Nonlinear focusing of DNA macromolecules

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The present paper reports the nonlinear electrophoretic focusing techniques developed after an original idea by Chacron and Slater [Phys. Rev. E **56**, 3436 (1997)]. Focusing of DNA molecules is achieved in an alternating nonuniform electric field, created in a wedge gel with hyperbolic boundaries. The fractions separated on such a wedge retained their rectilinear shape during the electrophoresis. Experiments with gel electrophoresis confirm the possibility of a noticeable nonlinear focusing of DNA molecules.

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I. INTRODUCTION

Gel electrophoresis is one of the most frequently used separation techniques for the biological macromolecules. Therein, the molecules migrate in a support medium (gel), being separated according to their mobility by an applied external electric field as determined by their charge and size.

During the electrophoresis, the separation is accompanied by diffusion of individual fractions due to the thermal nature of electrophoretic motion [1], thereby reducing the quality of separation. Alternatively, the so-called isoelectric focusing [2] provides very distinct bands of the separated fractions. Isoelectric focusing takes advantage of the charge dependence of biological molecules on the local *p*H of the separation medium. A drastic increase in the molecular concentration, or focusing, is observed at a point where the molecules have zero total charge, the so-called isoelectric point, therefore ceasing to migrate in an electric field. Unfortunately, isoelectric focusing is virtually unfeasible for DNA molecules.

A recent study of the nonlinear electrophoresis approach [3] along with an exact solution of the diffusion equation we obtained for the isofocusing method [4] enabled us to turn to the method of focusing of DNA molecules by nonlinear electrophoresis in a wedgelike medium.

A focusing method applicable when the particle velocity depends nonlinearly on an external applied field had been proposed earlier by Chacron and Slater [5]. The same authors discussed the possibility to focus the DNA molecules and obtained equations for the focus points.

The present paper reports results on nonlinear focusing in pulsed gel electrophoresis of DNA molecular fragments. Section II describes the nonlinear gel electrophoresis, and Secs. III and IV—a model of focusing in a gel wedge and the experiments in the nonlinear focusing of the DNA molecules.

II. NONLINEAR ELECTROPHORESIS

In relatively weak fields, the molecular drift velocity U_d is proportional to the external electric field E: $U_d=\mu E$, with the molecular mobility μ being virtually field independent. Macromolecules, initially in the form of a stochastic coil, start changing shape in an electric field, aligning gradually along the external electric field vector. Thus the molecular mobility in fact varies as a function of the electric field. McDonnell *et al.* [6] were among the first to report this dependence. Lumpkin *et al.* [7] discussed the nonlinear molecular mobility of DNA in gels in detail, concluding that the nonlinear mobility component $\Delta\mu$ in moderate fields is proportional to the field amplitude squared, $\Delta\mu\sim E^2$, being independent of the length of the molecule. The respective nonlinear velocity correction is proportional to the field cubed: $\Delta U_d\sim E^3$. A more realistic electric field dependence of DNA mobility, including fluctuations, yields the revised biased reptation model with fluctuations (BRF) [8]. The BRF theory proposes the following asymptotic dependence of the nonlinear velocity term:

$$\Delta U_d=\nu E|E|. \tag{1}$$

Recently, we studied the nonlinear properties of DNA in an electrophoretic experiment [3]. These experiments demonstrated that the drift velocity of a double-stranded DNA molecule in an electric field was distinctly nonlinear. The nonlinear drift velocities of longer molecules are higher than those of shorter molecules, depending on the molecular size in a complex manner. This pattern contrasts with that of conventional electrophoresis, where shorter molecules are always faster than longer ones.

An asymmetric periodic electric field (Fig. 1) with zero average value was used to observe the nonlinear drift velocity. As an example, we applied a field of 3.33 V/cm for 30 sec in one direction and then a field of 10 V/cm for 10 sec in

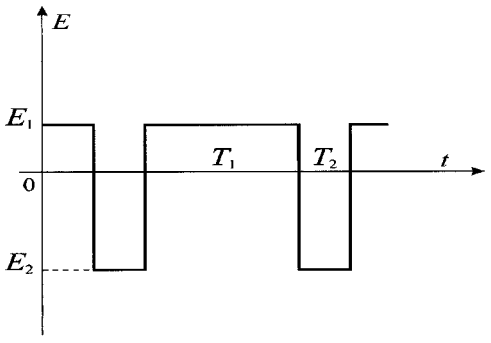

FIG. 1. Electric-field pulse sequence.

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the opposite direction. The average field value $\langle E \rangle$ is obviously zero, while the averaged $\langle E|E| \rangle$ is not. Alternating the electric field over several hours, we observed a noticeable advance of the DNA molecules, caused exclusively by the nonlinear velocity component.

In the course of several hours, after many periods of the electric field, we observed a noticeable movement of the DNA molecules, caused exclusively by the nonlinear velocity term. We have even managed to separate the molecules as a function of their nonlinear mobility.

Similar pulse sequences, with a close to zero average value, had been used in the zero integrated field electrophoresis technique [9] and also in Ref. [10]. In our opinion, such techniques should be more accurately named ''nonlinear electrophoresis'' (NEP), as they are essentially based on the nonlinearity of the drift velocity. The nonlinear drift velocity is the separation parameter in NEP experiments.

III. NONLINEAR FOCUSING IN A HYPERBOLIC WEDGE

A remarkable property of NEP is its capability to focus long molecules in a nonuniform electric field. The alternating field parameters for the nonlinear focusing are chosen so that the linear and nonlinear components of the average drift velocity would subtract, partially or completely. Hence the time-averaged drift velocity would be close to zero.

To focus DNA molecules by NEP, we used a nonuniform electric field, the amplitude of which decreased from one part of the gel to the other. The nonlinear term provided the major average velocity contribution in the high-field zone, whereas in the low-field zone, with nonlinearity virtually absent, the average drift velocity was determined exclusively by the linear velocity term. Next, we chose a variable voltage, so that the average field intensity would be a small positive value, while the averaged $\nu\langle E|E| \rangle$ would be negative.

In our experiments, the averaged molecular drift velocity in the high-field zone was negative, changing sign in the low-field zone. Thus the average molecular drift velocity would assume a zero value in some intermediate point. It is in this point, which we named a *virtual trap*, that the molecule is focused. Pronounced molecular focusing will be achieved in such a virtual trap, provided the molecular drift amplitude integrated over a period was considerably smaller than the bandwidth. Note that unlike conventional isoelectric focusing in constant fields, nonlinear focusing is achieved by averaging over a large number of field periods.

Focusing of molecules required the generation of nonuniform electric fields. In the literature, changes in the gel geometry were repeatedly proposed for producing nonuniform fields in order to improve separation [11–13]. However, the idea failed to find wide application due to two main disadvantages. First, the nonuniform electric fields affect the temperature distribution in the gel. Second, it is generally believed that the shape of separated fractions becomes curvilinear in a nonuniform electric field (''smiling effect''). These effects can impair the resolution of gel electrophoresis.

Therefore, the problem was to find a geometrical shape of the flat gel slab that would not affect the band shape. We found that a cuneiform gel slab with hyperbolic boundaries has suitable geometric form. Such a wedge (Fig. 2) with length L and transverse dimensions H_1 and H_2 was formed using special insulator inserts in the rectangular gel mold.

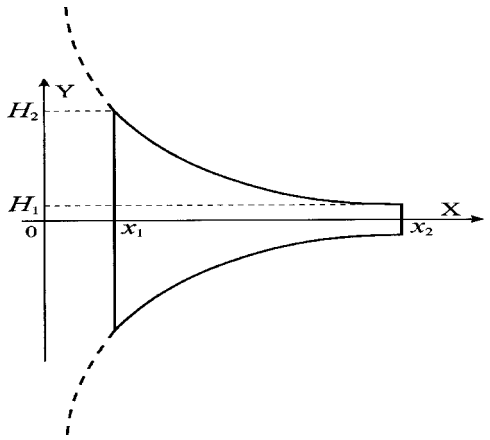

FIG. 2. Lateral boundaries of the wedge are hyperbolic segments. With a voltage V applied across the wedge, the axial electric field E_x will be linearly dependent on x: $E_x = 2Vx/L^2$. Here $x = 0$ corresponds to the infinite wedge width and L is the wedge length. Note that E_x is independent of y, keeping the separated bands straight.

A. Distribution of an electric field in the hyperbolic wedge

Let us consider the electric-field distribution and potentials in a hyperbolic wedge, neglecting the fringe and temperature effects at the gel boundaries. For an ''ideal'' infinitely expanded hyperbolic wedge, the potential distribution may be easily found using the theory of analytical functions. Since the potential of an electrical field satisfies the Laplace equation, the complex potential Φ may be considered. A solution of the Laplace equation for the hyperbolic boundaries is known [14]:

$$\Phi(z) = \varphi + i\psi = Az^2, \tag{2}$$

where $z = x + iy$ is a complex variable, the x axis coincides with the wedge symmetry axis, and A is determined from the boundary conditions as shown later. The real part φ of the complex potential describes the scalar potential of the electric field, while the imaginary part ψ describes the field lines and, in particular, the wedge boundaries. Using Eq. (2), the following equations for σ and ψ are obtained:

$$\varphi = Ax^2 - Ay^2, \quad \psi = 2Axy. \tag{3}$$

The constancy condition for ψ defines the wedge boundary lines in the x-y plane:

$$2Axy = \text{const}. \tag{4}$$

As seen from Eq. (4), the wedge boundaries have a hyperbolic shape.

The electric field formed in the hyperbolic wedge gel is determined by the gradient of φ, being described by the equations

$$E_x = -2Ax, \quad E_y = 2Ay. \tag{5}$$

Therefore, the x component of the electric field changes linearly along the wedge. The appearance of the E_y component of the field due to the gel wedge shape results in a transversal distension of the bands across the wedge axis of symmetry. However, the E_y is negligible in the vicinity of the symmetry axis. Note also that E_x is independent of y. Consequently, the band shapes during electrophoresis in the gel wedge remain rectilinear.

Note also that the field with decreasing intensity, obtained in the hyperbolic wedge gel, greatly improves the absolute resolution of the method without impairing the relative resolution of the conventional electrophoresis. This happens because the distances between the fractions are reduced proportionally to the narrowing of the bands themselves. However, this conclusion is only valid for the behavior of the drift velocity. Making use of the nonlinearity, when the particle mobility is field dependent, we can improve both the absolute and relative resolutions. Thus nonlinear focusing allows us to improve the relative resolution as well.

B. Description of the fraction drift considering the velocity nonlinearity

Let us consider a finite segment of the wedge (Fig. 2). Assume that the start of the gel corresponds to the coordinate $x = x_1$ and its end corresponds to the coordinate x_2, where $x_2 - x_1 = L$, the length of a wedge segment. The voltage $V = \Delta\varphi$ on the axis of symmetry between coordinates x_2 and x_1 equals

$$V = A(x_2^2 - x_1^2). \tag{6}$$

This equation allows us to find the constant A, determined by the voltage V and wedge geometry.

The constant A becomes a time-dependent function for a periodic voltage:

$$A(t) = V(t)/(x_2^2 - x_1^2). \tag{7}$$

The linear electric-field distribution obtained in Eqs. (5) allows us to calculate the fraction drift in the nonlinear electrophoresis experiment.

Let us now consider the drift velocity U_d of macromolecules in a nonuniform periodical electric field along the wedge axis of symmetry:

$$U_d = \mu E + \nu |E| E. \tag{8}$$

Averaging Eq. (8) over the electric-field period, we obtain

$$\langle U_d \rangle = \mu \langle E \rangle + \nu \langle |E| E \rangle. \tag{9}$$

Substituting Eq. (5) for the E_x, we obtain

$$\langle U_d \rangle = -2x\mu \langle V \rangle / (x_2^2 - x_1^2) - 4\nu x^2 \langle V|V| \rangle / (x_2^2 - x_1^2)^2. \tag{10}$$

Thus the equation for the coordinate of the fraction $x(t)$ averaged over the period becomes

$$\frac{dx}{dt} = \langle U_d \rangle = -\alpha x - \beta x^2, \tag{11}$$

where $\alpha = 2\mu \langle V \rangle / (x_2^2 - x_1^2)$ and $\beta = 4\nu \langle V|V| \rangle / (x_2^2 - x_1^2)^2$.

Integration of Eq. (11), provided that the linear μ and nonlinear ν molecular mobility coefficients as well as the electrical field averages $\langle V \rangle$ and $\langle V|V| \rangle$ are known, results in the fraction drift law

$$x(t) = \frac{\alpha}{C \exp(-\alpha t) - \beta}, \tag{12}$$

where C is a constant determined from the initial conditions $C = \alpha / x(0) + \beta$. Note that a similar equation had been obtained earlier in [5].

The solution obtained describes the averaged fraction drift. The most important feature of that drift is the fraction focusing at the points where the average drift velocity vanishes:

$$\langle U_d \rangle = \alpha x + \beta x^2 = 0. \tag{13}$$

According to Eq. (13), the focusing point x_f may be calculated as

$$x_f = -(x_2^2 - x_1^2) \frac{\mu \langle V \rangle}{2\nu \langle V|V| \rangle}. \tag{14}$$

Note that the asymptotic behavior of Eq. (12) at infinite times produces the same equation.

As evident from Eq. (14), hyperbolic wedge focusing always requires opposite signs of the $\langle V \rangle$ and $\langle V|V| \rangle$ average values. As an example we consider a periodic potential with period $T = 60$ sec. We first apply a positive voltage $V_1 = 90$ V for $T_1 = 10$ sec, and next the polarity is reversed, $V_2 = -20$ V for $T_2 = 50$ sec. In this case, the average voltage will be negative, $\langle V \rangle = (V_1 T_1 + V_2 T_2)/T = -13.3$ V. However, the $\langle V|V| \rangle$ average will be positive, $\langle V|V| \rangle = (V_1^2 T_1 - V_2^2 T_2)/T = 1016.6$ V^2.

Analysis of Eq. (14) demonstrates that the focusing points (virtual traps) depend on the ratio of the linear mobility coefficient μ to the nonlinear mobility coefficient ν and, also, on the electric field averages.

Thus fragments with different ratios of the linear to nonlinear mobility coefficients, μ/ν, would be focused at different points. Moreover, the focusing point position may be controlled by the periodic electric-field wave form.

IV. EXPERIMENT

To test the theoretical model, we separated *Hind*III-digested DNA of phage Lambda using NEP in agarose gel (Figs. 3 and 4).

Agarose (ultrapure DNA grade, BioRad Laboratories, Richmond, CA) was dissolved by boiling in tris-acetate buffer, containing 89 mM tris base, 89 mM acetic acid, and 2 mM ethylenediaminetetraacetic acid (EDTA). Upon cooling to 60 °C, 40 ml of agarose solution at a concentration of 0.75% (w/v) were poured into a wedged gel vessel with in-

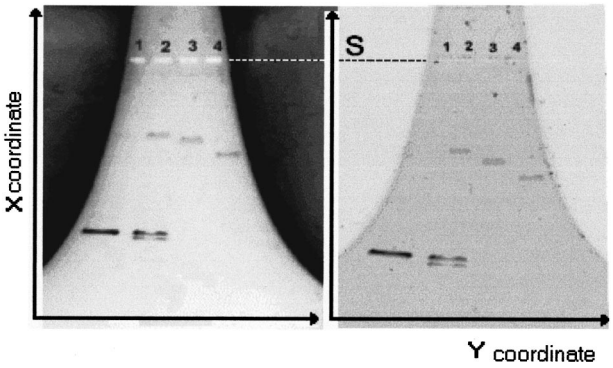

FIG. 3. Nonlinear focusing of *Hind*III lambda DNA fragments: the result of a 24-h experiment, at the left; the changes in the pattern produced after 35 h of NEP, at the right. The wedge length was $L=9$ cm, with wedge widths of $H_1=10$ cm and $H_2=2$ cm.

stalled plastic start slot former (designated as *s* in the figures). The gel slab formed was 4 mm high. The solidified gel was submerged in tris-acetate EDTA buffer, so that the buffer layer above the upper gel surface was at least 3 mm. The *Hind*III lambda DNA fragments dissolved in a loading buffer were introduced into the start slot. Electrophoresis was performed in a horizontal unit. The separated DNA fragments were stained with ethidium bromide and visualized with an UV transilluminator (BioDoc II/NT, Biometra Analytical GmbH). We used an agarose wedge with a length of 10 cm, larger transverse width of 10 cm, and smaller width of 2 cm.

A computer-controlled voltage source was used to maintain the potential difference. The control was accomplished using a LAB PC+ unit (National Instruments). The voltages at the hyperbolic wedge ends were controlled using two platinum measuring electrodes 0.2 mm in diameter. The electrodes were positioned precisely on the symmetry axis of the wedge 9 cm from one another and used exclusively for voltage monitoring. The computer-assisted system provided a specified potential wave form on the working electrodes during the entire electrophoresis session. Note that the voltage at the working electrodes is always higher than the measured voltage: the latter was monitored at 0.1 V accuracy and 0.01 sec time resolution. Additionally, currents were measured during the entire voltage period.

Figure 3 demonstrates the results obtained upon nonlinear focusing of DNA molecules. The gel contained four start slots (tracks) that were loaded with the 23.1 kilobase pairs (kbp) fraction of *Hind*III lambda phage DNA hydrolysate (slot 1), 23.1 and 4.4 kbp fractions (slot 2), 6.6 kbp fraction (slot 3), and 9.4 kbp fraction (slot 4). A field period comprised 90 V for 10 sec followed by 19.8 V for 50 sec in the opposite direction.

The DNA hydrolysate solution was not heated before the experiment. As a result, the second track contains a 27.5-kbp band that appeared through hybridization of 23.1- and 4.4-kbp fragments due to the ''sticky ends'' effect. Thus the second track reveals two similar fractions, corresponding to 27.5 and 23.1 kbp.

Figure 4 demonstrates 27.5 and 23.1 kbp fractions as two distinct well-separated bands after 48 h of NEP, thereby confirming the potential efficiency of nonlinear focusing.

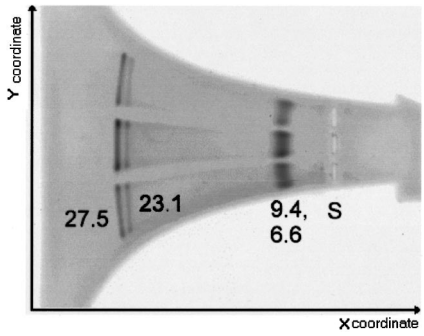

FIG. 4. Nonlinear focusing of DNA molecules exemplified by large DNA fragments of *Hind*III-hydrolyzed lambda phage DNA in a hyperbolic wedge. The pulse cycle comprised 81 V for 5 sec and 30 V for 15 sec in the opposite direction; 48 h NEP session.

In this experiment all of phage lambda DNA *Hind*III fragments were separated by NEP. The pulse cycle comprised 81 V for 5 sec and 30 V for 15 sec in the opposite direction. There is another band alongside the first two in the Fig. 4. A second conventional electrophoresis, performed in the orthogonal direction after the nonlinear focusing experiment, showed that this band corresponds to the lighter DNA fragments, of 9.4 and 6.6 kbp, present in the hydrolysate.

These experiments prove that nonlinear focusing is indeed possible for the phage lambda/DNA *Hind*III fragments.

V. CONCLUSION

An approach developed after an original idea by Chacron and Slater [5] for focusing DNA molecules, based on nonlinear electrophoresis and geometric trapping in nonuniform electric fields, was investigated.

The focusing of DNA molecules was carried out in an alternating nonuniform electric field, created by using a wedge gel with hyperbolic boundaries. It was shown that the fractions separated on such a wedge retain their rectilinear shape.

The drift velocity in the narrow part of the wedge, where the field is high, is dominated by the nonlinear component, being negative. However, in the wider part the fields are low: thus, the nonlinear mobility becomes negligible and the drift velocity reverts to positive. Thus focusing should occur in the central part of the wedge.

Gel electrophoresis experiments supported the possibility of a pronounced nonlinear focusing of DNA molecules. This nonlinear separation technique presents encouraging prospects for the preparative isolation of long DNA fragments and the development of new separation methods.

Nonlinear focusing provides an important addition to the existing set of molecular biology techniques. This method requires further experimental and theoretic studies and has promising potential for molecular biological investigations.

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[1] G. W. Slater, Electrophoresis **14**, 1 (1993).
[2] P.-G. Righetti, *Isoelectric Focusing: Theory, Methodology and Applications* (Elsevier, Amsterdam, 1983).
[3] L. L. Frumin, S. E. Peltek, S. Bukshpan, V. V. Chasovskikh, and G. V. Zilberstein, Phys. Chem. Commun. **11**, 1 (2000).
[4] L. L. Frumin, S. E. Peltek, and G. V. Zilberstein, J. Biochem. Biophys. Methods **45**, 103 (2000).
[5] M. J. Chacron and G. W. Slater, Phys. Rev. E **56**, 3446 (1997).
[6] U. W. McDonnell, M. N. Simon, and F. W. Studier, J. Mol. Biol. **110**, 119 (1977).
[7] O. J. Lumpkin, P. Dejardin, and H. Zimm, Biopolymers **24**, 1573 (1985).
[8] T. Duke, J.-L. Viovy, and N. Semenov, Biopolymers **34**, 239 (1994).
[9] C. Turmel E. Brassard, R. Forsyth, K. Hood, G. W. Slater, and J. Noolandi, in *Electrophoresis of Large DNA Molecules, Theory and Applications*, edited by E. Lai and B. W. Birren (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1990), p. 131.
[10] G. A. Greisss, E. Rogers, and P. Serwer, Electrophoresis **21**, 859 (2000).
[11] G. W. Slater and J. Noolandi, Electrophoresis **9**, 643 (1988).
[12] A. Olsson, T. Moks, M. Uhlen, and A. B. Gaal, J. Biochem. Biophys. Methods **10**, 83 (1984).
[13] W. Ansorge and S. Labeit, J. Biochem. Biophys. Methods **10**, 37 (1984).
[14] M. A. Lavrentiev and B. A. Shabat, *Methods of Analytical Function Theory* (Nauka, Moscow, 1987).