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Effects of Surface Geometry on Polyelectrolyte Adsorption

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Abstract : For the adsorption of polyelectrolyte at the surface of polyacrylamide gel particle, preferential adsorption of the large polyelectrolyte such as DNA is governed by the surface area of an adsorbent. The adsorption equilibrium constant can be varied by surface geometry of porous polymer, and it can be described as a function of ionic strength and surface area. Physical parameters affecting the adsorption were estimated using the theoretical governing equation of polyelectrolyte which electrophoretically moved along the column, and geometrical surface area was estimated by Waldman-Mayer's physical model. The separation of polyelectrolytes was studied using the physical parameters estimated by ionic strength and surface geometry.

1. Introduction

The gel electrophoresis commonly provides the highest resolution in the purification of polyelectrolytes, but it has extremely poor scaling properties so that it has been impossible to adapt this milligram bench technique to multigram preparative separations. On the other hand, gel chromatography has far superior scaling properties and a resolving power that is second only to gel electrophoresis. But the electrochromatography by the combination of electrophoresis with chromatography would show better separation, it may be because field-induced dispersion is virtually eliminated by proper manipulation of an electric field and amplify the resolving power of each while retaining the superior scaling properties of chromatography. Therefore, the separation process of electrochromatography needs to be known about 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 polyelectrolyte moves headfirst through the pores of the gel. This intraparticle convective velocity plays an important role to enhance the separation of polyelectrolytes in the column, many researchers have tried to innovate the ideas to create new materials or new apparatus for the intraparticle velocity.

In previous studies of convective-diffusive mass transport in the area of separation engineering, the packing materials with high porosity lead to an efficient separation in the chromatography due to convective velocity. For example, researchers[1, 2] proved that intraparticle convection effects in "Large pore" packing materials in the gel chromatography make the separation of proteins possible by reducing the broadness of peak in the packed column. Carta[3] analyzed the effects of intraparticle convection for the dynamic capacity of the adsorption bed using the LDF (Linear Driving Force) approximation in permeable support. Rodrigues et al.[4] investigated the intraparticle convection in "Large pore" packing material "Hyper D media" enhanced the performance for the separation of proteins in the chromatography column.

But the motivation of this paper is to separate large polyelectrolyte such genome DNA using the

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electrochromatography mentioned earlier. Simply speaking, the genome DNA can't enter the pores of gel particles packed in the column and they can be separated only through the jammed spaces between gel particles due to interactions of particles. In this case, the separation of genome DNA becomes predominated by the adsorption of gel particle. This phenomena of adsorption in the column plays very important role by the geometircal surface area. This kind of idea has rarely tried in the area of colloid science. Therefore, the objective of this paper is an initial step for genome DNA to separate using external interations between gel particles. In this case, the surface geometry of polymeric gel particle is important because the solute is DNA which contains lots of surface charges.

In the experiment performed in this study, the displacement of polyelectrolyte was observed visually in gel particle column. This method provides us a preliminary idea about the motion of polyelectrolyte in the column packed with gel particles under several experimental conditions such as salt, pH, electric field, particle size and polyelectrolyte concentration. For example, ionic strength effects on the displacement of polyelectrolyte can give crucial clue for estimating the adsorption equilibrium constant of polyelectrolyte.

Therefore, the adsorption equilibrium constant will be applied for the development of theoretical model using the semi-empirical model of conventional. The adsorption of polyelectrolyte which deals in this paper is the application of "Colloid and Interface Science". The adsorption by the Stern layer and an electric double layer will be considered to count the adsorped thickness onto the polymeric surface.

2. Theory

2.1 Estimation of equilibrium constant

The equilibrium constant between the gel particle and polyelectrolyte was semi-empirically estimated using the governing equation in the column. The propagation distance from the top of the column to the leading edge of polyelectrolyte displacement was measured in the column. It varies strongly depending on the salt concentration and surface area of the gel particle. The propagation speed of polyelectrolyte in the column for the equilibrium conditions is given by

$$\frac{\partial c}{\partial t}+u\left(\frac{V}{L}\right)\frac{\partial c}{\partial z}+\frac{1-\varepsilon}{\varepsilon}\frac{\partial n}{\partial t}=0 \qquad (1)$$

where the dispersion coefficient is negligible because the transport rate of polyelectrolyte is very slow in the presence of an electric field. The Langmuir adsorption formulation becomes the linear equilibrium relation because the adsorption of DNA is very strong.

$$n = Kc.$$

With boundary conditions

$$c(z,t) = c_0 \quad \text{for} \quad t/\sigma < z$$
$$c(z,t) = 0 \quad \text{for} \quad t/\sigma > z$$

The propagation speed is

$$\sigma(I,A) \equiv \frac{\partial t}{\partial z} = \frac{1+\left(\frac{1-\varepsilon}{\varepsilon}\right)K(I,A)}{u\left(\frac{V}{L}\right)} \qquad (2)$$

The equilibrium constant "K" is given by

$$K(I,A) = \frac{\sigma(I,A)u\left(\frac{V}{L}\right)-1}{\left(\frac{1-\varepsilon}{\varepsilon}\right)} \qquad (3)$$

where "A" is the external surface area of the gel particle and $U\left(=u\left(\frac{V}{L}\right)\right)$ is the free solution mobility in external space. The free solution mobility "U(I)" is strongly dependent upon the ionic strength of buffer solution. The ionic strength dependence of free solution mobility was calculated using the analytical solution of Overbeek[5] as discussed in following section.

2.2 Equilibrim constant vs. ionic strength

The equilibrium costant (K) for polyelectrolyte can be determined from

$$-RT \ln K = \Delta G^{\circ} \quad (4)$$

The electrostatic interaction between the solvent and the ionized dipole was adapted from the theory of Kirkwood.[6] The free energy of binding could be significant since the electrostatic force becomes large due to the large dipole moment of polyelectrolyte. In this case, the Debye-Huckel term is assumed to be negligible, the increase of hydrophobic free energy required for polyelectrolyte interactions in the presence of salt is related to the increase in the surface of contact between salt and polyelectrolyte. The interaction of polyelectrolyte can be related to the salt-induced increase in free energy by enlarging the surface cavities which contain the bulky solute molecules. Therefore, an overall expression for polyelectrolyte adsorption equilibrium constant can be described as a function of ionic strength as

$$RT \ln K = C + DI \quad (5)$$

where C and D are empirical constant.

2.3 Free solution mobility vs. ionic strength

The equation for the mobility of solute in free solution for non-symmetrical electrolyte was given by

$$U = \frac{e\zeta}{6\pi\eta}\left[f_1(\kappa a) - (z_- - z_+)\frac{e\zeta}{kT} f_2(\kappa a) - \frac{z_+ z_- + z_+ z_-}{z_+ + z_-}\frac{\varepsilon\kappa T}{6\pi\eta}\left(\frac{e\zeta}{kT}\right)^2 f_4(\kappa a)\right] \quad (6)$$

where can be expressed as $\rho = \frac{N_A e^2 z}{\lambda_0}$, λ_0 is the limiting equivalent conductance of the ions, it was obtained by Abramson.[7] $f_1(\kappa a)$ was previously calculated by Henry[8] as follows.

$$f_1(\kappa a) = 1 + \frac{(\kappa a)^2}{16} - \frac{5(\kappa a)^3}{48} - \frac{(\kappa a)^4}{96} + \frac{(\kappa a)^5}{96} - \left\{\frac{(\kappa a)^4}{8} - \frac{(\kappa a)^5}{16}\right\} e^{\kappa a}\int_{\kappa a}^{\infty}\frac{e^{-t}}{t}dt \quad (7)$$

where $f_2(\kappa a)$ and $f_4(\kappa a)$ were tabulated by Overbeek.[5] They can be determined from Tables of Overbeek.[1] The value of is 0.460 for 1:1 electrolyte.

The thickness (κ) of the diffuse layer is

$$\kappa^2 = \frac{e^2\sum_{i=1}^{N} z_i^2 C_{ai}}{\varepsilon kT} \quad (8)$$

where C_a is expressed as the number of ions per cubic meter, and C_a is related to the molar concentration M_i of the ions by $C_a = 1000MiNA$. The molarity of buffer ions was calculated from the dissociation constant for each acid and base.

2.4 Estimation of the external particle surface area

A random network of polymer chains has strongly irregular shapes in those regions where the chains are physically or chemically connected. Weakly hydrophobic polymers like polyacrylamide give rise to swelling of gel particles in solution as shown in Figure 1 and the degree of swelling is the highest at the side facing the solution. The surface areas of porous adsorbent can be different according to the gel concentration of gel particle. It is necessary to estimate the surface area because adsorption estimated through the geometrical model of Waldman-Meyer.[9] The geometrical parameters were experimentally measured in the chromatographic column by Waldman-Meyer.[9]

Porath[10] found that the pores in the gel filtration particle become narrower with depth and were thus visualized as conical cavities. Waldman-Meyer[10] has developed a conical shaped pore model as shown in Figure 1 and tested this model with experimental data. Total volume is described by the sum of polymer matrix volume (V_m), void volume (V_i). The void volume and polymer matrix volume were measured experimentally using the volume of elution curve which is used. The volume of conical pores in gel particle was written by Waldman Meyer[10] from simple geometric-consideration as

$$V_i = \frac{N\pi h R_x}{3} \quad (9)$$

The volume of the gel phase was given by

$$V_i + V_m = \frac{N\pi}{3}\left(h + \frac{r}{\sin\theta}\right)\left(R_x + \frac{r}{\cos\theta}\right)^2 \quad (10)$$

The angle of the conical pore was

$$\tan\theta = \frac{R_x}{h} = \frac{R_x + \frac{r}{\cos\theta}}{h + \frac{r}{\sin\theta}} \quad (11)$$

where "h" is the height of the cone "R_x" the radius of conical shape, "N" number of conical cavities, "r" the radius of the hydrated polymer which constitutes the matrix and is one-half of the solid angle subtending the cone. The geometrical dimensions for the present study were calculated from the above Eqs. (9)-(11) through measured experimental data of V_m and V_i. Table 1 lists the dimensions from the experimental data. The surface area was calculated from the data and Figure 2 shows that increasing the exclusion limit to the gel particle as decreasing exponentially the surface area of the gel particle. The conical pores in the polyacrylamide gel were found to be tapered with slight angle.

2.5 Semi-empirical equation of equilibrium constant

The measured equilibrium constant increases in accordance with increasing ionic strength. This can be described by Eq.(5) and it shows that the adsorption of DNA increases with higher ionic strength and less porosity of gel particle. This equation can be used as a guide to consider adsorption effects of DNA in the packed column. The empirical equation is given by

$$\ln K(I,A) = (-687510A+74.113)I+451.48A+0.52325 \quad (12)$$

where "A" is the surface area (cm^2) of a porous gel particle.

3. Experimentals

3.1 Materials

48.5 kbp DNA was purchased from Bethesda Research laboratories (BRL, Gaithersberg, MD) and T4, T5, and T7 DNA were purchased from Sigma Chemical Co.. DNA stock solution (400-500g/ml) stored in the refreigerator at 4C in Tris buffer (10mM Tris, 1mM EDTA and pH8.0) were used. The samples used in the gel column were prepared by diluting the stock solution with Tris buffer solution of same concentration to the desired concentration. The buffer solution used for DNA experiments in the gel was Tris Boric buffer (50mM tris base, 50mM boric acid and 1mM EDTA: pH8.0). Precipitated DNA was prepared by heating in the presence of 0.5M $MgSO_4$ at 6℃ for 10min.

3.2 Gel matrix

Polyacrylamide Bio-Gel filtration particles were obtained from BioRad Co.. These gel particles are normally nonionic in character, and they were found that do not distort under high electric fields. The size of particle diameters used was in the ranges of 40-180m. The size distributions for these gel matrix were not available from the manufacturer. P4, P6, P10 of polyacrylamide brand (BioRad. Co.) have exclusion limits of 2,000, 4,000, 10,000 respectively. Their pore size and surface area can be different as shown in Table 1.

To prepare the DNA-agarose gel for continuous gel electrophoresis, a weighted amount of agarose

Table 1. Comparison of Conical Shape Properties in Different Gel Particles

Type of Bio P Gel	P4	P6	P10	P30
Angle of conical shape (degree)	77	89	134	183
Bed porosity (ε)	0.309	0.401	0.324	0.330
Depth of conical shape (㎛)	62.6	52.8	39.9	42.7
Surface area of porous particle ($cm^2, \times 10^{-3}$)	0.314	0.275	0.127	0.057

was dissolved in a calculated volume of deionized water by boiling in the heater. The amount of solvent lost by evaporation was checked and found to be negligible. The agarose was Seakem LE agarose, purchased from FMC Co.. Typical properties include gelling temperature at 1% solution of 36-42C and electroendosmosis of 0.1-0.15. All other chemicals were reagent grade. Electrophoresis was performed in a horizontal gel apparatus and a vertical electrophoresis chamber purchased from BioRad Co.. Following electrophoresis, gels were stained with ethidium bromide (0.5mg/ml) and observed using an ultraviolet transilluminator.

Once the gel particles were settled, the column was inserted into a vertical BioRad electrophoresis

chamber. Electrophoresis was carried out at pH8.0. The temperature of the circulator was kept at 18C. The gel particle media used was impermeable to the DNA. The exclusion limit of Bio-p6-Gel is 5bp (from data of BioRad Co.,) in comparison with 410bp of 1% agarose gel (Edmondson et al.[11]). The columns were made of quartz glass in order to facilitate the detection of DNA under UV light.

3.3 DNA adsorption

The equilibrium studies were performed in the column with internal diameter of 0.25cm and with 12.5cm length. The adsorption of DNA was studied in buffer of different ionic strength. DNA displacement was a measurement of distance to the leading end of the DNA from the top of the column.

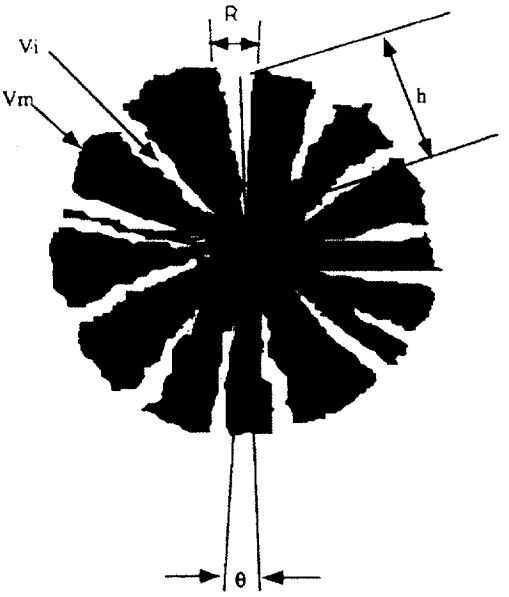

Fig. 1. Schematic representation of gel particle.

4. Results and Discussion

The DNA displacement measured in the packed column is the distance to the leading end from the top of column. DNA displacement was found to be strongly dependent on the total surface charge of the gel particles and the surface area of the gel particles. The polyacrylamide particles used in the present study are weakly hydrophobic and are made by copolymerization of acrylamide and N-N'-methylene-bis-acrylamide. Polyacrylamide is normally nonionic in solution, but it does have carboxylate groups and amide groups. It has a tendency to develop charges of anionic character through the dissociation of ionizable carboxylate groups of polymer.

DNA adsorption in the charged gel particles is proportional to the surface area of the gel particle. Polyacrylamide gel particles swell in solution and the pores are generally more open at the surface than those in dried state as shown in Figure 1. The polyacrylamide has a large swelling factor of 1.5 (Richards et al.[12]) in solution. The degree of swelling becomes highest at the gel surface facing the solution. The shape of the pores seems to be conical, and the external surface area of the gel particle varies with the exclusion limit. Therefore, DNA adsorption may be varied. In other words, less porous gel particles have large total surface charge and thus more DNA may adsorb onto the surface.

A method to estimate the external surface area is the geometrical exclusion model of Waldman-Meyer.[9] This allows the external surface area of the porous particle to be determined semi-empirically. The polymer matrix and inclusion volumes of the gel particles were measured. The geometrical dimensions such as diameter and depth of pore were estimated through Eqs. (9)-(11). The calculated results are listed in Table 1 for the different porous particles. It can be seen in Figure 2 that the surface area of the gel particles decreases exponentially as the exclusion limit increases.

The double layer surrounding the gel particle becomes thinner in higher ionic buffer solutions. The higher , the more counterions accumulate in the adsorption layer of the diffuse double layer. of Eq.(8) is a parameter describing the extent of screening of ionic charges by other ions. Thinner double layer enhance coion-dipole electrostatic attractions because equally charged gel particles can approach each other closely before

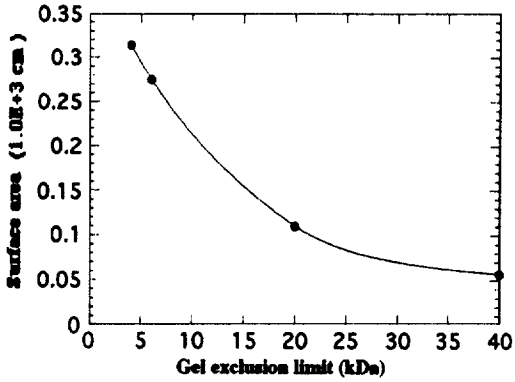

Fig. 2. Geometrical surface area in porous gel particle.

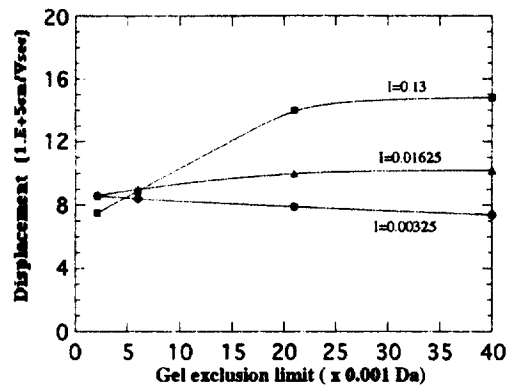

Fig. 3. Gel porosity vs. ionic strength at pH=8.2.

electrostatic repulsion is felt. Adsorption occurs on the external surface of gel particle because large DNA does not penetrate into inside the pore space. Therefore, the thickness of the adsorption layer is strongly dependent on the ionic strength and its value can be calculated numerically from Eq.(8). Surface charge due to adsorption tends to increase up to saturation of the surface. The adsorption layer thickness is reduced from 6 angstrom to 3.9 angstrom when the molar concentration of buffer solution is changed from 0.01M to 0.05M by Eq.(8). DNA adsorption may favor the accumulation of counterions close to the surface, and the electric double layer potential is reduced further away from the surface.

DNA adsorption increases exponentially due to an increase in charged surface area of porous gel particle. DNA displacement becomes less broad due to larger molecular adsorption on particles with larger surface area of gel particle. In highly dilute buffer solutions, decreasing the exclusion limit increases slightly DNA displacement as shown in Figure 3. The ionic interaction force becomes negligible, while approach between gel filtration particle and DNA becomes repelled each other. The electrical double layer of counter ions around a charged particle can be quite extensive, up to about a few nm, and DNA cannot approach each other very closely in this level of concentration. DNA adsorption significantly varies with different salt concentration and different surface area. At larger exclusion limits the dependence of DNA displacement is reversed with ionic strength, for example, increasing ionic strength increases the DNA displacement due to the increased the gel exclusion limit as shown in Figure 4. This displacement of DNA in column with large porous particles seems to be in agreement with the dependence of electrophoretic mobility in solution.

When 10l pulse of 500/ml DNA solution was loaded into the packed column in 0.0025-0.1M Tris-Boric buffer, pH 8.2 and 0.0025-0.4M $MgSO_4$, Increasing salt concentration in the eluent increased the equilibrium constant determined by elution column as shown in Figure 5. The slope of plot in different salt concentration was obtained by plotting the equilibrium constant vs. salt concentration. The slope of lot in TBE buffer

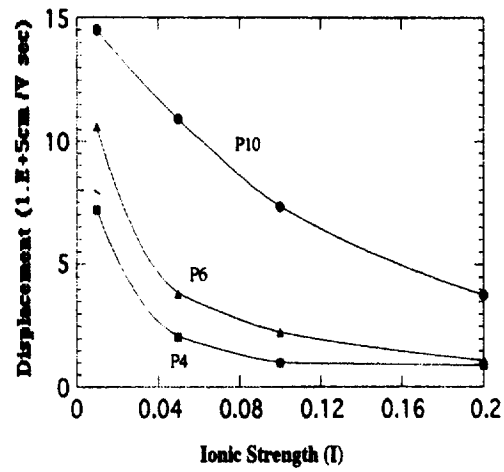

Fig. 4. DNA displacement as a function of ionic strength and gel exclusion limit.

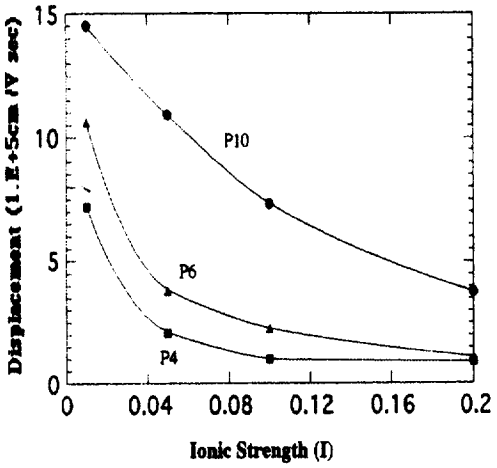

Fig. 5. DNA displacement in divalent ion in pH=8.2.

is 7.89, while that in magnesium sulfate solution is 14.19. DNA in high magnesium sulfate solution adsorbs more significantly onto the surface. This indicates that the repulsive hydration force significantly increases at the salting-out salt which has high surface tension increment. This case is equal to the phenomena of the increase of hydrophobic force. The use of divalent salt may promote DNA adsorption, and DNA displacement measured experimentally was significantly reduced with an increase of divalent ions as shown in Figure 5. These divalent ions may induce DNA adsorption by electrostatic interactions. The electric double layer is reduced in the divalent ions from Eq.(8).

The addition of magnesium sulfate in buffer increases free energy with increase of valence and of ionic strength due to high dissociation constant. Multivalent cations, for example, divalent cations of Mn^{++}, Ca^{++}, Mg^{++} and trivalent Al^{+3}, are used for promoting DNA adsorption. These metal ions can bind quite strongly to carboxylate groups of hydrolyzed polyacrylamide gel. The role of these ions is to serve as links between anions of DNA and negative surface charge sites of polyacrylamide gel particles. Therefore, the interactions of gel particle surface appears to be DNA binding onto the gel surface in which coulombic, hydrogen bonding, and hydrophobic interactions are amalgamated.

The effects of pH on DNA adsorption were performed for the separation of large DNA in the packed column. Although the net charge on the polyacrylamide is negative due to the carboxylic groups, the negative charge on the packing may be reduced in the acidic pH range. This in turn seems to increase electrostatic interactions with the cation of polyacrylamide gel because the anions under strong acid are reduced in the polyacrylamide gel. The displacement in low pH for DNA is connected with the deionization of phosphate groups whose pH is 1 to 3. This makes DNA displacement lower in the packed column as shown in Figure 6. At pH values exceeding 9, an increase in DNA displacement is attributed to the ionization of the acidic groups of DNA as DNA tends to be more anionic. Therefore, DNA displacement is related to the ionization of acidic and basic groups. An increase of DNA displacement can be attributed to the ionization of the functional groups of DNA. The electrostatic attraction of DNA to the gel particle can be controlled by adjusting the ionic strength of the solution rather than pH so that the DNA and gel have different electrical charges.

The physical effects of DNA displacement arise from an electric field, and DNA displacement seems to be between free solution and continuouse agarose gel as shown in Figure 7. Therefore, the physical interactions between them can be another important factor to separate large DNA due to the size of gel particles packed in the column. When different electric fields of 8 to

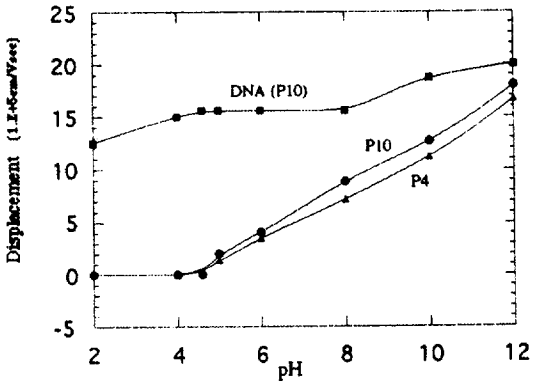

Fig. 6. pH effects of DNA displacement.

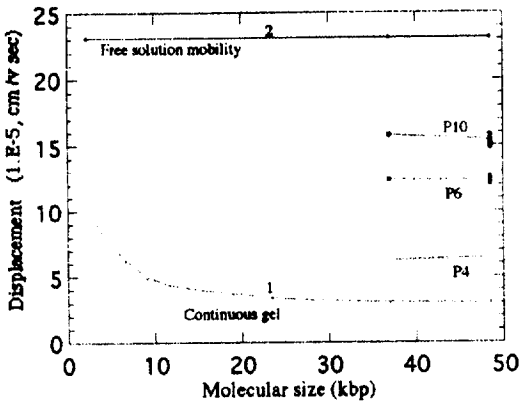

Fig. 7. Effects of DNA molecular size at pH=8.2, I=0.05M

24 V/cm were applied, the displacement of DNA in the packed column was observed to be independent upon an electric field. The electric field effects by different DNA concentration in the ranges of 1 to 430g/ml were investigated. Increasing DNA concentration may lead to DNA aggregation, it may affect DNA motion in the gel particle system. DNA in high concentration may promote aggregation by electrostatic interactions of DNA, and it may alter the radius of gyration and may take place more adsorption onto the gel particle from the increase of total charges.

It can be considered that the size of aggregated DNA may not grow big enough with DNA concentration and that the adsorption due to aggregation onto the gel surface may not be large enough. Theoretically, the dimensions of a random coil molecule in solution depend on both the solvent quality and the concentration of polyelectrolyte. The aggregation effects by DNA concentration up to 430μg/ml are not significant to improve DNA adsorption onto the gel surface. If DNA aggregation is a significant factor, the DNA adsorption may be increased due to high surface charges of DNA. In the space between gel particles was found to be bigger than area occupied by individual DNA. The external area was 1,000 times bigger than the area occupied by DNA. DNA displacement at elevated temperatures in the presence of divalent metal salt was found to be independent of an electric field, although we expected it to be varied by the increase of surface interactions due to be more aggregation.

5. Conclusion

The surface adsorption of large polyelectrolyte was studied with gel particles in the packed column. A factor affecting the transport of large DNA in the column packed with gel particles is adsorption, which can be different with pH, ionic strength and pore size of gel particle. DNA adsorption was found to be strongly dependent on the surface area of the gel particle as well as it could be enhanced by the kind of salt.

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