

THE EFFECT OF ELECTROPHORETIC CONVECTION FOR THE SEPARATION OF SOLUTE IN THE CHROMATOGRAPHY COLUMN (I)

Young Gyu Park[†]

Dept. of Chemical Engineering, Daejin University, 11-1 Sundan-Ri, Pochun-Kuen, Kyungki-Do 487-711, Korea
(Received 19 August 1998 • accepted 18 November 1998)

Abstract—A theoretical model has been derived in an electrophoretic packed column where an electric potential is applied to a column in the axial direction. The effect of electrophoretic convection in gel particles packed in the column significantly contributes to the separation of large polyelectrolytes because the conformation of polyelectrolyte quickly orients in the field direction. The dependence of the transport in the gel particle upon field intensity and molecular size aids in understanding the transport of polyelectrolyte in the packed column, since the convective velocity of polyelectrolyte is accelerated inside a porous gel particle. There are few convection studies of large polyelectrolyte in a column packed with porous gel particles under an electric field for the separation. Convective-diffusive transport of a large polyelectrolyte is analyzed using Peclet number described by electrophoretic mobility and diffusion coefficient measured experimentally. The separation of two different polyelectrolytes in the packed column is performed using a value of Pe_e/Pe_g of individual polyelectrolyte by molecular size and an electric field. The purpose of this paper is to study the separation of solute from a mixture in the column using the physicochemical properties in the gel particle which are measured experimentally.

Key words: Packed Column, Electrophoretic Convection, Large Polyelectrolyte

INTRODUCTION

The transport of polyelectrolytes in polymeric materials has important applications in a wide range of separation and purification processes. A number of theoretical and empirical approaches [Park, 1996; Ahn et al., 1993; Lu et al., 1992] have been made to separate polyelectrolytes using the bioseparation process. Although some studies have been devoted to the fundamental problems of a separation process such as the chromatography column, there have been only a few detailed studies in solute transport because both diffusion and electrophoretic intraparticle convection are present in the column.

It is widely acknowledged that gel electrophoresis provides the highest resolution in the purification of polyelectrolytes such as proteins and nucleic acids. However, it is also true that gel electrophoresis has extremely poor scaling properties so that it has been impossible to adapt this milligram bench technique to multigram preparative separations. On the other hand, chromatography has far superior scaling properties and a resolving power that is second only to electrophoresis. Therefore, we would hope that gel electrophoresis and chromatography could be combined in a manner that would amplify the resolving powers of each while retaining the superior scaling properties of chromatography.

O'Farrel [1985] initially attempted to separate proteins in the electrochromatography column. The problems which occurred were not effectively addressed until an electrophoretic column

was invented capable of focusing proteins at the interface between two different phases. In this application, field-induced dispersion is virtually eliminated by proper manipulation of the electric field. The successful combination of electrophoresis with chromatography suggests that large scale electrochromatography is feasible, but little is known about the underlying physics of the process or how it can be effectively scaled-up. In this paper, the basic understanding of the dynamics of solute in the gel particle is a key for the separation of polyelectrolytes in a packed column.

The intraparticle convective transport in the dynamic problem is of current interest in a number of separation processes. For example, Frey et al. [1993] have analyzed the convective effects that solutes enter the interior of the particles in a high performance liquid chromatography. Rodrigues et al. [1991] have studied the effects of intraparticle convection on protein separation of the packed column. Carta [1995] has analyzed the effects of intraparticle convection for the dynamic capacity of the adsorption bed using the LDF approximation in a permeable support. Dogu et al. [1989] have investigated the intraparticle diffusion coefficient in a single-pellet cell that displayed a significant hydrodynamic convective component inside porous solids. A number of analyses dealing with intraparticle convection have been attempted within a porous catalytic pellet [Nir and Pisman, 1977; Rodrigues et al., 1982; Cresswell, 1985].

In the case of a large polyelectrolyte, it is difficult for the polyelectrolyte to penetrate through the narrow pores of polymeric sieving materials because the radius of gyration of polyelectrolyte is relatively large compared to the pore size. Slow diffusive transport inside the polymeric sieving material causes

[†]To whom correspondence should be addressed.
E-mail: ypark@road.daejin.ac.kr

the peak of the elution curve to be broadened in the column, and the peak-broadening in the column may make two different polyelectrolytes difficult to separate. However, if two different polyelectrolytes are applied in the presence of an electric field in the column, polyelectrolytes can be separated as preventing the peak-broadening because they move readily through the sieving particle packed in the column. This implies that the electric field may increase the macroscopic diffusion of polyelectrolyte in the direction perpendicular or parallel to the electric field, as well as enhance the permeation of polyelectrolyte due to the electrophoretic convection inside the pore.

Use of an electric field in the separation process has been frequently employed for more delicate separations in the chromatography column [Rudge and Ladish, 1988] and the extractor [Ptassinski and Kerkhof, 1992]. The electrophoretic convection in the internal pores may make the separation of polyelectrolytes possible by reducing the broadness of peak in a packed column. The intention of this theoretical study is to examine the separation of two different polyelectrolytes in the presence of an electric field in the packed column. The purpose of this paper is to show how intraparticle mass transport by the electrophoretic convective velocity influences the separation of polyelectrolytes.

Theoretical methods to solve the electrophoretic mass transport problem are performed in the particle and the column domains. The mathematical emphasis in this paper is on developing the spectral expansion by a set of eigenvalues. The spectral evaluation on the transport differential operator is performed for the dynamic studies of the solute in the gel particle. The mathematical model for the particle domain is developed using a one-dimensional approximation. The relative effects of diffusional-electrophoretic convective transport are performed by varying the ratio ($\chi = Pe_f / Pe_g$) of the dimensionless Peclet number of the fluid to the solid phases. An operator theoretic method [Ramkrishna and Amundson, 1985] is used to solve the intraparticle mass transport problem. The limiting case of very slow transport inside the particle relative to the outside allows a decoupling of the particle from the column. The equation for the transport in the column is developed by the species mass balance equation in the packed column. The characteristic method [Rhee, 1986] is used to solve the transport problem in the column domain.

MATHEMATICAL MODEL

1. Model of Mass Transport in the Packed Column

The mathematical model for the packed column is based on the following assumptions. The electric field is applied to the x-direction in the electrophoretic chamber as shown in Fig. 1. A single particle considered for this study has a uniform stagnant boundary layer of the fluid phase and it is assumed to be dominant by convective velocity and diffusion in the x-direction in the column. The convection and diffusion in the column direction are assumed to be bounded by two boundary layer regions surrounding a gel particle. Therefore, the total fluxes over each internal boundary between each layer and the linear equilibrium conditions are assumed to exist and to be expressed in terms of the species distribution coefficient (β). The fluid

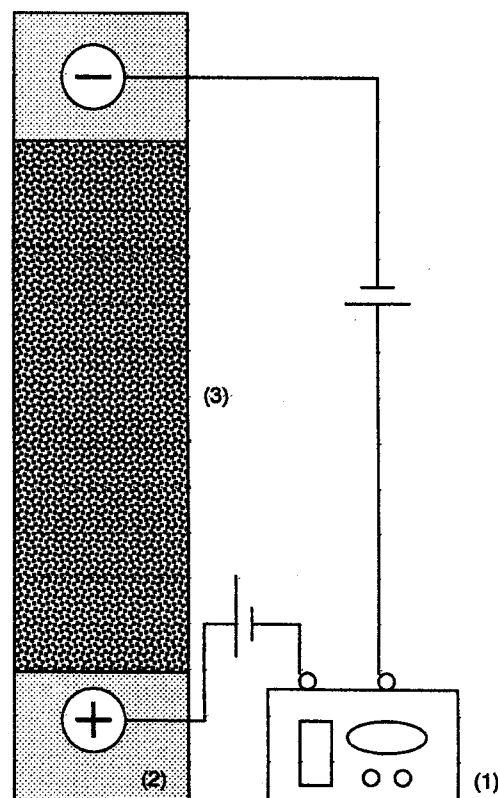


Fig. 1. A schematic of a packed column in the presence of an electric field.

(1): Electric power source, (2): Tris buffer solution, (3): column packed with agarose gel particles

velocity does not exist in the column and the solute transport at very slow flow velocity in the presence of an electric field in the column. Therefore, the axial dispersion is assumed to be neglected.

Fig. 2 is a schematic of the x-directional dimension in a gel particle. The mathematical models in the gel particle and in the column can be described as follows.

(1) The molar species continuity equation for solute

$$\frac{\partial c_{i,k}}{\partial t} = \nabla \cdot (D_{i,k} \nabla c_{i,k}) - \nabla \cdot (c_{i,k} z_{i,k} F u_{i,k} \nabla \Psi_{i,k}) \quad (1)$$

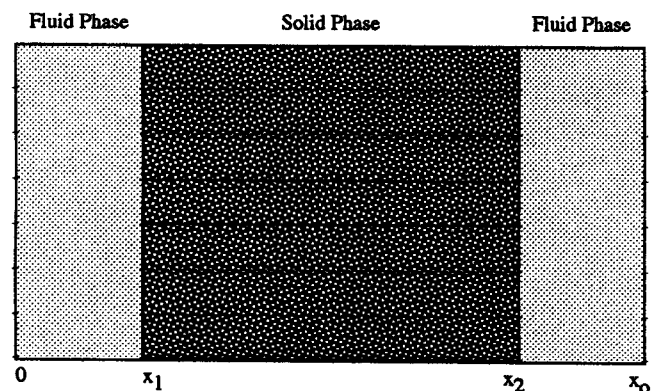


Fig. 2. A one-dimensional schematic picture of gel particle.

x_1 : thickness of upper fluid layer, x_2 : thickness of gel layer, x_0 : total thickness of gel particle and boundary layer

with boundary conditions and initial conditions

$$\begin{aligned} & -D_{i,k+1} \nabla c_{i,k+1} + c_{i,k+1} (u_{i,k+1} z F \nabla \Psi_{i,k+1}) \\ & = -D_{i,k} \nabla c_{i,k} + c_{i,k} (u_{i,k} z F \nabla \Psi_{i,k}) \\ & \beta_{i,k+1} c_{i,k+1} = \beta_{i,k} c_{i,k} \quad \text{at } x' = x'_k, \quad k=1, 2 \\ & c_{i,k}(t=0) = 0, \quad k=1, 2, 3 \end{aligned}$$

where subscript k denotes each phase of fluid-solid phase: for example, an upper boundary layer is in case of $k=1$, a lower boundary layer for $k=3$ and a gel layer for $k=2$.

(2) The molar species balance for the particular species in a packed column

$$\varepsilon \frac{\partial c_{i,b}}{\partial t} + \varepsilon (z_{i,f} F v_{i,f} \nabla \Psi_{i,f}) \frac{\partial c_{i,b}}{\partial z} + (1 - \varepsilon) \frac{\partial \langle c_{i,2} \rangle}{\partial t} = 0 \quad (2)$$

with boundary and initial conditions

$$\begin{aligned} c_{i,b}(0, t) &= c_o & \text{at } 0 < t < t_o \\ c_{i,b}(0, t) &= 0 & \text{at } t > t_o \\ c_{i,b}(z, t=0) &= 0 & \text{at } x \\ c_{i,b}(0, t) &= 0 & \text{at } t > 0 \end{aligned}$$

In the equations, the species molar concentration, the electrophoretic mobility, and the diffusion coefficient in the gel particle are given, respectively, by $c_{i,b}$, $u_{i,b}$, and $D_{i,b}$. v_i in Eq. (2) is the electrophoretic mobility in the fluid phase and it is assumed to be the same as that of free solution. F is the Faraday constant and $\Psi_{i,k}$ is an electrophoretic potential.

Eq. (2) is coupled with the effective distribution coefficient of particle phase defined as

$$\langle c_{i,2} \rangle = B(\tau) c_{i,b} \quad (3)$$

$\langle c_{i,2} \rangle$ is the average concentration in the fluid inside pores and the $B(\tau)$ denotes the effective distribution coefficient of solute in the presence of an electric field. In the absence of an electric field, the effective distribution coefficient is equal to a porosity of β . And the model Eqs. (1)-(2) are redescribed using dimensionless parameters and transformation variable as shown in Table 1.

The molar species equation at each layer can be generalized in each different k -th layer from Eq. (1) as below:

$$\frac{1}{\phi_{i,k}} \frac{\partial C_{i,k}}{\partial \tau} = \frac{\partial^2 C_{i,k}}{\partial x^2} - \frac{Pe_{i,k}^2}{4} C_{i,k}; \quad s_{k-1} \leq x \leq s_k \quad \text{for } k=1, 2, 3 \quad (4)$$

$$\begin{aligned} \frac{y_{i,k} \phi_{i,k}}{\beta_{i,k}} \left(-\frac{\partial C_{i,k}}{\partial x} + Pe_{i,k} \frac{C_{i,k}}{2} \right) &= \frac{y_{i,k+1} \phi_{i,k+1}}{\beta_{i,k+1}} \\ \left(-\frac{\partial C_{i,k+1}}{\partial x} + Pe_{i,k+1} \frac{C_{i,k+1}}{2} \right); &k=1, 2 \end{aligned} \quad (5)$$

$$C_{i,k} y_{i,k} \beta_{i,k} = C_{i,k+1} y_{i,k+1} \beta_{i,k+1} \quad (6)$$

where $y_{i,k} = \exp(Pe_{i,k} x_k/2)$ and boundary conditions at the edges of boundary layers are $C_1(0)=1$ and $C_3(1)=\exp(-Pe_f/2)$. For a single gel particle surrounded by two equal sized stagnant boundary layers, it is assumed that $x_k=s_1$, $\phi_{i,k}=1$, $Pe_{i,k}=Pe_f$ for $k=1$; and $x_k=s_2$, $\phi_{i,k}=\phi$, $Pe_{i,k}=Pe_g$ for $k=2$; and $x_k=1$, $\phi_{i,k}=1$, $Pe_{i,k}=Pe_f$ for $k=3$ and $x=0$ at $k=0$.

Table 1. Dimensionless variables and transformation variable

The dimensionless variables of Eqs. (4)-(8) are

$$\begin{aligned} C'_i &= \frac{c_i}{c_o}, \quad x = \frac{x'}{x_o}, \quad \tau = \frac{t D_k}{x_o^2}, \quad \phi = \frac{z_k u_k \nabla \Psi_k}{z_1 u_1 \nabla \Psi_1}, \\ Pe_k &= \frac{(z_k F u_k \nabla \Psi_k) x_o}{D_k}, \quad s_k = \frac{x_k}{x_o}, \quad Z = \frac{z}{L}, \quad \alpha = \frac{\varepsilon}{1 - \varepsilon} \end{aligned}$$

The transformation variable of Eq. (4) is

$$C = C' \exp \left(\frac{-Pe_k x}{2} \right)$$

The effective distribution coefficient of Eq. (3) can be defined by this relationship as follows.

$$B(\tau) = \frac{\int_0^{x'_1} c_{i,1} dx + \int_{x'_1}^{x'_2} c_{i,2} dx + \int_{x'_2}^{x'_3} c_{i,3} dx - (\int_0^{x'_1} c_{i,1} (Pe_f=0) dx + \int_{x'_1}^{x'_2} c_{i,2} (Pe_f=0) dx)}{(x'_2 - x'_1) C_o} \quad (7)$$

If an electric field is absent, the Pe_f is equal to be zero like the right hand side of Eq. (7). The total concentration in a gel particle described by Eq. (7) is coupled with Eq. (2) by the dimensionless form as

$$\frac{z_{i,f} F u_{i,f} \nabla \Psi_{i,f}}{L} \frac{\partial c_{i,b}}{\partial Z} + [1 + \alpha B(\tau)] \frac{\partial c_{i,b}}{\partial t} = -\alpha \frac{\partial B(\tau)}{\partial t} c_{i,b} \quad (8)$$

where dimensionless terms are in Table 1. The linear operator approach is employed in the present work to solve the model in a relatively straightforward way. The solution to Eq. (8) is developed by using the characteristic method [Rhee, 1986]. The solution is used to determine the effects of the intraparticle convective transport on the separation of two different components.

2. Electrophoretic Mobility in a Gel Particle

The electrophoretic mobility in a given gel particle is generally written as

$$\mu = \frac{Q(\eta, T, c)}{\xi(\eta, T, c)} g(L, E) \quad (9)$$

where L is the contour length of the polyelectrolyte, η the viscosity of the solvent, c the concentration of gel particle, $[C]$ the ionic strength of the solvent, and E the electric field strength. $\xi(\eta, T, c)$ is the frictional force per unit length of a polyelectrolyte, which depends on the viscosity, the temperature, and the gel concentration. $Q(T, [C])$, the effective charge of the polyelectrolyte, is sensitive to the temperature and the ionic strength of the solvent $[C]$. $g(L, E)$ is a function of the contour length and of the electric field. There are various theoretical predictions of the variation of $g(L, E)$ with the polyelectrolyte length. The migration of polyelectrolyte through the gel particle follows different laws according to the intensity of the electric field, the size of the molecule, and the concentration of the gel particle used.

When the contour length L of the fragment is smaller than its persistence length p , which is itself smaller than the average pore size $\langle a \rangle$ for most agarose gel particles, the fragment can be seen as a rod of negligible width and length that can enter all pores. When L is larger than the persistence length

p, the fragment folds on itself and takes the approximate shape of a globule with radius of gyration R_g given by [Slater et al., 1986]

$$R_g^2 = \frac{1}{3} pL \left\{ 1 - \frac{p}{L} + \frac{p}{L} \exp\left(-\frac{L}{p}\right) \right\} \quad (10)$$

This fragment is excluded from the pores of radius $a \ll R_g$. When $R_g > a$, large polyelectrolyte does migrate. This is explained by the reptation theory, which assumes that the molecule is moving headfirst through the pores of the gel instead of migrating as a globule hopping from one pore to the next. With this reptative motion, the mobility becomes [Shaffer et al., 1989]

$$\frac{\mu}{\mu_o} = \frac{\langle h_x^2 \rangle}{(Na)^2} \quad \text{for } R_g > a \quad (11)$$

where $\langle h_x^2 \rangle$ is the average distance between the ends of the fragment in the field direction, μ_o is the electrophoretic mobility in free solution and N is the number of pores necessary to house the fragment.

In the biased reptation model [Slater et al., 1986], the mobility is a function of $\Theta = qEa/2kT$. When the electric field ($N\Theta$) is small, reptation induces a negligible change in the value of R_g , i.e., the molecule retains a globular shape. In this case, the chain obeys the Kratky-Porod wormlike-chain formula, with persistence length, p and chain-contour length L , and mean-square end-to-end length is [Lumpkin et al., 1985]

$$\langle h_x^2 \rangle = 2p^2 \left[\frac{L}{p} - 1 + \exp\left(-\frac{L}{p}\right) \right] \approx \frac{1}{3} Na^2$$

and we get the mobility from Eq. (11) as follows.

$$\frac{\mu}{\mu_o} \approx \frac{1}{3N} \quad (12)$$

For large polyelectrolyte such that $N\Theta \gg 1$, the globule deforms and one cannot obtain an analytic expression for $\langle h_x^2 \rangle$. In this case, the most straightforward way to study the problem is through computer simulations. The result, consistent with approximate analytical expression [Slater et al., 1986; Lumpkin et al., 1985], is

$$\frac{\mu}{\mu_o} \approx \frac{\Theta^2}{9} \quad \text{for } N\Theta^2 \gg 1 \text{ and } \Theta \leq 1 \quad (13)$$

This indicates that the electrophoretic mobility of large polyelectrolyte in finite electric fields is independent of the size of the fragments. This is because large polyelectrolytes are stretched in the field direction, leading to a mobility that depends only on the electric force per unit length of molecule.

Concerning the influence of the electric field on the electrophoretic mobility, we compare our data with the theoretical predictions of Slater and Noolandi [1989], Lumpkin et al. [1985]. All these theories assume that the polyelectrolyte moves along its tube by Brownian motion biased by the electric field. They also postulate that the orientation of the head determines the orientation of the tube, and that there is no correlation between the successive tube segments. With these assumptions, they are found an expression for μ of the form

$$\frac{\mu}{\mu_o} = \frac{1}{N} + \frac{\Theta^2}{3} \quad (14)$$

SOLUTION METHODOLOGY

1. Spectral Solution in a Gel Particle

The initial boundary value problem for the concentration profile in the adsorbent particle reduces to

$$\frac{\partial U}{\partial \tau} = LU + B_k I \quad (15)$$

where L is represented as a 3×3 diagonal matrix having differential diagonal operations and I is a 3×3 diagonal unit matrix.

$$L_k = -\phi_{i,k} \left(\frac{\partial^2}{\partial d^2} - \frac{Pe_{i,k}^2}{4} \right); \quad k=1, 2, 3$$

U is a 3×1 matrix representing the eigenfunction (u_k). Their matrix forms are given by

$$L = \begin{bmatrix} L_1 & 0 & 0 \\ 0 & L_2 & 0 \\ 0 & 0 & L_3 \end{bmatrix} \quad \text{and} \quad U = \begin{bmatrix} u_1 \\ u_2 \\ u_3 \end{bmatrix}$$

and the domain associated with this differential operation is given in a Hilbert space H by [Ramkrishna and Amundson, 1985; Locke and Park, 1992]

$$\begin{aligned} D(L) &\equiv \{g \in H \text{ and } Lg \in H : u_1(0) = 0; u_{i,k}(s_k) y_{i,k}(s_k) \beta_k(s_k) \\ &= u_{i,k+1}(s_{k+1}) y_{i,k+1}(s_{k+1}) \\ \beta_{i,k+1}(d_{k+1}); \frac{y_{i,k} \phi_{i,k}}{\beta_{i,k}} v_{i,k} &= \frac{y_{i,k+1} \phi_{i,k+1}}{\beta_{i,k+1}} v_{i,k+1}; \\ k &= 1, 2, 3; u_3(1) = 0 \end{aligned}$$

where $\mu_{i,k} = \left(-\frac{\partial C_{i,k+1}}{\partial x} + Pe_{i,k+1} \frac{C_{i,k+1}}{2} \right)$, B_k is $Q_1[1 + \exp\{(Pe_g - Pe_i)(s_2 - s_1)\}] A_i \exp(-Pe_g)$, Q_1 is $\sqrt{\lambda - \frac{Pe_f^2}{4}}$, Q_2 is $\sqrt{-\frac{Pe_g^2}{\phi_{i,k}}}$ and u_k is an eigenfunction. A_i of B_k is written in Appendix.

The eigenvalue problem associated with the operator L , previously defined in (15), is given by $LU = \lambda U$. The above Eq. (11) yields the following two types of equations

$$\frac{d^2 u_{i,k}}{dx^2} + Q_{i,k}^2(\lambda) u_{i,k} = 0; \quad k=1, 2, 3 \quad (16)$$

where $Q_{i,k} = \sqrt{\lambda - \gamma_{i,k}}$, $\gamma_{i,k} = \phi_{i,k} \frac{Pe_{i,k}}{4}$

The solution of this problem is readily written as a spectral expansion in terms of the eigenvalues and eigenvectors of L . Eigenfunctions of L that belong to different eigenvalues are orthogonal each other. Furthermore, the set of eigenfunctions constitutes a complete set, and all non-zero eigenvalues of L are real and positive. The general solution of Eq. (16) can be written as

$$u_{i,k} = A_{i,k} \xi_{i,k}(Q_{i,k}, x) + B_{i,k} \zeta_{i,k}(Q_{i,k}, x) \quad (17)$$

where $\xi_{i,k}(Q_{i,k}, x)$ and $\zeta_{i,k}(Q_{i,k}, x)$ are either trigonometric functions (when the arguments are real) or hyperbolic functions (when the arguments are imaginary). With no loss of generality, we can take $\xi_{i,k}(Q_{i,k}, x) = \sin(Q_{i,k} x)$ and $\zeta_{i,k}(Q_{i,k}, x) = \cos(Q_{i,k} x)$. The u_k are eigenfunctions in the fluid phase when $k=1, 3$, while u_k are eigenfunctions in the solid phase when $k=2$. The eigenfunctions for various cases are normalized from the general solution given by Eq. (17). Eq. (17) can be rewritten as

$$u_{i,k} = \kappa [\xi_{i,k}(Q_{i,k}, x) + \frac{B_{i,k}}{A_{i,k}} \zeta_{i,k}(Q_{i,k}, x)] \quad (18)$$

The value of κ can be calculated by normalizing the eigenfunctions; its value is written in the appendix. The coefficients $B_{i,k}/A_{i,k}$ are determined by applying the corresponding inner product to the boundary conditions of Eq. (1) and their values are listed in the appendix.

The transient solution for the concentration profile in the gel particle can be represented in each phase by Eq. (15) to give a self-adjoint eigenvalue problem [Locke and Park, 1992] as

$$C_{i,k}(x, \tau) = \sum_{n=1}^{\infty} \frac{B_{i,k} u_{i,k}(x, \lambda_n)}{\lambda_n} [1 - e^{-\lambda_n \tau}]; \quad 1 = 1, 2, 3 \quad (19)$$

Eq. (2) in the gel particle domain and total flux boundary condition (3) is solved by the direct interactions resulting from the diffusive or convective coupling through adjoining boundaries between fluid and solid phases. An operator theoretic approach is used to solve the intraparticle mass transfer problem in the column. The dynamic behavior of heterogeneous phases is analyzed to seek the mass transfer of solutes in the column.

The mass transport inside the particle relative to transport outside the particle allows a decoupling of the particle problem to the column problem. The spectral evaluation of the transport differential operator can be performed for a parametric study of physical properties on the concentration profiles inside a gel particle. The spectral solution of the mathematical model in a gel particle domain requires accurate calculation of eigenvalues. The bisection method is used to calculate the eigenvalues between consecutive asymptotic lines which define the range over which to search for roots. The solution to the characteristic equation is described as combinations of $\sin(\cdot)$ and $\cos(\cdot)$ or $\sinh(\cdot)$ and $\cosh(\cdot)$. λ_n of eigenvalues can be calculated using the characteristic equation as below:

$$\Gamma_{i,k}(\lambda_n) = \Lambda_{i,k}(\lambda_n)$$

$$\text{where } \Gamma_{i,k} = \beta_{i,2} \phi_{i,2} \left(\frac{Pe_f}{Pe_g} \right) \left[Q_{i,2}^2 + \frac{Pe_g^2}{4} \left(1 - \frac{1}{\beta_{i,2} \phi_{i,2}} \right) \right]^2$$

$$\Lambda_{i,k} = \frac{Q_{i,1}}{\tan(Q_{i,1} d_1)} \left[\frac{Pe_g Q_{i,1}}{Pe_f \beta_{i,1} \phi_{i,1} \tan(Q_{i,1} d_1)} + \frac{2Q_{i,2}}{\tan[Q_{i,2}(d_2 - d_1)]} \right]$$

The time, τ , in Eq. (3) is associated with the dynamics of the particle for a given external concentration value. The time, τ ,

represents the time after the polyelectrolyte begins to penetrate into a gel particle. The time, t , in Eq. (8) represents the time after the sample on the column is injected. When a sample is transported to the direction of an electric field from the top of column, the polyelectrolyte in a certain gel particle is an empty state in the column before the polyelectrolyte penetrates into the gel particle. In this case, the relationship between two different transient times of τ and t is given by

$$\tau = t - \frac{Z}{(z_{i,f} Fu_{i,f} \nabla \Psi_{i,f})} \quad (20)$$

In order to solve Eq. (8) it is necessary to identify the slope of the characteristic curve which is given by

$$\frac{dt}{dZ} = \frac{[1 + \alpha B(\tau)]L}{z_{i,f} Fu_{i,f} \nabla \Psi_{i,f}} \quad (21)$$

The concentration along each characteristic curve is given by deriving $dc_{i,b}/dt$ from Eq. (8) and the concentration profile in the column is given as

$$c_{i,b} = c_{i,b}(\tau_0) \frac{1 + \alpha B(\tau_0)}{1 + \alpha B(\tau)} \quad (22)$$

There is a characteristic curve formed from the start as the state of concentration c_0 moves into the column, and the elution curve of polyelectrolyte in a column moves along a straight line. The characteristic equation is rewritten from Eq. (21) as

$$Z - Z_0 = \frac{z_{i,f} Fu_{i,f} \nabla \Psi_{i,f}}{\alpha L} \int_{\tau_0}^{\tau} \frac{dp}{B(P)} \quad (23)$$

Fig. 3 illustrates the structure of the case of a plug of polyelectrolyte injected into an initially empty column. The characteristics emanating from the $t=0$ axis for the initially empty column all have slope equal to zero due to $B(\tau)=0$, and concentration c_0 has zero concentration. The characteristics are given by Eq. (23) with $Z_0=0$ and $\tau_0=t_0$. To construct a particular characteristic in this region it is necessary to choose τ_0 . The position of the elution curve in the column can be calculated when τ varies from τ to some desired upper limit from Eq. (23); τ is calculat-

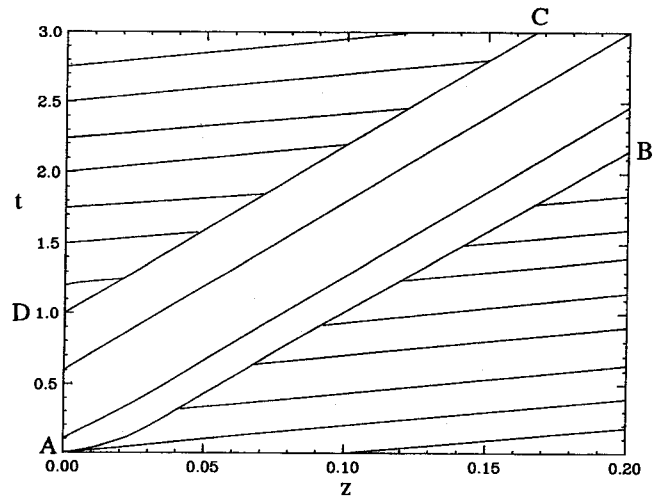


Fig. 3. Characteristic curve in a packed column at $Pe_f=6.9$, $Pe_g=1.2$, $\beta=0.210$, $\phi=0.35$.

ed from Eq. (20). The concentration profile is obtained from c_0 of AB as the characteristic curve of Fig. 3 emanates from the t -direction. The propagation speed of the polyelectrolyte along the packed column varies depending on the several regions of the characteristic curve on the t - Z plane. The polyelectrolyte does not exist below line AB and above line DC. The characteristic curves lie inside the region of ABCD. The elution curve of polyelectrolyte like DNA varies with a concentration which ranges from c_0 for AB to $c=0$ for the characteristic curve DC. As the polyelectrolyte moves along the column, the concentration profile of polyelectrolyte in the column propagates along a characteristic direction with the propagation speed of the reciprocal of dt/dZ of Eq. (21).

EXPERIMENTAL

The gelation process for agarose gel electrophoresis takes place within 15 min after casting in 60°C in order to measure the electrophoretic mobilities of DNA fragments. Agarose is chosen because of its good mechanical properties at low concentration. The large polyelectrolyte for this experiment is DNA such as λ phage HIND III, and Φ X HAE III DNA fragments are purchased from BRL. The range of molecular length is from 23,600 to 310 bp. The loading solution contains 20 % by weight of DNA, 10 % by weight of a dye solution (10 % bromophenol blue+50 % glycerol and 40 % EDTA). This buffer is used to prepare the loading DNA solution and to fill the electrophoretic tray. The pH of the buffer is 6.8. The electrophoretic apparatus is a BioRad Model 2303, with a useful gel length 25 cm. Two silver electrodes are inserted from the top, 8 cm apart, into the electrophoretic tray. The voltage between these electrodes is measured with a digital voltmeter, which determined that the average voltage drop during an experiment was less than 3 %. The temperature of the buffer was controlled within an accuracy of 0.2°C in order to keep the temperature with a digital thermometer connected to two thermocouples dipped in the apparatus.

Each experimental run is soaked for 30 min in a 2×10^{-5} M ethidium bromide solution to bind the intensity of the fluorescence of the ethidium bromide. Each electrophoretic mobility is determined with at least three measurements of the position of the bands at different time intervals. A linear fit is displacement vs. time, and the corresponding fitted lines. These lines never go through the origin. Invariably, the extrapolation to $t=0$ gives a finite positive value for the displacement. The electrophoretic mobility of DNA fragments is measured with a function of electric field and molecular size.

The gel particle is prepared with electrophoretic grade agarose from Bio-Gel-A-150m (BioRad) which fractionates in the range of 1,000,000-150,000,000. This agarose has very low electro-osmosis; measured electrical mobilities are thus not complicated by this effect. The gel particle, which has a diameter of 40 μ m, is packed in the column as shown in Fig. 1.

Once agarose gel particles are loaded in the column, the columns are inserted into the vertical BioRad electrophoresis chamber. DNA mobility in this study is a measurement of the distance to the leading end of the DNA from the top of the column. The electrophoretic mobility of DNA can be easily observ-

ed because it moves, having sharp band shape along the column under the fluorescence light.

The packed column used in this experiment is a cylindrical glass tube of 0.5 cm in diameter and 15 cm in length purchased from BioRad Co. as shown in Fig. 4. The eluent from the column is carried out at pH 7.2; the absorbance in the column is measured with a spectrophotometer at 340 nm in the gel scanning spectrophotometer (Shimadzu). The temperature of the circulator is kept at 18°C.

The bed porosity (ϵ) can be determined by measuring V_0 and V_T , where total bed volume V_T can be determined from the breakthrough response of urea, a small molecule that is completely included in all gel particles, and V_0 can be determined by using T4 DNA, a large molecule that is totally excluded from the gel particles. The bed porosity ϵ is equal to V_0/V_T . T4 DNA is purchased from Sigma Chemical Co. The mobility of DNA in free solution is 4.0×10^{-4} cm²/sec from Olivera [1969].

RESULTS AND DISCUSSION

1. Electrophoretic Convection in the Gel Particle

The contour length of large polyelectrolytes like DNA molecules that can be separated by conventional continuous field electrophoresis in agarose gels is about 300 times the diameter of the average pore in the gel matrix. The persistence length of polyelectrolyte in the usual gel media is 150 bp in case of DNA (bp: base pair); the radius of the unperturbed worm-like chains can be estimated by Eq. (10) and their results are listed in Table 2. The contour lengths are estimated assuming the value of 0.34 nm for the repeat per base pair and molecular weight. The pore size of the 2 % agarose gel particle is about 63 nm [Stellwagen, 1985]. The polyelectrolyte can be expected to extend through a significant number of pores, depending on its length. If the polyelectrolyte is very long, it can be regarded as a series of blobs, each consisting of several persistence lengths, linked from pore to pore. The size of the blobs is determined by the number of statistical segments that might be accommodated, assuming random orientation between segments, within each pore.

Previous studies [Slater et al., 1986; Lumpkin et al., 1985] have suggested that the mobility of the random worm-like chain conformation varies as the ratio of the mean square end-to-end length to the square of the contour length. The perturbation of molecular conformation by the field effectively results in a greater end-to-end length as shown in Table 2, in qualitative agreement with the observed increase of mobility in small electric fields as shown in Fig. 4. An electric field imposes a

Table 2. Conformational properties of DNA on a gel particle

Item	0.367 kbp DNA	1.01 kbp DNA	2.66 kbp DNA
Radius of gyration (R_g)	36.5 nm	70.5 nm	146.7 nm
Persistence (p)	51 nm	51 nm	51 nm
Contour length (L)	125 nm	343 nm	903 nm
Mean square end-to-end length ($\langle h^2 \rangle$)	7,548 nm	29,784 nm	86,904 nm
Pore size (a)	63 nm	63 nm	63 nm

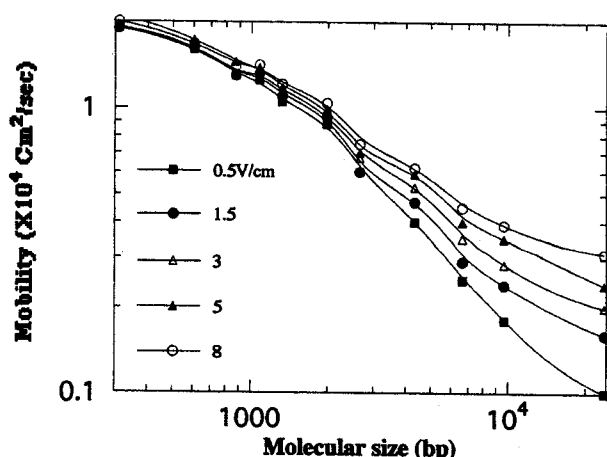


Fig. 4. Logarithm of the molecular length vs the electrophoretic mobility at various electric field strength for 2 % agarose concentration.

net translation in the field direction, but the mobility begins to be field-dependent if the polyelectrolyte is longer than about 6 persistence lengths, long enough to extend beyond one or two sphere-accommodating pores as shown in Fig. 4. This field-strength dependence is based on the supposition that the field alters the conformation of the molecule, with a stronger translational force on the perturbed conformation than on the original random conformation.

The low-field electrophoretic mobility of polyelectrolyte is inversely proportional to the contour length of the polyelectrolyte as shown in Eq. (12) if its conformation in the gel particle is not affected by the electric field. Mobility approximating this dependence on DNA length at low field strength has been reported by Stellwagen [1985] for fragments up to 13 kbp. However, many observations of mobility show that long molecules are clearly discriminated according to length at values of the applied field that give convenient velocities as shown in Fig. 4.

2. Effect of an Electric Field in the Gel Particle

The electrical field is assumed to be the same in the gel particle as in the fluid phase of boundary regions, because the current-carrying ions can readily penetrate the gel particle. Concentration profiles in the gel particle and in the boundary layer around gel particle can be calculated from the model Eq. (19). The dimensionless Peclet number in Eq. (19) is the major control variable of the electrophoretic convection and diffusion coefficient in a gel particle. Electrophoretic convective-diffusive transport in the gel particle can be analyzed through two different Peclet numbers in the fluid phase (Pe_f ; $Pe_f = Pe_s$) of bulk fluid and the solid phase (Pe_g ; $Pe_g = Pe_s$) of gel particle. Transient concentration profiles in the gel particle and in the boundary layer surrounding the gel particle are associated with the electrophoretic convective velocity in the column.

If the electric field is small enough, the reptation of polyelectrolyte induces a negligible change in the radius of gyration of polyelectrolyte, i.e., the polyelectrolyte retains a globular shape. In this case, the ratio (ϕ) of electrophoretic mobility of polyelectrolyte in a gel particle to that in free solution theoretically has a constant value smaller than 1/3 because the value of 1/3 can be obtained from the mobility equation of $\mu/\mu_0 = \langle \chi^2 \rangle /$

$(Na)^2$ derived by Eq. (11), where the average distance ($\langle \chi^2 \rangle$) between the ends of the polyelectrolyte in the field direction has a constant value of $Na^2/3$ and the number (N) of pores necessary to house the polyelectrolyte segment is approximately 1 because the polyelectrolyte fits into one pore. Since the diffusion coefficient in the gel particle is much smaller than that in the bulk phase, the ratio of Pe_f/Pe_g has a value of smaller than 1.0. Experimentally, the ratio (ϕ) of electrophoretic mobility is 0.35 in case of 1.01 kbp of DNA at 1 V/cm.

If an electric field is relatively high, the transport of polyelectrolyte through the gel particle can be increased. The transport of polyelectrolyte in a gel particle under the influence of an electric field has been theoretically predicted by Slater et al. [1989] and Lumpkin et al. [1985]. Eq. (14) indicates that the transport of polyelectrolyte in the gel particle varies by $1/N$ in a low electric field as well as by E^2 in an electric field with a high electric field and a large molecule. This principle is due to the conformational change of polyelectrolyte when a constant electric field is applied in ordered arrays of pores. The polyelectrolyte conformation at a higher electric field becomes extended and aligned, because the field biases the direction of a leading segment of polyelectrolyte. In particular the stretching time (t_{st}) of a DNA chain after an electric field in a gel particle is dependent on the length (n) of the DNA; its relationship is derived by Viovy [1987] as $t_{st} \propto n/E^2$. A small polyelectrolyte can transport through the gel particle with less serious change in polyelectrolyte conformation according to the equation derived by Viovy. This induces the fast dynamic speed of small polyelectrolyte in the gel particle. Larger polyelectrolyte slowly penetrates through the gel particle since the molecular size is big compared to the pore size.

Experimentally, the electrophoretic mobility of the DNA fragments is measured at 18°C as a function of field strength (from 0.25 V/cm to 8 V/cm). The electrophoretic mobility of the large DNA fragments ($N > 1,000$ bp) increases with the electric field. For small DNA fragments the electrophoretic mobility is field independent as shown in Fig. 4. An electric field effect is observed for these smaller fragments. The field dependence is largest at intermediate field values. In a high electric field of 10 V/cm and 2 % agarose gel, the electrophoretic mobilities are experimentally 1.3×10^{-4} , 2.0×10^{-4} cm²/V-sec. for 1.01 kbp and 0.367 kbp, respectively.

$B(\tau)$ of Eq. (7) is not only dependent on the electrical field, but it is also dependent on the internal and external rates of diffusion. This is because concentration gradients form inside and near the surface of the gel particle. The dependence of $B(\tau)$ on the electrical voltage is theoretically analyzed by the parameters in the gel particle as a function of $Pe_f/Pe_g (= \chi)$ in Fig. 5. Fig. 5 shows that total concentration profiles of 0.367 kbp significantly increase according to the electric field in comparison with those of 1.01 kbp. It is interesting to note that the effective distribution coefficient goes to the proper limiting value (β) of the porosity at the steady state when an electric field is not applied. This implies that it is equal to the volume of pore space to be accessible to the solute inside a gel particle. That is, β is a measure of the capacity of DNA in the gel. Another interesting point is that further increase of an electric field would have less effect on the electrophoretic mobility as shown in

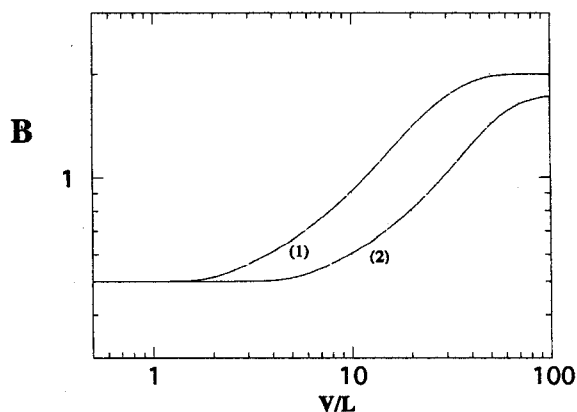


Fig. 5. Convective-diffusive effects of the effective distribution coefficient in a gel particle as a function of an electric field at $\beta=0.5$, $\phi=0.5$. (1) 0.367 kbp DNA ($\chi=Pe_r/Pe_g=0.037$), (2) 1.01 kbp DNA ($\chi=Pe_r/Pe_g=0.054$)

Fig. 5. The value of B in a high electric field increases until it reaches the limiting value of $1/\phi$. The rate of approach to the limiting value is strongly dependent on the electric field strength. The build-up of DNA in the gel particle is strongly dependent upon the value of $Pe_r/Pe_g(\chi)$. The DNA chain in the case that Pe_r is greater than Pe_g is rapidly stretched in the gel, the dynamic speed of DNA to approach the steady state is faster and the transport rate of DNA through the gel particle can be increased in a high electric field. This indicates that the total concentration inside a gel particle becomes steeper with the applied electric field. High Peclet number in the solid phase is an important role to enhance the concentration profiles in a gel particle due to the high convection in the gel.

3. Effect of Molecular Size in the Gel Particle

Since the electric forces deform the polyelectrolyte fragments so that segments of the polyelectrolyte chain occupy consecutive gel network pores in a random walk, the mobility of a larger polyelectrolyte chain becomes slower by a reciprocal-length relation. In a high electric field of 10 V/cm, transient concentration profiles can be calculated using Eq. (19) and the physical properties of Table 3. It is shown in Fig. 6 that the dynamic speed of 0.367 kbp DNA in the gel particle becomes faster as it approaches the steady state in comparison with 1.01 kbp. This indicates that smaller DNA migrates faster from the bulk phase into and through the gel particle due to an increase of electrophoretic convective velocity.

The polyelectrolyte can deform in order to squeeze through pores; the electrophoretic mobility decreases with the molecular size as shown in Fig. 4. The intraparticle transport by convec-

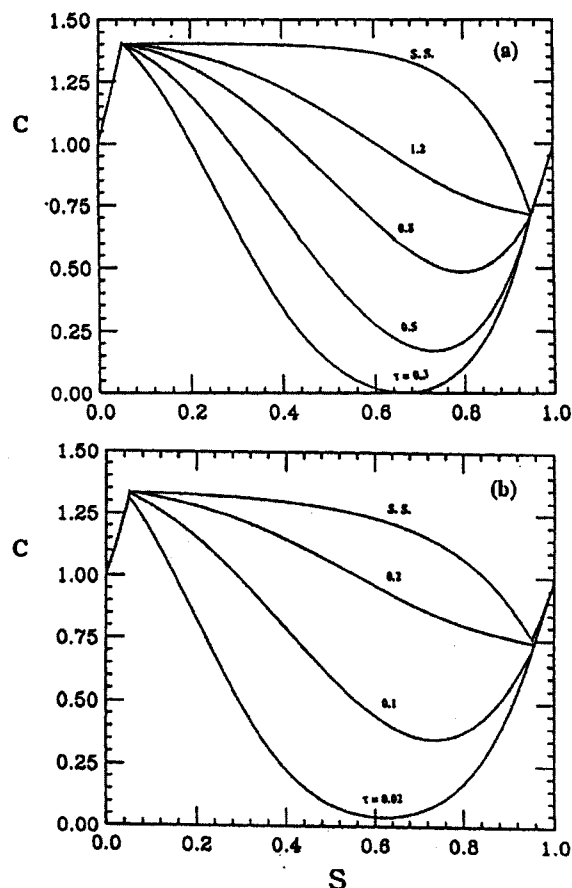


Fig. 6. Transient concentration profiles of DNA in a gel particle. Transient concentration profiles are plotted as dividing by porosity (β). Data measured are listed in Table 3. (a) 0.375 kbp DNA at $E=10$ V/cm, $Pe_r=6.9$, $x_0=1$, (b) 1.010 kbp DNA at $E=10$ V/cm, $Pe_r=1.9$, $x_0=1$

tion may be significantly dependent on a different polyelectrolyte-to-pore size. For example, 0.367 kbp DNA can penetrate the agarose gel particles to a much faster extent than 1.01 kbp DNA. The transient rate to approach the steady state in the gel particle is significantly reduced as polyelectrolyte penetrates into the gel particle farther than $x=s_g$, because the rate of transport in a gel particle is much smaller than that in free solution. In this case, the total concentration by convective-diffusive transport is derived by Eq. (7). If the size of polyelectrolyte is smaller than the persistence length of polyelectrolyte, the polyelectrolyte can bend within pores [Lumpkin et al., 1985]. The chain of polyelectrolyte retains its globular shape during electric migration without serious change of conformation inside pores. For example, the pore diameter of an agarose gel particle is greater

Table 3. Experimental results of DNA at 10 V/cm

Item	367 bp DNA	1.01 kbp DNA
Diffusion coefficient in gel particle (D_g) [Park, 1995]	4.0×10^{-9} cm ² /sec	1.6×10^{-9} cm ² /sec
Diffusion coefficient in free solution (D_f) [Park, 1995]	5.8×10^{-8} cm ² /sec	7.1×10^{-8} cm ² /sec
Porosity (β)	0.210	0.071
Mobility in free solution in pH 7.2 [Hervet et al., 1987]	4.0×10^{-4} cm ² /V-sec	4.0×10^{-4} cm ² /V-sec
Mobility in solid phase	2.0×10^{-4} cm ² /V-sec	1.3×10^{-4} cm ² /V-sec

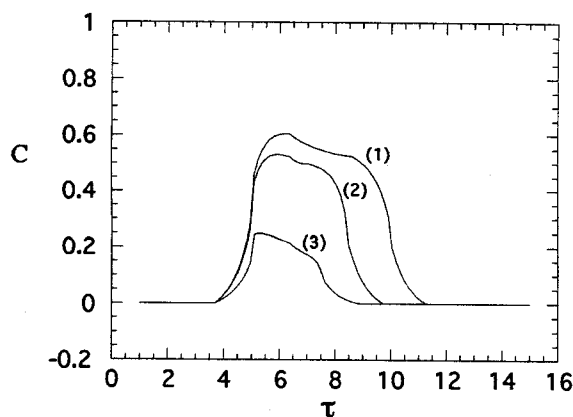


Fig. 7. Concentration profiles of DNA dispersed in the packed column.

(1) 0.367 kbp DNA ($\beta=0.261$, $\Phi=0.5$), (2) 1.01 kbp DNA ($\beta=0.261$, $\Phi=0.5$), (3) 2.66 kbp DNA ($\beta=0.261$, $\Phi=0.5$)

than 63 nm; the persistence length of DNA of about 51 nm is smaller than pore size. DNA can bend at an internal pore and it does not keep its overall globular shape in the presence of an electric field as the molecular size is bigger. But a large polyelectrolyte can deform in order to squeeze through the pore depending on the molecular size.

Fig. 7 shows how the concentration profiles of DNA in the column vary depending on the $Pe_g/Pe_f (= \chi)$ in cases of 0.367, 1.01, 2.66 kbp DNA. The higher χ is, the faster the velocity in the column. The concentration profile of 0.367 kbp DNA in the column occurs because the concentration inside a gel particle increases. This implies that a polyelectrolyte migrates faster from the fluid phase into and through the gel particle due to an increase of electrophoretic convective velocity. Depending on the electric field in comparison with 1.01 kbp as shown in Fig. 5, 0.367 kbp DNA reaches rapidly the limiting value of $1/\Phi$. This relationship can be used to separate different DNA in the column using the electrophoretic mass transport parameter, $Pe_f/Pe_g (= \chi)$.

4. Separation of Large Polyelectrolyte in the Column

As we mentioned before, the elution curve of polyelectrolytes in a column is closely related to the dynamic speed of polyelectrolyte in a gel particle. The separation criteria of the mixture of two components can be separated from band migrations using the ratio of Peclet numbers in the two phases. The ratio (χ) of Pe_f/Pe_g is related to the retention time of polyelectrolyte in the gel particle. Fig. 8 shows how one component can separate from the other component through the column. Note that when the ratio ($R = \chi_1/\chi_2$) of the two polyelectrolytes is equal to 1.0, they are inseparable because the transport properties of the polyelectrolytes such as diffusion and convection become identical to each other. But if the transport properties of the two different polyelectrolytes are different, their separation can be predicted as shown in Fig. 9. The separation of two different polyelectrolytes is predicted by the ratio $R = (Pe_g)_2/(Pe_g)_1$, in which $(Pe_g)_2/(Pe_g)_1 = 2.2$. For example, the χ values for 0.367, 1.01 and 2.66 kbp DNA at 10 V/cm are 0.037, 0.054 and 0.102, respectively. The subscript "1" represents DNA which is eluted

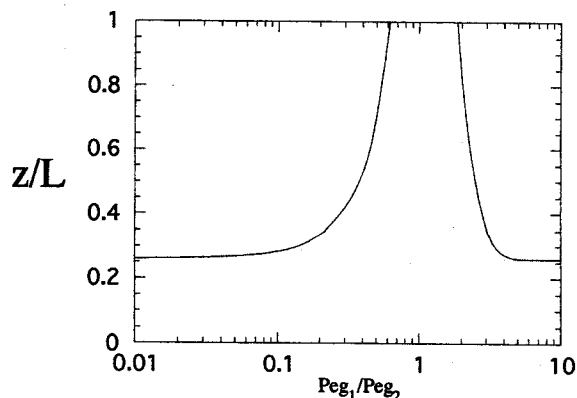


Fig. 8. Theoretical result for the separation of two different DNA at 10 V/cm when $Pe_{g1}/Pe_{g2}=2.2$.

L is the column length, z is a separation point in the column.

first, and subscript "2" is one which is eluted second. The distance to start the separation of two components in the packed column can be theoretically calculated from the dimensionless term, z/L . The separation point of two different DNA in the packed column can be predicted by different properties of the diffusion coefficient and electrophoretic convective velocity in the porous gel particle. The predicted separation distance in the column is 6.23 cm for the mixtures of 0.367 kbp DNA and 1.01 kbp DNA and is 5.21 cm for the mixtures of 0.367 kbp DNA and 2.66 kbp DNA at 15 cm column length under an electric field of 10 V/cm. Experimental measurement under the fluorescent light is performed to check this predicted separation point in the column when 0.367 kbp and 1.01 kbp DNA are loaded. The experimental separation point turned out to be 6.1 cm in the packed column. This implies that theoretical calculation by the model equation is relatively convincing.

Conclusively, the separation point of two different polyelectrolytes in a packed column can be predicted by the diffusion coefficient and convective velocity, measured experimentally. It is expected to be useful for designing a large-scale separation process using the packed column.

CONCLUSIONS

A model is formulated to predict the behavior of the transport of a column packed with gel particles. A complete analysis of the model equations is used in the transient state for a full description of the effects of system parameters, including diffusion coefficient, electrophoretic convection, and gel concentration, on the dynamic problem. The relative ratio of convection and diffusion in solid phase and fluid phase is a factor to determine the transport of polyelectrolyte inside a gel particle; the separation of two different polyelectrolytes is predicted by this ratio. The temperature dependence by the electric field leads to the result that the transport of polyelectrolyte in the gel is affected by the hydrodynamic drag in the pores. The findings in this paper are recommended as a useful guide for the analysis and the design of devices in the laboratory and other scales required for a variety of bioseparations.

ACKNOWLEDGEMENT

The author would like to acknowledge Dr. Lim for his financial assistance of the SCRI (USA).

APPENDIX

Unknown constants of Eq. (16)-(19) are written by

$$A_{i,1} = \frac{\beta_{i,2} \varphi_i \xi_{i,1}}{\beta_{i,2} \xi_{i,2} e^{\frac{(Pe_f - Pe_g)(d_2 - d_1)}{2}}} \frac{e^{\frac{(Pe_f - Pe_g)}{2}}}{\xi_{i,1}}$$

$$\varphi_i = \frac{2\beta_{i,2} \phi_{i,2} Q_{i,2}}{2 + \phi_{i,2} Pe_g Q_{i,2} \left(\frac{1}{\beta_{i,2} \phi_{i,2}} - 1 \right)}$$

The coefficients of Eq. (17) can be described as

$$\frac{B_{i,1}}{A_{i,1}} = \frac{\sin(Q_{i,1} d_1)}{\sin[Q_{i,2}(d_2 - d_1)] + \frac{B_{i,3}}{A_{i,1}} \cos[Q_{i,2}(d_2 - d_1)]}$$

$$\frac{B_{i,3}}{A_{i,1}} = \frac{2\phi_{i,2} \beta_{i,2} Q_{i,2} \tan(Q_{i,1} d_1)}{Q_{i,1} + \phi_{i,2} \beta_{i,2} Pe_f \left(\frac{1}{\beta_{i,2} \phi_{i,2}} - 1 \right) \tan(Q_{i,1} d_1)}$$

$$\frac{B_{i,3}}{A_{i,1}} = \frac{\frac{B_{i,2}}{A_{i,2}}}{\sin(Q_{i,1} d_1)}, \quad \frac{B_{i,2}}{A_{i,2}} = \frac{\left(\frac{B_{i,2}}{A_{i,1}} \right)}{\left(\frac{A_{i,2}}{A_{i,1}} \right)}$$

The normalization constant κ of Eq. (18) is calculated as

$$\kappa = \frac{1}{\sqrt{f_1 + \beta f_2 + f_3}}$$

$$f_1 = \frac{1}{2} \left[d_1 - \frac{\sin(2Q_{i,1} d_1)}{2Q_{i,1}} \right]$$

$$f_2 = \frac{1}{2} \left[\left(\frac{B_{i,1}}{A_{i,1}} \right)^2 + \left(\frac{B_{i,2}}{A_{i,1}} \right)^2 \right] (d_2 - d_1) + \frac{1}{4Q_{i,2}} \left[\left(\frac{B_{i,1}}{A_{i,1}} \right)^2 + \left(\frac{B_{i,2}}{A_{i,1}} \right)^2 \right] \cdot \sin[2Q_{i,2}(d_2 - d_1)] + \frac{1}{2Q_{i,2}} \left(\frac{B_{i,1}}{A_{i,1}} \right) + \left(\frac{B_{i,2}}{A_{i,1}} \right) (1 - \cos[2Q_{i,1}(d_2 - d_1)])$$

$$f_3 = \frac{1}{2} \left(\frac{A_{i,3}}{A_{i,1}} \right)^2 \left[d_1 - \frac{\sin(2Q_{i,1} d_1)}{2Q_{i,1}} \right]$$

NOMENCLATURE

- c_i : concentrations of polyelectrolyte in different phases [g/cm³]
 c_b : bulk concentrations of polyelectrolyte [g/cm³]
 d_i : dimensionless position of gel particle [-]
 D_i : diffusion coefficient of ionic species [g/sec cm²]
 D_k : diffusion coefficient in different phases [g/sec cm²]
 E : electric field [Volt]
 F : Faraday constant [-]
 $\langle h_2 \rangle$: mean square end-to-end distance [cm]
 k : each phase in the gel particle layer [-]
 L : column length [cm]
 n : pore number [-]
 N : number of base pair of DNA [-]
 Pe_g : Peclet number in gel particle [-]
 Pe_f : Peclet number in fluid phase [-]
 Q : total electric charge [Coulomb]
 r : radius of gyration of DNA [cm]
 R_c : column radius [cm]
 s : pore size [cm]
 t : time [sec]
 t_{str} : stretching time of DNA [sec]
 u : electrophoretic convective velocity in bulk fluid [cm/sec]
 u_k : electrophoretic migration of ionic species [cm]
 u_k : eigenfunction of Eq. (12) [-]
 V : electric voltage [volt]
 x : dimensionless spatial coordinate [-]
 x_o : total length of particle diameter and stagnant boundary layers [cm]
 z : column coordinate [-]
 z_k : valence of solute [-]
 Z : dimensionless column length [-]

Greek Letters

- ϕ : ratio of electrophoretic convection velocity [-]
 β : porosity of gel particle [-]
 β_{eff} : effective distribution coefficient of solute [-]
 ε : bed porosity [-]
 λ : eigenvalue [-]
 ξ, ζ : trigonometric function defined in Eq. (13) [-]
 τ : dimensionless time in the gel particle [-]
 Ψ : electrophoretic potential [-]

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