Macromolecular separation through a porous surface.

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Abstract

A new technique for the separation of macromolecules is proposed and investigated. A thin mesh with pores comparable to the radius of gyration of a free chain is used to filter chains according to their length. Without a field it has previously been shown that the permeability decays as a power law with chain length. However by applying particular configurations of pulsed fields, it is possible to have a permeability that decays as an exponential. This faster decay gives much higher resolution of separation. We also propose a modified screen containing an array of holes with barb-like protrusions running parallel to the surface. When static friction is present between the macromolecule and the protrusion, some of the chains get trapped for long durations of time. By using this and a periodic modulation of an applied electric field, high resolution can be attained.

1 Introduction

Recent advances in gel electrophoresis have dramatically improved the separation of DNA molecules. The use of pulsed electric fields has now made it possible to do routine separation
of mega-base DNA molecules. There are still improvements in the technique that would be highly desirable. Here a variant of electrophoresis is considered. Instead of separation through a gel, we consider the separation of molecules through porous surfaces, that look like fine screens.

The obvious advantage of such an approach is that separation does not require migration over distances long compared to chain length, as is necessary with gel electrophoresis. In that case typically a molecule drifts of order ten centimeters. Instead, separation can only occur at the screen itself. Thus effects of trapping that have caused problems might be avoided. One might also hope that separation utilizing a screen may be more rapid, as the total displacement of the molecule is much smaller.

Here we investigate two ways to separate macromolecules at a surface. In many respects the findings are encouraging, however there are still considerable technical difficulties that will be necessary to overcome in order to make such ideas practical. The most obvious difficulty is in actually manufacturing such a porous screen. For the separation of DNA, it would need to contain an array of holes of size of order a few thousand angstroms. Its thickness should also be of this order of magnitude.

The motion of a polymer through such a screen has been investigated previously\[1, 2\]. It was found that the permeability of the molecule decreases as $M^{-2}$ where $M$ is the molecular weight. It would be highly desirable to have a more rapid decrease, as this would increase resolution.

In this paper, first we present data for charged molecules moving near a pore in the presence of a pulsed electric field. With an appropriate configuration of pulses, we find the permeability of the device decreased very rapidly with chain length, as an exponential for long enough chains. This then gives rise to a sharp separation, one that is tunable, by varying the time-scale of pulses in the external field.

Second we present the outline of another device with even sharper resolution. It involves both holes and pegs, and may be impossible to fabricate with current technology. However because of the rapid development of fabrication techniques it may be practical in only a few years. Such a device might then have interesting applications.
2 Surfaces with holes

When a polyelectrolyte is close to a screen, it can pass through it by a process similar to reptation\(^3\). If a weak field is applied perpendicular to the surface, this will lead to some differential separation of chains according to their chain lengths. However the effect is a power law\(^1, 2\), and so is rather weak. Here we propose a method for increasing the power of separation dramatically.

If the field is made strong and at an angle, almost parallel to the surface, then the chain will find it difficult to pass through a pore. As the angle of the field becomes parallel and the electric field goes to infinity, the number of chains that pass through the surface go to zero. If the chain starts to pass through a pore, it will occasionally get hooked as shown in figure 1(a). A chain initially on the upper side of the screen is shown. At this point, the chain stretches, (b), creating a large tension which yanks the chain out of the pore (c). This was seen in simulations using a method described in detail below. This would suggest that the passage of long molecules would be greatly diminished by this effect.

However upon performing simulations, we also discovered that there is another mode of motion which allows long chains to occasionally pass. Once the chain has stretched as in (c), the leading end sometimes will pass though a pore (d), and the chain can thread itself through to the other side (e). This mode of motion is frequent enough to prevent this configuration from being an efficient separation technique. So although the total permeability was small, the dependence on chain length was still fairly weak.

We tried using baffles on the lower side of the screen to prevent this threading mode, but it was not effective. Once chain is threaded through a pore, it will continue to migrate through, even if it is running into a wall.

Instead we found that the following sequence of field pulses proved highly effective. Pulses were also crucial in improving gel electrophoresis\(^4, 5\) for reasons that are similar to those found here. A set of snapshots from the simulation are shown in figure 2. The length in monomer units, is 25.

1. At the beginning of a cycle the chain is close to the screen and is in a random coil
configuration. This is shown in figure 2(a) labeled “start”.

2. Then a field is applied, in these simulations, at a grade of 2 to 1. It hits the screen as shown in 2(a) and then flattens out and tumbles along the screen. As in the previous discussion, we see it often becomes hooked around a pore, see figure 2(b) and (c).

3. The field is suddenly reversed. In these simulations, for half the duration of the previous stage. The chain quickly moves of the surface of the screen, 2(d)

4. The field is turned off and the chain goes back to the shape of a random coil

In the cycle shown in the figure the chain doesn’t get through because the field is reversed before it has a chance to pass completely through. Often however it does manage to pass through the screen, in which case it is unlikely to be able to return to the other side, since the duration of the field reversal is for only a fraction of the time in the forwards direction.

The point of step 3, is to disentangle chains from the screen and move them away from it. There are more sophisticated ways to do this which may work better in practice. Instead of only reversing the field, a large electric could be first applied parallel to the surface. This has the effect of disentangling chains without letting a significant number from the lower side pass through. At that point the field could be reversed and this would insure that no chains were entangled with the surface.

The chains need to be a large distance, compared with the radius of gyration, from the surface before they are allowed to relax to random coils. Otherwise they can diffuse by Brownian motion to the surface while the coils are relaxing. Short and long chains migrate at almost the same rate in free solution as they act as free draining chains[13]. Therefore all the chains will move away from the surface by about the same distance. The relaxation time should be that for the longest chains, or at least it should be set at a relaxation time appropriate for chains longer than those that we desire to separate. A simple estimate shows that for the longest chains, this criterion is met for the design analyzed here. A chain drifts of order its radius of gyration in one relaxation time and the radius of gyration $R_g = N^{3/5}a$, $a$ being close to a persistence length and $N$ being the number of persistence lengths. Denoting the distance diffused by a chain $x$, we want to move the chains at a distance away from the screen $d \gg x$. 

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In one relaxation time a chain moves a distance of order its radius of gyration, \( x = N^{3/5}a \) so we require

\[
d >> N^{3/5}a.
\] (1)

To estimate \( d \), we use the fact that the time-scale for migration of a chain through the screen is such that a free chain will move of order its own length, or several times that. Therefore the reverse field will move a chain of order \( Na \). Therefore we need \( Na >> N^{3/5}a \), or

\[
N^{2/5} >> 1
\] (2)

This is well satisfied for chains longer than 100 kilobases.

With a mixture of different chain lengths, the shorter ones will diffuse more rapidly and have a larger population at the surface. In a time \( T \), their density \( \rho(N) \) is proportional to \( \exp(-d^2/DT) \) where \( D \), the diffusion coefficient is proportional \( 1/R_g \propto N^{-3/5} \). Therefore

\[
\rho(N) \propto \exp(-\text{const}.N^{3/5}).
\] (3)

So even if chains are present at the surface, their numbers decay very rapidly with chain length, so this effect by itself should provide excellent resolution.

In addition, if we wish to eliminate this effect, a large electric field parallel to the surface can be maintained during the relaxation stage. This inhibits any stray chains near the surface from passing through it. Then chains substantially shorter than 100 kilobases should be usable with this apparatus.

### 3 Simulation

Simulation results are shown in figure 3. The simulation technique used was similar to one used previously to study gel electrophoresis, and has had a number of successes predicting, or explaining the results of experiments on pulsed field gel electrophoresis most importantly predicting the motion later seen by fluorescence microscopy.

The polymer is represented as a freely hinged chain, with the distance between adjacent monomers constant. An off-lattice Langevin equation was solved for the chain. The dynamics
were that of a chain in the free draining limit, and no long range hydrodynamic interactions were included. Tension between neighboring monomers was introduced to keep their separation constant. By solving a tridiagonal matrix equation for each time step, the tensions were computed, enabling one to update the position of the monomers quite efficiently.

The screen here is represented by a linear array of pegs. We specified $F_i$, the repulsive force between the ith peg and a bead on the chain. $F_i$ was chosen so as not to allow crossing of chain through pegs. Several forms for $F_i$ have been tried for gel electrophoresis, all giving similar results. The one used in these simulations is the same as was used previously and has the form

$$F(r_i) = \begin{cases} \sigma \Delta r \left[ \frac{1}{\Delta r} - \frac{1}{r_c} \right]^2 & \text{for } r_c < \Delta r < 2r_c \\ 0 & \text{otherwise} \end{cases}$$

Here $\Delta r$ is the difference between the position of a bead and the center of a peg. $\sigma$ adjusts the strength of the potential, and $r_c$ represents the hard core radius, which the chain can never penetrate. For the results described in this paper, $\sigma = 0.195$, and $r_c = 0.2$, the link lengths equal 0.47, and the distance between the pegs equals 1. This corresponds to having about two persistence lengths of DNA between pores. With a larger pore separation, the results described are not expected to change qualitatively.

A random force was added representing thermal noise, and an electric field added as described above. The magnitude of the parameters chosen is fairly high for those typically used in electrophoresis experiments, fields of about 20V/cm, however lower fields should give similar behavior, but take longer to simulate.

In the beginning of a cycle, a chain was initially started in the shape of a random walk. The pegs were placed in the x-y plane along the line y=0 with a spaced by one unit. Then a downwards electric field in the (-2,-1) direction was applied for 160 time steps, then it was reversed for 80 time steps. If all monomers of the chain passed to the other side of the screen, the cycle was terminated and the passing of the chain through the screen was recorded.

The results of simulations at different chain lengths are shown in figure 3. 1000 cycles were performed for each data point. For long chain lengths the decrease is very sharp. The solid line is a fit to an exponential. The data appears to be falling off as an exponential.
Unlike the case of a constant applied field, the use of a pulsed field appears to enhance the resolution of this device significantly. There is some average time for the chain to get through the screen. Such dynamics have been investigated previously in connection with pulsed field gel electrophoresis[6]. The time scale for crossing the screen should be linear with the chain length. With a fixed duration on contact with the screen the probability of it passing through becomes very small.

A rough estimate of this probability is as follows. In order for a long chain to pass through, it must initially be highly stretched so that it can thread its way through quickly. The probability that a random walk has a radius of gyration comparable with chain length $N$ decreases exponentially with $N$.

4 Discussion

Recently an artificial array of pegs has been manufactured out of SiO$_2$ and used in studies of electrophoresis[14, 15]. DNA molecules separate well with such arrays. It would be interesting to try substituting a one dimensional array of pegs as was studied numerically in the last section.

Since such arrays are already realizable it does not seem too far-fetched to imagine arrays of pores, instead of pegs, etched in silicon SiO$_2$. With enough structural support, such arrays would then be usable on an entire two dimensional surface allowing much more material to pass through.

We envisage that in a the complete apparatus will have a large volume of chains above and below the surface. There are many possible scenarios for how the complete apparatus should work. We discuss below two ideas that may be useful in obtaining a realistic working device.

Chains are introduced into the upper half and will selectively migrate to the lower half. Once a chain is in the lower half, it quickly migrates away from the surface, as the average field points down. Even if a chain does reenter the upper half, this will be a sharply decreasing function of chain length with an even stronger chain dependence than eqn. (3) and will not
adversely effect the resolving power.

We first discuss a situation where the vertical thickness of the upper half of the device is quite small, of order of \( d \), see eqn. (1).

In this case the composition of chains in the upper half of the device will change quickly with time. If enough pulses are applied, all the chains on the surface will get through and the region near the surface will be depleted of chains. At some point we repopulate the chains. If the upper half is repopulated after \( m \) cycles, by introduction of more solution, then we can calculate, in steady state, how the concentration of chains, relative to the upper half, depends on chain length. If the probability that a chain gets through in one cycle is \( p \), then the probability that it will get through after \( m \) cycles is

\[
P_m = 1 - (1 - p)^m
\]

To gain an idea of how such an effect enhances resolution it is necessary to use the exponential fit of figure 3 as simulation results are not accurate enough at the chain lengths we now consider. Using this fit for the probability \( p \) as a function of chain length, we have computed \( P_m \) from eqn. (5) for \( m = 500 \) cycles. This is shown in figure 4. This has the fortunate effect of making the resolution even sharper.

It would be preferable not to have such a shallow upper half, as the periodic introduction of more chains complicates the procedure. So consider now the opposite limit of large vertical depth. In this case, with an average field pointing down, there is a problem. Long chains will pile up at the surface after many pulses. Therefore it would be better to have an average field of zero to overcome this difficulty. Note that it is possible to have an average field of zero and still separate chains. We refer the reader back to the last paragraph of section 2 where we suggested applying a large electric field almost parallel to the surface to inhibit possible drift of chains through pores during the relaxation period. If a large field that is almost parallel to the surface but tilted slightly vertically is applied, this will restore a uniform chain density in the upper half of the device. During this time there will be negligible drift of chains in the reverse direction, as discussed earlier.

To check that this inhibition takes place, we tried varying the strength of the vertical field
in our simulation and measuring the time it takes for a chain to pass through the screen in a constant field, for a chain of length 25. When the strength of the vertical field is decreased so the ratio of fields is 10 to 1, instead of 2 to 1, the time it takes to pass through the screen goes up by about two orders of magnitude. It is so low that it is very hard to gain adequate statistics to measure it accurately.

As time progresses, the depleted region will increase in size as the square root of time, due to diffusion of chains from far above the screen. As in the first scenario, we would like to repopulate chains on the surface. This could be accomplished by slow stirring, leading to similar behavior to figure 4.

5 Screens with Barbs

The screen studied in the preceding sections does not appear to be as selective as gel electrophoresis. However we speculate on how modifications of this basic idea might allow one to obtain similar resolution by using a more elaborate surface and a more complicated sequence of electric fields.

Figure 5 shows what is required to make this device. Besides having holes, cylinders with overhangs are created next to them as is shown by the structure in the front. Behind this is an alternative, cylinders that are tilted towards the horizontal.

The idea behind such a device is that static friction prevents a chain trapped in a U shape around a barb from moving if the two arms of the chain have almost the same length. This effect was predicted theoretically [6] and has been seen in fluorescence microscopy experiments [16].

A mixture of chains of different lengths, are pushed towards the surface by an electric field and then the electric field is directed almost parallel to the surface so that the chains migrate. The lower left hand picture in figure 5 shows the surface. Besides the array of barbs, there are baffles placed underneath them to hasten the curling of chains. Once a chain has fallen off a barb, it will fall onto a baffle and curl as it slides down. It will then fall on top of another barb where it has a large chance of being hooked by it.

The sequence of fields and motion of the chain during a cycle is as follows.
1. Occasionally the two arms of the chain are close enough together that the chain is pinned by static friction. The electric field is continuously rotated until it is pointing almost vertically up.

2. It is kept in this configuration for enough time to allow chains that are not pinned to slide off the barb. Now only those chains that have their lengths almost equal are left on the surface. The rest have migrated away from the surface.

3. The electric field is suddenly reversed. The chains on the surface now enter the hole beneath them. The duration of this portion of the cycle is crucial in determining which chains end up on the other side. If the chain is too long, the ends of it do not pass all the way through the hole, otherwise the whole chain passes to the other side, whereupon the chain ends can diffuse by Brownian motion laterally.

4. The field is reversed again. The chain shown is long enough so that it stays threaded. Therefore it ends up in the same configuration as in 2. If the chain were shorter. It would very likely become trapped on the other side. The important point is that chains longer than a certain threshold, depending linearly on the duration of step 3, will almost always end up on the upper surface.

After stage 4, the field can be set parallel to the surface towards the right, for a short duration, to allow chains to come off the barbs. At the point the entire process repeats itself.

The drawback to this technique is that it requires chains to become pinned. The number of such chains pinning per unit time is small, therefore the speed of separation should be slower than in the previous device that was analyzed. The advantage is that it is highly selective and by adjusting the cycle time as separation progresses, should be able to extract a very pure samples.

It should be noted that for ring polymers, such a technique with minor modifications can also be used. In this case it is expected to be far more efficient, as a large fraction of polymers entangled with barbs will be trapped.
6 Conclusion

In this paper separation of polymers was considered by using a permeable surface. The first device considered had a surface that is an array of pores of spacing, size and thickness of order several thousand Angstroms. Improvements in nano-lithography may make such devices practical. By repeatedly applying a sequence of pulses, it should differentially separate long for short chains. The critical chain length where separation occurs should depend linearly on pulse durations. One way to test out the ideas proposed in this paper would be to use a line of pegs similar to previous experiments on arrays of pegs[14, 15].

The second device would be more complicated to construct. In addition to the pores, it has an array of barbs protruding from the surface. Utilizing the fact that chains occasionally get pinned by static friction, a sequence of pulses can be devised to make it possible to very precisely separate chains according to their length. This device may be useful in applications where precise determination of chain size is needed, but it is not necessary to separate the entire mixture.

There are still many experimental problems that would need to be addressed to make these devices practical. However it might prove worthwhile investigating these ideas further experimentally.

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Figure 1: Behavior of a polyelectrolyte drifting along a surface with pores in the presence of an applied field. (a) The chain tumbles along the surface. (b) It gets hooked. (c) It stretches and disentangles from a pore. (d) Occasionally after (c), the leading end will thread itself through a pore. (e) After (d) it is able to pass to the other side.
Figure 2: Snapshots from the simulation described in the text of a chain near a porous surface.
(a) At the start of a cycle, the chain is initially away from the screen, but quickly migrates onto it.
(b) The chain becomes threaded through a pore. (c) It starts threading and begins to disengage from the surface. (d) The field is reversed and it migrates away from the surface.
Figure 3: The fraction of cycles where the chain completely passes through the screen. The chain length is given in units of the number of monomers. The solid line is an exponential fit to the data.
Figure 4: The fraction of chains that pass through a screen after 500 cycles.
Figure 5: Two possible ways of constructing a barb. In the front, two cylinders at right angles are fabricated. Beneath them is a pore. In the back, a cylinder is manufactured at an oblique angle. Beneath it is a pore.
Figure 6: *Lower left hand diagram:* The device looking vertically down onto the screen. Chains are occasionally hooked around barbs but can fall off landing on the baffle below. It then will curl up and slide off onto another barb. 1-4 show the sequence of pulses applied to the device as described in the text.