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Short-cut method for predicting the productivity of affinity chromatography

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ABSTRACT

A method was developed for predicting the productivity P (amount of the target protein recovered per unit volume per unit time) of affinity chromatography (AFC) and similar chromatographic operations. The constant-pattern approach was employed for describing the breakthrough curve. It was found that P increases with increasing u at low u regions and there is a maximum in the P–u curve. The u value that gives the maximum P (u_M) was derived as a function of the number of transfer units, n. The experimental breakthrough curves for several different packing media systems such as 40- and 10-μm porous particles, 2.5-μm non-porous particles and surface-derivatized membrane media were measured, from which the n values were determined. The u_M values were then calculated using the n values. It is suggested that the present method is useful for the rapid survey of the operating conditions in scaling-up AFC and similar chromatographic operations.

INTRODUCTION

Production-scale affinity chromatography (AFC) will play an important role in downstream treatment processing in biotechnology [1]. For this purpose, the productivity (protein recovered per unit time per unit column volume) must be increased by increasing the mobile phase velocity or by increasing the sample loading.

For isocratic elution chromatography of proteins, we developed a method for searching for the maximum sample feed volume $V_{F,M}$ that satisfies a specified purity ratio and recovery ratio for given column dimensions and at a certain linear mobile phase velocity u [2]. The productivity P is then obtained from $V_{F,M}$ as a function of u. The calculated P–u relationships have shown that there is a certain u value that gives the maximum productivity P_M for given column dimensions [2]. The existence of such P_M values in AFC was also pointed out by Katoh *et al.* [3], Kamiya *et al.* [4] and Afeyan *et al.* [5]. Most AFC separations have been operated at low flow-rates, as the productivity was not considered to be an important factor even when it is used in downstream processing in bioindustries. One of the reasons is that as most of the packing media used for AFC, such as agarose, are soft, the flow-rate cannot be increased at high-flow rates such as 1 $cm^3/cm^2 \cdot min$ [6]. Consequently, the working range of the linear flow-rate u is much lower than that which yields P_M.

Much effort has recently been devoted to the development of mechanically stable packing media and column designs for AFC. Linear velocities considerably above 1 $cm^3/cm^2 \cdot min$ are now possible. Hence it is important to examine how P is affected by operating and column parameters.

In this work, a short-cut method was developed for predicting the P–u relationships in AFC and similar chromatographic operations. The constant-pattern approach is employed for describing the breakthrough curve (BTC) [7–10]. The u value that gives P_M (u_M) is derived as a function of the number of transfer units, n. The breakthrough curves for various packing media systems such as 40- and 10-μm porous particles, 2.5-μm non-porous particles and surface-derivatized membrane media were measured. The n values determined from the experimental breakthrough curves were employed for calculating P_M and u_M.

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EXPERIMENTAL

The column chromatographic experiments were carried out with a peristaltic pump or an Altex Model 100 pump, a Tosoh UV8010 UV detector equipped with a preparative flow cell and a conductivity meter equipped with a flow cell (DS-8M, Horiba, Kyoto, Japan). The outputs of the UV detector and the conductivity meter were recorded with a strip-chart recorder. The temperature was maintained at 25°C.

The proteins used were human immunoglobin G (IgG) (Wako, Osaka, Japan), five-times crystallized ovalbumin (Seikagaku Kogyo, Tokyo, Japan), bovine serum albumin (fraction V) (Wako) and egg white lysozyme (Seikagaku Kogyo).

The following columns and packing media were employed: CM Toyopearl 650S (d_p = 40 μm) (Tosoh, Tokyo, Japan) packed into a column of d_c = 0.9 cm and Z = 2 cm (d_c = column diameter, Z = column length); a DEAE-Memsep 1000 cartridge and a protein A-Memsep 1000 cartridge (Millipore, Bedford, MA, USA) (d_c = 1.8 cm, Z = 0.5 cm, total bed volume, V_t = 1.4 ml [11]); a protein A-5PW column (Tosoh) (d_c = 0.5 cm, Z = 5 cm); protein A-Toyopearl 650 (Tosoh) packed into a column of d_c = 0.9 cm and Z = 2 cm; DEAE-5PW (Tosoh) (d_c = 0.75 cm, Z = 7.5 cm); and DEAE-NPR (Tosoh) (d_c = 0.46 cm, Z = 3.5 cm).

The buffer systems used were 14 mM Tris–HCl (pH 7.7) containing 0.03 M NaCl for the DEAE ion-exchange chromatographic (IEC) columns, that containing 0.1 M NaCl for the protein A AFC columns, and 10 mM phosphate buffer (pH 8) containing 0.03 M NaCl for the lysozyme–CM IEC column system.

The equilibrium protein concentration in the column stationary phase C_s was determined from the experimental breakthrough curves according to the following equation:

$$C_s = C_0(V_C - V_0)/V_S = C_0(V_b - V_0 - \int_{V_a}^{V_b} X\mathrm{d}V)/V_s \quad (1)$$

where C_0 is the sample (protein) concentration, V_C is the equilibrium adsorption volume (column adsorption capacity), $X = C/C_0$ is the relative concentration, V_a and V_b are the volume at which $C = 0$ (or $X = 0$) and $C = C_0$ (or $X = 1$) (see Fig. 1). $V_s = (V_t - V_0)$ is the column gel volume, V_t is the total column volume and V_0 is the column void volume. The column void fraction $\varepsilon = V_0/V_t$ was determined from dextran T2000 pulses (0.5–1.0 mg/ml) for the DEAE and CM Toyopearl columns. The ε of Memsep cartridges was taken to be 0.8 [11]. The ε values of other columns were assumed to be 0.4.

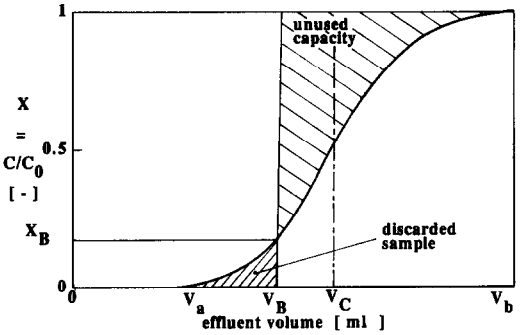

Fig. 1. Breakthrough curve (protein concentration–volume curve) at the adsorption stage for affinity chromatographic operation.

RESULTS

Let us examine the column operation in AFC, which consists of (i) sample charge, (ii) washing of contaminants, (iii) desorption of the target protein and (iv) re-equilibration of the column. At the sample charge stage, the sample solution is fed to the AFC column until the relative protein concentration ($X = C/C_0$) at the column outlet reaches a specified value (usually X = 0.05–0.1). This X value is referred to as the breakthrough concentration X_B and the volume at $X = X_B$ is called the breakthrough volume V_B (Fig. 1). Similar procedures are often employed for the stepwise elution IEC or hydrophobic interaction chromatography (HIC) of proteins. A typical example is the recovery of lysozyme from egg white with cation-exchange columns [12]. This type of operation by HIC is also effective in the initial stage of enzyme purification schemes [13].

As shown schematically in Fig. 1, at $V = V_B$ (or $X = X_B$), the amount of the protein applied to the column is C_0V_B, where C_0 is the protein concentration. The loss of the protein M is given by

$$M = \int_{V_a}^{V_B} C\,dV \tag{2}$$

Then, the total amount of the protein present in the column per unit column volume at $V = V_B$ is

$$(C_0 V_B - M)/V_t \tag{3}$$

where $V_t = A_c Z$ is the column volume, A_c is the column cross-sectional area and Z is the column length. Then, the productivity P is defined as

$$P = Q_R(C_0 V_B - M)/(t_C V_t) \tag{4}$$

If we consider that the amount of protein present in the mobile phase at $V = V_B$ will be lost during the washing period, V_B should be read as $V_B - V_o$.

As AFC is a batch operation, the separation time (cycle time) t_C is given as the sum of the time for (i) the sample charge (breakthrough) t_B, (ii) the wash-out of contaminants t_w, (iii) the desorption of the target protein t_d and (iv) the regeneration of the column t_r [3,4,7,8]:

$$t_C = t_B + t_w + t_d + t_r = t_B + \alpha(V_t/F) = t_B + (\alpha/\varepsilon)(Z/u) \tag{5}$$

where $u = F/(A_c\varepsilon)$ is the linear mobile phase velocity, F is the volumetric flow-rate and ε is the column void fraction. Although t_B is determined from the shape of the BTC, the time needed to complete the remaining three periods can be approximated to that needed to elute α times the total bed volume V_t. Q_R is the recovery ratio, which is 1.0 for ideal desorption and less than 1.0 for actual operation. It also reflects the loss of the protein during the washing period.

It is expected from eqns. 4 and 5 that at a given C_0 P will increase with increasing u (or F). However, as an increase in u causes the spreading of the breakthrough curve due to the stationary phase diffusion [7,8,14], V_B decreases and M increases. Eventually, $C_0 V_B - M$ is a decreasing function of F. Therefore, P increases with increasing u (or F) at low u regions because the decrease in t_C is larger than that of $(C_0 V_B - M)$. A further increase in u causes a much sharper decrease in $C_0 V_B - M$ than that of t_C. Consequently, the slope of the P–u curve gradually decreases with increasing u, and finally it becomes negative, as shown schematically in Fig. 2. This is the origin of the maximum P (P_M) in the P–u curve, as

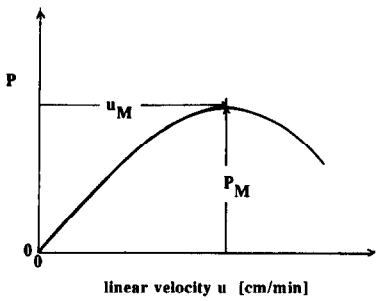

Fig. 2. Schematic diagram of the relationship between productivity P and linear velocity u.

shown by Katoh *et al.* [3], Kamiya *et al.* [4] and Afeyan *et al.* [5].

As the adsorption isotherm is highly favourable in the AFC, IEC and HIC of proteins, the constant-pattern approach can be applied for describing the BTC [3,4,7–10]. Thus, $X = C/C_0$ is a unique function of $T^* = n(T - 1)$, where n is the number of transfer units, $T = (tu/Z - 1)/(HK_0)$ is the dimensionless time, $H = (1 - \varepsilon)/\varepsilon$ is the phase ratio, $K_0 = C_{s0}/C_0$ is the distribution coefficient (the slope of the adsorption isotherm at $C = C_0$) and C_{s0} is the stationary phase protein concentration at $C = C_0$. Fig. 3 shows the X–T^* curve for irreversible adsorption [4,8–10].

$$X = 1 - [(2/3) - 0.273T^*]^2 \tag{6}$$

The use of other constant-pattern BTC equations is also possible. M^* and T_B^* are given by

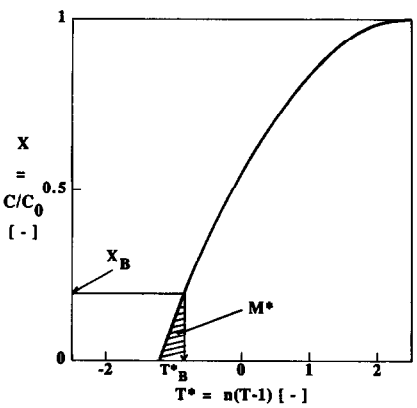

Fig. 3. Constant-pattern breakthrough curve calculated by eqn. 6.

$$M^* = \frac{n}{C_0(1-\varepsilon)K_0} \cdot \frac{M}{V_t} \tag{7}$$

$$T_B^* = \frac{n[(t_B u/Z) - (1 + HK_0)]}{HK_0} \tag{8}$$

Again, if we consider the loss of the protein in the mobile phase, t_B should be read as $t_B - (Z/u)$.

Inserting the above equations into eqn. 4 yields

$$P = Q_R C_0[\varepsilon + (1-\varepsilon)K_0 + \\ + (T_B^* - M^*)(1-\varepsilon)K_0/n]/t_C \tag{9}$$

t_C is also given by

$$t_C = (Z/u)[(T_B^* HK_0/n) + (1 + HK_0) + (\alpha/\varepsilon)] \tag{10}$$

The number of transfer units n is usually assumed to be proportional to Z/u or the height of a transfer unit $HTU = Z/n$ is proportional to u:

$$n = Z/HTU = D(Z/u) \tag{11}$$

where D is a proportionality factor (note that D is proportional to the overall volumetric mass transfer coefficient which is frequently used in fixed-bed adsorption [1,3,4]). The meaning of HTU is similar to that of HETP. Small HTU values imply a good column performance. Comparison of the analytical solution for the BTC at linear equilibrium [10] with a theoretical plate model yields $n = 2[HK/(1 + HK)]^2 N$, where N is the plate number [15].

Inserting eqns. 10 and 11 into eqn. 9 yields

$$P = \frac{(1 + HK_0) + HK_0(u/Z)(T_B^* - M^*)/D}{(1 + HK_0) + HK_0(u/Z)(T_B^*/D) + (\alpha/\varepsilon)} \cdot \\ \cdot Q_R \varepsilon C_0 (u/Z) \tag{12}$$

The maximum P (P_M) is obtained at $u = u_M$:

$$u_M = \frac{D}{2} \cdot \frac{(1 + HK_0)}{HK_0} \cdot \frac{Z}{(M^* - T_B^*)} \approx \frac{D}{2} \cdot \frac{Z}{(M^* - T_B^*)} \tag{13}$$

$$P_M = \\ \frac{1}{2} \cdot \frac{(1 + HK_0)}{(1 + HK_0)\left(1 + \dfrac{T_B^*}{M^* - T_B^*}\right) + \left(\dfrac{\alpha}{\varepsilon}\right)} \cdot \varepsilon C_0 Q_R u_M \tag{14}$$

Since $M^* - T_B^*$ is determined from the constant-pattern BTC as a function of X_B, P_M and u_M are readily calculated once parameters such as D, HK_0, ε, α and Z are known. The D value and HK_0 value (the adsorption isotherm) can be determined from experimental BTCs.

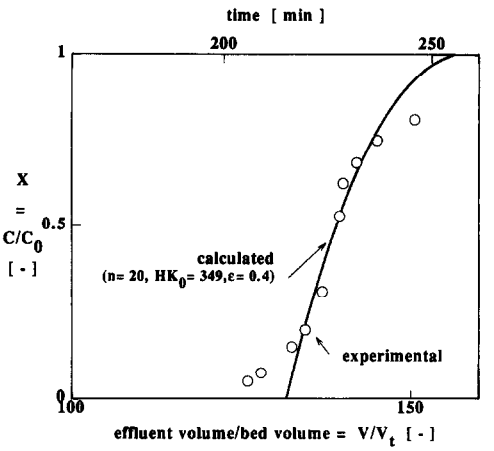

Fig. 4. Breakthrough curve for human IgG–protein A-5PW system (10-μm porous particles). $C_0 = 0.25$ mg/ml; $d_c = 0.5$ cm; $Z = 5$ cm; $V_t = 0.98$ ml; $F = 0.6$ ml/min.

It is also noted that P_M and u_M are proportional to Z. Thus u_M becomes smaller as the column length Z is decreased. This was already pointed out by Katoh *et al.* [3].

Kamiya *et al.* [4] calculated the purification rate *versus* flow-rate relationship for the BSA–Toyopearl 650M–anti-BSA column system, which shows a maximum. Their calculated results when converted according to our definition are that $u_M = 0.53$ cm/s

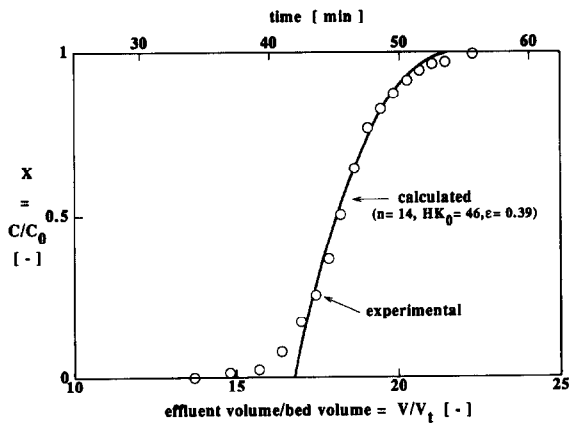

Fig. 5. Breakthrough curve for lysozyme–CM Toyopearl 650S system (40-μm porous particles). $C_0 = 3.5$ mg/ml; $d_c = 0.9$ cm; $Z = 2$ cm; $V_t = 1.27$ ml; $F = 0.51$ ml/min.

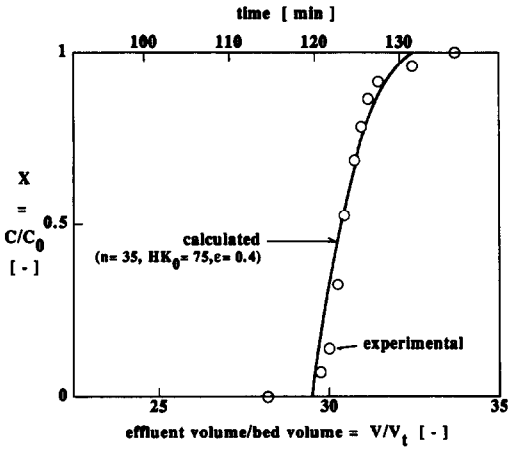

Fig. 6. Breakthrough curve for BSA–DEAE-5PW system (10-μm porous particles). C_0 = 1.0 mg/ml; d_c = 0.75 cm; Z = 7.5 cm; V_t = *3.3 ml;* F = 0.83 ml/min.

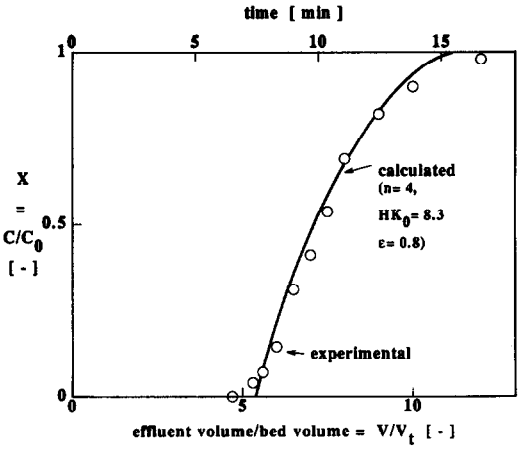

Fig. 8. Breakthrough curve for ovalbumin–DEAE-Memsep system (membrane medium). C_0 = 1.0 mg/ml; d_c = 1.8 cm; Z = 0.5 cm; V_t = 1.4 ml; F = 1.0 ml/min.

and $P_M = 2.5 \cdot 10^{-4}$ mg/s · ml. In their calculation, $\alpha = 35$, $Q_R = 0.9$, $(1 - \varepsilon)K_0 = 27$, $d_c = 0.46$ cm, $Z = 10$ cm, $C_0 = 0.05$ mg/ml, $D = 0.0912\ s^{-1}$ and $X_B = 0.1$. The $M^* - T_B^*$ value calculated by eqn. 6 is 1.056 for $X_B = 0.1$. Then, inserting these values into eqns. 13 and 14 and assuming that $\varepsilon = 0.38$, we obtain $u_M = 0.45$ cm/s and $P_M = 2.2 \cdot 10^{-4}$ mg/s · ml. These values are close to those mentioned above.

Figs. 4–8 show the experimental and calculated BTCs. The calculated curves are the best fit by eqn. 6, from which D values in eqn. 11 are determined. The results are summarized as follows.

Ovalbumin–DEAE-Memsep system (membrane medium): $u_M/Z = 1.93\ min^{-1}$, $HTU = 0.12\,(n = 4)$ at $F = 1$ ml/min ($u = 0.49$ cm/min), $HTU = 0.245u$, adsorption isotherm $C_s = 646C/(1 + 17.1C)$.

Lysozyme–CM Toyopearl 650S system (40-μm packing): $u_M/Z = 6.96\ min^{-1}$, $HTU = 0.14$ ($n = 14$) at $F = 0.51$ ml/min ($u = 2.1$ cm/min), $HTU = 0.068u$, adsorption isotherm $C_s = 440C/(1 + 4C)$.

Human IgG–protein A-5PW system (10-μm packing): $u_M/Z = 14.3\ min^{-1}$, $HTU = 0.25$ ($n = 20$) at $F = 0.6$ ml/min ($u = 7.64$ cm/min), $HTU = 0.033u$, adsorption isotherm not determined.

BSA–DEAE-5PW system (10-μm packing): $u_M/Z = 10.5\ min^{-1}$, $HTU = 0.21$ ($n = 35$) at $F = 0.826$ ml/min ($u = 4.67$ cm/min), $HTU = 0.045u$, adsorption isotherm not determined.

BSA–DEAE-NPR system (non-porous 2.5-μm packing): $u_M/Z = 33\ min^{-1}$, $HTU = 0.175$ ($n = 20$) at $F = 0.815$ ml/min ($u = 12.3$ cm/min), $HTU = 0.0142u$, adsorption isotherm not determined.

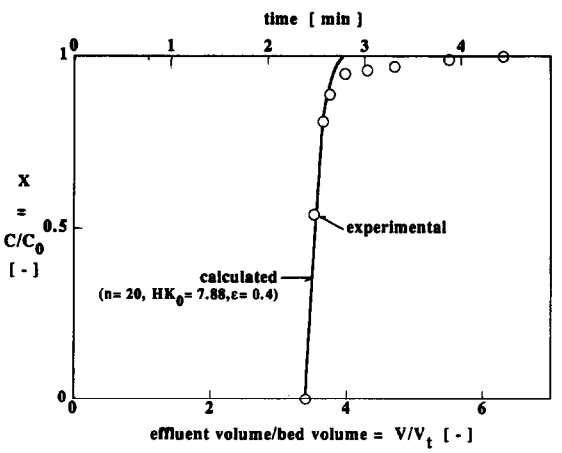

Fig. 7. Breakthrough curve for BSA–DEAE-NPR system (non-porous 2.5-μm particles). C_0 = 1.0 mg/ml; d_c = 0.46 cm; Z = 3.5 cm; V_t = 0.58 ml; F = 0.82 ml/min.

DISCUSSION

It is well known in elution chromatography that increasing u and/or increasing sample volumes result in a lowering of the resolution. This is the reason

why there is a maximum productivity P_M at a specified purity and recovery [2]. In AFC, P defined by eqn. 4 reflects the column gel bed utility at the breakthrough volume (time). High-performance AFC columns, in which mass transport rates between the mobile and stationary phases are very rapid, give high P values. Surface-derivatized membrane media, non-porous packings and perfusion chromatographic packings [5] are expected to be candidates for such very high performance methods.

The present results indicate that the performance of the membrane IEC employed ($HTU = 0.245u$) is not much higher than that of the conventional porous packing MPIEC ($HTU = 0.068u$). In addition, the relative column adsorption capacity V_C/V_t is much lower than that of the porous packing columns. However, we also observed that the resolution in linear gradient elution experiments using membrane IEC is higher than that with porous packing IEC (data not shown). The mass transfer mechanism in the membrane media should be investigated in more detail.

The performance of the non-porous high-performance liquid chromatographic (HPLC) column (DEAE-NPR) was the highest ($HTU = 0.0142u$). The n value is 20 even at such a high flow-rate as $u =$ 12 cm/min. However, the adsorption capacity is also lower than that of the porous packing columns.

In linear isocratic elution chromatography, HETP is proportional to d_p^2 at high flow-rates [14]. On the other hand, when we compare the performance of the 10-μm porous packing HPLC columns (HTU = $0.033u$ and $0.045u$) with that of the 40-μm porous packing MPLC column (HTU = $0.068u$), the HTU of the former is about half to two thirds of the latter. Detailed analysis on the stationary phase diffusion is needed to clarify this curous behaviour.

Although the purpose of this study was not to give a complete theoretical model and the experimental data that support it, the assumption of eqn. 11 may be a serious limitation of the present method. This assumption, which is often employed, is valid for the stationary phase diffusion control which may be allowable owing to the low molecular diffusion coefficient of proteins. However, the diffusion path is greatly reduced in the high-performance methods mentioned above, and further investigation is needed to examine the flow-rate dependence of n or HTU. This will be also useful for the investigation of the displacement chrimatographic separation of proteins.

SYMBOLS

A_c	column cross-sectional area (cm^2)
C	protein concentration at the column outlet or in the solution (mg/ml)
C_0	initial concentration (mg/ml)
D	parameter in eqn. 11 (s^{-1})
d_c	column diameter (cm)
d_p	particle diameter (μm)
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)
HTU	$= Z/n$: height of a transfer unit (cm)
K	= distribution coefficient
K_0	$= C_{s0}/C_0$: K at $C = C_0$
M	amount of the protein discarded before the breakthrough volume (mg)
N	$= Z/$HETP: total number of theoretical plates
n	number of the transfer unit
P	productivity defined by eqn. 4 (mg/min · ml)
P_M	maximum productivity in the P–u relationship (mg/min · ml)
Q_R	recovery ratio
T	$= [(tu/Z) - 1]/HK_0$
T^*	$= n(T - 1)$
T_B^*	T^* at $X = X_B$
t	time (min or s)
u	$= F/(A_c\varepsilon)$: linear mobile phase velocity (cm/min or cm/s)
u_M	u at $P = P_M$ (cm/min or cm/s)
V_B	breakthrough volume (ml)
V_0	$= V_t\varepsilon$: column void volume (ml)
V_t	$= A_cZ$: total column volume (ml)
X	$= C/C_0$
X_B	dimensionless breakthrough concentration
Z	column length (cm)
ε	void fraction of column

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