 show that the flow increased with concentration for a given solute. Similar results were obtained for the 3,500-MWCO membrane.

The slope of the lines in Fig. 4, in units of cm/min, can be converted to the flow (in units of cm³/min) by multiplying by the cross-sectional area of the tube (0.09 cm²). The students were provided with the cross-sectional area and instructed to calculate the flows, record them in a table (Table 1), and plot the flow against the concentration. The results for the 1,000-MWCO membrane are shown in Fig. 5. The data clearly show the linear relationship between flow and concentration (in mol/l) and that this relationship was different for urea, sucrose, and PEG. Similar qualitative results were obtained for the 3,500-MWCO membrane.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Membrane A: 1,000 MWCO</th>
<th>Membrane B: 3,500 MWCO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Δx/Δt cm/min</td>
<td>Qv cm³/min</td>
</tr>
<tr>
<td>Sucrose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25 M</td>
<td>0.462</td>
<td>0.0416</td>
</tr>
<tr>
<td>0.50 M</td>
<td>0.930</td>
<td>0.0837</td>
</tr>
<tr>
<td>0.75 M</td>
<td>1.328</td>
<td>0.1195</td>
</tr>
<tr>
<td>Urea, 1 M</td>
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<td>0.0022</td>
</tr>
<tr>
<td>PEG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0375 M</td>
<td>0.308</td>
<td>0.0277</td>
</tr>
<tr>
<td>0.075 M</td>
<td>0.810</td>
<td>0.0729</td>
</tr>
</tbody>
</table>

MWCO, molecular weight cutoff; PEG, polyethylene glycol. The position of meniscus was plotted vs. time to obtain velocity of column movement or slope ($\Delta x/\Delta t$), which was multiplied by cross-sectional area (0.09 cm²) to obtain flow ($Q_v$). Data were calculated from averages of 4 separate determinations obtained by students.

THINKING INTERLUDE

At this point, the students can be asked several questions:

1) Is there a relationship between the osmotic flow and the concentration of sucrose? If so, write an equation for this relationship.

2) Is there a relationship between the osmotic flow and the concentration of PEG? If so, write an equation for this relationship.

3) Is there a relationship between the osmotic flow and the concentration of urea? If so, write an equation for this relationship.

4) Are the relations between flow and concentration different for sucrose, PEG, and urea? Why?

In this section, the instructors helped the students realize that their observations were consistent with the relation

$$Q_v = -AL_p\sigma RT\Delta C$$

where $Q_v$ is the flow (measured in cm³/min), $A$ is the area (in cm²), $L_p$ is the hydraulic conductivity or
filtration coefficient, $\sigma$ is the reflection coefficient, $R$ is the gas constant, $T$ is the temperature, and $\Delta C$ is the concentration difference across the membrane. To calculate $\sigma$ from data of flow and $RT\Delta C$, it was necessary to first determine $A \times L_p$.

DETERMINATION OF $A \times L_p$

The pressure-driven flow of water across the model membrane was determined using the device shown in Fig. 6. The entire device was submerged in water, so water was in both the inner and outer compartments and there was no osmotic pressure difference. In this case, the net water flow is given by Eq. 3 (3). The device used to measure $A \times L_p$ was identical to that described earlier in Fig. 1, except that the outlet tube was replaced with a four-way stopcock, and the membrane was mechanically reinforced with stainless steel mesh. This allowed the development of a significant hydrostatic pressure difference across the membrane. Water was infused using a Harvard syringe pump at a constant and known rate. The pressure in the inside compartment rose, driving fluid out through the dialysis membrane. Eventually a steady state was reached in which the rate of infusion of water into the inner compartment was exactly equal to the rate of water exit through the dialysis membrane. This occurred at some pressure difference between the inner and outer compartments. This pressure was measured by a pressure transducer connected to a Grass polygraph. The plot of flow against pressure for the 3,500-MWCO and 1,000-MWCO Spectrapor membranes is shown in Fig. 7. The slopes of the lines give $A \times L_p$ (in units of cm³·mmHg⁻¹·min⁻¹). The area of the membrane was 90.5 cm², but this variable always appeared in conjunction with $L_p$, and the product $A \times L_p$ was the parameter of interest.

CALCULATION OF $\sigma$, THE REFLECTION COEFFICIENT

The students were asked to calculate the reflection coefficients for urea, sucrose, and PEG for both the 1,000-MWCO and 3,500-MWCO membranes by use of the flow rates they had calculated and shown in Table 1 and the value of $A \times L_p$ provided to them in the handout. The results of the calculations (Table 2) allow several conclusions. First, the reflection coefficient, $\sigma$, appears to be independent of the concentration of solute. Second, $\sigma$ depends on the solute, being smallest for urea, larger for sucrose, and larger still for PEG. Third, $\sigma$ depends on the membrane, being smaller in every case (urea, sucrose, and PEG) for the 3,500-MWCO membrane than for the 1,000-MWCO membrane. These observations make it clear that the phenomenon of osmotic flow depends on the characteristics of the membrane and of the solute.

The mechanistic interpretation of $\sigma$ depends on the kind of membrane involved in the osmosis. The microporous membrane is perhaps easier for students to visualize. In this model, the reflection
coefficient is viewed as being due to a steric hindrance of solute entry into a cylindrical pore oriented perpendicular to the surface of the membrane. Because a solute molecule is larger than the pore, then the solute cannot enter the pore, and all collisions of the solute with the membrane result in reflection of the solute molecule. The membrane is impermeant, and \( \sigma = 1.0 \) for this membrane. When the solute is smaller than the pore, it can travel across the membrane by going through the pore. Because the solute molecules are larger than the solvent water molecules, they are reflected back from the membrane more often than water. In this case, \( 0 \leq \sigma \leq 1.0 \), and the observed osmotic pressure is diminished. Various equations have been derived to relate \( \sigma \) to the geometric size and shape of hypothetical pores within membranes (3, 6, 10).

**DEMONSTRATION OF THE MAGNITUDE OF OSMOTIC PRESSURE**

In a separate demonstration, the device shown in Fig. 6 was filled with 1 M sucrose and placed in a beaker of pure water (Fig. 8). The outlet tube was closed to the outside air but connected by a fluid-filled cannula leading to a pressure transducer.
connected to a Grass polygraph. The demonstration was set up a few minutes before the laboratory, so that the students could see the full development of the pressure. The pressure increased nearly linearly to ~700 mmHg and then increased more slowly (Fig. 9).

The purpose of this demonstration was twofold, to demonstrate that the osmotic flow could produce a real pressure of considerable magnitude and to illustrate that the development of pressure in the bulk solution required time, whereas the development of osmotic flow at no pressure did not. The students were asked three questions in this regard:

5) How long did it take to establish steady-state flow for 0.75 M sucrose?

6) How long did it take to establish steady-state pressure with 1 M sucrose?

7) Explain the difference in the time to produce steady-state pressure compared with steady-state flow.

In the case of the flow experiments, the steady-state flow was established almost immediately, because all of the flow went down the calibrated tube and none went to expanding the compartment. In the case of the pressure demonstration, the osmotic flow caused an expansion of the inner compartment, which developed a pressure because of this expansion. Because the pressure results from this expansion, considerable fluid transfer was required for the pressure generation. Here the students were introduced to the concept of compliance, \( C = \frac{\Delta V}{\Delta P} \), the change in volume per unit change in pressure. The rapid development of steady-state osmotic flow is due to the fact that the pressure difference driving the flow is only within the pores of the thin dialysis membrane, and the compliance of this structure is small. The compliance of the whole inner compartment and dialysis membrane, however, is large, so a large volume must be transferred to produce the pressure. In both cases,
Setup for measuring the pressure developed by osmotic flow. After the inner compartment was filled with 1 M sucrose, the device was closed to air and inserted in a beaker full of water. The hose connected the inner compartment to a P23XL pressure transducer.

however, the pressure driving flow within the membrane itself was established rapidly.

KEY POINTS

This laboratory exercise illustrated the following key points for the students.

1) A membrane separating a solution from pure water will result in fluid movement from the pure water to the solution side. This is the principal observation of osmosis, but it is not an explanation of it.

2) This fluid movement can generate a pressure, which in this exercise was > 1,000 mmHg. The pressure that would be generated at equilibrium is the observed osmotic pressure. For a perfectly semipermeable membrane, one which does not let solute pass at all, the observed pressure at equilibrium would be the osmotic pressure. Because the membranes used in this laboratory exercise are not ideal semipermeable membranes, the pressure observed at equilibrium would be the effective osmotic pressure.

3) Osmotic flow depends on the concentration of solute (Figs. 4 and 5), because the effective osmotic pressure across the membrane is proportional to concentration (the van't Hoff Law), and the greater the osmotic pressure, the greater the flow.

4) Osmotic flow also depends on the kind of solute (Fig. 5). This is due to the different values of $\sigma$ for each solute-membrane pair. Thus $\sigma$ for PEG was
highest, \( \sigma \) for sucrose was next, and \( \sigma \) for urea was near zero.

5) The reflection coefficient, \( \sigma \), also depends on the characteristics of the membrane. The \( \sigma \) was always larger for the membrane containing small pores (the 1,000-MWCO membrane).

6) An equation can be written that describes fluid movement produced by both hydrostatic pressure (the kind of pressure produced by the weight of a column of fluid) and osmotic pressure. This equation is

\[
Q_v = AL_p(\Delta P - \sum \sigma \Delta \pi)
\]

Here the \( \Sigma \) indicates that the osmotic contributions of all solutes on both sides of the membrane need to be considered to obtain the total effective osmotic pressure difference across the membrane.

SUMMARY

The laboratory exercise described here provides a practical series of experiments that novice students can reliably perform within a 2-h laboratory period. The exercise provides a confirmation of van't Hoff's law while placing more emphasis on the osmotic flow than on the pressure. The students are able, within a 2-h period, to determine parameters, such as the reflection coefficient, which stress the interaction of both solute and membranes in generating the osmotic phenomenon.

The devices described here were designed and fabricated in collaboration with Mr. Tom Gentry in the Department of Biomedical Engineering at the Medical College of Virginia. Virginia Commonwealth University. The devices can be custom-made by contract with the Department of Biomedical Engineering. Arrangements can be made through the authors.

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