DESIGN OF AN INTEGRATED CONTINUOUS DOWNSTREAM PROCESS FOR EMERGING ACID-SENSITIVE ANTIBODIES BASED ON A CALCIUM-DEPENDENT PROTEIN A LIGAND

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Antibodies are used as therapeutics and for diagnostics of a variety of diseases, and novel antibodies are continuously being developed to find treatments for new diseases. Therefore, the manufacturing process must accommodate a range of antibody characteristics. Acid-sensitive antibodies, such as the IgG2 and IgG4 isotypes or the emerging bispecific antibodies, can severely compromise product purity and yield in the purification process due to the potential formation of aggregates. To address this problem, we have developed an integrated continuous downstream process for the purification of acid-sensitive antibodies at mild conditions (Figure 1).

A calcium-dependent Protein A-based ligand, called Z_{Ca}, was used in the capture step in a 3-column periodic counter-current chromatography operation. The binding of Z_{Ca} to antibodies is regulated by calcium, meaning that acidic conditions are not needed to break the interaction and elute the antibodies. Further, the virus inactivation was achieved by a solvent/detergent method, where the pH could remain unchanged. The polishing steps included a cation and an anion exchange chromatography step, and screening of the process conditions in the capture and polishing steps was performed to allow for a seamless integration of the process steps. The minimum pH in the process was 5.5, which is significantly higher than the pH values used in a traditional antibody purification process, ranging between 3.2 and 3.5.

The process was implemented at laboratory scale and run for 9 days obtaining a high yield, and a consistently high purity, including reduction values of the host cell protein and DNA concentrations similar to those obtained in state-of-the-art processes, as well as aggregate levels below the detection limit, which is attributed to the mild conditions used in the process. In addition, this has been the first time, to our knowledge, that the removal of solvent after a solvent/detergent-based virus inactivation has been addressed for the purification process of antibodies.

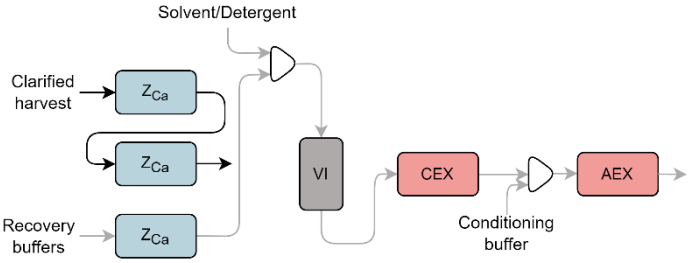

Figure 1. Downstream process diagram.