Biotechnol. Bioprocess Eng. **2000**, 5: 332-339

Separation of ΦX HAE III DNA with Electrochromatography

Young G. Park*

Department of Chemical Engineering, Daejin University, 11-1 Sundan-Ri, Pochun-Kuen, Kyungki-Do 487-711, Korea

Abstract Experimental and theoretical works were performed for the separation of large polyelectrolytes such as DNA in the column packed with gel particles under an electric field. This paper shows how intraparticle convection effects the separation of DNAs in the column because DNAs quickly oriented through the pores in the field direction. Dimensionless transient mass balance equations were derived considering diffusion and electrophoretic convection. The separation criteria is theoretically studied using two different Peclet numbers in the fluid and solid phases and these criteria were verified uing two different DNAs by electrophoretic mobilities measured experimentally, showing how the separation position of DNAs varies in the column according to values of Pe_f/Pe_s of individual DNA. Governing equations are simultaneously solved by operator theoretic and characteristic methods to yield the column response.

Keywords: packed column, convective electrophoretic velocity, DNA, peclet number

INTRODUCTION

The separation tools using polymeric materials such as gel electrophoresis and gel chromatography have important applications in a wide range of separation and purification of biomolecules. Commonly gel electrophoresis provides the highest resolution in the purification of polyelectrolytes such as protein and nucleic acid. However, it has extremely poor scaling properties so that it has been impossible to adapt this milligram bench technique to multi-gram preparative separations. On the other hand, gel chromatography has far superior scaling properties and a resolving power that is second only to gel electrophoresis. However, electrochromatography by combining of electrophoresis with chromatography would show better separation, perhaps because field-induced dispersion is virtually eliminated by proper manipulation of an electric field and amplifies the resolving power of each while retaining the superior scaling properties of chromatography. Therefore, what needs to be known about the separation process of electrochromatography is the underlying physics of the process or how it can be effectively scaled-up.

An important feature utilizing an electric field in the column is the presence of intraparticle convection because polyelectrolytes move headfirst through the pores of the gel. The theoretical model of electrochromatography has been rarely investigated under the consideration of intraparticle transport of both the diffusion and the convection by decoupling of the particle in the column. Previous researches of convective-diffusive mass transport were studied in the area of reaction engineering [1,2] and separation engineering [3-7]. The separation of proteins with electrochromatography was performed by several researchers [8-9], but has been rarely tried with DNA.

The conventional separation method in the case of large polyelectrolytes such as DNA causes the peak of the elution curve to be broadened in the column, and the peak-broadening in the column may make separating two different DNAs difficult to separate, maybe due to the fact that the radius of gyration of DNA is relatively large compared to the pore size of a sieving particle. Therefore, two different DNAs will be separated to prevent the peak-broadening in the presence of an electric field because they stretch and move readily through the sieving particle packed in the column, implying that the electric field may enhance the permeation of DNA due to the electrophoretic convection inside the pore. The examples using an electric field in the separation process were frequently employed for the more delicate separations in the chromatography column [10] and the extractor [11].

Therefore, it is necessary to prove that the beneficial effect of the intraparticle convective velocity is expressed by the occurrence of response curve in the column. The objective of this paper was to predict the separation criteria of two different DNAs in the column using the ratio ($\chi = Pe_f/Pe_g$) of dimensionless Peclet numbers which contained two different convective velocities in the fluid and in the solid phases of individual DNA, and to examine experimentally. Theoretical methods to solve the electrophoretic mass transport problem are performed by the operator theoretic method [12,13] and the characteristic method [14]. Therefore, basic understandings of the physical dynamics by solute

*Corresponding author
Tel: +82-31-539-1994 Fax: +82-31-536-6676
e-mail: ypark@road.daejin.ac.kr

surrounding the gel particle will be a key for the separation of DNA in the packed column.

THEORY

The theoretical model of the system as shown in Fig. 1(a) was developed with the following assumptions: The electric field is assumed to be uniformly applied to polymeric gel media as well as for the convective electrophoretic velocities in bulk fluid and intraparticle to be constant. The axial dispersion was assumed to be negligent because of the solute transport at very slow flow velocity in the column under an electric field. The electric field was assumed to be applied to the x-direction in the electrophoretic chamber and a single particle considered for this study has a uniform stagnant boundary layer of the fluid phase as shown in Fig. 1(b). The continuity equations of species i for the gel particle and packed column with boundary conditions are given as follows

$$\frac{\partial c_{i,k}}{\partial t} = D_{i,k}\frac{\partial^2 c_{i,k}}{\partial x'^2} - \left(z_i F u_{i,k}\frac{\partial \Psi_{i,k}}{\partial x'}\right)\frac{\partial c_{i,k}}{\partial x'} \qquad k = 1, 2, 3 \tag{1}$$

$$\varepsilon\frac{\partial c_{i,b}}{\partial t} + \varepsilon\left(z_i F v_{i,t}\frac{\partial \Psi_{i,t}}{\partial x'}\right)\frac{\partial c_{i,b}}{\partial x} + (1-\varepsilon)\frac{\partial \langle c_{i,b}\rangle}{\partial t} = 0 \tag{2}$$

Total flux boundary conditions and initial condition of gel particle are

$$-D_{i,k+1}\frac{\partial c_{i,k+1}}{\partial x'}\left(z_i F u_{i,k+1}\frac{\partial \Psi_{i,t}}{\partial x'}\right)c_{i,k+1} = -D_{i,k}\frac{\partial c_{i,k}}{\partial x'} + \left(z_i F u_{i,k}\frac{\partial \Psi_{i,t}}{\partial x'}\right)c_{i,k} \tag{3}$$

$$\beta_{i,k+1}c_{i,k+1} = \beta_{i,k}c_{i,k} \quad \text{at} \quad x' = x'_k,\ k = 1, 2 \tag{4}$$

$$c_{i,k}\,(t=0) = 0, \qquad k = 1, 2, 3 \tag{5}$$

with boundary and initial conditions in the electrochromatography

$$c_{i,b}\,(0,t) = c_o \quad \text{at} \quad 0 < t < t_o$$
$$c_{i,b}\,(0,t) = 0 \quad \text{at} \quad t > t_o$$
$$c_{i,b}\,(x,t=0) = 0 \quad \text{at} \quad x$$

Eq. (1) is coupled with the effective distribution coefficient of particle phase defined as

$$< c_{i,2} > = B(\tau)\, c_o \tag{6}$$

$$B(\tau) = \frac{\int_0^{x'_1} c_{i,1}dx' + \beta\int_{x'_1}^{x'_2} c_{i,2}dx' + \int_{x'_2}^{x'_0} c_{i,1}dx'}{(x'_2 - x'_1)c_o} - \frac{\left(\int_0^{x'_1} c_{i,1}(Pe_t = 0)dx' + \int_{x'_2}^{x'_0} c_{i,3}(Pe_t = 0)dx'\right)}{(x'_2 - x'_1)c_o} \tag{7}$$

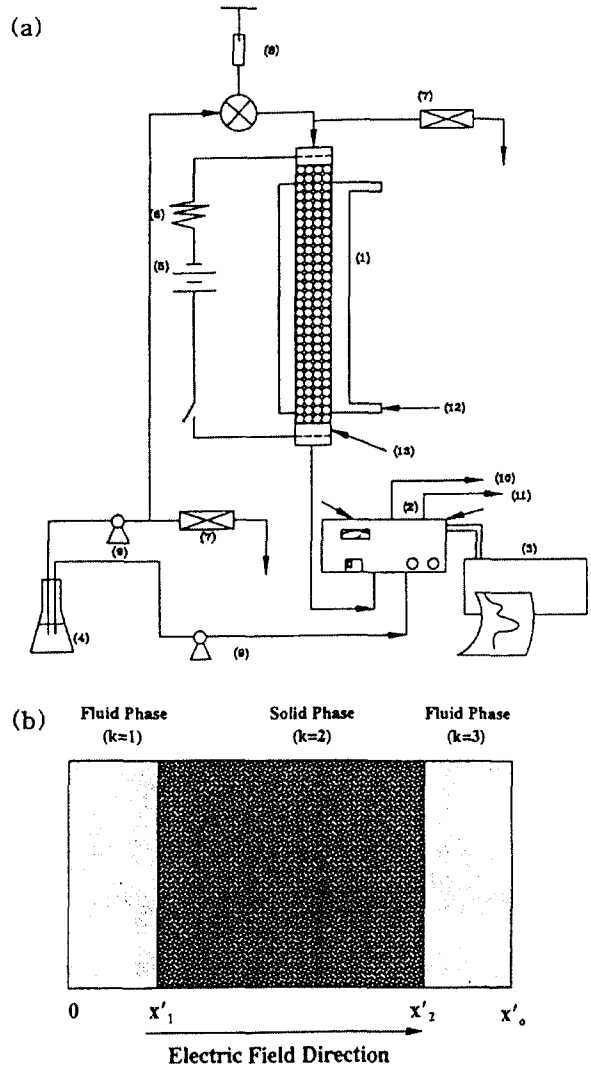

Fig. 1. (a) A schematic picture of a packed column in the presence of an electric field (Bio-Gel paricles are packed with size of less than 40 μm and the power conditions during the run can be altered with a range of up to 1,000 volts or 500 mA. Power supply has the ability to monitor current to the microampere level). (1) Main body of electrochromatography, (2) Controller, (3) Recorder, (4) Buffer solution, (5) Switch, (6) Electric power, (7) Vent valve, (8) Inlet of sample, (9) Pump, (10)-(11) Electric line, (12) Water jacket, (13) Column; (b) Schematic diagram for the gel particle in the rectangular coordinate.

The summation of integration of Eq. (7) is to get the total amount of solute held up by the action of the electric field. $B(\tau)$ denotes the total average concentration of the gel particle in the presence of an electric field. Its expression with respect to time is calculated from the solution of Eq. (1) and is introduced into Eq. (2) to solve the dispersion curve. It is given by the sum of the total solute associated with the solid phase (including that in the boundary layer) minus the amount that would be

in the boundary layer if no electrical field is applied, divided by the amount $(x_2'-x_1')c_o$, *i.e.* the amount in the particle when no electrical field is applied. The reason of the substraction in the denominator of Eq. (7) is to make $B(\tau)$ reduce to the proper limit of constant value when an electric field goes to zero. In this case of no electric field the effective distribution coefficient $B(\tau)$ comes to be β and it is a function of the applied electric field gradient V/L.

Fig. 1(b) shows that subscript k of Eq. (1) denotes each phase of the fluid-solid phase, for example, an upper boundary layer of rectangular coordinate is in the case of k=1, a lower boundary layer for k= 3 and a gel layer for k=2. $z_i F u_{i,k} \frac{\partial \Psi_{i,k}}{\partial x'}$ of Eq. (1) indicates the convective electrophoretic velocity, it redescribed as the electrophoretic mobility multiplied by the electric field per unit length as shown in Appendix I. And the electrophoretic mobility will be measured experimentally in the gel slab electrophoresis. In above Eqs. (1) and (2), $c_{i,k}$, $u_{i,k}$, $v_{l,f}$, $D_{i,k}$, F and $\psi_{i,k}$ are referred to "Nomenclature" and $<c_{i,2}>$ is the average concentration in the fluid inside pores. The solution of equations (1)-(7) was described in Appendix II.

MATERIALS AND METHODS

Apparatus of Electrophoresis Chamber

The gel particle was prepared with electrophoretic grade agarose from Bio-Gel-A-50m (BioRad) which fractionated in range of 200,000-1,000,000. The gel particles which has the diameter of 40 μm were packed in the column as shown in Fig. 1. After agarose gel particles were loaded in the column, the column was inserted into the vertical BioRad electrophoresis chamber. The electrophoretic mobility of DNA can be easily observed because it moved having a sharp band shape along the column under the fluorescence light. The packed column used in this experiment was a cylindrical glass tube of 2.0 cm in diameter and 30 cm in length designed specially by Duke Scientific Corp. The buffer solution in the electrophoresis chamber had at pH 7.2 and the absorbance in the column was measured with a spectrophotometer at 340 nm. The temperature of the circulator in the electrophoresis chamber was kept at 18°C.

Measurement of Physical Properties

The extraparticle void volume (V_o) was determined using T4 DNA, a large molecule which was totally excluded from the gel particles. V_T (total column volume) was measured in the absence of an electric field by injecting 100 μL of the sample containing the selected urea at a concentration in the range of 1 mg/mL and measuring the volume of the eluent required for the peak to emerge from the column. The extraparticle void fraction (β) was calculated from the relation of V_o/V_T. T4 DNA was purchased from Sigma Chemical Co. The mobility of DNA in free solution was 4.0×10^{-4} cm^2/Vsec from Olivera *et al.* [15]. The gel porosity (β) was estimated using the first moment equation of Rodrigues *et al.* [16] using the curve response measured by gel scanning spectrophotometer (Gilford Co.).

The diffusion coefficients of DNA in the free solution are obtained from Pecora [17] and those in a gel particle were estimated by assuming that the DNA would be spherical. If the pore size diameter "a" and the radius of gyration "R_g" are known, the diffusion coefficient of DNA can be described by the ratio of $\theta = R_g/a$. The diffusion coefficient at the value of θ can be estimated [18] by

$$\frac{D_k}{D_o} = \frac{6\pi(1-\theta)^2}{\frac{9}{4}\pi^2\sqrt{2}(1-\theta)^{-2.5}\left[1-1.2167(1-\theta)+1.5336(1-\theta)^2\right]+ \frac{6\pi(1-\theta)^2}{\left(-22.5083-5.6117\theta-0.3363\theta^2-1.216\theta^3+1.647\theta^4\right)}} \quad (8)$$

From above results, the experimental data are listed in Table 1.

ϕ is a relative ratio of mobilities in the gel phase and fluid phase, the mobility in free solution was referred from Olivera *et al.* [15] and the mobility in the gel particle was measured experimentally. The boundary layer thickness (δ) can be calculated when the sphere was immersed in a stagnant fluid and was obtained from the relationship, $\delta = D_k/k_f$, in which the mass-transfer coefficient was calculated when the mass-transfer Sherwood number was equal to 2.0 [19] at low velocity.

Measurement of Electrophoretic Mobility

The gelation process for 4% agarose gel elctrophoresis took place within 15 min after casting at 60°C, and the electrophoretic mobilities of DNA fragments were measured. Agarose was chosen because of its good mechanical properties at low concentration. Large polyelectrolytes used for this experiment were DNAs such as λ phage DNA and ΦX HAE III DNA fragments which were purchased from BRL (Bethesda Research Laboratories). The range of molecular length was from 23,600 to 310 bp (base pair). The loading solution contained 20% DNA by weight, 10% dye solution (10% bromophenol blue +50% glycerol and 40% EDTA by weight). Tris buffer was used to prepare the loading DNA solution and to fill the electrophoretic tray. The pH of Tris buffer was 6.8. The electrophoretic apparatus used was a Bio-Rad Model 2303, and the gel length loaded inside the apparatus was 20 cm. Two silver electrodes were inserted from the top, 8 cm apart, into the electrophoretic tray. The voltage between these electrodes was measured with a digital voltmeter, and 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 as shown in Fig. 1.

Table 1. Physical dimension for the size of DNA and porous gel particle

Item	0.367 kbp DNA	1.010 kbp DNA
Radius of gyration R_g (nm)	36.5	70.7
Persistence length p (nm)[17]	51.0	51.0
Contour length ℓ (nm)	125.0	343.0
Pore size diameter a (nm)	40.0	40.0
ℓ /p	2.5	7.0

Each experimental run was 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 was determined with at least three measurements of the position of the bands at different time intervals. The electrophorteic mobilities of DNA fragments were measured as a function of the electric field and molecular size.

Radius of Gyration of DNA

When the contour length "ℓ" of the fragment was smaller than its persistence length "p", which is itself smaller than the average pore size <a> for most agarose gel particle, the fragment can be seen as a rod of negligible width and length that can enter through all pores. When "ℓ" was larger than the persistence length "p", the fragment folded on itself and took the approximate shape of a globule with radius of gyration, R_g, given by [20]

$$R_g^2 = \frac{1}{3}P\ell\left\{1-\frac{p}{\ell}+\frac{P}{\ell}\exp\left(-\frac{\ell}{p}\right)\right\} \quad (9)$$

This fragment was excluded from the pores of radius because of a << R_g, where "a" is the pore size (diameter) of gel particle.

RESULTS AND DISCUSSION

Total Concentration in the Gel Particle

The pore size played an important role when DNA migrated electrophoretically with appreciable mobility through the gel and its size of 4% agarose gel particle was about 89 nm [21]. The contour length of DNA was generally much longer than the diameter of the average pore in the gel matrix. Table 3 shows their comparisons between the dimension of the gel matrix and DNA. The contour lengths in Table 2 are estimated, assuming the value of 0.34 nm for the repeat per base pair.

Experimental results of Fig. 2 show that the mobility begins to be field-dependent, DNA are clearly discriminated according to length at values of the applied field.

Table 2. Physical properties of DNA measured experimentally at 8 V/cm

Item	0.367 kbp DNA	1.010 kbp DNA
Diffusion coefficient in gel particle (cm^2/s)	1.37×10^{-8}	1.37×10^{-8}
Diffusion coefficient in free solution (cm^2/s)	15.8×10^{-6}	7.15×10^{-8}
Porosity (β)	0.210	0.125
Mobility in free solution in pH 7.2 (cm^2/V-s)	4.0×10^{-4}	4.0×10^{-4}
Mobility in gel particle in pH 7.2 (cm^2/V-s)	1.9×10^{-4}	1.2×10^{-4}

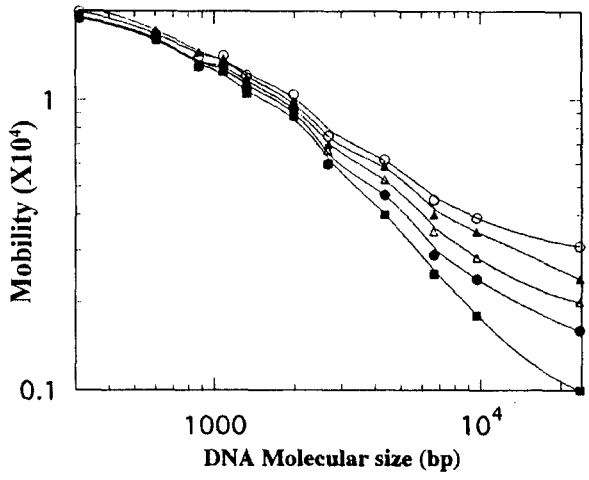

Fig. 2. Logarithm of the molecular length vs the electrophoretic mobility at various electric field strength for 4% agarose concentration (■: 0.5 V/cm; ●: 1.5 V/cm; △: 3 V/cm; ▲: 5 V/cm; O: 8 V/cm).

In model Eqs. (1)-(2), 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 through the gel particles. Total concentrations in the gel particle and in the boundary layer around gel particle can be calculated from Eq. (11) by two different Peclet numbers in the fluid phase (Pe_f; $Pe_1 = Pe_3$) of bulk fluid and the solid phase (Pe_g; Pe_2) of a gel particle.

If the electric field was small enough, DNA conformation in the gel particle was not affected by the electric field as shown in Fig. 2. Larger DNA (N>1,000 bp) slowly penetrated through the gel particle, the electrophoretic mobility of DNA fragments increased with the electric field. At a high electric field of 8 V/cm and 4% agarose gel, the electrophoretic mobilities were experimentally 1.2×10^{-4} and 1.9×10^{-4} cm^2/V-sec for 1.01 kbp and 0.367 kbp, respectively. Since the diffusion coefficient in the gel particle was much smaller than that in the bulk phase, the ratio of Pe_f/Pe_g had a value of smaller than 1.0. Experimentally, the ratio (ϕ) of electrophoretic mobility was 0.35 in the case of 1.01 kbp DNA at 1 V/cm in Fig. 2.

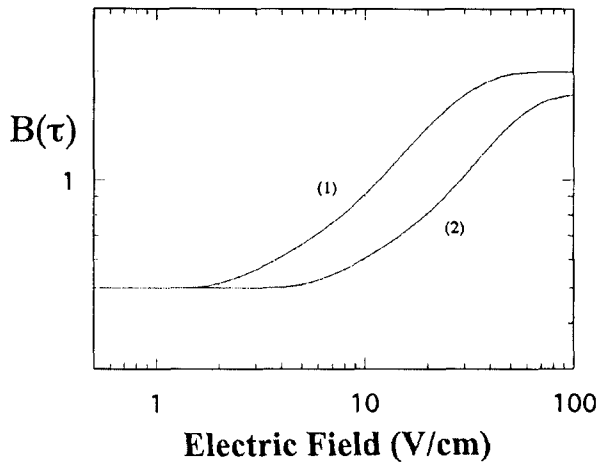

Fig. 3. Theoretical results of the effective distribution coefficient in a gel particle as a function of an electric field (1) χ = 0.182 (0.367 kbp DNA); (2) χ = 0.0026 (1.01 kbp DNA); (χ = Pe_f/Pe_g)

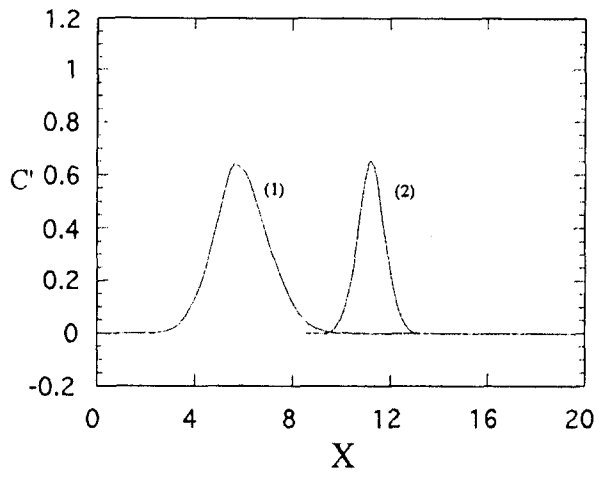

Fig. 4. Theoretical results of concentration profiles of DNA dispersed in the packed column (1) 1.01 kbp DNA (χ = Pe_f/Pe_g = 0.0026, β = 0.125, ϕ = 0.3); (2) 0.367 kbp DNA (χ = Pe_f/Pe_g = 0.182, β = 0.210, ϕ = 0.5).

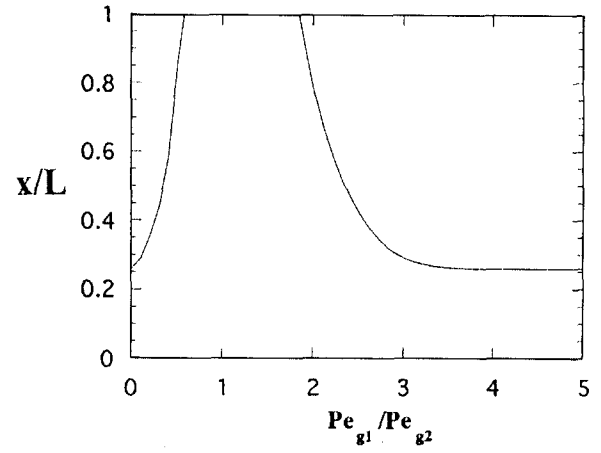

Fig. 5. Theoretical result for the separation of two different DNAs (Solid line indicates the separation point in the column to separate two different DNA at 8 V/cm. Pe_f, Peclet number in the fluid phase, is independent upon the electric field. L is the column length, x is the separation point in the column).

The average concentration of DNA in a gel particle, $B(\tau)$, was dependent on the electric field. The dependence of $B(\tau)$ on the electrical voltage was theoretically analyzed by the parameters in the gel particle as a function of $Pe_f/Pe_g(=\chi)$. Fig. 3 shows that the rate of approach to the limiting value is strongly dependent on the electric field strength and total concentration on the voltage gradient which is significantly dependent on the convective electrophoretic velocity of species in a porous gel particle. In the present analysis, it is interesting to note that total concentration of $B(\tau)$ goes to the proper limiting value of the porosity (β) at the steady state when an electric field is not applied, implying that $B(\tau)$ is equal to the volume of pore space to be accessible to the solute inside a gel particle and $B(\tau)$ is a measure of the capacity of DNA in a gel particle. The value of $B(\tau)$ in high electric field increased until it reached the limiting value of $1/\phi$. This result can indirectly compare with experimental results in which the electrophoretic mobility became constant in a higher electric field with molecular size as shown in Fig. 2.

Fig. 3 shows that the build-up of two different DNAs in the gel particle is strongly dependent upon the value of $Pe_f/Pe_g(=\chi)$. When the value of χ became larger, 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 the high electric field as shown in Fig. 3. This indicates that the total concentration inside a gel particle becomes steeper with the applied electric field in case of 0.367 kbp DNA compared with 1.01 kbp DNA as shown in Fig. 3.

Separation of Large DNA in the Column

Fig. 4 shows how the concentration profiles of DNA in the column vary depending on the Pe_f/Pe_g ($=\chi$) in the cases of 0.367 kbp and 1.01 kbp DNA. The higher Pe_f/Pe_g ($=\chi$) was, the faster the movement of DNA occurred along the column, implying that 0.367 kbp DNA would migrate faster from the fluid phase into and through the gel particle due to an increase of electrophoretic mobility. This relationship can be used to separate different DNA in the column using the electrophoretic mass transport parameter, $Pe_f/Pe_g(=\chi)$.

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 DNA in the gel particle. Fig. 5 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 different DNAs is equal to 1.0, they are inseparable because

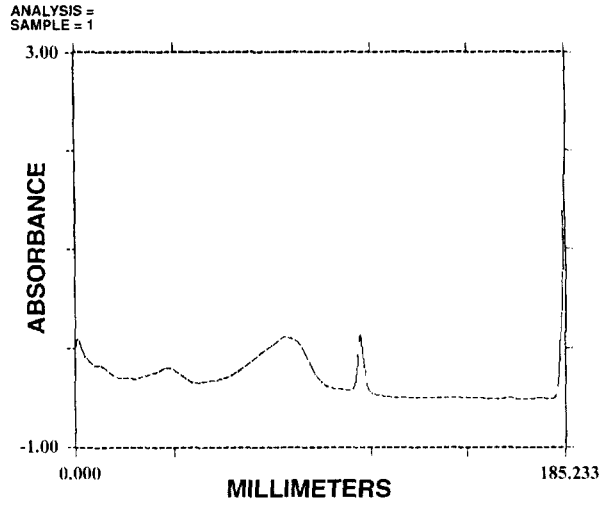

Fig. 6. Gel scanning experimental result for the separation of two different DNAs in the glass column packed with gel particles (A: 1.01 kbp DNA, B: 0.367 kbp).

the transport properties of DNA such as diffusion coefficient and convective electrophoretic velocity would become identical to each other. But if the transport properties of two different DNAs were different, their separation can be predicted as shown in Fig. 6. Therefore, the separation criteria can be compared with results separated experimentally from those predicted by the ratio $R=(Pe_g)_2/(Pe_g)_1$, in which $(Pe_f)_2/(Pe_f)_1=2.211$ for the separation of 0.367 and 1.01 kbp at 8 V/cm and an R value is 2.09.

The subscript "1" represents DNA which was eluted first, and subscript "2" is DNA which was eluted second. The distance to start the separation of two components in the column can be theoretically calculated from the dimensionless term, x/L. The separation point of two different DNAs in the packed column can be predicted by different properties of the diffusion coefficient and convective electrophoretic velocity in the porous gel particle. The predicted separation distance in the column was 10.4 cm for the mixtures of 0.367 kbp DNA and 1.01 kbp DNA at 30 cm column length under an electric field of 8 V/cm as shown in Fig. 4. Experimental measurement under the fluorescent light was performed to check this predicted separation point in the column when 0.367 kbp and 1.01 kbp DNAs were loaded. Fig. 6 shows an experimental separation result scanned along the column using a gel scanning spectrophotometer. The separation point in the experiment turned out to be 10.3 cm, which supported the theoretical calculation presented by the model equation.

CONCLUSION

A model was formulated to predict the behavior of the transport of a column packed with gel particles. A complete analysis of the model equations was done in the transient state for the full description of the effects of the system parameters including diffusion coefficient and convective electrophoretic velocity. The relative ratio of electrophoretic convection and diffusion in solid phase and fluid phase, Peclet number, was a factor determining the transport of DNA inside a gel particle, and the separation of two different DNAs was predicted by this ratio of $\chi=Pe_f/Pe_g$. The findings in this study provide a useful guide to the analysis and the design of devices in laboratories and other scales required for a variety of bioseparations.

Acknowledgements This work was supported by Korea Research Foundation Grant (KRF-98-020-E00039) and the Daejin University Research Grant in 1998.

Appendix I

The electrophoretic mobility of Eq. (8) can be solved by Poisson's equation. If electroneutrality was assumed and the diffusion coefficient was considered to be independent of the concentration, the multiplication of Eq. (1) by z_iF of the charge per mole, and summation of all species at steady state leads to

$$\frac{\partial^2\left(\sum_{i=1}^{N} FD_{i,k}c_{i,k}z_{i,k}\right)}{\partial x^2}=\frac{\partial\left(\sum_{i=1}^{N} F^2u_{i,k}c_{i,k}z_{i,k}{}^2\frac{\partial\Psi}{\partial x}\right)}{\partial x} \qquad \text{(A-1)}$$

the left hand side of Eq. (A-1) becomes zero by electroneutrality because of $D_i>>D_{d,k}$ and Eq. (A-1) reduces to

$$D_i\left[\sum_{i=1}^{N} c_{i,k}z_{i,k}+\frac{D_{d,k}}{D_i}c_{d,k}z_{d,k}\right]\cong D_i\sum c_{i,k}z_{i,k}\cong 0$$

where the subscript "d" denotes DNA with a small diffusion coefficient and all current-carrying species present in the solution have the same diffusion coefficient, D_i, Thus, the integration of Eq. (A-1) leads to Ohm's law [13] and an electrochemical potential term of Eq. (A-1) can be written as

$$\frac{\partial\Psi}{\partial x'}=\frac{I}{\sum_{i=1}^{N} c_iu_iz_i{}^2F}=\frac{I}{R}=\frac{V}{L} \qquad \text{(A-2)}$$

where I is the integration constant and it is equivalent to a constant current. R is the electrical resistance defined by Newman [10], V is the electrical field and L is the length of column. The convective electrophoretic velocity in the fluid phase $uzF\frac{\partial\Psi}{\partial x}$ is described $u\left(\frac{V}{L}\right)$. This term is the electrophoretic mobility of DNA measured experimentally in the gel.

Appendix II

The dimensionless formulation of equations (1)-(2) are redescribed by

$$\frac{1}{\phi_{i,k}}\frac{\partial C_{i,k}}{\partial \tau}=\frac{\partial^2 C_{i,k}}{\partial s^2}-\frac{Pe^2_{i,k}}{4}C_{i,k} \qquad (A\text{-}3)$$

$$s_{k-1}\le s\le s_k \quad for \quad k=1,2,3$$

$$\frac{y_{i,k}\phi_{i,k}}{\beta_{i,k}}\left(-\frac{\partial C_{i,k}}{\partial s}+Pe_{i,k}\frac{C_{i,k}}{2}\right)$$

$$=\frac{y_{i,k+1}\phi_{i,k+1}}{\beta_{i,k+1}}\left(-\frac{\partial C_{i,k+1}}{\partial s}+Pe_{i,k+1}\frac{C_{i,k+1}}{2}\right), \quad k=1,2$$

$$C_{i,k}y_{i,k}\beta_{i,k}=C_{i,k+1}y_{i,k+1}\beta_{i,k+1} \qquad (A\text{-}4)$$

$$C_{i,k}(t=0)=0 \quad for \quad k=1,2,3$$

Dimensionless equation in the column is given by

$$\frac{1}{L}\frac{\partial\left(C_{i,b}z_iFv_{i,f}\Delta\Psi_{i,f}\right)}{\partial X}+\left[1+\alpha B(\tau)\right]\frac{\partial C_{i,b}}{\partial t}=-\alpha\frac{\partial B(\tau)}{\partial t}C_{i,b} \qquad (A\text{-}5)$$

where the gradient of Eq. (A-5) indicates x-direction of rectangular coordinate and boundary and initial conditions are as follows.

$$C_{i,b}(0,t)=c_o \quad at \quad 0<t<t_o$$

$$C_{i,b}(0,t)=0 \quad at \quad t>t_o$$

$$C_{i,b}(x,t=0)=0 \quad at \quad x$$

where $y_{i,k}=\exp(Pe_{i,k}s_k/2)$. Dimensionless terms in the system, s, $Pe_{i,k}$, $C'_{i,k}$, $C_{i,b}$, X, α, ϕ, and τ, are x'/x'_o, $u(V/L)x_o/D$, c_i/c_o, x/L, $\varepsilon/(1-\varepsilon)$, $u_2(V/L)_2/u_1(V/L)_1$, tD/x'^2_o, respectively. It is assumed that $s_k=s_1$, $\phi_{i,k}=1$ and $Pe_{i,k}=Pe_f$ for $k=1$; and that $s_k=s_2$, $\phi_{i,k}=\phi$ and $Pe_{i,k}=Pe_g$ for $k=2$; and that $s_k=1$, $\phi_{i,k}=1$ and $Pe_{i,k}=Pe_f$ for $k=3$ and $x=0$ at $k=0$ for a single gel particle surrounded by two equal sized stagnant boundary layers. The transient solutions of Eq. (A-3) were represented in each phase as

$$C_{i,k}(x',\tau)=\sum_{n=1}^{\infty}\frac{\Omega_{i,k}U_{i,k}(x',\lambda_n)}{\lambda_n}\left[1-\exp(-\lambda_n\tau)\right]; \quad k=1,2,3 \qquad (A\text{-}6)$$

The eigenvalue λ and eigenfunction U was described by the characteristic equation [13].

The concentration profile of Eq. (2) can be calculated using the equations for the characteristic direction σ in the (x,t)-plane if the equations for the characteristic curves are put together [14],

$$\frac{dC_{i,b}}{dt}=-\frac{\alpha C_{i,b}\dfrac{\partial B(\tau)}{\partial t}}{1+\alpha B(\tau)} \qquad (A\text{-}7)$$

The concentration profile along each characteristic curve can be written as integrating Eq. (A-7) as follows.

$$C_{i,b}=C_{i,b}(\tau_o)\frac{1+\alpha B(\tau_o)}{1+\alpha B(\tau)} \qquad (A\text{-}8)$$

A characteristic curve is formed from the start as the state of concentration c_o moves into the column, and the concentration profiles of DNA in the column moves along the characteristic curve. The time, τ, represents the time after the DNA begins to penetrate into a gel particle. The time, t, in Eq. (2) represents the time after the DNA sample on the column is injected. When the sample is transported to the direction of an electric field from the top of the column, DNA in a certain gel particle is an empty state in the column before DNA penetrates into the gel particle. In this case, the relationship between two different transient times of τ and t is given by

$$\tau=t-\frac{x}{\left(v_{i,f}z_iF\dfrac{\partial\Psi_{i,f}}{\partial x}\right)} \qquad (A\text{-}9)$$

The structure of the characteristic curve of DNA injected into an initially empty column. The characteristics emanating from the $t=0$ axis for the initially empty column all had the slope equal to zero due to $B(\tau)=0$, and zero concentration of $C_{i,b}$ in Eq. (A-8) from $\tau_o=t_o$ at $X_o=0$. To construct particular characteristics in this region, it was necessary to choose τ_o. The position of DNA in the column can be calculated when τ varies from τ_o to some desired upper limit from Eq. (A-8).

NOMENCLATURE

a	: Pore size diameter, cm
$B(\tau)$	: Effective distribution coefficient of solute
c_i	: Concentration of DNA in the gel particle, g/cm^3
$<c_{i,2}>$	: Average concentration in the fluid inside pore, g/cm^3
C_i	: Dimensionless concentration in the gel particle
c_b	: Concentration of DNA in the boundary layer, g/cm^3
$C_{i,b}$	: Dimensionless concentration of DNA in the boundary layer
D_k, D_d	: Diffusion coefficient of DNA, cm^2/s
D_i	: Diffusion coefficient of species in the gel, cm^2/s
D_l	: Diffusion coefficient of ionic species, cm^2/s
D_o	: Diffusion coefficient of species in free solution, cm^2/s
E	: Electric field, V
F	: Faraday constant, C/equiv
k	: Each phase in the gel particle layer
ℓ	: Contour length, cm
L	: Column length, cm
N	: Number of species
p	: Persistence length of Eq. (9), cm
Pe_g	: Peclet number in gel particle
Pe_f	: Peclet number in fluid phase
R_g	: Radius of gyration of DNA, cm

s : Dimensionless x-coordinate
t : Time, s
u_i : Mobility of ionic species in the gel, cm^2/Vs
U_k : Eigenfunction of Eq. (A-6)
$v_{i,f}$: Mobility of ionic species in the external phase of gel particle, cm^2/Vs
V : Electric voltage, V
V_o : Void volume in the column, cm^3
V_T : Total volume in the column, cm^3
x' : Space coordinate in the gel particle, cm
x'_o : Total length of particle diameter and stagnant boundary layers, cm
x : Axial coordinate in the column
X : Dimensionless column length
z_i : Valence of solute

Greek Symbols

β : Porosity of gel particle
δ : Boundary layer thickness, cm
ε : Bed porosity
ϕ : Ratio of electrophoretic convection velocity
λ : Eigenvalue
μ : Mobility in the gel, cm^2/Vs
μ_o : Mobility in free solution, cm^2/Vs
τ : Dimensionless time in the gel particle
Ψ : Electrophoretic potential, V

REFERENCES

[1] Nir, A. and L. Pisman (1977) Simultaneous intraparticle forced convection, diffusion and reaction in a porous catalyst I. *Chem. Eng. Sci.* 32: 35-41.
[2] Staphanopoulos, G. and K. Tsiveriotis (1989) The effect of intraparticle convection on nutrient transport in porous biological pellets. *Chem. Eng. Sci.* 44: 2031-2039.
[3] Frey, D. D., E. Schweinheim, and C. Horvath (1993) Effect of intraparticle convection on the chromatography of biomolecules. *Biotechnol. Prog.* 9: 273-284.
[4] Carta, G. (1995) Linear driving force approximation for intraparticle diffusion and convection in permeable supports. *Chem. Eng. Sci.* 50: 887-889.
[5] Lu, Z., J. M. Lourero, M. D. Douglas, and A. E. Rodrigues(1992) Effect of intraparticle forced convection on gas desorption from fixed beds containing "Large-pore" ad-sorbents. *Ind. Eng. Chem. Res.* 32: 1530-1540.
[6] de la Vaga, R., C. Chenou, J. M. Loureiro, and A. E. Rodrigues (1998) Mass transfer mechanisms in Hyper D media for chromatographic protein separation. *Biochem. Eng. J.* 1: 11-23.
[7] Leitao, A. and A. Rodrigues (1998) Dynamic behabior of a fixed-bed biofilm reactor: analysis of the role of the intraparticle convective flow under biofilm growth. *Biochem. Eng. J.* 2: 1-12.
[8] Rudge, S. R., S. K. Basak, and M. R. Ladish (1993) Solute retention in electrochromatography by electrically induced sorption. *AIChE J.* 39: 797.
[9] Yang, T., C. J. Lee, and I. M. Chu (1998) A new approach to counteracting chromatographic electrophoresis. *Sep. Sci. Tech.* 33: 1819-1831.
[10] Newman, J. S. (1991) *Electrochemical System.* pp. 245-246. Prentice-Hall, Englewood Cliffs, NJ, USA.
[11] Ptassinski, K. J. and P. J. A. M. Kerkhof (1992) Electric field driven separations: Phenomena and applications. *Sep. Sci. Tech.* 27: 995-1003.
[12] Ramkrishna, D. and N. Amundson (1985) *Linear Operator Methods in Chemical Engineering with Applications to Transport and Chemical Reaction Systems.* Prentice-Hall, Englewood Cliffs, NJ, USA.
[13] Locke, B., P. Arce and Y. Park (1993) Application of self-adjoint operators to electrophoretic transport in a composite media II. *Chem. Eng. Sci.* 48: 4007-4015.
[14] Rhee, H., R. Aris, and N. Amundson (1986) *First-order Partial Differential Equations. Volume I, Theory and Application of Single Equation.* pp. 117-140. Prentice-Hall, Englewood Cliffs, NJ, USA.
[15] Olivera, B., P. Baine, and N. Davidson (1961) Electrophoresis of nucleic acids. *Biopolymer* 2: 245-259.
[16] Rodrigues, A. E., L. Zupping, and J. M. Loureiro (1991) Residence time distribution of inert and linearly adsorbed species in a fixed bed containing "large-pore" supports: Applications in separation engineering. *Chem. Eng. Sci.* 46: 2765-2773.
[17] Pecora, R. (1991) DNA: a model compound for solution studies of macromolecules. *Science* 251: 893-897.
[18] Deen, W. M. (1987) Hindered transport of large molecules in liquid-filled pores. *AIChE J.* 9: 1409-1425.
[19] Fogler, H. S. (1986) *Elements of Chemical Reaction Engineering,* pp. 523-528, Prentice-Hall, Englewood Cliffs, NJ, USA.
[20] Slater, G. W. and J. Noolandi (1986) On the reptation theory of gel electrophoresis. *Biopolymer* 25: 431-454.
[21] Hervet, H. and C. P. Bean (1987) Electrophoretic mobility of λ phage DNA fragments in agarose gel. *Bipolymer* 26: 727-742.

[Received May 26, 2000; accepted July 27, 2000]