Modeling the Electrophoretic Separation of Short Biological Molecules in Nanofluidic Devices

Modeling the Electrophoretic Separation of Short Biological Molecules in Nanofluidic Devices

Ghassan N. Fayad
e-mail: gfayad@alum.mit.edu

Nicolas G. Hadjiconstantinou[1]
e-mail: ngh@mit.edu

Department of Mechanical Engineering,
Massachusetts Institute of Technology,
Cambridge, MA 02139

*Via comparisons with rigid-rod and wormlike-chain Brownian dynamics (BD) simulations and the experimental results of Fu et al. (2006, "Molecular Sieving in Periodic Free-Energy Landscapes Created by Patterned Nanofilter Arrays," Phys. Rev. Lett., **97**(1), p. 018103), we demonstrate that, for the purposes of low-to-medium field electrophoretic separation, sufficiently short biomolecules can be modeled as point particles, with their orientational degrees of freedom accounted for using partition coefficients. This observation is used in the present work to build an efficient BD simulation method. Particular attention is paid to the model's ability to quantitatively capture experimental results using realistic values of all physical parameters.*
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Introduction

In this paper, we focus on the development and *validation* of a Brownian dynamics (BD) [1,2] simulation model for modeling the performance of nanofluidic separation devices for the separation of *short* $[L \leq 300\ \text{bp}(108\ \text{nm})]$ dsDNA molecules using nanofilter arrays [3,4]. These and similar [5] devices are replacing traditional molecular sieving approaches for biological molecule separation (e.g., gel electrophoresis [6]), and are of considerable interest in the context of chemical, biological and medical applications [7–9].

The particular nanofilter device of interest here consists of a large number $(N_p \sim 10{,}000)$ of alternating shallow and deep regions etched in a silicon wafer as shown in Fig. 1. Biological molecules (DNA, protein) of contour length L and persistence length $L_p \approx 54$ nm driven by an electric field through this periodic array of constrictions are size-separated, because their size-dependent mobilities result in size-dependent travel times. Typical dimensions for the shallow and deep region depths and period are $d_s \approx 55$ nm, $d_d \approx 300$ nm and $p \approx 1\mu$m, respectively [3]. This places the separation process in the Ogston regime, defined by $R_g \lesssim d_s$, where R_g is the radius of gyration, which for the wormlike-chain (WLC) Kratky–Porod model is given by Ref. [10]

$$R_g^2 = \frac{1}{3}L_pL - L_p^2 + 2\frac{L_p^3}{L} - 2\frac{L_p^4}{L^2}\left[1 - \exp\left(-\frac{L}{L_p}\right)\right] \qquad (1)$$

Despite their small sizes, these devices contain a very large number of solvent molecules making classical molecular dynamics simulations intractable. In other words, for an efficient model, some degree of coarse-graining is required. For the present application, BD simulations strike a good balance between fidelity and efficiency, and have already been used to model biological molecule separation [11–14]. Our approach places more emphasis on simplicity and efficiency which are essential in the context of device design and optimization.

Early studies of biomolecule separation using BD [11,12] focused on molecules that are sufficiently longer than L_p. In this limit, the separation mechanism is different from Ogston sieving and is known as entropic trapping [3]; moreover; as a result of the significantly larger molecule length, the BD models were of the freely-jointed bead-spring type. A BD study of short rodlike molecules in the geometry studied here has appeared in Ref. [13]; the focus of that paper was to demonstrate the feasibility of high-field electrophoresis and to highlight the importance of "torque assisted escape'' in the latter limit. More recently, Fayad and Hadjiconstantinou [14] developed a BD formulation based on the WLC [15] model in order to quantitatively model Ogston-regime separation; good agreement with the experimental results of Fu et al. [3] was observed.

Concurrently, Li et al. [16] developed a theoretical model describing the separation of short, essentially rigid biomolecules in the same type of device. In this model, valid under conditions typically satisfied by actual separation devices (e.g., sufficiently low electric field), biomolecules are modeled as point particles, with their rotational degrees of freedom captured using a partition coefficient. The basic premise is that under conditions for which rotational diffusion is much faster than the other transport timescales in the system, the resulting fast relaxation ensures that the rotational degrees of freedom of the molecule can be assumed to follow equilibrium (equiprobable) statistics. This observation enables one to calculate the entropic penalty associated with confinement by comparing the accessible (due to geometry) states to the total rotational states as drawn from the equilibrium distribution. Using this model, an effective (approximate) one-dimensional Fokker–Planck model was formulated and solved in Ref. [16], yielding an analytical description of the separation process. In a subsequent publication [17], Li et al. obtained a finite difference numerical solution of the Fokker–Planck description of the original (effectively two-dimensional) geometry, aiming to quantify the error associated with the dimensionality reduction introduced in Ref. [16]. Comparison of the two solutions shows that the one-dimensional model overestimates the molecule mobility at most by 10−20%.

Our objective here is to develop a model which incorporates the idea of partition coefficients within a flexible and efficient Fokker–Planck solution framework for treating engineering (multi-dimensional, geometrically complex) problems of practical interest. As we now show, this can be achieved by employing a point-particle BD formulation [1,2], which, as a particle-based method, is significantly more efficient in high-spatial dimensions and very convenient for simulating complex geometries. Specifically, in contrast to the analytical solution presented in Ref. [16], the three-dimensional BD model developed below does not make any simplifying assumptions regarding the system geometry, and is significantly more efficient than descriptions of equivalent fidelity, such as numerical solution of the Fokker–Planck equation. In fact, by comparing our simulation results with BD simulations of more complex models (rigid-rod, WLC) [14], as well as the experimental results of Fu et al. [3], we show that, *provided the molecules modeled are sufficiently short*, the partition-coefficient-based BD model

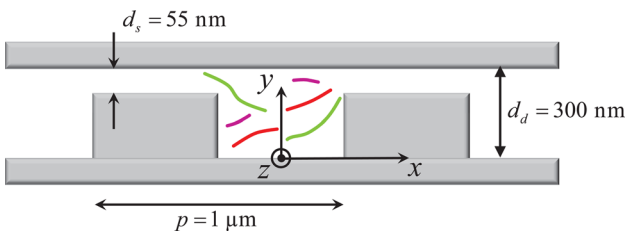

Fig. 1 Schematic of one period of the nanofilter array

[1]Corresponding author.
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entails virtually no loss in fidelity, while being significantly simpler and at least two-orders of magnitude more efficient compared to rigid-rod or worm-like-chain BD models.

We consider molecules of effective charge per unit length q' moving in the device shown in Fig. 1, under the action of an electric field of "average" magnitude E_{av}. The relative importance of advection to diffusion is quantified by the translational Péclet number

$$Pe_t = \frac{E_{av} p q' L}{k_B T} \tag{2}$$

where k_B is Boltzmann's constant and T is the temperature. For the short molecules ($L < 108$ nm) and small-to-moderate electric fields ($E_{av} \leq 63\,\mathrm{V/cm}$) considered here, the maximum value of this parameter is $Pe_t \approx 6$.

The effect of electric field torque on the molecule orientation can be quantified by the rotational Péclet number

$$Pe_r = \left(\frac{1-\varepsilon}{1+\varepsilon}\right)\frac{E_{av} q' L^2}{k_B T} \tag{3}$$

where $\varepsilon = d_s/d_d$. When the rotational Péclet number is small ($Pe_r \leq 1$), the torque due to electric effects is too small to bias the thermally induced distribution of rotational states, or in other words, the rotational diffusion is fast enough for the rigid molecule to sample all possible rotational configurations equiprobably. If additionally the characteristic rotational relaxation time for the molecules is much shorter than the average residence time in one device period [16], one can model the short rigid DNA molecules as Brownian point particles by augmenting their description with a position-dependent partition coefficient (see below) which includes information on the entropic barrier associated with the interaction between geometry and the molecule finite size.

The maximum value of the rotational Péclet number for the system considered here is $Pe_r \approx 0.4$, suggesting that the effect of torque is negligible. This has been verified by our own rigid-rod BD simulations in which neglect of the electric field torque for $Pe_r \lesssim 1$ was shown [18] to have a negligible effect. Also, ultimately, this assumption will be validated below by comparing our simulation results with rigid-rod and wormlike-chain BD simulations as well as experimental data.

When all accessible molecular configurations are equiprobable, the effect of the molecule finite size can be captured using a local orientational partition function $\kappa(\mathbf{r})$, where $\mathbf{r}$ is the position vector in space. This function measures the number of occurrences of mass centers at position $\mathbf{r}$ relative to that in free solution. The motivation for this formulation is that an orientational entropy may now be defined by

$$S(\mathbf{r}) = k_B \ln \kappa(\mathbf{r}) \tag{4}$$

and used to quantify the entropic barrier associated with entering the narrow regions of the device.

The latter is most conveniently approximated [16,19] by defining K_d and K_s to be the average partition coefficient for the deep and shallow region; thus respectively measuring the equilibrium probability of finding a molecule in the deep and shallow regions of the channel, relative to that in bulk solution. Mathematically, we can write

$$\boldsymbol{K}_d = \frac{\iiint_{V_d} \kappa(\mathbf{r}) d^3\mathbf{r}}{\iiint_{V_d} d^3\mathbf{r}} \tag{5}$$

$$\boldsymbol{K}_s = \frac{\iiint_{V_s} \kappa(\mathbf{r}) d^3\mathbf{r}}{\iiint_{V_s} d^3\mathbf{r}} \tag{6}$$

where V_d and V_s denote the volume of the deep and shallow regions of the nanofilter, respectively. These quantities can be evaluated either analytically when possible (e.g., Ref. [19]) or by direct enumeration (simulation) [16]. Let us also define $K = K_s/K_d$, which describes the ratio of probabilities of occurrence of the DNA molecule in the shallow region compared to the deep region (at *equilibrium*). Following these definitions, the change of free energy from deep to shallow region due to orientational effects is given by Refs. [3,16,19]

$$\Delta W = -T\Delta S \tag{7}$$

where the entropy term is given by $\Delta S = k_B \ln(K_s/K_d) = k_B \ln(K)$; hence the entropic contribution to the energy barrier is

$$\Delta W = -k_B T \ln(K) \tag{8}$$

Figure 2 shows a *schematic* of the *effective* free-energy landscape in the device. For simplicity, the two-dimensional electric field is approximated by an axial, piecewise constant effective field, which takes two values, namely E_d in the deep and E_s in the shallow region, respectively. The values E_d and E_s are given by

$$E_d = \frac{\varepsilon(1+\nu)}{\varepsilon+\nu} E_{av} \tag{9}$$

$$E_s = \frac{1+\nu}{\varepsilon+\nu} E_{av} \tag{10}$$

where $\nu = l_s/l_d$. We note that this approximation is not necessary nor related to the main modeling approximation which is the use of partition coefficients.

In our model, Brownian point particles move in the geometry of Fig. 1 (our simulations show [14] that using a more precise geometry representation has a small effect for short molecules) under the action of a driving electric field given by Eqs. (9) and (10). The diffusion coefficient of these molecules, $D(L)$, is assumed to follow the experimental results on short dsDNA molecules of Lukacs et al. [20]. The molecule effective charge per unit length, $q'(L)$, was determined as follows: the value $q'(L = 54\ \mathrm{nm})$, is chosen such that the simulation prediction matches the experimental result of Fu et al. for $E_{av} = 64\ \mathrm{V/cm}$ (for molecules of length $L = 54$ nm); $q'(L)$ is then allowed to vary weakly with L such that the resulting free-draining mobility is independent of the molecule length [14]. Electroosmotic flow was estimated [3] to be small and was thus not included in the model; inclusion of this effect is straightforward [16,21].

The equations of motion of these molecules are integrated using standard [1,2] BD methods. The entropic barrier that occurs at the transition from deep to shallow region is accounted for by allowing the Brownian particles to enter from deep to shallow region

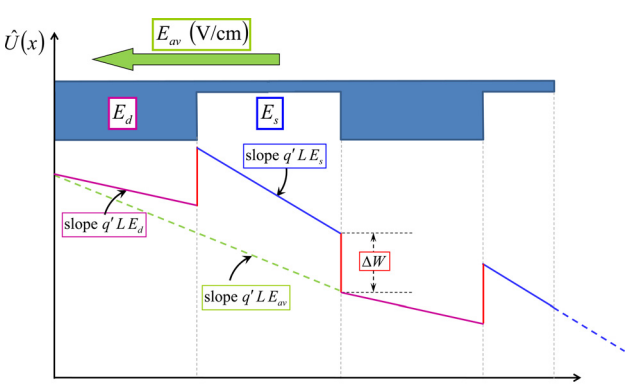

Fig. 2 Free energy landscape of a charged DNA molecule along the nanofilter channel

Table 1 Summary of partition coefficients [16]

DNA length (L) in nm	Partition coefficient (K)
20.25	0.8675
36	0.7844
54	0.6798
72	0.5800
81	0.5370
108	0.4425

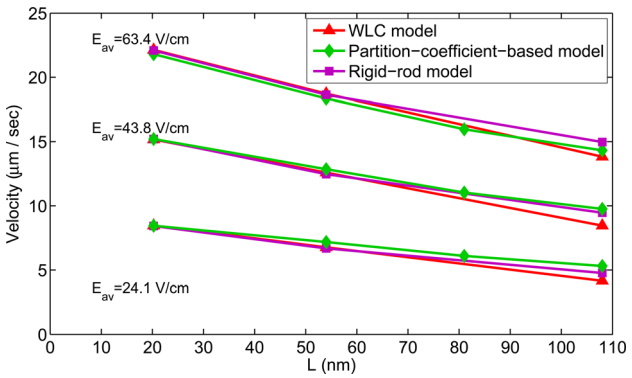

Fig. 3 Comparison between the rigid-rod, WLC and partition-coefficient-based models

with a probability $P_{\text{enter}} = K$. This is implemented in the following way: when a Brownian particle attempts to pass from the deep to the shallow region we draw from a uniform distribution a random number $r_{\text{rand}} \in [0, 1]$. If $r_{\text{rand}} > K$, the move is rejected (particle is specularly reflected at the boundary between the deep and shallow region); if $r_{\text{rand}} \leq K$, the move is accepted (the particle enters narrow region as intended). Table 1 gives the values of K used in this work; these were calculated numerically [16] using Eqs. (5) and (6).

Figure 3 compares the results obtained using the rigid-rod and WLC BD models presented in Ref. [14] and the partition-coefficient-based model presented here; the figure compares the travel velocity of DNA molecules as a function of their length for various electric fields. Very good agreement is observed between the rigid-rod model and the partition-coefficient-based model. Small discrepancies between these two and the WLC model appear for $L > L_p$, as expected. The overall agreement is quite remarkable given that, in addition to the partition coefficient, the electric field is also approximated using Eqs. (9) and (10) in the partition-coefficient-based simulation. Figure 4 compares the experimental data of Fu et al. [3] with the partition-coefficient-based model for the travel velocity of DNA molecules as a function of their length. Good agreement is observed.

By capturing the effect of the rotational degrees of freedom using an entropic penalty term, the resulting model is significantly simpler and more efficient than the rigid-rod and WLC models discussed in Refs. [14,18] with no perceptible reduction in fidelity (see Figs. 3 and 4). The improved efficiency is a result of a number of factors including:

(1) Reduction in degrees of freedom.
(2) A simplified boundary condition that is significantly more efficient to implement.
(3) A significantly larger time step compared to the rigid-rod model. This is possible because the rotational degrees of freedom-the limiting factor in the time step size-have now been eliminated.

These factors result in an implementation that is more than two orders of magnitude more efficient than the corresponding rigid-rod model (30 seconds of CPU time versus 7500 seconds of CPU time for the same benchmark problem). The speedup compared to the WLC model is even larger.

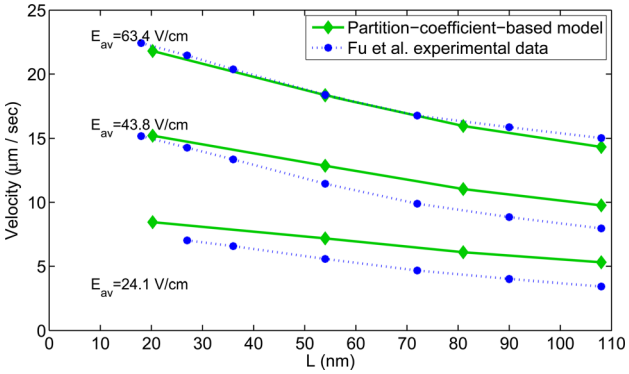

Fig. 4 Comparison between experimental data and the partition-coefficient-based model

In conclusion, we have developed, presented and validated an efficient method for modeling the electrophoretic motion of short DNA molecules. The model simulates molecules as Brownian point particles, and relies on the assumption $Pe_r < 1$, which for short biomolecules implies low to medium field applications ($Pe_r \propto (L/p)Pe_t$). The excellent agreement with rigid-rod and WLC BD simulations, as well as experimental data, suggests that this approximation is very reasonable for molecules that are on the order of or shorter than one persistence length. This study provides a path for extending theoretical work on the dynamics of Brownian particles [22,23] to short Brownian molecules in a variety of electrophoretic applications.

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