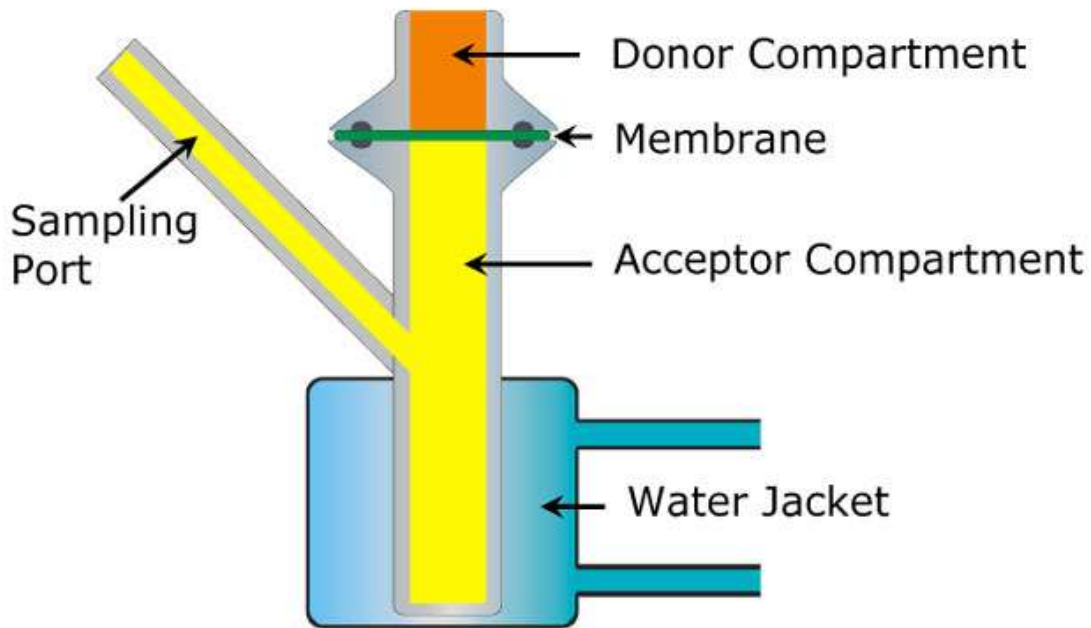


# Diffusion through a membrane

One of the most common measurements in pharmacy is the determination of drug permeability across a model membrane, such as skin, cornea, etc. The measurement is carried out either in Franz cell or in a side-by-side diffusion cell.



Franz cell. If the membrane is skin, *stratum corneum* points upwards.



A side-by-side diffusion cell. Total width ca. 9 cm. Chamber volume ca. 3 cm<sup>3</sup>.

Both the donor ( $\alpha$ ) and acceptor compartments ( $\beta$ ) are stirred so that ideally no concentration differences appear in their bulk solution. Samples are withdrawn from the acceptor compartment and its concentration determined with fluorescence or UV detection after separation with High Performance Liquid Chromatography (HPLC). Our task is to seek for the concentration as a function of time. Often the permeability is so low that the concentration of the acceptor compartment remains practically zero compared with the donor compartment. This is known as the **perfect sink condition**. In the beginning of an experiment there is a lag time during which the steady-state is reached. After that the concentration profile in the membrane is linear:

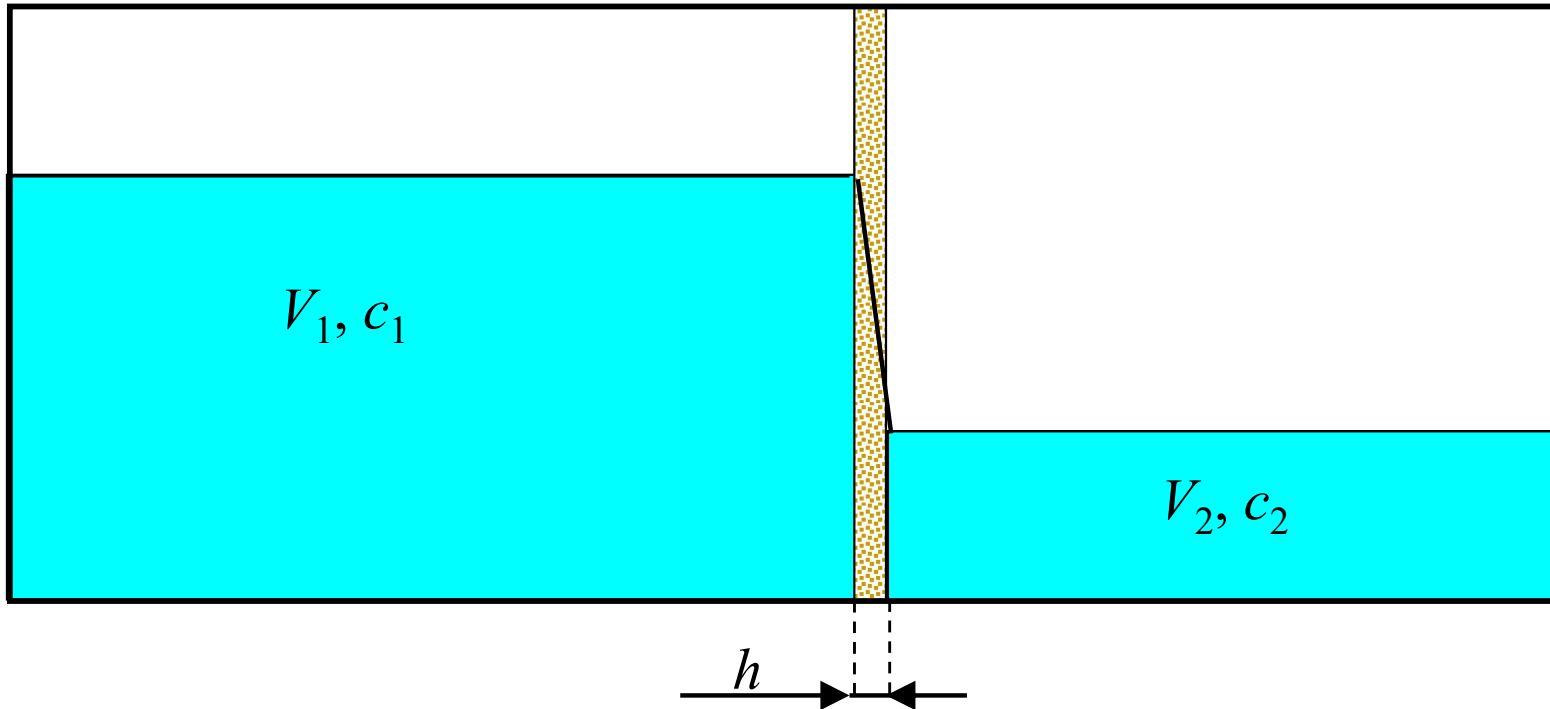
$$c(x) = c^\alpha + \frac{c^\beta - c^\alpha}{h} x \quad (8.1)$$

and the flux across the membrane is

$$J = -D \frac{c^\beta - c^\alpha}{h} \quad (8.2)$$

Example:

Membrane, thickness  $h$  and surface area  $A$ , separates two compartments with volumes  $V_1$  and  $V_2$ . Initially,  $c_1 = c_0$  and  $c_2 = 0$ . Calculate the concentrations  $c_1$  and  $c_2$  as a function of time.



This set-up corresponds to a typical drug permeation experiment.

Assume that both compartments are ideally stirred, i.e. they have a homogeneous concentration at all times. Mass balance:

$$V_1 c_0 = V_1 c_1(t) + V_2 c_2(t) \rightarrow c_2(t) = V_1/V_2 [c_0 - c_1(t)] \quad (8.3)$$

The volume of the membrane is thus neglected.

Diffusion takes place only in the membrane, and we already know the solution:

$$c = B_1 x + B_2 \quad (8.4)$$

With the boundary conditions ( $x = 0, c = c_1$ ) and ( $x = h, c = c_2$ )  $B_1$  and  $B_2$  can be found. The result is finally

$$c(x) = \frac{c_2 - c_1}{h} x + c_1 \Rightarrow \frac{\partial c}{\partial x} = \frac{c_2 - c_1}{h} = \frac{1}{h} \left[ \frac{V_1}{V_2} (c_0 - c_1) - c_1 \right] \quad (8.5)$$

Diffusion reduces the amount of the solute in compartment 1:

$$J = -\frac{V_1}{A} \frac{\partial c_1}{\partial t} = -D \frac{\partial c}{\partial x} = -\frac{D}{h} \left[ \frac{V_1}{V_2} c_0 - \left( 1 + \frac{V_1}{V_2} \right) c_1 \right] \quad (8.6)$$

Collecting the constants together,

$$\frac{dc_1}{dt} = \alpha - \beta c_1 \quad ; \quad \alpha = \frac{DAc_0}{V_2 h} \quad ; \quad \beta = \frac{DA}{h} \left( \frac{1}{V_1} + \frac{1}{V_2} \right) \quad (8.7)$$

This is an ordinary differential equation, its solution is

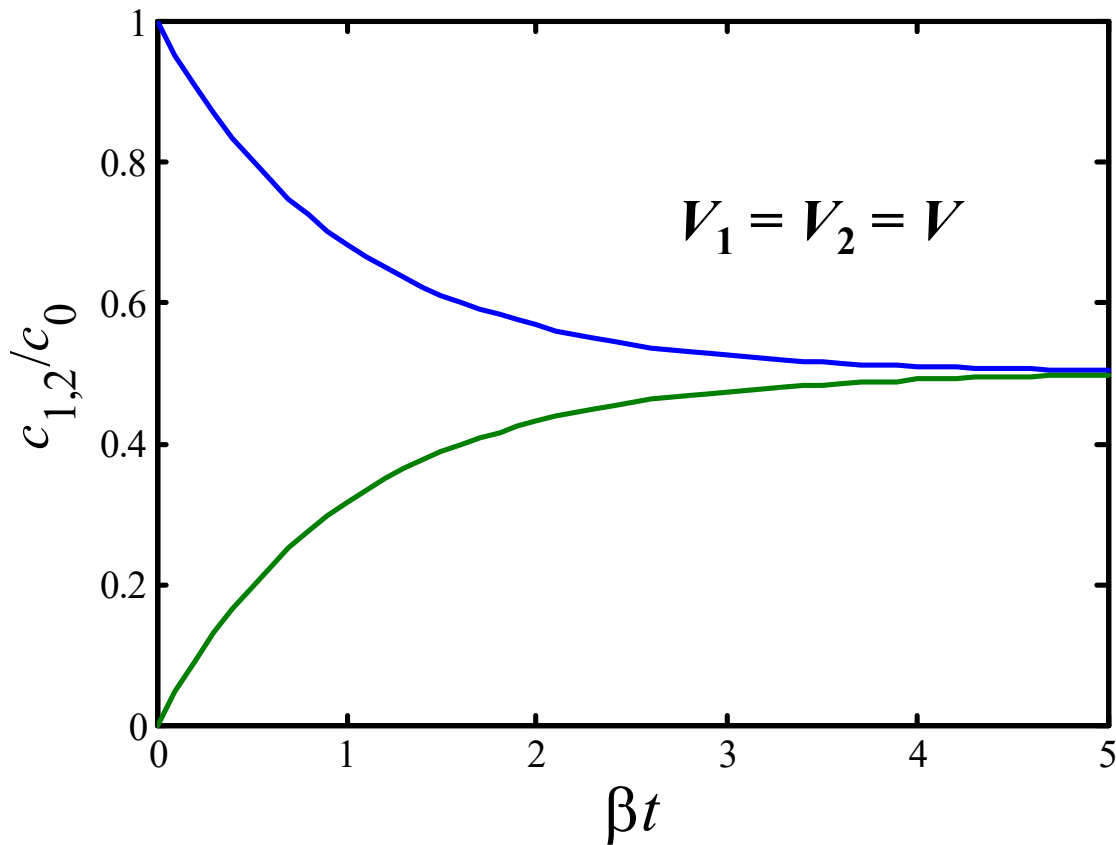
$$c_1 = \frac{\alpha}{\beta} + \left( c_0 - \frac{\alpha}{\beta} \right) e^{-\beta t} \quad ; \quad \frac{\alpha}{\beta} = \frac{c_0 V_1}{V_1 + V_2} \quad (8.8)$$
$$\frac{c_1}{c_0} = \frac{V_1 + V_2 e^{-\beta t}}{V_1 + V_2}$$

where the initial condition  $c_1(t = 0) = c_0$  has been applied. From mass balance (8.3) it is obtained

$$\frac{c_2}{c_0} = \frac{V_1}{V_2} \left( 1 - \frac{c_1}{c_0} \right) = \frac{V_1 (1 - e^{-\beta t})}{V_1 + V_2} \quad (8.9)$$

If  $V_1 = V_2$ , eq. (8.9) can be written in the form where a linear fit is possible:

$$\beta t = \ln \left( \frac{2c_2}{c_0} - 1 \right) \quad (8.10)$$

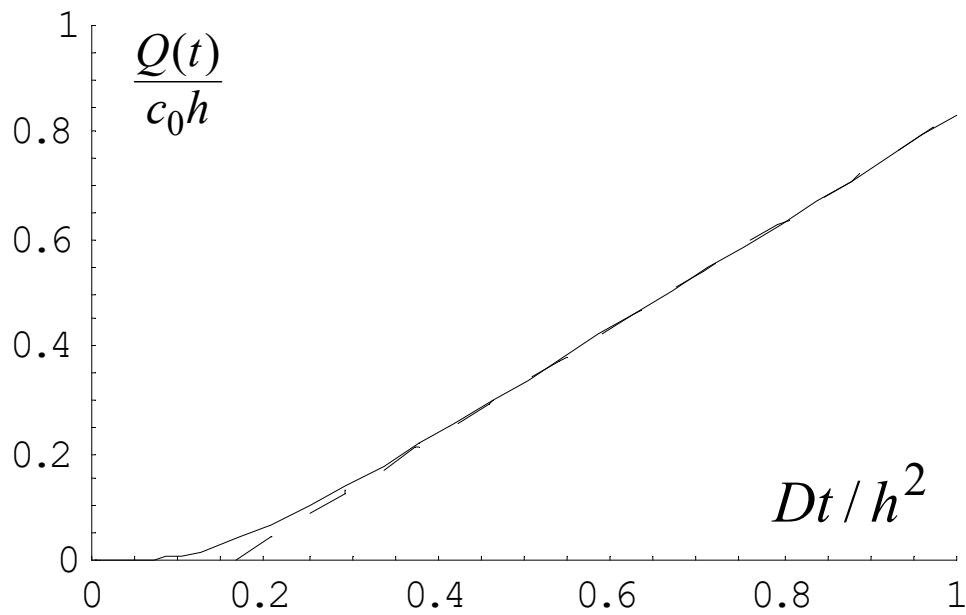


Aside plots of eq. (8.8) (descending) and (8.9) (ascending).

In the side-by-side cell  $V_1 = V_2 = V$ , and  $\beta$  is very small because the permeability  $K_p = D/h$  across a membrane is often low. Hence, exponent can be linearized, giving the practical result

$$\frac{c_2}{c_0} \approx \frac{DA}{Vh} t = K_p \frac{A}{V} t \quad (8.11)$$

Plotting the concentration of the acceptor compartment as a function of time, a straight line is obtained, giving the permeability from its slope.



In the beginning of the experiment there is the lag time before the steady-state is reached, but after that our solution is accurate. The full solution is obtained by solving Fick's 2. law (see textbook). The result is (perfect sink condition, donor at the constant concentration of  $c_0$ ):

$$\frac{Q(t)}{c_0 h} = \frac{Dt}{h^2} - \frac{1}{6} - \frac{2}{\pi^2} \sum_{k=1}^{\infty} \frac{(-1)^k}{k^2} \exp\left(-\frac{k^2 \pi^2 Dt}{h^2}\right) ; \quad Q(t) = \int_0^t J(h, u) du \quad (8.12)$$

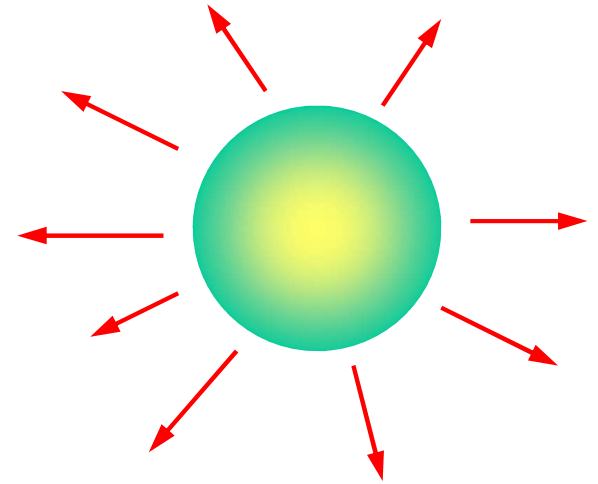
The series term vanishes rapidly, leaving a straight line that intercepts  $x$  axis at the point  $Dt/h^2 = 1/6$ . As a consequence, the lag time of diffusion is defined as

$$\tau = \frac{h^2}{6D} = \frac{h}{6K_p} \quad (8.13)$$

Since the slope of the straight line gives the permeability  $K_p$ , the actual membrane thickness  $h$  and furthermore  $D$  can be calculated from eq. (8.13).

## Dissolution of a spherical pill

The solution is searched in spherical geometry. During dissolution saturated concentration prevails on the pill surface at all times. Diffusion transports material from the surface into the bulk solution. Boundary conditions are thus  $(r = a, c = c^s)$  and  $(r = \infty, c = 0)$ . The solution was given earlier as  $c(r) = B_1 + B_2/r$ .



BCs tell that  $B_1 = 0$  and  $B_2 = c^s a$  ( $a = a(t)$  is the pill radius and  $c^s$  saturated concentration). Hence,

$$c(r) = c^s \frac{a}{r} \quad ; \quad J = -D \left( \frac{\partial c}{\partial r} \right)_{r=a} = \frac{Dc^s}{a} \quad (8.14)$$

The mass of the pill,  $m$ , is

$$m(t) = Mn(t) = \rho V(t) = \rho \frac{4}{3} \pi a(t)^3 \quad (8.15)$$

where  $M$  is molar mass,  $\rho$  density and  $V$  volume of the pill.



$$n = \frac{\rho}{M} \frac{4}{3} \pi a^3 \quad \Rightarrow \quad \frac{\partial n}{\partial t} = \frac{\rho}{M} 4\pi a^2 \frac{\partial a}{\partial t} = \frac{\rho}{M} A \frac{\partial a}{\partial t} \quad (8.16)$$

Dissolution reduces the amount of material of the pill:

$$-\frac{1}{A} \frac{\partial n}{\partial t} = J \quad \Rightarrow \quad -\frac{\rho}{M} \frac{\partial a}{\partial t} = D \frac{c^s}{a} \quad (8.17)$$

Initially, ( $t = 0$ ,  $a = a_0$ ). Integrating eq. (8.17) it is obtained

$$a = \sqrt{a_0^2 - 2Dc^s \frac{M}{\rho} t} \quad (8.18)$$

The characteristic feature of diffusion limited processes is their  $t^{1/2}$  dependence. The lifetime of the pill,  $\tau$ , is reached when  $a = 0$ :

$$\tau = \frac{\rho}{M} \frac{a_0^2}{2Dc^s} \quad \Rightarrow \quad a = a_0 \sqrt{1 - \frac{t}{\tau}} \quad (8.19)$$

Finally, the mass of the pill as a function of time is

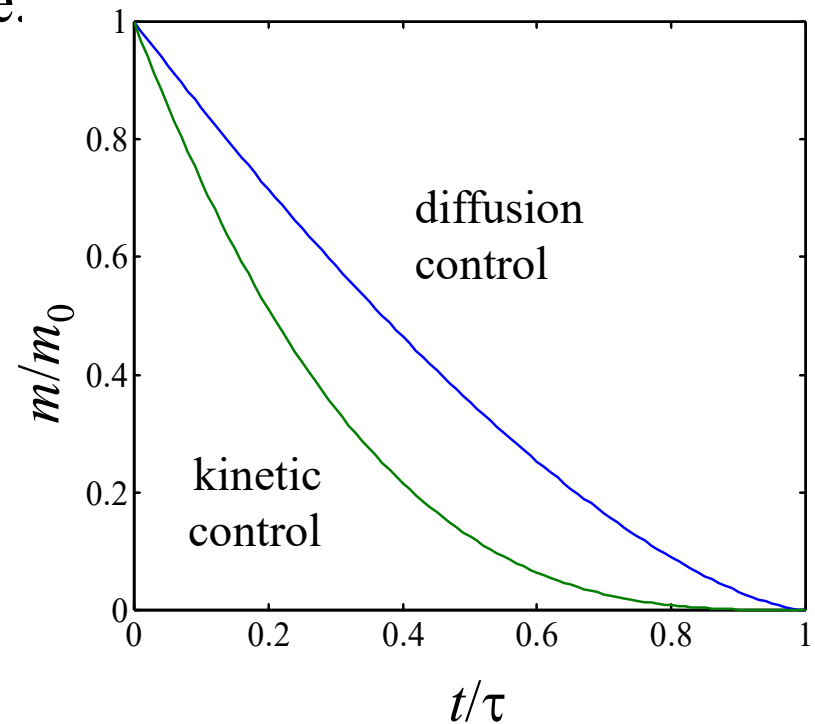
$$m(t) = \rho \frac{4}{3} \pi a(t)^3 = \rho \frac{4}{3} \pi a_0^3 \left(1 - \frac{t}{\tau}\right)^{3/2} \Rightarrow \frac{m(t)}{m_0} = \left(1 - \frac{t}{\tau}\right)^{3/2} \quad (8.20)$$

The plot below shows that the rate of dissolution is quite steady which is desirable from the point of view of drug delivery, for example.

Accurate solution is quite tedious; an interested reader may look at, e.g. Christensen et al. *J.Pharm.Sci.* **71** (1982) 694-9.

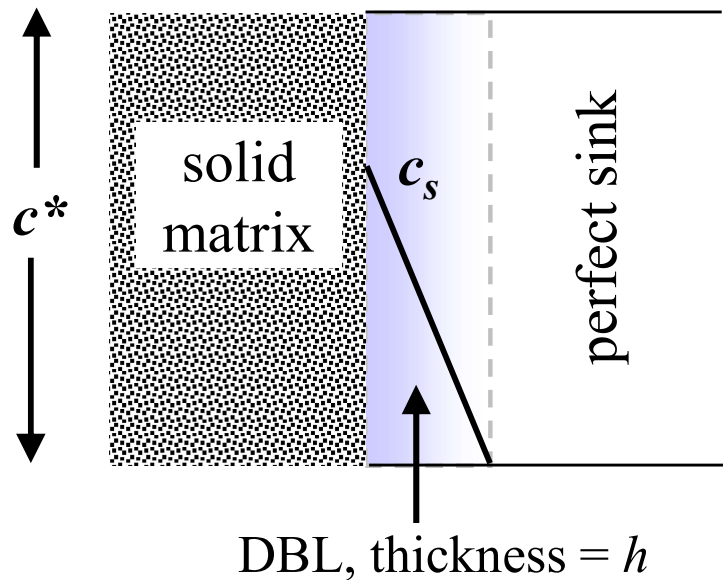
If dissolution takes place under kinetic control, viz  $-dm/dt = kA$  ( $k$  is the rate constant and  $A$  pill surface area,  $a = a_0(1 - t/\tau)$ ;  $\tau = a_0\rho/k$ . Now

$$\frac{m(t)}{m_0} = \left(1 - \frac{t}{\tau}\right)^3 \quad (8.21)$$



# Higuchi's dissolution model

Drug is often dispersed in solid form in a matrix, and dissolution begins when it is brought in contact with a liquid (water). Its saturated concentration is  $c_s$  but its content in solid form  $c^*$  can be substantially higher than  $c_s$ .



Higuchi modeled dissolution from a matrix assuming that at the point of dissolution saturated concentration prevails at all times and that drug is diffusing across the **diffusion boundary layer** (DBL) at steady-state. Looking at the picture aside, it is easy to see that the amount, removed from the matrix,  $Q$ , is

$$Q = c^* h - \frac{1}{2} c_s h \quad (8.22)$$

$$\frac{dQ}{dt} = \left( c^* - \frac{1}{2} c_s \right) \frac{dh}{dt} = D \frac{c_s}{h} = J \quad (8.23)$$

$h$  can be solved from the inner equality applying the initial condition  $h(t=0) = 0$ :

$$h = \left( \frac{Dc_s t}{c^* - (1/2)c_s} \right)^{1/2} \quad (8.24)$$

Inserting eq. (8.24) into (8.22) the result is obtained as

$$Q = [Dc_s(2c^* - c_s)t]^{1/2} \quad (8.25)$$

In the case  $c^* \gg c_s$ , eq. (8.25) is simplified into the form of

$$Q \approx [2Dc^*c_s t]^{1/2} \quad (8.26)$$

Diffusion controlled dissolution is, again, characterized by  $t^{1/2}$  dependence that provides a diagnostic criterion. In a general form,  $Q = kt^n$  where  $k$  is some constant. In cylindrical geometry the exponent  $n = 0.45$  and in spherical geometry  $0.43^*$ . Problem is actually more complex than presented here because of swelling of the matrix in water. In those cases the analysis above does not apply (non-Fickian diffusion), and other methods are needed.

\*P.L. Ritger, N.A. Peppas, *J.Contr.Rel.* **5** (1987) 23-36.

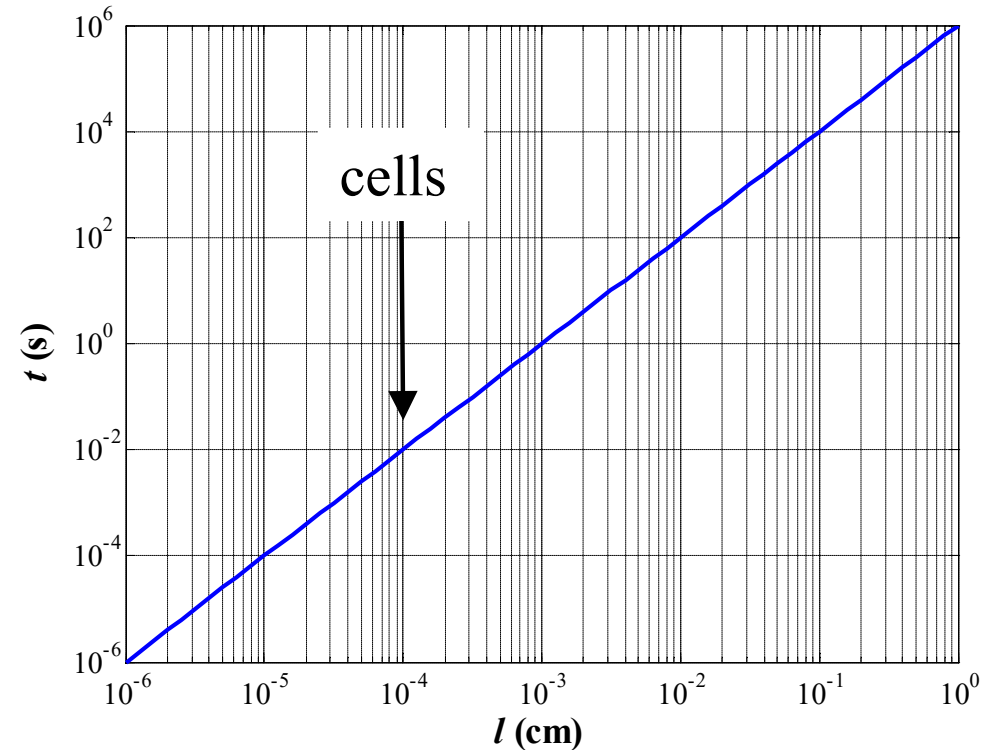
## Diffusion is rapid on the microscale

Einstein derived the distance  $l$  that a particle travels with Brownian motion in time  $t$ :

$$l \approx \sqrt{Dt} \quad \Rightarrow \quad t \approx \frac{l^2}{D} \quad (8.29)$$

Taking  $D = 10^{-6} \text{ cm}^2/\text{s}$ , the plot below can be drawn.

As can be seen in plot, travelling  $1 \text{ }\mu\text{m}$  takes only  $0.01 \text{ s}$ . This is important for the intracellular traffic that could not be diffusion controlled. If cells were, say,  $1 \text{ mm}$  across, the transport of nutrients or metabolites would take  $\sim 10,000 \text{ s}$ , making the cell metabolism hopelessly slow; perhaps life would not be possible.



# Fluorescence Recovery after Photo Bleaching (FRAP)

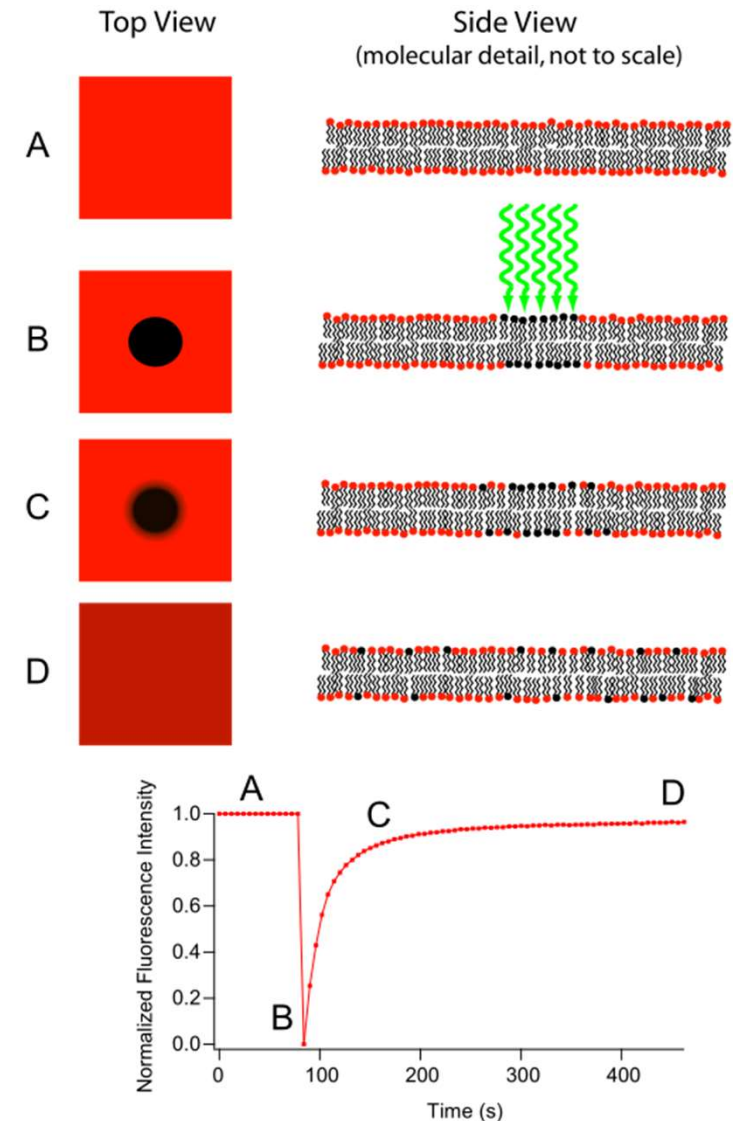
FRAP is a quick method the determination of the mobility of relatively large molecules. The molecules need to be fluorescent or fluorescence-labeled.

- A: Sample with a fluorescent molecule,  $\sim \mu\text{M}$
- B: The fluorescent molecule is destroyed from a small area ( $\phi \sim 10 \mu\text{m}$ ) with a powerful laser
- C: Fluorescence recovers due to diffusion
- D: In a ideal case phase A is recovered

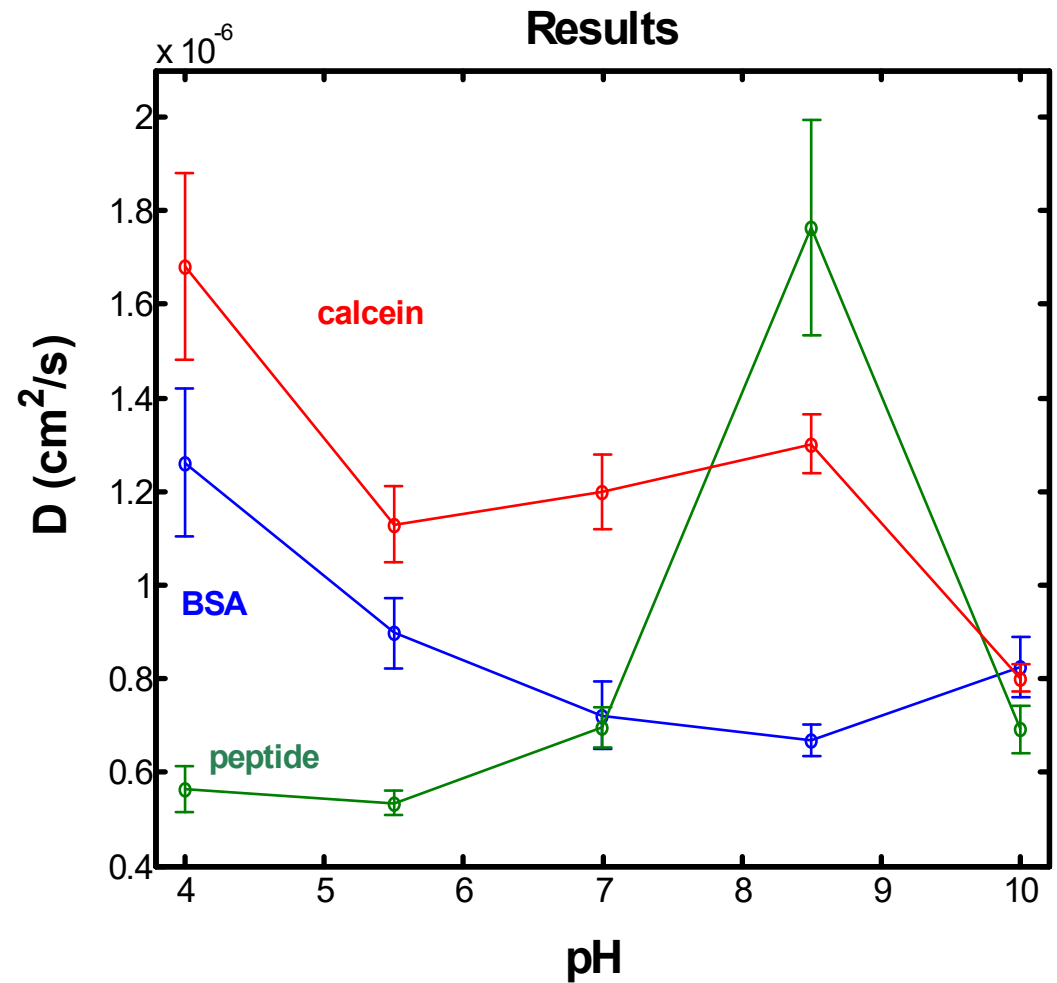
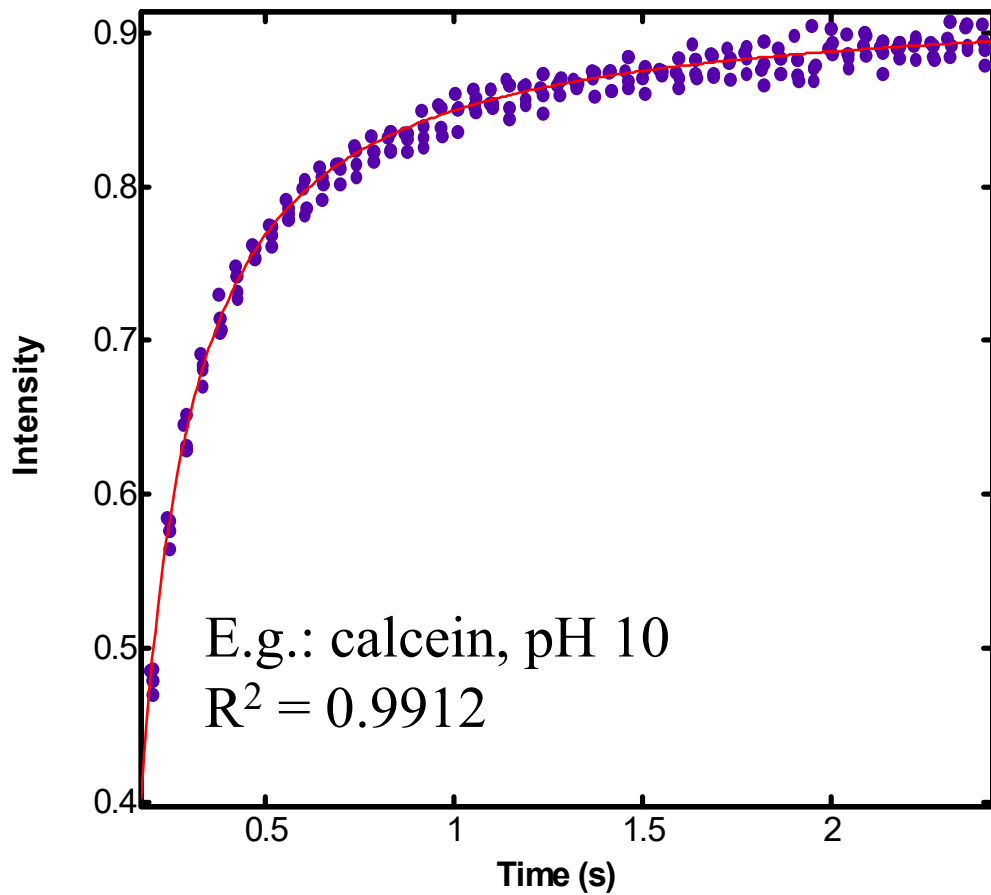
The shape of the phase C curve can be calculated from Fick's 2<sup>nd</sup> law (2D equation):

$$I = ae^{-b} [I_0(b) + I_1(b)] \quad b = \frac{r^2}{2Dt} \quad (8.30)$$

- $I$  = normalized fluorescence intensity  $\in [0,1]$
- $I_0$  and  $I_1$  = modified Bessel functions of the zeroth and first order, respectively
- $r$  = the radius of the destroyed area



# Fits of FRAP data, measured in a hydrogel (hydroxypropyl methylcellulose)



# Observations

- $D^{\text{gel}} \sim 10^{-6} \text{ cm}^2/\text{s}$  (?)
- The diffusion coefficient of BSA > Peptide (?)

But! Stokes - Einstein equation :  $D = \frac{k_{\text{B}}T}{6\pi\eta r}$

$\eta$  = viscosity

$r$  = molecule radius

$$\eta^{\text{gel}} \approx 4000 \text{ cP} \approx 4000 \cdot \eta^{\text{w}} \rightarrow D^{\text{gel}} \approx 1/4000 \cdot D^{\text{w}} \approx 10^{-10} \text{ cm}^2/\text{s}$$

MW of peptide  $\sim 1200$  and MW of BSA  $\sim 66400$

$$r \sim \text{MW}^{1/3} \rightarrow D_{\text{peptide}}/D_{\text{BSA}} \approx 3.8$$

Conclusion: hydrogel does not obey Stokes-Einstein equation, because it is a net-like structure.



# Goldman constant field approximation

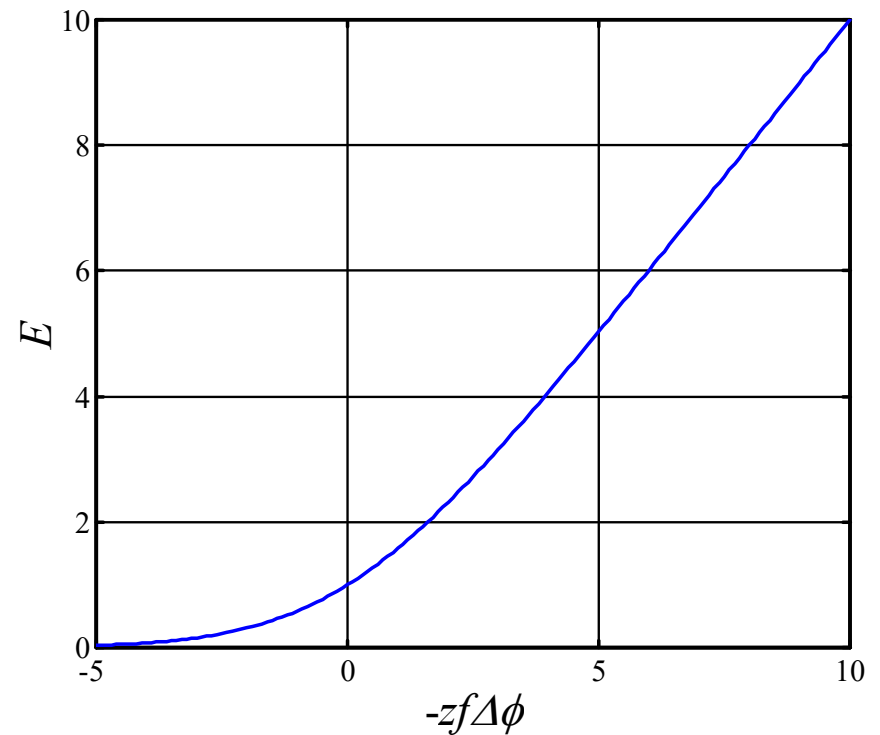
In biophysics **Goldman constant field approximation**  $\nabla\phi \approx \Delta\phi/h$  is usually applied.  $\Delta\phi$  is the potential drop across a membrane and  $h$  its thickness. The approximation is quite good for thin membranes with low charge density. With this approximation Nernst-Planck equation can be integrated. Denoting  $\Delta\phi = \phi^\beta - \phi^\alpha$

$$j = -\frac{D}{h} \frac{zf\Delta\phi}{e^{zf\Delta\phi} - 1} (c^\beta e^{zf\Delta\phi} - c^\alpha) = \text{EF} \cdot \frac{D}{h} (c^\alpha - c^\beta e^{zf\Delta\phi}) \quad (8.31)$$

Eq. (8.32) defines the iontophoretic enhancement factor:

$$\text{EF} \equiv \frac{zf\Delta\phi}{e^{zf\Delta\phi} - 1} \quad (8.32)$$

As shown aside,  $z\Delta\phi \approx -250$  mV already enhances the flux ten-fold. This is why iontophoretic delivery of drugs has received a lot of attention.



Electroosmosis is observed in several types of biomembranes. Therefore, membranes must include small water-filled capillaries (see picture below). The passive transport of molecules across a membrane can be divided into aqueous and lipid contributions:

$$J_p = J^w + J^o \quad (8.33)$$

As the conductivity of the lipid matrix is  $\sim 0$ , iontophoresis enhances only the aqueous route:

$$J_{IF} = J^w \cdot EF + J^o \quad (8.34)$$

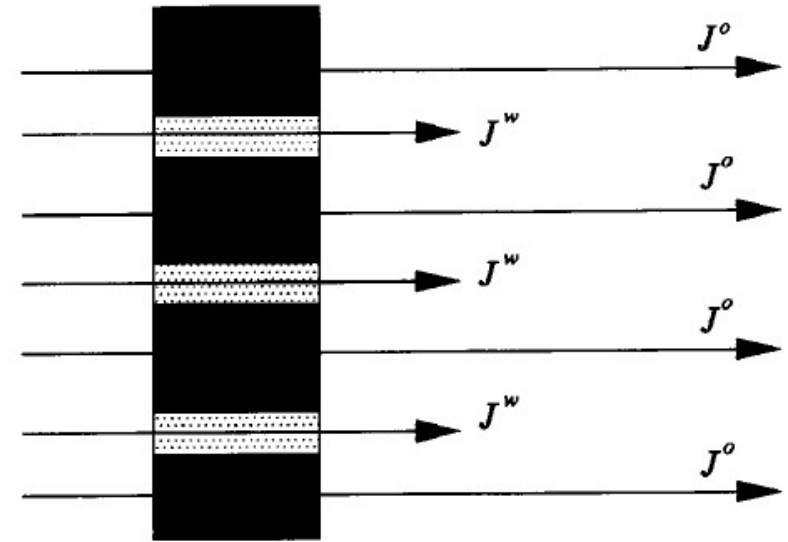
Measured iontophoretic enhancement factor  $EF_m$  is the ratio of the iontophoretic and passive fluxes:

$$EF_m = J_{IF}/J_p \quad (8.35)$$

Inserting eqs. (8.33) and (8.34) into eq. (8.35), the fraction of the aqueous route  $x$  is:

$$x = \frac{J^w}{J^w + J^o} = \frac{EF_m - 1}{EF - 1} \quad (8.36)$$

Because  $x$  is very low for lipophilic molecules their iontophoretic enhancement is not very reasonable. Eq. (8.36) thus provides a diagnostic criterion for the feasibility of iontophoretic delivery of a drug.



# Iontophoretic lag time

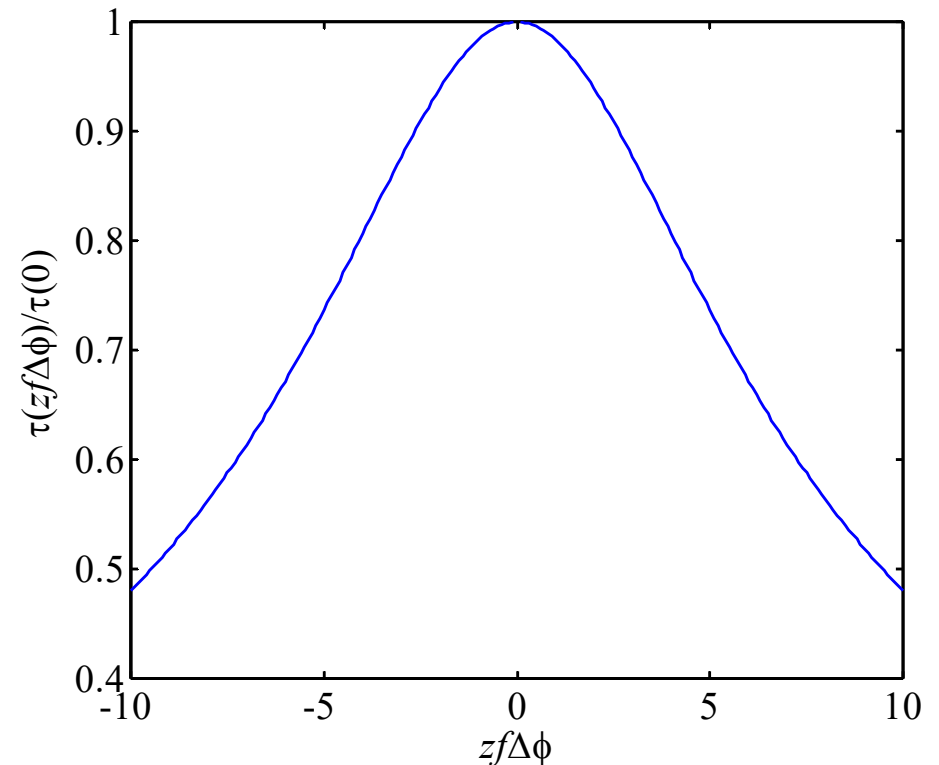
It is possible to calculate the lag time in an iontophoretic experiment by applying Goldman approximation in the time-dependent Nernst-Planck equation:

$$\frac{\partial c}{\partial t} = D(\nabla^2 c + z f \nabla c \cdot \nabla \phi) \approx D(\nabla^2 c + z f \nabla c \Delta \phi / h) \quad (8.37)$$

The solution is rather cumbersome (see textbook), and we content to give the ratio of the iontophoretic and the diffusion lag time (8.38) ( $y = z f \Delta \phi$ ):

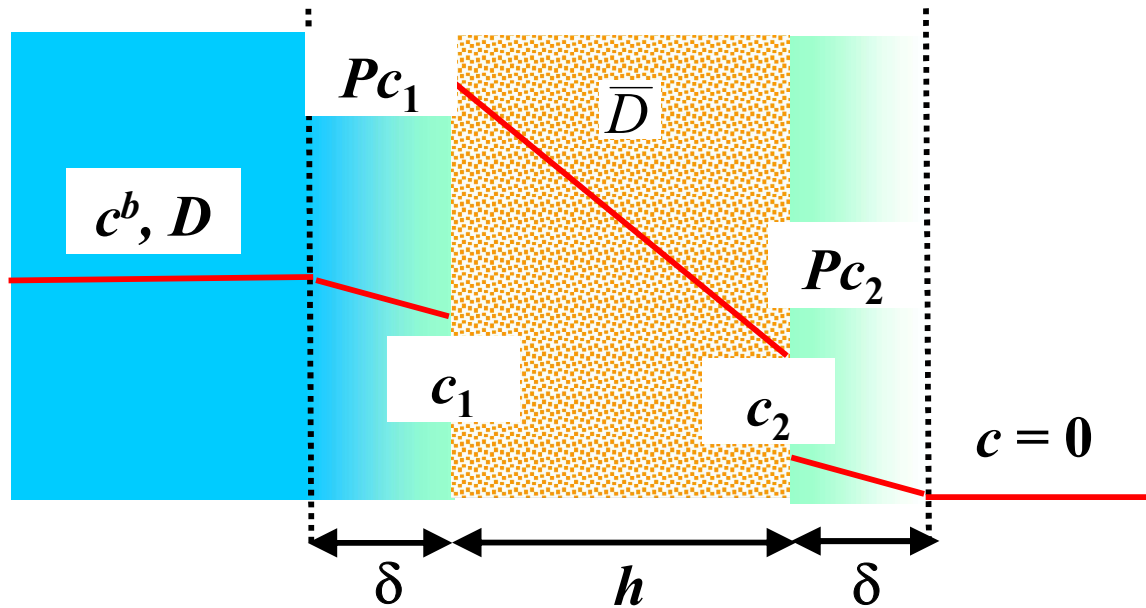
$$\begin{aligned} \frac{\tau(y)}{\tau(0)} &= 6 \frac{y \coth(y/2) - 2}{y^2} \\ &\approx \frac{6(y-2)}{y^2} \quad ; \quad y > 6 \end{aligned} \quad (8.38)$$

Iontophoresis also reduces the lag time.



## Some more transport

Let's consider steady-state transport as depicted below:



Membrane has the thickness  $h$ , and it is flanked by two diffusion boundary layers (DBL) of the thickness  $\delta$ . Partition coefficient between the solutions and the membrane is  $P$ .

$$J = \frac{D}{\delta} (c^b - c_1) = \frac{P\bar{D}}{h} (c_1 - c_2) = \frac{D}{\delta} c_2 \quad (8.39)$$

Let's eliminate  $c_2$  as the function of  $c_1$  from the latter equality:

$$c_2 = c_1 \left( 1 + \frac{D}{P\bar{D}} \frac{h}{\delta} \right)^{-1} \quad (8.40)$$

Inserting this to the former equality,  $c_1$  is obtained as the function of  $c^b$ :

$$c_1 = c^b \left( 1 + \frac{D}{PD} \frac{h}{\delta} \right) / \left( 2 + \frac{D}{PD} \frac{h}{\delta} \right) \quad (8.41)$$

Inserting eq. (8.41) into the transport equation (8.39) the result is:

$$J = c^b \left( 2 \frac{\delta}{D} + \frac{h}{PD} \right)^{-1} = K_p c^b \quad (8.42)$$

where  $K_p$  is permeability of the entire system. Interpreting  $D/\delta$  as the permeability of the DBLs and the membrane permeability as  $PD/h$ , it is obtained that

$$\frac{1}{K_p} = \frac{1}{K_p^w} + \frac{1}{K_p^m} + \frac{1}{K_p^w} \quad (8.43)$$

This result can be generalized for an arbitrary number of **sequential** transport steps. If a single step has the permeability  $K_{p,i}$  the total permeability  $K_p$  is

$$\left( K_p \right)^{-1} = \sum_i \left( K_{p,i} \right)^{-1} \quad (8.44)$$

Permeabilities of parallel processes are simply summed.

## Mediated transport

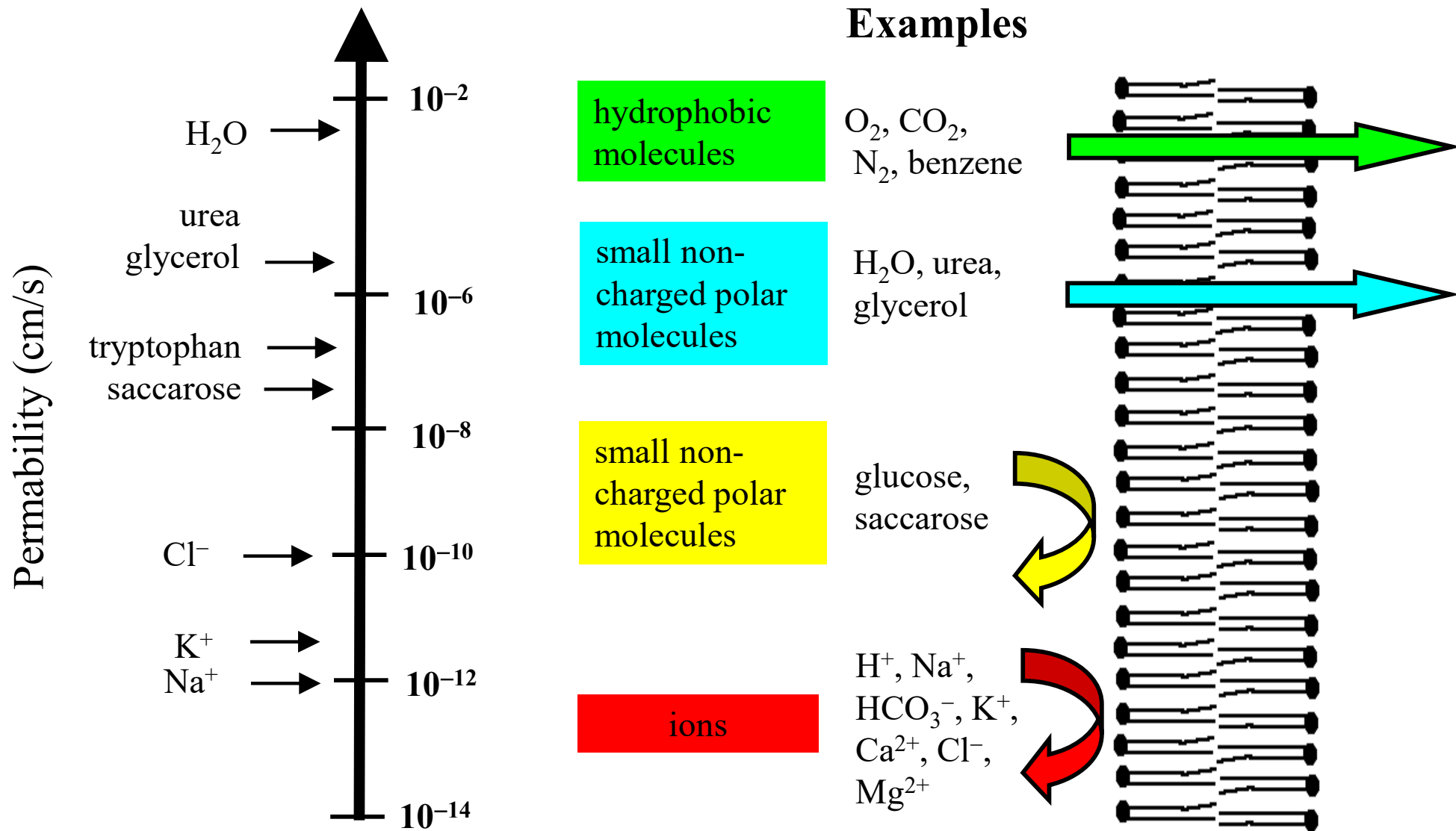
Most of transport across cell membranes takes place with other mechanisms than passive diffusion. Passing of small ions is practically impossible. Parsegian\* have calculated that the electrostatic energy needed to transfer an ion from water into the center of a membrane (thickness =  $h$ ) is

$$w_{el} = -\frac{z^2 e^2}{8\pi\epsilon_0 a} \left[ \left( \frac{1}{\epsilon_r^w} - \frac{1}{\epsilon_r^o} \right) + \frac{2a}{\epsilon_r^o h} \ln \left( \frac{2\epsilon_r^w}{\epsilon_r^w + \epsilon_r^o} \right) \right] \quad (8.45)$$

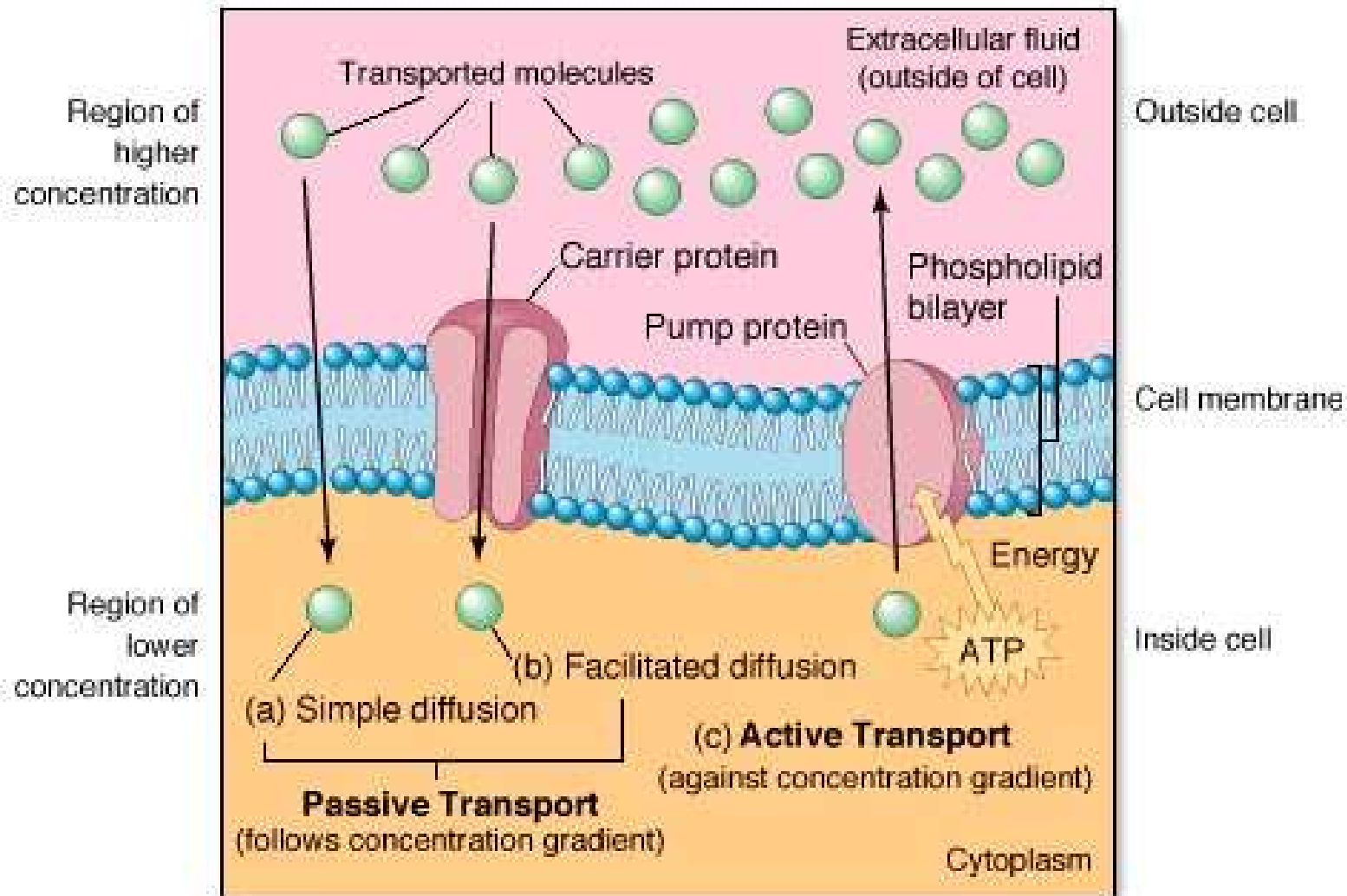
Lipids have  $\epsilon_r \approx 2$ , hence taking  $a = 0.1$  nm,  $z^2 = 1$  and  $h = 5$  nm, the energy is ca. 1.58 eV  $\approx$  153 kJ/mol. Eq. (8.45) is the Born model corrected for with a term taking into account the limited thickness of a membrane. Yet, 1.58 eV still is so high an activation energy that transfer is not possible.

The fact that ions are, however, transferring means that membranes have aqueous pathways and other mechanisms with which ions move passively or by utilizing ATP energy. According to Parsegian the energy barrier of an aqueous pore is  $118.6/b$  kJ/mol where  $b$  is the pore diameter ( $\text{\AA}$ ). Taking  $b = 5$   $\text{\AA}$ , required energy is only ca. 24 kJ/mol.

\*A. Parsegian, *Nature*, 221 (1969) 844-6.

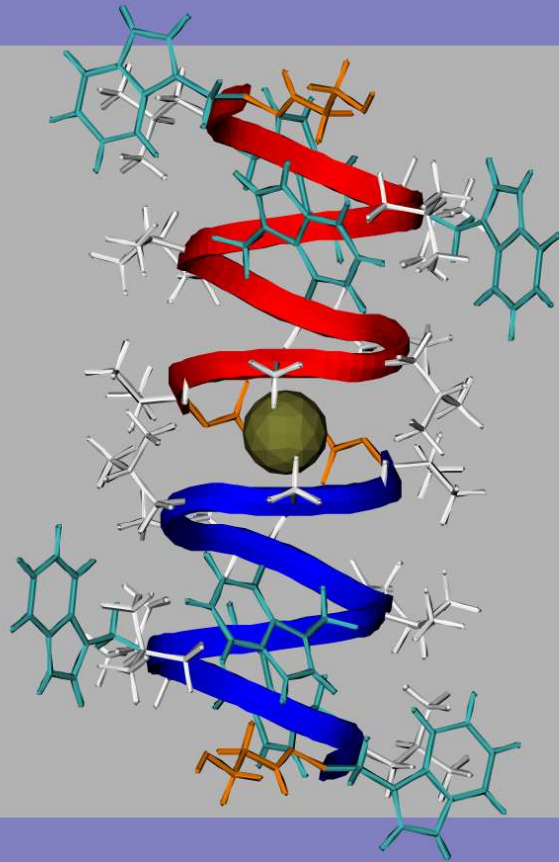


Passive permeability of molecules through lipid bilayer depends on the polarity and size. Ions do not pass it at all.



A well-known example of a protein forming an ion channel is gramicidin-A (gA) that allows the transport of  $\text{Na}^+$  and  $\text{K}^+$  but not  $\text{Ca}^{2+}$  due to its larger size.  $\text{Na}^+/\text{K}^+$  ATPase is a famous example of active transport with ATP energy.





Helical gA channel is composed of two protein units.

Two gramicidin-A molecules are needed to form an ion channel. The diameter of the channel is ca. 4 Å and length ca. 50 Å. Although transport takes place via passive diffusion, the narrow pore hinders diffusion which is basically Brownian movement. Additionally, transport is affected by the interaction of the charges of the ion and pore wall. Neglecting even the charges, the ionic concentration inside the pore is\*

$$\bar{c}_i = c_i(1 - \xi)^2 \quad (8.46)$$

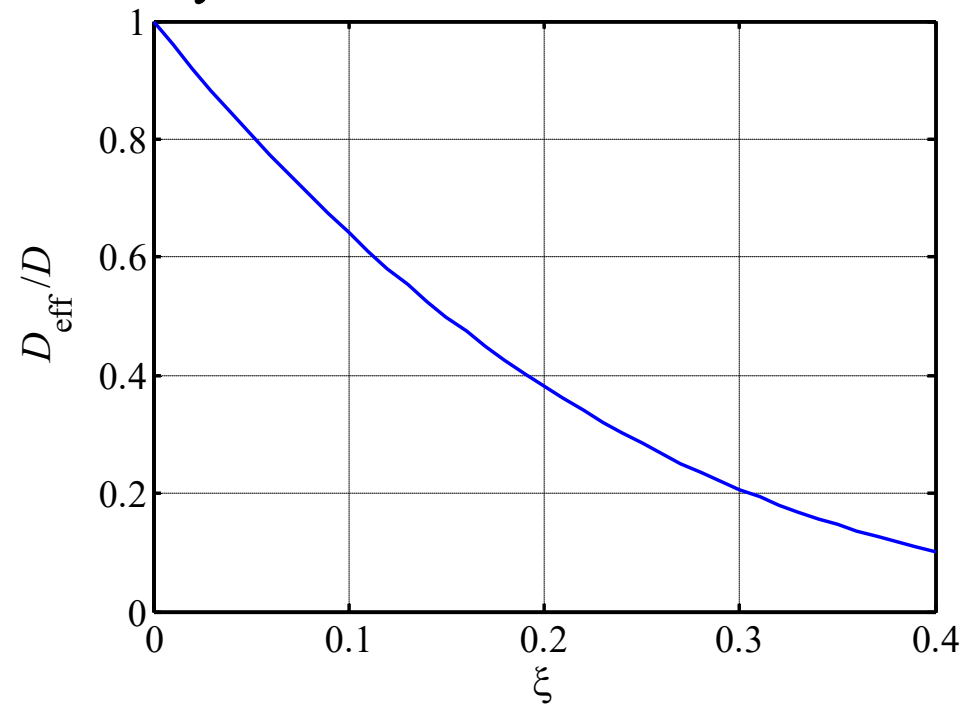
$\xi = a/r_p$  is the ratio of the ionic and pore radii. The effective diffusion coefficient of a non-charged species in a non-charged channel is obtained via **Renkin correction**\* if  $\xi < 0,4$ :

\*K.A. Johnson, et al., *Langmuir*, **5** (1989) 932-8.

$$\frac{D_{eff}}{D} = (1 - \xi)^2 (1 - 2.1044\xi + 2.089\xi^3 - 0.948\xi^5) \quad (8.47)$$

Ionic radius in an aqueous solution is a bit problematic quantity because an ion drags along its hydration water. This is why the mobility of, e.g.  $\text{Li}^+$  is lower than that of  $\text{Na}^+$  although its crystallographic radius is smaller. The ionic radius of  $\text{K}^+$  calculated from its mobility is 1.33 Å, making  $\xi = 0.665 > 0.4$ , beyond the applicability of Renkin correction. The same applies for  $\text{Na}^+$  and  $\text{Cl}^-$ . If  $\xi = 0.4$ ,  $D_{eff}/D \approx 0.1$ , which proves that the channel is slowing transport down significantly.

Although the rate of transport through a channel cannot be calculated accurately, it is clear that it is an order of magnitude lower than in free solution. In terms of the increase of the activation energy  $D_{eff}/D = 0.1$  corresponds to ca. 5.7 kJ/mol (at 298 K).



**Renkin correction**

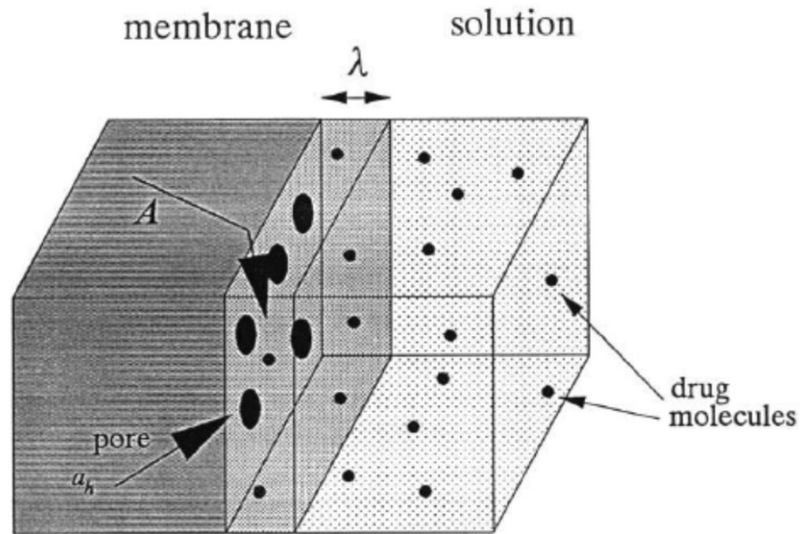
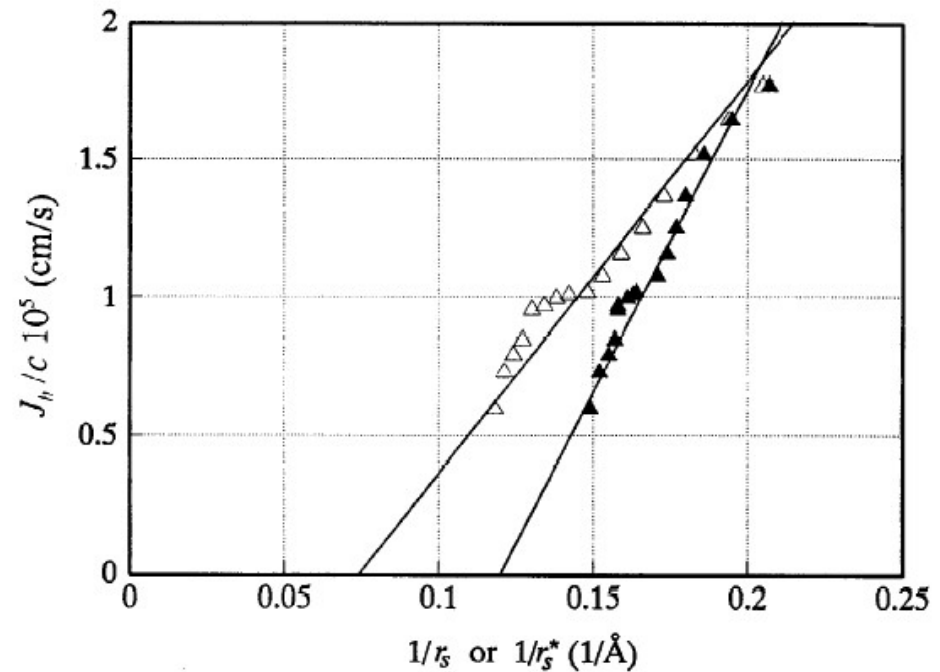


Fig. 1. The movement of drug molecules in a volume  $V$ . The drug molecules are a jump length  $\lambda$  away from the membrane surface with occasional holes.  $A$  is the surface area of the membrane and  $a_h$  the area of a hole.



If pores are sparse, like in the eye membranes, transport is no more driven by the concentration gradient (diffusion), but a molecule is passing through only when it hits the mouth of a pore. This process is more like **effusion** than diffusion. The rate of effusion is proportional to the collision frequency of molecules with the membrane and hence to its concentration, not its concentration gradient. A scheme of effusion in solution (left) and the flux through rabbit *conjunctiva* as the function of the inverse of the hydrodynamic ( $\blacktriangle$ ) and Stokes radii ( $\triangle$ ) of polyethylene glycols (right). Pore size in the membrane 8 – 14 Å. (K.M. Hämäläinen et al. *J. Contr. Rel.* **49** (1997) 97-104)