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PREDICTIVE MODEL FOR DESIGN OF A PACKED COLUMN: PARAMETER ANALYSIS

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Abstract—The effect of convective velocity in the packed column is presented. When an electric field is applied, the conformation of polyelectrolyte quickly orients in the field direction. The convective velocity of polyelectrolyte inside a porous gel particle is accelerated. 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. To date, few dynamic studies of polyelectrolyte in a porous gel particle have been attempted for the separation of polyelectrolyte in the packed column. Convective-diffusive transport of DNA is analyzed by physical properties measured experimentally, such as the diffusion coefficient, the electrophoretic mobility and the gel porosity. The purpose of this study is to show how the variation of physicochemical properties in the gel particle affects the separation of DNA from a mixture in the packed column. A theoretical model using the characteristic method is used to calculate the separation point in the packed column.

Key words: *Convective-diffusive Transport, Electrophoretic Mobility, DNA, Spectral Solution, Characteristic Method*

INTRODUCTION

The transport of polyelectrolytes in polymeric materials has important applications to a wide range of separation and purification processes. A number of theoretical and empirical approaches have been made to separate polyelectrolytes for applications of bioseparation processes using a packed column. In the case of a large polyelectrolytes, where it is difficult for the polyelectrolyte to penetrate through narrow pores of polymeric sieving materials because the radius of gyration of polyelectrolyte is relatively large in comparison with pore size. Slow diffusive transport inside the polymeric sieving material causes the peak of the elution curve to broaden in the column, and this may make different polyelectrolytes difficult to separate. However, a polyelectrolyte in the presence of an electric field moves head first through the obstacles of the sieving particle. The use of an electric field may increase the macroscopic diffusion of polyelectrolyte in a direction perpendicular or parallel to the electric field, as well as enhance the permeation of polyelectrolyte due to the electrophoretic convection inside a pore. The electrophoretic convection in internal pores may make the separation of polyelectrolytes possible by reducing the broadening of the peak in the packed column. The intention of this theoretical study is to examine the separation of two different polyelectrolytes in the presence of an electric field using a packed column.

Similar studies for convective effects in a porous medium have been conducted. Opong and Zydney [1991] have considered the convective-diffusive model to experimentally determine the hindrance factors in membrane separation process. Dogu et al. [1989] have investigated the intradiffusion coefficient in a single-pellet cell that displays a significant hydrodynamic convective component inside porous solids. The use of an electric field in the separation process has been frequently employed for the more delicate separations in the chromatography column [Rudges and Ladish, 1988] and the extractor [Ptassinski and Kerknf, 1992]. The convective transport of membrane system [Park and Lim, 1995] has been performed via interactions between fluid and solid phases. Although number of studies have been devoted to the fundamental problems of separation, there have been few detailed predictions of the dynamic behavior of a polyelectrolyte in the medium. The purpose of this study is to analyze the influences of electrophoretic mass transport of a column using several physical parameters of DNA.

Theoretical methods to solve the electrophoretic mass transport problem are performed in the particle and the column domains. The limiting case of very slow transport inside the particle relative to transport outside the particle allows a decoupling of the particle problem from column problem. The equation for transport in the column is developed by the species mass balance equation in the packed column. The characteristic method [Rhee et al., 1986] is used to solve the transport model in the column domain. A similar approach has been taken by Cooper [1972] to analyze irreversible equilibrium problems using an ion exchange column. The theoretical formulation for particle domain is obtained by spectral expansion using the operator theory [Ramkrishna and Amundson, 1985].

MATHEMATICAL FORMULATIONS IN THE GEL PARTICLE

It is assumed that a single particle considered for this study has a uniform stagnant boundary layer of the fluid phase. The molar species continuity equation is given as

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

where subscript k denotes each phase of many phases. The species molar concentration, the electrophoretic mobility, and the dif-

fusion coefficient in the gel particle are given respectively by $c_{i,k}$, $u_{i,k}$, and $D_{i,k}$. F is the Faraday constant. It is approximated in a one-dimensional rectangular system in which a gel particle (k=2) of solid phase is bounded by two boundary layer regions: an upper boundary (k=1) and a lower boundary (k=3). This assumption in a rectangular system does not give a change the qualitative results which will follow the same trend for spheres and cylinders. The molar species equation at each layer can be generalized in each different k layer from Eq. (1). Eq. (1) can be rewritten by using dimensionless variables and transformation variable. The dimensionless variables are tabulated in Table 1 and the following transformation variable is applied in terms of $C=C'\exp\left(-\frac{Pe_k}{2}x\right)$. The Eq. (1) by dimensionless terms can be rewritten as below

$$\frac{1}{\phi_k}\frac{\partial C_k}{\partial \tau}=\frac{\partial^2 C_k}{\partial x^2}-\frac{Pe_k^2}{4}C_k;\ a_{k-1}\le x\le a_k \text{ for } k=1,\ 2,\ 3 \tag{2}$$

Boundary conditions at the edges of boundary layers are

$$C_1(0)=1 \text{ and } C_3(1)=\exp(-Pe_f/2)$$

Internal boundary conditions in the interphase regions between the fluid and solid phases are described as dimensionless form using the total flux conditions of convective-diffusive transport and the equilibrium conditions as

$$\frac{y_k\phi_k}{\beta_k}\left[-\frac{\partial C_k}{\partial x}+Pe_k\frac{C_k}{2}\right]=\frac{y_{k-1}\phi_{k-1}}{\beta_{k-1}}\left[-\frac{\partial C_{k+1}}{\partial x}+Pe_{k+1}\frac{C_{k+1}}{2}\right] \tag{3}$$

$$C_k\ y_k\ \beta_k=C_{k+1}\ y_{k-1}\ \beta_{k-1}$$

The initial condition is

$$C_k(t=0)=0 \text{ for } k=1,\ 2,\ 3 \tag{4}$$

where $y_k=\exp(Pe_k\ x_k/2)$. For a single particle surrounded by two equal sized stagnant fluid layers, it is assumed that $x_k=a_1$, $\phi_k=1$, $Pe_k=Pe_f$ for k=1; and $x_k=a_2$, $\phi_k=\phi$, $Pe_k=Pe_g$ for k=2; and $x_k=1$, $\phi_k=1$, $Pe_k=Pe_f$ for k=3 and $x_0=0$. The transient solution for the concentration profile in the gel particle can be represented as the sum of the steady state term and transient term by the exponential function as

$$C_k(x,\ \tau)=C_k^{ss}(x)+C_k^{tr}(x,\ \tau);\ k=1,\ 2,\ 3 \tag{5}$$

where

$$C_k^{tr}(x,\ \tau)=\sum_{i=1}^{\infty}-\frac{B_{ki}u_{k,i}(x,\ \lambda_i)}{\lambda_i}\exp(-\lambda_i\tau)$$

$C_k^{ss}(x)$ can be calculated from ordinary differential equation of Eq. (2) and B_k is $Q_1[1+\exp\{(Pe_g-Pe_f)\ (a_2-a_1)\}]\ (A_3)\exp(-Pe_g)$, Q_1 is $(\lambda-Pe_f^2/4)^{1/2}$, Q_2 is $(\lambda-Pe_g^2/4\phi)^{1/2}$ and u_k of eigenfunctions and λ_i of eigenvalues can be calculated using the characteristic equation of below equation

$$\beta\phi\left(\frac{Pe_f}{Pe_g}\right)\left[Q_2^2-\frac{Pe_g^2}{4}\left(1-\frac{1}{\beta\phi}\right)\right]^2=\frac{Q_1}{\tan(Q_1\ a_1)}\left[\frac{Pe_gQ_1}{Pe_f\beta\phi\tan(Q_1a_1)}+\frac{2Q_2}{\tan\{Q_2(a_2-a_1)\}}\right] \tag{6}$$

A_3 can be calculated in terms of self-adjoint form of operator as

$$A_3=\frac{\beta\varphi\xi_1}{\beta\ \exp\left\{\frac{(Pe_f-Pe_g)(a_2-a_1)}{2}\right\}\xi_2+\varphi\zeta_2}\frac{\exp\left\{\frac{(Pe_f-Pe_g)}{2}\right\}}{\xi_1}$$

$$\varphi=\frac{2\beta\phi Q_2}{2+\beta\phi Pe_gQ_2\left(\frac{1}{\beta\phi}-1\right)}$$

$$\xi_1=\sin Q_1a_1,\ \xi_2=\sinh Q_2(a_2-a_1),\ \zeta_2=\cosh Q_2(a_2-a_1)$$

ELECTROPHORETIC CONVECTION OF SPECIES

The electric field in general is given by the solution of the Poisson's equation. If electroneutrality of chrged species in the gel region is assumed and if it is assumed that the diffusion coefficients are independent of concentration, the multiplication of Eqs. (1) by $z_i F$ of the charge per mole, and summing over all species [Newman, 1991] at steady state leads to

$$\nabla^2\left(F\sum_{i=1}^{N}D_{i,k}c_{i,k}z_{i,k}\right)=\nabla\left(F^2\sum_{i=1}^{N}c_{i,k}u_{i,k}z_{i,k}^2\nabla\Psi_k\right) \tag{8}$$

If a single species, denoted by subscript "d", of a polyelectrolyte with a small diffusion coefficient and all current-carrying species present in the solution have the same diffusion coefficient, (D_f), the left hand side of Eq. (8) becomes zero by electroneutrality because of $D_f \gg D_{d,k}$ and Eq. (8) reduces to

$$D_f\left[\sum_{i=1}^{N}c_{i,k}z_{i,k}+\frac{D^{d,k}}{D_f}c_{d,k}z_{d,k}\right]\cong D_f\sum_{i=1}^{N}c_{i,k}z_{i,k}\cong 0. \tag{9}$$

Thus, the integration of Eq. (8) leads to the Ohm's law [Newman, 1991] and an electrochemical potential term of Eq. (8) can be written as

$$\nabla\Psi=\frac{I}{\sum_{i=1}^{N}c_iu_iz_i^2F^2}=\frac{I}{\Omega}=\left(\frac{V}{x_o}\right) \tag{10}$$

where I is the current density, Ω is the electrical resistance defined by Newman [1991], V is the electrical field and x_o is the column length. The electrophoretic convection term in the fluid phase $u_f\ z_f F\nabla\Psi_f$ is described as $u_f\left(\frac{V}{x_o}\right)_f$. This term is equal to the electrophoretic mobility of species measured experimentally in the porous medium.

CHARACTERISTIC METHOD IN THE FIXED BED

The molar species balance for the particular species in a packed column is developed at very slow flow velocity in the presence of an electric field. Fig. 1 shows a schematic picture of packed column for this study. The axial dispersion is neglected as given by

$$\varepsilon\frac{\partial c_b}{\partial t}+\varepsilon(z_fFu_f\nabla\Psi_f)\frac{\partial c_b}{\partial z}+(1-\varepsilon)\frac{\partial c_2}{\partial t}=0 \tag{11}$$

If we input a square pulse of injection time t_o in the column of the empty state, the initial and boundary conditions for Eq. (11) are

$c_b(0,\ t)=c_o$ at $0<t<t_o$
$c_b(0,\ t)=0$ at $t>t_o$
$c_b(z,\ t=0)=0$ at $x>0$

Eq. (10) is coupled with the effective distribution coefficient of particle phase defined as

$$c_2=\beta_{eff}(\tau)\ c_b \tag{12}$$

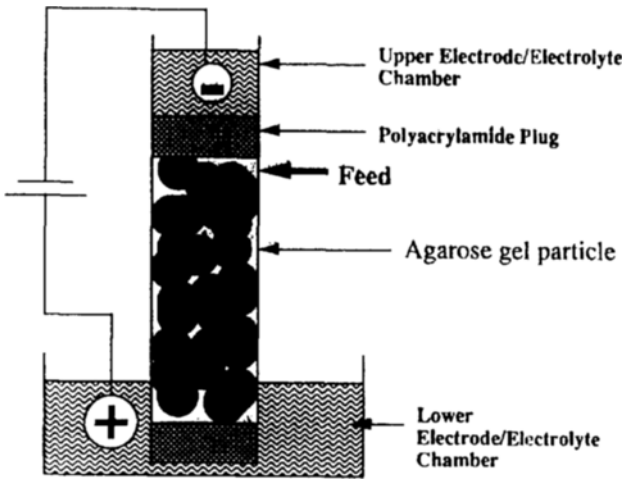

Fig. 1. A schematic picture of packed column in the presence of electric field.

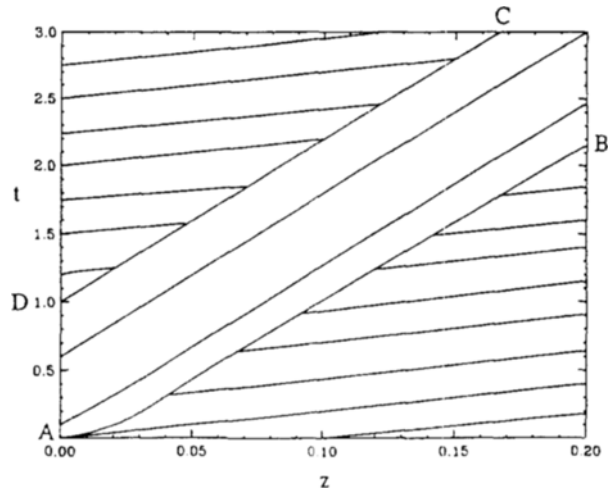

Fig. 2. Characteristic curve in a gel packed column at $Pe_f=6.9$, $Pe_g=1.2$, $\beta=0.158$, $\phi=0.32$.

Table 1. Dimensionless parameters

$a=\frac{x'}{x_o}$	$C'_i=\frac{c_i}{c_o}$
$\phi=\frac{u_g\left(\frac{V}{L}\right)_g}{u_f\left(\frac{V}{L}\right)_f}$	$\tau=\frac{t\ u_f\left(\frac{V}{L}\right)_f}{Pe_f x_o}$
$Pe_f=\frac{u_f\left(\frac{V}{L}\right)_f x_o}{D_f}$	$Pe_g=\frac{u_g\left(\frac{V}{L}\right)_g x_o}{D_g}$
$Z=\frac{z}{L}$	$\alpha=\frac{1-\varepsilon}{\varepsilon}$

The effective distribution coefficient $\beta_{eff}(\tau)$ can be defined as

$$\beta_{eff}(\tau)=\frac{\left[\int_0^{a_1} c_1dx+\beta\int_{a_1}^{a_2} c_2dx+\int_{a_2}^{1} c_3dx\right]-\left[\int_0^{a_1} c_1(Pe_f=0)dx+\int_{a_2}^{1} c_3(Pe_f=0)dx\right]}{(a_2-a_1)c_o} \tag{13}$$

The distribution coefficient β_{eff} represents the total concentration in a gel particle.

The total concentration in a gel particle described by Eq. (13) is coupled with Eq. (11) by the dimensionless form as

$$\frac{u_f\left(\frac{V}{L}\right)_f}{L}\frac{\partial c_b}{\partial Z}+\{1+\alpha\beta_{eff}(\tau)\}\frac{\partial c_b}{\partial t}=-\alpha\frac{\partial\beta_{eff}(\tau)}{\partial t}c_b \tag{14}$$

where dimensionless terms such as Z, α and τ are listed 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. (14) will be developed using the characteristic method [Rhee et al., 1986]. The solution will be used to determine the effects of the intraparticle convective transport on the separation of two different components.

The time, τ, in Eq. (13) 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. (14) represents the time after the sample on the column is injected. A gel particle does not exist any polyelectrolyte inside a gel particle until the polyelectrolyte arrives at that particle. Therefore, τ and t are related by

$$\tau=t-z/u_f(V/L)_f \tag{15}$$

In order to solve Eq. (14) it is first necessary to identify the slope of the characteristic curve which is given by

$$\frac{dt}{dZ}=\frac{\{1+\alpha\beta_{eff}(\tau)\}L}{u_f\left(\frac{V}{L}\right)_f} \tag{16}$$

The concentration along each characteristic curve is given by deriving dc_b/dt from Eq. (14) and the concentration profile in the column is given as

$$c_b=c_b(\tau_o)\left[\frac{1+\alpha\beta_{eff}(\tau_o)}{1+\alpha\beta_{eff}(\tau)}\right] \tag{17}$$

There is a characteristic curve formed from the start as the state of concentration c_o 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. (16) as

$$Z-Z_o=\frac{u_f\left(\frac{V}{L}\right)_f}{\alpha L}\int_{\tau_o}^{\tau}\frac{d\Theta}{\beta_{eff}(\Theta)} \tag{18}$$

Fig. 2 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 $\beta_{eff}(\tau)=0$, and concentration, c_b, has zero concentration. The characteristics are given by Eq. (18) with $Z_o=0$ and $\tau_o=t_o$. To construct a particular characteristic in this region it is necessary to choose τ_o. The position of elution curve in the column can be calculated when τ varies from τ_i to some desired upper limit from Eq. (18). t is calculated from Eq. (15). The concentration profile is obtained from c_o of AB as the characteristic curve of Fig. 2 emanates from the t-direction. The propagation speed of the polyelectrolyte along the packed column varies depending on the several regions of 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_o 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. (17).

PHYSICAL PARAMETERS FOR MODEL SIMULATION

Polyelectrolytes such as DNA are used for separation in the packed column. DNA is a flexible polymer exhibiting a variety of internal or intramolecular motions in the gel. The diffusion coefficient of DNA in the gel is rarely studied, because the twisting or torsional motion of DNA is very complicated in the presence of an electric field. The diffusion coefficient of DNA in the gel can be calculated, assuming constant persistence length. The mean displacement of the center of mass of DNA is written [Slater and Noolandi, 1986] as

$$\langle \dot{X}_m \rangle = \frac{\langle h^2 \rangle QE}{\Gamma^2 \xi} \tag{19}$$

where E, Q, and ξ are an electric field, the polyelectrolyte's total electric charge and the polyelectrolyte's translational frictional coefficient, respectively. Γ is the contour length. Q/ξ is independent of the chain length. Its value is found to be 5.08×10^{-3} cm^2/V sec [Holzwarth et al., 1989]. $\langle h^2 \rangle$ is the mean square end-to-end distance. $\langle \dot{X}_m \rangle/E$ is defined as the electrophoretic mobility of DNA in the gel [Viovy, 1987]. If the electrophoretic mobility of DNA is measured experimentally, the mean square end-to-end distance $\langle h^2 \rangle$ can be calculated. DNA fragments used for this study are 367 bp DNA and 1,010 bp DNA; bp denotes base pairs of DNA. The contour length for 367 bp DNA is 124.8 nm and that for 1,010 bp DNA is 343.4 nm [Pecora, 1991]. The electric charge is 5.7 electron charges per base pair. Their data are used to calculate the diffusion coefficient (D) in the gel particle as $D=KT/\xi$ [Viovy, 1987], K is the Boltzmann constant.

ϕ is a relative ratio of mobilities in the solid and fluid phases, and their data are measured experimentally. The electrophoretic mobilities of DNA in 2% agarose gel are measured by Park. λ Phage ΦX HAE III DNA is purchased from Bethesda Research Laboratories (BRL), Gaithersberg, MD. The range of molecular lengths is from 310 bp to 23,600 bp. Tris Boric buffer (50 mM tris base, 50 mM boric acid and 1 mM EDTA: pH 8.0) is used at pH=7.2 for this study. The free solution mobility of DNA is 3.0×10^{-4} cm^2/sec from Hervet and Bean [1987]. An important point is that the electrophoretic mobility in the free solution is independent of the molecular size. The diffusion coefficients of DNA in a free solution are obtained from Pecora [1991].

The moment equation is used for the evaluation of gel porosity. The surface concentration of the gel particle is assumed to be constant, and the thickness of boundary layer is assumed to be small enough to be negligible. The first moment (μ_1) defined by Suzuki and Fujii is used to estimate the gel porosity. It is obtained as

$$\mu_1 = \frac{1+\alpha\beta}{u_f\left(\frac{V}{L}\right)_f} \tag{19}$$

The bed porosity ε is obtained by measuring V_o and V_T. Total bed volume V_T, using urea, and V_o, using ferritin, are obtained experimentally (9). The bed porosity ε is equal to V_o/V_T. The simulation data for DNA are tabulated in Table 2.

Table 2. Physical properties of DNA in a gel packed column

Item \ DNA(Kbp)	0.367	1.01
Diffusion coefficient in free solution (cm^2/sec)	15.8×10^{-8}	7.15×10^{-8}
Diffusion coefficient in gel (cm^2/sec)	4.0×10^{-9}	1.67×10^{-9}
Porosity in gel (β)	0.250	0.158
Porosity in column (ε)	0.525	0.525

HEAT EFFECTS

Temperature influences the transport of DNA in the gel. The variation of the DNA mobility with the temperature is based on an Arrhenius equation. The difference of the DNA mobilities by temperature is due to the difference of the activation energy for the fluid viscosity. This is related to the hydrodynamic drag of the solvent. The temperature rise makes the interactions between the gel and the DNA weak due to low viscosity. Therefore, it is necessary to estimate how the temperature increases in the packed column under the electric field. The heat generation by an electrical current is given by $q=VI$, where I is the current in amperes and q is the rate of heat generation in watt or J/sec. The temperature rise in a column is estimated [Bird, 1960] by

$$\langle T \rangle - T_b = \frac{IVR_c}{2sLh}\left[1-\exp\left\{-\frac{zh}{R_c\rho u_f(V/L)_f C_p}\right\}\right] \tag{20}$$

where R_c is the column radius, T_b is the bulk fluid temperature, h is the overall heat transfer coefficient, C_p is the heat capacity of the material in the column, and ρ is the density of the column. The column length used in this experiment is 15 cm, and R_c is 1.1 cm, ρ is 1 g/cm^3, C_p is 1 cal/g-℃ [Weast, 1973]. The overall heat transfer resistance is the sum of the internal and external resistances and the conduction through the column. This is given [McCabe and Smith, 1976] by

$$\frac{1}{h} = \frac{R_{out}}{R_{in}h_{in}} + \frac{(R_{out}-R_{in})}{k_m}\frac{R_{out}}{R_{in}} + \frac{1}{h_{out}} \tag{21}$$

where h_{in} is the internal heat transfer coefficient, h_{out} is the external heat transfer coefficient, k_m is the thermal conductivity of the column, R_{out} is the external column diameter, R_{in} is the internal column diameter, and R_{in} is the logarithmic mean diameter. The thermal conductivity is 0.65-0.85 J/sec-m^2-℃, and the column thickness used is less than 1 mm. If the overall heat transfer is governed by conduction through the column wall, h calculated from Eq. (18) is 830 J/sec-m^2-℃.

RESULTS AND DISCUSSION

1. Transient Analysis 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 the

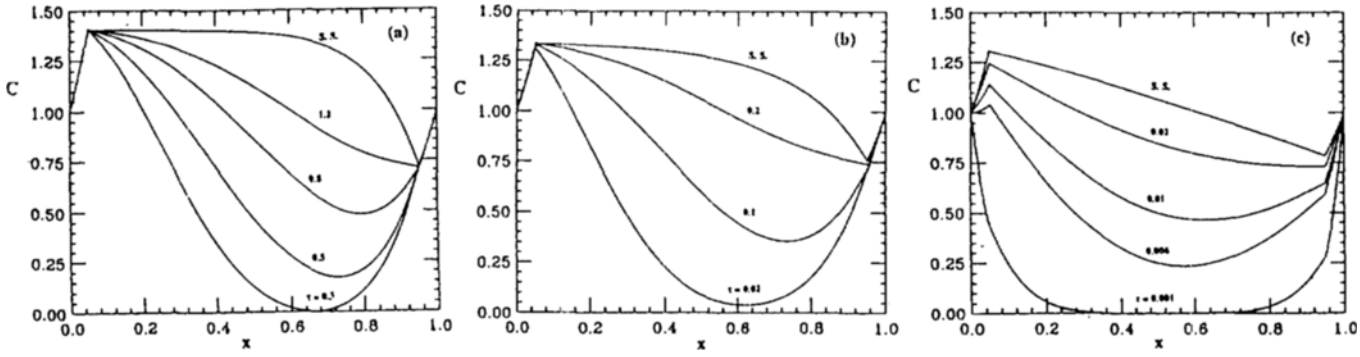

Fig. 3. Transient concentration profiles of DNA in a gel particle.
(a) 0.375 kbp DNA at E=4 V/cm, Pe_f=4.2, β=0.25, φ=0.67, (b) 0.375 kbp DNA at E=10 V/cm, Pe_f=4.2, β=0.25, φ=0.67, (c) 1.010 kbp DNA at E=10 V/cm, Pe_f=1.9, β=0.110, φ=0.43

gel particle can be calculated from the model Eqs. (2). The dimensionless Peclet number in the model Eq. (2) 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; $Pe_1=Pe_3$) of bulk fluid and solid phase (Pe_g; Pe_2) of the gel particle. Transient concentration profiles in the gel particle and in the boundary layer surrounding the gel particle are associated with the electrophoretic convective fluid velocity in the column.

In the case of small DNA such as 0.367 kbp DNA, the ratio of the radius of gyration (29.1 nm), [Pecora, 1991] to pore diameter (71 nm), [Stellwagen, 1985] of a 2% agarose gel particle is relatively small. If the electric field is small enough, the reptation of DNA induces a negligible change in the radius of gyration of DNA, i.e., the DNA molecule retains a globular shape. In this case, the average distance ($\langle h_x^2 \rangle$) between the ends of the DNA in the field direction has a constant value of $Na^2/3$ [Slater and Noolandi, 1986]. The number (N) of pores necessary to house the DNA segment is approximately 1 [Slater and Noolandi, 1989], since the DNA fits into one pore. The ratio of the electrophoretic mobility (o) of DNA in a gel particle to that in free solution theoretically has a constant value smaller than 1/3 from the mobility equation [$\mu/\mu_o = \langle h_x^2 \rangle/(Na)^2$] of Slater and Noolandi [1989]. Since the diffusion coefficient in the gel particle is much smaller than that in the bulk fluid phase, the ratio of Pe_g/Pe_f can be greater than 1. In the case that Pe_g is greater than Pe_f, the dynamic speed of DNA to approach a steady state is very slow in low electric field, as seen in the transient concentration profiles of Fig. 3(a). The transient rate to approach the steady state in the gel particle is significantly reduced as DNA penetrates into the gel particle farther than $x=a_1$, because the rate of transport in the gel particle is much smaller than that in free solution.

But the transport rate of DNA through the gel particle can be increased in a high electric field. The transport of polyelectrolyte in a gel under the influence of an electric field has been theoretically predicted by Slater and Noolandi [1989] and Lumpkin et al. [1985]. All these theories indicate that the transport of DNA in the gel 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 DNA when a constant electric field is applied in ordered arrays of pores. The DNA conformation at a higher electric field becomes extended and aligned, because the field biases the direction of the leading segment of the DNA molecule. The stretching time (t_{str}) of a DNA chain after an electric field is dependent on the length (N) of the DNA, $t_{str} \approx N/E^2$ [Viovy, 1987]. 0.367 kbp DNA can transport through the gel with less serious change in DNA conformation. This induces the fast dynamic speed of 0.367 kbp DNA in the gel particle. Larger DNA, like 1.01 kbp DNA, has the radius of gyration of 62nm, it reptates in a biased direction in the gel.

Since the electric forces deform the DNA fragments so that segments of the DNA chain occupy consecutive gel network pores in a random walk, the mobility of a 1.01 kbp DNA chain becomes slower by a reciprocal-length relation. In a high electric field of 10V/cm and 2% agarose gel particle, the electrophoretic convection terms [$u_f(V/L)_f$] obtained experimentally are 1.3×10^{-4}, 2.0×10^{-4} cm^2/V-sec for 1.01 kbp DNA and 0.367 kbp DNA, respectively. It is shown in Fig. 3(b) that the dynamic speed of 0.367 kbp DNA in the gel particle becomes faster as it approaches the steady state in comparison with Fig. 3(a). This is because the electrophoretic convective-diffusive flux of the DNA that is transported to the pores of the gel particle becomes faster. Therefore, fast dynamic speed of DNA in the gel particle is related to the magnitude of Pe_g induced by the electric field. The dynamic speed of 1.01 kbp DNA in the gel particle is much slower than 0.367 kbp DNA at 10 V/cm, as seen in Fig. 3(c). This result can be imagined from the relationship that the radius of gyration of 1.01 kbp DNA (62 nm) is bigger than that of 0.367 kbp DNA (29.1 nm) [Pecora, 1991]. The increase in transient concentration profiles in the gel particle arises due to the size of the DNA and the electric field strength. This relationship can be used to separate different DNA in the packed column.

Although high electric field plays significant role in enhancing the transport of DNA, the electrophoretic mobility is sensitive to the temperature rise, especially for the high molecular weight of DNA. When the temperature rise is calculated from Eq. (20) for the current and voltage used, the temperature could be generally expected to increase 0.3℃ to 5℃ in the ranges of the electric field 4-40 V/cm. The viscosity of bulk fluid in the gel medium is expected to decrease with increasing temperature. The DNA transport in the gel particle, using only relatively high molecular DNA, is increased more than 10% [Hervet and Bean, 1987] by less hydrodynamic drag due to low viscous bulk solution. Therefore, the temperature rise can be alleviated by using a smaller

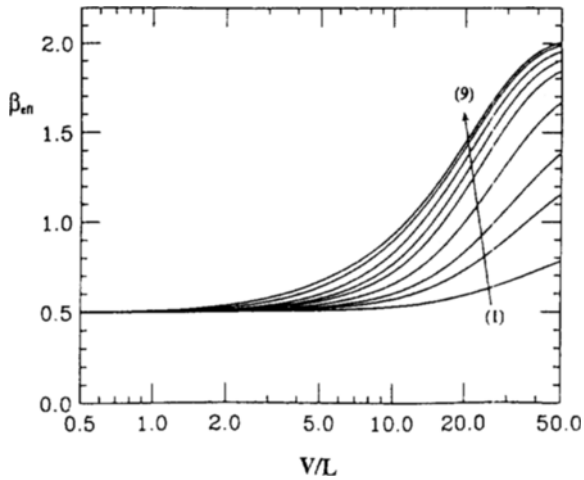

Fig. 4. Convective-diffusive effects of the effective distribution coefficients in a gel particle as a function of electric field at $\beta=0.5$, $\phi=0.5$.
Arrow direction indicates the increase of number ($r=Pe_f/Pe_g$). (1) r=0.01; (2) r=0.03; (3) r=0.05; (4) r=0.1; (5) r=0.2; (6) r=0.3; (7) r=0.5; (8) r=1.0; (9) r=2.0

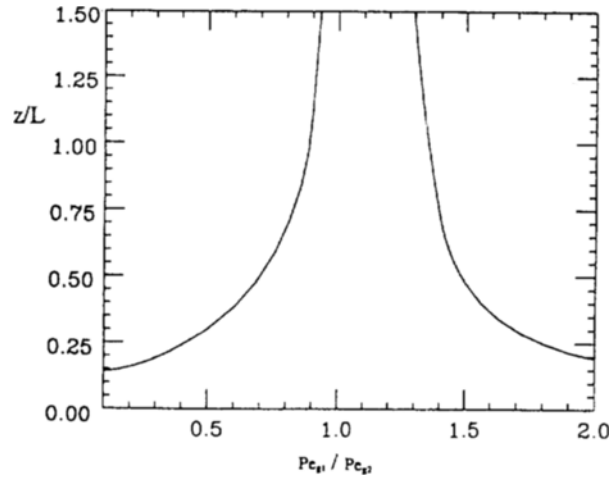

Fig. 5. Theoretical result for the separation of two different DNA at 10 V/cm when $Pe_{f1}=Pe_{f2}=2.2$
L is the column length and z is a separation point in the column. Pe_g and Pe_f are the dimensionless Peclet number defined in Table 1.

diameter of column and better cooling of the buffer solution. The use of a cool buffer solution would necessitate maintaining the non-variation of DNA mobility due to the temperature rise in the packed column.

2. DNA Transport in Internal Pore

If the pore diameter (a) of a gel particle is increased, the pore size can be much greater than the radius of gyration of the DNA. For large pores, the pore size ($a\approx128$ nm of 1% agarose gel particle) is much bigger than the persistence length ($p\approx50$ nm) of DNA, since DNA can bend within the pores. The DNA chain retains its globular shape during migration without serious change to conformation in internal pores. The DNA chain does not keep its overall globular shape in the presence of electric field. The DNA does not bend within the pores. DNA can deform in order to squeeze through a pore [Stellwagen, 1985], and the electrophoretic mobility of DNA decreases with the molecular size. If the pore diameter is small enough, a DNA chain can thread itself through consecutive small pores. If the length of this thread is long, it can happen that the two ends of a chain both advance in the same direction, while the middle is hung up on polymeric pores. This phenomenon has been proven experimentally by the entrapment of DNA in a high gel concentration [Holzwarth, 1989]. The DNA chains do not migrate in response to the applied electric field, but only diffuses by slow Brownian motion. This is called the self-trapping effect, and it can be effected using lots of small pores. The above results affect the electrophoretic intraparticle transport inside gel particle. The DNA concentration profiles in the pores can be considered as a function of the electrical field and pore size.

When the electrical field is applied, DNA migrates faster from the bulk fluid into and through the gel particle due to an increase in electrophoretic convective velocity. Fig. 4 shows the dependence of β_{eff} on the voltage gradient for the parameters that correspond to DNA accumulation. In the present analysis, equilibrium between the gel particle and bulk fluid has been assumed as the effective distribution coefficient, β_{eff}. β_{eff} 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 particles. The dependence of β_{eff} on the electrical voltage is theoretically analyzed by the parameters in the gel particle as a function of Pe_f/Pe_g in Fig. 4.

It is interesting to note that the effective distribution coefficient goes to the proper limiting value of β at a steady state when the electrical field is not applied. This is equal to the volume of pore space in the gel particle that is accessible to the DNA at zero electrical field. In other words, β is a measure of the capacity of the gel particle for the DNA. But in very high electric fields, the DNA would be expected to be completely oriented along the field direction, so that further increases in electric field strength would have no effect on the mobility. A theoretical calculation of the dependence of the relative mobility on electric field strength has been shown by Lumpkin et al. [1985]. The value of β_{eff} in a high electric field increases until it reaches the limiting value of $1/\phi$. The rate of approach to the limiting value of $1/\phi$ is strongly dependent on the electric field strength . The build-up of DNA in the gel particle is strongly dependent on the value of Pe_f/Pe_g. This indicates that the total concentration in the gel particle becomes steeper with the applied electric field. High Peclet numbers in the fluid phase become important in enhancing the concentrations in the gel particle. The relative ratio of convection and diffusion in both fluid and solid phases plays an important role in determining the transport of DNA in the gel particle. The separation of two different DNA can be theoretically achieved using the electrophoretic mass transport parameter, Pe_f/Pe_g.

3. DNA Separation

The elution curve of DNA in a column is closely related to the dynamic speed of DNA in a gel particle. The faster convective velocity leads to a rapid transport into a porous packing sorbent. The intraparticle transport by convection may be dependent on a different DNA-to-pore size. 0.367 kbp DNA can penetrate the agarose gel particles much faster extent than 1.010 kbp DNA. Pe_f/Pe_g can be used to calculate the separation criteria of the mixture of two components from band migrations using the ratio of the Peclet numbers in the two phases. The ratio (ρ) of Pe_f/Pe_g

is related to the retention time of DNA in the gel particle. Fig. 5 shows how one component can separate from the other component throughout the column. Note that when the ratio ($R=\rho_1/\rho_2$) of the two components is equal to 1.0, they are inseparable, because the transport properties of the components, such as convection and diffusion, become identical to each other. But if the transport properties of the two different components are different, their separation can be predicted as shown in Fig. 5. The ρ values for 0.367 kbp DNA and 1.010 kbp DNA at 10 V/cm are 0.037 and 0.054, respectively. The separation of two different DNA is predicted by the ratio $R=(Pe_g)_2/(Pe_g)_1$, in which the properties in the gel particle have $(Pe_f)_2/(Pe_f)_1=2.2$. The subscript "1" represents DNA which is eluted 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 7.75 cm for the mixtures of 0.367 kbp DNA and 1.010 kbp DNA at 15 cm bed length in the electric field of 4 V/cm. Conclusively, the separation point of two different DNA in a packed column can be predicted by the diffusior 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 bed 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 coefficients, 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 DNA inside a gel particle, the separation of two different DNA is predicted by this ratio. The temperature dependence by the electric field leads to the result that DNA transport in the gel is affected by the hydrodynamic drag in the pores. The findings in this paper recommend as a useful guide to the analysis and the design of devices in the laboratory and other scales required for a variety of bioseparations.

NOMENCLATURE

a_i : dimensionless positions in the gel particle
c_i : concentrations of polyelectrolyte in different phases
c_b : bulk concentrations of polyelectrolyte
C_p : heat capacity of the material in the column
D_f : ionic species diffusion coefficient
D_k : diffusion coefficient in the different phases
E : electric field
F : faraday constant
h : heat transfer coefficient
$\langle h^2 \rangle$: mean square end-to-end distance
I : current in ampere
k_m : thermal conductivity of the column
k : each phase in the gel particle
L : column length of composite media
N : pore number
Pe_g : Peclet number in gel particle
Pe_f : Peclet number in stagnant buffer solution
q : rate of heat generation
Q : total electric charge
R_c : column radius
t : time
t_{str} : stretching time of DNA
T_b : bulk fluid temperature
u : electrophoretic migration in the bulk fluid
u_k : electrophoretic migration
u_k : eigenfunction of Eq. (5)
V : electric voltage
x : dimensional spacial coordinate
x_t : total length of particle diameter and stagnant layers
$\langle \dot{X}_{cm} \rangle$: mean displacement of the center of mass
z : column coordinate
z_h : valence of solute
Z : dimensionless column length

Greek Letters

Φ : ratio of convective mobility in the gel to that in the buffer
β : porosity of the gel particle
β_{eff} : effective distribution coefficient of solute
ε : bed porosity
ρ : density of the column
λ : eigenvalue of Eq. (6)
τ : dimensionless time in the gel particle
μ_n : moment equation
Ψ : electrostatic potential
Γ : contour length
ξ_2, ζ_2 : functions defined at Eq. (6)

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