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Review

Simulated moving-bed chromatography for continuous separation of two components and its application to bioreactors

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ABSTRACT

Simulated moving-bed chromatography (SMBC) is a powerful technique to separate continuously two components in large amounts, and is useful on a preparative scale. Models for the calculation of the concentration profiles in SMBC are introduced, and also methods for design of the chromatograph and for the determination of operating conditions. Improved chromatographs developed to separate three components are described. The combination of chromatographic separation and an enzyme-catalysed reaction to improve the reaction performance is also described.

CONTENTS

1. INTRODUCTION

Gas and liquid chromatographic techniques are widely used for analyses and purifications in the laboratory. Chromatographic separation methods usually consume less energy than other separation methods such as distillation. The selection of suitable packing materials (hereafter referred to as the resin) can allow the separation of components that are difficult to purify by other methods. In particular, liquid chromatography is often carried out at room temperature, thus being suitable for the separation of heat-sensitive components. However, conventional types of chromatography are batchwise processes, which are not suitable for the separation

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of large amounts of feedstock. The following drawbacks should be overcome in industrial use: (i) the resin in a column is not always used efficiently, (ii) a large volume of eluent is needed to elute the separated components, resulting in products being diluted, (iii) highly purified products are not obtained when there are only slight differences in the affinities of the components for the resin and (iv) discontinuity of operation.

Continuous chromatographic techniques may be divided into two main types. One is continuous cross-current chromatography, where a fluid contacts vertically with the resin. A typical apparatus of this type is the rotating annular chromatograph [1–4]. Although this type of chromatography can separate multiple components, the performance is the same as that of conventional batchwise chromatography. This can be easily shown by replacement of the angular coordinate with the elution time in the mass balance equations [5]. The other is continuous counter-current chromatography, which can allow the separation of two components. A typical example of this type is the so-called hypersorption process, which was used in industry for the separation of low-carbon-number hydrocarbons about 40 years ago [6]. However, this process was not used for very long because of difficulties with the attrition of and movement problems with the resin. Some separation systems with this type of chromatography have been reported and are summarized in ref. 7.

Simulated moving-bed chromatography (SMBC) provides almost the same performance as a counter-current moving-bed chromatography without any actual movement of the resin [8–13]. In this paper, a mathematical model, design method and method for the determination of the operating conditions of SMBC are presented. The combination of chromatographic separation and an enzymatic reaction within the apparatus is also described.

2. SIMULATED MOVING-BED CHROMATOGRAPHY

2.1. Basic principle

The separation of components in conventional one-dimensional chromatography is achieved by their different migration rates in the column. Let us now consider components A and B, where A is more adsorptive than B. The adsorptive component A migrates in the column more slowly than the less adsorptive component B. If the resin particles packed in the column can be moved at a rate between the migration rates of A and B in the opposite direction to the fluid flow and the mixture of A and B is continuously introduced at the centre of the column, the components can migrate in different directions and their continuous separation is attainable. This is the principle of continuous counter-current chromatography. However, actual movement of the resin particles is usually difficult and is liable to cause their attrition and disturbance of the flow, as mentioned above.

To overcome these difficulties, a simulated moving-bed process has been developed. As shown in Fig. 1, a long packed column is divided into several columns that are connected together with tubes in series. The introduction and withdrawal points of the feed, desorbent, raffinate and extract streams are changed at given intervals to the next column in the direction of the fluid flow, resulting in a simulated counter-current contact of the fluid and the resin. This method is called simulated moving-bed chromatography and realizes the continuous separation of two components A and B without actual movement of the resin.

The SMBC system usually consists of four zones, I–IV, as shown schematically in Fig. 1, each of which has its inherent function. A sample solution including components A and B is fed at the feed point situated between zones II

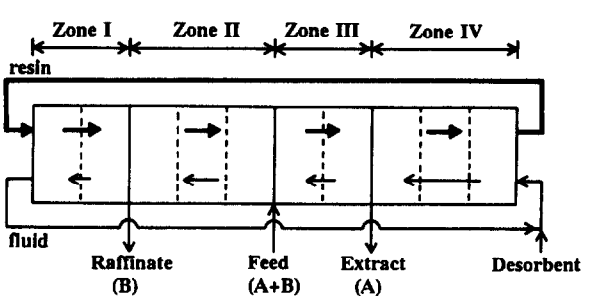

Fig. 1. Schematic diagram of a simulated moving-bed chromatograph.

and III. Component A is adsorbed on the resin in zone II and carried to zones III and IV by the simulated movement of the resin. The adsorption wave of less adsorptive component B can migrate to the withdrawal point of the raffinate stream within a switching interval of the introduction and withdrawal points and component B can be recovered in the raffinate stream. Hence zone II functions for the separation of the components. Zone III is a refining zone, where component B adsorbed partially in zone II is desorbed and returned to zone II. Component A adsorbed in zone II is mainly desorbed in zone IV to join the liquid stream passing through the zone, and is recovered in the extract stream. Zone I functions for adsorption of component B to suppress the dilution of component B in the raffinate stream.

An SMBC system consisting of three zones (correspond to zones II–IV in Fig. 1) has also been developed by Barker and co-workers [14–16]. In the SMBC system, zone IV was isolated and component A adsorbed in zone II was recovered as a product stream by elution with a large volume of desorbent or by increasing the temperature in the zone. They achieved success in the fractionation of dextran [17,18], glucose–maltose separation [7], etc., using this type of equipment [7].

2.2. Models for prediction of concentration profiles

Several mathematical models have been proposed to express changes in the concentration profiles in SMBC under any operating conditions. Ching and co-workers [19–21] proposed methods to predict the concentration profiles in SMBC based on an axial dispersion model and an ideal equilibrium plate model, and checked the effects of the operating conditions of the process on the concentration profiles by using McCabe–Thiele graphs [22].

Storti and co-workers [23,24] developed two mathematical models of counter-current adsorption processes: a detailed model and an equilibrium theory model. The latter neglects mass transport resistance and axial mixing phenomena and makes the mathematical treatment easy. However, the model has been shown to be useful for the optimum design of the counter-current unit and for the determination of the conditions to obtain the complete separation of any components into two fractions. Quantitative analysis can be effected through the detailed model, which takes into consideration both mass transport resistance and axial mixing phenomena.

We have presented two models to predict changes in the concentration profiles: an intermittent moving-bed model and a continuous moving-bed model [11]. These two models can be applied to adsorption systems not only with linear adsorption isotherms but also with nonlinear isotherms [12]. In both models, the adsorption rate process is represented by a linear driving force approximation [25] and axial dispersion of the fluid phase is ignored.

The intermittent moving-bed model faithfully expresses the manner of operation of SMBC and is useful in calculating unsteady-state profiles. SMBC is the same as conventional fixed-bed chromatography except at the moment of switching of the introduction and withdrawal points of the liquid streams. Mass balance equations referring to the concentrations of components in the mobile and stationary phases were formulated. The partial derivatives with respect to time are replaced by the forward type of finite-difference equations, whereas the derivatives for the distance are replaced by the backward type. A set of finite-difference equations are solved numerically with consideration of the boundary conditions at the introduction and withdrawal points located between two adjoining zones and of periodic rearrangement of dependent variables (concentrations) corresponding to the switching of the introduction and withdrawal points.

To estimate the steady-state profiles in SMBC, another model, termed the continuous moving-bed model, has been proposed. It is hypothesized in this model that the resin particles move continuously at a rate u_s, which is defined as L/T, where L is the length of each column and T is the switching interval of the introduction and withdrawal points of liquid streams. The mass balance equations at the steady state in terms of the concentrations of component k in the mobile phase C_k and in the stationary phase C_k^* can be

solved analytically and the concentrations at the outlet of zone n are represented by the concentrations at the inlet of the zone:

$$C_{kn,\text{out}} = \frac{\exp[\alpha_{kn}(1-\beta_{kn})N_n] - \beta_{kn}}{1-\beta_{kn}} \cdot C_{kn,\text{in}} + \frac{1-\exp[\alpha_{kn}(1-\beta_{kn})N_n]}{1-\beta_{kn}} \cdot C^*_{kn,\text{in}} \quad (1)$$

$$C^*_{kn,\text{out}} = \frac{\beta_{kn}\{\exp[\alpha_{kn}(1-\beta_{kn})N_n] - 1\}}{1-\beta_{kn}} \cdot C_{kn,\text{in}} + \frac{1-\beta_{kn}\exp[\alpha_{kn}(1-\beta_{kn})N_n]}{1-\beta_{kn}} \cdot C^*_{kn,\text{in}} \quad (2)$$

where N_n is the number of columns of zone n and the dimensionless parameters α_{kn} and β_{kn} are defined as follows:

$$\alpha_{kn} = K_{f,k} a_v L / u_n \quad (3)$$

$$\beta_{kn} = \frac{u_n}{u_s(1-\varepsilon_b)m_k} = \frac{\text{amount of solute transported by fluid phase}}{\text{amount of solute transported by solid phase}} \quad (4a)$$

$$= \frac{u_n/u_s(1-\varepsilon_b)}{m_k} = \frac{\text{slope of the operating line}}{\text{slope of the adsorption isotherm}} \quad (4b)$$

where a_v is the specific surface area, K_f the overall mass transfer coefficient, m the distribution coefficient and u_n is the superficial velocity of liquid flow at zone n in a hypothetical moving bed, and is calculated by $\nu_n - \varepsilon_b L/T$, where ν_n is the actual superficial velocity of liquid flow. The steady-state concentration profiles of the components can be obtained by solving a set of simultaneous algebraic equations (eqns. 1 and 2) with boundary conditions at the introduction and withdrawal points.

2.3. Method for determination of operating conditions

Many operating variables should be determined to fix the operating conditions or to design an SMBC system. Among the variables, the liquid flow-rate in each zone is the most important operating variable that directly affects the separation efficiency. A method has been developed for determining the flow-rate in each zone based on the physical meaning of the dimensionless parameter β_{kn}, which was defined by eqns. 4 for the case where the adsorption isotherms of two components are linear and independent of each other [11].

As mentioned above, each zone has its inherent function. Now let us consider the function of zone II again. As zone II is for adsorption of the adsorptive component A, the amount of A adsorbed and conveyed by the resin should be more than that transferred with the liquid flow. Therefore, β_{A2} should be less than unity as understood from the definition of β_{A2} (eqn. 4a). On the other hand, the less adsorptive component B should be transported to the withdrawal point of the raffinate stream and recovered there. Hence $\beta_{B2} > 1$ should be satisfied in zone II. A similar consideration for each zone gives the criteria shown in Table 1, which should be satisfied for separating components A and B successfully by SMBC.

An operating line for component k in zone n is obtained from a mass balance for the component from the inlet or outlet point to any point in the zone:

$$\rho(q_{k,\text{in}} - q_k) = [u_n/u_s(1-\varepsilon_b)](C_{k,\text{in}} - C_k) \quad (5)$$

where q is the amount adsorbed on the resin and ρ is the density of the resin. The concentration of the adsorptive component A is close to zero at the raffinate and desorbent points, and the

TABLE 1

CRITERIA FOR DETERMINING β_{kn} VALUES NECESSARY FOR OBTAINING A GOOD SEPARATION IN A SIMULATED MOVING-BED ADSORBER

n	Zone			
	I	II	III	IV
β_{An}	<1	<1	<1	>1
β_{Bn}	<1	>1	>1	>1

concentration of the less adsorptive component B is almost zero at the outlet point of the liquid in zone I and at the extract point under operating conditions for good separation. In this situation, eqn. 5 can be simplified to give the following two operating lines, both of which pass through the origin:

$$\rho q_B = [u_n/u_s(1-\varepsilon_b)]C_B \quad \text{(for zones I and III)} \tag{6a}$$

$$\rho q_A = [u_n/u_s(1-\varepsilon_b)]C_A \quad \text{(for zones II and IV)} \tag{6b}$$

If the adsorption isotherms are linear, the ratios $\rho q_A/C_A$ and $\rho q_B/C_B$ represent the distribution coefficients m_A and m_B, respectively. When an operating line lies over the adsorption isotherm of a component, desorption of the component occurs. The operating line should be located below the isotherm for the component being adsorbed. Therefore, the operating line in zones I and III should be drawn below and above the isotherm for component B, respectively. Similarly, the operating lines in zones II and IV should be located below and above the isotherm of component A, respectively. From these considerations, we can obtain the same criteria as shown in Table 1.

These criteria for determining the operating conditions are applicable for separation with any type of adsorption isotherm, although they cannot be used directly for non-linear adsorption isotherms. To extend the criteria to non-linear isotherms, a modified definition of β_{kn} is required:

$$\beta_{kn} = \frac{u_n}{u_s(1-\varepsilon_b)(\rho q_{k,F}/C_{k,F})} \tag{7}$$

where $\rho q_{k,F}/C_{k,F}$ is substituted for m_k in eqn. 4a.

As an example, let us consider the case with the adsorption isotherms shown in Fig. 2, which represent those for NaCl (component A) and glucose (component B) on Retardion 11A-8 [12], and determine the operating conditions for zone II. The isotherm of glucose is linear and that of NaCl is of the Langmuir type. They are independent of each other. An operating line in zone II intersects the isotherm of NaCl because

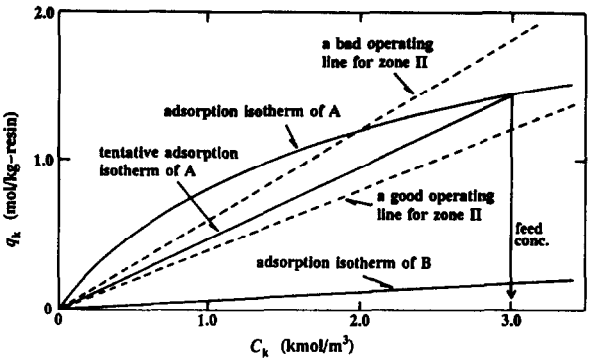

Fig. 2. Adsorption isotherms of NaCl (A) and glucose (B) on Retardion 11A-8 and operating lines for zone II for separating the two components successfully.

the isotherm is convex. As the NaCl concentration in the bed never exceeds its feed concentration because of the independence of the isotherms, the operating line connecting the origin and a point $(q_{A,F}, C_{A,F})$ may be considered to give its tentative upper limit. Based on the intermittent moving-bed model, computer simulation of the concentration profiles in the bed were made for the cases where the operating line for zone II was drawn below and above the tentative isotherm (the tentative upper limit of the operating line). Only when the operating line is located below the tentative isotherm is a good separation shown to be realizable. Under the conditions where the operating line is above the tentative isotherm NaCl is leaked into the raffinate stream. This suggests that the modified dimensionless parameter of eqn. 7 may be helpful for the determination of the operating conditions for the separation system with non-linear adsorption isotherms.

The method mentioned above was successfully applied to the determination of the operating conditions of SMBC for the glycerol–NaCl separation system, where the isotherm of glycerol (A) is linear and that of NaCl (B) is unfavourable [26]. From the method mentioned above, we can find a case where the concentration of a component is possible by SMBC when the adsorption isotherm of the component is dependent on another component. It was demonstrated experimentally that the adsorptive component phenylalanine was continuously recovered in the

extract stream at a concentration over its feed concentration for the separation system of phenylalanine and NaCl by using an adsorptive resin, Amberlite XAD-7 [27]. In the separation system, the adsorption isotherm of NaCl is linear and independent of the concentration of phenylalanine, whereas that of phenylalanine is expressed by a modified Langmuir equation and the amount adsorbed increases with increase in the concentration of NaCl. Fig. 3 shows the concentration profiles of the components in SMBC [27].

2.4. Method for design of the chromatograph

A simplified design method for SMBC has been proposed based on the continuous moving-bed model for the separation of two components, the adsorption isotherms of which are both linear and independent of each other [28]. By this method, the length of each column, the combination of the number of columns in each zone and the flow-rates of the liquid in each zone can be determined.

The dimensionless parameter α_{kn} can be expressed by eqn. 8 through integration of the mass balance equations in the liquid and solid phases at zone n:

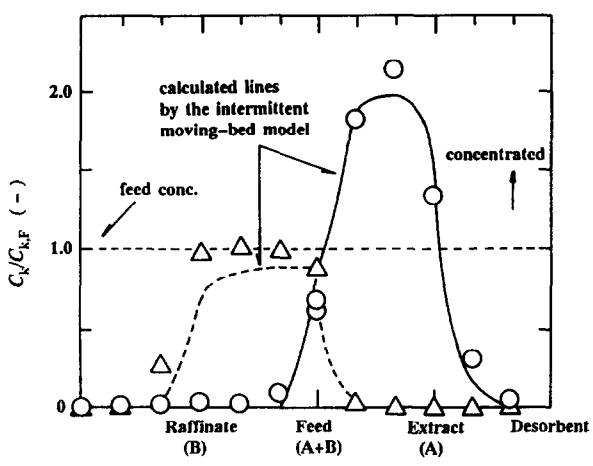

Fig. 3. Concentration profiles of (○) phenylalanine (A) and (△) NaCl (B) in a simulated moving-bed chromatograph. The concentration of phenylalanine in the extract stream is shown to be higher than its concentration in the feed stream.

$$\alpha_{kn} = \frac{K_{f,k} a_v L_n}{u_n} = \frac{1}{1-\beta_{kn}} \cdot \ln\left(\frac{C_{k,\text{out}} - C^*_{k,\text{out}}}{C_{k,\text{in}} - C^*_{k,\text{in}}}\right) \quad (8)$$

The following equation is also obtained from the material balance in zone n:

$$\beta_{kn}(C_{k,\text{out}} - C_{k,\text{in}}) = C^*_{k,\text{out}} - C^*_{k,\text{in}} \quad (9)$$

Considering the function of each zone that is fulfilled properly to give a good separation, the following simplified equations for α_{kn} can be obtained:

$$\alpha_{B1} = \frac{1}{1-\beta_{B1}} \cdot \ln\left[\frac{u_R}{u_F(1-\beta_{B1})}\right] \cdot \frac{C_{B,Ef}}{C_{B,F}} \quad (10a)$$

$$\alpha_{A2} = \frac{1}{1-\beta_{A2}} \cdot \ln\left[\frac{u_2 - u_3\beta_{A2}}{u_F(1-\beta_{A2})}\right] \cdot \frac{C_{A,R}}{C_{A,F}} \quad (10b)$$

$$\alpha_{B3} = \frac{1}{1-\beta_{B3}} \cdot \ln\left[\frac{-(u_2\beta_{B3} - u_3)}{u_F(1-\beta_{B3})}\right] \cdot \frac{C^*_{B,E}}{C_{B,F}} \quad (10c)$$

$$\alpha_{A4} = \frac{1}{1-\beta_{A4}} \cdot \ln\left[\frac{-u_E}{u_F(1-\beta_{A4})}\right] \cdot \frac{C^*_{A,R}}{C_{A,F}} \quad (10d)$$

where the subscripts F, R, E, D and Ef represent feed, raffinate, extract, desorbent and effluent (the liquid emerging from zone I), respectively. The length of each zone in SMBC and the operating conditions can be determined by using these equations according to the flow chart of the calculation in Fig. 4.

The concentrations of raw materials to be separated, their productivities and the concentrations of products are fixed. Assuming a moving rate of the resin u_s and liquid flow-rate in any zone, for example u_1, the liquid flow-rates in other zones can be calculated from the material balance. Then, substitution of very small values such as 10^{-4} for $C_k/C_{k,F}$ and $C^*_k/C^*_{k,F}$ in eqns. 10 yields α_{kn} values. The length of each zone can be given from the α_{kn} and the height of transfer unit $u_n/K_{f,k}a_v$. The length of each column is decided

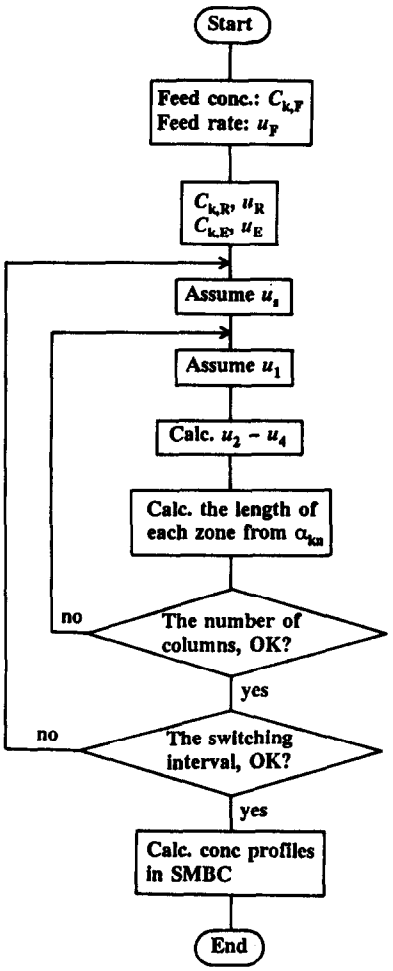

Fig. 4. Flow chart for simplified design method for a simulated moving-bed chromatograph.

so that the shortest zone may consist of at least two columns, because two columns are the minimum needed for achieving a good separation by SMBC [29]. If a zone requires an unreasonable number of columns, the value of u_1 may be changed and the calculation is repeated. The switching interval T is calculated from the u_s value. If the T value is extremely long or short, the u_s value should be changed and the calculation is repeated until a reasonable T value is obtained. The validity of the conditions thus obtained can be confirmed by calculation with the continuous moving-bed model.

2.5. Improved chromatography for continuous separation of three components

As mentioned above, SMBC is, in principle, a technique for the separation of two components or the fractionation of multiple components into two groups. The separation of multiple components into each component by SMBC requires some separation steps (the number of components to be separated − 1). Two methods have been developed to separate three components simultaneously by operations similar to SMBC.

One method uses columns with two kinds of resins, R_1 and R_2, whose functions are different from each other [30]. Fig. 5 shows the method schematically. Three components, A, B and C, are separated by the method. Component A is adsorbed neither on resin R_1 nor R_2 and is eluted from the raffinate stream. Component B is adsorbed on R_1 but not on R_2, while component C is adsorbed on R_2 but not on R_1. The resins R_1 and R_2 appear in zone III alternately. Zone IV is for desorption of components B and C adsorbed on R_1 and R_2, and the components are eluted alternately in the exact stream. The applicability of this method was illustrated for the separation system of starch, glucose and NaCl using four columns packed with a cation-exchange resin and an ion-retardant resin.

Another method has been proposed for separating three components using a resin [31–33]. Fig. 6 shows the concentration profiles of three components to be separated in the bed. Component M migrates at a rate between the migration rates of components A and B. The

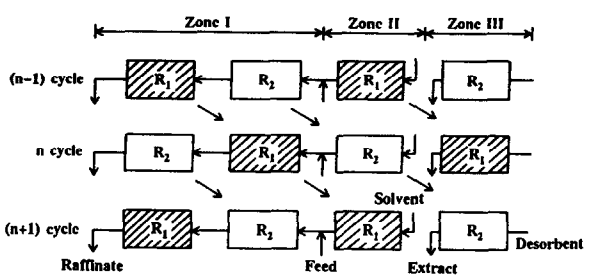

Fig. 5. Schematic representation of a chromatographic method for the separation of three components by using two kinds of resin, R_1 and R_2.

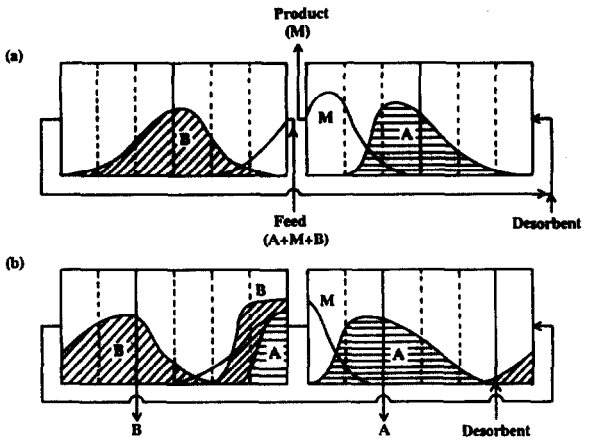

Fig. 6. Schematic representation of a chromatographic method for separating three components (new JO system).

system, denoted as a new JO system, possesses an additional outlet for component M and a stop valve. The switching interval T of the introduction and withdrawal points is determined so that the rate of movement of the resin is equal to the migration rate of component M. In the first step, mixture of the components and desorbent is fed from different points, the component M, which has been isolated from other components in the last step, is recovered, as shown in Fig. 6a. In the next step (Fig. 6b), simulated moving-bed operations are made without supplying the mixture. During this step, the most adsorptive component A and least adsorptive component B are eluted from the extract and raffinate streams, respectively. These steps are repeated alternately. This system has been confirmed on a pilot scale to be useful for the separation of three components, and it has been exploited commercially. This system can, in principle, be applied for the separation of four or more components by increasing the withdrawal points for separated components.

3. APPLICATION OF CHROMATOGRAPHIC SEPARATION TO BIOCHEMICAL REACTIONS

Although the type of bioreactor using immobilized enzymes is, in most cases, a fixed bed or stirred tank, it is not always better. The concept of a chromatographic reactor has been widely used in the field of catalysis, but there have been few applications to the biochemical field. Attempts to combine the chromatographic separation and an enzyme-catalysed reaction are described in this section.

3.1. Shift of reversible reaction toward favourable product

SMBC has been successfully used for production of a higher fructose syrup by separating fructose and glucose from their almost equimolar mixture, which is obtained through isomerization of glucose by glucose isomerase. The equilibrium constant of the isomerization is about 1. However, the process still consumes a relatively large amount of desorbent (water), which must be evaporated in a subsequent process. To decrease the amount of water consumed, a system has been proposed to produce higher fructose syrup by combining an adsorption process and an immobilized enzyme-catalysed reaction [34,35].

Fig. 7 shows the system schematically. Adsorption columns (A1–A9) are packed with the resin which adsorbs fructose selectively, and reactors (R1–R3) with immobilized glucose isomerase. The resin particles are moved, skipping the reactors in zone I, by a manner of simulated moving-bed operation. An equimolar mixture of glucose and fructose is introduced by a feed stream and flows in the adsorption column A4. As fructose in the mixture is adsorbed on the resin, the glucose content of the solution leaving the column becomes higher than the equilibrium

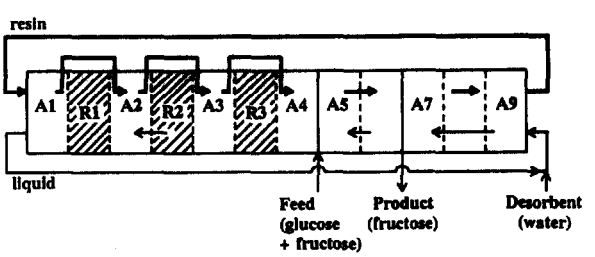

Fig. 7. Schematic representation of a system combining adsorption and immobilized enzyme reaction for shift of reversible reaction towards favourable product. A1–A9 are adsorption columns and R1–R3 are immobilized glucose isomerase reactors.

level. The solution is introduced into reactor R3 and glucose is converted into fructose there. Hence, by introducing the mixture through the adsorption columns and reactors alternately in zone I, most of glucose in the mixture is converted into fructose, which is adsorbed on the resin and conveyed to zones II and III. The liquid emerging from zone I contains very little of either glucose or fructose, and can be used as part of the desorbent, resulting in a decrease in the amount of make-up desorbent. Zones II and III have the same functions as zones III and IV of SMBC shown in Fig. 1, respectively. Fructose is recovered in the product stream. This system has been demonstrated experimentally to be practical. It has also been shown through computer simulations that this system consumes less desorbent than an SMBC process. Two models similar to the intermittent moving-bed model and the continuous moving-bed model for SMBC have been presented to calculate the concentration profiles in the system. The operating conditions for obtaining good productivity can be determined in a manner similar to that for SMBC.

3.2. Repeated use of free coenzyme by a reactor of the simulated moving-bed type

An immobilized conjugated enzymes reactor of the simulated moving-bed type has been developed to allow the repeated use of a free coenzyme [35,36]. A conjugated enzymes system consists of a main reaction, where the coenzyme is converted into another form, and a regeneration reaction of the coenzyme. Fig. 8 shows the operation of the reactor and the final states of location of the reactants in each step, assuming no zone spreading. The columns are packed with immobilized conjugated enzymes. The carrier of immobilized enzymes adsorbs a coenzyme more strongly than substrates and products. The operation of the reactor includes three steps, two of which, steps 1 and 2, are preliminary. In step 1 the mixture of coenzyme and substrates is fed to make the adsorption zones of a coenzyme in some columns in zone I, *e.g.*, three columns as shown in Fig. 8. Only this step includes the supply of the coenzyme. As the substrates and

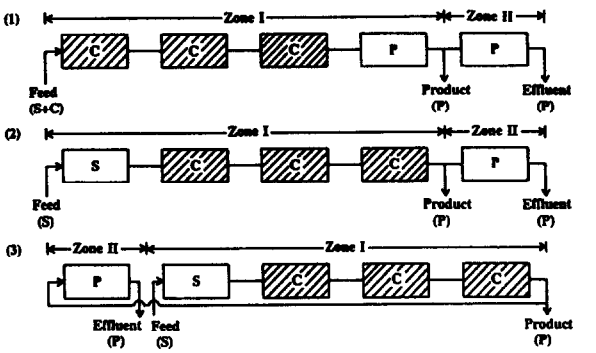

Fig. 8. Schematic representation of operations of a system for utilizing a free coenzyme effectively, and the locations of coenzyme (C), substrates (S) and products (P) at the end of each step.

products migrate faster than the coenzyme, the adsorption wave of the products reaches the withdrawal points earlier than that of the coenzyme, and the products are recovered from the product and effluent streams. In step 2, the feed is changed to the solution with only the substrates. This step is operated until the adsorption zone of the coenzyme migrates just at the distance of the one column. Step 3 is the main one, where the feed point and the withdrawal points are transported periodically to the next column in the same direction as the liquid flow. The substrates outrun the coenzyme, being converted into the products, which are recovered in the product stream. Part of the liquid emerging from zone I is introduced to zone II to replace the substrates by the products. The substrates recovered by the effluent stream are brought back to their reservoir. Direct introduction of the liquid emerging from zone II to zone I may be also possible. This step is repeated whenever the adsorption wave of the coenzyme migrates at the distance of one column. If there is no leakage of the coenzyme, the substrates can be converted continuously into the products without further addition of the coenzyme. This reactor was realized for reactions catalysed by hexokinase and pyruvate kinase, which required ATP (adenosine 5′-phosphate) as a coenzyme. The enzymes were immobilized on Amberlite XAD-2, which adsorbs ATP more strongly than substrates and products.

3.3. Repeated use of enzyme without its immobilization

Although many kinds of methods have been developed for the immobilization of enzymes, immobilization is not always successful. A new type of enzyme reactor, which allows free enzymes to be used repeatedly, has been developed based on the fact that enzymes migrate faster than low-molecular-mass substrates and products in a gel chromatographic column owing to their high molecular masses [35,37]. The reactor is constructed of at least two columns packed with a properly selected gel chromatographic resin. The simplest case of only two columns is described here, assuming that the distribution coefficient of an enzyme is zero. The dimensions of the columns are identical.

Fig. 9 shows schematically the reactor operation with six steps and the final states of the enzyme, substrate and product locations in each step. In step 1, the substrate solution is fed to column I so that the adsorption wave of the substrate will arrive at the exit of column II at the end of step 3. In step 2, the enzyme solution is fed, together with the substrate, to column I to fill the void of column I. The enzyme solution is supplied only in this step. These two steps are preliminary. In step 3, the enzyme located in column I at the end of step 2 is transferred to column II by supplying the substrate solution to column I. The enzyme outruns the substrate catalysing the reaction because of the faster migration rate of the enzyme. The purpose of step 4 is to recover the product from the top of column I by supplying the substrate solution from the bottom. During this step, the liquid in column II does not flow, but the enzymatic reaction proceeds. The functions of steps 5 and 6 are identical with those of steps 3 and 4, respectively, although the positions of feed and product streams are displaced. Steps 3–6 are repeated consecutively, the substrate being converted into the product without further addition of the enzyme if no enzyme leakage and denaturation occur. This reactor was successfully applied on a laboratory scale to the hydrolysis of maltose to glucose by glucoamylase using Bio-Gel P-10 as a gel chromatographic resin.

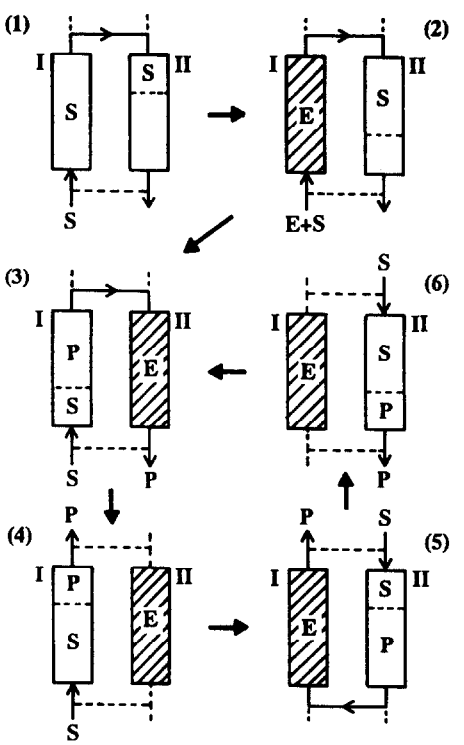

Fig. 9. Schematic representation of operations of a system for repeated use of enzyme without its immobilization, and locations of enzyme (E), substrate (S) and product (P) at the end of each step.

A chromatographic system of another type, combined bioreactor–separators, has also been developed for continuous dextran biosynthesis by dextransucrase and sucrose inversion by invertase [38–41]. Hence the combination of chromatographic separation and biochemical reactions has the potential to create new reactor systems with high performance. In the development of a new reactor system, understanding the reaction characteristics well is important.

4. SYMBOLS

a_v	specific surface area (m^2/m^3 bed)
C	concentration in the mobile phase (mol/m^3)
C^*	concentration equilibrium to q (mol/m^3)
K_f	overall mass transfer coefficient (m/s)
L	length of each column (m)

m distribution coefficient
N number of columns
q amount adsorbed (mol/kg)
T switching interval of the introduction and withdrawal points (s)
u_n superficial velocity of liquid flow in a hypothetical moving bed in zone n (m/s)
u_s hypothetical velocity of the resin flow ($=L/T$) (m/s)
v_n actual superficial velocity of liquid flow (m/s)
α_{kn} dimensionless parameter defined by eqn. 3
β_{kn} dimensionless parameter defined by eqn. 4
ε_b bed voidage
ρ apparent density of the resin (kg/m^3)

Subscripts

A adsorptive component
B less adsorptive component
D desorbent
E extract
Ef effluent
F feed
in inlet of the zone
k arbitrary component
n zone number
out outlet of the zone
R raffinate
1–4 zones I to IV

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