What can cell culture flocculation offer for antibody purification processes

"The eventual goal of the application of flocculation in monoclonal antibody processing is to develop a separation unit operation alternative to chromatography, with equivalent separation efficiency, high process capacity, good facility fit and sound process economics."

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Recent advances in cell culture processes have significantly increased monoclonal antibody (mAb) titers to levels as high as 30 g/l. Not unexpectedly, these improvements have resulted in a new set of starting conditions for downstream processing, including: elevated cell density levels (up to 10^8 cells/ml), increased solid content (packed cell volume, up to 40%) and increased soluble impurity levels such as host cell proteins (HCP) and high molecular weight species (HMW; up to 40%) [1–4]. New advances in mAb downstream processing are critically needed, in order to meet the significant challenges posed by increased cell culture productivity.

Purification productivity can potentially be improved in a number of ways: through process intensification by increasing chromatography column dimensions, operating in multiple production cycles, utilizing high capacity or mixed-mode chromatographic resins or membrane adsorbers or by reducing the number of necessary purification steps [5–8]. However, elevated levels of HCP and HMW in high cell density culture place greater demands on purification unit operations, often necessitating the use of novel or alternative technology to mitigate the higher burden placed on downstream steps [1–4,9].

Additionally, increases in cell quantity, cell debris and colloids from high cell density culture pose significant challenges to the harvest process, placing a greater burden on the centrifugation and/or clarification filter train. Culture suspensions containing high solid content with wide particle distribution and a high percentage of small particles further complicate the harvest unit operation, requiring significantly higher depth filter surface area which may exceed the existing filtration train capacity. Harvest operations have become extremely challenging, if not impossible because of these issues.

Flocculation has been widely used for many years in the chemical and food industries, as well as in wastewater treatment. In recent years, flocculation-based pretreatment of cell cultures to circumvent both harvest and purification issues experienced in mAb production has been explored and implemented [2–4,10–14], and is in effect a strategy with the potential to 'kill two birds with one stone'.

Many flocculation agents or flocculants, ranging from simple electrolytes to synthetic polyelectrolytes (or polymers) have been investigated. Flocculation is the agglomeration of particles (cells, cell debris or colloids in mammalian cell culture) caused by a bridging effect induced by the flocculant. Flocculation agents are adsorbed to particles largely through electrostatic attraction although additional interactive forces such as hydrophobic interaction or hydrogen bonding may be involved [2,15–16]. Flocculated cell culture has a higher mean particle size compared with untreated cell culture and increased mean particle size has been observed to consistently correlate with improved filterability. Notably, positively charged flocculants, such

as polyamines [12], calcium chloride/potassium phosphate [14], chitosan [13], polydiallyldimethyl ammonium chloride [3,4] and stimulus responsive polymer [2] have been shown to be successful in inducing flocculation, resulting in improved clarification efficiency, process yield and clearance of impurities during the primary mAb recovery process from mammalian cell culture.

It has been shown that flocculation of cell culture can enhance the removal of cells and cell debris via centrifugation, microfiltration or depth filtration and increase filtration throughput or capacity [2,13,17–18]. For example, chitosan used as a flocculant in mammalian cell culture significantly improved centrifugation efficiency as indicated by reduced centrate turbidity, resulting in a six- to seven-fold increase in the volumetric throughput of the subsequent depth filtration step [13]. In another case, depth filter capacity was increased by more than eightfold when cell culture was flocculated with a smart polymer [2]. In each case, process economics were improved due to reduction in filter costs and enhancement of process robustness, while process yield and product quality were not adversely affected.

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Removal of process or product-related impurities such as HCP, host DNA and HMW is an additional benefit that flocculation may provide. Introduction of a smart polymer directly into a bioreactor was demonstrated to result in reduced impurity levels in Protein A eluate, meeting the requirements of drug substance and, thereby, reducing the impurity load to subsequent purification steps [2]. Furthermore, a synergistic effect on impurity removal was observed when flocculation was combined with acid precipitation [2]. The combination of flocculation and acid precipitation may therefore be beneficial and should be explored if the target antibody is stable in tested acidic conditions.

Separation of soluble impurities from antibody product during harvest can reduce the overall impurity load to downstream purification steps, which may have several benefits. First, a higher product/impurity ratio could permit increased load on subsequent chromatographic steps, resulting in higher capacity chromatography unit operations. Second, a higher purity harvested culture fluid with lower centrate turbidity and soluble impurity levels can potentially increase the number of operation cycles and lifetime of chromatographic resins, particularly Protein A. Third, removal of impurities prior to Protein A could reduce the number of chromatographic steps and enable a two-column purification platform, with greater flexibility in the selection of the second chromatographic step. The second chromatography column could potentially be developed primarily for viral clearance purposes. Membrane chromatography suitable for high productivity or continuous processing might also be explored [5,19]. Therefore, introduction of flocculant into the harvest process could result in a simplified and more robust purification process and/or provide greater process flexibility for mAb downstream processing, potentially paving the way for the implementation of a fully disposable and/or continuous purification manufacturing process.

Finally, charged flocculants may serve as viral clearance agent through their ability to bind oppositely charged viruses [10,17], thus providing an orthogonal viral removal step, again adding flexibility to the manufacturing operation.

In summary, flocculation may enable the development of a robust harvest process with the benefit of removing potential obstacles to the downstream process. However, implementation of flocculation at commercial manufacturing scale should be assessed on a case-by-case basis. The compatibility of flocculation to harvest unit operation, disk stack centrifugation, depth filtration or microfiltration, should first be evaluated prior to implementation for an existing facility. Any cell culture, up to 2000 l, can be pretreated by flocculant directly in a bioreactor and then clarified by depth filter trains, making it attractive for high titer and low volume cell culture processes. When cell culture scale is larger than 2000 l, a high-capacity depth filtration device should be evaluated in order to ensure compatibility with flocculation. Due to the high packed cell volume, alternatively, settlement of cells and cell debris for high cell density culture can be evaluated to increase filtration capacity, and decrease filtration area. However, the settling behaviors of the flocculated culture need to be studied to ensure that the operation can be performed within a reasonable timeframe and without negatively impacting product quality. Novel strategies may also be investigated to recover the mAb product from settled cell pellets if needed.

If flocculation is to be considered for use in harvest operations, due diligence must be performed to verify its impact on downstream chromatography operations. Due to potential flocculant toxicity, residual flocculant levels in the drug substance should be assessed and acceptable clearance must be reached to ensure the safety of the drug product [2,20]. The obvious advantage of flocculation using stimulus responsive polymer

and precipitation using simple acids is that there is little concern on the residual level [2,14,18].

The eventual goal of the application of flocculation in mAb processing is to develop a separation unit operation alternative to chromatography, with equivalent separation efficiency, high process capacity, good facility fit and sound process economics. Shorter process development times and simpler scale-up procedures relative to a chromatography step could provide a solution in cases where mAbs or antibody-based molecules such as bispecific antibodies produced in high cell density culture demonstrate poor purification process performance in current platform downstream processes.

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