Gel electrophoresis of DNA in moderate fields: The effect of fluctuations

A. N. Semenov*
Physics Department, Moscow State University, Moscow 117234, Russia

T. A. J. Duke and J.-L. Viovy
Laboratorie de Physico-Chimie Theoretique, Ecole Superieure de Physique et de Chimie Industrielles de Paris, 10 rue Vauquelin, 75231 Paris Cedex 05, France

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The reptation model for gel-electrophoresis of DNA in a stationary field is considered. It is shown that tube-length fluctuations are of primary importance for the macromolecular dynamics in the region of moderate fields. Coupling between fluctuations and the chain conformation provides a mechanism of macromolecular orientation. It is predicted that the mobility in the "plateau" region is *linearly* (rather than quadratically as in the classical biased reptation theory) proportional to the electric field. This approach is also applied to the mobility minimum problem and to gel electrophoresis in tight gels (with pore sizes smaller than the Kuhn segment of DNA). It is shown that tube-length fluctuations do not suppress the minimum of the mobility. However, they do shift the minimum to lower molecular weights: the minimum corresponds to $M \propto \epsilon^{-1}$ rather than to $M \propto \epsilon^{-2}$, as predicted by the classical model, where $\epsilon \ll 1$ is the reduced field. It is also predicted that electrophoresis in tight gels is characterized by a number of regimes with different power dependencies of the mobility on the reduced field. The theoretical results are supported by computer simulation data and experimental evidence.

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I. INTRODUCTION

Dynamics of a single long polymer chain in a gel is a problem of fundamental interest. In particular, it can serve as a test of general concepts of polymer dynamics, e.g., the reptation model [1,2], which is very likely to be applicable to the gel case (in contrast to polymer solution or polymer melt dynamics where the reptation mechanism is not so apparent [3]). The problem of polymer dynamics in a gel under external (electric) field also attracts considerable attention mainly in connection with gel electrophoresis of DNA, a powerful technique for separation of DNA fragments according to their molecular weight [4].

Understanding of polymer dynamics under an external field had been substantially improved several years ago by application of the reptation concept to the theory of gel electrophoresis of DNA [5–8]. The so-called biased reptation model (BRM), which had been developed to describe the problem, implies that the polymer chain is constrained by the gel structure to move in a "tube," the basic motion being field-driven curvilinear diffusion along the tube axis (Fig. 1). The model has been definitely successful in predicting the general behavior of DNA mobility as a function of molecular weight [7,8].

The most important directions of recent work in this area are (1) the inclusion of fluctuations of the drift velocity and tube-length fluctuations [8–15]; (2) the study of the effect of inhomogeneity of the media [16–19]; (3) field

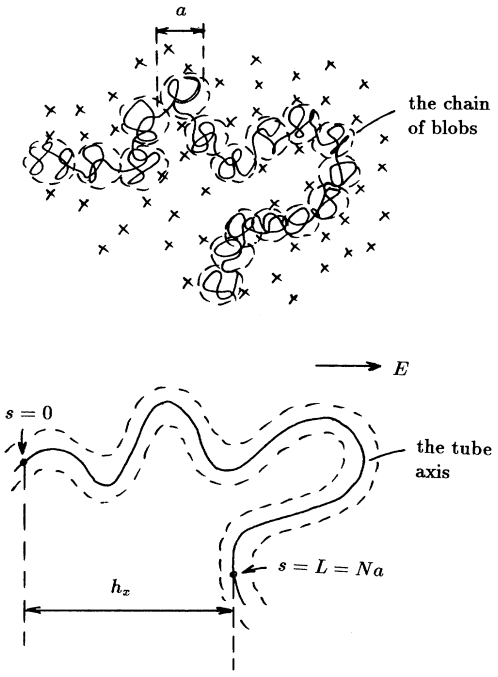

FIG. 1. DNA chain in a large-pore gel: the pore size $a \gg b$, where b is the Kuhn segment of DNA. The macromolecule is considered as a chain of blobs of size a, each blob being a nearly Gaussian coil consisting of approximately $(a/b)^2$ Kuhn segments. The gel subchains (schematically shown by crosses) confine the DNA chain in a tube of diameter $\sim a$; s is the coordinate along the tube axis, L is the total tube length, and h_x is the projection of the tube axis onto the field direction.

*Electronic address: sasha@ppl.phys.msu.su

inversion and cross-field electrophoresis and related problems [20–22]; (4) electrophoresis in strong fields, where a branched rather than a linear tube appears to be relevant (the branched structure can be considered as a result of the leakage of chain parts in the shape of "hernias" out of the original tube) [23–32].

The present paper is aimed at a detailed study of the effect of tube-length fluctuations only. We are not going to discuss or review any other effects and just assume that they are negligible. More specifically, we assume (i) that the gel is *homogeneous* on scales of an order or larger than macromolecular size, (ii) that the (electric) field acting on polymer links is uniform and stationary, (iii) that the field is weak enough for the effect of loops ("hernias") to be negligible. Yet the polymer chain dynamics even under these simplifying assumptions turns out to be rather complex [33]. We show that the effect of tube-length fluctuations results not merely in some small corrections but rather leads to an essential revision of a number of basic predictions of BRM.

The scope of the paper is the following. The next section is devoted to the description of BRM, and the main results obtained with the model. The approach called biased reptation with fluctuations (BRF) is outlined in Sec. III (the arguments are similar to those presented in Ref. [33]). The main result is that mobility of a long chain under constant field is *linearly* proportional to the field strength, see Eq. (18). The detailed quantitative analysis of the BRF is presented in Sec. IV; the results are compared with computer simulations data in Sec. V. The mobility minimum problem is discussed in Sec. VI. Here, we show that tube-length fluctuations shift the mobility minimum to the region of lower molecular weights [if the reduced field parameter, ϵ, defined by Eq. (3) is small]. The generalization of the main BRF results for tight (small porous) gels is considered in Sec. VII. We find a number of regimes where the mobility of a long DNA chain is proportional to ϵ, $\epsilon^{0.4}$, and ϵ^2 where ϵ is the reduced field.

II. BIASED REPTATION MODEL

Let us consider a polymer chain immersed in a gel, which is characterized by a single parameter, the average pore size a. The model assumes that the chain (DNA fragment) is entangled with the gel, i.e., the chain size, R is much larger than a; it is also assumed that the Kuhn segment of DNA, b is smaller than pore size: $R > a > b$. Thus, the DNA molecule can be considered as a flexible chain of blobs of size a, which is constrained to move in a virtual tube created by the gel environment (see Fig. 1). The tube length (the primitive path length [2]) is $L = Na$, where N is the number of blobs per chain ($N \gg 1$). The principle motion of DNA molecule is now one-dimensional (1D) curvilinear diffusion (Brownian motion) along the tube axis, lateral movements being forbidden. Complete conformational relaxation implies that the chain should move along the tube on a distance of the order of its length; thus the relaxation (disentanglement) time is [2,34]

$$\tau_{\rm dis} \sim L^2/D_{\rm 1D} = \tau_0 N^3, \tag{1}$$

where $D_{\rm 1D} = T/(\zeta N)$ is the curvilinear 1D diffusion constant, ζ is the effective friction coefficient per blob, and $\tau_0 = \zeta a^2/T$ is the blob time.

An electric field E induces additional directed drift of the chain provided that it is charged. The curvilinear drift velocity, $v_d = \dot{s}$ (here, s is a coordinate along the tube) is [6]

$$v_d = \epsilon \frac{h_x}{N\tau_0}, \tag{2}$$

where h_x is the projection of the end-to-end vector onto the field direction (see Fig. 1), ϵ is the reduced field parameter,

$$\epsilon = qEa/T, \tag{3}$$

and q is the effective charge per blob. Note that $\epsilon = E/E^{**}$ with $E^{**} = T/(aq)$.

Typical disentanglement time due to the drift is

$$\tau_d \sim \frac{L}{\langle v_d \rangle} \sim \frac{LN\tau_0}{\epsilon \langle |h_x| \rangle}. \tag{4}$$

Assuming Gaussian statistics, ($h_x \sim R = N^{0.5}a$), we find that the drift dominates over Brownian diffusion ($\tau_d < \tau_{\rm dis}$) if [36]

$$\epsilon > \epsilon_0 = N^{-3/2}. \tag{5}$$

Note that condition (5) is normally fulfilled in practice.

The center of mass velocity is

$$\dot{x} = v_d\, h_x/(Na). \tag{6}$$

Thus, electrophoretic mobility $\mu \equiv \langle \dot{x} \rangle / E$, which is of primarily importance for gel-electrophoresis experiments, is

$$\mu = \frac{\langle v_d\, h_x \rangle}{NaE} = \mu_0 \frac{\langle h_x^2 \rangle}{N^2 a^2}, \tag{7}$$

where $\mu_0 = q/\zeta$. So the mobility is determined by the tube conformation. If the field is weak enough, then the chain statistics is nearly Gaussian [37]: $\langle h_x^2 \rangle = Na^2/3$, and [6]

$$\mu/\mu_0 = 1/(3N). \tag{8}$$

However, stronger electric fields would induce some orientation of the leading blob, so that the tube will be elongated in the field direction (Fig. 2).

Slightly different treatments of the orientation effect were proposed [7,8] with similar results. The orientation distribution of the leading blob (see Fig. 2) was assumed to be governed by Boltzmann factor $\exp(-\epsilon \cos\theta)$ [7], where θ is the angle between the field direction and end-blob orientation. The mean orientation (the order parameter) of the end blob, which is then transferred by the drift to any other blob is

$$\eta \equiv \langle \cos\theta \rangle = \coth\epsilon - 1/\epsilon, \ \sim \epsilon \text{ for } \epsilon \ll 1. \tag{9}$$

Neglecting the Gaussian component of the end-to-end

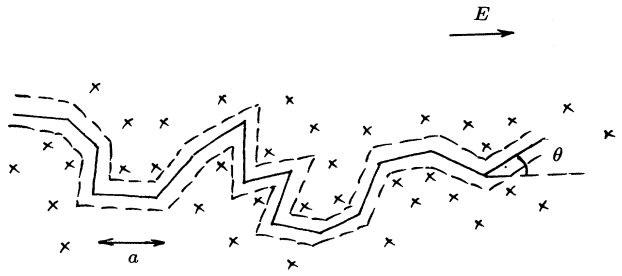

FIG. 2. The chain in a strong enough electric field, E, is elongated along the field direction (x axis); θ is the angle between the chain head segment and the field direction. The orientational order parameter is $\eta = \langle \cos\theta \rangle$.

vector, we find the mean (absolute) value of the end-to-end projection,

$$\langle |h_x| \rangle = Na\eta \sim Na\epsilon \quad \text{for } \epsilon \ll 1. \tag{10}$$

Clearly the effect of orientation is important if $|h_x| > R = N^{0.5}a$, i.e., if $\epsilon > N^{-0.5}$. Using eqs. (7, 10), we get [7]

$$\mu/\mu_0 \sim \begin{cases} N^{-1}, & N < N^* \quad (11a) \\ \epsilon^2, & N > N^*,\ \epsilon < 1 \quad (11b) \end{cases}$$

Here, $N^* \sim \epsilon^{-2}$ is the crossover chain length. Note that the crossover field $\epsilon^* \sim N^{-0.5}$ is well inside the region $\epsilon \gg N^{-3/2}$, where the Brownian longitudinal diffusion is negligible in comparison with the electrophoretic drift.

So the mobility as a function of molecular weight saturates at some field-dependent level, thus, reducing the resolution of electrophoretic technique in high molecular weight region. Although this behavior is definitely confirmed in experiments, the predicted field dependence of the mobility ($\mu \sim \epsilon^2$) is in rather poor agreement with experimental data [7,39]. In the next two sections, another theory for molecular orientation during electrophoresis is proposed. It is shown that tube-length fluctuations significantly enhance the orientation effect of the field leading to a qualitatively new dynamic behavior. One of the main results of the theory is the prediction of linear dependence of saturated mobility vs field ($\mu \sim \epsilon$), which is in much better agreement with experiments and computer simulations (see Sec. V).

One of the important successes of BRM is an explanation of the "band inversion" phenomenon [8–10]. In Sec. VI, we will show that although the general qualitative arguments for band inversion (see, e.g., [14]) based on BRM are correct, the effect of tube-length fluctuations is also very essential here and leads to a qualitatively different description of minimum of the mobility as a function of molecular weight.

III. THE BRF MODEL

The model of biased reptation with fluctuations (BRF) [33] takes into account that actual motion of the chain is not merely an electrophoretic drift along the tube axis but rather is a superposition of the drift and 1D Rouse motion. The latter results, in particular, in fluctuations of the chain density along the tube and in tube-length fluctuations. The relaxation time of a chain section consisting of m segments (blobs) is of order

$$\tau(m) \sim \tau_0 m^2.$$

During this time, the blobs in the section will move (along the tube) on a distance of order [40]

$$\Delta s(m) \sim a m^{0.5}.$$

Thus, the typical displacement of a blob along the tube during time t (here we assume that t is shorter than the Rouse time $\tau_R = \tau_0 N^2$) is [2]

$$\Delta s_f \sim a(t/\tau_0)^{1/4}. \tag{12}$$

The fluctuation, Δs_f for short enough time intervals is surely larger than the displacement due to the drift, $\Delta s_d \simeq v_d t$. On these short time scales ($t < t^*$, where t^* is estimated below) any blob, and, in particular, the leading-end blob rapidly *fluctuates* exploring different tube paths rather than moving with a constant velocity [Fig. 3(a)]. The typical length of the terminal sec-

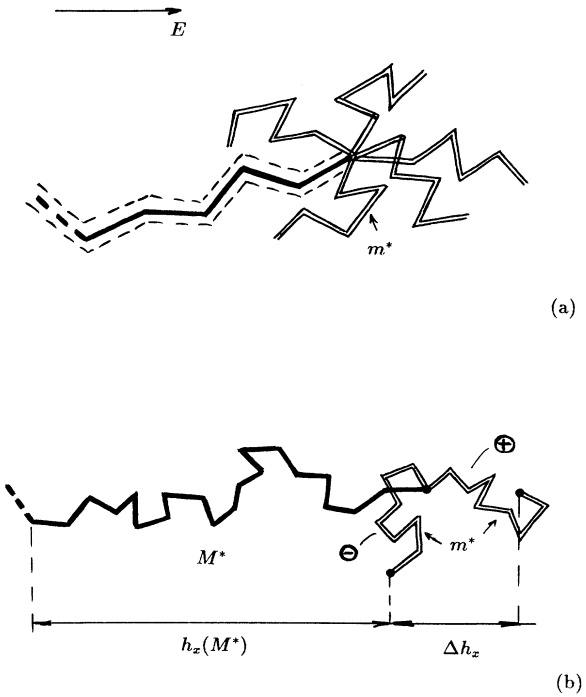

FIG. 3. (a) Alternative conformations that are being explored by the terminal section of the chain. The typical length of the rapidly fluctuating terminal section of the tube, shown in white, is of order of $s^* = m^* a$. The permanent tube part is shown in black. (b) The head part of the tube, M^*, which moves cooperatively during a time step, $\Delta t \sim t^*$. Two distinct conformations of the terminal m^* subchain (shown in white) correspond to forward (+) and backward (−) steps.

tion of the tube that can be reentered (and created once more) with these fluctuations is $s^* \sim \Delta s_f(t^*)$, where the crossover time t^* can be estimated using the condition

$$\Delta s_f(t^*) \sim \Delta s_d(t^*). \tag{13}$$

Using Eq. (12), we thus get

$$t^* \sim \left(\frac{a^4}{\tau_0 v_d^4}\right)^{1/3}, \quad s^* \equiv m^* a \sim \left(\frac{a^4}{\tau_0 v_d}\right)^{1/3}. \tag{14}$$

Now, we come to the central point of the BRF approach. We state that it is the terminal section of $m^* = s^*/a$ blobs rather than single end blob that must be in a quasiequilibrium with the electric field. Therefore, the terminal section must be slightly stretched by the total electric force $F = qEm^*$, the orientational order parameter being of order

$$\eta \equiv \langle \cos\theta \rangle \simeq \frac{Fa}{T} \simeq m^* \epsilon. \tag{15}$$

The order parameter (15) is much larger (by a factor of m^*) than that predicted by BRM [see (9)] using the assumption that only the orientation of the end blob is effectively influenced by the field.

Taking into account that $|h_x| \simeq Na\eta$ and using Eqs. (2) and (15), we get $m^* \sim \epsilon^{-0.5}$ and

$$\eta = \langle \cos\theta \rangle \sim \epsilon^{0.5}. \tag{16}$$

Comparing the last result with Eq. (9), we see that tube-length fluctuations significantly enhance the orientation order for weak fields ($\epsilon \ll 1$). Equation (16) is valid if (i) the Gaussian component of the end-to-end vector is negligible ($R = N^{0.5}a \ll Na\eta$) and (ii) the time t^* associated with backward movements of the terminal chain part is much smaller than the Rouse time τ_R . Both these conditions reduce to $N \gg N^*$ with

$$N^* \sim \epsilon^{-1}. \tag{17}$$

For $N \lesssim N^*$ both tube-length fluctuations and orientation effect of the field becomes negligible, the mobility being determined by the classical relation (8). Thus, using Eqs. (7) and (16) we get

$$\mu/\mu_0 \sim \begin{cases} N^{-1} & \text{for} \quad N < N^* \quad \text{(18a)} \\ \epsilon & \text{for} \quad N > N^*, \ \epsilon < 1. \quad \text{(18b)} \end{cases}$$

Note that the mobility of long chain varies *linearly* with the field in contrast with the BRM prediction (11); also, the limiting chain length N^* is inverse proportional to ϵ.

The derivation of the main results, Eqs. (16)–(18), was based on a "quasiequilibrium" ansatz for chain orientation. Below, we are going to show that the main results can be also obtained without this ansatz, using purely dynamical arguments. Let us concentrate on a 1D motion of the leading end of the chain (motion along the primitive path, or the tube) which is described by the function $s(t) \equiv s(0,t)$. Let us smooth the function over a time scale Δt. If $\Delta t \ll t^*$ [the time t^* is determined by Eq. (13)] then the motion can be described as a chaotic sequence of forward (along the tube) and backward movements (steps) which are due to tube-length fluctuations. On the other hand, for $\Delta t \gg t^*$, nearly all steps must be directed forward, with positive Δs. Let us choose $\Delta t \sim t^*$: now the chain end dynamics can be pictured as a sequence of mainly forward steps, yet with a noticeable fraction (say, 1/3) of backward steps. In other words, for $\Delta t \sim t^*$ each forward step of the leading end is tried a few (say, two) times. Now let us turn off completely the *direct* orientation effect of the field, which was considered by BRM as the main effect [see Eq. (9)]. As we demonstrated above, the direct effect is negligible in comparison with the fluctuation effect [compare Eqs. (9) and (16)]. Thus, we assume that each forward step implies a creation of a completely random (Gaussian) part of the tube consisting of $m^* = s^*/a$ segments (blobs). The real-space displacement of the end segment, Δx during the step should be of order of Gaussian size of m^* subchain:

$$\Delta x \sim d \equiv a\,(m^*)^{0.5}. \tag{19}$$

Note that Δx might be positive or negative with *a priori* equal probabilities. For simplicity, let us assume that Δx can take two values,

$$\Delta x = \pm d.$$

Once created, a different part of the tube can be destroyed by a subsequent backward step. An important point is that the probability of this backward step does *depend* on Δx. The probability is higher for $\Delta x < 0$ since the effective electrophoretic force is proportional to the end-to-end projection, h_x [see Eq. (2)] and, thus, the effective drift velocity (in forward direction) is locally smaller for the case $\Delta x < 0$. Thus, the probability p_+ that a step with $\Delta x > 0$ becomes permanent (will not be destroyed by subsequent backward step) must be a bit higher than the probability p_- for $\Delta x < 0$. Obviously the mean projection of the m^* terminate subchain onto the field direction is of order $d\Delta p$, where $\Delta p = p_+ - p_-$:

$$\langle \Delta x \rangle \sim d\ \Delta p. \tag{20}$$

The difference $\Delta p = p_+ - p_-$ must be of order of difference of effective curvilinear drift velocities for these two cases:

$$\Delta p \sim \frac{\Delta v_d}{v_d}, \tag{21}$$

where

$$v_d = \epsilon \eta a/\tau_0 \tag{22}$$

is given by Eq. (2). Note that here Δv_d is the difference between *local* curvilinear drift velocities of the chain end for the cases $\Delta x > 0$ and $\Delta x < 0$. On the time scale of one step, $\Delta t \sim t^*$ which is assumed to be much shorter than the Rouse time of the whole chain, $\tau_R = \tau_0 N^2$, distant chain parts move virtually independently, so that more oriented (along the field direction) parts move faster. On the other hand, a chain part consisting of M blobs should move cooperatively

if it's longest relaxation time $\tau(M)$ is shorter than Δt. Thus, the relevant length of the head part of the chain which must move cooperatively is given by the condition $\tau(M) = \tau_0 M^2 \sim \Delta t$, so that the number of blobs in the part is

$$M^* \sim (t^*/\tau_0)^{0.5}. \tag{23}$$

Therefore, Δv_d should be proportional to the difference between the end-to-end projections, Δh_x, of the head part of the chain (consisting of M^* blobs) for the cases of positive and negative "orientations" of the terminate m^* subchain [see Fig. 3(b)]:

$$\Delta v_d \sim \epsilon \frac{|\Delta h_x|}{M^* \tau_0} \sim \epsilon \frac{d}{M^* \tau_0}, \tag{24}$$

where we take into account that $|\Delta h_x| \sim d$. Now using Eqs. (20)–(24), we easily get

$$\langle \Delta x \rangle \sim \frac{d^2 \epsilon}{v_d M^* \tau_0}, \tag{25}$$

so that the order parameter is

$$\eta = \frac{\langle \Delta x \rangle}{a m^*} \sim \frac{(\epsilon \eta)^{2/3}}{\eta}. \tag{26}$$

After simple transformations, we finally obtain Eq. (16) which implies the final result for mobility, Eqs. (18).

Note that more generally, we can take into account both the fluctuation orientation effect and the direct orientation [Eq. (9)]: $\eta \sim \epsilon^{0.5} + \text{const} \times \epsilon$; however, for weak fields, $\epsilon \ll 1$, the second (direct) term could be neglected.

IV. QUANTITATIVE ANALYSIS OF THE BRF MODEL

Let us start with the basic equation for the 1D curvilinear dynamics of the chain:

$$\zeta \, \partial s(m,t)/\partial t = k \, \partial^2 s/\partial m^2 + qE \, \nu(m,t) + \Xi(m,t), \tag{27}$$

where m is the number of a blob (along the chain), $0 < m < N$, $s(m,t)$ is the position of the mth blob along the tube at the moment t, k is the effective longitudinal elastic modulus of the chain in the tube, $k \sim T/a^2$, $\nu(m,t)$ is the x component (projection onto the field direction) of the unit vector tangential to the tube axis at the point m, $\Xi(m,t)$ is the random thermal force acting on the segment m at the moment t. The random force is characterized by the correlation function,

$$\langle \Xi(m,t) \Xi(m',t') \rangle = 2T\zeta \, \delta(m-m') \, \delta(t-t').$$

Using reduced variables,

$$u = s/a, \quad \tau = t/\tau_0, \quad \kappa = ka^2/T, \quad \tau_0 = \zeta a^2/T, \tag{28}$$

we can rewrite Eq. (27) as

$$\partial u(m,\tau)/\partial \tau = \kappa \partial^2 u/\partial m^2 + f(m,\tau) + \xi(m,\tau), \tag{29}$$

where

$$f(m,\tau) = \epsilon \, \nu(m,\tau), \tag{30}$$

$$\langle \xi \rangle = 0,$$
$$\langle \xi(m,\tau) \xi(m',\tau') \rangle = 2 \, \delta(m-m') \, \delta(\tau-\tau'). \tag{31}$$

Note that κ is of order of unity.

Following [2], we assume that an effective constant force (of entropic nature) is applied to the chain ends; the force keeps the chain stretched along the tube. Thus, the boundary conditions for Eq. (29) are

$$\partial u/\partial m_{|m=0} = \partial u/\partial m_{|m=N} = \text{const} = 1. \tag{32}$$

We assume that the reduced field ϵ is small ($\epsilon \ll 1$); therefore, we expect that $\eta = \langle \nu(m,t) \rangle$ is also small. On the other hand, the chain is assumed to be long enough so that it does not retain its Gaussian conformation but rather is aligned along the field:

$$\langle h_x \rangle = Na\eta \gg N^{0.5} a. \tag{32'}$$

Under this condition, Eq. (7) can be rewritten as

$$\mu/\mu_0 = \eta^2. \tag{33}$$

Thus, in order to get the mobility, we have to analyze the dependence of the order parameter, η on ϵ.

The 3D dynamics of the polymer chain is completely defined by (i) 1D curvilinear reptation governed by Eqs. (29)–(32) and (ii) orientational properties of the (end) parts of the tube created by reptation. In the absence of the field, the end segments are oriented at random; the field induces some *a priori* orientation of these segments. We are going to show, however, that this direct effect of the field is negligible in comparison with *a posteriori* orientational order resulting from the coupling with 1D curvilinear motion. So, in the following consideration, we will completely neglect the direct effect of the field on the end parts of the chain.

From the consideration of Sec. III, one can gather that the fluctuation orientation effect is due to the coupling between chain orientation and the effective electric force which is locally proportional to the projection of a chain segment onto the field direction, $\nu = \cos\theta$. It is fluctuations of ν that are responsible for the orientation effect. We will show that these fluctuations can be considered as small perturbations.

The driving force for electrophoretic drift, f can be represented as a sum of two terms:

$$f(m,\tau) = f_0 + f_1(m,\tau), \tag{34}$$

where

$$f_0 = \langle f(m,\tau) \rangle = \epsilon \frac{\langle |h_x| \rangle}{Na} = \epsilon\eta \tag{35}$$

is the average force and $f_1 = f - f_0$. Thus, we represent the external force as a sum of an unknown con-

stant and some perturbation which depends on the segment position m, time τ and on the chain conformation Γ: $f_1 = f_1(m,\tau|\Gamma)$. Note that for the stationary electric field considered below f_1 does not *directly* depend on time τ, however, the force is still time dependent via time dependence of the chain conformation Γ.

At the present stage, we do not assume anything about the chain conformation. Thus, Eq. (34) can be considered as the definition of f_1 . Note also that no orientational order is induced without the perturbation (random contribution f_1) since the averaged force f_0 induces curvilinear drift with constant velocity $v_0 = f_0$, but does not imply any preferred direction. Therefore, if the random contribution were absent ($f_1 \equiv 0$), the chain would retain its equilibrium Gaussian statistics.

Now let us consider f_1 as a small perturbation (for a given averaged force, f_0) and analyze the first order of the corresponding perturbation scheme. In other words, let us consider a linear response of the chain conformation to the force f_1 (in Appendix A we show that the second-order and higher-order effects are negligible). Any quantity (in particular, the orientational order parameter, η) could be considered as a functional of $f_1(m,\tau|\Gamma)$. For $f_1 \equiv 0$ the order parameter is zero. Therefore, it is natural to expect that in the main approximation (for small f_1), the order parameter should be linear in f_1.

Using Eqs. (30) and (34), we write

$$f_1(m,\tau|\Gamma) = \epsilon\,\nu(m,\tau) - f_0. \tag{36}$$

The last equation can be formally represented as

$$f_1(m,\tau|\Gamma)$$

$$= \int_0^N dm' \int_{-\infty}^{\infty} d\tau'\; r(m',\tau')\, F_1(m,\tau;m',\tau'|\Gamma), \tag{37}$$

where

$$F_1(m,\tau;m',\tau'|\Gamma) = [\epsilon\,\nu(m',\tau') - f_0]\,\delta(m-m')\,\delta(\tau-\tau') \tag{38}$$

and the function $r(m',\tau') \equiv 1$. Equation (37) suggests a generalization of the problem for an arbitrary modulation function $r(m,\tau)$, which is assumed to be *a priori known*. The local order parameter,

$$\eta(m,\tau) \equiv \langle \nu(m,\tau) \rangle\,,$$

(here $\langle\,\rangle$ denote ensemble averaging) can be formally considered as a functional of the modulation "law," $r(m,\tau)$. In the main (linear) approximation, the relationship can be generally represented as

$$\eta(m,\tau) = \int dm'\,d\tau'\,K(m,\tau;m',\tau')\; r(m',\tau'). \tag{39}$$

The kernel $K(\cdots)$ represents a linear response for the segment m at the moment τ, to the local (random) force acted on the segment m' at τ'. Obviously, the function K depends only on the difference $\tau - \tau'$.

Let us *define* the function,

$$H(m',\tau) = \frac{1}{N}\int_0^N dm\; K(m,\tau;m',0), \tag{40}$$

which gives the averaged along the chain order parameter at τ induced by the "δ" force,

$$F_1(m,\tau;m',0|\Gamma) = [\epsilon\,\nu' - f_0]\;\delta(m-m')\,\delta(\tau) \tag{41}$$

which was applied to the m' segment at $\tau' = 0$ [here, $\nu' \equiv \nu(m',0)$]. Using Eq. (39) with $r(m,\tau) \equiv 1$, we thus get the total averaged order parameter:

$$\eta = \int_0^{\infty} d\tau \int_0^N dm'\; H(m',\tau). \tag{42}$$

Thus, in order to get the order parameter, we should consider the effect of a small instantaneous perturbation, Eq. (41).

While considering the function $H(m',\tau)$, we should take into account that the perturbation F_1, Eq. (41), is zero for $\tau < 0$. Therefore, the chain conformation is Gaussian for negative τ, so that $H(m',\tau) \equiv 0$ for $\tau < 0$. Also, the orientation of the m' segment at $\tau = 0$ should be quite random (isotropically distributed), implying uniform distribution density P for the x projection, $\nu' = \nu(m',0)$, of the unit vector tangential to the chain at the point m' at $\tau' = 0$:

$$P(\nu') = 0.5 \quad \text{for} \quad -1 < \nu' < 1. \tag{43}$$

Let $H(m',\tau|\nu')$ be the subaveraged orientation order parameter induced by the force F_1 , Eq. (41), with a given initial "orientation" of the m' segment, $\nu' = \nu(m',0)$. Obviously,

$$H(m',\tau) = \int d\nu'\,P(\nu')\,H(m',\tau|\nu'). \tag{44}$$

Note that at the moment $\tau = -0$ all segments (apart from the segment number m' which we will call the "oriented segment") must be randomly oriented. Therefore, the initial averaged along the chain order parameter is [41]

$$H(m',0|\nu') = \nu'/N. \tag{45}$$

The parts of the tube that are being created at the head of the chain or parts that are being lost at the tail are also oriented at random. Therefore, the mean order parameter can change only if the initially "oriented segment" is renewed [Fig. 4(a)] or removed at the tail [Fig. 4(b)].

Therefore,

$$H(m',\tau|\nu') = \frac{\nu'}{N}\,[1 - W(m',\tau|\nu')] \times\Theta\left(\tau - \left(\frac{N-m'}{f_0}\right)\right), \tag{46}$$

where $W(m',\tau\,|\nu')$ is the probability that initially oriented m' segment has been renewed during the time τ; $\Theta(\,)$ is the Heaviside function, and $(N-m')/f_0$ is the

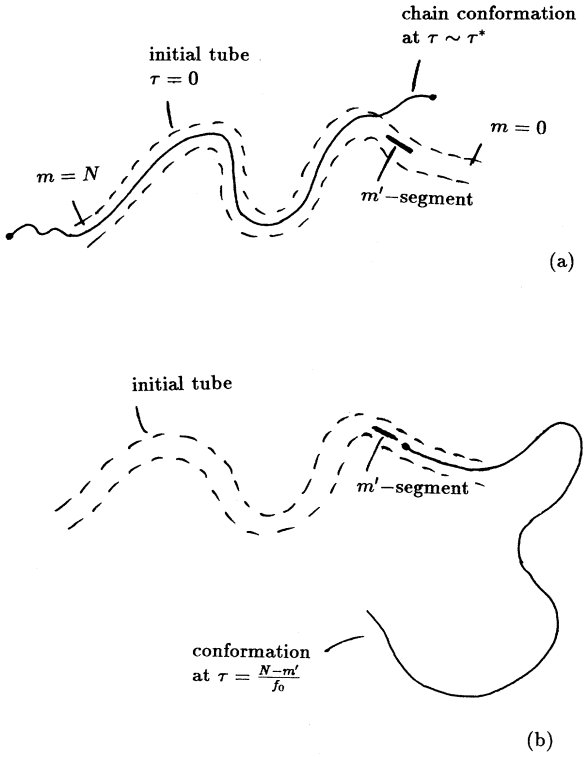

FIG. 4. Two possibilities to renew the head segment: (a) by backward motion on $m > m'$ steps along the tube; (b) by creeping along the tube on the distance $\Delta s > (N - m')a$.

mean time needed for the tail to drift up to "initially oriented" segment of the tube (here, we enumerate segments from the head, $m = 0$ to the tail, $m = N$).

In order to renew the oriented m' segment the chain end should move in a backward direction (along the tube) on the distance m' (see Fig. 4). This is almost impossible if m' is large enough. Using simple arguments of Sec. III [see derivation of Eq. (14)], we find that the backward motion is possible if

$$m' \lesssim m^* = f_0^{-1/3}\kappa^{-1/3}, \quad \tau \lesssim \tau^* = f_0^{-4/3}\kappa^{-1/3}. \quad (47)$$

Note that Eq. (47) is in agreement with (14), and $m^* = s^*/a$, $\tau^* = t^*/\tau_0$.

Thus, the function $\frac{\partial}{\partial\tau} W(m', \tau|\nu')$ is not negligible only for $m' \lesssim m^*$. Moreover, this function should tend to some constant limit for $\tau \gg \tau^*$:

$$W(m', \tau|\nu') \to w(m'|\nu') \equiv W(m', \infty|\nu') \text{ for } \tau \gg \tau^*. \quad (48)$$

The function $W(m', \tau|\nu')$ could be expanded in powers of the external force, $\epsilon\nu' - f_0$, which is essentially proportional to ϵ. In the first order, we write

$$W(m', \tau|\nu') = W_0(m', \tau) + [\epsilon\nu' - f_0]\, W_1(m', \tau) + O\left((\epsilon\nu' - f_0)^2\right), \quad (49)$$

where $W_0(m', \tau)$ is the probability of retaining (m'-segment) for reptation with constant drift velocity f_0.

Taking into account that $m' \lesssim m^* \ll N$ and $t(m') \gg \tau^*$, these conditions are ensured by Eqs. (5) and (32′)] we get [see Eqs. (46) and (48)]

$$\int_0^\infty d\tau\; H(m', \tau|\nu') \simeq \frac{\nu'}{f_0}\left[1 - w(m')\right]. \quad (50)$$

Using Eqs. (42)–(50), we get the general expression for the order parameter:

$$\eta = -\frac{\epsilon}{f_0}\int_0^N dm' \int d\nu'\, P(\nu')\, (\nu')^2\, w_1(m') = -\frac{\epsilon}{3f_0}\int_0^N w_1(m')\, dm', \quad (51)$$

where $w_1(m') \equiv W_1(m', \infty)$. Note that the upper limit of the last integral in the right hand side of Eq. (51) can be taken as infinity since $w_1(m') \to 0$ for $m' \gg m^*$.

Let us consider the 1D curvilinear motion of the leading chain end starting at arbitrary moment denoted as $\tau = 0$. On the time scale of order τ^* the end moves back and forth, thus creating and renewing the end parts of the tube. Obviously the maximum backward displacement of the end (for $0 < \tau < \infty$) must be achieved at some finite τ. Let $D(u, \tau)\, d\tau$ be the probability that the maximum backward displacement along the tube exceeds u and is achieved in the infinitesimal time interval $(\tau, \tau + d\tau)$. Obviously,

$$w(u|\nu') \equiv W(u, \infty|\nu') = \int_0^\infty D(u, \tau)\, d\tau. \quad (52)$$

Let $u_0(m, \tau)$ be a realization of the unperturbed process with constant drift velocity, i.e., a solution of Eq. (29) with $f(m, \tau) \equiv f_0$. The effect of the perturbative force F_1, Eq. (41), should result in additional displacement, $\Delta u(m, \tau)$ of the chain segments. Solving Eqs. (29) and (32) with $f = f_0 + F_1$, where F_1 is given by Eq. (41), we get

$$u(m, \tau) = u_0(m, \tau) - \Delta u(m, \tau),$$

$$\Delta u(m, \tau) = \frac{\epsilon\nu' - f_0}{\sqrt{4\pi\kappa\tau}}\left\{\exp\left[\frac{-(m - m')^2}{4\kappa\tau}\right] + \exp\left[\frac{-(m + m')^2}{4\kappa\tau}\right]\right\}. \quad (53)$$

Thus, in the first approximation, the end position is

$$u(0, \tau) = u_0(0, \tau) - \Delta u(0, \tau),$$

so that

$$w(u|\nu') = \int_0^\infty D_0[u + \Delta u(0, \tau), \tau]\; d\tau + O\left[(\epsilon\nu' - f_0)^2\right], \quad (54)$$

where $D_0(\cdots)$ corresponds to the unperturbed process u_0.

Substituting Eq. (53) into Eq. (54), we get

$$w(m'|\nu') = w_0(m') + [\epsilon\,\nu' - f_0]\; w_1(m') + O\left[(\epsilon\nu' - f_0)^2\right], \tag{55}$$

where

$$w_0(m') = \int_0^\infty D_0(m',\tau)\,d\tau$$

and

$$w_1(m') = \int_0^\infty \frac{\partial D_0(m',\tau)}{\partial m'}\,(\pi\kappa\tau)^{-0.5}\,\exp\left[-\frac{(m')^2}{\kappa\tau}\right]d\tau. \tag{56}$$

Taking into account that in Eq. (53) typical $m' \sim m^* \sim f_0^{-1/3}$ and $\tau \sim \tau^* \sim f_0^{-4/3}$ so that $m'^2/\tau \sim f_0^{2/3} \ll 1$, we simplify Eq. (56) as (see Appendix A for more details)

$$w_1(m') \simeq \int_0^\infty \frac{\partial D_0(m',\tau)}{\partial m'}\,(\pi\kappa\tau)^{-0.5}\,d\tau. \tag{57}$$

Using Eq. (51), we get, finally,

$$\eta \simeq \frac{\epsilon}{3\kappa^{0.5} f_0}\int_0^\infty D_0(0,\tau)\;(\pi\tau)^{-0.5}\,d\tau. \tag{58}$$

The integral in the right hand side of Eq. (58) is a function of only two parameters, f_0 and κ:

$$\int_0^\infty D_0(0,\tau)\;(\pi\tau)^{-0.5}\,d\tau = I(f_0,\kappa). \tag{59}$$

Note that $D_0(0,\tau)$ is essentially independent of N since the typical displacement, m^*, is much smaller than N. Using scaling (self-similar) properties of Eq. (29), we get the following scaling relation:

$$I(f_0,\kappa) = f_0^{2/3}\,\kappa^{1/6}\,I(1,1). \tag{60}$$

Here, $I(1,1)$ is a numerical constant.

Using Eqs. (35), (58)–(60), we get

$$\eta \simeq \left[\frac{I(1,1)}{3}\right]^{3/4}\kappa^{-1/4}\,\epsilon^{1/2}. \tag{61}$$

Equation (61) is the main result of this section. Using this equation and Eqs. (8) and (33), we find the following mobility behavior:

$$\mu/\mu_0 = \begin{cases} 1/(3N) & \text{for} \quad N \ll N^* & \text{(62a)} \\ C\,\kappa^{-0.5}\,\epsilon & \text{for} \quad N \gg N^*,\ \epsilon \ll 1, & \text{(62b)} \end{cases}$$

where

$$N^* = \frac{1}{3C\kappa^{-0.5}\epsilon} \tag{63}$$

and

$$C = \left[\frac{I(1,1)}{3}\right]^{3/2}. \tag{64}$$

Note that Eq. (62) is in apparent agreement with Eq. (18). Note also that an increase of the reduced longitudinal elastic modulus, κ, should suppress tube-length fluctuations and, thus, result in a decrease of the order parameter and the mobility.

Now, we have to evaluate the only unknown quantity $I(1,1)$. It hardly could be calculated analytically. However, this universal constant could be easily obtained from computer simulations corresponding to the following system of equations [compare with Eqs. (29)–(32)]:

$$\partial u/\partial t = \partial^2 u/\partial m^2 + 1 + \xi(m,t),\ 0 < m < \infty, \tag{65}$$

$$\partial u/\partial m_{|m=0} = 0,$$

$$\langle\xi\rangle = 0;\ \ \langle\xi(m,t)\,\xi(m',t')\rangle = 2\,\delta(m-m')\;\delta(t-t').$$

One can easily see that according to the definition, Eq. (59),

$$I(1,1) = \pi^{-0.5}\;\left\langle \tilde{t}^{\,-0.5}\right\rangle, \tag{66}$$

where $\tilde{t}$ is the time corresponding to the absolute minimum of $u(0,t)$ during semi-infinite period $0 < t < \infty$, with $t = 0$ being an arbitrary moment. Here $\langle\,\rangle$ means ensemble-averaged value with "equilibrium" initial conditions. The numerical result is $I(1,1) = 1.9 \pm 0.1$, so that

$$C \simeq 0.50. \tag{67}$$

In order to confirm the validity of the whole perturbation scheme, we need to estimate higher-order (second-order) corrections in random part of the driving force, f_1, and compare them to the first-order result, Eq. (61). The corresponding analysis is performed in the Appendix A.

V. COMPARISON WITH EXPERIMENTS AND COMPUTER SIMULATIONS

The relationship of the BRF predictions and experimental data for double stranded DNA in agarose gels has been already discussed in Ref. [39]. Here we review the discussion, trying to be as quantitative as possible.

Two regimes of reptation implied by Eq. (62) can be observed if $\epsilon \equiv E/E^{**} \ll 1$ and $N \sim 1/\epsilon$, i.e., for long enough DNA in weak enough fields. Thus low-field data of Heller *et al.* [43] and Slater *et al.* [42] are suitable for the comparison.

First, we should map the theoretical quantities N, ϵ, etc., to the experimental parameters — the Kuhn segment of DNA, b; the total contour length of the DNA fragment, L_0; the charge per Kuhn segment, q_0; and the field E. To do this, we use Eq. (3) and the following simple relations for the mean end-to-end distance,

$$\langle R^2\rangle = Na^2 = L_0 b, \tag{68}$$

and the total charge,

$$Q = q_0 L_0/b = Nq.$$

Taking into account that $\epsilon = E/E^{**}$ with $E^{**} = T/(aq)$, we thus get

$$N = L_0 b/a^2;\ q = q_0 a^2/b^2;\ E^{**} = \frac{Tb^2}{q_0 a^3}.$$

Using the experimental values for DNA in 1% agarose gel,

$$b \simeq 100\text{ nm};\ a \simeq 300\text{ nm};\ q_0 \simeq 30e, \tag{69}$$

we get

$$N \simeq \frac{L_0}{900\text{ nm}} \simeq \frac{L_0}{3000\text{ bp}};\ E^{**} \simeq 10\,\text{V/cm},$$

where bp means "base point."

The last theoretical parameter, the reduced longitudinal elastic modulus of the chain of blobs $\kappa = ka^2/T$, is not directly measurable. In order to calculate κ we note that the mean-square deviation of the primitive path length is [44]

$$\langle (\delta N)^2 \rangle = Ta^2 N/k = \frac{N}{\kappa}.$$

On the other hand, the model of independent slack or taut segments [see below, Eq. (71)] assumes that

$$\langle (\delta N)^2 \rangle = N_a \frac{f}{1+f}\frac{1}{1+f} = \frac{Nf}{1+f}.$$

Therefore,

$$\kappa = \frac{1+f}{f}. \tag{70}$$

Let us consider two sets of experimental observations of Heller *et al.* for 1% agarose gel: (1) for the weakest field, $E = 0.13\,\text{V/cm}$ and (2) for the longest DNA with $L_0 \simeq 50\,\text{kbp}$. For the first case, the mobility was observed to be inversely proportional to the molecular weight in the range $L_0 = 5$–$20\,\text{kbp}$, in agreement with Eq. (62a) [this equation can be applied since $L_0 < L_0^* \simeq 200\,\text{kbp}$, the numerical value of L_0^* being estimated using Eqs. (63) and (70)]. For the second case, the mobility varies linearly with the field in the range $E = 0.2$–$1.0\,\text{V/cm}$, in agreement with Eq. (62b) (note that $L_0 \simeq 50\,\text{kbp}$ corresponds to $\epsilon^* \sim 0.02$ and $E^* \sim 0.5\,\text{V/cm}$).

In order to test the BRF predictions more quantitatively, we have to turn to computer simulations results where the reduced modulus κ can be calculated precisely. Let us reconsider the results of careful numerical studies, reported in Refs. [29,35,39]. The model, that was described in detail in Ref. [35] represents a DNA molecule in a gel as a sequence of N_a segments of contour length a; each segment might be in two states: either slack or taut (coiled or extended). Only taut segments contribute to the tube (primitive path) length. If f is the ratio of the statistical weights of coiled and extended segments, then the number of primitive path segments is $N = N_a/(1+f)$. Taking into account that the total contour length is $L_0 = N_a a$, and using Eqs. (68) and (69), we get

$$f = a/b - 1 \simeq 2;\ N = \frac{N_a}{1+f} \simeq N_a/3. \tag{71}$$

The natural reduced field parameter used in computer simulations is defined as

$$\epsilon_a = q_a E a/T,$$

where $q_a = Q/N_a$. Using Eqs. (71), we get the following mapping between reduced fields used in the BRF theory and in computer simulations:

$$\epsilon = (1+f)\,\epsilon_a. \tag{72}$$

Using Eqs. (71) and (70) we rewrite the BRF prediction for the reduced mobility, Eq. (62b), as

$$\mu/\mu_0 = C\,[f(1+f)]^{0.5}\,\epsilon_a \simeq 1.2\,\epsilon_a \tag{73}$$

(for $N \gg N^*$).

The field dependencies of the reduced mobility obtained in computer simulations [39] for different chain lengths are shown in Fig. 5.

The BRF theoretical predictions, Eq. (73), are also shown in Fig. 5 by solid line. Note that for small ϵ_a, Eq. (73) is in a good agreement with computer simulation data (for $\epsilon N > 1$). However, for larger ϵ_a this equation somewhat overestimates the mobility. This difference can be explained as follows: for larger ϵ_a higher-order corrections to the main linear dependence of the mobility vs the field should become noticeable. As is shown in Appendix A [see Eq. (A13)] the first correction is expected to be negative. Taking this into account, we write instead of Eq. (73)

$$\mu/\mu_0 \simeq 0.50\,\kappa^{-0.5}\epsilon\left[1 - \beta\,\epsilon^{0.5}\right] \simeq 1.2\,\epsilon_a\left[1 - \sqrt{3}\beta\,\epsilon_a^{0.5}\right]. \tag{74}$$

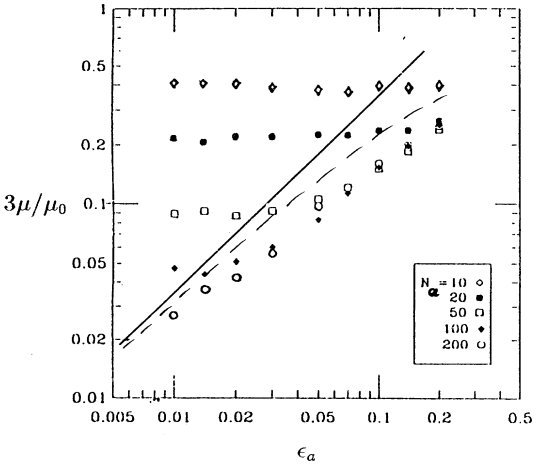

FIG. 5. The dependence of the reduced mobility, $3\mu/\mu_0$, on the reduced field, ϵ_a: ◇; •; □; ◇(filled); ○ — computer simulations data [39] for $N_a = 10$, 20, 50, 100, 200; solid line — theoretical prediction, Eq. (73); dashed line — the theory with correction, Eq. (74).

The dependence (74) with $\beta \simeq 0.7$ (this value is the best fit) is also plotted in Fig. 5 (dashed line); obviously the agreement between simulations and the theory, Eq. (74), is now good in the whole region. Note that the correction [second term in square brackets of Eq. (74)] leads to an effective decrease of the log-log incline, $\frac{\partial \ln \mu}{\partial \ln \epsilon_a}$: it is now slightly smaller than 1 (tending to 1 in the limit $\epsilon \to 0$), in agreement with computer simulations and in a marked disagreement with the previous (BRM) prediction ($\frac{\partial \ln \mu}{\partial \ln \epsilon_a} = 2$).

VI. MOBILITY MINIMUM PROBLEM

So far we have considered two limits: $\epsilon N \ll 1$ and $\epsilon N \gg 1$; in the latter regime the mobility nearly does not depend on molecular weight [see Eq. (62b)]. As was mentioned in the Introduction, the experimental observations suggest that the actual dependence of the mobility μ on the molecular weight (or N) is nonmonotonic. In order to account for this behavior theoretically, we have to consider the intermediate regime $\epsilon N \sim 1$.

Two main new difficulties arise in the BRF theory in the region $\epsilon N \sim 1$: (1) the fluctuation induced orientation of DNA chain essentially depends on molecular weight due to finite length effects, and (2) the drift velocity (which is proportional to f_0) is not nearly constant now; on the contrary, it fluctuates appreciably in parallel with fluctuations of the end-to-end projection $h \equiv h_x$. These two new effects are considered below.

A. Fluctuation-induced order parameter for small ϵ

Let us assume that the averaged along the chain driving force, f_0, is known. In the general case, this force is determined by the end-to-end projection, h_x [compare with Eq. (35) where time averaging was assumed]:

$$f_0 = \epsilon \frac{|h_x|}{Na}. \tag{75}$$

The order parameter near the leading end [47] for a given f_0 is defined by Eq. (58) of Sec. IV, which can be rewritten as

$$\eta = \frac{\epsilon}{3\sqrt{\kappa} f_0} \left\langle (\pi \tilde{\tau})^{-0.5} \right\rangle, \tag{76}$$

where $\tilde{\tau}$, here, is the time corresponding to the maximum *backward* displacement of the leading chain-end along the tube during the period $0 < \tau < \infty$ (the time origin, $\tau = 0$ being the arbitrary moment) [45]. The typical value of τ is [see Eq. (47)]

$$\tilde{\tau} \sim \tau^* = f_0^{-4/3} \kappa^{-1/3}.$$

The obtained equations for the order parameter, (76) and (58) are valid provided that $\tilde{\tau}$ is smaller that the longest relaxation time, Rouse time of the molecule in the tube: $\tilde{\tau} \ll N^2$. The derivation of Eq. (58) can be easily generalized for arbitrary $\tilde{\tau}/N^2$, the result is

$$\eta = \frac{\epsilon}{3f_0} \langle U(\tilde{\tau}) \rangle, \tag{77}$$

where $U(\tau)$ is the 1D shift of the chain end (along the tube) induced by the δ force applied at $\tau = 0$ to the end:

$$f(m,\tau) = \delta(m)\ \delta(\tau) \ \to \ U(\tau) \equiv u(0,\tau).$$

Using Eq. (29) with no random contribution ($\xi \equiv 0$) and with boundary conditions (32), we get

$$U(\tau) = \frac{1}{N} \varphi(\kappa \tau / N^2), \tag{78}$$

with

$$\varphi(x) \equiv \frac{1}{\sqrt{\pi x}} \sum_{i=-\infty}^{\infty} \exp(-i^2/x). \tag{79}$$

Note that for small x (large N), the N dependence of the function $U(\tau)$ is exponentially weak.

The statistics of $\tilde{\tau}$ is also determined by Eq. (29). Self-similar properties of this equation implies that the distribution density of the reduced quantity, $\kappa\tilde{\tau}\ /N^2$ depends only on a single parameter, $\kappa^{-1/2} f_0 N^{3/2}$. Therefore the averaged value, $\langle \varphi(\kappa\tilde{\tau}/N^2) \rangle$, also depends only on this parameter, $\langle \varphi \rangle = \Phi(\kappa^{-1/2} f_0 N^{3/2})$, so that

$$\eta = \frac{\epsilon}{3f_0 N} \Phi(\kappa^{-1/2} f_0 N^{3/2}). \tag{80}$$

The unknown function $\Phi(\,)$ cannot be calculated analytically; however, its asymptotics can be easily obtained using Eqs. (60) and (79):

$$\Phi(z) \simeq \begin{cases} 1 & \text{for } z \ll 1 \quad (81a) \\ 1.9\ z^{2/3} & \text{for } z \gg 1, \quad (81b) \end{cases}$$

where 1.9 is the value of $I(1,1)$. Note that the condition $z \equiv \kappa^{-1/2} f_0 N^{3/2} \gg 1$ coincides with $\tilde{\tau} \ll N^2/\kappa$, and simultaneously with the condition $\epsilon N \gg \kappa^{-0.5}$. In this regime, the order parameter predicted by Eqs. (80) and (81) agrees well with the results 58–61 of Sec. IV.

B. The effect of drift velocity fluctuations

In the region of low electric fields, the DNA molecule becomes less and less stretched: its conformation tends to that of a Gaussian chain. Thus noticeable fluctuations of the end-to-end distance, and, therefore, of the curvilinear drift velocity should be expected. An analysis of these fluctuations is a delicate problem since these fluctuations are superimposed on the tube-length fluctuations and on the random Brownian motion along the tube. It is good to start the analysis with a simpler model, assuming that the tube-length fluctuations are suppressed.

More specifically, let us consider the biased reptation model (BRM) without random diffusion: the only mechanism of the chain motion is a drift of the chain as a whole along the tube induced by electric field. Let us also switch off the direct orientation effect. We shall denote this simple model $[-F-D-O]$ (i.e., no tube-length

fluctuations, no random diffusion, no direct orientation). A dynamic equation for the end-to-end projection $h \equiv h_x$ for this model can be constructed following Doi *et al.* [10]. During a short time Δt, the chain will reptate along the tube on the distance,

$$\Delta s = v_d \Delta t, \tag{82}$$

where

$$v_d = v_d(h) = \frac{\epsilon\,|h|}{N\tau_0} \tag{83}$$

is the instantaneous curvilinear drift velocity, see Eq. (2). The change of the end-to-end projection is

$$\Delta h \equiv h(t+\Delta t) - h(t) = \Delta h_+ - \Delta h_-,$$

where Δh_+ is the x projection of the new part of the tube (created during Δt), and Δh_- corresponds to the part that disappeared at the chain tail. Assuming that $\Delta s \gg a$, so that the parts can be considered as Gaussian coils, we write

$$\left\langle (\Delta h_+)^2 \right\rangle = \left\langle (\Delta h_-)^2 \right\rangle = a\ \Delta s/3, \tag{84}$$

$$\langle \Delta h_+ \rangle = 0;\ \ \langle \Delta h_- \rangle = h\frac{\Delta s}{Na}. \tag{85}$$

Here, $h \equiv h(t)$, and we also assume that the mean end-to-end projection of a tube segment is h/N. Thus,

$$\left\langle (\Delta h)^2 \right\rangle = 2\ D^*\ \Delta t;\ \ \langle \Delta h \rangle = v^*\ \Delta t, \tag{86}$$

where

$$D^* = D^*(h) = \frac{a\ v_d(h)}{3}; \tag{87}$$

$$v^* = v^*(h) = -\frac{h}{Na} v_d(h). \tag{88}$$

Let $\rho(h,t)$ be the density distribution function of the end-to-end projection. Equation (86) implies the following diffusion equation for $\rho(h,t)$, which can be constructed in a standard way:

$$\frac{\partial \rho}{\partial t} = \frac{\partial}{\partial h}\left\{ \frac{\partial}{\partial h}\left[D^*(h)\ \rho \right] - v^*(h)\ \rho \right\}. \tag{89}$$

The stationary solution of Eq. (89) is

$$\rho(h) = \frac{\text{const}}{|h|}\ \exp\left(-\frac{3h^2}{2Na^2} \right). \tag{90}$$

Note that the distribution (90) is in agreement with the obtained in Ref. [10] if one neglects details concerning how to remove singularity at $h = 0$. Moreover, as has been shown by Viovy [14], the distribution (90) is exact for the case of no random diffusion. The result (90) can be also easily generalized in order to incorporate the direct orientation effect of the field, i.e., for the model $[-F - D + O]$ (see Ref. [10]). One of the important features of the distribution (90) is the singularity at $h = 0$, which is obviously unphysical. This singularity is not due to a failure of the solution (the solution is correct), but rather due to a failure of the *model*: for small h the random diffusion cannot be neglected.

Thus, let us turn to a more physical model $[-F + D - O]$ taking into account the random curvilinear diffusion. This model has been solved exactly using the method of "many-segment" distribution functions in Ref. [46]. In particular, it was shown that the stationary distribution function, $\rho(h)$, is not influenced by the field and remains Gaussian [48]:

$$\rho(h) = \text{const}\ \times \exp\left(-\frac{3h^2}{2Na^2} \right). \tag{91}$$

Let us analyze the applicability of Eqs. (82)–(89) to the model under consideration. For small enough fields, the random curvilinear diffusion dominates over the electrophoretic drift. As was shown in Sec. II, this is true if $\epsilon < \epsilon_0 = N^{-3/2}$. Therefore, the result (91) is quite expectable in this region. However for larger fields, $\epsilon \gg \epsilon_0$, the drift should dominate over random diffusion [see Eq. (5)]. Therefore, from the first sight, the above derivation for the $[- - -]$ model and the final result, Eq. (90), must be also applicable to the $[- + -]$ model in the region $\epsilon \gg \epsilon_0$. This conclusion is erroneous, however, since the correct stationary distribution, given by Eq. (91), is different.

The contradiction that we arrive at is rather subtle. It can be resolved as follows. We must take into account that with random curvilinear diffusion, the orientation of the leading end is not isotropic, but is coupled with the end-to-end vector, h (remember, however, that in the model under consideration the *direct* orientation effect of the field is switched off). Formally, this can be shown in the following way. Note that the dynamics with random diffusion (but without tube-length fluctuations) can be considered as a limiting case of more general dynamic implied by the BRF: we can switch off the tube-length fluctuations formally by taking longitudinal elastic modulus κ infinitely large, $\kappa \to \infty$. Substituting Eq. (75) in Eq. (80), we thus get the order parameter at the leading end for $\kappa \to \infty$,

$$\eta = \frac{a}{3h}\Phi(0) = \frac{a}{3h}. \tag{92}$$

Therefore, instead of $\langle \Delta h_+ \rangle = 0$ in Eq. (85), we shall have

$$\langle \Delta h_+ \rangle = \eta\ \Delta s = \frac{a\ v_d(h)}{3h}\ \Delta t,$$

and, thus Eq. (88) should be modified as

$$v^*(h) = v_d(h)\ \left(\frac{a}{3h} - \frac{h}{Na} \right). \tag{93}$$

The diffusion equation (89) with Eqs. (87) and (93) gives the correct Gaussian distribution (91) as a stationary solution. Thus, with random curvilinear diffusion the end-to-end vector distribution must be Gaussian even if globally the diffusion is negligible [i.e., in the region de-

fined by in Eq. (5)].

This result relies on the validity of Eq. (92) for the local order parameter, which implies that the diffusion locally (but not necessarily globally) dominates over the electrophoretic drift. The last condition (local dominance of the diffusion) means that one step along the tube due to random diffusion occurs faster than that due to the drift:

$$a/v_d \gg a^2/D_{1D}. \tag{94}$$

Using Eq. (83), we rewrite this condition as

$$\epsilon \ll \frac{1}{N^{0.5}}. \tag{95}$$

In the opposite regime, $\epsilon \gg N^{-0.5}$, the random diffusion is completely negligible, so that the models $[-F+D-O]$ and $[-F-D-O]$ becomes indistinguishable, the stationary distribution being given by Eq. (90).

Now we are in a position to consider the realistic $[+F+D+O]$ model which is virtually equivalent to $[+F+D-O]$. Using Eqs. (80), (75) in the general case (of arbitrary κ), we get instead of Eq. (92),

$$\eta = \frac{a}{3h}\Phi\left(\frac{\epsilon h N^{0.5}}{\kappa^{0.5}\, a}\right). \tag{96}$$

The above equation for the order parameter at the leading end implies the following effective velocity:

$$v^*(h) = v_d(h)\left[\frac{a}{3h}\Phi(\alpha h) - \frac{h}{Na}\right], \tag{97}$$

where $\alpha = \epsilon N^{0.5}/(a\,\kappa^{0.5})$. Using Eq. (89) with (87), (97), we get the following stationary distribution:

$$\rho(h) = \frac{\text{const}}{|h|}\ \exp\left(-\frac{3h^2}{2Na^2} + \Psi(\alpha h)\right), \tag{98}$$

where

$$\Psi(z) = \int \Phi(z)\frac{dz}{z}. \tag{99}$$

In the region $\epsilon \gg \epsilon^*$, the parameter $z \equiv \alpha h \gg 1$ is typically large, so that the second equation (81) is applicable. Using Eqs. (98), (99) and (81), we obtain

$$\rho(h) = \frac{\text{const}}{|h|}\exp\left[-\frac{3h^2}{2Na^2} + 2.85\left(\frac{\epsilon^2 h^2 N}{\kappa}\right)^{2/3}\right]. \tag{100}$$

The distribution (100) implies the following reduced mobility:

$$\frac{\mu}{\mu_0} = \frac{\langle h^2\rangle}{N^2a^2} = C\kappa^{-0.5}\epsilon\left[1 - \frac{N^*}{2N}\right], \tag{101}$$

where $C \simeq 0.50$ and $N^* \sim 1/\epsilon$ are defined by Eqs. (64) and (63). Equation (101) implies that the mobility increases with the chain molecular weight (with N) in the region of its validity, i.e., for $\epsilon \gg \epsilon^*$ that is for $N \gg N^*$. In the opposite limit, $N \ll N^*$, the mobility is a decreasing function of N being inverse proportional to the molecular weight [see Eq. (62a)]. Thus, for a given field, $\epsilon \ll 1$, the mobility as a function of N should attain a minimum at $N \sim N^*$. Note that the minimum position predicted here with BRF ($N \sim 1/\epsilon$) differs from the analogous prediction of the BRM, $N \sim 1/\epsilon^2$ [10,14].

The above consideration suggests that for a given electric field, ϵ, the reduced mobility defined as $\mu(N)/\mu(N^*)$ must be a universal function of N/N^*. This universal dependence cannot be calculated analytically. Numerical results for this function will be published elsewhere.

VII. ELECTROPHORESIS IN TIGHT GELS

So far, we assumed that the Kuhn segment of DNA, b, is smaller than the pore size, a. Let us consider the opposite regime of tight gels: $a \ll b$. It is convenient to *define* the size a as the mean distance between entanglements along the chain. In the regime $a \ll b$, each Kuhn segment is constrained by a lot of entanglements. Therefore, the tube that confines the DNA chain must be extremely thin and long: the tube diameter must be much smaller than the Kuhn segment, and the tube length, L, must be nearly equal to the chain contour length: $L \simeq L_0 = N_0 b$. In the slip-link version of the tube model [2] entanglements are substituted by small fixed rings enveloping the chain (Fig. 6), the distance between nearest rings being a.

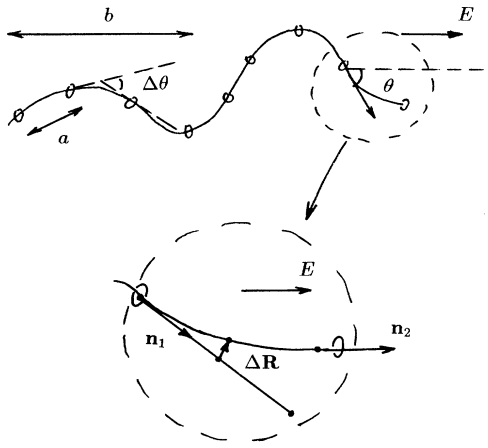

FIG. 6. The slip-link model of a persistent macromolecule in a tight gel. Entanglements are regarded as small rings through which the chain is freely drawn. The distance between the rings is a, $a \ll b$; $\Delta\theta$ is the angle between the neighboring segments; θ is the angle between the head segment and the field. The head segment deformed by the electric field, E, is shown in the inset. The unit vector tangential to the chain varies from $\mathbf{n}_1$ at the beginning to $\mathbf{n}_2$ at the end of the segment. The bending angle is determined by $\Delta\mathbf{n} = \mathbf{n}_2 - \mathbf{n}_1$. The displacement of the segment center of mass caused by the bend is $\Delta\mathbf{R} = \text{const} \times a\ \Delta\mathbf{n}/2$. The corresponding contribution to the potential energy of the segment in electric field is $\Delta F_e = -\mathbf{E}\cdot q\Delta\mathbf{R} \simeq -\epsilon(\Delta\mathbf{n})_x/2$, where a numerical constant is omitted.

Thus, the chain can be thought of as a sequence of $N = L/a$ linear (nearly straight) segments (blobs) of length a. The persistent flexibility mechanism [52] of the DNA chain implies that the typical angle between neighboring segments is $\Delta\theta \sim \sqrt{a/b}$, $\Delta\theta \ll 1$ (see Fig. 6).

The BRF predictions can be readily generalized for the case of tight gels. The basic concepts are the same as for large-pore gels. We are going to reveal the scaling behavior of the mobility using order-of-magnitude arguments.

One of the main new features is that the reduced longitudinal elastic modulus of the chain in the tube is now large: $\kappa \gg 1$. In order to estimate this modulus, we note that according to its definition the equilibrium mean-square fluctuation of the tube length is

$$(\delta L)^2 \sim La/\kappa.$$

The fluctuation δL for a short chain with $L \sim a$ can be readily estimated:

$$\delta L \sim L(\Delta\theta)^2 \sim a^2/b.$$

Therefore,

$$\kappa \sim \frac{La}{(\delta L)^2} \sim (b/a)^2. \tag{102}$$

For very small electric fields the chain should remain approximately Gaussian, therefore, its reduced mobility is [compare with Eq. (8)]

$$\mu/\mu_0 = \frac{\langle h_x^2 \rangle}{(Na)^2} = \frac{b}{3Na}. \tag{103}$$

A large enough field should induce an extension of the DNA chain, the mobility in this region being governed by the induced orientation order parameter, η: $\mu/\mu_0 = \eta^2$. The order parameter can be predicted using arguments presented in the second part of Sec. III. We have to take into account only two differences: (i) the typical spatial displacement of the chain end during the time τ^* is [compare with Eq. (19)]

$$\Delta x \sim d = \sqrt{abm^*}, \tag{104}$$

where the terminal "length" m^* is determined in Eq. (47); (ii) the Rouse time of M section of the chain is $\tau(M) \sim M^2/\kappa$, so that the number of segments in the part that moves cooperatively during τ^* is [compare with Eq. (23)]

$$M^* \sim (\kappa\tau^*)^{0.5}. \tag{105}$$

Using Eq. (25) together with Eqs. (22), (104), (105), (47), we get

$$\eta = \frac{\langle \Delta h_x \rangle}{m^*} \sim \frac{d^2\epsilon}{m^* v_d M^*} \sim \frac{b}{a}\frac{\epsilon^{2/3}}{\eta^{1/3}\kappa^{1/3}}. \tag{106}$$

Here and below, we take a as a unit length and τ_0 as a unit time.

After simple transformations, Eq. (106) gives $\eta \sim (b/a)^{1/4}\epsilon^{1/2}$, and

$$\mu/\mu_0 \sim \left(\frac{b}{a}\right)^{0.5} \epsilon. \tag{107}$$

The region of applicability of Eq. (107) is determined by two conditions: $M^* < N$, and $m^* > b/a$ (the last condition ensures that the terminal section is a Gaussian coil rather than a straight segment). Thus, Eq. (107) is applicable in the region

$$\left(\frac{b}{a}\right)^{0.5} \frac{1}{N} < \epsilon < \left(\frac{a}{b}\right)^{3.5}.$$

"Equilibrium" arguments of Sec. III suggest that direct field contribution to the order parameter dominates if $m^* < 1$, i.e., if

$$\epsilon\eta\kappa > 1. \tag{108}$$

A theory of direct orientation of a persistent chain in tight gels was proposed in Ref. [46]. We remind you, here, of the relevant points.

Let $f(\mathbf{n})$ be the density distribution function of the unit vector $\mathbf{n}$ tangential to the chain near its leading end. In order to set up an approximate equation for the time evolution of the distribution function, we consider the time interval Δt corresponding to one step on the entanglement length, a, along the tube:

$$\Delta t = a/v_d = (\epsilon\eta)^{-1} \tag{109}$$

(in dimensionless variables). The change of the end orientation, $\Delta\mathbf{n}$, during Δt is caused by thermal fluctuations and by electric force acting on the head segment: $\Delta\mathbf{n} = \Delta\mathbf{n}_f + \Delta\mathbf{n}_e$. The fluctuation contribution is characterized by the following moments [53,52]:

$$\left\langle (\Delta\mathbf{n}_f)^2 \right\rangle = 4a/b; \quad \langle \Delta\mathbf{n}_f \rangle = 0. \tag{110}$$

The field contribution, $\Delta\mathbf{n}_e$ can be obtained by minimization of the free energy associated with the head a segment:

$$F(\Delta\mathbf{n}) = \frac{b}{4a}(\Delta\mathbf{n})^2 - \frac{\epsilon}{2}(\Delta n)_x. \tag{111}$$

Here, the first term is the elastic energy due to end-segment bending, and the second term is the change of the potential energy of the segment in the electric field (see Fig. 6). Minimizing Eq. (111) under the obvious condition $\mathbf{n}\,\Delta\mathbf{n} = 0$, we get

$$\Delta\mathbf{n}_e = -\frac{a}{b}\frac{\partial U}{\partial \mathbf{n}}, \; U(\mathbf{n}) = -\epsilon n_x = -\epsilon\cos\theta, \tag{112}$$

where θ is the angle between $\mathbf{n}$ and the field direction (x axis).

Using Eqs. (109)–(112), we get the following dynamic equation for $f(\mathbf{n})$:

$$\frac{1}{\eta\epsilon}\frac{\partial f}{\partial t} = \frac{a}{b}\left[\nabla^2 f + \nabla(f\nabla U)\right], \tag{113}$$

where $\nabla \equiv \nabla_{\mathbf{n}}$ is the gradient in the orientation space.

The stationary solution of Eq. (113) is

$$f(\mathbf{n}) = \text{const} \times \exp(-U(\mathbf{n})),$$

which implies the order parameter

$$\eta \equiv \langle \cos\theta \rangle \sim \epsilon. \tag{114}$$

Thus, we get the reduced mobility in the region [see Eq. (108)] $1 > \epsilon > a/b$:

$$\mu/\mu_0 = \eta^2 \sim \epsilon^2. \tag{115}$$

In the last ϵ region that we have to consider, $(a/b)^{3.5} < \epsilon < a/b$, the terminal section is shorter than the Kuhn segment, but longer than the entanglement length: $a < m^* a < b$. Here, we also can use the scheme described just above with the only reservation: now the field-induced change of the end orientation is dominated by the fluctuation mechanism rather than the direct field effect. The change $\Delta\mathbf{n}_e$ can be obtained in analogy with $\langle \Delta h_x \rangle$ considered in the second part of Sec. III. Let Δt be the time corresponding to the displacement on the distance m^* (the terminate length) along the tube:

$$\Delta t \sim t^* \sim \frac{m^*}{\epsilon\eta}. \tag{116}$$

The typical change of orientation $\Delta\mathbf{n}$ during Δt is due to thermal fluctuations:

$$\langle (\Delta\mathbf{n})^2 \rangle = 4m^* a/b, \tag{117}$$

so that the typical "amplitude" in the x direction is

$$(\Delta n)_x \sim d = \sin\theta \left(\frac{m^* a}{b}\right)^{0.5}. \tag{118}$$

The mean value, $\Delta\mathbf{n}_e = \langle \Delta\mathbf{n} \rangle$ is [compare with Eqs. (20) and (21)],

$$\langle \Delta n_x \rangle \sim d\ \Delta p\ ,\ \Delta p \sim \frac{\Delta v_d}{v_d}. \tag{119}$$

The variation of the local drift velocity is [compare with Eq. (24)]

$$\Delta v_d \sim \epsilon \frac{\Delta h_x}{M^*}, \tag{120}$$

where Δh_x is the variation of the x projection of the terminate chain section associated with $\Delta\mathbf{n}$:

$$\Delta h_x \sim n_i^* \Delta n_x. \tag{121}$$

Now using Eqs. (118)–(121), we get

$$\langle \Delta n_x \rangle \sim d^2 \frac{\epsilon m^*}{M^* v_d},$$

$$\Delta\mathbf{n}_e = \langle \Delta\mathbf{n} \rangle \sim -\frac{a}{b\kappa\epsilon\eta} \frac{\partial U}{\partial \mathbf{n}}. \tag{122}$$

Here, we take into account that $v_d = \epsilon\eta$ and $M^* = \kappa (m^*)^2$.

Using Eqs. (116), (117), (122), we get [compare with Eq. (113)]

$$\frac{m^*}{\eta\epsilon} \frac{\partial f}{\partial t} = \left[\frac{m^* a}{b} \nabla^2 f + \text{const} \times \frac{a}{b\kappa\epsilon\eta} \nabla (f \nabla U) \right], \tag{123}$$

where const is a numerical constant. The stationary order parameter implied by Eq. (123) is

$$\eta = \langle \cos\theta \rangle \sim (\kappa\eta m^*)^{-1}. \tag{124}$$

Taking into account that $m^* \sim (\epsilon\eta\kappa)^{-1/3}$ we, thus, obtain the order parameter,

$$\eta \sim \epsilon^{0.2}\ (a/b)^{0.8}, \tag{125}$$

and the reduced mobility,

$$\mu/\mu_0 = \eta^2 \sim \epsilon^{0.4}\ (a/b)^{1.6}, \tag{126}$$

in the region $a/b > \epsilon > (a/b)^{3.5}$.

Equations (103), (107), (115), and (126) determine the mobility behavior for a small-pore gel. Note that the reduced parameters N and ϵ depend not only on the DNA molecular weight and electric field but also on the characteristic pore size, a. It is good to reformulate these results using natural variables,

$$L_0 = Na, \quad \epsilon_0 = \frac{q_0 E b}{T} = \left(\frac{b}{a}\right)^2 \epsilon, \tag{127}$$

which do not depend on a (here, L_0 is the chain contour length, and $q_0 = Qb/L_0$ is the charge per Kuhn segment). Thus, we have for $a < b$,

$$\mu/\mu_0 \sim \begin{cases} \frac{b}{L_0} \quad \text{if } \epsilon_0 < \epsilon_0^* & \text{(128a)} \\ \left(\frac{a}{b}\right)^{1.5} \epsilon_0 \quad \text{if } \left(\frac{b}{a}\right)^{1.5} \frac{b}{L_0} < \epsilon_0 < \left(\frac{a}{b}\right)^{1.5} & \text{(128b)} \\ \left(\frac{a}{b}\right)^{2.4} \epsilon_0^{0.4} \quad \text{if } \left(\frac{a}{b}\right)^{1.5} < \epsilon_0 < \frac{b}{a} & \text{(128c)} \\ \left(\frac{a}{b}\right)^4 \epsilon_0^2 \quad \text{if } \frac{b}{a} < \epsilon_0 < \left(\frac{b}{a}\right)^2, & \text{(128d)} \end{cases}$$

where

$$\epsilon_0^* \sim \begin{cases} \left(\frac{b}{a}\right)^{1.5} \frac{b}{L_0}, & L_0/b > \left(\frac{b}{a}\right)^3 & \text{(129a)} \\ \left(\frac{b}{a}\right)^6 \left(\frac{b}{L_0}\right)^{2.5}, & \left(\frac{b}{a}\right)^3 > L_0/b > \left(\frac{b}{a}\right)^2 & \text{(129b)} \\ \left(\frac{b}{a}\right)^2 \left(\frac{b}{L_0}\right)^{0.5}, & \left(\frac{b}{a}\right)^2 > L_0/b > 1. & \text{(129c)} \end{cases}$$

Thus the separation window $0 < \epsilon_0 \lesssim \epsilon_0^*$, where the mobility does depend on the molecular weight, becomes wider for smaller pore size, a [see Eqs. (129)]. Note that in order to minimize the separation time (which is roughly inverse proportional to the migration speed) one should use the largest possible field (within the separation window): $\epsilon_0 = \epsilon_0^*$. The migration speed $\dot{x} = \mu E$ is proportional to $(\mu/\mu_0)\epsilon_0$; using Eqs. (128a) and (129a), we get

$$\dot{x} \propto (\mu/\mu_0)\epsilon_0 \sim \left(\frac{b}{a}\right)^{1.5} \left(\frac{b}{L_0}\right)^2. \tag{130}$$

Therefore, we conclude that the minimum separation time in a tight gel can be attained using the combination of smaller gel pores and stronger fields.

The last conclusion is not valid for a large-pore gel.

For this case, using the following relation between the natural variables L_0 , ϵ_0, and N, ϵ:

$$L_0 = \frac{Na^2}{b}, \quad \epsilon_0 = q_0 \frac{Eb}{T} = \left(\frac{b}{a}\right)^3 \epsilon, \tag{131}$$

we can reformulate the final results for $a > b$, Eqs. (62), (63), as

$$\mu/\mu_0 \sim \begin{cases} \left(\frac{a}{b}\right)^2 \frac{b}{L_0}, & \epsilon_0 < \epsilon_0^* \quad (132a) \\ \left(\frac{a}{b}\right)^3 \epsilon_0, & \epsilon_0^* < \epsilon_0 < 1, \quad (132b) \end{cases}$$

where

$$\epsilon_0^* \sim \frac{b}{a}\frac{b}{L_0}. \tag{133}$$

The optimum migration speed is now proportional to $(\mu/\mu_0)\epsilon_0^* \sim (a/b)(b/L_0)^2$. Thus, the fastest separation in a gel with large pores ($a > b$) can be obtained by using the combination of larger pores and weaker fields.

VIII. DISCUSSION AND CONCLUSIONS

Recent experiments and computer simulations made clear that the reptation model oversimplifies the dynamics of DNA fragments in gels under strong electric fields ($\epsilon \gg 1$) [23,24,51,25,29,30]. Even for the simplest case of a stationary field, the behavior of DNA during gel electrophoresis is rather complicated, implying a quasiperiodic contraction and extension of the macromolecule [25]. These oscillations can be interpreted as huge tube-length fluctuations enhanced by the field which may actually result in effective destruction of the tube accompanied by formation of a more complex (fractal) structure [30].

In the opposite regime of weak fields, $\epsilon \ll 1$, considered in the present paper, the tube-length fluctuations are less pronounced, they are virtually not affected by the field. Thus, the tube model [2] is really applicable in this region (for $\epsilon \ll 1$). However, as a main qualitative result, we have shown that these tube-length fluctuations are still very important even for weak fields: they provide a completely different mechanism of the chain orientation. In Sec. III, we demonstrated that the field-induced orientation of DNA chain must be very much enhanced by the fluctuations: the direct contribution to the orientation order parameter, $\eta_{\text{direct}} \sim \epsilon$, can be neglected in comparison with the fluctuation part, $\eta_{\text{fluct}} \sim \epsilon^{0.5}$.

Note that the direct orientation effect can be switched off for a polymer chain with uncharged end segments (end blobs) [54]. The theory of Ref. [54] based on the BRM would predict a drastic difference between the mobilities of a normal (uniformly charged) chain and of a fragment with uncharged ends. This result is not supported by our theory: the BRF predicts quite a small mobility difference between the cases, of the order of $\Delta\mu/\mu \sim \eta_{\text{direct}}/\eta_{\text{fluct}} \sim \epsilon^{0.5}$. Thus, an experimental study of the effect of the uncharged end parts on the gel electrophoresis can serve as a sensitive test of the BRF theory [55].

A detailed analysis presented in Sec. IV B suggests that in the long-chain limit ($N \to \infty$), the order parameter and the mobility are regular functions of square root of reduced field, $\sqrt{\epsilon}$:

$$\eta = \text{const} \times \sqrt{\epsilon}\, f_\eta(\sqrt{\epsilon}); \quad \mu/\mu_0 = C\, \kappa^{-0.5} \epsilon\, f_\mu(\sqrt{\epsilon}), \tag{134}$$

where

$$f_\mu(\sqrt{\epsilon}) = 1 + \beta_1 \sqrt{\epsilon} + \beta_2\, \epsilon + \cdots . \tag{135}$$

As is shown in the Appendix A, the factor β_1 is likely to be negative. These results are both in qualitative and in good quantitative agreement with experimental measurements and computer simulations (see Sec. V).

Equation (134), which implies that the mobility nearly does not depend on the molecular weight, is valid for $\epsilon \gg \epsilon^* \sim 1/N$. For $\epsilon < \epsilon^*$, the mobility is molecular weight dependent, $\mu \propto 1/N$. The crossover field, $\epsilon^* \sim 1/N$ predicted by BRF is much weaker than that predicted by biased reptation without fluctuations approach ($\epsilon^* \sim 1/\sqrt{N}$). The crossover region $\epsilon \sim 1/N$ also corresponds to the minimum of the mobility as a function of molecular weight.

We predict that DNA fragments can be separated in a continuous field electrophoresis in the molecular weight range,

$$L_0 < L_0^* \sim \frac{b}{a}\frac{T}{q_0 E}, \tag{136}$$

where L_0 is the macromolecular contour length, q_0 is the charge per Kuhn segment, b. Thus, the separation range can be increased by using gels with smaller pore size, a, or by reducing the field strength (the latter being the more effective choice). Equation (136) is valid for $a > b$; for tight gels ($a < b$) the separation range is wider:

$$L_0 < L_0^* \sim \left(\frac{b}{a}\right)^{1.5} \frac{T}{q_0 E}. \tag{137}$$

The most effective separation in a tight gel can be obtained by using a combination of smaller pores and stronger fields.

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APPENDIX A: SECOND-ORDER CORRECTIONS IN F_1 TO THE MOBILITY

It is natural to expect that nonlinear effect of the electric field should result not only in a mean orientation of the chain, but also in some additional correlations between orientations of the chain segments (blobs). Hence,

the effective Kuhn segment may change ($a \to a^*$). With this change the basic equation (58) should be modified in accordance with note [41]. A renormalization of the constant $I(1,1)$ is thus expected:

$$I(1,1) \to I(1,1)\ \frac{a^*}{a}. \tag{A1}$$

Therefore, we can write [see Eqs. (58)–(60)]

$$\eta \sim \epsilon\, v_d^{-1/3}\ \frac{a^*}{a}, \tag{A2}$$

where $v_d = f_0$ is the mean curvilinear velocity of the chain end [here, we use reduced variables introduced in Eq. (28), and assume that $\kappa \sim 1$]. Hence, there are two main points worth checking: (i) the field-induced rigidity of the tube and (ii) a nonlinear effect of the random part of the driving force on the dynamics of the chain end.

Let us start with the first point. We define the effective Kuhn segment as

$$a^* = a \sum_n \left[\langle \nu(m'+n)\rangle - \eta\right], \tag{A3}$$

under the condition that $\nu(m') = 1$. Orientation of the segment m' is coupled with the orientation of any other segment only via additional driving force $f_1 \sim \epsilon$ acting on the m' segment. This force can change the mean velocity, v_d, of the chain end on the characteristic time scale $\tau^* \sim f_0^{-4/3} \sim \epsilon^{-2}$ [see Eq. (47)]. Using Eq. (53) and taking into account that the force is effective during a period $\tau \sim \tau^*$, we get the typical contribution to the end velocity induced by the force

$$\delta v \sim \frac{\epsilon}{\sqrt{\tau^*}} \sim \epsilon^2.$$

Using Eq. (A2), we get the following correction to the order parameter of the nth segment:

$$\delta\eta \equiv \langle \nu(m'+n)\rangle - \eta \sim \eta\ \delta v/v_d \sim \epsilon, \tag{A4}$$

where $n \neq 0$. Typical values of n should be of order of $m^* \sim f_0^{-1/3} \sim \epsilon^{-1/2}$, the typical range of backward displacement of the chain end [see Eq. (47)]. Thus, we can cut the sum (A3), excluding n with $|n| > m^*$. Substituting Eq. (A4) into (A3), we obtain

$$a^* = a\left[1 + O\left(\epsilon^{0.5}\right)\right]. \tag{A5}$$

Thus, the *relative* second-order correction to the order parameter and to the mean mobility

$$\delta\eta/\eta \sim \delta\mu/\mu \sim \epsilon^{0.5} \tag{A6}$$

is small.

Now, let us consider the second point and estimate the typical value of random contribution, δv, to the chain-end velocity due to random component of the driving force, f_1. Note that we are interested in the δv smoothed over characteristic time scale τ^*. Using Eq. (53), we get the typical contribution to the chain-end displacement, $\delta u_1(\tau)$, due to the random force acting on *one* tube segment that has been created at $\tau = 0$ (at a later moment τ' this segment should have the coordinate $m \simeq v_d\tau'$):

$$\delta u_1(\tau) \sim \epsilon \int_0^\tau d\tau'\ (\tau - \tau')^{-0.5} \exp\left[-v_d^2\tau'^2/(\tau - \tau')\right]. \tag{A7}$$

Note that the term in square brackets in the right hand side of Eq. (A5) is small since $\tau \sim \tau' \lesssim \tau^*$ and $v_0^2\ \tau^* \sim \epsilon \ll 1$. Therefore, we can rewrite the last equation as

$$\delta u_1 \sim \epsilon\ \tau^{0.5}. \tag{A8}$$

About $n \simeq v_d\tau$ new tube segments are created during some time τ. Forces, $f_1(m)$, $m = 0, 1, ..., n$, which act on different segments are almost uncorrelated since correlations between their orientations are negligible [see Eq. (A4)]. Therefore, the total δu induced by the force f_1 acting on all n segments is

$$\delta u \sim \delta u_1\ n^{0.5} \sim \epsilon v_d^{0.5}\tau.$$

Thus, the characteristic contribution to the end velocity,

$$\delta v \sim \delta u/\tau \sim \epsilon v_d^{0.5}\ ;\ \delta v/v_d \sim \epsilon/v_d^{0.5} \sim \epsilon^{1/4}. \tag{A9}$$

So, the relative fluctuation of the end velocity is small. Obviously, the relative correction to the mobility due to this fluctuation,

$$\delta\mu/\mu \sim \left\langle (\delta v/v)^2 \right\rangle \sim \epsilon^{0.5}.$$

Thus, we have checked that the second-order corrections to the mobility are always small.

Finally, we are going to describe one more important source of the next-to-main order correction to the mobility in the region $1 \gg \epsilon \gg \epsilon^*$. The basic expression (58) for the order parameter was derived using Eq. (56), where the factor $\exp\left[-\frac{(m')^2}{\tau}\right]$ was approximated by 1 (here, we assume that $\kappa \sim 1$). The relative first-order correction to this approximation is

$$(m')^2/\tau \sim f_0^{2/3} \sim \epsilon. \tag{A10}$$

Here, we take into account that $m' \sim m^*$, $\tau \sim \tau^*$ and also use Eqs. (35, 61). Note that the correction is negative.

The correction (A10) corresponds to typical values of m' and τ. A much larger (and also negative) correction stems from the region of relatively small m' and τ ($m' \ll m^*$, $\tau \ll \tau^*$), where the exponential factor provides an effective cutoff at $(m')^2 \sim \tau$. Note that for a given τ, the typical value of m' is of order of backward displacement of the leading end during the period τ, i.e. [see Eq. (12)],

$$m'(\tau) \sim \tau^{1/4}.$$

Therefore, the condition $(m')^2 \lesssim \tau$, specifying the region where the "exp" factor is nearly 1, can be rewritten as $\tau^{1/2} \lesssim \tau$, i.e., $\tau \gtrsim 1$.

Thus, the cutoff associated with the "exp" factor can be accounted for by changing the lower limit of integration in the right hand side of Eq. (58) from 0 to a value

of order of 1. This change implies a negative correction to η of order of

$$\Delta\eta \sim -\frac{\epsilon}{f_0} D_0(0,1). \tag{A11}$$

According to the definition, the quantity $D_0(0,1)$ must be of order of probability that the maximum backward displacement of the leading chain end is achieved during the first time step, i.e., for $\tau \lesssim 1$. In other words, $D_0(0,1)$ is roughly the probability that after the first time step the end will never return to its initial position, its displacement (along the tube) being always positive. This probability can be estimated as follows.

First, we note that a return to the initial position is nearly impossible for $\tau \gg \tau^*$, where the electrophoretic drift (in positive direction) dominates over random diffusion. On the other hand, for $\tau \lesssim \tau^*$ the drift is not important and can be neglected. Therefore, $D_0(0,1)$ should be of the order of the probability that during τ^* time-steps free end of the chain will never return to initial position along the tube. One-dimensional dynamics of the free end can be approximated by random walk on random walk [49]: $u(\tau) = u(\xi(\tau))$, where $u(\xi)$ and $\xi(\tau)$ are random walks. Thus, the probability that during τ steps $u(\tau) - u(0)$ is always positive is $p \sim p_1 p_2$, where p_1 is the probability that $\xi(\tau)$ will never return to $\xi(0)$, and p_2 is the same for $u(\xi)$. These probabilities are known [50]:

$$p_1 \sim \tau^{-1/2},\ p_2 \sim \xi_{\max}^{-1/2}.$$

Obviously, $\xi_{\max} \sim \tau^{1/2}$; thus,

$$D(0,1) \sim p(\tau^*) \sim (\tau^*)^{-3/4}. \tag{A12}$$

Now using Eqs. (A11), (A12) and (47), (61), we get

$$\Delta\eta \sim -\epsilon,\ \Delta\eta/\eta \sim -\epsilon^{0.5}.$$

With this correction the reduced mobility in the region $1 \gg \epsilon \gg \epsilon^*$ can be written as [compare with Eq. (62b)]:

$$\mu/\mu_0 = \eta^2 = C\kappa^{-0.5}\epsilon\left[1 - \text{const} \times \epsilon^{0.5}\right]. \tag{A13}$$

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[37] Volume interactions that generally tend to swell the chain can be neglected since DNA is locally very rigid: $d/b \simeq 0.02 \ll 1$ (here, $b \simeq 100$ nm is the Kuhn segment of DNA molecule and $d \simeq 2$nm is its diameter). More specifically the effect of volume interactions is negligible if the number of Kuhn segments per chain, $N_0 < (b/d)^2$ (see [38]). With numerical values quoted above, the last condition becomes $N < 800$.

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[40] Note that $\Delta s(m)$ is the typical value of the length-along-the-tube fluctuation of the m section.

[41] Note that we are considering, here, a freely jointed chain of segments (blobs). In the general case, some correlations of orientations of neighboring segments should be expected, so that the induced order parameter must be proportional to the effective Kuhn segment a^* of the chain (the primitive path):

$$H(m', 0|\nu') = \frac{a^*}{a}\nu'/N.$$

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[44] In order to derive this equation, we note that a tube-length fluctuation $\delta L = a\delta N$ implies with an additional elastic energy of longitudinal deformation: $\delta F_{\rm el} = 0.5k(\delta L)^2/N$. Note that the 1st term in the right hand side of Eq. (27) is in apparent agreement with this expression for $\delta F_{\rm el}$.

[45] Note that τ is the reduced time, $\tau = t/\tau_0$.

[46] A.N. Semenov and A.V. Subbotin, Vysokomol. Soedin., Ser. A **31**, 2561 (1989).

[47] In Sec. IV, we defined η as a time-averaged order parameter. Note, however, that the averaged order parameter is controlled by that of the leading end. In the general case, Eq. (58) gives the local order parameter near the leading end, which in the regime $\epsilon N \gg 1$ coincides with the averaged value, η. It is this local interpretation of Eq. (58) (with local η) that is using in the current section.

[48] In fact, Eq. (91) is valid for not very strong fields, see below Eq. (95).

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[55] Note, also, that BRF predicts a strong dependence of the mobility on the length, l, of the uncharged portion of the chain, the dependence being noticeable for l up to m^*a (m^* blobs). On the other hand, BRM would predict l dependence of μ only for $l \lesssim a$ (one end blob).