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Dosing considerations and impacts on the clarification of mammalian cell culture feed streams using poly—diallyldimethylammonium chloride flocculant in conjunction with Clarisolve® depth filters

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Background

A push for disposable technologies, coupled with increasingly higher cell culture densities, has placed a challenge on traditional solid-liquid separation technologies. To address this problem, flocculation, combined with Clarisolve® depth filters, has been utilized to enhance filtration capacity and quality. This work evaluates the application of poly-diallyldimethylammonium chloride (pDADMAC), as an effective pretreatment method for mammalian cell culture harvest. Clarisolve® filters were recently introduced as a novel graded depth filter designed for higher dirt holding capacity. One benefit of this technology is filtration train compression by combining both primary and secondary clarification in one device.

There are a variety of influences that may effect filtrate quality and this work begins to examine several of these properties. Cell culture conditions at harvest such as cell density and viability, mixing hold times, dosing and filtration sensitivity are studied to understand their impacts on filtration quality and throughput. Previous publications have demonstrated that pretreatment of cell culture reduces both HCP and DNA in the clarification pool. This often lowers the burden being placed upon downstream unit operations.

Furthermore, residual pDADMAC has been quantified using Surface Plasmon Resonance (SPR) and has been shown at optimal doses to be below acceptable toxicity limits.

Experimental Design

The CHO cell line used for all experiments is an internally developed non-producing host cell. In all instances pDADMAC is supplied by EMD Millipore (PN: 137069). Clarisolve® 40MS in µPod® device format are used for clarification. Particle size distribution (PSD) was characterized using a G400 Focused Beam Reflectance Measurement (FBRM) (Mettler-Toledo AC) with a 14/9.5mm probe. Pressure and flow are recorded with a Pendotech NFFSS.

Total Cell Density and Viability Impacts on Dosing

To understand impacts of pDADMAC dosing, raw cell culture can be treated and centrifuged to determine impacts of flocculation. Based upon filtrate quality, an optimum pDADMAC dose can be determined from these studies. This dose is subsequently used for depth filtration studies.

pDADMAC addition to the bioreactor can be normalized against the peak total cell density (PTCD) measurement. Figure 4 shows the relationship between pDADMAC addition and total cell density by evaluating supernatant quality. To reduce the cell concentration at harvest, serial dilutions of the harvest are made into basal growth media. These data suggests that normalizing pDADMAC addition to solids present in the bioreactor provides for a reasonable approach to pDADMAC addition. For a given sample, various cell counters will report different values. As such, caution and consistency should be exercised during dosing studies.

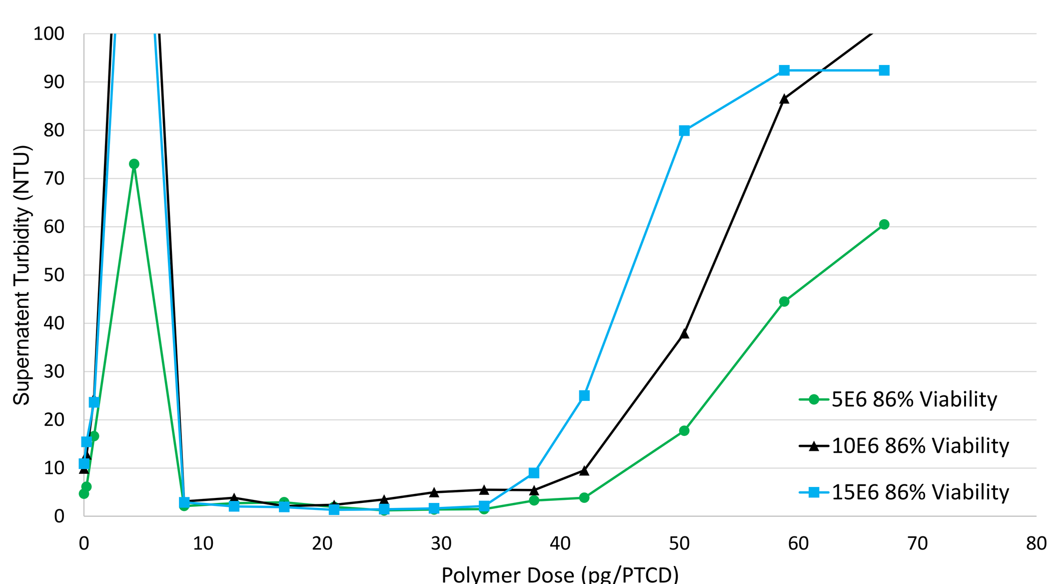


Figure 1: Total Cell Density Impacts on Dosing
From a representative harvest, dilutions are made into basal media to represent PTCD's of 15E6, 10E6 and 5E6 cells/mL. pDADMAC addition ranged from 0 to >150pg/PTCD.

Similarly, cell viability could impact pDADMAC dosing due to differences in the initial harvest PSD. Figure 7 indicates that over a range of viabilities, an optimum pDADMAC dose can be achieved; which provides for a more robust flocculation. Considerations for residual polymer should be weighed in addition to filtrate quality and impurity clearance.

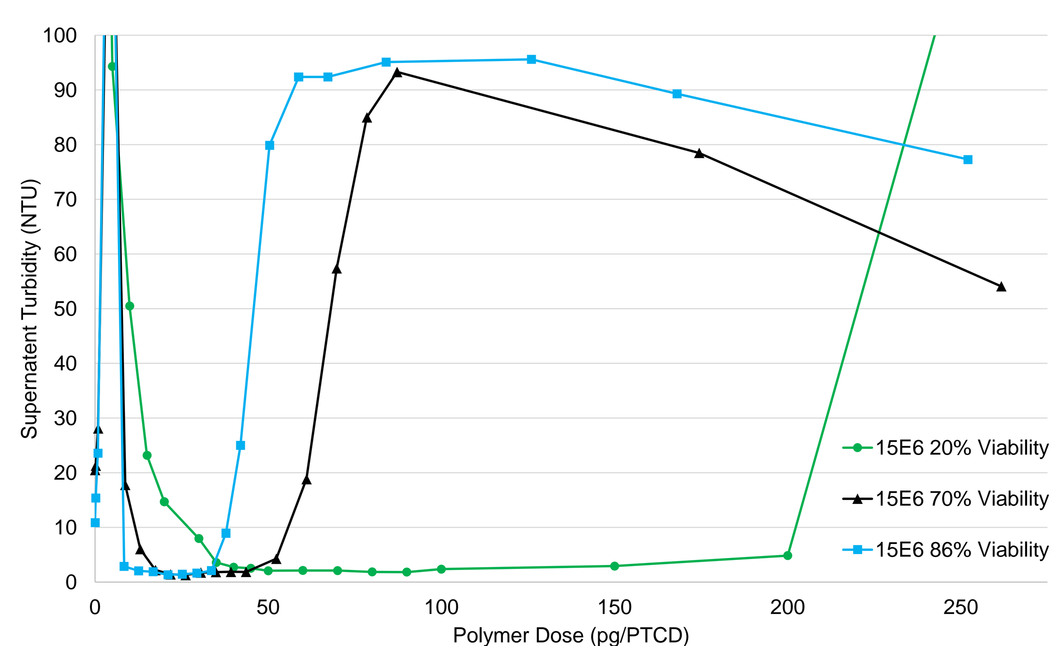
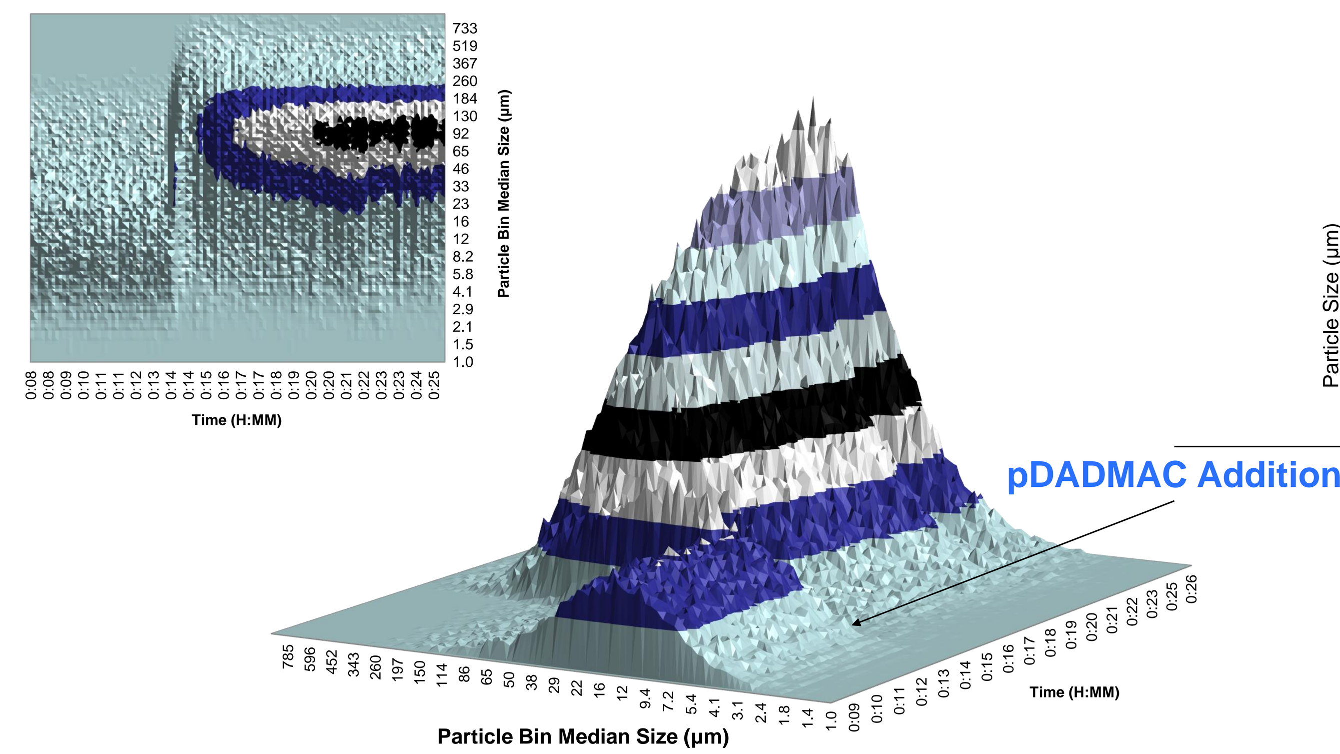


Figure 2: Cell Viability Effects on pDADMAC Dosing
At a similar PTCD of ~15E6 cells/mL, three cell viabilities, 20%, 70% and 86% were studied. PDADMAC addition ranged from 0 to >200pg/PTCD.

Mixing Hold Time

Sufficient mixing hold time is required to ensure adequate polymer dispersion and to allow for flocculation to reach a constant PSD. For unit operation simplicity, pDADMAC is dosed directly into the bioreactor. Particle size distribution is monitored in real time for the duration of the experiment. PSD data, presented as a 3D surface plot, shows the first 25 minutes of. data collected (Figures 3 & 4). A constant PSD is achieved within approximately 20 minutes of dosing. 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.



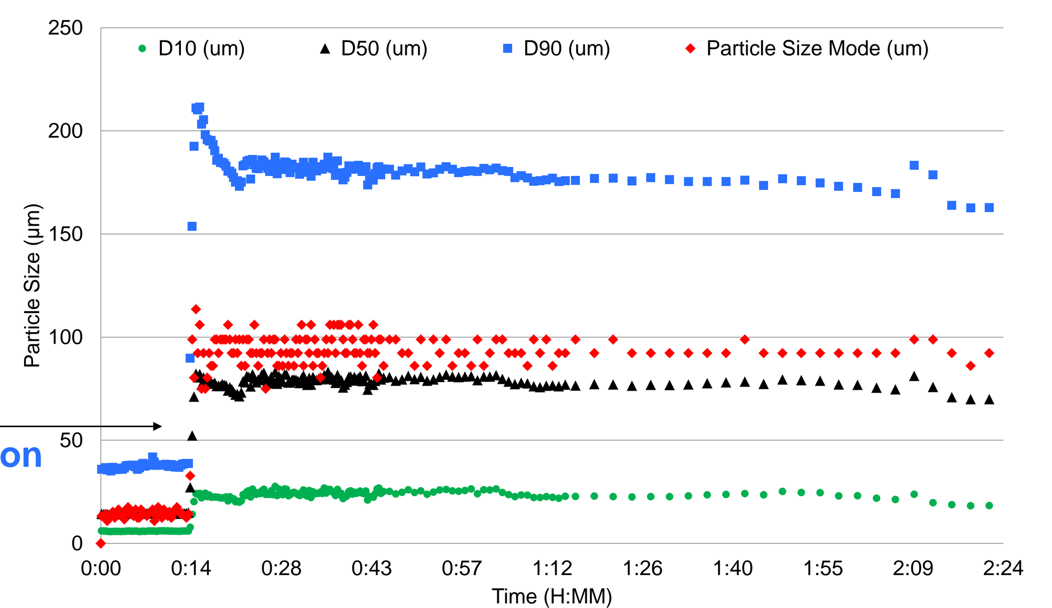


Figure 3 (Above and to the left): A Z-axis perspective of the PSD over time. A shift in the PSD around 14 minutes coincides with pDADMAC addition to the bioreactor.
Figure 4 (Above): Surface Plot of Particle Size Distribution vs Time
PSD data, presented as a 3D surface plot, shows the first 45 minutes of data collected.

Figure 5: Particle Size Distribution vs Time
D90 denotes where 90% of observed particles are equal to or smaller than the reported value. Likewise D50 and D10 denote values, where 50% and 10% respectively, of observed particles are equal to or smaller than the reported value.

Filtration Sensitivity

The effects of mixing hold times and flux rates on filtration capacity were studied in order to understand the operational window for clarification. In all instances the test conditions were run in duplicate. A sufficient mixing hold time is necessary to ensure that the dosed flocculating polymer has been sufficiently dispersed into the harvest and to allow for the flocculation to achieve a constant PSD.

Mixing hold times were varied between 0, 0.5, 1 and 2 hours. All devices had a constant flux of 150LMH. Over the harvest timeline, the filtrate quality and turbidity breakthrough profiles remained below 1 NTU for all evaluated devices. Filtration capacity varied by less than 5%. This data suggests that mixing hold time in the bioreactor does not impact filtration capacity.

Several flux rates, ranging between 75, 150 and 300LMH, were also examined to determine their impact on filtrate quality and capacity. The mixing hold time for all devices was 1 hour. In this evaluation, the turbidity breakthrough profiles remained below 1 NTU for all evaluated devices. Filtration capacity varied by less than 10%. Over the range of operating fluxes evaluated, only a minimal impact to filtration capacity was observed.

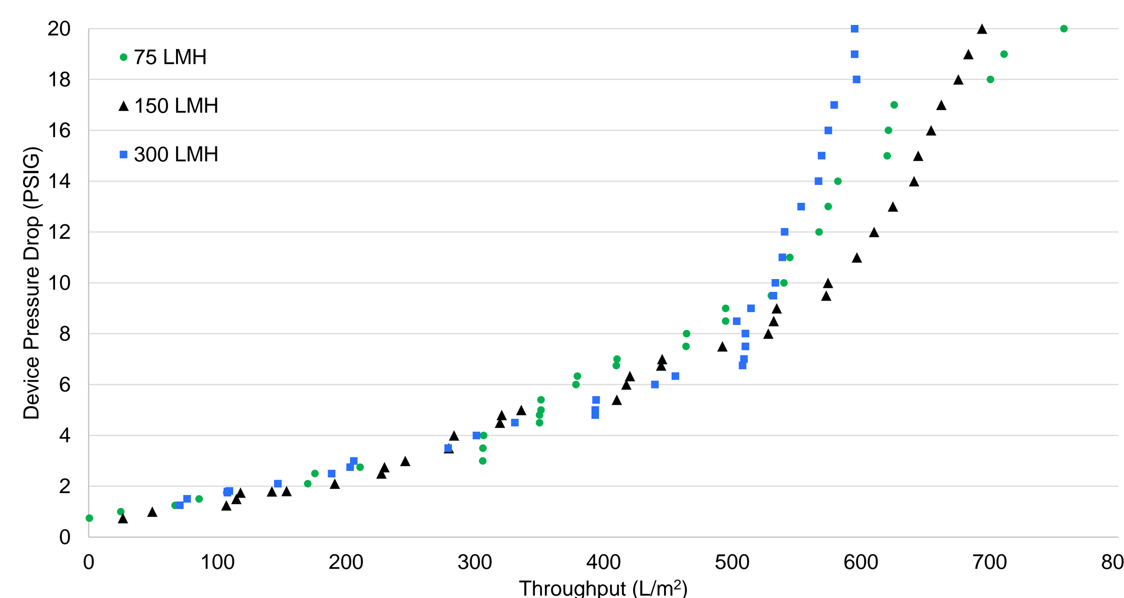


Figure 6: Flux Rate Relationship to Filtration Capacity
Flux rates of 75, 150 and 300 LMH were studied. The harvest for all devices was supplied by one bioreactor. The mixing hold time for all devices was 1 hour.

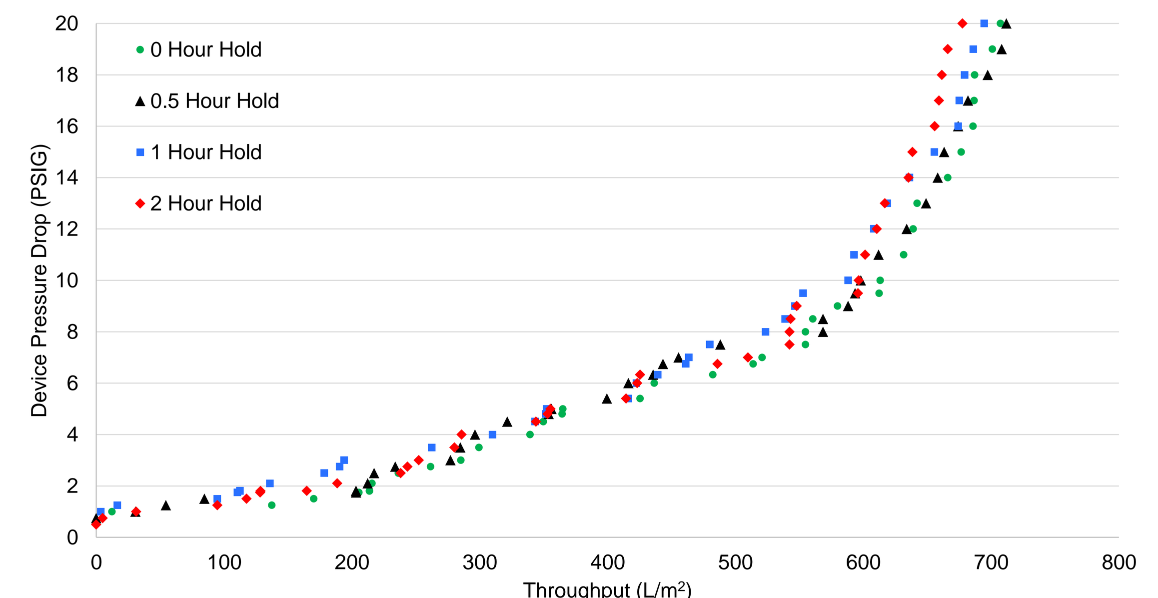


Figure 7: Influence of Mixing Hold Time on Filtration Capacity
Mixing hold times of 0, 0.5, 1 and 2 hours were studied. The harvest for all devices was supplied by one bioreactor. The Flux rate for all devices was 150LMH.

Dosing Sensitivity

To gain an understanding of the required precision during pDADMAC dosing to the bioreactor, polymer was added to three aliquots of a representative harvest. With the exception of polymer addition, all three aliquots were treated equally. The center point is defined as an optimal dose, based upon supernatant turbidity. pDADMAC was added at the optimal dose and ±20% of this value. The data suggests that minor deviations, with respect to an 'optimum dose', translates to no change in filtration performance. This provides for an additional degree of robustness with respect to this unit operation. Effects of dosing were not evaluated for residual pDADMAC or impacts to downstream unit operations.

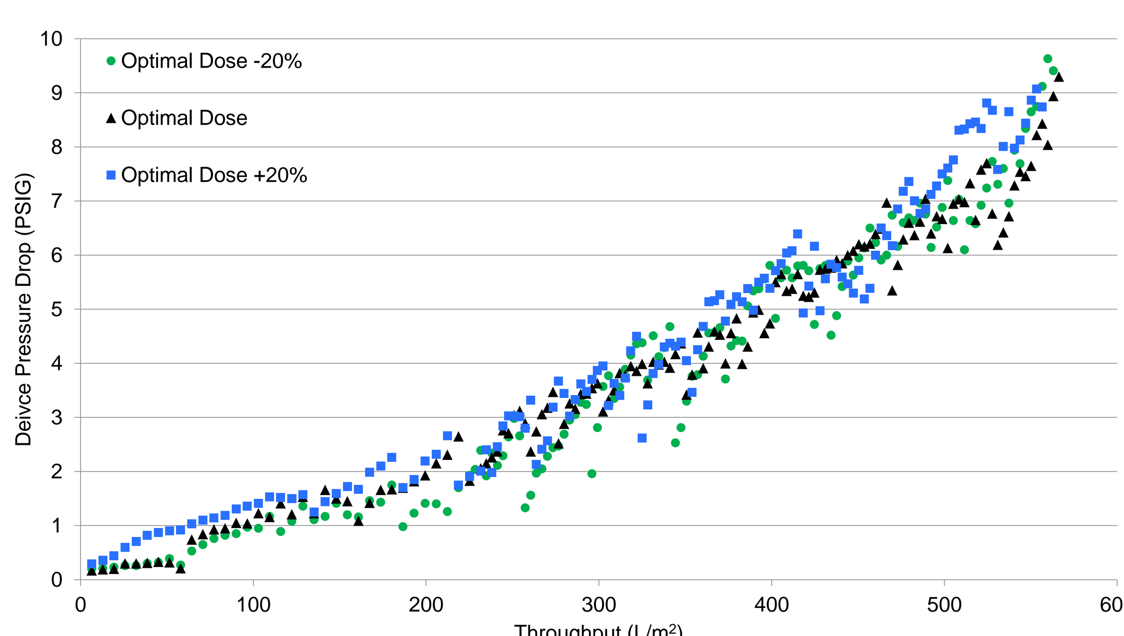


Figure 8: pDADMAC Dosing Sensitivity Analysis
Filtration performance comparison on varying pDADMAC dosing. pDADMAC was added at the optimal dose and ±20% of this value.

Summary

pDADMAC pretreatment of mammalian cell culture, coupled with Clarisolve® depth filters, provides for an effective pretreatment method by shifting the particle size distribution towards larger particles which can then be 'matched' to a graded depth filter designed for higher solids loading. The demonstration of consistent filtration performance, with flexibility around pDADMAC dosing, filtration flux rates and mixing hold times, provides for a robust clarification platform. Additionally, utilizing Clarisolve® depth filters for direct harvest, allows for a more compact clarification train, through the compression of primary and secondary filtration, and thus a smaller footprint. Future work is planned to examine impacts on shifts in the particle size distribution with respect to mixing energies and methodology. Additionally, further work is planned to examine the mechanisms of filtration at various flux rates.

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