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Review

Polymeric separation media for electrophoresis: cross-linked systems or entangled solutions

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ABSTRACT

The current status of separation media development for capillary electrophoresis is reviewed in light of well-known electrophoretic migration and entangled polymer solution theories. The relative strengths and weaknesses of crosslinked systems and entangled solutions are also examined. The residence time limit of DNA in a mesh is estimated and compared with the relaxation time of both a typical entangled polymer solution and a cross-linked gel. By advancing the concept of analyte residence time and life time of a characteristic network mesh, we can highlight the differences between the operative mechanism governing separation in these two types of electrophoresis media.

CONTENTS

1. INTRODUCTION

Capillary electrophoresis (CE) has found widespread applications in analytical and biomedical research. The scope and sophistication of CE are rapidly increasing. CE can perform analytical separations that are often substantially better than those using established chromatographic methods such as high-performance liquid chromatography (HPLC). Conventional electrophoretic methods are slow, labor-intensive, prone to relatively poor reproducibility and have limited quantitative capability. Furthermore, it has been difficult to fully automate the process. This situation has been greatly improved by the advent of CE. The major advantages of CE are that it can be fully automated, it offers high resolution, and it can quantitate minute amounts of samples [1]. These capabilities lie far beyond those of traditional electrophoretic methods.

CE has recently been used in the analysis of an extremely wide variety of molecules, including organic and inorganic anions and cations, drugs,

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dyes and their precursors, vitamins, carbohydrates, catecholamines, amino acids, proteins and peptides, nucleic acids, nucleotides, and oligonucleotides. Among standard separation methodologies, CE is the best known for its ability to separate a wide range of molecular weights. It is possible to separate in the same column species ranging in size from free amino acids to large proteins associated with complex molecular matrices. From the detection point of view, HPLC provides better concentration sensitivity while CE provides better mass sensitivity. However, initial attempts to resolve complex mixtures of biological macromolecules in open CE columns were disappointing. Proteins present a serious problem when using untreated fused-silica capillaries due to their adsorption onto the walls of the capillary. With oligonucleotides, the unfavorable charge-to-mass ratio tends to cause comigration of larger mixture components.

A highly innovation solution to overcome these difficulties was the development of gel-filled capillaries [2–8]. Remarkably high separation efficiencies have been obtained by gel-filled CE. To accomplish size selection in electrophoretic separation of mixtures of nucleic acids and sodium dodecyl sulfate denatured proteins, a cross-linked gel matrix is employed. However, the routine preparation of homogeneous stress-free gels in capillaries is difficult due to polymerization-induced shrinkage and appearance of bubbles inside the capillaries.

As an alternative to cross-linked gels, solutions of entangled polymer, such as polyethylene glycol, linear non-cross-linked polyacrylamides or (hydroxyethyl)cellulose (HEC) have been tested as macromolecular sieving media [9–14]. This approach provides ease of filling and flushing of the separation capillary after each analysis, thus avoiding the possible contamination of analyte from the previous run. However, entangled polymer solutions in the capillaries exhibit lower resolution than cross-linked gels.

The resolving power of CE using entangled polymer solution as the separation media is not good for large analyte molecules, presumably as a result of the relevant time scales of the sieving polymers and analyte molecules. The residence time (or passage time) of an analyte molecule in a mesh is controlled by the size and electrophoretic mobility of the analyte, mesh size of the network, and the imposed electric field strength. The life time of an entanglement is dictated by the mobility of strands forming the mesh, the network integrity, and the length and concentration of macromolecules constituting the network. In order to achieve good resolution, the relaxation time of the entangled polymer solution should be orders of magnitude greater than the residence time of the analyte molecules. Otherwise, an entirely different mechanism must be occurring to make separation of mixtures possible. These time-scale considerations are the focus of this paper.

2. BACKGROUND AND THEORY

2.1. Electrophoretic migration theory

There are two well-known theories for the migration of a flexible macromolecule through a polymer network: The Ogston model and the reptation model. Here, we briefly summarize these two models.

The Ogston model is schematically illustrated in Fig. 1. It assumes that the matrix consists of a random network of interconnected pores having an average pore size ξ. The migrating solute behaves as an undeformable particle of radius R_g. In this model, smaller molecules migrate faster because they have access to a larger fraction of the available pores, giving the following expressions [9,10,15]

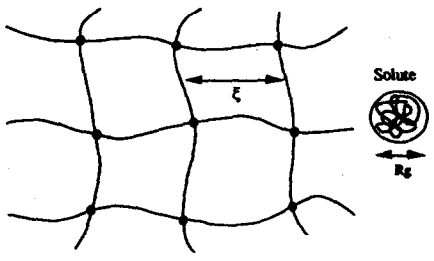

Fig. 1. Schematic of a solute migrating through a polymer network by the Ogston mechanism.

$$\mu = \mu_0 \exp[-Cb(R_g + r)^2] \quad (1)$$

or

$$\mu = \mu_0 \exp\left[-\frac{1}{4}\pi\left(\frac{R_g + r}{\xi}\right)^2\right] \quad (2)$$

where μ is the electrophoretic mobility of the analyte in the matrix, μ_0 is its free solution electrophoretic mobility, C is the concentration of polymer network, b is a constant and r is the thickness of the strand. This model, however, does not take into account for the electric field (E) effects on R_g. Therefore, eqns. 1 and 2 hold only in the limit of $E \rightarrow 0$. The Ogston model reduces to the celebrated Ferguson plot for $R_g \gg r$, where a plot of log (μ/μ_0) *vs.* C gives a straight line with a slope proportional to R_g^2.

The Ogston model assumes the migrating solute to be an undeformable spherical particle moving through a mesh network. Therefore, when $R_g > \xi$, this model ceases to be applicable. It is well known that a long and flexible chain molecules such as DNA can migrate even when $R_g \gg \xi$. This can be explained by the reptation model which assumes that the migrating solute moves as an unraveled coil from head to tail.

Assuming that one linear chain is moving in a given network, as shown in Fig. 2 [16]. The network is described by fixed obstacles O_1, O_2, etc. The chain is not allowed to penetrate any of these obstacles, but it can travel between them in a snakelike fashion. This motion is called "reptation". To understand the effect of the obstacles on the electrophoretic mobility, it is convenient to consider the chain to be contained within a certain imaginary tube [17]. In a unit time, parts of the tube are lost on the tail end while new parts of the tube are created on the headside. Lerman and Frisch [18] first introduced the reptation concept to the electrophoresis of biopolymers. In the limit of zero electric field strength,

$$\mu \approx \frac{1}{N} \quad (3)$$

where N is the number of repeat units of the analyte. Eqn. 3 suggests that the electrophoretic mobility μ is inversely proportional to the analyte molecular mass at low electric fields.

A modified reptation model that extends to strong electric fields is the biased reptation model. Under the influence of strong electric fields, the solute becomes more elongated. In the limit, the solute acquires a rod shape. This is schematically illustrated in Fig. 3. In the limiting of the biased reptation model, the electrophoretic mobility is no longer dependent on molecular size. Lumpkin expressed this effect by [23]

$$\mu \approx K\left(\frac{1}{N} + bE^2\right) \quad (4)$$

where K is constant and b is function of ξ, charge and persistence length of the migrating solute. As we can see from eqn. 4, the dependence of mobility on molecular size decreases when the electrical field or the molecular size increases. This is why DNA can be separated by using conventional electrophoretic technique at low fields up to approximately 20 000 base pairs [9].

2.2. Entangled polymer solution theory

To understand entangled polymer solutions, we need to introduce a simple scaling law by De Gennes [16]. Three regimes of polymer solutions can be imagined: (1) dilute solutions, (2) semi-

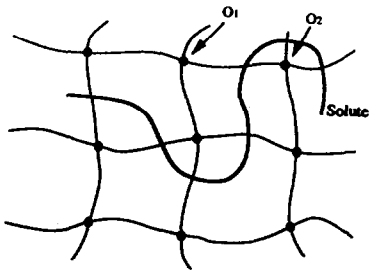

Fig. 2. Schematic of a solute migrating through a polymer network by the reptation mechanism.

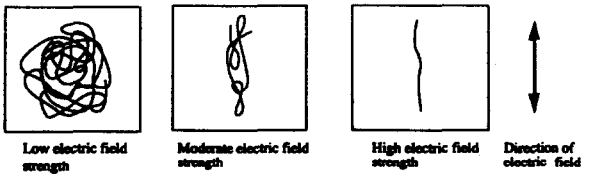

Fig. 3. Schematic of the elongation effect of the electric field on a migrating molecule.

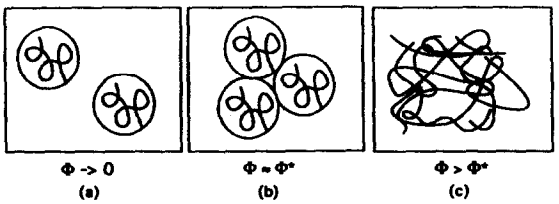

Fig. 4. Crossover between dilute and semi-dilute solutions: (a) dilute, (b) overlap, (c) semi-dilute.

dilute solutions and (3) concentrated solutions. Only dilute and semi-dilute solutions are important for separation. Transition from the dilute to the semi-dilute regime occurs at the overlap threshold. The overlap threshold where the polymer chains begin to be densely packed. This threshold is not sharp; it is more properly defined as a region of crossover between regimes (a) and (c) in Fig. 4. At the overlap threshold concentration, Φ^*, the bulk solution concentration is equal to the local concentration inside a single polymer chain. In an athermal solvent, this implies

$$\Phi^* \approx N_p^{-0.8} \tag{5}$$

where N_p is the number of segments in the polymer chain. Note that for large N_p, Φ^* is very small.

Now we discuss the concept of correlation length or mesh size (ξ), which characterizes an entangled polymer solution. This is illustrated in Fig. 5. The scaling law of ξ in the semi-dilute regime for an athermal solvent has been derived with the following assumptions: (i) For $\Phi > \Phi^* \xi$ depends only on concentration and not on the degree of polymerization N_p; (ii) for $\Phi \approx \Phi^*$ where the polymer chains are in contact but not yet interpenetrating ξ must be comparable with the size of a polymer chain R_g.

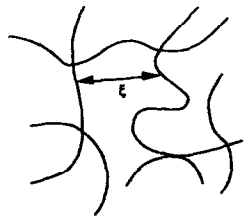

Fig. 5. Schematic of a characteristic mesh with size ξ.

The rescaling relation between ξ and Φ in an athermal solvent obeys the following form

$$\xi(\Phi) \approx a\Phi^{-0.75} \tag{6}$$

where a is a scaling constant. Eqn. 6 shows that ξ decreases rapidly with concentration.

Grossman and Soane [9,10] proposed the optimum conditions for CE using entangled polymer solutions to correspond to the lowest possible viscosity which maintains an entangled network, *i.e.* $\Phi \approx \Phi^*$. For such condition, combining eqns. 5 and 6 give

$$\xi(\Phi^*) \approx a\Phi^{0.6} \tag{7}$$

Eqn. 7 shows that in order to create a large ξ while minimizing the viscosity of the solution, one should choose a high-molecular-mass polymer. No consideration, however, was given to the integrity or longevity of the entangled network at the threshold condition. This aspect of the problem is now a subject of study.

3. DISCUSSION

We now closely scrutinize an entangled polymer solution mesh in contrast with a cross-linked gel mesh. This is illustrated schematically in Fig. 6. As we previously discussed, the entangled mesh is a spatial property, being temporally transient and not a permanent state. A given entangled mesh persists at best up to the relaxation time of the polymer chains constituting the mesh. The residence time of analyte molecule in this mesh is controlled by the size and electrophoretic mobility of the analyte, the mesh size of the network, and the imposed electric field strength. According to either the Ogston model

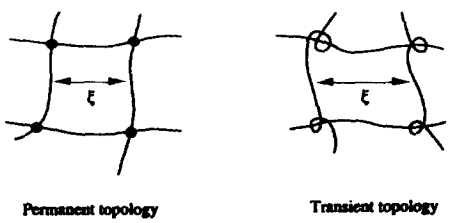

Fig. 6. Comparison of permanent network and transient network, corresponding to a cross-linked gel and a polymer solution.

or the reptation mechanism, in order to achieve good resolution, the relaxation time of the entangled polymer solution should be orders of magnitude greater than the residence time of the analyte molecules. In this manner, the mesh is maintained during transit of the analyte, so the matrix serves as an effective sieving medium.

It is well known that the relaxation times of polymer solutions and cross-linked gels can be measured by dynamic light scattering. A photon correlation function obtained by dynamic light scattering generates the underlying relaxation time spectrum. Such a spectrum often consists of slow and fast, and sometimes intermediate modes, indicative of different types of motion. We assume the mesh integrity is governed by the longest relaxation time, *i.e.*, the slow mode. In the ensuing comparison, the longest relaxation time is taken as the life time of the mesh. A typical DNA molecule is chosen as the model analyte. Specifically, the suitability of the gel and the solution networks as potential separation media for sequencing applications is investigated. The residence time of DNA can be estimated from the literature [9,10,19] by assuming two extremes (Ogston and biased reptation). In these two extremes, the residence time, τ_{res},

$$\left(\frac{\text{mesh size}}{\text{electrophoretic velocity}}\right)_{\text{Ogston}} < \tau_{res} < \left(\frac{\text{DNA contour length}}{\text{electrophoretic velocity}}\right)_{\text{biased reptation}} \quad (8)$$

where subscript Ogston means that the migrating solute is approximated as an undeformable particle (Ogston model). For biased reptation under the influence of large electric fields, the solute becomes more elongated, and the motion mimics that of a snake threading its way through the network. In the biased reptation mode, DNA moves by repeated stretching, slippage, relaxation and re-extension. The transient polymer network is momentarily under stress when interacting with the passing analyte. This is schematically illustrated in Fig. 7. Therefore, the correct time scale is estimated by employing the DNA contour length in the numerator of eqn. 8.

One can obviously question the driving force behind mesh destruction during electrophoresis.

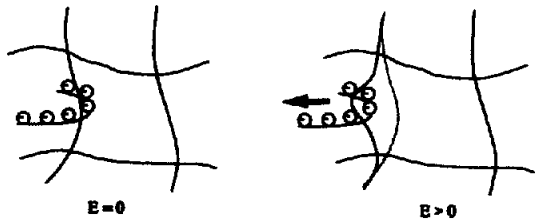

Fig. 7. Schematic of the transient interaction of a DNA molecule with one of the strands constituting and entangled mesh. Note the forces acting on the strand, causing dissociation of the mesh.

We propose at least two mechanisms. The first is the locally elevated osmotic pressure resulting from the increased local concentration of macromolecules during analyte passage. The second is the transient tethering the stretching action of the network strand as depicted in Fig. 7. Regardless of the origin of network disengagement around the mesh, it is the aforementioned two relevant time scales that govern the performance of the separation media.

The relaxation times of entangled polyacrylamide solution and cross-linked polyacrylamide gel are reported as following [20]:

$\tau = 5.9 \cdot 10^{-4}$ s (T = 3%, 25°C),

for entangled solutions

(9)

$\tau = 4.0 \cdot 10^{-3}$ s (T = 3%, 25°C),

for cross-linked gel

[T = (g acrylamide + g N,N′-methylenebisacrylamide)/(g acrylamide + g N,N′-methylenebisacrylamide + g H_2O)]. The calculated residence time limits of DNA are as follows [7,19]: For the two migrating extremes

Experimental results for 30 base pairs:

$1.5 \cdot 10^{-5}$–$1.8 \cdot 10^{-4}$ s

Experimental results for 100 base pairs:

$1.5 \cdot 10^{-5}$–$8.2 \cdot 10^{-4}$ s (10)

The calculated residence times of 100 base pairs are extrapolated from 30-base pair data based on a hydrodynamic diameter per base pair of 3.3 Å

[21,22]. The results show clearly that the relaxation time of the entangled polymer and residence time of DNA are very close. Therefore, sharp resolution cannot be expected for the high molecular mass of DNA (*e.g.* beyond 100 base pairs) using entangled polymers. The network imposing the sieving medium fails before the analyte moves through a mesh completely. This problem does not exist for chemically cross-linked systems.

As a final note, we point out other possibilities for different electrophoretic mobilities for analytes of varying sizes, even below the critical overlap threshold. An example is the momentary interaction of the analyte with the surrounding matrix molecules as it streams by the former. The longer the analyte, the higher probability for this type of interaction, with consequential retardation of mobility. It is yet unclear whether this type of interaction is sufficient for DNA sequencing applications.

4. CONCLUSIONS

We have reviewed the current status of CE and the basic theories related to the two key types of sieving media. We also have contrasted the relative strengths and weaknesses of cross-linked and entangled systems as sieving media by advancing the concepts of analyte residence time and life time of a characteristic network mesh. Our calculations show that it would be difficult to achieve high resolution for high molecular masses of DNA using entangled polymer solutions that are neither concentrated nor extremely viscous as the sieving media. We are in the process of developing novel polymer systems that possess low viscosity during fill but retain a high resolving power during analysis. Experimental results of such work will be reported in further publications.

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