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Free-flow electrophoresis with trapping by a transverse inhomogeneous field

(separation/DNA/polyelectrolytes)

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ABSTRACT **We propose an electrophoretic technique combining the use of a series of dielectric traps controlled by an ac electric field and conventional continuous field free-flow electrophoresis. From the theoretical model that we describe, one can expect, for DNA electrophoresis, an improvement of one to two orders of magnitude in the selectivities or experimental durations.**

The separation of biological macromolecules is a crucial problem in areas such as the pharmaceutical industry and biomolecular engineering. As an important example, human genome mapping requires the separation of large DNA molecules according to their sizes—from a few kilobase pairs (kbp) to a few tens of megabase pairs (Mbp). This is not possible by simple free-flow electrophoresis in aqueous solutions; indeed, electrophoretic mobility is independent of the molecular weight for long chains (1).

As an alternative, gel electrophoresis techniques were developed. Although it was soon recognized that continuous field devices have severe limitations (molecules of sizes larger than ≈50 kbp display the same mobility), pulsed-field methods allow for the separation, over a 1-day period, of DNA molecules of a few megabase pairs (2, 3). More recently (4), attaching a globular protein to one end of the chain has resulted in a spectacular increase in the accuracy of the separation for chains of intermediate size; the mobility decreases exponentially with the molecular weight in certain conditions.

However, free-flow electrophoresis can also be used with the addition of selective traps. Confinement effects, for example, could lead to separation (5). Here we propose the use of well-controlled geometries with transverse oscillating electric fields. The large paraelectric susceptibilities of polyelectrolytes lead to trapping phenomena in the strong field areas. The trapping time depends exponentially on the polarizability of the chains, which depends sharply on their length: we therefore expect good selectivity.

More precisely, we develop a model that describes the macromolecular drift and diffusion in a periodic structure, calculate the conditions for separation, and propose orders of magnitude considerations that demonstrate the efficiency and the feasibility of the technique.

The polyelectrolyte is considered as an undeformable charged object, which means that we neglect any conformational consideration. Its evolution in a force field $\mathbf{F}(\mathbf{x})$ is that of a Brownian point particle and is governed by a Fokker–Planck equation:

$$\frac{\partial P}{\partial t}(\mathbf{x}, t) = -\text{div}\, \mathbf{J}(\mathbf{x}, t)$$

$$\mathbf{J}(\mathbf{x}, t) = \frac{D^\circ}{kT} P(\mathbf{x}, t)\, \mathbf{F}(\mathbf{x}, t) - D^\circ\, \mathbf{grad}\, P(\mathbf{x}, t), \qquad [1]$$

where $P(\mathbf{x}, t)$ is the probability density of the particle at point $\mathbf{x}$ and time t.

D° is the coefficient characteristic of Brownian diffusion, which also applies for sedimentation and for all situations where the force field is of nonelectrical origin (6, 7). $\mathbf{F}$ is the force acting on the backbone plus counterion system, linked to the average velocity through Einstein's relation: $\mathbf{V} = D^\circ\mathbf{F}/kT$. When the force is due to an electric field $\mathbf{E}$, this defines a proportionality coefficient q between $\mathbf{F}$ and $\mathbf{E}$. The electrophoretic mobility μ (such that $\mathbf{V} = \mu\mathbf{E}$) verifies

$$\mu = \frac{qD^\circ}{kT}. \qquad [2]$$

It is important to point out that Brownian diffusion of a polyelectrolyte is similar to that of a hydrodynamically opaque sphere, because of the hydrodynamic interaction between the monomers (6–8). Therefore $D^\circ \simeq 6\pi\eta R_H$, with η the solvent viscosity and $R_H \simeq R(1 - \varepsilon)$, where R is the gyration radius of the macromolecule and ε is an usually small dimensionless factor accounting for interactions between the backbone and the counterions (7–10).

Electrophoretic motion is somewhat different: the counterions are systematically driven in a direction opposite to that of the backbone, and, because of their friction with the solvent, the whole polyelectrolyte becomes hydrodynamically transparent to the flow (6). The friction coefficient of the backbone is then (free-draining regime) proportional to its number of elements and therefore to its molecular weight (similar to neutral polymers in the Rouse description). The electric force acting on the backbone is also proportional to the molecular weight if it is homogeneously charged (which is the case for DNA), so the resulting mobility and the product qD° is size independent (for molecules large enough so that such scaling considerations are relevant) (1).

Finally we characterize the polarizability of the molecule in an electric field of frequency ω by a coefficient $\alpha(\omega)$ (we here neglect absorption and consider that α is real). In the presence of a field $\mathbf{E}^*(\omega) = \mathbf{E}^* \sqrt{2}\cos(\omega t)$, the polarization energy of the polyelectrolyte reads

$$\Delta W = \frac{1}{2}\alpha(\mathbf{E}^*)^2. \qquad [3]$$

To this energy corresponds a force $\mathbf{f} = -\mathbf{grad}(\Delta W)$, which drives the particle toward the regions of strongest field amplitude.

We now use this model to describe the one-dimensional motion of a molecule dragged by an electric field $\mathbf{E}$ and trapped in some places by an inhomogeneous orthogonal field, which oscillates at a frequency high enough not to

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substantially alter the direction of drift. We study here the case of a network where regions (of size a), in which an orthogonal field $\mathbf{E}^*$ has been established, alternate with regions (of size $\xi - a$) where no such field exists. The motion of the particle is then ruled by Eq. **1**, where the force $\mathbf{F}$ derives from the potential U depicted in Fig. 1. We consider only the regime in which particles, before crossing the trapping barriers, accumulate on distances very short compared to the size of the traps (a) and the periodicity of the network (ξ). With the notation $d = kT/qE$, this regime reads $d << a < \xi$ (regime 1). In such situations, the drift is strong enough that the particles visit the traps successively, and backwards motion can be omitted.

We thus have to consider the following formal problem: a Brownian particle under constant drift is trapped at periodic intervals (of size ξ) in identical traps. We call $\Psi(t)$ the probability distribution for the escape time t out of one of these traps and $\Phi(t')$ the probability distribution for the drift time t' from one trap to the next. Then that of the time to cross a region of length $L = N\,\xi$ is $P_L(t)$:

$$P_L(t) = \int \left(\prod_1^N dt_i\, dt_i'\, \Psi(t_i)\, \Phi(t_i') \right) \delta\left(t - \sum_1^N (t_i + t_i') \right). \quad [4]$$

This problem is most easily solved by considering the Laplace transforms of the probability distributions, defined as

$$\tilde{f}(E) = \int_0^\infty e^{-Et} f(t)\, dt. \quad [5]$$

Then, if P_L° is the value of P_L in the absence of traps (or $\mathbf{E}^* = \mathbf{0}$), one can transform Eq. **4** into

$$\tilde{P}_L(E) = (\tilde{\Psi}(E)\, \tilde{\Phi}(E))^N = (\tilde{\Psi}(E))^N\, \tilde{P}_L^\circ(E). \quad [6]$$

The moments of a distribution law $f(t)$ are given by

$$\langle t^n \rangle = (-1)^n \left. \frac{\partial^n \tilde{f}}{\partial E^n} \right|_{E=0}. \quad [7]$$

From Eq. **6** we then obtain the traversal time for a sample of size L and its variance S_L:

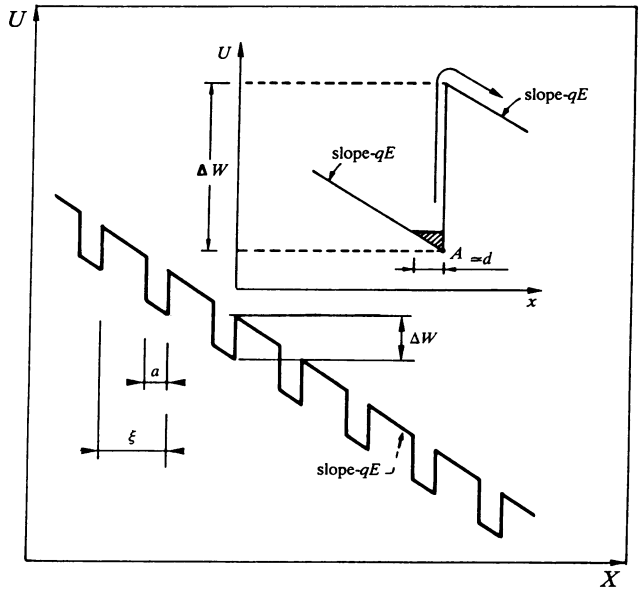

FIG. 1. Potential experienced by a molecule while drifting through the network. (*Inset*) Enlarged view of a trap and barrier.

$$\langle t \rangle = \frac{L}{\xi} \langle t_p \rangle + \langle t \rangle^\circ \quad [8]$$

and

$$S_L = \langle t^2 \rangle - \langle t \rangle^2 = \frac{L}{\xi} (\langle t_p^2 \rangle - \langle t_p \rangle^2) + S_L^\circ, \quad [9]$$

where $^\circ$ refers to the value in the absence of traps (free-flow electrophoresis value) and t_p refers to the escape time of a single trap. Calling V and D (respectively, V° and D°) the mean velocity and the diffusion coefficient in the presence (or in the absence) of traps and recalling that $S_L\, V^3 = 2\, D\, L$ for times long enough for the central limit theorem to apply (11), we get

$$V = \left\{ \frac{\langle t_p \rangle}{\xi} + \frac{1}{V^\circ} \right\}^{-1} = V^\circ \left\{ \frac{V^\circ \langle t_p \rangle}{\xi} + 1 \right\}^{-1} \quad [10]$$

and

$$D = V^3 \left\{ \frac{\langle t_p^2 \rangle - \langle t_p \rangle^2}{2\xi} \right\} + D^\circ \left(\frac{V}{V^\circ} \right)^3. \quad [11]$$

In both formulas, the first term of the right-hand side corresponds to trapping and predominates if natural diffusion is fast. Indeed, in the second term of Eq. **11**, if trapping is very efficient, V is much smaller than V°, and natural diffusion is no longer substantially responsible for the dispersion of similar particles: these are in quasi-permanence in a trap and do not have time to disperse while drifting to the following site.

We must now characterize the traps (Fig. 1 *Inset*) and $\langle t_p \rangle$ and $\langle t_p^2 \rangle$. If the energy barrier ΔW is somewhat larger than kT, the flux J of particles emerging from the trap can be obtained from a simplified version of Kramers model (see, for example, ref. 12) and reads $J = n/\tau$, where n is the number of particles in the trap (noninteracting and equivalent) and τ is given by

$$D^\circ \tau = \int_{\text{barrier}} e^{U/kT}\, dx \int_{\text{trap}} e^{-U/kT}\, dx. \quad [12]$$

In our case,

$$\int_{\text{trap}} e^{-U/kT}\, dx = \frac{kT}{qE}\, e^{-U_A/kT}$$

$$\int_{\text{barrier}} e^{-U/kT}\, dx = \frac{kT}{qE}\, e^{U_A/kT}\, e^{\Delta W/kT},$$

where U_A is the potential at the deepest point of the trap A, as depicted in the inset of Fig. 1.

Thus,

$$\tau = (D^\circ)^{-1} \left(\frac{kT}{qE} \right)^2 \exp(\Delta W/kT). \quad [13]$$

As explained, this result holds when $\Delta W >$ a few kT (regime 2). The number of particles varies according to

$$\frac{dn}{dt} = -J = -\frac{n}{\tau},$$

which leads to an exponential law for the escape time and

$$\langle t_p \rangle = \tau, \langle t_p^2 \rangle = 2\tau^2.$$

Thus, calling t° the mean drift time from a trap to the next one ($t^\circ = \xi/V^\circ$), we get from Eqs. **2**, **10**, and **11**

$$V = \frac{\xi}{\tau + t^\circ} \quad [14]$$

and

$$D = D^\circ \left\{ 1 + \frac{\xi}{2d} \left(\frac{\tau}{t^\circ} \right)^2 \right\} \left\{ 1 + \frac{\tau}{t^\circ} \right\}^{-3}. \quad [15]$$

According to Eqs. **13** and **14**, V is a function of the physical parameters μ, D°, and α. Consequently molecules characterized by different parameters will be separated. This holds even if they have the same electrophoretic mobility (and thus the same t°). This case, which as previously stated corresponds to large size DNA separation, is considered in the following. We describe the other physical parameters by scaling laws, as powers of a curvilinear size N of the polyelectrolyte chain (e.g., the number of base pairs): $\alpha \simeq N^\gamma$ and $D^\circ \simeq N^{-\nu}$. We also call P the dimensionless factor $\Delta W/kT$, which varies in the same way as α ($\simeq N^\gamma$). Then the variation of the trapping time $\tau = V^{\circ -1}\, d \exp(P)$ with N reads

$$\frac{d\tau}{\tau} = [-\nu + \gamma P] \frac{dN}{N}, \quad [16]$$

which leads to velocity variation

$$dV = \frac{V^2 \tau (\nu - \gamma P)}{\xi} \frac{dN}{N}. \quad [17]$$

This means that, on the average, after a drift distance x, molecules of sizes differing by δN will be a distance δx apart

$$\frac{\delta x}{x} = \left[1 + \frac{t^\circ}{\tau} \right]^{-1} [\nu - \gamma P] \frac{\delta N}{N}. \quad [18]$$

One achieves effective separation if δx is larger than the spread Δx due to dispersion: $(\Delta x)^2 = 2\,Dt = 2\,Dx/V$. Δx increases as the square root of the drift distance, whereas δx increases linearly. The separation therefore occurs after a distance x^* (and a time t^*) corresponding to $\delta x^* \cong \Delta x^*$, or

$$x^* \cong 2\,D/V \left[1 + \frac{t^\circ}{\tau} \right]^2 |\nu - \gamma P|^{-2} \left(\frac{\delta N}{N} \right)^{-2}. \quad [19]$$

To simplify the algebra, we consider the regime where dispersion is mainly the result of trapping: $(\tau/t^\circ)^2 \xi/d >> 1$ (regime 3). Note that this regime allows trapping and free-flow electrophoresis to contribute in comparable ways to the mean velocity (τ and t° of the same order) since $\xi >> d$ (regime 1). Then

$$x^* = \xi |\nu - \gamma P|^{-2} \left(\frac{\delta N}{N} \right)^{-2}, \quad [20]$$

$$t^* = \frac{x^*}{\xi} (\tau + t^\circ), \quad [21]$$

and

$$\delta x^* = (\xi x^*)^{1/2} \left[1 + \frac{t^\circ}{\tau} \right]^{-1}. \quad [22]$$

These formulas allow us to quantify the efficiency of the separation. To get a rough estimate, we use simple considerations to derive the orders of magnitude of the different parameters for the case of large DNA fragments. We call L the chain length, N its number of base pairs, p the persistence length (we shall here take a value corresponding to usual ionic strengths), and R the radius of the corresponding coil (estimated using Gaussian statistics). The diffusion coefficient D° is then approximated by that of a sphere of radius R and the polarizability by that of a spherical ionic cloud of the same dimensions (8) in a medium of relative permittivity ε of order 100. For the electrophoretic mobility we shall use the value experimentally obtained in ref. 1. Along these lines, we obtain the following:

chain length $L = 0.34\ N$ (nm)
radius $R^2 = 1/3\ p\ L \simeq 5\ N$ (nm^2)
diffusion coefficient $D^\circ = kT/\eta R = 2 \times 10^{-9}\ N^{-1/2}$ (m^2·s^{-1})
polarizability $\alpha = 4\pi\ \varepsilon\ R^3 \simeq 10^{-34}\ N^{3/2}$ (C·V^{-1}·m^{-2})
mobility $\mu = 2 \times 10^{-8}$ m^2·V^{-1}·s^{-1}
and $\gamma = 3/2$, $\nu = 1/2$, and $P = \Delta W/kT \simeq 10^{-10}\ N^{3/2}(E^*)^2$,

where E^* is expressed in V per cm. Note that in the present picture, Eq. **2** together with the independence of the electrophoretic mobility μ on N requires that the proportionality coefficient q scales as N^ν.

Current microelectronic technologies allow for the realization of geometries of very thin spatial resolution (a few thousand angströms). To illustrate our proposal, we choose an easily achievable periodicity: $\xi = 0.1$ mm.

We use this set of values to estimate the strength of fields and the time and the space necessary to separate chains of various sizes and with variable accuracy. A few results are gathered in Table 1 and Fig. 2. They indicate that with reasonable operating times and voltages (both dc and ac), quite efficient separation capabilities can be reached. Drift fields and drift distances comparable to those of pulsed-field gel electrophoresis, and therefore easily realizable, lead to times that are shorter by two orders of magnitude than those of pulsed-field gel electrophoresis for the same spatial resolution or to much better accuracy in comparable times. For example, 1-Mbp fragments of DNA differing by 10 kbp lie in two separated bands ≃2 mm apart after 1 hr, whereas the same result would typically require 100 hr with usual pulsed-field gel electrophoresis devices (see, for example, figure 3 in ref. 3). A more accurate resolution can be obtained at larger time expense.

Our model is oversimplified in several ways. One limitation results from the idealization of the electric field pattern: discontinuity is nonphysical. In fact E^* varies continuously from 0 to its maximal value over a characteristic length l, which on dimensional grounds is of the order of the device

Table 1. Values of various parameters as a function of DNA size (N) and field amplitudes (E and E^*)

N, Mbp	E, V/cm	E^*, V/cm	τ, s	t°, s	δN, kbp	x^*, mm	t^*, h	δx^*, mm
0.1	10	50	43	5	1	7.8	1.03	0.79
	20	50	11	2.5	1	7.8	0.28	0.71
1	10	9	17	5	10	7.4	0.44	0.66
	10	10	110	5	10	4.8	1.50	0.66
	10	11	900	5	10	3.2	8.06	0.56
	20	10	28	2.5	10	4.8	0.40	0.63
	50	10	4.4	1	10	4.8	0.07	0.56
	10	10			1	480	152	6.6
10	20	2	123	2.5	100	2.9	1.02	0.53
	20	1.8	11	2.5	100	4.5	0.17	0.55

In all cases, inequalities corresponding to regimes 1–3 are satisfied. Note the "short" amount of time required to separate out 1-Mbp DNA molecules.

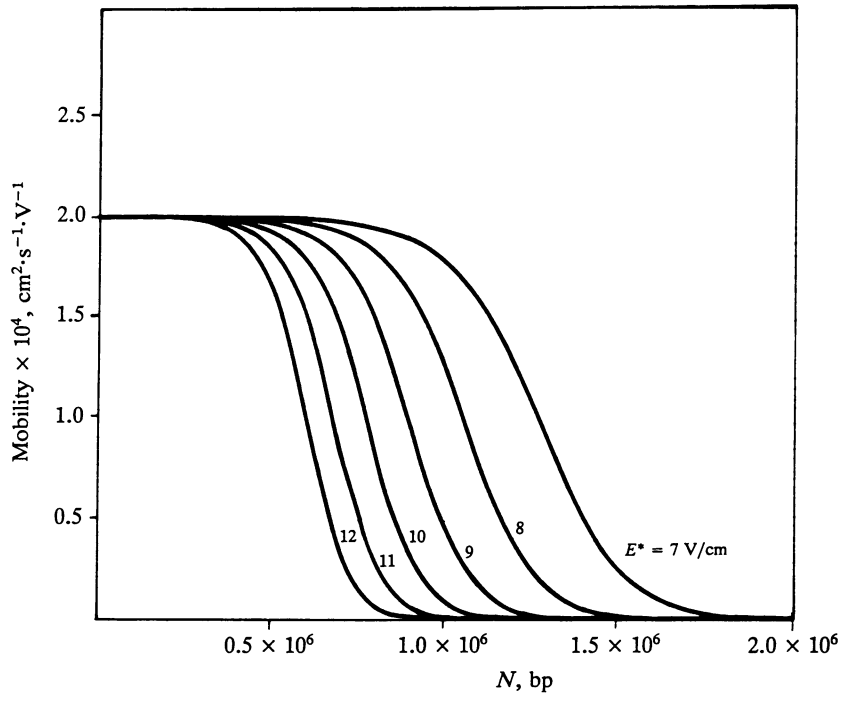

FIG. 2. Mobility of DNA molecules versus size N in the megabase-pair range for a drift dc field of 10 V/cm and a trapping ac field varying from 7 to 12 V/cm. Similar plots are obtained in the 0.1- to 10-Mbp ranges by changing the field amplitudes (see Table 1).

thickness e (for $a >> e$), which could easily be of the order of 10 μm. Field gradients in the thickness direction should also exist. Comparison to d shows that the polyelectrolyte no longer sees the trap as a vertical wall, but rather as a long slope (as long as ΔW is not so large that the trapping times would be expressed in years). In this regime, the prefactor and the argument of the exponential describing the trapping time depend on both E and E^* (or ω), and the form of the results is more complex. Calculations modeling the growth and decay of $(E^*)^2$ by a linear function or by a hyperbolic tangent show that the selectivity is somewhat smaller than in our simplified illustration, but remains interesting, and that the flexibility of the technique is preserved.

Moreover, field inhomogeneities along the thickness direction should attract the DNA fragments toward electrodes edges. Hence they should significantly reduce the characteristic size l to something like a passivation layer (which can be of the order of 5000 Å), giving, therefore, credit to our simple estimate.

Another possible limitation could come from the onset of convection currents due to heating. However, since typical geometries are fairly similar to capillary electrophoresis, hydrodynamic convection should not be a problem.

A more evident limit is the neglect of the conformational changes of the macromolecule. These have been observed experimentally (13) on time scales very comparable to those of the trapping process considered in the present article; the dynamics of this process therefore deserve a different description. However, although the detailed size dependence of the parameters controlling the effective mobility will be modified if this conformational change is taken into account, the selectivity should still be exponential, and the interest of the method preserved. In the same way, a more exhaustive description should include other regimes of spatial periodicity and transverse fields, where trapping and barrier crossing could follow different paths (one could for example imagine the extension of the molecule over a few traps; the dynamics are then possibly described by something similar to ref. 14, etc.).

The study of such regimes may be envisaged in the future, but the present description is a necessary starting point that exhibits the essential features of free-flow electrophoresis under an inhomogeneous transverse field, and points out its main characteristics: (*i*) an exponential dependence of trapping times and therefore a good selectivity and (*ii*) the possibility to control both the prefactor and the argument of the exponential by independent control of the drift field and of the transverse one (intensity and frequency), which confers an exceptional flexibility (Fig. 2).

We furthermore want to point out that the technique is not restricted to polyelectrolytes such as DNA. Other objects such as large proteins, chromosomes (15), cells, charged micelles, and latex spheres could also be separated that way. It is also worthwhile to note that the use of electric traps may be combined with other dc drifts, such as hydrodynamic flows, which opens the technique to uncharged particles.

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