Lecture 7 Viscosity + Diffusion

Physical Biology of the Cell: Chapters 12 & 13.
You will have read Purcell's excellent lecture "Life at low Reynolds Number" which introduces two very important points which we will explore:

i) for bacteria & molecules, all motion is diffusive. $F = ma$ + inertia is irrelevant.

ii) bacterial "hunting" strategies require active random motions on the 30 μm scale
- far enough to sense a gradient, escape diffusion
- if gradient is favorable → keep going.
- work of Howard Berg.

1. Navier - Stokes equation. "$F = ma$ for fluids"
Key equation of fluid mechanics.

Flow = velocity $(\vec{r}, t)$ vector field $\vec{v}(\vec{r}, t)$

Pressure = driving force = scalar $P(\vec{r})$

$$\frac{\partial \vec{v}}{\partial t} + (\vec{v} \cdot \vec{\nabla})\vec{v} = -\frac{1}{\rho}\vec{\nabla}P + \frac{\eta}{\rho}\nabla^2\vec{v}$$

$(\vec{v} \cdot \vec{\nabla})\vec{v}$: quadratic (nonlinear) term

ρ: density

$\frac{\eta}{\rho} = \nu$ = kinematic viscosity

This applies to Newtonian (incompressible) fluids obeying conservation law $\vec{\nabla} \cdot \vec{v} = 0$

Nonlinear equation:
- analytic solution only for a few cases
- boundary conditions can select:
  - laminar (layered) flow
  - turbulent flow with vortices
  - singularities

Left side of N-S equation = response

$\frac{\partial \vec{v}}{\partial t}$ classical acceleration

$(\vec{v} \cdot \vec{\nabla})\vec{v}$ gradients established within flow.

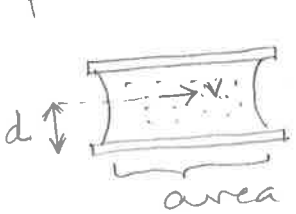
Right side of N-S equation = forces

$-\frac{1}{\rho}\vec{\nabla}P$ pressure differences and mass.

$\frac{\eta}{\rho}\nabla^2\vec{v}$ viscous friction forces.

2. Viscosity

Most easily defined with shear on moving plates. $\vec{v}$-field at surface follows along.



$$\text{shear} = \frac{F}{A} = \eta \frac{v}{d} = \eta \times \text{velocity gradient}$$

Definition assumes laminar flow $\Rightarrow$ small forces and velocity gradients

Viscosity η is a material property of fluid. associated with diffusion of faster and slower molecules.

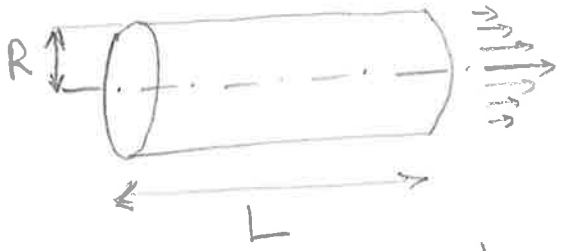
- can get very big when large molecules are dissolved eg honey, molasses
- can be highly temperature dependent eg glycerol, "glasses."
- [Purcell observation] pure simple liquids have lowest values, all similar

H_2O has $\eta = 10^{-3}$ Pa.s [SI unit]

3. Poiseuille solution

Laminar flow inside cylindrical tube.

Exercise in cylindrical coordinates...



ΔP = pressure drop.

Viscous drag up to stationary wall slows down flow there; max flow in centre.

$$v(r) = \frac{\Delta P}{4\eta L}\left(R^2 - r^2\right)$$ not derived

Strong radial dependence means total flow

$$Q = \int_0^R v(r)\, 2\pi r\, dr = \frac{2\pi\,\Delta P}{4\eta L}\left[\frac{R^2 r^2}{2} - \frac{r^4}{4}\right]_0^R = \frac{\pi\,\Delta P R^4}{8\eta L}.$$

R^4 dependence strongly limits flow of blood in capillaries vs arteries.

Small plaque build-up causes ΔP to rise quickly → BPM diagnosis of heart disease.

4. Reynolds Number.

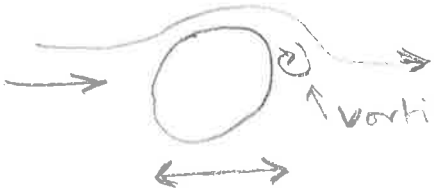
Dimensionless number to determine if flow is turbulent or not:

$$Re = \frac{\text{Kinetic Energy of flow}}{\text{Viscous Energy.}}$$

$Re < 10$ typically laminar flow.

$Re > 10$ beginning of turbulence (non-linear effects).

Consider an object trying to "swim" in water. Turbulent / laminar depends a lot on its size:



estimate $KE = mv^2 = \rho a^3 u^2$

viscous shear $\sim$ Force / area $= \eta u/a$. (def.)

Work done = force × dist $= \eta u/a \times a^2 \times a = \eta u a^2$.

$$Re = \frac{KE}{\text{Viscous work}} = \frac{\rho a^3 u^2}{\eta u a^2} = \frac{\rho}{\eta} a u.$$

$\rho = 10^3 \text{ kg m}^{-3}$ $\eta = 10^{-3}$ Pa.s. water.

i) animal swimming:
$a = 1$m $u = 1$m/s. $Re = 10^6$ turbulent.

ii) bacteria "swimming":
$a = 1\mu$m $u = 10\mu$m/s $Re = 10^{-5}$ laminar.

iii) molecule
$a = 1$ nm even smaller.

So in cellular biophysics we are <u>always</u> in the laminar flow regime.

5. Stokes equation

Inertial terms are small compared with visous.

Navier-Stokes $\rightarrow$ Stokes Equation

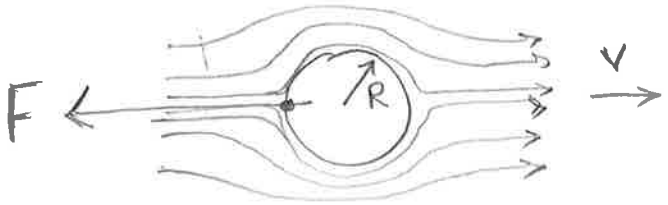
$\rho \to 0$ in $\cancel{\frac{\partial \vec{v}}{\partial t}} + \cancel{(\vec{v}\cdot\vec{\nabla})\vec{v}} = -\frac{1}{\rho}\vec{\nabla}P + \frac{\eta}{\rho}\nabla^2\vec{v}$

$$\boxed{\vec{\nabla}P = \eta \nabla^2 \vec{v}}$$ when $Re \ll 1$

Remarkable result: no time, no mass!

all non-linearities go away.

Exact solution for laminar flow around a sphere, Stokes drag force:

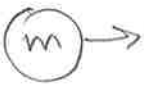


$$\vec{F}_{drag} = 6\pi\eta R\vec{v} = \gamma\vec{v}$$

For objects smaller than animals, we can always use this result, since we are always in the laminar limit.

i) Stopping distance

If a sphere starts off with velocity V_0 how far does it move by inertia?



$$F = m\frac{dv}{dt} = -6\pi\eta R v$$

$$v(t) = V_0 e^{-6\pi\eta R t/m} = V_0 e^{-t/\tau}$$

Relaxation time $\tau = m/6\pi\eta R$ ← $R = 1\,\mu m$

$$m = \frac{4}{3}\pi R^3 \rho \qquad \tau = \frac{4R^2\rho}{18\eta} \sim 10^{-12}\, 10^{6}\, s = 1\,\mu s$$

Distance $= V_0\tau \sim 10\,\mu m/s \times 10^{-6} = 10\,pm.$

But for bigger objects this grows as R^2.

ii) Propulsion. (Purcell + p502)

In low Re limit, "scallop" rule says single hinge motion just reciprocates. – no net motion.

2 hinges + sequence needed to move.

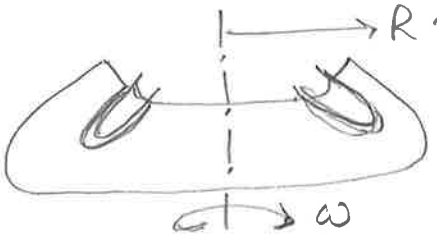
Bacterial flagellum much more effective

– rotates at 100 Hz

– screws its way through viscous medium.

6. Centrifuge.

Device for separating biomolecules based on size.



Rotate at high speed
100,000 rpm typically

Centripetal acceleration, $g_c = \omega^2 R$

$\omega = 2\pi \times 10^5/60 \sim 10^4$ $\quad g_c \sim 10^7\ ms^{-2}$

10^6 times greater than gravity $g = 9.81\ ms^{-2}$

For lamellar flow, no inertia.

$$F = \gamma v = m g_c.$$

$$v_{drift} = \frac{m}{\gamma} g_c = S g_c$$

$m/\gamma = S$ = sedimentation coefficient
is a property of the material, which can allow separations to be made.

Units of S are time, usually Svedburgs $= 10^{-13}$ s.

Ribosome has 30S & 50S subunits.

Strictly, buoyancy affects mass: For a sphere

$$S = \frac{(\rho - \rho_0)\frac{4}{3}\pi R^3}{6\pi\eta R} = \frac{2(\rho-\rho_0)R^2}{9\eta}$$

ρ = density, eg protein = 1.35 g/cc

ρ_0 = solvent eg 1 g/cc.

Quadratic dependence on R = big effect.

eg 50S subunit radius:

$$R^2 = \frac{9\eta S}{2(\rho-\rho_0)} = \frac{9 \times 10^{-3} \times 50 \times 10^{-13}}{2 * 0.4 \times 10^3} = (15\ nm)^2$$

a bit high.

$$v_{drift} = 50 \times 10^{-13} \times 10^7 = 50\ \mu m/sec$$
$$= 50\ mm \text{ in } 16\ min \text{ (typical spin)}$$

7. Centrifuge methods

i) crude separation methods (of cells)
slow spin, fixed time
debris (cell walls etc) → pellet.
repeat on supernatant, higher speed.
mitochondria, organelles, chromosomes.
higher speed, longer time
ribosomes, large proteins. etc.

ii) density gradient "ultracentrifuge"
"isopycnic" centrifuge.
Mix sample with CsCl ~ 1 Molar
Centrifuge long time to reach equilibrium.
Buoyancy of fractions → bands.
Salt reaches equilibrium quickly.
Proteins move at V_{drift}

iii) sucrose gradient
higher viscosity, so layer concentrations in tube. Add sample at top → bands.
Density and viscosity play role.

8. Diffusion (Ch 13, p509)

Diffusion is very effective for transport over short distances eg substrate or ATP → enzyme, but alternative methods needed for long distance:

eg giraffe blood circulation
neurotransmitters in nerve cells.

Final delivery is by diffusion, even when active transport takes place: 100 μm spacing of blood capillaries (mice or whales) for hemoglobin.

$t \approx L^2/D$ D = diffusion constant.

K^+ ion in H_2O 2000 $\mu m^2/s$.
Small protein H_2O 100 $\mu m^2/s$. = Large molecule
GFP protein in cytoplasm 7 $\mu m^2/s$.

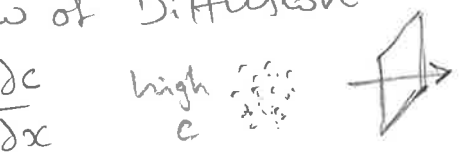
1 μm in 10ms, 100 μm in 100sec (graph).

slide

9. Fick's law of Diffusion

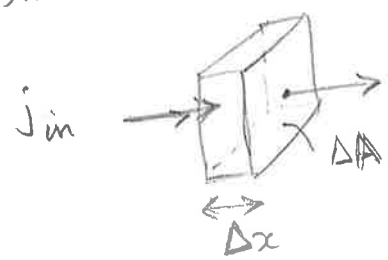
$$j = -D\frac{\partial c}{\partial x}$$

$$\vec{j} = -D\vec{\nabla} c$$



j = number / area / sec

Consider a small box



net flow into box $= j_{in} - j_{out} = -\frac{\partial j}{\partial x}\Delta x \Delta A$

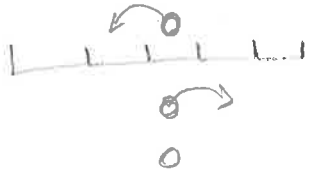
= rate of concentration rise inside $= \frac{\partial c}{\partial t}\Delta x \Delta A$

Conservation of mass / particles

$$\frac{\partial c}{\partial t} = -\frac{\partial j}{\partial x} = D\frac{\partial^2 c}{\partial x^2}$$ [or $\nabla^2 c$ in 3D.].

This is the Diffusion Equation.

ii) Can derive from random walk:
"Sum over microtrajectories"



	$k\Delta t$	$\Delta x = -a$
	$k\Delta t$	$+a$
	$(1-2k\Delta t)$	0

Difference equation relating site probabilities

$$p(x, t+\Delta t) = (1-2k\Delta t)\, p(x,t) + k\Delta t\, p(x-a, t) + k\Delta t\, p(x+a, t)$$

Taylor expand:

$$p(x, t+\Delta t) = p(x,t) + \Delta t \frac{\partial p}{\partial t}$$

$$p(x \pm a, t) = p(x,t) \pm a \frac{\partial p}{\partial x} + \frac{a^2}{2} \frac{\partial^2 p}{\partial x^2}$$

($\pm a \frac{\partial p}{\partial x}$ cancels out on substitution)

$$\Rightarrow \Delta t \frac{\partial p}{\partial t} = 2k\Delta t \frac{a^2}{2} \frac{\partial^2 p}{\partial x^2}$$

Which is the diffusion equation with $D = ka^2$

iii) Einstein (1905)
original derivation of D.E. based on integration over earlier concentrations and jumps of position, giving same result.

Diffusion equation occurs widely in physics.
T.D. Schrodinger equation, wave equation etc.
Solution depends strongly on boundary conds.

10. Greens function solution:

Superposition principle says that general solution of DE is a superposition (linearity)

Greens function is response to δ-function at $t=0$. : easy to add for general case.

$$c(x,t) = \frac{1}{\sqrt{4\pi Dt}} e^{-x^2/4Dt}$$ is a unit $\delta(x)$ at $t=0$.

$$\frac{\partial c}{\partial t} = \frac{1}{\sqrt{4\pi D}} \left(\frac{-1}{2t^{3/2}} + \frac{1}{t^{1/2}} \frac{+x^2}{4Dt^2} \right) e^{-x^2/4Dt}.$$

$$\frac{\partial c}{\partial x} = \frac{1}{\sqrt{4\pi D}} \frac{1}{t^{1/2}} \left(\frac{-2x}{4Dt} \right) e^{-x^2/4Dt}$$

$$\frac{\partial^2 c}{\partial x^2} = \frac{1}{\sqrt{4\pi D}} \frac{1}{t^{1/2}} \left(\frac{-2}{4Dt} + \left(\frac{2x}{4Dt} \right)^2 \right) e^{-x^2/4Dt}$$

$$= \frac{\partial c}{\partial t} \cdot \frac{1}{D}$$ as required.

Since this is a solution, all sums of such functions are also solutions.

Classical Gaussian:

i) normalised:

$$\int_{-\infty}^{\infty} e^{-x^2/4Dt} dx = \underbrace{\int_{-\infty}^{\infty} e^{-u^2} du}_{\sqrt{\pi}} \cdot \underbrace{\sqrt{4Dt}}_{dx = \sqrt{4Dt}\, du.} = \sqrt{4\pi Dt}$$

ii) Gaussian width:

$e^{-x^2/2\sigma^2} = e^{-1/2} = 0.6$ when $x = \sigma$

$\sigma = \sqrt{2Dt}$ broadens as $t^{1/2}$

$\sigma^2 = 2Dt$ is also the variance = (stan. dev)^2 :

$$\langle x^2 \rangle = \frac{1}{\sqrt{4\pi Dt}} \int_{-\infty}^{\infty} x^2 e^{-x^2/4Dt} dx = \frac{1}{\sqrt{4\pi Dt}} \frac{-\partial}{\partial \alpha} \underbrace{\int e^{-\alpha x^2} dx}_{\sqrt{\pi/\alpha}}.$$

where $\alpha = 1/4Dt$

$$\langle x^2 \rangle = \frac{1}{\sqrt{4\pi Dt}} \sqrt{\pi}\, \frac{\alpha^{-3/2}}{2} = \frac{1}{\sqrt{4Dt}} \frac{(4Dt)^{3/2}}{2} = 2Dt$$

This shows that the variance is just σ^2

11. Experimental observation of diffusion

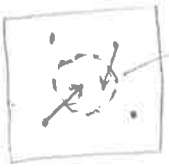
i) Particle tracking under a microscope

original measurements of Perrin (1920) to prove Einstein explanation of Brownian motion.

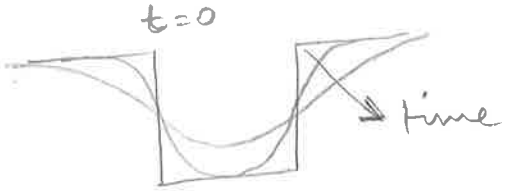
ii) FRAP

Fluorescence recovery after photobleaching

Dye molecule under powerful laser is ionised into higher electronic state, which no longer fluoresces.



bleached area

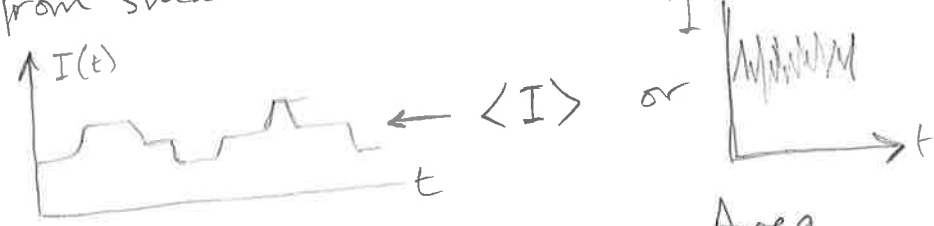


intuitive behaviour. Model as Fourier series in text p527 (ch 13).

iii) FCS.

Fluorescence Correlation Spectroscopy

Measure light intensity coming from small area in confocal microscope

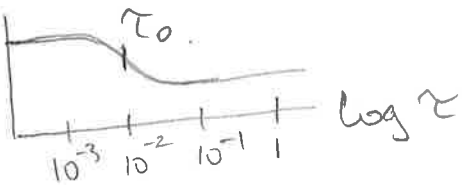


$$C(\tau) = \int I(t) I(t+\tau)\, dt$$

$$\tau_0 \sim \frac{\text{Area}}{D}$$

$$\Rightarrow D = A/\tau_0$$

↑ diffusing particle



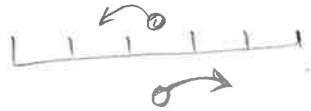
12. Smoluchowsky equation

Driven diffusion in external field.

"drunks on a hill" ⇒ less random.

Biased microtrajectories:

slide



$k_- \Delta t \quad \Delta x = -a$

$k_+ \Delta t \quad \Delta x = a$ } rates

$(1-(k_+ + k_-)\Delta t) \quad \Delta x = 0.$

Net drift $V = \langle \Delta x \rangle / \Delta t$

$= (k_+ a - k_- a) = (k_+ - k_-) a$

no drift if there is no bias.

Follow Taylor expansion as before, but linear term no longer cancels in difference equation

$$\Delta t \frac{\partial p(x,t)}{\partial t} = (k_- - k_+)\Delta t\, a \frac{\partial p}{\partial x} + (k_+ + k_-)\Delta t \frac{a^2}{2} \frac{\partial^2 p}{\partial x^2}$$

$$\frac{\partial p}{\partial t} = -V \frac{\partial p}{\partial x} + D \frac{\partial^2 p}{\partial x^2} \quad \text{where } D = (k_+ + k_-)\frac{a^2}{2}.$$

Smoluchowski equation

$p(x,t)$ = particle probability = concentration (x,t)

Usually applied to Brownian motion in a flow of velocity V.

Or in applied field of force F eg electric field.

$F = \gamma V \quad \gamma = 6\pi\eta R$ sphere in viscosity η

13. Einstein Relation

Brownian motion is driven by fluctuations eg collisions of water molecules with a small particle. $Re \ll 1$: $V = F/\gamma$

$F = -\frac{dU}{dx}$ coming from some potential $U(x)$.

Smoluchowski equation is Fick's law with a bias due to F.

slide. $$J(x) = -D\frac{dc}{dx} + \frac{F}{\gamma}c \quad \text{and} \quad \frac{\partial J}{\partial x} = -\frac{\partial c}{\partial t}$$

($-D\frac{dc}{dx}$: concentration gradient; $\frac{F}{\gamma}c$: bias force.)

Flux of particles due to conc. gradient + force.
In thermal equilibrium, flux is zero.

$$D\frac{dc}{dx} = \frac{F}{\gamma}c = -\frac{dU}{dx}\frac{c}{\gamma} \quad \text{using potential.}$$

$$\gamma D \frac{dc}{c} = -dU \quad \text{integrates to} \quad \gamma D \ln c = -U$$

$$\text{so} \quad \frac{c(x)}{c(0)} = \frac{e^{-U(x)/\gamma D}}{e^{-U(0)/\gamma D}}$$

This is in the Boltzmann form provided:

$$\boxed{\gamma D = k_B T}$$ Einstein relation

Remarkable result relating friction and diffusion
Both quantities are mediated by solvent particle interactions, but seem unrelated.
Diffusion is excited motion driven by molecular collisions = fluctuation
Viscous drag is the dissipation due to those same collisions.
"Fluctuation - Dissipation Theorem"

14. Stokes Einstein relation

Diffusion is a thermally excited process.
Properties / scaling understood by D.E.
But magnitude set by size of D.
Einstein relation scales this to the viscosity

Sphere in fluid of viscosity η has

$\gamma = 6\pi\eta a$ $\quad a$ = radius

$D = k_B T/\gamma = \dfrac{k_B T}{6\pi\eta a}$ Stokes-Einstein relation

a = 1nm (small protein) in water ($\eta = 10^{-3}$ Pa·s)

$k_B T = 4.1 \times 10^{-21}$ J $\quad D = 220\ \mu m^2/s$.

a = 10 nm (large protein) $\quad D = 22\ \mu m^2/s$.

15. Brownian Motion

movie

Direct observation by tracking in microscope

$MSD = \langle x(t) \cdot x(t+\tau) \rangle_t$ (if stationary)

$\langle\ \rangle_t$ = thermal average

slides

direct method to measure D.

Signature of driven Brownian motion:

if $x = v_{drift} t$

then $MSD \propto t^2$

Quadratic term measures drift.