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Numerical simulation of two-dimensional agarose gel electrophoresis of knotted and supercoiled DNA molecules

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Resume. The present work introduces a mathematical model, based on conservation laws and Maxwell-Stefan equations of mass transport, to analyze the electrophoretic mobility of two topological DNA families: supercoiled and knotted DNA molecules with the same total molecular mass. Comparison of experimental results and numerical simulations showed that our model is able to accurately reproduce two-dimensional (2D) agarose gel electrophoresis of supercoiled dimers and nicked knotted dimers under different conditions of electrostatic potential. The implementation of this mathematical approach could be useful to optimize conditions for the separation of different types of DNA topoisomers during electrophoresis.

Keywords. DNA topoisomers, electrophoresis, 2Dgels, electrophoretic mobility, Maxwell-Stefan equations)

1 Introduction

In our model, we considered the two-dimensional agarose gel electrophoresis as a two-phase process, where the agarose gel works as a homogeneous porous media and the DNA behaves as a fluid immersed in a resident fluid (running buffer) that migrates from one side to the other of the gel slab.

Agarose gel electrophoresis is a well-known method used for separating DNA molecules with different densities and shapes [13]. Two-dimensional (2D) agarose gel electrophoresis

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is the method of choice to separate the topoisomers of any duplex DNA ring [2, 9, 10]. It basically consists of two consecutive electrophoresis performed in one square gel slab. The first and the second electrophoresis are run at two orthogonal directions under different conditions. The first electrophoresis (known as first dimension) is usually performed in a low percentage agarose gel run at a relatively low voltage. The second dimension occurs perpendicular to the first one, but in a higher percentage agarose gel, and it is run at higher voltage. Negative charged DNA molecules migrate through a constant electric field established by electrodes connected to a voltage energy source. The agarose gel serves as a filter delaying the advance of the DNA molecules passing through its fibers. This method can be used to separate different families of DNA topoisomers. If a sample contains DNA molecules of the same molecular mass but different topology, 2D-agarose gel electrophoresis allows the identification of the topoisomers present in the sample since they exhibit different electrophoretic behavior [1, 4, 6, 9]. Supercoiled, catenated and knotted forms are clearly identified as they react in a different way to changes in the electrophoretic conditions.

Here we propose a mathematical approach that reproduces the results of two-dimensional agarose gel electrophoresis of two forms of circular DNA: supercoiled dimers (Sc Dimers) and knotted dimers (Kn Dimers), under different electrophoretic conditions [3]. The results obtained demonstrated that the electrophoretic mobility of Sc Dimers and Kn Dimers can be successfully reproduced by our mathematical model, even when a stronger electric field is applied during the second dimension.

1.1 Mathematical Modeling

Maxwell-Stefan equations were developed to study diffusion in multicomponent systems [8,12]. It considers all types of driving forces in mass transport: gravity, electrostatic potential, thermal gradient, pressure gradient, etc. In the case of agarose gel electrophoresis, the driving force is a constant electric field created by a continuous voltage energy source.

The Maxwell-Stefan equation for each component x_i with respect to the others x_j with $(x_i \neq x_j)$ is as follows:

$$\sum_{j=1, j\neq i}^{N} \frac{x_i x_j}{D_{ij}}(v_i - v_j) = -x_i \nabla_{T,p} \ln a_i - x_i \left(\frac{\overline{V}_i}{RT}\right) \nabla p - x_i z_i \left(\frac{F}{RT}\right) \nabla \phi, \qquad (1)$$

where N is the number of species being considered, ∇p is the pressure gradient, v_i is the velocity of the specie x_i, x represents the molar fraction (saturation), z_i the elemental charge of the components considered, D_{ij} the diffusivity of one of the component referred to the other, F the Faraday constant, R the ideal gas constant, T the absolute temperature, and $\nabla \phi$ the electrostatic potential applied.

In our model, we considered the two-dimensional agarose gel electrophoresis as a two-phase process, where the agarose gel works as a homogeneous porous media and the DNA behaves as a fluid immersed in a resident fluid (running buffer) that migrates from one side to the other of the gel slab.

In the electrophoresis process, the effect of the pressure and the chemical activity are neglected. The former since the process is developed in complete horizontal way, and the latter, because it has a very low influence in the electrophoresis process. If we denote the DNA by sub index α, and the running buffer as β, the constitutive the Maxwell - Stefan equations for the DNA and the buffer are:

$$\frac{x_\alpha x_\beta}{D_{\alpha\beta}}(v_\alpha - v_\beta) + \frac{x_\alpha v_\alpha}{D^*_{\alpha g}} = -x_\alpha z_\alpha \left(\frac{F}{RT}\right) \nabla\phi \tag{2}$$

$$\frac{x_\beta x_\alpha}{D_{\alpha\beta}}(v_\beta - v_\alpha) + \frac{x_\beta v_\beta}{D^*_{\beta g}} = -x_\beta z_\beta \left(\frac{F}{RT}\right) \nabla\phi \tag{3}$$

Equations (2) and (3) can be reduced to the following expression for the velocity of

$$v_\alpha = -\left(\frac{F}{RT}\nabla\phi\right) D_{\alpha g}\left(z_\alpha - z_\beta + \frac{z_\beta}{x_\alpha}\right) + \frac{v_\beta D_{\alpha g}}{D_{\beta g}}\left(\frac{x_\alpha - 1}{x_\alpha}\right), \tag{4}$$

The conservation law for continuity of fluid α give:

$$\partial_t x_\alpha - \partial_r(v^* x_\alpha) = 0, \tag{5}$$

where v^* is mean fluid velocity given by $v^* := x_\alpha v_\alpha + x_\beta v_\beta$ and ∂_r denotes the partial derivative with respect to the space variable, and t is the electrophoresis running time. This equation is complete with the constitutive equations introduced earlier. Combining the equations (4) and (5) we obtain the final equation that must be approximate in order to simulate the electrophoretic mobility. So, making the combining and neglecting some terms, it is obtained the following non-linear hyperbolic partial differential equation:

$$\partial_t x_\alpha + \partial_r f\left(x_\alpha, v_\alpha, v_\beta\right) = 0, \tag{6}$$

where $f\left(x_\alpha, v_\alpha, v_\beta\right) := \left(x_\alpha^2(v_\alpha - v_\beta) + x_\alpha v_\beta\right)$.

Some data, such as the electric field intensity E (V/m), the temperature T, the molar fraction of each topoisomer, and the migration velocity of each topoisomer v_α must be introduced *a priori* for this algorithm. The molar fraction of each topoisomer was obtained from densitometric analysis of the immunodetections. The velocity v_α was calculated by dividing the displacement of each of the topoisomers by the duration of electrophoresis. Then, we performed a polynomial regression that linked these speeds with the degree of complexity of topoisomers, ΔC. The buffer velocity v_β, was set as a multiple of the velocity v_α.

2 Results

In order to validate our model, we tried to reproduce the separation of knotted and supercoiled DNA molecules of the same size analyzed by two-dimensional agarose gel electrophoresis run according to standard conditions (first dimension run at 30 V in a

0.4% agarose gel; second dimension run at 150 V in a 1.0 % agarose gel) and under a stronger electric field during the second dimension (200 V and 1.0% agarose).

The immunograms obtained in the laboratory (left) and the images from our numerical simulation (right) are shown in figure 1.

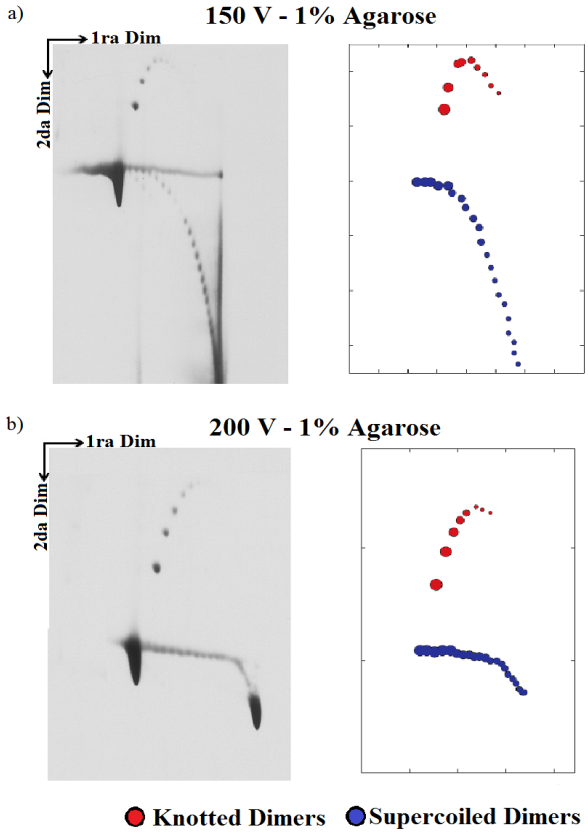

Figure 1: **2Dgel patterns of supercoiled and knotted dimers obtained experimentally and with the computational simulation:** a)two-dimensional electrophoresis where the 2^{nd} dimension run at 150 V and 1 % agarose; b) two-dimensional electrophoresis where the 2^{nd} dimension run at 200 V and 1 % agarose. The inmunotedection for these and other electrophoretic conditions were obteined from [3].

In the first dimension, both knotted, and supercoiled dimers increased their electrophoretic mobility with the value of ΔC. This was used to separate these molecules according to their complexity. However, the first dimension failed to separate molecules with the same ΔC but different topology. This separation was only possible during the second dimension of the agarose gel electrophoresis. The knotted molecules (red dots in Figure 1) migrated slower than the supercoiled molecules (blue dots). This migration pattern depends on the voltage applied during the second dimension. Under the standard voltage, the more complex knots migrated slower than simpler knots up to an inflection point, where mobility systematically increased with the average crossing number. This

inflexion point changed as we raised the voltage; it was located at $\Delta C = 5$, for 150V, and at $\Delta C = 6$, for 200V. In the case of supercoiled molecules, under standard conditions, their electrophoretic mobility during the second dimension increases along with the value of ΔC. However, for stronger electric fields (200 V), we found that the mobility of the topoisomers of low ΔC remained practically the same, while the mobility of those topoisomers with higher ΔC, increased linearly. These results showed that our mathematical approach can reproduce the electrophoretic behavior of the topoisomers analyzed under both conditions, standard and high voltage.

Comparisons of computer simulation's results with the experimental data are shown in Figures 2 and 3.

Figure 2 compares the results obtained from the simulation of the electrophoretic migration of knotted dimers run at 150 V and at 200 V during the second dimension. The simulation results were compared with the experimental values using the percentage error $e_r = \mid x_{exp} - x_{sim} \mid .100/x_{exp}$, where x_{exp} indicates electrophoretic mobility obtained experimentally and x_{sim} the electrophoretic mobility obtained from the simulation.

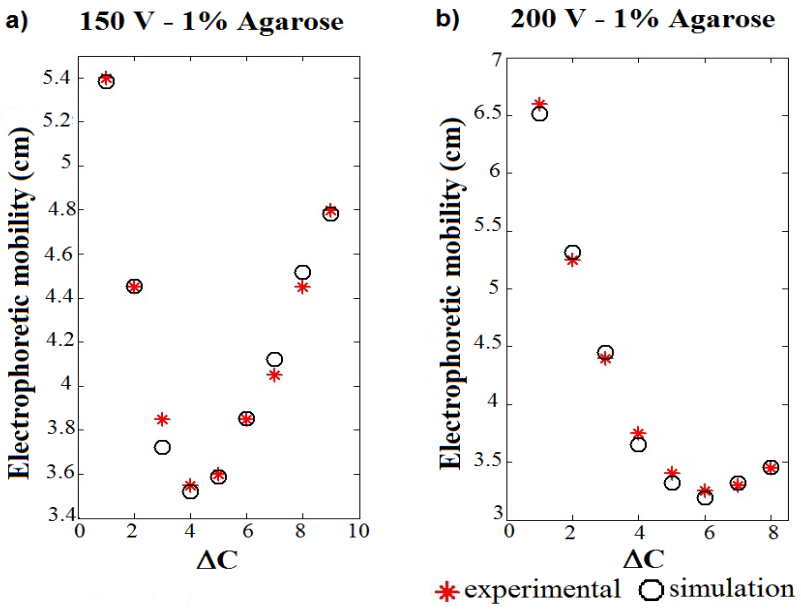

Figure 2: **Comparison of the experimental electrophoretic mobility of knotted dimers with the computational simulation:** a) Electrophoretic mobility with the 2^{nd} dimension run at 150 V. b) Electrophoretic mobility with the 2^{nd} dimension run at 200 V. The red (*) represent the experimental data and the black (o) correspond to the results of the numerical simulation.

The maximum percentage error between the simulated and the experimental values was $e_r = 2,167\%$ when the potential applied during the second dimension was 150 V, and $e_r = 2,10\%$ when the potential applied was 200 V.

Figure 3 compares the results obtained from the simulation of the electrophoretic migration of supercoiled dimers run at 150 V and at 200 V during the second dimension.

The maximum difference found between the numerical and the experimental data was $e_r = 2,54\%$ when the potential applied during the second dimension was 150 V, and $e_r = 1,02\%$ when the voltage was 200 V.

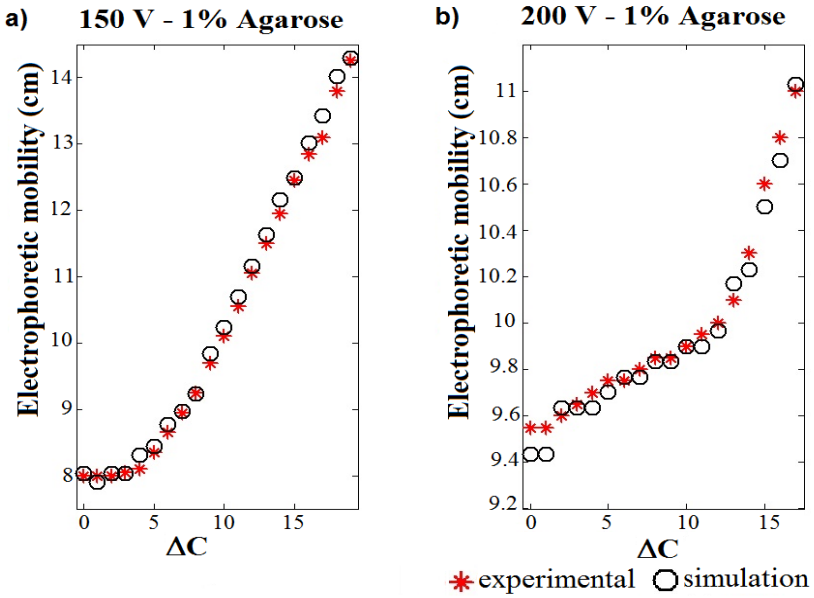

Figure 3: **Comparison of the experimental electrophoretic mobility of supercoiled dimers with the computational simulation:** a) Electrophoretic mobility with the 2^{nd} dimension run at 150 V b) Electrophoretic mobility with the 2^{nd} dimension run at 200 V. The red (*) represent the experimental data and the black (o) correspond to the results of the numerical simulation.

3 Conclusions

In this work we have shown that Maxwell-Stefan equations for mass transport are able to accurately reproduce the two-dimensional agarose gel electrophoresis of the knotted and supercoiled dimers under different electrostatic potential during the second dimension. This approach represents an advantage when it comes to simulating agarose gel electrophoresis, since it saves a lot of computational time when compared to other techniques.

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References

[1] J. Baxter, N. Sen, V.L. Martinez, M.E. de Carandini, J.B. Schvartzman, J.F. Diffley, and L. Aragon, Positive supercoiling of mitotic DNA drives decatenation by topoisomerase II in eukaryotes, *Science*, 2011. DOI: 10.1126/science.1201538.

[2] L. BELL, and B. BYERS, Separation of branched from linear DNA by two-dimensional gel electrophoresis, *Anal Biochem*, 1983. DOI:10.1016/0003-2697(83)90628-0.

[3] J. Cebrián, M.J. Kadomatsu-Hermosa, A. Castán, V. Martínez, C. Parra, M. J. Fernández-Nestosa, C. Schaerer, M.L. Martínez-Robles, P. Hernández, D.B. Krimer, A. Stasiak, and J.B. Schvartzman, Electrophoretic mobility of supercoiled, catenated and knotted DNA molecules, *Nucleic Acids Res*, 2015. DOI: 10.1093/nar/gku1255

[4] B. Laurie, V. Katritch, J. Sogo, T. Koller, J. Dubochet,and A. Stasiak, Geometry and physics of catenanes applied to the study of DNA replication, *Biophys J*, 1998. DOI: 10.1016/S0006-3495(98)77988-3

[5] C.C. Liu, B.M. Alberts, Type II DNA topoisomerases: enzymes that can unknot a topologically knotted DNA molecule via a reversible double-strand break, *Cell*, 1980. DOI: 10.1016/S0092-8674(80)80046-8.

[6] V. Lopez, M.L. Martinez-Robles, P. Hernández, D.B. Krimer, and J.B. Schvartzman, Topo IV is the topoisomerase that knots and unknots sister duplexes during DNA replication, *Nucleic Acids Res*, 2012. DOI: 10.1093/nar/gkr1237

[7] M.L. Martinez-Robles, G. Witz, P. Hernández, J.B. Schvartzman, A. Stasiak, and D.B. Krimer, Interplay of DNA supercoiling and catenation during the segregation of sister duplexes, *Nucleic Acids Res*, 2009 DOI: 10.1093/nar/gkp530.

[8] J.C. Maxwell, On the dynamical theory of gases; *Phil. Trans. R. Soc.*, 1867. DOI: 10.1098/rstl.1867.0004

[9] J. Roca, Two-dimensional agarose gel electrophoresis of DNA topoisomers, *Methods Mol Biol*, 2009. DOI: 10.1007/978-1-60761-340-4.

[10] J.B. Schvartzman, M.L. Martinez-Robles, P. Hernández, and D.B. Krimer, DNA replication and topology as visualized by two-dimensional agarose gel electrophoresis, *Plasmid*, 2010. DOI: 10.1016/j.plasmid.2009.11.001

[11] A. Stasiak, V, Katrich, J. Bednar, D. Michoud, and J. Dubochet, Electrophoretic mobility of DNA knots. *Nature*, 1996. DOI: 0.1038/384122a0.

[12] J. Stefan, Uber das Gleichgewicht und die Bewegung insbesondere die Diffusion von Gasgemengen, *Akad. Wiss. Wien*, 1871, 63: 63-124

[13] N.C. Stellwagen, Electrophoresis of DNA in agarose gels, polyacrylamide gels and in free solution, *Electrophoresis*, 2009. DOI: 10.1002/elps.200900052.