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Residence Time Distribution in the Chromatographic Column: Applications in the Separation Engineering of DNA

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Abstract Experimental and theoretical works were performed for the separation of large polyelectrolyte, such as DNA, in a column packed with gel particles under the influence of an electric field. Since DNA quickly orient in the field direction through the pores, this paper presents how intraparticle convection affects the residence time distribution of DNAs in the column. The concept is further illustrated with examples from solid-liquid systems, for example, from chromatography showing how the column efficiency is improved by the use of a n electric field. Dimensionless transient mass balance equations were derived, taking into consideration both diffusion and electrophoretic convection. The separation criteria are theoretically studied using two different Peclet numbers in the fluid and so lid phases. These criteria were experimentally verified using two different DNAs via electrophoretic mobility measurements, which showed how the separation position of the DNAs varies in the column in relation to the Peg/Pef values of an individual DNA. The residence time distribution was solved by an operator theory and the characteristic method to yield the column response.

Keywords: porous material, convective velocity, DNA, residence time distribution, HETP

INTRODUCTION

Gel electrophoresis can commonly derive high resolution in the purification process of polyelectrolytes, but adapting this milligram bench technique to multigram preparative separations has not been possible due to its extremely poor scaling. On the other hand, gel chromatography has far superior scaling properties and a resolving power that is second only to gel electrophoresis. However, electro-chromatography, the combination of electrophoresis with chromatography, might show better separation, as the field-induced dispersion can virtually amplify the resolving power of each, while retaining the superior scaling properties of chromatography. Therefore, the electro-chromatography separation processneeds to be investigated for the underlying physics of the process or how it can be effectively scaled-up.

An important feature, when utilizing an electric field in a column, is the presence of intraparticle convection, so knowledge of which polyelectrolytes move headfirst through the pores of the gel is required. The intraparticle convective velocity plays an important role in enhancing the separation of polyelectrolytes in the column, and a few studies have tried innovative ideas to create new materials or apparatus for its determination.

In the area of separation engineering, packing materials with high porosity have led to efficient chromatographic separations due to the convective velocity. For example, researchers [1-3] proved that the intraparticle convection effects in "large pore" packing materials used in gel chromatography make the separation of proteins possible by reducing the broadness of the peaks in the packed column. Carta [4] analyzed the effects of intraparticle convection on the dynamic capacity of the adsorption bed using the LDF (Linear Driving Force) approximation in permeable support. Rodrigues *et al.* [5-7] investigated the intraparticle convection in "large pore" packing materials, and found they enhanced the performance for the separation of proteins in the chromatography column, and influence the intraparticle convective flow on the dynamic response of a fixed-bed biofilm reactor.

Studies on the separation of polyelectrolytes in electro-chromatography have been performed with proteins [8,9] but have rarely been tried with DNA. The conventional method for large polyelectrolytes, such as DNA, caused the peak of the elution curve to broaden in the column, and these peak-broadening makes the separation of two different DNAs difficult. Therefore, in this study the DNAs will be separated, and the peak-broadening prevented by the presence of an electric field, as they stretch and readily move through the particles packed in the column. This implies that an electric field may enhance the permeation of the DNA due to the electrophoretic convection inside the pores. Examples using electric fields in the separation processes have frequently been employed for more delicate separations in process facilities [10,11].

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Theoretical models in electro-chromatography have rarely been investigated, taking into consideration the intraparticle transports of both diffusion and convection, by the decoupling of the gel particles from the column. There have been few theoretical studies accounting for the simultaneous convective-diffusive transports at the internal boundaries surrounding gel particle. The theoretical work in this paper was developed by using two different Peclet numbers and the total flux boundary condition at the interface between the different regions (fluid and solid phases) contained the diffusive and the convective components.

In this study we attempted to show how the intraparticle mass transport, due to the convective electrophoretic velocity, influences the separation of DNA on the column. Our motivation was to find an efficient column separation compared with a conventional separation column. The objectives of this paper were to understand the influence of intraparticle convection on the residence time distribution of DNA in the column, and to analyze the response of the column to an impulse input sample, and link the peak characteristics to a simple parameter for measuring the efficiency of a chromatographic column.

The residence time distribution was analyzed using the ratio ($r=Pe_g/Pe_f$) of the two Peclet numbers, which were described by the physical properties (diffusion coefficients and electrophoretic mobility) experimentally measured in the fluid and solid phases of an individual DNA. Theoretical methods for solving the electrophoretic mass transport problem were performed by the operator theory [12,13] and a characteristic method [14]. The separation criteria for the DNAs, using the ratio of the two different Peclet numbers, will be theoretically presented and experimentally examined.

THEORY

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

$$\frac{\partial^2\left(\sum_{i=1}^{N} FD_{i,k}c_{i,k}z_{i,k}\right)}{\partial x^2} = \frac{\partial\left(\sum_{i=1}^{N} F^2u_{i,k}c_{i,k}z_{i,k}{}^2\frac{\partial\psi}{\partial x}\right)}{\partial x} \quad (1)$$

the left hand side of Eq. (1) becomes zero due to electroneutrality, and because $D_l \gg D_{d,k}$ Eq. (1) reduces to

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

where subscript "d" denotes the DNA and "i" also the current-carrying species present in the solution which have the same diffusion coefficient, D_l. Thus, the integration of Eq. (1) leads to Ohm's law [13], so the electrochemical potential term of Eq. (1) can be written as

$$\frac{\partial\psi}{\partial x'} = \frac{I}{\sum_{i=1}^{N} c_iu_iz_i{}^2F} = \frac{I}{R} = \frac{V}{L} \quad (2)$$

where I is the integration constant, which is equivalent to a constant current. R is the electrical resistance, as defined by Newman [13], V the electrical field and L the column length. The convective electrophoretic velocity in the fluid phase, $uzF\frac{\partial\psi}{\partial x}$ is described as, $u\left(\frac{V}{L}\right)$ which is the same as the electrophoretic mobility of the DNA measured experimentally in the gel.

DNA transports across the boundary layer, penetrates into the gel particle and comes out across the boundary layer. The mathematical model of the system is based on the assumptions, that at the particle level, the mass transport occurs by intraparticle diffusion and convection when the slab geometry for the gel particles were considered, the axial dispersion was assumed to be negligible in the column due to the very slow flow velocity under an electric field.

The continuity equations of species i for the gel particles, and the packed column with boundary conditions, are given by Eqs. (3) and (4), respectively :

$$\frac{\partial c_{i,k}}{\partial t} = D_{i,k}\frac{\partial^2 c_{i,k}}{\partial x'^2} - u\left(\frac{V}{L}\right)\frac{\partial c_{i,k}}{\partial x'} \quad (3)$$

$$\varepsilon\frac{\partial c_{i,b}}{\partial t} + \varepsilon u\left(\frac{V}{L}\right)\frac{\partial c_{i,b}}{\partial x} + (1-\varepsilon)\frac{\partial\langle c_{i,b}\rangle}{\partial t} = 0 \quad (4)$$

The subscript k, in Eq. (3), denotes each phase of the fluid-solid phases. For example, an upper boundary layer of rectangular coordinate is k=1, a lower boundary layer k= 3, and k=2 denotes a gel layer. $<c_{i,2}>$ denotes the total average concentration of the solute inside the gel particles in the presence of an electric field. The total flux boundary conditions, and the initial condition of gel particles are:

$$-D_{i,k+1}\frac{\partial c_{i,k+1}}{\partial x'} + u_{i,k+1}\left(\frac{V}{L}\right)_{i,k+1}c_{i,k+1} = -D_{i,k}\frac{\partial c_{i,k}}{\partial x'} + u_{i,k}\left(\frac{V}{L}\right)_{i,k}c_{i,k} \quad (5)$$

$$\beta_{i,k+1}c_{i,k+1} = \beta_{i,k}c_{i,k} \quad \text{at } x'=x'_k, k=1, 2 \quad (6)$$

$$c_{i,k}(t=0) = 0 \quad , \quad k=1, 2, 3 \quad (7)$$

with the boundary and initial conditions in the column as follows.

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

Table 1. Dimensionless equations and dimensionless parameters in the governing equations

Dimensionless equations in a gel particle and in the column are given by

$$\frac{1}{\phi_{i,k}}\frac{\partial C_{i,k}}{\partial \tau} = \frac{\partial^2 C_{i,k}}{\partial s^2} - \frac{Pe^2{}_{i,k}}{4} C_{i,k} \quad s_{k-1} \le s \le s_k \quad \text{for} \quad k = 1,2,3$$

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

$$C_{i,k} y_{i,k} \beta_{i,k} = C_{i,k+1} y_{i,k+1} \beta_{i,k+1}$$

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

$$y_{i,k} = \exp(Pe_{i,k} s_k / 2)$$

$$\frac{1}{L}\frac{\partial (C_{i,b} z_i F v_{i,f} \nabla \Psi_{i,f})}{\partial X} + [1 + \alpha B(\tau)]\frac{\partial C_{i,b}}{\partial t} = -\alpha \frac{\partial B(\tau)}{\partial t} C_{i,b}$$

$$C_{i,b}(0,t) = c_0 \text{ at } 0 < t < t_0$$

$$C_{i,b}(0,t) = 0 \text{ at } t > t_0$$

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

Dimensionless terms in the system

$$s = \frac{x'}{x_0}, C_{i,k} = \exp\left(-\frac{Pe_{i,k}}{2} s\right)\frac{c_{i,k}}{c_0}, C_{i,b} = \frac{c_{i,b}}{c_0}, X = \frac{x}{L}, \alpha = \frac{1-\varepsilon}{\varepsilon}, \phi = \frac{u_g\left(\frac{V}{L}\right)_g}{u_f\left(\frac{V}{L}\right)_f}, \tau = \frac{t u_f \left(\frac{V}{L}\right)_f}{Pe_f x_0}$$

In Eqs. (1) and (2) above, $c_{i,k}$, $u_{i,k}$, $v_{i,f}$, $D_{i,k}$, F and $\psi_{i,k}$ are defined in the "Nomenclature" section. The dimensionless differential equations and their accompanying boundary and initial conditions in the gel particle are given by Table 1.

Moment Equation

The moments of the impulse response, *i.e.* the residence time distribution, are obtained by the van der Laan relation as follows.

$$\mu_n = \left[-\frac{d^n G(s)}{ds^n}\right]_{s\to 0} \tag{8}$$

The moment equations [16], using the response curve measured by gel scanning spectrophotometer (Gilford Co.), under the assumption that the thickness of boundary layer is small, are negligible. The transfer function G(s) of the chromatography is obtained by solving the system of PEDs in Eqs. (3) and (4) with Laplace transformation, i.e.

$$G(s) = \frac{\overline{C}}{\overline{C_0}} = \left\{1 + \varepsilon \frac{s}{u\left(\frac{V}{L}\right)} + (1-\varepsilon)\beta \frac{1}{Pe_g L} \sqrt{\frac{Pe_g{}^2}{4} + Pe_g \frac{L}{u\left(\frac{V}{L}\right)} s \frac{(\exp(2r_2)-1)(\exp(2r_1)-1)}{exr(2r_2) - \exp(2r_1)}}\right\} \tag{9}$$

where $r_1, r_2 = \frac{Pe_g}{2} \pm \sqrt{\frac{Pe_g{}^2}{4} + Pe_g \frac{L}{u\left(\frac{V}{L}\right)} s}$

The first moment (μ_1) obtained from the Laplaced transform equation and the standard deviation (σ), are described like Eqs. (10)-(11) using Eq. (9) by

$$\mu_1 = \varepsilon_c + \varepsilon_p(1-\varepsilon_c) \tag{10}$$

$$\sigma^2 = \mu_2 - \mu_1{}^2 = \left\{\frac{2\varepsilon_p(1-\varepsilon_c)x_0}{L} + \frac{2}{3}\varepsilon_p(1-\varepsilon_c)\alpha\right\} \left\{\frac{1}{\tanh(Pe_g)} - \frac{1}{Pe_g}\right\} \tag{11}$$

ε_c can be determined by measuring V_o and V_T as shown in section 2. The impulse response is not the residence time distribution since the boundary conditions of a total flux condition have been. In the residence time distri-bution theory it is assumed that the DNA entering the system cannot leave it again via the inlet section, and the DNA leaving the outlet section cannot enter the system again, so called, Danckwerts boundary condition.

The number of plates is an indication of the column efficiency, the plate, or the height equivalent one of a theoretical plate (HETP), in chromatography is a concept used to provide a measure of the peak's spreading, relative to the migration distance. The definition of HETP can be described by

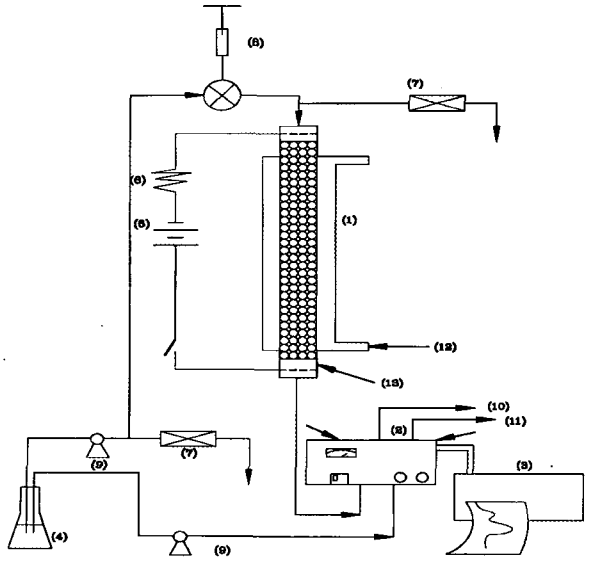

Figure 1. (a) A schematic picture of a packed column in the presence of an electric field (Bio-Gel paricles are packed with size of less than 40 μm and the power conditions during the run can be altered with a range of up to 1,000 volts or 500 mA. Power supply has the ability to monitor current to the 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.

$$\mathrm{HETP} = \frac{\sigma^2}{\mu_1^2} L = \frac{\left\{2\varepsilon_p(1-\varepsilon_c)x_0 + \frac{2}{3}\varepsilon_p(1-\varepsilon_c)\alpha L\right\}}{\left\{\varepsilon_c + \varepsilon_p(1-\varepsilon_c)\right\}^2}\left\{\frac{1}{\tanh(Pe_g)} - \frac{1}{Pe_g}\right\} \quad (12)$$

$$= \frac{\left\{2\varepsilon_p(1-\varepsilon_c)x_0 + \frac{2}{3}\varepsilon_p(1-\varepsilon_c)\alpha L)\right\}}{\left\{\varepsilon_c + \varepsilon_p(1-\varepsilon_c)\right\}^2} f(Pe_g)$$

MATERIALS AND METHODS

Electrophoresis Chamber

Gel particles were prepared with electrophoretic grade agarose from Bio-Gel-A-50m (BioRad), which fractionates in range of 200,000~1,000,000. The gel particles which had a diameter of 40 μm were packed in the column as shown in Fig. 1. After the agarose gel particles were loaded onto the column, the column was inserted into the vertical BioRad electrophoresis chamber. The electrophoretic mobility of the DNA could easily be observed as its movement had a sharp band shape along the column as viewed under fluorescence light. The packed column used in this experiment was a cylindrical glass tube, 0.2 cm in diameter and 20 cm in length, sepecially designed by Duke Scientific Corp. The buffer solution in the electrophoretic chamber was of pH 7.2, the absorbance in the column was measured at 340 nm, with a spectrophotometer. The temperature of the circulator in the electrophoresis chamber was kept at 18°C.

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

Item	0.367 kbp DNA	1.010 kbp DNA	λ DNA
Diffusion coefficient in gel particle(cm^2/sec)	0.81×10^{-8}	1.37×10^{-8}	1.00×10^{-9}
Diffusion coefficient in free solution(cm^2/sec)	15.8×10^{-8}	7.15×10^{-8}	4.9×10^{-8}
Porosity(ε_p)	0.210	0.125	0.057
Mobility in free solution in pH 7.2(cm^2/V · sec)	4.0×10^{-4}	4.0×10^{-4}	4.0×10^{-4}
Mobility in gel particle in pH 7.2(cm^2/V · sec)	1.9×10^{-4}	1.2×10^{-4}	1.1×10^{-5}

Measurement of Physical Properties

The extraparticle void volume (V_o) was determined using T4 DNA, a large molecule that was totally excluded from the gel particles. V_T, the total column volume was measured, in the absence of an electric field, by injecting a 100 μL sample containing urea at a concentration in the range 1 mg/mL, and measuring the volume of eluent required for the peak to emerge from the column. The extraparticle void fraction (ε_c) was calculated from the relation of V_o/V_T. The T4 DNA was purchased from Sigma Chemical Co. The mobility of the DNA in free solution, from Olivera *et al.*, was 4.0×10^{-4} cm^2/V · sec [15].

The gel porosity (ε_p) and diffusion coefficient (D_e) in gel were estimated from the moment equations of Rodrigues *et al.* [16] using the response curve measured by the gel scanning spectrophotometer (Gilford Co.) under the assumption that the thickness of boundary layer was small enough to be negligible. The experimentally estimated results of moment equations are listed in Table 2. ϕ is the relative ratio of the mobility in the gel phase and fluid phases, and the boundary layer thickness (δ) can be calculated for cases when a sphere is immersed in a stagnant fluid, and it is obtained from the relationship of $\delta = D_k/k_f$, where the mass-transfer coefficient is calculated for a mass-transfer Sherwood number equal to 2.0 [16], for low Reynolds numbers of an electric field.

Measurement of Electrophoretic Mobility

A 2% agarose solution was cast in an electrophoretic tray at 60°C, and cooled to room temperature within half

an hour. The DNA samples for this experiment were λ phage and ΦX HAE III DNA fragments, which were purchased from BRL (Bethesda Research Laboratories). The molecular lengths ranged from 310 to 23,600 bp (base pair). The loading solution contained 20% DNA by weight, and a 10% by weight dye solution (10% bromophenol blue +50% glycerol and 40% EDTA). The loading DNA solution was prepared in pH 7.2 Tris buffer, which was also used to fill the electrophoretic tray. The electrophoretic apparatus was a BioRad Model 2303, with a 20 cm gel length loaded inside the apparatus. Two silver electrodes, 8 cm apart, were inserted from the top into the electrophoretic tray. The voltage between the electrodes was measured with a digital voltmeter and the average voltage drop during the experiment was less than 3%. The temperature of the buffer was controlled to within an accuracy of ±0.2°C, and was maintained using a digital thermometer connected to two thermocouples dipped in the apparatus as shown in Fig. 1.

For each experimental run the gel was soaked for 30 min in a 2×10^{-5} M ethidium bromide solution to bind the intensity of the fluorescence. The electrophoretic mobility was determined from at least three measurements of the band positions obtained at different time intervals. The electrophoretic mobilities of the DNA fragments were measured as functions of the electric field and the molecular size.

Radius of Gyration of DNA

When the contour length, "λ", of a fragment was smaller than its persistence length, "*p*", which was itself smaller than the average pore size, <a>, of most of the agarose gel particles, the fragment was seen as a rod, with a negligible width and length, which can enter all the pores. When "λ" was larger than the persistence length "*p*", the fragment folds in on itself, and take the approximate shape of a globule with a radius of gyration, R_g, given by [20].

$$R_g^2 = \frac{1}{3} p\lambda\left\{1 - \frac{p}{\lambda} + \frac{p}{\lambda}\exp\left(-\frac{\lambda}{p}\right)\right\} \tag{13}$$

These fragments were excluded from the pores when a $\ll R_g$, where "a" is the pore size (diameter) of gel particle.

RESULTS AND DISCUSSION

Diffusivity Effects of DNA in the Presence of an Electric Field

The pore size plays an important role when DNA migrats electrophoretically, with appreciable mobility, through the gel, and its size in a 2% agarose gel particle was about 89 nm [21]. The contour length of the DNA was generally much longer than the diameter of the average pore in the gel matrix. Table 2 shows a comparison of the properties of the DNA molecules and pore sizes of gel particles. The contour lengths, shown in Table 3, were estimated assuming a repeat per base pair value of 0.34 nm.

Table 3. Physical dimension for the size of DNA and porous gel particle

Item	0.367 kbp DNA	1.010 kbp DNA	λ DNA
Radius of gyration R_g (nm)	36.5	70.7	364.2
Persistence length p(nm)[17]	51.0	51.0	51.0
Contour length λ(nm)	125.0	343.0	7854
Pore size diameter a(nm)	40.0	40.0	40.0
λ/p	2.5	7.0	154.0

The experimental measurements show that when the mobility begins to become field-dependent, the DNAs are clearly distinguished by their lengths at the specific applied field values. The electrophoretic mobility of DNA within an electric field was less varied with the smaller DNA molecular sizes compared to the larger sizes, as the small DNA molecules retain their globular shape [22], but those of the larger molecules (N>1,000 bp) increased with the electric field. Forexample, the electrophoretic mobilities in a high electric field, 8 V/cm, and a 2% agarose gel, were found experimentally, to be 1.2×10^{-4}, and 1.9×10^{-4} cm²/V-sec, for 1.01 and 0.367 kbp, respec-tively. Based on these physical properties as shown in Table 2, the Pe_g/Pe_f ratio has a value larger than 1.0, as the diffusion coefficient in the gel particles is much smaller than in the bulk phase, and the ratio (ϕ) of the electrophoretic mobilities was 0.35 in the case of the 1.01 kbp DNA at 1 V/cm.

The capacity of DNA in a gel particle is dependent on the electric field, so its dependence on the electrical voltage was theoretically analyzed by the parameters of the gel particles as a function of $Pe_g/Pe_f(=r)$. Fig. 2 shows that the rate of approach to the limiting value is strongly dependent on the electric field strength, and the total concentration of the voltage gradient is significantly dependent on the convective electrophoretic velocity of the species in a porous gel particle. In this analysis, it was interesting to note that total concentration in a gel particle goes to the proper limiting value of the porosity, ε_p, at the steady state when an electric field is not applied, implying that it was equal to the volume of pore space accessible to the solute inside the gel particle. The value for the total capacity in a high electric field increases, until it reaches the limiting value. This result compares with those of the moment analysis, where the diffusion coefficient became constant in the higher electric field, as shown in Fig. 3.

Fig. 3 shows the diffusion coefficients of the DNAs in

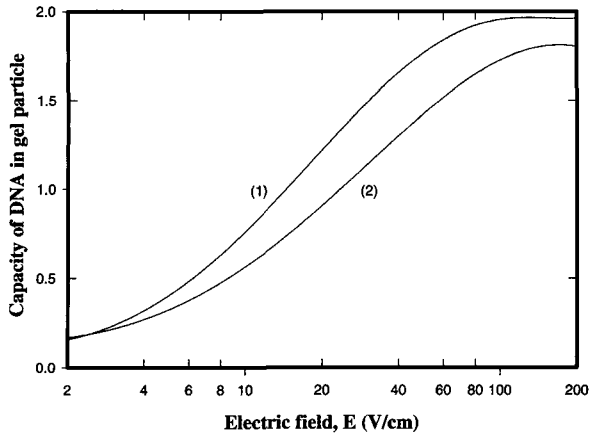

Figure 2. Theoretical results of the toal concentration in a gel particle as a function of an electric field (1) r=0.182 (0.367 kbp DNA); (2) r=0.0026 (1.01 kbp DNA); ($r=Pe_g/Pe_f$).

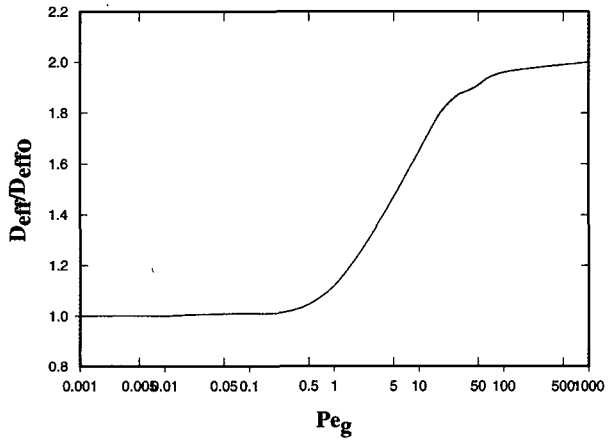

Figure 3. The convection effect in the diffusion coefficient of DNA in a gel particle.

the gel particles are strongly dependent on the $Pe_g/Pe_f(=r)$ value. When the r value became larger, the dynamic speed at which the DNA approaches the steady state is faster, and the transport rate of the DNA through the gel particles can be increased in high electric fields. This corresponds to the experimental results, where the total concentration inside a gel particle became steeper with the applied electric field, as in case of the 0.367 kbp DNA in compared with the 1.01 kbp DNA as shown in Fig. 2.

Analysis of Residence Time Distribution

The generalized van Deemter's equation will now be applied to the system, *i.e.* a packed column for measuring the physical parameters by a chromatographic technique. In the following calculations we refer to the data published by Rodrigues *et al.* [6,7], which relates to the experimental measurements of an inert tracer in the

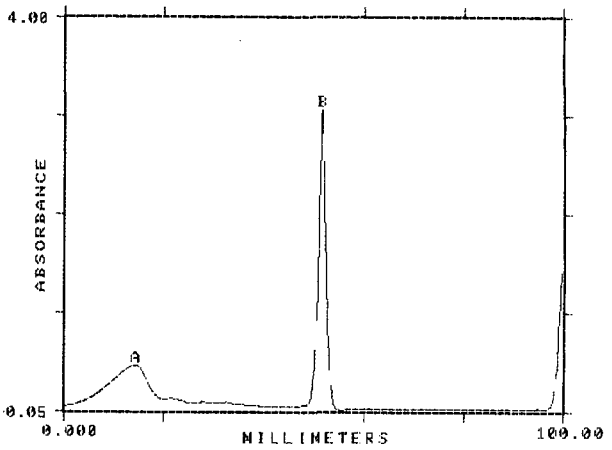

Figure 4. Experimental chromatogram scanned in the chromatographic column (A: 0.367 kbp DNA, B: bromphenol blue).

separation engineering. The column characteristics were: length, L=20 cm, the porosity, ε_p=0.210, and the tube diameter, d=0.2 cm. Particle characteristics were: d_p= 0.004 cm and average pore diameter, d_{pore}=400 Å.

Correlations for calculating the quantities involved in model parameters are:

(a) intraparticle velocity is

$$v = K\,(a_1\,U + a_2\,U^2) \tag{14}$$

where $U = u\left(\frac{V}{L}\right)$, $a_1 = 150\frac{(1-\varepsilon_c)^2}{d_p^2\varepsilon_c^2}$ and $a_2 = 1.75\rho\frac{1-\varepsilon}{\mu d_p\varepsilon_c^3}$ and K is the particle permeability according to Darcy's law for intraparticle convection flow *i.e.* $v = K/\mu(\Delta P/2\,L)$, and Ergun's law for the flow through the column, *i.e.*

$$\frac{\Delta P}{L} = \mu(a_1U + a_2U^2) \tag{15}$$

The performance of a linear chromatographic process is often measured by the height equivalent to a theoretical plate (HETP); the dependence of HETP on the superficial velocity U, for the case of a convectional supports is described by the van Deemter's equation, *i.e.*

$$\text{HETP} = A + \frac{B}{U} + CU \tag{16}$$

where the contributions of the statistical dispersion, molecular diffusion and mass transfer are A, B/U and CU respectively. At a sufficiently high superficial velocity the HETP increases linearly with U. In Eq. (16), C is given by:

$$C = \frac{\left\{2\varepsilon_p(1-\varepsilon_c)x_0 + \frac{2}{3}\varepsilon_p(1-\varepsilon_c)\alpha L\right\}}{\left\{\varepsilon_c + \varepsilon_p(1-\varepsilon_c)\right\}^2} f(Pe_g)$$

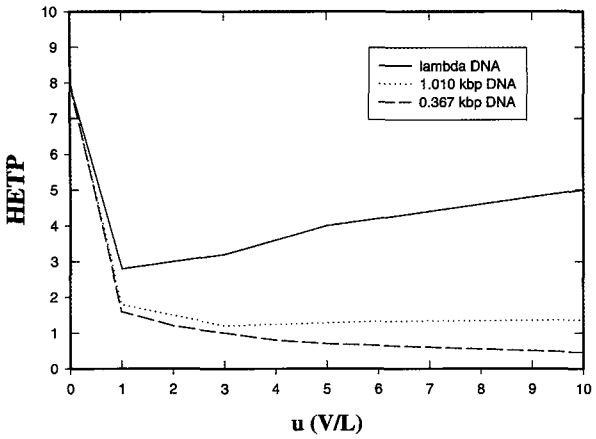

Figure 5. HETP vs as a function of the superficial velocity U for various DNA.

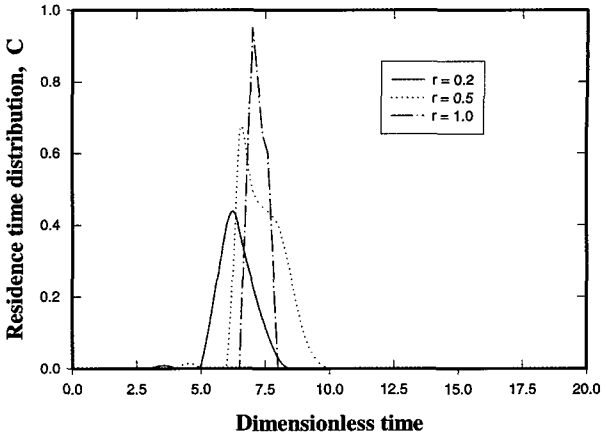

Figure 6. The convection effects of concentration profiles of DNA dispersed in the chromatographic column.

The parameters involved here are Pe_g, ε_p and ε_c. The term CU contains contributions from all the related with mass transfer kinetics, these being, Pe_g, intraparticle diffusion and intraparticle convection. The contribution of convection can be described by $f(Pe_g)$, which refers to the band spreading caused by pore diffusion. This term dominates the overall extent of band-spreading (plate height) under high superficial fluid velocity relative to solute diffusivity conditions.

There is a linear relation between the HETP and $f(Pe_g)$. If $Pe_g < 1$, $f(Pe_g)=1-1/Pe_g$, and if Pe_g is large, $f(Pe_g)=1$. So the analysis of the contribution of the term CU shows that for small values of U and hence small values of v and Pe_g, the HETP increases linearly with U; however, for large values of U, the term CU is proportional to U/v, *i.e.* proportional to $1/a_1 + a_2U$ and decreases with U. This is the reason for the HETP vs. U curve going to a minimum in the presence of intraparticle convection.

Fig. 5 shows the effect of intraparticle convection on the HETP for the electrophoretic separation system. As U increases, the HETP deviates from van Deemter's equation (the curve of HETP vs U linearly increases with U.) for conventional supports where intraparticle convection is not present. With the HETP vs U curve, the dimensionless parameter Pe_g changing, because the intraparticle velocity changes with the superficial velocity U according to Eq.(14), as shown in Fig. 5.

The typical data used in the following example are: column length 20 cm, particle diameter 40 μm, permeability 10^{-10} cm^2, effective diffusion coefficient (D_{eff}) 1.37×10^{-7} cm^2/sec, column porosity (ε_c) 0.4 and particle porosity (ε_p) 0.210. By changing ΔP, we get flow rates corresponding to the Reynolds number, based on the particle diameter, $Re < 0.1$, and then $\Delta P / L$ is proportional to U. Fig. 5 shows the HETP as a function of the superficial velocity for various DNAs.

It is well known that the minimum in a van Deemter's curve occurs at very low U values. The important feature of chromatography in the presence of an electric field is that the HETP is lower than in conventional chromatographic separation methods as the value of U increases. As the flow rate is increased still further, the intraparticle diffusion into the pores becomes rate limiting. Consequently, as with strictly diffusive (conventional method) transport, the plate height increases with superficial velocity. However, the plate height equation is only valid in the presence of an electric field in chromatography. Moreover, the fact the HETP changes little with the U value means the speed of an analysis can be increased. There is also no maximum in the v vs. U curve, which was observed in the electrophoretic separation system. Because of the HETP, the flow through the column is laminar as well as intraparticle convective flow, so $v=Ka_1U$. At high superficial velocities, the HETP leads to a limiting value, as determined by the column and particle porosities, and the particle permeabilities and diameters.

The improvement of column efficiency (HETP) due to intraparticle convection in the electrophoretic separation system can be seen in terms of residence time distributions. Consider the case of $r(=Pe_g/Pe_f)=1$ and let us look at the residence time distributions for a electrophoretic separation system. It is obvious that intraparticle convection leads to narrower and higher peaks due to an enhancement of diffusivity and transport rate becomes faster as shown in Fig. 6. It should be noticed by moment analysis that the controlling mechanism can change depending on the value of model parameters. The relative weight of $f(Pe_g)$ is the key factor to look at. The convection effects make the separation of DNAs easier and more efficient.

Separation of DNAs in the Column

Fig. 7 shows how the concentration profiles for the DNA on the column vary depending on the Pe_g/Pe_f ($=r$) for the cases of the 0.367, 1.01 kbp DNAs. The higher the Pe_g/Pe_f ($=r$), the faster the movement of the DNA along the column, implying that the 0.367 kbp DNA would migrate faster, from the fluid phase into and through the gel particle, due to the increase in

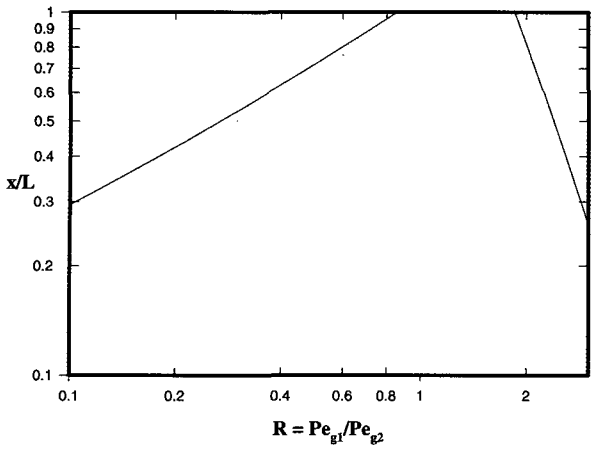

Figure 7. Theoretical result for the separation of two different DNAs. (Solid line indicates the separation point in the column to separate two different DNA at 8 V/cm. Pef, Peclet number in the fluid phase, is independent upon the electric field. L is the column length, x is the separation point in the column).

electrophoretic mobility. This relationship can be used to separate different DNAs on the column using the electrophoretic mass transport parameter, $Pe_g/Pe_f(=r)$.

The separation criteria of the two components can be obtained from the calculations of the band migration using the Peclet number ratio for the two phases. The Pe_g/Pe_f ratio is related to the retention time of a DNA in the gel particle. Fig. 7 shows how one component can be separated from another through the column. Note that when the ratio ($R= r_1/ r_2$) of the two DNAs is equal to 1.0, they are inseparable because the transport properties of DNAs, such as the diffusion coefficient and convective electrophoretic velocity, become identical. However, if the transport properties of two DNAs were different, their separation can be predicted, as shown in Fig. 6. Therefore, the separation results can be examined by a comparison of the experimental results, and those predicted by the $(Pe_g)_1/(Pe_g)_2$ ratio, as the physical properties of the DNA, *i.e.*, the diffusion coefficient and convective velocity, have constant values in the fluid phase, irrespective of the size of the DNA of the magnitude of the electric field.

The subscript "1" in Fig. 7 represents the DNA that migrated first, and the subscript "2" the DNA that migrated second, inside the column. The distance when the separation of the two components starts can be theoretically calculated from the dimensionless term, x/L. The separation point of the two DNAs can be predicted from the diffusion coefficient and convective electrophoretic velocity values of the porous gel particle. The R value was 2.09 for the 0.367 and 1.01 kbp DNA mixture, with an electric field of 8 V/cm. The predicted separation distance in the column, as shown in Fig. 7, was 92 mm with a 20 cm column length. An experimental measurement was also performed under fluorescent light to check the predicted separation point for the 0.367 and 1.01 kbp DNAs loaded onto the column. The results of the experimental separation were scanned along the length of the column using a gel scanning spectrophotometer. The separation point in the experiment turned out to be 92.5 mm, which supports the theoretical calculation presented by the model equation.

CONCLUSION

A model to predict the transport behavior in a column packed with gel particles was formulated. A complete analysis of the model equations was used in the transient state to a fully describe the effects of the system parameters, including the diffusion coefficient and convective electrophoretic velocity.

An analysis of the residence time distribution of DNA in a packed column support helps in the understanding of the efficiency enhancement of a column due to intraparticle convection. The use of peak characteristics, and their link to the concept of HETP, provides a tool for quantifying the efficiency of a column, in connection with an electrophoretic separation system, used to measure the effective diffusivities by chromatographic techniques.

The key factor in the analysis is the development of a generalized van Deemter's equation that clearly shows the intraparticle convection contribution. With chromatographic operations, in the presence of an electric field, there is an important deviation from the classic van Deemter's line, which means that, when the superficial velocity is increased, the intraparticle convection also increases, and thus the HETP decreases, relative to conventional van Deemter's conditions, and tends to a plateau. This fact is of great importance in improving the speed of a chromatographic operation by increasing the superficial velocity. These findings are supported by our experimental observations. The HETP *vs.* U curve goes through a minimum due to the relation between the intraparticle and superficial velocities, v and U, respectively.

The relative ratio of electrophoretic convection and diffusion between the solid and fluid phases, the Peclet number, became a factor in determining the transport of the DNA inside a gel particle, and the separation of the two DNAs was predicted by the $r=Pe_g/Pe_f$ ratio. A gel scanning spectrophotometer was used to experimentally examined the separation result, which was compared with the theoretical separation criteria. The findings in this study will provide a useful guide for the analysis, and design of devices, in the laboratory, and for the scale-up of a variety of bioseparations.

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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 pores, 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_I 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
λ Contour length, cm
L Column length , cm
N Number of species
p Persistence length of Eq.(13), 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/V · s
U Convective velocity, $u\left(\frac{V}{L}\right)$
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_c Column porosity
ε_p Gel porosity
ϕ Ratio of electrophoretic convection velocities
λ Eigenvalue
μ Mobility in the gel, cm^2/V · s
μ_o Mobility in free solution, cm^2/V · s
τ Dimensionless time in the gel particle
Ψ Electrophoretic potential, V

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