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# An Entropy-Based Approach to Introducing Osmotic Pressure

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**ABSTRACT** We present a new approach to teaching the concept of osmotic pressure in physical chemistry courses. Our route is different from the traditional derivation that hinges on equating chemical potentials. Instead, we resort to the equivalent, yet more intuitive, concepts of mixing entropy and free energy and use their relation to the second law of thermodynamics. Our strategy emphasizes the role of entropically driven forces, which are a principal, yet underappreciated, theme in physical chemistry and biophysics. In doing so, we have extended the available examples of entropic forces that can be introduced to undergraduate chemistry and biology students.

**KEY WORDS** osmotic pressure; entropic force; reversible work; free energy; instructional strategies

## I. INTRODUCTION

Many liquids spontaneously mix to form homogeneous solutions at thermodynamic equilibrium, with the predominant contribution to the free energy of mixing being the gain in entropy. This is an example of an entropic driving force often used to introduce the concept of entropy maximization to students studying thermodynamics for the first time; however, it is not immediately clear where in the mixing process a mechanical force can be generated. This contrasts many other examples where entropic forces have a clearer mechanical manifestation. These examples include the expansion of an ideal gas (1), elasticity of a rubber band (2, 3), macromolecular association through counterion release (4, 5), undulation forces acting between lipid membranes (6, 7), entropy of chelation in coordination complexes (8), and emergence of hydrophobic (9, 10) and depletion (11, 12) forces.

The entropic force driving the mixing of solutions becomes apparent by the action of an osmotic pressure. Historically, the concentration difference of solutes inside and outside of cells was first realized to produce a pressure gradient in plants, which leads to water flow (13–15). The driving force for this process is the tendency to increase entropy resulting from the mixing of solute and solvent. The flow of water continues until the concentrations equalize or until the force of hydrostatic pressure counteracts the flow. Thus, plant cells accumulate excess water that generates turgor pressure, defined as the hydrostatic pressure exerted by the cell's interior that presses the cell membrane against the cell wall (3). This force is generated by the difference in the osmotic pressure of the extracellular and intracellular

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media. The realization of an osmotic pressure and its analogy to the ideal gas law led to the first Nobel prize awarded in chemistry, which was presented to J.H. van 't Hoff in 1901 (16).

The value of introducing osmotic pressure to students can be emphasized by demonstrating its biological and biophysical significance. The intracellular osmotic pressure is actively regulated by transport of specific ions and other solutes across cell membranes (3, 17). This allows cells to remain in an isotonic state, whereby cells control water flux through the membrane to prevent the potentially lethal swelling or shrinkage of cells upon exposure to environments with different osmotic pressure (18). It was further found that unique cellular solutes, sometimes termed "osmolytes" or "compatible solutes," are tasked with regulating the osmotic pressure in cells (19, 20). For example, in marine animals (e.g., sharks), the combination of urea and trimethylamine N-oxide is found to counter the high salinity of the ocean (21). Other examples of osmoregulation include the activity of the antidiuretic hormone in human kidneys, the exertion of turgor pressure in plants, and the kinetic entrapment of water molecules in extremophilic microorganisms (17, 20, 22, 23).

Osmotic stress further underlies multiple biophysical mechanisms. Its action often dictates the structure of biological macromolecules under confinement, which is critically linked to their cellular function (24–26). For example, the folding of proteins and their self-association is affected by the addition of solutes (19, 27–29). Solutes occupy some of the space available to cellular macromolecules, reducing the overall number of possible configurations, thereby confining and crowding them; this consequence has therefore been coined the "crowding" or "excluded-volume" effect. The restriction of the volume available to macromolecules by added solutes generates a compacting force, sometimes termed a "depletion force," which in its simplest description is the osmotic stress (12). The osmotic pressure also plays a critical role as a relevant thermodynamic variable that governs the condensation of cellular organelles that lack membranes (30). Finally, the generation of osmotic pressure is used in a standard method that allows the measurement of intermolecular forces acting between macromolecules (31).

The link between water flow and osmotic pressure also has found widespread medical and technological implications. For example, the fluids used for irrigating wounds, kidney dialysis, and storage of contact lenses are all prepared with an osmotic pressure similar to that in cells, thereby preventing complications that may arise from secretion or absorption of excess water (32–34). In other medical treatments, the generation of an osmotic pressure gradient is beneficial. Examples include protection against cerebral edema by drawing water out of the brain (35) and the osmotically controlled rate of drug delivery into the gastrointestinal tract (36, 37).

Finally, students also can be made aware of the technological role of osmotic pressure in water desalination. In reverse osmosis, the pressure required to push water through a semipermeable membrane must be greater than the osmotic pressure gradient, which determines the energy consumption during water purification (38).

The classical experiment to measure the osmotic pressure, first introduced by botanist W. Pfeffer in 1877, includes a system containing two chambers: one holding an aqueous solution and the other pure water (13). The two chambers are separated by a semipermeable membrane that allows only water to pass through. The chemical potential of water in the chamber containing the solute is lower than that of pure water. Therefore, water passes spontaneously from the chamber containing the pure water toward the chamber with the solution. This process could potentially continue until all the water passes through because the chemical potential of the solution is always lower than that of pure water. However, if the level of the solution in the chamber rises because of the added water, the corresponding excess hydrostatic pressure counteracts the flow because the chemical potential increases under pressure. At equilibrium, the chemical potential of water in the two chambers is equal. The added hydrostatic pressure required to stop the flow of water is called osmotic pressure (1, 39, 40).

This description follows the standard way of introducing osmotic pressure to students in physical chemistry courses at the undergraduate level (1, 40, 41). It emphasizes the condition for equilibrium, requiring that the chemical potential of water be equal in the two chambers. It also directly relates osmotic pressure to hydrostatic pressure. In our experience, this correlation leads to students' bemusement because the addition of solute to water apparently has nothing to do with hydrostatic pressure. Even more relevant, the underlying thermodynamic mechanism that involves entropy and its maximization generally is not discussed when presenting osmotic pressure. Using chemical potentials, a concept that is nonintuitive to many students, further obscures the mechanism (42, 43). Indeed, many basic courses that introduce osmotic pressure, particularly to biology and biomedical majors, cannot assume students' prior knowledge of the chemical potential, making the discussion of the osmotic pressure even more challenging.

In recent years, we have adopted an alternative approach to presenting the osmotic pressure that emphasizes its entropic nature without directly involving the effect of hydrostatic pressure on the chemical potential. We show that the condition for equilibrium can be discussed in terms of reversible work required to transfer water to and from the solution. We also demonstrate the fundamental role of entropy in this process. This topic is presented after fundamental thermodynamic principles have been introduced, including the first and second laws, the Gibbs free energy, and some simple examples of Boltzmann entropy.

This approach circumvents the need to present chemical potentials before teaching osmotic pressure. In fact, in recent years, we have successfully presented the fundamentals of osmotic pressure to biomedical students without introducing the chemical potential. Moreover, we find that this approach is more readily understandable for most chemistry, biology, and biomedical students. An additional key advantage is that osmotic pressure can be presented as an example of entropic forces, with other examples being the expansion of an ideal gas, the elasticity of a rubber band, and the hydrophobic folding of a protein.

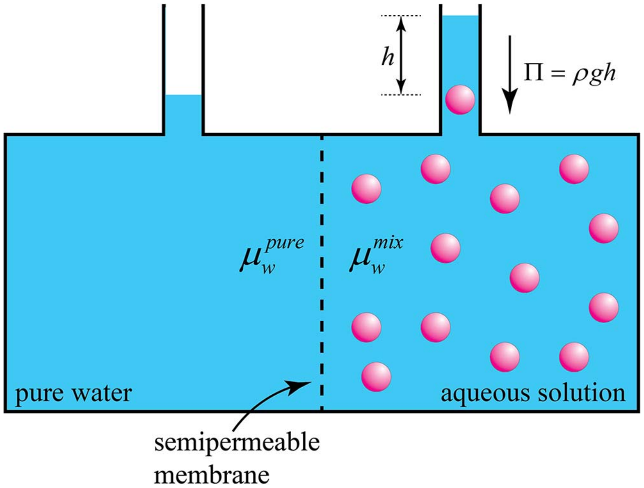

**Fig 1.** Schematic of pure water (left) and an aqueous solution (right) separated by a semipermeable membrane that allows only water to pass. The solute in the aqueous mixture is shown in pink. At equilibrium, the chemical potential of water is equal on both sides of the membrane. The difference in the solution levels in the two compartments results in a net applied pressure, $\Pi$, that counteracts the flow of water across the semipermeable membrane.

Here, we present our approach to introducing osmotic pressure. For comprehensiveness, we begin by recounting the current standard derivation found in most physical chemistry textbooks. We then introduce some necessary fundamental concepts that typically are presented earlier in a first course in thermodynamics or other introductory courses. These fundamentals include the derivation of expressions for the mixing entropy and free energy of ideal gases or solutions. In addition, we must familiarize students with the link between free energy and reversible work in isothermal processes. Having described these prerequisites, we present our new strategy to derive the osmotic pressure.

## II. STANDARD APPROACH: USING CHEMICAL POTENTIALS

In Figure 1, only water can pass through the semipermeable membrane so that at equilibrium, the chemical potential of water must be equal in the left (*l*) and right (*r*) compartments:

$$\mu_w^l = \mu_w^r \tag{1}$$

The chemical potential of water in an ideal mixture can be expressed as:

$$\mu_w^{mix}(T,P) = \mu_w^{pure}(T,P) + RT\ln x_w \tag{2}$$

where $\mu_w^{pure}$ is the chemical potential of pure water, $R$ is the gas constant, $T$ is absolute temperature, $P$ is pressure, and $x_w$ is the mole fraction of water in the mixture. This expression was proposed on the basis of experimental observations, culminating in Raoult's law (44). Many textbooks present Eq. 2 as an empirical finding without relating it to the free energy from which it can be derived (1, 40). Notably, Eq. 2 indicates that the chemical potential of water in a mixture will always be lower than that of pure water under the same conditions. This conclusion stems from the definite negative sign of the $RT\ln x_w$ term, which is related to the entropy gained (or reduction in free energy) from mixing solute and solvent. We will return to discuss this fundamental point in subsequent sections.

Combining Eqs. 1 and 2, we find that under the same temperature and pressure:

$$\mu_w^{l}(T,P) > \mu_w^{r,pure}(T,P) + RT\ln x_w \tag{3}$$

Thus, pure water from the left compartment should flow toward the mixture in the right compartment; however, when water is drawn from the left compartment, the water level, $h$, rises on the right. This leads to an added hydrostatic pressure, $\pi$, exerted on the solution. This pressure is proportional to the mass of the excess solution, $\rho\delta V$, where $\rho$ is the density of the mixture and $\delta V$ is the volume of excess solution. The weight of the excess solution column acts on a cross-sectional area $a = \delta V/h$ so that the acting pressure is $\pi = \rho g h$, with $g$ being the gravitational acceleration constant.

For dilute solutions, the excess water leads to a small added pressure, which increases the chemical potential of water by $\delta\mu_w \approx v_w\delta P$, where $v_w$ is the partial molar volume of water. At equilibrium, this increase in chemical potential exactly negates the decrease in chemical potential because of the $RT\ln x_w$ term so that Eq. 1 is satisfied, and

$$\mu_w^{l}(T,P) = \mu_w^{r,pure}(T,P) + v_w\delta P + RT\ln x_w \tag{4}$$

Realizing that $\mu_w^{l}(T,P) = \mu_w^{r,pure}(T,P)$ and that, by definition, $\delta P = \pi$,

$$v_w\pi = -RT\ln x_w \tag{5}$$

Finally, expressing $x_w$ by the mole fraction of the solute, $x_w = 1 - x_s$, and applying the approximation $\ln(1 - x_s) \approx -x_s$ for dilute solutions, we arrive at

$$v_w\pi = x_s RT \tag{6}$$

By further approximating $x_s/v_w$ with solute molar concentration, $C_s$, we arrive at the celebrated van 't Hoff relation for osmotic pressure (16, 45):

$$\pi = C_s RT \tag{7}$$

Eq. 7 strongly resembles the venerable ideal gas equation of state, $P = (n/V)RT$. This analogy was used by van 't Hoff to originally develop the expression for osmotic stress (45).

## III. INTRODUCTION TO MIXING ENTROPY

In our new approach to presenting osmotic pressure, we begin by familiarizing students with the concept of mixing entropy. We present two model systems that can be used to derive the expression for the entropy of mixing. The first involves mixing two ideal gases. This example avoids combinatorics and underscores the equivalence between mixing entropy and the entropy associated with expansion (46). It also clarifies the basis for the ideal gas analogy used originally by van 't Hoff to derive the expression for osmotic pressure (16). The second, more advanced example directly considers the condensed state by applying a lattice model to the mixing of two components to form an ideal solution. Both examples follow changes in the number of accessible microstates, $\Omega$, which is related to entropy, $S$, through Boltzmann's expression:

$$S = k_B\ln\Omega \tag{8}$$

where $k_B$ is Boltzmann's constant. We present both model systems and show that the commonly used term for ideal mixing entropy is recovered in both cases. The reader may choose to familiarize students with one or both examples.

## A. Mixing of ideal gases: a first example

Begin by describing the entropy of an ideal gas and how it changes with the volume in which it is confined. The number of microstates for one gas molecule, $\Omega_1$, increases linearly with volume, $\Omega_1 \sim V$, because the particle can be placed anywhere within the system. Thus, doubling the volume necessarily doubles the number of possibilities for placing the particle. Ideal gas particles do not interact, so a second gas molecule introduced into the system will have the same number of accessible states because it also can be placed anywhere within the volume. Thus, for each position of the first particle, the second particle can take any position. Correspondingly, the total number of microstates is proportional to the product of microstates of each particle, $\Omega_2 \sim \Omega_1^2$, so that $\Omega_2 \sim V^2$. To make the proportionality exact, one should also consider the indistinguishability of particles; this point is further discussed in the following section. By the same reasoning, the total number of microstates available to $N$ molecules is $\Omega_N \sim \Omega_1^N \sim V^N$, and the entropy of the gas has the following form:

$$S \sim Nk_B \ln V \tag{9}$$

Figure 2A shows the mixing of $N_{red}$ "red" ideal gas particles with $N_{blue}$ "blue" ideal gas particles, upon removing a partition. Before mixing, the two gases have the same temperature and number density. Because both gases do not interact, the total increase in entropy that results from mixing can be written as the sum of the individual changes in the entropy of each gas due to their expansion into the neighboring compartment:

$$\Delta S_{mix} = \Delta S_{red} + \Delta S_{blue} \tag{10}$$

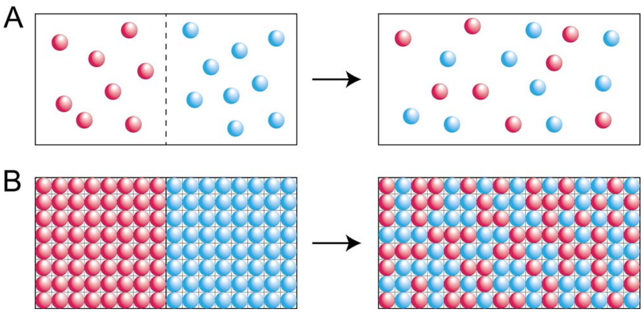

**Fig 2.** Two realizations of mixing entropy. (A) Mixing two ideal gasses. (B) Mixing two liquids to form an ideal solution. Both processes are isothermal and isobaric.

As the red gas expands to the right, the number of microstates for each red gas particle increases by a factor of $V_{\text{total}}/V_{\text{left}}$, where $V_{\text{left}}$ is the volume of the left compartment before mixing and $V_{\text{total}} = V_{\text{left}} + V_{\text{right}}$ is the available volume to the gas after expansion. Therefore, the change in the entropy of the red gas can be written as:

$$\Delta S_{red} = N_{red}k_B \ln \frac{V_{total}}{V_{left}} \tag{11}$$

Using a similar line of reasoning for the expansion of the blue gas, the entropy gained from mixing both gases is as follows:

$$\Delta S_{mix} = N_{red}k_B \ln \frac{V_{total}}{V_{left}} + N_{blue}k_B \ln \frac{V_{total}}{V_{right}} \tag{12}$$

Because the temperature and density of the gases are equal before and after mixing, we can replace volume ratios by mole fractions, $V_{left}/V_{total} = x_{red}$ and $V_{right}/V_{total} = x_{blue}$. Eq. 12 becomes:

$$\Delta S_{mix} = -N_{total}k_B(x_{red}\ln x_{red} + x_{blue}\ln x_{blue}) \tag{13}$$

where $N_{total} = N_{red} + N_{blue}$. Eq. 13 is the commonly known expression for the mixing entropy of two ideal gases (1). Moreover, because both gases are ideal, $\Delta H_{mix} = 0$, and the change in the Gibbs free energy due to mixing is simply:

$$\begin{aligned}\Delta G_{mix} &= -T\Delta S_{mix} = \\ &N_{total}k_BT(x_{red}\ln x_{red} + x_{blue}\ln x_{blue})\end{aligned} \tag{14}$$

The process of mixing reduces the free energy, $\Delta G < 0$. This is a result of the second law of thermodynamics that dictates that in any spontaneous process, entropy must increase. At constant temperature and pressure, this is equivalent to the minimization of *G*.

## B. Mixing ideal solutions: a second example

Figure 2B shows the isothermal mixing of $N_{red}$ "red" particles with $N_{blue}$ "blue" particles, on a lattice to form an ideal solution. The lattice is introduced as a means to derive a tractable number of microstates for the system, but the same result can be obtained from a less restrictive (off-lattice) representation (47). The number of microstates is equivalent to the number of ways the particles can be arranged on the lattice, considering that each type of particle is indistinguishable from other particles of the same color. In the mixture, combinatorics states that this number can be represented by the corresponding binomial coefficient:

$$\Omega = \binom{N_{total}}{N_{red}} = \binom{N_{total}}{N_{blue}} \tag{15}$$

Before mixing, both compartments contain only one kind of particle. Swapping between positions of two identical particles creates a state indistinguishable from that before the swap. Consequently, the binomial coefficient in these pure systems is simply unity. Using $S = k_B \ln \Omega$ and Eq. 15, we arrive at the change in entropy upon mixing:

$$\Delta S_{mix} = k_B \ln \frac{N_{total}!}{N_{red}!N_{blue}!} \tag{16}$$

Using the Sterling approximation, $\ln x! \approx x \ln x - x$, Eq. 16 reduces to Eq. 13, which also can be expressed in terms of the volume accessible to each component before and after mixing:

$$\Delta S_{mix} = N_{red} k_B \ln \frac{V_{total}}{V_{left}} + N_{blue} k_B \ln \frac{V_{total}}{V_{right}} \tag{17}$$

Eq. 17 emphasizes that the change in entropy of each component is related to the volume, similarly to the entropy of an ideal gas, so that in the limit of solutions dilute in one of the components:

$$S \sim N k_B \ln V \tag{18}$$

## C. Free energy as reversible work

Our new approach also requires students to be familiar with the link between free energy and reversible work in processes performed at constant temperature. One approach to derive this relation is as follows (1, 40). As with any process, the requirement of energy conservation applies also to reversible processes, so that:

$$dE - \delta Q_{rev} - \delta W_{rev} = 0 \tag{19}$$

where *E* is the total internal energy of the system, $Q_{rev}$ is the reversible heat exchanged with the surroundings, and $W_{rev}$ is reversible work (we defined positive work as work performed on the system, and heat transferred to the system as positive heat). Furthermore, for a reversible process, the Clausius inequality becomes an equality:

$$dS = \frac{\delta Q_{rev}}{T} \tag{20}$$

Combining Eqs. 19 and 20:

$$\delta W_{rev} = dE - TdS = dA \tag{21}$$

where *A* is the Helmholtz free energy. Therefore, for a reversible process at constant *T*, the change in free energy equals the total (sum of *PV* and non-*PV*) work. We note that the Helmholtz free energy is the most appropriate state function in our subsequent discussion because Eq. 21 requires only constant temperature. An analogous version of Eq. 21 that uses the Gibbs free energy *G*, instead of *A*, requires that in addition to temperature, the pressure will also be constant:

$$\delta W_{rev,\,non\text{-}PV} = dH - TdS = dG \tag{22}$$

with $\delta W_{rev,\,non\text{-}PV}$ involving only the work that is not associated with changes in system

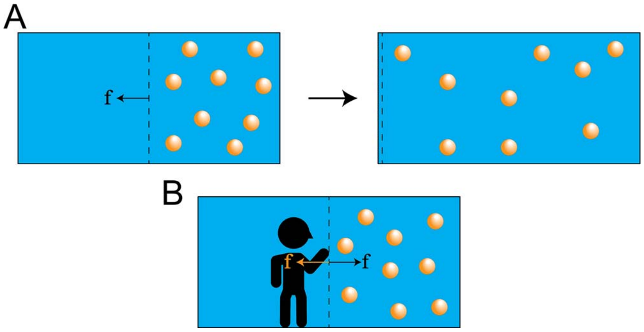

**Fig 3.** Osmotic pressure as an entropic force. (A) The solution on the right-hand side exerts a force, *f*, on a mobile semipermeable membrane and drives the membrane as far left as possible. (B) Virtual person applies an equal and opposing force, *f*, that prevents the membrane from moving. Orange circles represent the solute, and the blue background represents water.

volume. Because students are usually more familiar with the Gibbs free energy, we use $G$ in the following derivations, even though the requirement for constant pressure is only approximately satisfied in the systems discussed here. The same result can be obtained by using Eq. 21. Moreover, for an ideal solution, $\Delta H_{\text{mix}} = \Delta E_{\text{mix}} = 0$; therefore, in this case $\Delta A = \Delta G$, and either of the free energies can be used in the derivation.

## IV. NEW APPROACH TO OSMOTIC PRESSURE: USING MIXING ENTROPY

Figure 3A shows two compartments separated by a mobile semipermeable membrane that allows only water to pass through; the compartment on the left contains pure water, whereas the one on the right contains an ideal mixture of solute in water. Both compartments are maintained at equal temperature. Because the membrane is allowed to move, the partition will move to the left. This is because water will flow from the left compartment to the right compartment to increase the number of microstates of the system, which in turn increases its entropy and reduces its free energy. Initially, before the partition has time to move, the net accumulation of water in the right compartment increases the number density, thereby elevating the pressure in that compartment. This pressure gradient between the two compartments forces the partition to move to the left until it reaches the left wall. Stated differently, the partition moves because at any other position, the free energy can be further reduced by mixing more water with solute. The reverse process (water leaving the right compartment and moving to the left) is, in principle, possible; yet, it is highly improbable because it strongly (exponentially) reduces the number of available states. This process underlines the statistical nature of the second law.

To quantify the force exerted on the membrane, $f$, an external force is added that counteracts the increase in the volume of the right compartment. Imagine physically exerting this force on the membrane and stopping it from moving (Fig 3B). Infinitesimal changes in the position of the membrane, $dz$, require reversible work performed on the partition, $\delta W_{\text{rev}} = -fdz$. This reversible work is equal to changes in the free energy of the two-compartment system, $\delta W_{\text{rev}} = dG$. Using Eq. 22:

$$fdz = -dG = TdS - dH \tag{23}$$

Because for mixing of ideal solutions $dH = 0$, $fdz = TdS$. From Eq. 18, which corresponds to the entropy of a dilute component in an ideal solution, we obtain:

$$fdz = N_s k_B T d\ln V = \frac{N_s k_B T}{V} dV \tag{24}$$

where $N_s$ is the number of solute molecules. Recognizing that $dV/dz$ is the membrane cross-section area, $a$, and that $f/a$ is the pressure exerted on the membrane, $\pi$, we recover the expression for the osmotic pressure in Eq. 7:

$$\pi = \frac{N_s k_B T}{V} = C_s RT \tag{25}$$

This result is relevant for dilute solutions, where free energy depends most strongly on mixing entropy, which in turn depends linearly on solute concentration. Concentrated or nonideal solutions can be treated by a similar approach that uses the relevant expressions for $dG_{\text{mix}}$. In the Supplemental Material, we present two examples of how an expression for the osmotic pressure can be recovered from relevant expressions for the free energy of mixing corresponding to different kinds of solutions. These examples demonstrate how, for other types of solutions, interactions must be considered, and therefore $\Delta H_{\text{mix}}$ and $\Delta E_{\text{mix}}$ are generally nonzero. These additional derivations of osmotic pressure are presented as exercises, from which students can appreciate how intermolecular interactions between solution components introduce nonideality in the osmotic pressure. The derivations also introduce students to the virial expansion and its coefficients in the context of osmotic pressures.

## V. CONCLUDING REMARKS

We presented two approaches for deriving the equation for osmotic pressure. The standard approach, found in most textbooks and even on Wikipedia, is based on equating the chemical potential of water throughout the system (1, 40, 48). In contrast, our new approach directly invokes the role of entropy in generating the osmotic pressure. This approach offers several advantages over the standard method.

(a) Our derivation bypasses the requirement for students to be familiar with the concept of chemical potential, which, in our experience, is often introduced late in the course syllabus (or, as often happens, not at all) and is, many times, found to be nonintuitive by students (42, 43).

(b) Our approach emphasizes entropy as the underlying mechanism for the force generated by osmotic stress; this serves an opportunity to introduce students to the concept of entropically generated forces, which are valuable in multiple biophysical mechanisms.

(c) The starting point for our strategy uses the relevant expression for the free energy of mixing, and the approach is easily generalizable to other nonideal cases and affords a transparent way to include additional interaction terms and to appreciate their effect on the osmotic pressure.

(d) It is simple to generalize this approach to other entropic forces; for example, the force exerted by polymers acting as entropic springs follows a somewhat similar derivation (2, 3).

We encourage educators to implement our suggested derivation for osmotic pressure and witness first-hand whether students find it more approachable.

## SUPPLEMENTAL MATERIAL

All Supplemental Material is available at: https://doi.org/10.35459/tbp.2025.000282.S1.

## AUTHOR CONTRIBUTIONS

DH and IS were involved in the conceptualization of this article; IS, DH, and YNL completed the writing, reviewing, and editing; IS handled visualization concepts; and DH supervised the project and managed project administration and funding acquisition. All authors have read and agreed to the published version of the manuscript.

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## REFERENCES

1. Castellan, G. W. 1983. Physical Chemistry. Addison-Wesley Publishing Company, Reading, Massachusetts.
2. De Gennes, P.-G. 1979. Scaling Concepts in Polymer Physics. Cornell University Press, Ithaca, New York.
3. Phillips, R., J. Kondev, J. Theriot, and H. Garcia. 2012. Physical Biology of the Cell. Garland Science, New York, New York.
4. Harries, D., S. May, and A. Ben-Shaul. 2013. Counterion release in membrane–biopolymer interactions. *Soft Matter* 9:9268–9284.
5. deHaseth, P. L., T. M. Lohman, and M. T. Record, Jr. 1977. Nonspecific interaction of lac repressor with DNA: an association reaction driven by counterion release. *Biochemistry* 16:4783–4790.
6. Helfrich, W. 1978. Steric interaction of fluid membranes in multilayer systems. *Z Naturforsch A* 33:305–315.
7. Sornette, D., and N. Ostrowsky. 1994. Lamellar phases: effect of fluctuations (theory). In Micelles, Membranes, Microemulsions, and Monolayers. W. M. Gelbart, A. Ben-Shaul, and D. Roux, editors. Springer Science and Business Media, New York, pp. 251–302.
8. Schwarzenbach, G. 1952. Der chelateffekt. *Helv Chim Acta* 35:2344–2359.
9. Meyer, E. E., K. J. Rosenberg, and J. Israelachvili. 2006. Recent progress in understanding hydrophobic interactions. *Proc Natl Acad Sci U S A* 103:15739–15746.
10. Chandler, D. 2005. Interfaces and the driving force of hydrophobic assembly. *Nature* 437:640–647.
11. Sapir, L., and D. Harries 2015. Is the depletion force entropic? Molecular crowding beyond steric interactions. *Curr Opin Colloid Interface Sci* 20:3–10.
12. Asakura, S., and F. Oosawa. 1958. Interaction between particles suspended in solutions of macromolecules. *J Polym Sci* 33:183–192.

13. Pfeffer, W. 1877. Osmotische Untersuchungen: Studien zur Zellmechanik. Wilhelm Engelmann, Leipzig, Germany.
14. Eyster, H. C. 1943. Osmosis and osmotic pressure. *Bot Rev* 9:311–324.
15. Peters, W. S., and M. Knoblauch. 2022. How Münch's adaptation of Pfeffer's circulating water flow became the pressure-flow theory, and the resulting problems—a historical perspective. *J Plant Physiol* 272:153672.
16. van't Hoff, J. H. 1901. Osmotic pressure and chemical equilibrium. *Nobel Lecture* 13.
17. Alberts, B., R. Heald, A. Johnson, D. Morgan, M. Raff, K. Roberts, and P. Walter. 2022. Molecular Biology of the Cell: Seventh International Student Edition with Registration Card. WW Norton and Company, New York, New York.
18. Bourque, C. W. 2008. Central mechanisms of osmosensation and systemic osmoregulation. *Nat Rev Neurosci* 9:519–531.
19. Hochachka, P. W., and G. N. Somero. 2002. Biochemical Adaptation: Mechanism and Process in Physiological Evolution. Oxford University Press, Oxford, United Kingdom.
20. Burg, M. B. 1995. Molecular basis of osmotic regulation. *Am J Physio. Renal Physiol* 268:983–996.
21. Yancey, P. H., M. E. Clark, S. C. Hand, R. D. Bowlus, and G. N. Somero. 1982. Living with water stress: evolution of osmolyte systems. *Science* 217:1214–1222.
22. Olgenblum, G. I., B. O. Hutcheson, G. J. Pielak, and D. Harries. 2024. Protecting proteins from desiccation stress using molecular glasses and gels. *Chem Rev* 124:5668–5694.
23. Romero-Perez, P. S., Y. Dorone, E. Flores, S. Sukenik, and S. Boeynaems. 2023. When phased without water: biophysics of cellular desiccation, from biomolecules to condensates. *Chem Rev* 123:9010–9035.
24. Wennerström, H., and M. Oliveberg. 2022. On the osmotic pressure of cells. *QRB Discov* 3:1–10.
25. Zheng, S., Y. Li, Y. Shao, L. Li, and F. Song. 2024. Osmotic pressure and its biological implications. *Int J Mol Sci* 25:3310.
26. Zhou, H.-X., D. Kota, S. Qin, and R. Prasad. 2024. Fundamental aspects of phase-separated biomolecular condensates. *Chem Rev* 124:8550–8595.
27. Stewart, C. J., G. I. Olgenblum, A. Propst, D. Harries, and G. J. Pielak. 2023. Resolving the enthalpy of protein stabilization by macromolecular crowding. *Protein Sci* 32:e4573.
28. Minton, A. P. 2013. Quantitative assessment of the relative contributions of steric repulsion and chemical interactions to macromolecular crowding. *Biopolymers* 99:239–244.
29. Speer, S. L., C. J. Stewart, L. Sapir, D. Harries, and G. J. Pielak. 2022. Macromolecular crowding is more than hard-core repulsions. *Annu Rev Biophys* 51:267–300.
30. Zhou, H.-X., V. Nguemaha, K. Mazarakos, and S. Qin. 2018. Why do disordered and structured proteins behave differently in phase separation? *Trends Biochem Sci.* 43:499–516.
31. Parsegian, V., R. Rand, N. Fuller, and D. Rau. 1986. Osmotic stress for the direct measurement of intermolecular forces. In Methods in Enzymology, vol. 127. ed. Packer, L. Elsevier, Amsterdam, Netherlands, pp. 400–416.
32. Tyagi, R., K. Donaldson, C. Loftus, and J. Jallo. 2007. Hypertonic saline: a clinical review. *Neurosurg Rev* 30:277–290.
33. Szczotka-Flynn, L. B., E. Pearlman, and M. Ghannoum. 2010. Microbial contamination of contact lenses, lens care solutions, and their accessories: a literature review. *Eye Contact Lens* 36:116–129.
34. Wan, S., M. A. Roberts, and P. Mount. 2016. Normal saline versus lower-chloride solutions for kidney transplantation. *Cochrane Database Syst Rev* 8:CD010741.
35. Jha, S. K. 2003. Cerebral edema and its management. *Med J Armed Forces India* 59:326–331.
36. Schiller, L. R., M. Emmett, C. A. Santa Ana, and J. S. Fordtran. 1988. Osmotic effects of polyethylene glycol. *Gastroenterology* 94:933–941.
37. Gupta, B. P., N. Thakur, N. P. Jain, J. Banweer, and S. Jain. 2010. Osmotically controlled drug delivery system with associated drugs. *J Pharm Pharm Sci* 13:571–588.
38. Greenlee, L. F., D. F. Lawler, B. D. Freeman, B. Marrot, and P. Moulin. 2009. Reverse osmosis desalination: water sources, technology, and today's challenges. *Water Res* 43:2317–2348.
39. Dill, K., and S. Bromberg. 2010. Molecular Driving Forces: Statistical Thermodynamics in Biology, Chemistry, Physics, and Nanoscience. Garland Science, New York, New York.
40. Atkins, P. W. 1978. Physical Chemistry. Oxford University Press, Oxford, United Kingdom.
41. Levine, I. N. 2009. Physical Chemistry. McGraw-Hill Companies, New York, New York.
42. Job, G., and F. Herrmann. 2006. Chemical potential—a quantity in search of recognition. *Eur J Phys* 27:353–371.
43. Callen, H. B. 1991. Thermodynamics and an Introduction to Thermostatistics. John Wiley and Sons, Hoboken, New Jersey.
44. Raoult, F.-M. 1887. Loi générale des tensions de vapeur des dissolvants. *C R Hebd Seances Acad Sci* 104:1430–1433.
45. van't Hoff, J. H. 1995. The role of osmotic pressure in the analogy between solutions and gases. *J Membr Sci* 100:39–44.
46. Ben-Naim, A. 2012. Entropy and the Second Law: Interpretation and Misss-Interpretationsss. World Scientific Publishing Company, Singapore, Republic of Singapore.
47. Hill, T. L. 1986. An Introduction to Statistical Thermodynamics. Dover Publications, New York, New York.
48. Wikipedia. 2024. Osmotic pressure. Accessed 4 September 2024. https://en.wikipedia.org/wiki/Osmotic_pressure.