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Peak dispersion due to geometration motion in gel electrophoresis of macromolecules

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Abstract

A theoretical model for the motion of DNA chains through the gel under strong steady electric fields is proposed. It utilizes the geometration model of the motion, which is divided into three basic phases and described by the analytical equations. The model predicts in close quantitative agreement with the experimental observation that the gel electrophoretic mobility of DNA, in the limit of large chains and strong fields, reaches a plateau independent of DNA size and electric field. The predicted value of mobility is 4/9 of the free mobility of DNA. The calculated dispersion is proportional to the molecular size, which is strikingly opposite to the Brownian dispersion and also to the biased reptation dispersion but close to experimental observation in the pulsed field regime. The corresponding plate height due to DNA motion in the framework of obstacles is $H=0.0288l$, where l is DNA contour length. Finally the model allows the simulation of electrophoretic peak profiles that show a significant asymmetry, when the migration distance is $\leq 100l$. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Peak shape; Geometration motion; Mathematical modelling; DNA; Dispersion

1. Introduction

The most intensively studied quantity in the theory of electrophoresis of DNA fragments is, naturally, the electrophoretic mobility [1,2]. Selectivity of electrophoretic separation is directly proportional to the dependence of the mobility on molecular size and is decisive for a successful separation of DNA. Nevertheless, not only selectivity is responsible for resolution of two consecutive peaks in electrophoresis as the resolution consists of two factors, selectivity and efficiency. Consequently, it follows that efficiency of separation or peak broadening is also an important quantity and must be seriously taken into account. Unfortunately, calculation of peak broadening in the framework of existing models of DNA motion in a sieving matrix is even more difficult than the calculation of the electrophoretic mobility and published work on this question is rather scarce. Accurate and systematic experimental measurements of peak broadening for DNA fragments are scarce as well. This is rather surprising, since DNA fragments are polymers with a very well defined molecular mass and would be ideally suited for such measurements, unlike, e.g., proteins.

Considerations on dispersion could start basically from the Einstein relation between the diffusion coefficient D and mobility u

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$$D = u \cdot \frac{kT}{Mq_0} \quad (1)$$

Here q_0 is the relative charge per monomer unit, M is the molecular size in units, k and T are the Boltzman constant and absolute temperature, respectively. As the electrophoretic mobility of large-size DNA fragments presents a well-known velocity plateau, this simple relation would imply that $D \propto M^{-1}$. Published experimental results [3,4] disagree with this decrease of the diffusion coefficient with the molecular size. Therefore, more elaborate ways of accounting for the dispersion of charged polymers in sieving matrices has been searched for.

The theoretical calculations of peak broadening can be divided into two groups. The first general approach to peak dispersion was proposed by Giddings and Eyring [5] and was originally developed for explaining peak dispersion in chromatography due to a finite time of interaction of the analyte with the stationary phase. In this model it is supposed that a sample molecule in the column can be in either of two phases, mobile or stationary. In the mobile phase the molecule is supposed to undergo Brownian motion while in the stationary phase it remains at rest. The authors give explicit expressions for the dispersion in this model.

This approach was utilised by Chrambach's group for the description of peak dispersion in gel electrophoresis. These authors have performed experiments showing that the peak broadening of long DNA fragments or proteins migrating in gels by electrophoresis is significantly higher than the broadening due to a simple diffusion process [6–8]. Using basic features of the Giddings and Eyring's model [5], Weiss et al. [9] and Yarmola et al. [10] have also proposed a mathematical model able to explain basic features of the additional band broadening and even to explain peak asymmetry. This additional dispersion was ascribed to the finite time interaction of the analyte with the sieving matrix and called ''interactive dispersion''. In this model the authors suppose that the molecule of a polyelectrolyte in the sieving matrix can be in either of the two states, mobile or entangled. In the mobile state the molecule moves in the electric field and undergoes a Brownian motion, while in the entangled one it remains stationary until it eventually disentangles from the matrix obstacle. Entanglement and disentanglement times are supposed to be random variables.

In the second type of approach, the authors take explicitly into account the macromolecular nature of the moving analyte, and utilise existing models of polymer motion to calculate diffusion or dispersion coefficients that are directly related to peak dispersion. These models are particularly well suited for electromigration of DNA in sieving media. Along these lines, Zimm and Lumpkin [11] have utilised a reptation mechanism, having in mind that the classical reptation theory was not able to predict correctly the diffusion constant. Therefore they have extended the reptation model by adding a randomly fluctuating free energy of interaction between segments of the sample and the surrounding polymer matrix. Authors have been able to explain the strong dependence of the diffusion constant on chain length in regions where sample molecules fall into traps with a low free energy. In particular, they predicted that dispersion could be dramatically enhanced around the minimum of the mobility versus size curve.

However, it is not even necessary to invoke fluctuating interaction energies to account for enhanced peak dispersion in electrophoresis, as compared to the Einstein relationship prediction. The possibility of non-trivial enhanced band dispersion during gel electrophoresis, solely due to the specific non-linear features of the reptation process itself, was mentioned first by Adolf [12] in 1987, in an article which unfortunately remained relatively unremarked on by experimentalists. It was discussed later by Viovy [13], and finally studied in more detail by Slater [14], Duke et al. [1], Mayer et al. [15] and Viovy [16]. The basis of this approach is the notice that in relatively low fields, fluctuations in the end-to-end distance (projected on the direction of migration) remain of the order of $bN^{1/2}$, (where b is the Kuhn length and N the number of Kuhn segments in the chain). Therefore, the dispersion is typically of order $bN^{1/2}$ per reptation step (i.e., for a time step corresponding to the time necessary for the DNA chain to completely renew its conformation). The duration of the reptation step, however, is generally a decreasing function of the electric field, so that a field-driven dispersion, stronger than the Brownian

one, can occur. It also arises from the same approach that, depending on the reptation regime considered, the size-dependence of the dispersion coefficient jumps from ''Einstein'' M^{-1} scaling to $M^{-1/2}$, and then to M^0, i.e., a dispersion independent of size. The scaling predictions for the dependence of dispersion on field and pore size differ between the different treatments, due to different evaluations of the orientation factor in the reptation model, but the physical principle, and the size-scaling remain the same in the different models; for a more detailed analytical and numerical account, the reader is invited to refer the original articles. This mechanism of enhanced dispersion, is related with the non-linear nature of the biased reptation model, and is very specific of the reptation process.

The observation of DNA motion in a gel as revealed by fluorescence microscopy [17–20], though, has shown that a simple tube or reptation models cannot be applied for electrophoretic migration of DNA of moderate or large size [from about several thousand base pairs (bp) up to 200 kbp] in the high electric field regime (typically more than 1 or 2 V/cm). In this case, rather a different mechanism occurs, first predicted by Deutsch et al. [21–23] and called ''geometration''. For larger DNA molecules (>200 kbp) the motion is even more complicated, and involves the creation of branches and hernias during the movement [24]. Semenov and Joanny [25] have taken into account the branching of large DNA molecules in the framework of the biased reptation theory. They predicted that the longitudinal diffusion coefficient D_x, which is responsible for peak broadening, should scale as $D_x \propto N$, i.e., it is proportional to the molecule size.

Finally, Lee et al. [26] recently published a quasi-deterministic model for the gel electrophoresis of 10^6 bp-long DNA fragments in strong electric fields. Here the reptation concept is replaced by a random ''branching'' process inspired from the work of Duke et al. [1]. The dynamics, however, is described by deterministic mechanical equations without noise, and no fluctuations in tube orientations are considered. This model is surprisingly simple but still able to describe the main features of the motion of very large DNA in gels. It requires much less computational power than the freely-jointed chain of Deutsch, or the repton model of Duke. No considerations regarding peak dispersion were proposed in this article, but its simplicity makes it a good candidate for studying this problem.

The present paper aims at an understanding of peak dispersion of linear DNA in gel, under strong steady electric fields. We will utilise the geometration model by Deutsch [23] to derive an analytical description of the DNA motion in the matrix. For this, we will solve a few simple mechanical equations, analogously to the approach of Lee et al. [26]. Although the continuous dynamic equations in this approach are deterministic, instantaneous events (such as the collision of a molecule with an obstacle) involve some randomness. Reasonable assumptions on these events will allow us to derive the distribution function of time of the steps and, consequently, the velocity and dispersion of DNA electrophoretic peaks.

2. Theory

In the following, we will only consider peak dispersion arising from the geometration process. All other possible contributions that can appear in practice, like dispersion due to the finite size of the sample plug, thermal effects, adsorption at capillary wall, inhomogeneities in the sieving medium, heterogeneity of the sample, etc., are omitted. Assuming, as usually done in zone electrophoresis theory, that there is no coupling between these collective and interparticle processes and the intrinsic single molecule geometration process under study here, these different dispersion causes can be evaluated, if necessary, as the sum of all contributions.

2.1. Analytical description of the geometration model

We consider a linear molecule, with contour length l. The ''free'' velocity of the molecule (i.e., its velocity in the absence of interaction with the matrix, or when it is straight and aligned in the direction of the field) is called v_0. Following the geometration idea, the motion of the molecule in the matrix can be decomposed in several ''phases'':

2.1.1. First phase

The molecule, in a random coil conformation, encounters an obstacle at a random site along its length, and gets ''hooked''. Two arms of the molecule start to move at the same time in the direction of the electric field, see Fig. 1a. (In the most general case, for large chains more than two arms may grow. It was demonstrated by André et al. [27], however, that this complication only affects weakly the further dynamic evolution of the chain). In the ''strong field'' limit, entropic elasticity can be neglected, and both arms (of contour length l_1 and l_2, respectively), ''uncoil'' downfield with the same velocity v_0.

This first phase ends when the longer arm is stretched, i.e., the duration t_1 of the first phase is

$$t_1 = \frac{l}{v_0} \cdot \max\,[r, (1-r)] = \frac{l}{v_0} \cdot (0.5 + |r - 0.5|) \quad (2)$$

Here $r \in \langle 0,1 \rangle$ is a random number with the uniform density $f(r)$, which is given by relations

$$f(r) = 1 \text{ for } r \in \langle 0,1 \rangle \quad (3)$$

$$f(r) = 0 \text{ for } r < 0 \text{ or } r > 1 \quad (3a)$$

This uniform distribution reflects the assumption that the probability of hooking around the obstacle is the same at any site of the chain.

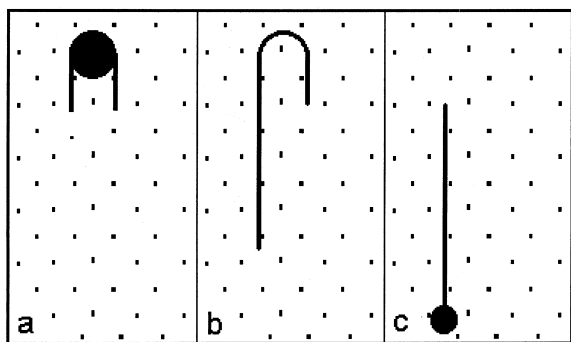

Fig. 1. Three phases of moving DNA through the gel. (a) First phase – DNA chain in random conformation encounters an obstacle and two arms start to grow, (b) second phase – both arms are fully stretched and DNA chain starts slide of the obstacle, (c) third phase – chain is released from the obstacle. When the head collides with another obstacle again, it assumes random conformation.

The distance x_C of the centre of mass from the obstacle is

$$x_C = \frac{l_1^2 + l_2^2}{2l} \quad (4)$$

so at time t_1 the migrated distance d_1 is:

$$d_1 = x_C(t_1) = 0.5l\left[r^2 + (1-r)^2\right] \quad (5)$$

Eventually the chain is left in a ''U-shape'', both arms fully extended and hanging on the obstacle in a random position with a tension maintaining the chain stretched.

It must be mentioned at this point that this is actually a somewhat simplified view: we already stated that we ignored the progressive build up of entropic tension, to replace it by an abrupt transition between a partly coiled state with no tension, and a fully stretched inextensible state. In addition, we ignored the fact that the two arms generally do not reach simultaneously full extension, so that when the first, shorter, arm is stretched, the growth of the longer arm is affected by the tension exerted by the first one. Also, we ignored hydrodynamic interactions between chain segments, both along one the same arm, and between the two arms. Previous detailed studies of polyelectrolyte collisions showed that such effects altered the final predictions in a relatively weak manner, see e.g., André et al. [27]. Therefore, we feel entitled to forget these complications in order to progress further in the understanding of the long-term behaviour, responsible of dispersion.

2.1.2. Second phase

The chain starts to slide around the obstacle (Fig. 1b). The longer arm drags the shorter one at a rate increasing with time. The drift velocity of the longer arm l_2 is proportional to the difference between the longer and shorter arm, $l_2 - l_1$, so

$$\frac{dl_2}{dt} = v_0 \cdot \frac{l_2 - l_1}{l} \quad (6)$$

The solution of Eq. (6) with the initial conditions for $t=0$, $l_2(0) = l(|r-0.5| + 0.5)$, $l_1(0) = l - l_2(0)$ is

$$l_2 = l\left[0.5 + |r - 0.5| \exp\left(\frac{2v_0}{l}\right)\right] \quad (7)$$

The overall duration of the second phase (ending when $l_2 = l$), is then

$$t_2 = \frac{l}{2v_0} \cdot \ln\left(\frac{1}{|2r-1|}\right) \quad (8)$$

At the end of this second phase the centre of mass is at a distance $l/2$ from the obstacle, so the distance travelled during the second phase, d_2, is

$$d_2 = 0.5l - 0.5l[r^2 + (1-r)^2] = lr(1-r) \quad (9)$$

2.1.3. Third phase

When the chain is released from the obstacle, tension disappears and it starts to fluctuate. Because of this fluctuation the collision section of the chain increases rapidly. For a relatively dense gel, having pore size much smaller than the radius of gyration of the chain at rest, we then expect that the chain will rapidly collide with an obstacle again, and start coiling at the "head" while the rest part of the chain is still moving down with the velocity v_0 (Fig. 1c). The downfield chain extension, l_2, decreases as $l_2 = l - v_0 t$. Eventually the entire chain gets in a "coil" conformation again and the process restarts at phase 1. Within this assumption of "quasi-instantaneous" collision for a tensionless (unhooked) chain, the time of this third phase is $t_3 = l/v_0$ and the distance travelled by the centre of mass is $d_3 = l/2$.

The overall duration of complete cycle, t, is then

$$\begin{aligned} t &= t_1 + t_2 + t_3 \\ &= \frac{l}{v_0}\left[\frac{3}{2} + |r-0.5| + \frac{1}{2}\cdot\ln\left(\frac{1}{|2r-1|}\right)\right] \\ &= h(r) \end{aligned} \quad (10)$$

and is a function of r, while the overall travelled distance d is

$$d = d_1 + d_2 + d_3 = l \quad (11)$$

and is constant (Table 1 summarises the derived relations).

The duration of a cycle $t = h(r)$, Eq. (10), is a random variable, which will be denoted T. The density of T, $g(t)$, can be derived when realising that the following relations can be written for the mean value $E(T)$ [28]:

$$\begin{aligned} E(T) &= \int_0^1 h(r)\, f(r)\, \mathrm{d}r = 2\int_0^{0.5} h(r)\, f(r)\, \mathrm{d}r \\ &= \int_0^{\infty} t\; g(t)\, \mathrm{d}t \end{aligned} \quad (12)$$

Here $f(r)$ is given by Eq. 3. We define $r = h^{-1}(t)$ as the inverse function of $h(r)$. In the interval $\langle 0,0.5\rangle$, $h(r)$ is monotonous so the density function $g(t)$ derived from Eq. 12 is:

$$g(t) = 2f[h^{-1}(t)] \cdot \frac{\mathrm{d}h^{-1}(t)}{\mathrm{d}t} \text{ for } t \in \left\langle \frac{2l}{v_0}, \infty \right) \quad (13a)$$

$$g(t) = 0 \text{ for } t \in \left\langle 0, \frac{2l}{v_0} \right) \quad (13b)$$

The density function $g(t)$ cannot be expressed explicitly. It is graphically depicted in Fig. 2, in v_0/l units (as obtained by numerical solution of Eq. 13). It should be noticed that $g(t) \to \infty$ for $t \to 2l/v_0$.

The mean value μ of the $g(t)$ distribution is, according to Eq. (12)

Table 1
Time and distance passed by the molecule in all phases of geometration motion

Phase	Time	Distance
1	$\frac{l}{v_0} \cdot (0.5 + \lvert r-0.5 \rvert)$	$0.5l[r^2 + (1-r)^2]$
2	$\frac{l}{2v_0} \cdot \ln\left(\frac{1}{\lvert 2r-1 \rvert}\right)$	$lr(1-r)$
3	l/v_0	$l/2$
Overall	$\frac{l}{v_0} \cdot \left[\frac{3}{2} + \lvert r-0.5 \rvert + \frac{1}{2} \cdot \ln\left(\frac{1}{\lvert 2r-1 \rvert}\right)\right]$	l

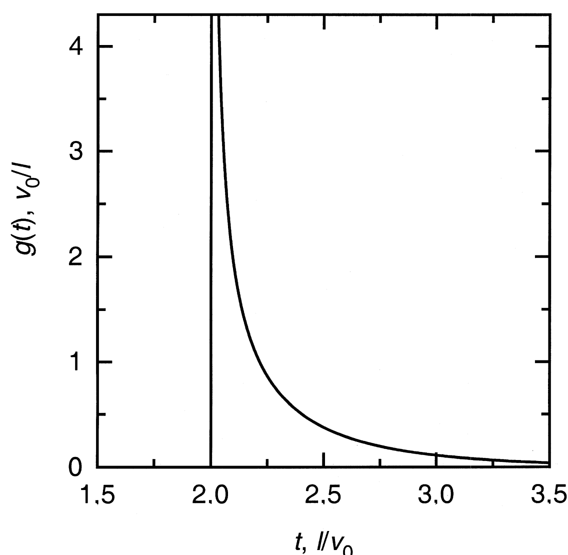

Fig. 2. Distribution function $g(t)$.

$$\mu = E(T) = 2\int_0^{0.5} h(r)\, f(r)\, \mathrm{d}r = \frac{2l}{v_0}\int_0^{0.5} [2 - r - 0.5 \ln(1-2r)]\, \mathrm{d}r = \frac{9}{4}\cdot\frac{l}{v_0} \quad (14)$$

The variance $\sigma^2 = E(T^2) - [E(T)]^2$ can also be easily derived when calculating $E(T^2)$:

$$E(T^2) = 2\left(\frac{l}{v_0}\right)^2 \int_0^{0.5} [2 - r - 0.5\ln(1-2r)]^2\, \mathrm{d}r = \frac{125}{24}\cdot\left(\frac{l}{v_0}\right)^2 \quad (15)$$

The formula for the variance finally comes out as

$$\sigma^2 = \left(\frac{l}{v_0}\right)^2\left(\frac{125}{24} - \frac{81}{16}\right) = \frac{7}{48}\cdot\left(\frac{l}{v_0}\right)^2 \approx 0.146\cdot\left(\frac{l}{v_0}\right)^2 \quad (16)$$

2.2. Calculation of migration time and peak dispersion

The long-distance motion of a DNA molecule in the framework of the geometration model is a series of repeated cycles comprising the above three phases. During each cycle the molecule travels a fixed distance l, but the duration of individual cycles is variable. The duration of the i-th cycle will be denoted t_i. To reach the detector, at a distance of $L = nl$ (where n is large and taken as natural number for simplicity), the molecule will need the overall time t_O,

$$t_O = \sum_{i=0}^{n} t_i \quad (17)$$

Naturally, the overall time t_O is a random variable, which will be denoted T_O here.

Since each cycle starts in a ''collapsed'' state, it is reasonable to assume that the process is stochastic and that the t_i values are uncorrelated. Then, the mean value of T_O is the sum of the individual mean values, $E(T_O) = n\mu$, and the variance is the sum of the individual variances, $D(T_O) = n\sigma^2$. In the framework of the present model it follows that the mean overall time $E(T_O)$ is

$$E(T_O) = \frac{9}{4}\cdot\frac{nl}{v_0} = \frac{9}{4}\cdot\frac{L}{v_0} \quad (18)$$

This implies that the mean travel time to the detector is size-independent. In other words, DNA molecules moving through gels by the geometration mechanism cannot be separated, in agreement with experimental evidence. The average velocity of the movement, $\bar{v}$, is:

$$\bar{v} = \frac{L}{E(T_O)} = \frac{4}{9}\cdot v_0 \quad (19)$$

This prediction is also in close quantitative agreement with the experimental observation that the gel electrophoretic mobility of DNA, in the limit of large chains and strong fields, reaches a plateau independent of DNA size and electric field, and equal to about 1/3 of the free mobility [29].

The dispersion of the overall time, $D(T_O)$, is, in the terms of separation science, the time-based peak dispersion, and will be denoted here as σ_t^2. It can be expressed as:

$$\sigma_t^2 = n\sigma^2 = n\cdot\frac{7}{48}\cdot\frac{l^2}{v_0^2} = \frac{7}{48}\cdot\frac{L}{v_0^2}\cdot l \quad (20)$$

Unlike the migration time, the dispersion is proportional to the contour length of the molecule. This dispersion originates from the randomness of the molecule motion. Interestingly, though, in the present case it is not related with random impulses from the low-molecular environment, as in Brownian motion, but with the randomness of the interactions of the analyte molecules with the surrounding heterogeneities (therefore, it is not thermally activated).

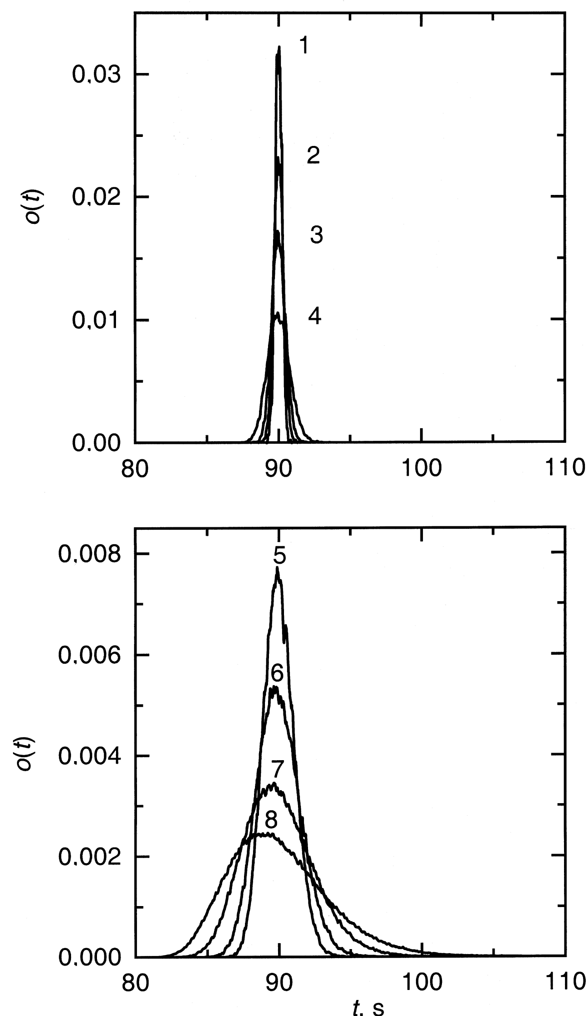

Fig. 3. Density function of the overall time, $o(t)$, for various contour lengths of DNA. Conditions: unretained velocity of DNA v_0, 0.5 mm/s; distance to the detector L, 20 mm; contour length l, 1 = 5 μm, 2 = 10 μm, 3 = 20 μm, 4 = 50 μm, 5 = 100 μm, 6 = 200 μm, 7 = 500 μm, 8 = 1000 μm.

For small peak widths (i.e., in the limit $L >> l$), the time-based and space-based dispersions are related by: $\sigma_x^2 = \sigma_t^2 \bar{v}^2$. So in the model,

$$\sigma_x^2 = \frac{7}{243} \cdot Ll \qquad (21)$$

Transformation of this expression using the well known relation between space-based dispersion $\sigma_x{}^2$ and the plate height H (given by $\sigma_x{}^2 = HL$), leads to

$$H = \sigma_x^2/L = \frac{7}{243} \cdot l \approx 0.0288l \qquad (22)$$

Eq. (22) expresses the contribution of random interactions of DNA molecules with the gel obstacles to the plate height, in the framework of the geometration mechanism.

2.3. Peak shapes

The peak profile, i.e., the time based signal measured by a detector located at the coordinate L, is proportional to the density function of the overall time T_O, which will be denoted $o(t)$. According to the central limiting theorem the density $o(t)$ converges to the normal distribution $N(n\mu, n\sigma^2)$ with increasing n. However, if n has a moderate value, the density $o(t)$ significantly differs from the normal distribution and has a nonsymmetrical shape. This is shown in Fig. 3, where the function $o(t)$ is depicted for various sizes of DNA fragments. All the curves were calculated by numerical simulation of Eq. (17), where t_i values were calculated from Eq. (10). Under given conditions ($v_0 = 0.5$ mm/s, $L = 20$ mm) it comes out that an apparent nonsymmetry is visible at curves 6–8, corresponding to a number of cycles $n = L/l \leq 100$. To fix ideas, for typical distance of a few cm travelled during agarose gel electrophoresis of large duplex DNA this corresponds to chain sizes of the order of 1 Mbp: this can explain why the observed band width of Mbp DNA in gel electrophoresis is generally rather large, whatever experimental precautions are taken.

3. Discussion

The present model leads to several simple predic-

tions for the steady-field mobility of large DNA in the strong field regime (geometration mechanism). As far as the mobility is concerned, the predictions are in good qualitative and quantitative agreement with experiment. The predicted dispersion is proportional to the molecular size, a prediction striking opposite to the Brownian dispersion one (in which it is inversely proportional to size) and also to the biased reptation ones, which range, depending on the regime, from an M^{-1} regime to M^0. This dispersion increasing with size seems at least qualitatively in agreement with experiments, although to our knowledge no detailed experimental evaluation of this quantity in the strong field regime was performed.

Of course, one has to realise that the regime investigated here corresponds, from the application point of view, to a relatively uninteresting region in which the velocity is not dependent on molecular size. It is, however, worth to notice that a dispersion increasing with DNA size also seems to be observed during the practically very important pulsed-field separations of Mbp DNA.

It is further stimulating to notice that even the very complicated movement of large DNA molecules can be described by means of a set of relatively simple equations, as was proposed by Lee et al. [26]. Therefore, we expect that the present model can be extended to pulsed-field situations, and that an equation analogous to Eq. (10) for $h(r)$ could be derived also for these more complicated situations (large DNA and pulsed fields). Therefore, we believe that the present work, and the rather good agreement of our predictions with experimental evidence, opens the route to studies of the mobility and peak dispersion in situations of extreme applied importance, such as pulsed-field electrophoresis of large DNA.

Appendix

Analytically solvable simplified model

Interestingly, a fully analytical solution can be derived, if a further rather mild simplification is included in the model: let us start, as previously, the first phase from a coiled molecule hooking around an obstacle at a random site of its length. Then both arms of the molecule start to move in the direction of the electric field. We now assume that the two arms grow at a speed proportional to their contour length as was originally considered by Deutsch [23]:

$$l_1 = v_0 r t \tag{23}$$

$$l_2 = v_0 (1-r) t \tag{24}$$

Then, both arms get fully stretched at the same time, and the first phase is finished when $l = l_1 + l_2 = v_0 r t_1 + v_0(1-r)t_1$. The duration of the first phase, t_1 is

$$t_1 = \frac{l}{v_0} \tag{25}$$

and is no more a random variable [another way to consider this simplification is to remark that it is formally equivalent to a preaveraging of the fluctuating variable t_1 in Eq. (2)].

The position x_C of the centre of mass with regard to the obstacle is

$$x_C = \frac{l_1^2 + l_2^2}{2l} \tag{26}$$

so at time t_1 it reaches the distance d_1,

$$d_1 = x_C(t_1) = 0.5l\left[r^2 + (1-r)^2\right] \tag{27}$$

The distance d_1 is the same as previously, see Eq. (5). The second and third phases are not modified as compared with the original model.

The overall time cycle t is then

$$t = t_1 + t_2 + t_3 = \frac{2l}{v_0} + \frac{l}{2v_0} \cdot \ln\left(\frac{1}{|2r-1|}\right) = h(r) \tag{28}$$

while the overall distance d is, as previously

$$d = d_1 + d_2 + d_3 = l \tag{29}$$

Using now the same approach as previously described, one can derive the density function for the duration T of the whole cycle:

$$g(t) = \frac{2v_0}{l} \cdot \exp\left[-\frac{2v_0}{l} \cdot \left(t - \frac{2l}{v_0}\right)\right] \quad \text{for } t \in \left\langle \frac{2l}{v_0}, \infty\right) \tag{30a}$$

$$g(t) = 0 \text{ for } t \in \left\langle 0, \frac{2l}{v_0} \right) \tag{30b}$$

In this ''preaveraged'' scheme, though, the distribution $g(t)$ can be expressed explicitly and it surprisingly comes out as an exponential distribution $E(A,\delta)$ with parameters A and δ,

$$g(t) = \frac{1}{\delta} \cdot \exp\left(-\frac{t-A}{\delta} \right),$$

where $\delta = l/2v_0$ and $A = 2l/v_0$. The mean value of the $E(A,\delta)$ distribution is $\mu = A + \delta = (5/2) \cdot (l/v_0)$ and the variance $\sigma^2 = \delta^2 = l^2/4v_0^2$.

The average velocity of the movement, $\bar{v}$, is then

$$\bar{v} = \frac{L}{E(T_O)} = \frac{2}{5} v_0 \tag{31}$$

and the dispersion σ_t^2

$$\sigma_t^2 = n\sigma^2 = n \cdot \frac{l^2}{4v_0^2} = \frac{L}{4v_0^2} \cdot l \tag{32}$$

Corresponding space based dispersion and plate height then comes out as

$$\sigma_x^2 = \frac{L}{25} \cdot l \text{ and } H = \frac{\sigma_x^2}{L} = \frac{l}{25}, \tag{33}$$

respectively.

Those values differ only slightly from the original derivations. This agreement suggests that further extensions of the model to more complex situations could valuably be attempted in the simplified ''preaveraged'' frame. It also provides further support to our approach, by showing that the predictions of the model are rather insensitive to the details of the local mechanisms chosen to describe macromolecular motion, and that the model is rather robust.

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