A FULLY CONTINUOUS AND MODULAR MONOCLONAL ANTIBODY PURIFICATION PROCESS WITH CAPTURE VIA PRECIPITATION

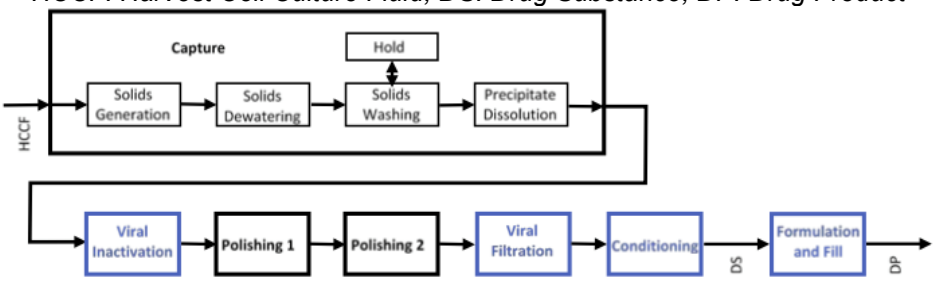
Todd M. Przybycien, Rensselaer Polytechnic Institute
Andrew L. Zydney, Pennsylvania State University
Steven M. Cramer, Rensselaer Polytechnic Institute
B. Wayne Bequette, Rensselaer Polytechnic Institute

Key Words: downstream processing, microfiltration, particle morphology, image analysis, process control

The cost and throughput bottlenecks in the current monoclonal antibody platform manufacturing process are the Protein A column chromatography capture step (1), which is limited in capacity and volumetric throughput and cannot be performed in a fully continuous mode. To relieve these bottlenecks, we have developed a fully continuous capture step based on precipitation paired with microfiltration (2) as a drop-in replacement for the Protein A-based capture step and have and demonstrated its performance with multiple industrial monoclonal antibody feeds. The selection of precipitation for the capture step is driven by increasing monoclonal antibody titers produced in the upstream process. As monoclonal antibody titers now routinely exceed 5 g/L, bulk separation techniques such as precipitation become more attractive relative to adsorptive separations such as bind-and-elute chromatography. Further, as the solubility of the monoclonal antibody is set by fixed concentrations of precipitants, raw material usage is essentially independent of titer.

We perform monoclonal antibody precipitation with inexpensive, reversible, and non-denaturing precipitants ($ZnCl_2$ and PEG-3350) in low-complexity continuous-flow tubular reactors with good selectivity and without capacity limitations. Continuous precipitation in a tubular reactor is the preferred format as the fluid shear history of the process stream determines the morphology of the precipitates. Tubular reactors offer well-defined fluid shear fields and narrow residence time distributions that are highly reproducible, facilitating scale-up. Precipitate generation is followed by a dewatering step to remove supernatant, one or more washing steps to remove residual supernatant and adherent impurities, and a re-dissolution step. We conduct the dewatering and washing steps in a fully continuous fashion using inexpensive static mixer/microfiltration module pairs. We can conduct the re-dissolution step at arbitrary concentrations up to the intrinsic solubility limit of the monoclonal antibody, resulting in highly concentrated, low volume process streams that can be purified in subsequent downstream processing steps with much smaller footprints. The precipitation-based capture step is a drop-in replacement for the Protein A-based capture step of the current platform process. Polishing operations are based on flow-through chromatography with minimal intermediate solution conditioning. The fully continuous, eminently scalable precipitation operation is compatible with current continuous upstream processes based on staggered fed-batch or perfusion bioreactors and with continuous operation of the remaining downstream process, including viral inactivation, polishing chromatography, viral filtration, and ultrafiltration/diafiltration conditioning steps.

Fully Continuous, Modular mAb Purification Process with Capture via Precipitation
HCCF: Harvest Cell Culture Fluid, DS: Drug Substance, DP: Drug Product



In this work we describe the performance of this system with several feed streams, comparing performance metrics to those of current platform mAb technology.

(1) Natarajan V and Zydney AL. Protein A chromatography at high titers. Biotechnology and Bioengineering. 110, 2445-2451 (2013). https://doi.org/10.1002/bit.24902
(2) Li Z et al. Continuous precipitation for monoclonal antibody capture using counter-current washing by microfiltration. Biotechnology Progress. 35(6), e2886 (2019). https://doi.org/10.1002/btpr.2886