ACS Macro Letters

Letter

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Efficient Separation of Long Polymer Chains by Contour Length and Architecture

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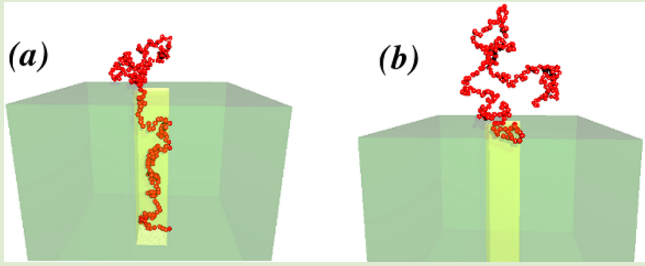
ABSTRACT: On the basis of theoretical considerations and computer experiment, we suggest a new technique for separation of polymer molecules. The method is based on filling an array of nanochannels with macromolecules whereby the subsequent ejection time depends strongly on small differences in the end-to-end distances of elongated configurations inside the nanotubes. In contrast to conventional methods for chromatographic separation, the efficiency of the proposed method *increases* with growing molecular length of the chains. The method appears promising also for the separation of ring from linear polymer chains.



All analytical chemists share the same dream: a separation method with perfect resolution. The best available resolution for polymers with relatively small molecular mass is provided by adsorption chromatography.[1] Oligomer mixtures can be separated into exact homologues up to several dozens of repeat units (the record separation goes up to 85 consecutive homologues).[2] For higher molecular mass, temperature gradient methods near the critical adsorption point are useful.[3] This method is based on weak adsorption and gives good resolution for flexible polymers with thousands of repeat units.[4,5] Samples with high molecular mass 10^5-10^6 are separated by gel chromatography[7,6] and field-flow fractionation.[8] Both methods exploit the depletion force produced by interaction of a polymer with an inert sorbent surface. The resolution is not very good because of the weak molecular mass dependence of the depletion force. Electrophoresis on gels (agarose)[9] and polymer matrices (polysaccharide, polyacrylamide)[10] is commonly used to separate DNA, including diagnostic purposes. Single-stranded and double-stranded DNA with lengths up to thousands of base pairs can be analyzed. The main difficulty is related to the effect of the polymer matrix density. For small DNA molecules the resolution is increased by increasing the density, but for large molecules the difference in mobility becomes small leading to poor separation.[11] Loading gels into capillaries also presents a serious problem.

During the last years a number of experiments on DNA electrophoresis in nanoslits[12] and nanochannels[13] have been reported. An important advantage of this approach is that no gel matrix is required. A reasonable resolution of small DNA fragments with 10−100 base pairs was demonstrated in 100 nm nanochannels in comparison with 50 μm microchannels.

Recently, a closely related technique for separation of DNA molecules,[14,15] based on confinement-induced entropic recoil of single molecules in a nanofluidic streamer, has been proposed. A dense array of nanopillars was fabricated by lithography, and DNA molecules were partially inserted into the dense array by a pulsed electric field. The pulse duration was adjusted to ensure that shorter molecules were fully inserted into the nanopillar region, while longer molecules remained partially outside on the other side of the interface. When the electric field was switched off the longer molecules quickly recoiled out of the dense region under the action of an entropic force due to a sharp interface between two regions with vastly different configuration entropy. The shorter molecules were recoiling much slower. A separation of DNA with 105 base pairs was demonstrated by this technique.

In this letter we propose a modified method to separating long polymer chains based on a strong molecular mass dependence of the ejection time of a polymer initially confined in a nanotube.[16,17]
The approach stems from the simulations and the analytical theory describing the ejection kinetics of a flexible chain.[16] In the initial configuration, one of the chain ends was restricted to touch the closed bottom of the tube of length L and diameter D (see Figure 1). Depending on the chain length N and on the tube parameters, the chain may be completely or only partially confined in the tube.

A typical variation of the mean ejection time τ with the polymer length N is displayed in Figure 2 for a tube diameter $D/a = 3$, where a is the segment length of the polymer, and

Received: June 18, 2013
Accepted: September 13, 2013
Published: September 18, 2013

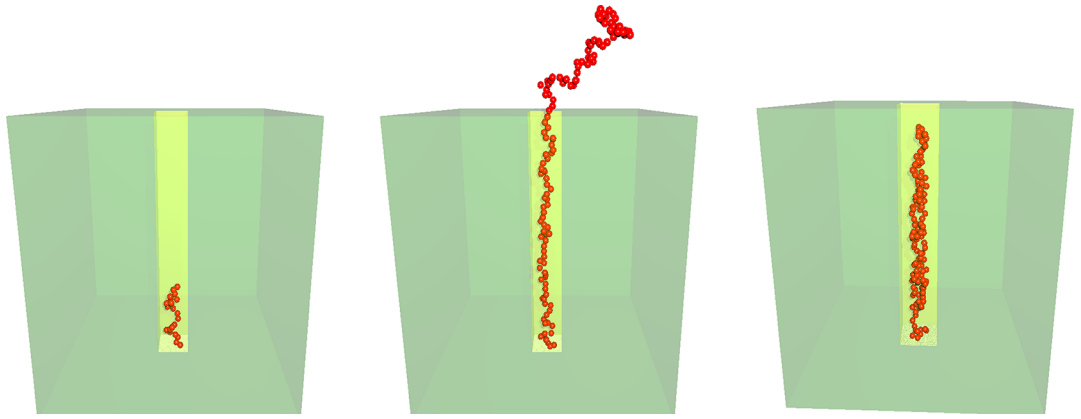


Figure 1. Initial configurations of linear chains with $N = 24$ (left) and $N = 128$ (middle) and of a ring polymer $N = 128$ (right), with one end touching the bottom of a tube. The tube diameter $D = 2$, and the tube length $L = 30$.

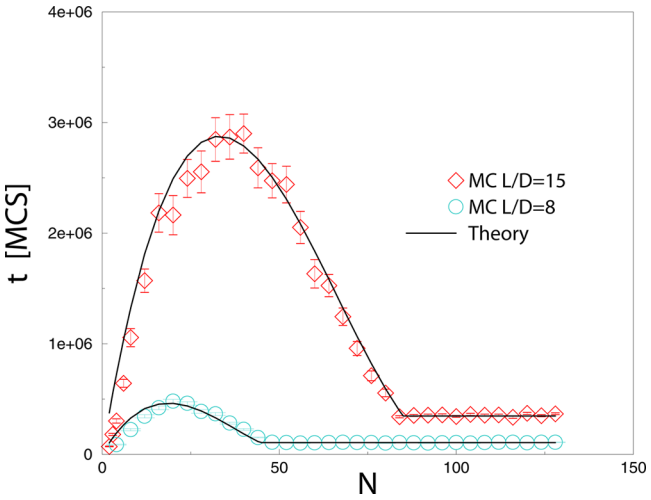


Figure 2. Mean ejection time τ of linear polymer chains vs N for $D/a = 3$, $L/D = 15$.

tube length $L/a = 8, 15$. The whole curve is nonmonotonic. For chains longer than a certain characteristic chain length, $N > N^*$, the ejection time, τ_{long}, is independent of N. It follows from the analytical theory that

$$N^* \propto \frac{L}{a}\left(\frac{a}{D}\right)^{2/3} \tag{1}$$

$$\tau_{long} = \tau(N^*) \propto \frac{\xi L^2}{k_B T}\left(\frac{a}{D}\right)^{5/3} \tag{2}$$

where ξ is the friction coefficient of a segment. The maximum ejection time

$$\tau_{max} \propto \tau_{long}\frac{L}{D} \tag{3}$$

corresponds to chain length $N_{max} = N^*/3$.

The scaling equations are based on the blob picture and imply that the tube is wide enough; i.e., each blob contains several segments. Simulation results demonstrate that the scaling theory works for tubes as narrow as 3 Kuhn segment lengths.

The physical reason for the distinction in chain lengths between $N < N^*$ and $N > N^*$ is that the initial configuration of long enough chains contains a free tail extending outside the tube confinement (Figure 1). The ejection kinetics is then driven by a constant entropic force produced by the tail. For chains shorter than N^*, the ejection process necessarily involves a diffusive initial stage which ends when a nucleus of a tail is formed outside that triggers the force-driven process. Typically, the diffusive stage is much slower and dominates the total mean ejection time. For the purpose of separation one can use the declining branch of the $\tau(N)$-curve after the maximum located at $N^*/3$ to N^*.

The resolution of the method is related to the slope of the curve, $d\tau/dN$, which has a simple analytical form following from the theory presented in ref 16. It is clear that the slope scales as

$$\frac{d\tau}{dN} \propto \frac{\tau_{max}}{N^*} \tag{4}$$

so that the absolute value of the slope is

$$\frac{d\tau}{dN} \propto \frac{\xi L^2}{k_B T} \tag{5}$$

The proposed method is based on the difference in the ejection times of two chains with a relatively close molecular mass. It is clear that the individual ejection times are described by a distribution which is relatively broad once a diffusive stage is involved. This limits the resolution in the relative ejection times, $\Delta\tau/\tau \approx 1$. The resolution in the relative difference in the chain length, $\Delta N/N$, is then estimated as

$$\frac{\Delta N}{N} \propto \frac{dN}{d\tau}\frac{\tau}{N} \propto \frac{k_B T}{\xi L^2}\frac{\tau}{N} \tag{6}$$

The resolution changes very significantly depending on the position along the $\tau(N)$ curve: if the ejection time is of the order of τ_{max}, then $\Delta N/N \approx 1$. However, as the ejection time decreases toward the minimum value, τ_{long}, the resolution is improved. The best resolution is achieved at the part of the curve close to N^*.

$$\left(\frac{\Delta N}{N}\right)_{min} \approx \frac{D}{L} \tag{7}$$

Thus, long and narrow nanotubes will potentially provide excellent resolution.

The separation idea, proposed above, applies equally well to ring polymers since the ejection time is strongly affected by small differences in the end-to-end distances of elongated configurations inside the tube. This was demonstrated by

dx.doi.org/10.1021/mz400314d | ACS Macro Lett. 2013, 2, 879−881

Langevin dynamics simulations, cf. J. Sheng et al.[17] The analytical theory remains essentially the same.

An important application of the proposed method would be the possibility of a very effective separation of linear and ring polymers with the same molecular mass. Their gyration radii in solution are very close, and standard separation methods are ineffective. However, the end-to-end distances of a strongly confined ring polymer in a tube are roughly only half that of a linear chain. If the tube parameters are such that the linear chain length is close to N^*, its ejection time will be $\tau_{lin} \approx \tau_{long}$. On the other hand, the residence time of the ring polymer will be $\tau_{ring} \approx \tau_{max}$, so that

$$\frac{\tau_{ring}}{\tau_{lin}} \approx \frac{L}{D} \tag{8}$$

The relative difference in the ejection time between a linear and a ring chain of the same molecular mass evaluated according to the analytical theory is shown in Figure 3.

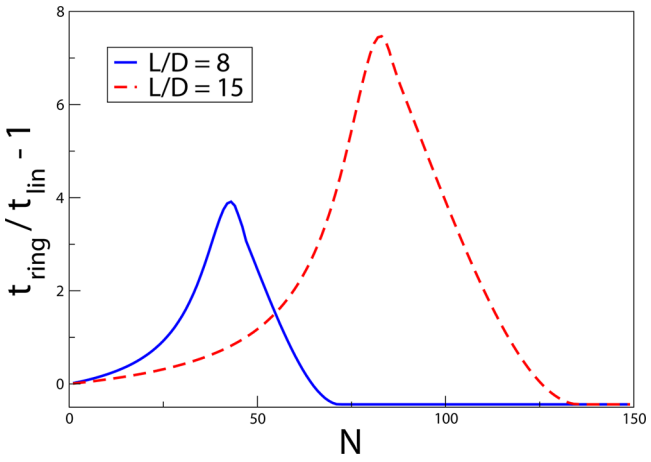


Figure 3. Ejection times ratio for ring vs linear polymers of the same length, N, according to theoretical prediction. The tube parameters are the same as in Figure 2.

Since the best resolution is always reached in the vicinity of the characteristic chain length $N^* \propto LD^{2/3}$, the choice of the tube parameters, L and D, is not arbitrary. According to the range of molecular masses of interest, the tube length must be chosen to ensure that the length of the confined chain configuration is close to L. Thus, the maximum resolution parameter is $L/D \approx N^*D^{-5/3}$ and for a fixed tube diameter increases linearly with the molecular mass of the chains to be analyzed. Interestingly, the resolution parameter is proportional to the total free energy of confinement and can be interpreted as the number of blobs in the initial chain configuration.

In contrast to other methods, the resolution improves for chains of larger molecular mass. Longer chains are best separated in longer tubes, and this allows a larger tube aspect ratio, L/D, thus improving resolution. This applies equally to separating a mixture of linear chains with different N, a similar mixture of ring chains, or a mixture of ring and linear chains with the same N. The last case is clearly illustrated by Figure 3. On the other hand, Figure 2 demonstrates that separation of chains of lengths $N > N^*$ between themselves is not possible since they all have the same ejection time.

A crucial aspect of the suggested separation approach is the achievement of proper initial chain configurations touching the bottom of the tube (see Figure 1). For charged linear and ring macromolecules, this may be done by applying electric field. Craighead and co-workers demonstrated experimentally that DNA molecules can be driven electrophoretically into narrow tubes[14] and into nanopollar arrays. Achieving the exact configuration that touches the tube bottom is a delicate question which may require adjusting the strength and duration of the field pulse. Basic tube geometry with one closed end employed in our simulations may also require some modification allowing an unimpeded passage of counterions. Driving uncharged polymers into a tube is more difficult to achieve experimentally and may require a preliminary chemical modification making a polymer chain telechelically active. On a futuristic note one can add that in living matter driving a DNA molecule into a strongly crowded conformation in a viral capsid is achieved very effectively by employing molecular motors.[18]

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Notes

The authors declare no competing financial interest.

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