CLARIFICATION AND CAPTURE OF A CHO-DERIVED MONOCLONAL ANTIBODY THROUGH FLOCCULATION AND AEX PROCESSES

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Flocculants have been widely used in the food and chemical industry, as well as in wastewater clarification. More recently, studies have shown the applicability of these agents in the clarification of mammalian cell suspensions as an alternative e.g. to centrifugation and microfiltration. Flocculants present several advantages such as low cost, process simplicity and facility to run under continuous mode. On the other hand, some flocculants have shown toxicity and thus their total clearance has to be demonstrated.

In this work, the cationic flocculants chitosan and poly diallyldimethylammonium chloride (PDADMAC) have been investigated and compared for the clarification of suspensions of CHO cells producing a recombinant humanized monoclonal antibody. Anion exchange (AEX) adsorption has been then evaluated for its capacity to remove host cell protein (HCP) and host cell DNA (HCDNA), with the final aim of in the future developing an integrated train of clarification and AEX capture within a tangential flow filtration (TFF) device.

CHO-DP12 cells (ATCC) were cultivated in batch mode in shake flasks at 37 °C and 185 rpm using the animal component free medium TC-LECC (Xell AG, Germany), reaching approximately 10 million cells/mL and 0.2 g/L mAb. After cultivation, 20 mL of cell suspension were put in contact with flocculants under different conditions of pH and of flocculant concentration, for 30 min at 100 rpm at room temperature. The samples were then let to sediment for 90 min and the turbidity was measured for 90 min at 600 nm. The AEX resin Q-Sepharose (GE Healthcare, Sweden) was equilibrated at pH 6.5 prior to adsorption studies with the clarified supernatant from the flocculation step. Adsorption was carried out in 1.5-mL tubes by mixing the resin at a 1:10 volumetric ratio with different dilutions of the clarified supernatant for up to 120 min at room temperature under agitation at 1200 rpm. Samples were monitored for IgG (PrA-HPLC), total proteins (BCA) and DNA (picogreen).

Regarding flocculation, when chitosan was used, no flocculation was observed in the concentration range of 0.01%-0.2% (10 to 200 pg per total cells), but did occur at lower flocculant concentrations. The best condition for chitosan was thus obtained at a concentration of 0.005% (5 pg per cell) and pH 6.5, resulting in 93.5± 1.1% IgG recovery and 98.5± 1.3% host cell DNA (HCDNA) removal. PDADMAC provided recoveries higher than 90% at concentrations between 0.0225% and 0.090%, with the latter concentration yielding an IgG recovery of 95.5± 2.4% and a HCDNA removal of 98.5%. For both agents, 30 min were sufficient to sediment all the flocculated particles under these best conditions.

Under the selected conditions for chitosan flocculation (pH 6.5 and 5 pg per cell), AEX studies were performed in order to determine the adsorption capacity at equilibrium and the residence time needed to maximize removal of HCP and DNA from the clarified supernatant. Equilibrium was reached after 10 min, and adsorption capacity at equilibrium was 11 mg/mL for total proteins of and 15 mg/mL for DNA. Although at pH 6.5 IgG adsorption was not expected to occur, approximately 5 mg/mL were adsorbed (as measured by Protein A-HPLC). Further studies are ongoing to improve IgG recovery in the adsorption step, but these first results show that these simple, low-cost techniques can be used to obtain a cleaner material for further purification steps. The next steps of this work will focus on integrating flocculation, AEX and TFF to obtain a particulate free stream.