

Development of Complex Curricula for Molecular Bionics and Infobionics Programs within a consortial* framework**

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**Molekuláris bionika és Infobionika Szakok tananyagának komplex fejlesztése konzorciumi keretben

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INTRODUCTION TO BIOPHYSICS

(Bevezetés a biofizikába)

COLLIGATIVE PROPERTIES

(Kolligatív sajátságok)

GYÖRFFY DÁNIEL, ZÁVODSZKY PÉTER

Introduction

- Colligative properties are properties of a dilute solution that depend only on the number of particles in the solution but do not depend on the properties of them, like mass or size etc.
- Colligative properties are:
 - Osmotic pressure
 - Lowering of vapour pressure
 - Elevation of boiling point
 - Depression of freezing point

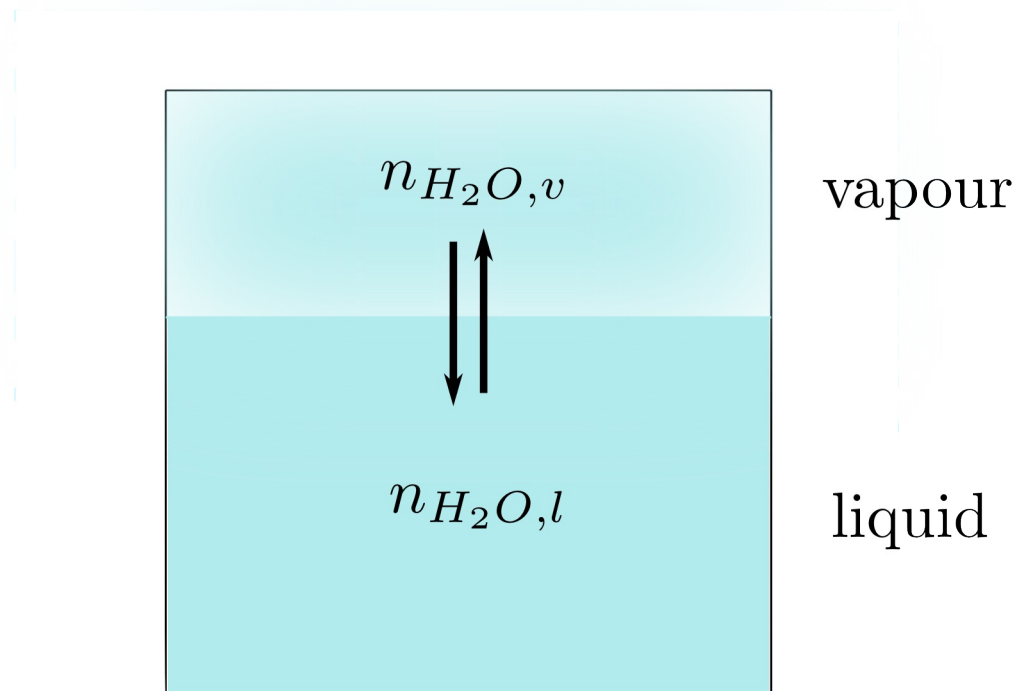
Osmotic pressure

- Osmotic pressure is one of the colligative properties of a solution
- To formalize it, let us set out from the known fundamental law:
 - In a heterogenous – consisting of more than one phase –, closed system at equilibrium, the chemical potential of an uncharged substance is the same in all phases between which it can pass freely

- Consider a closed box containing water in equilibrium with its vapour
- Suppose some vapour goes into the liquid phase (or vice versa)
- We would like to know what the free energy change of the system is

$$dG_{system} = \left(\frac{\partial G}{\partial n_l} \right) dn_l + \left(\frac{\partial G}{\partial n_v} \right) dn_v = \mu_l dn_l + \mu_v dn_v$$

Liquid-vapour system



- Since the system is closed, i.e. only energy exchange is possible between the system and its environment, we know that

$$n_l + n_v = \text{constant}$$

so

$$dn_l + dn_v = 0$$

and

$$dn_l = -dn_v$$

- Thus the free energy change is

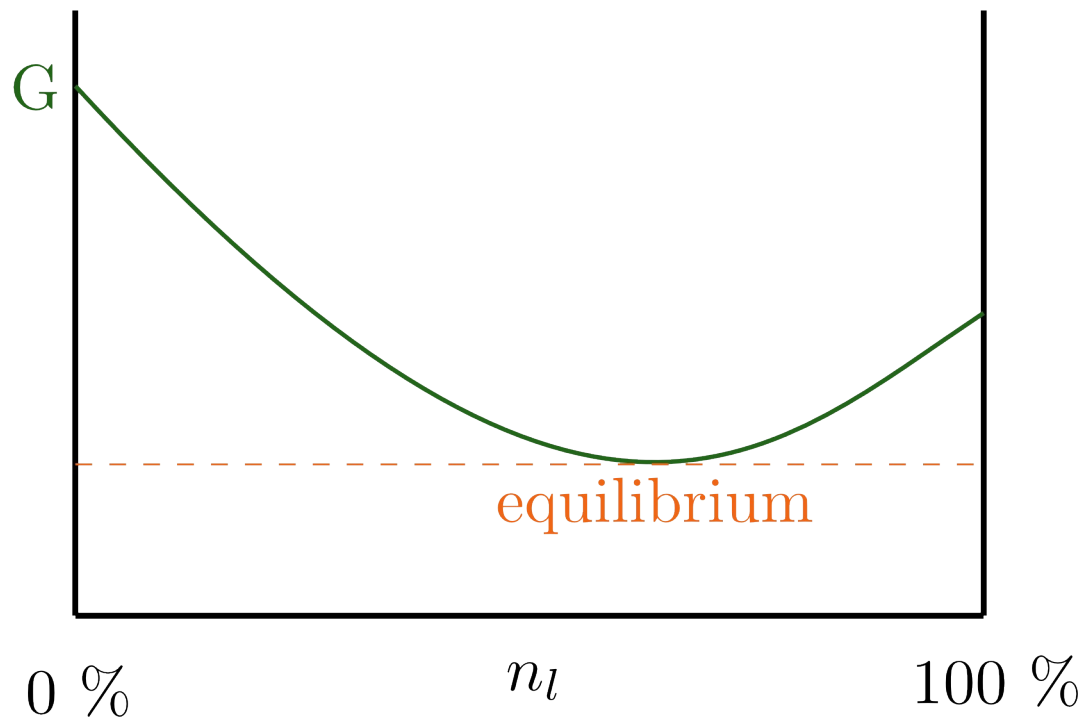
$$dG = \mu_l dn_l - \mu_v dn_v = (\mu_l - \mu_v) dn_l$$

based on which

$$\frac{dG}{dn_l} = \mu_l - \mu_v$$

- Let us plot the free energy, G , as a function of the number of moles in the liquid phase, n_l
- We can see that there pure liquid or pure vapour are both unstable conditions
- Therefore the free energy decreases moving away from either extreme
- Somewhere there is a minimum to this function
- By definition this is the equilibrium position
- At this point the slope of the curve equals zero

G vs. n_l



- Since at equilibrium the slope of the curve is

$$\left(\frac{\partial G}{\partial n_l}\right)=0$$

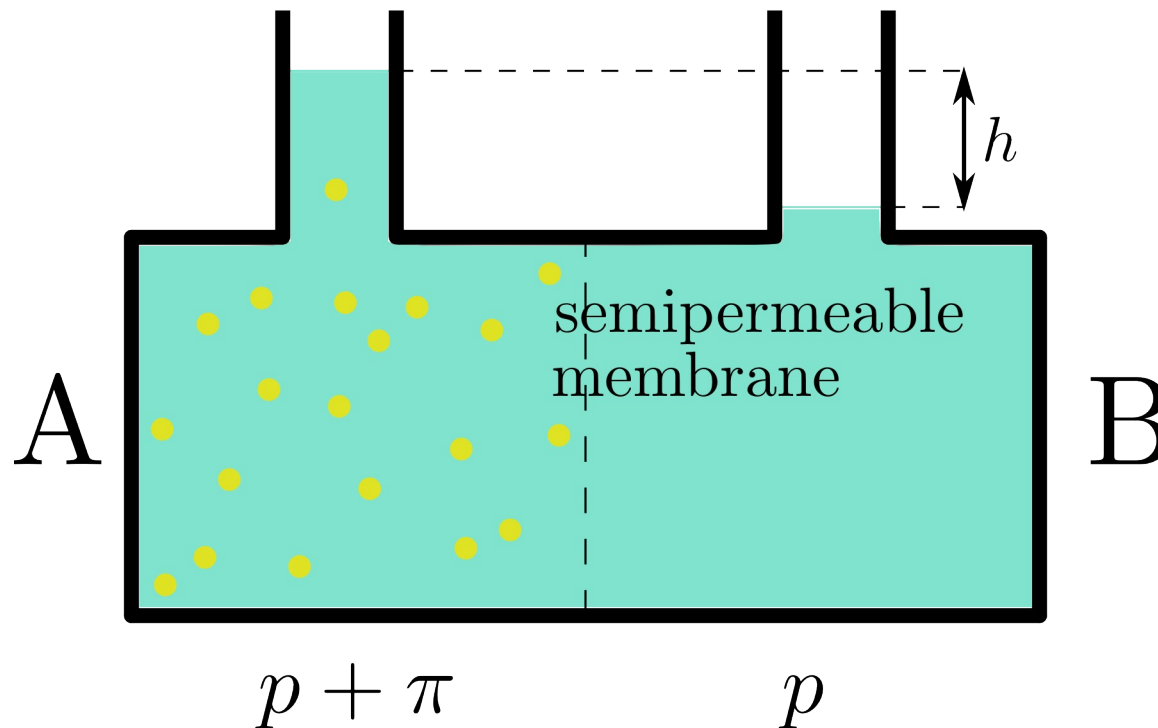
$$\mu_l - \mu_v = 0$$

and

$$\mu_l = \mu_v$$

- Now we will apply this fundamental law to osmotic pressure
- Let us consider the following figure
- In chamber A there are water and some polysaccharide to which the membrane is impermeable
- In chamber B there is pure water

Demonstration of the osmotic pressure



- Water will flow from side B to side A generating a pressure difference proportional to h , the difference in water levels in the standpipes at equilibrium
- The excess pressure on side A is the osmotic pressure and is signed by π

- As we know, the pressure is a function of depth

$$\pi (\text{Pa}) = h (\text{m}) \cdot \rho (\text{kg/m}^3) \cdot g (\text{m/sec}^2)$$

$$\text{Pa} = \text{N/m}^2$$

- π can be converted to atm by noting

$$1 \text{ atm} = 101325 \text{ Pa}$$

- For our case

$$\rho_{H_2O} = 1000 \text{ kg/m}^3$$

and

$$g = 9.80 \text{ m/sec}^2$$

- From the fundamental law discussed earlier, the chemical potential of water must be the same on both sides of the membrane
- This is not true for the polysaccharide which can not pass the membrane

- μ is a function of the temperature, the pressure and the composition of the solution

$$\mu_{1,A}(T, p + \pi, x_{1,A}) = \mu_{1,B}(T, p, x_{1,B})$$

- Since temperature is constant on both sides, we can consider μ to be a function of only the pressure and the composition

$$\mu = f(p, x)$$

- Making use of the known relationship, the chemical potentials are

$$\mu_{1,A}(p + \pi, x_{1,A}) = \mu_{1,A}^0(p + \pi) + RT \ln \gamma_{1,A} x_{1,A}$$

and

$$\mu_{1,B}(p, x_{1,B}) = \mu_{1,B}^0(p) + RT \ln \gamma_{1,B} x_{1,B} = \mu_{1,B}(p)$$

making use of the fact that

$$x_{1,B} = 1 \quad \text{and} \quad \gamma_{1,B} = 1$$

- According to the fundamental law

$$\mu_{1,A}^0(p + \pi) + RT \ln \gamma_{1,A} x_{1,A} = \mu_{1,B}^0(p)$$

so

$$\mu_{1,A}^0(p + \pi) - \mu_{1,B}^0(p) = -RT \ln x_{1,A} - RT \ln \gamma_{1,A}$$

- Now, we wonder how μ changes with p
- From basic thermodynamics, let us recall

$$\left(\frac{\partial G}{\partial p} \right)_{p, n} = V$$

and

$$\left(\frac{\partial G}{\partial n} \right)_{T, p} = \mu$$

- Since we can do partial differentiation in any order

$$\left(\frac{\partial \mu}{\partial p}\right) = \left[\frac{\partial}{\partial p} \left(\frac{\partial G}{\partial n}\right)_{T,p}\right]_{T,n}$$

$$\left[\frac{\partial}{\partial p} \left(\frac{\partial G}{\partial n}\right)_{T,p}\right]_{T,n} = \left[\frac{\partial}{\partial n} \left(\frac{\partial G}{\partial p}\right)_{T,n}\right]_{T,p}$$

$$\left[\frac{\partial}{\partial n} \left(\frac{\partial G}{\partial p} \right)_{T, n} \right]_{T, p} = \left[\frac{\partial}{\partial n} (V) \right]_{T, p}$$

$$\left[\frac{\partial}{\partial n} (V) \right]_{T, p} = \left(\frac{\partial V}{\partial n} \right)_{T, p} = \bar{V}$$

which is the partial molar volume

- For the standard chemical potential

$$\frac{\partial \mu_1^0}{\partial p} = \bar{V}_1^0$$

where $\bar{V}^0$ is the molar volume of the solvent

- After rearrangement we get

$$\partial \mu_1^0 = \bar{V}_1^0 \partial p$$

- Let us integrate $\partial\mu_1^0$ from p to $p+\pi$ assuming that water is incompressible, that is $\bar{V}_1^0$ is constant

$$\int_p^{p+\pi} \partial \mu_1^0 = \int_p^{p+\pi} \bar{V}_1^0 \partial p$$

- We get

$$\mu_1^0(p+\pi) - \mu_1^0(p) = \bar{V}_1^0 \cdot (p+\pi) - \bar{V}_1^0 \cdot p$$

- Finally we get

$$\bar{V}_1^0 \pi = -RT \ln x_{1,A} - RT \ln \gamma_{1,A}$$

- Since

$$x_1 + x_2 = 1,$$

$$x_1 = 1 - x_2$$

and thus

$$\bar{V}_1^0 \pi = -RT \ln(1 - x_2) - RT \ln \gamma_1$$

- Let us suppose that the concentration of polysaccharide is

$$w = 10 \text{ g/dm}^3$$

and the molecular weight of it is

$$M = 25000 \text{ Da}$$

so the molar concentration of it is

$$c = \frac{10 \text{ g/dm}^3}{25000 \text{ g/mol}} = 4 \cdot 10^{-4} \text{ mol/dm}^3$$

- Thus the mole fraction of the polysaccharide is

$$x = \frac{c}{c + c_{H_2O}} = \frac{4 \cdot 10^{-4} \text{ mol/dm}^3}{55.5604 \text{ mol/dm}^3} \approx 7 \cdot 10^{-6}$$

a very small number

- For small numbers

$$\ln (1 - \delta) \approx -\delta$$

and

$$\ln (1 + \delta) \approx \delta$$

if

$$\delta \ll 1$$

- Performing this approximation we get

$$\bar{V}_1^0 \pi = RT x_2 - RT \ln \gamma_1$$

- We know that

$$x_2 = \frac{n_2}{n_1 + n_2}$$

but since $n_1 \ll n_2$ we can write that

$$x_2 \approx \frac{n_2}{n_1}$$

- Thus

$$\bar{V}_1^0 \pi = RT \frac{n_2}{n_1} - RT \ln \gamma_1$$

from which

$$\pi = \frac{RT n_2}{n_1 \bar{V}_1^0} - \frac{RT \ln \gamma_1}{\bar{V}_1^0}$$

- Further simplifications can be performed

$$n_1 \bar{V}_1^0 \simeq V_1$$

and since the polysaccharide takes up only a small portion of the volume of the solution

$$V_1 \approx V$$

- Substituting these simplified expressions into the main equation we obtain

$$\pi = RT \frac{n_2}{V} - \frac{RT \ln \gamma_1}{\bar{V}_1^0} = RT c_2 - \frac{RT \ln \gamma_1}{\bar{V}_1^0}$$

- In the lab, we would weigh out a number of grams of polysaccharide and report its solution concentration in g/dm^3
- Let w_2 denote the weight of polysaccharide per liter
- Thus

$$c_2 = \frac{w_2}{M_2}$$

where M_2 is the molecular weight of the polysaccharide

- Substituting this expression of the molar concentration into the expression of the osmotic pressure we get

$$\pi = \frac{RT w_2}{M_2} - \frac{RT}{\bar{V}_1^0} \ln \gamma_1$$

- Now let us imagine that we are at great dilution
- Now

$$\gamma_1 \approx 1$$

and

$$\ln \gamma_1 \approx 0$$

thus

$$\pi = \frac{RT w_2}{M_2}$$

- We can solve this equation for the molecular weight of the polysaccharide

$$M_2 = \frac{RT w_2}{\pi}$$

so measuring the osmotic pressure of a solution of some substance its molecular weight can be determined

- Another common form of the equation at infinite dilution

$$\pi = RT c_2$$

- It can reminds us of the gas law

$$\pi V = n_2 RT$$

which holds for ideal solutions

- Let us see more about the *nonideality* term in the equation for osmotic pressure

$$\pi = \frac{RT w_2}{M_2} - \frac{RT}{\bar{V}_1^0} \ln \gamma_1$$

- Let us recall that if we plot μ_1 as a function of x_1 we get a straight line with slope $R \cdot T$ for the ideal case
- The distance between this line and the real curve is $-RT \ln \gamma_1$

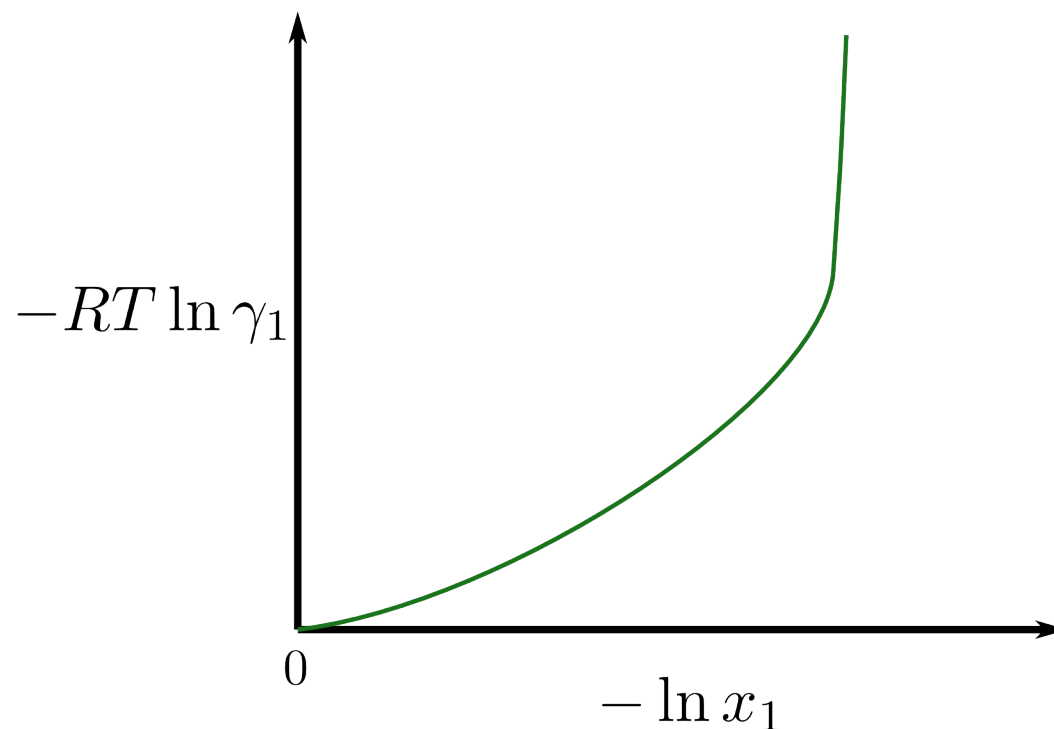
- Now, let us plot $-RT \ln \gamma_1$ as a function of $\ln x_1$
- The slope of the curve approaches zero as $\ln x_1$ approaches zero
- This is because the real and ideal curves coincide at

$$\ln x_1 = 0$$

since

$$\gamma_1 = 1 \quad \text{at} \quad x_1 = 1$$

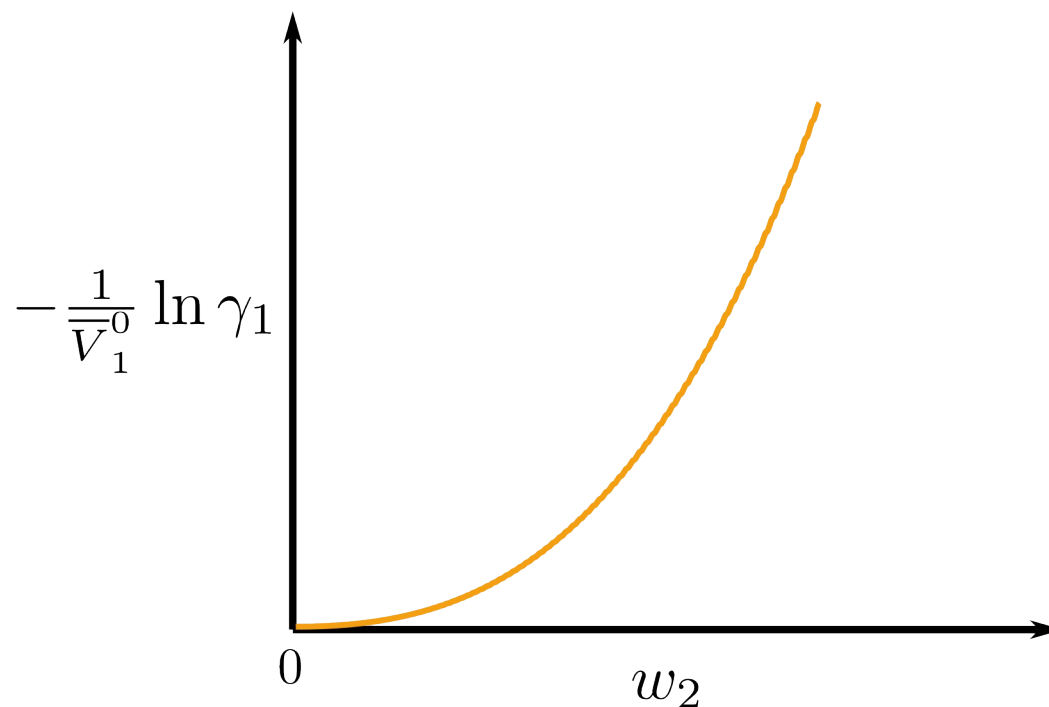
$-RT \ln \gamma_1$ vs. $\ln x_1$



- We can change the axes by multiplying the vertical axis by $1/(R \cdot T \cdot \bar{V}_1^0)$ and the horizontal axis by $55.56 \cdot M_2$ which converts x_2 to w_2

$$x_2 = \frac{n_2}{n_1 + n_2} \approx \frac{n_2}{n_1} = \frac{c_2}{c_1} = \frac{c_2}{55.56} = \frac{w_2}{55.56 M_2}$$

$$-1/\bar{V}_1^0 \ln \gamma_1 \text{ vs. } w_2$$



- We can use a Taylor expansion to express this function relating

$$-\frac{1}{\bar{V}_1^0} \ln \gamma_1$$

to

$$w_2$$

- The Taylor expansion is

$$-\frac{1}{\bar{V}_1^0} \ln \gamma_1 = B_0 + B_1 w_2 + B_2 w_2^2 + \dots$$

where B_i 's are constants

- This is an infinite series, but B_i gets very small further out

- Now we should evaluate what B_0 and B_1 are
- When

$$w_2 = 0$$

then

$$-\frac{1}{\bar{V}_1^0} \ln \gamma_1 = B_0$$

so

$$B_0 = 0$$

- B_1 is the initial slope

$$\frac{\partial \left(\frac{1}{\bar{V}_1^0} \ln \gamma_1 \right)}{\partial w_2} = B_1 + 2 B_2 w_2$$

which is also zero

$$B_1 = 0$$

- The curve starts off as a parabola

$$-\frac{1}{\bar{V}_1^0} \ln \gamma_1 = B_2 w_2^2 + B_3 w_2^3 + \dots$$

SO

$$\pi = RT \frac{w_2}{M_2} + RT \left(B_2 w_2^2 + B_3 w_2^3 + \dots \right)$$

- In general we do not know the values of B 's but this is the form of the equation
- Now, let us divide through by w_2

$$\frac{\pi}{w_2} = RT \left(\frac{1}{M_2} + B_2 w_2 + \text{'higher order terms'} \right)$$

where the higher order terms can be neglected

- Now the nonideality term is approximated by a constant B_2 times w_2

- This is the virial expansion
 - $1/M_2$ is the first virial coefficient
 - B_2 is the second virial coefficient
- Experimentally we can not measure π at infinite dilution
- We must measure π at finite concentration where nonideality is small but still present

Example 1

- Suppose we collected the following data:

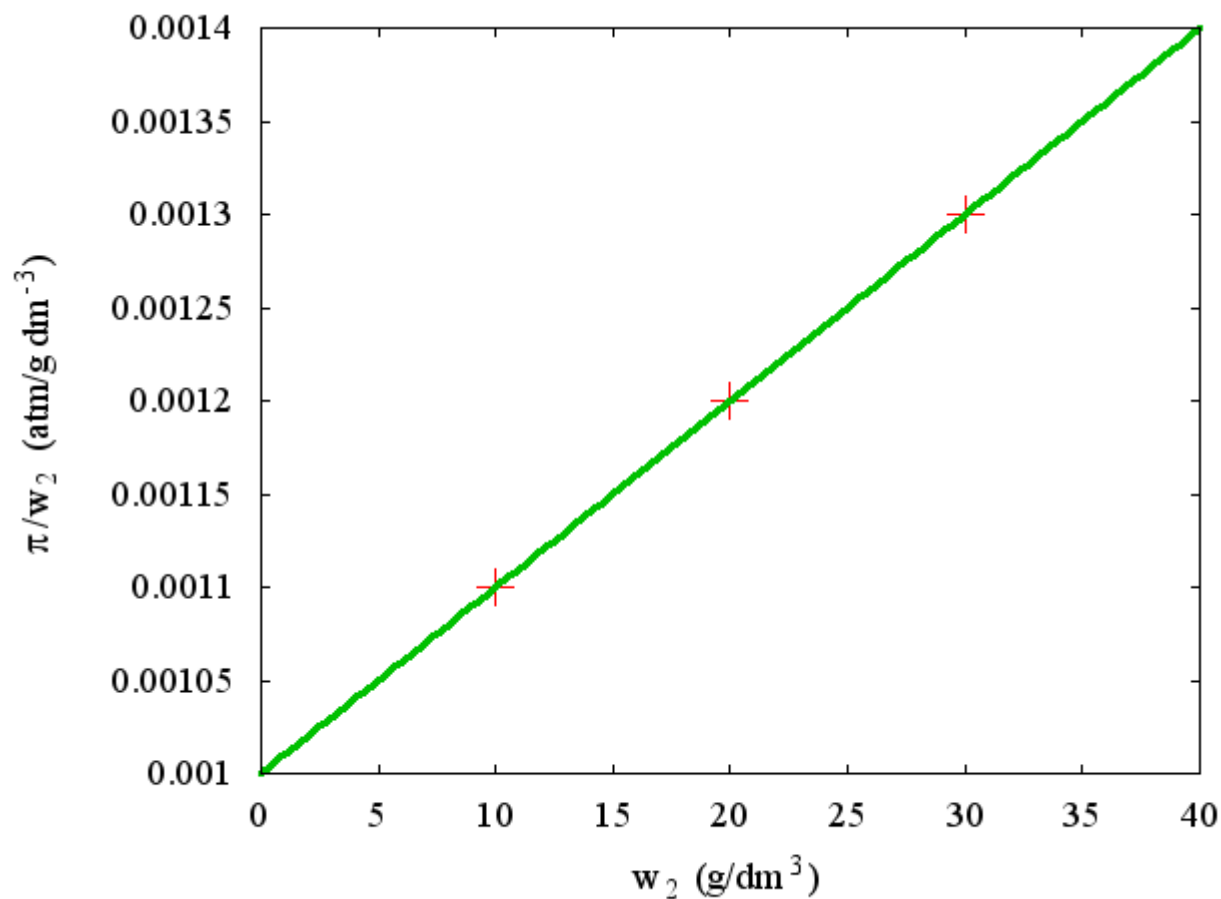
w_2 (g/dm ³)	π (atm)	π/w_2 (atm/g·dm ⁻³)
10	0.011	0.0011
20	0.024	0.0012
30	0.039	0.0013

- Let us plot π/w_2 as a function of w_2
- The extrapolated intercept gives us M_2 without the nonideality term

$$\text{intercept} = \frac{RT}{M_2}$$

- The slope of the curve is

$$\text{slope} = RT B_2$$



- The slope gives us the second virial coefficient
- The second virial coefficient tells us about forces between molecules
- When the slope is positive, the forces are repulsive
- A pure protein in a solution, at a pH which is not the isoelectric point, will have the same charge as all the other protein molecules
- These protein molecules will repel each other

- When the slope is negative, the forces are attractive
 - Such particles will dimerize (polymerize) at higher concentrations

- Two additional rules:
 - In an osmometer, if side A contains a polysaccharide at one concentration and side B contains a polysaccharide at a different concentration then

$$\pi = RT \Delta c$$

- If we put more polysaccharides on the same side of an osmometer then

$$\pi = RT \sum_i c_i$$

Macromolecules with bound dissociable ions

- Let us consider a container consisting of two chambers separated by a semipermeable membrane, called osmometer
- Let us put a protein in side A that dissociates to give one Na^+ ion
- There will be then the same number of proteins (negatively charged) as Na^+ ions

- According to this if

$$[P^-]_A = a$$

where $[P^-]_A$ is the molar concentration of the protein in chamber A, then

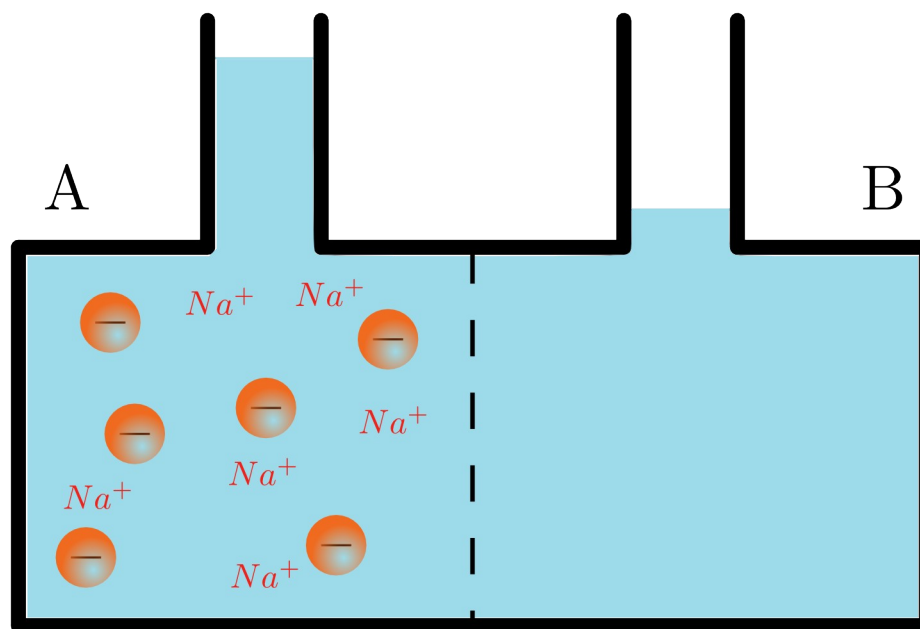
$$[Na^+] = a$$

- Therefore, now

$$\pi = RT \, 2 \, a$$

because π is a colligative property

Proteins with bound dissociable Na^+ ions



- Na^+ can diffuse across the membrane but the law of 'electroneutrality' states that there cannot be a finite (large) charge separation in solution
- On microscopic scale, we violate this law all the time (for example battery or the mitochondrial membrane etc.)

- If we measured M_2 without taking this into account, it will be too small by a factor of 2
- If the protein dissociates to give 10 Na^+ , then the M_2 would be off by a factor of 10

- Before we understood about polyelectrolytes, people thought proteins had a very small molecular weight
- Also the molecular weight appeared to vary a lot
- This leads to the *colloidal theory* of protein structure
- We can solve this problem by dumping a lot of salt into the system