Theory of DNA electrophoresis in physical gels and entangled polymer solutions

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A scaling theory is presented for the electrophoretic mobility of DNA in sieving media that form dynamically evolving meshworks, such as physical gels and solutions of entangled polymers. In such media, the topological constraints on the DNA's motion are perpetually changing as cross links break and rejoin or as the polymers diffuse. It is shown that if the rate of constraint release falls within a certain range (which depends on the field strength), fractionation can be extended to higher molecular weights than would be feasible using a permanent gel of equivalent pore size. This improvement is a consequence of the disruptive effect that constraint release has on the mechanism of molecular orientation. Numerical simulations support the predictions of the theory. The possibility of realizing such a system in practice, with the aim of improving on current electrophoresis methods, is commented upon. It is suggested that semidilute polymer solutions may be a versatile medium for the rapid separation of long single-stranded DNA molecules, and the particular quality of solution required is identified.

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I. INTRODUCTION

Separation of DNA by electrophoresis requires a sieving medium because the mobility of linear polyelectrolytes in free solution is independent of their molecular weight. The medium of choice has traditionally been a permanent gel, made from either agarose or polyacrylamide. Conventional gel electrophoresis provides excellent fractionation of molecules within a limited range of sizes. For short DNA fragments, the separation is supposed to be a consequence of the reduction in free volume available to the molecules as their size increases [1]. But for longer molecules, the ability of gels to provide good fractionation has been attributed to the way in which the mesh of cross-linked gel fibers obliges the DNA to wriggle end-on along a contorted path. The description of this process by the reptation model [2] is able to account for: (1) the inverse dependence of mobility on molecular weight that is observed experimentally at low field strengths [3,4] and (2) the transition to length-independent mobilities at high molecular weights, which is shown to be a consequence of the orientational effect of the field [5–7]. Recently, there has been considerable interest in replacing the gel with a concentrated solution of hydrophilic polymers [8–10]. This has been motivated, in particular, by the development of high-performance capillary electrophoresis [11], since the use of polymer solutions avoids the considerable technical difficulties associated with the manufacture of gel-filled capillaries and offers advantages of efficiency, flexibility, and convenience. The thinking is that the polymer molecules, being thoroughly entangled with one another, constitute a sort of mesh through which the DNA must pass [8,12,13]. The solution therefore acts rather like a gel, with the important exception that there are no permanent cross links and the mesh is constantly changing as the polymers diffuse. The system is not easy to analyze since the DNA may entrain the surrounding polymers thereby disturbing the network [13]. To build a firm foundation for a theoretical model, we propose first investigating the mobility of long-chain molecules in a somewhat simpler, but related, sieving medium: a temporary gel in which the cross links are perpetually breaking and rebonding as a result of thermal agitation. We shall be concerned with the case where the rate of rupture is rather slower than the rate of rejoining so that the fraction of broken links at any instant is small and the gel maintains its solidity.

Our analysis will be based on the reptation model, which is valid when the applied electric field is not too strong. The precise definition of this "weak field" condition is stated in Sec. II, where the parameters are defined. In Sec. III we recall the theory of "biased reptation including fluctuations," which accounts for the main features of the electrophoretic mobility of DNA in a permanent gel. The effect of cross-link rupture is calculated in Sec. IV where we conclude that certain types of temporary gel may provide better separations than permanent gels. The main predictions are checked by numerical simulation in Sec. V. Section VI provides a discussion of the practicality of performing electrophoresis in physical gels, and we conclude in Sec. VII with the suggestion that semidilute polymer solutions, which should offer the same advantages as temporary gels, may provide a versatile means of fractionating long single-stranded DNA molecules.

II. DEFINITIONS OF PARAMETERS

The molecular parameters relevant to the dynamics are the Kuhn length b of the polymer, the effective charge q_0 carried by each Kuhn segment, and the friction ζ_0 that a Kuhn segment experiences as it moves through the fluid. The permanent gel can be characterized by a single parameter, its average pore size a. For a reversible gel, the mean frequency w with which the cross links dissociate is

also required. Our aim is to establish expressions for the electrophoretic mobility of DNA molecules as a function of their contour length S (or, more conveniently, the number of Kuhn segments $N_0=S/b$), the applied electric field E, and the nature of the gel. Since we wish to emphasize the way quantities scale, numerical constants will be neglected.

The biased reptation model makes use of the "primitive path" representation of the polymer in a tube. The molecule is depicted as a chain of N segments, each of size equal to the tube diameter a. In the case $a > b$ where the molecule is flexible on the scale of the gel pores, each segment corresponds to a randomly coiled section of the molecule containing $p=(a/b)^2$ Kuhn lengths. So the effective charge and the friction per primitive chain segment are $q=pq_0$ and $\zeta=p\zeta_0$, respectively, and the total number of segments is $N=N_0/p$. In this article we shall restrict our discussion to this situation. The alternative case of "tight" gels where $a < b$, although readily investigated along the same lines, is a little more complicated and we prefer not to obscure the essential features of our analysis with too much detail.

We shall introduce some combinations of the parameters that play a special role in the theory. $\tau=a^2\zeta/kT$ is the Brownian relaxation time of a primitive path segment. Note that $\tau=p^2\tau_0$, where τ_0 is the relaxation time of a Kuhn segment. $\mu_0=q/\zeta=q_0/\zeta_0$ is the mobility that the molecule would have in free solution. $\varepsilon=qEa/kT$ is a dimensionless measure of the field strength; it is the typical energy change of a segment as it moves from one pore to another, relative to the thermal energy. It can be shown [14] that if $\varepsilon > 1$, the reptation model is no longer a valid description of the molecular motion, for the field is strong enough to pull loops of the molecule out of the tube. $\varepsilon < 1$ is therefore the "weak field" condition, mentioned above, that we shall be concerned with in this article.

III. PERMANENT GELS: BIASED REPTATION

Lumpkin and Zimm's analysis [4] of the driven reptation motion of a polymer shows that the mobility depends on the average conformation of the molecule, or more precisely on the projection h of the end-to-end vector in the field direction, according to the expression

$$\mu=\mu_0\langle h^2\rangle/(Na)^2 . \tag{1}$$

If the molecules maintain the Gaussian, random-walk conformation that they have when they are in thermal equilibrium, $\langle h^2\rangle \sim Na^2$ so that the mobility is inversely proportional to the molecular length. This is the reason for the ability of gel electrophoresis to separate molecules according to size. However, it is well known that there is a certain size limit beyond which all molecules migrate at the same speed, so that a separation can no longer be achieved. This is a consequence of the perturbational effect of the field on the molecular conformation: long molecules become oriented along the field direction. Duke, Semenov, and Viovy [7] have developed a theory for the orientation that illustrates that it depends on a competition between molecular drift through the gel and the longitudinal fluctuations (or "breathing mode") of the molecule in the tube (Fig. 1). Fluctuations release the end section of the chain from the tube, while the overall advance of the molecule through the gel seeks to imprison it. The faster the molecule is drifting, the shorter will be the length of the terminal section that has the opportunity to escape. It is only on the free section that the field can exert an orientational influence and the overall degree of alignment will depend on the total electrostatic force which is proportional to the length of the section.

The analysis may be summarized as follows. After a short time t, the number of primitive chain segments that will have escaped from the tube as a result of longitudinal fluctuations is

$$n_{\text{fluc}} \sim (t/\tau)^{1/4} . \tag{2}$$

During this time, the molecule will have drifted forward through a number of pores equal to

$$n_{\text{drift}}=t\dot{s}/a , \tag{3}$$

where $\dot{s}$ is the velocity of the molecule along the tube axis. Due to the different scaling of Eqs. (2) and (3), n_{fluc} is larger than n_{drift} at short times while the converse is true at long times; only on a time scale shorter than the crossover time t_{eq} can the fluctuations beat the drift. The condition

$$n=n_{\text{fluc}}(t_{\text{eq}})=n_{\text{drift}}(t_{\text{eq}}) , \tag{4}$$

then, specifies both the crossover time and the number n of segments immediately adjacent to the chain end that behave as though they are free of the tube. This terminal section is at liberty to explore the surrounding space. The sole constraint on its motion is that, being joined to the rest of the molecule that *is* hemmed in by the gel fibers, it is effectively anchored at one end. As the free section searches a path through the gel, its conformation will be affected by the external field, which tends to align it against the randomizing effect of Brownian agitation. The degree of orientation is proportional to the total force acting on the section so that, if θ is the angle that a segment makes with the field vector,

$$\langle\cos\theta\rangle \sim n\varepsilon , \quad \varepsilon < 1 . \tag{5}$$

But notice that n depends on the drift velocity $\dot{s}$ through Eq. (4), the drift velocity is proportional to the end-to-end

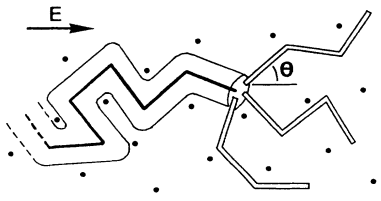

FIG. 1. Mechanism of orientation. In black: the major part of the molecule is trapped in a tube by the surrounding gel fibers. In white: alternative conformations of the rapidly fluctuation end section that is free of restrictions; owing to the influence of the electric field, the more oriented conformations are favored.

projection h,

$$\dot{s} = \varepsilon h / N\tau \ , \tag{6}$$

and the mean value of h depends on the orientation,

$$\langle h \rangle = Na \langle \cos\theta \rangle \ . \tag{7}$$

So the argument is circular and the set of Eqs. (2)–(7) must be solved self-consistently. A solution exists provided that $N > N^*$, given by

$$N^* \sim \varepsilon^{-1} \ . \tag{8}$$

[For shorter chains, the degree of orientation is not sufficient to outweigh the Gaussian component of the conformation so that Eq. (7) does not apply.] One finds that

$$n \sim \varepsilon^{-1/2} \ , \tag{9}$$

$$t_{eq} \sim \varepsilon^{-2} \tau \ , \tag{10}$$

so that the degree of orientation is

$$\langle \cos\theta \rangle \sim \varepsilon^{1/2} \ . \tag{11}$$

Thus, the prediction for the mobility of molecules in a permanent gel is

$$\mu/\mu_0 \sim \begin{cases} N^{-1} \ , & N < N^* \ , \quad (12a) \\ \varepsilon, & N > N^* \ . \quad (12b) \end{cases}$$

Molecules smaller than N^*, given by (8), remain Gaussian and have a mobility inversely proportional to their length. They can be separated. Molecules longer than N^* are oriented by the field and, as a consequence, have a length-independent mobility. They cannot be distinguished from one another by electrophoresis. Equations (12a) and (12b) have been confirmed by a detailed experimental study of the gel electrophoretic mobility of DNA in weak fields [15] and also by computer simulation [16].

IV. TEMPORARY GELS: CONSTRAINT RELEASE

Now we turn to the case of a reversible gel. How does rupture of the cross links affect the motion of the chain? Clearly, if the rate of dissociation is too high, the gel no longer provides sufficient topological constraints for the existence of a tube and the molecules will not reptate. If $w > \tau^{-1}$, the cross links are rupturing so quickly that the polymer is unable to equilibrate to its current environment before that environment changes. In this case, the gel imposes no topological constraint whatsoever on the polymer, which consequently moves as through it were in free solution. At slower rates $w < \tau^{-1}$, the gel fibers confine the molecule only temporarily. Although a tube exists, it is not immutable but gradually changes shape at each point along its length. In fact, the primitive path will move in a Rouse-like manner, for any pair of segments is free to change its conformation each time an adjacent cross link breaks. Since the rate of these local conformational changes is w, the effective Rouse diffusion coefficient of the primitive path is $D \sim a^2 w / N$. In the presence of an electric field that exerts force $F = NqE$ on the molecule, the mobility can be deduced from the Einstein relation $\mu E = FD/kT$. Thus,

$$\mu/\mu_0 \sim \begin{cases} 1, & w\tau > 1 \ , \quad (13a) \\ w\tau \ , & w\tau < 1 \ . \quad (13b) \end{cases}$$

The molecular mobility will be equal to the Rouse mobility of the primitive path, Eqs. (13a) and (13b), when this is faster than the reptative motion of the polymer along the (temporary) tube.

A far more interesting possibility arises when the rate of breakage is too slow to completely destroy the tube before the molecule has slithered out of it by reptation. For, in this case, the release of constraints may nevertheless be rapid enough to interfere with the process of orientation. There are two significant features about the mechanism of orientation. First, the process takes place on a particular time scale t_{eq}; the equilibration time of the fluctuating section that has the opportunity to escape the tube. Second, orientation arises out of the interplay between the electric force, which pulls on the free section of the chain, and the constraints imposed by the gel fibers, which hold back the rest of the molecule, effectively anchoring the location of one end of the free section. Clearly, if the cross links have a high probability of breaking within the equilibration time, the balance between force and constraints will be disrupted and the process by which orientation is generated will be destroyed. We therefore expect that if $w > t_{eq}^{-1}$, molecular orientation cannot be sustained, however long the polymers may be. From Eq. (10), this condition is $w > w^*$, where

$$w^* \tau \sim \varepsilon^2 \ . \tag{14}$$

In this case, there is no transition to the regime of reptation with orientation (12b) as the molecular size increases. Rather the molecules continue to reptate in Gaussian configurations with a mobility given by (12a). Evidently, at some point the reptative mobility, which decreases inversely with molecular length, becomes so small that it is outweighed by the Rouse-like drift of the molecule caused by constraint release (13). It is this factor that imposes the limit of separation, for the mobility then becomes length independent. Comparison of Eqs. (12a) and (13b) shows that Rouse drift dominates when $N < N^{**}$, given by

$$N^{**} \sim (w\tau)^{-1} \ . \tag{15}$$

An examination of Eqs. (15), (14), and (8) reveals, rather remarkably, that there is a range of dissociation rates, $\varepsilon^2 < w\tau < \varepsilon$, for which N^{**} is greater than N^*. By disrupting molecular orientation, a reversible gel can *extend the regime of length-dependent mobility.* The optimal choice of rupture frequency is $w = w^*$, which provides the maximal value of the separation limit

$$N^{**}_{max} \sim \varepsilon^{-2} \ . \tag{16}$$

As the dissociation rate decreases below w^* and the orientation mechanism becomes effective, one might expect the behavior to revert to that of a permanent gel.

But another intriguing possibility arises for $w < w^*$ is the condition for orientation to be *sustained* rather than *generated:* if a molecule is oriented, it will tend to remain so. But one should consider also the possibility that a molecule in a Gaussian configuration has no tendency to orient. Since a randomly coiled molecule has a lower drift velocity than an oriented one, the equilibration time of its fluctuating end section is longer, and so constraint release due to the breakage of cross links has more time to undo any nascent orientation. Thus, it is possible that a Gaussian chain will not spontaneously orient even though the oriented state, if attained, would be sustainable. Indeed, substituting $\langle h \rangle \sim N^{1/2}a$ for Eq. (7) and solving Eqs. (2)–(6), one obtains, instead of Eq. (10)

$$t_{eq} \sim \varepsilon^{-4/3} N^{2/3} \tau \ . \tag{17}$$

So we conclude that if $w > w^{***}$ given by

$$w^{***}\tau \sim \varepsilon^{4/3} N^{-2/3} \ , \tag{18}$$

molecules that are in a Gaussian configuration tend not to orient. This means that in the regime $w < w^*$ long chains have two alternative states. When $N > N^{***}$ given by

$$N^{***} \sim (w\tau)^{-3/2} \varepsilon^2 \ , \tag{19}$$

the molecule may be *either* oriented *or* Gaussian. Migration is more rapid in the first of these states than in the second, so the molecular mobility becomes double-valued. In practice, the molecule will persist in either one of the states until a chance fluctuation causes it to switch to the alternative one. The experimentally measured mobility would then depend on the fraction of time spent in each state. To estimate the likelihood of fluctuations of sufficient magnitude to cause a transition between oriented and Gaussian configurations or vice versa, we must look in a little more detail at the mechanism of orientation. Above, we showed that the orientation of new tube segments depends on the contour velocity of the chain along the tube. But, to be more precise, it depends only on the contour velocity of the section of the chain adjacent to the leading end whose Rouse time is equal to the equilibration time t_{eq} (the dynamics of the chain end is uncorrelated with the motion of more distant parts of the chain on the time scale in which orientation arises). The size of this section is $m = n^2$ segments. The orientation of new tube segments, then, is governed by the contour velocity of these m segments, which in turn depends on their own orientation. So transitions of the entire chain conformation may be triggered whenever chance fluctuations sufficiently alter the orientation of the m segments adjacent to the end. Consider first a molecule that is oriented along its entire length. Then $m \sim \varepsilon^{-1}$ and the random fluctuations in the projection of the m section (of magnitude $m^{1/2}a$) are not small relative to its mean value ($m\varepsilon^{1/2}a$). So transitions from oriented to Gaussian configurations are expected to be rather common. Now consider a molecule that is randomly coiled. Then $m \sim \varepsilon^{-2/3} N^{1/3}$; since the size of the m section increases with chain length N, the relative magnitude of fluctuations in its orientation decreases with N. So transitions from Gaussian to oriented conformations are expected to become less common at higher molecular weights. Together, these considerations imply that shorter molecules, $N \sim N^{***}$, are likely to make fairly frequent transitions between the two possible states, while longer molecules $N \gg N^{***}$ are likely to remain for long periods in the Gaussian state and make only rare, brief excursions to the alternative, oriented configuration. We infer that the molecular mobility may decrease rapidly as a function of size in the regime $N \gg N^{***}$, a feature that, by providing good selectivity over a narrow range of sizes, might be practically advantageous. But one should bear in mind that in the case where transitions due to thermal fluctuations become rare, molecules are likely to remain in a single state for a duration comparable to the time scale of the experiment. Then the fraction of time spent in each of the two alternative configurations will depend on both the initial conditions and the total duration of the experiment so that the measured mobility will be ill-defined. In practice, this will result in diffuse bands.

To summarize, the predicted mobility of molecules in a temporary gel is

$$\mu/\mu_0 \sim \begin{cases} 1 \ , & 1 < w\tau \ , & \text{(20a)} \\ N^{-1} \ , \ N < N^{**} \\ w\tau \ , \ N > N^{**} \end{cases} \varepsilon^2 < w\tau < 1 \ , \quad \begin{matrix} \text{(20b)} \\ \text{(20c)} \end{matrix}$$

$$\begin{cases} N^{-1} \ , \ N < N^* \ , & & \text{(20d)} \\ \varepsilon \ , \ N^* < N < N^{***} & w\tau < \varepsilon^2 \ , & \text{(20e)} \\ ? \ , \ N > N^{***} & & \text{(20f)} \end{cases}$$

with N^*, N^{**}, and N^{***} given by Eqs. (8), (15), and (19), respectively. The molecules migrate by reptation in re-

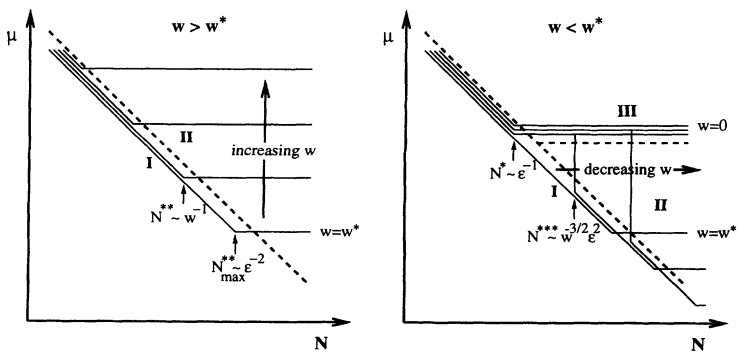

FIG. 2. Double-logarithmic plots showing the scaling of electrophoretic mobility with chain length at different values of the rate of cross-link dissociation. The regimes divided by the dashed lines are: I, reptation in Gaussian configurations; II, Rouse drift in Gaussian configurations; III, reptation in oriented configurations.

gimes b, d, and e and by Rouse drift in regimes a and c. Their conformation is Gaussian in regimes a–d, while in regime e they are oriented by the field. The question mark signifies that in regime f the mobility is double-valued: molecules may be either Gaussian, in which case their mobility is given by (20b) and (20c), or oriented, in which case their mobility is given by (20d). The scaling behavior of the mobility is shown graphically in Fig. 2.

It is worth mentioning that the potential of temporary gels to extend the range of fractionation is a prediction specific to the biased reptation theory including fluctuations [7]. Earlier versions of the biased reptation model [5,6] which did not take into account the fluctuation-induced molecular orientation, do not predict this effect.

V. NUMERICAL SIMULATION

These predictions have been tested by numerical simulation using the nonlocal defect hopping model [16–18] of polymer dynamics that has been successfully applied to a variety of other problems in gel electrophoresis. In addition to the usual set of rules, we added another rule that models the release of topological constraints as cross links rupture. During each time step of duration τ, and at each point on the chain, there is a probability $w\tau$ that two consecutive segments attempt to assume a new conformation by rotating through a randomly chosen angle about the axis joining their two ends (see Fig. 3). The attempt is accepted or rejected according to a Metropolis probability dependent on the consequent change in electrostatic energy.

The variation of mobility with chain length N is shown in Fig. 4 as a function of the dissociation rate w, for three different values of the reduced field strength ε. It is clear that an extension of the regime in which the mobility varies inversely with molecular length can be obtained for a particular range of values of w. This range becomes broader, and the maximal extension more significant, as the field is made weaker. From the graphs, we can identify w^*, the value of the dissociation rate that provides the largest range of length-dependent mobility, and the corresponding value $N^{**}_{\max}$ of the maximal limit of separation.

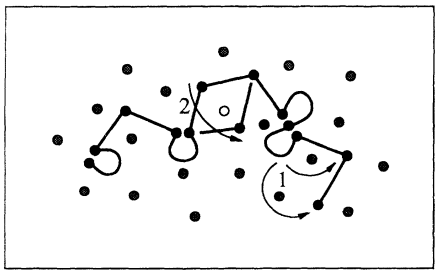

FIG. 3. Illustration of the model used to simulate the molecular dynamics. The DNA is represented as a primitive path with length defects. Circles indicate gel fibers that restrict the motion of the DNA. Reptation is modeled by the variable-range hopping of length defects along the primitive path (process 1). The release of a constraint (demarcated by the unfilled circle) allows the modification of the local conformation of the primitive path (process 2).

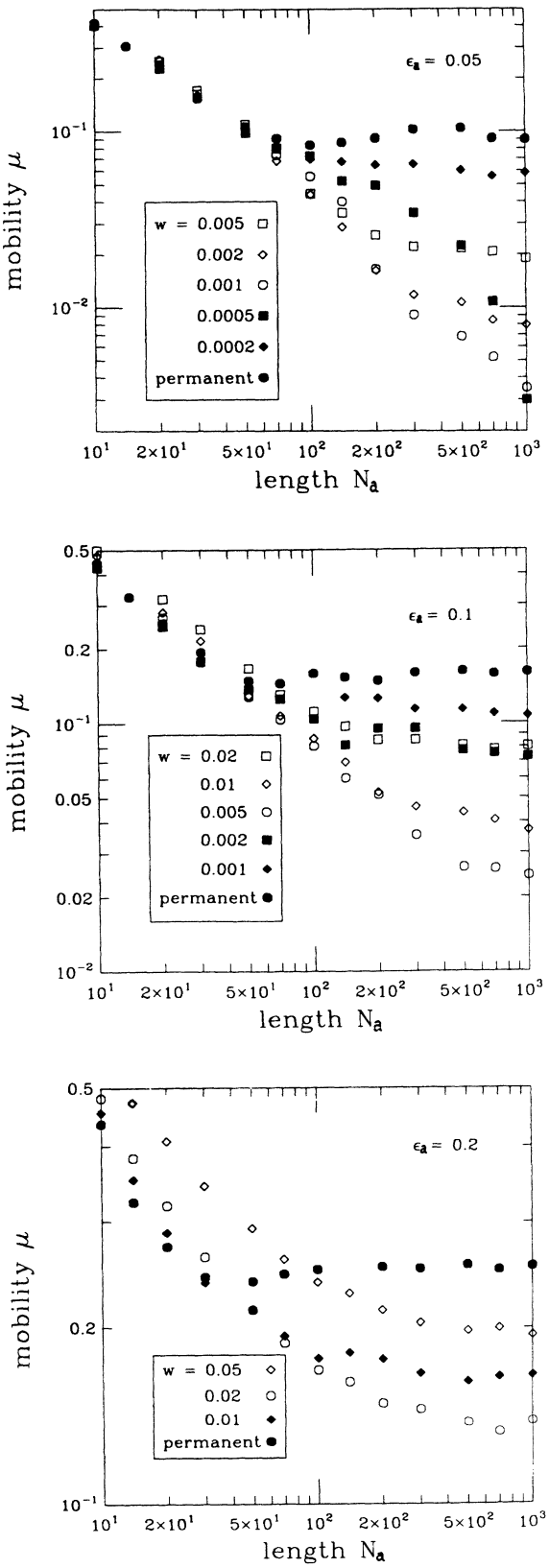

FIG. 4. Simulated length dependence of the mobility as a function of the dissociation rate for three different values of the electric field. The error in the data is less than 10% except for the points $\varepsilon = 0.05$, $w = 0.0005$, $N > 200$ (see text).

TABLE I. Values obtained from the simulation of the optimal rate of cross-link dissociation w^* and the corresponding limit of fractionation N^{**}_{max} at different values of the reduced field ε. The separation limit in a permanent gel, N^*, is shown for comparison.

ε	0.05	0.1	0.2
w^*	0.001	0.005	0.02
N^{**}_{max}	>1000	400	140
N^*	80	40	20

The trend in these values as the field strength varies, shown in Table I, is compatible with Eqs. (14) and (16). By observing a film of the simulation, one can readily confirm that molecules that lie in the regime of size-dependent mobility migrate principally by reptation in nearly Gaussian configurations [Fig. 5(b)]. If $w > w^*$, larger molecules that have a roughly size-independent mobility move by Rouse drift in Gaussian configurations [Fig. 5(c)] and their mobility is proportional to w, as predicted by Eq. (20c).

As w decreases below w^*, the general behavior approaches that of a permanent gel Eqs. (20d) and (20e) and long molecules reptate in oriented configurations [Fig. 5(a)]. At the lowest value of the field strength simulated, however, evidence was found of the transition to double-valued mobilities at large molecular sizes, Eq. (20f). The data for $\varepsilon=0.05$, $w=0.0005$ (just a little smaller than w^*) follow closely those of the permanent gel for chain sizes up to $N=200$, but deviate for longer molecules. Furthermore, the mobility of the long chains was found to vary considerably from one simulation to another. A plot of the center-of-mass motion (Fig. 6) confirms that the migration of these molecules is highly irregular, and that the instants of rapid migration correspond to an oriented molecular configuration while the intervals of slow movement correspond to coiled configurations. The

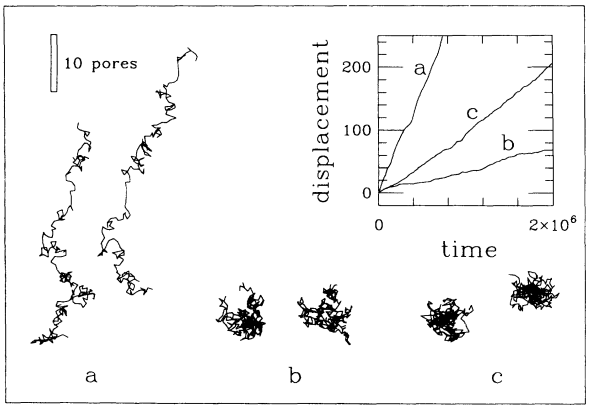

FIG. 5. Pair of successive molecular conformations (separated by a time interval of $50\,000\tau$) of a chain of length $N=500$ in a field of strength $\varepsilon=0.05$, at increasing values of the dissociation rate: (a) $w=0.0002$, the molecule is oriented and moves by reptation; (b) $w=0.001$, the molecule still migrates principally by reptation, but is closely Gaussian; (c) $w=0.005$, the molecule is randomly coiled and moves by Rouse drift. Inset: the displacement of the molecule with time for these three cases, showing the uniformity of the motion.

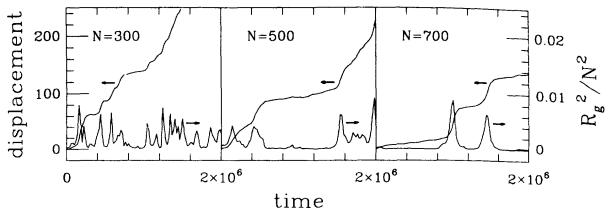

FIG. 6. The displacement of long chains with time for the case $\varepsilon=0.05$, $w=0.0005$ in which the molecules are bistable and alternate between periods of rapid migration in oriented configurations and slow migration in random conformations. Also shown is the radius of gyration of the chains.

fraction of time spent in each state was observed to vary with the chain length. For example, the molecule with $N=300$ made numerous transitions, the chain with $N=700$ became oriented just twice, each time only briefly, and the molecule of size $N=1000$ did not once succeed in orienting from its initial Gaussian configuration during the course of the simulation. This behavior is in accordance with the theoretical prediction of two different solutions for the mobility when the chain length is greater than N^{***}. As w decreases further below w^* the transition to double-valued mobility is no longer observed within the range of chain sizes investigated, as expected from Eq. (19).

VI. PRACTICAL REALIZATION

In order to determine whether or not the replacement of permanent gels by reversible ones could be practically advantageous, we need to consider the values of the physical parameters that would lead to improved separations to see if they correspond to a realizable system. Expressing Eqs. (8), (14), and (16) in terms of the molecular parameters and writing $E_0=kT/q_0b$, we have

$$N_0^* \sim (a/b)^{-1}(E/E_0)^{-1} \tag{21}$$

for the limit of separation in permanent gels, while the maximum limit for temporary gels is

$$N_0{}^{**}{}_{max} \sim (a/b)^{-4}(E/E_0)^{-2} \tag{22}$$

achieved when $w=w^*$ given by

$$w^* \sim (a/b)^2(E/E_0)^2\tau_0^{-1} . \tag{23}$$

Thus, the use of a temporary gel can extend the limit of separation by a factor up to $(a/b)^{-3}(E/E_0)^{-1}$. Clearly, the effect will be most significant using the combination of a weak field and a gel pore size not much larger than the Kuhn length.

Let us consider, specifically, the case of single-stranded DNA; owing to its great flexibility, this has a much higher value of E_0 than double-stranded DNA and consequently a low value of E/E_0 is more readily attainable. Presently, an experimental measurement of the Kuhn length of single-stranded DNA has not been obtained, but since it is expected to be flexible on the scale of just a few bases, a reasonable estimate would be $b \approx 3$ nm. The effective charge per Kuhn length, which will be lower

than the bare charge of one per base due to the screening of counterions, is likely to be $q_0 \approx e$. Then $E_0 \approx 10^5$ V/cm at room temperature. Furthermore, we can estimate the value of τ_0 from the mobility of DNA in free solution: since $\mu_0 = q_0/\zeta_0$ and $\tau_0 = \zeta_0 b^2/kT$, we have $\tau_0 = b/\mu_0 E_0$. Experimentally, $\mu_0 \approx 10^{-4}$ cm^2 V^{-1}s^{-1} so we deduce that $\tau_0 \approx 10^{-7}$ s.

Now, for the sake of definiteness, suppose that we choose a temporary gel with a pore size equal to 6 nm and use an electric field of 100 V/cm (conditions similar to those typically used in polyacrylamide electrophoresis). Then we see that by tuning the dissociation rate of the cross links to the appropriate value, we could increase the range of length-dependent mobility by a factor of about 100 over that obtainable using the equivalent permanent gel. However, such a large increase in the theoretical limit of separation may not count for much in real terms. In practice, fractionation of two species that differ only slightly in size requires that the spatial separation of their respective bands be greater than the band breadth, which is due to diffusion. Evidently, this condition is more rapidly satisfied the faster the molecules are migrating. In our example, the increase in the theoretical limit by a factor of 100 is achieved at the expense of slowing down the longest molecules by the same factor. So it is clear that the theoretical improvement could be turned to practical advantage only by performing separations over a very long duration of time. This suggests that a more advantageous choice of temporary gel would involve a compromise between extending the separation limit and shortening the run time. So suppose now that, still using a temporary gel with pore size 6 nm, we were to increase the field strength to 300 V/cm (a magnitude that can be sustained in thin capillaries without causing too much Joule heating). Then the theoretical separation limit would still be a factor of 10 higher than that provided by the equivalent permanent gel at $E = 100$ V/cm. Furthermore, owing to the elevated field strength, molecules of all sizes would be migrating three times more quickly. So, in this case, much of the improvement in the limit of separation—a factor of three at least—can be translated into practice without increasing the duration of the fractionation process. For this choice of field strength and pore size, the rate of cross-link rupture [Eq. (23)] that is required turns out to be $w^* \approx 300$ s^{-1}. Is this realizable? While it may be possible to construct such a physical gel, the twin requirements of the correct pore size and the correct dissociation rate are likely to be awkward to satisfy simultaneously, particularly since the latter has to be tuned rather finely. A much more promising candidate for a fractionating medium that could fulfill these requirements is a semidilute polymer solution.

VII. POLYMER SOLUTIONS

The semidilute regime of a polymer solution is that in which the molecular concentration c is high enough for the coils to overlap. The critical concentration above which the polymers interpenetrate is usually labeled c^*. When $c \gg c^*$ the polymers are well enmeshed with one another and form a network robust enough to resist deformation by the DNA which, influenced by the electric force, pushes against it. That is, we conjecture that the entanglement of any one polymer with its neighbors is sufficient to prevent it from being entrained by the DNA. Consequently, the DNA moves through the mesh of polymers just as it would through a gel. But the network acts like a temporary gel rather than a permanent one, for the thermal motion of the polymers ensures that the entanglements do not endure indefinitely. Indeed, each polymer is hemmed in by the surrounding molecules in just the same fashion as the DNA, and consequently diffuses by reptation. Since an entanglement is undone only when the end of a polymer passes by, the average lifetime of entanglements is equal to the tube renewal time (or reptation time) of the polymers. The inverse of this time, then, plays the role of the cross-link dissociation rate w of the temporary gel. The effective gel pore size is equal to the average distance between entanglements which, for a semidilute solution, is the screening length ξ.

Let us consider a solution of polymers in which each molecule contains N_p statistical segments of length b_p. If the solution were dilute, the radius of gyration R_p of the polymers would be

$$R_p \sim N_p^{3/5} b_p \ . \tag{24}$$

Here the excluded-volume exponent $\nu = \frac{3}{5}$ is that for a good solvent, which we shall suppose to be appropriate since for DNA fractionation we are concerned with hydrophilic polymers in aqueous solvents. When the number density of polymer segments c is higher than the critical value c^* given by

$$c^* \sim N_p / R_p^3 \tag{25}$$

the solution is semidilute and characterized by the screening length ξ above which the excluded volume interaction is screened [19,20],

$$\xi \sim (c/c^*)^{-3/4} R_p \ . \tag{26}$$

On length scales larger than ξ each polymer may be thought of as a chain of noninteracting subunits or "blobs" of size ξ (Fig. 7). The number N_{blob} of blobs on

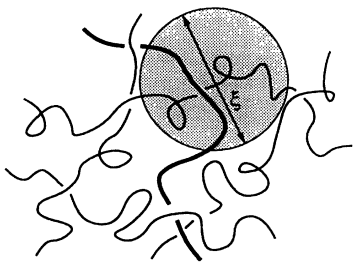

FIG. 7. Schematic representation of a single-stranded DNA molecule (thick line) threading through a mesh of chains (thin lines) in a semidilute polymer solution. The circle identifies one of the blobs into which the polymer molecules are conceptually divided.

each polymer is specified by the relation for the radius of gyration of a blob,

$$\xi \sim (N_p/N_{\text{blob}})^{3/5} b_p \ . \tag{27}$$

The reptation time τ_p of the polymers is

$$\tau_p \sim N_{\text{blob}}{}^3 \tau_{\text{blob}} \ , \tag{28}$$

where τ_{blob} is the blob relaxation time, so substituting from Eqs. (24)–(27) we obtain the expression for the rate of constaint release $w = \tau_p{}^{-1}$

$$w \sim (c/c^*)^{-15/4} \tau_{\text{blob}}{}^{-1} \ . \tag{29}$$

At this point, it may be worthwhile to demonstrate that the above arguments for the electrophoretic mobility of DNA in temporary gels can successfully be applied to semidilute polymer solutions. In the regime where constraint release causes the DNA to migrate by Rouse-like dynamics rather than reptation, its mobility in a temporary gel is given by Eq. (13b). Using this formula for a semidilute solution and Eq. (29) for the rate of constraint release, we obtain

$$\mu/\mu_0 \sim (c/c^*)^{-15/4} \tau/\tau_{\text{blob}} \ . \tag{30}$$

Now, we expect a polymer blob to have Zimm dynamics,

$$\tau_{\text{blob}} \sim \eta \xi^3/kT \ . \tag{31}$$

The charged DNA, on the other hand, behaves like a free-draining coil as a consequence of the counterion flow. So, the time τ associated with a tube segment [which comprises $p = (\xi/b)^2$ Kuhn segments] is the Rouse relaxation time

$$\tau \sim p^2 \tau_0 \ , \tag{32}$$

where τ_0, the relaxation time of a Kuhn segment, is given by

$$\tau_0 \sim \eta b^3/kT \ . \tag{33}$$

Thus, Eq. (30) for the mobility due to constraint release in semidilute solutions may be written

$$\mu/\mu_0 \sim (c/c^*)^{-15/4} \xi/b \ . \tag{34}$$

This is precisely the relation that we have obtained previously for this system [13], using a different line of approach based on a calculation of the rate of dissipation of energy in the polymer solution. This correspondence indicates that the arguments used above for a temporary gel can also be applied to polymer solutions. Thus we expect that for solutions, too, there will be a regime in which constraint release disrupts the orientation mechanism so that the regime of reptative separation is prolonged (a possibility that had not been investigated in Ref. [13]).

We shall now proceed to determine the size of the polymers and the concentration of the solution that are required to produce the values of w and ξ that, by preventing DNA orientation, provide a high value of the limit of fractionation. The condition $w = w^*$, on substitution from Eqs. (14), (26), (29), and (31)–(33), corresponds to a particular choice of the polymer size,

$$R_p/b \sim (E/E_0)^{-2/5} \ . \tag{35}$$

Remarkably, the size of polymer that provides the optimal rate of constraint release depends only on the field strength and is independent of the polymer concentration. Recall though that, in Sec. VI, we remarked that the range of fractionation is greater the smaller the mesh size. So, having chosen the polymer size according to (35), the concentration should be chosen [according to (26)] so as to make the mesh spacing ξ as close as possible to the DNA's Kuhn length b. This procedure will provide the maximal limit of fractionation for a given field strength.

Now let us consider the electrophoretic separation of single-stranded DNA in polymer solutions, using the values of the parameters estimated in Sec. VI. Again, we shall look at the practically relevant example of the use of an elevated field to provide a marked increase in the fractionation limit without increasing the run time. If the electric field strength is set at 300 V/cm, Eq. (35) indicates that the polymer size should be chosen to be $R_p \approx 30$ nm. Then Eq. (26) shows that the choice $c/c^* \approx 9$ would give $\xi/b = 2$, i.e., an effective pore size of 6 nm. This system would therefore be equivalent to that of the temporary gel discussed in Sec. VI which betters by a factor of at least three the practical fractionation limit of the equivalent permanent gel at 100 V/cm and yet requires no more run time. We remind readers that we have neglected numerical factors throughout, so these figures should be regarded as order-of-magnitude estimates rather than precise evaluations. Notice that both the radius of gyration and the concentration of the polymers are of magnitudes that are very easy to obtain in practice. We remark, though, one slight technical difficulty. Since the limit of fractionation is sensitive to the rate of constraint release and this, in turn, varies rapidly with polymer length, it is desirable that the polymers be as monodisperse as possible; a moderate degree of polydispersity would attenuate somewhat the fractionating capacity of the solution. With this proviso, semidilute polymer solutions appear to be a promising medium for the separation of long single-stranded DNA molecules, all the more so since they are well suited to the high-throughput capillary electrophoresis techniques currently being developed for sequencing applications.

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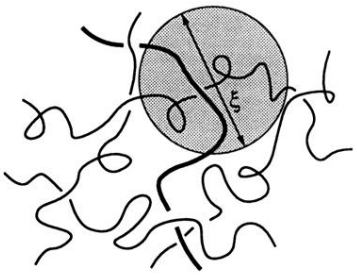

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