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MECHANISM OF SIMULTANEOUS SOLUTES SEPARATION AND CONCENTRATION BY SIZE EXCLUSION CYCLIC SEPARATION

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Abstract—The semicontinuous separation/concentration of two solutes with different molecular size by the size exclusion cyclic separation method is based on the opposite swelling responses of two gels to a temperature change. Experimental results of separation and concentration of solutes are verified qualitatively by the theoretical models based on the local equilibrium assumption. Separation of two solutes is shown by the breakthrough curves in coupled gel columns. In closed coupled columns, the volumetric space for the large molecules which are totally excluded from the gels becomes smaller, creating a concentrating effect as the gels swell when temperature changes. A mechanistic model is suggested to predict the large molecule concentration to increase to its solubility limit as cycle repeats.

INTRODUCTION

Cyclic separation is a separation method where a thermodynamic parameter, such as temperature, pressure and pH changes the equilibrium distribution coefficient of the solute cyclically. In parametric pumping, a method of cyclic separation, there is a synchronous change of flow direction and a thermodynamic variable. Details of parametric pumping and cyclic separation were presented elsewhere [1, 2].

Chromatography separates solutes according to the difference in their distribution between the mobile and stationary phases. In size exclusion chromatography, solutes with different molecular size are separated. Small molecules can diffuse into all of the pores while large molecules are excluded from the pores. Since only the volume outside the gel particles is available to large molecules, the largest molecules travel quickly through the column and exit first. The smallest molecules have the entire liquid volume, between and inside gel particles, available and thus take longest to exit the column. Size exclusion chromatography is commonly used for bioseparation, especially for protein desalting. Operating methods are discussed in a variety of articles and books [3, 4].

In spite of its separability with high resolution, chromatography finds limited use in the separation of very valuable products of small volume. It is due to its inherent thermodynamic demerits. In conventional chromatography, an eluent, supplied from external to the system as a separating agent, dilutes the separated solute solution. Removal of this mass agent costs some processes later. Different from other separation methods, such as filtration and extraction, only a portion of packed gels where solute bands stay is engaged in separation in chromatographic process. Above all, chromatography was invented and has been developed as a batch method, which is believed to be against the modern process concept.

The present author and Wankat [5] reported the separation of two solutes (Blue Dextran 2000 and nickel nitrate) in columns packed with Sephadex G-25 and Bio-Gel P-2. These gels show different thermodynamic behavior in water; Sephadex G shrinks and Bio-Gel P swells as temperature increases. Taking advantage of this opposing response to temperature change, the concept of parametric pumping was introduced to the conventional chromatography. The resulting semi-continuous size exclusion cyclic separation method not only separates but concentrates solutes, overcoming the chromatographic inherence.

In this paper, solute separating and concentrating mechanisms in the coupled column system are elucidated, based upon the experimental results.

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Table 1. Column elution studies for Sephadex G-25 (50-150 μm)

ε	0.928		0.893		0.825		0.777	
Temperature(℃)	5	45	5	45	5	45	5	45
Retention vol. (ml)								
Nickel nitrate	18.70	17.40	17.82	16.50	16.25	14.91	14.92	13.70
Blue dextran	7.81	8.15	6.89	7.67	5.51	6.00	4.61	5.02
Calc'd vel. (cm/min)								
Nickel nitrate	0.65	0.72	0.66	0.72	0.64	0.73	0.67	0.74
Blue dextran	1.56	1.52	1.71	1.55	2.00	1.82	2.16	2.02

Column length for gravity settling was 29.1 cm.
Flow rate ranged from 0.44 to 0.46 ml/min.

Table 2. Column elution studies for Bio-Gel P-2 (100-200 mesh)

ε	0.921		0.894	
Temperature (℃)	5	45	5	45
Retention vol. (ml)				
Nickel nitrate	22.88	18.21	22.04	17.75
Blue dextran	6.54	5.18	5.81	4.71
Calc'd vel. (cm/min)				
Nickel nitrate	0.47	0.60	0.47	0.58
Blue dextran	1.65	2.11	1.80	2.11

Column length for gravity settling was 25.4 cm.
Flow rate ranged from 0.45 to 0.46 ml/min.

EXPERIMENTAL

Size exclusion cyclic separation experiments for separating nickel nitrate and Blue Dextran 2000 have been studied. Cross-linked dextran gel (Sephadex G-25, 50-150 μm) from Pharmacia Fine Chemicals and polyacrylamide gel (Bio-Gel P-2, 100-200 mesh) from Bio-Rad Laboratories were used as the stationary phases. Two 1 cm I.D. chromatographic column, equipped with adjustable plungers and a water jacket (Column SR 10/50), manufactured by Pharmacia Fine Chemicals, were used for all experiments. Feed and effluents were pumped by a high-pressure Elalex pump and Milton-Roy minipumps. The entire system was operated automatically with the aid of three-way solenoid valves, pneumatically driven Cheminert six-position rotary valves and Chrontrol programmable timers.

Elution volumes and concentrations of nickel nitrate and Blue Dextran 2000 in aqueous solution were determined on and off line with Perkin-Elmer Lambda 1 single-beam spectrophotometer at wavelengths of 306 and 617 nm, respectively. The concentrations of both solutes were calculated by nonlinear regression since they both adsorb at both wavelengths.

1. Retention Volumes

Retention volumes of two solutes in Sephadex G-25 column are shown in Table 1 while those in Bio-Gel P-2 column are in Table 2. For Sephadex G-25 the retention volume for nickel nitrate and Blue Dextran decreases and increases, respectively, as temperature increases. For Bio-Gel P-2 the retention volumes for both solutes decrease as temperature increases. It is because both solutes move faster with temperature.

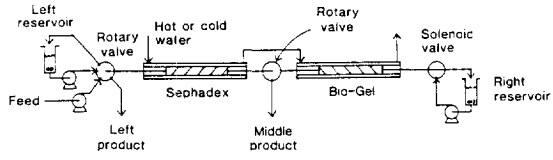

Fig. 1. Schematic diagram for continuous parametric pumping with coupled columns.

2. Coupled Columns System

Two columns packed with Sephadex G-25 and Bio-Gel P-2 separately are connected serially to form a coupled columns system (Fig. 1). Parametric pumping was performed semi-continuously with the coupled column system to take advantage of the differences in swelling behavior of the two gels. From the preliminary elution studies of these gels, Blue Dextran is expected to concentrate at the junction of the two columns (right side of the left column and left side of the right column). Nickel nitrate will concentrate in the left side of the columns. Fig. 2 shows the movements of the solute molecules along the columns in parametric pumping. In the coupled column experiments, both of the reservoirs and the gel columns were filled with water initially. The feed mixture of two solutes was introduced into the left end of the Sephadex G column as a pulse. While the mixture was pushed toward the right, the temperature of the columns was kept at 5℃. The middle product, which is abundant in the fast-moving Blue Dextran, was then withdrawn from the junction of two columns. After 10 minutes with no flow

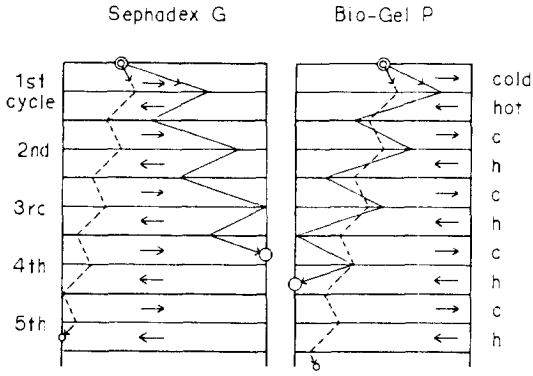

Fig. 2. Solutes migrations in Sephadex G and Bio-Gel P columns: ○ for large molecule and ◦ for small molecule.

to warm the columns to 45℃, the direction of flow was reversed. Withdrawing the left product from the left end of the Sephadex G column, which is abundant in nickel nitrate, completed the cycle. After 10 minutes to cool the columns down to 5℃, the feed mixture was injected to the left end of the columns to initiate the next cycle. The adsorption of Blue Dextran and nickel nitrate onto Sephadex G-25 and Bio-Gel P-2 was experimentally confirmed to be negligible [6].

RESULTS AND DISCUSSIONS

Separation performances are shown in Fig. 3. The three runs differ because the amount of left product was changed; run A has the smallest left product (0.47 mL/cycle) and run C has the largest (0.70 mL/cycle). During run A, a band of Blue Dextran, the concentration of which was increasing with the repeating cycles, moved left and right along the columns. In run B, the amount of left product was increased to 0.60 mL/cycle. The withdrawing period for the middle product was also changed to catch the maximum peak of Blue Dextran which had accumulated during run A. Blue Dextran concentration in the middle product at the first cycle in run B was 2.59 w/v%. That is more than 13 times the Blue Dextran concentration in the feed. The concentration of Blue Dextran decreased sharply and approached its steady state as cycle repeated. After the first 50 cycles in run C, concentration of nickel nitrate and Blue Dextran were constant in both products. The separation factor of the two solutes, α_{BN} was 1824. The mass balances of both solutes were met within 5% for the last cycles of both runs B and C.

1. Solute Separation

The breakthrough curves of two solutes are schematically drawn in Fig. 4. For the sake of simplicity, all the breakthrough curves are drawn, based upon the local equilibrium assumptions where mass transfer resistance and dispersion effect are neglected. The posing periods of 10 minutes between two half cycles

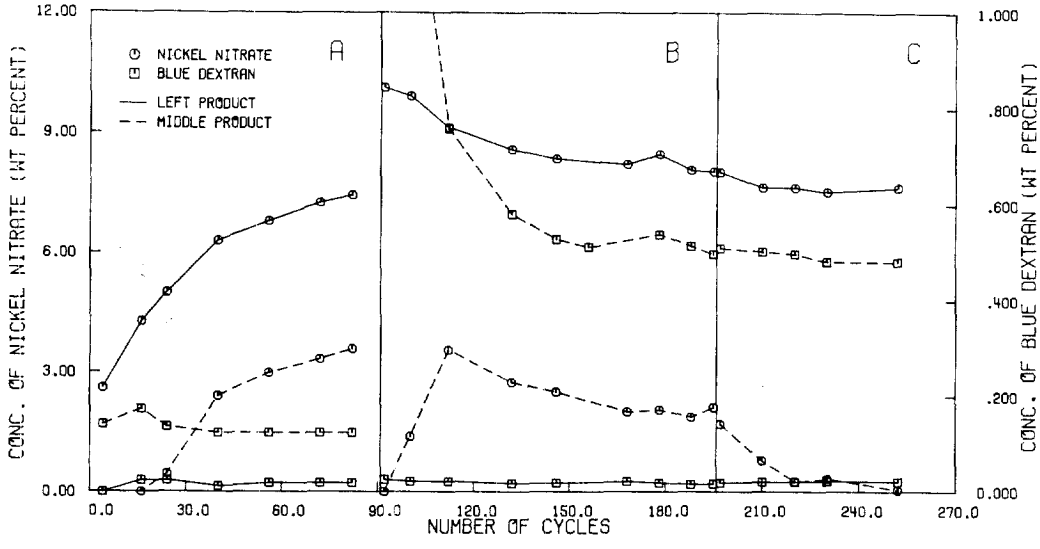

Fig. 3. Separation performance in continuous parametric pumping with coupled column: Sephadex G-25 (50-100 μm), ε= 0.808 (=25.7/31.8); Bio-Gel P-2 (100-200 mesh), ε=0.837 (=41.7/49.8); feed=1.27 mL/cycle, middle product =0.44 mL/cycle.

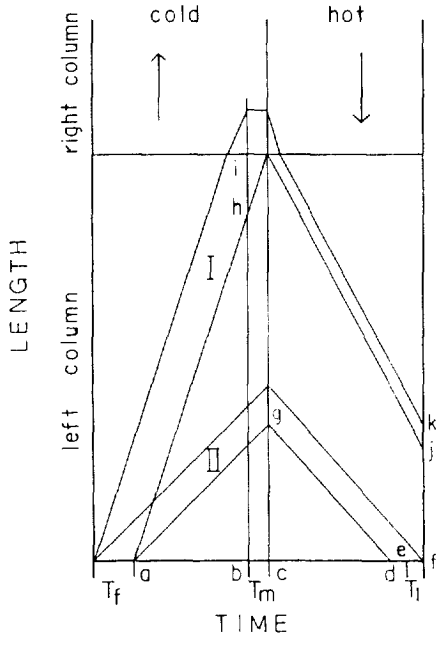

Fig. 4. Breakthrough curves of large molecule (I) and small molecule (II) in continuous coupled system: T_f, T_m and T_l are periods for the feed, middle product and left product, respectively.

are omitted in the figure. Solutes fed into the system during T_f follow the different paths [I for large molecule (Blue Dextran 2000), II for small molecule (nickel nitrate)] according to their speeds (Tables 1 and 2). Solution from the left and right reservoir drive the flow in the columns during the periods $\overline{ac}$ and $\overline{cf}$, respectively. A fraction of fast moving Blue Dextran band (T_m/T_f) exits from the junction of the two columns during $\overline{bc}$. It is equivalent to the portion of Blue Dextran in the Sephadex column ($\overline{hi}$) at the beginning of T_m (b). The Blue Dextran concentration of the middle product is the same as that of the feed solution. The remaining fraction ($1-T_m/T_f$) stays in the Bio-Gel column during T_m and returns back to the Sephadex column during the second half (hot) cycle. This band stays in the Sephadex column ($\overline{jk}$) at the end of the cycle and moves rightward during the next cold half cycle.

Small molecules stay in the Sephadex column during the whole cycle and leave as a left product during $\overline{ef}$. The concentration of left product is also same as that of feed solution. The remaining portion ($1-T_l/T_f$) enters into the left reservoir during $\overline{de}$. Judging from the local equilibrium theory, the left product concentration will be the same as that of the feed solution.

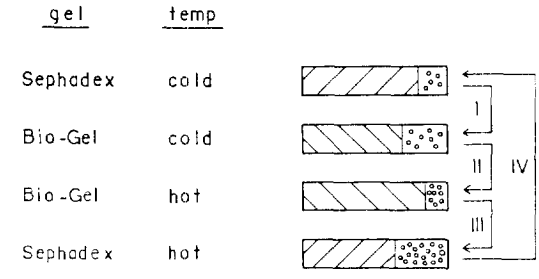

Fig. 5. Schematic drawings of solute concentration: ▨ for Sephadex and ▧ for Bio-Gel.

The concentration in the left reservoir will continuously increase to infinity. In fact, however, the nickel nitrate from the left reservoir disperses into the nickel nitrate band (II) in the column through the line $\overline{ag}$ and $\overline{gd}$ in Fig. 4, resulting in the increase of the left product concentration. The left product concentration is expected to be T_f/T_l times the feed concentration and that in the left reservoir is close to that value during the steady state operations.

As a result, the two solutes separate from each other and are obtained as pure solutes at different places and time. The probable contamination of each products by other solute caused by dispersion effect can be avoided by adjusting the length of the Sephadex column and the product withdrawing time. A system including two reservoirs and only one column (Sephadex) can separate the solutes but has limited concentrating effect. Concentrations of each products (left and right products) are inversely proportional to their volume fractions; Blue Dextran concentration of the right product is T_f/T_m times that of the feed solution and nickel nitrate concentration in the left product is T_f/T_l times that of the feed solution.

2. Solute Concentration

Opposing swelling responses of the two gels upon the temperature change can be exploited to yield a concentrating effect for large molecule. A mechanistic model to explain the concentrating effect of large molecule is schematically shown in Fig. 5 and Table 3. Rectangles in the figure represent the schematic cross-sectional view of the column. Shaded areas and circles are for volume occupied by gel particles and for large solute molecules, respectively. The figure shows the swelling (area increase) and the shrinking (area decrease) of Sephadex G and Bio-Gel P gels as temperature changes. The values of the interstitial porosities of gel columns [Sephadex, hot (0.306)>Bio-Gel, cold (0.294)>Sephadex, cold (0.281)>Bio-Gel, hot (0.247)] can be calculated from the experimental re-

Table 3. Changes of concentration and band length

Case	Situation	Concentration	Band length
I	bands transfer to Bio-Gel column at cold temperature	no change	decrease
II	temperature increase in Bio-Gel column	increase	no change
III	bands transfer to Sephadex column at hot temperature	no change	decrease
IV	temperature decrease in Sephadex column	increase	no change

sults (Tables 1 and 2, and Fig. 3). Solute molecules are large enough to be excluded totally from the gels and presented only in the right compartments (mobile phases). The length of the band of large molecule with a fixed volume decreases as it moves from the Sephadex to the Bio-Gel column during the cold half cycle (case I). It is because the cross-sectional area (interstitial pore volume) of the new (Bio-Gel) column is larger than that of the old (Sephadex) column. Note that both columns have the same diameter. This shortened band travels along the Bio-Gel column during the remaining cold half cycle. When the cold half cycle is completed (case II), there is a temperature increase during the posing period and a subsequent swelling of Bio-Gel gels. As both ends of the coupled columns are closed, this gel swelling causes the space available for the excluded molecules decrease, resulting in the increase of molecular population (concentration). Solvent (water) molecules of very small size are squeezed into the pores of swelling gel particles. The interstitial pore volume increases during the swelling process as the volume occupied by the gel materials is considered not to change much. The concentration increase of the large molecular solute is logically expected to be proportional to the decreasing ratio of interstitial pore volume. By the same reasoning, the length of solute band decreases (case III) and the solute concentration increases (case IV) in the following processes. The key to this concentrating mechanism stays in the fact that the decreases (2 times per cycle) of band length (increases of the interstitial pore volumes) repeatedly provide a room for further concentration of large molecules.

As cycle repeats, aforementioned band shortening and concentrating repeat by the factor of the multiplication of porosity ratios of each gel between two temperatures. As an example, the factor is 1.296 [=(0.306 /0.281)×(0.294/0.247)] when the degrees of compression (see Eq. (1) of [5]) are 0.808 and 0.837 for the

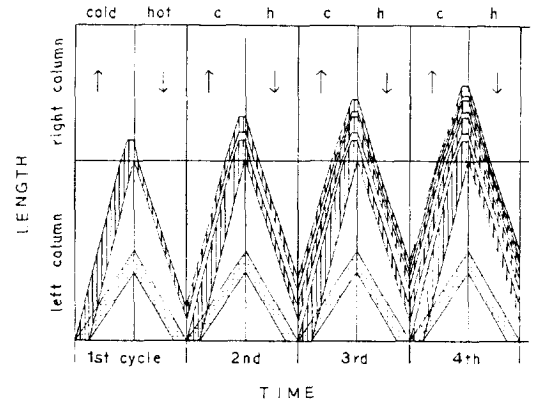

Fig. 6. Breakthrough curves of solutes in continuous coupled columns system.

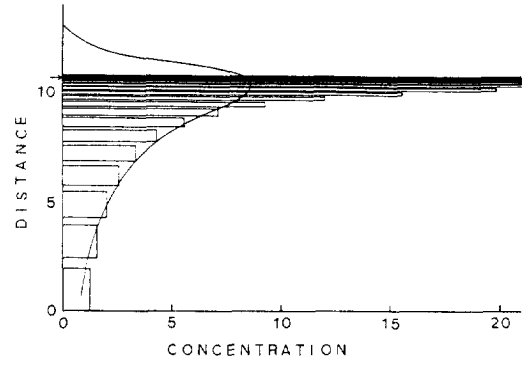

Fig. 7. Local accumulation and concentration of solute bands at infinite cycle: The curve is an approximate concentration profile at twentieth cycle.

Sephadex and the Bio-Gel columns, respectively.

Breakthrough curves for the first four cycles of the coupled columns system are shown in Fig. 6. At each cycle two solutes are separated from each other and obtained at the different places and times. The nickel nitrate (dotted area) concentration of the left product is T_f/T_i times the feed solution and the Blue Dextran (shaded area) concentration of the middle product is the same as that of the feed solution. The remaining portion of Blue Dextran not withdrawn as a middle product is accumulatively (spacewise) and repeatedly (time wise) concentrating in the Bio-Gel column. The breakthrough curve at the top during the fourth cycle has experienced 6 times (cyclically 3 times) of concentration and the one at the second top, 4 times, and so on.

3. Local Accumulations

The distances between the bands of large molecules

Table 4. Calculate solute concentration in Bio-Gel column

Band	Band front (cm)	Band length (cm)	Concentration (M)
1	1.95	1.95	1.19
2	3.93	1.51	1.54
3	5.47	1.17	1.98
4	6.66	0.91	2.56
5	7.58	0.70	3.31
6	8.30	0.54	4.28
7	8.85	0.42	5.52
8	9.28	0.33	7.13
9	9.61	0.25	9.21
10	9.86	0.20	11.90
11	10.06	0.15	15.37
12	10.22	0.12	19.84
15	10.50	0.05	42.75
20	10.68	0.0151	154
30	10.74	0.0012	1984
40	10.74	0.0001	25625
50	10.74	0.0000	330929

Column length (Sephadex G): 30 cm
ε: 0.808 (Sephadex G), 0.837 (Bio-Gel P)
Feed concentration: 1 M
Middle product time: 4 min

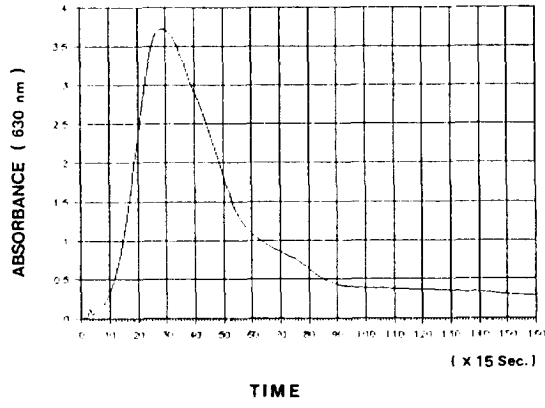

Fig. 8. Concentration profile of Blue Dextran 2000 after 16 cycles in continuous parametric pumping with the coupled column.

also decrease as the cycle repeats. Fig. 7 shows the location, length, and concentration of each solute band in the Bio-Gel column. The distance in the figure is measured from the left end of the column. Values in the concentration coordinates are the multiples of the Blue Dextran concentration of the feed solution. The lowest solute band near to the zero distance is formed by a fraction of Blue Dextran in the feed which is fed in the same cycle. The second lowest band is by the Blue Dextran fed in the previous cycle, and so on. The twentieth band is indicated by an arrow (10.68 cm). As the solute mass in each band (the area of a rectangle) is constant, the band length becomes taller (more concentrated) while the width becomes shorter as cycle repeats. The length, the distance, and the concentration of serial rectangles calculated from the breakthrough curves in Fig. 6 are presented in Table 4. The farthest distance a rectangle can reach is 10.74 cm. This was determined by taking the summation of the infinite geometrical series of the sum of the rectangle widths and the distances in between. Notice that only 10 cycles are needed to reach 90% (9.67 cm) of the maximum (ultimate) length reachable. The highest concentration at the 10th cycle is 12 times that of the feed solution. We see that the width of the rectangles and the distances between them decrease infinitely, while the length increases infinitely as cycle repeats. As a result, a short dense zone packed with many (ultimately, infinite number of) thin and tall rectangles forms. The concentrating solute band can be removed intermittently (once in some cycles) when the concentration reaches a proper value through the junction port at the middle. It should be before the solute concentration of the band reaches its solubility limit. The increasing viscosity of the solution in a band may hamper flow properties since the large molecular solute is commonly a polymer. In reality, the solute dispersion makes flat rectangles, cumulatively forming a skewed concentration profile, like the one in Fig. 7. A concentration profile obtained after 16 cycles of actual cyclic elution is shown in Fig. 8. The leading slope of the curve is sharp and the lagging one is slow as expected from the theoretical predictions. A detailed quantitative prediction of concentration profile of this accumulated solute band can be obtained by considering the dispersion effect in a column.

If a Bio-Gel column is shorter than the ultimate length reachable, solute accumulates in the right reservoir as cycle repeats. Actually, the solute molecules in the reservoir diffuse and disperse into the Bio-Gel column and eventually the middle product. At steady state, the concentration of the middle product is expected to be T_f/T_m times the feed concentration, as shown in Fig. 4.

4. Further Developments

The separating and concentrating effect becomes larger with the temperature difference. Some biologi-

cal materials (proteins for one), however, lose their activity at extreme temperatures. Gel combinations other than the Sephadex G-Bio-Gel P can be used for the coupled columns system as long as they have the opposing swelling behavior. Sephadex LH gels were experimentally observed to have a better (more) swelling-shrinking ability than Sephadex G gels.

The coupled columns system can be applied to any solutes separation where two solutes differ much in size. A mixed polymer and monomer solution is a possible example. A reaction with in-situ separation/concentration using the coupled columns system is being studied presently by this author. A polymer synthesized in an packed column can be simultaneously separated and concentrated in the same system, while unreacted monomers can be recovered and recycled to the system.

This system is based on size exclusion chromatography with temperature as a parameter. Other types of chromatography can be used to achieve the similar effects. Adsorption chromatography with temperature or pressure as a parameter, and ion exchange chromatography with pH are some of the possible analogies.

CONCLUSIONS

Two solutes with different molecular sizes are simultaneously separated and concentrated in a coupled gel column system which is operated cyclically with temperature as a parameter. In the coupled column system, the opposing swelling behavior of Sephadex G and Bio-Gel P with temperature change separates the solutes. Concentrations of the separated solutes are inversely proportional to their volume fractions with respect to the feed. Concentration of large molecules, which is uncommon to the conventional chromatography, was explained by a mechanistic model which is based on the local equilibrium assumption. The transferring bands of large molecule to the new gel column experience band shortening due to the larger interstitial pore volume. Solute concentrations of the shortened bands increase between each half cycle as the interstitial pore volume of the column decreases when packed gels swell upon temperature change. Repeating changes of temperature and flow direction concentrate the separated large molecular solute bands. The theoretical limit is infinity. In reality, the solute dispersion and diffusion makes the concentration profiles flat.

ACKNOWLEDGEMENT

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NOMENCLATURE

T : time period [min]

Greek Letters

ε : degree of compression

Subscripts

f : feed
l : left product
m : middle product

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