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Novel technology for protein capture: Mixed-mode expanded-bed adsorption

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For biopharmaceuticals downstream processes, it always requires highly productive and robust technologies to improve the process efficiency. Expanded-bed adsorption (EBA) is an innovative technology that allows capturing proteins directly from un-clarified feedstock, such as cell culture broth and homogenization. EBA technology combines solid-liquid separation with an adsorption step in a single-unit operation, aiming at increased overall yield, reduced operational time, and less requirements for capital investment and consumables. Mixed-mode chromatography (MMC) is a novel technology for bio-product separation, which combines multiple binding modes like hydrophobic and electrostatic interactions, hydrogen bonding, etc. High capacity, salt-tolerance, good selectivity and relatively low cost are the major advantages of MMC for direct capture process. In the present work two chromatographic techniques, EBA and MMC, were integrated to develop new separation technology, mixed-mode EBA, improving the protein capture efficiency and reducing the pretreatments on the feedstock, such as clarification, dilution and salt-adjustment. Several MMC ligands were coupled onto typical matrices (densified agarose beads) for EBA. The static adsorption, adsorption kinetics and dynamic binding were investigated, and the effects of pH and salt addition were evaluated. New technology was challenged with two typical biopharmaceutical processes, monoclonal antibody (mAb) capture from CHO cell culture broth and recombinant human albumin serum (rHSA) isolation from Pichia pastoris fermentation broth. After the optimization of operation conditions, high separation efficiency (purity, recovery, productivity) was obtained. The results demonstrated that mixed-mode EBA, combining the advantages of EBA and MMC, would be a promising new platform for protein capture with reduced feedstock pretreatments, high efficiency and relative low cost. New technology developed in the present work could also be expanded to other bio-product processes.

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Adsorptive removal of Triton X-100 from human plasma and its derivatives

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Viral transmission during the use of human plasma and its derivatives to treat various medical conditions can be fatal. Solvent/detergent treatment using non-ionic detergents like Triton X-100 inactivate the lipid enveloped viruses. However, the detergent interferes with downstream processing and analysis. Also, WHO permits a residual level of <25 ppm of Triton X-100 and thus it needs to be removed from post viral inactivation. Removal of Triton X-100 poses a challenge due to its low CMC and non-ionic character. Selective removal of Triton X-100 was studied using various hydrophobic resins screened on the basis of adsorption capacity, uptake kinetics and effect of plasma proteins on these parameters. Resins showing higher adsorption capacity and uptake rate with lower protein binding were selected for column studies. Breakthrough capacity of the shortlisted resins was determined at different flow rates and concentrations along with the effect of proteins. A simple and sensitive HPLC method was developed to detect Triton X-100 in the treated samples at ppm level. This research work asserted the impact of various resin characteristics and plasma proteins on selective detergent removal and thus the mechanism of adsorption of Triton X-100 onto these resins.

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