Sedimentation and electrophoresis methods

Schay G.

Physical basis of sedimentation methods

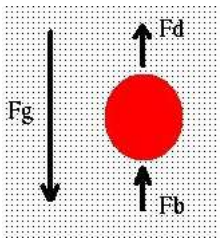
Goal: we would like to measure the mass of tiny particles

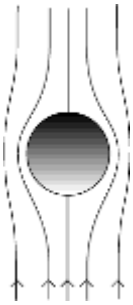
(this method originates long before the AFM, but is still in use)

Put the particle into a solvent/liquid, and see what happens:

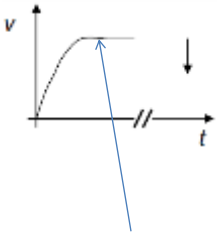
If it's density is higher than that of the liquid, it will sink, or settle down.

This is called **sedimentation**.





Fg : gravity force, Fd: drag force, Fb: buoyant force.



The particle will accelerate until the force equilibrium is not reached.

(or until the bottom of the holder tube is reached)

Here we have the force equilibrium

Newton-II. Law : $\Sigma F = m \cdot a$

and

$$\frac{\Delta v}{\Delta t} = a$$

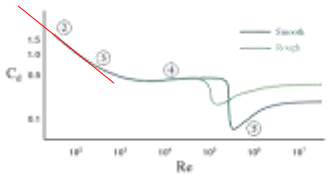
The drag force:

General equation: $F_d = \frac{1}{2}\rho v^2 \cdot C_d \cdot A$, where A is the cross-sectional area, and Cd is the **drag coefficient**.

At low speeds Cd ~ 1/Re, which means Fd is linearly proportional to the speed.

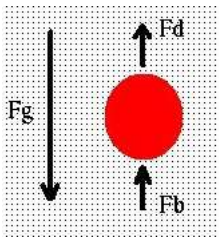
$$Re = \frac{v \cdot L}{\eta/\rho} = \frac{v \cdot L \cdot \rho}{\eta}$$

If we substitute this into the Eq. of Fd, then one can see that Fd at low speeds depends on the viscosity, and the diameter. (L is the characteristic length, in case of a sphere it is the diameter, but A also depends on L)



Double-log, and slope=-1

Fg : gravity force, Fd: drag force, Fb: buoyant force.



Newton-II. Law : ΣF = m·a
and
$\frac{\Delta v}{\Delta t} = a$

Fg = m·g
Fd = f · v (Re<0.1)
where f is the shape constant, embedding Cd.

From Archimedes's law we get: Fb = $\rho_{fluid} \cdot V_{particle}$, but $V_{particle} = m/\rho_{particle}$

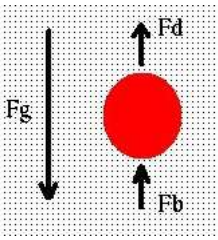
From the force equilibrium we get:
ΣF = 0, which means Fg-Fb-Fd = 0,
thus

$$f \cdot v = m \cdot g \cdot \left(1 - \frac{\rho_{fluid}}{\rho_{particle}}\right)$$

There is one problem with this:
If the particles are really small, then the Brownian motion will prohibit settling, and thus the method will not work.

Solution: Centrifuge!

Let's centrifuge the fluid+particles:



Fg : gravity force, Fd: drag force, Fb: buoyant force.

g = 9.8 m/s^2 would be the gravity force, but in the centrifuge
a = rω^2 is the acceleration felt by the particle. (ω is the angular velocity)

$$f \cdot v = m \cdot \boldsymbol{g} \cdot \left(1 - \frac{\rho_{fluid}}{\rho_{particle}}\right)$$

$$f \cdot v = m \cdot r \cdot \omega^2 \cdot \left(1 - \frac{\rho_{fluid}}{\rho_{particle}}\right)$$

We can rearrange such as:

$$S \equiv \frac{v}{r \cdot \omega^2} = \frac{m}{f} \cdot \left(1 - \frac{\rho_{fluid}}{\rho_{particle}}\right)$$

here S is the sedimentation coefficient. Unit is Svedberg, 1Sv = 10^{-13} s

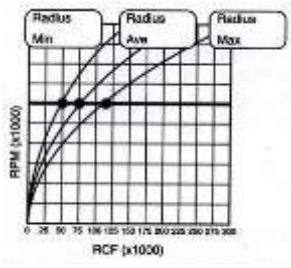
(Theodor Svedberg , Nobel prize 1926)

This shows, that mass and density paly a crucial role.
If the density is identical, then the bigger particle will sediment faster.

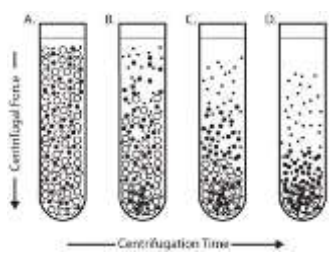
Useful equations

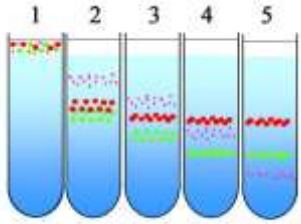
$\omega = 2\pi \left(\frac{rpm}{60}\right)$, rpm=revolutions per minute

RCF: relative centrifugal field
RCF = a = $r\omega^2 = 4\pi^2$ rpm^2/3600

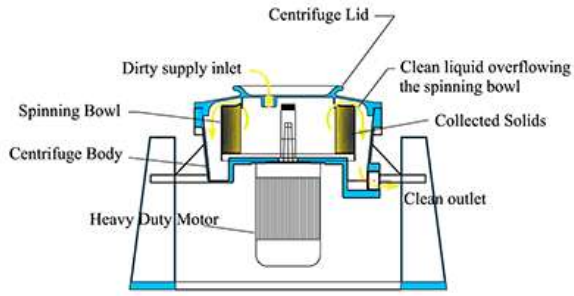


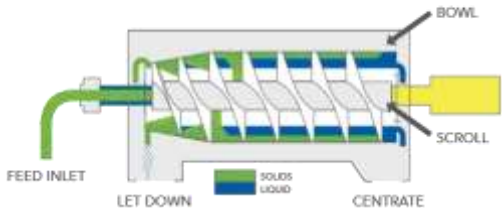
Since the terminal velocities are different, the particles segregate/separate by mass during the process

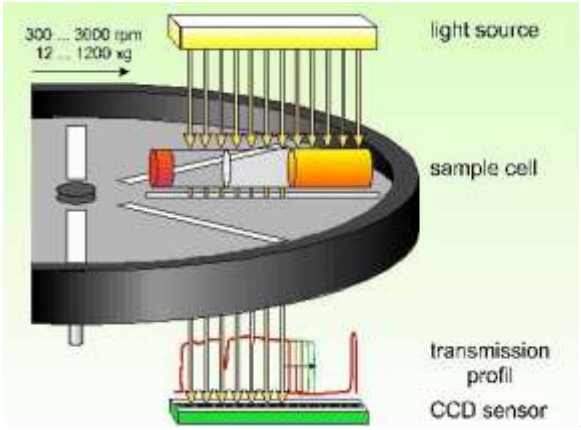




The centrifugal separator



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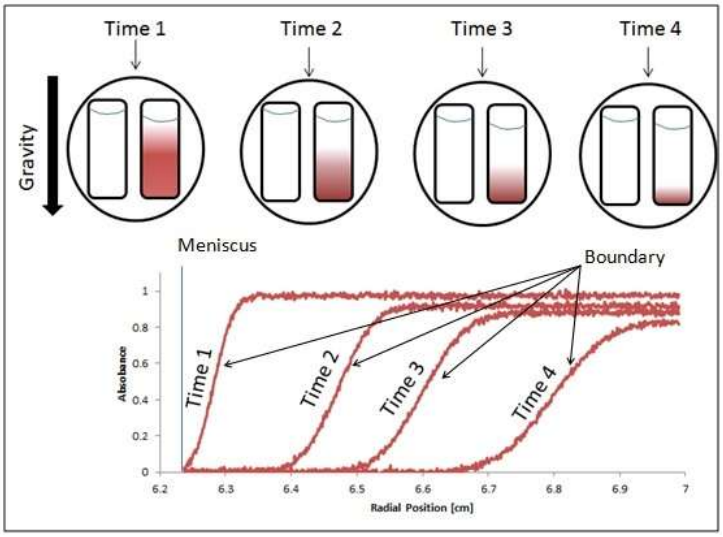
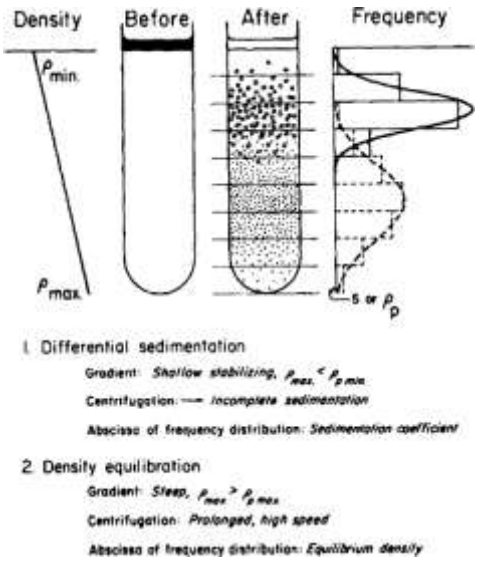

Time 1
Time 2
Time 3
Time 4
Gravity
Meniscus
Boundary
Absorbance
Radial Position [cm]

The only remaining unknown is the f: **form-factor**

But this is also in the diffusion:

$f = \frac{k\,T}{D}$ where D is the diffusion coefficient.

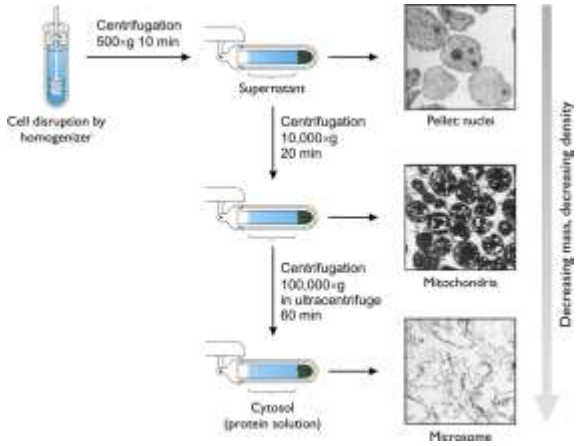
So we need to measure diffusion, in order to get the particle size.



It is possible to use a density gradient in the sedimentation medium.

In this case during centrifugation different density particles will stop settling at different positions.
This can be used also for separation:
Preparative or analytical ultracentifugation methods.

Differential centrifugation:
Separation based on the size of the particles.
This is not an equilibrium method.



Sedimentation equilibrium method

Here we wait, until the sedimentation and the Brownian motion reach an equilibrium.

This means, in equilibrium the net drag force is 0.

In thermal equilibrium, the Boltzmann distribution will describe the position of the particles in any force field:

$$\frac{n_1}{n_2} = e^{-\frac{\Delta E}{kT}}$$

In the energy term, we take into account the work of the forces.
If 1 and 2 denote distances r_1 and r_2 from the center of rotation, then

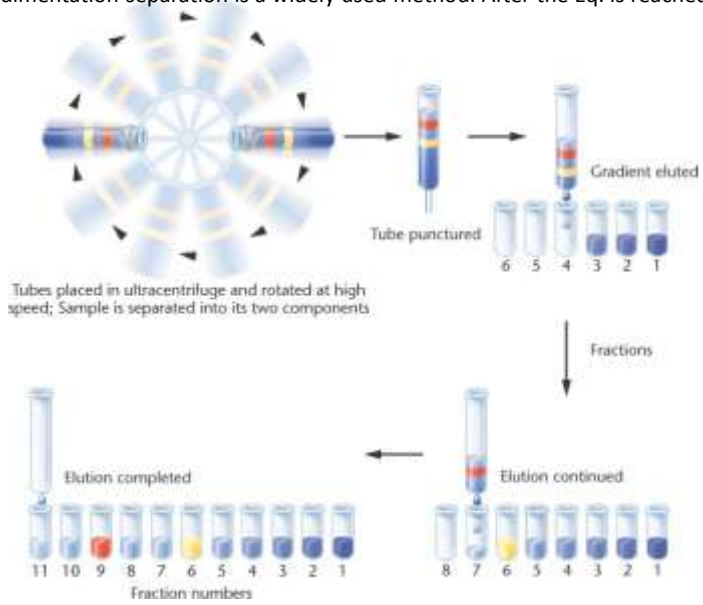
$$\Delta E = \frac{m}{2}\left({r_1}^2 - {r_2}^2\right)\omega^2\left(1 - \frac{\rho_{fluid}}{\rho_{particle}}\right)$$

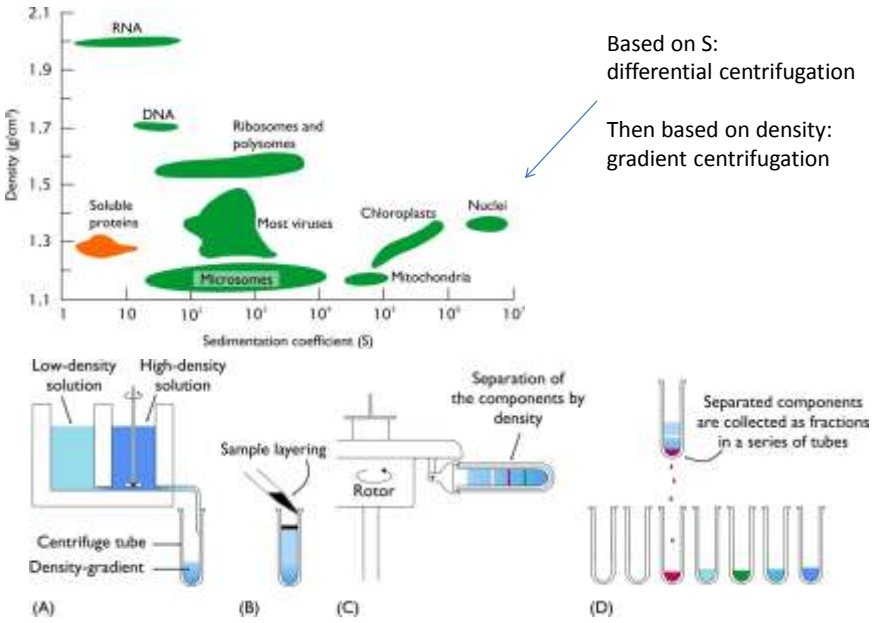
Substituting into the Boltzmann formula and taking the logarithm yields:

$$ln\left(\frac{n_1}{n_2}\right) = \frac{m}{2kT}({r_1}^2 - {r_2}^2)\omega^2\left(1 - \frac{\rho_{fluid}}{\rho_{particle}}\right)$$

We can measure the concentrations (n_1,n_2) the densities, and we know the radii, so the mass can be calculated.

Sedimentation separation is a widely used method. After the Eq. is reached:





Based on S:
differential centrifugation

Then based on density:
gradient centrifugation

Equilibrium separation method is based on the usage of a density-gradient

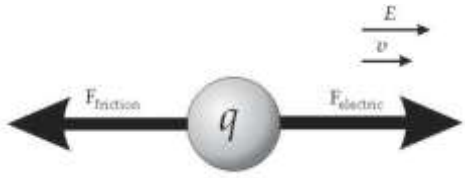
Electrophoretic methods

If a molecule is charged, and placed into an electric field, then a force will act on it.
This force (analogous to the sedimentation analysis) will cause a separation of the particles/molecules.
This is not an equilibrium method.

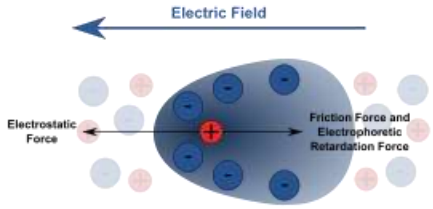
$$\mu_e = \frac{v}{E}$$

The electrophoretic mobility is defined by the velocity and the electric field creating that velocity.
This is specific for a given particle.

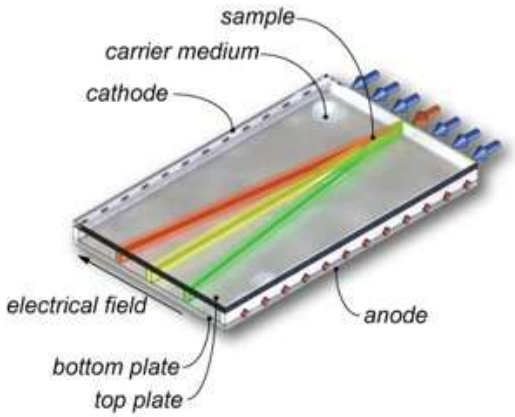
Forces in an uncharged solvent

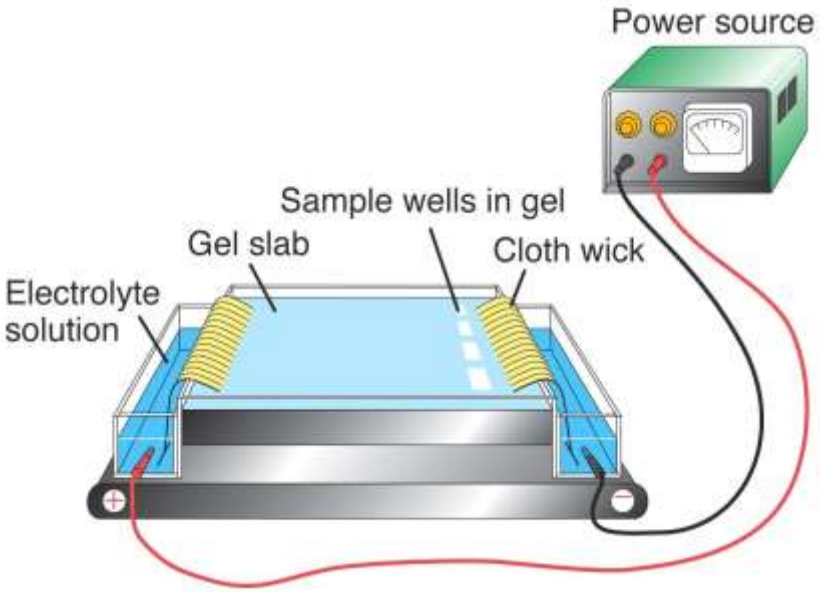


In a charged solvent there is retardation too:

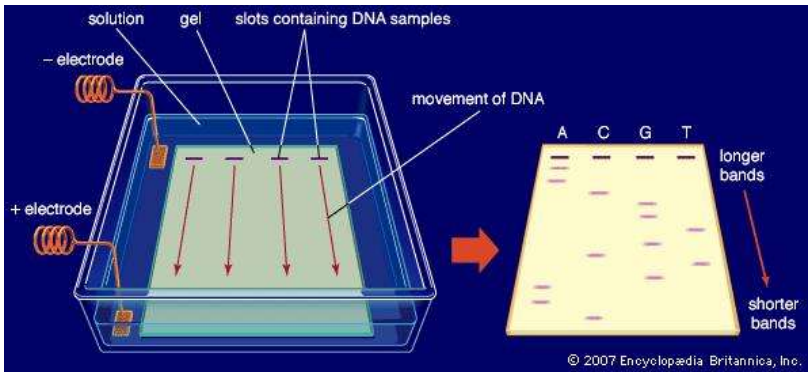


Free flow electrophoresis



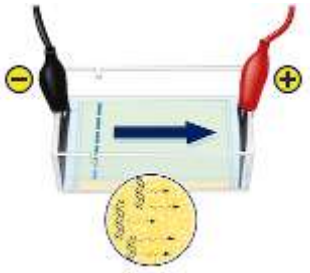


Gel electrophoresis

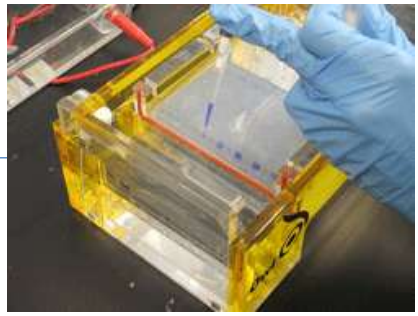


Gel electrophoresis

Run the gel



Load the gel



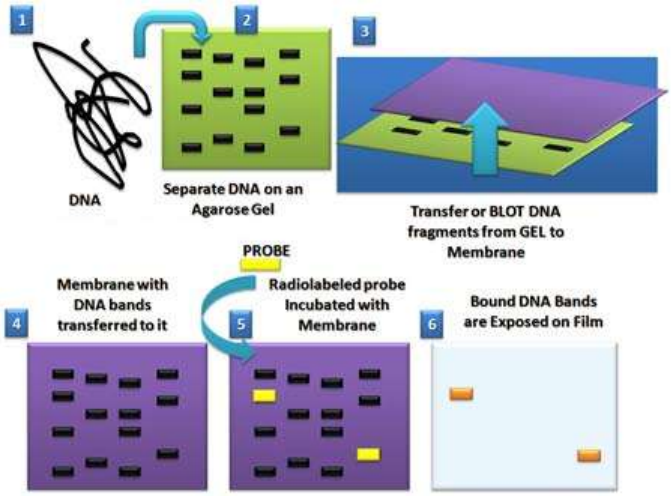
Label (here e.g. in-situ) and evaluate.

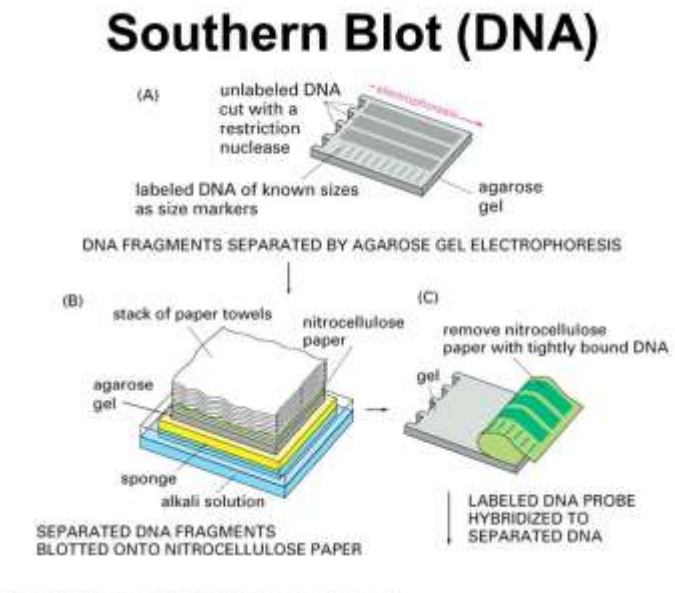
Labeling in a gel is not easy.

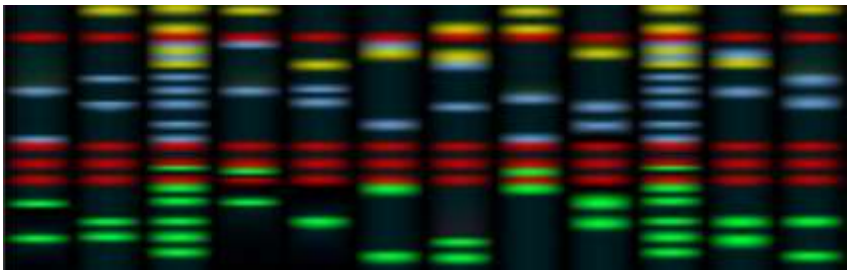
Blotting: one can transfer (and fix) the stripes on the gel
onto a vinyl, or other membrane.
Visualization by staining is then done on the membrane.

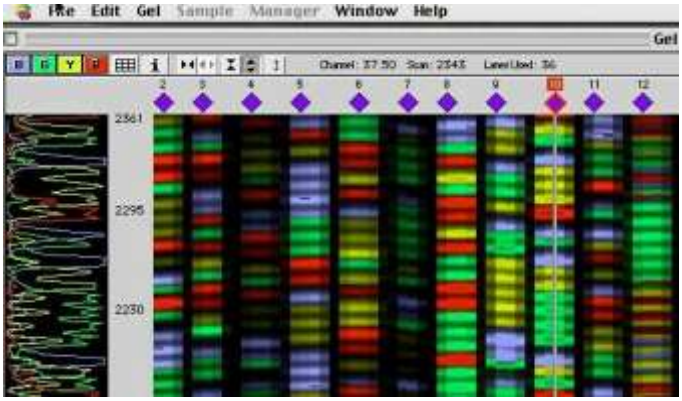
This enables the use of complicated chemical/biochemical reactions.

Southern blot (Edwin Southern)





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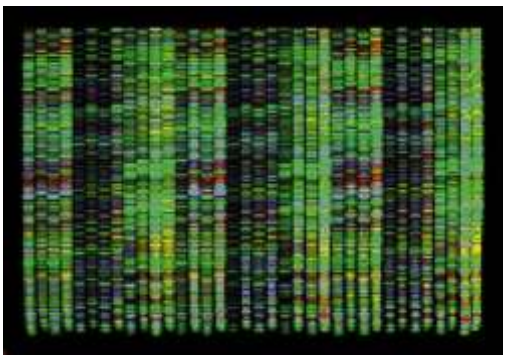

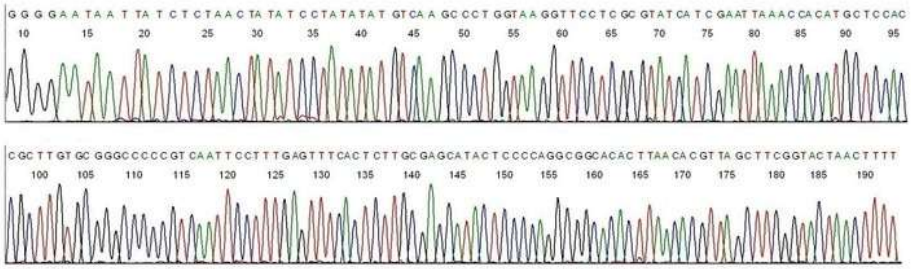

Borrelia burgdorferi CA382, complete genome
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Range 1: 445107 to 445291GenBankGraphics

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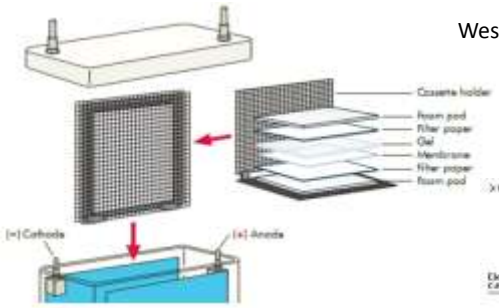
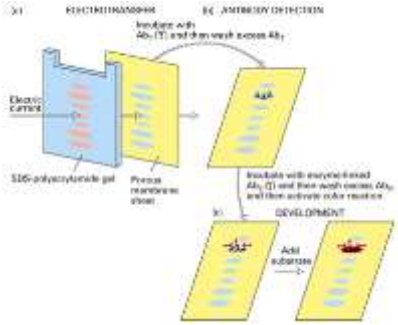
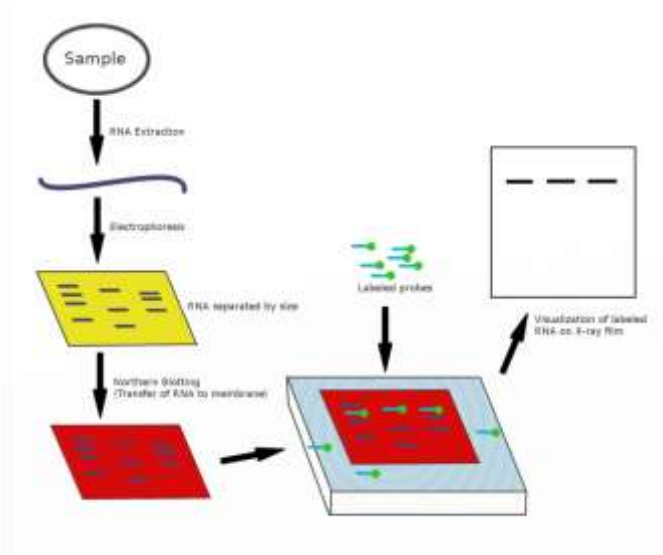
Western and Northern blotting: the names come from the play with the original name of the southern blot.

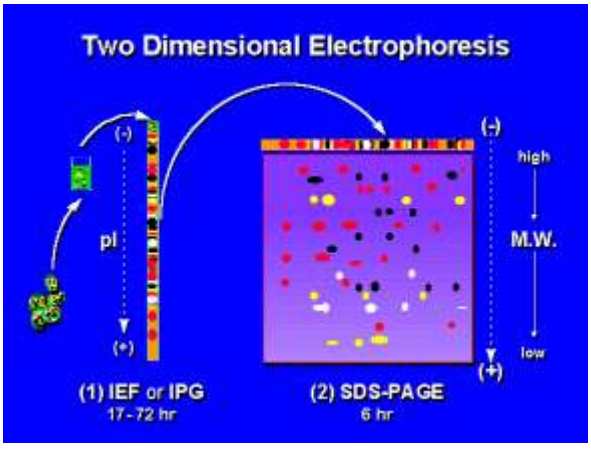


Western blot: protein detection



Northern blotting: detect RNA / expression levels

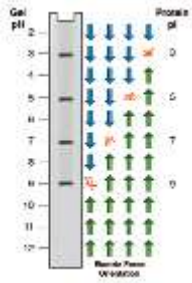


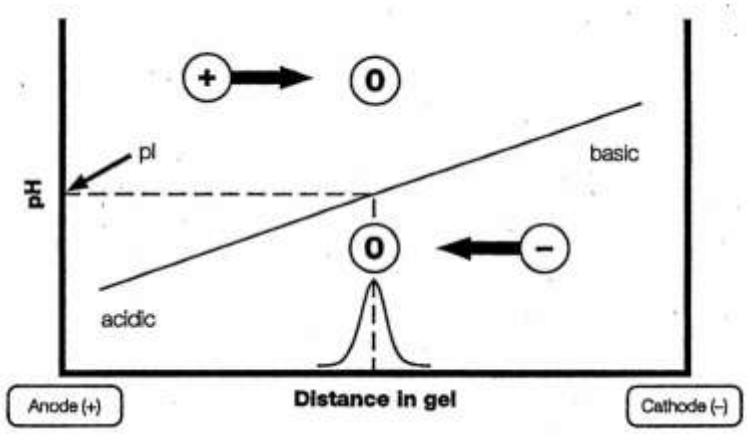


Isoelectric focusing

We use a gel, which has a pH gradient.
Due to the electrophoresis, the molecules will move towards the point in the gel, where the pH is equal to their isoelectric point.
At this point the molecules don't move any more, and are instead focused into sharp stripes.

The technique is capable of separating proteins differing in a single elementary charge.





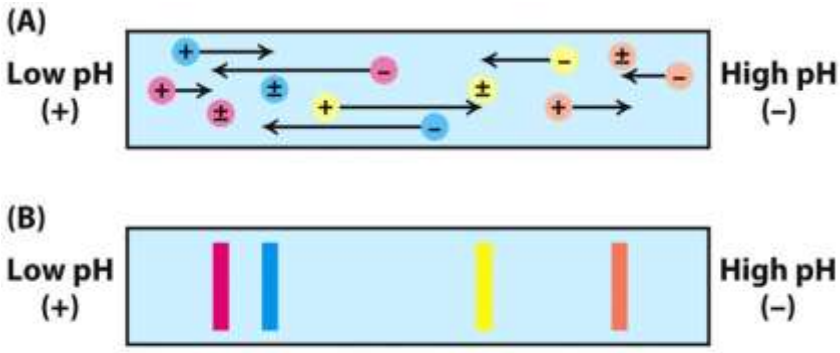


Figure 3.11
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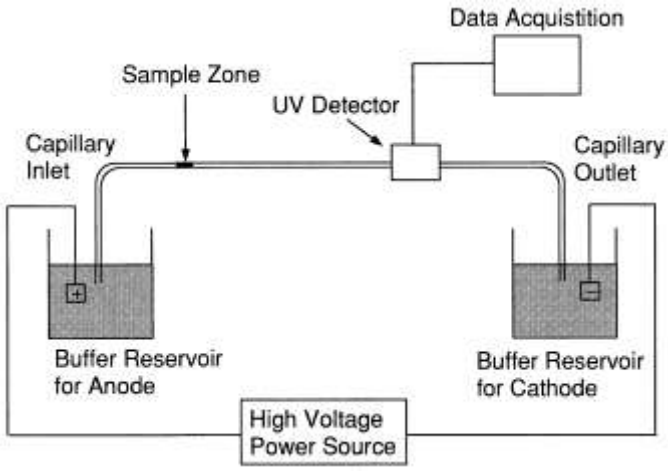
Capillary electrophoresis:
A chromatography technique, also useful for separation



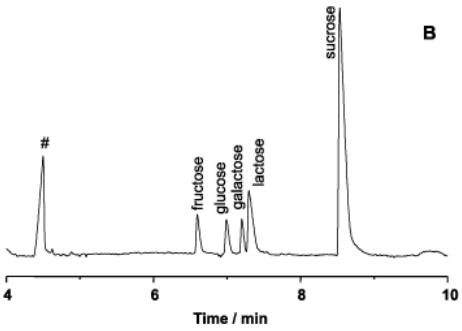


Figure 7. Nutritional Applications of Capillary Electrophoresis: analysis of carboxylic acids in wine (A) and carbohydrates in yogurt (B). (A) 10 mmol L^{-1} 3,5-dinitrobenzoic acid with 0.2 mmol L^{-1} CTAB, pH 3.6; 254 nm. (B) 15 mmol L^{-1} sorbate, 0.5 mmol L^{-1} CTAB and 35 mol L^{-1} NaOH; injection 3.4 kPa/15 s, 30 °C, –18 kV and 254 nm. (#) is a non-identified peak.

Automated parallel analysis