BIOSEPARATIONS SCIENCE AND ENGINEERING

Roger G. Harrison
University of Oklahoma, Norman, Oklahoma

Paul Todd
University of Oklahoma, Norman, Oklahoma

Scott R. Rudge
University of Oklahoma, Norman, Oklahoma

Demetri Petrides
University of Oklahoma, Norman, Oklahoma

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CHAPTER 7

Liquid Chromatography and Adsorption

Liquid chromatography and adsorption processes are based on the differential affinity of various soluble molecules for specific types of solids. In these processes, equilibrium is approached between a solid phase, often called the resin, or stationary phase, and the soluble molecules in the liquid phase. The solid phase is "stationary" because it is often packed in a fixed column. Since the liquid phase is often flowing past the solid phase, it is referred to as the mobile phase.

Chromatography is traditionally defined as a sorptive separation process in which a mixture of solutes in a feed solution is introduced at the inlet of a column containing the stationary phase and separated over the length of the column by the action of an elution solvent that is continuously fed to the column after the feed has been introduced. Figure 7.1 shows the method of separating solutes known as elution chromatography. By contrast, in pure adsorption processes, where there is no chromatographic effect of different solute bands being separated on the column, the stationary phase is generally uniformly saturated with the target solute before the introduction of an elution solvent which causes the solute to transfer into the mobile phase; this type of operation is sometimes referred to as on-off chromatography [1]. Chromatography is usually performed downflow through a stationary phase in a fixed bed, although a recent advance is to operate the column upflow during the feed step to allow the bed to expand with minimal mixing of the adsorbent phase, which can accommodate whole cells and cell fragments in the feed [2].

Besides the traditional elution mode for chromatography, other modes of chromatography are sometimes used, including the frontal mode and the displacement mode. In frontal chromatography, the target solute does not adsorb to the column, while some impurities are strongly retained by the stationary phase. The feed continues until the front of adsorbed impurities approaches the column outlet. An example of frontal chromatography would be the use of an anion exchanger to adsorb DNA from a protein product stream. A pH is chosen below the isoelectric point of the protein, making it positively charged and unable to bind to the anion exchange column. DNA is adsorbed because it is negatively charged at almost all pH values. This technique allows processing of large amounts of material with a relatively small column, because contaminants are usually present in small amounts relative to the product.
LIQUID CHROMATOGRAPHY AND ADSORPTION

Figure 7.1 Schematic illustration of elution chromatography. Three solutes, fed to a column containing a stationary phase, separate in the column as the elution solvent flows through the column. The three solutes separate as a result of differing affinities for the stationary phase.

In displacement chromatography, the column is loaded with the target solute to a fraction of its capacity and then is eluted by continuously introducing an even more strongly adsorbing substance, known as the displacer. For sufficiently long columns, the solutes in the feed become distributed in a pattern of adjacent pure solute bands, where each upstream solute acts as a displacer for each downstream solute located in the band immediately downstream [3].

Liquid chromatography is a commonly used separation process for biologics and fine and specialty chemicals, while adsorption is widely used for high throughput processes for small biomolecules such as antibiotics, sugars, and amino acids. As a unit operation, chromatography is a specialized version of adsorption-based separations, and many of the concepts taught in this chapter apply to both.

Adsorption is based on a variety of chemistries, some highly customized. At equilibrium, the individual components in the mixture to be separated are distributed between the stationary and mobile phases. This equilibrium can be modified by standard thermodynamic manipulations, such as temperature or composition (chemical activity) of the mobile phase. The most common thermodynamic variable to be modified is the composition of the mobile phase. Salt concentration, organic solvent concentration, and pH are common mobile phase thermodynamic characteristics that can be modified easily.

As in all separation operations, transport processes play a major role. Transport of chemical species from the mobile phase to the stationary phase takes place through a stagnant mass transfer layer at the surface of the particle, and solutes diffuse through pores to a binding site. The kinetics of the binding reaction can sometimes affect the separation. Axial and radial mixing can also be important factors in the separation, especially if the resin bed has not been well packed. Finally, the peripherals to the chromatography or adsorption column, such as the pumps, detectors, injectors, and filters, potentially add dead space and mixing volumes that affect the ultimate quality of the separation.
7.2 Adsorption Equilibrium

Adsorption equilibrium is described first in this chapter, followed by the dynamics of adsorption and chromatography. Both fixed-bed adsorbers and agitated-bed adsorbers are included. Various adsorbent types are discussed, as well as particle size, pressure drop, and equipment considerations. Scaleup as applied to both adsorption and chromatography concludes the chapter.

### 7.1 Instructional Objectives

After completing this chapter, the reader should be able to do the following:

- Write, plot, and use equations for adsorption isotherms.
- Derive the mass balance for fixed-bed adsorption and understand how it can be solved when simplifying assumptions are made.
- Calculate the shock wave velocity of a solute front when local equilibrium and negligible dispersion are assumed.
- Derive the mass balance for a series of agitated-bed adsorber columns.
- Assess chromatographic performance by elution peak analysis.
- Predict the chromatographic separation of solutes under assumptions of a linear isotherm, local equilibrium, and negligible dispersion.
- Explain the origins of band spreading in chromatography and its application to column optimization.
- Select resins based on the adsorption chemistry of the separands.
- Use particle size and pressure drop in the scaling of adsorption and chromatography.
- Use the length of unused bed (LUB) method to scale up a fixed-bed adsorber.
- Use constant resolution scaling rules for elution chromatography.
- Choose equipment for chromatographic separations including columns, packing procedures, pumps, and gradient makers.

### 7.2 Adsorption Equilibrium

Chromatography and adsorption work by the differential adsorption of species to a resin surface, or ligands, from a complex chemical mixture. The adsorption of a chemical species can be represented by the equilibrium reaction

\[ C + S \rightarrow K_{eq} \rightarrow CS \]  

(7.2.1)

where C is the dissolved chemical species, S is an adsorption site, CS is the chemical bound to the site, and \( K_{eq} \) is the equilibrium constant governing the reaction. The equilibrium constant for this adsorption is

\[ K_{eq} = \frac{[CS]}{[C][S]} \]  

(7.2.2)
Consider the three assumptions inherent in this representation of the adsorption reaction. First, it is completely reversible, and the chemical’s interaction with the adsorption site causes no alteration in its solution properties or solution state. Second, chemicals bind to sites in a one-to-one fashion, and they bind only to sites. In other words, the binding is “specific,” and there is no “nonspecific” binding, or interaction between molecules on the surface. Third, there is only one mode of binding to the site; all binding is equal and is described by a single value for $K_{eq}$. Despite these limiting assumptions, this “site model” of adsorption still serves as a very accurate description of adsorption in liquid chromatography.

In many cases, the concentration of adsorption sites is very much larger than the concentration of dissolved chemical species ($[S] \gg [C]$), and the equilibrium expression becomes

$$K_{eq} = \frac{[CS]}{[C]} \quad (7.2.3)$$

or,

$$[CS] = K_{eq}[C] \quad (7.2.4)$$

This representation is particularly common in analytical liquid chromatography, where sample volumes are very small and solutes typically dilute. This is known as linear equilibrium, because the concentration of the adsorbed species can be expressed as a multiple of the concentration of the dissolved species. There is no limitation to binding set by the inherent ability of the resin to bind the chemical. In other words, no saturation limit of the resin will be reached.

Chromatography in the linear limit is very suitable for analytical chromatography because it emphasizes the difference in $K_{eq}$ between species in order to separate them; these differences can be very small for very similar molecules. The purpose of analytical chromatography is to measure the concentrations of the various components of a mixture. To achieve this goal, “baseline resolution” (zero concentration between eluted separands) is critical, since determining the concentrations of two species that are not completely separated is less accurate. It is also critical that the concentrations of the various solutes do not affect their retention time, which is the case in linear equilibrium.

The linear isotherm approximation is less useful for preparative or industrial scale adsorption and chromatography. The most efficient operation uses all the adsorption sites available. In this case, the concentration of the empty adsorption sites available cannot be ignored. The concentration of unoccupied sites is not readily measurable, but the total number of sites is, so

$$S_{tot} = [CS] + [S] \quad (7.2.5)$$

where $S_{tot}$ is the total site concentration. Combining the general expression for $K_{eq}$, Equation (7.2.2), with Equation (7.2.5) for $S_{tot}$ gives

$$[CS] = \frac{K_{eq}S_{tot}[C]}{1 + K_{eq}[C]} \quad (7.2.6)$$

the well-known *Langmuir isotherm*. In the limit where $K_{eq}[C]$ is small in comparison to one, the denominator of Equation (7.2.6) goes to one, and the form of the linear adsorption equation is recovered [Equation (7.2.4)]. When $K_{eq}[C]$ is large in comparison to one, $[CS]$ is equal to $S_{tot}$; that is, the adsorption sites are saturated. The *Langmuir isotherm* [a plot of Equation (7.2.6)] is “concave downward,” having a linear slope in the low concentration range.
limit and a plateau as the resin becomes saturated (Figure 7.2). Equilibrium isotherms that are concave downward are considered to be favorable for adsorption. The Langmuir isotherm has often been used to correlate equilibrium adsorption data for proteins [4].

The consequences of the Langmuir isotherm for preparative and industrial chromatography are many. The adsorption of various species from a complex mixture is now seen as influenced not only by the equilibrium constants ($K_{eq}$'s), which are species specific, but also by the total binding capacity of the resin ($S_{eq}$), which is more specific to the resin than to the chemical species. This potentially decreases the differences between species in their chromatographic behavior at high concentrations. Baseline resolution of species is no longer generally attainable, and the concept of resolution begins to lose value in these applications. The goal of large-scale chromatographic separation is also different. Here, we are trying to purify one or possibly two major components. Purification is usually defined as a limit of particular contaminants, for example removal of DNA to less than 10 ppm. Measurement of contaminants is no longer the goal, and separation of contaminants from each other is also not important.

Another equilibrium isotherm that has proven useful is the empirical Freundlich isotherm [5]:

$$[CS] = K_{eq}[C]^{1/n} \quad n > 1 \quad (7.2.7)$$

With $n > 1$, this isotherm is concave downward (Figure 7.2). This isotherm can be explained as a result of energetic heterogeneity of the surface of the adsorbent. An expression of the Freundlich form results when a set of Langmuir isotherms (with different $K_{eq}$ values) is superimposed. The Freundlich isotherm has been used to describe the adsorption of a wide range of antibiotics, steroids, and hormones [4].

### 7.3 Adsorption Dynamics

A basic understanding of the dynamic behavior of adsorption processes is essential for the design and optimization of large-scale processes. The most important types of operation of adsorbers for biotechnology are fixed-bed columns and agitated-bed columns. The dynamics of each of these systems are analyzed.
7.3.1 Fixed-Bed Adsorption

The development of a mass balance is the starting point for analyzing fixed-bed adsorption. Simplifications of this mass balance lead to analytical solutions that give insight into the adsorption process.

**Mass Balance for Fixed-Bed Adsorption**

To understand the dynamics of a fixed-bed adsorption column, a mass balance is performed by considering a disk of cross-sectional area equivalent to that of the column (A), but differential in thickness (Δx). A species to be separated (called the separand) flows in and out of the disk by convection and the combined effects of molecular diffusion and mechanical dispersion. Note that the rate of convection into the disk is the interstitial velocity, that is, the velocity of fluid in the void fraction, ε. This convection rate is the superficial velocity (the flow rate divided by the cross section of the column) divided by the void fraction (= Q/εA). Within the volume of the disk, separand may accumulate within both the mobile and stationary phases. The mass balance for separand i can be written as follows:

\[
\begin{align*}
\text{(Rate of separand in)} - \text{(rate of separand out)} &= \text{(rate of accumulation of separand)} \\
\Delta x \Delta t \left( \frac{v}{\varepsilon} c_i - D_{\text{eff}} \frac{\partial c_i}{\partial x} \right)_{x,t} - \Delta x \Delta t \left( \frac{v}{\varepsilon} c_i - D_{\text{eff}} \frac{\partial c_i}{\partial x} \right)_{x+\Delta x,t} \\
&= \Delta x (c_i|_{x+\Delta x} - c_i|_x) + A(1 - \varepsilon) \Delta x (q_i|_{x+\Delta x} - q_i|_x) \\
&= A \varepsilon \Delta x A \varepsilon \Delta t (c_i|_{x+\Delta x} - c_i|_x) + A(1 - \varepsilon) \Delta x (q_i(t)|_{x+\Delta x} - q_i(t)|_x)
\end{align*}
\]

(7.3.1)

where:
- \( c_i \): concentration of separand i in the mobile phase = [C]_i,
- \( q_i \): concentration of separand i in the stationary phase averaged over an adsorbent particle = [CS]_i,
- \( \varepsilon \): void fraction (mobile phase volume/total column volume), commonly 0.3 to 0.4 in fixed beds,
- \( v \): mobile phase superficial velocity (flow rate divided by the empty column cross-sectional area, Q/A),
- \( D_{\text{eff}} \): effective dispersivity of the separand in the column,
- \( t \): time,
- \( x \): longitudinal distance in the column; \( x = 0 \) at column inlet.

Dividing by \( A \varepsilon \Delta x \Delta t \), and taking the limit as \( \Delta x \) and \( \Delta t \) go to zero, this mass balance becomes

\[
D_{\text{eff}} \frac{\partial^2 c_i}{\partial x^2} = \frac{v}{\varepsilon} \frac{\partial c_i}{\partial x} = \frac{\partial c_i}{\partial t} + \frac{1 - \varepsilon}{\varepsilon} \frac{\partial q_i}{\partial t}
\]

(7.3.3)

The right-hand side of this equation represents the accumulation rate of solute in any section of the column and recognizes that adsorption is an inherently unsteady state operation. The left-hand side of the equation represents the "in minus out" terms of the mass balance; solute moves through the column via the convection of the mobile phase (second term), or by diffusion or mechanical dispersion down a concentration gradient (first term). The term \( \partial q_i/\partial t \) represents the rate of mass transfer of the separand to an average particle in the...
7.3 Adsorption Dynamics

In general, the stationary phase and can be represented as

\[ \frac{\partial q_i}{\partial t} = f(x + q_i) \]  

(7.3.4)

where the subscripts represent separands i, j,.... This rate expression may be a linear driving force expression of the form

\[ \frac{\partial q_i}{\partial t} = K_\alpha (c_i - c_i^*) \]  

(7.3.5)

where \( K_\alpha \) is an overall mass transfer coefficient that includes both internal and external mass transfer resistance, and \( c_i^* \) is the liquid phase concentration that would exist at equilibrium with \( q_i \).

Assumption of Local Equilibrium and Negligible Dispersion

Since the overall pattern of mass transfer is governed by the form of the equilibrium relationship, the main features of the dynamic behavior can be understood without doing detailed calculations [5]. Thus, this first analysis of the mass balance for adsorption will assume equilibrium locally and ignore the dispersion term for simplicity. This allows us to focus on the velocity, \( u_i \), at which a solute traverses the column. Using an equilibrium isotherm relationship in the form \( q_i = f(c_i) \) (Figure 7.2), Equation (7.3.3) becomes

\[ \frac{\partial c_i}{\partial t} + \frac{v}{\varepsilon + (1 - \varepsilon)q'_i(c_i)} \frac{\partial c_i}{\partial x} = 0 \]  

(7.3.6)

where \( q'_i(c_i) \) is the slope of the equilibrium isotherm at concentration \( c_i \). If we let

\[ u_i = \frac{v}{\varepsilon + (1 - \varepsilon)q'_i(c_i)} \]  

(7.3.7)

then Equation (7.3.6) becomes

\[ \frac{\partial c_i}{\partial t} + u_i \frac{\partial c_i}{\partial x} = 0 \]  

(7.3.8)

We see that Equation (7.3.8) is identical in form to the equation of continuity for solute i moving at velocity \( u_i \) through a column with no packing [6]. Thus, the expression for \( u_i \) given by Equation (7.3.7) is the effective velocity of component i through the packed bed. When the equilibrium is linear for the solute, \( q'_i(c_i) = K_{eq,i} \), so that the effective solute velocity is independent of the concentration and is inversely proportional to \( K_{eq,i} \).

For preparative and industrial scale adsorption processes, where it is desired to use high adsorbent loadings, the equilibrium is nonlinear and generally the Langmuir isotherm is applicable. From Figure 7.2 for the Langmuir isotherm, it can be seen that \( q'_i(c_i) \) decreases with concentration; according to Equation (7.3.7), a decrease in \( q'_i(c_i) \) causes \( u_i \) to increase. This would result in the physically unreasonable overhanging concentration profiles shown in Figure 7.3. However, for the solute at the front that advances through the fixed bed, the concentration change is not continuous, and finite differences rather than
Figure 7.3 Shapes of shock wave concentration profiles (solid lines) and physically unreasonable overhanging concentration profiles for a favorable equilibrium isotherm (dashed lines): $c_1$: mobile phase concentration of solute; $c_{0i}$: solute concentration in feed to the adsorber.

differentials must be used for the change in $q_i$ with $c_i$, as follows:

$$u_{i,w} = \frac{\nu}{\varepsilon + (1 - \varepsilon) \frac{\Delta q_i}{\Delta c_i}}$$  \hspace{1cm} (7.3.9)

The resulting $u_{i,w}$ of the solute front is called the shock wave velocity, as the mathematics describing this phenomenon are similar to those describing acoustic waves and ocean waves. The calculation of the shock wave velocity is illustrated later (see Example 7.3).

In real systems, solute dispersion in the column makes perfect step changes in concentration impossible. However, when the equilibrium isotherm is favorable (i.e., concave downward, as for the Langmuir isotherm and the Freundlich isotherm), which is often the case for biological solutes, a solute wave front is self-sharpening. This can be explained from an examination of the effect of a favorable isotherm on the solute velocity $u_i$ determined by Equation (7.3.7): low concentrations ahead of the wave result in higher $q_i(c_i)$ and thus lower $u_i$, which high concentration within the wave give lower $q_i(c_i)$ and therefore higher $u_i$. These effects both work to sharpen the front. On the other hand, low concentrations that trail the front also give higher $q_i(c_i)$ and thus lower $u_i$, resulting in a broadening tail behind the sharp front. These effects are illustrated later in Example 7.4.

Assumption of a Linear Equilibrium Isotherm and Negligible Dispersion

If the equilibrium isotherm is linear and dispersion is neglected, an analytical solution of the mass balance for adsorption [Equation (7.3.3)] can be obtained. Although numerical solutions of this mass balance can be obtained, analytical solutions are valuable because they provide greater insight into the behavior of the system. The following equations describe adsorption assuming a linear adsorption isotherm, a linear driving force for the mass transfer rate, and negligible dispersion, respectively:

$$q_i = K_{eq,i}c_i$$  \hspace{1cm} (7.3.10)

$$\frac{\partial q_i}{\partial t} = K_v(c_i - c_i^*)$$  \hspace{1cm} (7.3.5)

$$\frac{\partial c_i}{\partial t} + \frac{1 - \varepsilon}{\varepsilon} \frac{\partial q_i}{\partial t} + \frac{\nu}{\varepsilon} \frac{\partial c_i}{\partial x} = 0$$  \hspace{1cm} (7.3.11)

The initial and boundary conditions for a column initially free of solute subjected to a step change in the solute concentration at the inlet at time zero are as follows:

$$t < 0 \quad q_i(0, x) = c_i(0, x) = 0$$  \hspace{1cm} (7.3.12)

$$t \geq 0 \quad c_i(t, 0) = c_{i0}$$  \hspace{1cm} (7.3.13)
7.3 Adsorption Dynamics

For the solution of these equations, it is convenient to write Equations (7.3.11) and (7.3.5) in terms of dimensionless variables:

\[
\frac{\partial \phi}{\partial \xi} + \frac{\partial \psi}{\partial \tau} = 0 \tag{7.3.14}
\]

\[
\frac{\partial \psi}{\partial \tau} = \phi - \psi \tag{7.3.15}
\]

where \( \phi = c_i / c_{i_0} \)
\( \psi = \bar{q}_i / \bar{q}_{i_0} \)
\( q_{i_0} = K_{eq,i} c_{i_0} \)
\( \xi = (k K_{eq,i} x / u)(1 - \varepsilon) \)
\( k = K_u / K_{eq,i} \)
\( \tau = k (t - x \varepsilon / u) \)

Equations (7.3.14) and (7.3.15) are exactly analogous to those used to describe heat transfer in a packed bed, and using classical methods the following solution was obtained [7]:

\[
\frac{c_i}{c_{i_0}} = e^{-\xi} \int_0^t e^{-u} I_0(2\sqrt{\tau \xi}) \, du + e^{-(\tau + \xi)} I_0(2\sqrt{\tau \xi}) \tag{7.3.16}
\]

where the Bessel function is given by

\[
I_0(2\sqrt{\tau \xi}) = \sum_{n=0}^{\infty} \frac{(\tau \xi)^n}{(n!)^2} = 1 + \tau \xi + \frac{\tau^2 \xi^2}{4} + \frac{\tau^3 \xi^3}{36} + \cdots \tag{7.3.17}
\]

The numerical evaluation requires tedious graphical integrations. Representative results of breakthrough curves using the results of graphical integrations [8] are shown in Figure 7.4.

\[\text{Figure 7.4 Adsorption breakthrough curves based on an analytical solution of the mass balance assuming a linear adsorption isotherm, a linear driving force for the mass transfer rate, and negligible dispersion. (Data from C. C. Fumas, "Heat transfer from a gas stream to a bed of broken solids," Trans. AIChE, vol. 24, p. 155, 1929.)}\]
Analytical solutions of the mass transfer equations for adsorption have been obtained with more realistic models, including intraparticle diffusion with and without external film resistance, but the solutions for the breakthrough curve are too cumbersome to be of much practical value [9].

**Determination of the Mass Transfer Coefficient from Adsorption Breakthrough Data**

A solution containing a biological compound was fed at a superficial velocity of 20 cm/h to a fixed-bed column containing an adsorbent until breakthrough (when $c_i/c_i^0 = 0.1$) was obtained 9.5 h from the start of feeding. The feed concentration was low enough that the equilibrium isotherm was linear, found previously in equilibrium experiments to be

$$g_i = 40c_i^*$$

where $g_i$ and $c_i^*$ have units of milligrams per milliliter. The column was 10 cm long, and the void fraction of the packing was 0.34. From this information, estimate the mass transfer coefficient $K_a$, assuming a linear driving force for the mass transfer rate.

$$\frac{\partial g_i}{\partial t} = K_a (c_i - c_i^*)$$

**SOLUTION**

We can solve this problem using the analytical solution of the breakthrough curve in graphical form (Figure 7.4) for the equations that describe mass transfer in adsorption, assuming a linear equilibrium isotherm, a linear driving force for the mass transfer rate, and negligible dispersion. At the column outlet, the parameters in the graphical solution shown in Figure 7.4 are as follows, using the definitions for the variables in Equations (7.3.14) and (7.3.15):

$$\phi = \frac{c_i}{c_i^0} = 0.1$$

$$\xi = \frac{K_a x}{v} (1 - \varepsilon) = \frac{K_a \times 10 \text{ cm}}{20 \text{ cm/h}} \times 0.66 = 0.33K_a$$

$$\tau = \frac{K_a}{K_{eq}} \left( t - \frac{x \varepsilon}{v} \right) = \frac{K_a}{40} \left( 9.5 \text{ h} - \frac{10 \text{ cm} \times 0.34}{20 \text{ cm/h}} \right) = 0.233K_a$$

where $K_a$ has units of hours$^{-1}$. We can rewrite these in terms of $K_a$ as

$$K_a = 3.03\xi = 4.29\tau$$

Finding $K_a$ is a trial-and-error solution. We select values of $\tau$ and $\xi$ that give $\phi = 0.1$ in Figure 7.4 and calculate $K_a$ using each of the two foregoing equations. This is continued
until the same $K_a$ is obtained for each equation. This procedure leads to

$$K_a \cong 105 \text{ h}^{-1}$$

This value of $K_a$ could now be used to predict breakthrough for other superficial velocities and column lengths.

**Constant Pattern Behavior**

In preparative and industrial adsorption processes, high feed concentrations, resistance to mass transfer, and axial mixing in the column lead to departures from ideal situations where a linear isotherm, local equilibrium, and negligible dispersion can be assumed. In the case of a favorable equilibrium isotherm (i.e., isotherms that are concave downward, which is the case for the Langmuir isotherm that can often be used for the adsorption of proteins), the concentration profile in the column develops in the initial region; and at some point from the column entrance, the profile propagates without further change in shape—thus the term “constant pattern.” This occurs because the dispersion in the column resulting from longitudinal dispersion and mass transfer resistance is opposed by the self-sharpening effect discussed earlier. When the equilibrium isotherm is highly favorable, the distance required to approach the constant pattern limit may be very small, a few centimeters to up to a meter [5]. The constant pattern approximation provides the basis for a very useful and widely utilized method for scaleup design using the concept of the length of unused bed (LUB) (see Section 7.8.1).

### 7.3.2 Agitated-Bed Adsorption

Agitated-bed adsorption processes have been developed to allow removal of a product secreted by the cells without first having to remove the cells. In this type of process, cell culture broth is passed through a series of agitated columns containing an adsorbent, as shown in Figure 7.5. Each column has screens at the inlet and outlet that are designed to retain the adsorbent within the column but allow the broth to pass through. When the concentration of the product in the effluent of the last column in the series reaches a certain value, the flow is stopped, and the lead column is taken out of the train. Periodic countercurrent operation is obtained by advancing each of the remaining columns in the train, placing a regenerated column of adsorbent in the last position, and starting the feed flow again. The lead column taken out of the train is washed with the adsorbent agitation to remove the broth solids, and the product is eluted from the adsorbent, usually in the fixed-bed mode.

This process has advantages over filtration, in that there is no filter aid to dispose of and it is not necessary to wash a filter cake containing the cells, so losses of the product

![Figure 7.5 Train of agitated-bed adsorption columns for the processing of cell culture broth.](image)
are often less. The equipment for this process is less expensive and easier to maintain than that used for centrifugation. The disadvantage is that expensive solid adsorbents are more easily fouled by the dirtier feed stream and so require harsher or more expensive regeneration procedures and more frequent replacement, compared to adsorbents utilized with streams with fewer impurities. Also, resin attrition can be an issue.

A useful mathematical model for this process has been developed [10]. The continuity equation for the \textit{n}th column in the train can be written for separand \(i\) as

\[
Qc_{i,n-1} - Qc_{i,n} = V_L \frac{dc_{i,n}}{dt} + V_R \frac{dq_{i,n}}{dt} \tag{7.3.18}
\]

where \(Q = \) volumetric flow rate
\(c_{i,n-1}, c_{i,n} = \) separand concentration in feed to and effluent from column \(n\), respectively
\(V_L = \) liquid volume in column
\(V_R = \) volume of adsorbent in column
\(q_{i,n} = \) separand concentration in adsorbent phase of column \(n\) averaged over an adsorbent particle
\(t = \) time

The rate of mass transfer of separand to the adsorbent phase is described by a linear driving force expression similar to Equation (7.3.5):

\[
\frac{dq_{i,n}}{dt} = K_s (c_{i,n} - c_{i,n}^*) \tag{7.3.20}
\]

where \(c_{i,n}^*\) is the separand concentration in the bulk liquid when it is at equilibrium with \(q_{i,n}\), and \(K_s\) is an overall mass transfer coefficient that can be correlated to experimental data as

\[
K_s = A \exp\left(-B \frac{q_i}{q_i^{\text{sat}}}ight) + D \exp\left(-E \frac{q_i}{q_i^{\text{sat}}}ight) \tag{7.3.21}
\]

Here \(A, B, C,\) and \(D\) are constants, and \(q_i^{\text{sat}}\) is the adsorbent phase concentration which is in equilibrium with the separand concentration \(c_i^{(n)}\) in the feed to the train of mixed columns. In the use of this model for the recovery of an antibiotic, equilibrium was modeled by the Freundlich isotherm written in the form

\[
c_{i,n}^{\text{eq}} = b q_i^{q}\tag{7.3.22}
\]

where \(a\) and \(b\) are constants.

Equations (7.3.19) to (7.3.22) constitute a set of mathematical relationships that govern the performance of each column in the train. These simultaneous equations can be solved by the Runge–Kutta numerical method [11] to predict the effluent and adsorbent concentrations as a function of time. Excellent agreement between predicted and experimental adsorption data for the recovery of the antibiotic novobiocin in a three-stage train has been obtained using this method [10].
7.4 Chromatography Column Dynamics

There are many methods of analysis for the dynamics of chromatography unit operations, too many to cover in detail in this textbook. To speak the language of chromatographers, the most relevant method to know is theoretical plate analysis. To be conversant with engineering methods of analysis, the application of mass balances, rate equations, and equilibria (Chapter 1) are the most relevant. A brief treatment of each of these methods is given here.

7.4.1 Plate Models

Plate models seek to explain the band broadening observed in chromatography by approximating a chromatograph as a series of well-mixed tanks at equilibrium. The terminology comes from analysis of distillation, where plates are sometimes used to hold vapor and liquid in contact to approach equilibrium at various temperatures and compositions. Just as distillation is often performed in a packed column but the concept of a "theoretical equilibrium plate" remains, so has it also come to symbolize resolving power of a chromatography column. A resemblance to the analysis of multistage extraction (Section 6.2) should be noted.

The original analogy was drawn by Martin and Synge [12] in 1941. By dividing the column into a series of imaginary well-mixed tanks at equilibrium and computing the mass balance around each, they deduced when the number of tanks became large, and the initial condition was a solute at a finite concentration in the first tank, that the concentration profile could be described by a Gaussian curve. Properties of a Gaussian curve are shown in Figure 7.6. The volume of the theoretical tanks (also called plates) is found by dividing the

![Diagram of Gaussian curve properties](image)

**Figure 7.6** Properties of a Gaussian peak \( \xi_{max} \), maximum peak height; \( \sigma \), standard deviation; \( w_i \), peak width at inflection points; \( w_h \), peak width at half-height; \( w \), peak width at base (base intercept); \( t_{re} \), average retention time. (Data from C. Horvath and W. R. Melander, "Theory of Chromatography," Fundamentals and Applications of Chromatographic and Electrophoretic Methods, Part A, E. Hefmann, ed., p A41, Elsevier Scientific, Amsterdam, Netherlands, 1983.)
column volume by the number of tanks, and, $H$, the height of the equivalent theoretical plate (HETP), can be expressed as

$$H = \frac{L}{N}$$

(7.4.1)

where $L$ is the length of the column and $N$ is the number of plates. For Gaussian peaks, the plate count ($N$) can be expressed as the squared average retention time divided by the variance of the peak [13]:

$$N = \frac{t_R^2}{\sigma^2} = \frac{t_R^2}{(w/4)^2}$$

(7.4.2)

where $w$ is the peak width at the base (the base intercept as shown in Figure 7.6). The determination of the number of theoretical plates is a primary method by which scientists compare the performance of different columns, packed by different methods or with different resins.

Peak width is used in the definition of resolution, $R_s$, which is a measure of the extent of separation of two peaks in a chromatography:

$$R_s = \frac{t_{R_2} - t_{R_1}}{\frac{1}{2}(w_2 + w_1)}$$

(7.4.3)

where $t_{R_1}$, $t_{R_2}$ = average retention time for separands 1 and 2, respectively

$w_1$, $w_2$ = peak width (time) for separands 1 and 2, respectively

Thus, resolution is increased by increasing the difference in the retention times and by decreasing the peak widths.

A low concentration of a moderately bound solute should be used to evaluate a column HETP. Linear response is desired from a molecule that will bind and release from the resin, so that kinetics and mass transfer are measured, as well as mechanical mixing that may be present in the column hydrodynamics. To be certain the solute is in the linear binding range, a variety of concentrations may be tried at first, to measure the effect of concentration on retention time and peak shape. Very often, a water-soluble aromatic compound like benzoic acid is used. A popular series of solutes are the “parabens,” methyl parabenzoic acid, ethyl paraben, and propyl paraben. HETP should also be measured under isocratic conditions (i.e., constant eluent buffer composition) to avoid influencing retention time and peak shape with a peak-sharpening gradient.

The undeniable simplicity of this plate model for evaluating the inherent separating power of a chromatography column accounts for its wide use in the field. Its primary use is in comparing multiple packings of the same column geometry with the same resin. Its applicability to the actual separation under study is always under debate, especially when the separation is at high solute concentration and large total solute load, and is eluted with a gradient.

7.4.2 CHROMATOGRAPHY COLUMN MASS BALANCE WITH NEGLIGIBLE DISPERSION

Chromatography separates solutes based on their differential binding to the resin. The separation is effected as the solutes exit the column outlet at different times. How the binding
of solutes to resins at equilibrium translates into an elution time difference is found in the mass balance. As the solute is carried by the mobile phase through the resin, each solute partitions between the mobile and stationary phases. The solutes that partition more strongly are retarded with respect to the flowing fluid, and they exit the column later (Figure 7.1). Solutes that do not partition to the stationary phase at all exit the column in the "void volume," which represents the mobile phase volume in the interstices of the packed resin.

The mass balance for chromatography is identical to that for adsorption, Equation (7.3.3). The first analysis of this mass balance for chromatography will ignore the dispersion term for simplicity. This allows us to focus on the peak position, or the rate at which a solute traverses the column. (Another way of doing this is by moment analysis, which is beyond the scope of this text.) By assuming local equilibrium and neglecting dispersion, we find that the effective velocity of a solute moving through the packed column is the same as that previously found in general for adsorption [Equation (7.3.7)]; this equation is useful in predicting the separation of different solutes, as is illustrated in Example 7.2.

**Chromatographic Separation of Two Solutes** Two solutes have linear equilibrium constants of $K_{eq,1} = 7.5$ and $K_{eq,2} = 7.8$, respectively. For a flow rate of 1.5 liter/min, in a column 63 cm in diameter, with a void fraction of 0.33, and local equilibrium, what column length is required to separate the two solutes by 5 min?

**Solution**

The effective velocity of solute $i$ for negligible dispersion is given by Equation (7.3.7) as

$$u_i = \frac{v}{\varepsilon \cdot (1 - \varepsilon) q_i'(c_i)}$$

For linear equilibrium,

$$q_i = K_{eq,i} c_i$$

$$q_i'(c_i) = K_{eq,i}$$

The superficial velocity is

$$v = \frac{1500 \text{ cm}^3}{\pi (31.5)^2 \text{ cm}^2} = 0.481 \text{ cm/min}$$

In moment analysis, the Laplace transform is taken with a Dirac delta function as the inlet condition ($x = 0$), and the derivatives of the transforms with respect to $s$ are evaluated at $s = 0$. The first moment corresponds to the elution time of the peak, the second to its dispersion, the third to the skew, and so forth.

For an excellent reference on moment analysis, see Kucera [14].
For solute 1, the effective velocity is therefore

\[
\nu_1 = \frac{0.481 \text{ cm/min}}{0.33 + (1 - 0.33)(7.5)} = 0.08982 \text{ cm/min}
\]

This same equation gives 0.08657 cm/min for solute 2. Translating solute velocities into elution times for a constant distance traveled \( L \),

\[
5 \text{ min} = L \left( \frac{1}{\nu_2} - \frac{1}{\nu_1} \right)
\]

Solving for \( L \) gives

\[
L = 12.0 \text{ cm}
\]

Note that four significant figures are used to calculate \( \nu_1 \) and \( \nu_2 \) to avoid error in calculating \( L \).

Equilibrium relations are usually more complicated than the linear case. For the nonlinear Langmuir equilibrium isotherm [Equation (7.2.6)], we obtain for the first derivative of \( q_i' \) with respect to \( c_i \),

\[
q_i' (c_i) = \frac{K_{eq,i} S_{tot}}{1 + K_{eq,i} c_i} = \frac{K_{eq,i}^2 S_{tot} c_i}{(1 + K_{eq,i} c_i)^2}
\]

As the concentration becomes higher, the value of the derivative becomes smaller and the solute velocity is higher. This makes sense, because to have a high mobile phase concentration, the stationary phase concentration must be near saturation, thus leaving no sites on the resin for the solute in the mobile phase to bind to. This holds true as long as the change in concentration is continuous. However, when a pulse injection is made, the resin immediately preceding the solute front is devoid of bound solute. In this case, the concentration change is not continuous but is a step change, and finite differences must be used for \( q_i' \) and \( c_i \), giving Equation (7.3.9) for the effective velocity of the solute. This velocity is called a shock wave velocity, as discussed for adsorption in Section 7.3.

**EXAMPLE 7.3**

Calculation of the Shock Wave Velocity for a Nonlinear Isotherm   Solute 1 has a Langmuir isotherm characterized by an \( S_{tot} \) of 120 \( \mu \)g/ml, and a \( K_{eq} \) of 7.5 ml/mg. Calculate the shock wave velocity for an injection of 1 mg/ml, and column conditions identical to those of Example 7.2.

**SOLUTION**

To use Equation (7.3.9) for the shock wave velocity, we need to know \( \Delta q \). For the Langmuir isotherm [Equation (7.2.6)], the resin concentration \( q = [CS] \) in equilibrium with concentration \([C] = 1 \text{ mg/ml} \) in the mobile phase is

\[
[CS] = \frac{K_{eq} S_{tot} [C]}{1 + K_{eq} [C]} = \frac{(7.5 \text{ ml/mg})(0.120 \text{ mg/ml})(1 \text{ mg/ml})}{1 + (7.5 \text{ ml/mg})(1 \text{ mg/ml})} = 0.106 \text{ mg/ml}
\]
Since the column initially has no solute,

\[
\frac{\Delta q_1}{\Delta c_1} = \frac{(0.106 - 0) \text{ mg}}{(1 - 0) \text{ mg}} = 0.106
\]

From Equation (7.3.9), we obtain

\[
\frac{v}{\varepsilon + (1 - \varepsilon) \frac{\Delta q_1}{\Delta c_1}} = \frac{0.481 \text{ cm}}{0.33 + (1 - \varepsilon)(0.106)} = 1.20 \text{ cm/min}
\]

This is over 10 times the solute velocity for the linear isotherm case given in Example 7.2. This is because the concentration of the solute is limited to 0.120 mg/ml on the stationary phase. For the linear case, a 1 mg/ml injection would lead to a 7.5 mg/ml concentration in the stationary phase.

The leading edge of a solute front derived from a Langmuir isotherm is a self-sharpening shock wave, which leads to the constant pattern behavior discussed earlier. The trailing edge, on the other hand, is a continuous diffuse wave. The trailing edge has a continuous concentration from the injection concentration to zero. To predict the elution profile of a peak, both the leading shock wave and the trailing diffuse wave must be calculated. This is done by solving Equation (7.3.7) for the trailing edge with respect to a range of mobile phase concentrations between the injection concentration and zero, and Equation (7.3.9) with respect to the shock front (as in Example 7.3). This is shown in Example 7.4.

**EXAMPLE 7.4**

**Calculation of the Elution Profile**  For an injection volume of 5 liters, and other conditions as stated in Examples 7.2 and 7.3, calculate the elution profile for solute 1.

**SOLUTION**

As determined in Example 7.3, the velocity of the shock front is 1.20 cm/min. The diffuse wave velocity is determined by selecting concentrations between 0 and the injection concentration of 1 mg/ml, and determining the velocity of each of these concentrations trailing the shock front. For example, at a solute concentration of 0.4 mg/ml, we can calculate \( dq_1/dc_1 \) from Equation (7.4.4):

\[
\frac{dq_1}{dc_1} = \frac{K_{eq,1}S_{tot}}{1 + K_{eq,1}c_1} = \frac{K_{eq,1}^2S_{tot}c_1}{(1 + K_{eq,1}c_1)^2} = \frac{7.5(0.120)}{1 + 7.5(0.4)} = \frac{(7.5)^2(0.120)(0.4)}{[1 + 7.5(0.4)]^2} = 0.056
\]

We can use Equation (7.3.7) to calculate the solute velocity:

\[
u = \frac{v}{\varepsilon + (1 - \varepsilon) \frac{dq_1}{dc_1}} = \frac{0.481}{0.33 + (1 - 0.33)(0.056)} = 1.31 \text{ cm/min}
\]
TABLE E7.4
Diffuse Wave Concentrations Following the Shock Wave for Example 7.3

<table>
<thead>
<tr>
<th>Solute concentration, $c_1$ (mg/ml)</th>
<th>Isotherm derivative, $dq/dc_1$</th>
<th>Solute velocity, $u_1$ (cm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8</td>
<td>0.018</td>
<td>1.40</td>
</tr>
<tr>
<td>0.6</td>
<td>0.030</td>
<td>1.37</td>
</tr>
<tr>
<td>0.5</td>
<td>0.040</td>
<td>1.35</td>
</tr>
<tr>
<td>0.4</td>
<td>0.056</td>
<td>1.31</td>
</tr>
<tr>
<td>0.23</td>
<td>0.121</td>
<td>1.17</td>
</tr>
<tr>
<td>0.1</td>
<td>0.294</td>
<td>0.91</td>
</tr>
<tr>
<td>0.03</td>
<td>0.779</td>
<td>0.86</td>
</tr>
<tr>
<td>0.005</td>
<td>0.836</td>
<td>0.54</td>
</tr>
<tr>
<td>0.001</td>
<td>0.887</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Figure E7.4 The elution profile calculated for a Langmuir isotherm. Conditions are given in the examples in the text.

For a flow rate of 1.5 liters/min, and an injection volume of 5 liters, the diffuse wave trails the shock by 3 min and 20 s [5 liters/(1.5 liters/min)]. Table E7.4 gives the solute velocities for various concentrations.

As can be seen, concentrations above 0.23 mg/ml run faster than the shock front. Since nothing can pass the shock front, these concentrations do not appear in the thermodynamic diffuse wave. They do, however, represent concentrations on the isotherm where the derivative is less than the slope of the chord that connects the shock concentrations. Concentrations of 0.23 mg/ml and less run behind the shock front, and thus constitute the diffuse wave. The shock front elutes from the 12.0 cm bed in 10.0 min. The diffuse wave is delayed by 3.33 min, as mentioned earlier, and then starts at approximately 0.23 mg/ml. The diffuse wave decreases in concentration to 0.1 mg/ml at 16.5 min, to 0.01 mg/ml at 24.8 min, to 0.005 mg/ml at 25.6 min, and to 0.001 mg/ml at 26.4 min. A representative graph is shown in Figure E7.4.

The Langmuir isotherm and other concave downward equilibrium conditions are very common for large-scale liquid chromatography. Analytical chromatography does not generally deal with concentrations large enough to generate a Langmuir isotherm. Ion exchange binding is related to, but not exactly the same as, the Langmuir isotherm. Concave
where $r =$ distance from center of spherical resin particle
$u_{i,p} =$ concentration of separand $i$ in the pore fluid based on a unit volume
of stationary phase (i.e., the solid matrix and the pore space)
$u_{i,s} =$ concentration of separand $i$ adsorbed on the internal surfaces of
the stationary phase based on a unit volume of stationary phase
$c_i =$ concentration of solute $i$ in the mobile phase
$D_p =$ effective separand diffusivity in the pore fluid
$k_a =$ forward rate constant of adsorption
$k_e =$ fluid phase mass transfer coefficient
$e^* =$ effective volume fraction of the stationary phase that is accessible
to the separand

For the case of isocratic elution, where the concentration of the elution solvent is constant,
a lumped-parameter type of solution in a simple form was obtained by Athalye et al. [17].
Their solution relied on using empirical correlations to predict the height equivalent of a
theoretical plate (HETP) for the column. The performance of chromatography columns can
be predicted using this model based on independent estimates of transport parameters. The
general solution of Rasmuson and Neremnius [18] involves a special numerical integration
method, to which the interested reader is referred. The procedure for calculating HETP
depends on the approach of van Deemter et al. [19], which is now presented.

While chromatography and adsorption can be modeled accurately, and elution profiles
solved exactly on high speed computers by means of finite element analysis, the most prac-
tical rule of thumb for estimating the impact of mass transfer and diffusion effects is the
van Deemter equation. Van Deemter et al. [19] determined empirically that the bandwidth
[measured as the height equivalent of a theoretical plate, or HETP—see Equations (7.4.1)
and (7.4.2)] can be related to the linear velocity in the column by the following expression:

$$H = A + \frac{B}{u} + Cu$$  \hspace{1cm} (7.4.11)

The HETP ($H$) has dimensions of length, and is usually reported in centimeters. The factor
$A$ is primarily due to dead volume and other sources of mechanical mixing and is thought
to be proportional to resin particle size. Molecular diffusion in the mobile phase is repres-
ented by $B$ ($\text{cm}^2 \text{s}^{-1}$). If the flow rate is on the order of the liquid phase diffusivity of the
solute, the peak broadens. This is almost never the case in liquid chromatography, but this
case is relevant for gas chromatography. Finally, mass transfer and intraparticle diffusion
are represented by $C$ (s), which is thought to be proportional to the square of the effective
liquid film thickness at the surface of the resin particle, divided by the effective diffusiv-
ity within the particle. Liquid chromatography is dominated by this mass transfer term. The
van Deemter equation is also important because it shows the efficiency of the separation,
represented by the lowest possible value of $H$, as a function of the productivity of the
column, represented by the highest possible value for $u$.

The van Deemter equation can be normalized as

$$H^* = A + \frac{b}{u^*} + cu^*$$  \hspace{1cm} (7.4.12)

where $H^* = H/d_p$ and $u^* = u d_p/D_m$, $d_p$ is the stationary phase particle diameter, and
$D_m$ is the diffusivity of the solute of interest [20]. The reduced velocity $u^*$ is also the
Figure 7.8 Typical van Deenert plot for three particle diameters: 50 μm (●), 25 μm (■), 10 μm (▲).

Péclet number for chromatography. The reduced plate height, $H^*$, is rarely below 2, that is, two particle diameters in a chromatography bed constitute a mixing zone. A typical van Deenert plot is shown in Figure 7.8.

When normalized in this manner, the data for many chromatographic applications fall very close to one another [20]. Giddings has given rules of thumb for parameter estimation for $a$, $b$, and $c$ [21]. While these rules of thumb are valuable for estimation of peak broadening in the design stage, data for individual applications should always be acquired.

The most efficient separation is achieved with the lowest possible plate height. The definition of efficient separation that is meant here is the separation with the least energy lost to entropy (or thermodynamically speaking, most reversible). More theoretical plates are contained in a given column length when the plate height is minimized. In liquid chromatography, $u^*$ is typically a large number, as diffusivities are on the order of $10^{-3}$ to $10^{-2}$ cm$^2$/s, while linear velocities are typically on the order of $10^{-5}$ cm/s, and the smallest resin diameter is $10^{-4}$ cm, so in the best case, $u^*$ is 0.1. If $a$, $b$, and $c$ are of the same order of magnitude ($b$ is commonly smallest), then no term in the van Deenert equation dominates. Resins smaller than 1 μm are not commonly available, since the practical limit of pressure drop and resin containment seems to have been reached at this particle size. Larger particle resins are often chosen because they are considerably cheaper, in which case $u^*$ approaches 100 and the $c u^*$ term in the van Deenert equation dominates the plate height. Clearly, small stationary phase particle diameter (1 μm) is desirable for efficient separations.

A comparison of separation efficiency to process cost has been developed for large-scale chromatography for biotechnological applications that gave an optimum particle diameter of 30 μm [22]. This work indicated that particle diameters in the range of 20 to 40 μm make good targets for scaleup of chromatographic processes. Comparisons of these types are subject to the economics of each particular case.

The van Deenert equation has been recast into dimensionless transport parameters by Athalye et al. [17] as follows:

$$
\frac{H}{d_p} = \frac{2}{\text{Pe}_z} + \frac{(1 - x)}{3(1 - \varepsilon)} \text{Re} \text{Sc} \left( \frac{1}{\text{Nu} + \frac{m}{10} + m'} \right)
$$

(7.4.13)
7.4 Chromatography Column Dynamics

where $Pe = dispersion~Péclet~number = d_p \mu / D_{eff}$

$x = \frac{\varepsilon}{\varepsilon + (1 - \varepsilon)(1 + K_{eq})}$, or the fraction of separand in the mobile phase at long times

$ReSc = (Reynolds~number)(Schmidt~number) = (d_p \mu \rho / \mu)(\mu / \rho D_m) = d_p \mu \varepsilon / D_m$, reduced velocity or diffusion Péclet number

$D_m = separand~diffusivity~in~unbounded~solution$

$Nu = Nusselt~number = d_p k_c / D_m$

$m = D_m / \varepsilon^2 D_p$

$m' = \frac{3}{5} m ([K_{eq}/(1 + K_{eq})]^2$

$Da = Damköhler~number = d_p^2 k_a / 4 D_p$

The physical significance of each term in Equation (7.4.13) is as follows:

- $Pe$ term: convective axial dispersion
- $Nu$ term: fluid phase mass transfer resistance
- $m$ term: intraparticle diffusion resistance
- $m'$ term: sorption kinetics resistance

Equation (7.4.13) is valuable because it enables the quantification of each contribution to the plate height, hence a conceptually useful sensitivity analysis.

7.4.4 GRADIENTS AND MODIFIERS

Chemical gradients are used to elute solutes that are bound tightly to the stationary phase. The constituents of the chemical gradient are chosen to compete with the target solute for binding sites on the surface of the stationary phase. This affects the binding equilibrium, such that, at different concentrations of the gradient-forming chemical, different binding profiles are given for the solute of interest.

**EXAMPLE 7.5**

**Equilibrium for a Protein Anion in the Presence of Chloride Ion** Derive the equilibrium for a monovalent protein anion on an ion exchanger in the presence of sodium chloride ion and plot isotherms for various concentrations of chloride ion.

**SOLUTION**

The equilibrium is described by the following two expressions

$\text{P}^- + \text{S}^+ \xrightleftharpoons{K_{eq,1}} \text{S} \cdot \text{P}$

$\text{Cl}^- + \text{S}^+ \xrightleftharpoons{K_{eq,2}} \text{S} \cdot \text{Cl}$

where $\text{P}^-$ is the protein anion, $\text{S}^+$ is an unoccupied binding site on the stationary phase, and sodium is a spectator counterion. The equilibrium expressions are

$$K_{eq,1} = \frac{[\text{S} \cdot \text{P}]}{[\text{P}][\text{S}]}$$

$$K_{eq,2} = \frac{[\text{S} \cdot \text{Cl}]}{[\text{Cl}][\text{S}]}$$
Mass balances on protein concentration, chloride concentration, and total sites apply.

\[ S_{\text{tot}} = [S] + [S \cdot P] + [S \cdot \text{Cl}] \]

The preceding three equations can be solved for the adsorbed protein concentration to give the "multicomponent" Langmuir isotherm:

\[ [S \cdot P] = \frac{K_{\text{eq},1}S_{\text{tot}}[P]}{1 + K_{\text{eq},1}[P] + K_{\text{eq},2}[\text{Cl}]} \]

where \( K_{\text{eq},1} \) is the equilibrium constant for the protein measured in the presence of chloride, and \( K_{\text{eq},2} \) is the equilibrium constant for chloride measured in the presence of protein.

As can be seen from the expression derived for \([S \cdot P]\), as the chloride concentration is increased, the concentration of bound protein decreases. A protein that is tightly bound at low ionic strength can be eluted efficiently by an increase in the salt concentration. With \( K_{\text{eq},1} = 7.5 \), \( K_{\text{eq},2} = 2.0 \), and \( S_{\text{tot}} = 120 \mu g/ml \), we find the binding isotherms shown in Figure E7.5, where the increasing chloride concentration is seen to decrease binding of the protein. This is the principle of gradient elution chromatography, as well as of the regeneration of columns in adsorption processes. Changing the thermodynamic conditions so that the equilibrium is shifted back in favor of the unbound species affects the elution of that species and the regeneration of the resin. The thermodynamic condition may be ionic strength or pH (shifting a multivalent ion to a monovalent or uncharged species), temperature, or solvent composition. Slowly changing the thermodynamic conditions, in a gradient in the mobile phase, can sharpen diffuse waves, as well as cause desorption of the bound species. A step change (step gradient) can regenerate a resin or displace bound constituents.

The rate of change of the gradient with time is important. Most gradients are linear—their rate of change is constant. In reversed-phase chromatography, a gradient of 1%/min is often used; that is, the percentage of organic modifier is increasing smoothly by 1% per minute with a constant flow rate. Another useful but less common gradient is exponential. This provides for a shallower gradient early in the chromatography, and a rapidly increasing gradient late. An exponential gradient is useful when one is separating a wide variety of molecules that range from very loosely bound to extremely tightly bound. Yet another technique is to run a linear gradient to the point of product elution, do an isocratic elution
7.5 Adsorbent Types

(i.e., constant composition of the elution solvent) through the product zone to maximize resolution, and follow by a gradient to regeneration conditions. Obviously, countless combinations and options exist.

There are two other special cases that arise from the equilibrium isotherm derived in Example 7.5. Ignore, for a moment, the identity of chloride in the equation, for this identity was assigned merely for purpose of example. The first is the situation of very large $K_{eq.1}$. In this case, the isotherm is very steep, approximating a square wave, and the chromatographic mode is called “on–off.” The isotherm remains steep until $K_{eq.1}/K_{eq.2}$ is of the order $K_{eq.1}/K_{eq.1}$, where $c_1$ and $c_2$ represent protein and salt anion concentration, respectively. Then solute 1 comes “off” the resin. Many proteins follow this case.

In the second case $K_{eq.2}$ is large, and greater than $K_{eq.1}$. In this case, solute 2 “displaces” solute 1 from the resin. Since solute 1 runs ahead of solute 2, and it has nonlinear binding itself, solute 1’s leading edge is sharpened by the shock wave effect, and its trailing edge is sharpened by the displacement affected by solute 2. This is the displacement chromatography technique that was discussed earlier.

7.5 Adsorbent Types

Many adsorbent resins have been developed for chromatographic separations. There are two basic resin materials, polymer and silica. Any type of chemistry can be conjugated to either resin material. Typically, however, silica resins have hydrophobic coatings and are used for reversed-phase chromatography. Polymer resins are used in aqueous applications and are conjugated with ion exchange, hydrophobic interaction, or affinity-type ligands.

The selection of the resin material defines many resin physical properties. The resin provides the surface area for the adsorption. Surface area is generally 100 to 1500 m²/g. The surface area on the outer surface of a 10 μm diameter solid sphere is 1.7 m²/g, so it follows that most of the surface area is in the internal porosity of the particle. Since this surface area is traditionally accessed by molecular diffusion (one class of resins, called perfusion resins, allows convection through the pores), the path length for this diffusion is important. The path length is defined as the radius of the resin, which is the maximum length a molecule will diffuse to gain access to the internal surface area of the resin. Therefore, both the diameter of the particle and its internal surface area are important for the resin performance.

7.5.1 Silica-Based Resins

Uncoated silica is compatible with water or organic solvent and serves as a good reversible adsorbent for hydrophilic compounds. An organic solvent is often used in the mobile phase, and water is added as the chromatography progresses. Silica is not typically stable at extremes of pH, especially basic pH (exceptions exist). Silica is available with high surface area and small particle size; being very rigid, it does not collapse under high pressures. Uncoated silica used in chromatography came to be known as “normal phase” chromatography. Silica resins are thought to denature some proteins and irreversibly bind others. However, silica-based chromatography is used for the purification of many commercial biotechnology products.
Silica particles coated with long-chain alkanes became widely available in the 1980s. This silica resin has a high affinity for hydrophobic molecules, which increases as the chain length of the bonded alkane increases. The bonding of organic layers to silica has become a science in its own right, and there are many varieties of the same chain length phase. Some phases are polymerized, some are simple monolayers, and some are “end capped,” which means that any uncovered surface of the silica is covered with an organic layer after the hydrophobic chain group has bonded. Each variety of resin may have different properties with respect to the mixture to be separated, and in many cases, different lots from the same supplier have different properties as well. The silica left bare around the bonded chains (in resins that are not end capped) often plays a role in the binding, and therefore the percent of the surface covered with each bonding reaction will lead to different properties for a particular resin type or manufacturer’s lot. Often, these imperfections in the manufacture of “reversed-phase” chromatography resins are important for the separation that can be achieved, but complicate the strict interpretation of the chromatographic result and can be difficult to reproduce. Reversed-phase chromatography is a very popular laboratory analytical technique and has been scaled up for the production of small molecules and peptides. Concerns about protein denaturation have slowed its introduction into protein manufacture; however, this is changing as the high resolving power of the technique is making it more and more attractive for high value added products.

7.5.2 POLYMER-BASED RESINS

Polymer-based resins are frequently used in industrial applications because of their high stability and low cost. The resins are manufactured by suspension polymerization, in which an emulsion of the polymer is made in an immiscible solvent, and a cross-linking agent is added. The reaction is allowed to proceed to completion, and then the particles are isolated from the suspension, washed, and frequently derivatized. Surfactants are often added in the suspension polymerization to control particle size; in general, however, polymer resins are larger (10–100 µm) than silica-based resins (1–25 µm). Polymer resins are also less rigid and not generally suitable for high pressure (>4 bar) applications (exceptions exist).

Two synthetic polymers that are commonly used are styrene divinylbenzene and polyacrylamide. Styrene divinylbenzene is very stable at pH extremes, and it has been used primarily as a support for ion exchange chromatography. While the backbone is somewhat hydrophobic, and nonspecific binding does occur, the stability and rigidity of this polymer make it a superior support for chromatography. Polyacrylamide is used less often. It is not used as a polymer solid but as a hydrogel, and its primary use in chromatography has been as a size exclusion gel. The cross-linking in polyacrylamide can be controlled very precisely by the amount of bisacrylamide added in the suspension mixture. The analogy to protein electrophoresis gels (Chapter 2) is apparent here.

Natural polymers such as agarose and dextran are also used in hydrogel form for low pressure chromatography resins. These polymers are naturally hydrophilic and are very compatible with protein and other biomaterials. Agarose can be cross-linked to form a reasonably rigid bead that is capable of tolerating pressures up to 4 bar. Dextran is less rigid and, like polyacrylamide, is used primarily in size exclusion. Dextran gels can be formed with very large pores, capable of including antibody molecules and virus particles. Both dextran and agarose have been derivatized with ion exchange groups, phenol groups,
antibodies, dyes, heavy metals, and other biological epitopes that allow very specific binding behavior for the target molecule.

7.5.3 **ION EXCHANGE RESINS**

Ion exchange resins are those that have been derivatized with an ionic group. The most commonly used ionic groups, listed in order of increasing \( pK \), are sulfoxy (SO\(_3\)), carboxyl (COO\(^-\)), diethylaminoethyl (DEAE) (2C\(_2\)H\(_3\)N\(^+\)HCl\(_2\)), and quaternary ethylamine (QAE) (4C\(_2\)H\(_3\)N\(^+\)). Thus sulfoxy is the most acidic, and QAE is the most basic. When the acidic ion exchangers are used at pH values above their respective \( pK \) values, they carry a negative charge and attract positive counterions. These are called cation exchangers. Conversely the basic ion exchangers are known as anion exchangers.

Sulfoxy and QAE are a strong acid and a strong base, respectively. Their \( pK \) values are close to 1 and 14, meaning that, for all practical purposes, they are fully ionized at all pH values. Carboxyl and DEAE are, respectively, a weak acid and base, with \( pK \) values closer to 4 and 10. These resins may be used in a pH gradient to ionize more or fewer of the ionic groups on the resin, thus effecting the separation. When the process calls for a pH shift, but separation of molecules along with that shift is not desirable, “strong” ion exchangers are preferred because their binding capacity is not likely to be altered.

Ion exchange resins are also always used in aqueous mobile phases, and they are almost always polymer resins. Water is the universal solvent for salts, which dissociate poorly in most organic solvents. The ability to manipulate the pH to effect separation also favors polymer matrices as backbone material. Finally, many common biological contaminants molecules bind nearly irreversibly to ion exchangers (see later). Therefore, to regenerate ion exchange resins, extreme pH values are often used, such as 1.0 M NaOH (pH 14), which would dissolve many common silica-based resins.

Ion exchange resins, once exclusively used to separate small ions, are now heavily used in the separation of proteins, peptides, nucleic acids, small polynucleotides, and other small molecules such as antibiotics. Proteins have distinct isoelectric points (see Chapter 1) based on the content and conformation of their charged amino acids, lysine, arginine, histidine, aspartic acid, and glutamic acid, in decreasing \( pK \) of the side chain group. Sometimes tyrosine deprotonates, depending on its microenvironment. Proteins have multiple charges and can be separated based on their number of charges, as well as their size, and sometimes charge heterogeneity. Nucleic acids, on the other hand, have a charge at each base, such that their charge is proportional to their mass. Since they have a negative charge at each base, they bind very well to anion exchangers, and not all to cation exchangers. Once bound to an anion exchange column, nucleic acids are very difficult to elute in an aqueous solution that does not simultaneously degrade them.

The selection of the buffer is an important consideration in the use of ion exchangers. It is advisable that the buffering species not interact with the adsorbent, which means that the charged form of the buffer should have the same sign as the charge on the adsorbent. It is also preferable that only simple anions be used in anion exchange (e.g., Cl\(^-\), acetate\(^-\)) and only simple cations (e.g., Na\(^+\), K\(^+\)) in cation exchange [23]. For example, when one is using DEAE-cellulose (an anion exchanger) at pH 8.0, a Tris-chloride buffer might be selected because it has HTris\(^+\) (noninteractive) and Tris (neutral) buffering species and Cl\(^-\) counterion.
7.5.4 Reversed-Phase Chromatography

Reversed-phase chromatography employs a hydrophobic phase bonded to the surface of the resin. Typically, reversed-phase resins are silica based. Reversed phase is so named because the partitioning of solutes between the mobile phase and stationary phase is opposite to that observed with bare silica. In other words, hydrophobic solutes bind in higher proportions in reversed phase, while hydrophilic solutes bind in higher proportion in “normal phase.” Solutes are typically introduced into reversed-phase columns in water, or with minimal amounts of organic solvent, so that most solutes partition to the stationary phase. The organic content of the mobile phase is slowly increased, typically as a percent of acetonitrile, methanol, or isopropanol, thereby decreasing the polarity of the mobile phase.

Hydrophobic phases that are bonded to silica are typically octyl (C₈), octyldecyl (C₁₈), phenyl, and methyl (C₁). The different chain lengths and densities (called “coverage” in the commercial literature) of the different bonded phases obviously lead to more or less hydrophobicity. The entire surface of the silica cannot be fully covered with a monolayer of the desired phase, however, because of steric effects. Bare silica remains exposed, and this bare silica can participate in the separation by interacting with hydrophilic molecules, or hydrophobic domains of large molecules, thereby altering the binding. The strategy employed for covering this exposed silica surface lends almost as much to the specifics of the separation achieved as the chain length of the bonded phase. Polymerized phases represent an attempt to cover the surface by polymerizing the alkyl chains together at their point of attachment to the silica. End-capped resins use a short chain length group, such as methyl or ethyl, to cover the unreacted surface sites. Resins that do not utilize either method are left so intentionally to take advantage of the “mixed-mode” separation that may result. The separation characteristics of these resins are difficult to reproduce precisely, leading to considerable variation in separation from one manufacturer’s lot of resin material to another.

Ions do not partition well in hydrophobic phases. It is common, therefore, to choose a counterion for reversed-phase chromatography. For biological mixtures, the counterion is nearly always a strong acid anion, such as trifluoroacetate, acetate, or chloride. The counterion has a strong effect on the separation, by partitioning along with the co-ion of interest. The counterion can be used to make solutes more or less hydrophobic and will not affect all solutes equally.

7.5.5 Hydrophobic Interaction Chromatography

Hydrophobic interaction chromatography is typically used for protein separations. It employs derivatized polymer resins, with phenyl, butyl, or octyl ligand groups, typically. Proteins adhere to the hydrophobic surface under high salt conditions and redissolve into the mobile phase as the salt concentration is reduced. Hydrophobic interaction chromatography differs from reversed phase in that the mobile phase is kept aqueous (polar), and the salt concentration is used to effect the partitioning to the surface. The mechanism of partition is related to precipitation as opposed to two-phase partitioning (reversed phase) or ionic interaction (ion exchange). This is due to the use of salts that strip proteins of their solvation water. As the concentration of “lyophilic” salts increases, the probability increases that the protein will aggregate, or nucleate on the surface of the resin [24]. Hydrophobic interaction chromatography is sensitive to pH, salt used, buffer type, and
temperature. Each of these must be carefully controlled to achieve reproducible separation but also represent an opportunity for increased selectivity.

7.5.6 Affinity Chromatography

Affinity chromatography takes advantage of biological interactions to effect binding of specific solutes. Antibodies, antigens, or dyes are conjugated to polymer resins for the purpose of binding specific solutes from a mixture. For instance, an antibody could be raised against a target protein. The antibody would be conjugated to a resin, usually via cyanogen bromide activation. The antibody then captures the solute out of the mixture, and the impurities flow through the column. The solute can be recovered by changing the pH, increasing the salt concentration, or adding a displacer, that is, a molecule that also has some affinity for the antibody, or other binding agent (affinity ligand) on the stationary phase.

Other examples of specific interactions that can be used to isolate proteins are enzyme–ligand, enzyme–cofactor, receptor–agonist (antagonist). In each case, one member of any of these pairs may be immobilized to a resin to isolate the desired partner. Affinity chromatography is often coupled with cloning techniques to synthesize the target molecule with an "epitope" or recognition sequence that can be captured by the affinity ligand. Affinity chromatography is used from small-scale research (e.g., high throughput screening) to large-scale purification.

7.5.7 Immobilized Metal Affinity Chromatography (IMAC)

Some proteins have high affinities for specific metals. This affinity may either be structural, as in the case of metalloproteins, which require metal centers for their biological activity, or based on the content of specific amino acid residues, such as histidine, tryptophan, and cysteine, which have increased affinity for transition metals such as nickel and copper. Techniques have been developed to immobilize metal ions with spacer arms onto polymer resins. These resins are referred to as IMAC resins, and they are used to purify proteins that have one of the two characteristics mentioned above. Genetic engineering has also been used to enable IMAC to be performed, with cloned target biomolecules being fused to polyhistidine tags (Section 1.4).

7.5.8 Size Exclusion Chromatography

Sometimes referred to as "gel filtration," size exclusion chromatography (SEC) separates solutes on the basis of their size. There is no derivatization of the polymer gel, and there is no binding between the solutes and the resin. Molecules larger than the largest pores in the gel (the exclusion limit) cannot enter the gel and are eluted first. Smaller molecules enter the gel to varying extents, depending on their size and shape, and thus are retarded on their passage through the bed. In general, SEC resins are hydrophilic polymer gels with a broad distribution of pore sizes. The pore size is dependent on the degree of polymerization of the gel. Size exclusion chromatography is a useful technique, especially for changing buffers, or for removing small molecules from protein solutions. Because of the lack of binding between the solute and the resin, however, the capacity of this technique is low. Size exclusion effects may be in action in all the above-described techniques, since all resins are macroporous.
Particle diameter also dominates the column pressure drop. The pressure drop is given by the Darcy equation [6]:

$$\frac{\Delta p}{L} = \frac{\mu v}{k}$$

(7.6.1)

where \(\Delta p\) is the pressure drop over column length \(L\), \(\mu\) is the viscosity of the mobile phase, \(v\) is the superficial velocity, and \(k\) is a constant. (In writing Equation [7.6.1], the term \(-\rho g\) on the left side in the complete form of the Darcy equation has been omitted because it is very small compared to \(\Delta p/L\).) A further correlation, known as the Blake–Kozeny equation [6], gives \(k\) as a function of resin particle size and void fraction:

$$k = \frac{d_p^2}{150 (1 - e)^2}$$

(7.6.2)

The Darcy equation applies for rigid particles, such as silicas. For polymer resins that are compressible, \(k\) is a function of the linear velocity and column diameter, and a plot of \(\Delta p\) versus \(v\) would be nonlinear over a sufficiently wide range of \(v\). Compressible gels get some of their hydrodynamic stability from the walls of the column; therefore the diameter of the column itself plays a role in the pressure drop. These factors need to be investigated for each application. As the stationary phase particle size is decreased, the pressure drop in the column increases as the inverse square. This increased pressure drop then requires additional power in pumping, as well as more specialized requirements for the construction of the column and its seals. The smallest particle sizes create inlet pressures of several hundred bars of pressure (several thousand psi) with a column length of 25 cm, and a velocity \(v = 0.01\) cm/s. Seals and columns that contain these pressures are typically of narrow bore (2.5 mm i.d.), since a small radius of curvature is more effective for holding pressure than a large radius. Large columns packed with small particles are built like pressure vessels, with large lug nuts closely spaced around the flange at the end pieces.

A further complication arising from small particles is containment. The column end pieces must contain the particles without plugging. Plugging can be caused by resin particles, fragments of particles generated through attrition, or other debris in the chromatography feed streams. Particles \(1 \mu m\) in diameter must be contained by \(0.25 \mu m\) frits. These frits become easy to foul. The smaller the particle size, the more finely the mobile phase must be filtered and held particle free.

The selection of the resin particle diameter is probably the most critical engineering choice to be made in chromatography design and scaleup. The resolution of the column is favored by small particle size, while the pressure drop is adversely affected. The smaller the particle size, the shorter the column can be made, while maintaining the same separation. To retain binding capacity, the same resin volume must be used. Thus, smaller particles may be packed in short, flat beds, resembling pancakes, while larger particles can be accommodated in long tubes. As the column narrows, linear flow rates increase for the same volumetric flow, thus adversely affecting both pressure drop and resolution. As the columns get shallow and broad, mechanical seals and good flow distribution become more difficult to attain.
7.7 Equipment

Equipment for adsorption and chromatography is similar in that a fixed column or columns are generally involved. The equipment for chromatography, however, tends to be more sophisticated than that for the fixed-bed adsorption process, since the scale of operation is generally smaller and the requirement for resolution is greater. This is reflected in the fact that fixed- and agitated-bed adsorption operations tend to be near the beginning of the purification process, while chromatography is generally at or near the end of the purification. This section describes some of the special design considerations required for adsorption and chromatography equipment.

7.7.1 Columns

Adsorption

Most fixed-bed adsorbers are cylindrical, vertical vessels through which feed, eluent, and regenerant streams are passed down through the bed of resin. There are two types of system to support fixed beds of adsorbents. One is a series of grids and screens, where each higher layer screen has successively smaller openings to prevent adsorbent from passing through and each lower layer has greater strength. In the other type of support, there is a graded system of particles such as ceramic balls. To prevent movement of the adsorbent at the top of the bed, a retention screen is usually installed on the top of the adsorbent and a layer of support balls placed on the top of the screen to hold the screen in place. Because the bed tends to expand and contract during operation of the process, the retention screen must be floating and not attached to the wall of the column. For large columns, it may also be necessary to have the inlet pipe branch into several pipes that are perforated along their length to ensure even distribution of the liquid across the column's cross section [25].

Chromatography

Chromatography columns are also cylindrical, vertical vessels designed to contain resin particles between 2 and 100 μm in diameter. Fluid is typically pumped downflow through chromatography columns, since air trapped beneath a column can cause voids that are ruinous for resolution. The frit, or in some cases netting, is critical for design of the columns. The frit is held in place by the end fittings on the column, often between gaskets, or incorporated into gaskets. A frit is critical at both the inlet and outlet of the column. The frit serves as a filter of last resort on the column inlet and holds the resin in place in the event the column is back-flushed. Polymer-based resins also swell under different conditions of ionic strength and pH, a phenomenon the inlet frit helps to contain.

The column comes in three basic types, fixed bed height, variable bed height, and axial compression. Fixed beds are used when a process is mature and the columns can be dedicated. The fixed bed is a tube that is fixed directly to end fittings. Threaded fittings are used on the analytical and preparative scales (up to 10 cm diameter); flanged connections are used for larger diameters. Fixed beds are also used for analytical high performance liquid chromatography (HPLC).
Variable or adjustable beds are common in pilot plants and development settings. The variable bed column is typically a tube that fits the outside diameter of the end fittings. The end fittings often consist of two flat plates sandwiching a gasket. When the end pieces are in place, the flat plates are compressed together, squeezing the gasket out against the tube. The top end piece may be placed at any level, depending on the volume of resin required for the process.

Axial compression columns are principally used for large-scale HPLC. When a rigid packing is used for chromatography, such as silica, packing efficiency is critical for high resolution. In this case, it has been found that slurry packing under high flow rate is not adequate. Axial compression columns address this by using a hydraulic piston as the inlet piece that compresses the resin along the length of the column. This not only causes the column to achieve the highest possible packing density, but also holds the resin in place as the action of the pump causes pressure pulses to wash through the column.

7.7.2 Chromatography Column Packing Procedures

Column packing is the most critical step in achieving high column performance in chromatography. There are three objectives in packing the column: to have the resin particles fully wetted, to have the particles fully disassociated from one another, and to achieve the highest possible packing density. To achieve the first objective, resin is slurry packed. This allows the resin to be in full contact with a solvent for an indefinite time prior to packing. It is critical to column capacity that the resin pores be full of solvent so that they are accessible to the process separands. Sometimes, especially at small scale, the resin is actively degassed, by sonication, vacuum, or heat. On larger scales, these methods are often impractical. In these cases, solvents such as ethanol that have high oxygen solubilities can be used to wet the particles.

Ensuring that the particles do not clump or form aggregates that can cause segregation during packing is goal number two. For this reason, solvents such as isopropanol are often added to the slurry. Isopropanol decreases the surface tension sufficiently to allow particles to disaggregate. Other agents, such as buffer salts, are sometimes used to effect particle-particle repulsive forces.

Finally, achieving the highest possible resin density is the ultimate packing goal. There are both chemical and mechanical considerations here. Chemically, particles should be in their least swollen state. If maximally swollen particles are packed, column conditions that cause the particles to subsequently contract will result in a bed that will no longer be packed efficiently, and voids will form. If particles are packed in their most contracted state, conditions that cause them to swell result in a more tightly packed column. High ionic strength is typically used to contract the particles. Attention must be paid to the strength of the seals when swellable resins are used. Resin can push through seals, crack the column, or occlude flow if allowed to swell too much. Highly swellable resins are generally avoided in large-scale processes. Mechanically, the particles should be packed quickly to deprive the slurry of the opportunity to settle and segregate according to particle size. The column should also be packed at or above the highest pressure (or flow rate) conditions expected for the column during normal processing. Finally, the inlet fitting should be placed as quickly and as evenly as possible, so that there are no biases in bed height and so that the pressure developed in the bed is captured mechanically in the seals.
Prior to the packing procedure, the resin is slurried at a packing density much lower than what will ultimately be achieved in the packed column. Therefore, a method is required for providing the extra volume to the column in which to slurry the resin. There are at least two common methods for doing this. The first is to temporarily increase the volume of the column, and the second is to use a secondary vessel. Adjustable-bed columns are easily adapted to the first method. Fixed-bed columns are often fitted with a packing extension tube to use this method. A secondary slurry vessel may also be used. This vessel is typically closed so that it can hold pressure. Then, clean solvent is pumped into the slurry vessel, displacing slurry to the column. Pumping slurries directly through a pump is not recommended because damage to both the resin and the pump may result.

7.7.3 Detectors

Detectors are selected to match the molecule in the application. Almost any detection method can be adapted to chromatography, from Fourier transform infrared spectroscopy to antibody conjugation techniques to mass spectroscopy. However, the most common are pH, conductivity, and light absorbance. Conductivity and pH are monitored to check the performance of the gradient, the loading of the column, the regeneration, and reequilibration. Automated chromatography equipment relies on these measurements to proceed to the next steps. Light absorbance, primarily in the ultraviolet range (280, 234, 229, or 214 nm, depending on the application) is used to monitor the effluent for evidence of the target molecule. All three measurements are noninvasive and use well-developed technologies. Other common detection methods in use in large-scale chromatography are refractive index, electrochemical detection, and light scattering.

Some applications are sufficiently sensitive to warrant on-line analytical detection. In these applications, a "split stream" is taken, and an analytical instrument, such as an HPLC, is injected with a sample. A purity can then be found for the sample, and a decision made to start, continue, or stop collection of the product. In these cases, the HPLC must be very fast compared with the large-scale chromatography, to permit assessment of purity of the target compound from contaminants and to allow a decision to be made before conditions have changed dramatically in the column effluent. HPLC methods can be developed that run in 1 or 2 min, when specific analytes are sought. In contrast, preparative scale chromatography cycles can last 4 to 8 h, depending on the optimization of the gradient elution sequence. For side stream analysis to be worthwhile, the preparative separation must give no apparent resolution of the target compound from a key contaminant that is present in levels of at least 1%. Also, there must be a requirement for purity of less than approximately 0.1% of this contaminant in the final product. When these conditions exist, and side stream detection must be applied, a high cost–low yield step is in store.

7.7.4 Chromatography System Fluidics

The fluidics of the chromatography system are always important in design and operation. Distribution of fluid across the column cross section is a key aspect in column efficiency and reproducibility. The pumps and tubing that feed the column are often part of this design.
Pumps

Pumps used in chromatography are typically positive displacement pumps. These provide reasonable pressures at reasonable cost. They have low shear, so do not pose a problem for sensitive biomolecules. Positive displacement pumps generally come in two varieties, peristaltic and rotary lobe. Peristaltic pumps use a rotating gear to compress flexible tubing through which the product stream flows. These are very familiar to biochemists and are useful for flow rates up to 10 liters/min. Rotary lobe pumps use two gears whose surfaces align such that at any rotation, they form a seal against each other, as well as against the interior of the pump housing. Rotary lobe pumps, uncommon below 1 liter/min, are almost always used at flow rates above 10 liters/min, where peristaltic pumps generally are not used. Rotary lobe pumps must be fed with positive pressure on the inlet, since they are not self-priming in general.

Gradient Makers

Most laboratory HPLC equipment allows programmed gradients; that is, the user selects a gradient type from a menu, and the gradient is mixed from two reservoirs in a dual-chamber pump. The two reservoirs contain the leanest concentration of salt or solvent and the richest concentration. The gradient is formed by the pump(s) selecting more or less volume from each reservoir as time progresses. The gradient can also be mixed from two tanks using CSTR principles; these principles were illustrated in Figure 5.1 and Equation (5.3.1), which gives the solute concentration at the outlet of the gradient mixer for a linear gradient. A linear gradient results if the rich tank is fed into the lean tank by gravity, while the lean tank (well mixed) feeds the chromatography system. In this case, both the rich tank and the lean tank decrease in volume at half the column flow rate each. It is important that the tanks be level with each other, be at the same height, and have the same cross section. To make an exponential gradient, the volume in the lean tank should remain constant, while the rich tank feeds at the same rate as the column flow rate.

7.8 Scaleup

The scaleup of adsorption and chromatography processes usually involves changes in flow rates and in the dimensions of the column. A safe way to scale up is to keep the column length constant, but this is sometimes not feasible. Because the objectives of adsorption and chromatography are usually different, the scaleup of these two operations is considered separately.

7.8.1 Adsorption

The scaleup of a fixed-bed adsorption focuses on the breakthrough curve for a single column, while scaleup of an agitated-bed process is concerned with breakthrough for the last column in a series of columns. The scaleup of mixing becomes important for the mixed-bed process. Scaleup for both types of adsorption process can generally be carried out based on laboratory data.
Fixed-Bed Adsorption

Several approaches can be used to scale up fixed-bed adsorption processes [26]. Two useful approaches are discussed here: (1) the length of unused bed (LUB) concept, which allows scaleup based on data from laboratory columns, keeping the particle size and superficial velocity constant, and (2) the computer simulation method, which also requires laboratory experimental data and constant particle size but does not require the superficial velocity to be constant.

LUB Method In discussing the LUB method of scaleup, it is necessary to define the break-point time \( t_b \) and the ideal adsorption time \( t^* \) on a breakthrough curve, which are indicated in Figure 7.9. The break-point time is usually taken at the relative concentration, \( c_i/c_{i0} \), where \( c_{i0} \) is the feed concentration) of 0.05 or 0.10 [27]. Since only the fluid last exiting the column has this concentration, the average fraction of the solute removed from the start of feeding to the break-point time is usually 0.99 or higher. The ideal adsorption time is the time for breakthrough that would occur if the solute were in perfect equilibrium with the bed of adsorbent, which would give a vertical breakthrough curve. For a symmetrical breakthrough curve, the ideal adsorption time is the time at which \( c_i/c_{i0} = 0.5 \). At the ideal adsorption time for a bed initially free of the solute to be adsorbed, based on a unit area of bed cross section,

\[
v c_i0t^* = L \rho_b q_{i, sat}
\]

(7.8.1)

where \( L \) = bed length

\( q_{i, sat} \) = average adsorbent phase concentration of solute \( i \) in equilibrium with feed concentration \( c_{i0} \), based on the adsorbent weight (weight of solute \( i \) per weight of adsorbent)

\( v \) = superficial velocity

\( c_{i0} \) = concentration of solute \( i \) in the feed

\( \rho_b \) = bulk density of the adsorbent

The ideal adsorption time is therefore given by

\[
t^* = \frac{L \rho_b q_{i, sat}}{v c_i0}
\]

(7.8.2)

The amount of the solute adsorbed at the break point can be determined by integrating the breakthrough curve up to time \( t_b \), as indicated in Figure 7.10. The width of the breakthrough curve defines the width of the mass transfer zone in the bed.

**Figure 7.9** Breakthrough curve for a fixed-bed adsorber, showing the break-point time \( t_b \), chosen at 5\% of the solute feed concentration, and the ideal adsorption time \( t^* \). The ratio of mobile phase concentration of solute to solute concentration in feed to the adsorber is \( c_i/c_{i0} \).
Figure 7.10 Integration of the breakthrough curve for a fixed-bed adsorber. The area of integration to the left of a vertical line at time $t$ is proportional to the amount of solute adsorbed up to that time. The ratio of mobile phase concentration of solute to solute concentration in feed to the adsorber is $c_t/c_0$.

For adsorption where the equilibrium isotherm is favorable, which is true for the Langmuir isotherm that can often be used for protein adsorption, the concentration profile in the mass transfer zone takes on a characteristic shape that does not change as the zone propagates through the bed [5]. At the break-point time, the adsorbent between the inlet of the bed and the beginning of the mass transfer zone is completely saturated (in equilibrium with the solute in the feed). The adsorbent in the mass transfer zone goes from being completely saturated to being almost free of solute, and the adsorbent could be assumed to be on the average about half-saturated. This would be equivalent to half the adsorbent in the mass transfer zone being saturated and the other half being unused. The scaleup principle is that the length of the unused bed in the mass transfer zone does not change as the bed length is changed [28].

The length of unused bed (LUB) can be determined directly from the breakthrough curve obtained experimentally. For this method, LUB is defined as [5]:

$$\text{LUB} = \left(1 - \frac{q_t}{q_{t,\text{sat}}}ight) L = \left(1 - \frac{t_b}{t^*}\right) L$$  \hspace{1cm} (7.8.3)

where $t_b$ and $t^*$ are stoichiometric times determined by integration of the breakthrough curve:

$$t^* = \int_0^\infty \left(1 - \frac{c_t}{c_{t,0}}\right) dt$$  \hspace{1cm} (7.8.4)

$$t_b = \int_0^{t_b} \left(1 - \frac{c_t}{c_{t,0}}\right) dt$$  \hspace{1cm} (7.8.5)

In scaleup calculations, the length of column required can easily be found by adding the LUB to the length calculated by assuming local equilibrium, with a shock wave concentration front [5].

**Example 7.6** Scaleup of the Fixed-Bed Adsorption of a Pharmaceutical Product  The breakthrough data given in Table E7.6 were obtained for the adsorption of a pharmaceutical product in a laboratory column (5 cm diameter $\times$ 15 cm high) at a feed flow rate of 400 ml/h and feed concentration of 0.75 U/liter, where U is units of biological activity of the pharmaceutical product. It is desired to scale up the process to operate in a column 30 cm high. What break-point time can be expected in the 30 cm high column? (Data from J. W. Chen, J. A. Buege, F. L. Cunningham, and J. I. Northam, "Scale-up of a column adsorption process by computer simulation," *Ind. Eng. Chem. Process Des. Dev.*, vol. 7, p. 1849, 1968.)
### TABLE E7.6

<table>
<thead>
<tr>
<th>( t ) (h)</th>
<th>( c_e ) (U/liter)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.01</td>
</tr>
<tr>
<td>26.7</td>
<td>0.49</td>
</tr>
<tr>
<td>32.0</td>
<td>0.39</td>
</tr>
<tr>
<td>36.0</td>
<td>0.53</td>
</tr>
</tbody>
</table>

\( \hat{t} \)

#### Figure E7.6
Breakthrough curve for the fixed-bed adsorption of a pharmaceutical product in Example 7.6.

**SOLUTION**

We use the LUB method, which involves graphical integrations of the breakthrough curve to determine the amount of solute adsorbed at various times. The breakthrough curve with concentration in dimensionless form is therefore plotted, with the curve extended to \( c_t/c_{t0} = 1.0 \) assuming that the curve is symmetrical about \( c_t/c_{t0} = 0.5 \) (Figure E7.6). By doing graphical integrations of this curve, we find the following:

Total solute adsorbed at saturation \( (c_t/c_{t0} = 1.00) \):

\[
Q c_0 \int_0^{44.0} \left( 1 - \frac{c_t}{c_{t0}} \right) dt = Q c_0 (31.5 \text{ h})
\]

Total solute adsorbed at the break point \( (c_t/c_{t0} = 0.05) \):

\[
Q c_0 \int_0^{21.5} \left( 1 - \frac{c_t}{c_{t0}} \right) dt = Q c_0 (21.2 \text{ h})
\]
where \( Q \) is the volumetric flow rate. Thus, \( t^* \) and \( t_b \) are 31.5 and 21.2 h, respectively, for this column. From Equation (7.8.3):

\[
\frac{\text{LUB}}{L} = 1 - \frac{Qc_0(21.2 \text{ h})}{Qc_0(31.5 \text{ h})} = 1 - 0.673 = 0.327
\]

\[
\text{LUB} = 0.327(15 \text{ cm}) = 4.90 \text{ cm}
\]

The adsorbent loading at saturation per total bed volume is

\[
\rho_v q_{i, \text{sat}} = \frac{v c_0 t^*}{L} = \frac{400 \text{ cm}^3 \text{ h}^{-1} \times 0.00075 \text{ U cm}^{-3} \times 31.5 \text{ h}}{3.14 \times 5^2 \text{ cm}^2 \times 15 \text{ cm}} = 0.0321 \text{ U cm}^{-3}
\]

For the column 30 cm high, the LUB does not change, so that

\[
\frac{\text{LUB}}{L} = \frac{4.90}{30} = 0.163
\]

The superficial velocity stays the same and is

\[
v = \frac{400 \text{ cm}^3 \text{ h}^{-1}}{3.14 \times 5^2 \text{ cm}^2} = 20.4 \text{ cm h}^{-1}
\]

From Equation (7.8.2),

\[
t^* = \frac{L \rho_v q_{i, \text{sat}}}{v c_0} = \frac{30 \text{ cm} \times 32.1 \text{ U liter}}{20.4 \text{ cm h}^{-1} \times 0.75 \text{ U liter}} = 62.9 \text{ h}
\]

Therefore, from Equation (7.8.3),

\[
t_b = t^* \left( 1 - \frac{\text{LUB}}{L} \right) = (62.9 \text{ h})(1 - 0.163) = 52.6 \text{ h}
\]

**Computer Simulation Method** In the design of a production scale adsorption process, it is often desirable to simulate the process on a computer based on laboratory or pilot plant data. An example of such a simulation for design and scaleup was carried out by Chen et al. [29] for the fixed-bed adsorption of a valuable pharmaceutical product. Their model neglected dispersion [Equation (7.3.11)] and used a linear driving force expression for the mass transfer rate [Equation (7.3.5)] and a combination of linear and Freundlich isotherms for the equilibrium relations. From a laboratory column, the following relationships for the mass transfer coefficient \( K_a \) were obtained:

\[
K_a = 1640 - 3700 \frac{q_i}{q_{i, \text{sat}}} \quad \text{for} \quad \frac{q_i}{q_{i, \text{sat}}} \leq 0.4 \quad (7.8.6)
\]

\[
K_a = 264 - 260 \frac{q_i}{q_{i, \text{sat}}} \quad \text{for} \quad 1 \geq \frac{q_i}{q_{i, \text{sat}}} > 0.4 \quad (7.8.7)
\]
The laboratory column experiments indicated that pore diffusion in the adsorbent was the controlling mechanism for mass transfer. The equilibrium isotherm relationships were

\[
c_i^* = 0.00006q_i \quad \text{for} \quad q_i \leq 20 \tag{7.8.8}
\]

\[
c_i^* = 2.74 \times 10^{-8} q_i^{3.57} \quad \text{for} \quad q_i > 20 \tag{7.8.9}
\]

Equations (7.3.5) and (7.3.11) were solved on a computer by the method of finite differences, subject to the mass transfer and equilibrium isotherm relationships for a step change in the concentration in the feed from 0 to \(c_{i0}\) and no solute on the adsorbent at time 0. This model was used to predict the operation of 24 and 40 in. columns, and excellent agreement was obtained between the model and experimental data for \(c_i\) versus time. Model predictions and experimental data are shown in Figure 7.11. Computer simulation was used to optimize process variables such as flow rate, temperature, cycle time, concentration of product in the feed stream, and amount of sorbent. Such a computer model of the sorption process also enables the ready evaluation of changes in the process variables after the plant process is put into operation.

**Agitated-Bed Adsorption**

The scaleup of a series of agitated columns containing adsorbent (Figure 7.5), operated in the periodic countercurrent mode, follows directly from the mathematical model presented for this process [Equations (7.3.19)-(7.3.22)] [10]. Besides the increases in the flow rate and volume that occur upon scaleup, the mixing patterns may also change. A change in the mixing during scaleup was demonstrated in a mixed-bed adsorption process applied to a fermentation broth containing an antibiotic [30]. For a change in column volume by a factor of 29 from the laboratory column 2 in. in diameter to the pilot plant column with a 6 in. diameter, the mixing changed from perfect mixing in the laboratory column to mixing characterized by the following equation in the pilot plant column, based on the following age distribution function that was determined:

\[
1 - \frac{c_i}{c_{i0}} = \exp \left[ -\frac{V_L}{V_P} \left( \frac{t}{\tau} - \frac{V_P}{V_L} \right) \right] \tag{7.8.10}
\]

where \(c_{i0}, c_i = \text{concentration of a nonadsorbing component } i \text{ in the feed or effluent, respectively}\)

\(V_L = \text{total volume of liquid in the column}\)
Figure 7.12 Mixing model based on the age distribution function determined experimentally for a pilot scale (6 in. diameter) agitated-bed adsorption column. Stagnant volume \( V_B = V_L - V_R - V_P \). The total volume of liquid in the column is \( V_L \).

\[
\begin{align*}
V_B &= \text{volume of back-mixed liquid in the column} \\
V_P &= \text{volume of liquid in plug flow in the column} \\
\tau &= \text{column residence time} \left( \frac{V_L}{Q} \right), \text{where } Q = \text{feed flow rate} \\
t &= \text{time from the start of a step change in the concentration of the nonadsorbing component from } 0 \text{ to } c_i
\end{align*}
\]

Equation (7.8.10) corresponds to a mixing model consisting of a plug flow region in series with a back-mixed region connected to a stagnant region (Figure 7.12) [31]. A model for nonideal mixing can be incorporated into the mathematical simulation and design of the large-scale process.

7.8.2 CHROMATOGRAPHY

Chromatography scaleup algorithms typically account for changes in bed height and diameter, linear and volumetric flow rate, and particle size. A general approach to scaleup is based on keeping the resolution constant. Yamamoto et al. [32, 33] have developed the following proportionality for resolution, \( R_s \), of proteins in linear gradient elution ion exchange chromatography and hydrophobic interaction chromatography:

\[
R_s \propto \left( \frac{D_m L}{g(V - V_0)u d_p^2} \right)^{1/2}
\]

(7.8.11)

where \( D_m \) = diffusion coefficient of the protein in solution
\( L \) = column length
\( g \) = slope of the gradient (change in concentration of gradient per volume of gradient)
\( V \) = column volume
\( V_0 \) = column void volume
\( u \) = interstitial fluid velocity
\( d_p \) = particle diameter

This equation has been found to be valid over a wide range of experimental conditions [32, 33]. To remove the volume terms from the expression for resolution, we make the definitions:

\[
\begin{align*}
\dot{Q} &= \frac{Q}{V} = \frac{Q}{V} \\
G &= V_L f_L
\end{align*}
\]

(7.8.12) \( \frac{\dot{Q}}{Q} \)

(7.8.13) \( \frac{\dot{Q}}{Q} \)
where \( Q \) is the inlet flow rate, \( \varepsilon \) is the column void fraction, and \( A_f \) is the column cross-sectional area. These substitutions lead to

\[
R_t \propto \left( \frac{D_{ms}^8}{G(1 - \varepsilon) \bar{Q}_d^2} \right)^{1/2}
\]

(7.8.14)

Thus, for scaleup with constant resolution from scale 1 to scale 2 for the same product and the same column void fraction, the scaleup equation is

\[
G_1 \bar{Q}_1 d_{p1}^2 = G_2 \bar{Q}_2 d_{p2}^2
\]

(7.8.15)

Thus, as the particle size increases on scaleup, the flow rate relative to the column volume must decrease and/or the gradient slope must decrease to maintain constant resolution, which seems correct intuitively.

In practice, the gradient and the stationary phase size and chemistry are not changed upon scaleup. This is because it is easy to develop lab scale processes that use the same resin and same gradient that can be used at the commercial process scale. Therefore, in practice, only the ratio between column volume and flow rate need be addressed:

\[
\frac{Q_1}{V_1} = \frac{Q_2}{V_2}
\]

(7.8.16)

Scaleup from a well-designed process development or preparatory column is reasonably straightforward. When the bed height can be maintained on scaleup, the mobile phase linear velocity remains the same, and the column is simply scaled by diameter. Scaling from a column 1 cm in diameter to column having a diameter of 10 cm would constitute a 100-fold increase in scale. This is the most conservative way to scale up a column but is not a necessary constraint.

**EXAMPLE 7.7**  Scaleup of a Protein Chromatography  A column 20 cm long, with an internal diameter of 5 cm, gives sufficient purification to merit scaleup. The column produces 3.2 g of purified protein per cycle, and a cycle takes 6 h, from equilibration through regeneration. You want a throughput of 10 g/h. What are the new column's dimensions if linear velocity is held constant?

**Solution**  As just discussed, for scaleup when the linear velocity is held constant, the column diameter is increased, and the column height is maintained the same. If the linear flow rate is held constant, then the cycle time cannot be altered. Thus, the scaled up column must produce 6 h/cycle \( \times 10 \text{ g/h} = 60 \text{ g/cycle} \). Since the flow rate is proportional to the throughput of protein,

\[
\frac{Q_2}{Q_1} = \frac{60 \text{ g/cycle}}{3.2 \text{ g/cycle}} = 18.75
\]
From Equation (7.8.16), the scaleup relationship when the gradient and the particle size are not changed upon scaleup, and since \( L_1 = L_2 \),

\[
\frac{Q_1}{Q_2} = \frac{V_2}{V_1} = \frac{\pi \left( \frac{D_2}{2} \right)^2 L_2}{\pi \left( \frac{D_1}{2} \right)^2 L_1} = \left( \frac{D_2}{D_1} \right)^2 = 18.75
\]

where \( D_1 \) and \( D_2 \) are the column diameters for columns 2 and 1, respectively. Since \( D_1 = 5.0 \text{ cm} \), we obtain

\[
D_2 = (18.75)^{0.5} D_1 = 21.6 \text{ cm}
\]

It is not always necessary to scale according to bed diameter. The flow rate may be normalized against the volume of the empty column, to give units of time\(^{-1}\). This normalized flow rate is held constant from one scale to another, as shown in Equation (7.8.16). The case given in Example 7.7 can be shown to be a specific case of this more general rule of thumb. Considerable research and practice indicates that this technique is equally effective and less restrictive compared to that just mentioned. In this case, column bed height may be increased or decreased, depending on the requirements of pressure drop and mechanical seals. A shallower bed gives a lower linear velocity and a wider diameter. A deeper bed gives a higher linear velocity (and higher pressure drop) and a proportionally narrower bed diameter. Therefore, bed height may need to be scaled on the basis of pressure drop constraints.

**Example 7.8**

Scaleup of Protein Chromatography using Standard Column Sizes  Consider the case given in Example 7.7. Available standard column diameters are 20 and 25 cm. What flow rates and bed depths would apply to each of these columns?

**Solution**

The column volumes for both columns are still 18.75 times that used in the laboratory column. Thus,

\[
V_2 = 18.75V_1 = 18.75 \left( \frac{\pi D_1^2}{4} L \right) = 18.75 \left( \frac{\pi (5^2)}{4} \times 20 \right) = 7359 \text{ cm}^3
\]

For a column 20 cm in diameter,

\[
L = \frac{V_2}{\pi D_2^2} = \frac{7359 \text{ cm}^3}{\pi (20)^2} = 23.4 \text{ cm}
\]

and for a column 25 cm in diameter,

\[
L = \frac{V_2}{\pi D_3^2} = \frac{7359 \text{ cm}^3}{\pi (25)^2} = 15.0 \text{ cm}
\]
In both cases, the gradient is also expressed in column volumes. The total gradient volume, that is, the total volume of eluent used to go from the leanest mobile phase condition to the richest, is expressed in terms of column volumes, and this is held constant on scaleup.

**Example 7.9**

**Scaleup of Elution Buffer Volumes in Protein Chromatography**  
An NaCl gradient is used to elute the product in Example 7.7. The gradient increases from 100 mM NaCl to 250 mM in 3 h. To form this gradient a 100 mM NaCl solution is combined with a 250 mM NaCl solution in a gradient mixer, in which the 100 mM NaCl solution is mixed (see Figure 5.1). The flow rate is 40 ml/min. What volumes of low salt and high salt eluent should be made for the scaleup column with an internal diameter of 20 cm?

**Solution**

The small-scale volume of each gradient buffer is 3.6 liters (40 ml/min x 180 min/2). The scaleup factor of 18.75 still applies, so that

Volume of each buffer at large scale = \((18.75)\times(3.6\text{ liters})\) = 67.5 liters

Suppose that in development, the product elutes in 60% of the gradient volume. The gradient may be truncated to save time and buffer volume. The gradient can be adjusted according to:

\[
G = \frac{c_{H} - c_{L}}{V} = \text{constant (7.8.17)}
\]

where \(c_{H}\) and \(c_{L}\) are the concentrations of the high strength and low strength buffers, respectively, and \(V\) is total gradient volume. Keeping the gradient slope constant is essential for maintaining resolution in chromatography.

Finally, pressure drop must be considered. Pressure drop is given by Darcy's equation [Equation (7.6.1)]. As columns increase in diameter, the pressure they can hold generally decreases (or the structural mass is increased, to provide the strength to maintain a seal). This is because as the radius of curvature increases, the force against the pressure decreases, which weakens the seal (think, e.g., about the stability of large bubbles in a glass of beer, compared with the smaller ones). It is possible to design the scaled-up column from the accessible pressure drop of the column.

**Example 7.10**

**Consideration of Pressure Drop in Column Scaling**  
Determine the minimum diameter possible for the columns analyzed in Example 7.8 and subject to the flow rate given in Example 7.9, when the column pressure should not exceed 300 kPa (28.8 psig), the maximum solution viscosity is 1.1 cp, and the void fraction in the column is 0.35. The resin particle size is 100 μm.
Pressure drop can be calculated using Darcy’s law (Equation (7.6.1)) by knowing $\mu$, $v$, $k$, and $L$. We can calculate $k$ from the Blake–Kozeny equation (7.6.2):

$$k = \frac{(100 \mu m)^2}{150 \times (1 - 0.35)^2} = 6.77 \mu m^2$$

The column volume must be 7359 ml (from Example 7.8), and the flow rate is 40 ml/min (from Example 7.9) times the scaleup factor of 18.75 (= 750 ml/min). We calculate the pressure drop for the column 20 cm in diameter and 23.4 cm in length, which would give the higher pressure drop of the two standard column sizes (20 and 25 cm):

Cross-sectional area = $\frac{\pi (20 \text{ cm})^2}{4} = 314 \text{ cm}^2$

$$v = \frac{750 \text{ cm}^3}{314 \text{ cm}^2} = 2.39 \text{ cm} \text{ min}$$

From Equation (7.6.1) the pressure drop is

$$\Delta p = \frac{\mu v L}{k} = \frac{0.011 \frac{g}{\text{cm s}} \times 2.39 \frac{\text{cm}}{\text{min}} \times 23.4 \text{ cm} \times \frac{1}{60 \text{ s}}} {6.77 \times 10^{-8} \text{ cm}^2} = 15.1 \text{ kPa}$$

Thus, the standard 20 cm diameter column would operate at well below the maximum allowable pressure.

### 7.9 Summary

The differential affinity of various solute molecules for solids of specific types is the basis for liquid chromatography and adsorption separation processes. Liquid chromatography is traditionally defined as the process where solutes are separated based on the speed at which they move through a column packed with adsorbent, also called the resin or stationary phase. By contrast, in pure adsorption processes the stationary phase is generally uniformly saturated with the target solute before an eluting solvent is introduced to cause the solute to transfer into the liquid or mobile phase.

- Different adsorption equilibrium constants make it possible to separate solutes from one another; equilibrium constants are controlled by solvent composition. In the reaction $C + S \leftrightarrow CS$ at equilibrium, the adsorption equilibrium constant is given by $K_{eq} = [CS]/[C][S]$, where $[S]$ is the concentration of vacant sites in the solid phase. If the total number of sites $S_{tot}$ is not much greater than $[CS]$, then sites are nearly saturated, and combining the site balance with equilibrium gives the Langmuir adsorption isotherm...
7.9 Summary

equation, which shows site saturation when [CS] is plotted versus [C]. The Langmuir isotherm is often used to describe the equilibrium data for proteins.

- A special case occurs when $S_{oi}$ is much greater than [CS]: without saturation, the plot is a linear adsorption isotherm, or $[CS] = K_{eq}[C]$. The linear isotherm is applicable to analytical chromatography.

- Another equilibrium isotherm that has proven useful is the Freundlich isotherm, which states that $[CS]$ is proportional to $[C]^{1/n}$, with $n > 1$. This isotherm has been used to describe the adsorption of a number of antibiotics, steroids, and hormones.

- As a solution containing a species to be separated, called a separand, is flowed into a packed bed, sites become occupied until they are saturated; then separand flows past this saturation zone to find new sites in a transition zone until the transition zone reaches the end of the bed, resulting in outflow of separand known as breakthrough. The analysis of breakthrough curves serves as the basis for scaling and optimizing separand isolation by packed-bed adsorption.

- The complete mass balance for the separand in a fixed-bed adsorber is given by

$$D_{eff} \frac{\partial^2 c_i}{\partial x^2} - \frac{v \partial c_i}{\partial x} = \frac{\partial c_i}{\partial t} + \frac{1 - \varepsilon}{\varepsilon} \frac{\partial q_i}{\partial t}$$

where $c_i$ is the concentration of separand in the mobile phase, $q_i$ is the concentration of separand in the adsorbent phase averaged over a particle, $D_{eff}$ is the effective dispersivity of the separand, $x$ is the distance from the bed inlet, $v$ is the superficial velocity, $\varepsilon$ is the void fraction of the bed packing, and $t$ is time. This mass balance also applies to chromatography.

- The mass transfer rate of separand into the adsorbent particle is often expressed in terms of a linear driving force as

$$\frac{\partial q_i}{\partial t} = K_a(c_i - c_i^*)$$

where $K_a$ is an overall mass transfer coefficient, and $c_i^*$ is the liquid phase concentration at equilibrium with $q_i$.

- By assuming local equilibrium and negligible dispersion, the mass balance can be solved to give the effective velocity of separand as

$$u_i = \frac{v}{\varepsilon + (1-\varepsilon)q_i'(c_i)}$$

where $q_i'(c_i)$ is the slope of the equilibrium isotherm at concentration $c_i$. When the equilibrium isotherm is nonlinear and favorable (e.g., a Langmuir isotherm) and there is a discontinuous change in concentration in the bed, $q_i'(c_i)$ becomes $\Delta q_i/\Delta c_i$, where the differences are taken across the discontinuity. The resulting velocity of the solute front is called the shock wave velocity.

- The mass balance for adsorption with the linear driving force expression for the mass transfer rate, a linear equilibrium isotherm, and negligible dispersion has been solved analytically to give an expression for the breakthrough curve.
• Chromatographic performance is assessed by elution peak analysis using a plate model, in which the number of theoretical plates is proportional to \( t_R^2/(w/4)^2 \), where \( t_R \) is average retention time and \( w \) is peak width. The resolution of two solutes is directly proportional to the difference in average retention times and inversely proportional to the sum of the peak widths.

• Equilibria for protein–salt ion exchange chromatography are calculated by using two equilibrium constants, one for protein and one for salt ion in the multicomponent Langmuir equation. This gives the protein adsorption isotherm as a function of salt concentration, which can be varied continuously in gradient elution chromatography. Gradient elution is thus distinguished from isocratic elution, in which no solute concentrations are varied.

• Band spreading in chromatography can be attributed to mechanical dispersion in the mobile phase, and molecular diffusion in the mobile and adsorbent phases. These factors govern the choice of particle size, pressure drop, and flow velocity. The impact of mass transfer and diffusion effects can be estimated by using the van Deemter equation.

• Adsorbents are selected on the basis of the adsorption chemistry of the separands, such as ion exchange, hydrophobic interaction, affinity reactions, metal affinity, and size exclusion.

• For a given mobile phase flow velocity, the required (Darcy's law) pressure drop is inversely proportional to the square of the particle diameter and directly proportional to column length and the viscosity and superficial velocity of the mobile phase. Small particle size, desired for high resolution, can lead to unreasonable pressure requirements.

• Numerous design considerations are required for adsorption and chromatography equipment.

• A common method to scale up fixed-bed adsorbers is the length of unused bed (LUB) method, which is dependent on the equilibrium isotherm being favorable (concave downward) and on the particle size and superficial velocity remaining constant.

• The scaleup of a series of agitated columns containing adsorbent follows directly from the mass balance for each column, linear driving force expression for the mass transfer rate, and equilibrium isotherm, which must be solved by numerical methods. For this system, scaleup of the mixing is an important consideration.

• The constant resolution scaling rules for gradient elution chromatography, in their simplest form, specify that the product of gradient slope, normalized flow rate, and particle diameter squared should be held constant. Since normalized flow rate includes column area, an efficient way to scale up is to increase column diameter.

**NOMENCLATURE**

- \( A \) constant in the van Deemter equation [Equation (7.4.11)] (cm)
- \( A_f \) cross-sectional area of column (cm²)
- \( B \) constant in the van Deemter equation [Equation (7.4.11)] (cm² s⁻¹)
- \( c \) concentration of a solute in the mobile phase (M, or g liter⁻¹)
- \( c^* \) equilibrium concentration of solute in the mobile phase (M, or g liter⁻¹)
- \( c_0 \) feed concentration of a solute in the mobile phase (M, or g liter⁻¹)
7.9 Summary

- \( C \) chemical species in the mobile phase
- \( C \) constant in the van Deemter equation [Equation (7.4.11)] (s)
- \( CS \) chemical species adsorbed to an adsorption site
- \( d_p \) stationary phase particle diameter (cm)
- \( D \) column diameter (cm)
- \( Da \) Damköhler number \( = d_p^2 k_a / 4 D_p \) (dimensionless)
- \( D_m \) molecular diffusivity of separand in unbounded solution (cm\(^2\) s\(^{-1}\))
- \( D_{eff} \) effective dispersivity in the column (cm\(^2\) s\(^{-1}\))
- \( D_p \) molecular diffusivity of separand in the pore fluid (cm\(^2\) s\(^{-1}\))
- \( E \) dispersion coefficient of separand, as a result of back mixing in the fixed-bed and nonuniformity of the velocity (cm\(^2\) s\(^{-1}\))
- \( g \) slope of the elution gradient (M liter\(^{-1}\), or g liter\(^{-2}\))
- \( g \) gravitational acceleration (9.866 m sec\(^{-2}\))
- \( G \) normalized slope of the gradient (M, or g liter\(^{-1}\))
- \( H \) height equivalent to a theoretical plate (HETP) (cm)
- \( H(x,t) \) dispersion function (dimensionless)
- \( H^* \) normalized height equivalent of a theoretical plate \( (H/d_p) \)
- \( I_0 \) Bessel function (dimensionless)
- \( k \) constant in the Darcy equation [Equation (7.6.1)] (cm\(^2\))
- \( K_a / K_{eq} \) forward rate constant of adsorption (s\(^{-1}\))
- \( K_c \) fluid phase mass transfer coefficient (cm s\(^{-1}\))
- \( K_a \) overall mass transfer coefficient (s\(^{-1}\))
- \( K_{eq} \) equilibrium constant (units vary)
- \( L \) length of the column (cm)
- \( m \) \( D_m / \epsilon P \) (dimensionless)
- \( m' \) \( \frac{1}{2} m [K_{eq} / (1 + K_{eq})]^2 \) (dimensionless)
- \( N \) number of theoretical plates
- \( Nu \) Nusselt number \( = d_p k_a / D_m \) (dimensionless)
- \( \Delta p \) pressure drop (kPa)
- \( Pe \) dispersion Péclet number \( = d_p u / D_{eff} \) (dimensionless)
- \( q \) concentration of a solute in the stationary phase averaged over an adsorbent particle (M, or g liter\(^{-1}\))
- \( Q_{tot} \) concentration of solute on the stationary phase at equilibrium with feed concentration \( c_0 \), averaged over an adsorbent particle (M, g liter\(^{-1}\), or g g\(^{-1}\))
- \( Q \) flow rate (ml min\(^{-1}\))
- \( \bar{Q} \) (flow rate)/(column volume) (min\(^{-1}\))
- \( r \) radial distance in the stationary phase (cm)
- \( R_e \) chromatographic resolution (dimensionless)
- \( Re \) Reynolds number \( = d_p u \rho / \mu \) [Equation (7.4.13)] (dimensionless)
- \( S_{tot} \) total concentration of adsorbent sites (ng ml\(^{-1}\))
- \( Sc \) Schmidt number \( = \mu / \rho D_m \) (dimensionless)
- \( t \) time (s)
- \( t_R \) retention time (s)
- \( t^* \) ideal adsorption time (s)
- \( u \) velocity of fluid through the interstices of the bed (cm s\(^{-1}\))
- \( u_i \) solute i effective velocity (cm s\(^{-1}\))
- \( u_{i,sh} \) solute i shock wave velocity (cm s\(^{-1}\))
- \( v \) mobile phase superficial velocity (cm s\(^{-1}\))
- \( V \) volume of fixed-bed column (ml)
- \( V_L \) volume of back-mixed liquid in column (ml)
- \( V_k \) liquid volume in column (ml)
- \( V_P \) volume of liquid in plug flow column (ml)
- \( V_R \) volume of adsorbent in column (ml)
- \( V_0 \) column void volume (ml)

\[ \frac{V_L + V_R}{2} \text{ total granular volume (ml)} \]
peak width at base (base intercept) (s)
concentration of separand in the pore fluid
based on a unit volume of stationary phase (i.e., the solid matrix and the pore space) (M, or g liter$^{-1}$)
concentration of separand $i$ adsorbed on the internal surfaces of the stationary phase based on a unit volume of stationary phase (i.e., the solid matrix and the pore space) (M, or g liter$^{-1}$)
longitudinal distance in the column (cm)
fraction of separand in the mobile phase at long times \[ e/e + (1 - e)e^*(1 + K_{eq}) \]

**Greek Letters**

\begin{itemize}
  \item $\gamma$ \hspace{1cm} $1 + K_{eq}(1 - e)/e$
  \item $\varepsilon$ \hspace{1cm} column void fraction (dimensionless)
  \item $\varepsilon^*$ \hspace{1cm} effective volume fraction of the stationary phase that is accessible to the separand (dimensionless)
  \item $\xi$ \hspace{1cm} \( k_{eq} = \xi/\nu \) \hspace{1cm} (dimensionless)
  \item $\mu$ \hspace{1cm} viscosity of mobile phase (g cm$^{-1}$ s$^{-1}$)
  \item $\rho$ \hspace{1cm} density of fluid (g cm$^{-3}$)
  \item $\rho_c$ \hspace{1cm} bulk density of adsorbent (g cm$^{-3}$)
  \item $\sigma$ \hspace{1cm} standard deviation for a peak (s)
  \item $\tau$ \hspace{1cm} \( k_{eq} = \tau/\rho_c \) \hspace{1cm} (dimensionless)
  \item $\tau_r$ \hspace{1cm} column residence time \( (V_L/Q) \) (s)
  \item $\phi$ \hspace{1cm} $c/c_0$ (dimensionless)
  \item $\psi$ \hspace{1cm} $q/q_0$ (dimensionless)
\end{itemize}

**Subscripts**

\begin{itemize}
  \item $i, j$ \hspace{1cm} components $i, j$
  \item $n - 1, n$ \hspace{1cm} columns $n - 1, n$ in a series of columns
\end{itemize}

**Table P7.1**

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<th>$q$ (mg/g)</th>
<th>$q'$ (mg/ml)</th>
</tr>
</thead>
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<td>3.20 x 10$^{-2}$</td>
</tr>
<tr>
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</tr>
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<td>1.00</td>
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</tr>
</tbody>
</table>

Three Binding Solute

- Derive the isotherms for three binding solutes that all compete for the same sites on the resin. If $K_{eq} < K_{eq}' < K_{eq}''$, qualitatively describe the effects of the following situations:
  - (a) $c_1$ is very small compared to $c_2$ and $c_3$.
  - (b) $K_{eq} > K_{eq}''$.
  - (c) $c_1$ is low initially, and increases throughout the elution process.

Solute Binding to Two Binding Sites

- Derive an isotherm for a single solute molecule that binds simultaneously to two binding sites. An example of this would be a divalent cation binding to a carboxymethyl-derivatized resin. Use this expression to generate an example isotherm.

Dispersion versus Molecular Diffusivity

- What is the difference between the effective diffusivity $D_{eff}$ and molecular diffusivity $D_m$? What are the units for each?

Prediction of the Break-Point Time in Fixed-Bed Adsorption

For low concentrations, the equilibrium for the antibiotic novobiocin and Dowex 21K anion exchange resin is linear,

\[ q = 125c_i^* \]

for $q$, and $c_i^*$ in units of milligrams per milliliter. For the range of concentrations where this isotherm is valid, the mass transfer coefficient $K_a$ averages 82 h$^{-1}$. Assuming a linear isotherm, estimate the break-point time (where $c_i/c_0 = 0.05$) in a fixed-bed adsorber with a bed length of 20 cm and superficial velocity of 40 cm/h. (Data from P. A. Boller, F. L. Cunningham, and J. W. Chen, "Development of a recovery process for novobiocin," *Biotechnol. Bioeng.* vol. 15, p. 533, 1973.)
7.7 Separation of Two Solutes Having Linear Equilibrium Two solutes have a linear equilibrium with the stationary phase. Their equilibrium constants $K_{eq}$ are 6.5 and 6.6, respectively. The task is to separate 20 ml of the mixture on a column 5 cm in diameter. The flow rate is 10 ml/min. Determine the minimum column length required to just separate the two compounds, with $e = 0.3$.

7.8 Calculation of the Shock Wave Velocity A solute has a Langmuir isotherm characterized by an $S_{sat}$ of 120 $\mu$g/ml and a $K_{eq}$ of 60 ml/mg. Calculate the shock wave velocity for an injection of 1 mg/ml and column conditions identical to those presented in Problem 7.7.

7.9 Using the LUB Method to Scale Up Fixed-Bed Adsorption Breakthrough data (see Table P7.9) have been obtained for a fixed-bed adsorption process with a weakly anionic adsorbent using a feed of filtered fermentation broth containing the antibiotic cephalosporin at a concentration of 4.3 g/liter. The bed is 1 m long $\times$ 3 cm diameter, and the superficial velocity is 2 m/h. It is desired to scale up this process to a bed length of 3 m using the same superficial velocity. Use the LUB method to estimate the break-point time, defined here as occurring when $c_t/c_{0,0} = 0.1$, for the large column. (Data from P. A. Belter, E. L. Cussler, W.-S. Hu, Bioseparations, p. 174, Wiley-Interscience, New York, 1988.)

7.10 Removal of a Low Level Contaminant One mode in which adsorbents are used is to strip a low level contaminant like DNA from a product solution. No other solution components are negatively charged at the operating pH for the column.

(a) If an anion exchanger is used for this purpose, determine the total bed capacity for DNA given these parameters: bed volume = 2 liters, $S_{sat} = 2$ mg/ml, $K_{eq} = 2$ ml/mg, and DNA concentration $= 5 \mu$g/ml.

(b) Determine the bed capacity for the same conditions except that the DNA concentration is 100 $\mu$g/ml.

7.11 Chromatography Scaleup It is desired to scale up the throughput by a factor of 150 for a linear gradient ion exchange chromatography of a product protein from the laboratory to the plant. The conditions for the laboratory chromatography are the following: 1.0 cm bed diameter (ID) $\times$ 20 cm bed height, 20 $\mu$m particle size, and 30 cm/h superficial velocity. The particle size of the same type of ion exchange resin available for the plant operation is 40 $\mu$m. Two columns are available in the plant: one column 14.0 cm diameter (ID) $\times$ 50 cm high, and another column 18.0 cm diameter (ID) $\times$ 50 cm high. To keep the resolution for the chromatography constant in the plant, which column should be used? For this column, what should be the resin bed height and the superficial velocity and what do you estimate the pressure drop to be? The viscosity of the mobile phase is 1.0 cp, and the void fraction for resin in the plant column is 0.33.


**Sequence**

1 MATERIAL GENGEPTRVVAKDGKLKLG-
SOPSIKLDRGQSTQPRGKTFD

52 APPACLPKATRKLGTNVNATEKSVKT-
---------------S-S-------------
KGPLKQKQSCSFAKMKTEKTCVAKS

106 SVPAASDAYPEIEKEFPPNFLD-
FEFDLPEEIQAHLPLGVPV.MILDEER

157 ELEKLFQGLGPSVKMPSPPWESNLY-
LQSTOPSSILSTDVQELPPVCCDIDI

**TABLE P7.9**

<table>
<thead>
<tr>
<th>$t$ (h)</th>
<th>$c_t$ (g/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.7</td>
<td>0.2</td>
</tr>
<tr>
<td>6.5</td>
<td>0.4</td>
</tr>
<tr>
<td>7.3</td>
<td>1.0</td>
</tr>
<tr>
<td>7.8</td>
<td>1.8</td>
</tr>
<tr>
<td>8.1</td>
<td>2.7</td>
</tr>
<tr>
<td>8.7</td>
<td>3.8</td>
</tr>
<tr>
<td>9.3</td>
<td>4.2</td>
</tr>
<tr>
<td>10.3</td>
<td>4.3</td>
</tr>
</tbody>
</table>
**Research Production Process**

A PCR-amplified gene was cloned into a modified pET28a vector to express recombinant protein in *E. coli*. Protein more than 90% pure was obtained by affinity purification with Ni-nitrioltriacetic acid (NTA) beads followed by Resource Q column chromatography.

In addition to the traditional process and host-cell-related impurities, the following substance-related impurities are present in the process:

**Impurity 1:** Des-Met (N-terminal methionine deleted for the desired protein)

**Impurity 2:** C-terminal cleavage product (cleavage between lysine 9 and glutamic acid 10)

1 ENGEPGTRVVKDKGLGSPSIKAL-DGRSQVSTPRFGKTFD

43APACLPKATRKLGVNATEKSVK-

TKGPLKQKQPSFCSSKMMTEKTCVKAKS

97 SVPA.sdDADAPIEIEKFPPFNP.LDFPLPEEHQIAHLPLSGVPMLDEER

148 ELEKLFQQLGPPSPVKMPSPPWESNL-LOSPSSILTLDVELPPVCCDDI

**Impurity 3:** N-terminal cleavage product (cleavage between lysine 9 and glutamic acid 10)

1 MATLIYVDK

**Impurity 4:** Misfolded isoform

1 MATLIYVDKENGEPGTRVVKDKGL-

KLGSPSIKALDGRSQVSTPRFGKTFD

52 APPACLPKATRKLGVNATEKSVK-

TKGPLKQKQPSFCSSKMMTEKTCVKAKS

106 SVPA.sdDADAPIEIEKFPPFNP.LDFPLPEEHQIAHLPLSGVPMLDEER

157 ELEKLFQQLGPPSPVKMPSPPWES-

NLLQSPSSILTLDVELPPVCCDDI

**Impurity 5:** Covalent dimer between cysteine 56 on adjacent molecules

Information about chromatography adsorbents can be found through links to various companies at the textbook website (www.biosep.ou.edu). There is also a link to a spreadsheet to approximate protein charge as a function of pH at the textbook website.

### 7.13 Specification of Equipment for Chromatography

**Steps (Mini-Case Study)** Use the purification steps selected in Problem 7.12, to choose equipment and specify column scale for the chromatography steps, given the following considerations:

- **Fermentation titer:** The *E. coli* process produces 500 liters of product at a concentration of 2.5 g/liter. One fermentation cycle is 48 h, and the success rate is 90% (failure means that the entire fermentation batch had to be dumped); 50% of the recovered protein is the target product.
- **There will be a need for 3000 g for clinical trial material for *E. coli* product, produced over three or more batches.**
- **There are 3 months available to campaign the clinical material, starting after the Christmas shutdown.**
- **Each purification step gives a yield of 90%, and there is an additional 10% penalty every third step.**
- **The protein degrades with the following zero-order kinetics (independent of concentration):**

\[
\text{rate} \frac{\text{g}}{\text{day}} = -50e^{-1000/T} \quad T \text{ in K}
\]

The degradation product is a deamidation of asparagine 11 and must be purified away if it exceeds 0.1%.

**General Plant Resources**

- **There is one 500-lier fermentor available.**
- **Fermentation products can be delivered to the first separation step free from solids.**
- **There are five columns available in the manufacturing area (see Table P7.13).**

### TABLE P7.13

<table>
<thead>
<tr>
<th>Number of columns</th>
<th>Diameter (cm)</th>
<th>Minimum/maximum length (cm)</th>
<th>Maximum pressure (bar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>5/20</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>63</td>
<td>7/30</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>12/50</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>30/100</td>
<td>100</td>
</tr>
</tbody>
</table>
• One chromatography cycle takes 6 h starting from storage or cleaning solutions, 4.5 h when one is resuming from the elution step.
• A water for injection (WFI) still is available that produces 200 liters/h.
• There are two 4°C cold rooms.
• Two crossflow filter housings are available, one capable of holding 50 ft² of membrane, the other capable of holding 200 ft².
• Any tank you size can be made available.

Factors to Consider
• Compatibility with current processes (what's currently used in manufacturing?)
• Cleaning and storage requirements (ease of accommodation in the plant, handling issues, etc.)
• Preferred vendor (experience, reliability, delivery, price, validation package?)
• Future scalability

References


