Sedimentation and electrophoresis methods

Schay G.

Physical basis of sedimentation methods

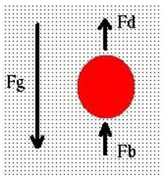
Goal: we would like to measure the mass of tiny particles

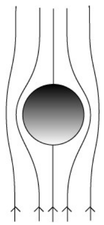
(this method originates long before the AFM or resonance methods, but is still in use)

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.

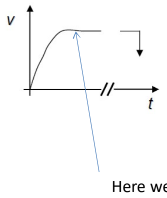
This is called **sedimentation**.





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

Drag: a force (Fd) acting on a moving object (usually in a fluid of given viscosity) , working against the movement. Fd ~ v,η,size



The particle will accelerate until the force equilibrium is reached.

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

Here we have the force equilibrium

Newton-II. Law : ΣF = m·a

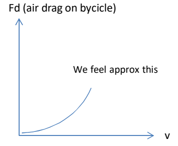
and

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

At force equilibrium a=0.

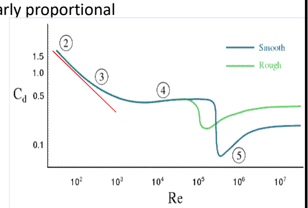
The drag force:

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**.

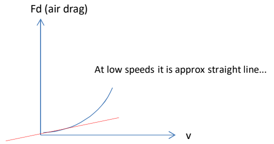


At low speeds Cd ~ 1/Re, which means Fd is linearly proportional to the speed.

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

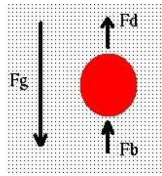


Double-log, and slope=-1



N.B.: Re is the Reynolds number

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 = g · ρ_{fluid} · $V_{particle}$, but $V_{particle}$ = m/$\rho_{particle}$
so Fb =m · g · $\frac{\rho_{flui}}{\rho_{particle}}$
From the force equilibrium we get:
ΣF = 0, which means Fg-Fb-Fd = 0,
(or Fd = Fg-Fb) thus

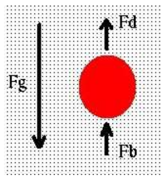
$$f \cdot v = m \cdot g \cdot \left(1 - \frac{\rho_{fluid}}{\rho_{particle}}\right)$$

V≠v (Volume, velocity)

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!

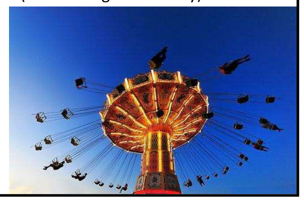
Let's centrifuge the fluid+particles:



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

g = 9.8 m/s² would be the gravity force, but in the centrifuge
a = rω² 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:

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

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

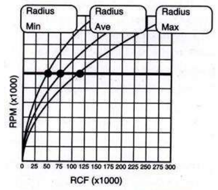
(Theodor Svedberg , Nobel prize 1926)

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

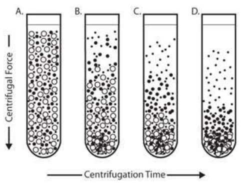
Useful equations

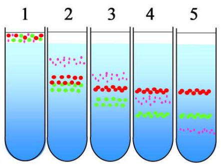
$\omega = 2\pi \left(\frac{rpm}{60}\right)$, rpm=revolutions per minute

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



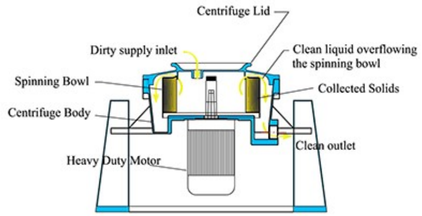
Since the terminal velocities are different, the particles segregate/separate by mass during the process



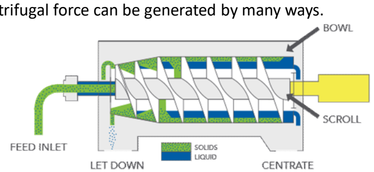


There is always an optimal centrifugation time!
Too short: no separation
Too long: every size reaches the bottom, also no separation.

The centrifugal separator



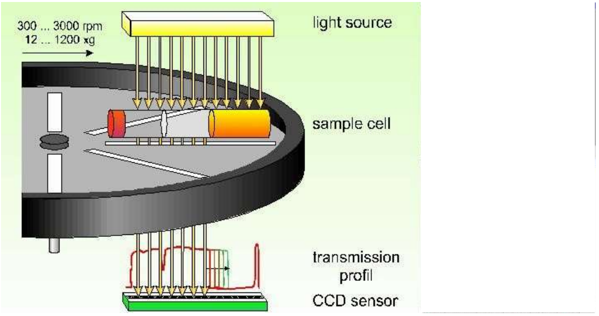
The centrifugal force can be generated by many ways.



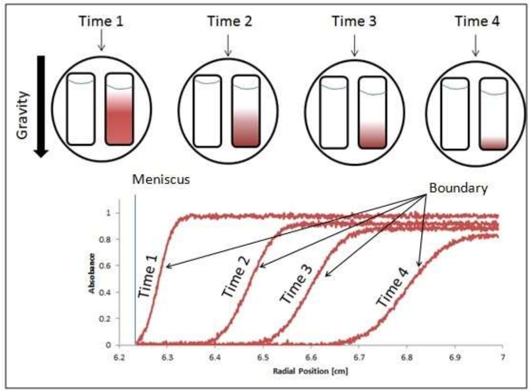
Rotating the mixture, or rotating the container, both, etc..

Everyday life:
Fruit centrifuge.

Quantitative methods
We want to measure during centrifugation



Centrifuging a mono-component system +fluid

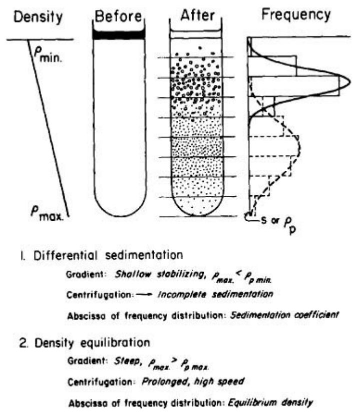


The only remaining unknown is the f: **form-factor**

But this is also in the diffusion:

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

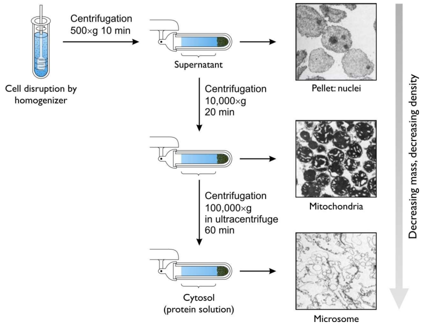
So we need to measure diffusion, in order to get the particle size.



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. (so there will be a concentration profile in the tube)
We spin with medium speed, so there is a sedimentation, but not a complete pellet formation

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

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

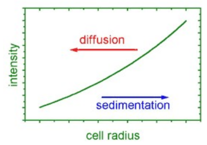
Extension material

Substituting into the Boltzmann formula and taking the logarithm yields:

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

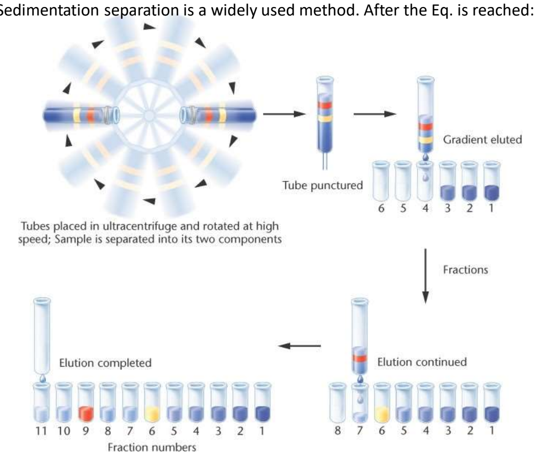
We can measure the concentrations (n_1,n_2) the densities, and we know the radii, so the mass can be calculated.

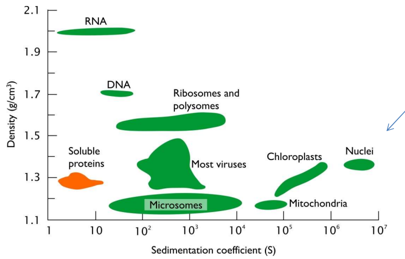
We do need the density, if that is unknown then at least 2 different solvents have to be used, so 2 independent equations can yield the 2 unknowns (m and density)



Extension material

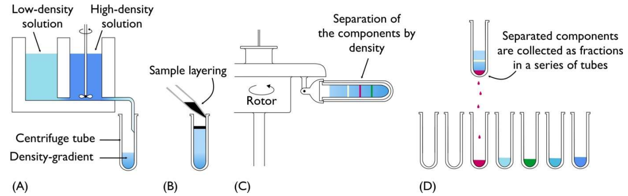
Sedimentation separation is a widely used method. After the Eq. is reached:





Based on S:
differential centrifugation

Then based on density:
gradient centrifugation



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

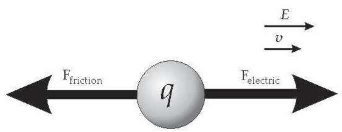
Electrophoretic methods

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.

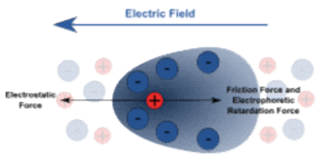
$$\mu_e = \frac{v}{E}$$

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

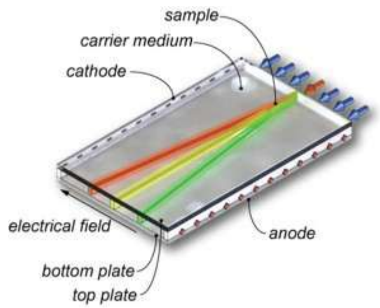
Forces in an uncharged solvent

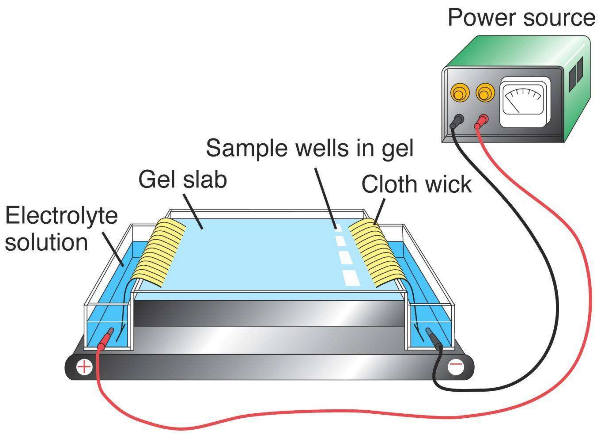


In a charged solvent there is retardation too:

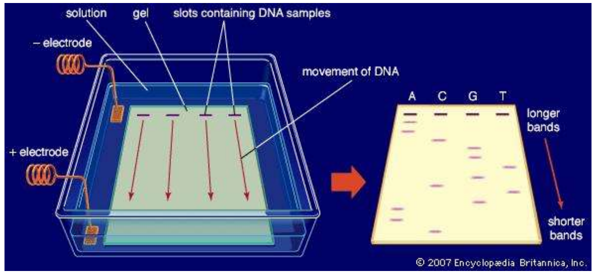


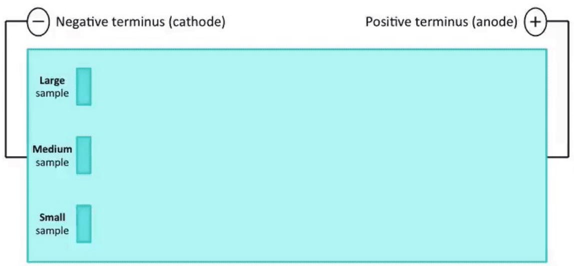
Free flow electrophoresis





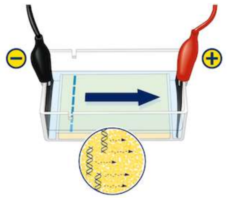
Gel electrophoresis



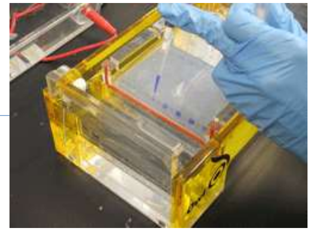


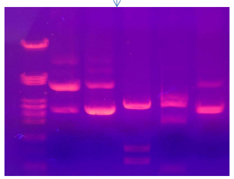
Gel electrophoresis

Run the gel



Load the gel





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

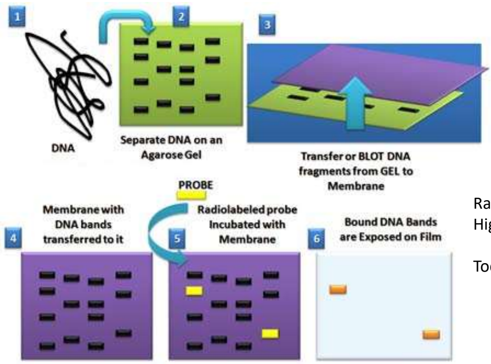
Labeling in a gel is not easy.

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.

Since the membrane has a higher density and viscosity than a gel,
the diffusion is much less, so during the chemistry the bands will not “smear”
as much.
Blotting is almost always done if the labeling takes considerable time (more than 1-2min)

Southern blot (Edwin Southern)

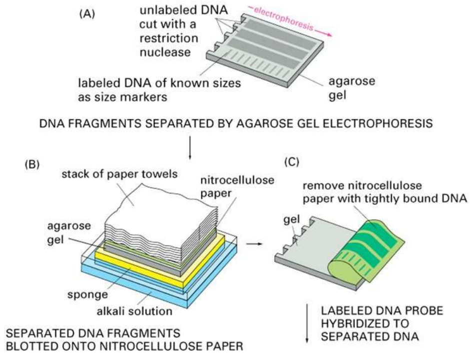


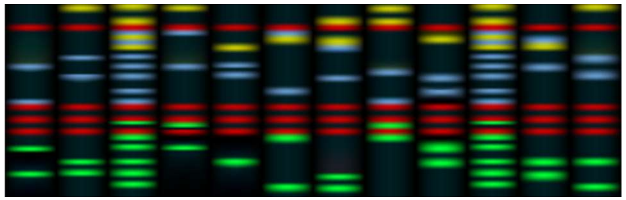
Radiolabeling:
High sensitivity!

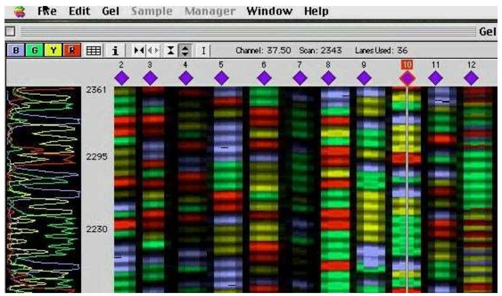
Today: fluorescence versions

Some blotting details…

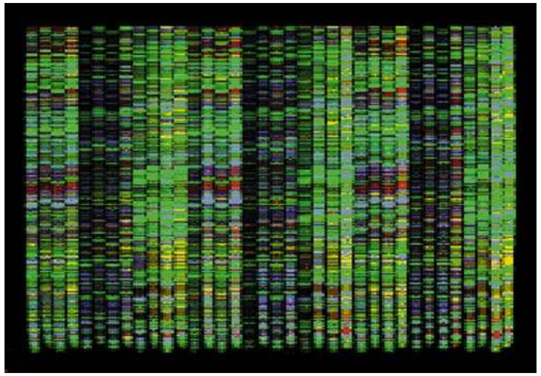
Southern Blot (DNA)





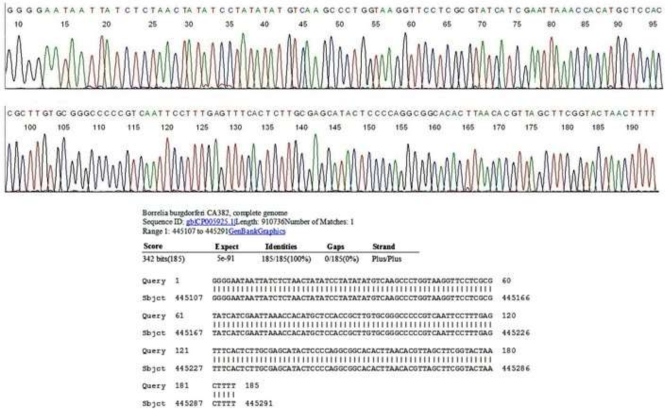


Fluorescence is much easier, and can be automated…



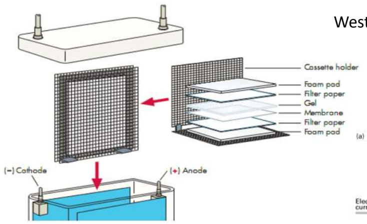
On a good quality gel a LOT of stripes can be differentiated...

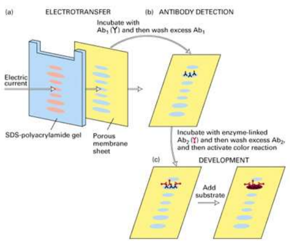
Used extensively in sequencing (getting the base sequence of DNA/RNA)



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

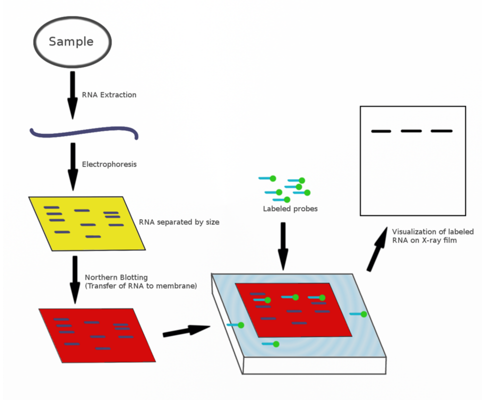
Western blot: protein detection

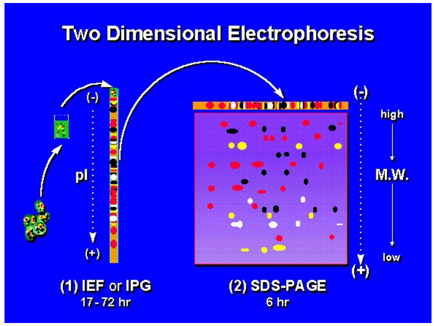




Proteins do not pair like DNA, but antibodies can be used for selective detection.

Northern blotting: detect RNA / expression levels



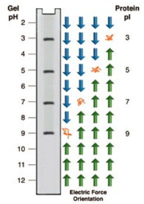


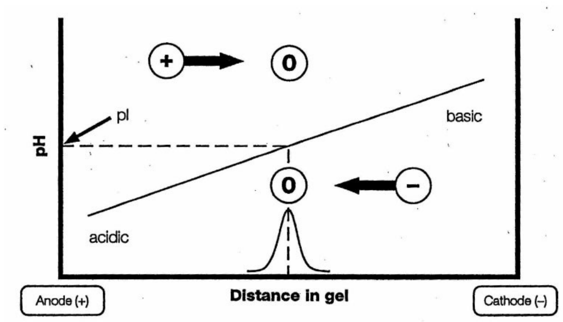
Here we make TWO runs, the second goes 90 deg to the first one.
The chemical/physical conditions are different

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.





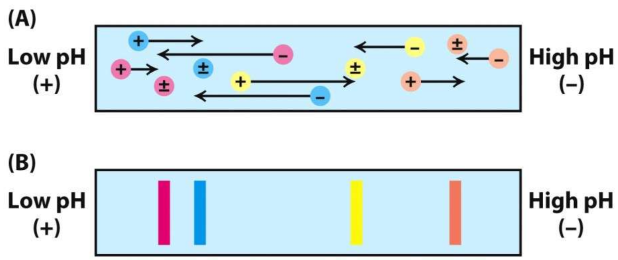
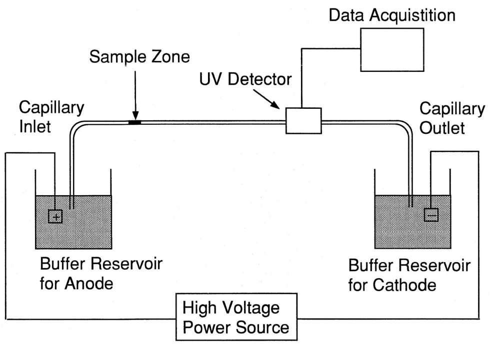
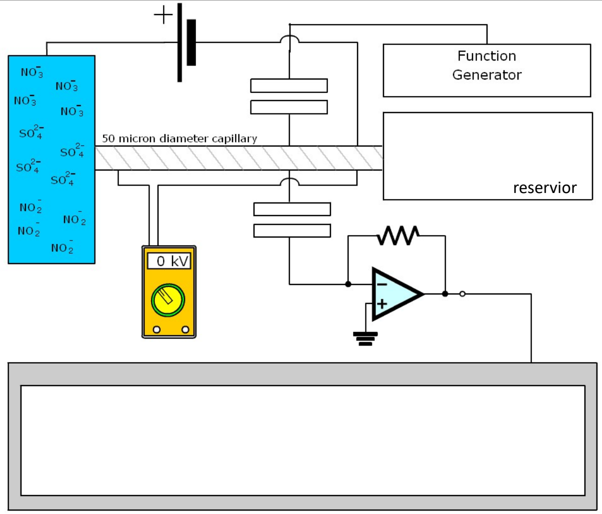
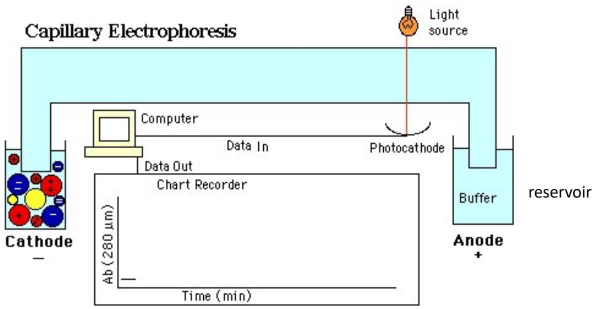


Figure 3.11
Biochemistry, Seventh Edition

Capillary electrophoresis:
A chromatography technique, also useful for separation







When pressure injection is used then all charges flow to the reservoir, but the flow rate is influenced by the size AND charge.

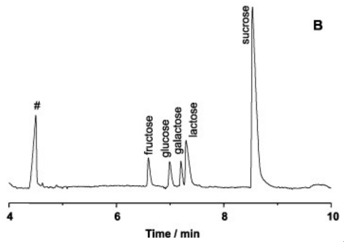


Figure 7. Nutritional Applications of Capillary Electrophoresis: analysis of carboxylic acids in wine (A) and carbohydrates in yogurt (B). (A) 10 mmol L^{-1} 3,5-dinitrobenzoic acid with 0.2 mmol L^{-1} CTAB, pH 3.6; 254 nm. (B) 15 mmol L^{-1} sorbate, 0.5 mmol L^{-1} CTAB and 35 mol L^{-1} NaOH; injection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

Automated parallel analysis