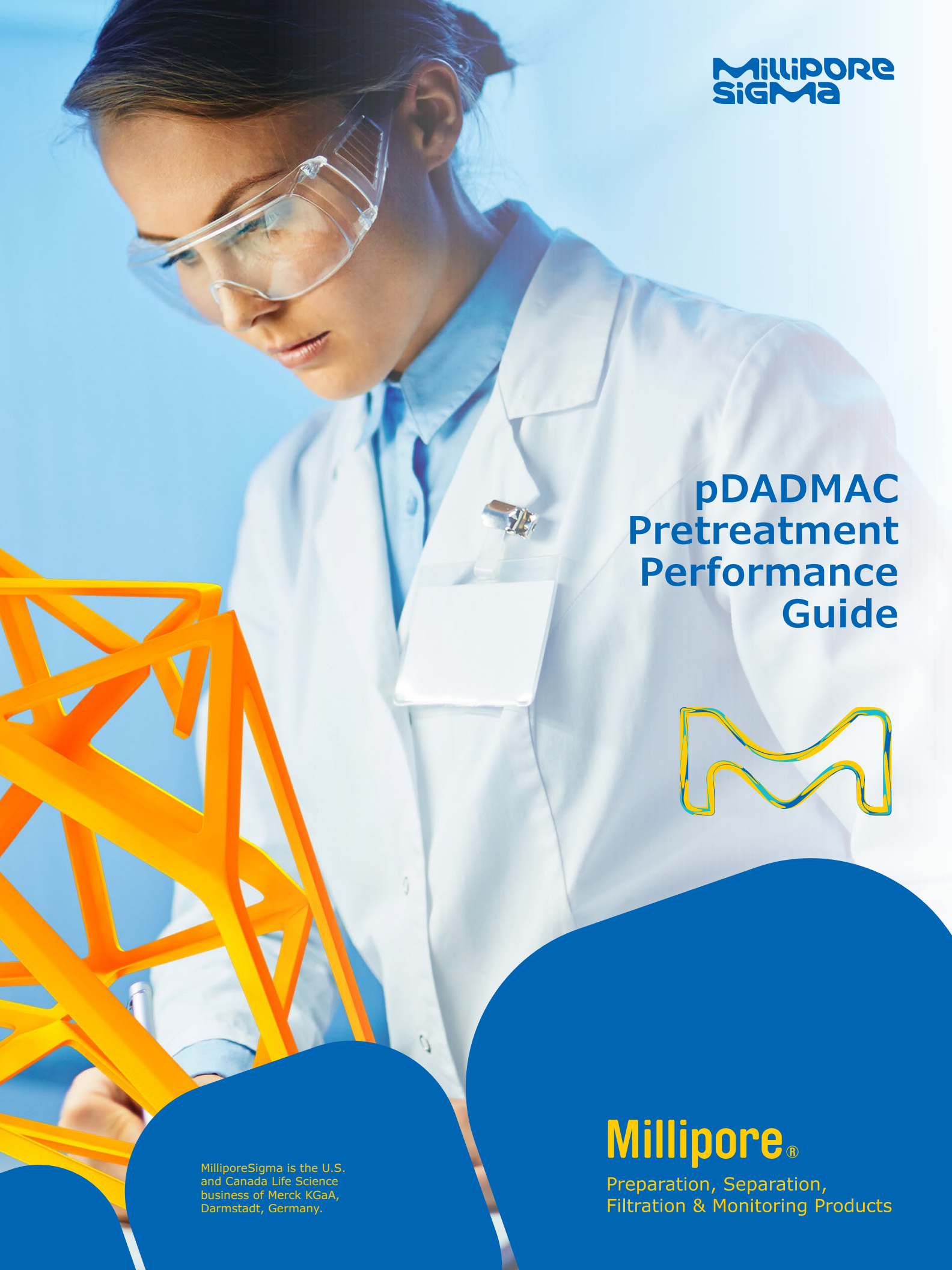

pDADMAC Pretreatment Performance Guide

MilliporeSigma is the U.S. and Canada Life Science business of Merck KGaA, Darmstadt, Germany.

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Preparation, Separation, Filtration & Monitoring Products

This pDADMAC Pretreatment Performance Guide is a reference document to provide assistance with evaluating different pretreatment technologies used in conjunction with Clarisolve® depth filters for cell culture harvest clarification. This version focuses on pretreatment with pDADMAC flocculant for clarification with Clarisolve® 40MS depth filters. This guide includes general guidelines on various aspects of this platform.

The results in this guide are intended as general examples and are not to be construed as product claims or specifications. These results summarize outcomes and observations obtained in application studies conducted with the specific model streams and detailed experimental conditions. Therefore, all test results should be confirmed by the end user using feed stream and process conditions representative of the specific application.

Table of Contents

Introduction

Introduction to Pretreated Feed Streams using Poly diallyl dimethyl ammonium chloride (pDADMAC) Cationic Polymer

The current evolution to high cell density and high-product titer recombinant protein cell cultures is placing a larger burden on traditional downstream clarification and purification operations. To alleviate potential bottlenecks, various types of pretreatment are being employed to make the clarification process more efficient. pDADMAC, a cationic polymer, is an effective flocculating agent that can be used for the pretreatment of recombinant protein feed streams. Upon addition to the cell culture feed stream in a concentration between 0.01 and 0.05%, pDADMAC rapidly flocculates the negatively charged cells and cellular debris into larger particles via an ionic interaction mechanism. This in turn can enhance the efficiency of the depth filtration step and subsequent purification steps.

Typically, pretreatment based purification process development starts with identifying how much polymer stock solution needs to be added to induce effective flocculation (often referred to as dosing studies). Once the dosing concentration is identified, other considerations for the pretreatment step may include how to add the flocculant, how long to mix and at what speed. Detailed studies to help identify ranges for these variables are provided in this document.

Once the pretreatment process is established, the next step is to identify the ideal depth filter to use for clarification and how much filter area is needed. Standard Pmax™ and Tmax™ studies can be carried out at bench scale to identify the area requirements. Information on scalability safety factors can come in handy to scale the process from bench to pilot to process scale.

Another consideration to keep in mind is how the novel pretreatment process affects the subsequent step; namely, whether any residual polymer induces continued precipitation in the filtrate and whether the treatment enhances the capture chromatography or Protein A step, if the modality is monoclonal antibody. You may also consider analysis for residual polymer.

Lastly, if the process is intended for implementation in a stainless-steel facility, cleanability should be considered and evaluated.

This guide provides a compilation of various studies to provide guidance for these considerations related to implementing pDADMAC based flocculation processes.

Summary of Studies

Flocculation Optimization

Dosing

Variability in cell culture conditions (density, viability, host cell) requires empirical evaluation of flocculant concentration to harvest cell culture for optimum results. Cationic polymers, like Poly diallyl dimethyl ammonium chloride (pDADMAC), bind to negatively charged cells and too little or too much polymer results in ineffecient flocculation. A brief testing method is outlined for initial weight to weight (w/w) dosing evaluation and testing. A method for normalizing the polymer to cell density is also presented.

Addition

Flocculant addition at bench scale is often performed as a one time bolus addition. Known quantity of polymer stock is dispensed directly into cell culture using a pipette. At pilot and process scale it is not feasible to perform flocculant addition as a bolus due to closed processing requirements. At these scales, addition of cationic polymers, such as pDADMAC, is performed using a pump to dispense the flocculant via feed port.

This study compared gradual pump and direct bolus addition of flocculant. These two methods of addition were compared based on chord length distribution and their impact on Clarisolve® 40MS depth filter capacity. Additionally, filtrate turbidity was investigated.

Mixing Evaluation

At pilot and process scale pDADMAC flocculation can be carried out directly in a single-use bioreactor or in a dedicated single-use mixing system. Differences in vessel geometry and impeller type between different mixing systems can make the scale-up challenging. In order to simplify mixing scale up during flocculation from bench to pilot scale comparative plots for tip speed, power to volume ratio and Reynolds number were generated for Mobius® 200 L Bioreactor, Mobius® Mix 200 mixing system and bench scale top mount marine impeller. These plots can be used to identify suitable mixing speeds for different mixing systems.

Effect of mixing on pDADMAC flocculation at low power to volume ratios ranging from 0.13 w/m^3 to 2 w/m^3 was evaluated using a top mount impeller. This was done to confirm if recommended mixing time (refer to next chapter for clarity) of 30 minutes prior to filtration was sufficient under reduced mixing conditions and to investigate if mixing at low power to volume ratios can impact subsequent depth filtration by Clarisolve® 40MS depth filter. Comparison of mixing of a top mount impeller and an equivalent size stir bar was also performed to investigate impact of two different mixing methods.

Continued Precipitation

Following depth and sterile filtration of pDADMAC treated feeds, low levels of residual pDADMAC may remain prior to further downstream purification. This clarified material may be stored overnight or loaded over multiple days directly onto Protein A. This delay in loading onto Protein A allows for continued precipitation and may pose operational challenges. This study sought to understand the effects of pDADMAC precipitation coupled with Clarisolve® 40MS depth filter against non-pretreated depth filtrate under ambient and cold room conditions. In addition, examination of secondary clarification to reduce potential precipitation was investigated and reported.

Effects of Pretreatment on Protein A Performance

A CHO derived mAb feed (mAb02) was clarified using traditional cellulosic and diatomaceous earth based Millistak® depth filtration (D0HC followed by X0HC), and pDADMAC flocculation followed by Clarisolve® 40MS filtration. These clarified streams were repeatedly purified using Protein A resin to assess resin longevity, product quality, including HCP removal, and yield over the course of a purification campaign.

Platform Scalability

Scalability across the Clarisolve® device family is an important consideration for the transfer of clarification from process development to large scale implementation. This section evaluates the scalability of Clarisolve® devices across the range of configurations offered including µPOD®, Lab scale Pod and Process scale Pod formats. It is recommended to perform intermediate, confirmatory scaling studies to validate the results of small scale tests.

Cleaning (pDADMAC)

Implementation of pDADMAC flocculation in fixed stainless steel infrastructure requires demonstration of successful cleaning validation. pDADMAC flocculated cell culture soil behaves differently from non-flocculated cell culture soil and cleaning evaluation is necessary to identify Clean-In-Place (CIP) process parameters suitable for effective cleaning. In this work, coupon based small scale cleaning evaluation was performed for cleaning of pDADMAC flocculated CHO cell culture from stainless steel surfaces. CIP process parameters which were investigated include cleaning chemistry, concentration, contact time and temperature.

Validation Services

The purpose of this study is to quantitate the residual level of unbound pDADMAC in sample matrices from post-Protein A eluates and/or drug product.

Flocculation Optimization

Dosing

Introduction

Flocculation of cell culture media with pDADMAC requires scoping studies to determine the optimum dose of pDADMAC to get consistent clarification performance and efficiency. Initial methods to identify pDADMAC addition utilize a weight to volume (w/v) addition of pDADMAC, while a dosing regime based on the ratio of pDADMAC to total cell density can be utilized.

Methods

Scoping studies consist of direct addition of pDADMAC to cell culture in 50 mL centrifuge tubes, followed by centrifugation at 3000 RPM for 5 minutes. Dosing studies based on w/v are conducted at a range of 0.02% – 2% w/v, while dosing studies based on pg pDADMAC/Total Cell Density (TCD) are conducted from 0 – >150 pg/TCD. Following centrifugation, supernatant turbidity is evaluated as an analog of flocculation efficiency.

Small scale depth filtration studies are conducted by direct addition of the prescribed amount of pDADMAC followed by mixing. Clarisolve® 40MS µPOD® filters are equipped with pressure transducers and flushed with water (600 LMH, 100 L/m^2). During clarification operation, pressure and flow are monitored.

Results and Discussion

One method for testing flocculation conditions is to conduct small scale testing of cell culture in centrifuge tubes and evaluate pDADMAC in concentrations ranging from 0.02–2 w/v percent, or higher depending on cell culture conditions. The pDADMAC treated cell culture can be centrifuged and evaluation of the supernatant turbidity provides the basis for initial dosing. In general, the lowest pDADMAC dose with corresponding low supernatant turbidity is chosen for depth filter evaluation. An example of this type of dosing is shown in **Figure 1**.

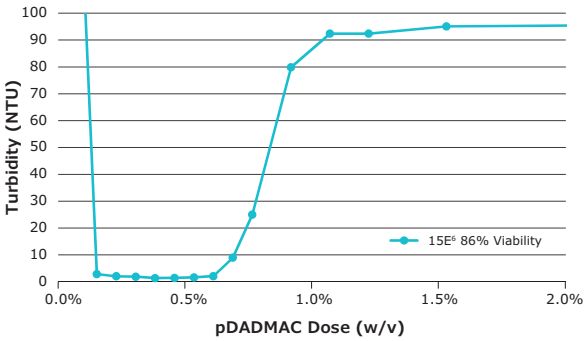

Figure 1. Total Cell Density Impacts on Dosing

Dosing of pDADMAC can also be attributed to the cell density or mass present. An alternative to dosing based on w/v utilizes a measurement for cell density. While a variety of different data points could be used, we investigated the use of pg pDADMAC to TCD for direct dosing into the bioreactor. **Figure 2** shows the results of **Figure 1** presented as turbidity vs. TCD for comparison. To evaluate if this method would be applicable to a variety of different cell densities, harvest cell culture was diluted into basal media to present total cell densities across a broad range and dosing from 0 to >150 pg/TCD was evaluated in a centrifuge scoping study. **Figure 3** shows the relationship between pDADMAC addition and total cell density by evaluating supernatant quality. Similar to cell density, cell viability could also impact the dosing range of pDADMAC to the bioreactor.

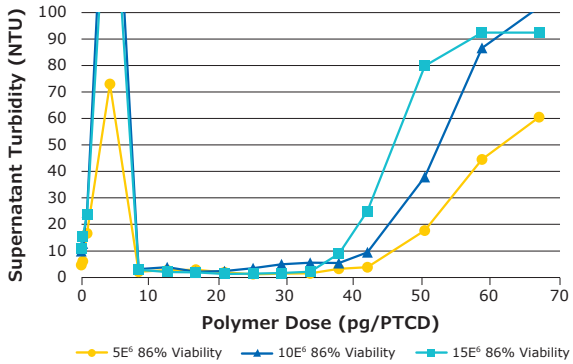

Figure 2. Total Cell Density Impacts on Dosing

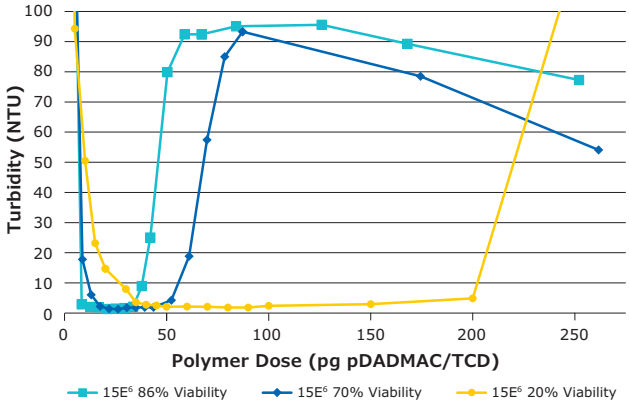

Figure 3. Cell Viability Effects on pDADMAC Dosing

Figure 3 examines the effects of cell viability on dosing effectiveness in a centrifuge study by evaluating 20%, 70% and 86% cell viability at a similar TCD of ~15E6 cells/mL across the dosing range. These results indicate that a dose of 30 pg/TCD is effective in reducing supernatant turbidity across a broad range of cell culture conditions. An additional variable that may be present is in regard to the precision of bioreactor dosing with

pDADMAC. **Figure 4** illustrates the effects of dosing on filtration performance at the optimum dose based on supernatant turbidity and also ±20% of this value. This data suggests that minor deviations, with respect to the 'optimum dose' are well tolerated when comparing filtration performance and in all cases filtrate turbidity was less than 10 NTU. One consideration for pDADMAC dosing that was not evaluated is the effect of residual pDADMAC on downstream unit operations.

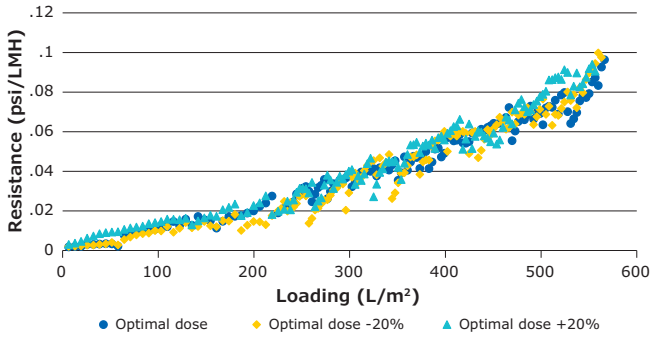

Figure 4. pDADMAC Dosing Sensitivity Analysis

Polymer Addition

Introduction

At development scale, pDADMAC flocculation is often performed by a bolus addition. During GMP manufacturing, addition of pDADMAC as a bolus might not be feasible and flocculant addition may be performed through a feed port using a pump. Two methods of addition were compared based on chord length distribution and their impact on Clarisolve® 40MS depth filter loading capacity and filtrate turbidity. Chord length distribution was measured using Mettler Toledo Focused Beam Reflectance Measurement (FBRM) G400 probe.

Methods

Cell culture (1.5 L) was dispensed in a 3 L polypropylene vessel. The impeller was assembled at a defined depth and set to 150 rpm. Particle Track G400 FBRM probe was submerged to half height to collect particle distribution data. Schematic of the setup is shown in **Figure 5**. For bolus one time addition of 10% pDADMAC polymer stock solution (at 30 pg/cell dosing) was made with a pipette and change in particle distribution was tracked. In case of pump fed method, 10% pDADMAC polymer stock solution volume equivalent to 30 pg/mL dosing was pumped in using a Watson Marlow pump installed with 0.76 mm ID tubing. For each experiment, the data was collected for at least 30 minutes starting from end of complete pDADMAC addition. Chord length distribution was measured for two cases with starting point being end of pDADMAC addition. With the exception of pDADMAC addition method, both feeds were treated equally. At the end of mixing both feeds were clarified using Clarisolve® 40MS depth filter and pool samples were taken for turbidity.

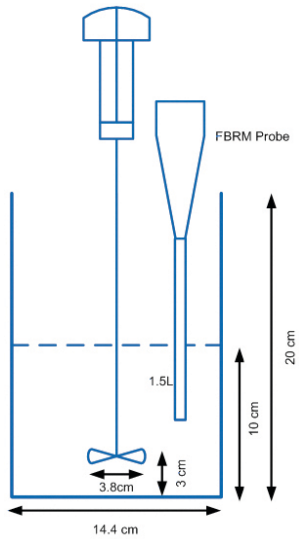

Figure 5. Schematic of mixing setup

Results and Discussion

Both median chord length as well as mean square chord length were a close match suggesting the method of addition did not affect the chord length distribution. Profiles for median chord length and mean square weighted chord length for comparison of method of addition of pDADMAC bolus versus pump fed are shown in **Figure 6 and 7** respectively.

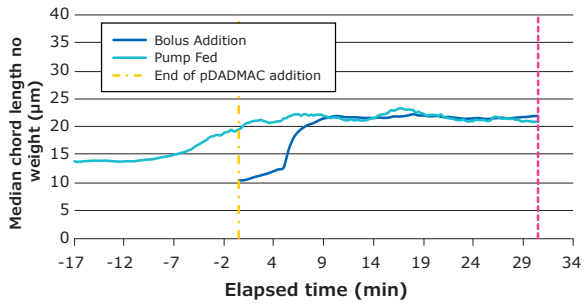

Figure 6. Comparison of method of addition of pDADMAC bolus versus pump fed: median chord length

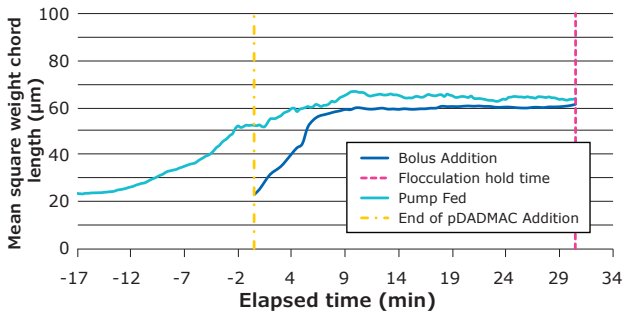

Figure 7. Comparison of method of addition of pDADMAC bolus versus pump fed: mean square chord length

There was no significant difference observed between Clarisolve® 40MS depth filter loading capacity and filtrate turbidity. **Figure 8** shows differential pressure profiles for method of addition, bolus and pump fed. Summary of loading capacities and pool turbidity is provided in **Table 1**.

Method of pDADMAC Addition	Loading at 10 psi (L/m²)	Pool Turbidities (NTU)
Bolus	297	3.37
Pump Fed	297	3.45

Table 1. Summary of loading capacity and pool turbidity for method of addition, bolus and pump fed

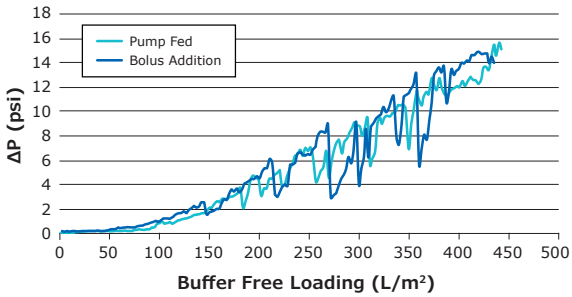

Figure 8. Clarisolve® 40MS depth filter differential pressure profiles for method of addition: bolus and pump fed

Mixing Evaluation

Mixing Hold Time

Introduction

Sufficient mixing hold time is required to ensure adequate polymer dispersion and to allow for flocculation to reach a constant particle size distribution (PSD). This experiment aims at determining the amount of hold time needed to reach a constant PSD.

Methods

For unit operation simplicity, pDADMAC is dosed directly into the bioreactor as a bolus. Particle size distribution is monitored in real time for the duration of the experiment.

Results and Discussion

A constant PSD is achieved within approximately 20 minutes of dosing as shown in **Figure 9**. It's likely that various cell types, flocculants, bioreactor geometries, mixing energies, etc., could impact the time required until a constant PSD is achieved. An achieved PSD shift, for a given harvest, is likely dependent upon the system's mixing energy. Energy input into the bioreactor during cell growth is maintained during pDADMAC addition and is fixed throughout the harvest.

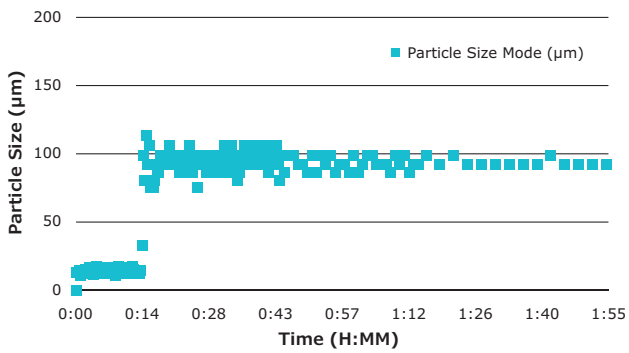

Figure 9. Particle Size Distribution vs Time

Mixing Speed

Introduction

Comparative plots for tip speed, power to volume ratio and Reynolds number were generated for Mobius® 200 L Bioreactor, Mobius® MIX 200 mixing system and bench scale top mount marine impeller to assist with mixing scale up.

Effect of mixing speed on pDADMAC flocculation at low power to volume ratios ranging from 0.13 w/m³ to 2 w/m³ was evaluated using top mount impeller. Comparison of mixing of a top mount impeller and an equivalent size stir bar was also performed to investigate impact of two different mixing methods.

Methods

Calculations for Reynolds number, power to volume ratio P/V (W/m³) and tip speed (m/s) were performed based on equations 1, 2 and 3.

$$Re_{impeller} = \frac{D^2.n.\rho}{\mu} \quad (1)$$

$$Power = N_p.n^3.D^5.\rho \quad (2)$$

$$Tip\ Speed = \pi.D.n \quad (3)$$

D = Diameter of impeller (m)
n = Revolutions per second (s^{-1})
ρ = Density (Kg/m³) 1.006 Kg/m³ (Cell culture media with 10% FBS)
μ = Viscosity (Pa.s) 0.00113 Pa*s (Cell culture media with 10% FBS)
Np = Impeller power number (for top mount impeller value assumed to be 0.3)

Comparison was performed assuming the volume to be 1.5 L for bench scale, 185 L for Mobius® Power MIX 200 L mixer (actual volume processed in current study), and 200 L for Mobius® Bioreactor. For density and viscosity representative values of cell culture media with 10% Fetal Bovine Serum were used. The plots for power to volume ratio, tip speed and Reynolds number are shown in **Figures 11, 12 and 13** respectively.

1.5 L of cell culture was dispensed in a 3 L polypropylene vessel. The impeller was assembled at a defined depth and set to defined rpm. Particle Track G400 FBRM probe was submerged to half height to collect particle distribution data. Schematic of the setup is shown in **Figure 10**. Bolus or pump fed addition of 10% pDADMAC polymer (at 30 pg/cell dosing) was made and change in particle distribution was tracked. For each experiment the data was collected for at least 30 minutes starting from end of complete pDADMAC addition.

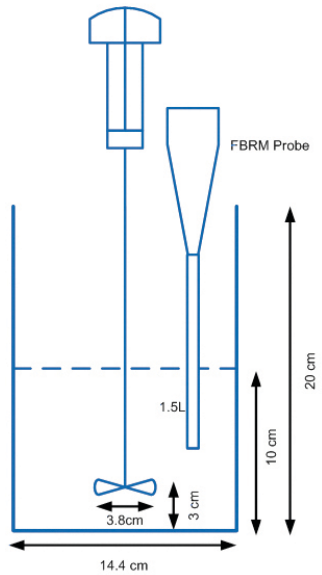

Figure 10. Schematic of mixing setup

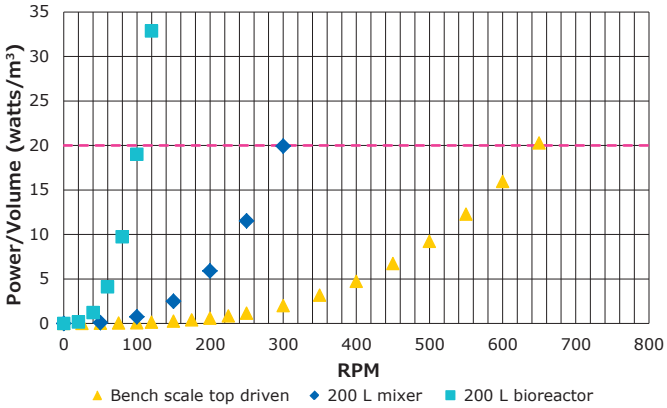

Figure 11. Power to volume ratio for bench scale system, Mobius® Power MIX 200 L mixer and Mobius® 200 L Bioreactor

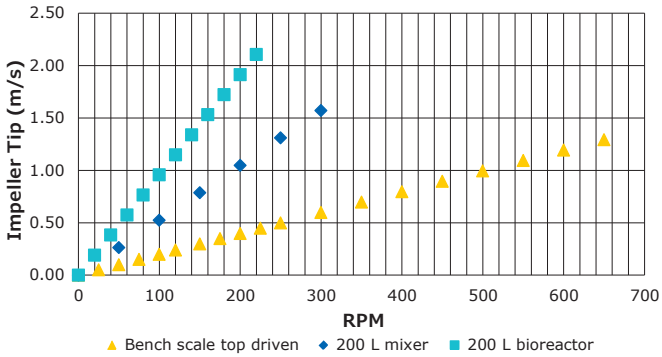

Figure 12. Tip Speed for bench scale system, Mobius® Power MIX 200 L mixer, and Mobius® 200 L Bioreactor

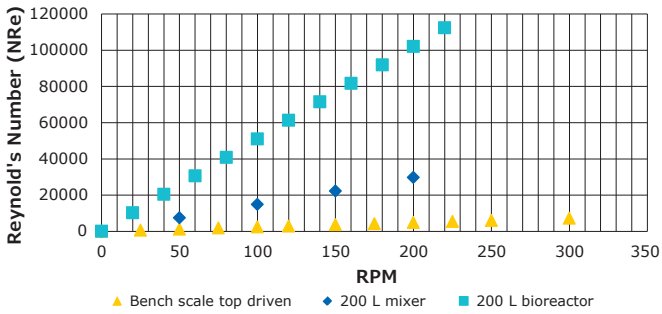

Figure 13. Reynold's Number for bench scale system, Mobius® Power MIX 200 L mixer, and Mobius® 200 L Bioreactor

Results and Discussion

The effect of mixing speed during flocculation at three different speeds 120, 150 and 300 rpm on median chord length and mean squared chord length profiles are shown in **Figure 14 and 15** respectively. Slower mixing speed took longer time for the floc size to stabilize and resulted in larger floc as compared to faster mixing speeds.

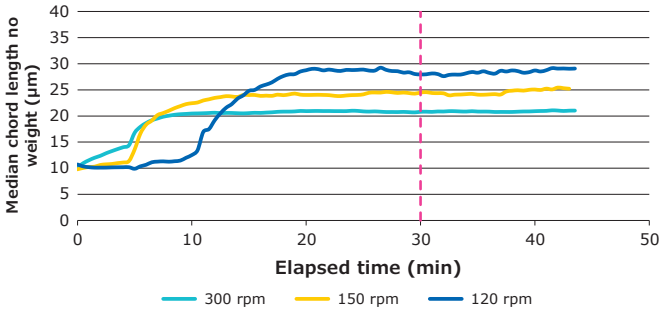

Figure 14. Effect of mixing speed: median chord length

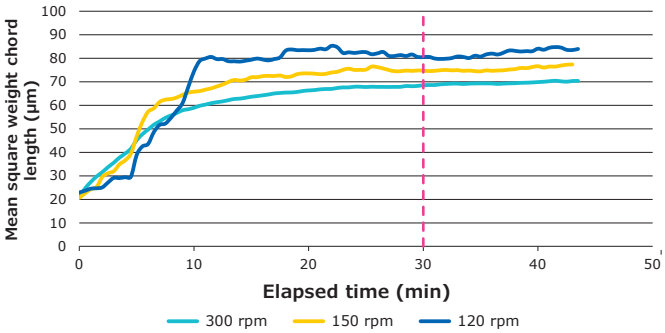

Figure 15. Effect of mixing speed: mean square weight chord length

After 30 minutes of mixing, each feed stream was clarified using Clarisolve® 40MS depth filter. No impact of mixing speeds during flocculation was observed on loading capacity and filtrate turbidity. **Figure 16** shows differential pressure profiles for three mixing conditions. Summary of loading capacities and pool turbidity is provided in **Table 2**.

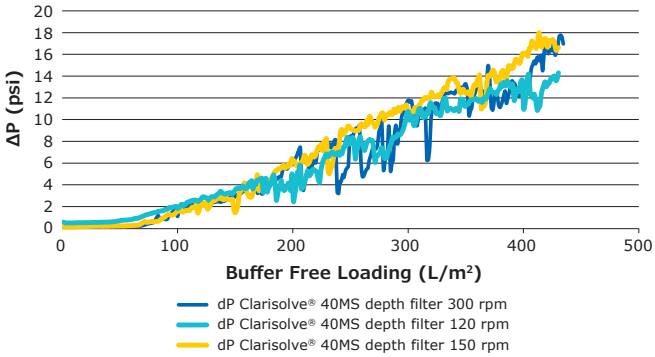

Figure 16. Differential pressure profiles for Clarisolve® 40MS depth filter for three different mixing speeds

Impeller Mixing Speed	P/V (W/m³)	Loading at 10 psi (L/m²)	Pool Turbidities (NTU)
120 rpm	0.127	297	3.72
150 rpm	0.249	281	3.79
300 rpm	1.99	294	4.72

Table 2. Summary of loading capacity and pool turbidity at different mixing speeds

Comparison of Top Mount Impeller vs. Stir Bar

Introduction

Mixing for bench scale flocculation studies are often carried out using a stir bar instead of an impeller. Currently there is no existing data to establish equivalence of mixing performance of stir bar and a top mount impeller in a flocculation process. This experiment was performed to demonstrate the equivalence of the two aforementioned mixing methods.

Method

A top mount marine impeller mixing performance was compared with same diameter stir bar run at 150 rpm in same vessel. Ratios for comparing impeller and stir bar geometry and impeller placement are shown in **Table 3**. The change in chord length distribution was compared after a bolus addition of pDADMAC was made. After 30 minutes, stable median chord length distribution was reached and both feeds were clarified with Clarisolve® 40MS depth filters.

Ratio of impeller diameter to vessel diameter	0.3
Impeller placement*	0.3
Ratio of stir bar to vessel diameter	0.3
Stir bar placement*	0.0

*Distance from base/height at total working volume

Table 3. Geometric comparison of top mount impeller and stir bar

Results and Discussion

Comparison of median chord length and mean squared chord length are shown in **Figure 17 and 18** respectively. The chord length distributions were comparable for both top mount impeller and stir bar. Stir bar median chord length were slightly shorter compared to top mount impeller potentially due to the shear caused due to stir bar being in contact with the vessel base.

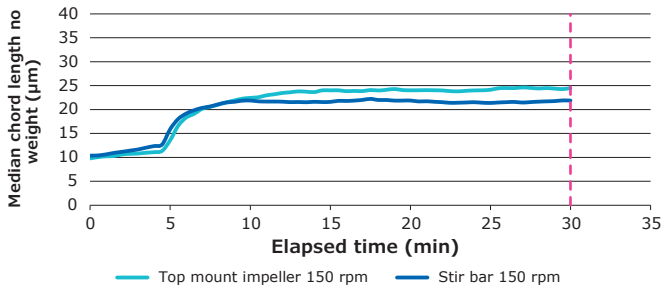

Figure 17. Comparison of top mount impeller versus stir bar on median chord length

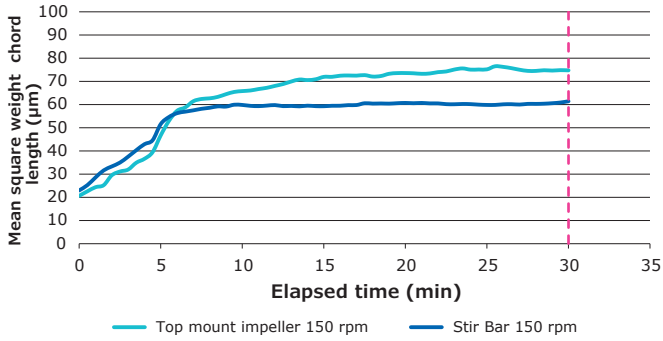

Figure 18. Comparison of top mount impeller versus stir bar on mean square weight chord length

There was no difference observed between the Clarisolve® 40MS depth filter loading capacity and filtrate turbidity when comparing the mixing using top mount impeller and stir bar. **Figure 19** shows differential pressure profiles for mixing using top mount impeller versus stir bar. Summary of loading capacities and pool turbidity is provided in **Table 4**.

Mixing type	Loading at 10 psi (L/m²)	Pool Turbidities (NTU)
Stir bar	297	3.37
Top Mount impeller	280	3.79

Table 4. Summary of loading capacity and pool turbidity for flocculation process using top mount impeller versus stir bar

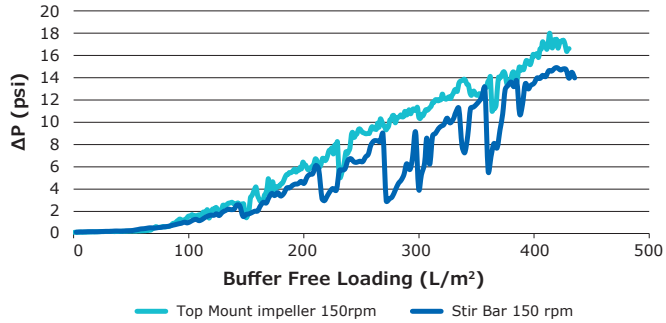

Figure 19. Clarisolve® 40MS depth filter differential pressure profiles for flocculation process using top mount impeller versus stir bar

Continued Precipitation

Introduction

Cell culture pretreated with pDADMAC has the potential to precipitate even after clarification due to residual polymer in the filtrate. This poses operational challenges during the Protein A loading step. This experiment was conducted in order to determine the impact flocculation has on precipitation rates compared to non flocculated clarified feed stream.

Methods

The Protein A loading can be performed in ambient or cold room temperatures depending on molecule stability. To understand the impact of pDADMAC flocculation on continued precipitation, we carried out experiments to compare traditional clarification and pDADMAC flocculation based clarification. Additionally, we also evaluated impact of secondary clarification post Clarisolve® 40MS depth filter for pDADMAC flocculated streams. The experiments were carried out under ambient as well as cold room conditions. The clarified, sterile filtered test samples were stored under ambient and cold conditions (4–8 °C) to mimic actual manufacturing and turbidity was measured over 60 hours.

Results and Discussion

Turbidity profiles for feed streams stored under ambient and cold room conditions are shown in **Figures 20 and 21** respectively.

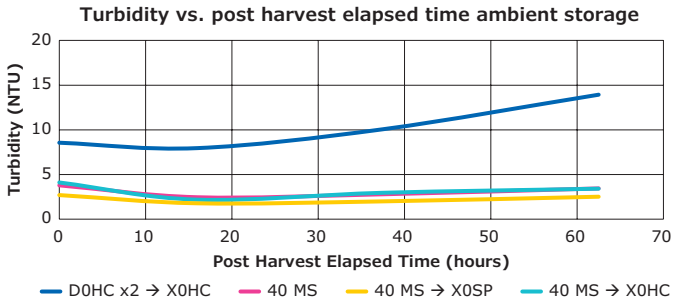

Figure 20. Continued precipitation under ambient conditions

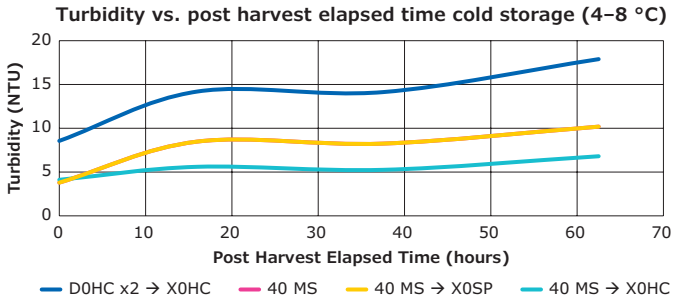

Figure 21. Continued precipitation under cold conditions

The data suggests continued precipitation was less severe in case of pDADMAC flocculated stream as compared to traditional clarification. Secondary clarification—post Clarisolve® 40MS depth filter—using Millistak+® depth filter with X0HC media series and Millistak+® HC Pro depth filter with X0SP media series, was able to further reduce continued precipitation. These trends were consistent under both ambient as well as cold storage conditions. The precipitation is dependent on concentration and stability of impurities, target molecule and other cell culture components under given storage condition and may vary based on feed stream.

Effects of Pretreatment on Protein A Performance

Introduction

Following clarification and sterile filtration, clarified cell culture is purified using Protein A chromatography. These experiments assessed how different clarification methods affected the performance of the resin over repeated purification cycles. Yield, column backpressure, leached Protein A and host cell protein concentrations were tracked over the course of the purification campaign. The Millistak+® D0HC + X0HC derived stream (non-flocculated feed) was loaded onto and eluted off the same column 100 times, while the pDADMAC and Clarisolve® 40MS derived stream (flocculated feed) was purified 160 times.

Methods

Both streams were purified with the use of hand packed Eshmuno® A resin columns, an AKTA™ chromatography system, and a standard purification method. The columns used in these experiments were first characterized before being exposed to clarified cell culture. Their dynamic binding capacity at 10% breakthrough ($DBC_{10\%}$) was determined using purified mAb02. The pressure drop across the resin bed was measured using pressure transducers coupled to a data acquisition system. These metrics were tracked over the course of the purification campaign. For each purification cycle, clarified cell culture loading was set to 80% of $DBC_{10\%}$.

Results and Discussion

mAb yield remained high, above or around 95%, over the course of both purification campaigns as seen in **Figure 22**.

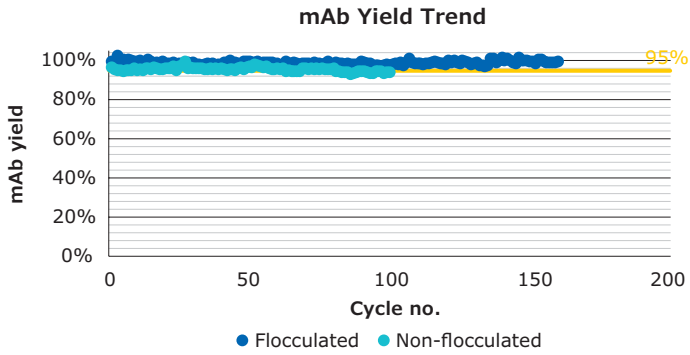

Figure 22. The evolution of yield over the course of the campaign

$DBC_{10\%}$ dropped faster in the case of the non-flocculated feed, but still remained above 80% capacity of the naive column as seen in **Figure 23**. Based on the data, the lifespan of the Protein A resin (as determined by dynamic binding capacity) was extended in the case of pDADMAC flocculation and Clarisolve® 40MS clarification.

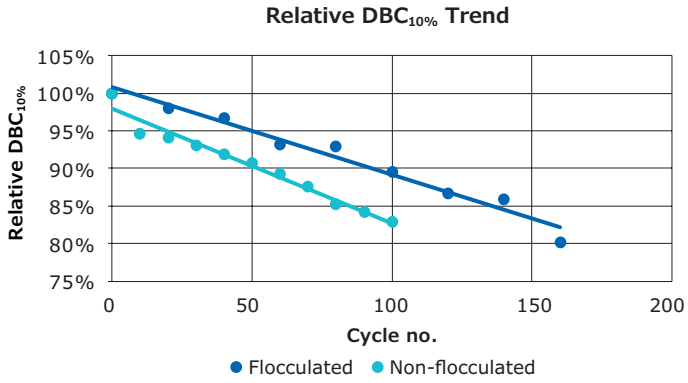

Figure 23. The evolution of dynamic binding capacity over the course of the campaign

Column backpressure increased considerably faster with the non-flocculated feed, reaching a maximum increase of about 42% at the end of the campaign (100 purification cycles). Such a dramatic increase can be prohibitive especially at process scale.

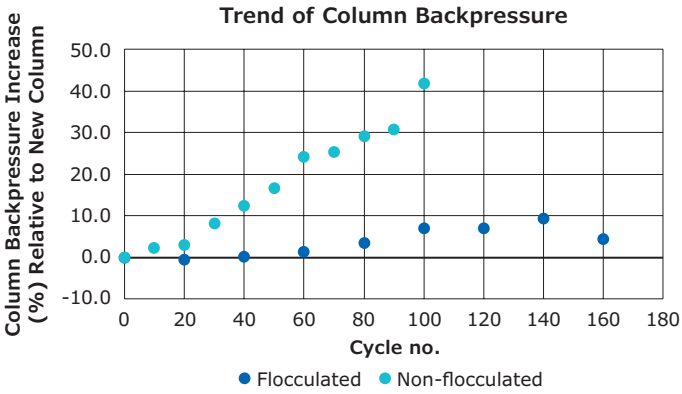

Figure 24. Evolution of backpressure over the course of the campaign

Host cell protein clearance over the Protein A column was greater in the case of pDADMAC flocculated feed. Leached Protein A levels remained within acceptable levels.

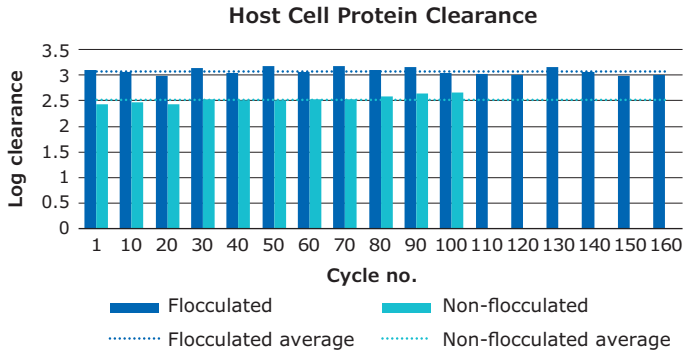

Figure 25. Host cell protein clearance over the course of the campaign

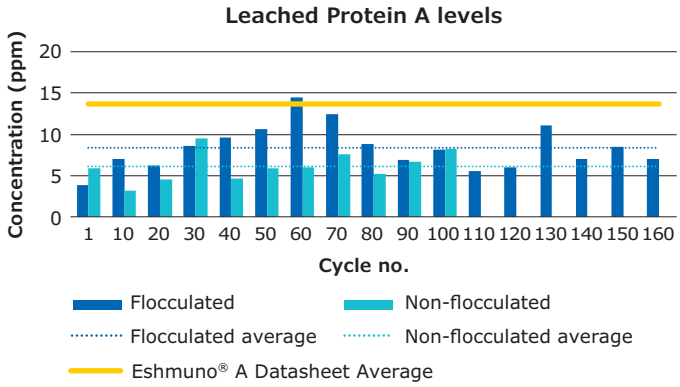

Figure 26. Measured leached Protein A concentrations in the eluate

Pretreatment Platform Scalability

Introduction

In each capacity study outlined in this report, lot matched media are utilized in a given study. This allows for evaluation of device contributions to capacity rather than potential variability among different media lots. Specific pretreatment methods were utilized for various grades of Clarisolve® depth filters. These studies compare device capacity from small scale (µPod®), intermediate scale (Lab scale Pod) and process scale (Process scale Pod) devices.

Methods

Cell Culture Pretreatment with pDADMAC: Cell culture (mAb02, 185 L) with a peak cell density of 16.3 x 10^6 cells/mL, a harvest cell density of 11.0 x 10^6 cells/mL, and 85% viability. Cell culture was transferred to a 200 L Mobius® mixer and mixed at a setting of 200 rpm. pDADMAC (925 mL, 0.05%) was added to the cell culture and mixed for a duration of greater than thirty minutes.

Device Operation: All depth filters were flushed with RO/DI water at 600 LMH for not less than 100 L/m^2. Following cell culture pretreatment, an aliquot of cell culture was removed for µPod® filter (CS40MS01L3) and Lab scale Pod (CS40MS01H1) and (CS40MS02H1) bench testing, while Process scale Pods (CS40MS01F-X and CS40MS03F1-X) were fed directly from the Mobius® mixer. A flux of 150 LMH was utilized for feed processing with inline pressure monitoring for each device.

Results and Discussion

Clarisolve® 40MS Depth Filter Devices

Observed results from µPod® devices are show in **Table 5**, with capacity (L/m^2) presented at 15 psid.

Device	Capacity at 15 psi (L/m^2)
µPod® A	507
µPod® B	516
µPod® C	500
µPod® D	481

Table 5. Clarisolve® 40MS Depth Filter in µPod® Format Capacity

Figure 27 summarizes the data from **Table 5** by plotting pressure (psig) vs. throughput (L/m^2) for µPod® devices.

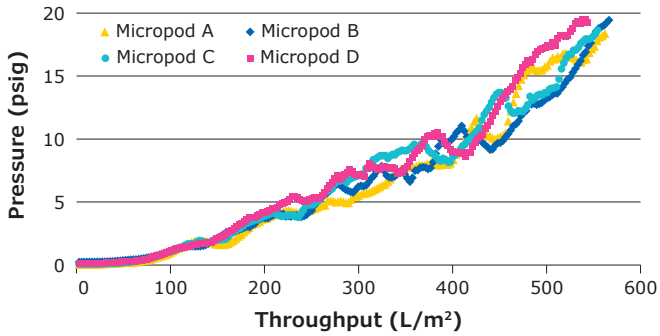

Figure 27. Clarisolve® 40MS Depth Filter in µPod® Format Pressure vs. Loading

Results from Lab scale Pod are shown in **Table 6**, again with capacity (L/m^2) presented at 15 psid.

Device	Capacity at 15 psi (L/m^2)
0.014 m^2 LSP A	444
0.014 m^2 LSP B	414
0.027 m^2 LSP A	378
0.027 m^2 LSP B	400

Table 6. Clarisolve® 40MS LSP Depth Filter Capacity

Figure 28 outlines the data from **Table 6** by plotting pressure (psig) vs. throughput (L/m^2) for LSP devices.

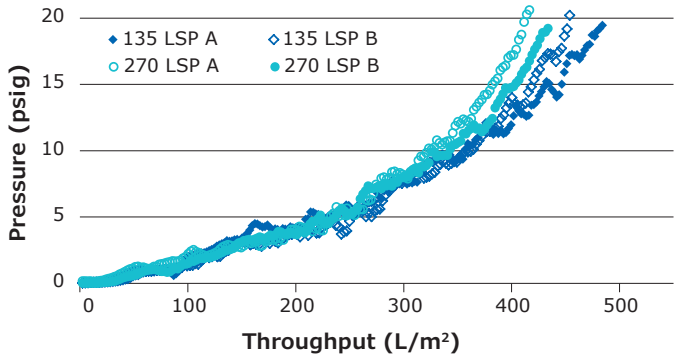

Figure 28. Clarisolve® 40MS LSP Depth Filter Pressure vs. Loading

Analysis of the Process scale Pod results are shown in **Table 7** with capacity (L/m²) presented at 15 psid.

Device	Capacity at 15 psi (L/m²)
0.11 m² PSP A	441
0.11 m² PSP B	446

Table 7. Clarisolve® 40MS PSP Depth Filter Capacity

The data from **Table 7** is presented as pressure vs. throughput in **Figure 29**.

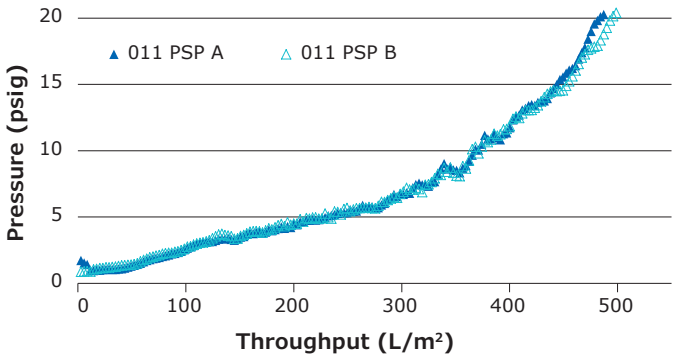

Figure 29. Clarisolve® 40MS PSP Depth Filter Pressure vs. Loading

Average capacity for each device type is shown in **Table 8**.

Device	Capacity at 15 psi (L/m²)
µPod®	501
0.014 m² LSP	429
0.027 m² LSP	389
0.11 m² PSP	444

Table 8. Clarisolve® 40MS Validated Device Average Capacity Comparison

Cleaning (pDADMAC)

Introduction

Customers implementing pDADMAC polymer flocculation in fixed stainless steel infrastructure require a Clean-In-Place (CIP) strategy to successfully clean vessels and equipment internals post use. Lack of a CIP strategy can make pDADMAC flocculation not feasible in fixed stainless steel infrastructure. Preliminary findings suggest cleaning can be challenging with conventional caustic based CIP processes. The key purpose of the study was to demonstrate that flocculated cell culture soils can be successfully cleaned using commercially available cleaning chemistries while staying within the process constraints applicable to most end users. The study also provides a brief overview of a standard coupon based scale down evaluation. This study was done in collaboration with Steris Corporation.

Methods

The study was performed at bench scale using 3”x6” 304 stainless steel finish 2 B coupons. An internal monoclonal antibody (mAb02) expressing CHO cell culture harvest was used for the study. Two soils were evaluated for the study. Details of the soils evaluated during the course of the study are provided in **Table 9**.

Flocculant	Feed	Description
N/A	mAb02 CHO harvest	Negative Control
pDADMAC	pDADMAC flocculated feed (30pg/cell)	Standard flocculated feed

Table 9. Summary of soils

mAb02 CHO harvest was used directly with no flocclulant as a negative control for soil. pDADMAC flocculated CHO cell culture harvest was prepared on site at Steris as shipping flocculated feed may drastically alter the nature of soil. Dosing was performed at 30 pg pDADMAC/cell mL. The sample were allowed to stir for 45 minutes prior to being used as a soil for the coupons. Coupons were dip coated with the cell culture and allowed to dry for 16 hours under ambient conditions.

Standard screening method for coupon study is based in agitated immersion. Control parameters include cleaning chemistry, concentration, time and temperature. Soiled coupons are suspended in a vessel with defined volume of the cleaning chemistry and at given concentration controlled at defined temperature. Coupons are removed at defined time points to test for cleanability. Agitated immersion would mimic a static soak on a process scale. In addition, the optimal chemistry also gets tested using two different methods, mechanical spray wash and cascade flow.

Visual inspection, a water break free test, residual weight analysis, and a total organic carbon (TOC) test were used to evaluate cleaning details.

Results and Discussion

Cleaning chemistries, CIP 100, Proklenz One and CIP 150 were effective in cleaning the untreated harvest (no pDADMAC added) at 1% v/v at 60 °C within 15 minutes of contact time. **Table 10** shows the results for agitated immersion.

Cleaner	Concentration	Time/Temperature	Visual Observation	Water Break Free
CIP 100 Detergent*	1% v/v	15 min/60 °C	Visually clean	Yes
CIP 100 Detergent*	0.5% v/v	15 min/60 °C	Visually clean	Yes
CIP 150 Detergent*	1% v/v	15 min/60 °C	Visually clean	Yes
Proklenz One Detergent*	1% v/v	15 min/60 °C	Visually clean	Yes

*CIP 100, CIP 200, CIP 150 and ProKlenz One are tradenames of commercial cleaning clemistries from Steris Corporation.

Table 10. Untreated mAb02 CHO harvest: Agitated immersion

CIP 100 was selected for evaluation of cleanability using the other two contact methods. Mechanical spray washer was able to clean the coupon with CIP 100 concentration of 1% v/v at 60 °C within 15 minutes of contact time. The spray pressure was set at 11 psi. **Table 11** shows the results for Mechanical spray washer.

Cleaner	Concentration	Time/Temperature	Visual Observation	Water Break Free
CIP 100 Detergent	1% v/v	15 min/60 °C	Visually clean	Yes

Table 11. Untreated mAb02 CHO harvest: Mechanical spray washer (11 psi)

Cascade flow was able to clean the coupon with CIP 100 concentration of 1% v/v at 60 °C within 15 minutes of contact time. The cascade flowrate was set at 0.5 gal/min. **Table 12** shows the results for Cascading Flow at 0.5 gal/min.

Cleaner	Concentration	Time/Temperature	Visual Observation	Water Break Free
CIP 100 Detergent	1% v/v	15 min/60 °C	Visually clean	Yes

Table 12. Untreated mAb02 CHO harvest: Cascade flow (at 0.5 gal/min)

Water and three concentration of sodium hydroxide were used as controls for cleaning chemistry. 2% w/v and 5% w/v sodium hydroxide was able to clean the untreated CHO harvest soiled coupons at 60 °C within 15 minutes of contact time. Water and 0.2% w/v concentration of sodium hydroxide were found to be ineffective to achieve cleaning. Results are summarized in **Table 13**.

Cleaner	Concentration	Time/Temperature	Visual Observation	Water Break Free
Deionized (DI) Water	—	120 min/60 °C	Heavy residue	No
NaOH	2% w/v	15 min/60 °C	Visually clean	Yes
NaOH	5% w/v	15 min/60 °C	Visually clean	Yes
NaOH	0.2% v/v	120 min/60 °C	Trace	No

Table 13. Untreated mAb02 CHO harvest: Control chemistries (agitated immersion)

Confirmatory runs were performed using CIP 100 in all three contact modes. Gravimetric method and TOC was used to confirm cleanability. Results are shown in **Table 14**.

Process	Pre Coating Coupon Weight	Coated Area	Dry Coating Weight	Post Cleaning Coupon Weight	Residue	TOC-TOC_{blank} (ppb)
Agitated Immersion CIP 100 Detergent 1% v/v 60 °C, 15 min	80.9385 g	91 cm^2	0.0351 g 0.39 mg/cm^2	80.9385 g	0.0 mg	73
Spray Wash at 11 psi CIP 100 Detergent 1% v/v 60 °C, 15 min	80.7236 g	91 cm^2	0.0455 g 0.50 mg/cm^2	80.7236 g	0.0 mg	0
Cascading Flow at 0.5 gal/min CIP 100 Detergent 1% v/v 60 °C, 15 min	82.3716 g	91 cm^2	0.0508 g 0.56 mg/cm^2	82.3716 g	0.0 mg	0

* TOC_{blank} =328 ppb

Table 14. Untreated mAb02 CHO harvest: Confirmatory experiments

pDADMAC flocculated mAb02 CHO harvest was the flocculated test soil evaluated during the study. Cleaning chemistries CIP 100, Proklenz One and CIP 150 were tested. Only CIP 100 was found to be effective in cleaning the pDADMAC flocculated harvest at 1% v/v at 60 °C within 60 minutes of contact time. **Table 15** shows the results for agitated immersion.

Cleaner	Concentration	Time/Temperature	Visual Observation	Water Break Free
CIP 100 Detergent	1% v/v	60 min/60 °C	Visually clean	Yes
CIP 100 Detergent	1% v/v	30 min/70 °C	Visually clean	Yes
CIP 150 Detergent	1% v/v	120 min/60 °C	Trace	No
Proklenz One Detergent	1% v/v	120 min/60 °C	Light residue	No

Table 15. pDADMAC flocculated mAb02 CHO harvest: Agitated immersion

CIP 100 was selected for the evaluation of cleanability using the other two contact methods. Mechanical spray washer was able to clean the coupon with CIP 100 concentration of 1% v/v at 60 °C within 30 minutes of contact time. The spray pressure was set at 11 psi. **Table 16** shows the results for Mechanical spray washer.

Cleaner	Concentration	Time/Temperature	Visual Observation	Water Break Free
CIP 100 Detergent	1% v/v	30 min/60 °C	Visually clean	Yes

Table 16. pDADMAC flocculated mAb02 CHO harvest: Mechanical spray washer (11 psi)

Cascade flow was able to clean the coupon with CIP 100 concentration of 1% v/v at 60 °C within 45 minutes of contact time. The cascade flowrate was set at 0.5 gal/min. **Table 17** shows the results for Cascading Flow at 0.5 gal/min.

Cleaner	Concentration	Time/Temperature	Visual Observation	Water Break Free
CIP 100 Detergent	1% v/v	45 min/60 °C	Visually clean	Yes

Table 17. pDADMAC flocculated mAb02 CHO harvest: Cascade flow (at 0.5 gal/min)

Water and two concentration of sodium hydroxide were used as controls for cleaning chemistry. 2% w/v and 5% w/v sodium hydroxide as well as water were not able to clean the pDADMAC flocculated CHO harvest soiled coupons at 60 °C even after 120 minutes of contact time. Results are summarized in **Table 18**.

Cleaner	Concentration	Time/Temperature	Visual Observation	Water Break Free
Deionized (DI) Water	—	120min/60 °C	Heavy residue	No
NaOH	2% v/v	120min/60 °C	Heavy residue	No
NaOH	5% v/v	120min/60 °C	Heavy residue	No

Table 18. pDADMAC flocculated mAb02 CHO harvest: Control Chemistries (agitated immersion)

Confirmatory runs were performed using CIP 100 in all three contact modes. Gravimetric method and TOC was used to confirm cleanability. Results are shown in **Table 19**.

Process	Pre Coating Coupon Weight	Coated Area	Dry Coating Weight	Post Cleaning Coupon Weight	Residue	TOC-TOC_{blank} (ppb)
Agitated Immersion CIP 100 Detergent 1% v/v 60 °C, 60 min	81.02236 g	91 cm^2	0.0341 g 0.37 mg/cm^2	81.0236 g	0.0 mg	0
Spray Wash at 11 psi CIP 100 Detergent 1% v/v 60 °C, 30 min	80.9538 g	91 cm^2	0.0350 g 0.38 mg/cm^2	80.9538 g	0.0 mg	0
Cascading Flow at 0.5 gal/min CIP 100 Detergent 1% v/v 60 °C, 45 min	81.2632 g	91 cm^2	0.0539 g 0.59 mg/cm^2	81.2632 g	0.0 mg	0

Table 19. pDADMAC flocculated mAb02 CHO harvest: Confirmatory experiments

Conclusion and Discussion

CIP strategies for cleaning stainless steel soiled with pDADMAC polymer flocculated feeds were identified. No issues were observed in cleaning non-flocculated CHO cell culture harvest which was used as negative control. Cleaning can be performed using CIP 100 at 1% v/v concentration at 60 °C using agitated immersion, spray washing (11 psi) or cascade flow within 15 minutes of contact time. 2% and 5% sodium hydroxide also successfully cleaned the non-flocculated cell culture at 60 °C using agitated immersion in 15 minutes.

Cleaning of stainless steel soiled with pDADMAC flocculated feed can be performed using CIP 100 at 1% v/v concentration at 60 °C using agitated immersion, spray washing (11psi) or cascade flow for 60 minutes, 30 minutes and 45 minutes contact times respectively. Sodium hydroxide up to 5% concentration with agitated immersion at 60 °C was not effective.

It is recommended to chase the dosing lines with conditioning buffer (50 mM Tris pH) to ensure all polymer gets accurately dispensed and to clean the lines simultaneously. It is also recommended to keep the dispense lines filled with buffer used to chase the polymer and not to run dry. This would help with ensuring there is no back flow.

Due to the unique nature of each cell culture flocculated feed, the cleaning strategy would need to be reevaluated and customized.

Summary

Overall Summary

This performance guide provides a detailed look at the impact of certain variables on the effectiveness of cell culture pretreatment and depth filter clarification as well as scalability and cleaning of pretreated feed streams. The results confirmed that following our recommendations for pretreatment addition, depth filter installation and use at all scales and cleaning leads to a robust clarification process. The results are summarized in the following text and table.

Flocculation Optimization

- Dosing of pDADMAC based on weight to total cell density offers a more accurate strategy. Dosing +/- 20% of the optimal dose had no impact on supernatant turbidity levels or depth filter performance.
- Low cell viability appeared to have an impact on supernatant turbidity and required a higher dose of polymer to reach sufficient levels of flocculation.
- There was no significant depth filter performance differences when comparing bolus and pump fed addition of polymer.

Mixing

- Within the limits of the experiment, there was no impact of mix speed on filtration capacity or turbidity.
- Slower mixing required a longer time for the floc size to stabilize but this time is within the recommended mixing time.
- Type of mixing has no impact on depth filter performance.

Continued Precipitation

- Flocculated feed streams showed less precipitation with post-depth filtration hold time compared to feed without pretreatment.
- The addition of a secondary depth filter further reduced this precipitation.
- Operation or storage temperature did not impact continued precipitation.

Effects of Pretreatment on Protein A Performance

- Clarified cell culture derived from pDADMAC flocculation and Clarisolve® 40MS filtration allowed for 30% more purification cycles on Protein A resin compared to cell culture clarified using traditional cellulosic/diatomaceous earth depth filters based on dynamic binding capacity
- Column backpressure remained considerably more stable in the case of pDADMAC flocculation and Clarisolve® 40MS filtration
- Lower host cell protein concentrations in the pDADMAC flocculated and clarified cell culture resulted in greater clearance over the Protein A column
- Leached Protein A levels in the eluate remained at acceptable levels

Scalability

- The test results demonstrate linear scalability for Clarisolve® 40MS Depth Filter with pDADMAC treated feed for all scales from µPod® to Process scale Pod formats.

Cleaning (pDADMAC)

- Only CIP 100 demonstrated the ability to effectively clean the system when the harvest was treated with pDADMAC.

It should be noted again that these tests should be repeated on the end user's feed stream to confirm the trends are the same.

Quantitation Screen of Residual pDADMAC

Our Validation Services provides a service to quantitate the residual level of unbound pDADMAC in sample matrices from post-Protein A eluates and/or drug product. We will first test post-Protein A eluates. If significant matrix interference is observed in the post-Protein A eluates, only then would we test the drug product. We therefore recommend sending both post-Protein A elute sample and drug product sample. Sample pretreatment, if necessary, is limited to dilution or protein precipitation. Validation Services and our internal testing partners perform this study with the LC-MS method specified in the application note AN33330000. Report will include description of samples submitted, quantitation of pDADMAC, and a chromatogram overlay including the standard. Final reports are issued electronically in a PDF format. The average timeline for this study is ~6 – 8 weeks after both Purchase Order and samples are received. Catalog Number: VSPDADMAC.

Please submit a quote request for pDADMAC quantitation services at **EMDMillipore.com/validation-quote-request**

Variable	Outcome
Flocculation Optimization – Effect of cell density	Dosing based on weight of total cell density offers a more accurate strategy.
Flocculation Optimization – Dosing polymer at +/- 20% target dose	No impact on depth filter performance (loading, pressure, and turbidity).
Flocculation Optimization – Effect of viability (20–86%)	Lower viability appeared to have an impact on supernatant turbidity and required a higher dose of polymer to reach an effective level of flocculation.
Method of flocculant addition	No impact on depth filter performance.
Mixing – Speed	No impact of mix speed on filtration capacity or turbidity. Slower speeds required longer time to stabilize but this time is within the suggested mixing time.
Mixing – Type of mixer	No impact on depth filter performance.
Continued precipitation – effect of hold time	Less post-depth filter precipitation with flocculation than without. Addition of a secondary depth filter further reduced this precipitation.
Continued precipitation – effect of hold temperature	No significant impact on continued precipitation.
Scalability – Clarisolve® 40MS Depth Filter w/pDADMAC pretreatment	The results demonstrated similar performances for all scales from µPod® to Process scale Pod formats.
Cleaning-pDADMAC pretreatment	Only CIP 100 demonstrated the ability to effectively clean the system when the harvest was treated with pDADMAC.

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