GEL ELECTROPHORESIS

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Abstract

Electrophoresis is the main metod for separating large polyelectrolyte molecules, primarily used on DNA, in biochemistry and medicine research. An overwiev of the models of physical mechanisms that are behind this phenomena will be presented. The prominent role is taken by reptation mechanisms, by which large flexible polyelectrolytes thread their way through the pores of the gel matrix. There are also other approaches that have their own advantages and disadvantages. Next to gel media, we also know different kind of electrophoresis technics, such as capilary electrophoresis or "electrophoresis on chips".

Contents

1 Gels

A polymer gel is a three-dimensional network of polymer chains joined together at a number of connection sites. The connections may be due to covalent chemical bonds or physical interactions such as hydrogen bonds or electrostatic forces.

Some ionic polymer gels have the ability to swell and shrink in an electric field. The volume change can be measured in few orders of magnitude. The process occurs in order to restore equilibrium between free energy (ionic forces, elastic energy, excluded volume...) and outer forces.[1] These properties are of great use in engineering field such as switches, memories, mass exchange devices – absorbing/desorbing materials,... If used in liquids, such behavior is accompanied by mass exchange between outer solution and gel interior.[2] These properties are presented more detailed in the seminar by colleague Jernej Mazej: "Fizika superabsorberskih gelov".

For studying charged particle transport under external fields we do not want any of the previously mentioned phenomena to occur. So we need gels without ionizable groups inside polymer network that are showing no drastic volume change in response to stimulation and are chemically inactive.[3] We also want uniform structure of the gel matrix with well defined pore size. Therefore non-ionic gels such as agarose (pore size 200-500 nm) and polyacrylamide (10-100 nm) have shown as most appropriate for separation of DNA and other protein molecules.[4]

2 Polyelectrolytes and electrophoresis

The term 'electrophoresis' originally referred to the migration of charged particles in electrical field. The alternative term 'ionophoresis' has been reserved for the migration of lower molecular weight substances in stabilized media such as gels and powders. Today the general term electrophoresis covers all applications regardless of the material being studied and medium being used.[5]

2.1 Electrophoresis and electroosmosis

If we put a charged object in a solution, it is screened by a diffusive layer of inverse charged solution. Using mean-field theory, the equilibrium distribution can be calculated using Debye-Huckel theory (linearized Poisson-Boltzmann equation). The fields are screened with exponentially decreasing potential $V_p(x) = V_0 e^{-\kappa x}$ with Debye screening length of

$$\kappa^{-1} = \left(\frac{\varepsilon\varepsilon_0 kT}{e^2 z^2 C}\right)^{1/2} \qquad ez, C - \text{charge and bulk concentration of ions.} \tag{1}$$

If the outer electrical field is present, it exerts a force on the object, but it also acts on the surrounding ions in Debye layer in the opposite direction.(Fig. 1) The calculations give simple results for two limits[4]. If Debye layer is larger than particle size $\left(\kappa^{-1} > R\right)$ the mobility (for sphere with charge $Q = 4\pi R^2 \sigma$) is

$$\mu = \frac{v}{E} = \frac{Q}{6\pi\eta R} = \frac{2R\sigma}{3\eta} \qquad \eta - \text{viscosity}, \sigma - \text{surface charge density.} \tag{2}$$

If $\kappa^{-1} \ll R$ the velocity becomes independent of particle size and shape $\mu = \frac{\varepsilon\varepsilon_0\xi}{\eta}$($\xi$ is surface potential). In the Debye-Huckel limit, the mobility is a function of the surface charge density.

$$\mu = \frac{\sigma}{4\pi\eta\kappa}. \tag{3}$$

The later case is the one of interest for biomolecule separation. We see that we can not perform electrophoretic separation by size in the free fluid media.

We can get another conclusion from the fact that this description of the particle movement is only relative to the surrounding fluid. Consider that the particle is not mobile (for instance glass wall in the water solution). The electric force will still act on counterions (in the opposite

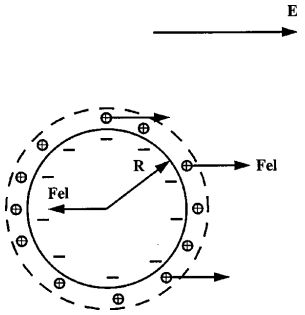

Figure 1: particle of radius $R > \kappa^{-1}$ during electrophoresis in an electrolyte.

direction as on the obstacle) which will move and drag the surrounding fluid with them. Flow of the fluid induced this way is called electroosmotic flow and can have drastic consequences on the performance of electrophoretic separation, so it has to be thoroughly controlled (for instance, by use of neutral gels,...).

2.2 Polyelectrolytes

Not all polymers contain electrical charged monomers. However, there are some polymers whose monomers may lose low molecular weight ions and become charged. Polymers of this sort are polyelectrolytes, and the ions breaking off are known as counterions.[6]

The simplest of polyelectrolytes are polyacrylic and polymethacrylic acids. When in solution in water, if an alkali is added, the monomers of these polymers can become negatively charged by dissociation of hydrogen ions. Biopolymers, such as DNA and proteins, also become polyelectrolytes when dissolved in water. DNA's chain has a large negative charge, whereas the monomers in proteins can be neutral or carry a positive and a negative charge. The last ones are therefore called polyampholytes.

Polymers most often separated by electrophoresis are DNA molecules and proteins. DNA is a perfect example as it occurs naturally in totally monodisperse charge distribution and in a huge range of sizes, from few base pairs (bp) to several hundreds of Mbp. Apart from DNA, RNA exists only in single stranded form. Because the aromatic parts of the nucleotides are rather hydrophobic, they tend to self hybridize and form secondary structures. By doing so they do no longer have the "smooth" chain conformation, and easily get entangled and trapped in the gel matrix. That leads to poor resolution. Same goes for single stranded DNA. For better results in these cases higher temperatures and special solvents are used. Proteins do not have such a uniform density of charge, which makes them less attractable for modelling. Oppose to the DNA they occur in nature as globular objects with many different acidic or alkaline groups attached to their tercirary structure. It is possible to destroy this structure by performing electrophoresis in the presence of strong surfactant (for instance sodium dodecyl sulfate), which transforms the protein into linear uniformly charged polyelectrolyte.[4]

2.2.1 Conformations of polymers

Consider a polyelectrolythe made of a sequence of monomers of typical size d. In practice they form persistent chains (persistent length $l_p \gg d$, l_p for DNA is about $30 - 60\,\mathrm{nm}$). So we can represent them as flexible cylinders of diameter d and contour length L. We introduce the Kuhn length $l = 2l_p$ as for the conformation of $L > l$, the chain behaves as a random walk of Kuhn

segments[4,6] with an average end-to-end distance R_N and a radius of gyration R_g related by[1,6]

$$R_N = \sqrt{6}R_g = lN_K^{1/2} = (lL)^{1/2} \quad (4)$$

$$R_g^2 = \frac{1}{N}\sum_{k=1}^{N}(r_{mean} - r_k)^2 = \frac{1}{6}l^2N \quad (5)$$

where we have introduced number of Kuhn segments $N_K = L/l$. For long chains excluded-volume interactions (two monomers cannot occupy the same position simultaneously) cause extra repulsion, which expands the chain further.

Until only small local nonelectrical forces act on each monomer (Brownian forces,...), that cause low velocities, the counterion cloud remains in quasistatic equilibrium, and behavior is the same as of a neutral polymer: the coil acts as an object of size R_g.

2.2.2 Polyelectrolytes in electrical field

Now we apply electrical field. In electrophoresis buffers, κ^{-1} is small so we are in regime of Eq. (3). From there we can derive similar expression for velocity

$$v = \frac{F_{el}}{\zeta} = \frac{QE}{\zeta}$$

where we need to estimate the friction of the polyelectrolyte ζ. As this is the regime of small velocities, i.e. low Reynolds numbers, the hydrodynamic equations can be linearized. So using this, we can superpose the flow of pure electrophoretic ($F_{ext} = 0$) and pure nonelectrophoretic ($E = 0$)cases. This way we merge the electrophoretic and electroosmotic effects. The mobility can be expressed by the balance of forces[4]

$$F_{ext} - \zeta\left(v - \mu_0 E\right) = 0 \quad (6)$$

where ζ is the friction of polyelectrolythe in the conformation it reaches in steady state. The evaluation of conformation of polyelectrolyte is simple in limit cases of very weak fields (small deformation) and strong fields (fully stretched chain). From this we can evaluate for instance the stall force ($v = 0$) for small fields as

$$F_{stall} = \zeta\mu_0 E \approx \eta R_g \mu_0 E. \quad (7)$$

What is interesting is that Eq. (6) does not explicitly use the charge of the polyelectrolyte. It actually represents the combination of the direct force exerted on the molecule and of the inert action of counterions.

3 Electrophoresis of globular particles

Early applications involved relatively small electrolytes, notably proteins in their native state. In such cases, it was found empirically that the ratio of the gel electrophoresis mobility μ to the free-solution mobility μ_0 (mobility without presence of gel, as calculated in Eq. (3)) was reasonably described as

$$\ln\left(\mu/\mu_0\right) = -K_r c, \quad (8)$$

where c is gel concentration and K_r (the retardation coefficient) is roughly proportional to the particle radius squared.[7] This approach was applied to the macromolecules, assuming that the particle radius in this case was the macromolecule radius of gyration R_g. While the approach worked well for smaller and coiled molecules, it failed to describe the behavior for large molecules.[1]

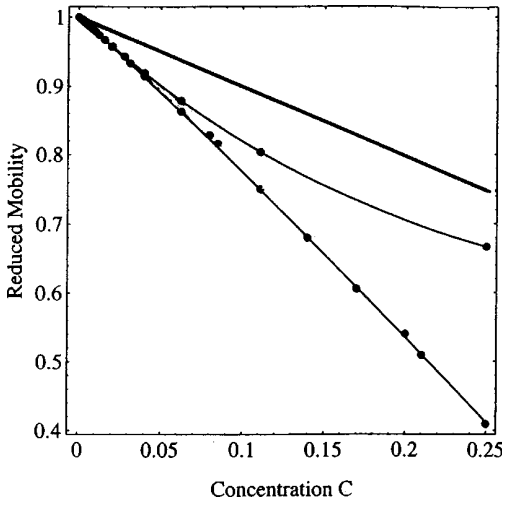

Figure 2: Reduced mobility vs obstacle concentration, as predicted in the Slater-Guo lattice model. Curves represent, from top to bottom, the free-volume model, the periodic gel, and the random gel.

3.1 Free-Volume model

In this straightforward approach the gel is assumed to act as a sieve with a distribution of pore sizes, and the separation is viewed as a kind of electric-field-driven filtration. The modeling is made on assumption that the ratio of mobility μ/μ_0 is the fractional volume (f) available to the particle in the gel[4]

$$\frac{\mu}{\mu_0} = f. \tag{9}$$

If we represent the gel and the moving particle by simple geometrical objects, we can calculate f exactly by the canonical statistics of intersections between the particle and gel components distributed randomly. If we consider our system as particles with excluded area S in a suspension of long fibres of radius r with total length j per unit volume[8], excluded volume of the system (per unit volume) is jS. So the fractional volume is expressed as

$$f = \exp\left(-jS\right). \tag{10}$$

For spheres with radius R, $S = \pi\left(2R + r\right)^2$. This expression combined with Eq. (8) lead to $K_r = c\left(R + r\right)^2$. The model can be expanded for particles in more complicated suspensions, of combination of fiber, planar and pointlike objects.[9] In this model, optimal separation is expected when the particle size is comparable with the average pore size of the gel.

3.2 Slater-Guo model

The two authors proposed a simple square lattice (2D) model[10,11] in which key parameters such as gel concentration, available free volume, pore size,... are trivial, and dynamic parameters such as diffusion coefficient or electrophoretic mobility can be calculated exactly. Obstacles in the lattice can be distributed periodically or randomly and can occupy one or more lattice sites. Moving particles are also inscribed on one or multiple sites in the lattice, and hard-core excluded-volume interactions are applied. Jump probabilities (in the absence of obstacles) for the directions perpendicular or parallel to the field are about 25%, but slightly changed due to field effect. If we consider field pointing in x direction we get jump probabilities

$$P\left(\pm y\right) = \frac{1}{4}, \quad P\left(\pm x\right) = \frac{1/2}{1 + \exp\left(\mp 2\varepsilon_r\right)} \approx \frac{1 \pm \varepsilon_r}{4} + O\left(\varepsilon_r^2\right) \tag{11}$$

where $\varepsilon_r = \frac{QEa}{2kT}$ is reduced electric field, Q is particle charge and a lattice period. It also has to be mentioned that the average jump time is affected by the field[4]. Jumps to sites occupied

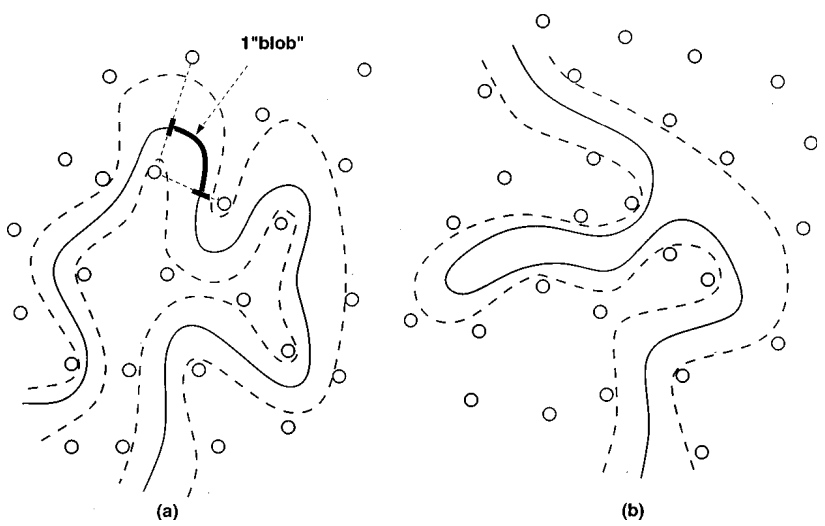

Figure 3: Schematic representation of the chain (solid line) and its "tube" (dotted lines) in the gel (bold circles). This particular figure corresponds to a rather stiff chain; (a) chain without a loop or hernia; (b) chain with a loop or hernia.

by obstacles are forbidden. Expansion of the mobility in the concentration of obstacles leads to solutions of the form[4]

$$\mu/\mu_0 = 1 - (\pi - 1)\, c + a_2 c^2 + ..., \tag{12}$$

where the linear term is independent, while a_2 depends on crystallographic arrangement of obstacles (periodical lattices) or on degree of randomness (random lattices). This is not the lattice equivalent of free-volume model prediction (Eq. (9))

$$\mu/\mu_0 = 1 - c + ... \tag{13}$$

The free-volume model always overestimates the mobility (Fig. (2)). Actually it corresponds to 'annealed' gel (in which obstacles would be moving rapidly as compared with the particles). More detailed model (more parameters, extended to 3D lattices) was also proposed later, but the concept remains similar.

4 Electrophoresis of non-globular particles

Previous description (Eq. (8)) proposes smaller mobility for large DNA molecules (large R_g), but we do not see this behavior, especially in high fields, where molecules change their globular unperturbed conformation.

4.1 Reptation model

The motion of long molecules can be considered as snakelike along the virtual tube, created by obstacles. Assume that the gel is rigid and composed of uniform pores. The section of a chain in one pore is called a blob. The molecule can leave the tube either by sliding motion and creation of new tube parts (blobs) on the ends, or by the creation of loops or "hernias".[1,4,6] Fig. (3)

4.1.1 Reptation in a field: biased reptation model (BRM)

When an electric field is applied (let's say in x direction), all blobs are subjected to the electric force. For a tube not fully aligned to the field direction, the field tends to push the polyelectrolyte against the gel fibers and to "pull" hernias out of the tube, against the entropic barriers discussed

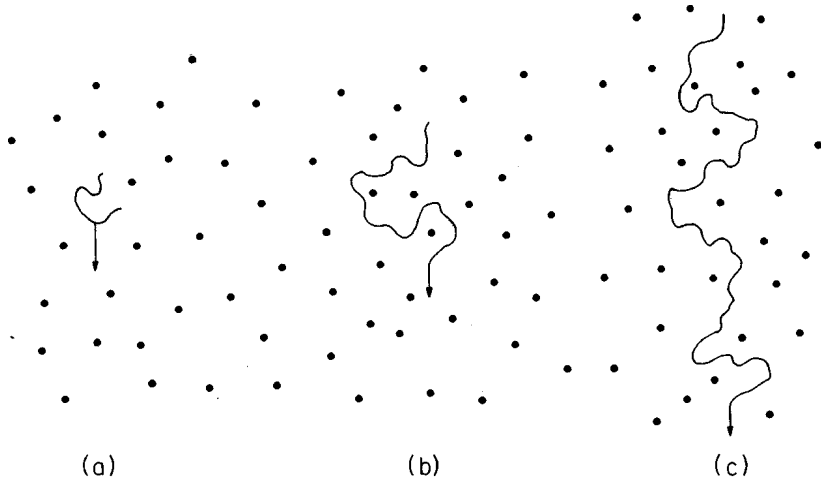

Figure 4: Different regimes of migration in constant-field electrophoresis: (a) Ogston sieving; (b) reptation without orientation; (c) reptation with orientation.

above. Qualitatively, one expects that this tube leakage will remain improbable as long as the external force is smaller than the entropic (thermal) force. Assuming that the polyelectrolyte velocity in the gel is small compared to the free-flow velocity, one can use the stall force in Eq. (7) as a good approximation for evaluating electrohydrodynamic forces on the blob. (hydrodynamic interactions over distances larger than b (blob size) are screened by the gel, so b is the relevant scale for the application of Eq. (7). Therefore the condition of weak external force can be expressed as[4]

$$\varepsilon = \frac{\eta b^2 \mu_0 E}{kT} \ll 1, \tag{14}$$

where ε is the ratio to the thermal energy, of the electrostatic potential energy associated with the displacement of one blob by a distance equal to its size. For the DNA in the 1% agarose $\varepsilon = 1$ occurs at about $10\,\mathrm{V/cm}$. To get rid of the pore size, we get another parameter, dependent of the Kuhn Length

$$\varepsilon_k = \frac{\eta l^2 \mu_0 E}{kT} = \varepsilon \left(\frac{l}{b}\right)^2. \tag{15}$$

In this simplest model, we are only considering average molecule orientation. The nonrandom force is the therefore sum of electrohydrodynamic forces on all blobs, projected onto the tube axis,

$$F_{eff} = \mu_0 \eta R_x, \tag{16}$$

where R_x is the projection of the end to end vector onto the field direction. The instantaneous velocity in the field direction can be derived as[4]

$$v_x = \mu_0 E \left(\frac{R_x}{Nb}\right)^2. \tag{17}$$

We see that the key dimensionless parameter is $\frac{R_x}{Nb}$, which we introduce as molecule orientation $H^2 = \left\langle R_x^2/N^2b^2 \right\rangle = \left\langle \cos^2\theta \right\rangle$, this is ratio of end-to-end vector to size of all occupied blobs. The description based on the assumption that the average molecule orientation was given by the force balance (Brownian - electrical) on the scale of one blob was suitable for experiments, when proposed, but not correct for flexible chains at scaling level.

4.1.2 Biased reptation with fluctuations (BRF)

For flexible chains the "direct" orientation term used in the biased reptation model is dominated by another term, coupling between local chain orientation and fast fluctuations in the position of

the end of the chain. In other words, internal modes can not be ignored. When taking all this into account, we get a more complex model which gives computational results for mobility in the two limits[4]

$$\frac{\mu}{\mu_0} = \begin{cases} 1/3\ N, & N\varepsilon < 1 \\ H^2 \approx 0.5\varepsilon, & N\varepsilon > 1 \end{cases} \tag{18}$$

from which an interpolation formula was proposed, very close to Eq. (18)

$$\frac{\mu}{\mu_0} = \left[(3N)^{-2} + (2\varepsilon/5)^2\right]^{1/2} \tag{19}$$

The crossover from unoriented reptation ($N < N^*$) to oriented fractionation ($N > N^*$) occurs at $N^* = \left(\frac{5}{6}\right)\varepsilon^{-1}$. The model should also predict that for $\varepsilon > 1$ mobility should saturate (it can not exceed free mobility) and become field independent, so it is introduced phenomenologically

$$\frac{\mu}{\mu_0} = \left[(3N)^{-2} + \left(\frac{2\varepsilon}{5 + 2\alpha\varepsilon}\right)^2\right]^{1/2}, \tag{20}$$

where α is the limiting ratio of free mobility to in-gel mobility for large gels and large fields. It is consistently found (experiments and simulations) to be $\alpha \approx 3$. Since BRF is based on a molecular approach, it can be rewritten in molecule parameters

$$\frac{\mu}{\mu_0} = \left[\left(\left(\frac{b}{l}\right)^2 \frac{1}{3N_k}\right)^2 + \left(\frac{2\varepsilon_k\,(b/l)^2}{5 + 2\alpha\varepsilon_k\,(b/l)^2}\right)^2\right]^{1/2}. \tag{21}$$

So if electrophoresis works well for smaller molecules, it becomes inefficient in case of huge molecules, all with the same mobility. So we need to find some other mechanism for their separation.

4.1.3 Pulsed-field electrophoresis

Even if the long chains have the same velocity, that is only after some time when they reach field oriented state. This orientation, however, is only progressively built up by reptation after the onset of the field. Therefore, a chain initially oriented at random starts its motion at a size-dependent velocity and reaches its steady state, size-independent, velocity only after a time of the order of the reptation time. Since the reptation time is itself size dependent, by pulsing the field at a given frequency, one can discriminate between "long" chains that cannot reach the steady-state velocity during one period and "short" ones that travel most of the time at the steady-state velocity.[4,12,13] (Fig. (5))

By increasing by about two orders of magnitude the size of DNA molecules that could be separated in gel electrophoresis, pulsed-field electrophoresis revolutionized molecular genetics and enabled genetic mapping, having a resolution of several Mbp (Mega base-pairs), and detailed analyses of small DNA fragments, down to the ultimate single base resolution necessary.

Pulsed-field electrophoresis methods can be classified into three "families":

- Crossed-field gel electrophoresis in which at least two field orientations are used. Most often two homogeneous electric fields at a fixed angle, generated by a series of electrodes clamped to regulated potentials, are used. We can also change the orientation of the field by rotating the electrodes or the sample.

- Field-inversion gel electrophoresis methods, in which the field is alternately applied in the two directions along a single orientation. One can distinguish unbiased field-inversion electrophoresis, in which the field amplitude is the same in the two directions (but the durations of the forward and backward pulses are different, to create a nonzero average field), and biased field-inversion electrophoresis, in which the forward and backward pulses have different amplitudes [a particular case of the latter is "Zero-Integrated Field" or ZIFE, in which the product of pulse time by field amplitude just compensates between the forward and the backward pulses.].

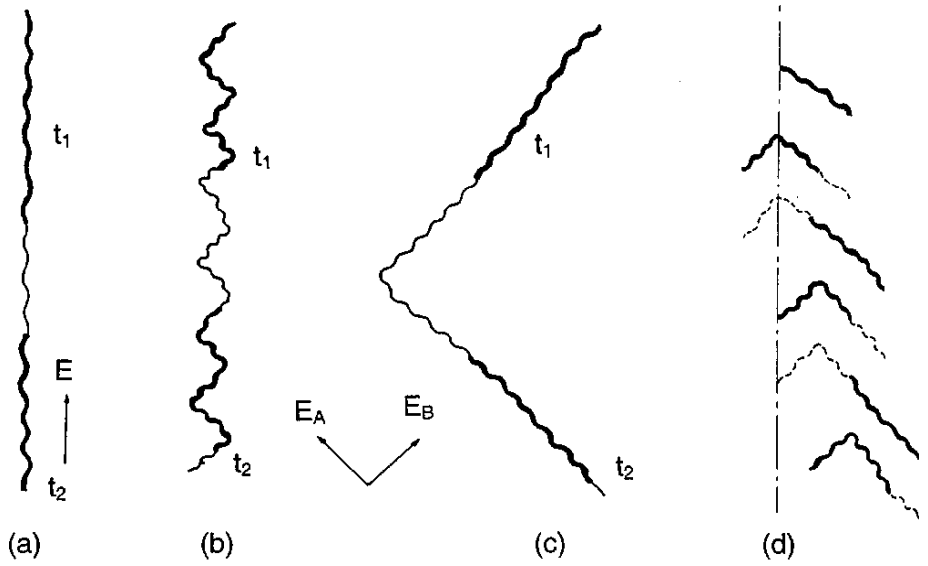

Figure 5: Different regimes of migration in crossed fields. Bold lines represent chain conformations at different times t_1 and t_2 , while the fine lines represent the tube. (a) Permanent field; (b) short-pulse regime ($t_{or} > t_p$), for field angles up to 90°. The chain cannot reorient fully during one pulse; (c) long-pulse regime ($t_{or} < t_p$), showing the path as a sequence of fully oriented sections; (d) Ratchet migration mechanism for crossed fields at obtuse angles, when head/tail exchange occurs at the end of each pulse. Subfigures from top to bottom alternatively represent the position of the chain at the end of pulses pulling to the right, then at the end of the next pulse to the left, etc. Dashed lines are guides for the eye, representing the position at earlier stages. Straight dot-dashed line represents the direction of average field.

- Intermittent-field methods in which the field is just interrupted periodically.

In cross fields, the separation power decreases abruptly at angles below 90°. If we solve BRM or BRF model equations in time dependent regime we get orientation time $t_{or} \propto NE^{-1}H^{-1}$.

Let us consider crossed fields, with the same amplitude E, at an angle ϕ that are alternately applied during a time t_p to a mixture of DNA molecules. The smaller chains with $t_{or} \ll t_p$ rapidly reach steady-state mobility at the beginning of each pulse: they spend most of the duration of pulse oriented, and migrate at the steady-state, constant-field velocity v_c. The macroscopic velocity along the diagonal x is obtained by projecting the zigzag path (and considering Eq. (17)):

$$v\left(t_{or} \ll t_p\right) = v_c\left(E\right)\ \cos\left(\phi/2\right) \approx \mu_0 E\ H^2\left(E\right)\ \cos\left(\phi/2\right). \tag{22}$$

Longer chains do not have the time to reorient completely during the pulse, so their behavior is sensitive to the effective field

$$v\left(t_{or} \gg t_p\right) = v_c\left(E\cos\left(\phi/2\right)\right)\ \cos\left(\phi/2\right) \approx \mu_0 E\ H^2\left(E\cos\left(\phi/2\right)\right)\ \cos\left(\phi/2\right). \tag{23}$$

There are some effects though that BRM and BRF can not explain, for instance both predict that pulsed field effects should vanish for field inversion (no reorientating). Also presence of orientation overshoots and hernias were observed but not predicted. Thus new theoretical models for separation mechanisms were proposed.

4.2 Other separation mechanisms

4.2.1 Migration in high fields

Biased reptation was only sufficient at fields up to the range of $\varepsilon \sim 1$. This is the order of most applications except of pulse-field electrophoresis separation on Mbp DNA. In this regime the tube

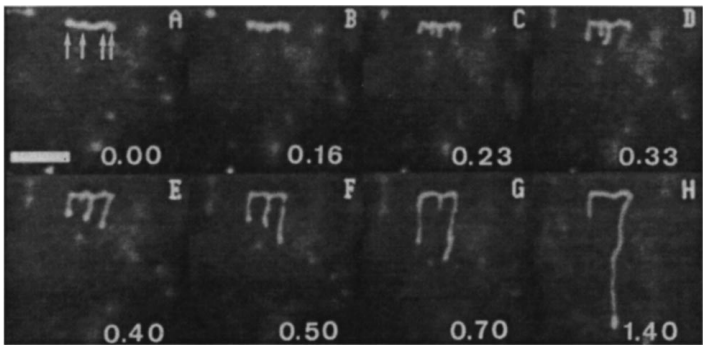

Figure 6: Evolution of a T2 molecule (160 kb) subjected to an electric field in the vertical direction starting at time 0, after having been aligned in the horizontal direction. Bar = 54 microns. The molecule does not move as a whole, reflecting the existence of topological (tube) constraints. However, several loops along the path start to grow in (C). The number of loops progressively decreases, at the same time as the length of the loops increases. Finally, the chain ends up in a U shape (G), before becoming fully aligned in the field direction.

constrains might be violated. The approaches were mostly of computer modelling and due to high number of free parameters, they are mostly restricted to 2D environments (although the same physics should apply). Let's just mention 2 of them, Lakes-straits model[14,15] and Repton model[16]. What they have in common is that the molecule is thought as a chain of internal nodes (lakes) catched in pores, and the only dynamics interesting is the exchange of short length of chain dl between the lakes under influence of entropic free energy and outer field.

For constant field mobility the predictions of BRF were recovered. But this models also explained effects, BRF was unable to. Under continuous field tubelength fluctuations and U-shape conformations with lots of hernias are observed. In pulsed fields, the reduced mobility occurs when the pulse time is close to the orientation time. This occurs due to field cycles during which the molecule makes no progress. During the pulse the chain extends in a field direction to form U shaped configuration, with lots of hernias pointing in field directions, but not do not have the time to pull free into a fully oriented tube. Hernias play the most important role. Each one of them represents a local head of the molecule (as seen in Fig. (6)). And what makes the long relaxation time is the rope-over-a-pulley process, when larger Hernias feed on the smaller ones, until one of them eventually wins.

4.2.2 Entropic trapping

All different kind of process is seen in very small fields, although it was overlooked for long time, as such small fields do not have experimental use for polymer separation. The physical basis of this phenomenon is actually very simple. When a flexible polymer is located in a random environment with a distribution of pore sizes and an average pore size comparable to the polymer radius of gyration or somewhat smaller, there is competition between distribution of the chain length among several pores (the formation of a "tube", responsible for reptation) and squeezing of the whole chain into a single pore. In the latter process, of course, the entropy loss will be minimum in the largest pores, and generally there is a range of polymer sizes for which a finite fraction of the pore-size distribution is able to "house" the whole chain at an entropy cost smaller than that of a tube conformation (and also, obviously, smaller than in other smaller pores). In general, these larger pores are localized, so their centers constitute local minima for the part of the free energy that is of entropic origin (hence their name "entropic traps").

4.3 Other methods of electrophoresis

Apart of gel electrophoresis, we also know other methods. One is capillary electrophoresis. The separation is performed in a narrow channel (usually silica capillary with diameter $50 - 100\mu m$ and a length between $10 - 50cm$). If we try to use gel media we encounter numerous problems as clogging, gel breakage and bubble formation. But there is a possibility of using fluid media that are impossible to use in macroscopic format. Anyway most of the separation physics has to be reinvented for capillary electrophoresis. The first advantages are excellent heat dissipation and though reduction of convection, allowing use of high fields ($100 - 1000V/cm$). The method also has its problems, for instance wall-analyte interaction, differences in path travelled by different analytes due to capillary bending, and of course wall-induced electroosmosis. For silica capillaries at neutral or alkaline pH, walls are negatively charged, and the direction of electroosmosis is opposite to the mobility of DNA. Since the electroosmotic mobility is generally larger than that of electrophoresis, analytes migrate from the cathode to the anode, and the order of migration of particles is reversed (those with the lowest electrophoretic mobility migrate fastest). This can be an advantage or a disadvantage, depending on the application.

A very exciting method is "electrophoresis on chips" [17]. We can transport the analyte through the microchannels comprising custom-made arrays of obstacles, engraved in silicon. This microfabricated arrays can also be seen as ideal 2D "artificial gels". It offers lots of possibilities to study migration mechanisms in gels due to controlled environment and simpler structure. Indeed the observations shoved great matching and conformation of mechanisms applying to migration in gels. It was even shoved that if field is strong enough ($\varepsilon > 1$), and the channels are aligned to the field direction, the lateral loops do not have the time to form and the chain moves straight along one channel and is only weakly stretched. The behavior under these conditions resembles closely to the predictions of the simple biased reptation model! In pulsed fields too this system mimic biased reputation predictions of zigzag movement at acute angles, and a "ratchet" migration with head-tail reversal at obtuse angles. This indicates of great possible improvements under these conditions; large pores allow fast migration; less conformation fluctuations, from what one can expect more uniform velocities and less band broadening; as there is little chain stretching, consequentially less chain trapping and/or breakage it should allow much faster separation of large DNA.

5 Conclusions

As electrophoresis is an important tool in many sciences, it also got attention from physicists in explaining the mechanisms behind it. Many of the observed phenomena (in this seminar only few of them were presented), especially for uniform gels with pore sizes larger than Kuhn length and smaller than radius of gyration, has been at least qualitatively understood through models, although none of the models gives us the whole picture by itself. This is due to number of degrees of freedom and therefore numerical complexity. In addition to the difficulties inherent in the electrohydrodynamics of saline solutions, one has to deal with polymers having numerous coupled degrees of freedom. A further complication lies in the appearance not only of a large range of characteristic scales, but also of a large range of energies. During gel electrophoresis, polyelectrolytes are generally very far from thermodynamic equilibrium so that perturbation approaches are tricky to use. Also Brownian motion still plays an essential role in local processes coupled to the large-scale dynamics, so that purely deterministic solutions are insufficient. With methods for direct observation on molecular scale (such as videomicroscopy) the models can be directly tested for their accuracy. One of the main aspects that was all the time ignored by the local force picture are hydrodynamic interactions. They are screened by the gel, so their influence in gel might not be so important, but the local picture change if we want to understand separations in fluid media, for instance capillary electrophoresis.

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