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# Predicting the performance of gel-filtration chromatography of proteins

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ABSTRACT

A method for determining the column and operating variables for the preparative separation of proteins by gel-filtration chromatography (GFC) of proteins is presented. The recovery of bovine serum albumin monomer from its dimer and higher molecular weight aggregates on high-performance GFC columns of various dimensions (column diameter $d_c = 0.75$–$10.8$ cm, column length $Z = 10$–$80$ cm, particle diameter $d_p = 10$–$17$ $\mu$m) was chosen as a model separation system.

In the calculation method, the maximum sample feed volume $V_{F,M}$ that satisfies a specified purity ratio $Q_P$ and recovery ratio $Q_R$ for a given column length $Z$ and particle diameter $d_P$ at a certain linear mobile phase velocity $u$ was sought. The productivity $P$, defined as the amount of the recovered protein per unit column volume per unit time, was then calculated. The effects of $Z$ and $d_P$ on the $P$–$u$ relationship were examined. It was found that in general $P$ increases with increasing $u$ and the slope of the $P$–$u$ curve becomes steep with decreasing $d_P$ and/or $Z$. A maximum in the $P$–$u$ relationship was observed when the separation was difficult.

The results show that it is not advantageous to employ larger particle diameter packings and/or a longer column in scaling-up. It is rather recommended that a short column packed with small gels be operated at relatively low flow-rates in the preparative GFC of proteins. It is also suggested that the calculation method presented is useful for scaling up GFC columns.

## INTRODUCTION

Gel-filtration (also called size-exclusion or gel-permeation) chromatography (GFC)[1] is an efficient method not only for analytical separations but also for preparative (large-scale) separations of proteins and other biological products[2,3]. GFC is classified as linear isocratic elution liquid chromatography (LC), in which the composition of the mobile phase (elution buffer) is constant and the distribution coefficient of a solute between the mobile and stationary phases ($K$) is not dependent on the solute concentration. In this type of LC the elution curve is nearly Gaussian

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when the sample feed volume $V_F$ is small, as in the analytical separation. Such an elution curve can be easily characterized by the HETP–$u$ relationship ($u$ = linear mobile phase velocity) and the $K$ value measured experimentally at small $V_F$ values.

However, in preparative separations, $V_F$ is increased in order to maximize the productivity at a specified purity. This results in a change in the Gaussian-shaped elution curve and overlap of the elution curves. Therefore, the calculation of the whole elution curves by a model which considers the effect of $V_F$ in addition to the other operating and column variables is needed in order to predict the performance of GFC.

Many researchers have reported on the optimization, the maximization of the throughput and the scaling rules (*e.g.*, refs. 4–7), but it seems that the development of a "user-friendly" computer program that can be run on a personal computer is needed for this purpose.

In this paper, a method for determining the column and operating variables for preparative separations of proteins by GFC is presented. The recovery of bovine serum albumin (BSA) monomer from its dimer and higher molecular weight aggregates on high-performance (HP) GFC columns of various dimensions (column diameter $d_c$ = 0.75–10.8 cm; column length $Z$ = 10–80 cm; particle diameter $d_p$ = 10–17 $\mu$m) was chosen as a model separation system.

The elution curves for large sample volumes were calculated numerically on the basis of the HETP–$u$ data and the $K$ values obtained at small sample volumes. The calculated curves were compared with the experimental curves for various $V_F$ values. Such a calculation procedure is used for searching for the maximum sample feed volume $V_{F,M}$ that satisfies a specified purrity ratio $Q_P$ and recovery ratio $Q_R$ for a given column length $Z$ and particle diameter $d_p$ at a certain $u$. The experimental data for HETP–$u$ and $K$ values obtained at small $V_F$ values are used in the calculation. The productivity $P$, defined as the amount of the recovered protein per unit column volume per unit time, was then calculated.

This calculation method was coded in FORTRAN and BASIC so that it can be run on a personal computer. The program can also search for the $V_F$ and $u$ values that can give the maximum productivity $P_M$ at a certain $Z$ and $d_p$. The effects of $d_p$, $Z$, $Q_P$ and $Q_R$ on $P$ were examined.

## THEORETICAL

### *Calculation of the elution curve*

Although GFC is modelled most rigorously by a set of partial differential equations that consider axial dispersion, stationary (gel) phase diffusion, fluid-film mass transfer and distribution of a solute[8,9], the analytical solution is complicated and difficult to calculate owing to its oscillating nature[10]. For the HPGFC columns employed here, the following solution can be employed (the details of the comparison of various models and the validation of the use of the following solution will be described elsehwere[11]):

$$C/C_0 = f(N,T) - f(N,T - T_0) \tag{1}$$

where $f(x,y) = \mathrm{erfc}[(x/2y)^{1/2} - (xy/2)^{1/2}]/2$, $T = tu/[Z(1 + HK)]$ and $T_0 = t_0u/[Z(1 + HK)]$; erfc is the error-function complement, $C$ is the solute concentration at the outlet of the column, $C_0$ is the initial concentration, $t$ is the time from the start of the sample injection, $t_0 = V_F/F$ is the sample injection time, $F$ is the volumetric flow-rate and is related to $u$ as $u = F/(A_c\varepsilon)$, $A_c$ is the column cross-sectional area and $\varepsilon$ is the void fraction of the column. $H = (1 - \varepsilon)/\varepsilon$ is the parameter describing the ratio of the column gel volume to the void volume. The total number of theoretical plates $N$ ( $= Z$/HETP) in eqn. 1 is considered to be the sum of the contributions of various parameters affecting the zone spreading, such as the axial dispersion, the stationary phase diffusion and the fluid-film mass transfer. This concept has already been propounded by many researchers (*e.g.*, refs. 12–17). The HETP equation derived from the moment equations of the Kubin–Kucera model[8,9] is given with reduced variables as[17]

$$h = A^* + B^*/v + C^*v + D^*v \qquad (2)$$

where $h =$ HETP$/d_p$ is the reduced HETP and $v = ud_p/D_m$ is the reduced velocity ($D_m$ is the molecular diffusion coefficient). This has the same form as the van Deemter equation[15]. Under the conditions employed here, the contribution of the second (molecular diffusion) and the fourth (fluid-film mass transfer) terms to the total $h$ value can be ignored[17]. Then eqn. 2 becomes

$$h = A^* + C^*v \qquad (3)$$

where $A^*$ is the (constant) axial dispersion term and $C^*v$ is the stationary phase diffusion term. The experimental results were predicted well by this equation, as shown previously[18].

Another important parameter in eqn. 1, $K$, can be related to the peak retention time of the elution curve $t_M$ at small $V_F$:

$$t_M - V_F/(2F) = (Z/u)(1 + HK) = (Z/u)(1 + k') \qquad (4)$$

Once the elution curves at small $V_F$ have been measured as a function of $u$, the HETP–$u$ relationship and the $K$ values can be obtained from the peak width and $t_M$.

*Calculation of the productivity*

We adopted the following definition for the productivity $P$:

$$\begin{aligned} P &= [(\text{recovery ratio})(\text{sample feed volume})]/[(\text{column void volume})(\text{cycle time})] \\ &= Q_R V_{F,M}/[V_0(V_0/F)] \end{aligned} \qquad (5)$$

where $V_{F,M}$ is the maximum $V_F$ value that satisfies a specified purity ratio $Q_P$ and recovery ratio $Q_R$. $Q_P$ and $Q_R$ are defined as follows:

$$Q_R = m_d/(V_F C_{0,d}) \qquad (6)$$

$$Q_P = m_d/(m_d + m_c) \qquad (7)$$

where $m$ is the amount of the recovered fraction. The subscripts c and d imply a contaminant and desired solutes, respectively. The cycle time in eqn. 5 is the time needed for eluting one column void volume. If we multiply $(1 + H)$ by $V_0/F$, it will be the time needed for eluting one column volume.

If we consider repetitive injections, the cycle time should be modified[5]. When the zone spreading is ignored (ideal case), $Q_R = 1.0$ and $V_{F,M}$ is the difference in the elution volumes of the two substances, which is equal to $V_{F,M} = [V_0 + K_d(V_t - V_0)] - [V_0 + K_c(V_t - V_0)] = (V_t - V_0)(K_d - K_c)$. Then, inserting the above two relatioships into eqn. 5 yields the ideal $P$ value $P_I$ (here, $K_d > K_c$ is assumed. If $K_d < K_c$, then $K_d - K_c$ should be read as $K_c - K_d$):

$$P_I = [(V_t - V_0)(K_d - K_c)]/[V_0(V_0/F)] = H(K_d - K_c)u/Z \tag{8}$$

This equation implies that $P$ is proportional to $u$ and the inverse of $Z$.

We search for the maximum sample feed volume $V_{F,M}$ that satisfies a specified purity ratio $Q_P$ and recovery ratio $Q_R$ for a given $Z$ and $d_p$ at a certain $u$. The calculation scheme is summarized as follows (the experimental results for the $h$–$v$ relationships and $K$ values are used; the subscript t means a tentative value):

(1) Set $K_c$, $K_d$, $\varepsilon$, $d_p$, $D_{m,c}$, $D_{m,d}$ and $Z$, and specify $Q_R$ and $Q_P$.

(2) Set $u$ and determine $N_c$ and $N_d$ from the $h$–$v$ curves.

(3) Set a tentative value of the sample volume $V_{F,t}$.

(4) Calculate the elution curves by eqn. 1.

(5) Calculate $Q_{P,t}$ as a function of $Q_{R,t}$ for the desired substance. [The elution curves of the target protein and the contaminant are integrated from the rear or the front end of the curve to give $m_c$ or $m_d$ as a function of time (in this study, from the rear end of the BSA monomer curve). $Q_P$ and $Q_R$ are then calculated from these values on the basis of eqns. 6 and 7. Then, from the $Q_P$–time and $Q_R$–time relationships, the $Q_P - Q_R$ relationship is obtained (see Fig. 3).]

(6) Compare $Q_{P,t}$ with $Q_P$ at $Q_{R,t} = Q_R$.

(7) If $Q_{P,t} > Q_P$, increase $V_{F,t}$; if $Q_{P,t} < Q_P$, decrease $V_{F,t}$.

(8) Repeat (4)–(7) until $(1 - V_{F,old}/V_{F,new}) < 0.01$.

(9) Set this $V_F$ to be $V_{F,M}$ and calculate $P$ by eqn. 5.

Although the above scheme treats the two elution curves, it can be extended to more than two curves. This program is now commercially available from Nihon Kagaku Gijyutu Kensyusyo (Tokyo, Japan) as JUSE-BIOLC[19].

## EXPERIMENTAL

TSK G-3000SW HPGFC columns of various dimensions were employed: column A, $Z = 30$ cm, $d_c = 0.75$ cm, $d_p = 10$ $\mu$m; column B, $Z = 67.5$ cm, $d_c = 0.75$ cm, $d_p = 10$ $\mu$m; column C, $Z = 67.5$ cm, $d_c = 2.15$ cm, $d_p = 13$ $\mu$m; column D, $Z = 60.0$ cm, $d_c = 5.5$ cm, $d_p = 17$ $\mu$m; column E, $Z = 80$ cm, $d_c = 10.8$ cm, $d_p = 17$ $\mu$m; column F, $Z = 10$ cm, $d_c = 4.5$ cm, $d_p = 17$ $\mu$m.

The apparatus used was a CCPE pump (Tosoh) and a UV-8010 UV detector (Tosoh) for columns A, B, C and F and a CCP-8070 pump (Tosoh) and a UV-8070 UV detector (Tosoh) for columns D and E.

The mobile phase (elution buffer) was 10 m$M$ phosphate buffer (pH 6.8) containing 0.3 $M$ sodium chloride or 0.2 $M$ phosphate buffer (pH 6.8). Bovine serum albumin (BSA) (Cohn Fraction V, Sigma, A8022) dissolved in the buffer solution was used as a sample. The concentration of BSA was 0.2–0.5% in most experiments. The experiments were carried out at 20–25°C.

The peak retention time, $t_M$, and the peak width at $C = 0.368\ C_M$, $w$, were measured from the elution curve at small $V_F$ values ($C_M$ = the maximum peak height in the elution curve). The HETP values were calculated according to the equation

$$\mathrm{HETP} = Z[(w^2/8 - t_0^2/12)/(t_M - t_0/2)^2] \tag{9}$$

The HETP–$u$ relationships were then converted to $h$–$v$ relationships with the $d_p$ value and the $D_m$ value calculated using the equation presented by Young *et al.*[20]. The void fraction of the column, $\varepsilon$, was determined from the $t_M$ of Blue Dextran 2000 pulses. The $Q_P$ and $Q_R$ values and the initial concentration of each component contained in the sample ($C_0$) were determined from the area of the analytical chromatogram using a TSK G-3000SWXL HPGFC column (30 × 0.75 cm I.D.) with $F$ = 0.4 ml/min and $V_F$ = 0.1 ml.

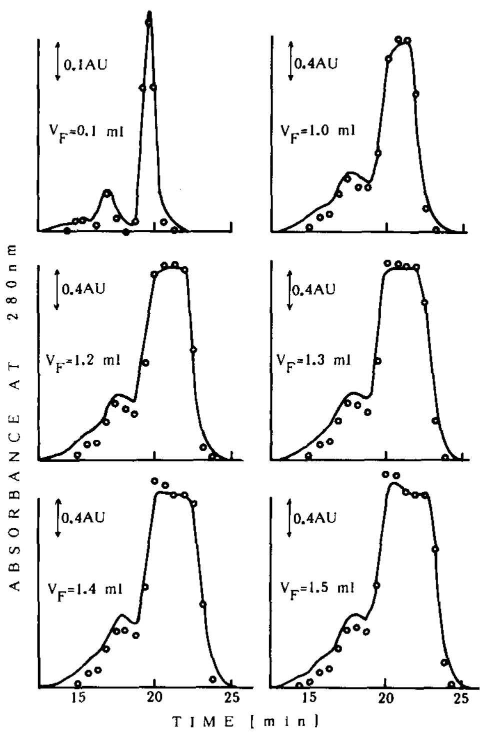



Fig. 1. Experimental and calculated elution curves for various sample volumes ($V_F$). Curves, experimental elution curves (detector response at 280 nm). Column A (30 × 0.75 cm I.D., $d_p$ = 10 $\mu$m); BSA concentration, 0.5%; $F$ = 0.4 ml/min. ○, Calculated results (the sum of monomer, dimer and aggregate curves). Data used for the calculation: $\varepsilon$ = 0.37, $K_{\text{monomer}}$ = 0.35, $K_{\text{dimer}}$ = 0.22, $K_{\text{aggregates}}$ = 0.14, $C_{0,\text{monomer}}:C_{0,\text{dimer}}:C_{0,\text{aggregates}}$ = 1:0.22:0.08 (determined from the chromatogram by analytical HPGFC). $N$ values for monomer, dimer and aggregates were determined from the $h$–$v$ relationship; $h = 4 + 0.09v$ shown in Fig. 2. The calculated $Q_R$ value at $Q_P$ = 0.99 is 0.97 at $V_F$ = 1.0 ml, 0.88 at $V_F$ = 1.2 ml, 0.82 at $V_F$ = 1.3 ml, 0.77 at $V_F$ = 1.4 ml and 0.72 at $V_F$ = 1.5 ml.

## RESULTS

### *Elution curves and h–v relationships*

Experimental and calculated elution curves on a small-scale column at various $V_F$ values are shown in Fig. 1. The HETP values of BSA monomer were determined from the peak width of the elution curve at small $V_F$. Then, the HETP–$u$ relationships were converted to $h$–$v$ relationships. As shown in Fig. 2, the $h$–$v$ relationships can be described by the equation

$$h = 4 + 0.09v \tag{10}$$

regardless of the particle size, $d_p$, as shown in a previous study[18]. This is the basis for examining the effect of $d_p$ on $P$ in the following section.

When $V_F$ is increased, the elution curve of BSA monomer becomes flat-topped, as shown in Fig. 1. It is interesting that the shape of the elution curves varies markedly with a small change in $V_F$ in the range 1–1.5 ml.

No substantial difference is found in the experimental elution curves at different sample concentrations (0.25–1%), as shown in Fig. 3.

We calculated the elution curves with eqn. 1 based on the assumption that they can be described by the sum of the three components, monomer (mol.wt. 69 000), dimer (mol.wt. 150 000) and higher molecular weight aggregates (mol.wt. 300 000), although there may be several different molecular weight aggregates and other contaminants such as globulins. A further assumption is that the $h$–$v$ relationships are similar for the three components. The calculated points are in fairly good agreement with the experimental curves in Figs. 1 and 3. The calculation shows that the small peak maximum observed on the left-hand side of the monomer curve in Fig. 3 is caused by the sum of the three peaks and is not the true peak. The calculated $Q_P$–time and $Q_R$–time curves in Fig. 3 and the calculated $Q_R$ values at $Q_P = 0.99$ given in the legend of Fig. 1 clearly illustrate the general relationship between $Q_P$ and $Q_R$. For example, in Fig. 1 when $Q_P = 0.99$ and $Q_R = 0.90$ are required, the $V_{F,M}$ value may be between 1.0 and 1.2 ml. This is the principle of the present method of determining $P$, which will be shown in the next section.

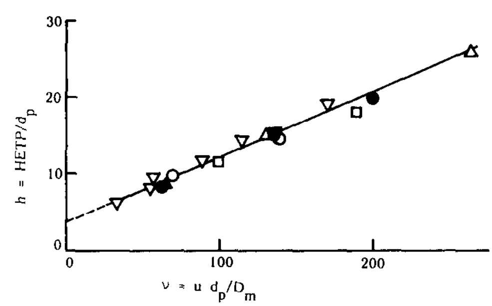



Fig. 2. Relationship between $h$ and $v$ for BSA monomer on columns of various dimensions. ▽ = Column A (30 × 0.75 cm I.D., $d_p$ = 10 μm); ○ = column B (67.5 × 0.75 cm I.D., $d_p$ = 10 μm); □ = column C (67.5 × 2.15 cm I.D., $d_p$ = 13 μm); △ = column D (60.0 × 5.5 cm I.D., $d_p$ = 17 μm); ● = column E (80 × 10.8 cm I.D., $d_p$ = 17 μm). BSA concentration, 0.2–0.5%; $V_F$ = 0.003–0.01 $V_t$. For other conditions, see Experimental.

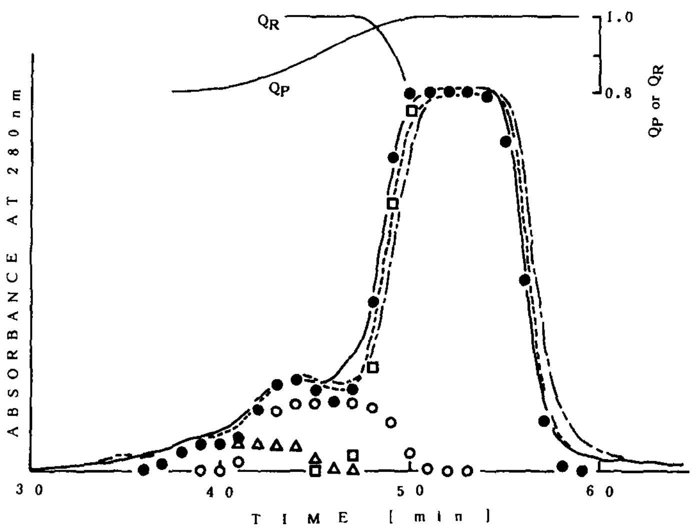



Fig. 3. Experimental and calculated elution curves for various sample concentrations on a large-scale column. Column E (80 × 10.8 cm I.D., $d_p$ = 17 $\mu$m); $V_F$ = 695 ml; $F$ = 93 ml/min. — = Experimental results, 0.25% BSA; --- = experimental results, 0.5% BSA; -·- = experimental results, 1.0% BSA; ● = calculated results for the sum of monomer, dimer and aggregate curves; △ = calculated results for aggregates; ○ = calculated results for dimer; □ = calculated results for monomer. Data used for the calculation: $\varepsilon$ = 0.36, $K_{monomer}$ = 0.39, $K_{dimer}$ = 0.26, $K_{aggregates}$ = 0.17, $C_{0,monomer}$:$C_{0,dimer}$:$C_{0,aggregates}$ = 1:0.18:0.07. Note that these values are different from those used in Fig. 1 owing to the inter-lot variation of GFC packings and BSA samples. $N$ values were determined by the same procedure as that in Fig. 1. The calculated $Q_P$–time and $Q_R$–time curves are also shown.

*Relationship between P and u*

Fig. 4 shows the calculated relationship between $P$ and $u$ for various combinations of $Z$ and $d_p$ at $Q_R = Q_P = 0.99$. Let us examine the $P$–$u$ curve for the shortest column, *i.e.*, 17 $\mu$m particle size and 10 cm long column. $P$ increases with increasing $u$ in the range $u$ = 0 to 0.6 cm/min and shows a maximum value $P_M$ at $u$ = 0.6 cm/min (hereafter, this $u$ is called $u_M$). Above $u_M$, $P$ drops rapidly to zero (the $u$ value at which $P$ is almost equal to zero is designated $u_c$). This is explained as follows: $N$ decreases with $u$ as predicted by eqn. 3. Therefore, when $u$ is increased $V_F$ should be reduced in order to satisfy $Q_P$ and $Q_R$. Below $u_M$ the decrease in cycle time ($V_0/F$) is larger than that in $V_F/V_0$. This results in an increase in $P$ with $u$. On the other hand, $P$ decreases with $u$ above $u_M$ as $V_F/V_0$ decreases much more rapidly than $V_0/F$. Above $u_c$, a specified $Q_P$ is not obtained even at the limit of $V_F = 0$.

Although no substantial $P_M$ is observed for the other three $P$–$u$ relationships, the slope of the curve decreases gradually with increasing $u$. For the same $d_p$, $P$ for a shorter column is higher than that for a longer one in the range $u < u_M$. It should be also noted that $P$ is markedly increased with decrease in $d_p$.

The filled circles in Fig. 4 are the experimental results obtained with column F ($d_c$ = 4.5 cm, $Z$ = 10 cm and $d_p$ = 17 $\mu$m). The $Q_P$ and $Q_R$ values were obtained from the HPGFC trace for the recovered fraction. Fig. 5 shows the elution curves obtained with column F, which corresponds to $P_M$ in Fig. 4. Although the $Q_P$ and $Q_R$ values are

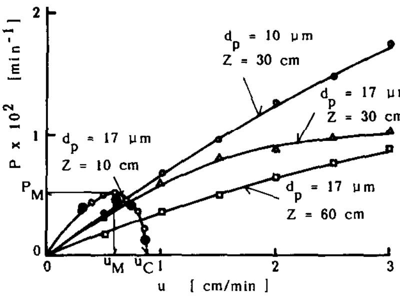



Fig. 4. Relationship between $P$ and $u$. The data used in the calculation are the same as in Fig. 3; ●, experimental results with column F (10 × 4.5 cm I.D., $d_p$ = 17 $\mu$m). $Q_R$ = 0.94–0.95 and $Q_P$ = 0.95–0.96 for these experimental results determined from the chromatogram obtained by analytical HPGFC.

lower than those in Fig. 4, it is seen that such a short column can separate BSA monomer fairly well.

Fig. 6 shows the calculated relationship between $P_M$ and $Q_P$ at $Q_R$ = 0.99 and $P_M$ and $Q_R$ at $Q_P$ = 0.99 for $Z$ = 10 cm and $d_p$ = 17 $\mu$m. $P_M$ increases with decreasing $Q_P$ or $Q_R$, but $P_M$ is more sensitive to $Q_P$.

Fig. 7 shows the calculated relationship between $P_M$ and $Z$ at $Q_P = Q_R$ = 0.99. The slope of the $P_M$–$Z$ relationship decreases with increasing $Z$.

## DISCUSSION

The recovery of protein monomer from its aggregates is a very important process in biotechnology[2]. As this process is usually performed in the last stage of purification, the amount of monomer is much larger than that of the aggregates and other contaminants. Therefore, in the production process, the overloading conditions can be

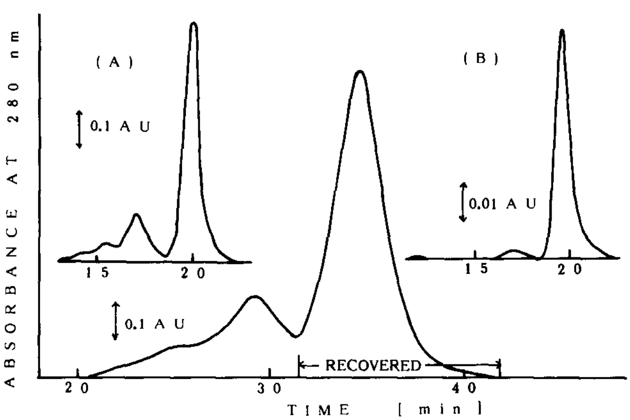
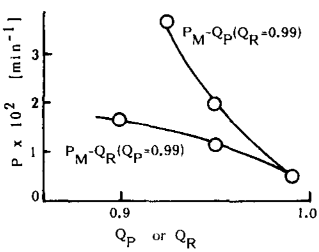



Fig. 5. Experimental elution curves on a 10.0 × 4.5 cm I.D. column ($d_p$ = 17 $\mu$m) and the purity of the recovered fraction checked by the analytical column. Column F; 0.5% BSA; $V_F$ = 3.73 ml; $F$ = 2.7 ml/min. The insets show the chromatograms of (A) the sample and (B) the recovered fraction by the analytical column (30 × 0.75 cm I.D. TSK G-3000SW XL); $F$ = 0.4 ml/min; $V_F$ = 0.1 ml. From the analytical chromatogram, $Q_P$ and $Q_R$ of the recovered fraction were determined as 0.95 and 0.96, respectively.



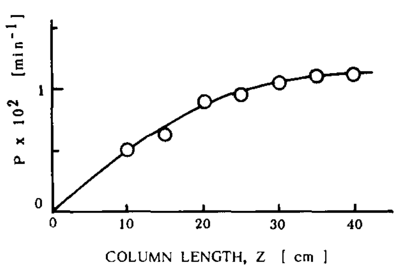




Fig. 6. Calculated relationships $P_M$–$Q_R$ at $Q_P$ = 0.99 and $P_M$–$Q_P$ at $Q_R$ = 0.99. $d_p$ = 17 $\mu$m; $Z$ = 10 cm; $\varepsilon$ = 0.36; other data used in the calculation as in Fig. 3.

Fig. 7. Calculated relationship between column length and $P_M$. $d_p$ = 17 $\mu$m; $\varepsilon$ = 0.36; $Q_P$ = $Q_R$ = 0.99; other data used in the calculation as in Fig. 3.

chosen as shown in ref. 2. For the determination of the column dimensions and operating variables for such conditions, the method presented here is considered useful.

The HETP–$u$ relationship for BSA dimer and aggregates were not determined easily from the experimental elution curves as each curve contains the other components. We assumed that the $h$–$v$ relationships for BSA dimer and for aggregates are the same as that for BSA monomer. This may be a crude approximation, although the calculated elution curves on the basis of this approximation are in fairly good agreement with the experimental curves shown in Figs. 1 and 3.

The purpose of this work was not to determine the optimum conditions for the system chosen in this study but to present a method for calculating the productivity at a specified purity and recovery. By using such a calculation program, we can survey the general trends of the effect of the column dimensions and particle diameter on the $P$–$u$ relationship with the HETP–$u$ relationship and $K$ values obtained with a given small-scale column. This will reduce the number of experiments that are needed in scaling up GFC columns.

Although it is difficult to establish a general strategy for maximizing $P$ from the present results, some interesting findings were obtained. $P_M$ exists in the $P$–$u$ relationship when the separation is difficult, *i.e.*, with columns of small $N$. In general, when $Z$ is increased, $F$ must be increased in order to obtain a similar $P$ value. However, $F$ cannot be increased beyond a certain $F_c$ value, especially in the case of soft GFC gel columns owing to compression of the gels[3]. Even for rigid gel columns such as HPGFC columns, $F_c$ is designated by the manufacturer. In some instances there is a pressure limit for the apparatus. Hence, even when the calculated $P$–$u$ relationship has no maximum as shown in Fig. 4, the $P$ value at $F = F_c$ becomes $P_M$.

Hence, $P_M$ for a given separation system (solutes–gel media) becomes:

$$P_M = f(Q_P, Q_R, d_p, Z, F_c) \tag{11}$$

It is known[3] that $F_c$ decreases with increase in $d_c$ and $Z$:

$$F_c = f(d_c, Z) \tag{12}$$

These equations indicate that $P_M$ for a specified $Q_P$ and/or $Q_R$ is a complicated function of $d_p$, $d_c$ and $Z$. However, as the present calculation method is easily performed on a personal computer, the user may determine the column and operating variables so that the specifications are fulfilled.

It is often said that $d_p$ should be increased in scaling up GFC columns. However, both $Z$ and $F$ must be increased in order to compensate for the loss in $P$ due to the increase in $d_p$. As stated above, an increase in $Z$ sometimes conflicts with an increase in $F$. Hence it is desirable that the column with small $d_p$ be operated at low flow-rates in order to increase $P$.

As GFC is linear isocratic LC, the solute concentration $C_0$ has no effect on $P$ in the calculation. In this study, the effect of $C_0$ was not observed for the experimental elution curves with $C_0$ = 0.25–1.0%, as shown in Fig. 3. However, it should be borne in mind that the separation efficiency often decreases drastically when the sample concentration is so high that the viscosity of the sample is much higher than that of the mobile phase[1,21].

SYMBOLS

| | |
|---|---|
| $A_c$ | column cross-sectional area ($cm^2$) |
| $A^*$ | axial dispersion term in eqn. 2 |
| $B^*/v$ | molecular diffusion term in eqn. 2 |
| $C$ | solute concentration at the column outlet (% or $M$) |
| $C_M$ | maximum peak concentration of the elution curve (% or $M$) |
| $C_0$ | initial concentration (% or $M$) |
| $C^*v$ | stationary phase diffusion term in eqn. 2 |
| $D^*v$ | fluid (stagnant)-film mass transfer term in eqn. 2 |
| $D_m$ | molecular diffusion coefficient ($cm^2/s$) |
| $d_c$ | column diameter (cm) |
| $d_p$ | particle diameter ($\mu$m) |
| $F$ | volumetric flow-rate (ml/min) |
| $H$ | $= (1 - \varepsilon)/\varepsilon = (V_t - V_0)/V_0$ |
| HETP | height equivalent to a theoretical plate (plate height) (cm) |
| $h$ | $=$ HETP$/d_p$, reduced plate height |
| $K$ | distribution coefficient (see eqn. 4 for the definition) |
| $k'$ | capacity factor |
| $m$ | amount of recovered fraction (g or mol) |
| $N$ | $= Z/$HETP, total number of theoretical plates |
| $P$ | $= Q_R V_{F,M}/[V_0(V_0/F)]$, productivity ($min^{-1}$) |
| $P_M$ | maximum productivity in the $P$–$u$ relationship ($min^{-1}$) |
| $Q_P$ | $= m_d/(m_d + m_c)$, purity ratio |
| $Q_R$ | $= m_d/(V_F C_{0,d})$, recovery ratio |
| $T$ | $= t/[(Z/u)(1 + HK)]$ |
| $T_0$ | $= t_0/[(Z/u)(1 + HK)]$ |
| $t$ | time from the start of the sample injection (min or s) |
| $t_M$ | peak retention time (min or s) |
| $t_0$ | sample injection time (min or s) |
| $u$ | $= F/(A_c\varepsilon)$, linear mobile phase velocity (cm/min or cm/s) |

$V_F$ $= Ft_0$, sample feed volume (ml)
$V_{F,M}$ maximum $V_F$ value that satisfies $Q_P$ and $Q_R$ (ml)
$V_0$ $= V_t\varepsilon$, column void volume (ml)
$V_t$ $= A_cZ$, total column volume (ml)
$w$ peak width measured at $C = 0.368C_M$ (min or s)
$Z$ column length (cm)
$\varepsilon$ void fraction of column
$\nu$ $= ud_p/D_m$, reduced velocity

## REFERENCES


1 H. Determann and J. E. Brewer, in E. Heftmann (Editor), *Chromatography*, Van Nostrand Reinhold, New York, 3rd ed., 1975, p. 362.
2 J. M. Curling (Editor), *Methods of Plasma Protein Fractionation*, Academic Press, Oxford, 1980.
3 J. C. Janson and P. Hedman, *Adv. Biochem. Eng.*, 25 (1982) 43.
4 E. Glueckauf, *Trans. Faraday Soc.*, 51 (1955) 34.
5 J. R. Conder and M. K. Shingari, *J. Chromatogr. Sci.*, 11 (1973) 525.
6 J. H. Knox and H. M. Pyper, *J. Chromatogr.*, 363 (1986) 1.
7 P. C. Wankat and Y.-M. Koo, *AIChE J.*, 34 (1988) 1006.
8 M. Kubin, *Collect. Czech. Chem. Commun.*, 30 (1965) 1104.
9 E. Kucera, *J. Chromatogr.*, 19 (1965) 237.
10 A. Rasmuson, *AIChE J.*, 31 (1985) 518.
11 S. Yamamoto, M. Nomura and Y. Sano, in preparation.
12 H. R. Halvorson and G. K. Ackers, *J. Polym. Sci., Part A-2*, 9 (1971) 245.
13 E. N. Lightfoot, R. J. Sanchez-Palma and D. O. Edwards, in H. M. Schoen (Editor), *New Chemical Engineering Separation Techniques*, Interscience, New York, 1962, p. 99.
14 G. Houghton, *J. Chromatogr.*, 15 (1964) 5.
15 J. J. van Deemter, F. J. Zuiderweg and A. Klinkenberg, *Chem. Eng. Sci.*, 5 (1956) 271.
16 S. R. Rudge and M. R. Ladisch, *Biotechnol. Prog.*, 4 (1988) 123.
17 S. Yamamoto, K. Nakanishi and R. Matsuno, *Ion-Exchange Chromatography of Proteins*, Marcel Dekker, New York, 1988.
18 S. Yamamoto, M. Nomura and Y. Sano, *J. Chromatogr.*, 394 (1987) 363.
19 *User's Manual for JUSE-BIOLC Software*, Nihon Kagaku Gijyutsu Kensyujyo, Tokyo, 1989 (in Japanese).
20 M. E. Young, P. A. Carroad and R. L. Bell, *Biotechnol. Bioeng.*, 22 (1980) 947.
21 S. Yamamoto, M. Nomura and Y. Sano, *J. Chem. Eng. Jpn.*, 19 (1986) 227.