2001 Bioinformatics and Genomics Symposium and the 2001 Annual Meeting of the American Electrophoresis Society

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and the
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The chemical engineering background provides an excellent foundation for the study of a wide-range of problems and systems. Of particular note is the significant effort to apply core principles of chemical engineering science to the study of biological systems and to the development of new technologies for the study of biological systems. The training in kinetics, thermodynamics and transport phenomena, coupled with a familiarity with applied mathematics and computer programming skills affords the chemical engineer an opportunity to make important contributions in these areas. At the same time, life science-based research is evolving more rapidly than ever before. Large datasets, the need to integrate information across many levels and the desire to have improved technologies for the study of biomolecules have helped bring the life science community in close contact with the chemical engineering community.

Presented in these pages are a collection of particularly interesting contributions from two separate, but related, topical conferences. Although only programmed for three years, the Bioinformatics and Genomics Topical has become a staple of the life science-based programming at the AIChE Annual Meeting and has drawn a large audience of chemical engineers and life scientists alike. Further, this programming has enjoyed ongoing financial support from a few key industrial partners. The presentations which make up the Bioinformatics and Genomics Topical detail technology development, data analysis techniques and attempts to solve key problems of interest to the biotechnology and bioengineering community.

The American Electrophoresis Society held it's annual meeting in collaboration with the AIChE this year. The Electrophoresis Society's meeting has the theme of Electrophoresis in Biotechnology and Bioengineering and thus serves naturally to bring together the programming efforts of these two groups. Of particular note this year are the large number of papers dedicated to proteomics and several contributions in the realm of nanobiotechnology. Further, the partnership between AIChE and AES has provided an opportunity to solicit a more broad range of contributions that would be possible for either group alone.

I would like to take this opportunity to express my sincere thanks to the authors, session chairs and AIChE staff which make these Topical Conferences possible. Further, I would like to express a special thank you to the following: Prof. Vassily Hatzimanikatis, co chair of the Bioinformatics and Genomics Topical, Prof. Pedro Arce, co chair of the Electrophoresis Meeting, Prof. Frank Witzmann, President of the American Electrophoresis Society, Joan Stevenson, Joe Cramer and Jeff Wood for their unparalleled support and to Dr. Sangtae Kim for his ongoing commitment to these programming areas.

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DIFFUSION AND ELECTROPHORESIS IN GELS: GOING BEYOND STANDARD OGSTON AND FREE VOLUME MODELS

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Prepared for presentation at the 2001 Annual Meeting, Reno, Nevada, Nov. 4-9:
Bioinformatics and Genomics: Plenary Session

Key words: gel electrophoresis; diffusion theory; computational science; Monte Carlo methods.

Abstract of the plenary talk

This presentation will start with an overview of the recent progress made in the development and application of our lattice model of low-field gel electrophoresis (the so-called "Ogston regime"). This model, which allows numerically exact results to be obtained, can also be used to study a wide range of diffusion problems in a variety of disciplines. In particular, we will look at the dynamics of oligomers and spherical analytes in ordered and disordered gels, including the case of a thermodynamic ratchet system. We will then discuss how the model can be extended to treat the following situations: electric field lines going around non-conducting obstacles; inhomogeneous systems; high electric field intensities; and the limit to the continuum.

***

1. Introduction

Over the last decade or so, our group has been developing a lattice model of low-field electrophoretic migration [1] and diffusion [2] in gels (i.e., in quenched systems of obstacles). The original version of the model applied to hard, rotationally isotropic analytes, and it neglected hydrodynamic interactions and intra-molecular degrees of freedom. The problem was then reduced to the solution of large systems of linear equations, which can be solved to provide numerically exact electrophoretic mobilities (or diffusion coefficients). The possibility of calculating exact mobilities for model sieving systems makes it possible to compare our results to the predictions of various free-volume theories, thus providing a precise and critical test of the fundamental hypotheses behind such models.

More recently, we generalised our model along the following directions [1,2]: a) we added attractive analyte-gel interactions to investigate their effect on sieving; b) we treated the case of oligomers (small macromolecules with internal degrees of freedom, including rod-like objects); c) we

studied the continuum limit where the lattice parameters become negligible compared to the size of the fibres; d) we examined some situations close to the percolation limit of the sieve; e) we compared the cases of conducting and non-conducting obstacles; and f) we compared various gel sieving structures. These studies both demonstrated the utility of this mathematical/numerical approach and the limitations of our current understanding of this class of problems. In the next sections, we will review some of the key elements of the model, and we will discuss how the model can be further generalised to treat exciting new problems, including problems related to micro- and nano- fluidics.

2. Ogston, sieving, electrophoresis and diffusion

The central problem that we are studying is that of the migration of a small particle (the "analyte") in a porous system (e.g., a gel) under the action of both Brownian forces and an external field $E \geq 0$. It is hard to overemphasize the importance of this problem in science. For example, it is related to the lateral diffusion of proteins in biomembranes, various gel electrophoresis and chromatography techniques, the diffusion of drugs in organs or out of a drug delivery system, and the passage of analytes through filtration membranes. According to the Nernst-Einstein theorem, which applies in the weak field limit, the diffusion coefficient D and the mobility μ (which is the ratio between the net velocity V and the applied field E) must satisfy the relation:

$$\frac{D}{\mu} = \frac{k_B T}{Q} \quad ; \qquad E \to 0 \tag{1}$$

where Q is the charge conjugated to the field E, T is the temperature and k_B is Boltzmann's constant. Therefore, calculating one of the low-field transport properties (D or μ) gives us the other. Alternatively, eq 1 imposes a constraint on our theoretical models. In a high field situation, one must replace the = sign by a $\geq$ sign and both D and μ become field-dependent, thus indicating that diffusion and randomness dominate when a thermodynamic system is brought far from equilibrium. Of course, what constitutes a high field is system-dependent. In the case of electrophoresis, it is well-kown that the mobility μ of small particles is essentially field-independent in most cases (the same is true for long DNA molecules if the field is low). This means that eq 1 must be valid for many electrophoresis problems. Therefore, what we learn about one class of problems (e.g., electrophoresis) also applies to the other (e.g., diffusion).

The migration of an analyte in a porous system of volume concentration φ is a complicated and old problem. Not surprisingly, there are literally thousands of papers about this topic, many of which were published in discipline-specific journals. Many models share one element: they assume that $D(\varphi)$ or $\mu(\varphi)$ are simple (and often universal) functions of the occupied volume fraction φ. Perhaps the simplest such model is the so-called Ogston model of gel electrophoresis, which assumes that:

$$\frac{\mu(\varphi)}{\mu(0)} = f(\varphi) \tag{2}$$

where $f(\varphi)$ is simply the fractional volume of the gel available to the analyte during the migration (e.g., $f(\varphi)=1-\varphi$ for a point-like analyte). Note that it is assumed here that ALL of the gel-analyte interactions can be captured by the purely geometric parameter $f(\varphi)$, which can indeed easily be calculated for any analyte/gel combinations. One rather unsatisfying aspect of such assumptions is that two gels or porous systems offering the same fractional free volume $f(\varphi)$ to a given analyte should equally affect the mobility $\mu(\varphi)$ or diffusion coefficient $D(\varphi)$ of this analyte, no matter what their internal structures are. In particular, the percolation properties of the two gels are irrelevant! Clearly, these models are "mean-field" models that average over the details of the system. Our goal is to go beyond such models to capture the subtle effects of the architecture of the porous medium on particle dynamics.

3. Mathematical methods and previous results

Our approach was originally to study the problem using rather standard and well-tested Monte Carlo methods where a particle is allowed to move on a lattice, step by step, and the probabilities of moving along the different directions are functions of the field intensity. When a particle moves onto a site occupied by an obstacle, it returns to its original site and the clock advances (this models hard-core repulsion). However, we found how to "solve" the Monte Carlo simulation exactly. More precisely, we found a way to write the dynamics that the Monte Carlo simulations are modeling as a matrix equation. The solution of this equation gives the probability of presence of the analyte on each lattice site (the lattice is finite and we use periodic boundary conditions to mimic macroscopic systems). In order to obtain the mean velocity of the analyte in the lattice system, we then use these computed probabilities together with the mean velocity on each lattice site. It is important to note that this approach actually gives numerically exact mobility *ratios* $\mu^*(\varphi)=\mu(\varphi)/\mu(0)$ (or, alternatively, diffusion coefficient ratios $D^*(\varphi)$) in the limit of *vanishingly* weak external fields. We refer the reader to our publications for further details.

We demonstrated that the exact diffusion coefficients $D^*(\varphi)$ calculated this way agree perfectly with the existing Monte Carlo simulation data and the exact analytical theories. Since we can calculate both the mobility $\mu^*(\varphi)$ and the free volume $f(\varphi)$ exactly for a wide variety of systems, our results demonstrate very convincingly that eq 2 is not generally valid. For example, a better approximation, for three dimensional gel systems and in the limit of the continuum, is given by expressions such as

$$\mu^*(\varphi) \cong \frac{1}{1+\frac{2}{3}f(\varphi)} \tag{3}$$

Interestingly enough, although the fundamental assumption of the Ogston model is invalid, the data analysis methods based on this model (e.g., the so-called Ferguson plot) remain useful because they all apply to low concentration gels.

Recently, Locke and Trinh [3] suggested that perhaps the failure of our models to confirm eq 2 was due to the fact that we had used straight field lines throughout the gel, thus implicitly assuming that the gel fibers were conducting. It is indeed probable that most materials forming porous systems would not be conducting, thus leading to field lines that go around the obstacles. However, if eq 2 were valid for the electrophoretic mobility, the fundamental Nernst-Einstein relation (eq 1) would be violated since we know that eq 2 is not valid for diffusion. Re-analysis of our results confirmed that in the limit of weak field intensities, our numerical results and eq 1 remain valid for non-conducting obstacles.

Essentially, our method requires that we enumerate all the possible "states" of the system and the various dynamic connections between them: this constitutes the linear equations of motion (or master equations) whose solution give the probability of being in each of these states in the steady-state. Since it is easy to calculate the velocity of the analyte for each state, the solution of the master equations directly gives the mean velocity. In fact, we wrote programs that use the gel structure as the only input: they then generate the matrix equations, solve them and provide the user with the exact mobility (or diffusion coefficient). Therefore, it is possible to generalize the method to any problem with a finite number of states (hence the need for periodic boundary conditions). For example, the method can be used to study small polymers (a state is then the probability of being in a given conformation and at a given location), attractive gel-analyte interactions, multiple particles moving simultaneously, etc. The limits are usually imposed by the available computing resources since the enumeration may require >1GB of memory for large problems, leading to matrices with more that one million rows and columns.

4. Prospects

In our opinion, our method can easily replace a large number of Monte Carlo simulations currently being used in various fields of science and technology. For example, we compared our approach to the Monte Carlo results obtained by various groups that use very large computers. We typically obtain 3 to 10 more digits in 30 minutes on a regular Pentium processor. Unless it is impossible to enumerate all the possible states of a system, our approach will beat Monte Carlo simulations for the simple reason that it solves the very same problem without using a slowly converging stochastic process.

At the current time, we have to complete the development of the model along three different lines. First, we must find a way to reduce the size of the relevant part of the phase space in order to study even larger problems. Second, we must find a way to add hydrodynamic interactions between the analyte and the medium. Finally, and more importantly perhaps, we have to generalize the approach to high-field situations. These are not easy tasks; for instance, a correct treatment of these problems must be able to conserve the correct superposition of the field-driven drift and the Brownian motion. As an example, the well-known Metropolis algorithm does not work in the high field limit because once all the jumps point in the field direction, the diffusion coefficient is zero while the velocity ceases to increase with field intensity.

A simple, fast and reliable method to calculate mobilities (or diffusion coefficients) may allow us to study a variety of problems in many scientific disciplines. Of great interest are problems related to micro- and nano- fluidics. For example, a number of new separation ideas based on ratchet processes, entropic traps and lateral diffusion sieves have recently been proposed. We are currently studying these problems using our method.

5. Conclusions

Experimentally, it is impossible to establish the validity of eq 2 because the free volume $f(\varphi)$ is not normally available. Monte Carlo simulations offer more control, but the error bars do not allow us to study the subtle effects of gel architecture on sieving and diffusion. Our approach clearly established that eq 2 is not valid, and that one must go beyond mean field models. This is actually excellent news since it means that one can actually design gels to perform non-trivial sieving/sorting tasks.

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[1] See our 8-part series "An Exactly Solvable Ogston Model of Gel Electrophoresis", G.W. Slater et al:
I: Electrophoresis (1996) 17, 977-988; II: Electrophoresis (1996) 17, 1407-1415;
III: J. Chromatography A (1997) 772, 39-48; IV: Electrophoresis (1998) 19, 1560-1565;
V: Electrophoresis (2000) 21, 823-833; VI: Electrophoresis (2001) 22, 673-683;
VII: Macromolecules (2001) 34, 3437-3445; VIII: Electrophoresis (2001) 22, 2631-2638.

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