from Bioseparation Processes in Foods, Rakesh K. Singh and Syed S. H. Rizvi, ed. Marcel Dekker, NY.

4
Scale-Up Techniques in Bioseparation Processes

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INTRODUCTION

Chromatography plays a major role in the downstream processing of biological materials encountered in the manufacture of food, pharmaceutical, and biotechnology products (Ladisch and Wankat, 1988; Velayudhan et al., 1993; Colin, 1993). Here, we offer approaches to scaling-up linear chromatography in chromatographic separations based on isocratic elution, as well as illustrate a nonideal, gradient-induced peak deformation effect, which may occur upon scale-up of gradient chromatography.

Linear behavior is associated with gel permeation chromatography of proteins, analytical chromatography carried out at low solute concentrations, and special cases of chromatography where linear equilibria appear to exist at high sorbent loadings. In these cases, the analysis of Lapidus and Amundson (1952) is applicable and facilitates calculation of elution profiles. The Peclet number, Pe, is a bridge between the plate


count, N, used by chromatographers, and the continuum approach where Pe = 2N.

The industrial process designer often requires a first estimate of the size of chromatography equipment for cost-estimating purposes and will usually have limited data based on chromatograms obtained on a bench scale. If a separation process can be identified as approximating linear chromatography behavior, the column’s length and diameter for a given throughput and particle size can be calculated. Calculation of representative elution profiles are also possible.

The classical and most direct description of scale-up principles is based on maintaining resolution between a two-component system in linear chromatography, where product would constitute one peak and all other components the second peak. An example of such a system is desalting, the separation of lower molecular weight components from a protein peak by size exclusion. The method can be extended to a ternary system consisting of the desired product, all the light-end impurities lumped into a second component, and all the heavy-end impurities lumped into a third component. Then the resolution described below pertains to the component that elutes closest to the product peak.

Scale-up of a given chromatography separation has the objective of maintaining the resolution between components. Resolution (R<sub>s</sub>) in linear systems is a function of the plate count and capacity factors, and may be written as:

\[
R_s = \frac{1}{4} (\alpha - 1) \sqrt{N \left( \frac{k_i^2}{1 + k_i^2} \right)}
\]  

(1)

where \( \alpha \) is the separation factor between the two components, and \( k_i \) is the capacity factor of the product.

Plate count is defined as:

\[
N = \frac{L}{H} = 16 \left( \frac{t_i}{t_w} \right)^2
\]  

(2a)

where L is the column length, H is the plate height, \( t_i \) is the retention time, and \( t_w \) is the width of a Gaussian peak width (illustrated in Fig. 4.1). The base of the peak is often ambiguous, hence motivating calculation of plate-count based on the width at half-height.

\[
N = 5.54 \left( \frac{t_i}{t_w_{1/2}} \right)^2
\]  

(2b)

Chromatographic peaks may often be significantly skewed so that the assumption of a Gaussian shape no longer applies and another approach


to calculating plate count must be used. Foley and Borsey (1983) suggest that the peak asymmetry be characterized by measuring the parameters A and B at a point 10% above the peak's base (Fig. 4.2) and then using the equation:

$$N = \frac{41.7 \left( \frac{A}{W_{0.1}} \right)^2}{B/A + 1.25} \tag{2c}$$

where $W_{0.1}$ is the peak width at 10% height.

Peak shape is also affected by solute concentration and the mobile phase stationary phase equilibrium. As described by Colin (1983), the amount of broadening depends on the injected quantity and some physicochemical parameters of the chromatographic system, such as the chemical nature of the mobile and stationary phases, the specific surface area of the stationary phase, and temperature. Of particular importance is the type of the distribution isotherm. It is recalled that this isotherm relates the concentration of the solute in the stationary phase to that in the mobile phase. Most often in liquid chromatography, the isotherm is of the Langmuir type, as illustrated in Fig. 4.3a. As can be seen, there is a plateau indicating the existence of a maximum solute concentration on the stationary phase (saturation capacity). The slope of the isotherm at
REFERENCES


a given mobile phase concentration is the capacity ratio, $k'$, of the solute at this concentration. Accordingly, $k'$ decreases with increasing concentration or increasing injected quantity and the peak maximum moves to shorter retention times. The peak actually becomes triangular, the end of the peak always appearing at the same time [Fig. 4.3b].

The plate count is a measure of the dispersion in a column; the higher the plate count, the sharper the peak. The capacity factor is defined as:

$$k' = \frac{V_r - V_o}{V_o} = \frac{t_r - t_0}{t_0}$$  \hspace{1cm} (3)

where $V_o$ is the retention volume and $t_0$ is the retention time of an excluded solute (such as a high molecular weight dextran). It should be noted that under overloaded column conditions, when linear equilibrium cannot be assumed, the amount of sample loaded and solute-solute interactions affect scale-up.

A systematic approach to estimating column length, diameter, and separation time is presented for cases where pore diffusion alone, mass transfer alone, and pore diffusion and mass transfer acting together are
<table>
<thead>
<tr>
<th>Purification conditions</th>
<th>Peptides</th>
<th>Fatty acids</th>
<th>Steroids</th>
</tr>
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<tbody>
<tr>
<td>Initial purity (%)</td>
<td>11</td>
<td>27</td>
<td>90</td>
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<tr>
<td>Final purity (%)</td>
<td>85</td>
<td>99</td>
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<tr>
<td>Production (kg/y)</td>
<td>14.4</td>
<td>2000</td>
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<tr>
<td>Recovery ratio (%)</td>
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<tr>
<td>Type of packing</td>
<td>C&lt;sub&gt;18&lt;/sub&gt; 12–45 μm</td>
<td>C&lt;sub&gt;18&lt;/sub&gt; 25–40 μm</td>
<td>Silica 10 μm</td>
</tr>
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<td>Amount of packing (kg)</td>
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<td>19</td>
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<tr>
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<td>Labor time per year</td>
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<tr>
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<td>Solvent composition</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;CN–H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;CN</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;Cl–CH&lt;sub&gt;3&lt;/sub&gt;OH</td>
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<tr>
<td>Flow rate (L/hr)</td>
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<td>200</td>
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<tr>
<td>Operation Cost ($/yr × 10&lt;sup&gt;3&lt;/sup&gt;)</td>
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<td>Total</td>
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<tr>
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<tr>
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<td>10.2</td>
<td>10.2</td>
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<tr>
<td>Site preparation</td>
<td>26.5</td>
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<tr>
<td>Total Purification cost ($/kg)</td>
<td>7576</td>
<td>220</td>
<td>101</td>
</tr>
</tbody>
</table>

Source: Colín (1993).

This brief description of costs illustrates the importance of overall process design in the cost of purification. The guidelines provided earlier in this chapter represent a starting point for scale-up, with a more detailed analysis required if the initial results appear promising.

ACKNOWLEDGMENTS

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FIG. 4.3 Elution profiles as a function of isotherm shape. (a) Langmuirian equation and (b) corresponding elution runs as a function of sample concentration. (Reprinted with permission from Colin, 1993.)
the product peak to become concentrated. At the same time, the normal process of gradient elution throughout the column could separate the desired product from its impurities. The result is a concentrated pure band of desired product.

COSTS

Purification costs depend on labor, stationary phase, solvent, maintenance, equipment, energy, and related costs. An approximate breakdown for small molecules has been given by Colin (1993), as summarized in Table 4.4. While actual costs will vary from one type of separation to another and is a function of scale, the data in Table 4.4 provides useful insight into the relative importance of the different cost components. In all three cases, solvent and labor costs seem to account for a substantial fraction of both operational and total costs. Automated control and process layout are shown to be important issues in this context.

A case study for the purification of α-galactosidase (Porter and Ladisch, 1992) indicates similar trends. Equipment costs appear to follow exponential scaling rules (Fig. 4.11), while labor and solvent costs are again a significant factor.

![Graph depicting equipment cost as a function of volume and column type.](image)

**FIG. 4.11** Column hardware costs as a function of volume and column type. 1989 retail catalog prices. Marshall Swift index = 895.1. (Reprinted with permission from Porter and Ladisch, 1992.)
the primary determinants in affecting solute dispersion. The simplest case, size exclusion chromatography (SEC), is considered first, followed by other types of linear isocratic elution chromatography and, finally, gradient elution chromatography.

**SCALING-UP SIZE EXCLUSION CHROMATOGRAPHY (PORE DIFFUSION CONTROLS)**

Since there is no sorptive mechanism in SEC, the process is inherently linear and thus lends itself to scale-up. Assuming that a reasonably rigid matrix is used, the work of Yamamoro et al. (1986, 1987) indicates that, even for 40-μm particles, pore diffusion seems to be the dominant contributor to band spreading. Assuming that the controlling mechanism does not change upon scale-up, it is then possible to describe the scaling process when the desired separation at bench scale has already been achieved and the bench-scale parameters are known (Snyder et al., 1972; Ladisch et al., 1984; Ladisch and Wankat, 1988; Wankat and Koo, 1988). Then, assuming that the velocity ratio \( u_x/u_b \)—the subscript x refers to large-scale, b to bench-scale separation—is determined from pressure drop considerations, scale-up reduces to specifying the column dimensions and the separation time on a large scale, given the ratio of particle sizes \( d_{p,x}/d_{p,b} \) and sample sizes \( V_{x,x}/V_{x,b} \). The ratio \( d_{p,x}/d_{p,b} \) is usually determined by commercial availability; the ratio \( u_x/u_b \) is then fixed by pressure-drop considerations; and the ratio \( V_{x,x}/V_{x,b} \) is arrived at through design considerations. We assume that the plate count \( N \) (and therefore the resolution, which is proportional to \( \sqrt{N} \)) is the same at bench scale and at large scale for pore diffusion as the controlling process. The plate count (Ruthven, 1984; Gibbs and Lightfoot, 1986):

\[
N_{\text{pore diffusion}} \sim \frac{L}{u_d d_p} \quad (4)
\]

results in the length ratio of:

\[
\left( \frac{L_x}{L_b} \right) = \frac{u_x}{u_b} \left( \frac{d_{p,x}}{d_{p,b}} \right)^2 \quad (5)
\]

where the ratios on the right-hand side of Eq. (5) are known. The column diameter \( D_c \) is then specified by requiring that the ratio of the sample volume to the column volume be the same at the bench and the large scale. Thus,

\[
\left( \frac{V_{x,x}}{V_{x,b}} \right) = \left( \frac{V_{p,x}}{V_{p,b}} \right) \quad (6)
\]
where $\lambda$ is a dimensionless measure of gradient steepness ($\lambda = \beta L/u$). Thus, as the gradient slope $\beta$ becomes steeper and the initial concentration $C_0$ becomes lower, shock formation within the column becomes more likely. It therefore becomes possible for a feed component to straddle the modulator shock layer. Since this feed component would thus be divided into two regions with markedly different modulator concentrations, its profile would be deformed so that a shoulder or a "false peak" could develop, as shown in Fig. 4.10 (Velayudhan and Ladisch, 1991). This effect has been reported in the literature in the context of stepwise elution, where the modulator shock layers are intentionally fed into the column as steps (Jandera and Churasek, 1985). Such shoulders could erroneously be attributed to impurities or on-column reactions. Thus, gradient deformation as a result of self-interference could cause some of the unusual effects that have been observed experimentally in preparative gradient elution.

An even more important consequence for preparative gradient elution is that the formation of a modulator shock layer allows for the possibility of simultaneous concentration and purification. If the experimental conditions are suitably manipulated, the desired product will be found in the vicinity of, or just behind, the modulator shock. This will cause

![Graph](image_url)

**FIG. 4.10** Simulation of binary feed mixture (solid and dashed lines) in reversed-phase chromatography with acetonitrile as the modulator (dotted line). The first peak straddles the acetonitrile shock layer and exhibits a leading shoulder, while the second peak elutes far away from the shock layer and has the expected shape. (Reprinted with permission from Velayudhan and Ladisch, 1991.)
and hence,

$$\left( \frac{D_{L,S}}{D_{L,b}} \right) = \left[ \frac{L_S}{L_S} \left( \frac{V_{q.b}}{V_{q.S}} \right) \right]^{1/2}$$

(7)

where the length \((L_S/L_s)\) ratio has already been determined by Eq. (5). The ratio of separation times is:

$$\left( \frac{L_S}{L_b} \right) = \left( \frac{L_S}{L_b} \right) \left( \frac{u_b}{u_s} \right)$$

(8)

The assumption that one mechanism dominates band spreading allows the scale-up to be determined from equations involving operational parameters without prior knowledge of solute properties such as the pore diffusivity.

SCALING-UP LINEAR ISOCRATIC ELUTION CHROMATOGRAPHY (MASS TRANSFER AND/OR PORE DIFFUSION CONTROLS)

Pore Diffusion Control

As before, we assume linear chromatography. Band spreading in linear chromatography has been extensively studied, and many results are available for the contributions from the various nonequilibrium terms (Güdöng, 1965; Ruthven, 1984). Under realistic conditions it seems likely that film mass transfer and pore diffusion will be the dominant contributors, although it is conceivable that eddy diffusion could begin to increase in importance as the particle size becomes larger. Under conditions of linear isocratic elution, slow-binding kinetics are unlikely to be the controlling mechanism. If we assume that either pore diffusion or mass transfer is the single controlling nonequilibrium mechanism, it becomes possible to derive scaling rules in an analogous manner to that used for SEC previously. When pore diffusion is the controlling mechanism, the scaling equations are identical to those given above for SEC [Eqs. (5), (7), and (8)].

Mass Transfer Control

When mass transfer is dominant over other causes of dispersion, the plate count is given by:

$$N_{\text{mass transfer}} \sim \frac{k_{\text{eff}} L}{u d_p}$$

(9)
in detail by Snyder (1980) and Jandera and Churacek (1985). However, these studies neglect the binding of the modulator itself to the stationary phase. At least in instances where the modulator alters solute retention by competing directly with the solutes for binding sites on the stationary phase, the modulator must be at a sufficiently high concentration to produce competitive interference with the solutes. It follows that the modulator undergoes self-interference, resulting in a deformation of the gradient shape because the modulator concentration is in the nonlinear region of its isotherm. Such self-interference should occur in ion exchange chromatography, where salts are used as modulators, and in reversed-phase chromatography, where organic modifiers are used to modulate retention. Hydrophobic interaction chromatography could be different in that the modulator salt achieves its alteration of solute retention in an indirect manner, rather than by direct competition with the solutes for binding sites (Röttger et al., 1980).

In the most extreme cases of gradient deformation, a shock layer, i.e., a narrow region of column in which the modulator concentration changes sharply, could develop. Nonequilibrium effects such as mass transfer and pore diffusion will increase the width of the modulator shock layer (Rhee and Amundson, 1972). In reversed-phase chromatography, the adsorption of the modulator, e.g., acetonitrile, is likely to be well described by the Langmuir isotherm (McCormick and Karger, 1980). For such an isotherm:

\[
q_m = \frac{a_m C_m}{1 + b_mC_m} \quad (43)
\]

where \(C_m\) and \(q_m\) are, respectively, the mobile and stationary phase concentrations of the modulator and \(a_m\) and \(b_m\) are the Langmuir parameters. The governing equation then becomes (Aris and Amundson, 1973):

\[
\left(1 + \frac{\phi_m C_m}{(1 + b_mC_m)^2}\right)\frac{\partial C_m}{\partial t} + \partial \frac{\partial C_m}{\partial x} = 0 \quad (44)
\]

with the boundary condition:

\[
C_m = C_m^0 + \beta t \quad \text{at} \quad x = 0 \quad (45)
\]

for a linear gradient, where \(\beta\) is the gradient slope at the column inlet, and \(\phi\) is the column phase ratio.

A shock will form within the column only if the following condition (Velayudhan and Ladisch, 1992) is met:

\[
(1 + b_mC_m^0)^3 < 2\phi_m b_m \lambda \quad (46)
\]
Many correlations for $k_{ST}$ exist, but they tend to fall into two broad classes: the first (Goto et al., 1983) is:

$$Sh \sim \text{Re}^{1/2} \text{Sc}^{1/8} \quad (10)$$

where $Sh$ is the Sherwood number, $Re$ the particle Reynolds number, and $Sc$ the Schmidt number; in the second class, the Sherwood number (Wilson and Gearkoplis, 1966) is given by:

$$Sh \sim \text{Re}^{1/3} \text{Sc}^{1/9} \quad (11)$$

Wakao and Funakazi (1978), in a critical review, have pointed out the need to account accurately for dispersion when estimating mass transfer effects and suggested a correlation of the form:

$$Sh = 2.0 + 1.1 \text{Re}^{0.6} \text{Sc}^{1/3} \quad (12)$$

There is a constant additive term in their correlation, but this should be negligible for liquids given the large Schmidt number (typical values in the range of $10^3$). We approximate this for analytical simplicity by an expression of the form given by Eq. (10) and obtain:

$$N_{\text{mass transfer}} \sim \frac{L}{u^{1/2}d_p^{3/2}} \quad (13)$$

This is similar in form to correlations of Grushka et al. (1975), Pieri et al. (1985), and Huber (1973).

Defining the plate count (and the resolution) to be the same at both bench scale and large scale gives:

$$\frac{L_x}{(u_x d_{p,x})^{1/2}} = \frac{L_b}{(u_b d_{p,b})^{1/2}} \quad (14)$$

As before, we assume that the ratios $u_x/u_b$, $V_{p,x}/V_{p,b}$, and $d_{p,x}/d_{p,b}$ are specified. Then Eq. (14) gives:

$$\frac{L_x}{L_b} = \left(\frac{u_x}{u_b}\right)^{1/2} \left(\frac{d_{p,x}}{d_{p,b}}\right)^{3/2} \quad (15)$$

As before, the constant ratio of sample volume to column volume now specifies the diameter of the large-scale column relative to the bench scale $d_{p,b}$:

$$\frac{D_{x,b}}{D_{x,b}} = \left(\frac{L_b}{L_x V_{p,b}}\right)^{1/2} \quad (16)$$

Finally, the ratio of separation time is given by:

$$\frac{t_x}{t_b} = \frac{L_x}{L_b u_x} \quad (17)$$
GRADIENT ELUTION

Gradient elution chromatography is characterized by the addition of a component to the mobile phase that alters, or modulates, the retention of the feed components. These additives, or mobile phase modifiers, are fed into the column such that their concentration at the column inlet changes with time. Commonly used modifiers include salts in ion exchange and hydrophobic interaction chromatography and organic modifiers in reversed-phase chromatography.

Again we restrict our attention to feed concentrations in the linear region of their adsorption isotherms. It is often the case that feed components migrate with respect to the modulator gradient until their capacity factors (which are functions of position and time) become quite low, thereby providing justification for the assumption of linear sorption. The modulation of solute retention by the gradient, as described by retention time and bands spreading, has been described and summarized.
SCALE-UP WHEN MASS TRANSFER AND PORE DIFFUSION ARE OF COMPARABLE MAGNITUDE

It is conceivable that mass transfer and pore diffusion could be of comparable magnitude, so that neither is dominant. Further, the expressions in Eqs. (5)-(8) and (15)-(17) are valid only under the assumption that the dominant mechanism does not change on going from the bench- to the large-scale system. This assumption could break down, for instance, when the particle size at the large scale is much greater than at the bench scale, particularly when mass transfer is the controlling mechanism at a small scale: pore diffusion is more likely to dominate for very large particles. Thus, a plate count based on composite resistance is needed. Since variances are additive under linear chromatography, it follows that:

\[ N_{\text{composite}} = A \frac{L}{ud_p} + B \frac{L}{u^{1/2} d_p^{3/2}} \]  

(18)

The consistancy of \( N \) gives, for pore diffusion controlling at the large scale and both mass transfer and pore diffusion affecting the bench scale:

\[ A \frac{L_n}{u_n (d_{p,n})} = A \frac{L_n}{u_b (d_{p,b})^{1/2}} + B \frac{L_n}{u_b^{1/2} (d_{p,b})^{3/2}} \]  

(19)

In this case, the solute parameters (which are embedded in \( A \) and \( B \)) enter the scaling equations. As before, the ratios \( u_n/u_b \), \( d_{p,n}/d_{p,b} \), and \( V_{d,n}/V_{d,b} \) are assumed known, to give:

\[ \frac{L_n}{L_b} = \frac{B' + A \frac{u_n}{u_b} \left( \frac{d_{p,n}}{d_{p,b}} \right)^{1/2}}{A} \]  

(20)

where \( B' = B u_n d_{p,b} \cdot {1/2} \) and \( A \) and \( B \) are given by (Ruthven, 1984):

\[ A = 1.1 \text{ Sc}^{-1/6} \sqrt{D_m} \]  

(21)

\[ B = \frac{2}{15} \frac{\varepsilon_m}{(1 + \varepsilon_m) \rho_i} \frac{1}{D_p} \]  

(22)

The equations for the diameter and the retention time of the large column remain Eqs. (16) and (17) where \( \varepsilon_n \) is the extraparticle void fraction and \( \varepsilon_p \) is the porosity of the stationary phase particle.

The capacity factors are often assumed to be the same for a given type of resin. Pieri et al. (1983) have presented a semi-empirical equation from Grushka et al. (1975):

\[ N = \frac{D''}{m} \left( \frac{L}{u^{1/2} d_p^{3/2}} \right) \]  

(23)
where $D_s$ is the apparent diffusivity of the solute in the support particle. This equation must be accompanied by additional boundary conditions for the individual support particles. These conditions are given as:

$$\frac{\partial n}{\partial t} = \frac{6k_m}{d_p} \left( c - \frac{n}{k} \right), \quad r = \frac{d_p}{2}, \quad x, t > 0$$

(41)

and:

$$n < \infty, \quad r = 0, \quad x, t > 0$$

(42)

where $n_r$ is the particle surface concentration, $d_p$ is the particle diameter, and $k_m$ is the mass transfer coefficient from the mobile phase to the particle surface. A "shrinking core" model has been developed in which solute diffuses into and out of the resin and is also retained by "reaction" on resin active sites. This model seems to be appropriate for ion exchange chromatography and has been applied to the adsorption of bovine serum albumin.
where $D$ is the effective diffusion coefficient, $L$ is the column length, $u$ is the linear velocity, $d_p$ is the particle size, and $m$ and $n$ are empirical constants. The value of $n$ ranges between 0.4 and 0.6 and is generally taken to be 0.5. The empirical constant $m$ is an inverse measure of column efficiency.

Equation (23) follows a relation presented by Snyder (Grushka et al., 1975):

$$h = mn^u$$  \hspace{1cm} (24)

where $h$ is the reduced plate height $L/N_d$, and $v$ is the reduced velocity $ud_p/D$ at relatively high reduced velocities where kinetic and mass transfer effects dominate.

Equation (23) provides an estimate of how column length, particle size, and linear velocity affect the plate count. These three parameters are often changed during the scale-up of a particular separation, particularly since particle size may be increased during scale-up to prevent high pressure drops. Examples of use of Eq. (23) include purification of pheromones on silica gel (Pieri et al., 1983) and ion exclusion chromatography of sulfuric acid from glucose (Neuman et al., 1987).

The impact of increasing particle size on decreasing plate count is well known. In the case of linear chromatography, Eq. (23) helps to anticipate changes in plate count of glucose on columns packed with a gel-type, styrene-divinylbenzene, sulfonated stationary phases of two different particle sizes (10–30 and 207–1190 μm) as indicated in Table 4.1. The equilibrium for glucose is linear. The decrease in plate count can be estimated by rearranging Eq. (23) to give:

### Table 4.1 Scaling Guidelines (Linear Systems): Specify $N$ and Resolution to Be Same on Both Process and Large Scale—$d_{p,0}/d_{p,a}$; $V_{col,a}/V_{col,b}$; $u/a/u_b$; $L_b$; $t_b$ are Known

<table>
<thead>
<tr>
<th>Calculate large-scale parameters</th>
<th>Pore diffusion</th>
<th>Mass transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length: $L_a = L \left( \frac{u}{a/u_b} \right)^n \left( \frac{d_p^m}{d_{p,a}^m} \right)^n$</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Col. Diam.: $D_a = \left[ \frac{D_f}{L_a/L_b} \left( \frac{V_{col,a}}{V_{col,b}} \right)^n \right]^{1/2}$</td>
<td>0.5</td>
<td>—</td>
</tr>
<tr>
<td>Run Time: $t_a = \frac{t_b}{L_a/L_b} \left( \frac{u}{a/u_b} \right)^n$</td>
<td>—</td>
<td>—</td>
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</tbody>
</table>

$x$: Large scale; $b$: Bench scale; $u =$ column velocity; $d_p =$ particle diam.; $L =$ column length; $V_e =$ feed volume.
### Table 4.3 Calculated Peclet Numbers and Dispersion Coefficients

<table>
<thead>
<tr>
<th>Figure</th>
<th>Temp (°C)</th>
<th>$v_h$(% of $v_o$)</th>
<th>V/mL</th>
<th>Pe</th>
<th>D(cm²/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.6a</td>
<td>55</td>
<td>10</td>
<td>204</td>
<td>138</td>
<td>0.271</td>
</tr>
<tr>
<td>4.6b</td>
<td>55</td>
<td>25</td>
<td>220</td>
<td>200</td>
<td>0.182</td>
</tr>
<tr>
<td>4.6c</td>
<td>55</td>
<td>50</td>
<td>219</td>
<td>109</td>
<td>0.343</td>
</tr>
</tbody>
</table>

Source: Neuman et al. (1987).

**FIG. 4.7** Experimental results for separation of sulfuric acid from glucose by IR-118H at temperatures of (a) 27, (b) 55, (c) 68, and (d) 81°C with water as eluent. Particle size of 297–1190 µm. Superficial eluent velocity of 0.59–0.63 cm/min. (Reprinted with permission from Neuman et al., 1987.)
\[ \frac{D' L}{m} = \frac{L}{\alpha d p^{-n} N} \]  

(25)

If the empirical constants, \( m \) and \( n \), and \( D' \), the diffusion constant, are assumed to be equal in both cases, then:

\[ \frac{D' L}{m_1} = \frac{D' L}{m_2} \]  

(26)

or

\[ \frac{L}{\alpha d p^{-n} N_1} = \frac{L}{\alpha d p^{-n} N_2} \]  

(27)

Rearranging to solve for \( N_2 \), the plate number in the bench scale column, yields:

\[ N_2 = \frac{N_1 \alpha d p^{-n} L}{\alpha d p^{-n} L} \]  

(28)

If a value of 0.5 is used for \( n \) (this becomes a case of mass transfer control [see Eq. (13)] and the parameters listed in Table 4.1 are used in Eq. (28), then the predicted value of \( N_2 \) is 40. This is the same magnitude of order as the measured plate count of 59.5 as listed in Table 4.2. Hence, the empirical Eq. (23) gives some basis with which to anticipate an increase in peak broadness (i.e., decrease in plate count) due to an increase in particle size. If peak retention times of the two components are known as well, resolution can be estimated from Eq. (1). The calculated resolution reflects average plate count and capacity factors and leads to an estimate of column length and consequently column diameter for a given volume.

**Table 4.2 Comparison of Analytical and Bench-Scale Separation of Sulfuric Acid from Glucose**

<table>
<thead>
<tr>
<th></th>
<th>Analytical</th>
<th>Bench scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionic form</td>
<td>H⁺</td>
<td>H⁺</td>
</tr>
<tr>
<td>Column length</td>
<td>60 cm</td>
<td>61 cm</td>
</tr>
<tr>
<td>Column i.d.</td>
<td>0.6 cm</td>
<td>2.5 cm</td>
</tr>
<tr>
<td>Column volume</td>
<td>16.97 cm³</td>
<td>309 cm³</td>
</tr>
<tr>
<td>Particle size</td>
<td>20–30 μm</td>
<td>297–1180 μm</td>
</tr>
<tr>
<td>Linear velocity</td>
<td>1.41 cm/min</td>
<td>0.61 cm/min</td>
</tr>
<tr>
<td>Plate count</td>
<td>4184</td>
<td>59.5</td>
</tr>
</tbody>
</table>
centrations of up to 30 mg/mL. This facilitated calculation of column length and diameter using Eqs. (5)--(8). Scale-up of this separation to a scale utilizing 40 L of stationary phase was carried out and shown to perform as expected.

In the classical continuum model of Lapidus and Amundson [Eqs. (30)--(33)], a relationship is not drawn between dispersion and other column parameters, such as eluent velocity or resin particle diameter. Subsequent continuum models have addressed this by substituting a kinetic relationship in spherical coordinates to describe diffusion into and out of a stationary particle, given by:

\[
\frac{\delta n}{\delta t} = D \left( \frac{\delta^2 n}{\delta r^2} + \frac{2}{r} \frac{\delta n}{\delta r} \right)
\]  

(40)
Column volume, in turn, is specified in part by the volume of sample loaded. Once volumes and throughputs are known, costs can be estimated. Scaling rules and guidelines for estimating costs primarily for **linear systems** can be found in the literature (Ladisch et al., 1984; Tice et al., 1987; Ladisch and Wankat, 1988; Wankat and Koo, 1988).

**PLATE COUNT VERSUS CONTINUUM APPROACH**

A comparison between plate and rate theories presented earlier (Rudge and Ladisch, 1988; Velayudhan and Ladisch, 1995) is reviewed below.

Giddings arrived at an algebraic expression for the height of a theoretical plate based on the random walk approach:

\[ H = \frac{B}{v} + C_v + \frac{1}{A} + \frac{1}{C_{n,v}} \]  \hspace{1cm} (29)

where \( v \) is the eluent interstitial velocity. The \( B \) term accounts for longitudinal diffusion in the mobile phase. \( C_v \) is related to pore diffusion and adsorption-desorption processes, and \( A \) and \( C_n \) are proportional to hydrodynamic column processes due to eddy dispersion and mixing in the mobile phase. The result is that each constant is a grouping of physical constants with at least one adjustable parameter, such as porosity. The determination of these parameters requires approximately 100 experimental observations for statistically significant results. This expression becomes identical in form to one from van Deemter et al. (1956) when the \( C_{n,v} \) term can be neglected.

The continuum approach facilitates calculation of a complete elution profile for a solute based upon a mass balance on the solute, and either an equation of state describing the stationary phase capacity or a kinetic relationship describing the rate at which solute approaches equilibrium with the sorbent. One early continuum model is that of Lapidus and Amundson (1952), who proposed a mass balance on a single solute:

\[ D \frac{\partial^2 c}{\partial x^2} = v \frac{\partial c}{\partial x} + \frac{\partial c}{\partial t} + \frac{1}{\epsilon_0} \frac{\partial n}{\partial t} \]  \hspace{1cm} (30)

where \( n = \left(1 - \epsilon_0\right)q \) and either an equilibrium relationship:

\[ c = kn \]  \hspace{1cm} (31)

or a kinetic relationship:

\[ \frac{\partial n}{\partial t} = k_1 c - k_2 n \]  \hspace{1cm} (32)
reduced skewing of the acid peak as the temperature increased. A likely explanation for the decrease in skew was identified as being the decrease in viscosity of the sample with increasing temperature, reducing lingering of the sample as it lavered on, or eluted by, water. This example illustrates that care must be taken in identifying probable causes of peak skew: pure diffusion and mass transfer phenomena may not be the only explanations.

Peak skewing occurs as a result of thermodynamic factors when samples of increasing concentration are separated. A dramatic example is given (Ladisch et al., 1991) for the separation of phenylalanine (Phe) from its phenyl methyl ester of aspartic acid (i.e., Aspartame®). In this case, the chromatogram for a sample containing 50 mg/mL of each component at a sample volume equivalent to 80% of the total column (extra + intra particle) void volume showed significant skew (Fig. 4.8), while a sample with 10-fold lower concentration showed well-shaped peaks (Fig. 4.9). This example illustrates that peak skew is likely for high-concentration samples (Ladisch et al., 1991). However, it is unlikely that such a separation would be run at these relatively high concentrations, and hence, linear chromatography conditions would probably be applicable. This separation was carried out using a methacrylic, macroporous, polymeric sorbent with a surface area of about 450 m²/g for which linear equilibria between the mobile and stationary phases was observed at con-
where \( k_1 \) and \( k_2 \) are lumped kinetic coefficients, to describe solute/sorbent interactions and \( n \) represents the amount of adsorbate on the adsorbent, moles per unit volume of bed as packed. An analytical solution for each case was derived, with the assumption of a semi-infinite column. Their solution for the equilibrium model reduces quite nicely when an initially clean column and a constant inlet concentration are assumed. These conditions are represented mathematically as:

\[
\begin{align*}
c &= 0, & t &= 0, & x > 0
\end{align*}
\]  
and:

\[
\begin{align*}
c &= c_i, & x &= 0, & t > 0
\end{align*}
\]

This relationship is given as:

\[
\frac{C}{C_i} = 0.5 \left[ 1 + \text{erf} \left( \frac{\sqrt{t}}{4\sqrt{D}} - x \sqrt{\frac{\gamma}{4D}} \right) \right] + \exp \left( \frac{v x}{D t} \right) \text{erfc} \left( \frac{\sqrt{t}}{4\sqrt{D}} + x \sqrt{\frac{\gamma}{4D}} \right)
\]  
where \( \text{erf} \) is the error function, \( \text{erfc} \) is the complementary error function, and \( \gamma = 1 + \frac{(1 - \epsilon_s) k}{\epsilon_s} \). The solution of Lapidus and Amundson gives no information on the origin of second-order dispersion, but simply assigns a number \( D \) to quantify it. The dispersion coefficient and plate number are related through the Peclet number:

\[
2N = Pe = \frac{v L}{D}
\]  
where \( Pe \) is the Peclet number and \( L \) is the length of the column. A different form comes from combining the van Deemter equation with Eq. (36):

\[
D = \frac{v L}{2N} = \frac{1}{2} \frac{v H}{L} = \frac{1}{2} \frac{v B}{v + C v + A}
\]  

where \( A, B, \) and \( C \) are on the order of \( 10^{-1}, 10^{-2}, \) and \( 10^{-1}, \) respectively (units consistent with \( v \) in cm/min). If a rough estimate of van Deemter coefficients can be obtained, the dispersion coefficient can be estimated for different flow rates. The Lapidus and Amundson solution can then be used to generate an elution profile such as shown for \( \beta \)-lactoglobulin elution from a Sephadex column (Fig. 4.4) at eluent velocities ranging from 0.17 to 0.85 cm/min. A reasonable fit is obtained at the higher flowrates, with significant deviation occurring at the lowest chromatographic velocity of 0.17 cm/min.
An alternate representation of this model was applied by Neuman et al. (1987) for sulfuric acid–sugar separation by ion exclusion, which in this case represents an example of linear equilibrium for glucose (Fig. 4.5). The effluent concentration profile is given by

$$\frac{c}{c_F} = \frac{1}{2} \left[ 1 + \text{erf} \left( \frac{Pe^{1/2} (v - \bar{v})}{2 \sqrt{v^2}} \right) \right] - \frac{1}{2} \left[ 1 + \text{erf} \left( \frac{Pe^{1/2} (v^0 - \bar{v})}{2 \sqrt{v^0}} \right) \right]$$

(38)

where $v^0$ is the sample volume, $\bar{v}$ is the effluent volume, $\bar{v}$ is the volume of feed required to saturate the column, and $v_e$ is the extraparticle void volume (124 mL) for a column with 309 mL bed volume. (Note $\text{erf}(-x) = -\text{erf}(x)$.) This is related to the extraparticle void volume $V_e$ and the stationary phase volume by:

$$\bar{v} = V_e + KV.$$

(39)

The values of parameters that resulted in the fit of Eq. (38) to the data (Fig. 4.6) are summarized in Table 4.3 and show reasonable agreement. The data and calculated result are consistent with a sample volume of up to 50% of the void fraction of the column. This illustrates that a large sample volume does not necessarily result in a skewed peak. Separation of the glucose from sulfuric acid is shown to give similar peak shapes for the glucose at temperatures ranging from 27 to 81°C (Fig. 4.7), with