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Preparative Chromatographic Separation: Simulated Moving Bed and Modified Chromatography Methods

Yi Xie[1], Yoon-Mo Koo[2]*, and Nien-Hwa Linda Wang[1]

[1] School of Chemical Engineering, Purdue University, West Lafayette, IN 47907, USA
[2] Department of Biological Engineering, ERC for Advanced Bioseparation Technology, Inha University, Incheon 402-751 Korea

Abstract Chromatography has been the method of choice for the separation of complex biological mixtures for analytical purposes, particularly for the last fifty years. Its use has recently been extended to preparative separation where the productivity relative to the amount of resin and solvent used is a matter of concern. To overcome the inherent thermodynamic inefficiency of batch chromatography, as exemplified by the partial temporal usage of the resin and dilution of the product with the solvent, chromatography has been continually modified by separation engineers. Column switching and recycling represent some of the process modifications that have brought high productivity to chromatography. Recently, the simulated moving bed (SMB) method, which claims a high separation efficiency based on counter-current moving bed chromatography, has become the mainstay of preparative separation, especially in chiral separation. Accordingly, this paper reviews the current status of SMB, along with several chromatographic modification, which may be helpful in routine laboratory and industrial chromatographic practices.

Keywords: SMB, column switching, recycle, RSEC, two-way chromatography

INTRODUCTION

For many biochemicals derived from chemical synthesis, semi-synthesis, natural products, fermentation broths, tissues, tissue cultures, and other sources, adsorption and chromatography processes are required to produce sufficiently pure products for human use [1]. As a general rule, the separation of a product from closely similar impurities (purification) is the most difficult and costly step, therefore, chromatographic steps usually represent the major fraction (>90%) of the production cost.

In conventional batch chromatography, a single column, packed with adsorbent particles and with one inlet and one outlet port, is used for adsorptive separation. A small pulse of the feed mixture is then injected into the column, followed by the continuous infusion of a desorbent or solvent. Since different solutes migrate at different speeds, they are separated as they migrate through the column. The individual bands are then collected as products at the outlet ports. Any overlapping bands are either recycled or discarded as waste. A sailing analogy for this process is shown in Fig. 1(a). To obtain a high purity (>99%) and high yield (>99%), complete separation is required, which involves the consumption of a large amount of solvent. Since a significant portion of the column is not used during the batch operation, the column utilization is also inefficient.

* **Corresponding author**
Tel: +82-32-860-7513 Fax: +82-32-875-0827
e-mail: ymkoo@inha.ac.kr

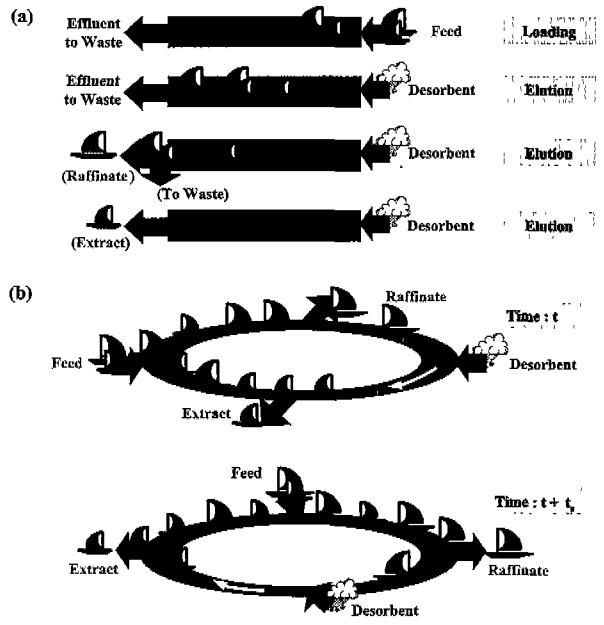

Fig. 1. Analogy of chromatography process with sailing boats. Small green boats stand for the high affinity component and the large red boats stand for the low affinity component: (a) batch chromatography, (b) simulated moving bed.

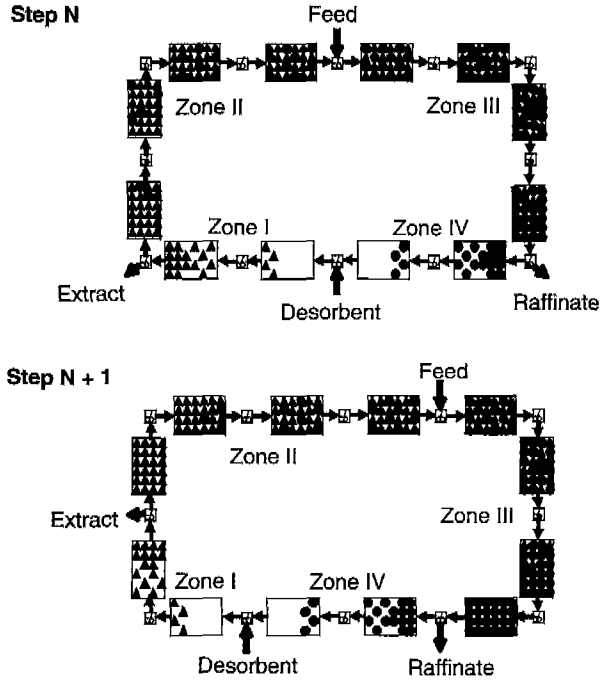

Fig. 2. Diagram of a four-zone SMB at two consecutive switching steps. Circles: affinity component. triangles: high-affinity component.

Continuous countercurrent operations have been widely used in the chemical industry, such as absorption and heat exchange, as they maximize the driving forces for mass or heat transfer. The application of a countercurrent operation to batch chromatography resulted in the first continuous countercurrent chromatography process, the Hypersorption process [2]. However, it is not easy to maintain a stable solid phase velocity. In addition, the adsorbent has a short lifetime due to attrition.

An alternative to a countercurrent flow is to simulate adsorbent movement by periodically moving the input and output ports through a ring fixed bed while keeping the bed stationary, *i.e.* simulated moving bed (SMB). Broughton and Gerhold developed the first SMB process and applied it to hydrocarbon separation [3]. In a standard four-zone SMB, a series of adsorbent columns are connected to form a circuit, which is divided into four zones by two inlet ports and two outlet ports (Fig. 2). The four ports are then periodically moved along the desorbent flow direction to follow the migrating bands. If the flow rates and port movement speed are properly designed, the feed is always added to regions where the two bands overlap, while the products are always withdrawn from regions where the two bands are separated. As such, a high purity (>99%) and high yield (>99%) can be achieved. Since the two bands overlap within the column circuit, the loading (or throughput per bed volume) of SMB is at least an order of magnitude higher than batch chromatography. Furthermore, a short solute migration distance from the feed port to the outlet ports prevents product dilution, thereby resulting in a significant reduction in desorbent consumption. A sailing analogy for conventional chromatography is shown in Fig. 1(b).

APPLICATIONS

Binary Separation

The enrichment of fructose from a fructose-glucose mixture to produce high-fructose corn syrup (HFCS) is one of the most successful industrial applications of binary separation using SMB. Fructose and glucose are both isomers and can be efficiently separated by cation exchange [4,5]. Because of the commercial importance of HFCSs and their linear adsorption behavior within a wide concentration range, a large amount of research work has already been conducted regarding fructose-glucose separation. The first successful application of SMB to this type of separation was reported by Bieser and deRosset [6]. The process was named Sarex, which is one of the series of SMB processes developed by Universal Oil Products (UOP, Plaines, Illinois, USA). It was later found that the Sarex process is unnecessarily sophisticated and less economic than a process developed by Illinois Water Treatment (IWT, now US Filter, Chicago, Illinois, USA) [7]. Among other research groups, Ching and his colleagues conducted extensive studies on the separation of fructose-glucose, including adsorbent selection [8], process comparisons [9], SMB experiments [4,10,11] and process modeling [12].

As Ruthven and Ching originally pointed out in their review paper in 1989 [7], the emphasis of applying SMB process has shifted from the petrochemical and food industry to the production of more valuable pharmaceuticals and bioproducts. Since the 1990s, SMB processes have been introduced to chiral separation, which is an important step in drug development. The first example of chiral separation using SMB was published by Negawa and Shoji [13], where a Chrialcel OD resin was used to separate 1-phenylethanol enantiomers. The results showed that the SMB process increased the productivity 60-fold and reduced the eluent consumption 86-fold, when compared to batch chromatography. Some other pioneer researchers in the field of chiral SMB include Ching and Nicoud, for exmple, Praziquantel, an anthelmintic drug, was purified by Ching *et al.* [14], while a chiral epoxide 1a,2,7,7a-tetrahydro-3-methoxynaphth-(2,3b)-oxirene was separated from its enantiomer using cellulose triacetate (CTA) as the stationary phase and methanol as the eluent by Nicoud *et al.* [15]. Many chiral systems have been tested using SMB since 1992, and these SMB applications to chiral separation have already been reviewed by two different groups, Schulte *et al.* [16] and Juza *et al.* [17]. Both review papers summarize the chiral systems reported in literature, chiral stationary phases and associated companies.

The high efficiency of an SMB lowers the cost of

chiral separation, which has attracted more and more pharmaceutical companies. For example, since 1998, UCB Pharma of Belgium and Daicel Chemical of Japan, two major pharmaceutical companies, have been using SMBs to produce multi-tons of pure enantiomers from two different chiral drugs [18]. In 1999, Novasep (Vandoeuvre-lès-Nancy, France) installed a production-scale SMB for chiral separation at Aerojet Fine Chemicals (Sacramento, CA, USA) [18]. To date, this SMB with 800-mm columns is the largest SMB for chiral separation in the world.

Multicomponent Separation

Most current applications of SMBs are focused on binary separations with only limited studies reported in literature. The engineers in UOP successfully applied their Sorbex group of SMBs to multicomponent separations, such as the purification of *p*-xylene using a Parex adsorbent and ethylbenzene using an Ebex adsorbent from a mixture of C_8 aromatics [19]. In this case, since *p*-xylene has the highest-affinity for the Parex adsorbent, it is collected at the extract port of the Parex SMB, and since ethylbenzene has the lowest affinity for the Ebex-type resin, it is collected at the raffinate port of the Ebex SMB. As such, these separations are essentially pseudo-binary separations. However, deRosset *et al.* did not disclose how the zone flow rates and switching times are selected to achieve a purity separation with such a system.

The idea of multicomponent separation using a single SMB was first proposed by Szepesy *et al.* [20] and then realized by Hashimoto *et al.* [21]. The process for a ternary separation involves four columns, which are packed with two different resins — Resin 1 and Resin 2. A column packed with Resin 1 is followed by a column packed with Resin 2. Component A has the highest affinity for Resin 1; component B has the highest affinity for Resin 2; and component C has the lowest affinity for both resins. Component C moves with the desorbent solution and is recovered at the raffinate port. Components A and B move with the resins and are collected at the extract port alternately. However, the process is limited due to the difficulty in finding resins that satisfy the aforementioned conditions.

Hatanaka and Ishida [22] proposed a process to fractionate a multicomponent mixture using a single SMB unit. The process includes a combination of a parallel desorbent flow and serial recycle flow, and distributes the components into different columns. The process is essentially a batch elution process with column switching. To obtain high-purity products, complete separations are needed. However, when the process was tested with numerical simulations, it produced a purity of less than 93% and 74% to 96% yield. A similar idea was pursued by Ching *et al.* [23], who pointed out that this type of column switching process does not have the advantages associated with a conventional SMB process.

Another method using a single SMB unit to separate a multicomponent mixture was introduced by Matsuda

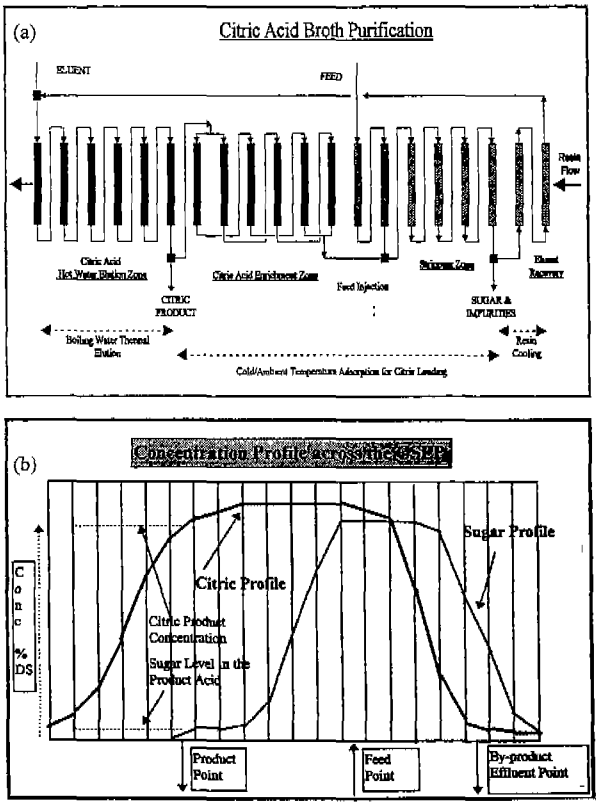

Fig. 3. ISEP/CSEP continuous separation. (a) Column arrangement for citric acid separation. (b) Concentration profile across CSEP.

[24] from the Organo Corp. (Tokyo, Japan). The basic idea is partial feed, and the process is divided into two steps within a cycle. During the first step, the feed is loaded into the SMB and intermediate-affinity component is collected from the column upstream from the feed port. During the second step, the feed is stopped and the highest-affinity and lowest-affinity components are collected at the extract port and raffinate port, respectively. Although the experimental data show a 97% purity and 98% yield for the intermediate component, a low throughput is still expected from this semi-continuous process.

The SMB unit used in multicomponent separations is not limited to a conventional 4-zone SMB. Kishihara *et al.* [25] applied a 9-zone SMB to realize a continuous separation of sucrose, glucose, and fructose. The 9-zone SMB is actually a combination of a 4-zone ring and 5-zone ring. An internal bypass stream containing a partially separated mixture is introduced from one ring to the other. The 5-zone ring separates the lowest-affinity component from the highest-affinity component, then the 4-zone ring separates the two adjacent components. Similarly, Wooley *et al.* [26] used a 9-zone SMB (ISEP from Advanced Separations Technology Inc. Lakeland., FL) to recover glucose and xylose from a biomass hydrolyzate. In this case, the recovery is 88% and the purity

near 100%. Yet the drawback of a 9-zone SMB is that the switching times for the two rings are the same. When compared with a tandem SMB with two separated rings, a 9-zone SMB has one less degree of freedom and therefore a higher solvent consumption and lower throughput per bed volume [27,28].

Because of the unique valve design of ISEP (or CSEP), an SMB with more than 9 zones and flexible wiring to control the flow direction can be designed [29]. An example of a separation process of citric acid from its fermentation broth is shown in Fig. 3(a). Different flow patterns, such as serial flow, parallel flow, and reversed flow, can all be implemented in this SMB separation process. The citric acid is eluted with hot water and harvested at the extract port, whereas the sugars and other impurities are recovered at the raffinate port at room temperature. The concentration profiles across the unit are shown in Fig. 3(b). Potentially, another zone (regeneration zone) can be added in front of the hot water elution zone to remove any extremely high affinity impurities. The drawback of ISEP/CSEP is that the central valve has to be customized for different processes, since the number of columns is limited by the number of valve ports. Also, because of the moving characteristics of the columns in ISEP/CSEP units, the scale-up of such a process is difficult. As such, a special column moving scheme is needed in a large-scale ISEP/CSEP, plus, the cost of a large-scale ISEP/CSEP valve is high.

Design

SMB has many advantages over batch chromatography. However, the design of an SMB, which involves more than 10 design parameters, is more complicated than that of batch chromatography. When developing SMB processes, one of the key issues is the determination of the zone flow rates and switching time. Various methods for SMB design have been reported in previous literature, such as the safety margin method [9,30], triangle theory [31-33], and standing wave design [24,35].

Safety Margin Method

SMB is similar to distillation in terms of its countercurrent operation. A McCabe-Thiele diagram is widely used in the design and analysis of a distillation process. It can also be used to design an SMB. Since an SMB usually has four zones and each zone can have a different flow rate from the others, there are four operation lines in a McCabe-Thiele diagram [7]. These operation lines are determined by the flow ratio of the solid phase and liquid phase. The flow ratio γ is defined as

$$\gamma = \frac{(1-\varepsilon_b)K\nu}{\varepsilon_b u_{0,CMB}} \tag{1}$$

where ε_b is the inter-particle voidage; K is the isotherm equilibrium constant; ν is the hypothetical solid velocity; and $u_{0,CMB}$ is the hypothetical interstitial velocity of the liquid phase in a continuous counter-current mode [9]. In order to achieve separation between component A (high affinity) and component B (low affinity), a McCabe-Thiele diagram for an SMB shows that the following inequalities should be satisfied:

$$\begin{array}{lll} \text{Zone I} & \gamma_A<1, & \gamma_B<1 \\ \text{Zone II} & \gamma_A>1, & \gamma_B<1 \\ \text{Zone III} & \gamma_A>1, & \gamma_B<1 \\ \text{Zone IV} & \gamma_A>1, & \gamma_B>1 \end{array} \tag{2}$$

If a safety margin (α), which is greater than unity, is added to the above inequalities, Eq.(2) can be rewritten as follows in terms of the zone flow rates and solid flow rate:

$$\begin{array}{ll} \text{Zone I} & F^{I}/S = K_A\alpha \\ \text{Zone II} & F^{II}/S = K_B\alpha \\ \text{Zone III} & F^{III}/S = K_A\alpha \\ \text{Zone IV} & F^{IV}/S = K_B\alpha \end{array} \tag{3}$$

where F represents the zone flow rate, S stands for the hypothetical solid flow rate, and the superscript stands for the zone number.

With the inclusion of the safety margin method, Ching *et al.* designed an SMB process based on a Sorbex system to separate fructose and glucose. Product purities and recoveries of 85-95% were obtained. Although this method is easy to use, the choice of the safety margin is empirical. As the safety margin increases, the purity and yield both increase [19]. As the safety margin approaches unity, Eq. (3) can be used to determine the operating conditions for an ideal system (*i.e.* no axial dispersion and mass transfer effects).

Triangle Theory

To obtain complete separation conditions for binary splitting between adjacent components in a multicomponent mixture, Storti *et al.* [31] developed the triangle theory using the framework of the equilibrium theory for multicomponent separation in a true continuous countercurrent system [36]. In the triangle theory for an SMB, the flow ratio (m) of the net liquid phase flow rate to the solid phase flow rate is defined as follows [32]:

$$m = \frac{Ft_s - V\varepsilon^*}{V(1-\varepsilon^*)} \tag{4}$$

where t_s is the port switching time; V is the bed volume; and ε^* is the total bed voidage ($= \varepsilon_b + (1-\varepsilon_b)\varepsilon_p$, where ε_p is the adsorbent particle porosity). The triangle theory specifies a triangle region in the plot of m_2-m_3 (the flow ratios of zone II and zone III) for an ideal system. The triangle region includes an infinite number of feasible operating conditions to ensure a 100% purity and yield for both the raffinate and extract products (Fig. 4). Regions for a pure raffinate product or extract product are also specified. The vertex in the triangle region provides the optimal operating conditions for the

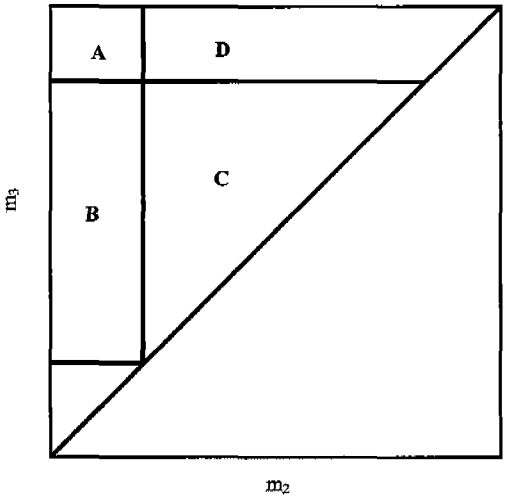

Fig. 4. Schematic plot of m_2-m_3 plane. Region A: no pure outlet, region B: pure raffinate only, region C: pure raffinate and pure extract, region D: pure extract only.

ideal system.

Later, Mazzotti *et al.* [37] proposed a key component approach to simplify the design method of Storti *et al.* [31]. In the key component approach, the binary splitting between the product and its adjacent component is treated as if other components are absent. The key component approach was later used to design a vapor-phase SMB for paraffin separation [38]. Recently, Migliorini *et al.* [33] modified the key component approach by taking all the components into consideration. They found that under a nonlinear condition, the key component approach can lead to a poor separation performance. Migliorini *et al.* also extended the triangle theory to design an SMB process with a temperature gradient [39]. The results showed that the temperature gradient operation of an SMB is feasible and may have a better productivity and less solvent consumption than an isothermal operation.

However, the design of an SMB as proposed by the above researchers - Storti *et al.* [31], Mazzotti *et al.* [32], and Migliorini *et al.* [33], does not allow for cross-contamination in the first ring of a tandem SMB. Cross-contamination refers to the distribution of either the low-affinity impurity or the high-affinity impurity between the raffinate and extract streams. Hritzko *et al.* [27,28] found that if cross-contamination is allowed in the first ring, the yield and throughput are higher and the solvent consumption is lower.

Standing Wave Design

In order to design an SMB process for linear, ideal, and nonideal binary separation, Ma and Wang developed the standing wave design [34]. This design is based on an analysis of concentration wave velocities, which can be calculated from the solute movement theory [40]. In SMB systems, the separation is governed

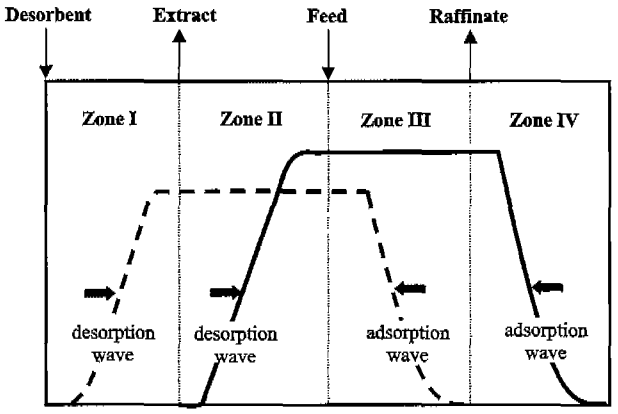

Fig. 5. Illustration of standing wave design. Solid line: low-affinity component, dashed line: high-affinity component. Arrows point to the standing wave in each zone.

by the individual concentration wave velocities related to each port. As such, separation will occur if the flow rates in the four zones are chosen such that the average port velocity is slower than the migration speed of the low affinity solute and faster than the migration speed of the high affinity solute. Moreover, if certain adsorption and desorption waves migrate at the same velocity as the average port velocity, they normally remain standing during a switching period, thereby assuring a high purity and high yield [34]. The standing wave at each zone is shown in Fig. 5.

For an ideal binary system, the standing wave conditions are given by

$$\begin{aligned} \nu - \frac{u_0^{\mathrm{I}}}{1+P\delta_2} &= 0 \\ \nu - \frac{u_0^{\mathrm{II}}}{1+P\delta_1} &= 0 \\ \nu - \frac{u_0^{\mathrm{III}}}{1+P\delta_2} &= 0 \\ \nu - \frac{u_0^{\mathrm{IV}}}{1+P\delta_1} &= 0 \end{aligned} \tag{5}$$

where the subscripts 1 and 2 stand for the low affinity solute and high affinity solute, respectively; the superscripts, I, II, III, and IV stand for the four zones; ν is the average port moving velocity, defined as L_c/t_s, where L_c is the single column length; u_0 is the interstitial velocity; P is the phase ratio, defined as $(1-\varepsilon_b)/\varepsilon_b$; and δ is defined as $\varepsilon_p+(1-\varepsilon_p)K$. This set of equations corresponds to the boundary values of all the feasible zone flow rates and port movement velocity that guarantee complete separation. The solution of Eq.(5) coincides with the vertex of the triangle region defined by the triangle theory. Equation 5 is also consistent with Eq.(3) as the safety margin (α) in Eq.(3) approaches unity.

In practice, large particles are usually used in low-pressure SMB systems and axial dispersion and mass transfer effects are significant in such systems. To ob-

tain a high purity and high yield, the axial dispersion and mass transfer effects have to be overcome by modifying the zone flow rates and switching time of the ideal design. For a nonideal system, the standing wave conditions are given by

$$\begin{aligned}
\nu-\frac{u_0^{\mathrm{I}}}{1+P\delta_2}&=-\frac{\beta_2^{\mathrm{I}}}{(1+P\delta_2)L^{\mathrm{I}}}\left(E_{b2}^{\mathrm{I}}+\frac{P\nu^2\delta_2^2}{k_2^{\mathrm{I}}}\right)\\
\nu-\frac{u_0^{\mathrm{II}}}{1+P\delta_1}&=-\frac{\beta_1^{\mathrm{II}}}{(1+P\delta_1)L^{\mathrm{II}}}\left(E_{b1}^{\mathrm{II}}+\frac{P\nu^2\delta_1^2}{k_1^{\mathrm{II}}}\right)\\
\nu-\frac{u_0^{\mathrm{III}}}{1+P\delta_2}&=\frac{\beta_2^{\mathrm{III}}}{(1+P\delta_2)L^{\mathrm{III}}}\left(E_{b2}^{\mathrm{III}}+\frac{P\nu^2\delta_2^2}{k_2^{\mathrm{III}}}\right)\\
\nu-\frac{u_0^{\mathrm{IV}}}{1+P\delta_1}&=\frac{\beta_1^{\mathrm{IV}}}{(1+P\delta_1)L^{\mathrm{IV}}}\left(E_{b1}^{\mathrm{IV}}+\frac{P\nu^2\delta_1^2}{k_1^{\mathrm{IV}}}\right)
\end{aligned} \tag{6}$$

where L is the zone length, E_b is the axial dispersion coefficient, and k is the lumped mass-transfer parameter defined as,

$$\frac{1}{k_i}=\frac{R^2}{15\varepsilon_p D_{pi}}+\frac{R}{3k_{fi}} \qquad (i=1,2) \tag{7}$$

where R is the radius of the resin particle, D_p is the intra-particle diffusivity, and k_f is the film mass-transfer coefficient. β is the logarithm of the ratio of the highest concentration to the lowest concentration of a standing wave in a particular zone. β is the index of the product purity and yield; the larger the β value, the higher the product purity and yield [34,35 ,41].

The standing wave design provides unique solutions for nonideal systems. No sophisticated process model or trial and error procedure is needed. Based on the standing wave design, Wu *et al.* [41] and Xie *et al.* [35] successfully designed an SMB process to separate a mixture of two amino acids. As a result, high-purity (96 - 99%) and high-yield (96 - 99%) products were obtained. The standing wave design was also extended to linear, nonideal, multicomponent systems [27,28] and nonlinear, ideal, binary systems [42].

Modeling

In general, the modeling of an SMB process can be categorized into two major types: (1) an equivalent countercurrent model, in which the solid phase and liquid phase move countercurrently, and (2) a simulated moving bed model, in which the periodic port movement is considered [7,43,44]. The interstitial velocities of the liquid phase of these two types are related by the following equation:

$$u_0^{\text{type 1}}=u_0^{\text{type 2}}-\nu \tag{8}$$

Both models have been previously reported in literature [10,45]. The type 1 model is simpler, however, the type 2 model can closely describe the real SMB process and is more physically meaningful than type 1 model. If three or more columns per zone are used in an SMB, type 1 and type 2 models provide a similar prediction [46]. However, if less than three columns per zone, in particular one column per zone, are used in an SMB, the prediction of a type 1 model will significantly deviate from that of a type 2 model.

The SMB models can be further itemized in terms of an ideal or nonideal system. For ideal systems, a single countercurrent column model was proposed and analytically solved by Rhee *et al.* [36]. Later, Storti *et al.* [31] extended the model from a single countercurrent column to four countercurrent sections, considering the concentration changes at the joint nodes between adjacent sections. The solution of the ideal model for four countercurrent sections led to the development of the aforementioned triangle theory.

For nonideal systems, three types of models have been reported in previous literature: (1) the equilibrium stage model, (2) dispersed plug flow (equilibrium dispersive) model, and (3) general rate model. These models are widely used in the simulation of single column chromatography processes. Since an SMB can be considered as a single chromatography column with four inlet and outlet ports along the column, these single column models can be readily extended to an SMB. The equilibrium stage model is based on the plate theory for chromatography [48]. The column is divided into small stages. At each stage, the mobile phase and stationary phase are assumed to be in equilibrium. The axial dispersion and mass transfer effects are lumped into the number of plates. The equilibrium stage model for an SMB is usually used to predict the steady state operation (or column profiles) of an SMB [4,12,49]. The dispersed plug flow model, considers the axial dispersion and lumped mass transfer [4,34,49]. For linear isotherms, the dispersed plug flow model can be solved analytically for a steady state operation, while for nonlinear isotherms, the model has to be solved numerically. Unlike the dispersed plug flow model, in which a lumped mass transfer rate is adopted, the general rate model distinguishes between the film mass transfer and the intra-particle dispersion [42,52,53]. As a result, the general rate model is more rigorous than the dispersed plug flow model, although the former requires more computational time.

MODIFIED CHROMATOGRAPHY METHODS

Column Switching Methods

In chromatography, only a portion of the packed resin is involved in active separation, as such, it is a thermodynamically inefficient method, which is time consuming and has a high solvent usage. Column switching has been used for years in gas and liquid chromatography to improve the inherant drawbacks of chromatography. Wankat [54,55] previously reviewed column switching techniques in detail. The basic apparatus is illustrated in Fig. 6(a). When a multicomponent mixture is to be separated, fast moving solutes which separate in column A are withdrawn at product loca-

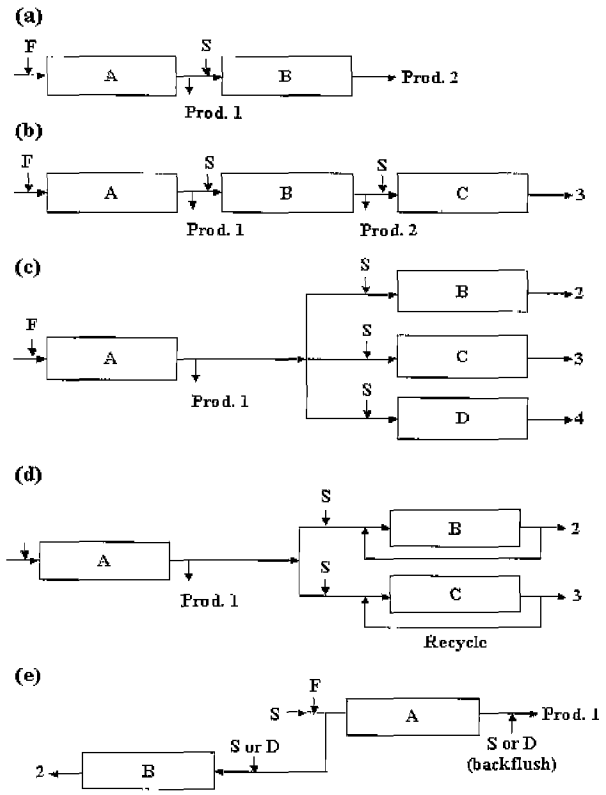

Fig. 6. Schematic of column switching systems. (a) Basic system, (b) series arrangement, (c) parallel arrangement, (d) parallel arrangement with recycle, (e) for backflush material (With permission from author [55]).

tion 1. Those solutes which do not completely separate, are then sent to column B to be removed at product location 2. Slowly moving solutes which separate in column A can be removed at a later time at product location 1. A fresh solvent can also be inserted into column B, as shown in Fig. 6(a). The two columns can contain the same or different packings, and can be based on totally different partition mechanisms. The idea of different packings can also be extended to several columns connected together in either a series (Fig. 6(b)) or parallel arrangement (Fig. 6(c)). In addition, fully automated systems have been developed, and semipreparative-scale and large-scale units are now commercially available.

The solute movement theory demonstrates the reason why column switching is helpful. This is illustrated in Fig. 7 for the equipment shown in Fig. 6(a) where columns A and B contain different packings. Solute 1 is removed after column A, while solutes 2 and 3, which are not separated, are sent to column B where they are easily separated. Component 4 is removed from column A. In this application, a fresh solvent or carrier gas is added to column B after the peaks of solutes 2 and 3 have entered that column. The next feed pulse can be input into column A much sooner than if a single long column A were used, thereby resulting in a higher throughput. When a particular pair of components is very difficult to separate, the use of a precolumn to remove all other components is recommended. The use of a single short column to remove solutes 1 and 4 in Fig. 7 reduces their zone spreading. The throughput can be further increased by partially separating the components and recycling the overlapping peaks. The recycling can be either back to the initial column or around a single column, as shown in Fig. 6(d).

The column switching system shown in Fig. 6(c) can be used instead of recycling. For example, for a three-component feed, all the columns in a parallel arrangement are usually packed with the same packing. A front cut of the first product of pure solute 1 is removed after column A. The overlapped portion of solutes 1 and 2, which is normally recycled, is then sent to column B, which gives solutes 1 and 2 as products. The second product of pure solute 2 is withdrawn after column A, and the following overlapped portion of solutes 2 and 3 is then sent to column C where the products are pure solutes 2 and 3. The third product of pure solute 3 is then recovered from column A, and the recycle from the overlap with the next pulse is sent to column D.

Column switching methods can be employed with a backflush. For example, rapidly moving solutes are sent to column B, see Fig. 6(a), while column A is backflushed to remove the other solutes. The backflushed material can also be sent to another column, as shown in Fig. 6(e), to separate certain slowly moving components. This column can utilize the same or a different packing. Either a solvent or a desorbent can be used for the backflush.

Some applications of column switching techniques include the determination of 5-fluorouracil and its metabolite in urine using HPLC [56], D-amino acids in milk [57], insulin in biological samples using reversed-phase HPLC [58], and group-type separation of different PAH classes using C_{18}-modified silica and polystyrene packings [59].

Chromatography with Recycling

In batch chromatography, the simplest way to obtain a product is to make a single cut between each component. In both linear and nonlinear elution chromatography, the peaks are spread because of diffusion and dispersion in the column or isotherm effects. In order to achieve high purities, the two peaks must be quite well separated. This requires either long columns, or short feed pulses, or both, to reduce the feed throughput. However, this problem can be solved by recycling [55]. When a rather poor peak resolution is obtained, the overlapping portion of the peaks can be collected separately and recycled (Fig. 8). The principles for recycling are the same for all linear systems. For example, a single feed pulse involves three fractions of product A, a recycle, and product B. With multiple feed pulses, as shown in Fig. 8, an additional recycle is applied between the two feed pulses.

Usually the recycle stream is collected as a single

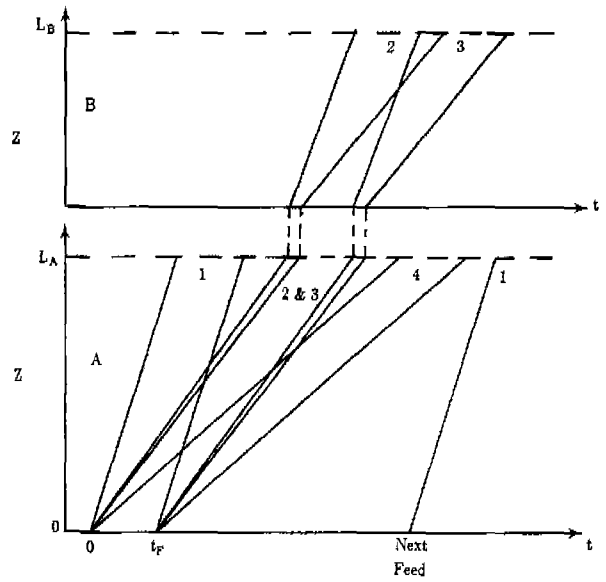

Fig. 7. Solute movement diagram for column switching system of Fig. 1(a). Isotherm are linear (With permission from author [55]).

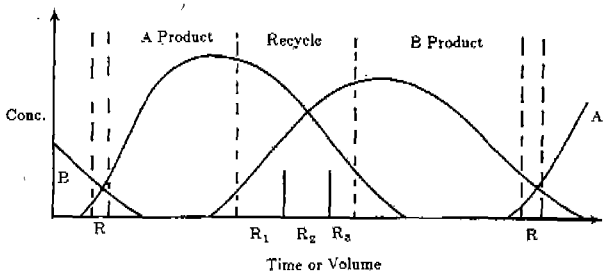

Fig. 8. Chromatogram of recycle system for nonlinear two-component system (With permission from author [55]).

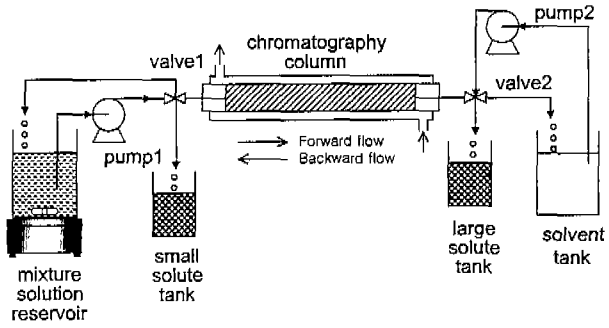

Fig. 9. Schematic representation of Reciprocating Size Exclusion Chromatography for on-line separation of solutes of different size.

mixed fraction. This recycle stream can be input before, with, or after the feed. Since the recycle stream is somewhat diluted, it is possible to mix the recycle with a concentrated feed to obtain a net feed of the desired concentration. The optimum recovery ratio r, defined as the ratio of the mass material recovered to the mass of the total feed, which is the fresh feed plus the recycle, is around 0.5 to 0.6 [60]. The usual recycle arrangement loses some of the separation that has already been obtained, since streams with different concentrations are mixed together. As such, better results can be obtained by subdividing the cycle into fractions which are recycled separately [61]. For example, the recycle stream in Fig. 8 can be subdivided into three fractions. Fraction R_1 has a higher concentration of solute A and can be injected either before the next feed pulse or mixed with the first portion of the fresh feed. Fraction R_2 has about the same proportion of A and B as the feed and can be either mixed with the fresh feed or mixed with the second portion of the fresh feed. Fraction R_3 has a higher ratio of B to A than the feed and needs to be placed at the tail end of the fresh feed. Although this procedure can increase productivity, it is more complicated and requires more separators. Improved models for recycling chromatography are already commercially available [62].

Reciprocating Chromatography

Reciprocating Size Exclusion Chromatography (RSEC)

A modified operation of size exclusion chromatography, RSEC, was developed [63] to recover large molecules on-line from the mixture solution. The on-line recovery of large molecules from the mixture is more of a challenge, compared to the routine practice of filtration where only small molecules are isolated from the mixture. RSEC is operated semi-continuously, based on an elution in the frontal mode, where solutes of different sizes in a step feed proceed along the column, forming their own fronts. The frontal mode operation is considered to give a higher separation capacity than the peak mode in preparative chromatography

In the RSEC system (Fig. 9), both large and small molecules are isolated from the mixture by repeating cycles of feeding the mixture solution. The large molecules in the band, moving ahead of the mixture solution, are isolated into the large solute tank during the forward flow period in the frontal mode. The solvent eluted before the large molecules is gathered in the solvent tank, reserved for the backward flow in the second half of the cycle. The remaining band of the mixture solution in the column at the end of the first half of the cycle is returned to the reservoir during the second half of the cycle. The band of slow-moving small molecules, following the mixture solution during the backward flow period, is then recovered in the small solute tank. Thus, one full cycle consists of solvent gathering in the first half, resulting in the recovery of the large molecules, and the return of the mixture solution to the reservoir in the second half, resulting in the recovery of the small molecules.

The packed column is initially filled with pure solvent, while the reservoir is filled with the feed mixture solution with known concentrations. Pumping the feed

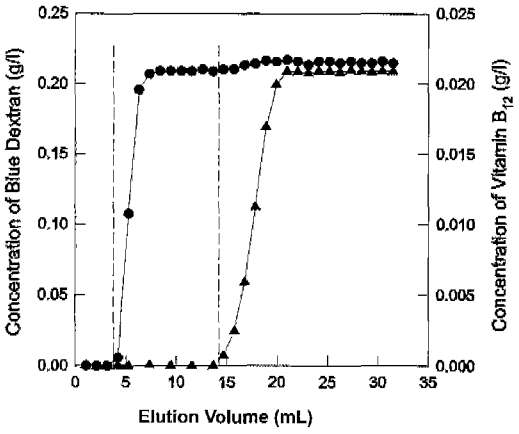

Fig. 10. Frontal elution curves of Blue Dextran and vitamin B_{12} during the first half cycle.

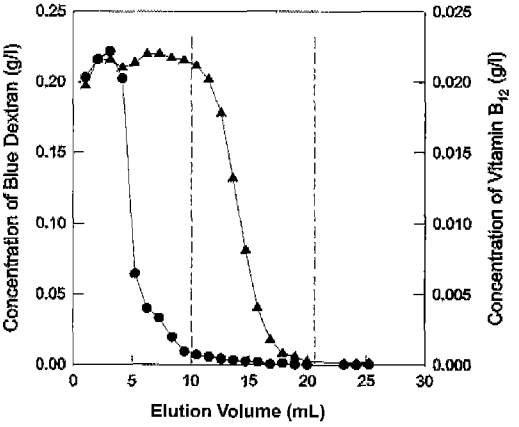

Fig. 11. Frontal elution curves of Blue Dextran and vitamin B_{12} during the second half cycle.

mixture solution into the packed column initiates the first half of the cycle. The retention volumes of Blue Dextran and vitamin B_{12} in this column are estimated from the frontal elution curves, as shown in Fig. 10. Blue Dextran is recovered as the second fraction, following the first fraction of the pure solvent. The mixture solution remaining in the column at the end of the first half of the cycle is then pumped backward to the reservoir during the second half of the cycle. The frontal elution curves at the left end of the column are shown in Fig. 11. The zero in this figure is the time when the second half of the cycle starts. Following the first portion of the mixture solution, which is returned to the reservoir, the slow-moving small solutes are recovered as a pure solution.

The concentration changes in the reservoir and recovered solutions with the repeating cycles are shown in Fig. 12. As the cycles are repeated, the concentration of the recovered Blue Dextran decreases along with the concentration of Blue Dextran in the reservoir. In the current example, the recoveries of Blue Dextran and

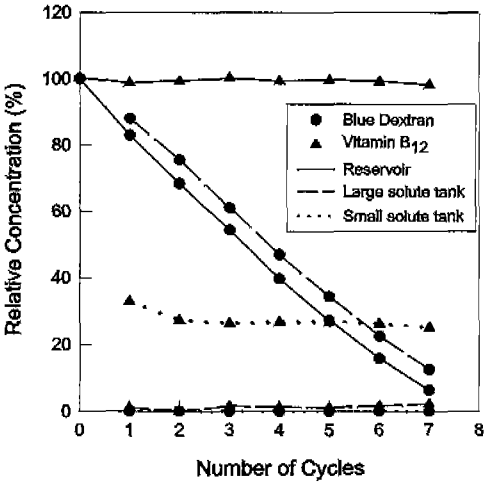

Fig. 12. Concentrations of Blue Dextran and vitamin B_{12} in the reservoir and recovery tanks.

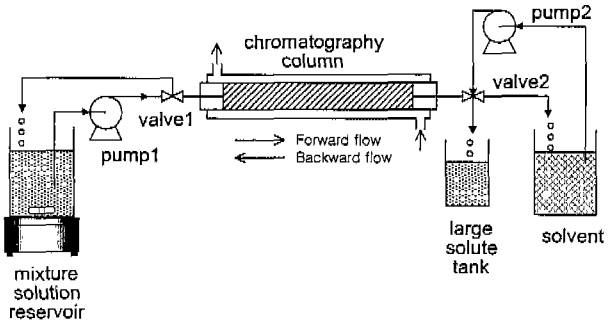

Fig. 13. Schematic representation of RSEC with temperature swing.

vitamin B_{12} after 7 cycles were calculated to be 89% and 50%, respectively.

Mathematical simulations, based on the local equilibrium model, were carried out to compare the separation productivities between RSEC and batch SEC with repeated feeds and diffusivity as a variable [64]. The separation with RSEC was found to be better than that with SEC with repeated feeds in the range of high diffusivity of either solute.

RSEC with Temperature Swing

RSEC can also be operated with a swing between two temperatures in a synchronous way with the flow direction to recover a large solute on-line from the mixture, in addition to the small solute concentration, as shown in Fig. 13. The concentration of small solutes in RSEC with a temperature swing is possible by taking advantage of the temperature-dependent swelling properties of a porous gel. These effects are generated by a synchronous change in the flow direction and temperature, based on the temperature-dependent swelling properties of porous gels [65-67].

The elution curves of Blue Dextran and nickel nitrate

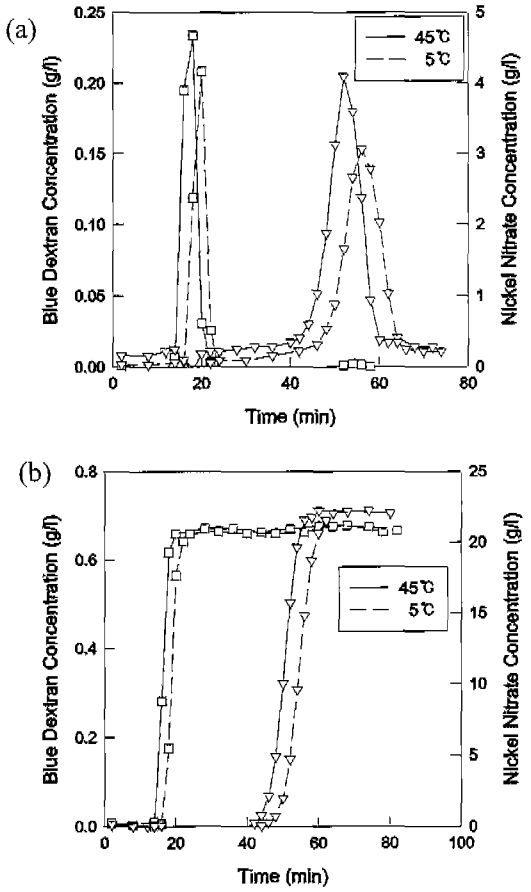

Fig. 14. Separation behaviors of solutes in RSEC at two temperatures in peak mode (a) and frontal mode (b).

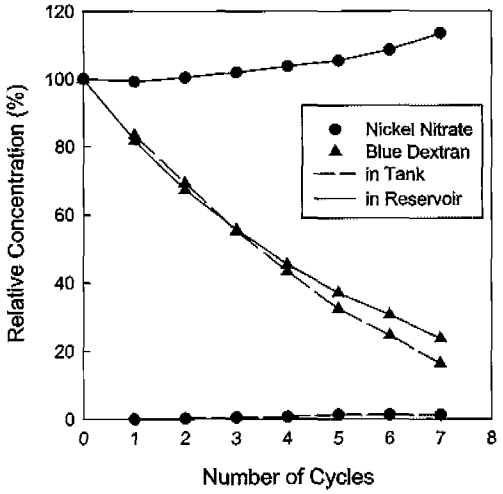

Fig. 15. Concentrations of Blue Dextran and nickel nitrate in reservoir and tank.

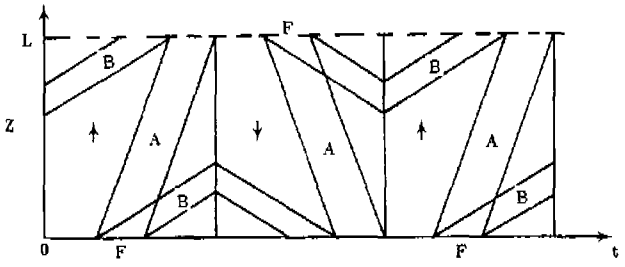

Fig. 16. Solute movement diagram for two-way SEC with feed from both ends. Shown for linear isotherms. (A): high MW species, (B): low MW species (With permission from author [55]).

in an SEC column, packed with Bio-Gel P-4, were experimentally obtained in the peak mode at two temperatures (Fig. 14(a)). Since the solutes in this system moved faster at a higher temperature, RSEC with a temperature swing was designed and operated to yield a better separation performance. In the first half of the cycle of pumping the solution from the reservoir to the column (forward flow) at 5°C, Blue Dextran was recovered as the second fraction, following the first fraction of pure solvent (Fig. 14(b)). After 10 min of waiting for temperature equilibration at 45°C, the pure eluent was pumped back (backward flow) to return the unseparated mixture solution to the reservoir during the second half of the cycle. The next cycle then started after waiting 10 min for equilibration at 5°C.

With the repeating cycles, the concentration of the recovered Blue Dextran decreased as the concentration of Blue Dextran in the reservoir decreased (Fig. 15). Blue Dextran was recovered as a pure solution. The concentration of nickel nitrate in the reservoir increased as expected. The recovery of Blue Dextran, calculated from the amount left in the reservoir, was 76% after 7 cycles. The concentration of nickel nitrate was also measured to be 13% higher than the initial concentration.

The enhanced separation in RSEC with a temperature swing, when compared to RSEC without a swing, is caused by the gel swelling with a temperature change. In reciprocating chromatography, based on partition mechanisms other than size exclusion, such as adsorption and ion-exchange, the inclusion of a temperature swing will produce better separation as the temperature change affects the adsorption isotherms, which in turn affects the retention volume of the solutes in the column in a favorable manner. In fact, any thermodynamic parameter, other than temperature, such as pH and ionic strength, can be employed with RSEC to improve the separation performance, as long as it can affect the adsorption isotherms.

Two-way Chromatography

In two-way chromatography [55], the flow is first up the column, which is then reversed. The products are withdrawn from both ends and sometimes, from the middle of the column. Two-way chromatography has been applied to SEC for the commercial purification of

whey proteins [68]. The main purpose of the flow reversal is to keep the nets supporting the gel free of precipitated protein, which would appear to result in a 10% increase in capacity. The system uses an alternating feed from both ends. The fast moving, high molecular weight proteins are removed in the same direction as the feed pulse, whereas the slow moving, low molecular weight salts and lactose are backflushed (Fig. 16). Note that the feed is introduced several minutes after the flow direction is reversed. For nonlinear competing solutes, flow reversal and intermediate withdrawal may also be useful [69].

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