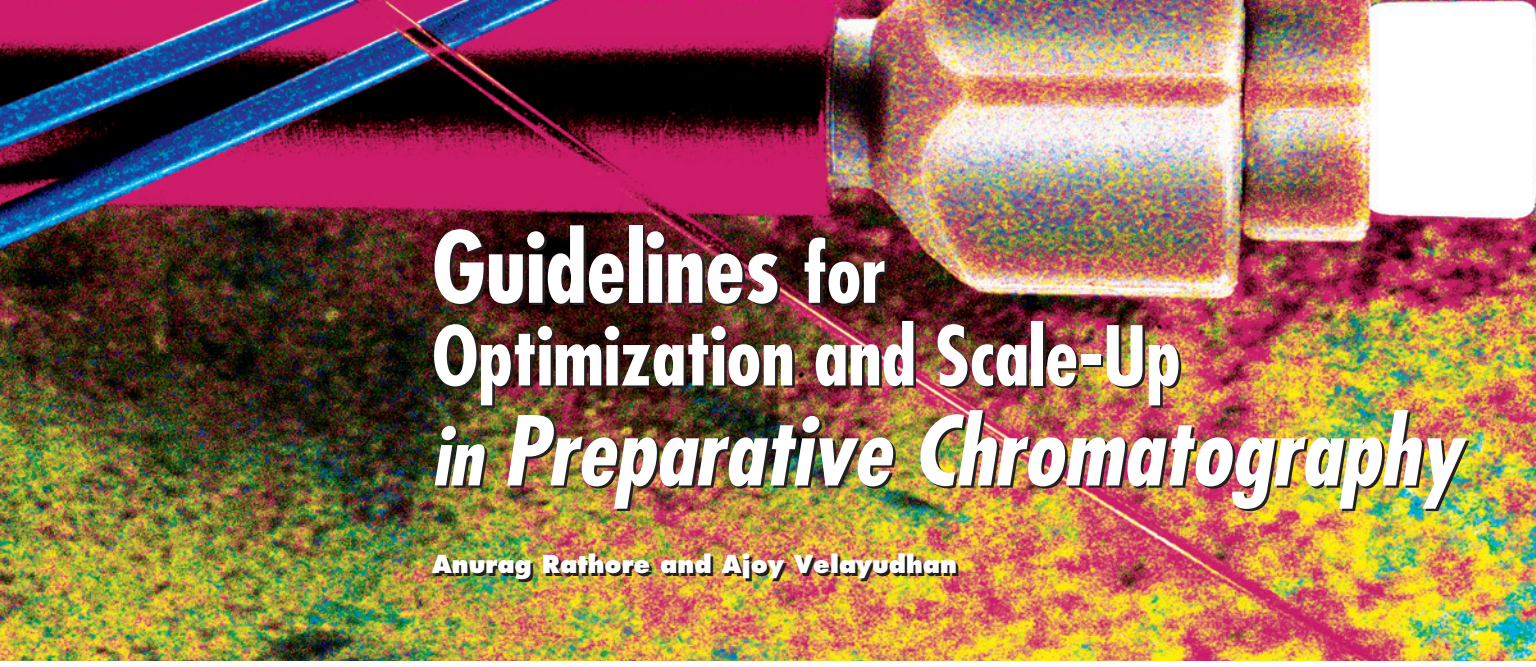

Guidelines for Optimization and Scale-Up in Preparative Chromatography

Anurag Rathore and Ajoy Velayudhan

Optimizing your purification and separation process when your complex biological feedstock is ready for scale-up can be daunting. Your design process can be streamlined, simplified, and made cost efficient if you supplement your empirical approach with the theoretical and experimental tools presented in this article.

Preparative chromatography is the dominant technique for purifying biological compounds in production, especially in the pharmaceutical and biotechnological industries. Our theoretical understanding of chromatography tools has deepened during the past 20 years. Nevertheless many obstacles prevent us from making purely theoretical predictions that realistically mimic a process-scale run using biological feedstock. New theoretical advances can, however, support and supplement purely empirical approaches to scale-up and optimization.

In this article we examine state-of-the-art theoretical and experimental methods for scale-up and optimization, and offer some guidelines for streamlining the design process. We present the elements of a method development strategy for bench-scale use, a simple quantitative scale-up calculation, and a summary of practical considerations in scale-up. More detailed and sophisticated scale-up considerations are available in several books (1–3) as well as in the recent chromatography literature.

INTERACTION AND OPERATION MODES

The various kinds of chemical interactions used in chromatography for selective separations are called *modes of interaction*. Examples include electrostatic interactions in ion-exchange or ion chromatography and hydrophobic interactions in reversed-phase or in some affinity-chromatography separations. Once a mode of interaction has been chosen, the various separation methods (isocratic or gradient elution, stepwise elution, displacement, or frontal analysis) are conveniently called *modes of operation* (4). For many separations, the best mode of interaction is easily specified, and scale-up or optimization focuses on choosing the mode of operation.

The appropriate mode of interaction for a given feedstock is often clear, and the choice lies only in picking the best mode of operation. In industrial practice, several constraints often restrict the selection to isocratic elution, sequential stepwise elution, or gradient elution.

Thermodynamically driven separations. The most effective form of sequential stepwise elution involves choosing appropriate levels for the mobile phase modulator (for example, salts in ion-exchange or organic solvents in reversed-phase) in each step so that only desired product, free of impurities, elutes in one of the steps. (If several components of the feedstock are to be recovered, each component must elute alone in its own modulator step.)

Under these conditions, selectivity becomes irrelevant because the components are never found together. Hence the column plate count becomes unimportant. This sort of sequential stepwise elution schedule, which can be called thermodynamically driven, can be used when feed components exhibit *on–off* binding, that is when feed components are either very strongly bound or almost completely unbound. This kind of "all-or-nothing" adsorption can be used for macromolecules (5,6) and is often exploited in solid-phase extractions. Under these conditions, large particles can be used with impunity, both at bench and at process scales. The only problem in scaling up such separations is the possibility of overloading the column too heavily. Because the product is selectively displaced

from binding sites by more retentive impurities, an overloaded column can cause the product to move faster than expected and emerge in more than one step of the stepwise elution schedule. That problem can be avoided by reducing the loading or increasing the column length.

Kinetically driven separations. Many separations of practical importance cannot be run in a sequential stepwise manner. The product peak comigrates with one or more impurities implying that kinetic factors will have an effect in determining the extent of mixing between adjacent peaks in the chromatogram, and therefore affects recovery and production rates (1). *Gradient elution* is a kinetically driven separation method that is particularly important for biological products. The primary step for using gradient-elution chromatography is screening a variety of resins so that the optimal resin is chosen. That choice is made by ensuring that the objective is clear, identifying the nature of the separation problem, looking for literature precedents, and using prior experience with the product. Input from your chromatographic media and instrumentation vendors can also be useful at this stage.

LAB-SCALE PROCESS DEVELOPMENT

The thermodynamic component of method development can be simple and rapid. For many separations, the choice of the stationary-phase method is more important than the choice of mobile phase (particularly for ion-exchange runs in which standard salts are used as mobile phase modulators). Of course, specific binding often requires the use of special additives to the mobile phase, but this article refers only to the stationary phase to help make the general approach clear.

Holding one phase constant. To develop the most efficient separation process, we use a standard mobile phase, then rapidly and equitably screen a variety of stationary-phase resins reducing the problem to one of "resin screening." Once resin candidates are available, we screen to select the best resin for performing a particular separation — perhaps the most important step in method optimization (3,7–11).

Resin screening. The algorithm shown in Figure 1 and the screening approach we suggest in the "Two-Stage Approach to Resin Screening" sidebar use ion-exchange chromatography in the gradient mode of operation, but the decisions in that figure and in the resin selection steps can be generalized to other contexts.

In most cases, the primary criterion for resin screening is selectivity. However other screening

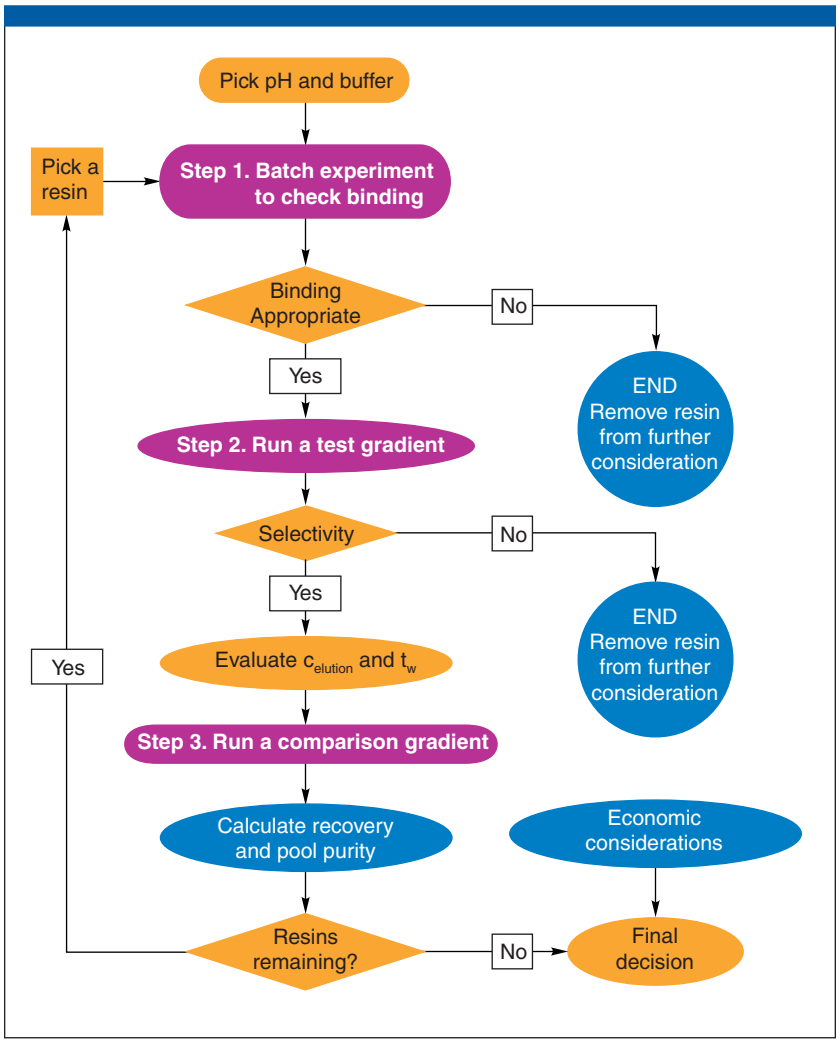

Figure 1. Resin screening protocol (7)

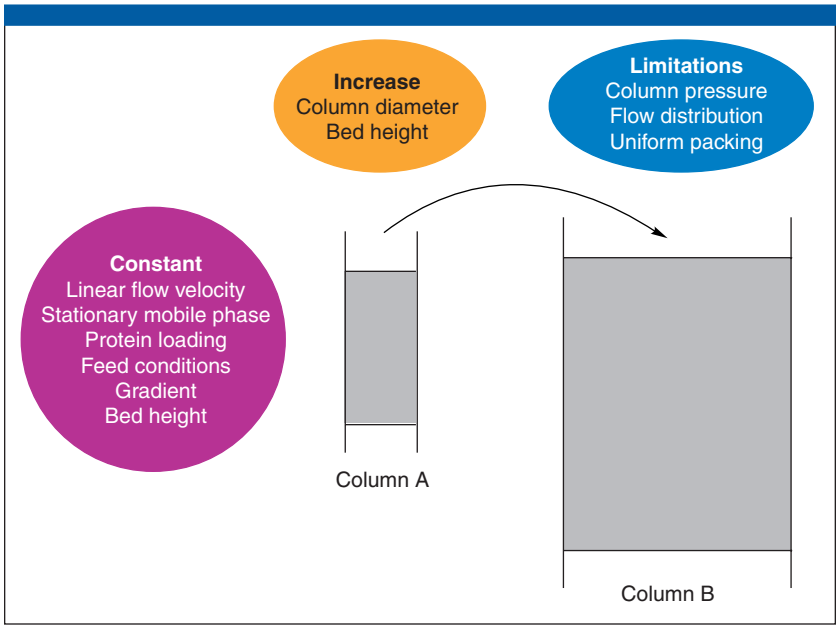

Figure 2. Scaling up from bench or laboratory production to pilot plant production

A Two-Stage Approach to Resin Screening

To develop the most efficient separation process, we use a standard mobile phase, then rapidly and equitably screen a variety of stationary-phase resins reducing the problem to one of resin screening as shown in Figure 1. Once resin candidates are available, we use a two-stage process to select the best resin for performing a particular separation. In our example, we have used ion-exchange chromatography in the gradient operation mode, but the decisions in these resin selection steps can be generalized to other contexts.

Stage 1

Pack all stationary phases into columns of identical size. If possible, run all columns at the same flow rate. (Running at the same flow rate is not always practical. For instance, if the particle sizes available for different stationary phases are markedly different, pressure drop constraints can limit the range of flow rates.)

Run a test gradient that spans a wide range of modulator level to facilitate retention. Make the gradient as shallow as practicable to get as much resolution simultaneously as possible under these conditions.

Exclude stationary phases that exhibit little or no product retention at this stage. You can also exclude stationary phases in which little resolution is found between the product and the primary impurities. That decision, however, should be made carefully because the test gradient may be a poor indicator of a sorbent's resolution. In other words, a sorbent may provide poor resolution of the product under the test gradient, but high resolution under another gradient, so excluding a resin at this stage should usually be based on additional supporting data.

Stage 2

In this stage of resin selection, parameters such as the feed loading and equilibration buffer should be kept the same for all stationary phases. If the same flow rate was used for all runs in Stage 1, then maintain that flow rate at this stage. If different sorbents were run with different flow rates, use the same flow rate used in Stage 1 for each sorbent in this stage.

Determine a tailored comparison gradient for each of the stationary phases remaining. The comparison is intended to show each sorbent under its most effective conditions for the given feed mixture. The comparison gradient is centered around the modulator concentration at which the product eluted in the test gradient in Stage 1. By making the assumption that, all other parameters being constant, the band spreading of the peaks is inversely proportional to the gradient slope, we get equation 1.

$$\alpha \times w = \beta \times m \quad (1)$$

where α and w are respectively the gradient slope and the product peak width in the test gradient, and β and m are the corresponding parameters in the comparison gradient. If we require that all comparison gradients have the same time (for standardization), then the starting and ending modulator concentrations (c_x and c_y respectively) can be determined from the following equations (2 and 3)

$$c_x = c_{elution} - \left(\left(\frac{\alpha}{2}\right)\left(\frac{n}{m}\right)\left(\frac{t_w F}{V}\right)\right) \quad (2)$$

$$c_y = c_{elution} + \left(\left(\frac{\alpha}{2}\right)\left(\frac{n}{m}\right)\left(\frac{t_w F}{V}\right)\right) \quad (3)$$

where $c_{elution}$ and t_w are respectively the concentration and the time at which the center of the product peak eluted in the test gradient, n is the number of column volumes in which the comparison gradient is run, F is the flow rate, and V is the column volume. If the beginning concentration c_x is found to be negative, it is set to zero. Now, the usual quantitative parameters of production rate and recovery and purity can be used to determine which stationary phase is best.

The assumption that peak width is inversely proportional to the gradient slope is an approximation and will be invalid or fail in some situations (if there is significant competition among the product and impurities for binding sites on the adsorbent, for instance). However, the assumption is likely to be a reasonable approximation for many separations. More detailed methods of this kind can be established (15), but usually require more data for each sorbent. Similarly, optimal gradients have been determined in normal and reversed-phase systems through numerical optimization of the governing equations (16); this optimization is a significant advance in the field, but is not yet at the level of accessibility in which industrial practitioners would use it routinely. Our two-stage approach was chosen for its simplicity and for its ease of use in an industrial context (7).

criteria can also be identified and used for particular separation problems. The general resin selection approach that we describe has two stages that focus on a comparison gradient as shown in the resin screening sidebar. A comparison gradient is an equitable way of comparing different stationary phases for a given feed mixture because each stationary phase has a gradient that is optimized for its particular retention pattern. With that comparison available, the typical quantitative parameters (production rate, product recovery, and product purity) can be used to determine which stationary phase is best.

Additional resin selection considerations. The simple approach we outline provides a rapid selection tool for choosing the best resin. If the chromatography step of process development is intended for preparative-scale operation, particularly for commercial manufacture, other issues must be addressed before final resin selection: the resin cost, the physical and chemical stability of the resin at bed height, the number of cycles the resin will be reused at the manufacturing plant, the media availability for commercial-scale demand, the resin lifetime, the leaching of ligands, the regulatory support files offered by the vendor, and the batch-to-batch variation in resin quality, among others.

Optimization of kinetics at bench-scale. Once the stationary and mobile phases have been chosen, the optimal kinetics or operating conditions need to be determined. These are the studies that determine particle size, column dimensions, optimal gradient slope, and feed loadings.

Operating conditions can be optimized by using experiments to evaluate the effect of different operating parameters that affect resin performance selectivity and protein loading. These parameters can include mobile-phase conditions (pH, organic content, and buffer composition, for example) and gradient (slope) design. Optimum mobile-phase conditions and the gradient design are then chosen from the experimental data obtained.

Flow velocity and protein loading are then evaluated for their effect on separation quality. Resin performance, bed height, protein loading, and flow velocity should offer satisfactory resolution and cycle times. Laboratory experiments should be done at the bed height that will be used at pilot scale to obtain comparable column performance data after scale-up. A detailed analysis of the interaction among these kinetic parameters is complicated. In industrial practice, a heuristic approach similar to the one we describe is often used and is likely to produce effective, if not necessarily optimal, operating conditions in the

Figures 2 and 3 illustrate a common method for scale-up for pilot operations: handling increased volume by increasing column diameter, keeping column volume proportional. But such simple "volumetric" scale-up is not always appropriate. The quantitative analysis we describe can be used to show whether a volumetric scale-up approach can be used.

Because the quality of the separation achieved at small scale needs to be maintained but the optimal operating conditions for different scales of operation are unknown, we settle for determining effective, near-optimal operating conditions at bench scale. Effective scale-up rules should then produce comparable results at larger scale.

The usual approach to scale-up is to hold the plate count constant and increase the feed volume and column volume proportionately.

If subscript *b* describes the parameters at bench scale and *l* the parameters at large-scale, we have equations 4 and 5

$$N_l = N_b \quad (4) \qquad \frac{V_{feed,l}}{V_{column,l}} = \frac{V_{feed,b}}{V_{column,b}} \quad (5)$$

where N is the number of plates, V_{feed} the feed volume, and V_{column} the column volume.

If band spreading is dominated by pore diffusion, as is often the case (1,2), then the plate count can be described by using equation 6

$$N = K\frac{L}{u d_p^2} \quad (6)$$

where L is the column length, u is the mobile phase linear velocity, and d_p is the particle diameter. The proportionality constant K includes geometrical factors (such as phase ratio) and thermodynamic factors (such as retention factor). The plate count can also be derived from the van Deemter equation (14), when A and B terms in the van Deemter equation are negligible relative to the C term, and the C term dominated is by pore diffusion.

By combining equations 4 and 6, we get

$$\frac{L_l}{u_l d_{p,l}^2} = \frac{L_b}{u_b d_{p,b}^2} \quad (7)$$

Equation 7 represents one constraint on the three variables L_l, u_l, $d_{p,l}$. Because this approach is based on mimicking large-scale results from bench-scale results, the variables L_b, u_b, $d_{p,b}$ are assumed to be known.

Using Darcy's Law in the following form (equation 8) can help us quantify the pressure drop across the columns

$$u = k\left(\frac{d_p^2}{\mu}\right)\left(\frac{\Delta p}{L}\right) \quad (8)$$

where k is the permeability *without* any dependence on particle diameter, (which has been factored out) and μ is the mobile phase viscosity. In general, the best results are obtained at the maximum allowable pressure drop (1). If the maximum permissible pressure drop is different at bench and large scales, we use equation 9

$$\frac{u_l L_l}{d_{p,l}^2} = P\left(\frac{u_b L_b}{d_{p,b}^2}\right) \quad (9)$$

where P is the ratio of maximum pressure drops at large and bench scale. More commonly, in cases where P=1, the result becomes equation 10

$$\frac{u_l L_l}{d_{p,l}^2} = \frac{u_b L_b}{d_{p,b}^2} \quad (10)$$

Dividing our combined equation (equation 7) by the equation above (equation 10) gives the simple expression shown in equation 11

$$u_l = u_b \quad (11)$$

Thus equality between plate counts and between maximum pressure drops leads to equality between mobile phase velocities across scales. Substituting equation 11 into either our combined equation 7 or the equation for different maximum pressure drops at bench and large scales (equation 10), gives us the familiar result in equation 12.

$$\frac{d_{p,l}^2}{L_l} = \frac{d_{p,b}^2}{L_b} \quad (12)$$

Typically, the choice of particle size is limited during large-scale processing by cost or availability. Once a particle size is chosen, the previous equation specifies the column length.
An approximate theoretical calculation for the optimal length $\frac{d_p^2}{L}$ is given in the article by Guiochon et al. (1). That equation can also be used to give us another estimate of the column length, given the particle size.

To determine the column diameter at large scale, equation 5 can be rewritten as equation 13.

$$\frac{V_{feed,l}}{V_{feed,b}} = \sigma = \frac{V_{column,l}}{V_{column,b}} = \left(\frac{L_l}{L_b}\right)\left(\frac{D_{c,l}^2}{D_{c,b}^2}\right) \quad (13)$$

In equation 13, σ is the scale-up factor (which must be specified before scale-up can begin), and D_c is the column diameter. Because the column length at large-scale has been determined from equation 12, the column diameter can be obtained from equation 14.

$$\frac{D_{c,l}}{D_{c,b}} = \left(\sigma\frac{L_l}{L_b}\right)^{\frac{1}{2}} \quad (14)$$

In cases in which the particle diameter is kept constant during scale-up, we obtain the scale-up results by "volume overloading" (from equation 12) and the column length remains constant (in addition to the particle diameter and the mobile phase velocity). So scale-up in those cases consists simply of increasing the column diameter by a factor of $\sqrt{\sigma}$.

The results in these equations have been obtained for pore diffusion as the process that dominates band spreading. Generalizations of the method to mass-transfer as the controlling step, and the effects of nonlinear adsorption, are summarized in a recent review (17). This approach remains valid for many kinds of overloaded separations. Further discussions on the effects of nonlinear adsorption on scale-up can also be found in the literature (1,2).

These calculations assumed that the phase ratio remained constant upon scale-up. That is not always a good assumption when the overall scale-up factor is very large (above 100). A recent example of such variations in phase ratio is described by Heuer et al. (18). In practice, it is worthwhile to estimate the phase ratio experimentally at each scale and use the results above with caution if the phase ratio changes appreciably with scale.

Experimental techniques to avoid changes in packing structure on scale-up include axial compression, radial compression, and annular expansion (mixed radial and axial compression). Useful reviews of these packing methods are found in several references (19–21). In many cases, the approaches suggested have resulted in improved column performance.

Practical Scale-Up Guidelines

Scaling up chromatography columns — each with its own special requirements and biological product — means coping with the key issues that surround various column parameters.

Bed stability (physical)

- Bed instability is caused by lack of support from the column wall and is an issue with columns of >25–30 cm, packed with compressible media (24).
- Bed instability leads to possible redistribution of particles during operation and maldistribution of flow across the column.
- Bed stability is very important for nonrigid gel media and instability can restrict maximum usable bed height (24).
- To improve bed stability, stacked columns can be used so that the pressure difference across each section is reduced (25).

Bed stability (chemical)

- Chemical bed instability can be caused by leaching of ligands (affinity chromatography) or deterioration of reused matrices from harsh cleaning or regeneration conditions (silica packings at high pH) or irreversible binding at the packing surface (24).
- Chemical bed stability becomes a critical issue in commercial manufacturing because the media is typically reused 50–200 times.

Product loading

- Beyond a point, increasing the product loading (mg product per mg adsorbent) reduces resolution.
- It is common to determine the maximum product loading for which acceptable resolution is achievable at lab scale, and then operate the large-scale column at 80–90% of that maximum.

Gradient slope

- Gradient elution often provides higher efficiency, concentrated products, and reduced process time and solvent consumption.
- At large scale, forming accurate and reproducible gradients can be challenging.

Flow distribution

- In large columns, it is difficult to achieve uniform flow distribution; failure to do so can lead to peak tailing.
- The use of a flow distributor at the column inlet is the most common solution for achieving uniform flow distribution (24).

Packing quality

- In large columns, obtaining a homogeneously packed column is very difficult.
- Channeling in the column leads to peak broadening or peak splitting.
- Common solutions to this problem are packing with axial compression and the use of self-packing columns (21).

System design

- Considerable dead volume can arise out of piping, valves, flow meters, air sensors, and other utilities in a large-scale chromatography system.
- Extra-column volume leads to dilution, higher pressure drops, and is a source of peak broadening.
- Bypassing air traps and filters during sample loading and choosing tube diameters to achieve turbulent flow and reduce undesirable axial mixing can help (26).
- It is better to design the system so that all inlet sources are at or above column inlet and all outlet sinks are at or below column outlet, ensuring minimal hydrostatic head on the column.

Fraction collection

- Peak width and shape at large scale can fluctuate because of lot-to-lot variations in media and in reused media, so the need for fraction collecting should be reexamined.
- If fractions need to be collected, the detector must be kept close to the fraction collector to make the collection accurate, and the collection scheme should be robust enough to suit the manufacturing environment.

Media availability

- Other factors that must be considered when choosing the media for large-scale operations include long-term availability of the required amounts of media, cost, lot-to-lot consistency, resin lifetime, and supporting documentation to aid the regulatory filing (27).

Costing

- Cost analysis needs to be done when moving to large scale.
- Key contributions to cost are raw materials (including resin costs), facility costs, and capital costs.

hands of an experienced practitioner. Detailed analyses of such optimizations have been published elsewhere (12).

A QUANTITATIVE MODEL FOR SCALE-UP

The performance of a chromatographic column depends on a variety of design and operating factors. To facilitate scale-up, you need to maintain kinetic (particle size, pore size, ligand chemistry, temperature, mobile phase) and dynamic (bed height, flow velocity, packing density) equivalence between the chromatography columns used in the laboratory and those used in the pilot plant.

Kinetic and dynamic equivalence can be maintained by using the identical stationary and mobile phases in the two columns and operating them at identical bed height, linear flow velocity, protein loading (mg protein per mL resin), feed conditions, gradient length, and slope (3). To handle the increased load volume on a column at pilot scale, the most common procedure is to increase the column diameter so that the column volume increases proportionately (13). This keeps the residence time and the stability of the product constant.

Scouting experiments (to find optimal conditions) in the laboratory are typically done in small columns to minimize consumption of stationary and mobile phases; this also permits several runs

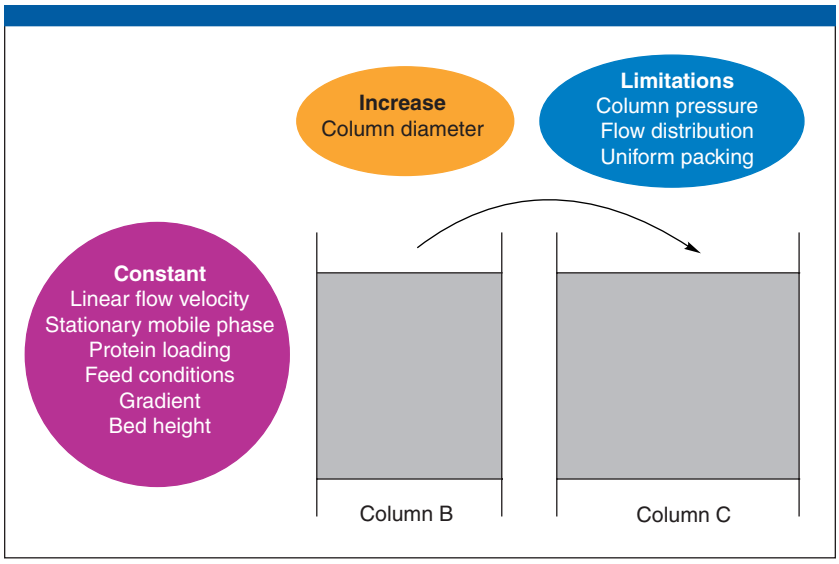

Figure 3. Scaling-up from pilot plant production to commercial production

to be done simultaneously. However, the bed height must remain constant during scale-up, so the best scouting approach is to perform the final optimization steps at the bed height that will be used later at pilot plant and commercial scale (See Figures 2 and 3).

These general considerations are frequently used in industry as the basis for scale-up. But such simple "volumetric" scale-up is not appropriate in all cases. The quantitative analysis we describe in the "Scale-Up Calculations" box can be used to show when volumetric scale-up can be used.

SCALE-UP CONSIDERATIONS

The basic idea behind scale-up is to preserve the quality of the separation achieved at small scale (14). Implicit in our approach to scale-up is the admission that we are not yet able to determine optimal operating conditions a priori for different scales of operation. So we settle for determining effective, near-optimal operating conditions at bench scale. Effective scale-up rules should then produce comparable results at larger scales. A typical scale-up from laboratory to pilot plant is on the order of 50- to 100-fold. And that increase is frequently followed by another 10- to 50-fold scale-up from pilot plant to final commercial manufacturing scale.

Many parameters affect the success of scale-up, including the resin stability (physical and chemical), the product, the equipment (flow distributor, fraction collector, packing quality, system design), and the operating conditions (product loading, gradient slope). The "Practical Scale-Up Guidelines" sidebar summarizes the various issues that are associated with these parameters and points to the pitfalls that can be encountered during scale-up.

Although isocratic and gradient elution are the most common modes of operation (with displacement chromatography having a small but significant role), there are other modes that can be used at a large scale. Frontal chromatography has always played an important role at large scales, under the guise of "adsorption steps" in a variety of applications, especially in the chemical industry. Because feed introduction in isocratic, gradient and displacement runs are nothing more than frontal chromatography — clearly an important part of a run. The "Comparison of EBA and SMB" box summarizes the key issues for two other modes of chromatography: expanded bed adsorption (EBA) and simulated moving bed (SMB) chromatography. Although the basic scale-up rules we have outlined apply to both EBA and SMB techniques, the additional considerations for these two modes of operation are discussed in the sidebar.

Just as each mode of operation has its practical considerations, there are many practical issues

Comparison of EBA and SMB

Expanded-bed adsorption (EBA) and simulated moving bed (SMB) chromatography are two different modes of operation that have some additional considerations.

Expanded-Bed Adsorption Chromatography

- EBA consists of specially designed particles fluidized in a column with controlled flow distribution to provide large numbers of plates with minimal back mixing (28).
- Stability of the expanded bed is a critical element for a successful scale-up of an EBA column and is affected by the flow distribution, flow velocity, composition and properties of the feed, particle size distribution of the packing, and feed–media interactions (28).

Simulated Moving Bed Chromatography

- Mobile phase and feed components are injected into and withdrawn from a ring of chromatography columns at points that are rotating between the columns during the process.
- SMB is emerging as the mode of choice for enantiomeric separations (29).
- Stability of the different zones depends on an accurate and precise control of operating flow rates and valve switching times of the feed and eluent streams (29).

Comparison of Different Chromatography Interaction Modes

Each mode of interaction has various practical considerations during scale up.

Ion-exchange chromatography (IEC)

- IEC is commonly used for protein separation because of its high dynamic capacities, low relative media costs, the ability to use simple buffers, high usable flow rates, robustness, scalability, and ease of operation.
- Scale-up is relatively easy to perform following the guidelines mentioned in this article and in the literature (2).

Hydrophobic interaction chromatography (HIC)

- Denaturation of the product can result from the use of high salt concentrations.
- High viscosity of the mobile phase can reduce the accuracy and reproducibility of the gradient formation and limit the maximum bed height that can be used (30).
- HIC separations are typically sensitive to temperature variations in buffers or in the surrounding environment.

Size-exclusion chromatography (SEC)

- In most cases, the maximum bed height and column diameter that can be used are limited by the physical stability of the medium. This is commonly resolved by using a series of smaller columns.
- Although separation time increases linearly with bed height as with other modes, the resolution only increases as a square root of bed height.
- Resolution depends on the feed volume (relative to the column size). It has been suggested that desirable cycle time and resolution can be obtained when scaling up an SEC column by selecting the bed height and linear flow velocity, then varying the feed volume to fine-tune the resolution. The column diameter is changed to meet productivity requirements (31).
- Typically, to achieve an acceptable separation, feed volume needs to be 1–5% of column volume.

Reversed-phase chromatography (RPC)

- RPC is popular for separating small molecules and is limited in protein applications because of issues about the use of organic or volatile solvents in purification processes (such as protein denaturation and unfolding, waste handling, possible need for explosion-proof equipment, and environment concerns).

Affinity or metal (chelate) chromatography

- These techniques offer short processing times and high specificity and resolution and are particularly useful when the target is present in low concentrations in a complex mixture.
- Media are generally expensive and can be unstable under certain operating conditions (ligand leaching).
- Resins are often compressible, so physical stability of the bed can be an issue (32,33).

peculiar to each mode of interaction. The general features of the various modes are presented briefly in the "Comparison of Different Chromatography Interaction Modes" sidebar.

SCALE-UP EXAMPLE

Figure 4 shows an example of scale-up of an anion-exchange chromatography column. Both the separations were performed, as suggested in Figure 2, with identical stationary and mobile phase conditions, linear flow velocity, protein loading, feed conditions, and gradient slope. However, the column volume increased 600-fold between lab scale (1.6 cm diameter, 5 cm height, 10-mL volume) and pilot scale (20 cm diameter, 20 cm height, 6.3 L volume). Purity of the different column fractions, as measured by the analytical anion-exchange chromatography assay, is plotted in Figures 4a and 4b. The scale-up was successful because the elution patterns for both the product and the impurities are identical. Purity for the final column pools was estimated to be 94% at lab scale and 99% at pilot scale. The step yield for the column also compared favorably between the two scales (51% at lab scale and 61% at pilot scale). This example underlines the importance of following the above-mentioned guidelines for scale-up of a chromatography column.

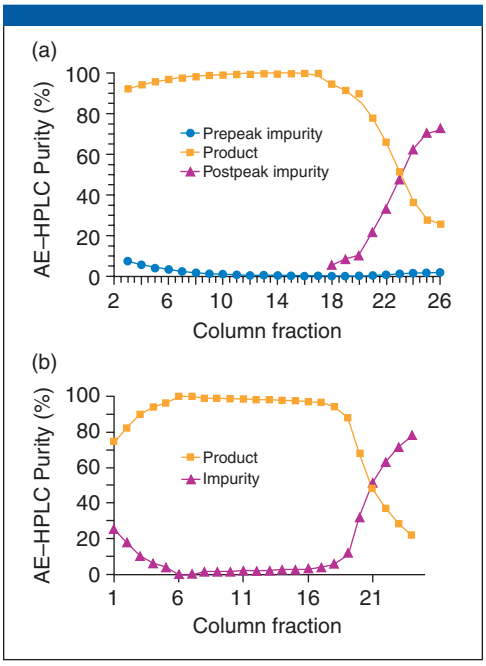

Figure 4. Scale-up of an anion-exchange (AE) column from laboratory production to pilot plant scale; (a) purity of column fractions by AE high performance liquid chromatography (HPLC) at lab scale to (b) purity of column fractions by AE-HPLC at pilot plant scale

CHROMATOGRAPHY COLUMN SCALE-UP

This article provides an overview of the basic principles and practices of scale-up in preparative chromatography. Modes of interaction and modes of operation are defined to clarify the options available during scale-up. Because it is first necessary to optimize a bench-scale separation before attempting to scale it up, attention is first fo-

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cused on method development at bench-scale. Thermodynamically driven separations are based on finding an appropriate sequential stepwise elution schedule and are trivial to scale up. Kinetically driven separations are exemplified by gradient elution, with resin screening on a rational footing. Finally we presented a simple scale-up method and practical considerations that often arise during scale up of a column. ***BPI***

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