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Electric field mediated DNA motion model

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Abstract

Understanding the motion and the governing equations of a molecule's path in tissue is an ultimate requirement for the repeatable, site specific delivery of molecules [Joseph D. Hickey. Modelling the Motion of Ions and Molecules in Electroporation and Electrophoresis Field Conditions. University of South Florida, College of Arts and Sciences, Department of Physics, Tampa, Florida, 2003., Joseph D. Hickey and Richard Gilbert. Modeling the electromobility of ions in a target tissue. *DNA and Cell Biology*, 22 (12) (2003) 823–828.]. This paper describes a computationally efficient mathematical model and simulation technique for the examination of DNA fragments in a 1% agarose gel. The speed of the individual DNA fragments through the agarose gel was described through two parts. The maximum velocity was calculated using the Coulombic force divided by Stoke's law and that value was retarded by an exponential rate equation.

The simulation utilizes previously published techniques modified for this specific application [Joseph D. Hickey and Richard Gilbert. Fluid flow electrophoresis model. *Bioelectrochemistry*, 63 (2) (2004) 365–367., Joseph D. Hickey and Richard Gilbert. Modeling the electromobility of ions in a target tissue. *DNA and Cell Biology*, 22 (12) (2003) 823–828.]. Five representative DNA fragment sizes that span the resolution of a 1% agarose gel were chosen for this analysis. The speeds corresponding to these five DNA fragment sizes were converted into discrete values and used in a 50 step simulation. The resultant error comparing the simulation with experimental distance was 7.76%. Through a 1-D optimization procedure, this error was reduced to 3.02% for a 52 step simulation.

Keywords: Electrophoresis; Drug and gene delivery; Computationally efficient simulation

1. Introduction

Electrophoresis is a technique used for the directed motion of charged species in an applied electric field. Two common applications of electrophoresis in the biosciences are the delivery of genes to cells and the separation of DNA. The effectiveness of electrogenetherapy, the delivery of genes to cells through applied electric fields, is increased by the addition of electrophoretic chase pulses after the application of initial electroporation pulses [5,6,8]. DNA and protein electrophoresis are common laboratory procedures that utilize the driving force of an applied electric field acting on charged molecules suspended in a retarding media. The force imbalance induces velocity differences between different species as a function of respective charge and radii.

Although electrophoresis is a common laboratory procedure and the corresponding physics is well understood, the literature is currently lacking a computationally efficient method for predicting the molecular motion of large molecules in diverse matrices influenced by electric fields. A small molecule simulation has previously been described by Hickey and Gilbert [2,4] but this method is not sufficient for handling multiple species with different charges and radii simultaneously [1]. The creation of a model that describes the motion of DNA in an electrophoresis gel is the first step towards the better understanding of site specific electric field mediated delivery of drugs and genes into tissue.

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2. Modeling electrophoresis in gels using a force model

Understanding the motion and the governing equations of a molecule's path in tissue is an ultimate requirement for the repeatable, site specific delivery of molecules in tissue [3,4,7]. Producing an accurate, descriptive, two dimensional large molecule through tissue model from first principles is a very complex and difficult task due to a lack of literature data to support model precepts. A 1% agarose gel was ran using DNA standards at $6.56\frac{\mathrm{V}}{\mathrm{cm}}$, for 1 h in a 1x TAE buffer [10]. DNA standards HyperLadder I, made by Bioline USA Inc., Canton Ma., catalog number BIO-33025 and a 100 bp ladder made by Bayou Biolabs, Harahan, La., catalog number L-101 were chosen for repeatability of the experimental data and for the coverage of the 1% agarose gel functional range.

The motion of the DNA fragments in an agarose gel was modeled using Lagrangian mechanics treating the moving elements as existing in a constant velocity steady state. The DNA fragments were assumed to be prolate spheroids traveling through 1-D tubes, similar to reptation DNA motion models, traveling along their minor axis. The Coulombic force from the applied electric field acting on the charged molecules was assumed to be the sole driving force. Two retarding forces were considered. The first was the hydrodynamic retarding force described by Stoke's law and the second was the retarding force due to the interaction of the 1-D gel tubes with the DNA spheroid's minor axis radius.

$$S = \frac{zeE}{6\pi\eta r_{\mathrm{pore}}} - S_{\mathrm{gel}} e^{\frac{-\mathrm{bp}_{\mathrm{gel}}}{\mathrm{bp}}} \tag{1}$$

Eq. (1) calculates the speeds at which the different DNA fragments travel through an agarose gel. The first section of the equation describes the Coulombic, zeE, z is the charge of the ion, e is the fundamental charge of an electron, E is the applied electric field across the electrophoresis chamber, and the Stoke's law, $6\pi\eta r_{\mathrm{pore}}$ interaction, η is the viscosity of the electrophoresis buffer. The variable r_{pore} is the radius of the lower range DNA fragment for that specific agarose gel concentration. For a 1% gel, r_{pore} has a value of 4.827 nm [1]. This value was computed using a packing factor analysis method, each base pair of each DNA fragment was modelled as cylinder with a length of 2.1 nm and a width of 0.34 nm [9,11]. A 100 base pair, bp, fragment is the lower separation limit of a 1% agarose gel, its equivalent resting diameter was chosen as the diameter of the 1-D tubes being used as a model for the polymeric knot network.

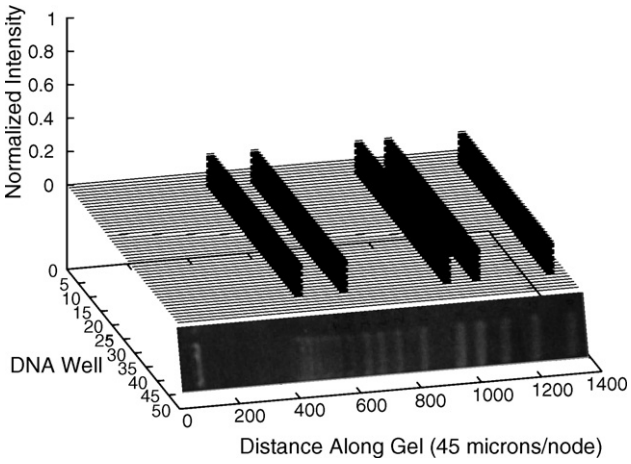

Fig. 2. DNA fragment final distribution. The final frame in the simulation showing the correlation between the predicted and experimental DNA fragment band placements.

The second section of Eq. (1) is in the form of a retarding rate. The variable $\mathrm{bp}_{\mathrm{gel}}$ is the base pair value where the agarose gel takes a greater effect, this can be seen in Fig. 1 where curvature is introduced on the left hand side of the graph. The variable S_{gel} is the speed that corresponds to the $\mathrm{bp}_{\mathrm{gel}}$ base pair value of 750 bp, and for the 1% agarose gel this value is $4.92\frac{\mathrm{V}}{\mathrm{cm}}$. These values were calculated using a golden section error minimization routine using multiple iterations of random initial guesses to reduce the possibility of arriving at a localized minima. As seen in Fig. 1, this model visually tracks the experimental data quite well and the R^2 value for the fit is 0.9941.

3. Simulating the motion of DNA fragments in agarose gels

The mathematical model described in Section 2 was used in conjunction with nodal analysis techniques to simulate the

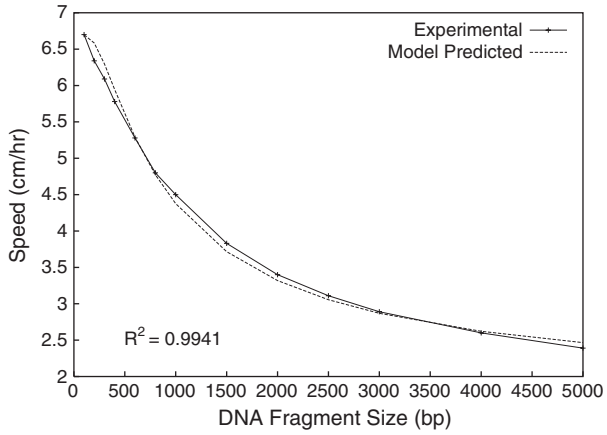

Fig. 1. Experimental vs model predicted speeds. Comparison of the experimentally measured DNA fragment speeds to the Eq. (1) predicted speeds.

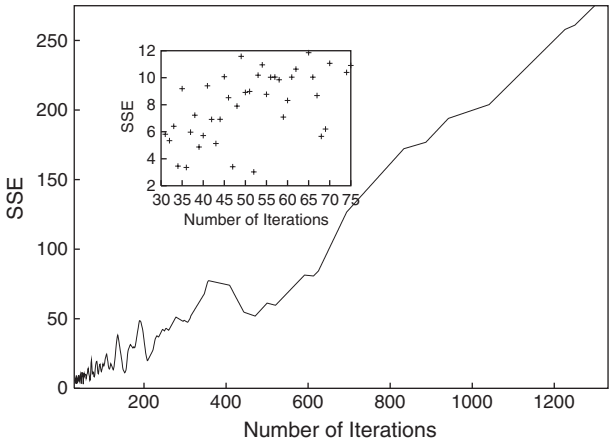

Fig. 3. Results of optimization procedure. 1-D optimization algorithm results predicting the optimal number of steps for the simulation.

motion of DNA fragments in a 1% agarose gel. Five representative DNA fragment sizes were chosen, 200, 600, 1000, 2500 and 5000 bps. These sizes span the resolution of a 1% agarose gel and they show the ability of the mathematical model.

The general techniques used in the creation of this simulation are covered in two papers previously published [2,4]. The specifics are listed here. The array used for this simulation was 1331×50 elements. This size was selected because it provided the required resolution and had similar dimensional shape to a single well of an agarose gel. Both the agarose gel and the applied electric field were considered to be without anisotropy. The initial DNA fragment distribution was in a well at the cathode side of the array. Intensity rather than concentration was chosen for the descriptive variable for two reasons. First, intensity and distance traveled are the two measurable qualities from an electrophoresis gel. Second, plotting concentration would have required five overlapping concentration plots because the initial concentration was equally distributed into the five representative fragment sizes described in the paragraph above.

The flow rules for this model were chosen to be unidirectional. This followed from the assumption that the agarose gel was created from a series of 1-D tubes. The DNA fragments separated entirely due to their different speeds predicted by Eq. (1). By converting the continuum space of the agarose gel into a discrete space of 50 by 1331 nodes and using the know values of time and total distance travelled for each DNA fragment, the speed of the fragments was converted from $\frac{\text{cm}}{\text{s}}$ to $\frac{\text{nodes}}{\text{time}}$ where each node represented a linear distance of 45 μm. The five transformed speeds were used along with the nodal length of the array and a 50 step iteration to simulate the motion of the DNA fragments as a function of time. The final result is shown in Fig. 2. The sum error comparing the experimental to the simulation for the five fragment sizes is 7.76%.

4. Conclusion

This paper described a novel computationally efficient method for the simulation of DNA in a 1% agarose gel. This method utilized previously described techniques [2,4] for the simulation portion of the research. The sum of the error in the final result is 7.76%. While this seems large for numerical analysis it is consistent with the errors incurred in laboratory grade DNA gel electrophoresis. For the same size array this error can be reduced to 3.02% for a 52 step simulation. This value was calculated using a 1-D optimization procedure. The result of that procedure is shown in Fig. 3. Alternatively, a 2-D optimization procedure could be implemented that examines not only the number of array iterations but also the length of the array. This could possibly reduce the error but it may increase the computational intensity of the simulation.

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