ENCYCLOPEDIA OF

BIOPROCESS TECHNOLOGY: FERMENTATION, BIOCATALYSIS, AND BIOSEPARATION

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See also ADSORBENTS, INORGANIC; ADSORPTION, EXPANDED BED; ADSORPTION, PROTEIN, BATCH; ADSORPTION, PROTEINS WITH SYNTHETIC MATERIALS; CHROMATOGRAPHY, SIZE EXCLUSION; MEMBRANE CHROMATOGRAPHY.

CHROMATOGRAPHY, RADIAL FLOW

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KEY WORDS

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Protein
Purification
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Scale-up
Separation

OUTLINE

INTRODUCTION

The production of a modern biotechnology product, typically a recombinant protein, requires a multistage downstream process. Such a process usually centers on two or more liquid chromatography steps. As production scales escalate, the columns used become larger and more expensive. It is not uncommon to have bed volumes reaching hundreds of liters at industrial scales.

Radial flow columns were first used for gas–solid catalytic reactions in large packed beds. They were designed to increase gas flow rate and reduce pressure by increasing the cross-sectional flow area. Radial flow chromatography (RFC) columns first entered the commercial biotechnology market in the mid-1980s (1). RFC was intended as an alternative to the conventional axial flow chromatography (AFC) for preparative- and large-scale applications. In a radial flow chromatography column (Fig. 1), the mobile phase flows in the radial direction, not in the axial direction. The mobile enters from the outside tube and merges into the center tube (Fig. 2). In comparison to a slim AFC column, an RFC column provides a relatively larger flow area and a shorter flow path. It allows a higher volumetric flow rate with a lower bed pressure. The effect is equivalent to using a short pancakelike AFC column (Fig. 3). Pancakelike AFC columns are quite common in industrial applications. They are available from most major commercial column vendors. In scale-up to accommodate large feed loads, it is impractical to increase the column height of AFC columns by too much because excessive bed pressure drop will result. This is especially troublesome when soft gels are used. Thus, pancakelike AFC columns are used.

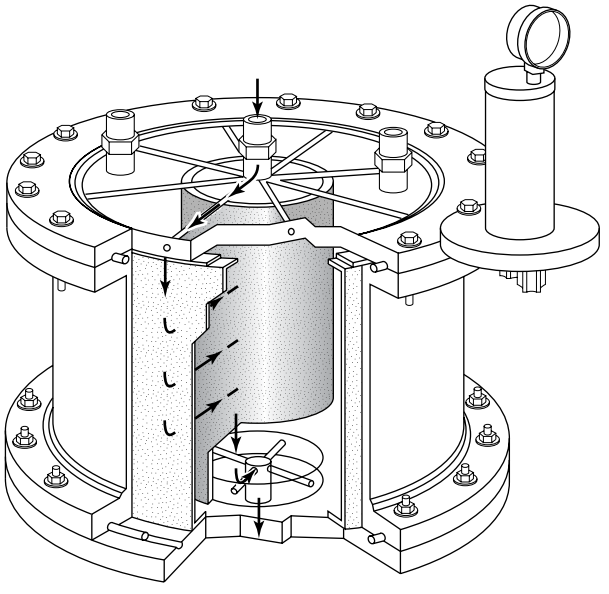


Figure 1. Diagram of an acrylic Superflo® column. *Source*: Courtesy of Sepragen Corp.

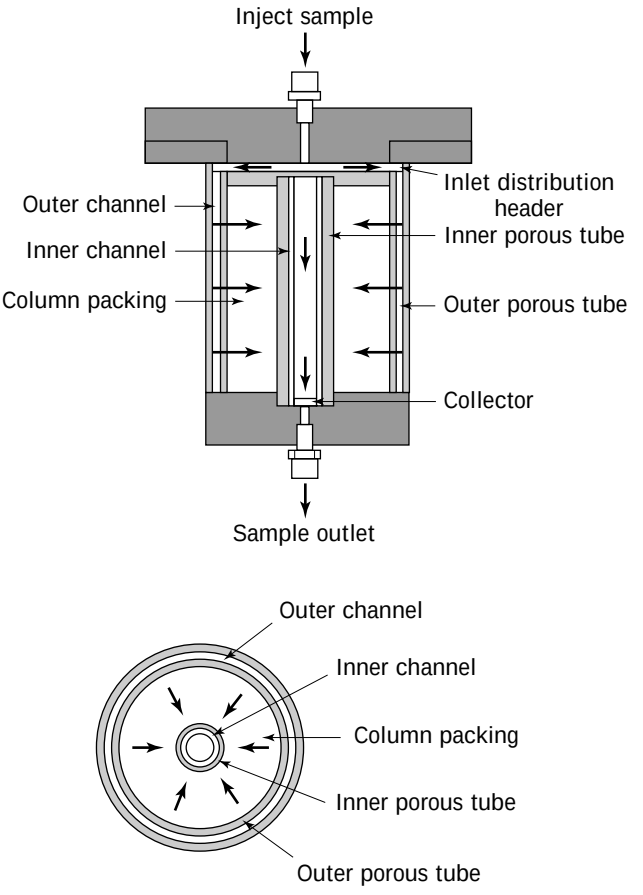


Figure 2. Anatomy of a Superflo® column. *Source*: Courtesy of Sepragen Corp.

This article is devoted to the discussion of the applications of RFC columns in bioseparations and RFC modeling and scale-up issues. Applications examples will be provided based on existing literature. A general rate model will be presented for the modeling of RFC. Experimental and theoretical comparisons between AFC and RFC will be discussed.

RADIAL FLOW COLUMN CONFIGURATIONS

In 1947, Hopf (2) described a radial chromatography device. The device had a feed pipe in the center. The outward liquid flow in the radial direction was forced by the centrifugal force when the device was rotated. He called this device a chromatofuge. Such a device was obviously too complex and expensive for large-scale industrial application, and thus it was not adopted in biotechnology.

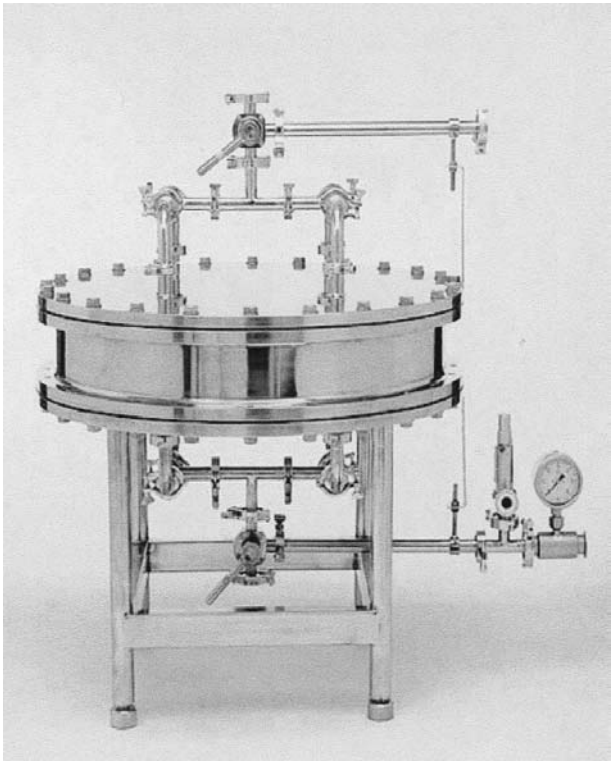


Figure 3. A large-scale pancakelike axial flow column. Courtesy of Pharmacia Biotech, Inc.

To date, two commercial companies have marketed RFC columns. The first one is CUNO, Inc., in Connecticut. They marketed the Zetaffinity series of preparative-scale radial flow cartridges, which looked like a spiral-wound filtration cartridge (Fig. 4). Such a design obviously was rooted in the fact the CUNO is a major manufacturer of industrial filtration systems. The packing for CUNO's RFC cartridges

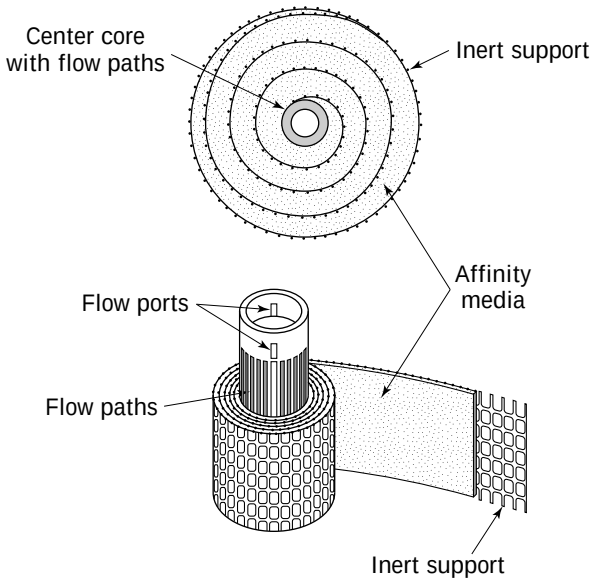


Figure 4. Structure of a Zetaffinity® cartridge.

was based on modified cellulose (3). These cartridges were used for affinity chromatography, hence the trade name Zetaffinity. CUNO discontinued its Zetaffinity product line in 1991.

The second vendor and manufacturer of RFC columns is Sepragen Corporation in Hayward, California. Their RFC product line carries the trade name Superflo®. Sepragen markets unpacked RFC columns ranging from 50 mL to 200 L. Figure 5 shows several Superflo columns with a similar diameter but different column heights. Superflo columns come in stainless steel, acrylic, polycarbonate, and polyethylene. They can sustain a pH range of 2 to 12 and a maximum pressure of 50 psi. They are used by major biopharmaceutical companies at production scales. Sepragen's president owns a U.S. patent (4) on the design of unpacked RFC columns. Figure 1 shows an acrylic RFC column with inward radial flow. The feedstream enters from a center input port at the top. It is then distributed through several flow channels to the outer shell and subsequently enters the packing media in the radial direction toward a center collection tube. The effluent exits the tube through an outlet port at the bottom. The two ports next to the center input port at the top in Figure 1 are packing ports used during media packing. A bubble trap with a pressure gauge sits on the edge of the column's top lid. Figure 2 is an anatomical view of a Superflo column. Superflo columns usually use inward flow instead of outward flow because it is more difficult to distribute outward flow without increasing flow distortion caused by gravity. The other reason for not using outward flow is because inward flow provides slightly sharper peaks than outward flow based on computer simulation (5). Outward flow is usually used for the packing or regeneration of a RFC column.

PACKING PROCEDURES FOR RFC COLUMNS

Because of their structure, CUNO's Zetaffinity cartridges do not require special packing procedures. Sepragen's Superflo columns are packed by first displacing air using outward flow with a buffer solution. Packing slurry is then pumped into the column through the two packing ports on the column top surface (Fig. 1). Excess buffer is squeezed out of the column through the input port on the column top surface. After that, the column is ready for use.

PRESSURE DROPS OF RFC COLUMNS

Because of a short flow path and a low pressure drop, both CUNO's and Sepragen's RFC devices exhibit a highly linear relationship between bed pressure drop versus flow rate. Figure 6 shows a linear relationship between bed pressure and flow rate for an 800-mL Zetaffinity cartridge studied by Huang et al. (3).

Figure 7 shows the pressure drops for a Superflo 20 L column and a Superflo 50 L column packed with several different soft-gel chromatographic media (6). It indicates that the pressure drops are quite low at relatively high flow rates. At lower flow rates, the pressure drop curve is linear. This behavior is similar to that of short pancakelike AFC columns.

Figure 5. Superflo columns. *Source*: Courtesy of Sepragen Corp.

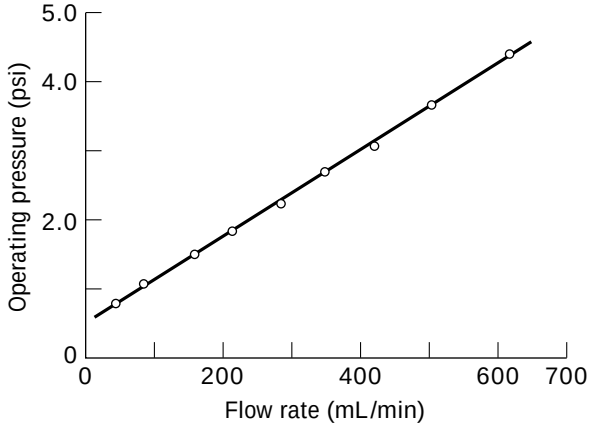


Figure 6. Pressure drop vs. flow rate for an 800-mL Zetaffinity cartridge.

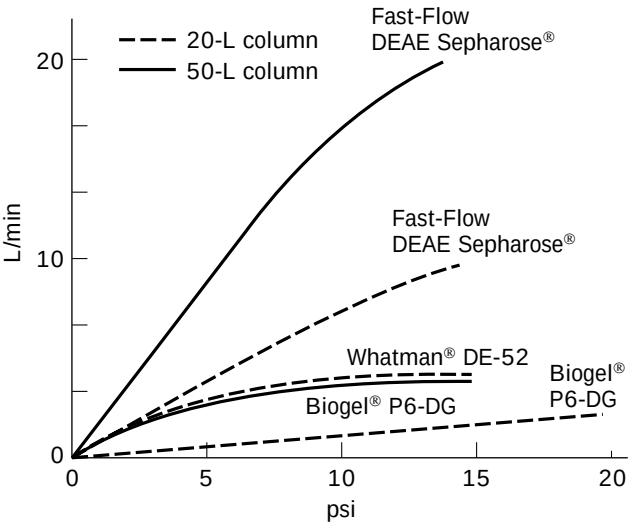


Figure 7. Pressure drop vs. flow rate for 20-L and 50-L Superflo® columns. *Source*: Courtesy of Sepragen Corp.

COMPARISON OF RADIAL FLOW AND AXIAL FLOW COLUMNS

Saxena and Weil (7) did an experimental case study of the comparison of RFC and AFC. They used a 100-mL axial flow glass column (Econocolumn®) with 2.5-cm i.d. from BioRad Laboratories (Richmond, Calif.) and a Superflo-100 RFC column from Sepragen with a bed volume of 100 mL. Both columns were packed with QAE cellulose with the following procedures. The Superflo column was packed using a 25% slurry (with 0.5 M NaCl) that was pumped in through the two packing ports at a flow rate of 30 mL/min. The final QAE cellulose density after packing was 6 mL per dry gram. The Superflo column was packed in about 20 min. The AFC column was packed using 50% slurry added from the top of the column. After the liquid was drained and the bed was settled, additional slurry was added. This process was repeated until the same amount of QAE cellulose was packed into the AFC column. The two columns were used to separate two 10-mL ascites fluid samples. The samples were pretreated by dialyzing the ascites fluid with 10 mM phosphate buffer of pH 8.0 for 2 days with three changes. Precipitates and debris were then removed by centrifugation. Stepwise salt gradients were used. Figure 8 shows the comparison between the 100-mL

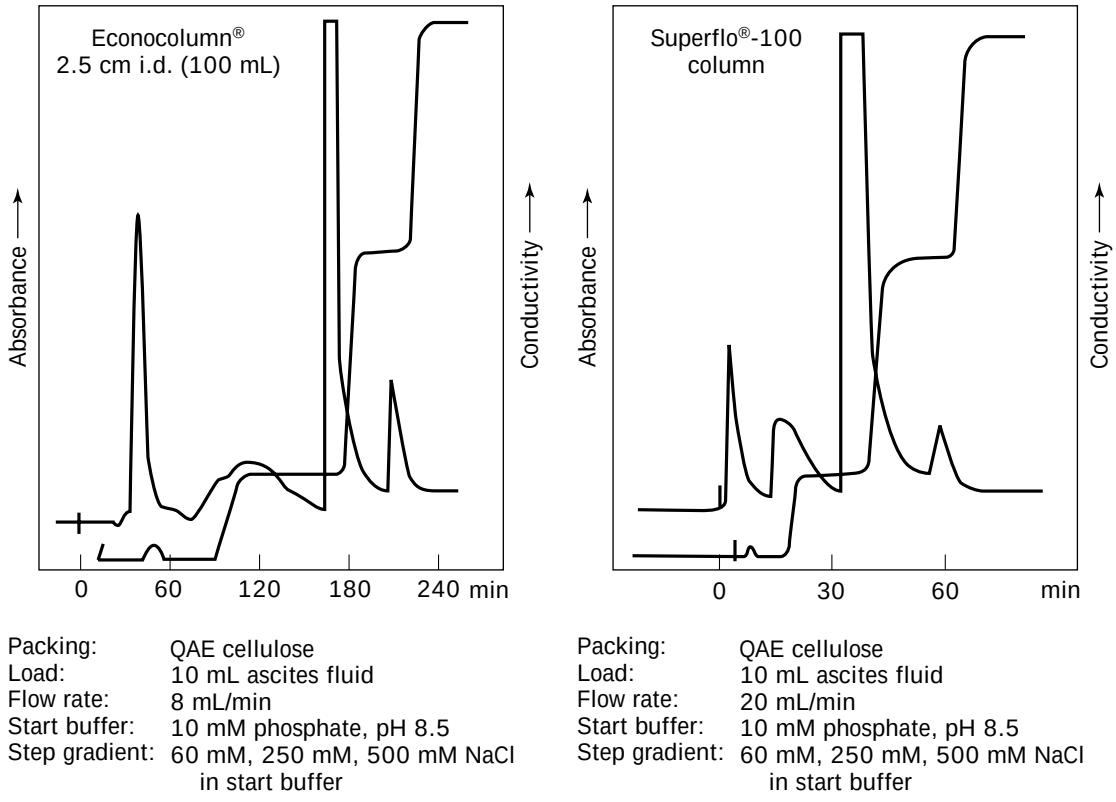


Figure 8. Comparison of AFC and RFC columns. *Source*: Courtesy of Sepragen Corp.

AFC column and the 100-mL Superflo-100 RFC column. From Figure 8, it can seen that the RFC column achieved similar separation results with much less time. In this case, the two columns were equivalent only in bed volume. A stricter comparison should use a pancakelike, short AFC column with its bed height about the same as the packing's thickness in radial direction for the RFC column, and both columns should have equal bed volume. The case study discussed next is close to satisfying these conditions.

Tharakan and Belizaire (8,9) used a 50-mL RFC column with a bed height of 0.95 cm and packing thickness of 3.0 cm in the radial direction and a 50-mL AFC column with a bed height of 2.8 cm. Both columns were packed with Sepharose CL2B resin-containing monoclonal antibody from Pharmacia (Piscataway, N.J.). They studied the purification of a protein called factor IX. Their experimental results indicated that the two columns gave similar purification results. This was expected because the AFC column was pancakelike. Tharakan and Belizaire (8) also packed the same column with S-200 Sephacryl size-exclusion gel from Sigma Chemical Co. (St. Louis). The protein band was more diffused using RFC than AFC.

Lane et al. (10) compared the performance of a Superflo-100 RFC column and a 6.6 $\times$ 4.4 cm i.d. AFC column for the separation of egg-white proteins. Both columns had a nominal bed volume of 100 mL. They were packed with the same media for comparison. Two anion-exchange cellulose media were tested, Whatman DE52 and QA52 from Whatman Specialty Products Division (Maidstone, U.K.). Egg whites were first separated from fresh hen eggs and then treated with a buffer. After treatment with a cell debris remover, egg-white suspensions were filtered using filter paper. The samples for chromatography had a protein concentration of 14 mg/mL. Sample load volume was 40 mL. After sample loading, the column was washed with a buffer. Elution was carried out using a linear gradient of 0 to 0.5 M NaCl in 0.025 M tris/HCl buffer at pH 7.5. Various flow rates were tested, ranging from 5 to 50 mL/min for the AFC column and 5 to 150 mL/min for the RFC column. Figures 9 and 10 represent typical results obtained by Lane et al. (10). They indicate that the AFC column gave slightly sharper peaks and faster elution times for both DE52 and QA52 media at a flow rate of 25 mL/min.

PROS AND CONS OF RADIAL FLOW COLUMNS

RFC columns provide a short flow path and a large cross-sectional area. This has the same effect as short pancakelike AFC columns. However, RFC columns occupy considerably less floor space. Both RFC and pancakelike AFC columns face flow distribution problems. According to Sepragen, its Superflo columns have better flow distribution than typical large pancakelike columns.

Compared to long AFC columns, RFC columns produce smaller pressure drops, thus enabling larger volumetric flow rates. If soft gels are used as separation media, the low pressure drop of RFC columns helps prevent bed compression (11,12). RFC is especially suitable for affinity chromatography using soft gels. In affinity chromato-

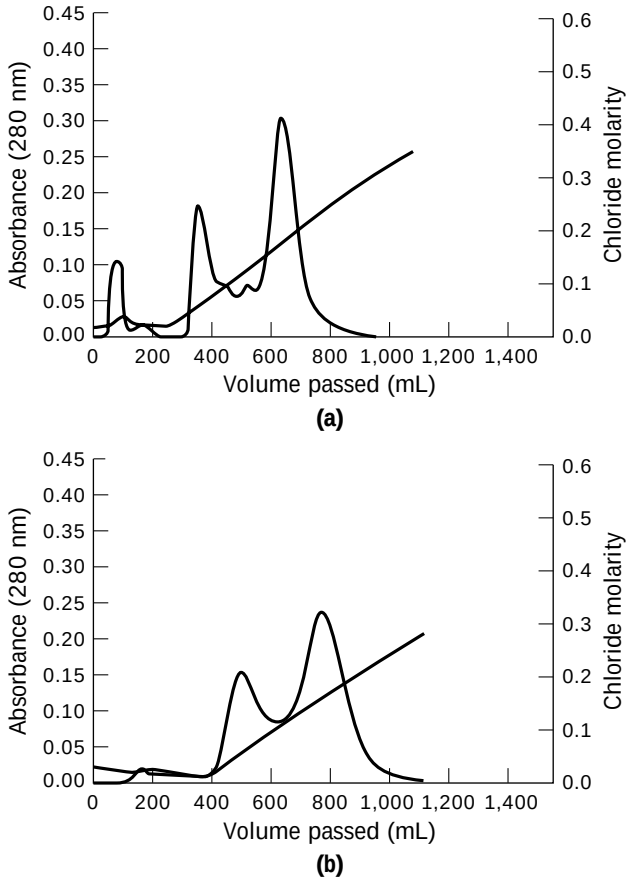


Figure 9. Comparison between a 100-mL AFC column (a) and a 100-mL RFC column (b) packed with DE52 in the separation of egg-white proteins.

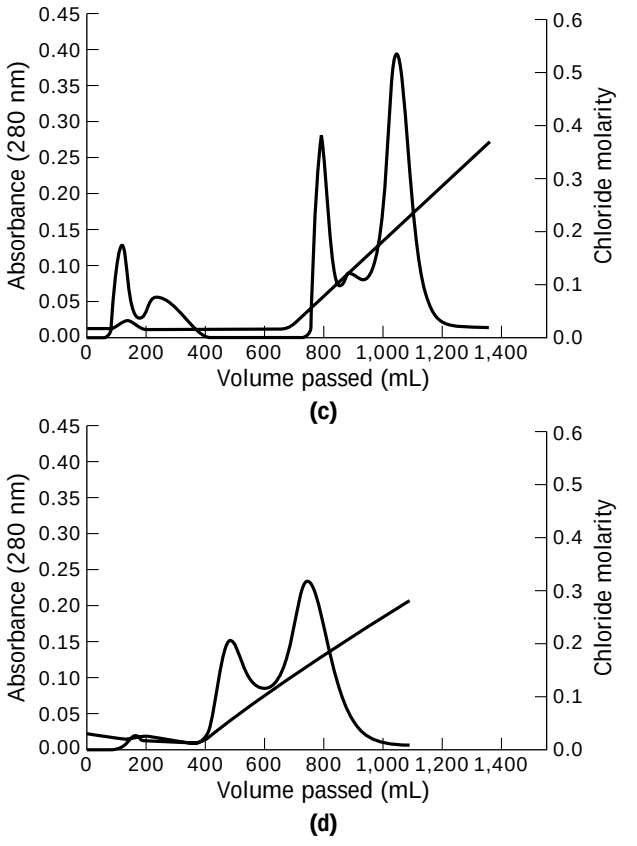


Figure 10. Same as Figure 9, but the columns were packed with QA52.

graphic operations, dilute feeds are typically used. Because of the extremely high affinity between the product and the gel matrix, very high flow rates are permitted without sacrificing column resolution. RFC is also a good choice for strong reversed phase, hydrophobic interaction and ion-exchange media.

Scale-up of RFC columns are relatively more straightforward because it is usually done by increasing the column height. To a certain extent, this does not seems to increase flow distortion problems in practice. If an AFC column is scaled up by increasing diameter, the flow distribution behaves quite differently from a small AFC column for which plug flow is easy to achieve. Thus, predicting actual performance of a large pancakelike AFC column from a small one is difficult.

The disadvantage of the RFC column is primarily its limited resolution caused by a short flow path. If the flow path is increased to a large extent, there will be flow distribution problems in the radial direction because of gravity. High resolution for more demanding separations can only come from using AFC columns with sufficiently large column length. This is precisely the reason why RFC has no use in analytical HPLC. RFC is not suitable for separations in which solute-stationary interactions are weak. For example, RFC is not a suitable choice for size-exclusion chromatography (SEC), because SEC depends strongly on the length of flow path for its resolution. RFC's short flow length cannot meet the demand. It is also expected that RFC is not suitable for other chromatography with weak solute-stationary phase interactions.

If mechanically strong packing materials, such as silica-based particles, are used, a longer column flow path can be used because the bed can sustain a much higher pressure. If a relatively high resolution is desired, RFC would be at a disadvantage compared to AFC since RFC is limited by its short flow path.

APPLICATION EXAMPLES

Applications Using Zetaffinity Cartridges

Huang et al. (3) studied several Zetaffinity cartridges from CUNO that contained modified cellulose-based affinity media. Table 1 lists the dimensions of three Zetaffinity cartridges tested by Huang et al. Figure 11 shows a chromatogram for the removal of proteases from human plasma using an 800-mL Zetaffinity cartridge with a flow rate of 100 mL/min (3). The cartridge contained modified cellulose with para-aminobenzamidine (PAB) as ligand. A 70% efficiency of protease removal was achieved in a single pass. The treated plasma was expected to have a three-fold increase in stability. The same type of 800-mL Zetaf-

Table 1. Dimensions of CUNO Radial Flow Cartridges Tested by Huang et al. (3)

Parameter	Small	Medium	Large
Nominal size	250 mL	800 mL	3200 mL
Bed volume	210 mL	810 mL	3020 mL
Outer diameter	7.0 cm	12.7 cm	12.7 cm
Inner diameter	0.6 cm	0.9 cm	0.9 cm
Height	6.4 cm	6.4 cm	23.8 cm

finity cartridge loaded with 1,260 mg PAB ligand was also used to purify crude trypsin purchased from Sigma Chemical. The data in Figure 12 were obtained by Huang et al. (3) using a flow rate of 295 mL/min.

Planques et al. (13) used a 250-mL Zetaffinity cartridge from CUNO packed with modified cellulose as chromatography media. They first chemically treated the cartridge to couple the media with L-lysine. This affinity chromatography media was able to bind with a human plasminogen protein. After centrifugation and microfiltration, human plasma was diluted with a buffer and then applied to the cartridge at a flow rate of 20 mL/min. After washing and elution, a 85% recovery yield and an increase of 110-fold in specific activity were achieved.

Applications Examples Using Sepragen's Superflo Columns

Akoum et al. (14) used a Superflo-400 RFC column packed with histidyl-Sepharose gel for the purification of myxalin, a glycopeptide with anticoagulant property. The feed for the column was obtained from a fermentation broth with the microorganism *Myxococcus xanthus*. Before the feed was applied to the column, it was clarified and concentrated using centrifugation, microfiltration, and reverse osmosis. A relative short processing time was achieved.

Strætkvern et al. (15) used a 60-mL, 2.2-cm i.d. AFC column, a Superflo-250 column (250 mL in bed volume), and a 2,500-mL AFC column for the separation of DNase from the extracts of cod pyloric cacca. The packing medium was Q-Sepharose Fast Flow anion exchange gel from Pharmacia. Column dimensions and operating conditions are listed in Table 2. Table 3 is a summary of their experimental results. Their results indicate that the RFC column required much less time and achieved higher productivity. The superior performance in this case should not be inter-

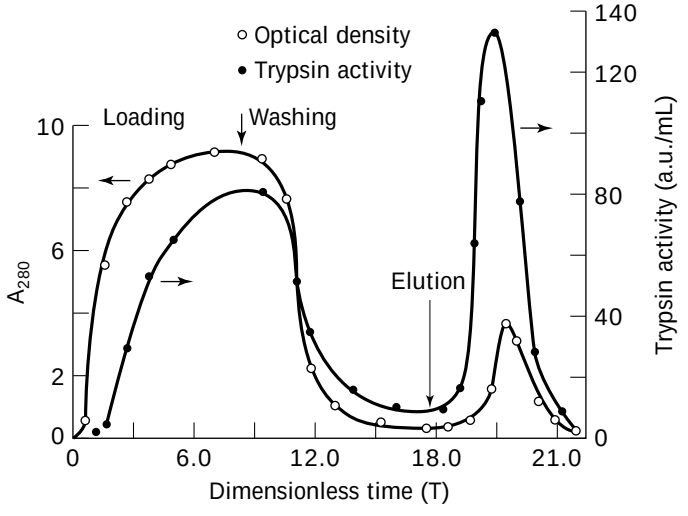


Figure 11. Protease removal from human plasma using a Zetaffinity® cartridge. a.u., affinity unit.

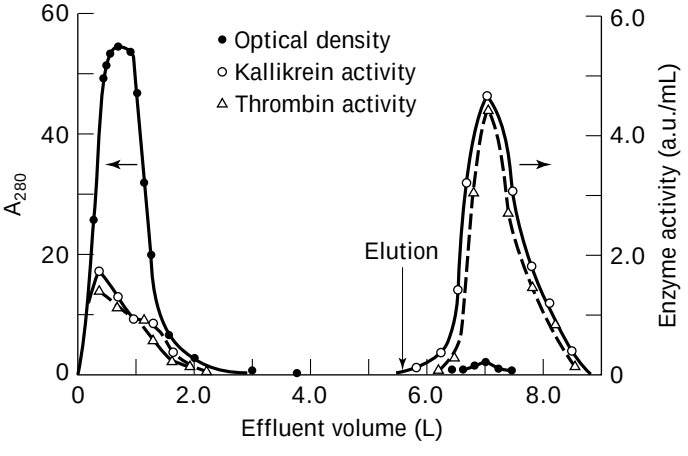


Figure 12. Trypsin purification using a Zetaffinity® cartridge. a.u., affinity unit.

Table 2. Columns Used by Strætkvern et al. (15)

Parameter	Small column	Medium column	Large column
Column type	Axial flow	Radial flow	Axial flow
Column volume (mL)	60	250	2,500
Cross-sectional flow area (cm^2)	3.8	120 (outer)	154
Volumetric flow rate (L/h)	0.6	17.4	20.9
Sample volume (L)	0.033	0.135	1.32
Protein concentration (mg/mL)	2.6	2.6	2.4
Scale-up factor	1	4	40

Table 3. Purification Results Obtained by Strætkvern et al. (15)

Parameter	Small column	Medium column	Large column
Elution volume (L)	0.20	0.87	3.57
Protein (mg)	10	44	350
Total activity (units $\times 10^{-6}$)	20	78	783
Specific activity (units $\times 10^{-6}$ mg^{-1})	2.00	1.77	1.35
Yield (%)	100	107	76
Purification (fold)	20	17	13
Cycle time (h)	1.3	0.25	1.4
Productivity (units $\times 10^{-6}$ $h^{-1} \cdot mL^{-1}$ gel)	0.256	1.25	0.135
Productivity ($mg \cdot h^{-1} \cdot mL^{-1}$ gel)	0.13	0.70	0.1

preted as a fixed rule here because the AFC columns they used were not equivalent pancakelike columns.

Weaver et al. (16) used a 10-L Superflo RFC column packed with Q-Sepharose Fast Flow medium from Pharmacia for the separation of uridine phosphorylase from total crude extracts of *Escherichia coli*. After fermentation, 375 L of broth was concentrated to 10 L. It was then washed with 50 L of 20 mM K_3PO_4 buffer containing 1 mM $MgCl_2$. After adding 20 μg lysozyme per milliliter of broth, 100 mg of DNase, and 100 mg of RNase, the cells were homogenized using a bead mill. The supernatant was diluted to 30 L and then applied to the 10-L RFC column. The feed was recirculated back to the column at a flow rate of 1.3 L/min for 3 h and then discharged. The column was washed with three different buffer solutions to remove bound lipid and hydrophobic proteins. Elution was then carried out using a buffer containing 0.225 mM NaCl. The collected effluent was 50 L. The final product after dialysis had a purity of 85% and a recovery yield of 82%.

McCartney (17) used two Superflo-100 RFC columns packed with S-Sepharose FF (Pharmacia) ion-exchange media in tandem for the purification of an undisclosed recombinant protein from *E. coli*. The system was able to process 4 L feed in less than 2 h with a concentration factor of 64 times.

Saxena et al. (12) applied a Superflo-1500 column (1,500 mL in bed volume) packed with immobilized Protein-A Sepharose in the purification of an antimelanoma IgG2A antibody from ascites fluids. Sample was loaded with a flow rate of 104 mL/min. After loading, the column was washed with a buffer using a flow rate of 170 mL/min. Elution was carried out with a flow rate of 92 mL/min. An actual recovery of 3.1 g of the antibody with a product purity greater than 97% was achieved in 3.5 h. A Superflo-1500 packed with riboflavin immobilized on a Sepharose-4B matrix via an epoxy linkage was used by the same researchers to obtain a crude preparation of a riboflavin-binding protein (12). The entire run was carried out using a flow rate of 350 mL/min. They also used a Superflo-200 column packed with antiricin B-chain antibody immobilized on cross-link agarose (12). The flow rate used was 45 mL/min. A 100% yield was achieved with a product amount of 2.1 g and a purity of 100%.

MATHEMATICAL MODELING OF RADIAL FLOW CHROMATOGRAPHY

In 1950, Lapidus and Amundson (18) proposed a simplified theoretical model for RFC. Their model ignores radial diffusion in the bulk-fluid phase and intraparticle diffusion. It is similar to that used by Rachinskii (19). Inchin and Rachinskii (20) subsequently included molecular diffusion in the bulk-fluid phase. Lee et al. (21) proposed several single component rate models for the comparison of statistical moments for RFC and AFC. They included radial dispersion, intraparticle diffusion, and external mass transfer effects. Kalinichev and Zolotarev (22) performed an analytical study on moments for single component RFC in which they treated the radial dispersion coefficient as a variable.

A rate model for nonlinear single component RFC was solved numerically by Lee (23) by using the finite difference and orthogonal collocation methods. His model considered radial dispersion, intraparticle diffusion, external mass transfer, and nonlinear isotherms. It used averaged radial dispersion and mass transfer coefficients instead of treating them as variables. A nonlinear model of this kind of complexity has no analytical solution and must be solved numerically.

Realistic modeling of RFC should treat the radial dispersion and external mass transfer coefficients as variables rather than constants because the linear flow velocity (v) in the RFC column changes continuously along the radial coordinate of the column. Without this distinctive important feature the curvature in the flow path is lost, and thus the column can be imaginatively cut and spread out to become exactly like a pancakelike AFC column.

General Rate Model for Multicomponent RFC

Gu et al. (24,25) presented a general rate model for RFC in which radial dispersion and external mass transfer coefficients are treated as variables rather than constants. The model was solved numerically using finite element and the collocation methods for discretizations of the bulk-fluid phase and the particle phase governing partial differential equations (PDEs), respectively. Figure 13 is an anatomy of a RFC column for the purpose of modeling. The following basic assumptions are made in order to formulate a general rate model for RFC.

1. The column is isothermal.
2. The porous particles in the bed can be treated as spherical and uniform in diameter.
3. The concentration gradients in the axial direction are negligible. This means that the maldistribution of radial flow is ignored.
4. The fluid inside particle macropores is stagnant, that is, there is no convective flow inside macropores.
5. An instantaneous local equilibrium exists between the macropore surfaces and the stagnant fluid in the macropores.
6. The film mass transfer theory can be used to describe the interfacial mass transfer between the bulk-fluid and particle phases.
7. The diffusional and mass transfer coefficients are constant and independent of the mixing effects of the components involved.

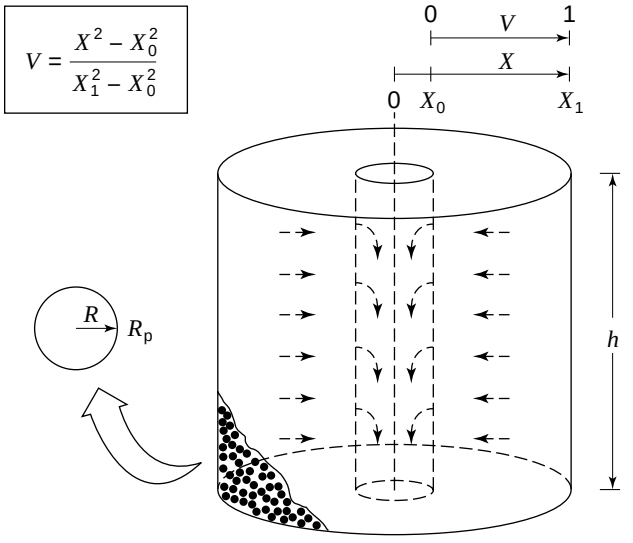


Figure 13. Anatomy of an inward-flow RFC column.

Based on these basic assumptions, equations 1 and 2 are formulated from the differential mass balance for each component in the bulk-fluid and particle phases. In equation 1, the plus sign represents outward flow, and −v is inward flow.

$$-\frac{\partial}{\partial X}\left(D_{bi}X\frac{\partial C_{bi}}{\partial X}\right) \pm v\frac{\partial C_{bi}}{\partial X} + \frac{\partial C_{bi}}{\partial t} + \frac{3k_i(1-\epsilon_b)}{\epsilon_b R_p}(C_{bi} - C_{pi,R=R_p}) = 0 \quad (1)$$

$$(1-\epsilon_p)\frac{\partial C_{pi}^*}{\partial t} + \epsilon_p\frac{\partial C_{pi}}{\partial t} - \epsilon_p D_{pi}\left[\frac{1}{R^2}\frac{\partial}{\partial R}\left(R^2\frac{\partial C_{pi}}{\partial R}\right)\right] = 0 \quad (2)$$

in which C_{pi}^* is related to C_{pi} via isotherm (24).

The initial conditions for the PDE system are at

$$t = 0,\ C_{bi} = C_{bi}(0, X) \quad (3)$$

and

$$C_{pi} = C_{pi} = C_{pi}(0, R, X) \quad (4)$$

The boundary conditions are at the inlet X position

$$\partial C_{bi}/\partial X = (v/D_{bi})[C_{bi} - C_{fi}(t)] \quad (5)$$

and at the outlet X position

$$\partial C_{bi}/\partial X = 0 \quad (6)$$

Equations 1 and 2 can be written in dimensionless forms as follows.

$$-\frac{\partial}{\partial V}\left(\frac{\alpha}{Pe_i}\frac{\partial c_{bi}}{\partial V}\right) \pm \frac{\partial c_{bi}}{\partial V} + \frac{\partial c_{bi}}{\partial \tau} + \chi_i(c_{bi} - c_{pi,r=1}) = 0 \quad (7)$$

$$\frac{\partial}{\partial \tau}[(1-\epsilon_p)c_{pi}^* + \epsilon_p c_{pi}] - \eta_i\left[\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial c_{bi}}{\partial r}\right)\right] = 0 \quad (8)$$

In equations 1 to 7, the dimensionless variable, $V = (X^2 - X_0^2)/(X_1^2 - X_0^2) \in [0, 1]$, is based on the local volume averaging method [24]. $\alpha = 2\sqrt{V + V_0}(\sqrt{1 + V_0} - \sqrt{V_0})$ is a function of V.

The dimensionless initial conditions are at

$$\tau = 0,\ c_{bi} = c_{bi}(0, V) \quad (9)$$

and

$$c_{pi} = c_{pi}(0, r, V) \quad (10)$$

The dimensionless boundary conditions are

$$\partial c_{bi}/\partial V = Pe_i[c_{bi} - C_{fi}(\tau)/C_{0i}] \quad (11)$$

At the inlet V position, for frontal adsorption, $C_{fi}(\tau)/C_{0i} = 1$. For elution,

$$C_{fi}(\tau)/C_{0i} = \begin{cases} 1 & 0 < \tau < \tau_{imp} \\ 0 & \text{otherwise} \end{cases}$$

After the introduction of a sample in the form of a rectangular pulse, it component I is displaced, $C_{fi}(\tau)/C_{0i} = 0$. If component I is a displacer, $C_{fi}(\tau)/C_{0i} = 1$. At the outlet V position, $\partial c_{bi}/\partial V = 0$. For the particle phase governing equation, the boundary conditions are at r = 0

$$\partial c_{pi}/\partial r = 0 \tag{12}$$

and at r = 1

$$\partial c_{pi}/\partial r = Bi_i(c_{bi} - c_{pi,r=1}) \tag{13}$$

Note that all the dimensionless concentrations are based on C_{0i}, that is, the maximum of the feed profile $C_{fi}(\tau)$ for each component.

The radial dispersion coefficient (D_{bi}) depends on the linear velocity (v). In liquid chromatography, it can be assumed (5,22,25) that $D_{bi} \propto v$. Thus, $Pe_i = v\,(X_1 - X_0)/D_{bi}$ can be considered constant in liquid RFC. The variation of Bi_i values observes the following relationship:

$$Bi_i \propto k_i \propto v^{1/3} \propto (1/X)^{1/3} \propto (V + V_0)^{-1/6} \tag{14}$$

If $Bi_{i,V=1}$ values are known, Bi_i values anywhere else can be obtained from equation 15.

$$Bi_{i,V} = [(1 + V_0)/(V + V_0)]^{1/6} Bi_{i,V=1} \tag{15}$$

χ_i can be calculated from Bi_i using its definition $\chi_i = 3Bi_i\eta_i(1 - \epsilon_b)/\epsilon_b$.

Numerical Solution

The PDE system of the governing equations is first discretized. The finite element and orthogonal collocation methods are used to discretize the bulk-fluid phase and the particle phase governing equations, respectively. The resulting ordinary differential equation (ODE) system is then solved using the public domain ODE solver called DVODE written by Brown et al. (26). The compiled Fortran program is free to academic researchers. Both DOS and Windows 95 versions are available. Information on the software is available at *http://www.ent.ohiou.edu/~guting/CHROM/*.

A study of the effects of treating D_{bi} and k_i as variables compared to treating them as constants was carried out by Gu et al. (5,25). The comparison between RFC and AFC was also studied through computer simulation. Figure 14 shows that outward flow gives slightly sharper concentration profile (5). The figure also shows that RFC gives similar concentration profiles as AFC with equivalent physical parameters. These theoretical results support the experimental results obtained by Tharakan and Belizaire (8).

SCALE-UP OF RFC COLUMNS

One of the advantages of RFC columns is the relative ease for scale-up. In Sepragen's Superflo column series, increasing the column height is a rather safe way to accommodate an increase of sample size to a certain degree. However, one must keep in mind that maldistribution in radial flow may deteriorate slightly. If the bed thickness in the radial direction is increased, bed pressure usually increases proportionally. The performance prediction will be less reliable if the column diameter is increased. Figure 15 shows that a 15-fold increase of sample load and bed column produced very similar performances when DEAE cellulose is used to separate an ascites fluid (27,28). Figure 16 is an example with a 50-fold increase of sample load and bed column (27). These two examples are very successful examples.

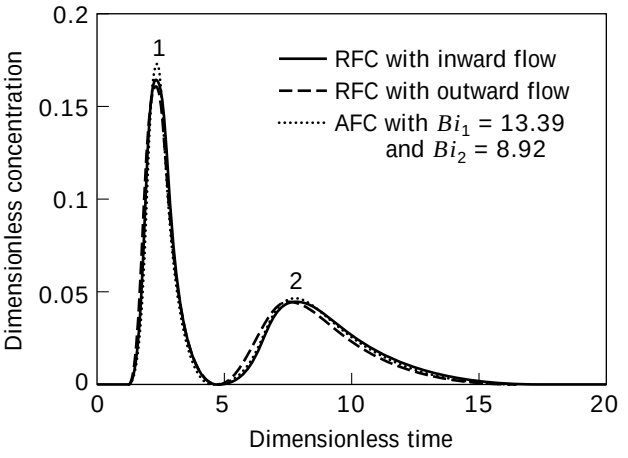
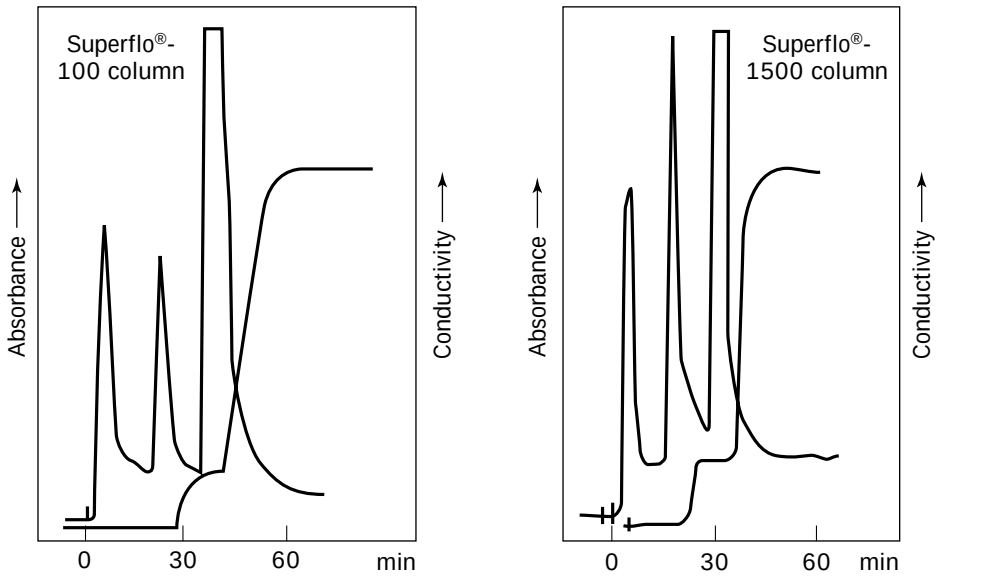


Figure 14. Simulated comparison for inward- and outward-flow RFC and AFC.

The mathematical model introduced above can be used to help the scale-up process. Before a column is bought, its performance can be predicted using computer simulation. Isotherm data must be obtained experimentally or be supplied by the vendor of the packing material. Mass transfer parameters can be estimated by using existing correlations (24).

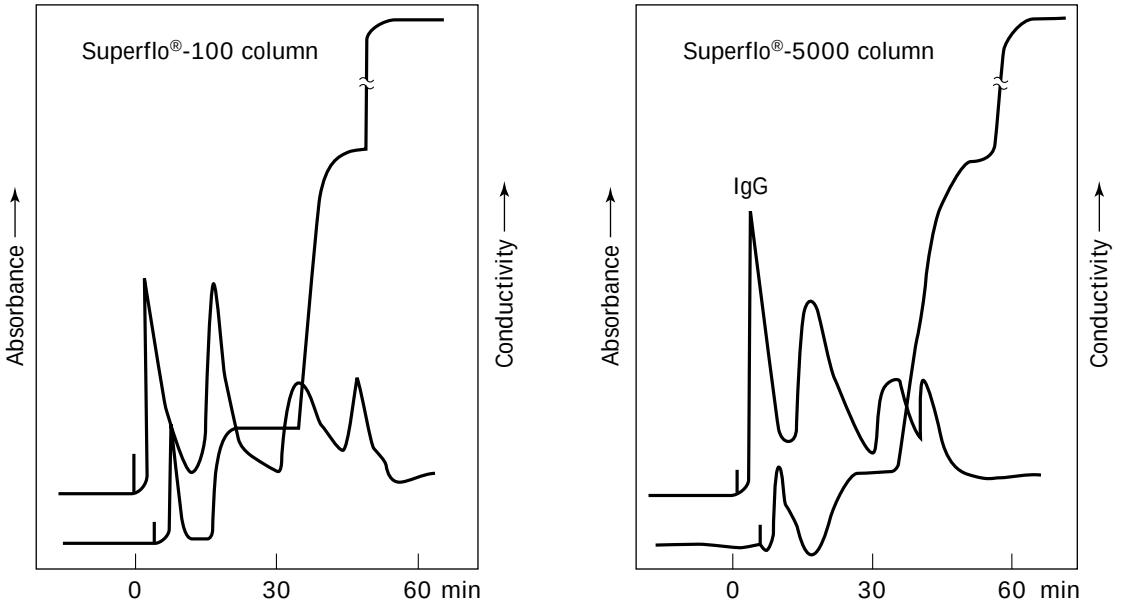
CONCLUSIONS

RFC columns have a short flow path and a large flow area, resulting in a small bed pressure. They are especially suitable when soft-gels are used. Because of its limited resolution, RFC should be used in chromatographic separations involving strong solute–stationary phase interactions, such as affinity chromatography, strong anion or cation exchange, strong reversed phase, and strong hydrophobic interaction chromatography. RFC is not suitable for SEC and other chromatography with weak solute–stationary phase interactions. RFC is an attractive alternative to AFC in preparative- and large-scale separations. Both experimental and theoretical modeling indicate that to a large extent an RFC column behaves much like a pancake-style AFC column with the same packing volume and with its bed height about the same as the packing's radial thickness in RFC column. However, RFC has a much



Packing:	DEAE cellulose	Packing:	DEAE cellulose
Load:	10 mL ascites fluid	Load:	150 mL ascites fluid
Flow rate:	10 mL/min	Flowrate:	150 mL/min
Start buffer:	10 mM phosphate, pH 8.5	Start buffer:	10 mM phosphate, pH 8.5
Step gradient:	60 mM, 500 mM NaCl in start buffer	Step gradient:	60 mM, 500 mM NaCl in start buffer

Figure 15. A 15-fold scale-up example using Superflo® columns.



Packing:	DEAE cellulose	Packing:	DEAE cellulose
Load:	10 mL cell culture fluid (murine IgG)	Load:	500 mL cell culture fluid (murine IgG)
Flow rate:	10 mL/min	Flow rate:	500 mL/min
Start buffer:	10 mM phosphate, pH 8.5	Start buffer:	10 mM phosphate, pH 8.5
Step gradient:	60 mM, 250 mM, 700 mM NaCl in start buffer	Step gradient:	60 mM, 250 mM, 700 mM NaCl in start buffer

Figure 16. A 15-fold scale-up example using Superflo® columns.

smaller footprint, and it seems to handle flow distribution better than very wide pancakelike columns.

NOMENCLATURE

Bi_i	Biot number of mass transfer for component i, $k_i R_p(\epsilon_p D_{pi})$
C_{0i}	Concentration used for nondimensionalization, $\max\{C_{fi}(t)\}$
C_{bi}	Bulk-fluid phase concentration of component i
C_{fi}	Feed concentration profile of component i, a time-dependent variable
C_{pi}	Concentration of component i in the stagnant fluid phase inside particle macropores
C^*_{pi}	Concentration of component i in the solid phase of particle (based on unit volume of particle skeleton)
c_{bi}	$= C_{bi}/C_{0i}$
c_{pi}	$= C_{pi}/C_{0i}$
c^*_{pi}	$= C^*_{pi}/C_{Oi}$
D_{bi}	Axial or radial dispersion coefficient of component i
D_{pi}	Effective diffusivity of component i, porosity not included
k_i	Film mass transfer coefficient of component i
Pe_i	Peclet number of radial dispersion for component i, $v(X_1 - X_0)/D_{bi}$
R	Radial coordinate for particle
R_p	Particle radius
r	$= R/R_p$
t	Dimensional time ($t = 0$ is the moment a sample enters a column)
v	Interstitial velocity
V_0	$X_0^2/(X_1^2 - X_0^2)$
V	Dimensionless volumetric coordinate
X	Coordinate in the radial direction for a RFC column.

Greek Letters

α	$= 2\sqrt{V + V_0}(\sqrt{1 + V_0} - \sqrt{V_0})$ for RFC
ϵ_b	Bed void volume fraction
ϵ_p	Particle porosity
η_i	Dimensionless constant, $\epsilon_p D_{pi} L/(R_p^2 v)$
χ_i	Dimensionless constant for component i, $3Bi_i\eta_i(1 - \epsilon_b)/\epsilon_b$
τ	Dimensionless time, vt/L
τ_{imp}	Dimensionless time duration for a rectangular pulse of the sample

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See also CHROMATOGRAPHY, COMPUTER-AIDED DESIGN; CHROMATOGRAPHY, ION EXCHANGE; CHROMATOGRAPHY, SIZE EXCLUSION.