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Maintenance of column performance at scale

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Abstract

Pack-in-place column packing methods were developed for Q Sepharose Big Beads at 40 cm I.D. and scaled up to 200 cm I.D. in Chromaflow columns. The efficiency and asymmetry of the packed bed were evaluated as a function of test velocity and sample volume. The performance of the packed beds at both scales approached the theoretical limits of column performance ($H_{red}=2$ and $A_f=1$) expected in small analytical columns. The packing strategy was effective for scale up and the stability of the packed beds, the effectiveness of the column design with respect to the mobile phase distribution system and the stability of the media to the pack-in-place technology, are presented.

Keywords: Column performance; Preparative chromatography

1. Introduction

Production of therapeutic bio-molecules requires the use of chromatography at large scale. In the past most texts and review treatments of chromatography were from the analytical perspective but recently, reflecting the success of the biotechnology industry in production, testing and marketing bio-molecules, the focus has expanded to include larger scale applications [1–3]. Still, due to the proprietary nature of most biological manufacturing scenarios, detailed descriptions of process design, improvements and scale-up are rare [4–10]. As a result, recovery scientists faced with scaling up a chromatographic process find that they need to rely as much on their own experience as on the small literature base. Rigorous mathematical analysis of column packing and the behavior of chromatography beds is known at small scale [11,12]. Analysis of columns packed at larger scale has been done but only one diameter was tested [13].

The growth of biotechnology means that engineers are becoming involved in the early stages of design of chromatography scale up [14]. Various models are proposed to help in the design of chromatography scale up [15,16]. In this report we have optimized a method at a one scale and transferred it to a larger scale.

Changing scale is a fundamental problem in bio-molecule manufacturing.

There are several reasons to be concerned about the ability to change scales in the purification of a bio-molecule. At small scale the need for more material in order to develop an assay will require a change in scale. Uncertain market demands for a candidate molecule that will force a change in scale at the process development phase. As a candidate molecule moves through clinical trials, more material is required and this requires a change in scale.

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The main effect of changing scale is to increase throughput. Three factors need to be controlled in doing this. It is assumed that the separation is already worked out, so in scaling up it is essential to maintain the resolution. In this regard close attention must be paid to the scaled up aspect ratio, the V_{ext} of the larger bed, the gradient slope if necessary and the linear velocity of the fluid phase. Column capacity also must be scaled and this means that the ratio of sample load to column volume needs to be scaled as well. The yield of the process needs to be maintained after scale up as well. This means that column efficiency needs to be maintained. All of these variables are impacted by column packing procedures.

In scaling up, other parameters outside of the chromatography have some importance. The robustness of the separation protocol is a factor that needs to be assured. The feedstock often changes as fermentation or cell culture processes are scaled up from bench scale bioreactors to large-scale production vessels. As a result, the level of contaminants change, the product concentration can fluctuate and the volumes are certainly different. Larger scale chromatography requires a larger scale support system as well. Large columns require increased tank capacity for storage of buffer and cleaning solutions. Large columns can weigh several tons and require hoisting and safety equipment beyond what is ordinarily installed in small facilities. Overall the facility capabilities need to match the large-scale operation.

Maintaining resolution and throughput in chromatographic separations during scale up can be difficult unless the performance of the packed beds at the larger scale are comparable to the smaller scale columns. Developing robust packing procedures at larger scale to gain equivalent performance can be time consuming and costly. Packing methods developed at smaller scale or in different column formats may not be applicable at larger scale due to limitations of the hardware design or packing technique. Maintaining the appropriate efficiency and asymmetry during scale up requires not only appropriate equipment and packing methods but also requires appropriate test equipment and procedures to effectively evaluate column performance.

The use of retractable nozzles in the column end cells for introducing media into the column has given rise to a variety of packing modalities which can be generally referred to as ''pack-in-place'' techniques. Selecting a particular pack-in-place technique is dependent on the type of media to be packed, the bed geometry required, the type of equipment used to deliver the media into the column, and the specific capabilities of the column (e.g., fixed bed, manual variable adapter or hydraulic assisted adapter, pressure rating) and the stability of the media to the packing conditions. There is no universal pack-in-place method. Further, not all pack-in-place methods can be scaled up and most scalable techniques will require minor modifications to produce well packed beds.

Q Sepharose Big Beads is a spherical crosslinked agarose based ion-exchange medium with a particle size distribution between 100 and 300 μm and $d_{50} \simeq$ 190 μm specifically designed for capture steps using crude or viscous feed stocks. Column beds packed with this medium will tolerate moderate axial compression and relaxation without degradation of the media, formation of voids and cracks in the packed bed, or significant differences in packed bed performance, due in part to the elastic like properties of the agarose. The packing strategy developed for Q Sepharose Big Beads with Chromaflow columns relies on pumping the appropriate amount of media into the column at a flow velocity near the critical velocity. The bed is formed in a slightly over compressed mode with a small gap between the packed bed and the column end cell when the packing is terminated. The bed is allowed to relax back against the end cell.

Sending a pulse of a permeable tracer solute through the column and measuring the resulting zone broadening is common for the evaluation of packed beds. The ''pulse'' method in theory is quite easy to perform but in practice it can be very difficult to achieve accurate and reproducible results with large-scale equipment designed for preparative rather than analytical application. The pulse should be applied with an asymmetry factor (A_f) close to 1 if the A_f of the packed bed is to be evaluated accurately [17]. Similarly, the determination of efficiency (H) of the packed bed is very sensitive to sample volume (V_s), test velocity (μ), and extraneous system volumes (V_{ext}) between the point of injection and the monitor array [18]. However, A_f should be insensitive to V_s,

μ, and V_{ext} providing the column distribution system is adequate and there in no significant band distortion in V_{ext}. Another method that can be used to qualify packed beds is residence time distribution (RTD) analysis [19]. This method relies on formation of a step gradient with the test solute and is often better suited for use with large-scale systems.

The optimized pack-in-place procedure for Q Sepharose Big Beads in Chromaflow columns of 40 and 200 cm I.D. and the evaluation of packed beds are presented below.

2. Experimental

2.1. Media

Q Sepharose Big Beads was prepared as a 40–50% slurry in water or 50 m*M* NaCl at 4°C for packing. A minimum of 1.25 times V_c of settled media was prepared for column packing (80 l for 40 cm I.D. at $L=50$ cm; 1855 l for 200 cm I.D. at $L=47$ cm).

2.2. Columns

A Chromaflow CFV 400 Mark II equipped with 50 μm stainless steel screens and a PS2A packing station was used for packing method development at 40 cm I.D and $L=50$ cm. A Chromaflow CFV 2000 equipped with 50 μm stainless steel screens and custom packing station equipped with a 78 mm I.D. Sand Pipper S30 diaphragm pump was used for packing method optimization at 200 cm I.D and $L=47$ cm.

2.3. Column packing

Briefly, column packing is a five-step procedure. The empty column is filled with slurry in the upflow direction with the top nozzle in the unpack position and the bottom nozzle in the pack position. When slurry emerges from the top nozzle, the top nozzle is switched to the run position and air is purged out. The top nozzle is in the run position and the bottom nozzle is in the pack position still. In the third step, the top nozzle is switched to the pack position and the bottom nozzle is switched to the run position. In this step the column is packed in the downward direction. In the fourth step the packing is stopped when a small clearing zone is seen in the column, The top nozzle is switched to the run position. Both nozzles are in the run position now. The packing pump is stopped. The last step is bed expansion in which the packed bed expands to fill the small gap. This process has been illustrated elsewhere [20].

2.4. Testing

All columns were equilibrated in 25–100 m*M* NaCl prior to testing. Flow velocity during testing ranged from 10 to 250 cm/h. At 40 cm I.D., a 10

Table 1
Packing data summary for CFV 2000 optimization

Pack No.	Initial pack flow (l/min)	Slurry (%)	Bed height (L) (cm)	Compression factor (C_F) (%)	V_c (l)	V_s (l)	Flow (l/min)	H (μm)	H_{red} (H/187 μm) $d_{50}=187$	A_f	V_r (l)
1	360	50	50	1.17	1573	50	72	4630	24.8	2.8	1212
2	410	38	47.5	1.23	1496	30	33.5	4608	24.6	3.7	1088
3	414	ND	47	1.26	1481	30	53	ND	ND	>3	1028
4	240	45	47	1.14	1481	15	58	4082	21.8	3.3	1088
5	350	42	47	1.17	1481	30	56	3300	17.6	2.6	1070
6	220	47	47	1.15	1481	30	55	2857	15.3	2.0	1129
7	240	44	47	1.14	1481	30	53	4425	23.7	4.6	1109
8	320	45	47	1.13	1481	30	55	1634	8.7	1.5	1235
9	250	46	47	1.13	1481	30	55	822	4.4	0.9	1425
10	295	46	47	1.12	1481	30	50	759	4.1	0.9	1429
11	295	46	47	1.1	1481	30	50	904	4.8	0.9	1305

ND: Not determined.

m*M* Standard BioProcess system with UNICORN v3.1 control (Amersham Pharmacia Biotech) was used for column qualification with 1% acetone and 0.5 *M* NaCl as test solutes. At 200 cm I.D., a custom 53 mm I.D. monitor array under UNICORN v3.1 control and a Viking IC20S rotary lobe pump were used for column qualification with 0.5 *M* NaCl as the test solute. V_{ext} for both test systems was $<0.05\%$ V_t.

3. Results and discussion

The column packing strategy described above, developed at 40 cm I.D. was successfully scaled up to 200 cm I.D. Summaries of the 11 packs performed at the larger scale are presented in Table 1. The column was purged with mobile phase prior to packing in Packs 1–6. Packs 7–11 were packed according to the method described above. A decrease in flow velocity was required to maintain a compression factor (C_F=settled gel volume/V_c) of 1.11 to 1.13 in the larger column as shown in Fig. 1. The drop in packing flow velocity required to maintain the desired C_F was greater than would be predicted by the loss of wall effect [16]. Differences in C_F obtained during optimization studies at 200 cm I.D. with different initial packing flow velocities and methods are readily observed in the pressure flow characteristics of the resulting packed beds (Fig. 2a) and are very reproducible when the desired flow profile during packing is maintained (Fig. 2b).

The efficiency and asymmetry of the packed beds obtained with the optimized packing procedure using V_s=1% V_c and a flow velocity of 40 cm/h is given in

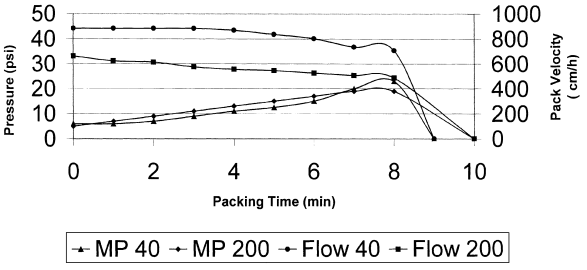

Fig. 1. Packing profiles for Q Sepharose Big Beads in 40 and 200 cm I.D. columns. MP is mobile phase pressure in p.s.i. measured at the column inlet. Flow is flow velocity in cm/h.

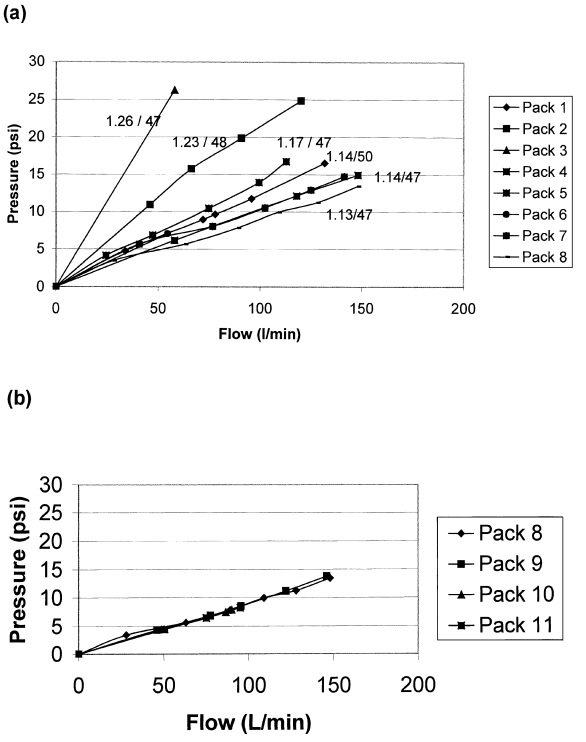

Fig. 2. Pressure flow characteristics of packed beds of Q Sepharose Big Beads. Panel (a) shows the packed bed pressure flow profiles for Q Sepharose Big Beads in the 200 cm I.D. column for different bed compression factors. The upper number of the pair (for example 1.26/47 for pack number 1) refers to the compression factor; the bottom number refers to the final bed height. Compression factor is the ratio of gravity settled media volume to final packed bed volume. Final bed height was determined by visual inspection. Panel (b) shows the pressure flow profiles for Q Sepharose Big Beads in the 200 cm I.D. column at a bed height of 47 cm at optimal compression factor of 1.11 to 1.13.

Fig. 3. Both scales of operation showed very similar trends of H increasing as a function of flow and A_f being independent of flow. Similarly, the distribution analysis (Fig. 4) showed similar trends in the insensitivity of A_f to V_s and the increase in H with increasing V_s. The results show that the maximum efficiency of H_{red}=2 is readily attained at either scale of operation with test velocities below 20 cm/h and V_s=1% V_c. The insensitivity of A_f to test velocity and V_s show the effectiveness and scalability of the distribution systems used in the Chromaflow columns.

The optimized packing methodology was repeated

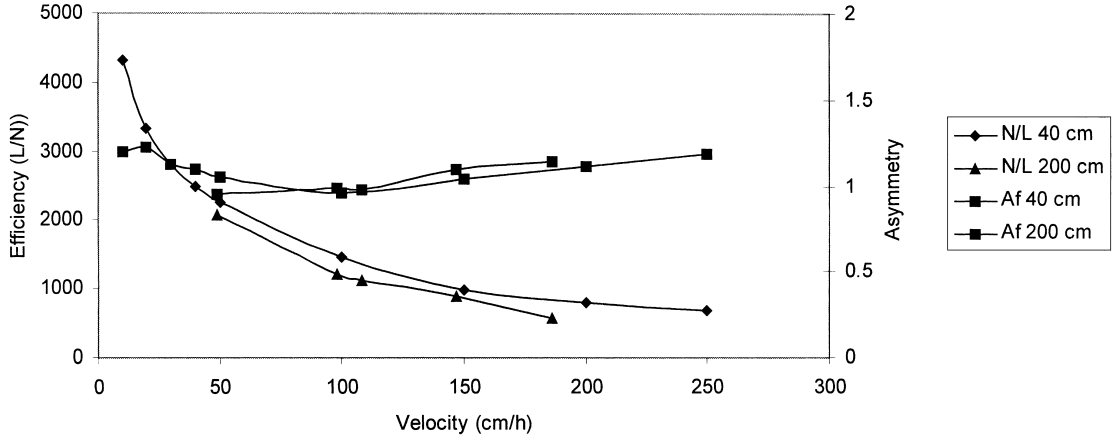

Fig. 3. Van Deemter analysis for Q Sepharose Big Beads packed in two columns, a 40 cm I.D. column with a bed height of 50 cm and a 200 cm I.D. column with a bed height of 47 cm.

three times to show reproducibility of the packing methodology (packs 8–11). The only differences in the packing procedure during this reproducibility testing was that the flow-rate during packing ranged from 250 l/min in pack 9 to 295 l/min in packs 10 and 11. The results from these packs are shown in Table 2. Although, the flow-rates and sample volumes were slightly different during evaluation of the three packs, the asymmetry showed only a 2.5% variation between the three packs. With available data, the efficiency and peak retention volume are found to be within 10% variance across these three packs,. If corrected for flow-rate and sample volume, the variance for efficiency and peak volume is less

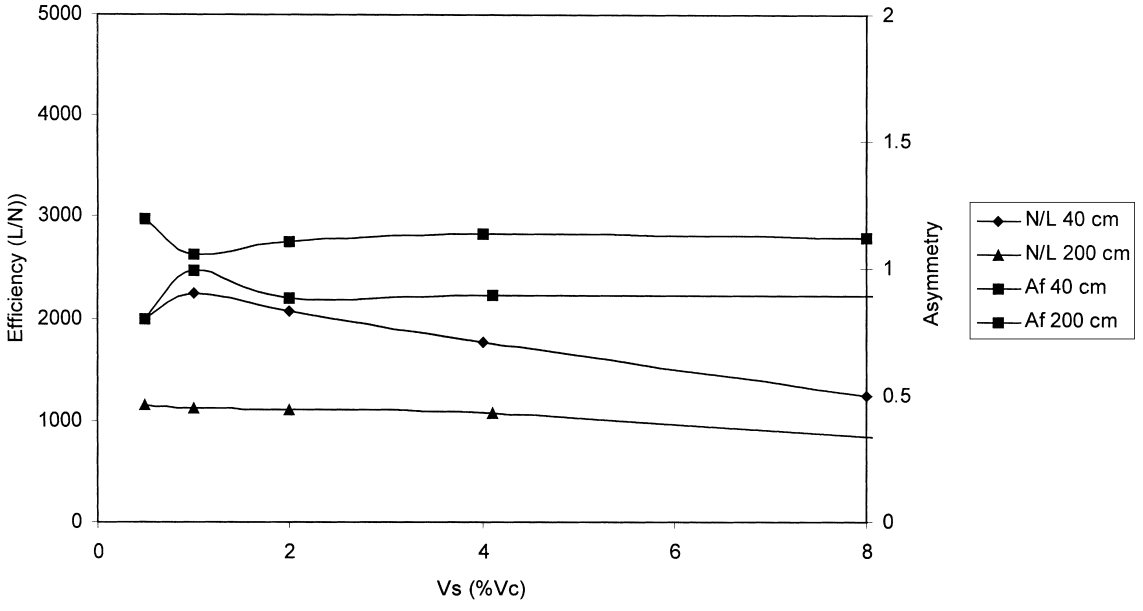

Fig. 4. An analysis of the distribution system in the column for Q Sepharose Big Beads packed in two column geometries, a 40 cm I.D. column with a bed height of 50 cm and a 200 cm I.D. column with a bed height of 467 cm.

Table 2
Reproducibility of optimized packing methodology

Pack No.	Air feed (bar)	Packing flow (l/min)	Slurry (%)	Compression factor	V_s (% V_c)	N/L	H_{red}	A_f	Test velocity (cm/h)	V_r (% V_c)
9	2.8	250	46	1.13	1.1	1180	4.5	0.97	109	86
10	3.1	295	46	1.12	2.0	1318	4.1	0.95	98	96
11	3.1	295	46	1.10	1.0	1296	4.4	0.99	98	88

Table 3
Stability testing

Pack 11	V_s (% V_c)	N/L	H_{red}	A_f	Test velocity (cm/h)	V_r (% V_c)
Before	1.0	1296	4.4	0.99	98	88
After	1.0	1064	5	1.00	102	86
% Variance	0	9	6	0.5	2	0.5

than 2.5%. These results indicate that the packing methodology is very reproducible.

The stability of pack 11 was examined by flowing the mobile phase at 90 l/min (maximum intended operational flow) for 16 h and then testing the bed for efficiency and asymmetry. The results of the stability test are presented in Table 3. The low variation noted during testing indicates the optimized packing methodology produces stable bed configurations.

The stability of the Q Sepharose Big Bead particles to 11 cycles of packing and unpacking in the CFV 2000 was tested by comparing the particle distribution analysis of samples taken before and after the 11 packs. There was no significant change in the particle size distribution (data not shown).

The comparison of the pulse method and RTD analysis for packs 8 and 11 of the CFV 2000 is shown in Table 4. The values of H for the RTD analysis are slightly less than for the pulse method which is typical. The A_f determined by the two methods are in good agreement.

4. Conclusions

In order to maintain column performance after scaling up it is essential to develop the correct packing procedure at scale. It is necessary to do a thorough evaluation of packing flow profiles considering the performance of the column with all processing fluids and temperatures. In scaling remember to specify the system capabilities for the highest flow-rate, this is often a packing flow-rate and not any of the running flow-rates. This is often overlooked. It is necessary to determine the appropriate compression factor of the media in use. This is also often overlooked. Media suppliers often times only estimate this value. In order to scale up successfully, compression factor needs to be de-

Table 4
Pulse and RTD analysis in CFV 2000

Pack No.	V_s (% Vc)	N/L	H_{red}	A_f	Test velocity (cm/h)	V_r (% V_c)
8 Pulse	1	677	7.9	1.51	108	83
8 RTD	75	517	10.3	1.63	108	84
8 RTD	75	416	12.9	1.59	161	90
11 Pulse	1.0	1064	5.0	1.00	102	86
11 RTD	75	718	7.4	1.37	100	86

termined. In order to evaluate the success of the scale up appropriate testing protocols must be developed. Since the scale up will require different equipment it is necessary to take the hardware effects into consideration by running a Van Deemter analysis and a distribution analysis.

5. Nomenclature

A_f	Peak asymmetry factor
C_F	Compression factor
d_{50}	Average particle diameter
H	Plate height (L/N)
H_{red}	Reduced plate height (H/d_{50})
V_c	Geometric column (bed) volume
V_{ext}	Extra-column volume
V_r	Retention volume
V_s	Stationary phase volume
V_t	Total liquid volume
μ	Mobile phase velocity (cm/h)

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