

Development of an ADC Process with Single-Use Membrane Technology

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Abstract

Membrane chromatography is routinely used to remove host cell proteins, viral particles, and aggregates during antibody downstream processing. The application of membrane chromatography to the field of antibody-drug conjugates (ADCs) has been applied in a limited capacity and in only specialized scenarios.

Here, we utilized the characteristics of the membrane adsorbers, Sartobind[®] S and Phenyl, for aggregate and payload clearance whilst polishing the ADC in a single chromatographic run. The Sartobind[®] S membrane was used in the removal of excess payload while the Sartobind[®] Phenyl was used to polish the ADC by clearance of unwanted DAR species and aggregates. The Sartobind[®] S membrane reproducibly achieved log-fold clearance of free payload with a ten membrane volume wash. Application of the Sartobind[®] Phenyl decreased aggregates and higher DAR species while increasing DAR homogeneity. The Sartobind[®] S and Phenyl membranes were placed in tandem to simplify the process in a single chromatographic run. With the optimized binding, washing and elution conditions, the tandem membrane approach was performed in a shorter timescale with minimum solvent consumption with high yield. The application of the tandem membrane chromatography system presents a novel and efficient purification scheme that can be realized during ADC manufacturing.

Introduction

The development of cation-exchange and hydrophobic interaction membrane chromatography for site-specific ADC process is described using an engineered cysteine-mAb with Pyrrolobenzodiazepine(PBD)-dimer as a model conjugation system. Key process parameters such as product yield, efficiency of free PBD-dimer and aggregate removal were evaluated.

Process Diagrams

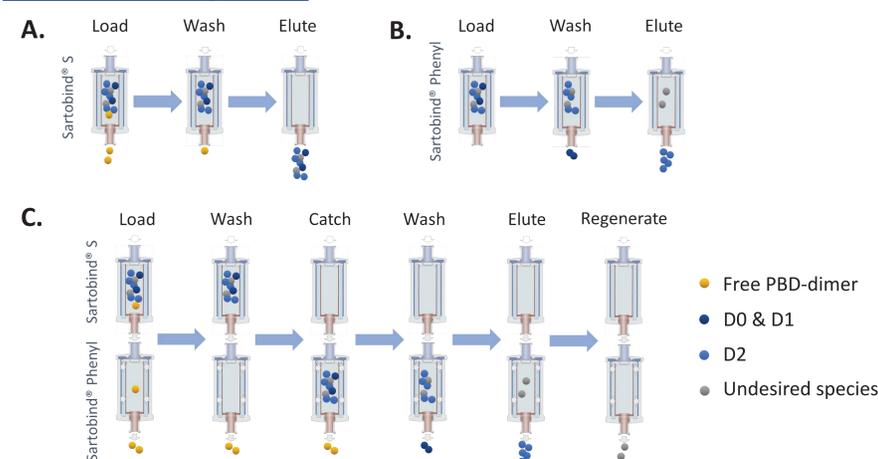


Figure 1. Process diagram. (A) Bind/Elute using Sartobind[®] S to remove residual payload, and (B) Bind/Elute using Sartobind[®] Phenyl to remove aggregate in stand-alone model; (C) Simplified process to purify target ADC with Sartobind[®] S and Phenyl in tandem model.

Results

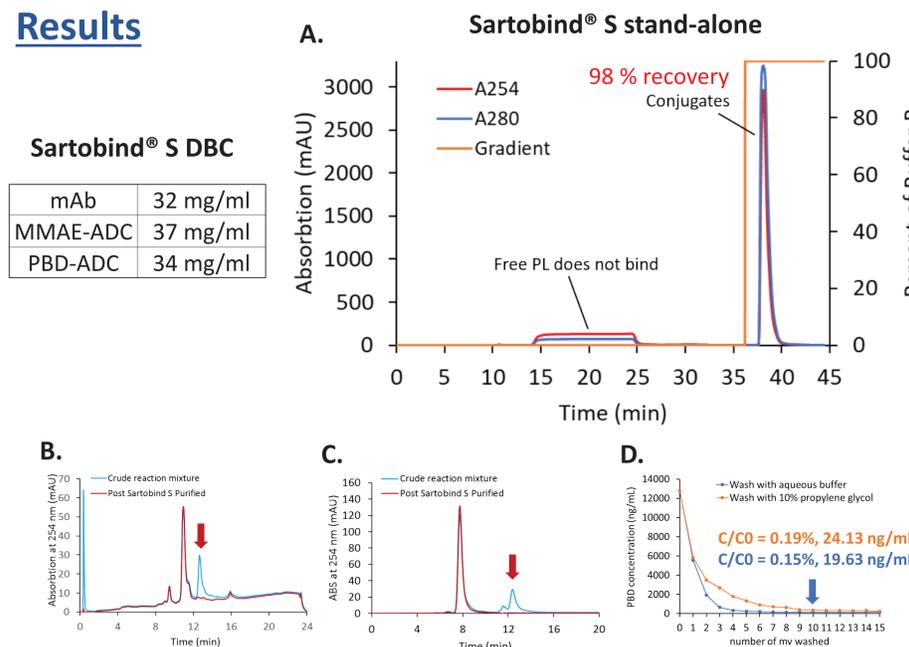


Figure 2. (A) Removal of residual PBD using Sartobind[®] S in stand-alone model. The quenched reaction mixture (gram scale) was loaded to a 150 mL Sartobind[®] S membrane, then washed with 10 mv binding buffer prior to elute. (B) HIC and (C) SEC profiles showing the removal of free PBD dimer. (D) LC-MS method showing PBD dimer removal efficiency with Sartobind[®] S.

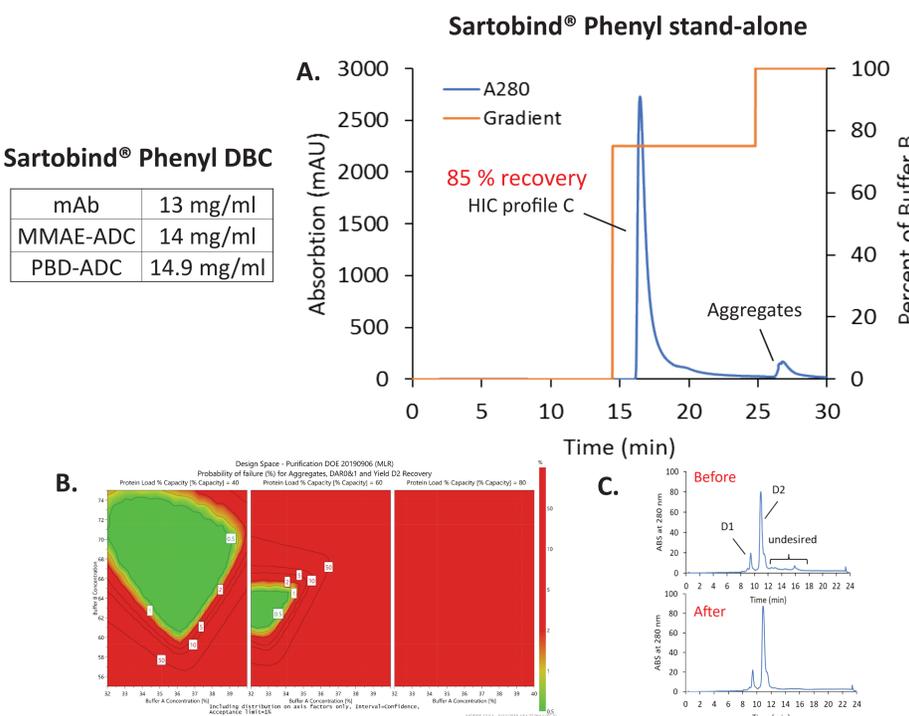


Figure 3. (A) Purification of engineered Cysteine-mAb-PBD ADC using Sartobind[®] Phenyl in stand-alone model. The Sartobind[®] S purified material (gram scale) was loaded to a 150 mL Sartobind[®] Phenyl membrane, then targeted and undesired ADC species were eluted sequentially. (B) DoE study to optimize the aggregates removal with Sartobind[®] Phenyl purification conditions. (C) HIC profile of ADC prior/pos Sartobind[®] Phenyl purification.

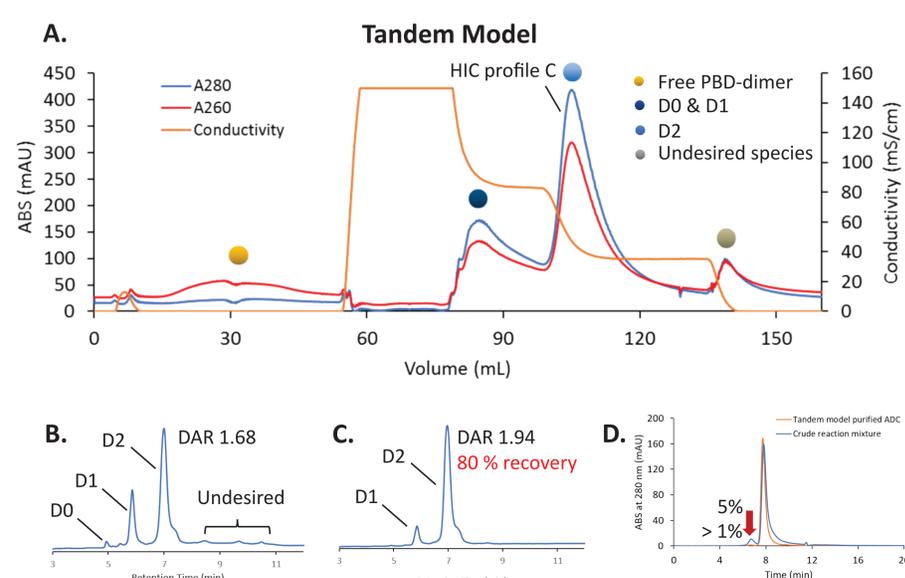


Figure 4. (A) Purification of engineered Cysteine-mAb-PBD ADC in tandem model. The quenched reaction mixture (gram scale) was loaded to Sartobind[®] S which was tandemly connected to Sartobind[®] Phenyl. The conjugation species were separated through loading, washing, and multiple elution steps. (B) HIC profile showing the crude reaction mixture contains ADC variants with a range of DAR, residual PBD dimer, aggregates, and organic solvent. (C) HIC profile of the tandem method purified ADC. The DAR of the ADC increased from 1.68 to 1.94. (D) SEC profile showing the percentage of aggregate dropped from 5 % to less than 1 % after tandem model purification.

Conclusions

- Membrane based process consumed less buffer, shortened process and hold times (less GMP scientist FTE days) which can reduce the cost and time for a cGMP manufacturing campaign.
- Membrane devices are scalable single-use, closed systems that improve manufacturing safety, eliminating the need of packing, qualification, and cleaning validation studies associated with resin-based column chromatography.
- Removal of free payload and undesired conjugate species by membrane chromatography presents a novel and efficient process that directly translates into improved efficiency both during process development and cGMP manufacturing.

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