

## INVITED COMMENTARY

# Kinetic model of osmosis through semipermeable and solute-permeable membranes

F. Kiil

University of Oslo, Institute for Experimental Medical Research, Ullevål University Hospital, Oslo, Norway

Received 3 October 2001,

accepted 19 April 2002

Correspondence: Dr Fredrik Kiil,  
Institute for Experimental Medical  
Research, Ullevål University  
Hospital, Kirkeveien 166, N-0407,  
Oslo 4, Norway.

### Abstract

The gas analogy of the van't Hoff equation for osmotic pressure  $\Delta\pi = RT/\bar{V}$ , where  $R$  is gas constant,  $T$  absolute temperature and  $\bar{V}$  mole volume of water, remained unexplained for a century because of a few misconceptions: (1) Use of supported membranes prevented the recognition that osmotic forces exert no effect on the solid membrane. During osmotic flow frictional force of solvent within membrane channels equals osmotic kinetic force  $\pi$  at the interface against the solution containing impermeant solute. (2) Retrograde diffusion of water is much less than osmotic flow even when  $dx$  in the gradient  $dc/dx$  approaches zero. (3) The gas analogy was thought to be accidental. Actually, the internal kinetic pressure is  $P = RT/\bar{V}$ , because intermolecular forces cancel out at the liquid interface, just as within a gas. The kinetic osmotic pressure is the difference in solvent pressure across the interface:  $\pi = RT/\bar{V} - (RT/\bar{V})X_1 = (RT/\bar{V})X_2$ , where  $X_1$  and  $X_2$  are the mole fractions of water and impermeant solute, respectively. Integration gives  $\pi = -(RT/\bar{V})\ln X_1$ , identical to the thermodynamic equation. This equation is correct up to 25 atmospheres, and up to 180 atmospheres by assuming that a sucrose molecule binds 4 and a glycerol molecule 2.5 water molecules. For solute-permeable membranes, the reflection coefficient  $\sigma$  can be calculated by formulas proposed for ultrafiltration. Because the fraction  $(1-\sigma)$  of solute concentration behaves as solvent, osmosis may well proceed against the chemical potential gradient for water. The analogy to an ideal gas applies because  $\pi = -(RT/\bar{V})\ln X_1$  is the small difference between enormous internal solvent pressures.

**Keywords** diffusion, glycerol, intermolecular forces, reflection coefficient, sucrose, van't Hoff.

In all living matter osmotic forces account for water transport through membranes more permeable to water than to one or more of the solutes facing the membrane. Osmotic flow or osmosis occurs into the compartment with lowest solvent concentration and highest concentration of solutes that do not permeate the membrane.

Some membranes are semipermeable to all solutes, such as the water channels of aquaporin AQP1 which reside permanently in the apical and lateral cell membranes of proximal tubules (Nielsen & Agre 1995). These hour-glass shaped water channels are at the

narrowest no more than 3 Å wide (Agre *et al.* 2002). As the effective diameter of H<sub>2</sub>O is 3 Å, the water channels permit the passage of H<sub>2</sub>O in a single file, but rejects Cl<sup>-</sup>, which is almost spheroid in shape and has an effective diameter of 3.6 Å. Continuous transport of NaCl is therefore for electrostatic reasons excluded although the unhydrated Na<sup>+</sup> is smaller than the water molecule.

An example of a solute-permeable membrane is the tight junctions encircling epithelial cells at the luminal surface in proximal tubules. With gap distance of 6–7 Å

they permit the passage of NaCl, but these slits are almost impermeable to mannitol, which has an effective Einstein–Stokes molecular diameter of 8 Å.

The golden age of osmosis research lasted a decade from Pfeffer in 1877 had published a study of osmotic pressure of sugar solutions until van't Hoff and Arrhenius in 1887 concluded that osmotic pressure is the consequence of bombardment of the semipermeable membrane by molecules and ions. Since then there has been much bewilderment concerning the physical nature of osmosis.

The purpose of this commentary is to show that a few misconceptions, which have been held up to the present day, have delayed progress in understanding the molecular mechanisms of the osmotic force. First, the principal difference between osmotic and hydrostatic pressures in their effect on the membrane have remained remarkably unrecognized. Second, the distinction between osmosis and diffusion has not been clear. A persistent hypothesis is that diffusional exchange of solvent and solute at the pore opening generates osmosis (Comper & Williams 1990). Third, the central problem since van't Hoff has been to provide alternative explanations to his bombardment hypothesis. Some including van't Hoff assumed that his equation applies only to very dilute solutions, whereas others felt that the gas analogy is accidental. Fourth, a long-lived hypothesis has been that there exists a causal relationship between osmotic pressure and vapour pressure. Finally, attention has been focused on solute-permeable membranes and the possible valve-like function of impermeant solute molecules on the pore opening of an osmotic membrane (Dainty & Ferrier 1989, Janáček & Sigler 2000).

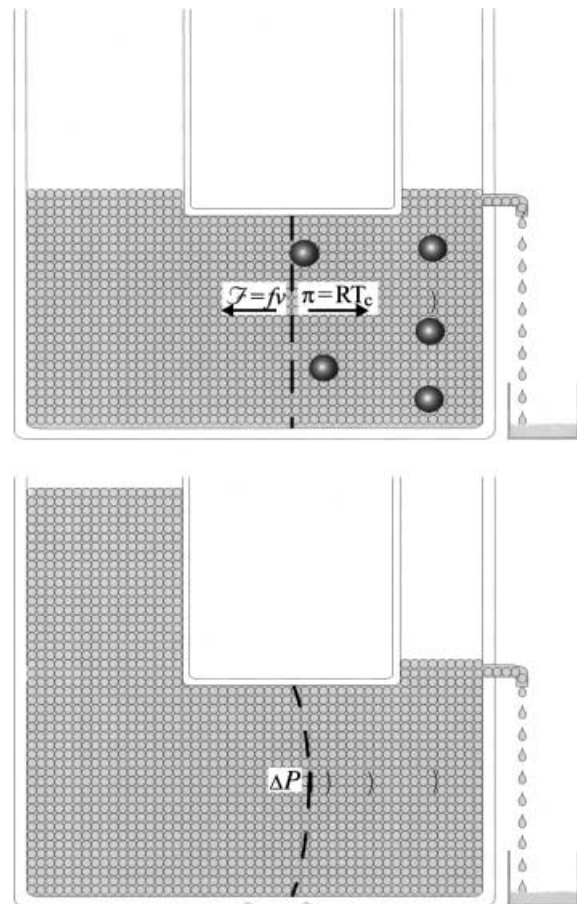
The model discussed in this commentary states that a kinetic osmotic pressure  $\pi$  (force per area) is generated in the liquid-filled pores or slits of the osmotic membrane at the interface between solvent in the channels and impermeant solutes. Although  $\pi$  may be many atmospheres, it constitutes a small difference between vast internal kinetic pressures of solvents on either sides of the osmotic membrane. Finally it is shown that by recognizing that solvent includes permeant solutes, the same rules apply to semipermeable and solute-permeable membranes.

#### *Difference between osmotic and hydrostatic pressures*

Water transport through natural and artificial membranes follow common rules, but a fundamental property of osmotic membranes has been overlooked, possibly because of the choice of equipment. Wilhelm Pfeffer, a German botanist, constructed in the 1870s the first effective osmometer by allowing solutions of potassium ferrocyanide to diffuse from one side and copper sulphate from the other side into the wall of

unglazed porous porcelain pots. Where the solutions met within the pore channels, a membrane of copper ferrocyanide was formed that was permeable to water but not to most solutes. Because of the small channel diameter, the membranes withstood hydrostatic pressures of several atmospheres. Later modifications of supported osmotic membranes tolerate hydrostatic pressure differences of hundreds of atmospheres.

Figure 1 illustrates the fundamental difference between osmosis and hydraulic flow. A thin unsupported semipermeable membrane separates a compartment containing a solution from a compartment of pure water. Even with osmotic kinetic energy equivalent to a pressure of several atmospheres and large water transport there is no displacement or distention of the membrane if there is no hydrostatic pressure difference across the membrane (Kiil 1982). In contrast, as illustrated in the bottom panel, attempts to raise the



**Figure 1** Comparison of effects of osmotic vs. hydrostatic pressure on a semipermeable membrane. Osmosis does not displace the membrane because the osmotic force  $\pi$  at the interface and the frictional force  $F$  in the membrane channels are of the same magnitude. A hydrostatic pressure difference ( $\Delta P$ ) acts also on the solid membrane.

fluid transport to similar rates by applying potential energy, causes bulging. Thus hydrostatic forces act both on the solid membrane and on the liquid in the channels, whereas osmotic forces act exclusively on the liquid in the channels.

An osmotic force increases the velocity of flow through a membrane channel until a steady state is reached when the osmotic force and the opposing frictional force are equal:

$$\pi + F = 0. \quad (1)$$

In this equation the frictional force may be proportional to a friction coefficient  $f$  and the velocity  $v$ :  $F = -fv$ . The minus sign arises because the frictional force acts in opposite direction to the flow. At equilibrium no net force displaces the membrane. Thus, by using supported membranes it was impossible to know whether osmotic flow had any effect on the membrane. Therefore, the gas analogy based on the assumption that solute molecules just as gas molecules cause a pressure by bombarding the membrane was not definitely ruled out.

That osmotic flow does not displace the membrane is of fundamental biological significance. For instance, in the first part of the distal tubules, luminal osmolality is less than 50 mOsm  $\text{kg}^{-1}$ , whereas the osmolality of the peritubular fluid may be 300 mOsm  $\text{kg}^{-1}$ . Thus the osmotic pressure required for water transport through the epithelium is equivalent to a hydrostatic pressure of 5000 mmHg (torr) or 6.5 atmospheres. Much smaller hydrostatic pressure differences would disrupt the tubular epithelium.

### Difference between osmosis and diffusion

Thermodynamic derivations of equations for osmosis can only be undertaken at osmotic equilibrium. Osmotic equilibrium is reached when the excess in hydrostatic pressure on the solution side of the membrane, which is impermeable to solutes, prevents flow through the membrane from the compartment containing pure water. In a solution the chemical potential of water is reduced. The hydrostatic pressure difference, called osmotic pressure,  $\Delta\pi$ , restores the chemical potential of the solvent water. From the definition of the chemical potential an equation for osmotic pressure was derived (Gouy & Chaperon 1888):

$$\Delta\pi = -(RT/\bar{V})\ln X_1 \quad (2)$$

with approximations  $\Delta\pi \approx (RT/\bar{V})X_2$ , and the van't Hoff equation  $\Delta\pi \approx RT\Delta c$ . In these equations  $R$  is the gas constant,  $T$  is absolute temperature,  $\bar{V}$  is the volume of one mole of solvent (18 mL for  $\text{H}_2\text{O}$ ),  $\ln X_1$  is the natural logarithm of the mole fraction of the solvent,  $X_2$  is the mole fraction of the solute, and  $\Delta c$  is the

difference across the membrane in molar concentration of solutes to which the membrane is impermeable.

This equation is unchallenged. It should be noted that osmotic equilibrium is reached for the solvent, in this case water which passes through the membrane, whereas no equilibrium is reached for impermeant solute. Physically there is no distinction between solute and solvent and between molecules and ions in their thermal behaviour. Because of the strong repulsive forces at close contact molecules and ions move like perfectly elastic particles at speeds proportional to the absolute temperature. Solute molecules which behave like water molecules by moving through an osmotic membrane are therefore in this connection included in the concept solvent.

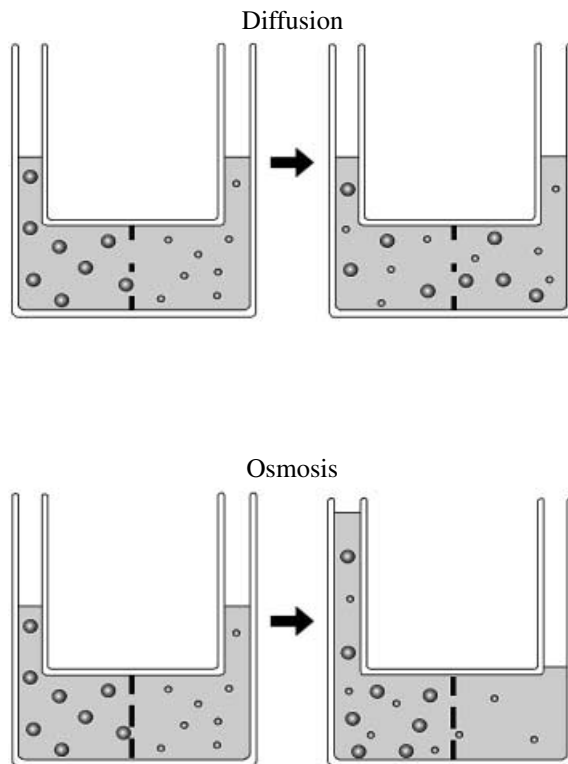
Adolf Fick, a prosector in anatomy, discovered in the 1850s that the laws valid for conduction of heat and electricity also apply to diffusion. Although diffusion is much more rapid in a gas than in a liquid, Fick's laws apply to all fluids. To derive an expression for the diffusion coefficient  $D$  in Fick's equation  $J = -Ddc/dx$ , Einstein (1905) assumed that each particle is driven through a homogenous medium by a force according to van't Hoff's equation. By introducing Stokes' law the diffusion coefficient becomes

$$D = RT/6\pi r\eta N_A, \quad (3)$$

where  $\eta$  is viscosity,  $r$  is radius of the particle and  $N_A$  is Avogadro's number  $6 \times 10^{23}$ . A year later Smoluchowski (1906) derived a similar equation by basing his calculations on random collisions. Smoluchowski derived the equation without invoking the van't Hoff equation. As derivations based on random movement and the van't Hoff equation led to the same result, the suspicion was raised that diffusion and osmosis might express different sides of the same kinetic phenomenon. Another long-lasting problem has been whether osmosis is a function of the membrane or of the composition of the liquid as Einstein's use of the van't Hoff equation might suggest.

Figure 2 shows that it is the bore of the membrane channels that decides whether diffusion or osmosis takes place. Initially the compartments have the same composition. In the upper panels the channels of the membrane are as permeable to solute as to water. Equilibrium is reached when the composition of the fluid is the same on both sides of the membrane because of diffusion of solutes and solvent through the membrane. By osmosis (lower panels), no equilibrium is reached. All solvent would eventually be transported into the solution. However, osmosis may be stopped by the hydrostatic osmotic pressure  $\Delta\pi$  (lower right).

In the diffusion process thermal random movements of solute and water molecules cause a net transport along the concentration gradients  $dc/dx$ . Diffusion is



**Figure 2** Effect of altering channel bore. Upper panel: Through a membrane with wide channels diffusion of solutes and water takes place at equal concentration gradients and diffusion coefficients until the composition of the solution is equal in both compartments. Lower panel: By rendering the membrane channels impermeable to large solutes, but wide enough to permit the passage of water and small solutes (solvent), the hydrostatic pressure increases in the compartment of large solutes until the osmotic pressure  $\Delta\pi$  is reached, when the chemical potentials of solvent are equal in both compartments.

essentially a one to one exchange of molecules. If a solute diffuses in one direction at a diffusion coefficient  $D$ , water moves in the opposite direction from lower to higher solute concentrations at the same concentration gradient  $dc/dx$  and at same diffusion coefficient  $D$ . Provided their molecular volumes are not widely different, the difference in volume flow in either direction is negligible (Bearman 1961).  $D$  is inversely related to  $r$  (eqn 3). Accordingly, retrograde diffusion of water is most rapid when the solute is a small molecule, such as tritium.

As solute diffusion and retrograde water diffusion are numerically equal, the volume flows caused by osmosis and diffusion may be compared. For instance, across tight junctions of proximal tubules osmotic flow is  $2 \text{ nL min}^{-1} \text{ mm}^{-1}$  tubular length, and transjunctional diffusion of NaCl is  $200 \text{ pmol min}^{-1} \text{ mm}^{-1}$  tubule length (Kiil 2002). Back-diffusion of water at a rate of  $0.0035 \text{ nL min}^{-1} \text{ mm}^{-1}$  tubule length is less than 0.2%

of osmotic flow. Thus, the transport capacity of osmosis is orders of magnitude larger than diffusion of water. On the other hand, Comper (1994) argued that osmotic flow several times more rapid than measured by tracer diffusion of water is no valid argument against osmosis as a diffusion process. His argument is that in tracer experiments diffusion is estimated over the whole thickness of the membrane as compared with the very sharp concentration gradient during osmosis.

If along a tube the concentration falls from  $c$  to zero, the rate of transport is proportional to  $dc/dx$  and therefore inversely related to the length  $\Delta x$  of the tube. Rather than regarding diffusion as a random walk, diffusion may be attributed to a virtual force which drives each particle down the concentration gradient. According to Sten-Knudsen (1978) an expression for this virtual force is

$$X_{\text{diff}} = -(RT/N_A c)dc/dx. \quad (4)$$

This diffusion force becomes as large as the osmotic force expressed by the van't Hoff approximation  $\pi = RTc$ , when

$$RT \int_0^{\Delta x} dx = -RT \int_0^c N_A dc,$$

which gives

$$\Delta x = 1/N_A c. \quad (5)$$

Alternatively the diffusion length  $\Delta x$  which raises diffusion to  $2 \text{ nL min}^{-1} \text{ mm}^{-1}$  through the tight junction may be estimated. In either case  $\Delta x$  is  $<10^{-10} \text{ \AA}$ . It is therefore not possible to induce osmosis by reducing the diffusion distance. Moreover, the change of dimension of the proportionality factor from  $\mu\text{m}^2 \text{ s}^{-1}$  to  $\mu\text{m} \text{ s}^{-1}$  indicates that diffusion and osmosis are not commensurable.

### The gas analogy

The problem which stirred the scientific community for several generations was the interpretation of the data published by Pfeffer (1877). By immersing his ceramic porous pot in water and filling it with solutions of sucrose (cane sugar) Pfeffer measured  $\Delta\pi$  at various sugar concentrations and temperatures. By recalculating Pfeffer's data by converting grams to moles van't Hoff (1886) derived an equation for osmotic pressure, which originally was written  $\Delta\pi = iRT/\bar{V} = iRT\Delta c$ . Van't Hoff introduced the factor  $i$ , inspired by de Vries' observations on plant cells. For undissociated solutes, such as sugars,  $i = 1$  but is close to whole numbers 2 for NaCl and KCl and is close to 3 for electrolytes with three ions. The dissociation hypothesis proposed by Arrhenius (1887) provided an explanation of

de Vries' and Pfeffer's data. Thus the van't Hoff equation may be written  $\Delta\pi = RT/\bar{V} = RT\Delta c$ , if the molar concentration difference  $\Delta c$  across the membrane includes all particles (molecules and ions) that do not permeate the membrane.

Because of the analogy to the equation for an ideal gas  $p = RT/\bar{V}$ , where  $p$  is the gas pressure and  $\bar{V}$  is the gas volume containing one mole, it was natural to seek explanations in terms of the kinetic gas theory. Both van't Hoff and Arrhenius believed that the osmotic pressure is generated by the impact of solute molecules hitting the membrane. The equation for an ideal gas does not apply to real gases because of intermolecular forces. Even with the reservation that the van't Hoff equation is valid only for dilute solutions, the gas analogy seemed accidental, as the intermolecular forces in liquids are many orders of magnitude greater than in gases. Nevertheless, the gas analogy continued to impress for decades. Ehrenfest (1915) wrote: 'The fact that the dissolved molecules of a dilute solution exert on a semipermeable membrane – in spite of the presence of the solvent – exactly the same pressure as if they alone were present, and that in the ideal gas state – this fact is so startling that attempts have repeatedly been made to find a kinetic interpretation that was as lucid as possible'. Although well understood thermodynamically, attempts to understand the phenomenon of osmosis kinetically continued to fail – until the problem more or less went into oblivion or was considered obsolete.

In their derivation of equations for the diffusion coefficient neither Einstein nor Smoluchowski were bothered by the fact that large intermolecular forces exist between the molecules in real gases and that the intermolecular forces are several orders of magnitude greater in a liquid. The explanation is that molecules within a gas or a liquid behave as if no intermolecular forces exist. Recognizing that the interface in membrane channels are within the liquid, the analogy to an ideal gas becomes understandable. In retrospect, it appears that all relevant information was available long before the van't Hoff equation was derived. The story goes that Pfeffer asked Clausius, the founder of the kinetic gas theory, whether he could explain that a 1% tasteless sugar solution caused water transport into his ceramic pot until the liquid column was two storeys high. Clausius held it impossible. Nevertheless, Clausius' equation for the pressure of real gases permits the elimination of intermolecular forces. They lead to the understanding that osmotic pressure is the difference in partial kinetic pressure of solvents on either side of the interface (Kiil 1982). Another key to understanding the nature of osmosis was held, but not used, by van der Waals, who was a colleague of van't Hoff at the University of Amsterdam.

An equation for real gases, known as van der Waals' equation, first appeared in his doctoral thesis 'On the continuity of the gaseous and liquid state' (van der Waals 1873) 13 years before van't Hoff proposed  $\pi = iRT/V$  for the osmotic pressure of dilute solutions. Van der Waals' equation for the internal kinetic pressure of a fluid may be written

$$P = RT/(\bar{V} - b) - a/\bar{V}^2 \quad (6)$$

where the factors  $a$  and  $b$  are related to intermolecular forces and compressibility, respectively. Isotherms based on van der Waals' equation predicts the critical temperature for liquidation of real gases, an observation that strengthened the view that gases, vapours and liquids are of the same nature: molecules in random collisions possess equal kinetic energy at equal temperature in a solution and in the saturated vapour above the solution. In van der Waals' equation  $a/\bar{V}^2$  cancel out in the interior of a gas, so that the real kinetic pressure inside a gas is  $RT/(\bar{V} - b)$ . (Landau *et al.* 1967)

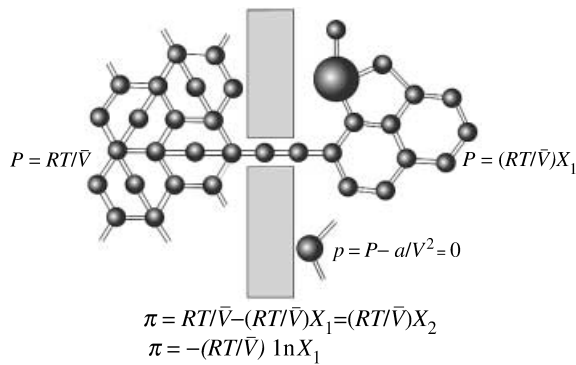
Factor  $b$ , an index of compressibility, is for many real gases four times larger than the actual volume of the gas molecules. This correction would give negative volume for water, which compared with gases is incompressible. By setting  $b = 0$ , the volume is the molar volume of water:  $\bar{V} = 18$  mL.

Although the kinetic energy of water molecules is not altered by transition from vapour to liquid at identical temperature, the reduction in molar volume to  $\bar{V} = 18$  mL greatly increases the attractive intermolecular van der Waals' forces. They are inversely related to the sixth power of the distance between the molecules and millions of times larger than in a gas. If intermolecular forces cancel out in a liquid, the internal kinetic pressure becomes

$$P = RT/\bar{V}, \quad (7)$$

in complete analogy to the ideal gas equation. This extrapolation from gas to liquid suggests remarkably high internal pressures of a liquid. Thus, if van der Waals equation is valid for liquids, such as water, the internal kinetic pressure is  $P = RT/\bar{V} \approx 1400$  atmospheres or a water column  $\approx 1450$  m high. The pressure would be even higher if  $b > 0$ . Internal kinetic pressures of this magnitude seems as a great overstatement. On the other hand, no alternative explanation has been given for the fact that a 10% difference in solvent concentration at the interface causes an osmotic pressure of 140 atmospheres.

Figure 3 illustrates the difference between forces acting on the solid wall and on the liquid at the interface of a single-file channel. At the walls of the container (and at the surface of any pressure recorder) asymmetry of the intermolecular forces attract molecules into the solution. The intermolecular force at the wall,  $a/\bar{V}^2$ ,



**Figure 3** Osmotic kinetic pressure. In a liquid the kinetic pressure  $P$  cannot be measured, because cohesive intermolecular forces ( $a/V^2$ ) of equal magnitude draw molecules into solution. Within the liquid intermolecular forces cancel. In the left water compartment including the single row of water in the membrane channel the kinetic pressure is  $P = RT/\bar{V}$ , where  $R$  is the gas constant,  $T$  absolute temperature and  $\bar{V}$  is the mole volume of water (18 mL). In the solution the partial pressure of water is  $P = (RT/\bar{V})X_1$ . The kinetic osmotic pressure  $\pi = (RT/\bar{V})X_2$  is the difference in  $P$  across the interface, which after integration becomes  $\pi = (RT/\bar{V})\ln X_1$ .  $X_1$  and  $X_2$  are mole fractions of water and solute, respectively.

equals the internal kinetic pressure  $RT/\bar{V}$ . Kinetic pressure can therefore not be measured. The pressure that is recorded in a liquid is exclusively due to the potential energy or weight of the liquid column above the recording level.

In contrast, within liquids each molecule is surrounded symmetrically by other molecules, just as in gases. Because of the symmetry intermolecular forces cancel out. In the channels of a semipermeable membrane the liquid is continuous. Each molecule is bound to its neighbour by intermolecular forces, even when the water molecules are lined in a single file. At the interface between solvent in the channel and solution there is an abrupt change in partial kinetic pressure of water. Intermolecular forces are equal on both sides of the interface and cancel out. The difference in partial kinetic solvent pressure constitutes the kinetic osmotic pressure  $\pi$  that forces solvent out of the channels into the solution. As intermolecular forces cancel out, the difference between the partial pressures is according to eqn 7:

$$P_{\text{water}} - P_{\text{solution}} = \pi = RT/\bar{V} - RTX_1/\bar{V}$$

$$= RTX_2/\bar{V}$$

where  $X_1 = n_1/(n_1 + n_2)$  and

$$X_2 = 1 - X_1 = n_2/(n_1 + n_2). \quad (8)$$

The kinetic osmotic pressure  $\pi$  can be measured at osmotic equilibrium when  $\pi = \Delta\pi$ . Alternatively, in biological examinations, the kinetic osmotic pressure

can be estimated by raising the concentration of impermeant solutes on the solvent side of the membrane until osmotic equilibrium is reached. In contrast, the internal kinetic pressure is not measurable.

The number of solute particles,  $n_2$ , appears both in numerator and denominator of  $X_2$ . For this reason the kinetic osmotic pressure is not directly proportional to  $n_2$ . The additional effect on  $\pi$  of raising  $n_2$  at constant solvent number  $n_1$  is slightly higher at low than at high solute concentration. By integrating  $(RT/\bar{V})dx/(n_1 + x)$  between  $x = 0$  and  $x = n_2$  (Kiil 1989), the result is a logarithmic expression

$$\pi = \int_0^{n_2} (RT/\bar{V})dx/(n_1 + x) = -(RT/\bar{V}) \ln X_1, \quad (9)$$

in agreement with the thermodynamic derivation (eqn 2). The basic condition for a kinetic derivation is therefore fulfilled.

In the molar volume of water  $\bar{V} = 18$  mL each molecule occupies a cubicle with side length  $3 \text{ \AA}$ . The collision distance is about  $1 \text{ \AA}$ , or less than the effective radius of the water molecule. By such oscillations momentum is exchanged at frequencies ranging between  $10^{12}$  and  $10^{13}$  per second. This unconceivable kinetic behaviour is mediated by Piet Hein (1966): ‘Nature, it seems, is the popular name for milliards and milliards and milliards of particles playing their infinite game of billiards and billiards and billiards’.

In Nature’s billiards water molecules within a single file channel may at the interface exchange momentum  $10^{13}$  times per second. In a solution containing  $0.5 \text{ mM}$  of impermeant solutes the frequency of momentum exchange of water molecules is 90 millions  $\text{s}^{-1}$  lower. A deficiency of 90 millions  $\text{s}^{-1}$  in every channel may seem large in order to generate an osmotic pressure of  $10 \text{ mmHg}$ , but the fractional deficiency is only  $0.000009$  of the total frequency.

I want to emphasize that it is the difference in solvent concentration across the interface that causes osmosis and not the hit of individual solute molecules on the channel openings. Much effort has been wasted in attempts to explain the behaviour of impermeant solute molecules: ‘A solute molecule present at the pore mouth will obviously retard water flow from the solution into the pore, and will exert a force of attraction on water in the pore’ (Dainty & Ferrier 1989). A century before, van’t Hoff (1894) expressed similar views after abandoning his bombardment hypothesis. Since then the hypothesis of blockade of pore openings by solute molecules has reappeared several times, most recently advocated by Janáček & Sigler (2000). By focusing on the behaviour of solute molecules it has been forgotten that the century-old thermodynamic derivation (eqn 2) strongly suggests that the only function of impermeant

solute molecules is to reduce the mole fraction of the solvent.

Although not interfering with exchange of momentum at the interface, the vast intermolecular forces are of fundamental significance for osmotic flow by providing the cohesive continuity of liquids. Because there is no external kinetic pressure, intermolecular and thermal kinetic forces are of the same magnitude. The magnitude may be measured as the negative pressure required to break a water column. Hundreds of atmospheres are needed to disrupt the gas-free water column in thin Z-shaped tubes during centrifugation (Briggs 1950). Because of the continuity generated by intermolecular forces, solvent molecules are pulled along the membrane channels by the osmotic force at the interface and thrust into the solution against a hydrostatic pressure, consistent with the word osmosis derived from a Greek word meaning push or thrust.

#### Vapour pressure and deviation from $X_1$

Almost all estimates of osmotic pressure of solutions exposed to a semipermeable membrane are based on measurement of the depression of the freezing point. The depression is a function of the saturated vapour pressure above a solution. It has therefore been believed that there exists a causal relationship between vapour pressure and osmotic pressure, but the relationship can be attributed to analogy (Kiil 1989).

Water molecules in saturated vapour above a solution of non-volatile solutes have the same mean kinetic energy and distribution of energy as the molecules in the solution. To evaporate, water molecules must be in surface position and possess sufficient kinetic energy directed out of the solution. In a solution with a fraction  $X_2$  of non-volatile solutes the fraction of water molecules available to evaporation is  $X_1$ . Above the solution the vapour pressure  $p_1$  builds up until energy equilibrium is reached at saturation, when equal number of water molecules evaporate and return anywhere to the surface of the solution. At saturation the vapour pressure is reduced from  $p_0$  for pure water ( $X_1 = 1$ ) to  $p_1$  above the solution. This relationship is expressed as

$$p_1 = p_0 X_1, \quad (10)$$

usually called Raoult's law after the French chemist, who demonstrated the validity of this relationship for several solvents and solutes in dilute solution before van't Hoff derived his equation. The common feature is that only the fraction  $X_1$  of water molecules in surface position influences evaporation, and only the fraction  $X_1$  of water molecules at the interface influences osmotic pressure.

When solute concentration is raised, Raoult's law and the equation for osmotic pressure no longer apply. The

deviation from ideality takes place at identical  $X_1$ . For a sucrose solution this happens at an osmotic pressure of  $\approx 25$  atmospheres. At higher sucrose concentrations osmotic pressure is higher and vapour pressure is lower than predicted from equations 2 and 10. Thus the actual mole fraction of water is lower than  $X_1$ . Fewer water molecules possess sufficient energy to evaporate. Fewer water molecules oppose the pressure exerted by the water molecules in the channels of a semipermeable membrane.

It is sometimes stated that Raoult's law is valid 'because the number of solvent molecules per unit area of the surface of the liquid is less owing to the presence of solute molecules' (Rock 1983). According to this view it is the area occupied by solute molecules that counts. However, small and large solute molecules affect evaporation and osmosis in the same way provided they are non-volatile and impermeant. As small and large solute molecules possess the same kinetic energy, the simplest explanation of non-ideal behavior is that each solute molecule binds a certain number  $\alpha$  of water molecules, which alters  $X_1 = n_1 / (n_1 + n_2)$  to the modification  $X_{x1} = (n_1 - \alpha n_2) / (n_1 + n_2 - \alpha n_2)$ , where the number  $\alpha$  is dimensionless.

*Uncharged solutes.* By assuming that each molecule of sucrose (cane sugar) binds  $\alpha = 4.2$  water molecules, the calculated osmotic pressure coincides over a pressure range between 25 and 180 atmospheres with directly observed osmotic pressure and with calculated osmotic pressure based on the the reduction of saturated vapour pressure (Kiil 1989). From osmolalities measured by depression of the freezing point (Handbook of Chemistry & Physics 1982) it can be calculated that the number of water molecules is  $\alpha \approx 2.5$  for glycerol and  $\alpha = -0.2$  for urea, suggesting slight evaporation of urea.

Thus the number of water molecules bound to solute particles seems to vary greatly between solutes. By binding the number of solvent particles is reduced. Thus osmotic pressure and the depression of the freezing point are colligative properties: dependent on the number of solvent particles but not on their chemical nature.

The conventional thermodynamic approach to correct deviations from Raoult's law has been to introduce concepts such as activity,  $a_w$ , and activity coefficient  $\gamma = a_w / X_1$  or osmotic coefficient  $\phi = \ln(a_w / X_1)$ . In comparison, the advantage of  $X_{x1}$  is that  $\alpha$  is calculated from measurement of freezing point depression at only one molar concentration, permitting calculation of  $X_{x1}$  at other concentrations. On the other hand,  $a_w$  must be calculated from data obtained at each molar concentration and approaches  $a_w = X_1$  at very low concentration.

**Charged solutes.** To explain the effect on osmotic pressure Poynting (1896) proposed binding of water to electrolytes, such as NaCl, as an alternative to Arrhenius dissociation hypothesis. Diffusion experiments indicate that the sodium ion has a hydration number of 1.2 and that the chloride ion is not hydrated. An alternative to hydration is that water surrounding cations is more organized than in bulk solution (Cussler 1984). The effect of hydration on osmotic transport is not clear; the difference in reflection coefficients between NaCl and NaHCO<sub>3</sub> for the passage of the tight junctions of the proximal tubules seems related to the difference in anion size rather than to the hydrated sodium ion, whose effective diameter would be larger than the anion diameters (Kiil 2002).

Within cells water may appear both as monomers and organized with more gel-like structure around the cytoskeleton. Because of the dipole moment water molecules may be organized in several layers along densely charged membranes and render the membrane impermeable to water (Pollack 2001). At the interface between free and organized water with differences in density and chemical potential, solutes may cause water transport and complex secondary effects, which are called micro-osmosis (Wiggins 1995).

A hypothesis of ionic interactions developed by Debye & Hückel in 1923 and improved by Onsager in 1926 is considered among the highlights of classical physics, but applies only to very dilute solutions with complete solute dissociation (Fried *et al.* 1977). Electrolytes cause some order into the chaos of thermal collisions because anions and cations attract each other. A central ion tends to be surrounded by a group of ions of opposite sign. At higher concentrations than 10 mM electroneutral ion pairs start forming. A ratio between osmolality (measured by freezing point depression) and molarity:  $\text{Osm kg}^{-1}/\text{M} = 1.85$  suggests that 15% of NaCl is undissociated ion pairs.

For NaCl the ratio  $\text{Osm kg}^{-1}/\text{M} = 1.85$  stays constant for concentrations ranging between 140 mM and 1 M. Below 140 mM the ratio increases and becomes 1.9 at 50 mM and 2.0 at 10 mM. Above 1 M the ratio exceeds 2.0 at 2.5 M and 2.4 above 4.5 M. Thus the behaviour of NaCl in solution seems complex and is far from understood.

### Solute-permeable membranes

Staverman (1951) derived in terms of irreversible thermodynamics the reflection coefficient  $\sigma$ . Since then attempts have been made to offer a physical interpretation in terms of friction coefficients along the membrane channels, leading to elaborate formulas (Kedem & Katchalsky 1961, Ginzburg & Katchalsky 1963, Hill 1989). Finkelstein (1987, 1989) is with good reason

critical to the introduction of frictional coefficients into formulas for the reflection coefficient and feels that phenomenological coefficients provide no information about the physical nature of osmosis. In comparison, a definition of  $\sigma$  in terms of the kinetic model is simpler:  $(1 - \sigma)$  is the fraction of the solute concentration that behaves as solvent by giving off momentum at the interface of the membrane channels, whereas the other fraction,  $\sigma$ , does not. Solutes with reflection coefficient  $\sigma = 0$  behave completely as solvent. A membrane is semipermeable to a solute with reflection coefficient  $\sigma = 1$ .

Across a solute-permeable membrane with pure water in one compartment and a dissolved solute with reflection coefficient  $\sigma$  in the other compartment, the equation for the kinetic osmotic pressure is

$$\begin{aligned}\pi &= RT/\bar{V} - (RT/\bar{V})(X_1 + (1 - \sigma)X_2) \\ &= \sigma(RT/\bar{V})X_2,\end{aligned}$$

which by integration yields

$$\pi = -\sigma(RT/\bar{V})\ln X_1. \quad (11)$$

For solute-permeable membranes the van't Hoff equation is written  $\Delta\pi = \sigma RT\Delta c$ .

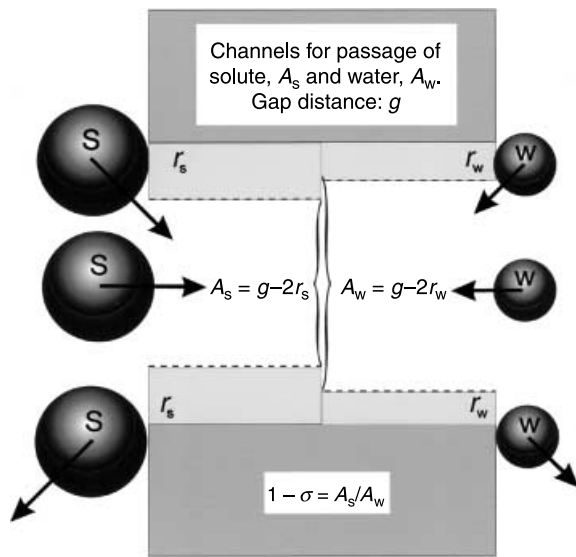
Equations for the reflection coefficient consistent with the kinetic definition may be derived in analogy with derivation of the sieving coefficient of ultrafiltration (Solomon 1968), but there is an important difference. Whereas the kinetic osmotic pressure is a function of the momentum given off at the interface between solvent and impermeant solute, a sieve separates fine from coarser particles independent of the thermal molecular activity.

Figure 4 illustrates solute (S) and water (W) molecules with effective radii of  $r_s$  and  $r_w$  at the openings of a short slit with gap distance  $g$ . Both solute and water molecules give off their momentum to the fluid inside the slit if the distance from the solid wall is larger than the radius of the molecules. Because of differences in molecular size the channel available for passage of solute molecules ( $A_s$ ) is more narrow than the available channel for passage of water molecules ( $A_w$ ). Compared with water molecules only a fraction  $A_s/A_w = (1 - \sigma)$  of the solute molecules give off their momentum as solvent. This event is independent of the friction solvent particles may encounter during the passage of the water channels.

The root-mean-square speed of water molecules is about  $650 \text{ m s}^{-1}$ , 100 000 times more rapid than the highest velocity of transjunctional volume flow in the proximal tubules. The osmotic impact is therefore the same whether the solution is transported to the interface by convection or diffusion, or is stationary (osmotic equilibrium).

In ultrafiltration solute and water molecules approach the membrane channels by convection at the velocity of





**Figure 4** Kinetic derivation of osmotic reflection coefficient ( $\sigma$ ). A fraction of solute molecules ( $1 - \sigma$ ) behaves as water molecules (solvent) by giving off their momentum to molecules within the channel.

the liquid. Convective flow may be laminar with a parabolic flow pattern with the highest speed centrally. Such uneven distribution is taken into account in Renkin's formula (Renkin & Gilmore 1973) and may be applied to osmotic flow through the tight junction, but certainly not to the single-file transport of water molecules through the channel of AQP1. In addition to dimensions of the channel, passage of ions will be influenced by electrical charges in the walls. For instance, positive charges resist the passage of protons in the water channel of AQP1 (Agre *et al.* 2002).

A paradox in transport through solute-permeable membranes was reported by Meschia & Setnikar (1958) and has been the source of much confusion. In a study on collodian membranes separating a urea solution ( $\sigma_{\text{urea}} = 0$ ) from a dextran solution ( $\sigma_{\text{dextran}} = 1$ ), but of lower molar concentration, they demonstrated that osmosis proceeds from the urea to the dextran solution against the chemical potential gradient for water. This experiment led to doubts whether irreversible thermodynamics reflects reality.

In a book about the theory and reality of water movement through membranes, Finkelstein (1987) claimed that water is driven along the membrane channels by a hydrostatic pressure gradient; water drags urea because the pressure gradient forces the solution towards the membrane side. A defender of irreversible thermodynamic interpretations comments: 'How it forces it out of the pores against the large opposing pressure gradient is left for the hapless reader to figure out' (Caplan 1987). Irreversible

thermodynamists too, have been unable to provide a tenable explanation, although the founder of this discipline, Lars Onsager, was on the right track (Kiil 1982). In a more extensive comment Essig & Caplan (1989) states that the gain in free energy from water moving up its chemical potential gradient is more than compensated by the loss in free energy from urea moving down its chemical potential gradient. To underline this view they offer the thought experiment that the channels of the membrane have been narrowed so that even urea does not pass, with the consequence that water flow through the membrane is reversed. As the concentration difference of water between the two compartments has not been altered, they feel that they 'must invoke the interaction of water and urea flows, whose contribution has been abolished'.

To dissolve this difference in opinion between defenders of irreversible thermodynamic and hydrodynamic interpretations of osmosis it is sufficient to recall that urea with  $\sigma_{\text{urea}} = 0$  behaves as water. In the thought experiment with  $\sigma_{\text{urea}} = 1$ , urea has become a solute. Solvents, including the fraction  $1 - \sigma$  of any solute, are always transported from a higher to a lower chemical potential. This rule applies even when water is not among the solvents. Although the term solvent drag has been widely used, it is misleading. Water does not drag urea more than urea drags water. Both are as solvents dragged by the kinetic osmotic force at the interface.

The rule that solvent comprises solutes behaving as water at the interface has two other applications: osmosis proceeds between solutions of equal osmolality if they contain solutes with different reflection coefficients. Solutes with low reflection coefficient may be transported against its concentration gradient, provided the osmotic transport exceeds retrograde transport by diffusion. In these examples it is assumed that solutes behaving as water molecules by giving off their momentum at the interface also pass through the membrane channels. However, in hour-shaped channels a dissociation between these effects is feasible.

In a macroscopic tube liquid transport usually takes place along a hydrostatic pressure gradient. A problem is whether this rule also applies to osmosis. If there were a hydrostatic pressure gradient  $\Delta P/\Delta x$  through a membrane, as Finkelstein (1987) believes, the pressure would be more and more negative along the channel until the interface is reached. At osmotic equilibrium  $\Delta P/\Delta x$  approaches infinity, and the hypothetical negative hydrostatic pressure inside the channel at the interface would equal the positive hydrostatic pressure of the solution. In contrast, in terms of the kinetic model solvent molecules having large cohesive intermolecular forces are towed through the membrane channels by the kinetic pressure difference at the interface. The cohesive

forces between particles are anywhere alike, and the internal kinetic pressure is the same along the train. The kinetic force at the interface is therefore likely to cause a transport that is not dependent on a hydrostatic pressure gradient along the channel.

Kinetic and potential energy is interchangeable, but whether the frictional forces are the same for osmotic and hydrostatic forces is not clear. A reduction in osmotic flow was attributed by Vegard (1908) to the dilution of impermeant solutes contiguous to the semipermeable membrane by water entering through the membrane. Also later investigators have been unable to produce identical flows across supported membranes exposed to identical hydrostatic and osmotic pressures (House *et al.* 1981). Nevertheless, conventional formulas including Starling's equation for transcapillary transport, are based on the assumption that the conductance is the same whether induced by (colloid)-osmotic or hydrostatic pressure differences.

I want to thank my successor as director of the institute, Ole M. Sejersted, for providing a 'think-box' and for his helpful comments. I also want to thank Morten Ræder, Theis Tønnessen and Geir Christensen for their comments, and Per Kr. Lunde and Sonja Flagestad for other help in preparing the manuscript.

## References

- Agre, P., King, L.S., Yasui, M. *et al.* 2002. Aquaporin water channels – from atomic structure to clinical medicine. *J Physiol* **542**, 3–16.
- Arrhenius, S. 1887. Über die Dissociation der in Wasser gelösten Stoffe. *Z Physik Chemie* **1**, 631–648.
- Bearman, R.J. 1961. On the molecular basis of some current theories of diffusion. *J Phys Chem* **73**, 1961–1968.
- Briggs, L.J. 1950. Limiting negative pressure of water. *J Appl Physics* **21**, 721–722.
- Caplan, S.R. 1987. Theoretical reality. *Nature* **329**, 400.
- Comper, W.D. 1994. The thermodynamic and hydrodynamic properties of macromolecules that influence the hydrodynamics of porous systems. *J Theor Biol* **168**, 421–427.
- Comper, W.D. & Williams, R.P.W. 1990. Osmotic flow caused by chondroitin sulfate proteoglycan across well-defined Nucleopore membranes. *Biophys Chem* **36**, 215–222.
- Cussler, E. 1984. *Diffusion. Mass Transfer in Fluid Systems*. Cambridge University Press, Cambridge, pp. 525.
- Dainty, J. & Ferrier, J. 1989. Osmosis at the molecular level. *Stud Biophys* **133**, 133–140.
- Ehrenfest, P. 1915. *Collected Scientific Papers*. M.J. Klein (ed.), 1959. North Holland, Amsterdam, p. 364.
- Einstein, A. 1905. Über die von der molekular-kinetischen Theorie der Wärme geforderte Bewegung von in ruhenden Flüssigkeiten suspendierten Teilchen. *Ann Physik* **17**, 549–560.
- Essig, A. & Caplan, S.R. 1989. Water movement: does thermodynamic interpretation distort reality? *Am J Physiol* **256**, C694–C698.
- Finkelstein, A. 1987. *Water Movement through Lipid Bilayers, Pores, and Plasma Membranes. Theory and Reality*. John Wiley & Sons, New York, 228 pp.
- Finkelstein, A. 1989. Water movement: does thermodynamic interpretation distort reality? *Am J Physiol* **256**, C699.
- Fried, V., Hamerka, H.F. & Blukis, U. 1977. *Physical Chemistry*. Macmillan Publishing Co, New York, pp. 983.
- Ginzburg, B.Z. & Katschalsky, A. 1963. The frictional coefficients of the flows of non-electrolytes through artificial membranes. *J Gen Physiol* **47**, 403–418.
- Gouy, M. & Chaperon, G. 1888. Sur l'équilibre osmotique. *Ann Chim Phys* **6**, 120–132.
- Hein, P. 1966. *Atomyriades. Grooms*. Borgens Pocketbooks, Copenhagen, 24 pp.
- Hill, A.E. 1989. Osmotic flow equations for leaky porous membranes. *Proc R Soc Lond* **237**, 369–377.
- van't Hoff, J.H. 1886. Une propriété général de la matière diluée. *Svenska Vet Akad Handl* **21**, 1–49.
- van't Hoff, J.H. 1894. Wie die Theorie der Lösungen entstand. *Ber Dtsch Chem Ges* **27**, 6–19.
- House, C.R. 1981. Unstirred layers and osmotic flow across artificial membranes. In: H.H. Ussing, N. Bindsvlev, N.A. Lassen & O. Sten-Knudsen (eds) *Water Transport Across Epithelia*, pp. 120–129. Munksgaard, Copenhagen.
- Janáček, K. & Sigler, K. 2000. Osmosis: membranes impermeable and permeable for solutes, mechanism of osmosis across porous membranes. *Physiol Res* **49**, 191–195.
- Kedem, O. & Katchalsky, A. 1961. A physical interpretation of the phenomenological coefficients of membrane permeability. *J Gen Physiol* **45**, 143–179.
- Kiil, F. 1982. Mechanism of osmosis. *Kidney Int* **21**, 303–308.
- Kiil, F. 1989. Molecular mechanisms of osmosis. *Am J Physiol* **256**, R801–R808.
- Kiil, F. 2002. Mechanisms of transjunctional transport of NaCl and water in proximal tubules of mammalian kidneys. *Acta Physiol Scand* **175**, 55–70.
- Landau, L.D., Akhiezer, A.I. & Lifshitz, E.M. 1967. *General Physics. Mechanics and Molecular Physics*, p. 145. Pergamon Press, Oxford.
- Meschia, G. & Setnikar, J. 1958. Experimental study of osmosis through a collodion membrane. *J Gen Physiol* **42**, 429–444.
- Nielsen, S. & Agre, P. 1995. The aquaporin family of water channels in kidney. *Kidney Int* **48**, 1057–1068.
- Pfeffer, W. 1877. *Osmatische Untersuchungen* W. Engelmann, Leipzig.
- Pollack, G.H. 2001. *Cells, Gels and the Engines of Life*, pp. 305. Ebner & sons, Seattle.
- Poynting, J.H. 1896. Osmotic pressure. *Philosop Mag J Sci* **42**, 289–301.
- Renkin, E. & Gilmore, J. 1973. Glomerular filtration. In: J. Orloff & R. Berliner (eds) *Handbook of Physiology. Section 8: Renal Physiology*, pp. 185–248. American Physiological Society, Washington D.C.
- Rock, P.A. 1983. *Chemical Thermodynamics*, p. 342. Oxford University Press, Oxford.
- Smoluchowski, M.V. 1906. Zur kinetischen Theorie der Brownschen Molekularbewegung und der Suspensionen. *Ann Physik* **21**, 756–780.

- Solomon, A. 1968. Characterization of biological membranes by equivalent pores. *J Gen Physiol* **51**, 335–364.
- Staverman, A. 1951. The theory of measurement of osmotic pressure. *Recueil des Travaux Chimiques des Pays-Bas* **70**, 344–352.
- Sten-Knudsen, O. 1978. Passive transport processes. In: G. Giebisch, D.C. Tosteson & H.H. Ussing (eds) *Membrane Transport in Biology*, **1**, pp. 5–113. Springer Verlag, Berlin.
- Vegard, L. 1908. On the free pressure in osmosis. *Proc Camb Phil Soc* **15**, 13–23.
- deVries, H. 1883. Sur la force osmotique des solutions diluées. *Compt Rend* **97**, 1083–1087.
- deVries, H. 1888. *Osmotische Versuche mit lebenden Membranen*. *Phys Chem* **2**, 415–419.
- van der Waals, J.D. 1873. Over de Continenteit van den Gas, PhD Thesis. University of Leiden, Leiden, Netherlands.
- Wiggins, P.M. 1995. Micro-osmosis in gels, cells and enzymes. *Cell Biochem Function* **13**, 165–172.