18

THE DESIGN AND ECONOMICS OF LARGE-SCALE CHROMATOGRAPHIC SEPARATIONS

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18.1 INTRODUCTION

Chromatography ("color writing") was the fanciful name given in 1906 by the botanist Mikhail Tswett to the adsorptive separation method he employed to separate plant pigments on columns of calcium carbonate using petroleum ether as the eluting solvent [1]. Chromatography is a differential migration process; components of a mixture that distribute differently between a particulate[1] adsorbent and a fluid separate because they move at different rates through the fluid-perfused[2] adsorbent bed. If conditions are chosen correctly, the individual species in the mixture emerge at the outlet of the chromatographic column in pure bands.

The broad array of sorbent and fluid combinations available today make chromatography a versatile and widely applied analytical and preparative purification tool at the laboratory, pilot, or industrial scale for virtually any class of pharmaceutical compound, particularly when gentle, but nonetheless highly selective, separation conditions are required. Chromatography is an enabling technology in biotechnology—practically all industrial biopharmaceutical purification processes contain one or more chromatography steps. While employed less frequently in small-molecule drug (API) manufacturing processes, it is nevertheless heavily used in early stages of API development, and does find important industrial applications in natural product and chiral separations. In addition, it is indispensable for the large-scale purification of synthetic peptides. In this chapter, the focus is on chromatography as it is practiced in the purification of small-molecule drugs and peptides.

Chromatography is considered by many to be an expensive step; a useful tool to obtain from milligrams to a few hundred grams of intermediates or drug substances for deliveries early in development, but ultimately a step that must be superseded in favor of more cost-effective methods (extractions and crystallizations, or improved synthesis routes) before commercialization of the process. However, done properly, chromatography is economically competitive; indeed with modern equipment, method optimization, and solvent-sparing technologies—including solvent recycling and continuous multicolumn chromatography techniques—chromatography is both effective and efficient for industrial scale purifications.

This chapter will begin with an outline of the key design elements in chromatography and a discussion on fundamental chromatographic relationships such as retention and selectivity. The various available chromatographic chemistries will then be discussed. This will be followed by brief sections on chromatographic operating parameters, the choice of the mode of operation including multicolumn systems, choice of equipment, scale up, a short section on parametric design space, and a discussion on chromatographic economics.

18.2 KEY DESIGN ELEMENTS

Choice of the combination of the adsorbent (also known as chromatographic media or the stationary phase) and the fluid

[1] Monolithic adsorbents are becoming increasingly popular in the literature, but casting monoliths at the industrial scale remains a challenge.
[2] The fluid may be a gas, a supercritical fluid, or a liquid; for this chapter, the discussion will be confined to liquid chromatography.

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(or mobile phase) is the first design element in chromatography. This choice sets the chromatographic "chemistry," dictating the type of physicochemical interactions that take place within the system as well as the means to manipulate their intensity to achieve the desired separation goals. Many types of adsorbents with a wide array of surface chemistries are available today, and several are prepared with large-scale applications in mind, that is, they are manufactured under controlled conditions in large batch sizes. The mobile phase, an aqueous or solvent-based mixture that could also contain other components such as acids, bases, buffers, or salts, is selected to be compatible with the adsorbent, and acts to mediate adsorption and release of the separating species from the stationary phase. Manipulation of the mobile phase composition is the primary means to control retentivity—a measure of the strength of adsorption of the separating species on the adsorbent—and obtain selectivity—a measure of the difference in retentivities of separating species, which is the key to effecting the desired separation. Solubility of the separating species in the mobile phase is also an important consideration; high solubility is desirable to achieve high productivity. A classification of the various adsorbents and compatible mobile phases on the basis of the underlying physicochemical factors governing retention is provided in Section 18.3.

Another important design element is the choice of operating mode. Most chromatographic separations are carried out in the elution mode, where the separating species move through the system in the presence of all components of the mobile phase. However, there are other means to carry out chromatography. In the displacement mode, one or more mobile phase components (introduced after feeding the separating species) binds tightly to the adsorbent, swamping available binding sites so the separating species move ahead of it. Continuous chromatographic techniques are operating modes in their own right; simulated moving bed (SMB) chromatography and the multicolumn solvent gradient process (MCSGP) have become more popular in recent years as applications for large-scale chromatographic processes increase. All of these are discussed in more detail in Section 18.5.

Once the choice of media and operating mode is determined, the chromatographic process is defined by its operating parameters such as the column dimensions, the adsorbent particle size, mobile phase flow rate, the operating temperature, and other factors pertinent to the operating mode (e.g., cycle and column switching times in simulated moving bed systems). Variation of these operating parameters would form the basis for process and economic optimization as well as the regulatory design space for a chromatographic process. Operating parameters and design space are also discussed later in this chapter.

18.3 FUNDAMENTAL CHROMATOGRAPHIC RELATIONSHIPS

18.3.1 Chromatographic Velocity

Chromatographic operations take place in a column packed with particles. The volume fraction of the interstitial space between the particles in a randomly packed bed, or the interstitial porosity, ε_e, typically has a value of about 0.4. The totally porous sorbent particles commonly used in chromatographic applications often have an internal void fraction, ε_i, of approximately 50%, so that the packed bed has a total porosity, ε_T ($=\varepsilon_e + (1-\varepsilon_e)\varepsilon_i$), of roughly 0.7. For a fluid flow rate F in a bed of cross-section area A, three flow velocities[3] may be defined and are related as follows

$$\frac{F}{A} = u_s = \varepsilon_e u_e = \varepsilon_T u_0 \tag{18.1}$$

Here, u_s is the superficial velocity, u_e is the interstitial velocity, and u_0, the average velocity of an unretained molecule that explores the entire void space, is known as the chromatographic velocity. For modeling purposes, it is assumed that the mobile phase migrates through the system at the chromatographic velocity.[4]

18.3.2 Operating Pressure

Chromatographic operations are mostly carried out at a fixed velocity or flow rate. The pressure drop, ΔP, across a packed column is related to the chromatographic velocity, u_0, the column length, L, the adsorbent particle diameter, d_p, and the mobile phase viscosity, η, via Darcy's law as

$$\Delta P = \frac{L u_0 \eta \varphi}{d_p^2} \tag{18.2}$$

Where the factor φ is a proportionality factor related to porosity given by the Cozeny–Karman equation

$$\varphi = 180 \frac{(1-\varepsilon_e)^2}{\varepsilon_e^2} \frac{\varepsilon_T}{\varepsilon_e} \tag{18.3}$$

For the typical values of the porosities given above, this factor has a numerical value of approximately 710, while in practice it may vary from 600 to 1000 depending on the exact porosities, as well as the regularity and roughness of the particles. In equation 18.3, the variables can be expressed in any consistent units; the equation is also consistent without the need to add any correcting factors if the pressure drop is

[3] In this analysis, we assume one-dimensional axial flow in a cylindrical column; radial flow systems exist but will not be considered here.

[4] Strictly speaking, in the surface layer near the sorbent, the composition of a multicomponent mobile phase is often different from that in the bulk and changes in mobile phase composition can lead to difference in migration rates of the solvent components themselves, but this subtlety can often be ignored in practice.

expressed in bars, the velocity in centimeters per second, the column length in centimeters, the particle size in micrometers, and the viscosity in centipoise.

18.3.3 Mass Balance Equation

A differential one-dimensional mass balance for a retained species in a chromatographic system can be written in simplified form as

$$\frac{\partial c}{\partial t} + \phi \frac{\partial q}{\partial t} + u_0 \frac{\partial c}{\partial x} = D_e \frac{\partial^2 c}{\partial x^2} \tag{18.4}$$

Here, the parameters t and x are time and distance along the column, respectively, c is the concentration of the species in the mobile phase, q is its concentration in the stationary phase, and ϕ is the ratio of stationary to mobile phase volumes (i.e., $(1-\varepsilon_T)/\varepsilon_T$). In this simplified view, all dispersive effects, including molecular diffusion, transport of the species to and within the particle and any dispersive influence of slow adsorption kinetics are lumped into the effective dispersion coefficient, D_e.

18.3.4 Retention and Retention Factor

A system of mass balance equations (one for each migrating species), with suitable initial and boundary conditions, serves as an adequate model for chromatography [2]. The adsorbed concentration q is related to the mobile phase concentration via an equilibrium relationship known as the adsorption isotherm. In general (and very often in the practical case of preparative chromatography that is carried out at high concentrations of the migrating species), the adsorption isotherm of each species is dependent not only on its own concentration in the mobile phase but also on that of all the other species present. Consequently, migration through the system is concentration dependent; the system of equations is nonlinear, and must be solved numerically. On the other hand at low concentrations, it can be assumed that the distribution coefficient ($K = q/c$) for each species is an independent constant. In this circumstance, in an ideal system without dispersion, the mass balance equation reduces to

$$\frac{\partial c}{\partial t} + \frac{u_0}{(1+\phi K)} \frac{\partial c}{\partial x} = 0 \tag{18.5}$$

This is a one-dimensional wave equation with propagation velocity—or the migration velocity of a species through the system—of $u = u_0/(1 + \phi K)$. The product ϕK is known as the retention factor, k'. Despite being defined only at low concentrations in limited circumstances, k' is a key factor in the understanding and characterization of chromatographic behavior.

Conditions where the distribution coefficient K of each migrating species is independent of concentration (the Henry's law region) are known as "linear" (or "analytical" conditions, as these are conditions under which chromatographic analyses are carried out). A system in which the mobile phase composition is kept constant over time is termed "isocratic." In a linear isocratic system, if the mixture to be separated is injected to approximate a δ-function, the retention factor k' of each separating species can be determined from its retention time, t_R, (the time of elution of the center of gravity of the migrating component) and the dwell time, t_0 (the time of elution of an unretained component), as follows [3]

$$k' = \phi K = \frac{t_R - t_0}{t_0} \left(= \frac{V_R - V_0}{V_0} \right) \tag{18.6}$$

(V_R and V_0 are the corresponding elution and dwell volumes; $V_R = Ft_R$, where F is the mobile phase flow rate, and $V_0 = Ft_0 = \varepsilon_T V_C$, where V_C is the total column volume.)

The distribution coefficient K is a thermodynamic property, related to the free energy of adsorption, and as a result k' is a function of temperature via the relationship[3]

$$\ln k' = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} + \ln \phi \tag{18.7}$$

Here, ΔH and ΔS are the enthalpy and entropy of adsorption, T is the temperature in degrees Kelvin, and R is the universal gas constant. Values of k' can increase or decrease with increasing temperature depending on the sign of the enthalpy of adsorption; in most instances the latter is true as adsorption is often enthalpically favored (i.e., ΔH is negative), but there are important exceptions, such as in hydrophobic interaction chromatography of proteins, and in other individual cases, where the opposite can hold.

18.3.5 Selectivity

The power of a chromatographic system to discriminate between two species is quantified by the ratio of their retention factors, termed the "selectivity," $\alpha (= k'_1/k'_2$, where the subscripts refer to the two species and $k'_1 > k'_2$, so that $\alpha > 1$). The type of system chemistry, the mobile phase composition, and the temperature are major factors that influence selectivity. In industrial applications, concentrations are usually high, so that Henry's law no longer applies and distribution coefficients as well as selectivities can be nonlinear functions of the concentration of all the locally present separating species. Nevertheless, the first step in designing a chromatographic separation is to operate in the Henry's law regime to find conditions (i.e., the right stationary and mobile phase composition) that maximize selectivity. To achieve this, one must understand how retention and selectivity can be manipulated in the context of the various chromatographic chemistries. A discussion on some of the more popular chemistries is given in Section 18.4.

18.3.6 Efficiency

During chromatography, dispersive effects counteract the effectiveness of the separation; chromatographic efficiency thus increases when dispersion is decreased. Contributions to dispersion arise from flow anastomosis, molecular dispersion, transport in and out of the particle, and slow adsorption kinetics. Efficiency is characterized by the so-called plate number, N, which arises from a model that treats the chromatography column as a series of stirred cells containing equal amounts of stationary phase. Based on this model applied in the Henry's law adsorption regime, the plate number is related to the width of a chromatographic peak[5] by the relationship[3]

$$N = 5.54\left(\frac{t_R}{w_{1/2}}\right)^2 \tag{18.8}$$

The plate number is related to the lumped dispersion coefficient in equation 18.4 as follows [4]

$$N = \frac{u_0 L}{2D_e} \tag{18.9}$$

The larger the number of plates, the more closely the system approximates plug flow, and the more efficient it is.

The height equivalent of a theoretical plate, $H(=L/N)$, is a related measure of the efficiency. The plate height is a function of the particle size and flow velocity, as elucidated first by van Deemter and his colleagues [5]

$$H = Ad_p + \frac{BD_m}{u_0} + \frac{Cd_p^2}{D_m}u_0 \tag{18.10}$$

Here, A, B, and C are constants, with typical numerical values of about 1.5, 0.8, and 0.3, respectively [6] and D_m is the molecular diffusivity of the separating species in the mobile phase. The first (or A) term of this equation is independent of flow rate and arises from flow nonuniformity within the packed bed. The second (or B) term is a result of molecular dispersion and rapidly becomes insignificant as flow velocity increases. The third (or C) term is a consequence of diffusion of the separating molecule in and out of the stagnant fluid within the particle pore space. It increases linearly with flow rate and is usually the most significant factor related to band spreading. Contributing factors to the plate height, such as diffusion through the boundary layer around the particle, and the effect of slow adsorption kinetics, are usually of less importance and have thus been ignored in the simplified equation presented above.

[5] The peak is theoretically the output of a δ-function input to the system; in practice a small analytical size injection is employed. The peak approximates a Gaussian distribution. The width at half height, $w1/2$, is more convenient to use than the width at the baseline.

The van Deemter equation can be written in dimensionless form by introducing the reduced plate height, $h(=H/d_p)$, and the reduced velocity, $\nu(=u_0 d_p/D_m)$ so that

$$h = A + \frac{B}{\nu} + C\nu \tag{18.11}$$

Figure 18.1 shows the van Deemter curve and indicates the approximate practical range for the reduced plate height for small molecules (of 15–80, assuming diffusivities in the $2–5 \times 10^{-6}$ cm^2/s range, flow velocities from 0.1 to 0.3 cm/s, and a 10 μm particle diameter). With similar flow velocities and particle size, peptides have a higher reduced velocity range because of their lower molecular diffusivities. This shows that in practice, the B term reduces to zero and that band spreading is dominated by diffusion through the sorbent particle.

Since in practical cases diffusion through the particle is the major contributing factor to the plate height (and thus to N), another useful scaling factor related to efficiency, termed the Lightfoot number, Li, can be constructed by taking the ratio of the characteristic diffusion time across a particle ($t_{diff} = {d_p}^2/D_m$) and the dwell time in the system ($t_0 = L/u_0$) so that

$$\mathrm{Li} \equiv \frac{t_0}{t_{diff}} = \frac{LD_m}{u_0 d_p^2} \tag{18.12}$$

18.3.7 Operation at High Concentration

As mentioned earlier, at high concentration Henry's law no longer applies, and the adsorption isotherm of each species is generally a nonlinear function of the concentration of all the species present. Migration of the components under these conditions is concentration dependent.

While many forms of the adsorption isotherm are possible depending on the molecular properties of the mixture and the sorbent, in many cases the sorbent displays a finite maximum capacity for the adsorbing species. In this situation, compounds vie for a limited number of sites on the sorbent and the adsorption is competitive. At elevated concentrations then, relatively fewer sites are available and the slope of the adsorption isotherm decreases, implying that regions of higher concentration migrate faster. In this circumstance, the low concentration at the leading edge of a concentration front tends to move more slowly than the high concentration of its trailing edge; this has a self-sharpening effect, countering the dilutive effect of dispersion and leading to a sharp shock wave. The opposite is true for the rear, producing a long dilutive tail. Peaks that at low concentration appear symmetrical become triangle shaped at higher concentrations. This effect is illustrated in Figure 18.2. In multicomponent systems, which are of more interest in practice,

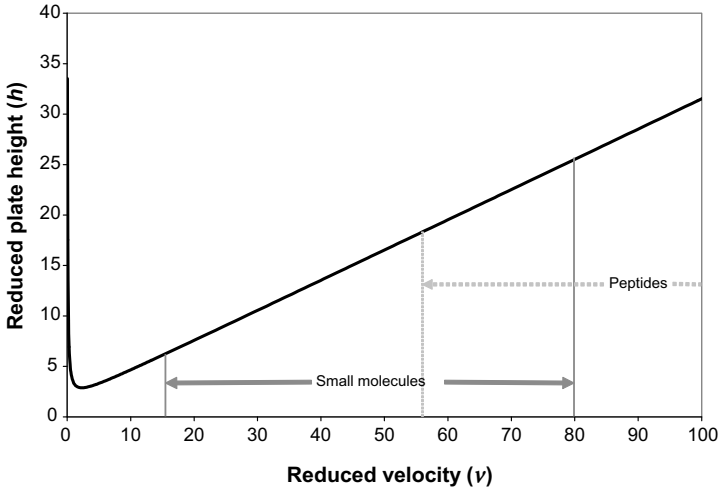

FIGURE 18.1 van Deemter plot of reduced plate height versus reduced velocity showing typical operating regimes for small molecules and peptides as described in the text.

strongly bound compounds displace and thus suppress the binding of more weakly adsorbed species. Under the right conditions in a chromatographic system, this results in more weakly bound components being pushed ahead of more strongly bound ones; some concentration can occur as a result. This so-called "displacement effect" is beneficial if an early eluting component is the target of the purification. Unfortunately, competitive adsorption also leads to a perverse "tag-along" effect; weakly bound components act to suppress somewhat the binding of strongly bound components, accelerating their motion and dragging them ahead, reducing the extent of separation. The effect is shown for two components eluting individually and together in Figure 18.3.

It is worth noting that not all systems display competitive binding, and the displacement and tag-along effects are by no means universal. Some systems display cooperative binding; the chromatographic effects in such circumstances are the inverse of those discussed above. Some systems show a combination of these types of binding, and the resulting peak shapes in chromatography can be quite complex.

One practical consequence of operating at high concentration is that the neat symmetric separated peaks one is used to seeing in analytical separations disappear. Nonspecific detection at the end of the column (usually by UV light absorption), often shows an undifferentiated blob. Once properly characterized, such detection can indeed be used to govern collection of pure fractions, but at least in early

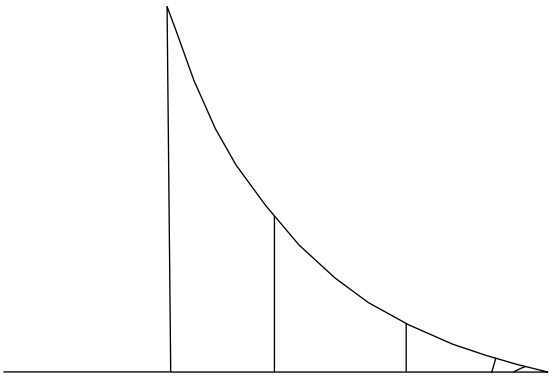

FIGURE 18.2 Schematic showing overlaid elution profiles resulting from injections of increasing concentration of a single species when adsorption is competitive. The elution profiles appear as a series of nested near-triangular shapes, with sharp fronts and extended tails. The end of each tail coincides with the retention time of the species at infinite dilution.

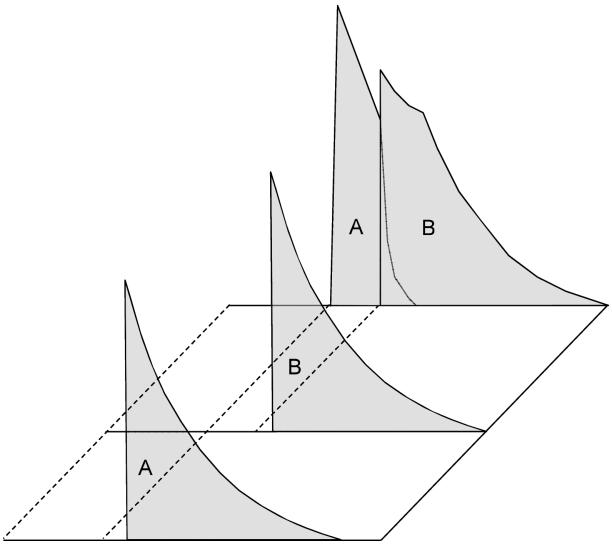

FIGURE 18.3 Schematic showing elution profiles of separate injections of two species A and B, and of a mixture of A and B. In the mixed injection, the peak shape of each species is altered by the presence of the other: A is compressed and concentrated by the displacement effect, and the front end of B elutes a little earlier than in the single component case because of the tag-along effect.

stages of process development there is no substitute for collecting multiple fractions of the emerging eluent stream and analyzing these to understand how well the separation has progressed.

18.3.8 Load

The load on a column is the quantity of feed, expressed conveniently either as total solids or as the amount of the desired product in the feed mixture, introduced into the system per injection. A dimensionless load parameter, Γ, can be defined as the column load divided by the mass of sorbent, and this can be used as a scaling factor in conjunction with the plate number, as discussed later.

18.4 CHROMATOGRAPHIC ADSORBENT CHEMISTRIES AND BASIS OF RETENTION

18.4.1 Normal Phase Chromatography

"Normal phase" chromatography implies use of a polar stationary phase, usually unmodified porous macroreticular silica, with an organic solvent blend as the mobile phase. Mobile phase solvents covering the spectrum from the very nonpolar (heptane), to the very polar (methanol) are used. The silica surface is populated with silanol groups with a distribution of activities that depend on their structure [7], and water can bind strongly at the most polar sites. As a consequence, minor fluctuations in the water content of nominally dry solvents can have a profound effect on retention. Deliberate addition of some water (usually about 0.5% by volume, although higher amounts up to 7% have also been used [8, 9],) to the mobile phase can provide uniformity and quell variations in retention.

Retention is manipulated by changing the mobile phase blend; addition of polar solvents generally decreases retentivity. Good guides to selection of solvents to maximize selectivity are available [10]. Solvents used have been grouped according to their electron donor, electron acceptor and dipole properties, and blends of solvents with broadly different properties often leads to the best selectivity. Selection of the appropriate solvent blend can be assisted by the use of thin-layer chromatography (TLC) on silica-coated plates, which are in common use in organic chemistry laboratories. Translation from TLC to column chromatography may require some reduction in the proportion of the most polar component of the blend to increase retentivity.[6]

[6] In TLC, practitioners like to elute components so that they migrate to about half the length of the plate, so that the "retardation factor" R_F (distance migrated/plate length) is ~0.5. Retention factor and retardation factors are related ($k' = 1/R_F - 1$), so the corresponding k' is ~1. For column chromatography, $k' > 3$ is often desirable, hence the need to increase retention while translating from TLC to LC.

In normal phase chromatography with bare silica as the stationary phase, the layer of solvent closest to the silica surface has a different composition from the bulk. Equilibration of the surface layer upon changes in the bulk takes time, presumably because of the heterogeneity of active sites on the silica surface. As a result, most normal phase separations on silica are carried out isocratically (i.e., without changing the solvent composition during the chromatography).

Because many molecules of interest are very soluble in organic solvents, and because silica is relatively inexpensive and has high adsorptive capacity, normal phase chromatographic processes can be run at high concentrations, resulting in productive and cost-effective separations. On the other hand, the surface energy of bare silica can be influenced by many factors (trace metals, for instance), and a normal phase system can suffer from variability for a variety of reasons, including variations in silica manufacturing, or fluctuations in the crude feedstock which may contain components that bind practically irreversibly to the silica and can slowly change the character of the surface. Bonded phases with more homogenous surfaces are available that reduce or eliminate these sensitivities, and these can also be used in a gradient mode (i.e., where the composition of the mobile phase is changed during the chromatographic run). Phases with diol and amino functions are available that have niche applications (for instance, for separation of molecules containing polyols) with selectivities that differ considerably from bare silica. Bonding chemistry adds to the cost of the adsorbent but this may be offset by a longer column lifetime under the right conditions.

18.4.2 Reversed-Phase Chromatography

So-called "reversed-phase" chromatography is carried out with nonpolar stationary phases using a mixture of water with a miscible solvent as the mobile phase (the polarity of the system is reversed compared to the "normal" phase described above). Molecules separate from each other on the basis of their hydrophobicity; more hydrophobic species are more highly retained. Porous macroreticular silica-based stationary phases whose surfaces have been modified by bonding an alkyl group, such as octadecyl(C18), octyl (C8), or butyl(C4), are most commonly used, although a wide variety of others, including macroreticular polymer-based adsorbents, are available. Commonly employed water-miscible solvents used in the mobile phase are acetonitrile, methanol, ethanol, isopropanol, and more rarely, tetrahydrofuran. Mobile phases are often buffered or acidified to influence retention of ionizable species. Organic or inorganic acids (e.g., acetic, trifluoroacetic, methanesulfonic, and phosphoric acids) can interact with basic groups on the separating species, forming ion-pairs and altering their hydrophobicity, and simultaneously suppress ionization of

residual (unbonded) silanols on the stationary phase surface, reducing ionic interactions. For separation of ionizable species, manipulation of the nature of the ion-pairing agent and the pH can have a profound effect on selectivity.

For a given pH and ion-pairing agent, the retention factor in reversed-phase chromatography decreases with the increasing volume percent of miscible organic solvent in the mobile phase, ψ. In such a system, the relationship between k' and ψ can be adequately described over a broad range of ψ by the expression

$$\ln k' = \ln k'_0 - S\psi \qquad (18.13)$$

Here, k'_0 is the retention factor extrapolated to zero organic content. The slope of the plot, S, correlates roughly with molecular weight, large molecular weight species having larger S values, although there is often considerable variation for species with similar molecular weight. The relationship breaks down at high organic content, where retention can sometimes increase with increasing ψ. Figure 18.4 shows the k' versus ψ relationship for several compounds in a hypothetical mixture.

Reversed-phase is the most widely used form of chromatography on an analytical scale and is also used extensively in industrial applications. Practically every peptide manufacturing process, for instance, includes a reversed-phase chromatography step.

18.4.3 Chiral Chromatography

Perhaps the most important development in the field of chromatography over the past two decades has been the introduction of stationary phases with chiral selectivity. Such phases have seen increasing use on an industrial scale for the separation of enantiomers, providing an alternative to stereoselective synthesis or classical resolution techniques. The phases contain a chiral selector (for instance, substituted cellulose or amylose) bonded or coated onto a chromatographic particle (usually porous macroreticular silica). A broad variety of chiral stationary phases (CSPs) are available utilizing different selectors and these can be operated in either the reversed- or normal-phase modes, depending on their design and solvent compatibility. Choice of the best phase for an application is typically a process of screening a broad range of CSPs under analytical conditions for the ones with the best selectivity. Since selective adsorption capacity can be limited on CSPs, it is worth choosing a few different CSPs to see which one works best under the higher-concentration conditions intended for the application.

CSPs are expensive relative to normal- or reversed-phase sorbents, and separation design efforts often focus on minimizing the amount of stationary phase. In chiral separation applications, the intent usually is to separate enantiomers; presence of other impurities is incidental. For this reason, simulated moving bed chromatography and related multicolumn technologies, which are ideally suited to binary separations, have seen increasing use in the pharmaceutical industry.

18.4.4 Other Chemistries

While most applications of large-scale chromatography for separation of small molecules employ the types of phases discussed above, other types of chemistries are also sometimes employed. Notable among these are phases containing weak or strong anionic or cationic ionized groups bonded on macroreticular silica or polymeric particles, commonly

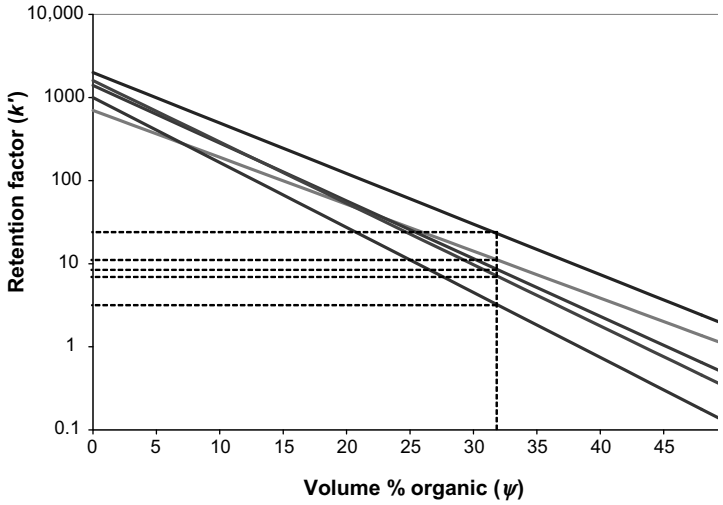

FIGURE 18.4 Plots of retention factor of various components of a mixture versus the organic solvent volume percentage in the mobile phase in a reversed-phase chromatography system, where there is typically a straight line relationship between log k' and ψ.

referred to as ion exchange or electrostatic interaction phases. These are usually operated with aqueous or hydro-organic mobile phases containing salt and buffers for pH control. Salt screens electrostatic interactions, so retention on these phases decreases with increasing mobile phase salt content. pH effects ionization of the separating molecules as well as the ionizable groups on the stationary phase surface and is thus a useful means to manipulate retention.

Hydrophilic interaction chromatography (HILIC) uses polar stationary phases and water-miscible solvents; retention increases with increasing polarity and increases in the water content of the mobile phase result in a decrease in retention. Other types of chemistries, such as those used for hydrophobic interaction chromatography (HIC) where weakly hydrophobic surfaces are employed, or for size exclusion chromatography (SEC), where molecules are not retained but separate on the basis of size, are usually applied only in large molecule separations.

18.5 OPERATIONAL ASPECTS

18.5.1 Chromatographic Applications

Chromatographic systems can be used in several ways in a chemical process. One simple application is for removal of impurities—a process stream is run through a chromatographic column and one or more impurities are adsorbed on the bed. Once the bed is saturated, it is either discarded, or regenerated, reequilibrated, and returned to use. This requires conditions where the impurities are tightly bound and the product is minimally affected by the sorbent. Conditions for this simple operation can often be found if the product and the impurities are not very closely related; separating neutral impurities from a charged product, for instance.

Another application is the inverse operation; a dilute process stream is run through a chromatographic column to capture the product. The product is then desorbed in a concentrated form with a suitable eluent. For example, a clarified aqueous fermentation broth may be run through a reversed phase system to capture the product, which is then eluted in concentrated form in methanol.

A chromatographic system is convenient tool for carrying out certain solvent switches. For example, if it desired to change the product solvent from aqueous acetonitrile to methanol, the product could be captured on a reversed-phase column. This may require dilution of the stream with water to create strong binding conditions for the product. The column could then be washed with dilute aqueous methanol (maintaining binding conditions) to remove acetonitrile, and the product can then be eluted in methanol. A similar product-capture/wash/elute approach can also be used to desalt a process stream. Yet another variation is the use of a reversed-phase system to effect a counterion switch, an approach often used after the purification of peptides. For example, a peptide purified by reversed phase chromatography using trifluoroacetic acid (TFA) as the ion-pairing agent (often a good choice to obtain selectivity for reverse-phase separation, but usually not desired in the final product) can be switched to another counterion form, say acetate, by (a) capturing it (after suitable aqueous dilution) on a reversed-phase column, (b) washing under binding conditions with streams containing first ammonium acetate to remove the TFA and then acetic acid to wash away excess ammonium ion, and finally (c) eluting the peptide by increasing the organic solvent content of the acetic acid containing mobile phase.

By far the most useful application of chromatography, of course, is the purification of a product from closely related impurities; for instance, separation of a desired peptide from a dozen or more synthesis impurities. Such impurities can have differences as subtle as, say, a beta-aspartic acid substituted for aspartic acid in the original sequence, resulting in an isomer with an extra methylene group in the peptide backbone. The following operational modes are employed for such applications.

18.5.2 Elution Chromatography

Elution is the most familiar mode of operation for chromatography; species migrate through the column and separate in the presence of a mobile phase that directly mediates retention. When the mobile phase composition is held unchanged, this is known as "isocratic" elution. When the mobile phase composition is varied during the migration, this is known as "gradient" elution.

The motivation for changing the mobile phase composition in a gradient elution process lies in the potentially wide range of retentivities of all the components in the mixture to be separated. An increase in the strength of the mobile phase over time ensures that the most strongly retained component will elute off the column within a practicable time period. The difference between operating under isocratic and gradient conditions in a reversed-phase system can be understood by examining the $\ln k'$ versus ψ plot in Figure 18.4. For a successful isocratic operation, a single solvent strength must be selected that affords selectivity between the desired compound and the other components of the mixture and also enables elution within a reasonable time frame. For instance, operating at 32 vol% organic, the first and last components to elute would have k's of about 3 and 23, respectively. A gradient in solvent strength would hasten the elution of the more strongly absorbed compounds.

Gradients are most commonly either linear or stepwise, and they are usually formed by blending two streams, changing the blend composition appropriately over time. Some equipment allows formation of convex or concave gradients, but this is not recommended if transfer between different brands of equipment is anticipated.

When the mobile phase strength increases over time, the front and rear of a migrating peak are often in different solvent environments, so that retention is lower at the rear of the peak than the front. This results in a peak "compression" that acts counter to and somewhat mitigates the dilution that is always observed under isocratic conditions. Reducing dilution can have significant impact on solvent volumes and processing of the collected purified material.

18.5.3 Displacement Chromatography

Under conditions of competitive binding and at high column loads, the displacement effect mentioned earlier leads to concentration of early eluting, weakly bound species displaced by more strongly bound components. Displacement chromatography, introduced in the 1940s by Tiselius [11], and developed further in more recent years by the academic groups of Horváth [12] and Cramer [13], among others, exploits this effect to the fullest extent possible. A mixture introduced into the column under strong binding conditions (e.g., low salt content in an ion-exchange system, where k' is high), is pushed through the column not by the customary means of changing the eluent strength, but by introducing a solution containing a species that is more strongly bound than any of the mixture components, called the displacer. Velocity of the displacer front can be manipulated by adjusting its concentration. The displacer swamps the binding sites on the sorbent, acting almost like a piston to move the other species ahead of it. The mixture species, concentrated and moved forward by the action of the displacer, then act to displace each other in order of binding strength, until—after development of the displacement train over a sufficient column length—they separate into contiguous concentrated bands of individual components. The displacer does not mix with the separating components except by dispersion at the end of the separation train. The displacer must be removed from the column before it can be reused. This can be accomplished by changing the eluent strength or other conditions such as the pH to facilitate washing the displacer from the column.

This mode of separation is ideally suited for economical preparative separations, since it perforce runs at high concentration and makes efficient use of the capacity of the stationary phase. However, relatively few displacement separations have been implemented. One reason may be that development of a displacement separation is often time-consuming and can require expert attention. Some headway in widening the use of this technique is being made, particularly in ion-exchange chromatography applications [14].

18.5.4 Multicolumn Chromatography

It has long been recognized by chemical engineers that the most effective mass transfer between two phases takes place when they are contacted in a continuous, countercurrent manner. In a chromatographic system with a particulate solid sorbent, actual movement of the solid would disrupt the packing structure negating any potential efficiency gains. Nevertheless, many of the benefits of countercurrent solid motion can be achieved by simulating the solid motion by appropriately switching inlet and outlet positions in a multicolumn system. When the inlet and outlet ports are simultaneously switched in the direction of fluid flow, as shown in Figure 18.5, the sorbent appears to flow in the opposite

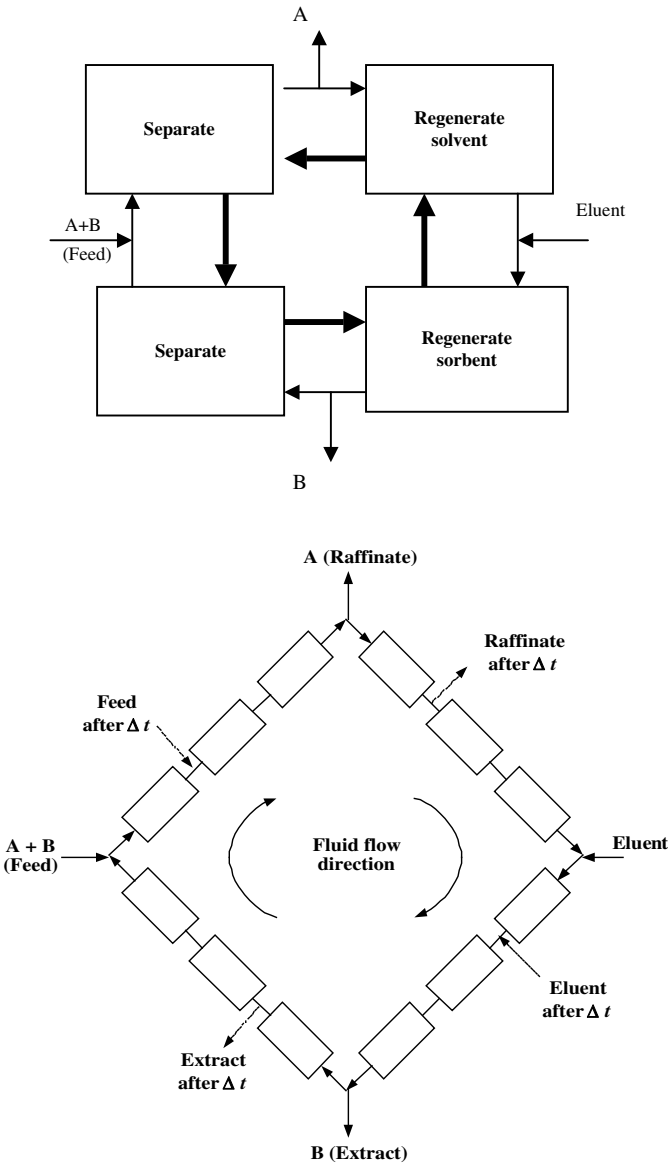

FIGURE 18.5 The four-zone simulated moving bed chromatograph. *Top*: Shows a schematic of the four-zone moving bed—dark arrows show the desired direction of solid flow. *Bottom*: Shows how simulation of the solid movement is carried out in a 12 columns system with 3 columns in each zone. The position of each of the external flow switches by one column after each switching period Δt. Flow within the column ring is driven by a pump.

direction. Simulated moving beds of this kind have been used in multiton industrial operations such as the purification of *p*-xylene from C8 fractions since the early 1960s, with installed capacity exceeding 10^7 tons/year [15]. Over the last two decades, simulated moving beds and related multicolumn chromatography (MCC) technologies, have been increasingly employed in pharmaceutical applications.

The configuration shown in Figure 18.5 shows two inlet streams (one for the feed mixture, the other for the eluent) and two outlet streams (one for the separated early eluting compound(s), known as the raffinate, the other for the separated late eluting compound(s), known as the extract). The system has four zones, two separation zones on either side of the feed inlet, a solvent recycle zone that strips the early eluting compound from the solvent, sending it toward the raffinate, and a sorbent recycle zone that strips the late eluting compound from the sorbent, sending it toward the extract. The presence of two outlet streams makes the technique naturally suited to binary separations, and it thus comes as no surprise that in the pharmaceutical arena the simulated moving bed technology is used most widely for enantiomer purifications. There are three major operating advantages over conventional chromatography: (a) the stationary phase is used to maximum capacity, minimizing sorbent requirements; (b) operation can be adjusted to obtain close to quantitative yields at the targeted purity, minimizing loss of precious product, and (c) internal recycle of the solvent minimizes the solvent consumption [16].

Various efficiency modifications have been introduced recently into the repertoire of multicolumn operations. A process employing asynchronous column switching, known as "Varicol," enables the assignment of a fractional part of a physical column to a given separation zone, reducing the number of columns required and thus the column hardware needed [17]. Appropriately timed flow rate changes within a switching period (termed "power feed") have also led to significant improvement in MCC efficiency [18]. A recent development that is expected to significantly impact the future design of nonbinary large-scale gradient elution separations is the introduction of a technology termed the "multicolumn countercurrent solvent gradient purification (MCSGP)" process [19]. This process can employ as few as three columns in a semicontinuous countercurrent operation that in principle could be adapted to work for any existing gradient elution separation. The major advantage of the process is that the semicontinuous operation enables near quantitative yield of the desired compound at the target purity. Unlike the more traditional four-zone binary systems, the MCGSP does not afford significant solvent savings. Nevertheless, yield considerations can be a sufficient driver to implement such a scheme.

Many of the advantages of the simulated moving bed can also be realized using a single or dual column multiinjection steady recycle process invented by Charles Grill in the mid-1990s [20]. This process has now been commercialized [21].

18.6 EQUIPMENT

18.6.1 Columns

Until the introduction of large-scale high performance chromatographic equipment in the 1980s, industrial chromatographic separations were carried out in large columns packed literally with tons of relatively large sorbent particles (c. 100 μm diameter or larger). One consequence of large particle size was the need for significant column lengths (several meters) to achieve separations. Poor distribution in such columns created inlet and outlet flow development zones, creating serious inefficiencies and making column aspect ratio an important factor in scale-up. Modern high performance columns, now available from several vendors, have excellent flow distributors, so that performance is virtually independent of column diameter. Sorbent particle diameters used today are usually between 10 and 20 μm. While these provide high efficiency so that column lengths of less than 1 m (and usually $\ll$50 cm) are sufficient for many separations, they demand proper packing techniques and relatively high operating pressure, of up to about 100 bar. One technology that has solved these issues and so has dominated the large-scale column market is the so-called dynamic axial compression (DAC) column. A hydraulic piston inside the column is used initially to compress a slurry of sorbent particles into a firmly packed bed, and pressure is maintained on the bed during column operation, ensuring that any holes or pockets that may arise from bed subsidence over time are eliminated, ensuring efficient long-term operation. It is not unusual for a DAC column of 1 m diameter to show the same efficiency as an analytical column packed with the same sorbent. This makes scale up facile, as the only important scaling parameter is the cross-section area of the column. Figure 18.6 shows an example of a large-scale DAC column. The tall gray assembly is part of the hydraulic system that transmits pressure to the column piston. For unpacking, the bottom flange of the column is opened and the piston is driven downwards to force the sorbent out. Column packing and unpacking of the few tens of kilos of sorbent is thus straightforward, taking only a few hours (including all preparation time) in an industrial setting.

18.6.2 Pumps, Detectors, and Controllers

High-performance chromatography requires pumping of the mobile phase at a fixed flow rate through the system at pressures up to 100 bar. Industrial chromatography pumping systems almost universally employ double (or sandwich) diaphragm positive displacement pumps. The two diaphragm

FIGURE 18.6 A 1 m internal diameter dynamic axial compression column for large scale high-performance liquid chromatography. The gray cylinder above the column is part of the hydraulic system to maintain pressure on the piston during packing an operation. Components of the piston assembly are seen in the foreground. Photograph courtesy of Novasep Inc.

design assures that failure of a single diaphragm will not contaminate the process side with oil or fluid from the pump. Continuous monitoring of the space between the diaphragms for rupture provides assurance that any failure will be detected immediately.

It is advisable to use separate pumps for feed and mobile phase streams, although some pumping designs use the same pump for both.

A common feature in pumping system designs is a gradient forming system that enables blending of two (or more) inlet streams to vary mobile phase composition for gradient elution operation. Valves and mass flow meters along with electronic controllers are often used to achieve the gradient. Gradient composition monitoring and control using PAT systems, such as near IR probes with feedback control, are available commercially.

The effluent stream is usually monitored by a detector; signals from the detector can be used to decide when to collect fractions. The most commonly employed detector is the variable wavelength UV detector, although a plethora of others (conductivity detectors, for instance) can be used depending on the application. Some systems take a small slipstream to pass through a detector, others use a full-flow detector cell. Under the high concentration conditions typical in preparative chromatography, it is common for the UV absorption signal to saturate at the wavelengths and cell path lengths commonly used in analytical chromatography. Often sensitivity needs to be dampened to properly capture the emerging peak and make useful fraction collection decisions. One simple way to accomplish this in practice is to use a UV wavelength far from the absorption maxima of the compound.

Fraction collection is accomplished by directing flow to different collecting vessels. Decision making on fraction collection can be triggered by the detector signal. As mentioned earlier, UV signals from industrial chromatographs do not look quite as simple as those in analytical systems, so some logical rules based on time, flow volume, and thresholds of UV signal and/or UV slope can be employed to start and end fraction collection periods. Most commercial equipment manufacturers supply software with sophisticated fraction collection algorithms.

Pump valves and detectors are often mounted in one assembly, called a pumping skid. Control of the skid is accomplished with the aid of a programmable logic controller (PLC) with appropriate software running from a PC. Fully automatic operation is possible. Electronic controls are either located remotely from the skid or in an appropriately purged box to ensure the unit is explosion proof. In explosion proof equipment, valves are activated pneumatically.

Equipment for multicolumn units likewise involves a combination of columns, pumps, valves, and process analytics specific to the design of the units. Figure 18.7 shows a series of columns intended for use in a Varicol MCC at industrial scale.

18.7 SCALE-UP

With good radial flow distribution, inlet and outlet flow development zones are practically eliminated and chromatography can be genuinely described by a one-dimensional (axial) mass balance such as that in equation 18.4. The simplest possible scale-up paradigm under these circumstances is to maintain the same sorbent particle size, column length, and chromatographic velocity across scales, setting all other parameters proportional to the column cross-section area. This will ensure practically identical pressure drop, column efficiency (plate number), separation quality, and specific production rate (production rate normalized to sorbent mass) across scales. An example showing such a scale up from a standard 0.46 cm internal diameter (ID) column to a 45 cm ID column is shown in Table 18.1.

If identical particle sizes are not employed, similar quality of separation can be obtained by keeping the dimensionless number Li and the normalized feed load constant across scales. Rearranging equation 18.2 to solve for the chromatographic velocity u_0 and substituting the result into the expression of Li from equation 18.12 one obtains

$$\mathrm{Li} = \frac{\left(L/d_\mathrm{p}^2\right)^2}{\Delta P}\left(\frac{\eta\varphi}{D_\mathrm{m}}\right) \tag{18.14}$$

FIGURE 18.7 A six-column simulated moving bed unit. Each column has 1 m internal diameter. The unit was designed to operate in a Varicol process. Photograph courtesy of Novasep Inc.

TABLE 18.1 Example of Scale-Up Keeping Particle Size and Column Length Fixed

Parameters	Small Scale	Scale Factor	Large Scale	Comments
Key Scale Up Parameters				
Column diameter	0.46 cm		45.00 cm	
Column cross section	0.17 cm^2	9,570	1590.43 cm^2	Basis for scale up 9569.94
Parameters that must be Fixed to Enable Scale-Up based on Cross-Section Area				
Particle size	10.00 µm		10.00 µm	
Column length	25.00 cm		25.00 cm	
Parameters that Scale with Cross-Section Area				
Mass sorbent in column	2.53 g	9,570	24.25 kg	Packed density of 0.61 g/cm^3
Flow rate	1.50 mL/min	9,570	14.35 L/min	
Feed injected per run	25.00 mg	9,570	239.25 g	Load factor held constant
Important Parameters that Remain Unchanged in this Scale-Up Paradigm				
Chromatographic velocity	0.215 cm/s		0.215 cm/s	
Pressure drop across column[a]	40 bar		40 bar	Equation 18.2: $\varphi = 710$; $\eta = 1.05$ cP
Feed composition	–		–	
Mobile phase composition	–		–	
Run time	25 min		25 min	
Gradient program	–		–	
Detector settings	–		–	
Temperature	30°C		30°C	
Performance Attributes				
Yield	85%		85%	
Production rate	1.22 g/day		11.71 kg/day	Feed injected/day × Yield
Specific production rate	0.48		0.48	kkd = kg produced/kg sorbent/day
Mobile phase used per day	2.16 L		20,671 L	
Specific solvent consumption 1	1.76 L/g		1.76 L/g	No recycle
Specific solvent consumption 2	N/A		176 L/kg	90% recycle

[a]There will be additional pressure drop caused by flow in piping, not accounted for here.

TABLE 18.2 Productivity Comparison for Columns Packed with 10 and 60 Particles

	Case 1	Case 2	Comments/Calculations
Scaling Calculations			
Particle size [μm]	10	60	
Pressure drop [bar]	40	3	3 bar typical of low pressure equipment
Column length [cm]	25	246	Calculation based on scaling parameter
$(L/dp^2)^2/\Delta P$ [dyne^{-1}]	15,625,000	15,625,000	Scaling parameter
Productivity Calculations: Columns Packed with 1 kg of Sorbent in Both Cases			
Linear flow velocity, u_0 [cm/s]	0.215	0.059	Equation 18.2: $\varphi = 710$; $\eta = 1.05$ cP
Dwell time, t_0 [min]	1.94	69.89	$t_0 = L/u_0$
Run time, t_R [min]	25	900	Case 2 based on ratio of dwell times
Column ID that contains 1 kg sorbent [cm]	9.14	2.91	Packed density of 0.61 g/cm^3
Feed injected per run [g]	9.9	9.9	From Table 18.1, keeping feed to sorbent mass constant
Yield	85%	85%	Based on Example Table 18.1
Production rate [g/day]	484.70	13.46	A 36-fold difference
Column Dimensions and Sorbent Quantities Required to Obtain the Same Productivity			
Column diameter [cm]	45	86	
Column volume [L]	40	1,431	Must have 36 × volume to maintain equal productivity
Sorbent mass to pack column [kg]	24.25	873.15	
Feed injected per run [g]	239.25	8613	
Number of injections/day	57.6	1.6	$\# = 24 \times 60/t_R$
Production rate [kg/day]	11.71	11.71	= # × feed injected per inj* Yield
Specific productivity	0.48	0.013	kg produced/kg sorbent/day

The viscosity η and diffusivity D_m are constants across scales as long as temperature is maintained constant. The bed permeability φ should remain constant across scales as long as similar sorbent particle geometry (e.g., spherical particles) is maintained. Thus, the pertinent invariant scale factor when sorbent particle size is not held constant is $(L/d_p^2)^2/\Delta P$.

Table 18.2 shows an example of two systems with different particle sizes, 10 and 60 μm, respectively, where the factor $(L/d_p^2)^2/\Delta P$ is held constant. Separation quality (not quantified in the table) and important performance characteristics such as solvent consumption are expected to be identical in both cases, but specific production rates are dramatically different. The smaller particle size sorbent has an increased specific productivity proportional to the square of the ratio of particle sizes (a factor of 36 in the example). If the same production rate was required in the two cases, the quantity of 60 μm sorbent required would be 36-fold that in the 10 μm system. Dimensions of identically performing columns packed with the two different particles are also given in Table 18.2. This example illustrates the incentive for using smaller particles and higher pressures for chromatographic operations.

18.7.1 Scaling of Gradients

Solvent gradients should be expressed in scalable terms. For example, in reversed-phase chromatography, varying solvent composition from X% to Y% in a straight-line manner over several column volumes (CVs) of flow (rather than expressing it as A% of solvent A to B% of solvent B over 20 min) enables seamless scale up.

18.8 DESIGN SPACE

The so-called design space is the window within the operating parameter space in which acceptable process performance is achieved. The goal of this section is to provide the reader with some appreciation for the various operating parameters and the sensitivity of conventional chromatographic operations to these parameters. Multicolumn systems are not discussed. The manner of defining the design space for regulatory agencies—carrying out design of experiments, etc—is not within the scope of this discussion.

Figure 18.8 shows a view of a chromatographic process illustrating the various process parameters as well as salient product quality and process performance attributes. Design issues associated with some of the parameters, for instance sorbent selection, column dimensions and efficiency, have been discussed earlier in this chapter. Some of the other parameters are discussed below.

18.8.1 Process Quality Attributes

Critical quality attributes of the product stream from a chromatographic process are product purity, which must be above a minimum target (e.g., >98.5%), with key individual

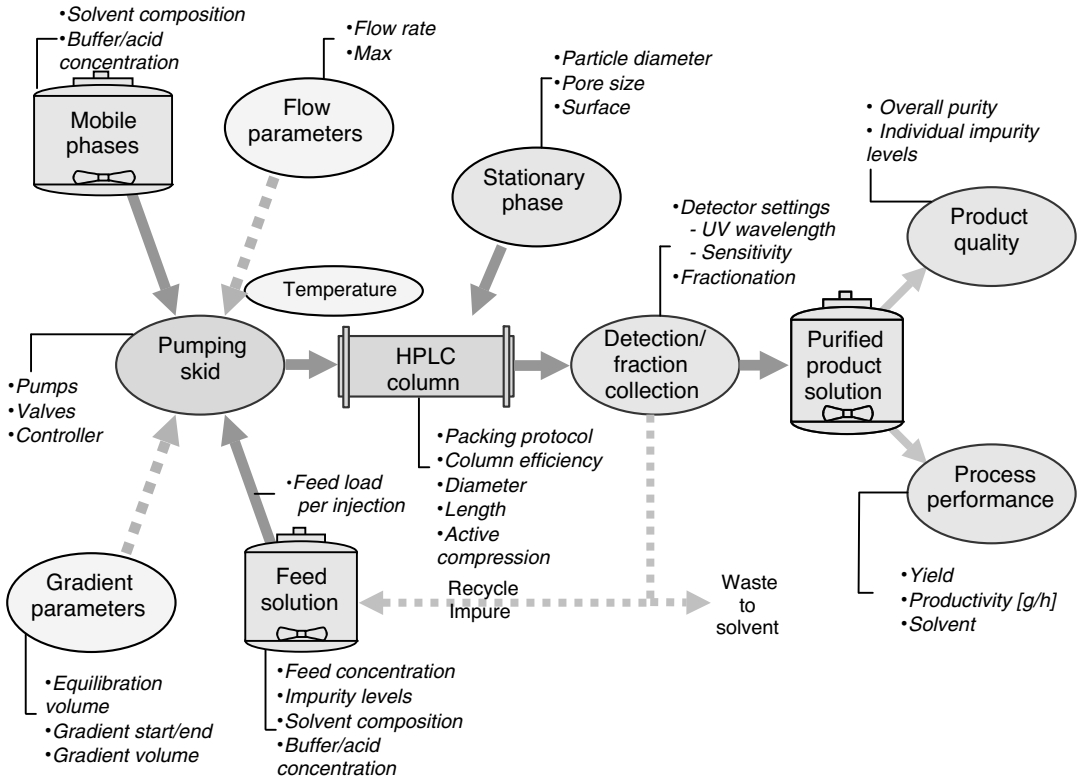

FIGURE 18.8 Overview of chromatographic operating parameters, product quality attributes, and process performance measures.

impurities maintained below prespecified maximum limits. The concentration in the product stream is a quality attribute, but a wide range is usually acceptable and thus it is not critical.

18.8.2 Process Performance Attributes

Key process performance (and indeed process economic) measures are (a) yield, calculated as the moles of desired product recovered at or above the target quality as a percentage of the moles introduced in the crude feed, (b) process productivity, which is simply a mass rate of production, expressed in gram or kilogram/hour (a specific production rate, normalized to the mass of sorbent, is sometimes a useful measure), and (c) solvent consumption, most usefully expressed in terms of each solvent species consumed per unit mass of product. These parameters are discussed further in Section 18.9.

18.8.3 Feed Parameters

As shown in Figure 18.8, feed concentration, impurity levels and the feed solution characteristics, including solvent composition, buffer, or counterion concentration, and pH are important factors. The rule of thumb is that for the same feed load, better results are obtained the higher the feed concentration. Nonetheless, feed concentration is usually more critical in isocratic elution than in gradient elution chromatography. This is because in gradient elution, feed introduction is usually under relatively strong binding conditions, enabling concentration of the feed at the column inlet regardless of its concentration. There is usually no such opportunity under isocratic conditions, unless the feed solvent composition is manipulated to afford somewhat stronger binding conditions than the eluent itself. (In reversed-phase chromatography, this would imply using lower organic modifier in the feed than in the eluent.) Feed solubility is of course a strong limiting factor influencing concentration; some practitioners using isocratic elution chromatography manipulate feed solvent composition to provide more solubility at the expense of strong binding (e.g., raising the methanol content in normal phase chromatography) [9]. The pH can also play a powerful role; there is at least one example where a pH mismatch between feed and eluent has been exploited to enhance separation [22]. Criticality and sensitivity of the process to these parameters needs to be experimentally explored during process design.

18.8.4 Mobile Phase Parameters

Solvent composition (i.e., the blend of different solvents employed), buffer concentration and pH are key mobile

phase parameters. In systems, where two or more mobile phase streams are blended by the gradient-forming system in the pumping skid, a wide composition range may be permissible depending on whether or not appropriate process analytical technology is employed to monitor or control the gradient. Heats of mixing and gas evolution upon solvent blending (gas solubility may change upon blending and solvents that are not degassed may outgas as a consequence) need to be accounted for. Typically outgassing occurs only at low pressures at the column outlet and may or may not interfere with detection. Prior degassing of solvent streams may not be required if the system has sufficient pressure at the detector cell to prevent outgassing.

18.8.5 Fraction Collection

Issues with UV or other detection of product in the column effluent have been discussed above. Proper collection and combining of fractions containing purified material is critical to the success of the chromatographic process. Ideally, a process should be designed to collect one purified fraction and perhaps one or two lower purity fractions that could be recycled. The remainder of the process stream can be sent to solvent recovery units. Collection of a single pure fraction is possible only after all the nuances of the chromatographic operation have been fully understood and a fail-safe rich cut collection algorithm is devised that can handle all possible variation in feedstock purity and any minor drifts in retention caused by operating within a parameter ranges. Until sufficient process history is available, it is prudent to collect multiple fractions; combination of *contiguous* fractions at different purity levels *in their entirety* to achieve a rich cut at the desired purity is operationally the same as taking a single rich fraction, and thus does not carry the stigma associated with blending of poor and high quality materials.

18.8.6 Column Lifetime

An important design and economic parameter is column lifetime. Some minimum lifetime should be specified before commercial implementation of a process, and this can be obtained from process history or from multiple small-scale injections on a small scale. Minimum plate count and selectivity criteria, measured with a test tracer solution (either a small injection of product or other marker compounds), should be in place to define acceptable column performance, and these should be tied to process performance. For small-molecule applications, it should be feasible to extend the use of a column beyond the existing historic lifetime based on a tracer test. Since column performance may diminish for mechanical reasons (poor maintenance of bed integrity owing to lack of sufficient axial pressure, for instance), the sorbent may be unpacked and repacked on multiple occasions. For regulatory purposes, some limits for such operations must be defined. It is also useful to establish whether or not the feed components can eventually poison the sorbent (by irreversible binding, for instance). In addition, depending on nature of further downstream operations, it may be important to develop tests for column leachables and demonstrate absence or establish acceptable limits of these compounds in the process stream.

18.8.7 Temperature

Column and solvent temperature is an important parameter. Ideally isothermal conditions should be maintained, as retentivity is temperature dependent as shown in equation 18.7. However, during operation of larger columns, a small increase (1–3°C) between the column skin temperature and the solvent inlet temperature is useful to overcome frictional heating effects and within the column and related wall effects [23].

18.9 ECONOMICS

Given the many parameters associated with design of a chromatographic process, rigorous economic assessment and optimization of chromatography is a complex task. Key economic parameters, such as return on capital and Lang factors (the ratio of total installation cost to cost of equipment), vary from company to company, so that outcomes of net present value (NPV) assessments of the same separation problem may lead to different conclusions at different locations. Nevertheless, cost drivers for chromatographic operations are common to most other processes; in an industrial manufacturing operation the overall cost, expressed per kilogram or ton of product purified, is the sum of costs of amortized capital, labor, consumables, waste, and product loss. Intangibles not usually taken into account because they are hard to quantify are development opportunity costs. For instance, a chiral separation is typically easier and quicker to implement and scale up than an asymmetric synthesis. If the unwanted enantiomer can be racemized, the major objection to a separation approach (automatic 50% yield loss associated with the unwanted enantiomer) may be mitigated. With commensurate resources for optimization and engineering, ultimate process costs of the chiral chromatography and a more elegant asymmetric synthesis may not be significant, but the time talented chemists spent on the synthesis development could have been used more fruitfully elsewhere. Only organizations that develop sufficient competence in chromatographic operations would have the confidence to exploit the technology to its full potential.

Capital and labor costs are usually specific to the site of operation. In this author's opinion, cost for large-scale high performance chromatographic equipment and installation is not prohibitive compared to new installation of other process

equipment. There is sufficient competition among vendors and important equipment designs are now off patent. The key economic concerns for the chromatography development engineer should be the minimization of cost of consumables and product loss. Several good literature references for quantitative optimization are available [24,25].

18.9.1 Stationary Phase

During development, cost of the stationary phase can be high. Sorbent usually is dedicated to a particular product and, since product failure rates are high, there is limited opportunity to spread the cost of the phase over a long period of time. On the other hand, during commercial operation, sorbent costs, while significant, are not usually a limiting factor since column lifetime can be quite long. For instance, chiral stationary phase lifetimes of several years have been reported. Unpublished reports suggest stationary phase costs in such applications can be held below $\$10\,kg^{-1}$ purified [26].

18.9.2 Solvent Consumption

Solvent volumes employed in chromatography are indeed high. Operating a 45 cm ID column at 10 L a minute involves handling 4000 gal a day. However, measures can be taken to drastically limit solvent *consumption* and costs by implementing solvent recycle procedures. At full production, it is reasonable to expect upwards of 90% solvent recycle (on the organic component; water is rarely recycled; in some instances this may give organic solvent rich mobile phases the economic edge, since disposal costs for aqueous waste must be considered).

For separations where multicolumn techniques, such as simulated moving bed technology or multiinjection steady state recycle technology can be employed (mostly for chiral separations) solvent consumption can be drastically reduced compared to column chromatography. Coupled with solvent recycle, use of SMB technology may consume less solvent than a conventional three-step classical chiral resolution by crystallization with a tartrate salt, and the total cost for a simulated moving bed operation can be competitive with or lower than such a process [27]. New chiral stationary phases with high adsorption capacity are becoming available in the market; use of these phases is expected to improve the economics of chromatographic separation of enantiomers even further.

18.9.3 Product Loss

For high value products, yield loss can have a significant economic impact. In conventional chromatography (e.g., a gradient elution separation of peptides), yield losses can be minimized by recycling impure fractions. Regulatory agencies expect defined criteria for choosing fractions for recycling, and defined limits on the number of times recycle is allowed. Multicolumn techniques have a distinct advantage since high yield is an inherent attribute of continuous processes. An example showing yield increase from 85% in a conventional gradient peptide purification to $>95\%$ in an MCSGP process, with a concomitant 25-fold productivity increase, has been published [27].

18.10 CONCLUSIONS

With modern high performance equipment and improvements in operating strategies, including the increasing use of multicolumn technologies, robust and economic large-scale chromatographic processes can be designed for purification of a wide variety of pharmaceutical compounds.

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