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Improving affinity chromatography resin efficiency using semi-continuous chromatography

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ABSTRACT

Protein A affinity chromatography is widely used for purification of monoclonal antibodies (MAbs) from harvested cell culture fluid (HCCF). At the manufacturing scale, the HCCF is typically loaded on a single Protein A affinity chromatography column in cycles until all of the HCCF is processed. Protein A resin costs are significant, comprising a substantial portion of the raw material costs in MAb manufacturing. Cost can be reduced by operating the process continuously using multiple smaller columns to a higher binding capacity in lieu of one industrial scale column. In this study, a series of experiments were performed using three 1-ml Hi-Trap™ MabSelect SuRe™ columns on a modified ÄKTA™ system operated according to the three Column Periodic Counter Current Chromatography (3C PCC) principle. The columns were loaded individually at different times until the 70% breakthrough point was achieved. The HCCF with unbound protein from the column was then loaded onto the next column to capture the MAb, preventing any protein loss. At any given point, all three columns were in operation, either loading or washing, enabling a reduction in processing time. The product yield and quality were evaluated and compared with a batch process to determine the effect of using the three column continuous process. The continuous operation shows the potential to reduce both resin volume and buffer consumption by ~40%, however the system hardware and the process is more complex than the batch process. Alternative methods using a single standard affinity column, such as recycling load effluent back to the tank or increasing residence time, were also evaluated to improve Protein A resin efficiency. These alternative methods showed similar cost benefits but required longer processing time.

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1. Introduction

The standard process for the purification of a monoclonal antibody (MAb) from harvested cell culture fluid (HCCF) involves three main chromatography steps: Protein A affinity (ProA), cation exchange, and anion exchange chromatography [1]. The affinity resin used during the first chromatography step represents a substantial portion of the raw material cost in biologics manufacturing. Improving ProA efficiency during the purification process can reduce the manufacturing cost of MAbs. Using three Column Periodic Counter Current Chromatography (3C PCC) can potentially improve efficiency and lower the resin volume requirements for affinity chromatography.

Affinity chromatography separates proteins from impurities based on a reversible interaction between a protein and a specific ligand coupled to a chromatography matrix [2]. In the batch process, the ProA column is equilibrated followed by HCCF loading, which is based on the binding capacity of the ProA resin. Typically, the column is loaded up to 90% of 1% breakthrough (BT) capacity. Since ProA resin works on a bind and elute principle, the impurities flow through while the protein of interest stays bound to the column. The HCCF load is followed by wash cycles to remove impurities. The protein is then eluted with buffer at a specific pH and conductivity, followed by column regeneration to prepare the column for the next cycle [3]. The process is repeated until all of the HCCF is purified.

Because HCCF is loaded only up to 90% of 1% BT in the batch process, the column is underutilized (the safety factor is used to account for loss of capacity over resin reuses, resin ligand density variability and binding capacity differences due to packing variability). When HCCF is loaded on the column, the protein binds to the top of the column due to the resin's strong affinity for protein. As the top of the column becomes saturated, some of the protein flows through the column and is captured by the unsaturated portion at bottom of the column. This results in the complete utilization of the binding capacity of the top of the column. However, the BT (beyond 1% BT) from the bottom of the column is not captured and is lost in waste, resulting in underutilization of resin at the bottom

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of the column [4]. This underutilization of resin also results in excess buffer consumption because it is used to wash/elute part of the resin (bottom part of the column) without protein.

In this study, we evaluated various methods, including the 3C PCC process (developed by Karol Lacki at GEHC) and alternative approaches to the batch process (recycle load effluent back to load tank, increased residence time) to improve protein capture. The 3C PCC principle using a custom modified ÄKTA™ explorer system, enables efficient utilization of the ProA resin, which results in using less buffer as well as a reduction in processing time [5]. The technology utilizes a simulated moving bed concept [6], where all columns are being used in series or parallel depending on the processing step. The use of multiple columns in this manner allows the columns to be loaded to a higher binding capacity. This technology also has the potential to reduce buffer consumption and processing time [6] because all columns are used simultaneously. The simulated moving bed concept has been used in the pharmaceutical and small molecule industry [7–12], but neither the simulated moving bed nor 3C PCC technology is commonly used for MAb purification.

Alternative methods using a modified batch process were also evaluated for comparison to the 3C PCC method and to potentially provide different options to improve ProA efficiency in MAb manufacturing. This improved efficiency may support the use of 3C PCC in existing manufacturing facilities to purify MAbs with high HCCF titers [13–15].

2. Materials and methods

2.1. Equipment and materials

2.1.1. 3C PCC system

An ÄKTA explorer chromatography system from GE Healthcare (Uppsala, Sweden) was modified to operate as a 3C PCC system (Fig. 1). The system comprised an ÄKTA Purifier 100 (Part# 28-4062-66), two additional UV monitors (part# 18-1108-35), an additional Pump P-900 (2) (part# 18-1114-00), an AD900 analog/digital converter to allow for the use of additional components (part# 18-1148-62), and additional eight-port valves (part# 18-1108-41). Three columns (1-ml, bed height of 2.5 cm) packed with MabSelect SuRe™ (HiTrap™, GE Healthcare) were used during system evaluation. The system was controlled using UNICORN software (v 5.11) with a customized strategy (GE Healthcare) for 3C PCC.

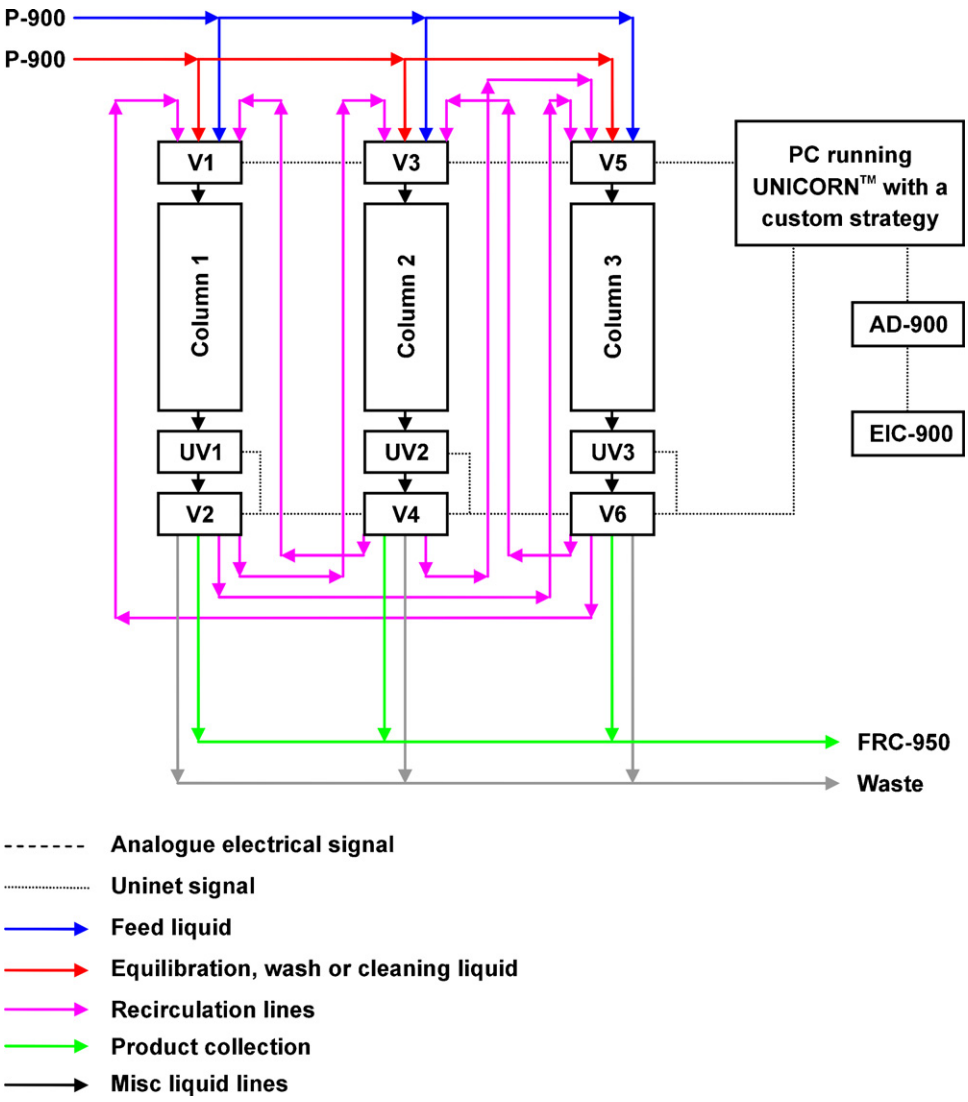

Fig. 1. Modified 3C PCC system schematic (copyright GE Healthcare).

2.1.2. Modified batch processes (recycling effluent, increasing residence time) system

The ÄKTA system described in Section 2.1.1 was also used to evaluate modified batch processes. However, for the modified batch process, only one 1-ml column packed with MabSelect SuRe (HiTrap, GE Healthcare) was used instead of three, and only one UV monitor was attached to the system.

2.1.3. Affinity resins

Two resins, MabSelect SuRe (GE Healthcare, Uppsala, Sweden) and ProSep® vA (Millipore Corporation, Billerica, MA), were evaluated during this study.

2.1.3.1. MabSelect SuRe™. The load flow rate of the MabSelect SuRe varied from 12 to 60 column volumes (CV)/h for a low-titer MAb (MAb1) and 12 to 30 CV/h for a high-titer MAb (MAb2) to include flow range for a typical batch process. The flow range for the high-titer MAb is low, due to equipment limitations. The flow rates for buffer steps were kept constant for different runs (Wash 1, Wash 2, Wash 3, and regeneration: 30 CV/h; elution: 24 CV/h) similar to the batch process.

2.1.3.2. ProSep-vA™. The ProSep vA (7.8 ml) column with a bed height of 10 cm was loaded with MAb2 at a flow rate of 12 CV/h. Other processing steps were loaded at flow rates varying from 6 to 30 CV/h (Wash 1, Wash 2, Wash 3: 15 CV/h, regeneration: 30 CV/h, elution: 6 CV/h).

2.2. Purification process

2.2.1. MAbs and antibody purification process

MAbs were selected ranging from low (1 g/L) to high (4 g/L) cell culture production titers (MAb1 and MAb2, respectively) to test the high and low range of feed concentrations for the 3C PCC technology. The buffers used were similar to those used for the batch process. The columns were equilibrated with 25 mM Tris/25 mM sodium chloride, washed 0.4 M phosphoric acid, eluted with 0.1 N acetic acid (pH 2.9), and regenerated with 0.1 N sodium hydroxide for MabSelect SuRe and 0.1 M phosphoric acid for ProSep-vA [16–18].

2.2.2. Three column periodic counter-current chromatography (3C PCC)

The 3C PCC system was operated continuously with three 1-ml columns instead of one (3-ml) column. Similar to the batch process, the HCCF was loaded (loading temperature of 15–25 °C) onto Column 1 until the column reached 1% BT capacity (Fig. 2A). In the meantime, Columns 2 and 3 were equilibrated and were ready to accept a HCCF load. When 1% BT was achieved, the effluent from Column 1 was diverted to Column 2 to capture the unbound protein from Column 1 (Fig. 2B). The HCCF was loaded on Column 1 until 70% BT was achieved. Thereafter, the load was directed to Column 2, and wash cycles began for Column 1 (Fig. 2C). Because some protein is lost during washing, the wash effluent from Column 1 was directed to Column 3 to capture lost protein. Subsequently, Column 1 was eluted, regenerated, and equilibrated (Fig. 2D). All three columns underwent these steps at different times during the process resulting in equivalent elution from each of the three columns.

2.2.2.1. Switch time calculation. Switch time is the time at which direction of any flow changes (e.g., redirection of Column 1 effluent from waste to Column 2 when Column 1 reaches 1% BT capacity). Since, columns are used in series or parallel depending on processing step, it is critical that the duration of processing steps match, which is accomplished by calculating switch time. The method for determining the switch times is based on finding two characteristic

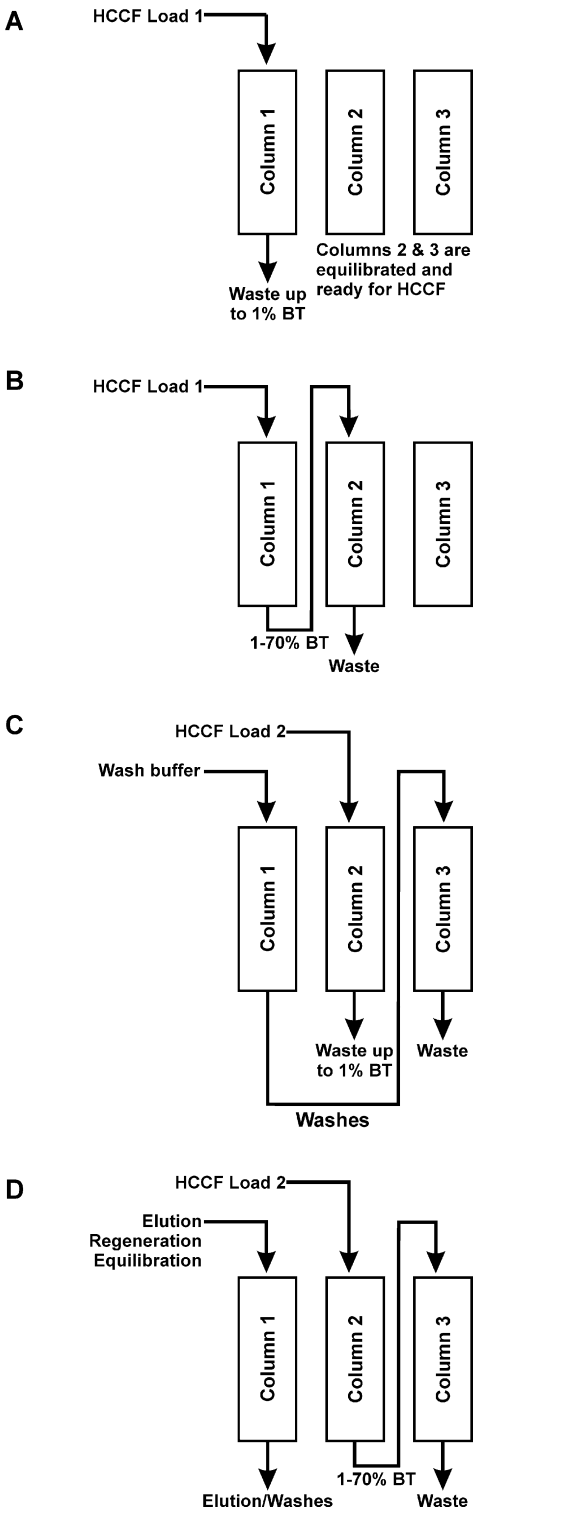

Fig. 2. 3C PCC process description. (A) *Step 1*: The harvested cell culture fluid (HCCF) is loaded onto Column 1 until the column reaches 1% breakthrough (BT) capacity; Columns 2 and 3 are equilibrated and are ready to accept HCCF load. (B) *Step 2*: Once 1% BT is achieved, the effluent from the Column 1 is diverted to Column 2 to capture unbound protein from the Column 1. The HCCF is loaded on Column 1 until 70% BT is achieved. (C) *Step 3*: The load is directed to Column 2 and Column 1 undergoes wash cycles. Because some protein is lost in washes, the wash effluent from Column 1 is directed to the Column 3 to capture the protein lost in washes. (D) *Step 4*: Finally, Column 1 is eluted, regenerated, and equilibrated while Columns 2 and 3 are being loaded.

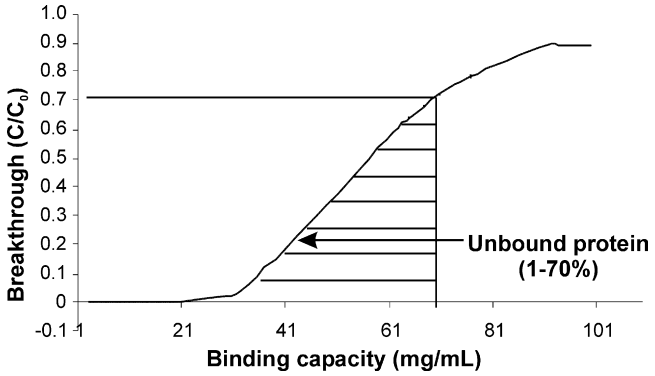

Fig. 3. Breakthrough curve–dynamic binding capacity is calculated similar to the batch process to calculate 1% BT as well as the 70% BT point at different flow rates for each molecule. Unbound protein (protein that gets carried over to the next column) is calculated by integrating the area under the curve (shown by shaded area).

times: a time necessary for reaching 1% BT and time necessary for reaching 70% BT. The dynamic binding capacity, or BT, for the 3C-PCC process is calculated similar to that of the batch process [12,13]. Similar to batch process, the HCCF with known titer/concentration was loaded on to the column and fractions of the affluent were collected. The affluent samples were analyzed for concentration to calculate 1% and 70% breakthrough (the ratio of concentration at time "$t = t$" and concentration at time "$t = 0$"). The 1% BT and 70% BT were determined at different flow rates for each MAb. The specific BT percentage of 70% was chosen arbitrarily. The number can be increased or decreased based on risk versus additional cost savings by going to higher number. In this study, 70% BT resulted in >40% resin and buffer reduction, based on the analysis discussed in Section 4.1. These switch times were used to determine six other switch times for a total of eight switch times in the process. The switch times will vary based on MAb and titer. Low titer MAb would have longer load time versus low load time for higher titer MAb. The load time would also impact the load flow rates which will be discussed in Section 3.

Unbound protein (protein that gets carried over to the next column) was calculated by integrating the area under the curve (Fig. 3). In addition, the amount lost in washes was also analyzed using an antibody concentration assay described in Section 2.3. The BT curve and wash-step data were used to calculate characteristic switch times that were required to program a UNICORN method. The spreadsheet required to calculate switch time is provided by GEHC as part of 3C PCC system.

2.2.3. Consistency and ProSep vA runs

Consistency runs ($N = 10$) were performed for one MAb (MAb 2) to evaluate consistency in results using the 3C PCC system. The high-titer MAb was selected to represent titers of new MAbs in development. Additionally, experiments were also performed with the 3C PCC system with a different affinity resin (ProSep vA) to evaluate the feasibility of using the technology for different resins.

2.2.4. Modified batch processes to achieve higher resin-binding capacity

In addition to the 3C PCC process, two other modified batch processes (a recycling process and an increased residence time process) were also evaluated to achieve higher resin-binding capacity.

2.2.4.1. Recycling effluent to the original tank. The recycling process involved working with the typical one column, similar to the batch process. However, instead of HCCF loading only up to 1% BT capacity, the column was loaded to 70% BT capacity. The effluent was directed to waste up to 1% BT (Fig. 4A). The flow through after 1% BT was redirected to the original tank to capture the protein lost from 1% to 70% BT capacity (Fig. 4B). After 70% BT, the HCCF load to the column was stopped. The column was then washed, eluted, regenerated, and equilibrated (Fig. 4C). These processing steps were repeated until the entire HCCF load was purified.

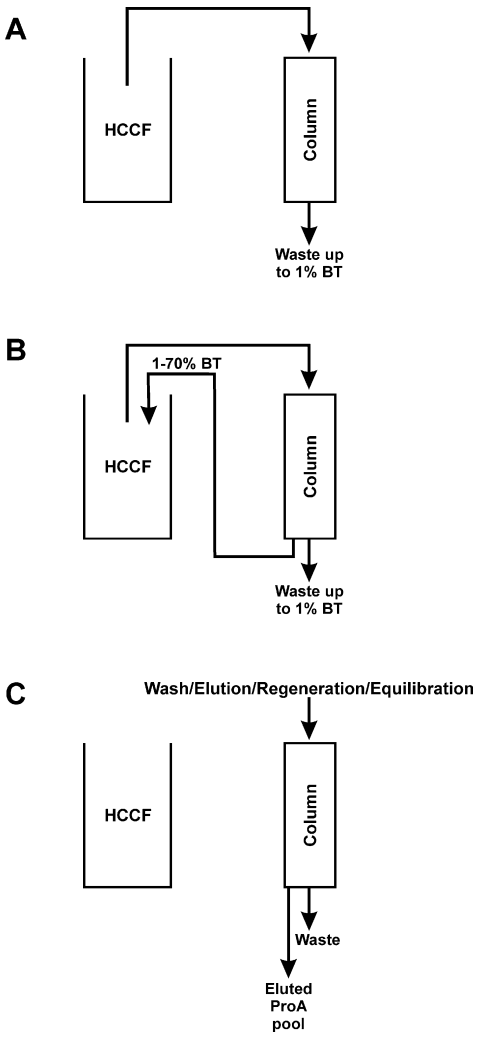

Fig. 4. (A) *Step 1*: The harvested cell culture fluid (HCCF) is loaded onto the column until the column reaches 1% breakthrough (BT) capacity. (B) *Step 2*: The flow through after 1% BT is redirected to the original tank to capture the protein lost from 1% to 70% BT capacity. (C) *Step 3*: The column is washed, eluted, regenerated, and equilibrated.

2.2.4.2. Increasing residence time. The increased residence time process used the standard one column, similar to the batch process. However, the HCCF was loaded at a low flow rate (1.8 CV/h) to increase the residence time, which can result in an increase of the resin-binding capacity similar to the 3C PCC process.

2.2.5. Downstream chromatography runs

The purified ProA pools generated with the 3C PCC process and batch process were purified through downstream chromatography steps (cation exchange and anion exchange chromatographies) to ensure similar step yields and product quality as those of the batch process.

2.2.6. Resin reuse studies

Resin reuse studies were performed with 5 ml column (bed height of 2.5 cm) to evaluate resin lifetime with higher loading capacity up to 70% BT. These studies evaluated column performance

by repeating the processes under the same conditions for a number of cycles. In this study, we chose 250 cycles which is typical for a batch process. The parameters compared across the cycles included host cell protein (HCP), Chinese hamster ovary (CHO) DNA, leached ProA, insulin, and aggregates.

2.3. Analytical methods

The antibody concentration of HCCF was measured using a 2.1 cm × 30 cm POROS column (Applied Biosystems, Foster City, CA) on an Agilent 1100 HPLC (Agilent Technologies, Santa Clara, CA). Buffer A (100 mM sodium phosphate, 250 mM sodium chloride, pH 6.3), Buffer B (2% acetic acid, 100 mM glycine), and Buffer C (0.1 M phosphoric acid, 205 CAN) were used, and the total run time was 4.5 min. The protein concentration in the purified pool was measured using the Agilent 8453 (Agilent Technologies, Santa Clara, CA) spectrophotometer at 280 nm. Multi-product enzyme-linked immunosorbent assay methods were used for HCP, and leached ProA analysis. TaqMan polymerase chain reaction was used for CHO DNA analysis and interlaced size exclusion chromatography was used for analysis of aggregates in the purified pool. The elution pool was not analyzed for virus removal. However, this should be tested if the user intends to claim viral or aggregate clearance from the ProA step.

3. Results and discussion

The evaluation comprised runs at different flow rates (12–60 CV/h) for two different MAbs (MAb1, MAb2). Performance was evaluated by comparing the 3C-PCC process performance, as determined by step yield, HCP and UV profile, to a single-column batch process. Consistency runs with MabSelect SuRe and performance of ProSep vA are also discussed. The results of alternative methods (effluent recycle to tank and increase in residence time) to achieve higher binding capacity are shown next as compared with the 3C PCC process. The downstream purification of ProA pools from all processes and resin reuse studies is also discussed.

3.1. 3C PCC results

3.1.1. MAb1 results

The low-titer MAb1 was purified using HiTrap 1-ml MabSelect SuRe columns at load flow rates ranging from 12 to 60 CV/h to evaluate high and low range inclusive of typical batch conditions.

3.1.1.1. Step yield and product quality. Product quality and step yield results from the 3C PCC system were compared with the batch process (Table 1). The step yield was >98% for load flow rates ranging from 12 to 30 CV/h and was comparable to the historical batch process. The HCP was also comparable to the batch process (<10,000 ppm). Historical HCP data ($N = 3$) for MAb1 in the batch process using MabSelect SuRe ranges between 6000 and 13,000 ppm (nanogram HCP/milligram protein concentration) with a step yield >98%. At higher flow rates (42 CV/h), the column binding capacity is reduced (as expected and reported in the literature [5]). Hence, a large amount of protein was moved from one column to subsequent column during the 1–70% BT step. Due to this, the time required for the subsequent column to reach 1% BT (when load was directed to the subsequent column) was shorter. In parallel to this part of the loading stage, the wash from the previous column was loaded onto the third column. However, the time required for Wash 1 was greater than that required by the subsequent column to reach 1% BT, preventing the third column from accepting the load from the subsequent column. Hence, due to this time constraint, the wash from the previous column was not loaded onto the third column resulting in protein loss during the wash step, resulting in a step yield loss (81.6%).

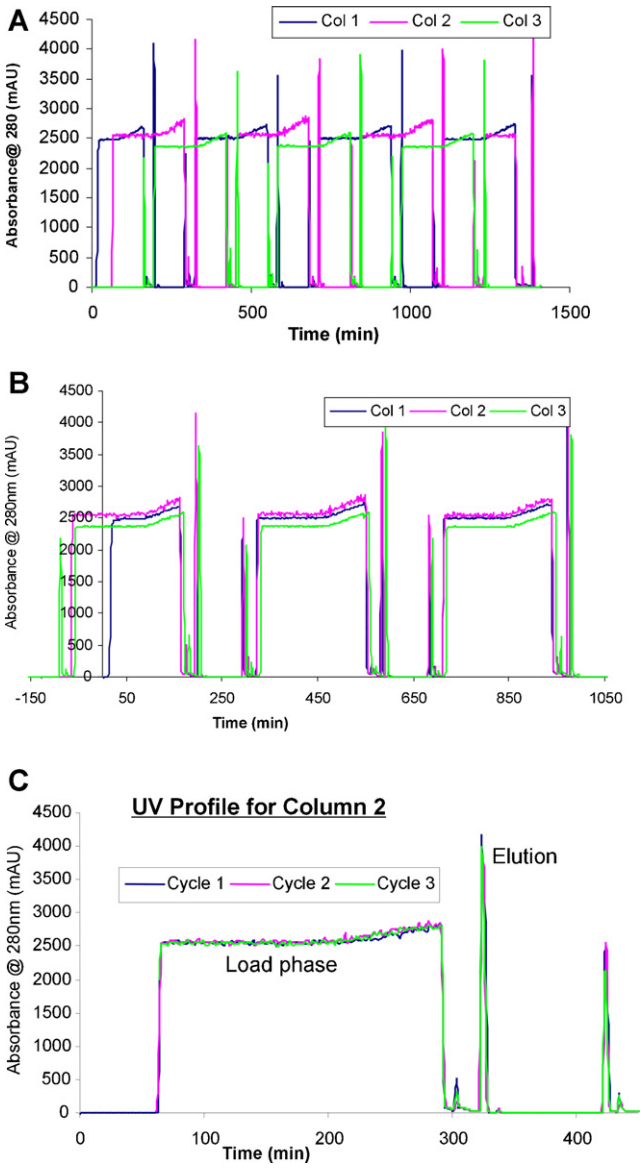

Fig. 5. UV profiles for the three columns. (A) The switch times occurred as expected. Column 2 comes online as soon as Column 1 reaches 1% BT at approximately 63 min. HCCF load to first column stops when 70% BT is achieved at approximately 161 min. Similarly Column 3 comes on line as soon as Column 2 reached 1% BT capacity at 192 min proving that the switching is occurring as expected. (B) Similar UV profiles were observed with runs at other flow rates. All three columns performed consistently over the course of different cycles. (C) The UV profiles were also monitored to ensure consistent column performance over a course of different cycles. The column performed consistently over the different cycles.

The operation at higher flow rates (60 CV/h) was not feasible. The protein lost from 1 to 70% BT and the amount of protein in washes was more than the protein required to reach 1% BT capacity. Hence, the 3C PCC system was limited to operation at low flow rates (12–30 CV/h) only.

3.1.1.2. UV profile. The first run was performed at 24 CV/h to simulate batch process conditions. The UV profile was monitored to ensure the accuracy of switch times for the various steps. The switch times were monitored for all runs, however, UV profiles for only one flow rate (24 CV/h) are presented. The switch times occurred as expected, based on the calculations (Fig. 5A). Column 2 came online as soon as Column 1 reached 1% BT. HCCF load to Column

Table 1
Comparison of product quality and step yield results for MAb1 from the 3C PCC system with those of the batch process. The load flow rate was varied from 12 to 60 CV/h to evaluate high and low range inclusive of current conditions. The step yield and HCP were comparable to batch process at 12–30 CV/h. At higher flow rates, the column binding capacity is reduced (as expected). Hence, a large amount of protein was lost from one column to subsequent column during 1–70% step. Due to this, the time required for subsequent column to reach 1% (when load was directed to subsequent column) was low. In parallel to this step, the wash from previous column is loaded onto the third column. However, time required for wash was higher than that required by subsequent column to reach 1% BT preventing third column from accepting load from subsequent column. Hence, the wash from previous column was not loaded onto third column resulting in protein loss in wash step resulting in step yield loss (81.6%). The protein loss to subsequent column at 60 CV/h was higher than 1% BT, hence run could not be performed.

Load flow rate (CV/h)	Breakthrough (g/L)			Wash 1 in series (Wash 1 loaded on column)	Wash 1 protein (g/L)	Step yield (%)	HCP (ppm)
	1%	70%	Protein to subsequent column (during 1–70%)				
12	38	53	6	Yes	2	99.4	8000
24	22	49	9	Yes	3	100.0	4750
30	25	50	15	Yes	1.5	98.0	6000
42	18	43	12	No	1.5	81.6	5000
60	14	44	16	No	1.5	–	–
Historical data ($N = 3$)	21	N/A	N/A	N/A	N/A	>98%	<10,000

CV, column volume; HCP, host cell protein.

Table 2
Comparison of product quality and step yield results for MAb2 from the 3C PCC system with those of the batch process. The load flow rate was varied from 12 to 30 CV/h. The step yield and HCP were comparable to batch process at 12 CV/h. At 18 CV/h, only 2 out of 5CV's were loaded onto next column due to high protein loss (13 g/L) to subsequent column during 1–70% step resulting in low step yield (90.1%). At 30 CV/h, the protein loss to subsequent column is same as 1% BT, hence run could not be performed.

Load flow rate (CV/h)	Breakthrough (g/L)			Wash 1 in series	Wash 1 protein (g/L)	Step yield (%)	HCP (ppm)
	1%	70%	Protein to subsequent column				
12	30	59	12	Yes	7	100	1000
18	25	55	13	First 2 CVs of total 5 CVs	7	90.1	1600
30	20	51	20	Yes	8	–	–
Historical data ($N = 3$)	35	N/A	N/A	N/A	N/A	>97%	<2000

CV, column volume; HCP, host cell protein.

1 stopped when 70% BT was achieved. Similarly, Column 3 came on line as soon as Column 2 reached 1% BT capacity proving that the switching was occurring as expected. Similar UV profiles were observed for runs operated at flow rates of 12–60 CV/h. All three columns performed consistently over the course of different cycles (Fig. 5B). The UV profiles were also monitored to ensure consistent column performance over a course of different cycles. The column performed consistently over different cycles (Fig. 5C), verifying that column performance was not affected by loading to the higher binding capacity of 70% BT.

3.1.2. MAb2 results

The high-titer MAb2 was purified using the HiTrap 1-ml MabSelect SuRe columns at load flow rates ranging from 12 to 30 CV/h.

3.1.2.1. Step yield and product quality. The product quality and step yield results from the 3C PCC system were compared with those of the batch process (Table 2). The step yield was >98% at 12CV/h, which is comparable to the historical data ($N = 3$) for a batch process. The HCP data were comparable to the batch process. The HCP ranged between 900 and 1400 ppm with a step yield of >98.0% for the batch process. However, at a load flow rate of 18 CV/h, only two out of five Wash 1 CVs were loaded onto the next column due to high protein loss (13 g/L) to the subsequent column during the 1–70% step which resulted in a low step yield (90.1%) as a result of protein loss in Wash 1.

Similar to MAb1, the operation at higher flow rates was not feasible. The cumulative mass of MAb2 present in the effluent from Column 1 during operation was between 1 and 70% BT and the amount of protein present in the washes was more than the protein required to reach 1% BT capacity.

Since the system was limited at a high flow rate for both low and high titer MAbs (1–4 g/L), a software modification was made to enable pausing the buffer and load pump, independent of each

Table 3
Evaluation of 3C-PCC process consistency for the purification of MAb2, as compared with ten runs were performed at a flow rate of 12 CV/h with MAb 2 to ensure process consistency. The step yields and product quality for all ten runs were consistent and comparable to the historical batch process data. The deviation is due to variation in analytical methods as well as HCCF HCP values (feed stock was not same for all the runs). The step yields were greater than 98% and the HCP values were within acceptable range.

Run number	Step yield (%)	Pool protein concentration (g/L)	HCCF HCP (ppm)	Pool HCP (ppm)
1	99	11.77	250,000	2300
2	109	12.28	250,000	3300
3	115	13.78	340,000	3400
4	110	13.07	340,000	2000
5	100	11.42	370,000	2400
6	101	11.54	370,000	2000
7	110	12.62	370,000	2100
8	101	12.34	130,000	2600
9	103	12.17	120,000	1900
10	104	12.58	120,000	1800
Historical data ($N = 3$)	>98%	5–7	N/A	<2000

HCCF, harvested cell culture fluid; HCP, host cell protein.

Table 4
Comparison of product quality and step yield results for MAb2 from the recycling process with those of the batch process. The load flow rate was varied from 12 to 30 CV/h for Mab2. As seen in 3C PCC experiments, there is protein loss during wash cycle. Hence, Wash 1 was collected separately and loaded at the end of the run as last cycle. The step yield increased from 90% to 100% when Wash 1 was loaded onto the column. The step yield was greater than 98% at higher flow rates of 18–30 CV/h. The HCP was comparable to the batch process at flow rates of 18–30 CV/h. The other product quality attributes were also comparable to historical batch process.

	12 CV/h	12 CV/h	18 CV/h	30 CV/h	Historical data ($N = 3$)
Wash 1 loaded on column	No	Yes	Yes	Yes	N/A
Step yield (%)	90	100	100	98	>97
HCP (ppm)	<2000	<2000	<2000	<2000	<2000
% aggregate	N/A	0.78	1.39	1.22	0.8–1.1
Leached Protein A (ppm)	N/A	<4.18	<3.01	<3.0	2–3
Insulin (ppm)	N/A	<0.04	<0.04	<0.04	<0.01
CHO DNA (ppm)	N/A	0.03	0.08	0.02	0.1–0.2

CHO, Chinese hamster ovary; HCP, host cell protein.

other. This pause enabled holding the load step until the subsequent column was ready, and it resulted in a step yield increase from 90.1% to 97.0%. The HCP was <3000 ppm, which was comparable to the batch process.

Leached protein was also analyzed to evaluate the impact of increased HCCF load hold time on leached ProA. The leached ProA (5.7 ppm) was within acceptable limits (<30 ppm), eliminating any concerns about the effect of increased HCCF hold time on the column. The impact of a load time increase of 10% (~5% increase in total time) was minimal.

3.2. Consistency and ProSep vA runs

3.2.1. Consistency results on MabSelect SuRe

Ten runs were performed with MAb 2 at a flow rate of 12 CV/h to ensure process consistency. The step yields and product quality for all 10 runs were consistent and comparable to historical batch process data (Table 3). The deviation was due to variation in analytical methods, as well as HCCF HCP values (feed stock was not the same for all of the runs). The step yields were >98%, and the HCP values were within an acceptable range (<2000 ppm).

3.2.2. ProSep vA results

Similar step yield and product quality results and the increase in binding capacity (~45%) showed that the 3C PCC process can be used with different ProA resins. The step yield of 98% and HCP value of 1700 ppm were comparable to MabSelect SuRe, as well as the batch process (data not shown).

3.3. Modified batch processes to achieve higher resin binding capacity

3.3.1. Recycling effluent to the original tank

3.3.1.1. Step yield and product quality. The product quality and step yield results from the recycling process were compared with the batch process (Table 4). As seen in the 3C-PCC experiments, there is protein loss during wash cycle. Hence, Wash 1 was collected separately and loaded at the end of the run as the last cycle. The step yield increased from 90% to 100% when Wash 1 was loaded onto the column. The step yield was >98% at higher flow rates of 18–30 CV/h. The HCP was comparable to the batch process at all flow rates. The other product quality attributes, including HCP DNA, insulin, aggregates, and leached ProA, were also comparable to the historical batch process.

3.3.2. Residence time

The MAb2 HCCF load flow rate on a 1-ml MabSelect SuRe was reduced to 1.8 CV/h to maximize residence time. The conditions of the other processing steps (Wash 1, Wash 2, Wash 3, regeneration: 30 CV/h, elution: 24 CV/h) were kept similar to the 3C PCC process. The step yield (97%) and HCP (3000 ppm) at 1.8 CV/h were comparable to the batch process (data not shown). However, in spite of the lower load flow rate, the binding capacity was 40 g/L compared with 70% BT capacity of 50 g/L at 18 CV/h for the 3C PCC process. In addition, processing time also increased, which makes the increased residence time process a relatively difficult technology to implement in a manufacturing facility.

3.4. Downstream purification

The step yields and product quality across the purification path were comparable to historical batch process data. The protein pools from affinity chromatography using the 3C PCC process and the recycling process were purified through the downstream chromatography purification steps (cation exchange and anion exchange chromatographies) to ensure that there was no negative impact on these downstream steps.

3.5. Resin reuse

Similar to the standard single-column process, the 3C PCC process can be used for 250 cycles without any significant step yield loss or impact on product quality. The high-titer MAb2 was used to evaluate the feasible resin reuse cycles with this technology. The MabSelect SuRe column was loaded with MAb2 up to 70% BT capacity. The column was sanitized with 0.5 N sodium hydroxide after every six cycles, similar to the standard single-column process. There was a 10% decrease in protein concentration which is comparable to the batch process (Fig. 6). Also, the product quality was consistent over different cycles and is comparable to the batch process. The % aggregates were slightly higher compared to historical which can be contributed to sample handling. However, the difference is insignificant and within acceptable limit of less than 5%.

This is a significant result, as resin reuse is a critical component of this study to completely evaluate the benefit of the 3C PCC technology or modified methods that load to higher binding capacity. If the number of cycles obtained with 3C PCC technology or modified methods that load to higher binding capacity is less than the batch process, the resin-saving benefit gained with the new technologies would be nullified.

4. Comparison of 3C PCC and modified batch processes to batch process

The processes were compared for their performance and potential cost savings.

4.1. Economic evaluation

An economic evaluation of the 3C PCC process and the alternative processes was performed and compared with that of the batch

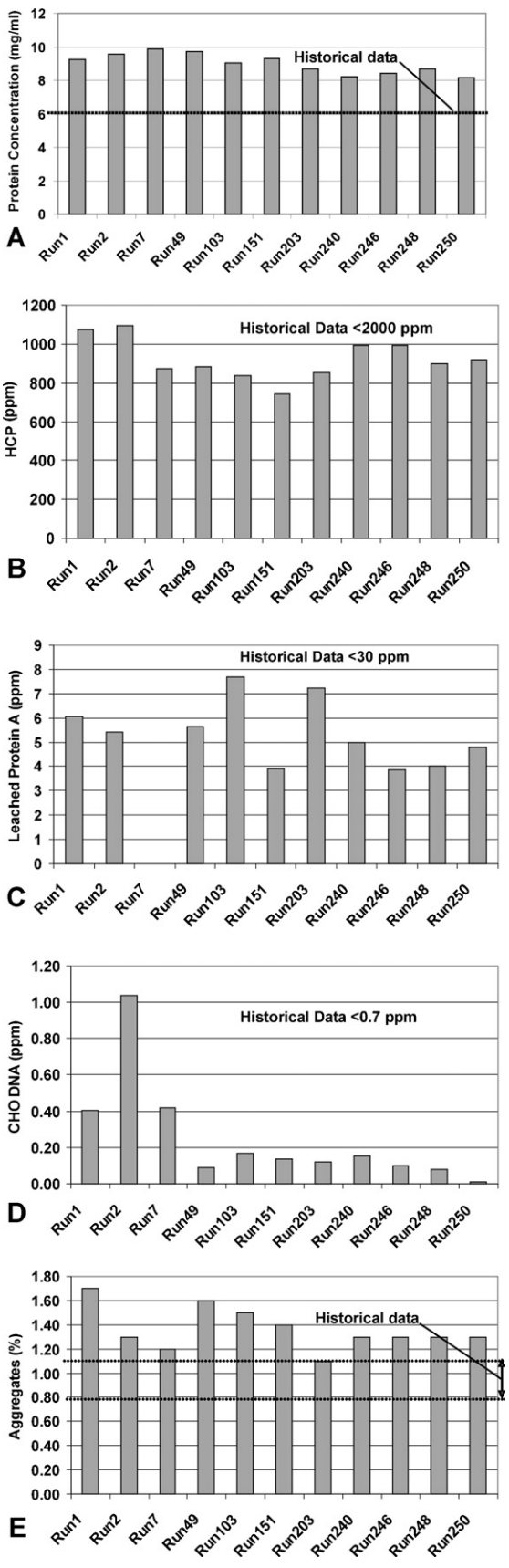

Fig. 6. Resin reuse studies were performed with high-titer MAb2. (A) There was a 10% decrease in protein concentration, which is comparable to the batch process. (B–E) Product quality is consistent over different cycles and is comparable to the batch process.

process. The factors considered for economic evaluation included: (a) process conditions which included batch size (as a function of fermenter size and titer), step yields, cycle time (as a function of resin binding capacity and batch size), number of columns (three small columns for 3C PCC versus one large column for batch process), (b) labor hours for different unit operations like column packing/unpacking, cycle time, number of cycles per run, (c) capital cost which included design engineering cost, column cost, software development cost, equipment cost, instrument control cost, construction and changeover cost, (d) process change cost due to regulatory impact for existing molecules, process development and process validation, (e) consumable cost, (f) inflation, and (g) runs per year.

The cost of resin per gram bulk (based on cost of the resin, number of cycles, binding capacity, compression factor, and step yield) would be approximately 40% lower using the 3C PCC process compared with the batch process. The traditional hastealloy columns were assumed for financial evaluation for all processes. A more comprehensive analysis was also performed using a period of 15 years. A conservative model was used for the new technologies. Inflation, labor escalation, and discount rate were accounted for in the cost calculations. The implementation of either the 3C PCC process or the recycling process was favorable. The cost savings ranged from $5 to $11 million over 15 years depending on implementation scenarios considering the number of sites and type of manufacturing (clinical, commercial, or both). Additionally, the use of multi-column chromatography increases the flexibility of using pre-packed columns because smaller columns are used instead of one large column. It may be especially beneficial in the manufacture of clinical products, where industrial scale columns are used for a few cycles and then discarded.

4.2. Considerations and implementation scenarios

The results show that higher loading options increase the efficiency with comparable step yield and product quality for all systems. However, the 3C PCC system is relatively more complex due to additional valves and pumps that increase the probability of mechanical failures.

The recycling process is easier to implement in an existing manufacturing facility; however, it is limited by variability in the titer for each HCCF load cycle. Recycling the effluent back to the original tank results in dilution of the original stock and, hence, a different titer for each load cycle. Also, the processing time for this method is greater when compared with the 3C-PCC process, and is equivalent or greater when compared with the batch process.

The other alternative, increasing residence time, is not a viable option due to increase in processing time beyond typical acceptable manufacturing ranges. However, for manufacturing facilities designed for titers of 1–2 g/L, retrofitting existing systems with a 3C PCC system or a recycling system may allow the purification of high-titer molecules without adding tanks or increasing the size of the chromatography columns.

5. Conclusions

This study showed that the efficiency of affinity chromatography can be improved using multi-column chromatography (3C PCC). The multi-column chromatography and modified batch processes have the potential to save approximately 40% on the cost of resin, buffer, and processing time. The results showed similar step yield and product quality compared with the traditional single-column process. In addition, the higher binding capacity had no impact in the number of cycles. The resin can be used for 250 cycles using 3C-PCC technology, which is similar to the batch process. In addition, due to reduced number of process cycles, the elution

pool is reduced in both cases (3C PCC and the recycling process), leading to a reduction in both processing time and raw materials for downstream purification processes. However, both the 3C PCC and the recycling processes are more complex operations than the batch process. Also, the work was performed at lab scale. The technology would have to be evaluated at pilot scale before implementing it in manufacturing. In addition, process validation for marketed products would be a challenge because there are no large-scale data for biological processes using these technologies. The best starting point for their implementation would be with a new molecule in the clinical testing stage. In all cases, due to reductions in resin, buffer consumption, and processing time, these technologies provide the option of using existing manufacturing facilities for increasing HCCF titers. Their implementation would be a function of resin reuse, economic analysis, and facility requirements for individual manufacturers.Acknowledgements

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