Biotechnology and Bioprocess Engineering
https://doi.org/10.1007/s12257-024-00099-1

RESEARCH PAPER

Design and optimization of a continuous purification process using ion-exchange periodic counter-current chromatography for a low-titer enzyme

Kwanyoung Ko[1] · Min-Jung Kim[1,2] · Dasom Kim[1] · Kangyun Seo[1] · Sangho Lee[2]

Received: 4 December 2023 / Revised: 19 March 2024 / Accepted: 25 March 2024

Abstract
A continuous purification process can be beneficial to the purification of biologics due to its higher productivity and efficiency than a conventional batch purification process. However, regulatory issues and lack of established cases render deployment of the continuous process difficult in industrial settings. Here we report a case study for design and optimization of an advanced continuous process for purifying a low-titer enzyme as a model biologic. To convert a conventional batch process to an advanced continuous one in purification of biologics, conventional unit operations (UOs), including ultrafiltration/diafiltration (UF/DF) and batch chromatography, were replaced by advanced ones such as in-line dilution conditioning (IDC) and periodic counter-current chromatography (PCC). The UF/DF UO was changed to IDC UO to adjust pH and conductivity. The mixing ratio of the sample and the conditioning buffer in IDC was determined by experiments with three buffers. PCC was optimized with two variables, column height and sample loading residence time, as the delta pressure in the columns was less than 1.0 bar. A graph indicating the operating area was plotted to efficiently control the PCC. Although the sample volume increased in IDC, PCC had a complementary advantage in that purification was performed faster than batch chromatography. We observed at least 25% increase in economic advantage when the advanced continuous process was applied to purify a low-titer enzyme. We propose not only a continuous process with the substitution of UF/DF and batch chromatography with IDC and PCC but also a method to optimize PCC by plotting operating areas.

Keywords Low-titer enzyme · Integrated Continuous process · In-line dilution conditioning · Periodic counter-current chromatography

1 Introduction

In many industries, such as petrochemical, food, and chemical ones, a batch process has been converted to a continuous one [1, 2]. Continuous processes can improve the productivity and reduce cost and equipment footprint [3]. Recently, they have been studied in the pharmaceutical industry primarily to reduce costs due to frequently changing market demand [4, 5]. However, regulatory issues and lack of established cases render deployment of the advanced continuous process difficult in industrial settings. A primary regulatory hurdle in the implementation of continuous manufacturing processes is the challenge of integrating and validating equipment capable of real-time quality monitoring. Due to the need for investment in new facilities and research, there are still limited cases of applying continuous processes to drug development in the pharmaceutical industry [6].

A typical biopharmaceutical manufacturing process is composed of two main stages: The upstream process (USP), which involves cultivating cells in a bioreactor to express proteins, and the downstream process (DSP), which entails the separation and purification of the target protein from the culture medium containing the expressed proteins. In the early stages of pharmaceutical continuous process development, USP studies were mainly conducted by replacing fed-batch cultures with perfusion cultures. Although the productivity of USP was increased by running perfusion cultures,

Kwanyoung Ko and Min-Jung Kim have equally contributed to this work.

✉ Sangho Lee
sangholee@skku.edu

[1] R&D Center, GC Biopharma, Yongin 16924, Korea

[2] Department of Biological Sciences, Sungkyunkwan University, Suwon 16419, Korea

Published online: 04 April 2024

the cost of DSP to purify high-productivity USP products soared [7]. Subsequently, the advanced continuous process in DSP was studied in the capture step (1st chromatography) by replacing batch chromatography with multi-column chromatography. This process leads cost reduction with high productivity in DSP [8–10].

Periodic counter-current chromatography (PCC) employing multi-columns allows cost reduction and productivity increase by maximizing resin capacity. Studies on PCC have been conducted using many types of equipment such as AKTA PCC 75 and CaptureSMB as well as the multi-column counter-current solvent gradient purification (MCSGP) process. The dynamic binding capacity (DBC) for PCC where a sample is continuously loaded into serial columns is higher than that for batch chromatography. The residence time (RT) can be reduced because the volume of each column in PCC is smaller than that in the batch process [11–13].

Most studies on PCC have focused on high-titer antibodies (> 5 g/L) with perfusion culture in USP and protein A resin in DSP [14–16]. However, the application of PCC to low-titer enzymes has rarely been reported. To facilitate the application of ion-exchange multi-columns to purify low-titer native enzymes and to verify economic advantages in non-antibodies continuous processing cases, we designed and optimized an advanced continuous processing method for low-titer enzymes using in-line dilution conditioning (IDC) and PCC.

2 Materials and methods

2.1 Biologic sample

In this experiment, the utilized protein was a low-titer natural enzyme with a molecular weight of approximately 60 kDa. It was characterized by the attachment of various glycans, conferring an isoelectric point (pI) that varied between 5.5 and 6.5. The starting material was 4 L of clarified culture media (Harvest) and the target protein concentration was 0.19 mg/mL.

2.2 Description of the conventional batch process

The conventional batch process consisted of ultrafiltration/diafiltration (UF/DF) and ion exchange chromatography (Table 1). For UF/DF step, membrane with a 30 kDa cutoff (P3C030C01, Merck) was used that concentrates 13-fold and exchanges buffer pH and conductivity. The retentate of UF/DF unit operation (UO) was injected for anion exchange chromatography (Q Sepharose Fast flow, 17-0510, Cytiva) with a column volume of 53 mL. The target protein was obtained by increasing the sodium chloride molarity at the elution step.

Table 1 Information of start material and conventional batch process conditions

Start material	Parameters
	Titer: 0.19 g/L
	Harvest volume: 4 L
	pH ≒ 7.2
	Conductivity ≒ 10 mS/cm
Unit operation	Step
UF/DF	Ultrafiltration (≥ 13X)
	Diafiltration (≥ 3 DV)
Q chromatography (column volume: 53 mL)	Sample load (unbound)
	Unbound wash (5 CV)
	Elution (5 CV)
	Column wash (5 CV)
	CIP (5 CV)

UF/DF ultrafiltration/diafiltration, *CIP* cleaning-in-place

2.3 Description of the advanced continuous process

In the advanced continuous process, the IDC UO was used instead of the UF/DF UO of the conventional batch process (Fig. 1). The sample which was conditioned by the IDC was directly injected into the PCC. IDC featuring in-line liquid mixing has advantages over UF/DF because IDC is simpler. For this mixing, two pumps (EW-77916-20, Masterflex) were operated and connected to the harvest sample and conditioning buffer. After finding the conditioning buffer, the mixing ratio of the sample and the conditioning buffer was determined. And then, a multi-column process was performed using AKTA PCC 75 (29457820, Cytiva). Finally, the performance was compared to that of the conventional batch process.

2.3.1 IDC buffer scouting

The pH and conductivity of the starting material (harvest) were about 7.2 and 9.7 mS/cm, respectively. To adjust the sample pH to 7.5 and conductivity to less than 6 mS/cm, the conditioning buffer was researched. The pH values of 7.5, 7.7, and 8.0 were tested in a 10 mM-Tris buffer in the conditioning buffer. The sample and buffer in-line were mixed using two pumps. The pH and conductivity were observed as the ratio of the flow rates of the two pumps changed.

2.3.2 Breakthrough (BT) curve test

To confirm the DBC of resin and determine the process parameters in PCC, a BT curve test is needed. The BT curve is the same as that of the DBC test of batch chromatography.

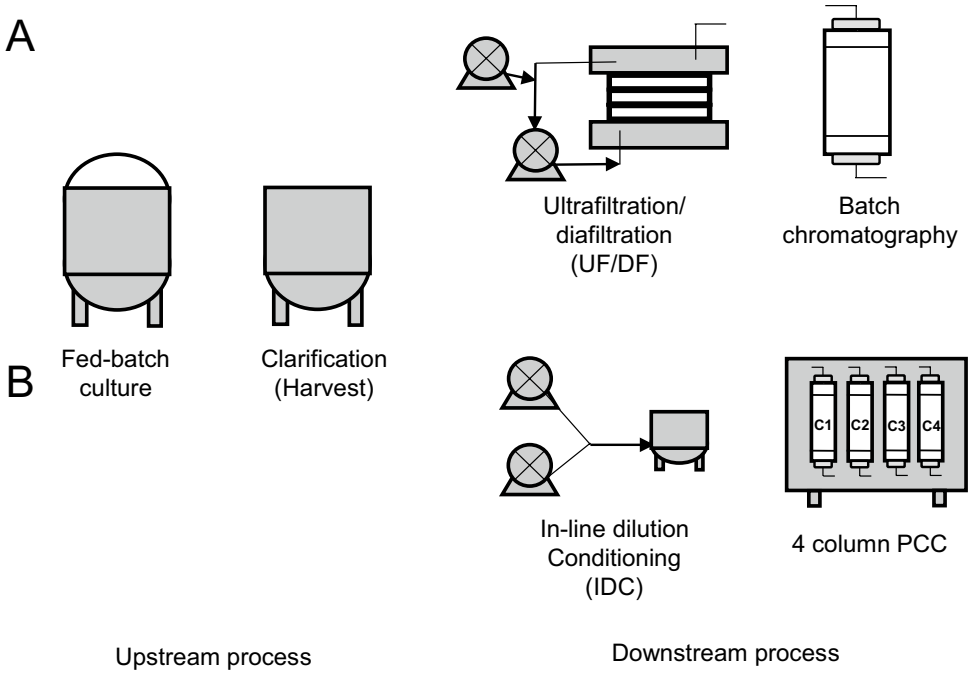


Fig. 1 Process diagrams of two processes. **A** Conventional batch process including UF/DF and batch chromatography. **B** Advanced continuous process including IDC and 4 column-PCC. UF/DF: ultrafiltration/diafiltration, IDC: in-line dilution conditioning, PCC: periodic counter-current chromatography

The Hitrap Q 1 mL column (17-1153-01, Cytiva) was used for the test. The chromatographic process was the same as that in Table 1, with a RT of 1 min, and the load volume injected into the column was 1600 mL. By inserting a BT curve into the method design tool (MDT) of AKTA PCC 75, a design space plot of the PCC was obtained. The plot shows the correlation between the sample loading RT in the PCC and the BT (%) portion in the sub-loading column. The PCC development was based on this plot.

2.3.3 PCC parameter development

This study aimed to transform conventional batch process into an advanced continuous one. In the case of batch chromatography, it must be replaced by a PCC. We established PCC process parameters with similar performance to the batch chromatography. A 4-column PCC was used, and column height and sample loading RT were set as process parameters. Owing to the variation in height when the columns are packed, it is quite possible that different delta pressures will occur in the columns. Therefore, the delta pressure for the four columns was set to under 1 bar.

2.3.4 Holding vessel design between IDC and PCC

A holding vessel is essential for correcting the flow-rate error of the two pumps in the IDC step. It can delay the process just by filling a tank when any accident occurs during manufacturing and provide sufficient mixing with the sample and buffer by holding in the tank. Determining an appropriate vessel volume is important to achieve a stable process. We estimated the vessel volume according to Eqs. (1)–(4):

$$F_A + F_{\mathrm{B}} \geq F_{\mathrm{PCC\ load}} \tag{1}$$

$$F_{\mathrm{accumulation\ in\ vessel}} = F_A + F_{\mathrm{B}} - F_{\mathrm{PCC\ load}} \tag{2}$$

$$V_{\mathrm{accumulation\ in\ vessel}} = F_{\mathrm{accumulation\ in\ vessel}} \cdot t_{\mathrm{PCC\ load}} \tag{3}$$

$$V_{\mathrm{vessel}} \geq V_{\mathrm{max,accumulation}} \tag{4}$$

F_A is the flow rate of the sample, F_{B} that of the dilution buffer, and F_{PCC} that of the PCC load; V is the vessel volume; and t_{PCCload} is the total time of sample loading of the PCC.

2.4 Analysis

The yield and purity of the target proteins were compared by the two processes. Reversed-phase high-performance liquid chromatography (RP-HPLC) was used to measure the concentration of the target protein. The gradient elution method was applied by mixing acetonitrile (1.00030.4000, Merck) and water (AH365-4, BURDICK & JACKSON). The purity was analysed with size exclusion (SE)–HPLC with phosphate buffer saline using the (17-516, Lonza) isocratic method. Finally, sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed to

compare band patterns with those of processes in a 4–12% Bis–Tris gel (NP0321BOX, Invitrogen).

3 Results

3.1 Overview of advanced continuous process

We developed the advanced continuous process for purification of a low-titer native enzyme with 5.5–6.5 of pI value in capture step (Fig. 1). We used cultures from fed-batch reactors in USP and an ion-exchange resin in DSP. An IDC UO was used for sample preparation. IDC is usually used so that the target concentration of buffer can be prepared by controlling the mixing ratio of a stock solution and water. By extending this principle, the pH or conductivity of a sample can also be conditioned. We changed the conventional batch process, which included UF/DF and ion-exchange chromatography (Fig. 1A), to an advanced continuous process with IDC and PCC (Fig. 1B). The combination of the simple IDC UO and PCC UO has the advantage of increasing efficiency by eliminating the complicated UF/DF UO. AKTA PCC 75 was used for the multi-column process. It can execute three or four column processes and minimize the loss of target proteins in the process by a post-load wash (PLW) step. This step prevents the yield loss from system hold-up by washing a column to a tandem column when the loading valve is switched [11, 12]. In addition, it uses a MDT that calculates PCC run parameters from a BT curve test which facilitates PCC design [13]. In addition, the PCC operating area was plotted and verified to account for scale-up. Moreover, we analysed the impact of costs by plotting the correlation among resin reuse number, resin re-packing, and total cost for managing the process.

3.2 Optimization of parameters in advanced continuous process

Before developing the advanced continuous process, the conventional batch process was conducted as the control to compare process performance. The harvest was concentrated to approximately 13-fold and exchanged to threefold with a diafiltration buffer via UF/DF. The step yield of UF/DF was 80.4%. The concentrate was purified by batch chromatography with a yield of 86.8%. The purity of the final product was 62.6%, and the overall yield and purity were 69.8 and 62.6%, respectively (Table 2). Once we established a customized advanced continuous process, we set out to optimize parameters for IDC and PCC.

3.3 Optimization of IDC parameters in advanced continuous process

To find a suitable conditioning buffer that adjusts pH 7.5 ± 0.2 and a conductivity of less than 6 mS/cm, three buffers were used (Table 3). The mixing ratio range between the sample and buffer was determined by controlling the pump flow rate (Table S1). The pH was adjusted to 7.5 ± 0.2, regardless of the mixing ratio of the sample and buffer. However, we believed that using a conditioning buffer with a pH of 7.5 may have hindered the control of the process because all conditions were below pH 7.4. Based on these results, the minimum pH was set above 7.5. The ratio of 1:0.9 of the sample to buffer was sufficient to meet the conductivity. The operating range of mixing was set above 1:1, considering the safety margin. The conditioning buffer had a pH of 7.7 to 8.0, 10 mM Tris, and the mixing ratio was set above 1:1.

3.4 Optimization of PCC parameters in advanced continuous process

A BT curve test was performed to determine the PCC process parameters by confirming the DBC. After preparing the sample with a 1:1 mixture of harvest and pH 7.7 buffer, 1,600 mL was loaded with a RT of 1 min (1 mL/min). The flow-through was collected per 40 mL, and each fraction was quantitatively analysed using by RP-HPLC. The C/C_0 value reached 1.0 at 400 mL loading corresponding to 10 fractions (Fig. 2A). The values of C/C_0 were entered into the MDT in the AKTA PCC 75. The correlation between the BT portion and sample loading RT was plotted (Fig. 2B). Because the red zone (lower zone) is an impossible condition for the PCC operation, the user can operate the PCC after the operating condition in the green zone (upper zone) is determined [13].

Table 2 Results of conventional batch process

Unit operation	Volume (mL)	Concentration[a] (mg/mL)	Total amount (mg)	Yield (%)	Purity (%)
Start material	4000	0.190	760.0	–	–
UF/DF	310	1.972	611.3	80.4	–
Q chromatography	265	2.002	530.5	86.8	62.6
Overall				69.8	62.6

UF/DF ultrafiltration/diafiltration

[a]Protein concentration

Table 3 Sample dilution buffer scouting for IDC

Buffer no	pH	Concentration[a] (mM)
1	7.5	10
2	7.7	
3	8.0	

IDC in-line dilution conditioning

[a]Tris buffer was used for the scouting

Moreover, the conditions were calculated from MDT for the maximum capacity (BT 100%) and maximum productivity (BT 5%) (Table 4). The higher the titer of the sample was, the higher productivity in Table 4 would be, because PCC would purify much amounts of proteins during the same time. Other studies with high-titer sample naturally concluded to have higher productivity in PCC than batch mode [14–16]. Therefore, this study is valuable because we show the application case of continuous process with a low-titer sample and low productivity.

Because one of the advantages of PCC is that less reagents are needed, the total resin volume of the 4-column PCC was set under batch mode (53 mL). The column height (H) was set as a variable, whereas the inner diameter (I.D.) of the column was set to 1.6 cm to consider scale-up. Parameters for IDC and PCC had to be optimized simultaneously. The flow rates of the two pumps were set to 5 mL/min and the sample loading flow rate was 8.66 mL/min in PCC. Based on the flow rates, the holding vessel volume was set to 2 L. The volume can be varied to control the two flow rates. The volume of the vessel was fixed with a large safety margin because we controlled the two flow rates manually. However, the volume of the vessel could be reduced by controlling the flow rates automatically. We designed a PCC with a delta pressure (ΔP) of < 1.0 bar for process safety because the critical pressure of the resin used is < 3 bars [17]. ΔP was checked with an equilibrium buffer because the correlation between the BT portion and sample loading RT graph does not reflect the delta pressure in the column depending on the geometry (H and I.D.). When the sample loading RTs were 1.0 and 1.5 min, ΔP values were approximately 1.2 and 0.8 bar, respectively.

Run 1 was tested with maximum column volume of 52 mL (13 mL/column × 4 columns). The condition for the run 1 was determined as 13 mL of each column volume and 1.5 min (8.66 mL/min) of sample loading RT. Other steps, such as unbound wash, elution, column wash (CW) and cleaning-in-place (CIP), were set with a RT of 2.0 min and the same injection volume of buffers as in batch mode. The value of BT in PCC was set at 5%, which was the maximum productivity condition, because the volume of load material increased by approximately 40-fold compared that in the conventional method due to the replacement of UF/DF UO

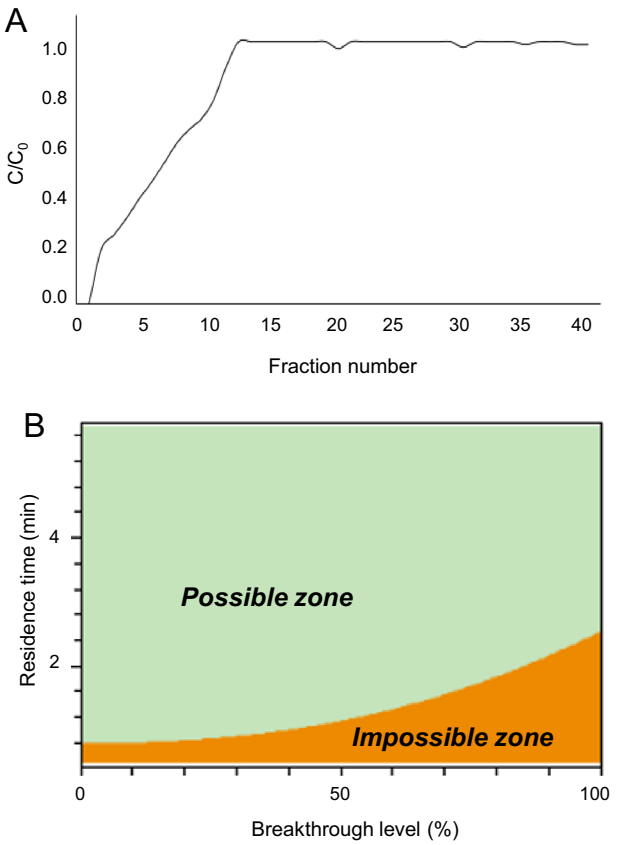


Fig. 2 Results of experimental C/C_0 curve and simulation in MDT. **A** A C/C_0 curve from the breakthrough curve test. **B** A plot of sample residence time as a function of breakthrough level. The possible zone of PCC operation is coloured as green. MDT: method design tool, PCC: periodic counter-current chromatography

by IDC step. The conditions of the run 1 had column geometry (I.D./H = 1.6/6.5) and 1:1 mixing in in-line conditioning with 4 L of starting material. The pH and conductivity of the load sample into PCC were 7.34 and 5.18 mS/cm by IDC step, respectively. Three periodic peaks were observed (PLW, elution, and CW) in a chromatogram of PCC. PLW is a specific tool in AKTA PCC 75 that prevents the loss of target proteins in the pathways. Eleven eluents were purified

Table 4 Optimized PCC conditions for max productivity or max capacity calculated from MDT in AKTA PCC 75

Parameter	Max productivity	Max capacity
Sample loading residence time (min)	0.75	2.5
Breakthrough (%)	5	100
Productivity (g/L/h)	1	< 1
DBC (g/L)	4	15
Capacity utilization (%)	29	100

PCC periodic counter-current chromatography, *MDT* method design tool, *DBC* dynamic binding capacity

in the run 1 unlike the conventional batch process which has just one eluent, and the process yield and product purity were 70.2 and 69.3%, respectively. The yield was similar to the conventional batch process, and the purity was slightly higher than before. The results of the run 1 were better than those of the conventional batch method. The comparison of chromatogram between two processes was performed (Fig. 3). Figure 3A indicates chromatogram of purification that has a different number of eluates, and Fig. 3B represents that the purity of the advanced continuous process was higher.

A higher mixing ratio in the IDC step and a faster sample loading RT in the PCC were tested in run 2. The harvested sample and conditioning buffer were mixed at a ratio of 1:2. To set the sample loading RT to 1 min, the column height was lowered to 5.0 cm while maintaining the same inner diameter. The maximum sample loading RT was treated for 1 min by considering both the maximum productivity condition (Table 4), and the safety margin. The delta pressure in the column was approximately 0.7–0.8 bar at 1 min of sample loading RT (10 mL/min) using equilibrium buffer. For in-line conditioning, pumps A and B were operated at 3.5 and 7.0 mL/min, respectively. Then, the sample was injected into the PCC at 10 mL/min. After IDC step, a pH of 7.49 and a conductivity of 3.87 mS/cm were measured. Although 4 L of the starting material was also used in the run 2, the PCC load sample was much larger than that in the run 1 due to the mixing ratio. Fifteen eluents were prepared with a yield of 75.0% and purity of 64.9% (Fig. S1). The yield was higher than run 1 with a decrease in resin volume. The reason for the yield increase is to strongly capture the target proteins to the ligand by decreasing the conductivity in ion-exchange chromatography. In addition, the impurities in the conditioned sample might be higher than in conventional batch process because there was no separation in IDC step, unlike UF/DF UO. Because the process under study was a capture chromatography focused on the recovery of the target protein, the mixing ratio in IDC was set to 1:2 after the run 2. The performance of the advanced continuous process was verified in runs 1 and 2. In terms of column height and sample loading RT, the operating range was rigid because there were only two points.

To obtain the operating range as an area, run 3 was performed with a column height of 5 cm and 1.2 min of sample loading RT (8.33 mL/min). To avoid a longer process time, the sample loading RT was determined to be close to 8.66 mL/min. Therefore, the harvest and conditioning buffers were mixed at 3 and 6 mL/min, respectively, and the PCC loading flow rate was 8.33 mL/min in the run 3. The results of IDC step were a pH of 7.60 and conductivity 3.13 of mS/cm. The yield and purity were 73.3% and 64.7%, respectively, for 14 eluents, similar to the run 2 (Fig. S2). After the run 3 was performed, the operating area could be plotted as a triangle in the graph, indicating the correlation between sample loading RT and column height.

To determine the working area, after selecting any point in the triangle, the process was operated as run 4. We used 3.4 L of the harvest, and the mixing ratio in the IDC step was 1:2. Each column volume in the PCC was 11 mL (I.D./H = 1.6/5.5), and the sample loading RT was 1.25 min (8.8 mL/min). The conditioned sample had a pH of 7.50 and a conductivity of 3.37 mS/cm. The analysis showed that the yield and purity were 78.6% and 63.8%, respectively, and the triangular zone proved to be appropriate (Fig. S3).

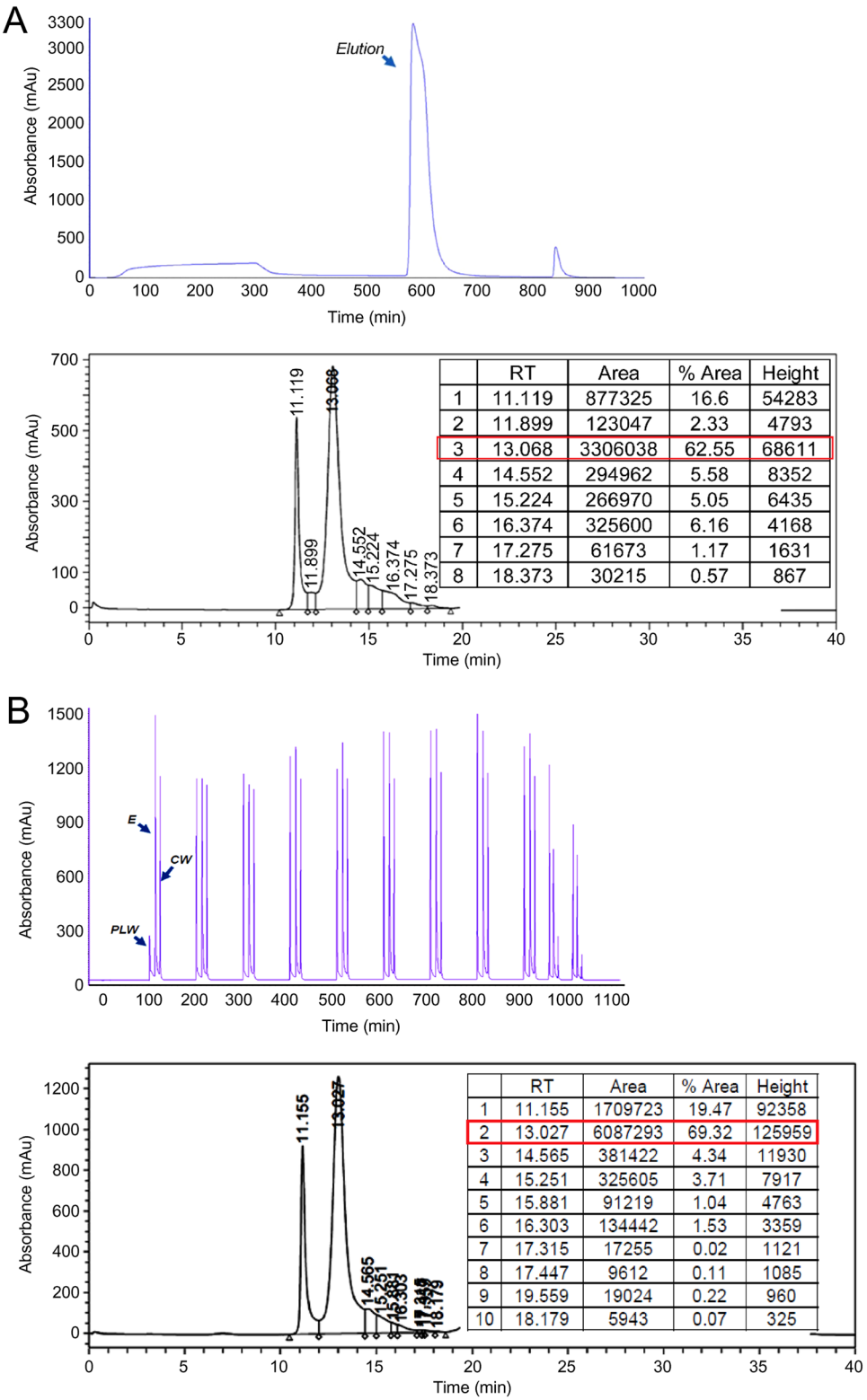
The operating range and results of yield and purity of advanced continuous process were compared to those of the conventional batch process (Fig. 4). The operating range was plotted according to the column height and sample loading time. We separated out three risk zones from the triangular range: a high-pressure risk zone, a risk zone with longer process time (non-effective process), and a non-recommendation zone with harsh conditions in terms of flow rate and column capacity (Fig. 4A). Because the results of all operating conditions were higher than those in conventional batch purification, the superiority of advanced continuous process regarding the results and cost was demonstrated (Fig. 4B and C). The SDS-PAGE results revealed that all products were equal in their sizes and the purity was similar (Fig. S4). The results of the advanced continuous process 4 runs showed that the quality was the same or better than that of the conventional method under all conditions (Table 5).

In this study, it was confirmed that the UF/DF UO for sample preparation and the batch chromatography in the conventional batch process can be converted to the advanced continuous process including IDC and PCC UO. The application of the new process enables to automate manufacturing process from a bioreactor to a capture chromatography. Automated operation can reduce manual errors and prevent contamination in pharmaceutical manufacturing.

3.5 Cost comparison of advanced continuous process with conventional batch one

We compared the cost of the two types of process as the advanced continuous process might be not cost effective because we had to purify too low-titer samples. As the amount of sample is increased, more reagents are also needed. Therefore, we had to verify the effectiveness in terms of cost. The cost was calculated based on the amounts of reagents used (Table 6). The cost standard was set to the resin of the conventional batch process. The amount of resin in the advanced continuous process was applied to minimum volume. The membrane cost was the highest. When comparing UF/DF with IDC, the cost decreased by 756-fold.

In case of chromatography, PCC could save 13% of cost than that in batch mode, which was calculated by using Eqs. (5)–(6):



	RT	Area	% Area	Height
1	11.119	877325	16.6	54283
2	11.899	123047	2.33	4793
3	13.068	3306038	62.55	68611
4	14.552	294962	5.58	8352
5	15.224	266970	5.05	6435
6	16.374	325600	6.16	4168
7	17.275	61673	1.17	1631
8	18.373	30215	0.57	867

	RT	Area	% Area	Height
1	11.155	1709723	19.47	92358
2	13.027	6087293	69.32	125959
3	14.565	381422	4.34	11930
4	15.251	325605	3.71	7917
5	15.881	91219	1.04	4763
6	16.303	134442	1.53	3359
7	17.315	17255	0.02	1121
8	17.447	9612	0.11	1085
9	19.559	19024	0.22	960
10	18.179	5943	0.07	325

Fig. 3 Results of conventional batch and advanced continuous processes. **A** Chromatogram of a column (top side) and result of the purity (bottom side) in conventional batch process. **B** Chromatogram of 4-columns PCC (top side) and result of the purity (bottom side) in advanced continuous process. PCC: periodic counter-current chromatography, RT: residence time

$$\text{Cost of conventional process} = 30\alpha\,(\text{membrane}) + \alpha\,(\text{resin}) + 0.25\alpha\,(\text{Buffer}) \quad (5)$$

$$\text{Cost of advanced process} = 0.75\alpha\,(\text{resin}) + 0.25\alpha\,(\text{Buffer}) \quad (6)$$

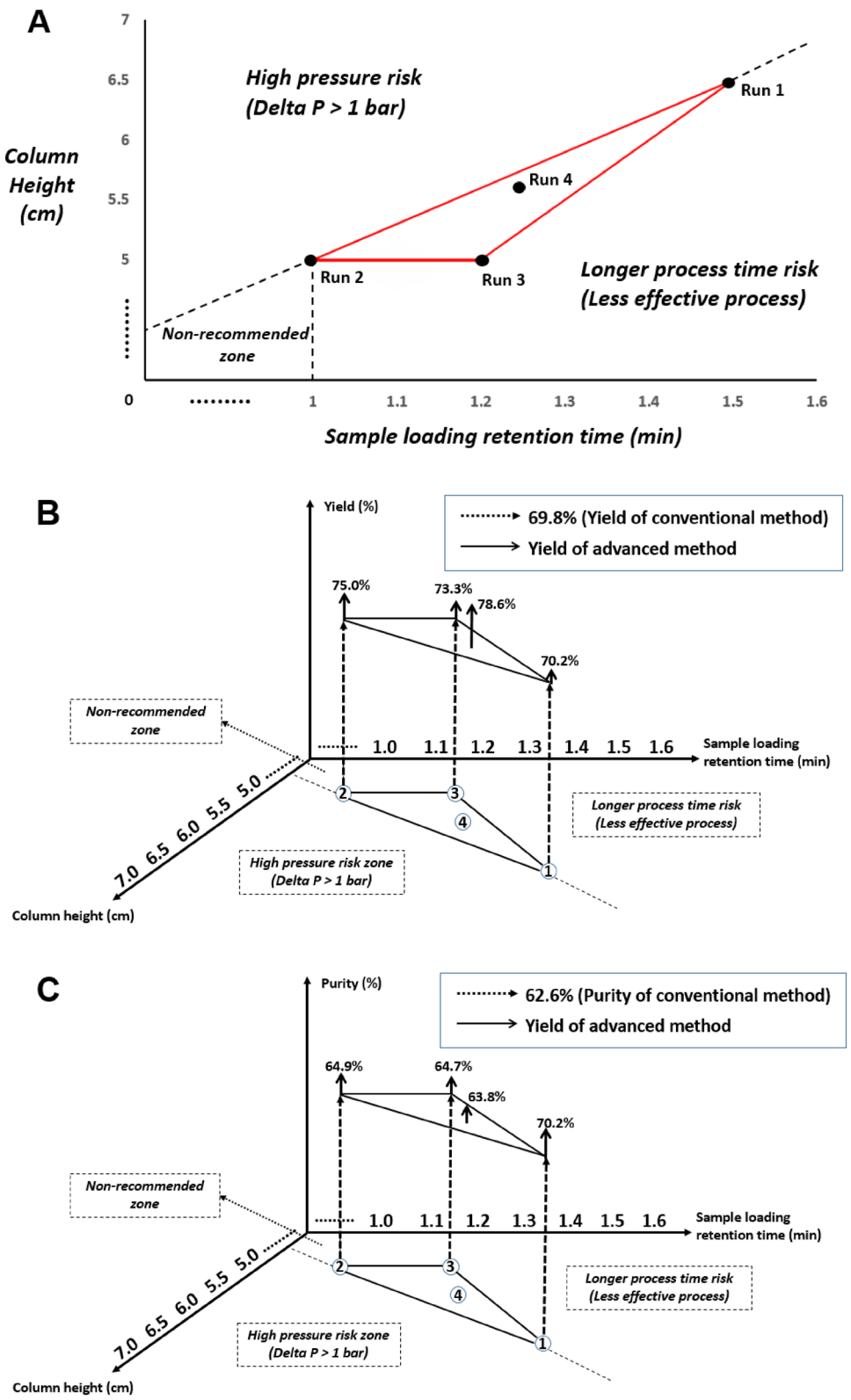


Fig. 4 Comparison of advanced continuous process with conventional batch process by operating range, yield and purity. **A** Operating range of periodic counter-current chromatography (PCC) with the combination of column height and sample loading residence time. **B** Yield with PCC 4 runs versus that with conventional process. **C** Purity with PCC 4 runs versus that with conventional process

The advanced continuous process could reduce the cost by 31 times when comparing two processes just with one batch. However, because this benefit was derived from just one batch, we attempted to adjust the reuse numbers of the membrane and the resin. Then, we compared the total cost ratio for managing the process and

Table 5 Results of advanced continuous process

Run no	IDC conditions				PCC running conditions			Product quality	
	Start material	Dilution buffer	Mixing ratio	PCC load sample	Column height (cm)	Sample loading RT (min)	Breakthrough (%)	Yield (%)	Purity (%)
1	pH 7.2 Cond. 9.7[a]	pH 7.7 10 mM Tris	1:1	pH 7.34 Cond. 5.18	6.5	1.5	5	70.2	69.3
2			1:2	pH 7.49 Cond. 3.87	5.0	1.0	5	75.0	64.9
3			1:2	pH 7.60 Cond. 3.13	5.0	1.2	5	73.3	64.7
4			1:2	pH 7.50 Cond. 3.37	5.5	1.25	5	78.6	63.8

PCC periodic counter-current chromatography, *IDC* in-line dilution conditioning, *RT* residence time

[a]Unit of conductivity: mS/cm

Table 6 Comparison of cost between advanced continuous process with conventional batch process by unit operations

Process	Unit operation	Material	Cost
Conventional process	UF/DF	Membrane	30.00α
		Buffer	0.24α
	Anion exchange (batch)	Resin	1.00α
		Buffer	0.10α
	Sub-total		31.34α
Advanced process	IDC	Buffer	0.04α
	Anion exchange (PCC)	Resin	0.75α
		Buffer	0.21α
	Sub-total		1.00α

UF/DF ultrafiltration/diafiltration, *IDC* in-line dilution conditioning, *PCC* periodic counter-current chromatography

calculating the cost effectiveness between two processes. We assumed run number as r, reuse number of the resin as k, and re-packing number of the resin as c. The value of c was defined as r/k ($n \geq 1$). The number of membrane reuse was set permanently; thus, we did not consider the change of membrane.

In case of the cost of buffer, because two types of process had the same cost, the buffer cost was neglected while comparing the cost ratio. Moreover, the resin of advanced continuous process was reused four times than that of conventional batch process because the PCC consisted of four columns and periodic elution. Therefore, if the reuse number of the resin is too small, it would be not cost effective. In considering these points, Eqs. 5–6 were rearranged by the total cost Eqs. (7)–(8):

$$\text{Total cost of conventional process} = 30 + \sum_{r=1}^{\infty} \alpha \quad (7)$$

$$\text{Total cost of advanced process} = \sum_{r=1}^{\infty} 0.75\alpha * 4 \quad (8)$$

Based on these equations, the impact of the resin reuse number and the re-packing number on the total cost was investigated (Fig. 5A). In terms of the resin re-packing number, it was effective if the re-packing number was less than 14 while performing r batches (Fig. 5B). When it was higher than 8, we found that the advanced continuous process was cost effective (Fig. 5C). Although the resin reuse test was not conducted, we could reason that the number is at least 12 considering the runs 1–4 (total 50 eluates). We concluded that the advanced continuous process could save 25% costs compared to the conventional batch process cost when k was 12 (Fig. 5C). The gain will be larger as k increases.

We analysed the total cost for both processes-the conventional batch process and the advanced continuous process-by UOs (Fig. 6 and Table 6). The initial operation, UF/DF in the conventional batch process and IDC in the advanced continuous process, was critical in determining the total cost: the improvement of cost saving was over 750-fold (30.24α for the conventional process vs. 0.04α for the advanced process). The overall cost saving by employing the advanced continuous process was over 30-fold (31.34α vs. 1α).

To confirm cost effectiveness, we compared the productivity index (Table 7). The productivity index was calculated by dividing amount of product by process time, number of the laborer, and process cost. We compared the results of the conventional batch process (Table 1) and run 1 of the advanced continuous process. The whole process time of the conventional batch process was 15 h, shorter about 30% than advanced continuous process (22 h). However, more laborers and UOs were needed in the conventional process, counterbalancing the advantage in terms of the process time. By contrast, the advanced continuous process required only 2

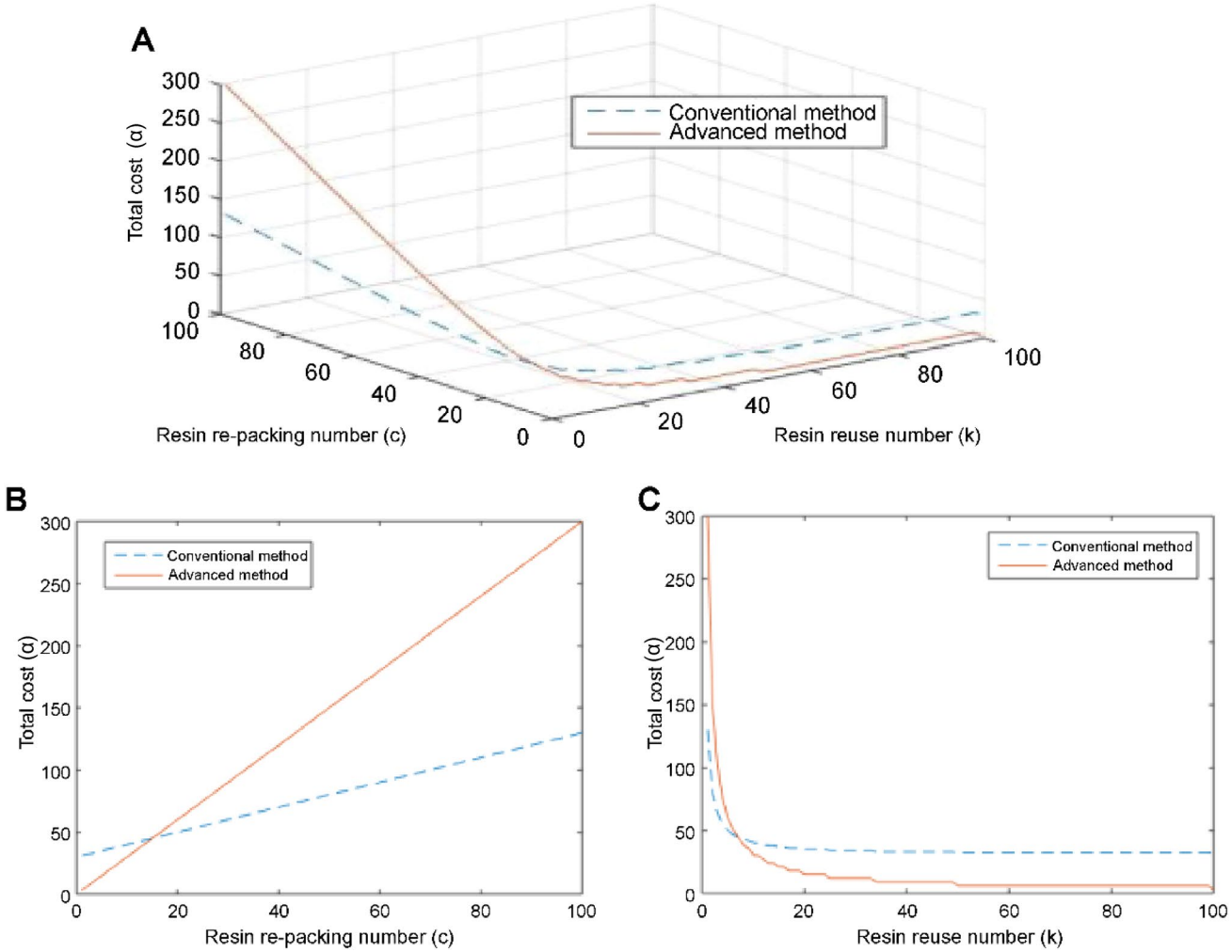


Fig. 5 Results of cost analysis. **A** Total cost according to resin re-packing number and resin reuse number. **B** Total cost according to resin re-packing number. **C** Total cost cording to resin reuse number

laborers although the whole process time was longer than that of the conventional batch process because IDC & PCC could be operated automatically. The productivity index was 0.28 in the conventional batch process and 12.1 in the advanced continuous process. In short, the continuous process is more than 43 times more effective than the conventional one. The main key factors for superior productivity index for the advanced continuous process were the number of labourer and the process cost due to automation. Application of the advanced continuous process enabled us to purify the low-titer enzyme.

4 Discussion

In this study, a conventional batch process that included UF/DF and batch chromatography was converted to PCC by IDC. The pH and conductivity of the sample were adjusted by mixing with a conditioning buffer in the ratio of sample to buffer (1:1–1:2) instead of UF/DF. The results of advanced continuous process were better than those of the conventional batch purification. Because the yield and purity of advanced continuous process were 0.4–10.8% and 1.2–6.7% higher, respectively, conversion to continuous process has advantages not only automation but also quality improvement. To control the advanced continuous process, the operating area was plotted from three experiments with two variables: column height and sample loading RT. This area was demonstrated with an arbitrary point run (run 4). Further, we confirmed that it is possible to save the cost by adopting advanced continuous processes. Our results could help scale-up study of IDC because real-time monitoring system could be adopted in larger scales to set-up the mixing condition in IDC. Also, the result suggests that advanced continuous purification have the economic advantage of diminishing UO (UF/DF) and decreasing resin volume by up to 25%. Replacing the UF/DF with the IDC not only reduces

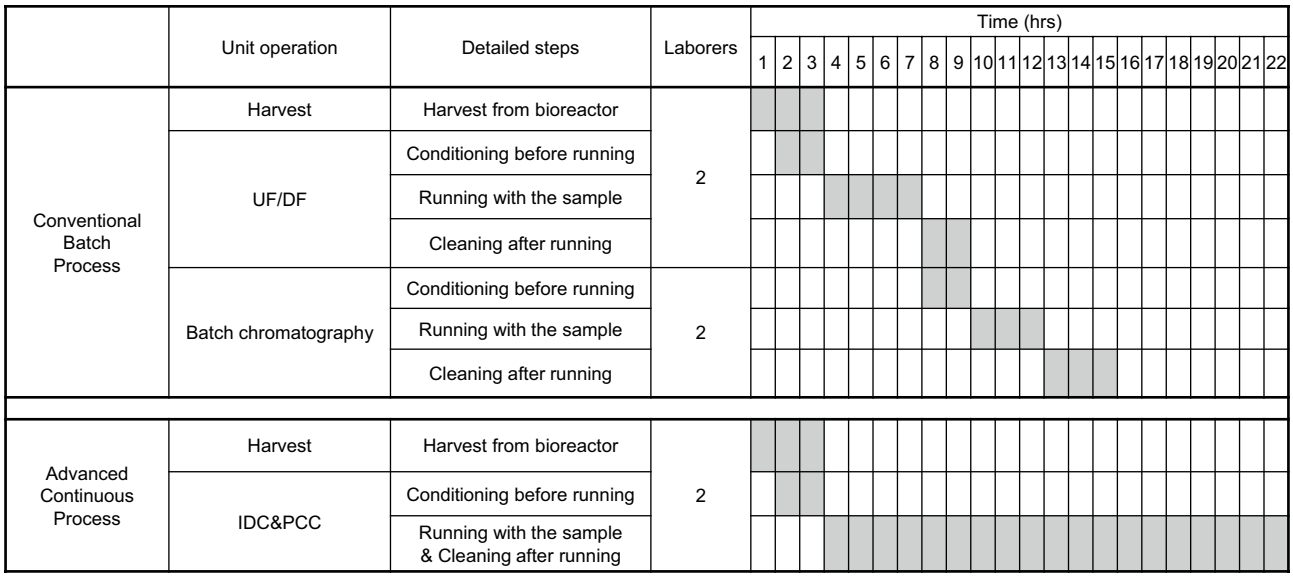


Fig. 6 Comparison of process time and number of laborers between conventional batch process and advanced continuous process. UF/DF: ultrafiltration/diafiltration, IDC: in-line dilution conditioning, PCC: periodic counter-current chromatography

Table 7 Comparison of productivity index between conventional and advanced processes

Process	Process time (h)	Number of laborer	Process cost (α)	Amount of product (mg)	Productivity index[a]
Conventional process (Table 1)	15	4	31.34	530.5	0.28
Advanced process (run 1)	22	2	1.00	533.5	12.10

[a] $\text{Productivity index} = \frac{\text{Amount of product}}{(\text{Process time})(\text{Number of laborer})(\text{Process cost})}$

manpower and time required for membrane maintenance, but also offers advantages in terms of cost. Additionally, it aids in complying with regulatory requirements for pharmaceutical approval, including control over batch-to-batch variation of membrane filters, process validation, and data integrity issues. The transition to the IDC involves the adoption of system automation technologies, thereby enhancing compliance with regulatory standards for pharmaceutical approval.

This study could give a guidance for developing continuous process in a capture step, which containing fed-batch bioreactor, UF/DF, and chromatography with low-titer enzymes. Because GC Biopharma has various modalities apart from antibodies, we intend to expand our advanced continuous process study to vaccines, plasma-derived materials, mRNA and so on.

The advanced continuous process presented here has advantages in terms of cost and convenience. By eliminating the need for UF/DF UO, costs are much reduced. This shows that low-titer enzymes can also be used as the advanced continuous process with economic advantages. Process control was simplified by the introduction of IDC step. In addition, a new PCC development approach is proposed to determine the triangular operating zone, which has advantages. One of advantages is that it can be scaled up by changing the column diameter, and the other that the sample loading RT can be controlled according to the column height. Because columns must be packed by hand, it is possible that the height does not match the target. The triangle allows process control regardless of the deviation from the column packing. Moreover, implementing cost analysis, we could know that costs will be reduced. The biggest factor to reduce the cost is to eliminate the unit process (UF/DF), and the other is that the volume is decreased in transforming batch chromatography to PCC.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12257-024-00099-1.

Acknowledgements This work was supported by GC Biopharma.

Data availability Due to company policy, data used in research cannot be provided to other researchers.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval Neither ethical approval nor informed consent was required for this study.

References

1. Anderson NG (2001) Practical use of continuous processing in developing and scaling up laboratory processes. Org Process Res Dev 5:613–621. https://doi.org/10.1021/op0100605
2. Laird T (2007) Continuous processes in small-scale manufacture. Org Process Res Dev 11:927. https://doi.org/10.1021/op700233e
3. Ötes O, Flato H, Winderl J et al (2017) Feasibility of using continuous chromatography in downstream processing: comparison of costs and product quality for a hybrid process vs. a conventional batch process. J Biotechnol 259:213–220. https://doi.org/10.1016/j.jbiotec.2017.07.001
4. Farid SS (2007) Process economics of industrial monoclonal antibody manufacture. J Chromatogr B Anal Technol Biomed Life Sci 848:8–18. https://doi.org/10.1016/j.jchromb.2006.07.037
5. Konstantinov KB, Cooney CL (2015) White paper on continuous bioprocessing. J Pharm Sci 104:813–820. https://doi.org/10.1002/jps.24268
6. Hernandez R (2015) Continuous manufacturing: a changing processing paradigm. BioPharm Int 28:20–27
7. Gomis-Fons J, Andersson N, Nilsson B (2020) Optimization study on periodic counter-current chromatography integrated in a monoclonal antibody downstream process. J Chromatogr A 1621:461055. https://doi.org/10.1016/j.chroma.2020.461055
8. Aumann L, Morbidelli M (2007) A continuous multicolumn countercurrent solvent gradient purification (MCSGP) process. Biotechnol Bioeng 98:1043–1055
9. Baur D, Angarita M, Müller-Späth T et al (2016) Optimal model-based design of the twin-column CaptureSMB process improves capacity utilization and productivity in protein A affinity capture. Biotechnol J 11:135–145. https://doi.org/10.1002/biot.201500223
10. Godawat R, Brower K, Jain S et al (2012) Periodic counter-current chromatography—design and operational considerations for integrated and continuous purification of proteins. Biotechnol J 7:1496–1508. https://doi.org/10.1002/biot.201200068
11. Chen CS, Konoike F, Yoshimoto N et al (2021) A regressive approach to the design of continuous capture process with multi-column chromatography for monoclonal antibodies. J Chromatogr A 1658:462604. https://doi.org/10.1016/j.chroma.2021.462604
12. Pagkaliwangan M, Hummel J, Gjoka X et al (2019) Optimized continuous multicolumn chromatography enables increased productivities and cost savings by employing more columns. Biotechnol J 14:e1800179. https://doi.org/10.1002/biot.201800179
13. Cytiva (2020) ÄKTA pcc chromatography systems. https://cdn.cytivalifesciences.com/api/public/content/digi-18245-original. Accessed 11 Jan
14. Baur D, Angarita M, Müller-Späth T et al (2016) Comparison of batch and continuous multi-column protein A capture processes by optimal design. Biotechnol J 11:920–931. https://doi.org/10.1002/biot.201500481
15. Baur D, Angelo JM, Chollangi S et al (2018) Model assisted comparison of protein A resins and multi-column chromatography for capture processes. J Biotechnol 285:64–73. https://doi.org/10.1016/j.jbiotec.2018.08.014
16. Guo J, Jin M, Kanani D (2020) Optimization of single-column batch and multicolumn continuous protein A chromatography and performance comparison based on mechanistic model. Biotechnol J 15:e2000192. https://doi.org/10.1002/biot.202000192
17. Nweke MC, McCartney RG, Bracewell DG (2017) Mechanical characterisation of agarose-based chromatography resins for biopharmaceutical manufacture. J Chromatogr A 1530:129–137. https://doi.org/10.1016/j.chroma.2017.11.038