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CHROMATOGRAPHY, RADIAL FLOW

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INTRODUCTION

The production of a modern biotechnology product, frequently a recombinant protein, requires a multistage downstream process because the feedstock is usually a liquid that is dilute in product concentration and contains many impurities some of which are unknown chemical compounds. Such a process typically centers on two or more liquid chromatography steps in order to achieve the desired purity. As production scales escalate, the chromatography columns used become larger and more expensive. It is not uncommon to have column bed volumes of 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). They were marketed as an alternative to the conventional axial flow chromatography (AFC) for preparative- and large-scale applications. These columns were not configured for analytical applications because RFC columns do not offer any advantages for such purposes. In an RFC 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 larger flow area and a shorter flow path (i.e. radial bed length). It allows a higher volumetric flow rate with a lower bed pressure. The effect is equivalent to using a short pancake-like AFC column (Fig. 3). Pancake-like 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 compress the gel too much and make fluid flow more difficult. This is especially troublesome when soft gels are used. Thus, pancake-like AFC columns are used despite their short flow paths.

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

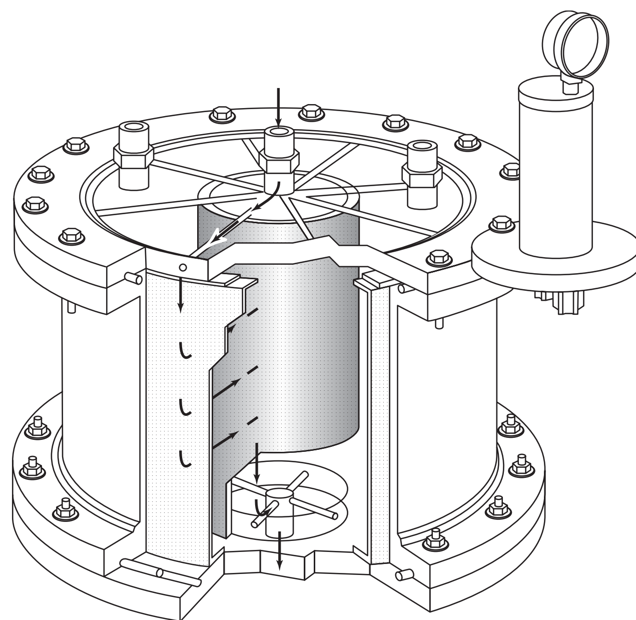

Figure 1. Diagram of an acrylic Superflo column. (Courtesy of Sepragen Corp.)

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 driven by the centrifugal force when the device was rotated. Because of this, 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 applications.

To date, only a few commercial companies have marketed RFC columns. The first one was the CUNO, Inc. in Connecticut. They marketed the Zetaffinity series of preparative-scale radial flow cartridges that looked like a spiral-wound microfiltration cartridge (Fig. 4). Such a design obviously was rooted in the fact that the CUNO is a major manufacturer of industrial filtration systems. The packing for CUNO's RFC cartridges was fabric-like modified cellulose (3) instead of adsorbent particles. These cartridges were used for affinity chromatography with a trade name of Zetaffinity. CUNO discontinued its Zetaffinity product line in 1991.

The second vendor and manufacturer of RFC columns is the Sepragen Corporation (http://www.sepragen.com) 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 2–12 and a maximum pressure of 50 psi. They are used by some biopharmaceutical

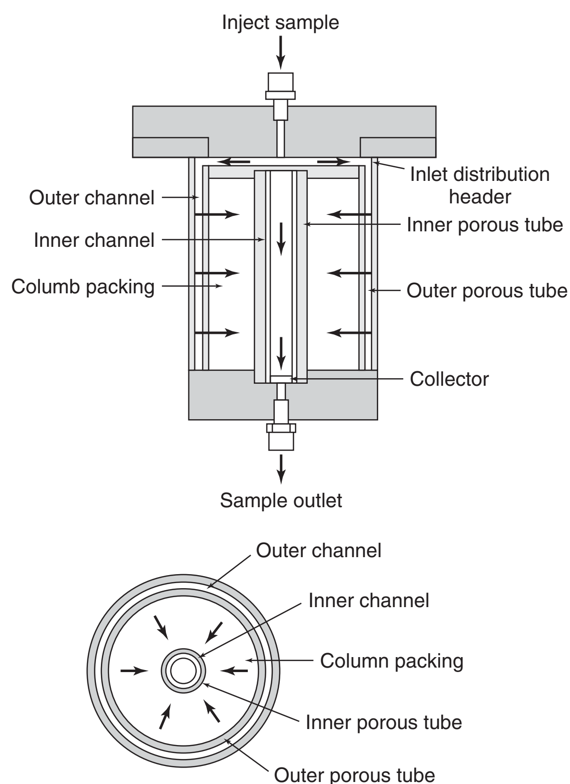

Figure 2. Anatomy of a Superflo column (Courtesy of Sepragen Corp.)

companies at production scales. Vinit Saxena of Sepragen owns a US patent (4) on the design of unpacked RFC columns. Figure 1 shows an acrylic RFC column with inward radial flow. The feed stream 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 Fig. 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. Another reason for using inward flow is that it provides slightly sharper peaks than outward flow based on computer simulation (5). Outward flow is usually used for the packing or regeneration of an RFC column.

Founded in 2002, PROXCYS Downstream Biosystems (http://www.proxcys.nl) in Emmen, The Netherlands, is a new supplier of preparative- and large-scale radial flow chromatographic columns. PROXCYS offers radial flow columns ranging from 50 mL to 1200 L. Figure 6 shows a CRIO Series CA-601 S 5-L radial flow column. PROXCYS also markets AXCIS columns that are hybrid radial flow columns that allow particle-containing crude feeds

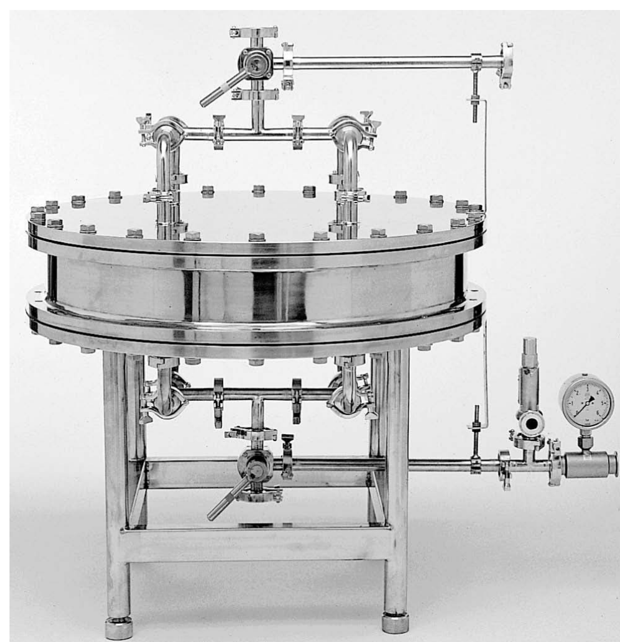

Figure 3. A large-scale pancake-like axial flow column. (Courtesy of General Electric. ©General Electric.)

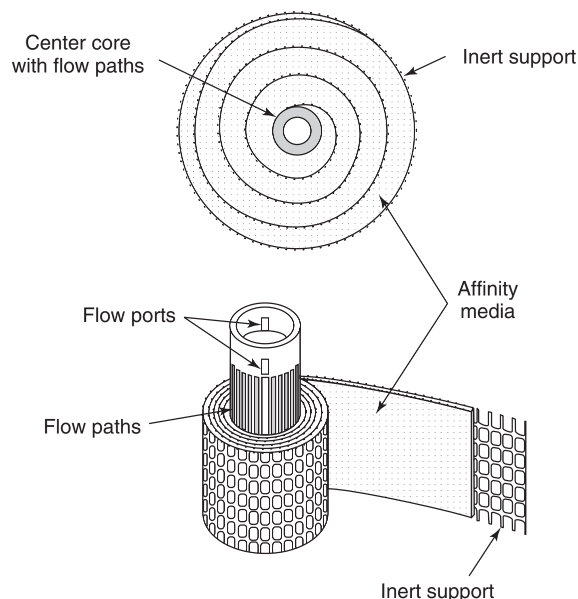

Figure 4. Structure of a Zetaffinity cartridge.

much like the so-called expanded bed chromatography columns that were first commercialized by Pharmacia in Piscataway, New Jersey (now part of GE Healthcare). The difference is in flow direction with the latter being axial flow.

Both Sepragen and PROXCYS market small "slice" radial flow columns. They look like a carved out portion of an RFC column resembling a slice of a cake with its

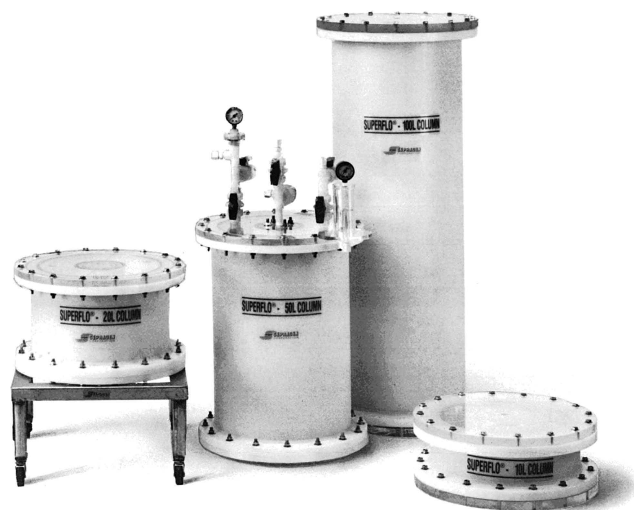

Figure 5. Superflo columns. (Courtesy of Sepragen Corp.)

Figure 6. PROXCYS CRIO Series CA-601 S 5-L radial flow column. (Courtesy of PROXCYS Downstream Biosystems.)

center beveled. Figure 7 shows a 100-mL CRIO-MD 121 column from PROXCYS. The CRIO-MD series columns look much like Sepragen's Wedge columns. Such columns can be used for small-scale applications and in scale-up and scale-down investigations. They are somewhat like a conical column with axial flow that was studied by Pfeiffer (6) because both types of columns have a narrower bed downstream.

In addition to the three companies above, Ngo and Khatter (7) mentioned the use of 100- and 500-mL Avid AL radial flow columns made by BioProbe International

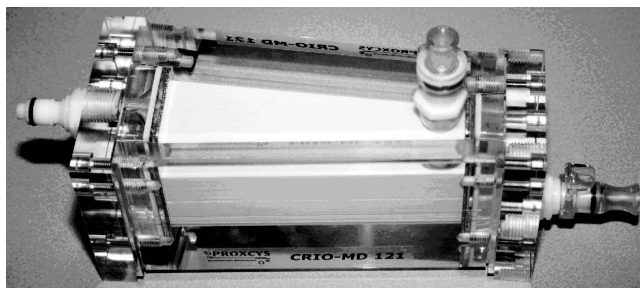

Figure 7. PROXCYS CRIO-MD 121 (100 mL) radial flow column. (Courtesy of PROXCYS Downstream Biosystems.) (This figure is available in full color at http://mrw.interscience.wiley.com/emrw/9780470054581/home.)

in California, USA. The columns are no longer being marketed. BIA Separations (http://www.biaseparations.com) in Austria markets 8, 80, and 800 mL tube-shaped monolithic columns for ion exchange, reversed phase, affinity, and other applications. Harrison Research, Inc. (http://www.harrisonresearch.com) in California, USA, sells preparative centrifugally accelerated thin-layer chromatography devices with radial flow.

PACKING PROCEDURES FOR RFC COLUMNS

Because of their structure, CUNO's Zetaffinity cartridges do not require packing by the end user. 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 cover (Fig. 1). Excess buffer is squeezed out of the column through the input port on the column's top cover. A detailed packing procedure for Superflo columns was provided with illustrations by Wallworth (8). PROXCYS' columns are slurry packed using an automated process using a pump. Toward the end of the packing process, the bed pressure starts to take off sharply, this is when packing should stop. Unlike Superflo columns, PROXCYS' columns have packing ports very close to the center frits. PROXCYS claims that this packing port arrangement achieves better packing results. Packing of large radial flow columns remains a technical challenge. Users may shy away from RFC columns that do not resolve the undesirable channeling problem during packing. Munson-McGee (9) investigated packing problems in a 1.5-L Superflo 1500 radial flow column packed with ion exchange resins, and also mathematically modeled pressure and velocity distribution.

PRESSURE DROPS OF RFC COLUMNS

Because of a short flow path and a low pressure drop from the column inlet to outlet, both CUNO's and Sepragen's RFC devices exhibit a highly linear relationship between bed pressure drop and flow rate. Figure 8 shows a linear relationship between bed pressure and flow rate for an 800 mL CUNO's Zetaffinity cartridge studied by Huang *et al*. (3).

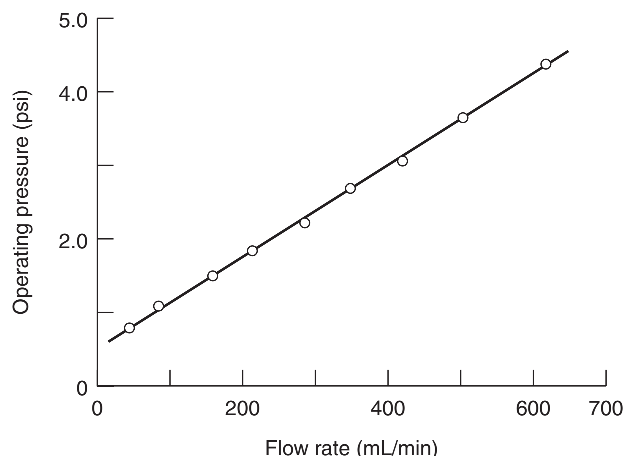

Figure 8. Pressure drop versus flow rate for an 800 mL Zetaffinity cartridge.

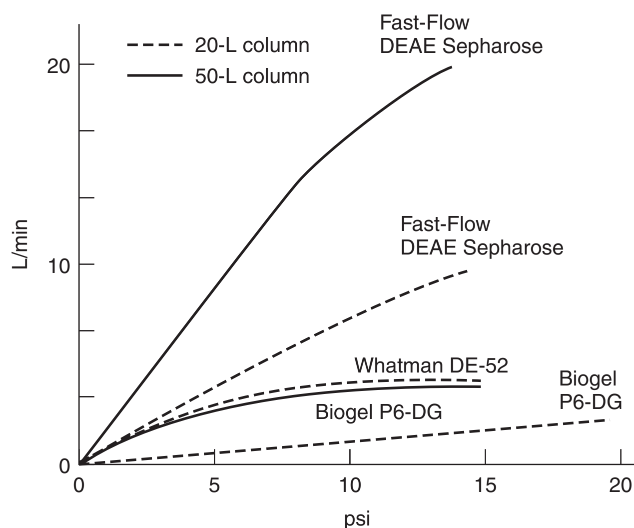

Figure 9. Pressure drop versus flow rate for 20 and 50 L Superflo columns. (Courtesy of Sepragen Corp.)

Figure 9 shows the pressure drops for a Superflo 20 L column and a Superflo 50 L column packed with several different soft-gel chromatographic media (10). The figure indicates that the pressure drops are quite low at high flow rates. At lower flow rates, the pressure drop curve is linear. This behavior is similar to that of short pancake-like AFC columns.

It would be interesting to measure the pressure distribution inside an RFC column. Unfortunately, such data are not available. With a constant volumetric flow rate, the interstitial linear fluid velocity increases in inward flow toward the center because the cross-sectional area for flow becomes narrower. The increased velocity results in higher kinetic energy for the fluid. The kinetic energy gain by the fluid is offset by a reduced local pressure as dictated by Bernoulli's principle in fluid mechanics. On the other hand, the increased velocity considerably increases friction loss that requires a larger pressure drop to overcome. The consequences from the increased velocity compromise each other, and thus the net effect on local pressure is unknown. For soft-gel columns, bed compression is unavoidable even in low-pressure RFC. If the pressure drop per unit radial length progressively increases toward the center in inward flow, gel density increases. This helps balance the gradual reduction of gel volume toward the center. Pfeiffer (6) demonstrated this for a conical column with its narrower end downstream. The same phenomenon in RFC columns needs to be proven experimentally.

COMPARISON OF RADIAL- AND AXIAL FLOW COLUMNS

Saxena and Weil (11) did an experimental case study to compare RFC with AFC. They used a 100-mL axial flow glass column (Econocolumn) with 2.5 cm i.d. from Bio-Rad Laboratories in Hercules, California (http://www.bio-rad.com), and a Superflo-100 RFC column from Sepragen with a bed volume of 100 mL. Both columns were packed with quaternary aminoethyl (QAE) cellulose with the following procedures. The Superflo column was packed using 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 m*M* 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 10 shows the comparison between the 100-mL AFC column and the 100-mL Superflo-100 RFC column. From Fig. 10, it can be 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 pancake-like short AFC column with its bed height about the same as the packing thickness in radial direction for the RFC column, and both columns should have equal bed volumes. The case study described next is close to satisfying these conditions.

Tharakan and Belizaire (12,13) 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. 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 like a pancake. Tharakan and Belizaire (12) also packed the same column with S-200 Sephacryl size exclusion gel from Sigma Chemical Co. (St. Louis, Missouri). The protein band was more diffused using RFC than AFC.

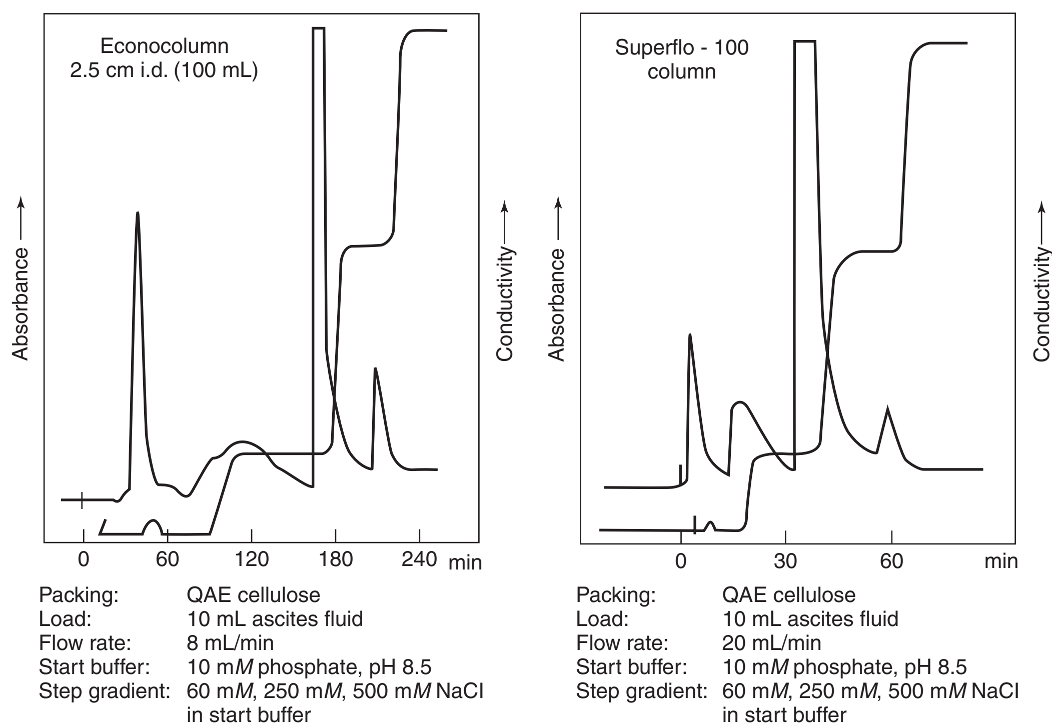

Figure 10. Comparison of AFC and RFC columns. (Courtesy of Sepragen Corp.)

Cabanne *et al*. (14) compared a 120-mL AFC column with a 120-mL RFC column (CRIO-MD 62 from PROXCYS). Both columns had an identical flow path length of 6 cm. They showed slightly better performances for the RFC column.

Lane and coworkers (15) compared the performance of a Superflo-100 RFC column and a 6.6 × 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. One was Whatman DE52 and the other was QA52 from Whatman Specialty Products Division (Maidstone, UK). Egg whites were first separated from fresh hen eggs and then treated with a buffer. After being treated with a cell debris remover, egg-white suspensions were filtered using a filter paper. The samples for chromatography had a protein concentration of 14 mg/mL. The sample loading volume was 40 mL. After sample loading, the column was washed with a buffer. Elution was carried out using a linear gradient of 0–0.5 *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 11 and 12 represent typical results obtained by Lane *et al*. (15). 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 RFC COLUMNS

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

Compared to long AFC columns, RFC columns produce smaller pressure drops, and thus enabling larger volumetric flow rates. If soft gels are used as separation media, the low pressure drop of RFC columns helps relieve bed compression (16,17). RFC is especially suitable for affinity chromatography using soft gels. In affinity chromatographic operations, dilute feeds are typically used. Owing to the extremely high affinity binding 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 is more straightforward since it is usually done by increasing the column height that is comparable to increasing the column diameter of an AFC column. To a certain extent, this does not seem to increase flow distortion problem 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 pancake-like AFC column from a much thinner one is problematic.

The disadvantage of an RFC column is primarily its limited resolution due to a short flow path. If the flow path is increased to a large extent, there will be flow distribution problems in the radial direction due to gravity. High resolution for more demanding separations can only come from using AFC columns with a sufficiently large

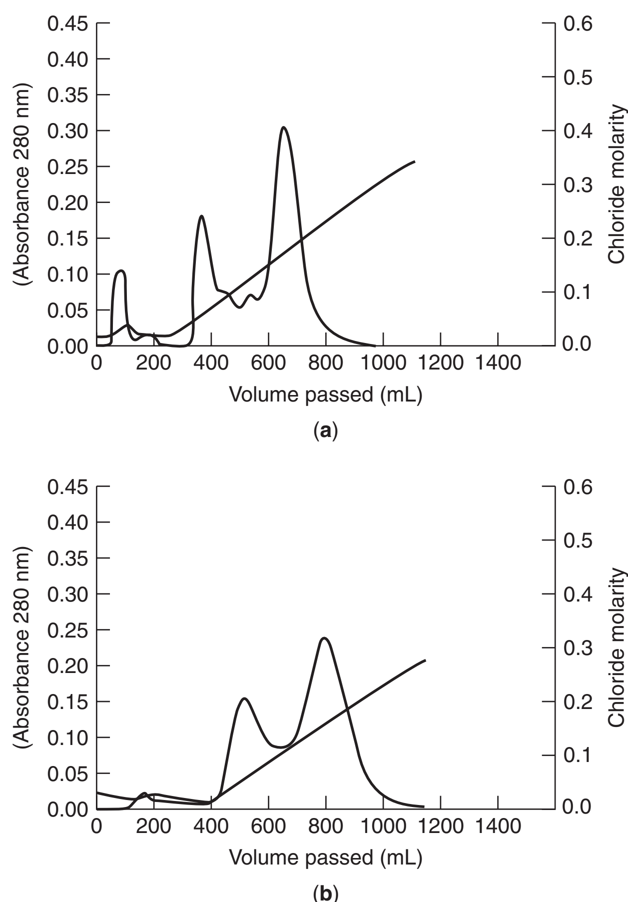

Figure 11. Comparison between a 100-mL AFC column (a) and a 100-mL RFC column (b) packed with DE52 in the separation of egg-white proteins.

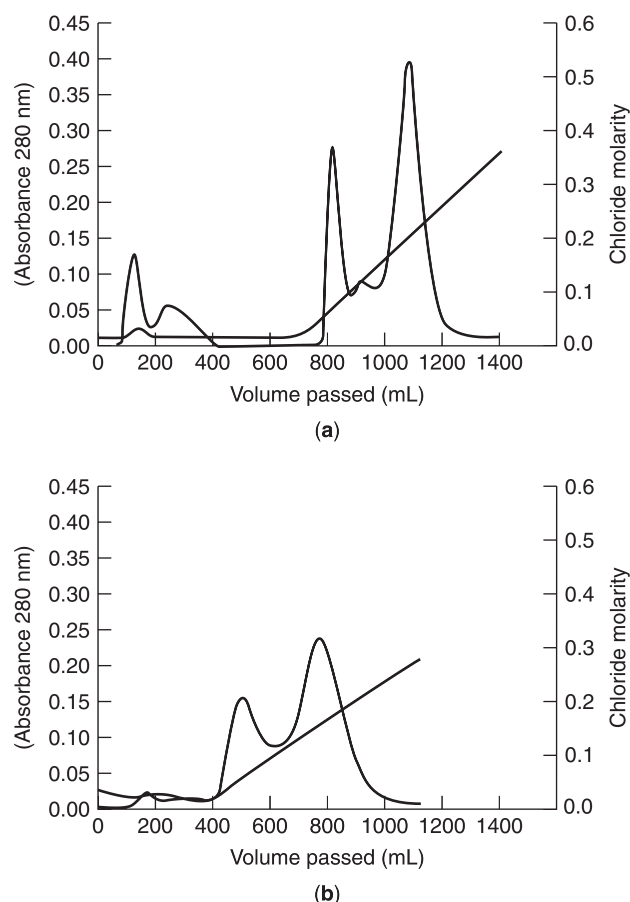

Figure 12. Comparison between a 100-mL AFC column (a) and a 100-mL RFC column (b) packed with QA52 in the separation of egg-white proteins.

column length. This is precisely the reason why RFC has no use in analytical high performance liquid chromatography (HPLC). RFC is not suitable for separations in which solute–stationary interactions are weak or there is no specific binding. 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 with no specific binding. RFC's short flow length cannot meet the demand.

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

Application Examples Using RFC Columns Packed with Membrane Sheets

Huang *et al*. (3) studied several Zetaffinity cartridges from CUNO with modified cellulose-based affinity media. Table 1 lists the dimensions of three Zetaffinity cartridges tested by Huang *et al*. Figure 13 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 *p*-aminobenzamidine (PAB) as ligand. Protease removal was achieved with 70% efficiency in a single pass. The treated plasma was expected to have a threefold increase in stability. The same type of 800 mL Zetaffinity cartridge loaded with 1260 mg PAB ligand was also used to purify crude trypsin purchased from Sigma Chemical. Figure 14 shows the results obtained by Huang *et al*. (3) using a flow rate of 295 mL/min.

Planques *et al*. (18) 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 protein called

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

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

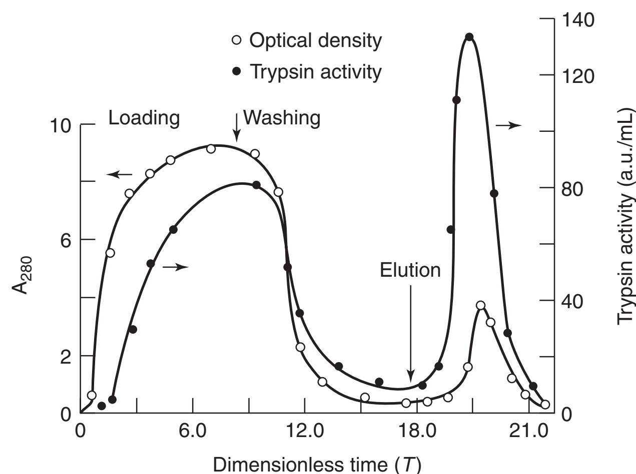

Figure 13. Protease removal from human plasma using a Zetaffinity cartridge.

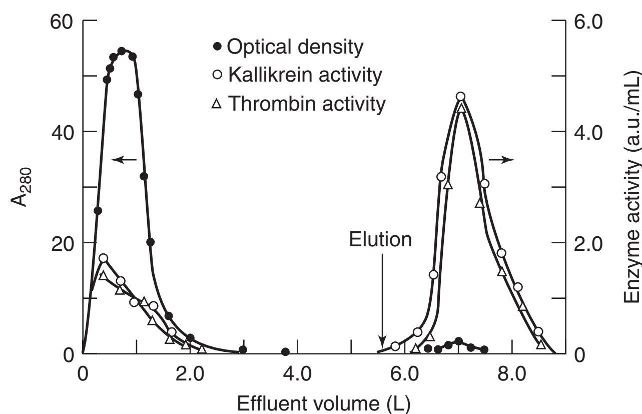

Figure 14. Trypsin purification using a Zetaffinity cartridge.

human plasminogen. After centrifugation and microfiltration, human plasma was diluted with a buffer and then fed to the cartridge at a flow rate of 20 mL/min. After washing and elution, recovery yield of 85% and an increase of 110-fold in specific activity were achieved.

Wang and coworkers (19) fabricated a radial flow column with an i.d. of 7 cm and height of 2 cm to purify human urinary kallikrein from pretreated human urine. The perforated center cylinder was wrapped with a QAE ion exchange membrane sheet. Thus, the column was similar to a CUNO's Zetaffinity cartridge. Sun *et al*. (20) used a small RFC column packed with cellulose membrane covalently linked with diethylaminoethyl (DEAE) for the purification of human prothrombin from Nitschmann fraction III. The column was purchased from the Institute of Chemical Physics, Chinese Academy of Sciences.

Applications Examples Using Sepragen's Columns

Akoum and coworkers (21) used a Sepragen's 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 *Myxococcus xanthus* fermentation broth. Before the feed was applied to the column, it was clarified and concentrated by using centrifugation, microfiltration, and reverse osmosis. A relatively short processing time was achieved.

Strætkvern and coworkers (22) used a 60 mL, 2.2 cm i.d. AFC column, a Superflo-250 column (250 mL bed volume), and a 2500-mL AFC column for the separation of deoxyribonuclease (DNase) from the extracts of cod pyloric caeca. 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 a higher productivity. The superior performance in this case should not be interpreted as a fixed rule here because the AFC columns they used were not equivalent pancake-like columns.

Table 2. Columns Used by Strætkvern *et al.* (22)

Parameter	Small Column	Medium Column	Large Column
Column type	Axial flow	Radial flow	Axial flow
Column volume (ml)	60	250	2500
Cross-sectional flow area (cm^2)	3.8	120 (outer)	154
Volumetric flow rate (h^{-1})	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.* (22)

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

Weaver and coworker (23) 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 broth was concentrated to 10-L. It was then washed with 50-L of 20 m*M* K_3PO_4 buffer containing 1 m*M* $MgCl_2$. After adding 20 μg of lysozyme per milliliter of broth, 100 mg DNase, and 100 mg 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 m*M* NaCl. The collected effluent was 50 L. The final product after dialysis had a purity of 85% and a recovery yield of 82%.

McCartney (24) used two 100-mL 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 and coworkers (17) used a Superflo-1500 column (1500 mL in bed volume) packed with immobilized Protein-A Sepharose in the purification of an anti-melanoma IgG2A antibody from ascites fluids. The sample was loaded at a flow rate of 104 mL/min. After loading, the column was washed with a buffer at a flow rate of 170 mL/min. Elution was carried out at 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 (17). The entire run was carried out using a flow rate of 350 mL/min. They also used a Superflo-200 column packed with anti-ricin B chain antibody immobilized on cross-link agarose (17). The flow rate used was 45 mL/min. Yield was achieved with a product amount of 2.1 g and a purity of 100%.

Sun *et al*. (25) used a 15-mL AFC column a 50-mL Superflo radial column, both packed with DEAE Sepharose Fast Flow media, to separate human prothrombin from Nitschmann fraction III derived from fractionation of human plasma for albumin and IgG with the Nitschmann method. The AFC column was loaded with 50 mL sample and RFC column with 200 mL sample. A slightly better peak resolution was achieved using the RFC column. The same researchers also used the same columns to purify human fibrinogen from Nitschmann fraction I (26). Tseng *et al*. (27) successfully utilized a 100-mL Superflo radial flow column from Sepragen packed with DEAE-52 cellulose for the purification of human salivary cystatin SN (CsnSN), a member of the cystatin superfamily of cysteine proteinase inhibitors. Levison (28) reported a comparison of the performances of a 100-mL Superflo-100 column, a 10-L Superflo-10L column, and a 100-mL AFC column, all packed with DE52 for the separation of egg-white proteins. The chromatograms resemble each other indicating a linear scale-up from 100 mL to 10 L.

Applications Examples Using PROXCYS' Columns

So far, the aforementioned paper by Cabanne *et al*. (14), which compared a 120-mL AFC column with a 120-mL RFC column (CRIO-MD 62 from PROXCYS), appears to be the only published example. Because PROXCYS is a newcomer in the RFC market, more published case studies using their columns have not appeared in the open literature yet.

Applications Examples Using RFC Columns with Monolithic Packing

In recent years, several research groups have attempted to pack RFC columns with monolithic media that have proven to reduce band spreading in AFC. Gustavsson and Larsson (29) fabricated a 65 mL RFC column packed with a single piece of superporous agarose gel (considered a type of monolithic chromatography media). After the gel was derivatized with Cibacron Blue 3GA affinity ligands, the column was used to separate dehydrogenase from a 200 mL crude bovine lactate dehydrogenase extract with excellent results. Yang *et al*. (30) obtained a monolith from the polymerization of glycidyl methacrylate and ethylene dimethacrylate in the presence of porogens. The polymer was modified with DEAE weak anion exchange groups before being packed into a 38 mL radial flow column. The pressure drop of the column showed a linear relationship with flow rate and its value was 1.7 MPa at the highest tested flow rate of 50 mL/min.

Hahn *et al*. (31) studied dispersion effects in a preparative polymethacrylate monolith with radial flow having dimensions of 15 mm outer in diameter, 1.5 mm in inner diameter, and 45 mm in bed height. It should be noted that for rigid monoliths, RFC unlikely would provide any major advantages over AFC.

An Example of Continuous Radial Flow Chromatography

Lay *et al*. (32) reported the use of RFC in continuous mode. They constructed an RFC column with rotating annulus and eight input ports equally spaced on the outer cylinder as shown in Fig. 15. The bed packed with 500 mL of DEAE Sepharose Fast Flow anion exchanger was divided into four neighboring zones in a full circle: feed zone, wash zone, elution zone, and a second wash zone. The column was used to separate bovine serum albumin (BSA) from lactoferrin achieving a separation factor of 4.78 for BSA. Continuous RFC is the equivalent of continuous annular chromatography that was reviewed by Hilbrig and Freitag (33). They differ in flow direction with the latter using axial flow.

MATHEMATICAL MODELING OF RADIAL FLOW CHROMATOGRAPHY

In 1950, Lapidus and Amundson (34) proposed a simplified theoretical model for RFC. Their model ignores radial diffusion in the bulk-fluid phase and intraparticle diffusion. It is similar to a model used by Rachinskii (35). Inchin and Rachinskii (36) subsequently included molecular diffusion

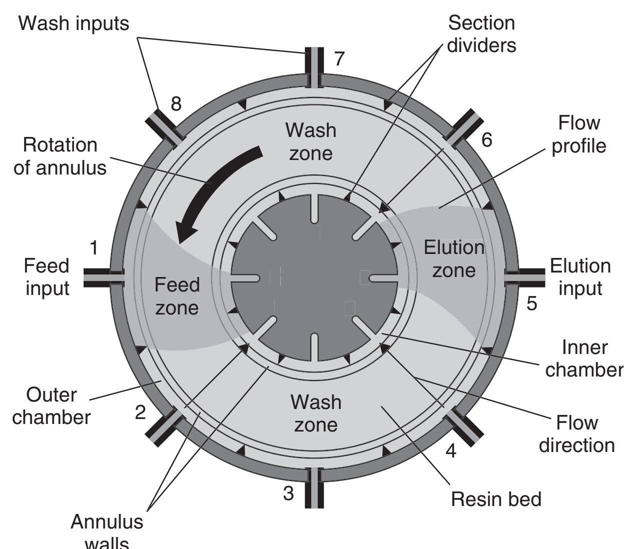

Figure 15. Schematic of a continuous RFC column. (Source: Ref. 32.)

in the bulk-fluid phase. Lee *et al*. (37) 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 (38) performed an analytical study on moments for single component RFC in which they treated the radial dispersion coefficient as a variable. Duong and Shallcross (39) presented a model for ion exchange RFC, which considered radial dispersion, external film mass transfer mechanisms, and binary cation exchanges. The model was used to predict experimental breakthrough curves.

A rate model for nonlinear single component RFC was solved numerically by Lee (40) by using the finite difference and orthogonal collocation methods. His model considered radial dispersion, intraparticle diffusion, external film 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. Recently, Lay *et al*. (32) modeled a continuous RFC column shown in Fig. 15. Their model that considered dispersion in radial and angular directions, intraparticle diffusion, and interfacial film mass transfer and a second-order kinetics involving BSA and NaCl was solved using the explicit finite difference method implemented in MATLAB (http://www.matlab.com). The second-order kinetics leads to the Langmuir isotherm when the forward and backward reactions are at equilibrium. The radial and angular dispersion terms were found to be negligible for their column with a short radial flow path of 3 cm, but intraparticle diffusion was important. Model predictions matched experimental data satisfactorily for the separation of BSA from lactoferrin.

Realistic modeling of RFC should treat the radial dispersion and external film mass transfer coefficients as variables rather than as constants because the linear flow velocity (v) in the RFC column changes continuously along the radial coordinate of the column. Without this distinctive 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 pancake-like AFC column.

General Rate Model for Multicomponent RFC

Gu and coworkers (41,42) presented a general rate model for RFC in which radial dispersion and external mass transfer coefficients were treated as variables rather than as constants. The model was solved numerically. Figure 16 shows the anatomy of an 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.

On the basis of these basic assumptions, Equations (1) and (2) are formulated from the differential mass balance for each component in the bulk-fluid and particle phases, respectively. In Equation (1), "+v" represents outward

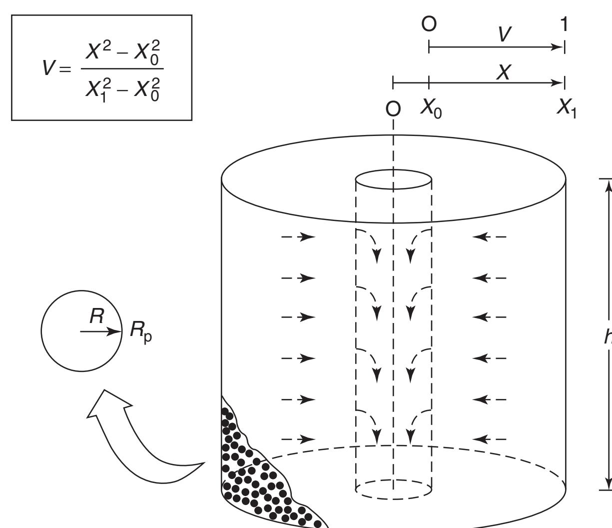

Figure 16. Anatomy of an inward flow RFC column.

flow and "−v" inward flow.

$$-\frac{1}{X}\frac{\partial}{\partial X}\left(D_{\mathrm{b}i}X\frac{\partial C_{\mathrm{b}i}}{\partial X}\right)\pm \mathrm{v}\frac{\partial C_{\mathrm{b}i}}{\partial X}+\frac{\partial C_{\mathrm{b}i}}{\partial t}+\frac{3k_i(1-\varepsilon_{\mathrm{b}})}{\varepsilon_{\mathrm{b}}R_{\mathrm{p}}}(C_{\mathrm{b}i}-C_{\mathrm{p}i,R=R_{\mathrm{p}}})=0 \quad (1)$$

$$(1-\varepsilon_{\mathrm{p}})\frac{\partial C^*_{\mathrm{p}i}}{\partial t}+\varepsilon_{\mathrm{p}}\frac{\partial C_{\mathrm{p}i}}{\partial t}-\varepsilon_{\mathrm{p}}D_{\mathrm{p}i}\left[\frac{1}{R^2}\frac{\partial}{\partial R}\left(R^2\frac{\partial C_{\mathrm{p}i}}{\partial R}\right)\right]=0 \quad (2)$$

In Equation (2), $C^*_{\mathrm{p}i}$ is related to $C_{\mathrm{p}i}$ via isotherm (41).

The initial conditions for the partial differential equation (PDE) system are as follows:
At $t=0$,

$$C_{\mathrm{b}i}=C_{\mathrm{b}i}(0,X) \quad (3)$$

and

$$C_{\mathrm{p}i}=C_{\mathrm{p}i}(0,R,X) \quad (4)$$

The boundary conditions are as follows:

$$\text{At the inlet } X \text{ position}: \quad \partial C_{\mathrm{b}i}/\partial X=(v/D_{\mathrm{b}i})[C_{\mathrm{b}i}-C_{\mathrm{f}i}(t)] \quad (5)$$

$$\text{At the outlet } X \text{ position}: \quad \partial C_{\mathrm{b}i}/\partial X=0 \quad (6)$$

Equations (1) and (2) can be written in dimensionless forms as follows:

$$-\frac{\partial}{\partial V}\left(\frac{\alpha}{Pe_i}\frac{\partial c_{\mathrm{b}i}}{\partial V}\right)\pm\frac{\partial c_{\mathrm{b}i}}{\partial V}+\frac{\partial c_{\mathrm{b}i}}{\partial \tau}+\xi_i(c_{\mathrm{b}i},c_{\mathrm{p}i,r=1})=0 \quad (7)$$

$$\frac{\partial}{\partial \tau}\left[(1-\varepsilon_{\mathrm{p}})c^*_{\mathrm{p}i}+\varepsilon_{\mathrm{p}}c_{\mathrm{p}i}\right]-\eta_i\left[\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial c_{\mathrm{p}i}}{\partial r}\right)\right]=0 \quad (8)$$

In Equation (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 (41). Parameter $\alpha=2\sqrt{V+V_0}(\sqrt{1+V_0}-\sqrt{V_0})$ is a function of V, in which $V_0=X_0^2/(X_1^2-X_0^2)$.

The dimensionless initial conditions are as follows:

$$\text{at } \tau=0, \quad c_{\mathrm{b}i}=c_{\mathrm{b}i}(0,V) \quad (9)$$

and

$$c_{\mathrm{p}i}=c_{\mathrm{p}i}(0,r,V) \quad (10)$$

The dimensionless boundary conditions are as follows:

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

At the inlet V position, for frontal adsorption, $C_{\mathrm{f}i}(\tau)/C_{0i}=1$; for elution,

$$C_{\mathrm{f}i}(\tau)/C_{0i}=\begin{cases}1 & 0\le\tau\le\tau_{\mathrm{imp}}\\ 0 & \text{otherwise}\end{cases}.$$

After the introduction of a sample in the form of a rectangular pulse,

if component i displaced, $C_{\mathrm{f}i}(\tau)/C_{0i}=0$;
if component i is a displacer, $C_{\mathrm{f}i}(\tau)/C_{0i}=1$.

At the outlet V position, $\partial c_{\mathrm{b}i}/\partial V=0$. For the particle phase governing equation, the boundary conditions are as follows:

$$\text{At } r=0, \quad \partial c_{\mathrm{p}i}/\partial r=0 \quad (12)$$

$$\text{At } r=1, \quad \partial c_{\mathrm{p}i}/\partial r=Bi_i(c_{\mathrm{b}i}-c_{\mathrm{p}i,r=1}) \quad (13)$$

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

The radial dispersion coefficient $D_{\mathrm{b}i}$ depends on the linear velocity v. In liquid chromatography, it can be assumed (5,38,42) that $D_{\mathrm{b}i}\propto v$. Thus, $Pe_i=v(X_1-X_0)/D_{\mathrm{b}i}$ can be considered constant in liquid RFC. The variation of Bi_i values observes the following relationship:

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

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

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

Parameter ξ_i can be calculated from Bi_i using its definition $\xi_i=3Bi_i\eta_i(1-\varepsilon_{\mathrm{b}})/\varepsilon_{\mathrm{b}}$.

Numerical Solution

The PDE system of the governing equations is first discretized to become an ordinary differential equation (ODE) system. The finite element and orthogonal collocation methods are used to discretize the bulk-fluid phase and the particle phase governing equations, respectively. The resulting ODE system is then solved using a public domain ODE solver called *double-precision variable-coefficient ordinary differential equation solver* (DVODE) developed by Brown *et al.* (43). The Microsoft Windows-based executable software program is free to any academic researchers for teaching and research. Information on the software is available at http://www.ent.ohiou.edu/~guting/CHROM/.

A study of the effects of treating $D_{\mathrm{b}i}$ and k_i as variables compared to treating them as constants was carried out by Gu *et al.* (5,42). The comparison between RFC and AFC was also studied through computer simulation. Figure 17 shows that inward flow in RFC gives slightly sharper concentration profiles (5,42) than outward flow. The small difference is theoretically predicted, but it may not show up experimentally, especially in columns with a large X_0 or V_0 value. 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 (12).

SCALE-UP OF RFC COLUMNS

One of the claimed 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 in sample size to a certain degree. However, one must keep in mind that flow distribution in radial flow may deteriorate. If the bed thickness

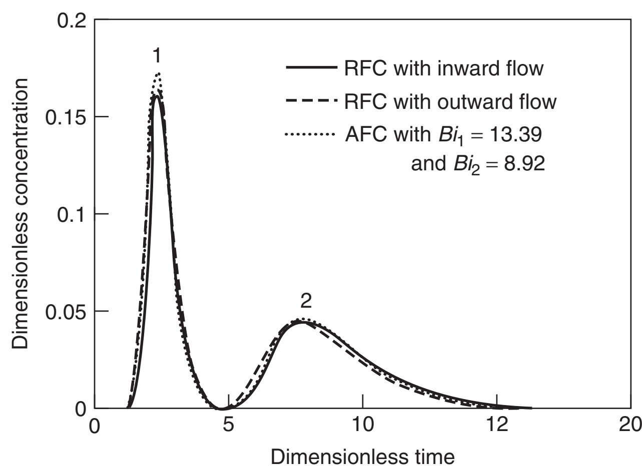

Figure 17. Simulated comparison for inward and outward flow RFC and AFC.

in the radial direction is increased, bed pressure usually increases proportionally. Figure 18 shows that a 15-fold increase in sample load and bed column produced similar performances when DEAE cellulose was used to separate an ascites fluid (11,44). Figure 19 is an example with a 50-fold increase in sample load and bed volume (11). These two examples are very successful examples.

The performance prediction will be difficult if the RFC column diameter is increased, which is equivalent to increasing column length in AFC. This kind of scale-up will change elution times and peak shapes. The mathematical model described above can be used to help the scale-up process. Before a column is bought off the shelf or custom built, its performance can be predicted using computer simulation-based different column dimensions and operating conditions. Isotherm data must be obtained experimentally or be supplied by the vendor of the packing material. To simplify the problem, only one or two key components should be used in computer simulation. Various mass transfer parameters used in the model can be estimated by using existing correlations (41).

CONCLUSIONS

RFC columns have a short flow path and a large flow area, resulting in small bed pressure. They are specially suited for pressure sensitive soft gels, although they are also used for rigid particles. Owing to its limited resolution, RFC should only be used in preparative- and large-scale chromatographic separations based on 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 forms of chromatography with weak solute–stationary phase interactions. RFC is not a replacement for AFC, but rather an alternative to AFC in preparative- and large-scale separations. Both experimental results and theoretical modeling indicate that 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 smaller footprint, and it seems to handle flow distribution better than very wide pancake-like columns.

Acknowledgments

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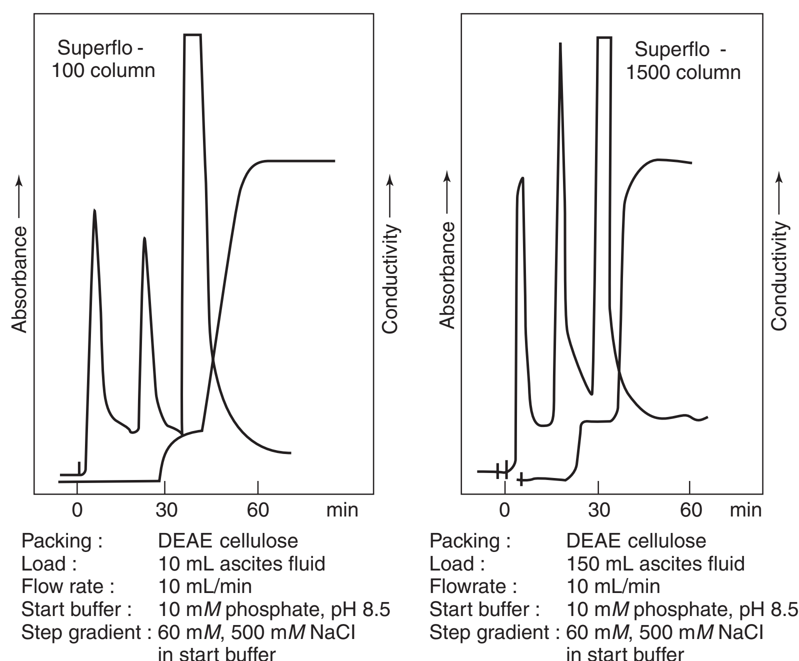

Figure 18. A 15-fold scale-up example using Superflo columns.

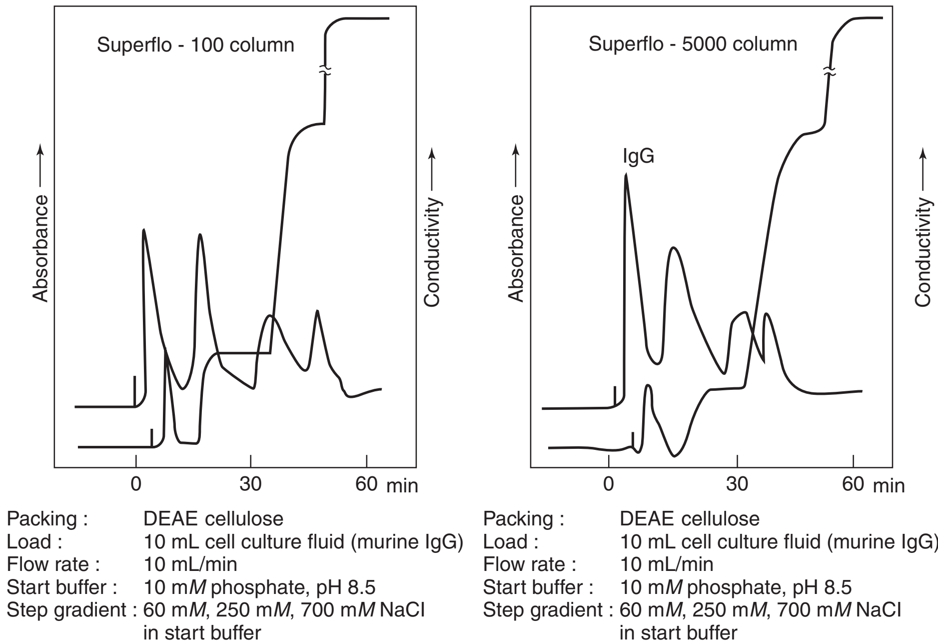

Figure 19. A 50-fold scale-up example using Superflo columns.

NOMENCLATURE

Bi_i	Biot number of mass transfer for component i, $k_i R_p/(\varepsilon_p D_{pi})$
C_{0i}	concentration used for nondimensionalization, $\max\{C_{fi}(t)\}$
C_{bi}	bulk-fluid phase concentration of component
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_{0i}
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	dimensionless volumetric coordinate, $(X^2 - X_0^2)/(X_1^2 - X_0^2)$
V_0	$X_0^2/(X_1^2 - X_0^2)$
X	coordinate in the radial direction for an 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, $\varepsilon_p D_{pi} L/(R_p^2 v)$
ξ_i	dimensionless constant for component i, $3Bi_i\eta_i(1-\varepsilon_b)/\varepsilon_b$
τ	dimensionless time, vt/L
τ_{imp}	dimensionless time duration for a rectangular pulse of the sample

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