RANDOM WALKS
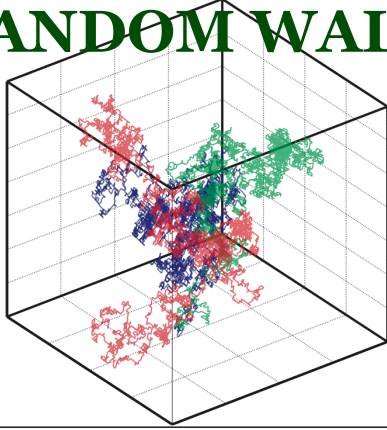

Brownian Motion: oil globules in suspension

Brownian Motion of a Conidial Spore

10 micron · 10 seconds · 20 sec · 30 sec · 40 sec · 90 sec · 140 sec · 190 sec · 240 sec · 0 sec

Brownian Motion: random walk simulation

Brownian Motion: random walk dispersion

Diffusion works best at small distances.

average displacement = $(6 \cdot D \cdot t)^{1/2}$
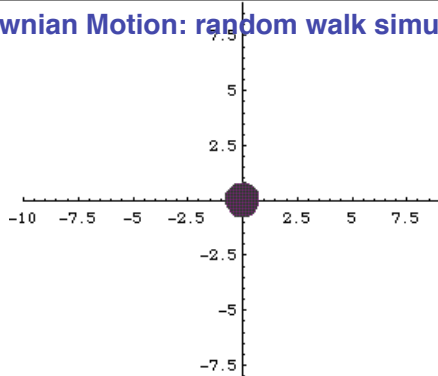

Neurospora: tip expansion

Expansion at the tip must be coordinated with the incorporation of new material at the tip.

A signaling control system is essential:

• **a growth sensor sensitive to cell expansion**

• **a signal transducer.**

The signal transducer is a tip-high Ca^{2+} gradient.

The other physical mechanism underlying tip expansion is related to the feedback mechanisms. How does the hyphal tip 'know' it is expanding, to allow for continued incorporation of cell wall and membrane at the appropriate, controlled rate to match the rate of expansion? We have explored two aspects of this. One is a polar gradient, tip-high, of Ca2+ in the cytoplasm at the tip. The other is identifying the mechanisms used to generate and maintain that tip-high Ca2+ gradient.

Neurospora: Ca²⁺ gradient

Growing hyphae of *Neurospora crassa* have a tip-high Ca^{2+} gradient. The magnitude of the gradient is correlated with growth rate.

The elevated Ca^{2+} will mediate vesicle fusion during tip expansion.

Silverman-Gavrila LB and RR Lew (2003) Calcium gradient dependence of *Neurospora crassa* hyphal growth. Microbiology (*SGM*). 149:2475-2485.

Neurospora hypha do exhibit a tip-high Ca2+ gradient, as imaged with Ca2+ sensitive fluorescent dyes. If we quantify the gradient as the difference in concentration at the tip versus behind the tip.

endoplasmic reticulum

SPRAY

Ca^{2+} → + → Ca^{2+} ← ATP

calcium sequestration ADP + Pi

Ca^{2+}

IP$_3$ RECEPTOR

calcium release

Ca^{2+} IP$_3$

+

vesicle fusion

wall vesicles

STRETCH-ACTIVATED PHOSPHOLIPASE C

The predictions of Ca^{2+} distribution versus time based on a steady supply of Ca^{2+} at the tip and removal behind the tip.

$$[Ca^{2+}] = \frac{M}{2(\pi Dt)^{1/2}} \bullet e^{(-x^2/4Dt)} + [Ca^{2+}]_{basal}$$

The Ca^{2+} gradient was initially fit to obtain an estimate of the diffusion coefficient (5.6 μm^2 sec^{-1}) using a 4 second time interval, when the hyphae would have grown about 1.2 μm. *In dilute CaCl$_2$ solutions, the diffusion coefficient for Ca^{2+} is about 775 μm^2 sec^{-1}. Intracellular Ca^{2+} diffusion coefficients are much smaller, 2-15 μm^2 sec^{-1}.*

Calcium Distributions: in conidia and hyphae

Organellar Ca^{2+} and cytoplasmic [Ca^{2+}] exhibit time dependent random walks.

Permeability: Diffusion through a membrane

c$_o$ (outside concentration)

c$_i$ (inside concentration)

Flux will depend upon the ability of the particle to enter the membrane (partitioning)

d (distance)

Partitioning, $K_p = \dfrac{c_{(membrane)}}{c_{(aqueous)}}$

$J = D\dfrac{K_p}{d}[c_{outside} - c_{inside}]$

where $D\dfrac{K_p}{d} = P$,

units of $\dfrac{\frac{cm^2}{sec}}{cm}$, or cm•sec^{-1}

Permeation through a membrane depends directly upon the ability of the molecule to partition into the membrane

Relations between permeability, oil/water partitioning and molecular weight

Source: Collander, R. (1954) The permeability of *Nitella* cells to non-electrolytes. Physiologia Plantarum 7:420–445.

There is a closer correlation when molecular weight and oil/water partitioning are factored together.

Pressure is one way to determine the permeability of the cellular plasma membrane to a solute molecule. Below, sucrose is impermeable, but ethanol is permeant.

Hydrostatic Pressure: Using the pressure probe

Hyperosmotic Shock: Hyphal Shrinkage

RANDOM WALKS

They *permeate* the physical foundations of biological phenomena
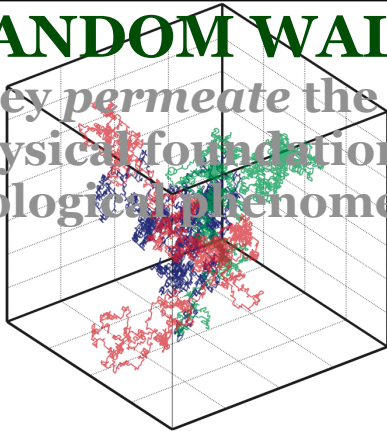
LECTURE TWO MOVEMENT OF MOLECULES THROUGH THE MEMBRANE. *

To describe movement of molecules, we need a framework. The one which is usually used is Flux (J)

$$J = \frac{\text{mol of substance}}{\text{sec} \cdot \text{cm}^2}$$

1 cm
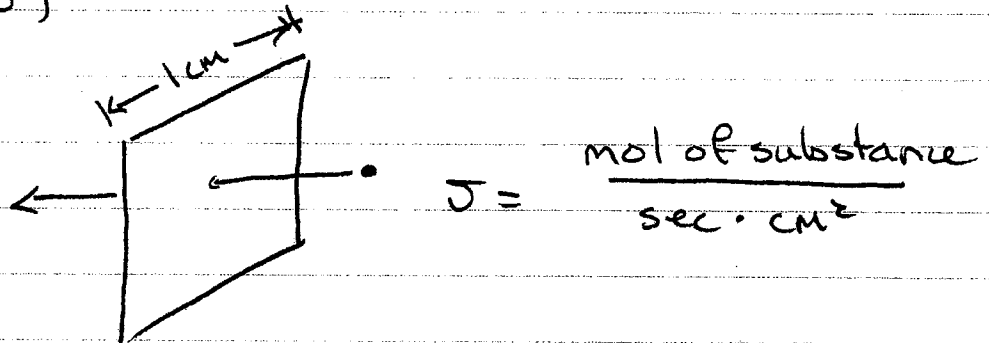
Flux, J, depends upon the concentration of the substance, or rather, the difference in concentration between two regions.

$$\frac{\Delta C}{\Delta X} \propto J$$

C (concentration)

J

α

J

J

X (distance)
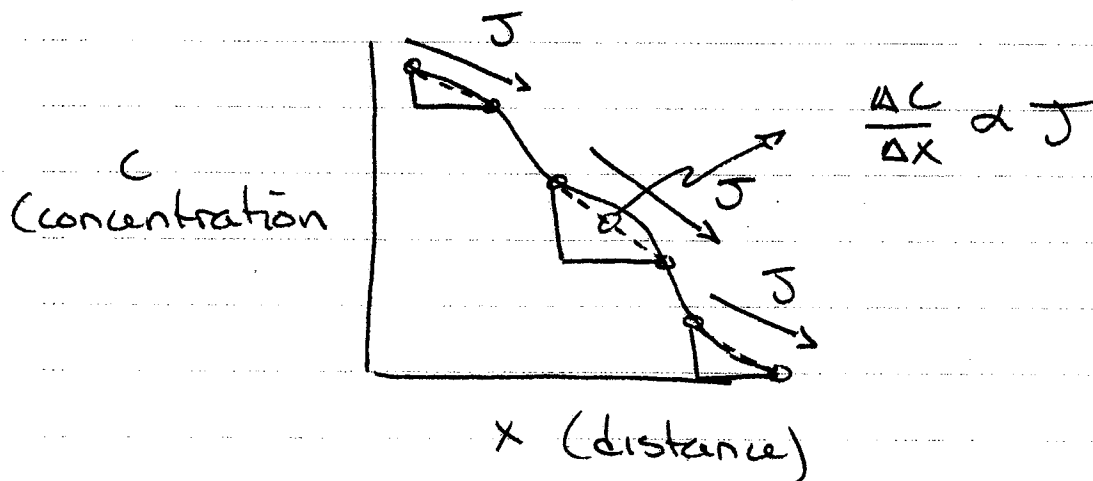
more accurately, we can consider the slope at a point tangent to the C versus x curve.

*reading (required) Berg, H.C Random Walks in Biology, Chapters 1 & 2.

$$J \propto dc/dx$$
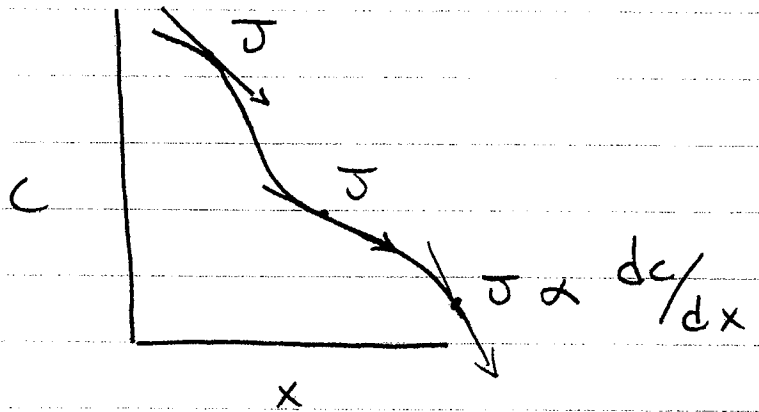
units of $\dfrac{dc}{dx}$ are $\dfrac{mol/cm^3}{cm}$ $\dfrac{\frac{1}{cm}}{\frac{1}{cm}} \rightarrow mol/cm^4$

to arrive at units of J $\left(\dfrac{mol}{cm^2 \cdot sec}\right)$, a coefficient, D (cm^2/sec) must be included:

$$J = -D \cdot \dfrac{dc}{dx}$$

$$\left(\dfrac{cm^2}{sec}\right)\left(\dfrac{mol}{cm^4}\right) \rightarrow \dfrac{mol}{sec \cdot cm^2}$$

D is known as the diffusion coefficient

The above is known as Fick's law of diffusion and follows a general form:

$$Flow = \overset{resistance}{\underset{to\ flow}{}} \cdot driving\ force.$$

Ohm's law can be put in this form:

$$I = gV$$

$$\llcorner where\ g = \dfrac{1}{R}$$

4

To understand how a concentration gradient results in a flux requires a mechanistic description of the movement of the molecule of interest.
This requires analysis using a random walk.

Starting with a line (that is, a one dimensional case)

$$N(x) \qquad N(x+\delta) \qquad \text{number of particles}$$

$$x \qquad x+\delta \qquad \text{distance}$$
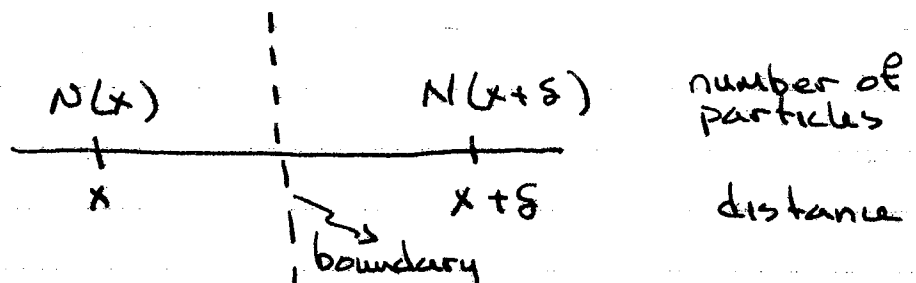
boundary

- with $N(x)$ particles at x, and $N(x+\delta)$ particles a $x+\delta$.

- How many particles will move across the boundary from point x to point $x+\delta$ in a given time τ?

- If the probability for a particle to move to the left is the same as the probability to move to the right, then at a time $t+\tau$, half the particles at x will move to $x+\delta$, and half the particles at $x+\delta$ will have moved to x.

$$\tfrac{1}{2}N(x)$$

$$N(x)$$

$$x \qquad x+\delta$$
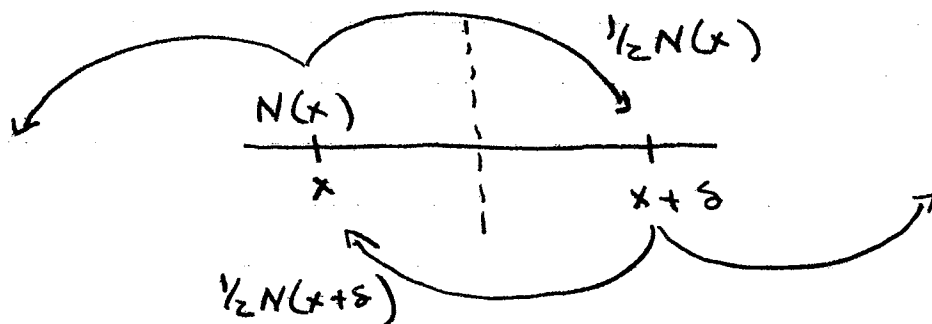
$$\tfrac{1}{2}N(x+\delta)$$

Number of particles x to $x+\delta$ $\qquad \tfrac{1}{2}N(x) - \tfrac{1}{2}N(x+\delta)$

Notation

the net number of particles $\rightarrow -\tfrac{1}{2}\left[N(x+\delta) - N(x)\right]$

5

Random Walk in Three Dimensions
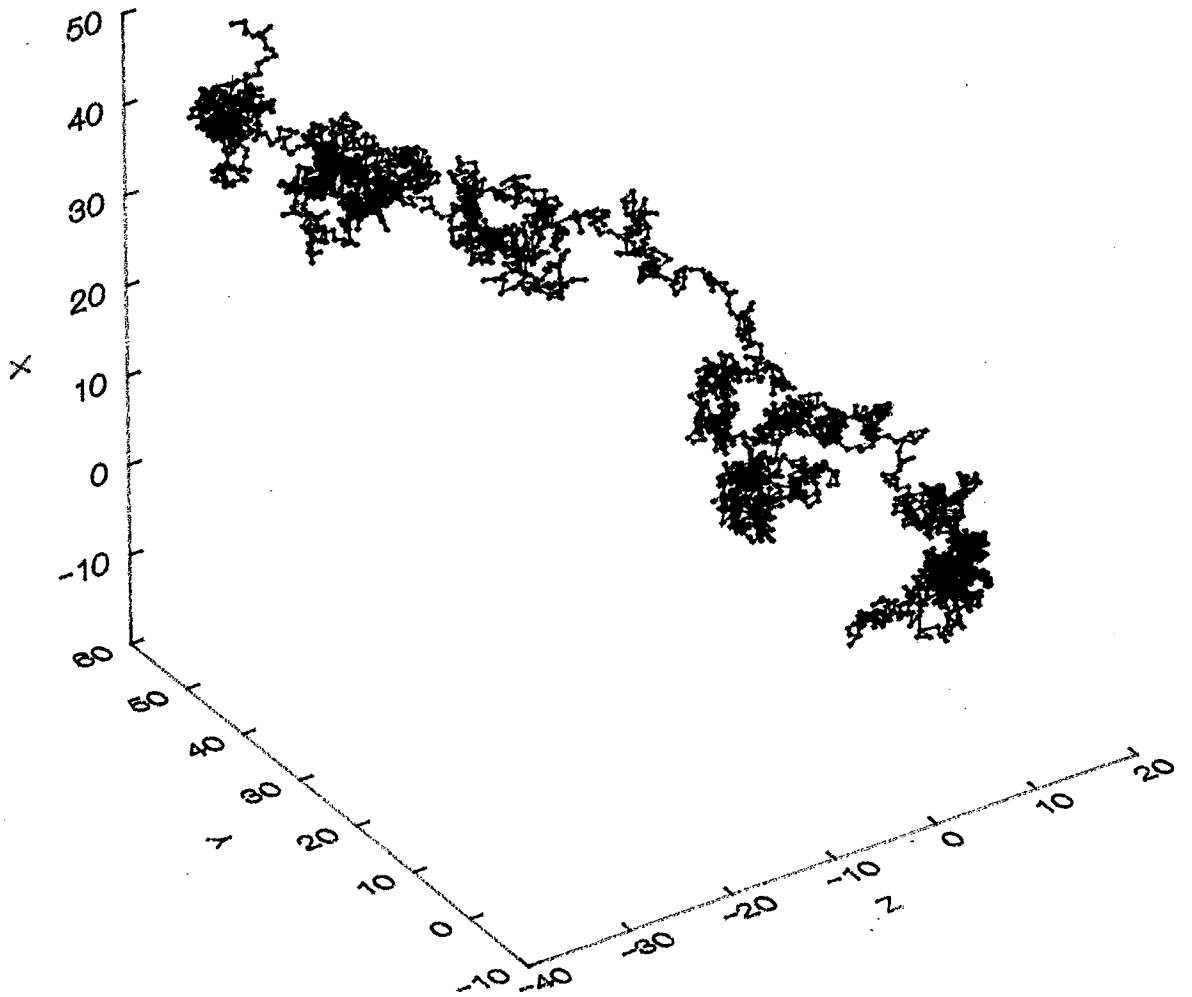
The net number of particles going from
x to $x + \delta$ will be:

$$-\frac{1}{2}\left[N(x+\delta) - N(x)\right]$$

To obtain flux, we have to divide by the
area, A; and the time interval τ

$$J_x = -\frac{1}{2}\left[N(x+\delta) - N(x)\right]\Big/ A\tau$$

multiplying by δ^2/δ^2

$$J_x = -\frac{1}{2}\frac{\delta^2}{\boxed{\delta^2}\,\boxed{A}\tau}\left[N(x+\delta) - N(x)\right]$$

units of volume are $\delta \cdot A$

...

$$J_x = -\frac{1}{2}\frac{\delta^2}{\tau}\frac{1}{\delta}\left[\underbrace{\frac{N(x+\delta)}{\delta \cdot A}}_{C(x+\delta)} - \underbrace{\frac{N(x)}{\delta \cdot A}}_{C(x)}\right]$$

so we have $\quad J_x = -\frac{1}{2}\frac{\delta^2}{\tau} \cdot \frac{C(x+\delta) - C(x)}{\delta}$

7

If we take the term:

$$\frac{C(x+\delta) - C(x)}{\delta}$$

to the limit where $\delta \to 0$,

then

$$\frac{C(x+\delta) - C(x)}{\delta} = \frac{dC}{dx},$$

by definition.

So

$$J_x = -\frac{1}{2}\frac{\delta^2}{\tau} \cdot \frac{dC}{dx}$$

where $\frac{1}{2}\frac{\delta^2}{\tau}$ is the Diffusion
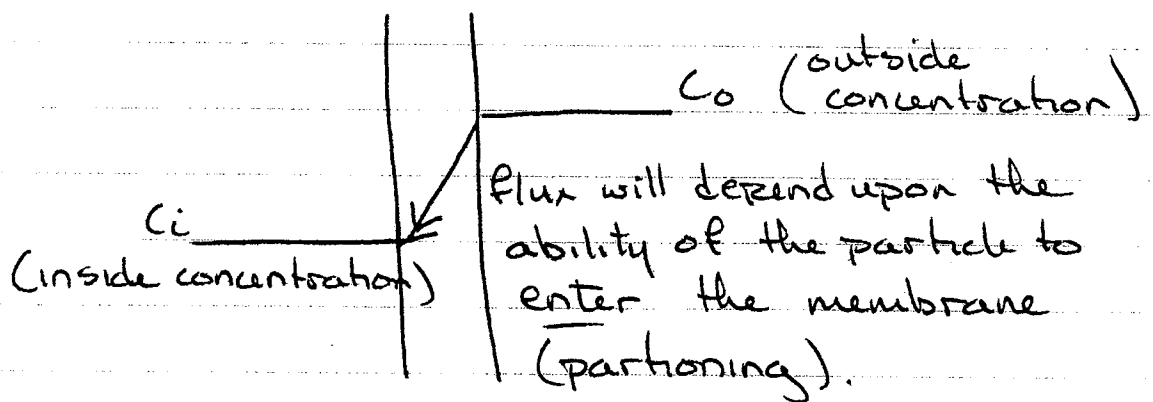
coefficient, D (units of $^{cm^2}/_{sec}$)

Key points:

1) Flux depends upon random movements of particles; net movement is due to the fact that there are more particles in particular regions of space.

8

2) The speed at which this occurs depends upon the Diffusion coefficient, whose units, cm^2/sec, imply:

The amount of space a particle will explore in a small unit of time, τ.

Therefore, a property of the particle.

In addition to flux, $J = -D \frac{dc}{dx}$, we have to consider movement through the membrane:

C_o (outside concentration)

C_i (inside concentration)

Flux will depend upon the ability of the particle to enter the membrane (partioning).

$k-d x$
distance

partitioning, $K_p = \frac{C(membrane)}{C(aqueous)}$

so, flux becomes

$$J = D \frac{K_p}{d} \cdot [C_o - C_i]$$

$\underbrace{\qquad}$
P (permeability coefficient)

9

Fick's First Law of Diffusion dominates descriptions of molecular transport. It is a phenomenological equation; that is, it is based upon experimental results and lacks a theoretical underpinning. The equation is:

flux, J (mol cm^{-2} sec^{-1})

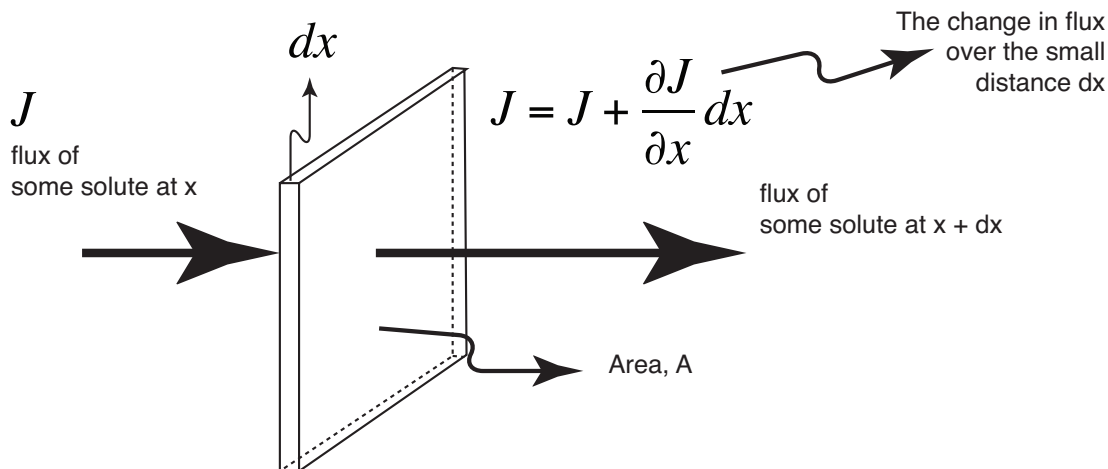concentration gradient, dc/dx
with units of (mol cm^{-3})/(cm),
or mol cm^{-4}.

The movement of molecules depends upon the concentration gradient of molecules. This is described by Fick's First Law of Diffusion.

$$J = -D \bullet \frac{dc}{dx} \Rightarrow (\frac{cm^2}{sec})(\frac{mol}{cm^4}) \Rightarrow (\frac{mol}{sec \bullet cm^2})$$

Diffusion coefficient with units of cm^2 sec^{-1}

The diffusion equation, $J = -D(\partial c/\partial x)$, does not account for changes in concentration that will occur as molecules move fom one location to another, in accordence with the flux, J. (Instead, it assumes a steady state, in which the change in concentration over time is zero: $\partial c/\partial t = 0$.).
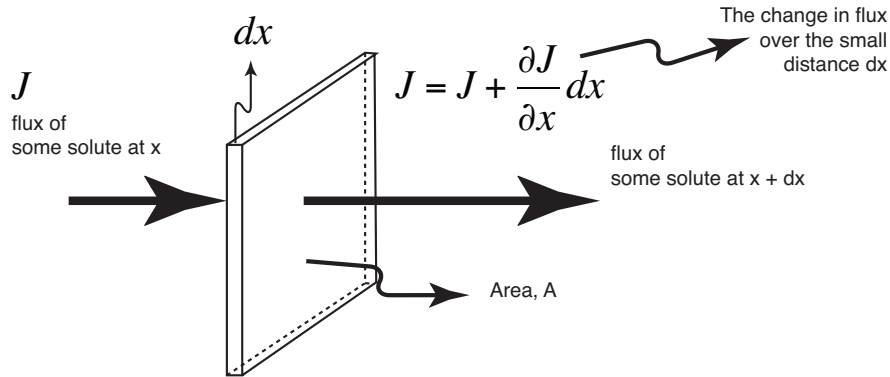
How do we account for the non-steady-state time dependence of diiffusion? The derivation of a general equation relies upon the assumption of conservation of mass[1].

Consider the changes in flux, J, with respect to distance $(\partial J/\partial x)$ through a small volume element of width x+dx and area A:

dx

The change in flux over the small distance dx

J

flux of
some solute at x

$$J = J + \frac{\partial J}{\partial x} dx$$

flux of
some solute at x + dx

Area, A

The change in the amount of solute in the volume element, A•dx, is equal to the amount flowing in, J•A minus the amount flowing out, (J+(∂J/∂x)dx)•A per unit time. Note that the change in the amount of solute in the volume element A•dx can be expressed as $\partial c/\partial t$, multiplied by the volume element A•dx.

[1]Noble, PS (1974) Introduction to Biophysical Plant Physiology. WH Freeman and Co. pp 9–19.

The change in flux over the small distance dx

$$J = J + \frac{\partial J}{\partial x}dx$$

J
flux of some solute at x

flux of some solute at x + dx

Area, A

dx

$$\frac{\partial c}{\partial t}dx \bullet A = (J \bullet A) - \left(J + \frac{\partial J}{\partial x}dx\right) \bullet A$$

$$\frac{\partial c}{\partial t}dx \bullet A = (J \bullet A) - (J \bullet A) - \left(\frac{\partial J}{\partial x}dx \bullet A\right)$$

$$\frac{\partial c}{\partial t}dx \bullet A = -\frac{\partial J}{\partial x}dx \bullet A$$

The change in the amount of solute in the volume element A•dx is equal to the amount moving into the volume element minus the amount moving out of the volume element

dividing by dx • A : $\dfrac{\partial c}{\partial t} = -\dfrac{\partial J}{\partial x}$

This is known as the Continuity Equation and is based on conservation of mass: that matter can be neither created nor destroyed.

Substituting the continuity equation, $(\partial c/\partial t) = -(\partial J/\partial x)$, into the flux equation, $J = -D(\partial c/\partial x)$, yields:

$$\frac{\partial c}{\partial t} = -\frac{\partial}{\partial x}\left(-D\frac{\partial c}{\partial x}\right)$$

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2}$$

This is known as the Fick's Second Law of Diffusion. It describes how the concentration of the solute changes with position and with time as a result of diffusion. The solutions of this equation depend upon the geometry. Books are devoted to solutions to the diffusion equations[1].

[1]Crank, J (1975) The Mathematics of Diffusion, Second edition. Clarendon Press, Oxford.

From an historic point of view, it's worthwhile to explore the method Adolf Fick used to establish the veracity of what we now know as Fick's Laws of Diffusions[1].

Fick noted that the underpinning theory should be identical to that obtained for the diffusion of heat in a conducting body (developed by Fourier), and Ohm's Law describing the diffusion of electricity in a conductor.

For solute diffusion through a series of concentration strata (c_1 through c_n) varying with height (h_n), Fick invoked conservation of mass:
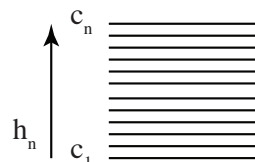
$$\frac{\partial c}{\partial t} = -k \, \frac{\partial^2 c}{\partial h^2}$$

That is, the change in concentration will depend upon the second derivative of concentration with respect to distance, multipled by k, a constant dependent on the nature of the substance. Note that this is suitable for a simple system, in which the geometry and volume of each stratum is the same.

To test this, Fick used an apparatus in which a steady state concentration gradient was created between solid salt and pure water. He then measured the specific gravity at various depths. Allowing the system to reach a steady state, where $\partial c/\partial t$ would be zero, leads to a solution of the second derivative equation: a linear gradient: $c = a \cdot h + b$.
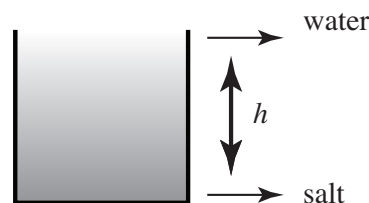
From $\partial/\partial h \ (a \cdot h + b) = a$,
and $\partial/\partial h \ (a) = 0$.
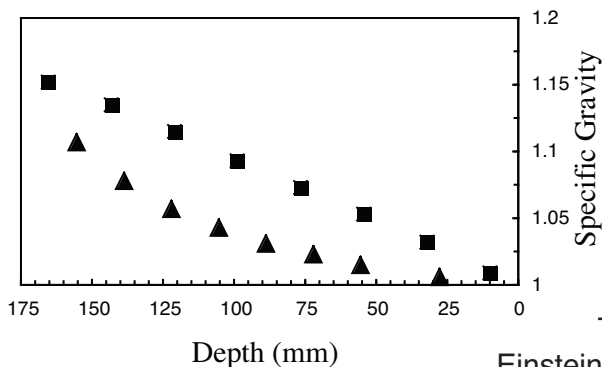
In fact, this is what he found (Fick's data is graphed to the left) (square symbols). He performed a further test with a more complex geometry (a funnel), in which the steady state solution is a non-linear concentration gradient (triangle symbols).

This was one of the starting points for Einstein's elucidation of the molecular motion underlying diffusion. The other was the behaviour of particles in solution: Brownian Motion.

[1]The original english article (Fick, A: On liquid diffusion.) (an abstract of a longer German article) was published in 1855, and republished in the Journal of Membrane Science 100:33–38 (1995).
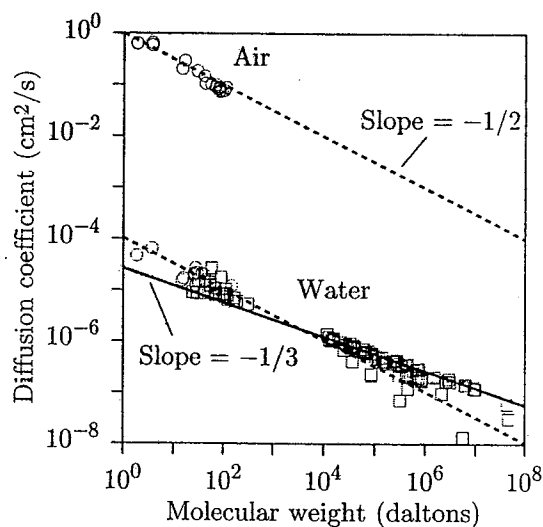
Figure 3.11 The measured diffusion coefficient D is plotted in logarithmic coordinates as a function of the molecular weight M for 19 gases diffusing in air and for 123 solutes diffusing in water. The data are from several sources (Tanford, 1961; Cohn and Edsall, 1965; Cussler, 1984; Lide, 1990). The measurements of solutes in water were made at temperatures in the range of 20-25°C and are extrapolated to infinite dilution. Those in air were made at atmospheric pressure and at temperatures in the range of 0-26.1°C. Circles represent gases diffusing either in air or in water; squares represent other solutes.

Table 3.3 Diffusion coefficients of selected molecules in air (above line) and water (below line). All these are included in Figure 3.11, which also gives citations. M is the molecular weight; D is the diffusion coefficient.

Molecule	Medium	Temp. (°C)	M (g/mol)	D (cm^2/s)
Hydrogen	Air	0	2	6.11×10^{-1}
Helium	Air	3	4	6.24×10^{-1}
Oxygen	Air	0	32	1.78×10^{-1}
Benzene	Air	25	78	9.60×10^{-2}
Hydrogen	Water	25	2	4.50×10^{-5}
Helium	Water	25	4	6.28×10^{-5}
Oxygen	Water	25	32	2.10×10^{-5}
Urea	Water	25	60	1.38×10^{-5}
Benzene	Water	25	78	1.02×10^{-5}
Sucrose	Water	25	342	5.23×10^{-6}
Ribonuclease	Water	20	13,683	1.19×10^{-6}
Hemoglobin	Water	20	68,000	6.90×10^{-7}
Catalase	Water	20	250,000	4.10×10^{-7}
Myosin	Water	20	493,000	1.16×10^{-7}
DNA	Water	20	6,000,000	1.30×10^{-8}
Tobacco mosaic virus	Water	20	50,000,000	3.00×10^{-8}

since $\dfrac{D k_P}{d}$ has units of $\dfrac{\frac{cm^2}{sec}}{cm}$

P has units of cm/sec

Typical permeability coefficient values :

water	PC membrane	2×10^{-4} to 3.4×10^{-4}
	red blood cell	1.2×10^{-3}
urea	PC membrane	4×10^{-6}
	red blood cell	7.7×10^{-7}
glycerol	PC membrane	5.4×10^{-6}
	red blood cell	1.6×10^{-7}
Na^+		$10^{-11} - 10^{-14}$
Cl^-		10^{-11}
H^+/OH^-		$10^{-4} \sim 10^{-8}$

In general, neutral solutes are relatively permeable, depending upon molecular size and ability to partition into a hydrophobic environment.

H^+/OH^- is a notable exception amongst charged solutes.

11

Typical values for permeability coefficients

Membrane permeabilities of selected solutes in *Chara*, *Nitella*, human erythrocytes, and artificial membranes[1].

Solute	molecular weight	olive oil : water partition coefficient	*Chara ceratophylla*	*Nitella mucronata*	Human erythrocyte	Artificial lipid membrane
water	18	$1.3 \cdot 10^{-4}$	$2.5 \cdot 10^{-3}$	$1.2 \cdot 10^{-3}$	$2.5 \cdot 10^{-3}$	$2.2 \cdot 10^{-3}$
formamide	45	$1.1 \cdot 10^{-6}$	$2.2 \cdot 10^{-5}$	$7.6 \cdot 10^{-6}$	$1.1 \cdot 10^{-6}$	$1.0 \cdot 10^{-4}$
ethanol	46	$3.6 \cdot 10^{-2}$	$1.6 \cdot 10^{-4}$	$5.5 \cdot 10^{-4}$	$2.1 \cdot 10^{-3}$	
ethanediol	58	$4.9 \cdot 10^{-4}$	$1.1 \cdot 10^{-5}$		$2.9 \cdot 10^{-5}$	$8.8 \cdot 10^{-5}$
butyramide	87	$1.1 \cdot 10^{-6}$	$5.0 \cdot 10^{-5}$	$1.4 \cdot 10^{-5}$	$1.1 \cdot 10^{-6}$	
glycerol	92	$7.0 \cdot 10^{-5}$	$2.0 \cdot 10^{-7}$	$3.2 \cdot 10^{-9}$	$1.6 \cdot 10^{-7}$	$5.4 \cdot 10^{-6}$
erythritol	122	$3.0 \cdot 10^{-5}$			$6.7 \cdot 10^{-9}$	

[1] compiled by Weiss TF 1996 Cellular Biophysics. Volume I: Transport. MIT Press. Original citations are Collander R 1954 The permability of Nitella cells to non-electrolytes. Physiol. Plant. 7: 420–445, and Stein WD 1990 Channels, Carriers and Pumps. Academic Press.

formamide

ethanediol

butyramide

glycerol

erythritol
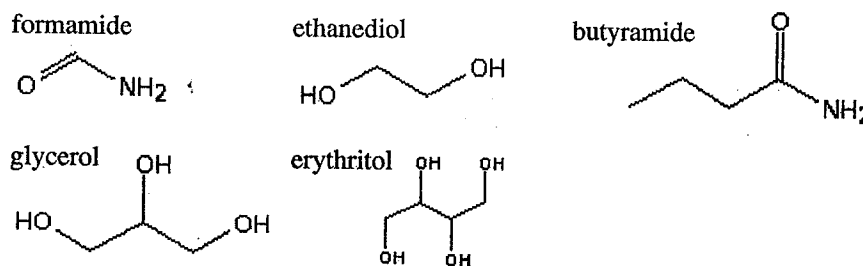
For comparison, permeability coefficients for ions are much lower. In an artificial membrane:

Na^+ 10^{-11} to 10^{-14} cm/sec
Cl^- 10^{-11}
H^+/OH^- 10^{-4} to 10^{-8}
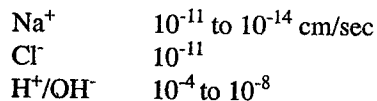
In general, neutral solutes are relatively permeable, depending upon molecular weight and their ability to partition into a hydrophobic environment. Charged molecules are barely capable of partitioning into hydrophobic enviroments. H^+/OH^- is a notable exception among charged molecules.
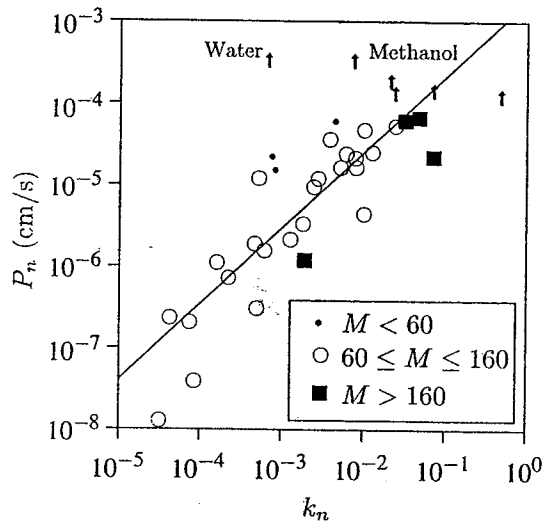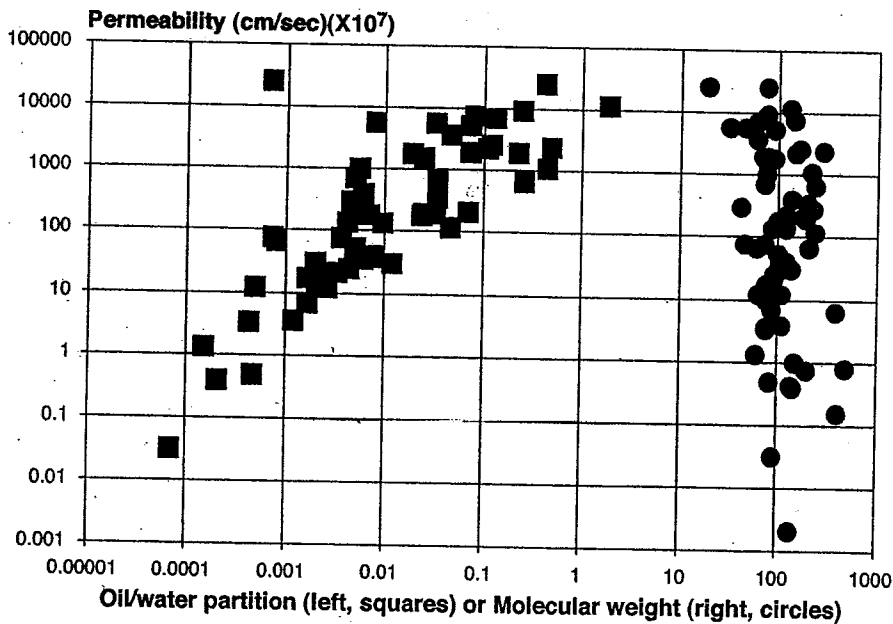
12

Figure 3.37 The permeability, P_n, of the membrane of the cell of the plant *Chara ceratophylla* versus the olive oil:water partition coefficient, k_n, for a number of solutes plotted on double logarithmic coordinates (adapted from Collander and Bärlund, 1933 Tables 6 and 10). The symbols are used to indicate the molecular weights of the solutes. The points with arrows indicate that the permeability of that solute was too large to be measured, but is above the value at the base of the arrow. The regression line has the equation $\log P_n = 0.930 \log k_n - 2.74$ with a correlation coefficient of 0.86.

Table 3.6 Membrane permeabilities of selected solutes in *Chara*, *Nitella*, human erythrocyte, and artificial lipid membranes (Collander, 1954; Stein, 1990). M is the molecular weight, and k is the olive oil:water partition coefficient.

Solute characteristics			Membrane permeability (cm/s)			
Name	M	k	Chara ceratophylla	Nitella mucronata	Human erythrocytes	Artificial lipid
Water	18	1.3×10^{-3}	6.6×10^{-4}	2.5×10^{-3}	1.2×10^{-3}	2.2×10^{-3}
Formamide	45	1.1×10^{-6}	2.2×10^{-5}	7.6×10^{-6}	1.1×10^{-6}	1.0×10^{-4}
Ethanol	46	3.6×10^{-2}	1.6×10^{-4}	5.5×10^{-4}	2.1×10^{-3}	
Ethanediol	58	4.9×10^{-4}	1.1×10^{-5}		2.9×10^{-5}	8.8×10^{-5}
Butyramide	87	1.1×10^{-6}	5.0×10^{-5}	1.4×10^{-5}	1.1×10^{-6}	
Glycerol	92	7.0×10^{-5}	2.0×10^{-7}	3.2×10^{-9}	1.6×10^{-7}	5.4×10^{-6}
Erythritol	122	3.0×10^{-5}			6.7×10^{-9}	

13

Permeability of Chara cells. The compounds, molecular weight, permeability, and oil/water partition data are shown. From Collander, R. (1954) The permeability of *Nitella* cells to non-electroytes, Physiol. Plant. 7:420-445.

Compound	MW	Perm	O/W
Deuterium hydroxide	19	25000	0.0007
Ethyl acetate	88		2.5
Methyl acetate	74	25000	0.43
sec.-Butanol	74	9300	0.25
Methanol	32	5700	0.0078
n-Propanol	60	7200	0.13
Ethanol	46	5500	0.032
Paraldehyde	132	12000	1.9
Urethane	89	5200	0.074
iso-Propanol	60	3800	0.047
Acetonylacetone	144	7500	0.081
Diethylene glycol monobutyl ether	162	2600	0.12
Dimethyl cyanamide	70	1900	0.073
tert-Butanol	74	1900	0.23
Glycerol diethyl ether	148	2300	0.11
Ethoxyethanol	90	1800	0.019
Methyl carbamate	75	1600	0.025
Triethyl citrate	276	2400	0.5
Methoxyethanol	76	990	0.0056
Triacetin	218	1100	0.44
Dimethylformamide	73	705	0.0049
Triethylene glycol diacetate	234	661	0.033
Pyramidone	231	655	0.26
Diethylene glycol monoethyl ether	134	406	0.006
Caffeine	194	357	0.033
Cyanamide	42	292	0.0045
Tetraethylene glycol dimethyl ether	222	285	0.0056
Pinacol	118	229	
Diacetin	176	209	0.071
Methylpentanediol	118	191	0.024
Antipyrene	188	192	0.032
iso-Valeramide	101	182	0.023
1,6-Hexanediol	118	177	0.0068
n-Butyramide	87	139	0.0095
Diethylene glycol monomethyl ether	120	134	0.0042

Compound	MW	Perm	O/W
Trimethylcitrate	234	121	0.047
Proprionamide	73	79	0.0036
Formamide	45	76	0.00076
Acetamide	59	66	0.00083
Polyethylene glycol monoethyl ether	200	66	
Succinamide	99	54	0.0049
Glycerol monoethyl ether	120	40	0.0074
N,N-Diethyl urea	116	39	0.0076
1,5-Pentanediol	104	34	0.0061
Dipropylene glycol	134	31	0.002
Glycerol monochlorhydrin	110	30	0.012
1,3-Butanediol	90	24	0.0043
2,3-Butanediol	90	21	0.0034
1,2-Propanediol	76	17	0.0017
N,N-Dimethyl urea	88	15	0.0023
1,4-Butanediol	90	14	0.0021
Ethylene glycol	62	12	0.00049
Glycerol monomethyl ether	106	12	0.0026
N,N-Dimethyl urea	88	12	
1,3-Propanediol	76	10	
Ethyl urea	88	6.6	0.0017
Polyethylene glycol diacetate	380	6.3	
Thiourea	76	3.6	0.0012
Diethylene glycol	106	3.8	
Methyl urea	74	3.2	0.00044
Urea	60	1.3	0.00015
Triethylene glycol	150	1	
Polyethylene glycol diacetate	480	0.8	
Tetraethylene glycol	194	0.71	
Dicyanodiamide	84	0.46	0.00047
Hexanetriol	134	0.42	
Hexamethylene tetramine	140	0.39	0.00021
Polyethylene glycol monoethyl ether	400	0.15	
Glycerol	92	0.032	0.00007
Pentaerythritol	136	.002	

Permeability (cm/sec)(X10⁷)

Oil/water partition (left, squares) or Molecular weight (right, circles)

14

For animal & plant cells, charged solute
concentrations vary widely between the inside
and outside environments

Animals	K^+	Na^+	Ca^{2+}	ψ
intracellular	high	low	low	~60 mV
extracellular	low	high	high	0

Plants	K^+	Na^+	Ca^{2+}	ψ
intracellular	high	varies	low	-200 mV
extracellular	low	low	high	0

The presence of a
voltage difference affects ion movements, and
therefore must be considered as an additional
driving force on the flux, J. To do this,
we start with the concept of a chemical potential
μ, where flux,

$$J \propto \frac{d\mu}{dx}$$

where $\frac{d\mu}{dx}$, the chemical potential gradient

replaces concentration

Ion	$[Ion]_{extracellular}$ (mM)	$[Ion]_{intracellular}$ (mM)	$\dfrac{[Ion]_o}{[Ion]_i}$	Equilibrium Potential (mV)
ANIMAL				
Na^+	145	12	12	+67
K^+	4	155	0.026	−98
Ca^{2+}	1.5	$<10^{-7}$ M	$15 \cdot 10^6$	+395
Cl^-	123	4.2	30	−90
NITELLA (FRESH WATER ALGAE)				
Na^+	1.0	15	0.067	−65
K^+	0.1	120	$8.3 \cdot 10^{-4}$	−169
Ca^{2+}	1.0	$<10^{-7}$ M	$15 \cdot 10^6$	+395
Cl^-	1.5	75	0.02	+93
VALONIA (MARINE ALGAE)				
Na^+	490	40	12.25	+60
K^+	10	435	0.023	−90
Ca^{2+}	1.0	$<10^{-7}$ M	$15 \cdot 10^6$	+395
Cl^-	560	140	4.0	−33
HIGHER PLANT				
Na^+	10	15	0.67	−10
K^+	10	180	0.056	−69
Ca^{2+}	10	$<10^{-7}$ M	$1 \cdot 10^8$	+440
Cl^-	1.5	75	1.35	+7

Sources: (animal) Hille, Bertil (1984) Ionic Channels of Excitable Membranes
(other) Luttge and Higinbotham (1979) Transport in Plants

16

In this case,

$$J = \text{mobility} \left(\frac{CM}{s}\right) \cdot \text{activity} \left(\frac{\text{mole}}{cm^3}\right) \cdot \underbrace{\text{driving force}}$$

where mobility $\rightarrow u$, activity $\rightarrow c$, driving force $\rightarrow d\mu/dx$

$$= u \cdot c \cdot \frac{d\mu}{dx}$$

now,

$$\mu = RT (\ln c) + zF\Psi$$

temperature $\nearrow$, Faraday constant $\nearrow$ (F), gas constant $\searrow$ (R), ion valence $\searrow$ (z)

so

$$d\mu = RT \, d(\ln c) + zF \, d\Psi$$

where $d(\ln c) \rightarrow \frac{1}{c} dc$

$$\frac{d}{dx} \, \text{of} \, d\mu = \frac{RT}{c} \frac{dc}{dx} + zF \frac{d\Psi}{dx} = \frac{d\mu}{dx}$$

so

$$J = -u \cdot c \cdot \left[\frac{RT}{c} \frac{dc}{dx} + zF \frac{d\Psi}{dx} \right]$$

$$= -(uRT)\left(\frac{dc}{dx}\right) - zFuc\left(\frac{d\Psi}{dx}\right)$$

the Nernst-Planck equation   17

Thermodynamic "Refresher"

Gibbs free energy   temperature

$$G = U + PV - TS$$

internal
energy   pressure
and volume   entropy

The "d" operation

$$dG = dU + PdV + VdP - TdS - SdT$$

At constant temperature & pressure $\leftarrow$ simplifying.

$$dG = dU + PdV - TdS$$

see diagram for Gibbs minima at equilibrium.

The chemical energy, $\mu = \left(\dfrac{\partial G}{\partial n}\right)$

the partial derivative acknowledges that μ is for one molecular species, all others being maintained constant.

a reference
chemical potential.

     activity   pressure   mass

$$\mu = \mu^* + RT \ln a + \bar{V}P + zFE + Mgh$$

(liq phase)

volume per
mole
(essentially
unchanged in liquids)

electrical
potential

height

For our purposes, we are interested in a change in μ (thus, μ^* cancels out) and only activity (≈ concentration) and electrical potential.
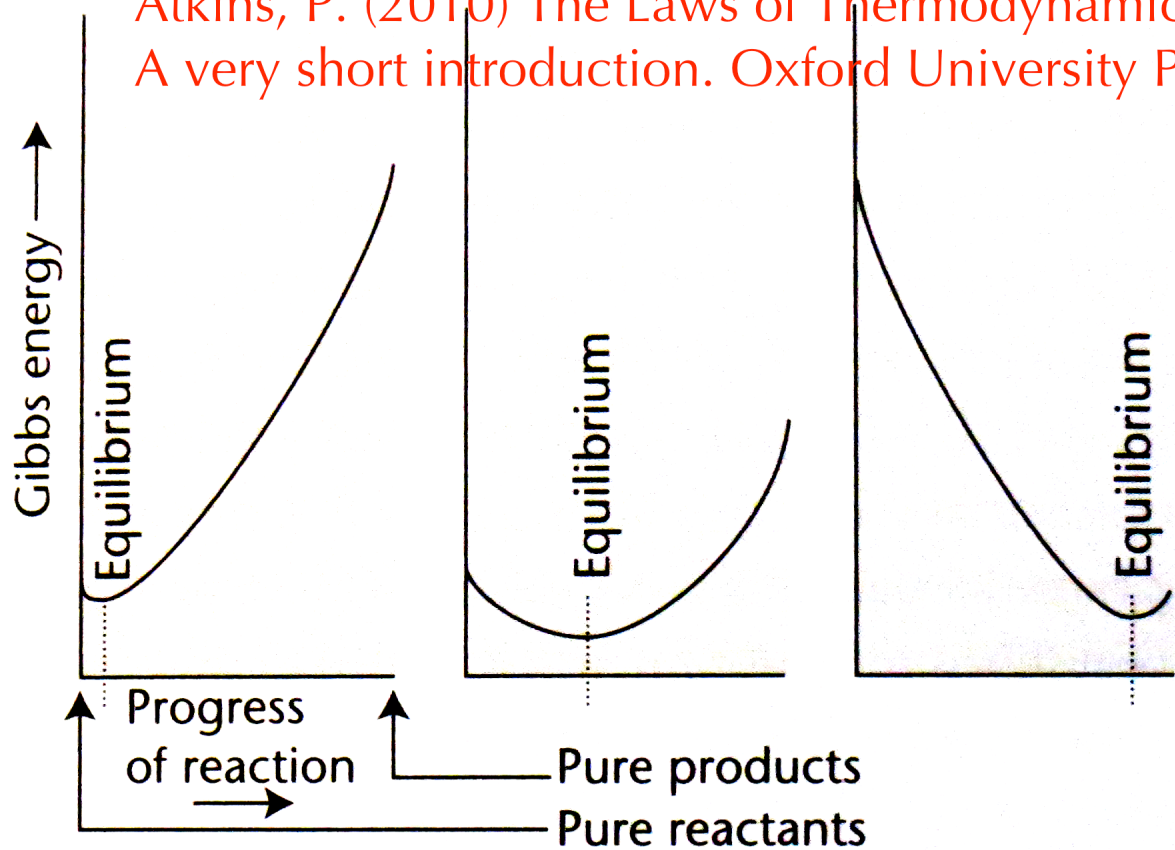
19. The variation of the Gibbs energy of a reaction mixture as it changes from pure reactants to pure products. In each case, the equilibrium composition, which shows no further net tendency to change, occurs at the minimum of the curve

In this formalism, the diffusion coefficient

$$D = uRT \qquad \text{units } cm^2/s$$

known as the Einstein equation, which describes the ability of the chemical species to 'expose' space on the basis of its mobility, u, and its kinetic energy RT (analogous to kT, which defines the velocity of the chemical species.

In the form:

$$J = -(uRT)\left(\frac{dc}{dx}\right) - zFuc\frac{d\psi}{dx}$$

the equation isn't useful because we need to know what $\frac{dc}{dx}$ and $\frac{d\psi}{dx}$ actually are.

To do this, we need to integrate over the boundary conditions of the membrane.

To start with, we assume that the gradient of the electrical potential, $d\psi/dx$ is constant:

slope, $\dfrac{d\psi}{dx} = \dfrac{\psi^o - \psi^i}{\Delta x}$

$$= \dfrac{\psi}{L}$$
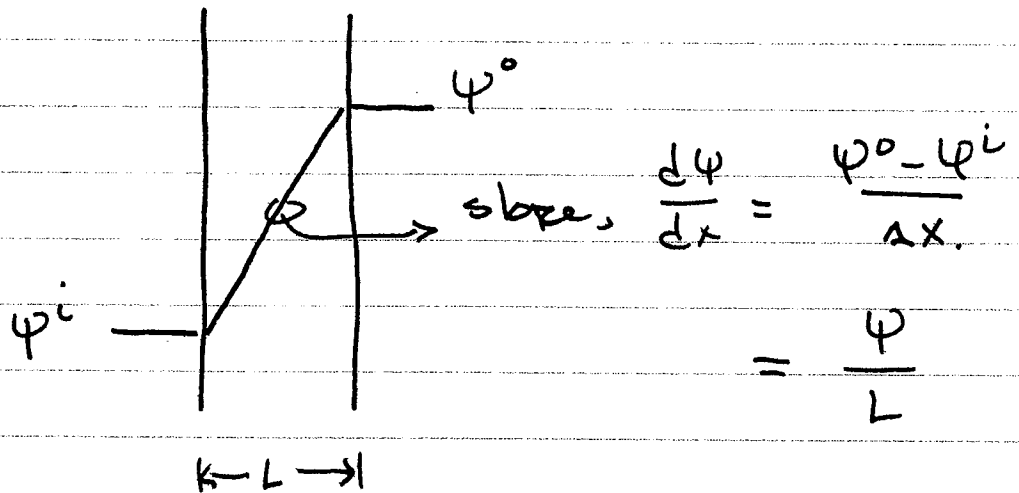
so,

$$J = -D\left(\dfrac{dc}{dx}\right) - zFuc\,\dfrac{\psi}{L}$$

re-arranging

$$\dfrac{-D}{J + zFuc\,\dfrac{\psi}{L}}\,dc = dx$$

we can integrate over the boundary conditions:

C^o concentration outside
C^i concentration inside
$0 \ \& \ L$ width of the membrane.

19

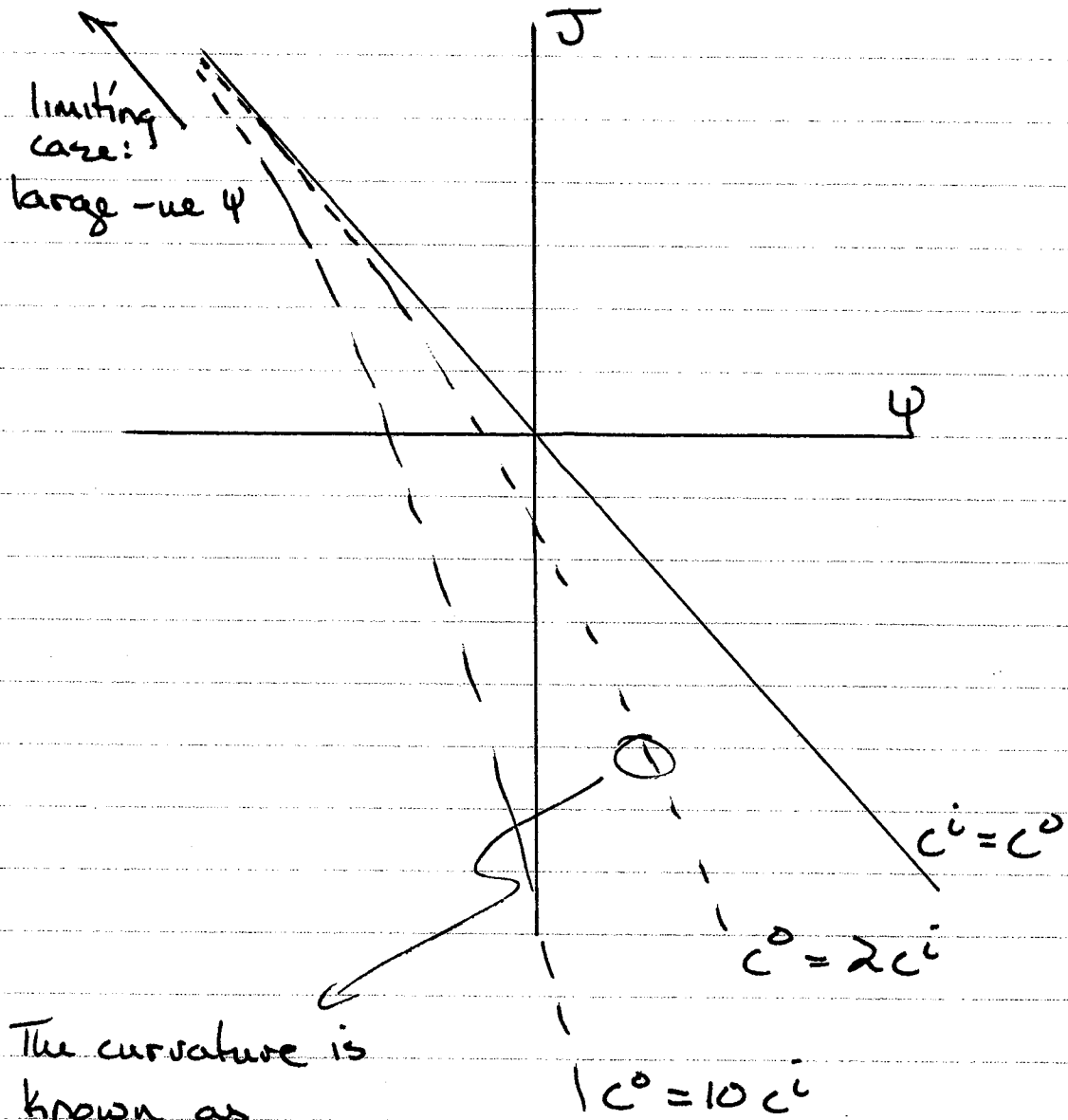$$\int_{c^o}^{c^i} \frac{-D}{J + zFuc\,\Psi/L} = \int_0^L dx$$

which yields:

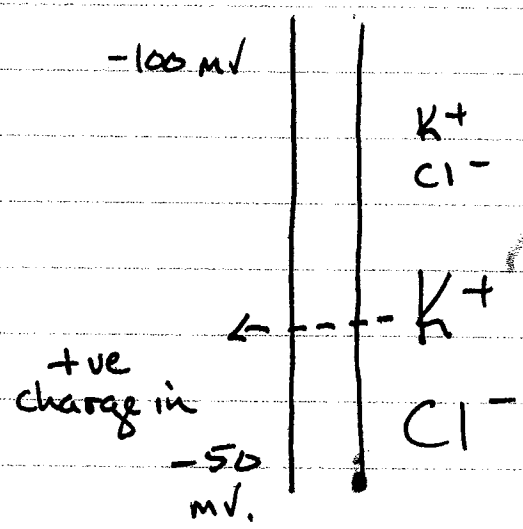$$J = -P\left(\frac{zF\Psi}{RT}\right) \frac{[c^o - c^i \exp(zF\Psi/RT)]}{[1 - \exp(zF\Psi/RT)]}$$
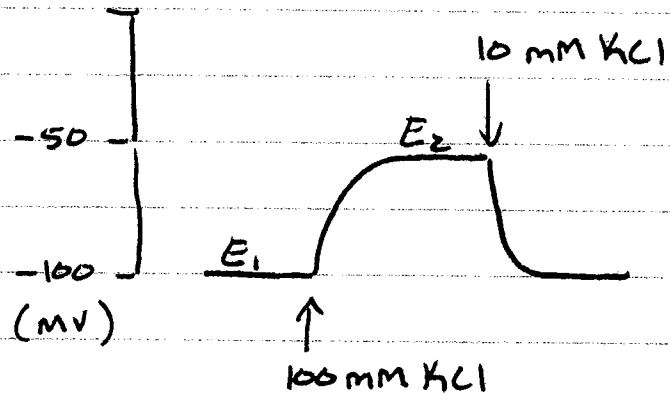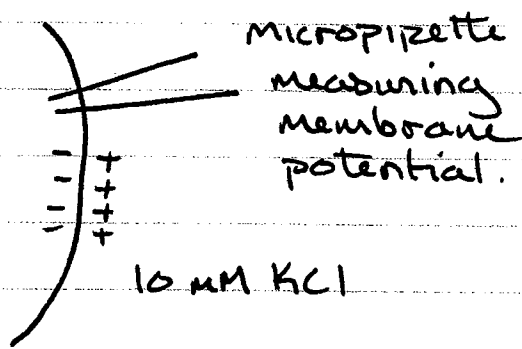
where $P = D/L$

This is called the Goldman constant field equation. Constant field because we assume $d\Psi/dx$ is constant.

We can now predict flux, J, as a function of the difference in concentration and potential.

Flux (out to in)

$c^i < c^o$

$c^i > c^o$

(a) Nernst potential (flux is zero)

* $J = -P(c^o - c^i)$ (in to out)

21

limiting
case:
large −ve ψ

J

ψ

$c^i = c^o$

$c^o = 2c^i$

$c^o = 10 c^i$

The curvature is
known as
Goldman rectification

Now, we can consider two cases.

Case One   flux is 0   (J=0)

$$0 = -\frac{PzF\psi}{RT} \left[\frac{c^o - c^i \exp(zF\psi/RT)}{1 - \exp(zF\psi/RT)} \right.$$

$$0 = c^o - c^i \exp(zF\psi/RT).$$

solve for ψ

$$\frac{c^o}{c^i} = \exp(zF\psi/RT)$$

$$\ln \frac{c^o}{c^i} = zF\psi/RT.$$

$$\frac{RT}{zF} \ln \frac{c^o}{c^i} = \psi \qquad \text{The Nernst equation.}$$

Uses

1) Identification of permeant ions.

example. effect of KCl on membrane potential of
           cell.

23

micropipette
measuring
membrane
potential.

10 mM KCl

$E_2 - E_1 = \frac{RT}{zF} \ln \frac{c_2^o}{c^i} - \frac{RT}{zF} \ln \frac{c_1^o}{c^i}$

$= \frac{RT}{zF} \left[\ln \frac{c_2^o}{c^i} - \ln \frac{c_1^o}{c^i} \right]$

$= \frac{RT}{zF} \ln \frac{\frac{c_2^o}{c^i}}{\frac{c_1^o}{c^i}} = \frac{RT}{zF} \ln \frac{c_2^o}{c_1^o} \cdot \frac{\cancel{c^i}}{\cancel{c^i}}$
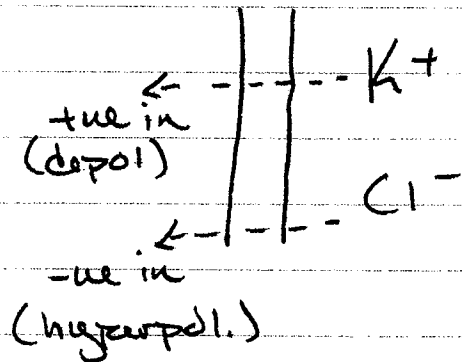
24

$$E_2 - E_1 = \frac{RT}{zF} \ln \frac{c_2^o}{c_1^o}$$

$$\frac{c_2^o}{c_1^o} = \frac{\cancel{\$} \, 100}{10} \quad ; \quad z = 1 \quad ; \quad \frac{RT}{F} = 25 \text{ mV}.$$

$$E_2 - E_1 = (25) \ln[10] = 58 \text{ mV}.$$

If Cl^- was permeant:

+ve in
(depol) $\leftarrow$ - | - | - - K^+

-ve in $\leftarrow$ - | - | - - Cl^-
(hyperpol.)

the potential would hyperpolarize by 58 mV.

∴ K^+ is permeant, Cl^- is not permeant.

Nernst potentials often identify permeant ions.
so E_K, E_{Cl} are commonly coorch considered

Nernst potential   Nernst potential
for K^+ for Cl^-

Case Two the potential, ψ, is zero.

$$J = -P\,\frac{zF\psi}{RT}\left[\frac{c^o - c^i\,\exp\left(zF\psi/RT\right)}{1 - \exp\left(zF\psi/RT\right)}\right]$$

for small potentials (approaching zero)

$$\exp\left(zF\psi/RT\right) = 1 + zF\psi/RT$$

so...

$$J = -P\,\frac{zF\psi}{RT}\left[\frac{c^o - c^i\left(1 + zF\psi/RT\right)}{1 - \left(1 + zF\psi/RT\right)}\right]$$

$$J = -P\,\frac{zF\psi}{RT}\cdot\frac{c^o - c^i\left(1 + zF\psi/RT\right)}{-zF\psi/RT}$$

$$J = -P\;\;c^o - c^i - c^i\cdot zF\psi/RT$$

for $\psi = 0$.

$$J = -P\left(c^o - c^i\right)$$

the equation for an uncharged solute.
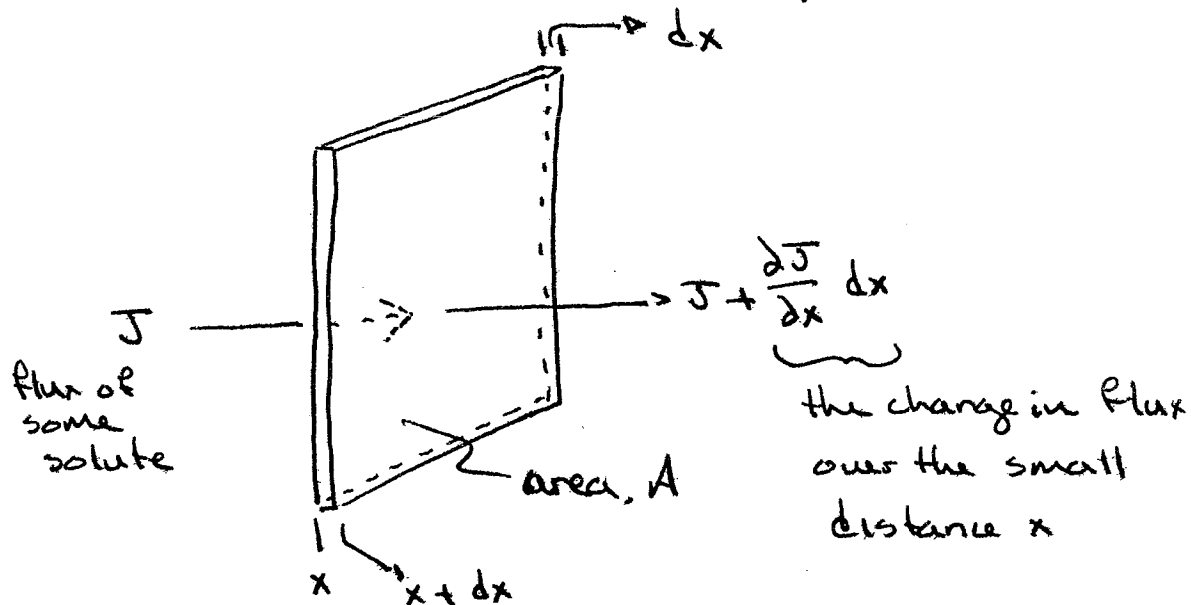
26

The Time Dependence of Diffusion

The diffusion equation, $\bar{J} = -D \frac{\partial c}{\partial x}$

does not account for the changes in concentration that will occur as molecules move from one location to another, in accordance with the flux, $\bar{J}$.

(Nota bene Instead, it assumes a steady state, in which $\partial c / \partial t = 0$)

How then, do we account for the non-steady state time dependence of diffusion?

One approach considers changes in flux, $\bar{J}$, with respect to distance x ($\partial \bar{J} / \partial x$).

J
flux of some solute

$\bar{J} + \frac{\partial \bar{J}}{\partial x} dx$

the change in flux over the small distance x

area, A

x   x + dx

At x, the flux is $\bar{J}$

At x + dx, the flux has changed to $\bar{J} + \frac{\partial \bar{J}}{\partial x} dx$

27

The change in the amount of solute in the
volume element $A\,dx$

<div style="text-align:center">area, A   small distance x</div>

is equal to the amount flowing in, $\bar{J}\cdot A$
minus   the amount flowing out, $\left(J + \frac{\partial J}{\partial x}\,dx\right)\cdot A$
per unit time

Now the change in the amount of solute in the
volume element $A\cdot dx$ per unit time can be
expressed as $\partial c/\partial t$, times the volume element
$A\cdot dx$.

That is $\quad \frac{\partial c}{\partial t}\,A\cdot dx = J\cdot A - \left(J + \frac{\partial J}{\partial x}\,dx\right)A$

$$\frac{\partial c}{\partial t}\,A\cdot dx = \underbrace{J\cdot A - J\cdot A}_{\text{cancel out}} - \frac{\partial J}{\partial x}\,dx\cdot A$$

$$\frac{\partial c}{\partial t}\,\underbrace{A\cdot dx}_{} = -\frac{\partial J}{\partial x}\,\underbrace{dx\cdot A}_{}$$
<div style="text-align:center">divide out</div>

And, $\quad -\frac{\partial J}{\partial x} = \frac{\partial c}{\partial t}$ This is known as
the continuity
equation.

<div style="text-align:center">if flux declines concentration
must increase</div>

Continuity refers to the idea that matter is
neither created or destroyed.

We can substitute the continuity equation

$$-\frac{\partial J}{\partial x} = \frac{\partial c}{\partial t}$$

into the flux equation

$$J = -D\frac{\partial c}{\partial x}$$

$$-\frac{\partial}{\partial x}\left(-D\frac{\partial c}{\partial x}\right) = \frac{\partial c}{\partial t}$$

or,

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2}$$

This is known as Fick's second law of diffusion.

It describes how concentration of the solute changes with position and time as a result of diffusion.

Examples of the time dependence of concentration are shown on the overheads.

29

2.2. Method of reflection and superposition

2.2.1. *Plane source*

It is easy to see by differentiation that

$$C = \frac{A}{t^{\frac{1}{2}}} \exp(-x^2/4Dt),$$ (2.1)

where A is an arbitrary constant, is a solution of

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2},$$ (2.2)

which is the equation for diffusion in one dimension when D is constant. The expression (2.1) is symmetrical with respect to $x = 0$, tends to zero as x approaches infinity positively or negatively for $t > 0$, and for $t = 0$ it vanishes everywhere except at $x = 0$, where it becomes infinite.

$$C = \frac{M}{2(\pi Dt)^{\frac{1}{2}}} \exp(-x^2/4Dt),$$ (2.6)

and this is therefore the solution which describes the spreading by diffusion of an amount of substance M deposited at time $t = 0$ in the plane $x = 0$. Fig. 2.1 shows typical distributions at three successive times.
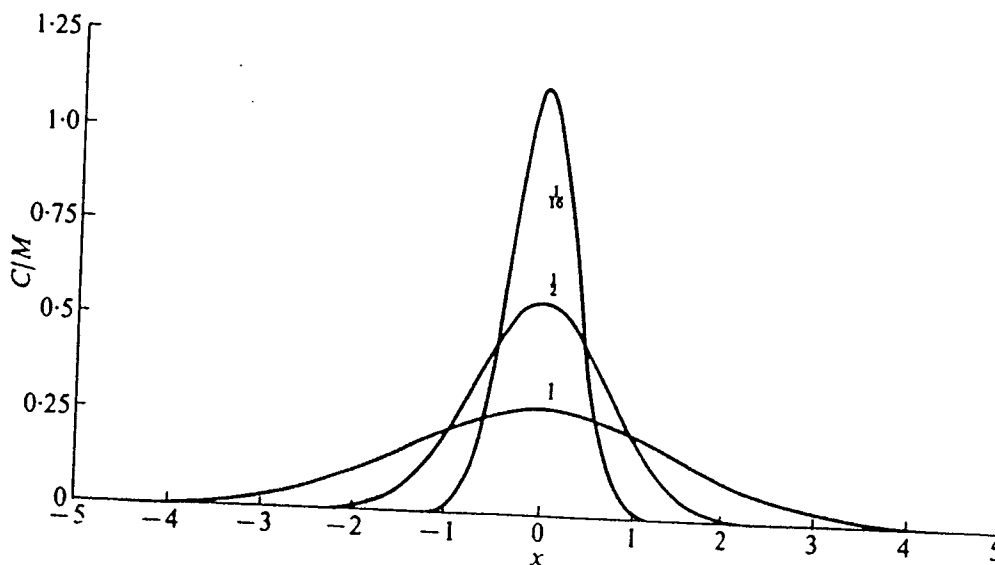

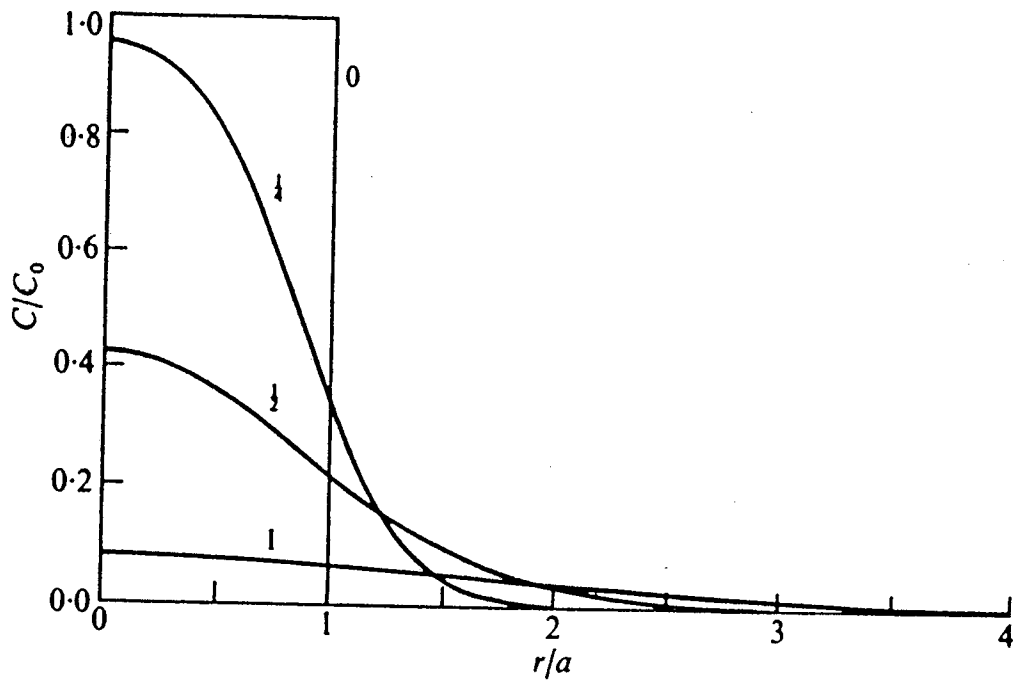FIG. 2.1. Concentration–distance curves for an instantaneous plane source. Numbers on curves are values of Dt.

30

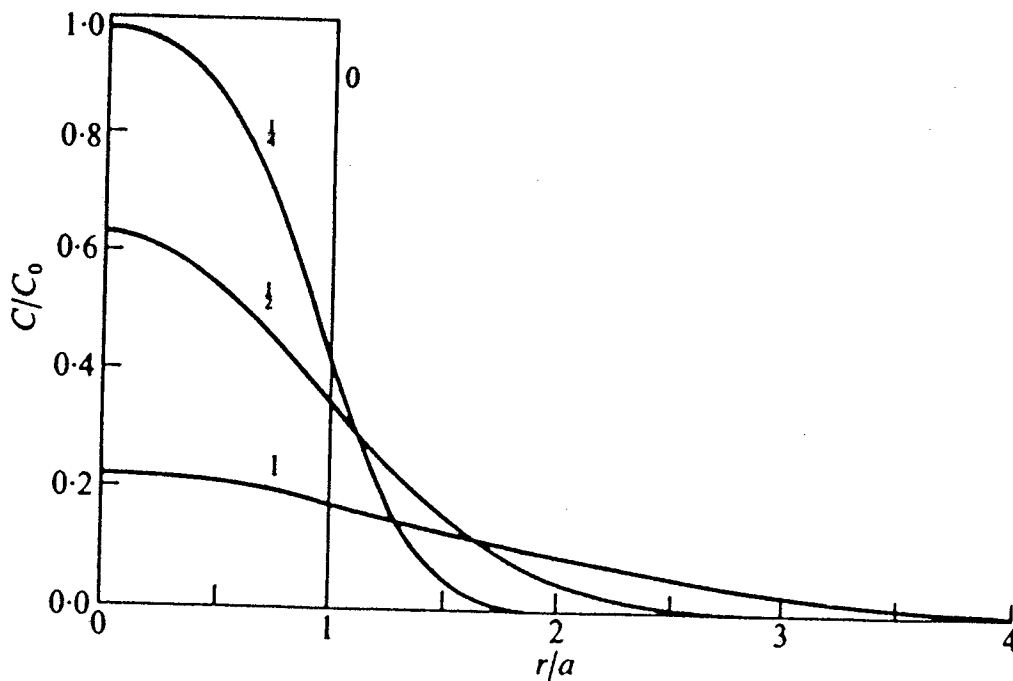FIG. 3.1. Concentration distributions for a spherical source. Numbers on curves are values of $(Dt/a^2)^{\frac{1}{2}}$.

FIG. 3.2. Concentration distributions for a cylindrical source. Numbers on curves are values of $(Dt/a^2)^{\frac{1}{2}}$.

31

Diffusion: The Einstein connection.

The phenomenon of particle motion had been explored by many scientists since its first description by Robert Brown (hence the term "Brownian motion"

Einstein's use of molecular theory validated its use elsewhere. Explicit in his work were calculations of Avogadro's number (molecules/mole) and the dimensions of the molecule. Both of great importance to Physicists

A key element of his analysis was the use of the mean square displacement of the particle over time (Elementary Theory of the Brownian Motion pp. 80-81)

To use his formalism:

$$D = \frac{1}{2} \frac{\Delta^2}{\tau}$$

"coefficient of diffusion

unit time

Δ signifies the length of the path described on average by a solute molecule during the time τ in the direction of the x-axis.

Nota bene: To account for the conundrum that positive and negative displacements will, when averaged together, be "0", the mean of the squares must be used. That is, $\Delta = \sqrt{\Delta^2}$

32

Solving $D = \frac{1}{2} \frac{\Delta^2}{T}$ for Δ

yields $\Delta = \sqrt{2DT}$

This is testable, and indeed displacement of the particle is dependent on the square root of time ($\sqrt{T}$ above).

A more modern formalism is $\langle x^2 \rangle^{1/2} = (2Dt)^{1/2}$ (see Berg, Random Walks in Biology)

This relation can be expanded to the x, y & z coordinates of three dimensional space. Following Berg:

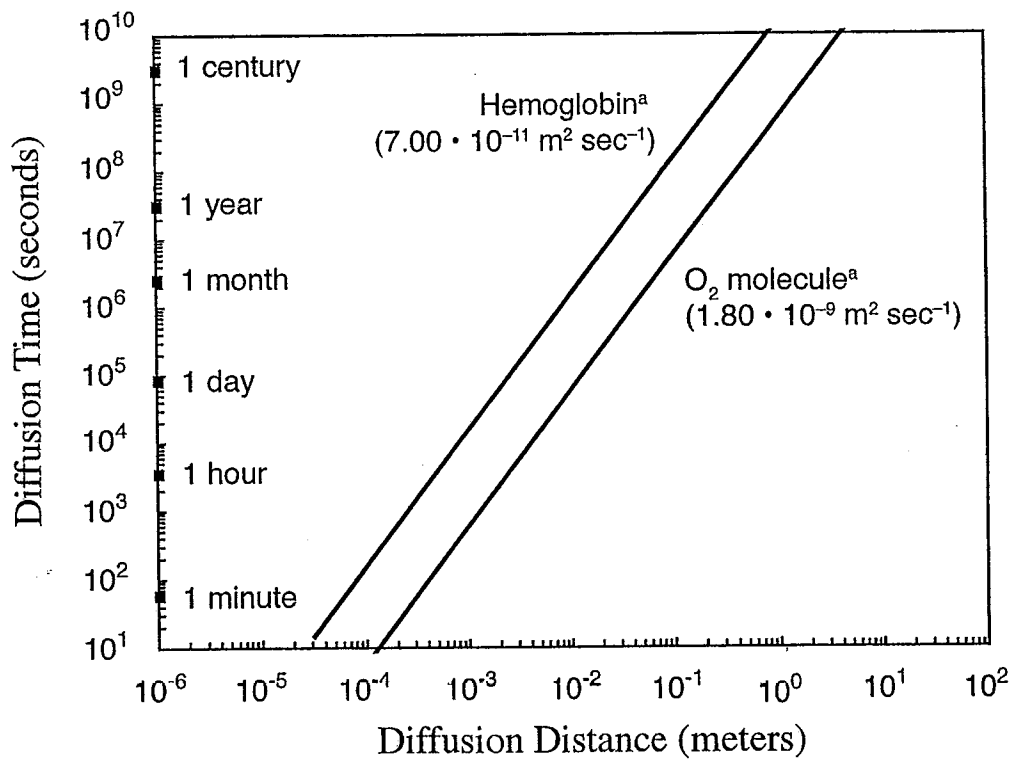$$\langle x^2 \rangle = 2Dt, \quad \langle y^2 \rangle = 2Dt \quad \text{&} \quad \langle z^2 \rangle = 2Dt$$

Then, from $r^2 = x^2 + y^2$ $\quad \langle r^2 \rangle = 4Dt$

or $\quad r^2 = x^2 + y^2 + z^2 \quad \langle r^2 \rangle = 6Dt$

Average Diffusion distance in three dimensions is shown, versus the diffusion time, for oxygen & hemoglobin - two players in oxygen transport

$$L = \sqrt{6 \cdot D \cdot t}$$

Diffusion works best at small distances.

Diffusion graph showing Diffusion Time (seconds) on the y-axis (10^1 to 10^10, with markers for 1 minute, 1 hour, 1 day, 1 month, 1 year, 1 century) versus Diffusion Distance (meters) on the x-axis (10^-6 to 10^2). Two lines are plotted: Hemoglobin[a] ($7.00 \cdot 10^{-11}$ m² sec⁻¹) and O₂ molecule[a] ($1.80 \cdot 10^{-9}$ m² sec⁻¹).

[a]Brouwer ST, L Hoof, F Kreuzer (1997) Diffusion coefficients of oxygen and hemoglobin measured by facilitated oxygen diffusion through hemoglobin solutions. Biochim Biophys Acta. 1338:127-136.

34