CONTINUOUS SEPARATION AND CONCENTRATION OF PROTEINS USING AN ANNULAR CHROMATOGRAPH

YOUICHI TAKAHASHI AND SHIGEO GOTO

Department of Chemical Engineering, Nagoya University, Nagoya 464-01, Japan

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A continuous rotating annular chromatograph with rotating feed nozzles and product collectors was used to separate two kinds of proteins, (myoglobin and hemoglobin). Simultaneously, both proteins could be concentrated by using two kinds of eluents. The cation exchange resin, CM-Sepharose CL-6B was used as an adsorbent. The adsorption forces of both proteins on CM-Sepharose CL-6B were dependent on the value of pH in the buffer solution. Therefore, both proteins could be separated and concentrated by changing pH in the eluent. Experimental results could be predicted by numerical solutions.

Continuous chromatographic systems have been developed for preparative separations. Moving chromatographic beds and simulated moving beds are mostly restricted to two-components separation¹⁾. On the other hand, a continuous rotating chromatograph proposed by Martin⁵⁾ can be used to separate multicomponents. Begovich *et al.*²⁾ used a rotating annular bed with stationary feed stream and product collection port to separate metal ions. Recently, De Carli *et al.*³⁾ have used the same apparatus as Begovich *et al.*²⁾ to separate three amino acids (glutamic acid, valine and leucine). Simultaneously, each of the amino acids could be concentrated in stepwise elution by using four kinds of solutions, such as feed mixture, displacer solution, regenerant and rinse. In this case, a dilute feed solution was introduced from a fixed nozzle and amino acids were adsorbed. Amino acids were desorbed from the adsorbent by a displacer solution. Three amino acids could be separated and concentrated by the difference of adsorption force on the cation exchange resin. However, the components could not be separated completely and the concentration ratio was low.

In our previous papers^{4,6-8)}, a continuous rotating annular chromatograph (abbreviated as CRAC) with rotating inlet nozzles and outlet collectors was proposed. This system is the opposite of the device of Begovich *et al.*²⁾ and has the following advantages. Since a weighty bed need not be rotated, rotating power can be saved and scaling up of the device is easy. The bed can be easily kept at constant temperature with a thermostat jacket. This is especially important for biological separations which require accurate temperature control. Sodium chloride and

methanol were separated by action of ion exclusion using CRAC and cation exchange resin as an adsorbent⁴⁾. Three kinds of amino acids (aspartic acid, glutamic acid and glycine) were separated by the difference of adsorption force for isocratic elution using CRAC⁸⁾. CRAC with two nozzles was used for non-isocratic elution to separate three kinds of amino acids (glutamic acid, glycine and valine)⁶⁾. In isocratic elution, since the adsorption force of valine on the adsorbent was much stronger than that of the other two amino acids, valine was eluted too late and the peak of valine was overlapped with that of the other amino acids after one rotation. Therefore, to weaken the adsorption force of valine on the adsorbent, another eluent was used and was introduced through another rotating nozzle in non-isocratic elution. Three kinds of amino acids could be separated very well in non-isocratic elution. Also, a single component of an amino acid (valine) was concentrated continuously using CRAC⁶⁾. In this case, dilute solution of valine was used as feed and the eluent was introduced from nozzles.

In this work, two components of proteins (myoglobin and hemoglobin) could be separated and concentrated simultaneously by using CRAC.

1. Principle

Figure 1 shows the principle of concentration and separation using CRAC. Feed solution containing dilute Solute-a and Solute-b is supplied into the whole annulus and two kinds of eluents are introduced through rotating nozzles. The products are collected by collectors which rotate as the same angular speed as the nozzles. For the feed solution in the annular bed, a feed flow zone is formed and the adsorbent is saturated with Solute-a and Solute-b from the top of

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Fig. 1. Principle of continuous separation and concentration using rotating annular chromatograph (CRAC)

- Region A: Saturated Solute-a and Solute-b in feed flow zone
 Region B: Saturated Solute-a in feed flow zone
 Region C: No solutes in feed flow zone
 Region D: Concentrated Solute-a in Eluent-1 flow zone
 Region E: Saturated Solute-b in Eluent-1 flow zone
 Region F: No solutes in Eluent-1 flow zone
 Region G: Concentrated Solute-b in Eluent-2 flow zone
 Region H: No solutes in Eluent-2 flow zone

the annular bed as shown in Region A. In this zone, the adsorption force of Solute-a and Solute-b is very strong. Since the adsorption capacity of Solute-a is smaller than that of Solute-b, Solute-a can flow down faster than Solute-b. Then, Region B will appear. On the other hand, when one eluent fluid (called Eluent-1) is introduced from rotating nozzles, an Eluent-1 flow zone appears. The adsorption force of Solute-a is weakened but that of Solute-b is still strong in this zone. Solute-a on the adsorbent is transferred to Eluent-1 fluid at the front of Eluent-1 flow zone and is moved down to the exit. Then one dilute component of Solute-a in the feed can be concentrated as shown in Region D. The adsorbent is still saturated with Solute-b in the Eluent-1 flow zone as shown in Region E. When another eluent fluid (called Eluent-2) is introduced from rotating nozzles, an Eluent-2 flow zone appears. The adsorption force of Solute-b is finally weakened in this zone. Solute-b on the adsorbent is transferred to Eluent-2 fluid at the front of the Eluent-2 flow zone and is moved down to the exit as shown in Region G. Contrarily, no solutes are contained in Regions C, F and H of the respective feed, Eluent-1 and Eluent-2 flow zones. The height $Z_{\min}$ indicates the minimum requirement for complete separation and concentration. Regions A, B and E with saturated solutes do not exist in the lower part from $Z_{\min}$.

2. Theory

The mathematical description of the separation and concentration of two components in CRAC can be expressed by the following equations. When radial gradients in velocity and concentration are assumed to be negligible, the mass balance on an element in CRAC yields

$$\omega \frac{\partial C}{\partial \theta} + \frac{u \partial C}{\varepsilon_B \partial z} + \frac{3\bar{D}(1-\varepsilon_B)}{R_p \varepsilon_B} \frac{\partial \bar{C}}{\partial r} \bigg|_{r=R_p} = D_L \frac{\partial^2 C}{\partial z^2} \quad (1)$$

The mass balance in the particle gives

$$\bar{D} \left(\frac{\partial^2 \bar{C}}{\partial r^2} + \frac{2}{r} \frac{\partial \bar{C}}{\partial r} \right) = \omega \frac{\partial \bar{C}}{\partial \theta} \quad (2)$$

The boundary conditions are

$$\bar{D} \frac{\partial \bar{C}}{\partial r} \bigg|_{r=R_p} = k_f(C - C^*) \quad \text{at } r=R_p, \quad \theta > 0 \quad (3)$$

$$\frac{\partial \bar{C}}{\partial r} = 0 \quad \text{at } r=0, \quad \theta > 0 \quad (4)$$

$$C = \bar{C} = 0 \quad \text{at } z \geq 0, \quad \theta = \theta_{E1} + \theta_{E2} \quad (5)$$

$$C = 0 \quad \text{at } z=0, \quad 0 \leq \theta \leq \theta_{E1} + \theta_{E2} \quad (6)$$

$$C = C_0 \quad \text{at } z=0, \quad \theta_{E1} + \theta_{E2} < \theta \leq 2\pi \quad (7)$$

In this work, the zero of angular coordinate indicates the leading point to introduce Eluent-1. As shown in Fig. 1, the angular distance of Eluent-1 and Eluent-2 injections, θ_{E1} and θ_{E2} , are expressed by $2\pi Q_{E1}/(Q_{E1} + Q_{E2} + Q_F)$ and $2\pi Q_{E2}/(Q_{E1} + Q_{E2} + Q_F)$ respectively. Feed solution containing two components is introduced everywhere else.

The equations of adsorption isotherms of two components may be given as follows:

$$\bar{C} = f_1(C) \quad \text{in the feed flow zone} \quad (8a)$$

$$\bar{C} = f_2(C) \quad \text{in Eluent-1 flow zone} \quad (8b)$$

$$\bar{C} = f_3(C) \quad \text{in Eluent-2 flow zone} \quad (8c)$$

where the adsorption force in the feed flow zone should be much stronger than that in either Eluent-1 or Eluent-2 flow zone. The adsorption isotherms of both components were assumed to have no interaction with each other.

It is assumed that the angular distance of the Eluent-1 and Eluent-2 flow zone in Fig. 1 are constant at any axial coordinates. Then the Eluent-1 and Eluent-2 flow zones are expressed by

$$\theta_D(z) < \theta < \theta_D(z) + \theta_{E1} \quad \text{at } z > 0 \quad (9a)$$

for Eluent-1 flow zone

$$\theta_D(z) + \theta_{E1} < \theta < \theta_D(z) + \theta_{E1} + \theta_{E2} \quad \text{at } z > 0 \quad (9b)$$

for Eluent-2 flow zone

where the angular delay, $\theta_p(z)$, is a function of the axial coordinate from the top of the bed and is given by $\omega z \varepsilon_B / u$. The feed flow zone is the whole annular bed except for the Eluent-1 and Eluent-2 flow zones.

Numerical solutions were obtained by the orthogonal collocation method.

3. Experimental

3.1 Apparatus

The CRAC apparatus used in this work was the same as that described in the previous paper⁷⁾. In this work, two kinds of eluents were used and were introduced through two rotating nozzles. The feed solution containing two kinds of dilute proteins was supplied everywhere else in the top of the annular bed. The angular distance between the two nozzles was 45 degrees. Eluent-1 was introduced through the first nozzle, Eluent-2 through the second.

The adsorbent was packed in the annular bed. The height of the adsorbent bed was 30 cm. Glass beads were packed to a height of 4.5 cm on top of the adsorbent bed to prevent overflow and mixing of the eluents. The nozzles were embedded 3.0 cm deep in the bed of glass beads. Thicknesses of 2 and 5 mm were used in this work. 60 evenly spaced small stainless steel tubes (I.D. 2 mm, length 30 mm) were attached to the bottom of the annular bed to facilitate the flow of fluids at a constant velocity around the annular bed. The product was collected by using a drain saucer with 45 evenly spaced sampling tubes. After steady state was reached, the tubes were settled to collect samples.

3.2 Operation

A cation exchange resin, CM-Sepharose CL-6B (Pharmacia Fine Chemicals Co. Ltd.), was used as the adsorbent. The average diameter of the adsorbent was 7.4×10^{-5} m. The feed solution contained myoglobin and hemoglobin, both at concentrations of 0.01 kg/m^3 in 0.05 M sodium phosphate buffer. The same buffer was used as Eluent-1 and Eluent-2 respectively. The values of pH were adjusted to pH 5.3, 6.0 and 7.2 for the feed solution, Eluent-1 and Eluent-2, respectively. Temperature in the annular bed was kept at 288 K by circulating water into the jacket. The exit concentrations of proteins were measured by a UV-meter (Simadzu Co. Ltd.). The exit pH values were measured by a Compact pH meter (Horiba Co. Ltd.).

4. Results and Discussion

4.1 Adsorption isotherms

Figure 2 shows the adsorption isotherms of two proteins on the cation exchange resin CM-Sepharose CL-6B at 288 K. They were measured in buffer solutions of pH 5.3, 6.0 and 7.2 by the batch method. The equations could be expressed as Freundlich type and are listed in Table 1. The adsorption force of

Fig. 2. Adsorption isotherms

Table 1. Equations of adsorption isotherms of proteins

	Myoglobin	Hemoglobin
pH 5.3	$\bar{C} = 125C^{0.78}$	$\bar{C} = 114C^{0.83}$
pH 6.0	$\bar{C} = 7.48C^{0.93}$	$\bar{C} = 117C^{0.95}$
pH 7.2	$\bar{C} = 0$	$\bar{C} = 0$

myoglobin at pH 6.0 was much smaller than that at pH 5.3. On the other hand, the adsorption force of hemoglobin at pH 6.0 was slightly smaller than that at pH 5.3. The adsorption isotherms of both proteins at pH 7.2 were nearly zero. Therefore, both proteins could be adsorbed in the solution at pH 5.3. At pH 6.0, myoglobin could be desorbed in the solution but hemoglobin could not. Both proteins could be completely desorbed in the solution at pH 7.2.

The behavior of adsorption isotherms may be explained by isoelectric points (abbreviated as pI) of proteins. If the value of pH in the solution is lower than pI, the ionic state of protein is cationic and thus the protein can be adsorbed on the cation exchange resin. When a solution with higher pH than pI is used as an eluent the protein becomes anionic and can be desorbed from the cation exchange resin. Therefore, multicomponent mixtures of proteins can be separated and concentrated by changing pH or the ionic strength in solutions.

4.2 Elution curves in CRAC

Figure 3 shows the elution curves in CRAC. The circle and square marks indicate the concentrations of myoglobin and hemoglobin respectively. The solid lines indicate the calculated results for both proteins. The triangle marks and dotted lines indicate the pH of solution at the exit.

Myoglobin was eluted first and hemoglobin later. The pH profile has two bands. The first band, at pH 6.0, was formed by Eluent-1. The second band, at pH 7.2, was formed by Eluent-2. As shown in the pH profile, myoglobin and hemoglobin were concentrated by Eluent-1 and Eluent-2 respectively. Both proteins were completely separated and concentrated.

Fig. 3. Elution curves in CRAC

The calculated profiles are much sharper than the experimental ones. For myoglobin and hemoglobin, the maximum ratios of experimental results are about 10 and 15 while those of calculated results are about 47 and 50. The reduction of maximum ratio in experimental results may be due to the mixing of the solutions at the exit of the annular column. Since the colors of myoglobin and hemoglobin were red and the cylinder of the annular bed was made of transparent acrylic, the bands of solutes in the annular bed could be observed visually. The bandwidths of Regions D and G in Fig. 1 were about 4 and 6 mm for myoglobin and hemoglobin respectively. On the other hand, the distance between the exit tubes at the bottom of the annular column was 5.2 mm—too wide to distinguish the bandwidths of both proteins. Therefore, the concentrated solution of proteins was mixed with a dilute one at the exit of the annular column. If the mixing of solutions around exit tubes can be taken into account, the calculated profiles may agree with the experimental one. However, since the flow patterns near the exit are complex it is difficult to simulate them exactly. If the distance between exit tubes can be made much narrower, the experimental maximum ratio may become closer to the calculated one.

4.3 Effect of rotating speed

Figure 4 shows the effect of the rotating speed on concentration profiles while the feed flow rate, Q_F , Eluent-1 flow rate, Q_{E1} , and Eluent-2 flow rate, Q_{E2} , are held constant. With increasing rotating speed the peak heights of both proteins became lower, the bandwidths wider and the peak positions later. Therefore, to recover higher-concentration proteins the rotating speed should be reduced. When the rotating speed was too low, however, Eluent-1 and Eluent-2 flow zones could not be formed sufficiently. Therefore, a lower limit of desirable rotating speed exists.

4.4 Effect of thickness of annular bed

Figure 5 shows the elution curves using annular

Fig. 4. Effect of rotating speed

Fig. 5. Effect of thickness of annular column

columns of two bed thicknesses, while superficial velocity, u , and rotating speed, ω , are held constant. The experimental results agreed closely. Therefore, when a thicker annular column was used, more feed solution could be treated. However, if the thickness is too great, Eluent-1 and Eluent-2 flow zones will not be formed effectively.

4.5 Effect of column height

Regions D and G in Fig. 1 could be visually observed by their red color. The bandwidths became wider as the height increased. The part of the annular bed below $Z_{\min}$ is not needed for concentrating solutes. The value of $Z_{\min}$ can be calculated as follows.

Figure 6 shows the calculated results at various heights of the annular column for the same experimental conditions as in Fig. 3. To simplify this figure, only the lower parts of calculated curves ($C/C_0 \leq 5$) are shown. At $z = 0.025$ m, myoglobin and hemoglobin appear before 360 degrees since Region A in Fig. 1 exists. Both elution curves are broad and overlap each other. At $z = 0.05$ m, the peak of myoglobin becomes sharp but the elution curve of hemoglobin is still broad and overlaps that of myoglobin since Region B exists. At $z = 0.075$ m, the front part of the hemoglobin curve is still broad. At $z = 0.1$ m, the peaks of both proteins are sharp and are separated completely. Therefore, in this condition,

Fig. 6. Effect of column height

the minimum height, $Z_{\min}$, is about 0.1 m.

Conclusion

Using CRAC, two components of proteins (myoglobin and hemoglobin) could be separated and concentrated simultaneously. The adsorption force was dependent on the pH value. Experimental results could be predicted by the numerical solutions.

If three or four eluents are used in this system, multi-components can be separated and concentrated. Suitable eluents should be selected to desorb only one solute from other solutes.

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Nomenclature

C	= concentration in liquid phase	[kg/m ³]
$\bar{C}$	= concentration in adsorbent phase	[kg/m ³]
C_0	= feed concentration	[kg/m ³]
C^*	= equilibrium concentration	[kg/m ³]
$\bar{D}$	= intraparticle diffusivity	[m ² /s]
D_L	= axial dispersion coefficient	[m ² /s]
k_f	= liquid-film mass transfer coefficient	[m/s]
Q_{E1}	= Eluent-1 flow rate	[m ³ /s]
Q_{E2}	= Eluent-2 flow rate	[m ³ /s]
Q_F	= feed flow rate	[m ³ /s]
R_p	= radius of adsorbent particle	[m]
r	= radial coordinate	[m]
u	= superficial velocity	[m/s]
z	= axial coordinate	[m]
ε_B	= interparticle void fraction in the bed	[—]
θ	= angular coordinate	[deg or rad]
ω	= rotating speed	[deg/s or rad/s]

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