Korean J. Chem. Eng., **19**(4), 663-668 (2002)

Separation of Mixed Solutes Using Reciprocating Size Exclusion Chromatography: Computer Simulation Based upon Experimental Parameters

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(*Received 22 January 2002 • accepted 22 March 2002*)

Abstract−Mixed solutes of different molecular size were separated by reciprocating size exclusion chromatography and the results were compared with conventional size exclusion chromatography with repeated feeds using computer simulation. Operational conditions, such as overlapping between elution curves and diffusivities of solutes, were varied in simulation. Simulation showed the separation efficiency was higher in RSEC than SEC with repeated feeds in the region of high diffusivity of either solutes at low degree of overlapping. Further study to improve RSEC by employing temperature swing is being made.

Key words: Chromatography, Reciprocating Size Exclusion Chromatography, Computer Simulation, On-line Separation

INTRODUCTION

Chromatography has been a leading method in biotechnological separation, especially in protein separations [Janson, 2001; Hong and Row, 2002]. Conventional size exclusion chromatography (SEC) is operated in peak mode, by which solutes of different molecular size are separated from each other and exit from the column as separated peaks. Since conventional size exclusion chromatography has several limitations, such as low productivity and batch operation, it is mainly applied for analytical use, not much for preparative use. For preparative purposes, novel reciprocating size exclusion chromatography (RSEC) was developed [Chang and Koo, 1999]. Solute separation in RSEC is achieved by frontal mode operation, and solutes of different size proceed along the column, forming fronts of their own. The fact that the preceding front in a column is pure in large molecules, and is far ahead of the following front of small molecules triggered the concept of the RSEC to recover large molecules from the mixture solution on-line. RSEC was applied to recover large molecules on-line from the mixture solution [Kim et al., 2000]. And RSEC and SEC with repeated feeds were applied to separate mixed solutes of large molecules (Blue Dextran) and small molecules (vitamin B_{12}) [Kim et al., 1999]. A comparative study of RSEC and SEC with repeated feeds to separate mixed solutes showed that recovery rate of large molecules in RSEC was higher than that in SEC with repeated feeds. But recovery rate of small molecules in SEC with repeated feeds was higher than that in RSEC.

The separation of the RSEC was expected to be enhanced by changing the operating conditions, such as diffusivities of solutes and timing schedules. In this study, experimental comparisons between RSEC and SEC with repeated feeds were made along with the computer simulation to predict their performances with varying operating conditions. Operational parameters and basic data for simulation were obtained from experiments.

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EXPERIMENT

1. Materials

A commercial polyacrylamide gel, Bio-Gel P-10 (Bio-Lad, fractionation range: 1,500-20,000), was packed in a water-jacketed column (Pharmacia SR 10/50). The packed gel layer was compressed from both sides of the column by using plungers, with the degree of compression of 0.82. The final length of the compressed gel was 24.5 cm. The temperature of the column was kept at 5 °C using a constant-temperature water bath (Cole-Parmer) and a water jacket.

Elution behavior in an SEC column [1 cm (ID)×24.5 cm] was tested by using Blue Dextran (Pharmacia, MW: 2,000,000) and vitamin B_{12} (BDH Laboratory Supplies, MW: 1355.38) as standard materials for large and small molecules, respectively. The feed concentrations of Blue Dextran and vitamin B12 were 0.2 and 0.02 g/*l*, respectively. Distilled water was used for the eluent. The eluent flow rate was 0.42 ml/min in both directions. The reciprocating operation was carried out by using a high-pressure pump (Eldex) and a multi-way valve (Cole-Parmer) on each side of the gel-packed column, controlled by a multiport programmable timer (ChronTrol).

2. Operation

In general, size exclusion chromatography is operated in peak mode, where solutes of different molecular size in a pulse of feed are separated from each other and exit from the column as separate peaks. In SEC with repeated feeds (Fig. 1), the feed mixture was fed to the column as a pulse, followed by an eluent. The isolated band of the small molecules (vitamin B_{12}), following the band of pure large molecules (Blue Dextran), was isolated into the small solute tank. The durations of the feed pulse and the elution in each cycle were scheduled so that the band of pure small molecules was touched at the base line by the following band of pure large molecules from the next cycle at the exit of the column. In SEC with repeated feeds, the cycle time was defined as the time interval between elution curves of neighboring cycles.

In frontal mode, solutes of different size in a step feed proceed along the column, forming fronts of their own. In RSEC (Fig. 2), the large molecules (Blue Dextran) were isolated from the mixture

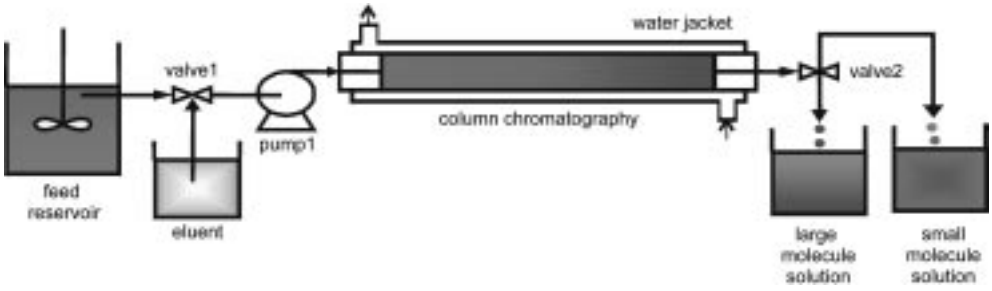

Fig. 1. Schematic drawing of SEC with repeated feeds.

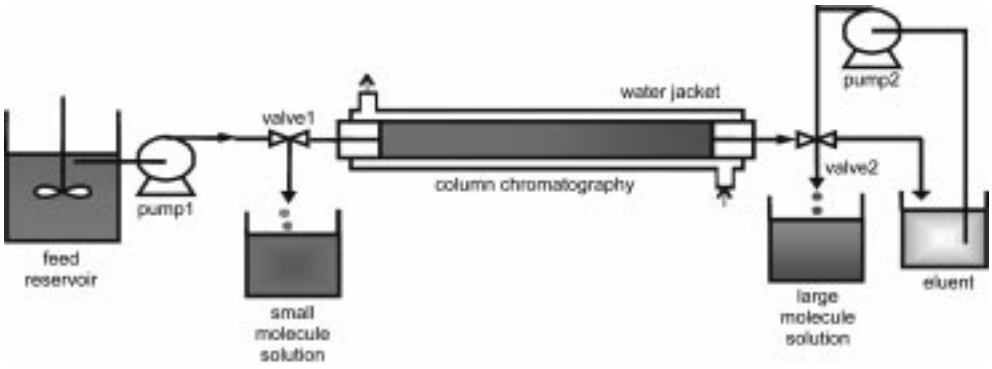

Fig. 2. Schematic drawing of RSEC.

by repeating cycles of feeding mixture solution. The large molecules were isolated into a large solute tank during the forward flow period in frontal mode. The following slow-moving portion of unseparated mixture solution, which remained in the column, was returned to the reservoir by backward flow. During the backward flow period, the slow-moving small molecules (vitamin B_{12}) lagged behind the unseparated mixture solution and were isolated into the small solute tank at the left side of the column in Fig. 2. In RSEC, the cycle time was defined as the time interval from the beginning of the elution front of fast-moving large molecules during the forward flow to the end of the elution curve of slow-moving small molecules during the backward flow. The solvent eluted before the band of large molecules during the first half cycle was gathered in the solvent reservoir and reused as eluent for the backward flow during the second half cycle.

MATHEMATICAL MODELING

Mathematical modeling was required to predict the separation behavior of RSEC and SEC with repeated feeds. For this purpose, we used the local equilibrium model to represent the mass balance in the column [Jonsson; Pharmacia]. A model equation based upon local equilibrium model was used for computer simulation.

$$\frac{\partial C}{\partial t}+\frac{(1-\varepsilon)}{\varepsilon}\gamma K_d\frac{\partial C_s}{\partial t}+\rho_s\frac{(1-\varepsilon)(1-\gamma)}{\varepsilon}\frac{\partial N}{\partial t}+u\frac{\partial C}{\partial z}-(E_D+D_M)\frac{\partial^2 C}{\partial z^2}=0 \tag{1}$$

The first term represents accumulation of solute in mobile phase, the second term represents accumulation of solute in stationary liquid phase, and the third term is adsorption related term of solute, representing accumulation of solute in stationary phase of gel. The fourth and fifth terms express convective mass transfer and mass transfer by axial dispersion and diffusion, respectively. The third term can be omitted on the assumption that there is no adsorption between solute and gel in SEC, that is, solute is separated only by pore size of gel and size of solutes. Assuming that the solute concentration in stationary liquid phase of gel is same as that in mobile phase in the case of rapid equilibrium, the mass balance equation of SEC is simplified as follows [Koo, 1985].

$$\left(1+\frac{(1-\varepsilon)}{\varepsilon}\gamma K_d\right)\frac{\partial C}{\partial t}+u\frac{\partial C}{\partial z}-(E_D+D_M)\frac{\partial^2 C}{\partial z^2}=0 \tag{2}$$

$$K_d=\frac{V_e-V_o}{V_s}=\frac{V_e-V_o}{V_t-V_o-V_g} \tag{3}$$

If Blue Dextran is large enough to be excluded from gel pore and vitamin B_{12} is small enough to pass the gel pore, K_d value of Blue Dextran and vitamin B_{12} becomes 0 and 1, respectively. Therefore the mass balance equation of Blue Dextran and vitamin B_{12} is expressed as the following equations.

$$\text{Blue Dextran: } \frac{\partial C}{\partial t}+u\frac{\partial C}{\partial z}-(E_D+D_M)\frac{\partial^2 C}{\partial z^2}=0 \tag{4}$$

$$\text{vitamin } B_{12}\text{: } \left(1+\frac{(1-\varepsilon)}{\varepsilon}\gamma\right)\frac{\partial C}{\partial t}+u\frac{\partial C}{\partial z}-(E_D+D_M)\frac{\partial^2 C}{\partial z^2}=0 \tag{5}$$

The followings are the initial and boundary conditions of the system.

$$\text{Initial condition: } C_i(z,0)=0 \tag{6}$$

$$\text{Boundary condition: } C_i(0,t)=C_i^o,\ 0<t\le t_{inj} \tag{7}$$

$$\frac{\partial C_i}{\partial z}=0,\ z=L \tag{8}$$

E_D and D_M, axial dispersion and eddy dispersion, respectively, were summed into $D_{a,i}$ assuming rapid equilibrium between stationary phase and mobile phase.

$D_{a,j}$ value was evaluated as follows [Row, 1999].

$$R = \frac{2\Delta t}{w_1 + w_2}\left(\text{if } w_1 = w_2 = w, R = \frac{\Delta t}{w}\right) \quad (9)$$

$$N_1 = \left(\frac{T_r}{\sigma}\right)^2 = 16 \times \left(\frac{T_r}{w}\right)^2, \quad (10)$$

$$N_1 = 5.54 \times \left(\frac{T_r}{w_{1/2}}\right)^2 \text{ (in case of excessive tailing of peak)} \quad (11)$$

$$H = \frac{L}{N_1}, D_{a,i} = \frac{HL}{2T_o} = \frac{Hu}{2} = \frac{Lu}{2N_1}, T_o = \frac{L}{u} \quad (12)$$

COMPUTER SIMULATION

Mathematical modeling based upon local equilibrium model was applied to size exclusion chromatography. Fortran language, Microsoft Fortran Powerstation 4.0, was used for numerical analysis of the chromatographic system. The Thomas Method was applied to obtain the concentration profiles. Crank & Nicolson Method was used for numerical calculation of elution curves. Product mass was obtained by multiplying elution volume and concentration of product by using the Simpson Method [Riggs, 1995].

The diffusivities of Blue Dextran and vitamin B_{12} were determined from the experimental elution curve with feed pulse of 1.5 min. $D_{a,i}$ values of Blue Dextran and vitamin B_{12} were calculated from equations, and ε, γ and u values were obtained from experiments, as shown in Table 1.

Calculated elution curve in simulation was compared with experimental one in Fig. 3. The simulated elution curves for RSEC and SEC with repeated feeds are given in Figs. 4 and 5, respectively. Period of feed pulse for complete separation between Blue Dextran and vitamin B_{12} in SEC with repeated feeds was experimentally determined to be 10.0 min with the given column of 24.5 cm. This duration of the feed pulse in each cycle was determined so that the peak of pure separated solutes in the elution curve were overlapped by 0.005% of initial concentration of solutes from the base line with the peaks from next cycle. As 0% is unable to be defined, 0.005% was chosen for complete separation of solutes.

Table 1. Operational parameters for simulation

ε	0.382
γ	0.795
u	1.4 cm/min
$D_{a,BD}$	0.060 cm^2/min
$D_{a,vitB12}$	0.082 cm^2/min

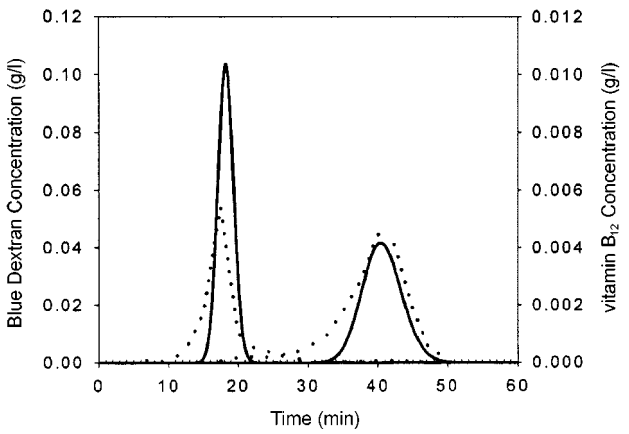

Fig. 3. Experimental and Simulated SEC elution curve with pulse input of 1.5 min.
—: Calculation, ·····: Experiment

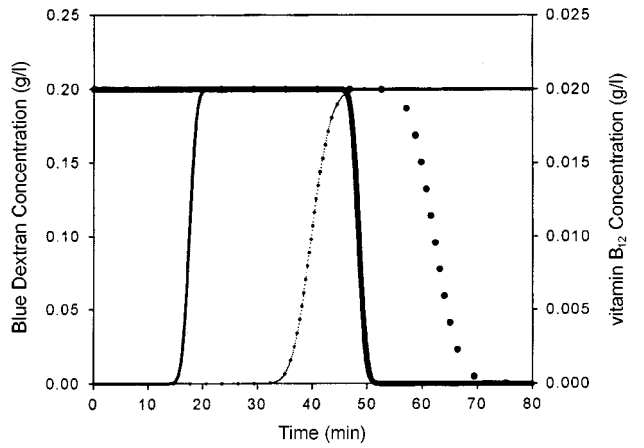

Fig. 4. Simulated elution curves of Blue Dextran and vitamin B_{12} for RSEC.
—: Blue Dextran, Forward, ·····: vitamin B_{12}, Forward, —: Blue Dextran, Backward, ·····: vitamin B_{12}, Backward

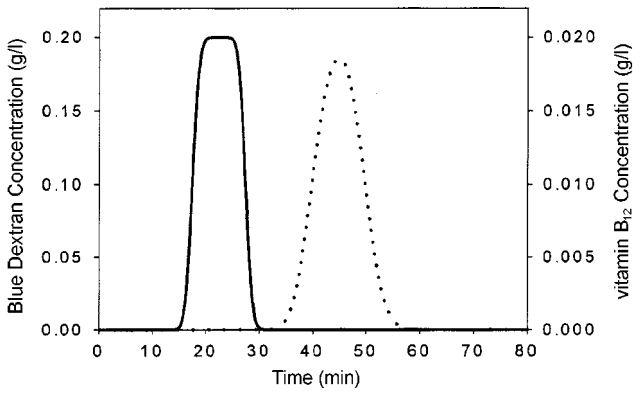

Fig. 5. Simulated elution curves of Blue Dextran and vitamin B_{12} for SEC with repeated feeds.
—: Blue Dextran, ·····: vitamin B_{12}

RESULTS AND DISCUSSION

The degree of overlap between each peak of two solutes in the elution curve was varied by changing the period of feed pulse. The degree of overlap and diffusivities of solutes have a direct effect on separation behavior. Experimental comparisons between RSEC and SEC with repeated feeds (SEC-RF) were made along with the computer simulation to predict their performances with varying these operating conditions.

The feed time was varied so that the peak of pure separated sol-

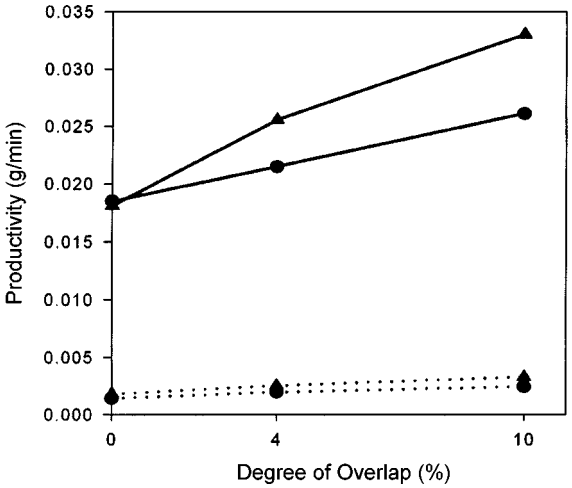

Fig. 6. Comparisons of simulated separation productivities between RSEC and SEC-RF with varying degrees of overlap.
●: RSEC, ▲: SEC-RF, ——: Blue Dextran, ·····: vitamin B_{12}

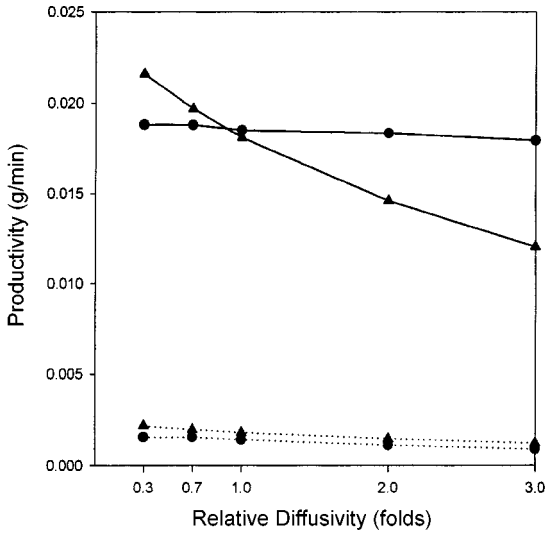

Fig. 7. Comparisons of simulated separation productivities between RSEC and SEC-RF with varying values of diffusivity of Blue Dextran.
●: RSEC, ▲: SEC-RF, ——: Blue Dextran, ·····: vitamin B_{12}

utes in elution curve was overlapped from 0.005 (complete separation) to 4 and 10% of initial concentration of solutes from the base line with the peaks from the next cycle. Productivity (g/min) was defined as the mass of separated product during a cycle per cycle time. In this study, productivity of solute separation represents the separation efficiency. With increasing degree of overlap, the purity and the yield of separated products decrease. The differences in purity and yield between RSEC and SEC with repeated feeds were assumed to be negligible with varying degree of overlap. The simulation results of RSEC and SEC with repeated feeds for productivities with varying degrees of overlap are given in Fig. 6.

Productivities of vitamin B_{12} were higher in SEC with repeated feeds than in RSEC in all degrees of overlap, simulated. This trend was also true with Blue Dextran, except the range near complete separation that is 0% degree of overlap. Though the differences in productivity between RSEC and SEC with repeated feeds with Blue Dextran were larger than those with vitamin B_{12}, the ratio of productivities between RSEC and SEC with repeated feeds was similar in both Blue Dextran and vitamin B_{12}.

In SEC with repeated feeds, the productivity increase is mainly due to the increase of the period of feed pulse which is allowed by increasing the degree of overlap between elution curves of Blue Dextran and vitamin B_{12} with the column of given length (Fig. 5). The productivity increase in RSEC is caused by the increase of product recovery time, which is possible with higher degree of overlap. The product recovery time in RSEC is the time between the edge of pure product and the borderline between the pure product and unseparated zone (Fig. 6). The time location of this borderline moves away from the edge of pure product as the degree of overlap increases. As mentioned earlier, the increase in productivity in both systems is accompanied by the decreases in purity and yield of the separated products.

Diffusion of each solute molecule will broaden elution peaks, and affect the performance of RSEC, as well as SEC-RF. The effect of diffusivities of solutes on both systems was compared with varying diffusivities in simulation. In these simulations, the degree of overlap between elution curves of Blue Dextran and vitamin B_{12} was fixed at 0.005%.

First, the value of diffusivity of Blue Dextran was changed without changes in other values, including the diffusivity of vitamin B_{12} (Fig. 7). As the diffusivity of Blue Dextran increases, the productivities of both solutes decrease in both systems. The productivities of Blue Dextran in RSEC were higher than in SEC with repeated feeds when the value of diffusivity of Blue Dextran was larger than the real value, and vice versa. The productivities of vitamin B_{12} in RSEC were less than in SEC with repeated feeds in all ranges. The fast decrease in productivity of Blue Dextran in SEC with repeated feeds with increasing diffusivity of Blue Dextran is due to the combined effect of less feed amount per cycle and increased cycle time caused by the broadening of elution curves of Blue Dextran and vitamin B_{12}. Howevr, the slow decrease in Blue Dextran productivity in RSEC is due to the fact that the feed amount per cycle does not change. In this case, though, the cycle time also increases according to the broadening of pure Blue Dextran during the forward flow by high diffusivity of Blue Dextran. The comparable decrease in vitamin B_{12} productivity between RSEC and SEC with repeated feeds is ascribed to that the period of product recovery of vitamin B_{12} was shortened due to the broadening of unseparated zone during the backward flow.

The value of diffusivity of vitamin B_{12} was also changed while that of Blue Dextran was fixed at the real value (Fig. 8). The productivities of Blue Dextran in RSEC were higher than in SEC with repeated feeds when the diffusivity of vitamin B_{12} was larger than the real value, and vice versa. The productivity of vitamin B_{12} in RSEC was lower than in SEC with repeated feeds in most ranges. The trend of decrease in productivities with increasing diffusivity of vitamin B_{12} was the same as that of Blue Dextran, except that the decreasing rate of Blue Dextran productivity in RSEC was higher. This is because the period of product recovery of Blue Dextran was shortened due to the broadening of unseparated zone during the forward flow.

The concentration of product with original diffusivities of both

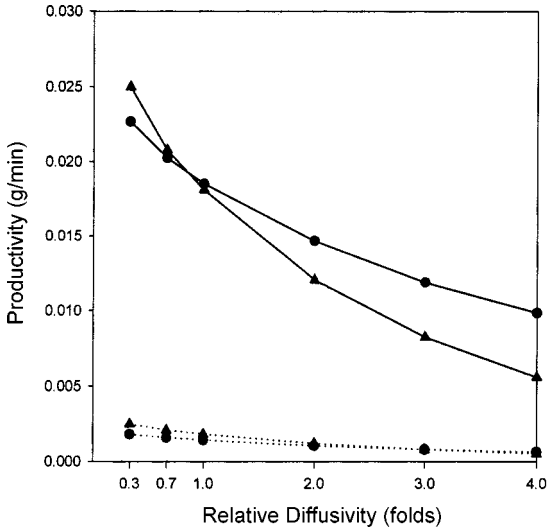

Fig. 8. Comparisons of simulated separation productivities between RSEC and SEC-RF with varying values of diffusivity of vitamin B_{12}.
●: RSEC, ▲: SEC-RF, ——: Blue Dextran, ·····: vitamin B_{12}

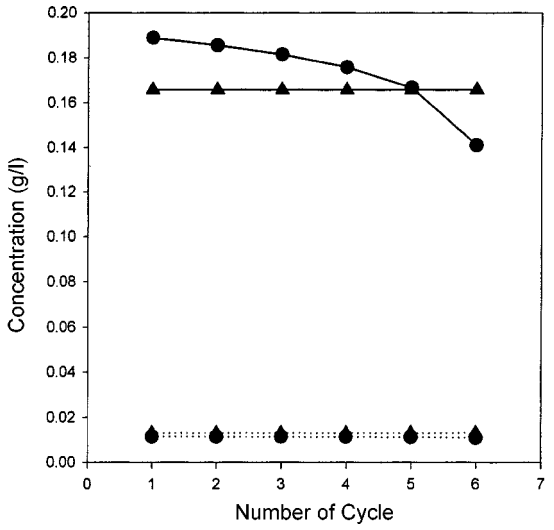

Fig. 9. Comparisons of simulated product concentration between RSEC and SEC-RF.
●: RSEC, ▲: SEC-RF, ——: Blue Dextran, ·····: vitamin B_{12}

solutes at complete separation was compared in Fig. 9. The concentration of separated Blue Dextran as a product in RSEC was higher than in SEC with repeated feeds, with fast decrease as cycle repeats. The concentration of separated vitamin B_{12} was higher in SEC with repeated feeds during whole cycles. The fast decrease of product concentration of Blue Dextran in RSEC is caused by the fact that Blue Dextran concentration in the feed tank decreases as cycle repeats.

As a conclusion, RSEC shows advantage over SEC with repeated feeds in the recovery of large molecules, such as Blue Dextran, when diffusivity of large molecules is relatively high and the degree of overlap between each elution curve of Blue Dextran and vitamin B_{12} is low. The period of product recovery can be increased by changing temperature synchronously with flow reversal. The performance of RSEC is believed to get better in the sense of productivity along with purity and yield by employing this temperature swing, which is being further studied.

NOMENCLATURE

C : solute concentration in the mobile phase
C_i : concentration of solute *i*
C_s : solute concentration in the stationary phase
$D_{a,i}$: apparent dispersion coefficient of *i*
D_M : molecular dispersion
E_D : eddy dispersion
H : HETP
K_d : distribution coefficient
L : column length
N : solute concentration in the solid phase
N_1 : the number of theoretical plates
R : peak resolution
T_r : retention time of solute
ΔT : the difference of retention time of two solutes
t : time
t_{inj} : feed injection time
u : velocity of mobile phase
v : interstitial velocity of fluid
V_e : elution time
V_o : void volume
V_t : volume of total column
V_s : volume of stationary phase
V_g : volume of gel matrix
w : peak width
$w_{1/2}$: width at ½ height of peak
z : axial distance

Greek Letters

ε : interstitial void fraction
σ : standard deviation ($=\frac{1}{4}w$)
γ : intrastitial void fraction
ρ_s : density of the solid phase

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