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Continuous precipitation for monoclonal antibody capture using countercurrent washing by microfiltration

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Abstract

There is renewed interest in the possibility of using precipitation for initial capture of high-value therapeutic proteins as part of an integrated continuous downstream process. Precipitation is greatly facilitated by the high product titers now achieved in most cell culture processes, in sharp contrast to chromatographic processes whose performance is reduced at high titers. The current study used a combination of reversible cross-linking (zinc chloride, $ZnCl_2$) and volume exclusion (polyethylene glycol) agents to precipitate a monoclonal antibody product directly from harvested cell culture fluid using a continuous tubular precipitation reactor. The precipitates were then dewatered and continuously washed using tangential flow filtration, with a countercurrent-staged configuration used to reduce the amount of wash buffer required and increase host cell protein removal. Long-term operation was achieved by operating the membrane modules below the critical filtrate flux to avoid fouling. Experimental results demonstrate the feasibility of this fully continuous integrated precipitation process at bench scale, with design calculations used to explore the key factors affecting the performance of this system for initial antibody capture.

KEYWORDS

antibody, continuous processing, microfiltration, precipitation, staging

1 | INTRODUCTION

Precipitation is one of the earliest developed techniques used for large-scale protein purification because of its low cost and the flexibility to fine-tune the separation based on the choice of precipitation methods. Plasma proteins are still purified using precipitation, now in combination with chromatographic polishing steps, with the different fractions (products) obtained by proper choice of ethanol concentration, solution pH, temperature, and salt concentration.[1,2] This is facilitated by the high concentrations of several key plasma proteins including albumin, IgGs, fibrinogen, alpha-1-antitrypsin, and apolipoprotein.[3] However, the very low titer of early recombinant proteins (<0.1 g/L) made target protein precipitation impractical if not impossible. Instead, the initial capture was performed using bind-and-elute chromatography, for example, Protein A affinity chromatography is currently used for the initial capture of essentially all monoclonal antibody (mAb) products.[4,5]

The significant increase in product titer over the past two decades, with concentrations above 5 g/L now fairly routine for mAbs,[6] has led to a renewed interest in the potential use of precipitation for antibody capture. Previous studies have examined the use of cold ethanol and $CaCl_2$,[7] polyethylene glycol (PEG) and $CaCl_2$,[8] PEG and low pH,[9] and $ZnCl_2$ and PEG[10] for antibody precipitation. More recently, Burgstaller et al.[11] achieved high recovery of a monoclonal antibody product from harvested cell culture fluid (HCCF) by continuous precipitation using a combination of 2 mM $ZnCl_2$ with 7% PEG with molecular weight of 6 kDa. The precipitate was then concentrated and washed using tangential flow filtration with 0.2 μm pore size hollow fiber membranes.

The mAb was recovered in the washed product stream at a yield of 95% with 97% purity, with a significant reduction in high molecular weight impurities.

The objective of this study was to examine some of the key design variables in a fully integrated continuous precipitation process for the purification of a mAb using the $ZnCl_2$–PEG system. However, in contrast to Burgstaller et al.,[11] the $ZnCl_2$ and PEG were added sequentially (instead of at the same time) to better control the yield and morphology of the precipitated mAb. The precipitates were then directly and continuously dewatered using a hollow fiber membrane module without any retentate vessel (hold tank) as used by Burgstaller et al.[11] In addition, the washing step was performed using a two-stage countercurrent configuration without any $ZnCl_2$ or PEG to significantly reduce the buffer requirements/costs while enhancing removal of host cell proteins (HCPs). We also employed an inline resolubilization step using a low pH glycine buffer, providing a final product stream that would be suitable for subsequent purification as part of a fully integrated continuous downstream process. Model calculations were used to obtain additional insights into the overall system performance.

2 | MATERIALS AND METHODS

2.1 | Cell culture

Experiments were performed with a monoclonal antibody produced in Chinese hamster ovary cells in a 2 L perfusion bioreactor. Cell culture fluid was harvested (HCCF) around day 6 using tangential flow filtration with 0.2 μm pore size hydrophilized polyethersulfone (PES) hollow fiber membranes in a MiniKros® module with 470 cm^2 membrane area (Repligen Corporation, Rancho Dominguez, CA). The mAb titer was around 4.6 g/L as determined by a Cedex Bio HT analyzer (Roche CustomBiotech, Indianapolis, IN).

2.2 | Precipitation conditions

Small-scale batch precipitation experiments were performed using mAb that was purified in batch mode using Protein A chromatography. Data were obtained over a range of $ZnCl_2$ (Sigma-Aldrich, St. Louis, MO) concentrations from 2.5 to 20 mM, and with PEG having number average molecular weight of 3,350 g/mol (Sigma-Aldrich, St. Louis, MO) at concentrations from 2 to 15 wt%. In each case, the $ZnCl_2$ was added first followed approximately 30 s later by the PEG; note that all reported concentrations are based on the final solution volume after addition of both precipitants. The precipitates were centrifuged and the mAb concentration in the supernatant was determined by a Cedex Bio HT analyzer. The mAb yield was calculated by using a simple mass balance.

2.3 | Continuous precipitation

The continuous precipitation–dewatering–washing–resolubilization was conducted using the experimental system shown schematically in Figure 1, consisting of a series of static mixers and hollow fiber membrane modules. The tubular precipitation reactor was constructed from silicone tubing with an inner diameter of 0.48 cm = 3/16 in. (Masterflex L/S Platinum-Cured Silicone Tubing, model 96416-15, Cole-Parmer, Vernon Hills, IL) fitted with a static mixer (Koflo Corporation, Cary, IL). The HCCF was continuously fed to the static mixer through a Y-shaped connector with barbed hose fittings using a peristaltic pump (Watson-Marlow Fluid Technology Group, Wilmington, MA). $ZnCl_2$ was mixed with the HCCF at the Y connector to initiate precipitate nucleation, with PEG added as a volume exclusion agent between the first and second static mixers to facilitate the growth of the precipitates.

Most continuous precipitation experiments were performed by pumping HCCF into the precipitation reactor at a flow rate of 5 mL/min, where it was combined with 1 mL/min of a 0.1 M $ZnCl_2$ solution. A 17.5 wt% PEG solution was added between the two static mixers to achieve final concentrations of about 2.3 g/L for the mAb, 10 mM for the $ZnCl_2$, and 7 wt% for the PEG. The total residence time in the tubular precipitation reactor was only 30 s, which is much smaller than the 2.5 to 22.5 min used by Burgstaller et al.[11] The more efficient precipitation in this work is likely because of the sequential addition of the $ZnCl_2$ and PEG (these were added simultaneously by Burgstaller et al.); both studies were performed with approximately the same mAb concentration (between 2 and 3 g/L).

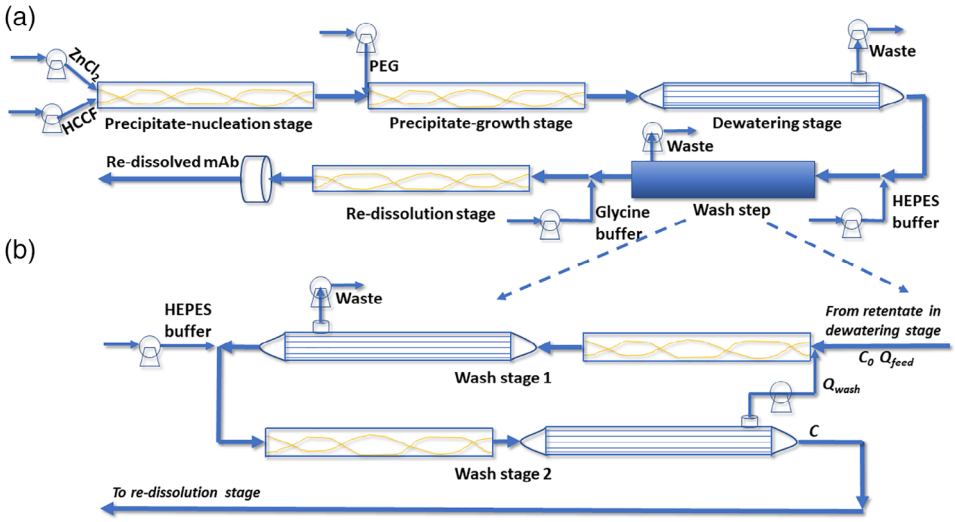

FIGURE 1 Schematic of (a) continuous precipitation system and (b) expanded view of the two-stage countercurrent washing step

The precipitates were concentrated with 0.2 μm hydrophilized PES hollow fiber membranes in a MidiKros® module with 140 cm^2 membrane area and 1.8 mL internal (lumen) volume (Repligen Corporation, Rancho Dominguez, CA). Soluble impurities were further removed by washing using 50 mM HEPES buffer (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) at pH 7.0 without any added $ZnCl_2$ or PEG. HEPES was used as the buffering species, rather than phosphate or Tris, as it has minimal interactions with zinc ions.[12,13] The lower panel in Figure 1 shows an expanded view of the two-stage countercurrent washing configuration. Fresh HEPES buffer was added to the second stage with the permeate from that stage, containing a relatively low concentration of HCP (CHOP) and DNA, recycled back to the first stage. The dewatered precipitated protein was fed to Wash Stage 1, mixed with the permeate from Stage 2, and then concentrated by the hollow fiber module. The retentate from Stage 1 was then mixed with the fresh HEPES buffer and fed to Stage 2. Small static mixers (around 20 cm in length) were used between the hollow fiber modules to insure complete mixing of the wash buffer and precipitate. Experiments were also performed with just a single washing stage in which fresh wash buffer was directly mixed with the precipitated protein.

The precipitated protein was resolubilized by inline addition of 2 M glycine at pH 3.2; this low pH disrupted the crosslinking by the Zn^{2+} causing nearly complete dissolution of the precipitated protein. The resolubilized mAb was then collected through a double-layer Sartoclear® depth filter (DL60, 8 μm/0.8 μm, Sartorius Corporation, Bohemia, NY) to remove any insoluble material.

The entire system was first filled with buffer and all flow rates were allowed to stabilize. The feed was then switched to HCCF, the precipitation was started, and the entire process was allowed to equilibrate. Samples were obtained approximately 10 min after initiation of the precipitation.

2.4 | Critical flux experiments

The critical flux for the protein precipitate in the hollow fiber module was evaluated using the flux-stepping procedure described previously by Li and Zydney.[14] The precipitated protein was generated continuously using the tubular precipitation reactor shown in Figure 1a, with the critical flux for the dewatering stage evaluated by removing the wash and redissolution modules (with the retentate outflow simply discarded). A peristaltic pump (Watson-Marlow Fluid Technology Group, Wilmington, MA) was placed on the permeate exit line from the hollow fiber module to control the filtrate flux, while the transmembrane pressure (TMP) was monitored by placing SciLog pressure sensors (Parker Hannifin Corporation, Cleveland, OH) on both the feed and permeate lines. The TMP was evaluated as a function of time during constant flux operation, with the filtrate flux increased stepwise to determine the onset of fouling.

2.5 | Analytics

The mAb concentration and purity were evaluated by size exclusion chromatography (SEC) and a CHOP ELISA. The product purity was calculated from the SEC chromatograms as the ratio of the monomer peak area to the sum of all peak areas. Note that the $ZnCl_2$ appeared to have some interaction with the column, resulting in an unexpected peak tailing as reported previously by Burgstaller et al.[11]

3 | RESULTS AND DISCUSSION

3.1 | Precipitation

The appropriate conditions for performing the precipitation were evaluated using small-scale batch precipitation experiments. High mAb yields (>97%) were obtained at $ZnCl_2$ concentrations above 15 mM (even in the absence of PEG). The $ZnCl_2$ concentration could be reduced to 10 mM by adding 7 wt% PEG because of the combination of the crosslinking and volume exclusion effects. A more detailed discussion of the thermodynamics of the precipitation process will be provided in a future publication. All subsequent precipitation experiments were performed using these latter conditions.

The results from the batch experiments were then confirmed by performing continuous precipitation in the tubular reactor with the flow rates of HCCF, $ZnCl_2$, and PEG adjusted to give final concentrations of 10 mM $ZnCl_2$ and 7 wt% PEG. The solution became turbid immediately after mixing HCCF with the $ZnCl_2$, that is, just after the entrance to the first static mixer, reflecting the rapid kinetics of precipitation using $ZnCl_2$ as a cross-linking agent. The overall mAb yield for the precipitation step was greater than 97%, with the precipitates remaining stable for at least 24 hr during batch holding (which was the maximum holding time examined). The collected precipitate looked fluffy when observed in a graduated cylinder; it settled to approximately one-half the original volume after 24 hr. The average size of the precipitates was on the order of 1 μm based on the initial settling velocity.

3.2 | Critical flux

In order to insure stable operation of the dewatering and washing steps over extended periods of time, it is desirable to operate the hollow fiber membrane modules below the critical flux, which is defined as the value of the filtrate flux at which membrane fouling first becomes significant, in this case defined as an increase in TMP of more than 50 kPa in 24 hr (i.e., >2 kPa/h). The critical flux in tangential flow microfiltration is governed by the mass transfer characteristics of the membrane module, possibly in combination with any long-range interactions (e.g., electrostatic repulsion) between the precipitate and the membrane surface.[15]

Typical data from the critical flux experiments with the precipitate formed with 10 mM $ZnCl_2$ and 7 wt% PEG in a MidiKros® hollow fiber membrane module containing 0.2 μm hydrophilized PES hollow fiber membranes with 140 cm^2 membrane area are presented in Figure 2. The TMP is shown as a function of time, with each interval corresponding to a different (constant) value of the filtrate flux (labeled directly above the pressure data). The TMP was below 6.9 kPa (1.0 psi) throughout the entire experiment, reflecting the high permeability of the 0.2 μm hollow fiber membrane (around 70 $L/m^2/h/kPa$).

The TMP for the first two intervals remained essentially constant over each 40-min filtration (variation less than 0.5 kPa/h); the greater TMP for the periods with higher filtrate flux is as expected. However, the TMP at a permeate flux of 30 L/m^2/h (8.3 μm/s) increased by nearly 1.5 kPa over 24 min, corresponding to a rate of more than 3.7 kPa/h. Thus, the critical flux for this experiment was greater than 26 L/m^2/h, the highest flux at which the TMP remained stable, but below 30 L/m^2/h, which was the first flux at which the TMP showed a clear increase with time during the constant flux filtration.

The critical fluxes for a series of hollow fiber modules, with different number, length, and inner diameter for the hollow fibers, are summarized in Table 1. All of the data were obtained using the same concentrations of mAb, $ZnCl_2$, and PEG as determined previously. In each case, the critical flux was evaluated from the flux-stepping experiments at a feed flow rate of 10 mL/min using the average of the filtrate flux values determined just above and below the critical flux. The critical flux for Module A was 28 L m^{-2} h^{-1}. Module B was designed with fewer fibers but with 65-cm fiber length; however, this caused more than a 50% reduction in the critical flux even though Module B had a higher shear rate. This behavior is in contrast to previous results in which the critical flux increased with increasing shear rate,[14] suggesting that the difference in critical flux between Modules A and B was not because of the change in back mass transfer. Instead, the lower critical flux in Module B may be due to the large axial pressure drop (ΔP) in the longer hollow fibers, which was more than 10 times that in Module A (because of the combination of the higher shear rate and the longer fiber length). The large ΔP leads to a high inlet TMP, which would in turn cause the local flux near the module inlet to exceed the critical flux for fouling even though the average filtrate flux in Module B was fairly low. Critical flux experiments were thus performed with Module C having fibers with larger ID (1.0 mm) but shorter length. The critical flux in Module C was slightly smaller than that in Module A (24 L m^{-2} h^{-1} vs 28 L m^{-2} h^{-1}), which is probably because of the lower shear rate. It was not possible to operate Module C at higher shear rates as this would have required much larger volumetric feed flow rates. Additional studies will be required to fully characterize the critical flux behavior for the precipitated mAb as a function of the module geometry and shear rate.

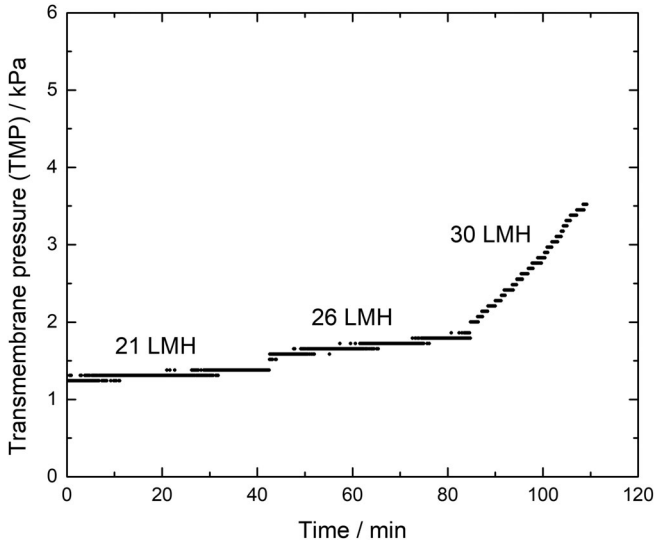

FIGURE 2 Transmembrane pressure as a function of time during critical flux experiments with precipitate formed from a mixture of 2.3 g/L mAb, 7 wt% PEG, and 10 mM $ZnCl_2$. The permeate flux values for each interval are labeled above the TMP data

Based on the results in Table 1, the dewatering step used Module A with the filtrate flux maintained at a value of 26 L m^{-2} h^{-1}, corresponding to a permeate flow rate of 6.1 mL/min. This provided a 2.5-fold increase in the concentration of the precipitated mAb while removing approximately 60% of the HCPs (CHOP) and DNA (assuming no retention of either impurity by the membrane).

3.3 | Washing

Further reductions in the concentration of soluble impurities (e.g., CHOP, DNA, and excess $ZnCl_2$ and PEG) were achieved by "washing" the precipitated mAb, that is, by diluting with buffer and then reconcentrating the precipitated mAb using a hollow fiber membrane module. In order to reduce costs and eliminate the need to remove $ZnCl_2$ and PEG later in the downstream process, the washing was performed using HEPES buffer without any $ZnCl_2$ or PEG. Initial experiments used 6 mL/min of HEPES, providing a 2.5-fold dilution of the dewatered feed (which was at a flow rate of 4 mL/min). The wash buffer and precipitate were mixed using a short static mixer, with a residence time around 20 s, followed by reconcentration (back to the initial flow rate of the precipitate of 4 mL/min) in a separate hollow fiber module, providing additional reduction in CHOP by approximately 60% (again assuming no retention). The permeate from the washing stage was initially clear, but it did become turbid upon incubation overnight, likely because of further precipitation caused by the $ZnCl_2$ remaining in the permeate solution.

Although it would be possible to increase CHOP removal simply by increasing the amount of added buffer, very high dilution ratios would be needed to achieve the high levels of purification typical for an initial capture step based on affinity chromatography, and the large quantity of added buffer could lead to resolubilization of the precipitated mAb (prior to the included resolubilization step), which would reduce the final product yield. Alternatively, the wash buffer can be effectively "re-used" by performing the washing in a countercurrent-staged configuration as shown in Figure 1b. In this case, fresh wash buffer was added into the second stage of the two-stage washing process, with the permeate from this stage (which contained only a low concentration of CHOP and DNA) recycled back to the first stage. The retentate, which contained the precipitated protein, flowed from Stage 1 to Stage 2. This type of countercurrent staging has been discussed in some detail by Nambiar et al.[16] in the context of diafiltration for mAb formulation and by Dutta et al.[17] in the context of continuous countercurrent tangential chromatography.

The extent of impurity removal (R) in this type of countercurrent-staged washing is given as[16]:

TABLE 1 Critical flux of hollow fiber membranes with different fiber geometries. Each module contained 0.2 μm hydrophilized polyethersulfone hollow fiber membranes

Module	Fiber ID (mm)	Surface area (cm^2)	Shear rate (s^{-1})	Number of fibers	Fiber length (cm)	Critical flux (L m^{-2} h^{-1})
A	0.5	140	300	45	20	28
B	0.5	92	1,500	9	65	<13
C	1.0	88	120	14	20	24

$$R = \frac{C_0}{C} = \frac{\alpha^{N+1} - 1}{\alpha - 1}, \quad (1)$$

with

$$\alpha = \frac{Q_{wash} S}{Q_{feed}}, \quad (2)$$

where C_0 is the concentration of soluble impurities in the feed entering the washing step, C is the concentration of soluble impurities in the final retentate leaving the washing step, Q_{feed} is the feed flow rate, Q_{wash} is the buffer flow rate used for washing, S is the sieving coefficient (transmission) for the impurity through the hollow fiber membrane, and N is the number of stages (modules) in the washing step. For example, a flow ratio of 9:1 (α = 9) in the wash step would provide a 10-fold reduction in the concentration of a soluble impurity for a single stage (assuming S = 1). However, a two-stage system using the same value of α would provide a 91-fold reduction in impurity level, and this would be increased to more than 800-fold using a three-stage system, all with the same quantity of fresh wash buffer.

Figure 3 shows results for the CHOP removal after the continuous integrated dewatering (2.5-fold concentration) and either a one- or two-stage washing step using α = 1.5 (wash buffer flow rate of 6 mL/min with a feed flow rate of 4 mL/min assuming S = 1). The fold removal was defined as the ratio of the CHOP mass flow rate in the feed from the bioreactor divided by the CHOP mass flow rate leaving the wash step, with the CHOP concentrations determined by a CHOP ELISA. The CHOP removal for the single stage washing was only R = 6.6, but this increased to R = 10 when using the two-stage countercurrent washing. Note that it was not possible to operate at higher values of α using the available hollow fiber membranes without extensive fouling since these high conversions would have required operation at a filtrate flux above the critical flux. The $ZnCl_2$ concentration after the two-stage washing should be <0.9 mM assuming no Zn retention, while the PEG concentration would be reduced to below 0.6 wt%.

The solid curves in Figure 3 are model calculations accounting for the 2.5-fold concentration in the dewatering step, with the degree of CHOP removal in the washing step given by Equation (1) assuming 100% CHOP transmission through the membrane. The experimental results are in good agreement with the model calculations, providing a framework to design/optimize the countercurrent staged washing step to achieve the desired degree of CHOP removal. More than 100-fold CHOP removal could be obtained using a three-stage washing step with α = 3, although we were unable to explore these conditions experimentally because of material constraints with the HCCF.

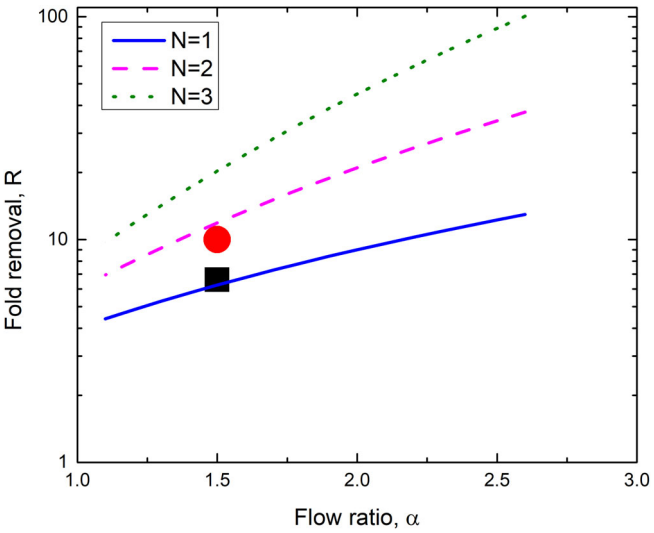

FIGURE 3 Experimental data and model calculations for CHOP removal after dewatering (2.5-fold concentration) and countercurrent washing with 1, 2, or 3 stages

We would expect similar levels of removal for DNA and other low molecular weight species as the 0.2 μm pore size hollow fiber membranes should have minimal retention of all soluble components. Note that solute retention due to adsorptive phenomenon would lead to similar, or potentially greater, degrees of overall impurity removal, although this type of "fouling" could lead to changes in both TMP and small solute retention over time.

3.4 | Redissolution

The precipitated mAb was resolubilized by lowering the pH to disrupt the cross-linking associated with the Zn^{2+} cations. A 2 M glycine buffer at pH 3.2 was mixed inline with the washed precipitate as shown previously in Figure 1a. The required amount of redissolution buffer was first determined by performing a series of batch titration experiments with the results summarized in Figure 4. In this case, the precipitates were collected from the retentate of the dewatering step (without washing), mixed with different amounts of glycine, and the pH and percentage of resolubilized mAb were evaluated after incubation for 30 min. The addition of higher relative amounts of glycine provided a lower pH and in turn a greater yield of soluble mAb. More than 90% mAb recovery was achieved when the pH was reduced to below 5. These experiments provide a conservative estimate on the amount of glycine needed for redissolution since the Zn^{2+} and PEG

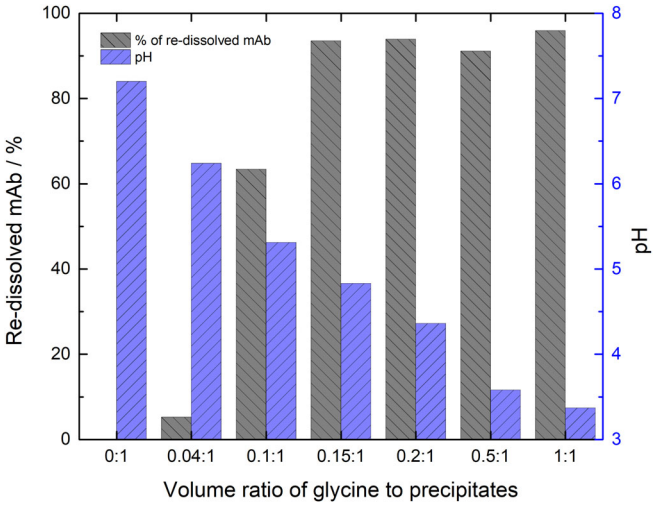

FIGURE 4 Redissolution of precipitated mAb using batch titration with 2 M glycine buffer at pH 3.2

concentrations in the actual process will be reduced by the washing step.

The continuous redissolution was performed using a small static mixer, with length chosen to provide a residence time of around 20 s; this was more than sufficient to obtain a nonturbid solution as determined by visual inspection and then confirmed by dynamic light scattering (DLS). The size distribution of the resolubilized product, as determined by DLS, was very similar to that seen in the initial HCCF feed. The redissolution used a glycine buffer flow rate of 2 mL/min, which achieved a pH of 3.5 in the resolubilized product stream. This low pH would greatly facilitate the implementation of an inline virus inactivation step (immediately after the resolubilization) using a continuous flow reactor.[18]

3.5 | Integrated continuous process

The operating conditions for the integrated continuous precipitation–dewatering–washing–resolubilization process are summarized in Table 2. The washing was conducted using the two-stage countercurrent configuration shown in Figure 1b with α = 1.5. The 2 M glycine resolubilization buffer was added inline at a flow rate of 2 mL/min, providing a resolubilized mAb at a pH of approximately 3.5, well suited for a subsequent inline virus inactivation step. The resolubilized mAb was passed directly through a double-layer Sartoclear® depth filter to remove any insoluble material.

The estimated volumes and residence times for each step in the continuous process are summarized in Table 3. The approximate dead-volumes for the different units accounted for the static mixers, tubing, and hollow fiber membrane modules. The volumes ranged from 2 mL for the dewatering and redissolution steps to 11 mL for the two-stage countercurrent washing, giving a total dead-volume of approximately 20 mL. The residence times in each step were estimated assuming a linear variation of the flow rate through the hollow fiber modules (from initial feed flow rate to exit retentate flow rate).

TABLE 2 Operating conditions and CHOP concentrations for the integrated continuous process

Stream	Flow rate (mL/min)	CHOP (ppm)	mAb concentration (g/L)
HCCF	5	130,000	4.6
0.1 M $ZnCl_2$	1	–	–
17.5 wt % PEG	4	–	–
Feed to dewatering step	10	130,000	2.3
HEPES wash buffer	6	–	–
Resolubilization buffer	2	–	–
Solubilized mAb	6	14,000	3.1

TABLE 3 Volumes and estimated residence times for the different steps in the integrated continuous precipitation process

Step	Step volume (mL)	Residence time (s)
Precipitation	5	30
Dewatering	2	17
Two-stage washing	11	74
Redissolution	2	20

This gives a total residence time of 140 s (<2.5 min) for the entire system compared to as much as 20 min (or more) for just the precipitation step in the work by Burgstaller et al.[11]

The integrated process was run continuously for 1 hr; longer operating times were not practical because of the limited amount of feed material available. This 1-hr process yielded more than 1.1 g of resolubilized mAb using 60 mL of the 0.1 M $ZnCl_2$, 240 mL of the 17.5 wt% PEG, 360 mL of the HEPES wash buffer, and 120 mL of the glycine redissolution buffer. Thus, the total wash buffer usage was 0.32 L/g of mAb, which is about twice the wash buffer utilization in a typical Protein A chromatography column (0.15 L/g) assuming six column volumes for the wash with a dynamic binding capacity of 40 g/L.

The entire system was operated without any intermediate feed pumps, with a total pressure drop of only 50 kPa = 7.3 psi (evaluated from the difference between the pressure at the inlet to the tubular precipitation reactor and that at the outlet from the final depth filter). There was no evidence of any membrane fouling, as the TMPs in the dewatering and wash modules remained stable throughout the process at values less than 2 kPa (<0.3 psi). The CHOP and mAb concentrations were also stable based on off-line assays for samples collected at multiple time points during the process. The final mAb concentration was 3.1 g/L and the overall mAb yield was 80%. The mass balance closure was within ±2%, with 98% mAb yield in the precipitation step and 82% yield in the washing. The significant loss of mAb in the permeate from the wash step was due to resolubilization of some of the precipitated protein upon removal of the Zn^{2+}. This mAb loss could be reduced by adding $ZnCl_2$ to the wash buffer as per the work by Burgstaller et al.[11] The final CHOP concentration was 14,000 ppm (mass of CHOP per mass of mAb), corresponding to a

10-fold reduction in CHOP levels relative to the mAb. Monomer purity was 89% as determined by the main peak in SEC; the actual monomer purity is likely higher than this due to peak tailing associated with the $ZnCl_2$.[11] Based on the results from the washing step, we would expect the PEG concentration to be <0.6 wt% and the Zn^{2+} to be <0.9 mM. These would be further reduced as part of the subsequent purification steps in the downstream process, for example, anion/cation exchange chromatography and the final ultrafiltration/diafiltration.

4 | CONCLUSIONS

Although several recent studies have demonstrated the feasibility of using precipitation for the initial recovery/capture of mAb products, the work presented in this article provides the first demonstration of a fully integrated continuous process for precipitation, dewatering, washing, and resolubilizing a mAb without any intervening hold steps. The precipitation was conducted in a tubular precipitation reactor with sequential inline addition of $ZnCl_2$ (a cross-linking agent) and PEG (a volume exclusion agent), enabling the use of very short residence times (<30 s) in the precipitation step, significantly reducing the overall system hold-up volume.

The precipitated mAb was dewatered and washed using hollow fiber membrane modules, with the washing conducted using a countercurrent two-stage washing configuration. To the best of our knowledge, this is the first reported use of this type of staged operation as part of a continuous precipitation process. The dewatering and two-stage wash step were able to remove 90% of the HCPs using a wash buffer flow rate of 6 mL/min, which is only 20% more than the HCCF feed flow rate. Much higher levels of CHOP removal could be obtained using higher flow rates of wash buffer and/or more stages as per the model calculations presented in Figure 3. However, this would require the use of hollow fiber modules that could be operated with higher conversion (ratio of permeate to feed flow rates); additional experimental studies are required to identify opportunities to increase the critical flux with precipitated proteins.

The mAb yield for the fully integrated process was 80%, with most of the mAb loss occurring in the wash step, likely because of premature resolubilization of the mAb by the wash buffer (which was free of both $ZnCl_2$ and PEG). The mAb yield could be increased by the addition of small amounts of $ZnCl_2$ to the wash buffer to reduce redissolution of the precipitate. The system was run at steady state for 1 hr, providing a total of 1.1 g of purified mAb using about 0.32 L of wash buffer per g of mAb. Each hollow fiber membrane module processed 600 mL of the precipitate, giving a loading of 43 L/m^2, with no evidence of fouling; the TMP remained below 2 kPa (<0.3 psi) throughout the process. Scale-up of this system could easily be achieved using larger area membrane modules. The final resolubilized product was depth-filtered to remove any insoluble material and was obtained at pH 3.5, making it directly suitable for processing in a continuous virus inactivation reactor in combination with appropriate polishing operations, for example, flow-through membrane chromatography. Thus, the precipitation system examined in this study could provide the basis for a low-cost fully integrated continuous process for purification of mAb products. Such a system would be linearly scalable in terms of membrane area of hollow fiber modules by changing the number of fibers. Future studies will be required to demonstrate the full potential of this downstream processing platform.

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CONFLICT OF INTEREST

The authors declare that they have no potential conflict of interest.

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