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

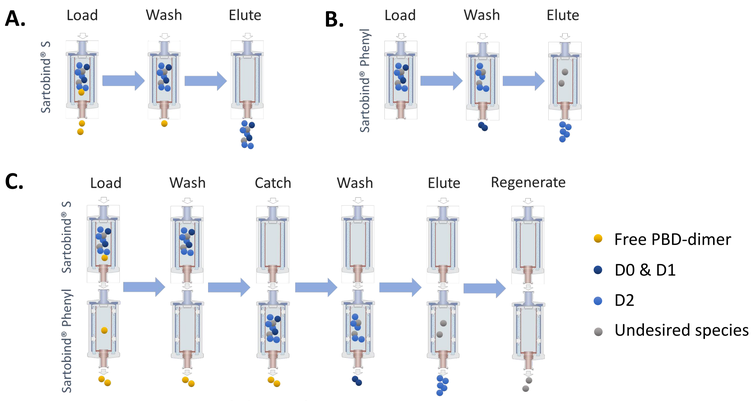

Figure 1. Process diagram. **(A)** Bind/Elute using Sartobind® S to remove residual payload, and **(B)** Bind/Elute using Sartobind® Phenyl to remove aggregate in stand-alone model; **(C)** Simplified process to purify target ADC with Sartobind® S and Phenyl in tandem model.

Results

Sartobind® S DBC

mAb	32 mg/ml
MMAE-ADC	37 mg/ml
PBD-ADC	34 mg/ml

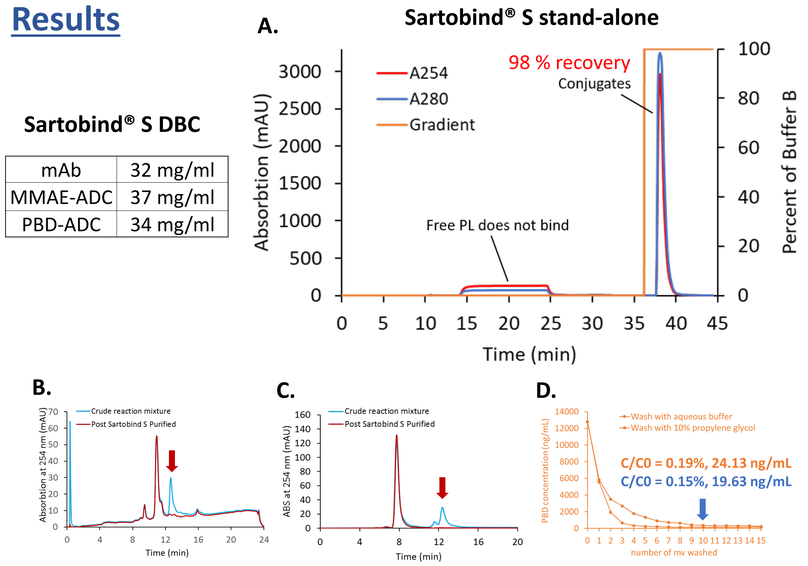

Figure 2. (A) Removal of residual PBD using Sartobind® S in stand-alone model. The quenched reaction mixture (gram scale) was loaded to a 150 mL Sartobind® S membrane, then washed with 10 mv binding buffer prior to elute. **(B)** HIC and **(C)** SEC profiles showing the removal of free PBD dimer. **(D)**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

Sartobind® Phenyl DBC

mAb	13 mg/ml
MMAE-ADC	14 mg/ml
PBD-ADC	14.9 mg/ml

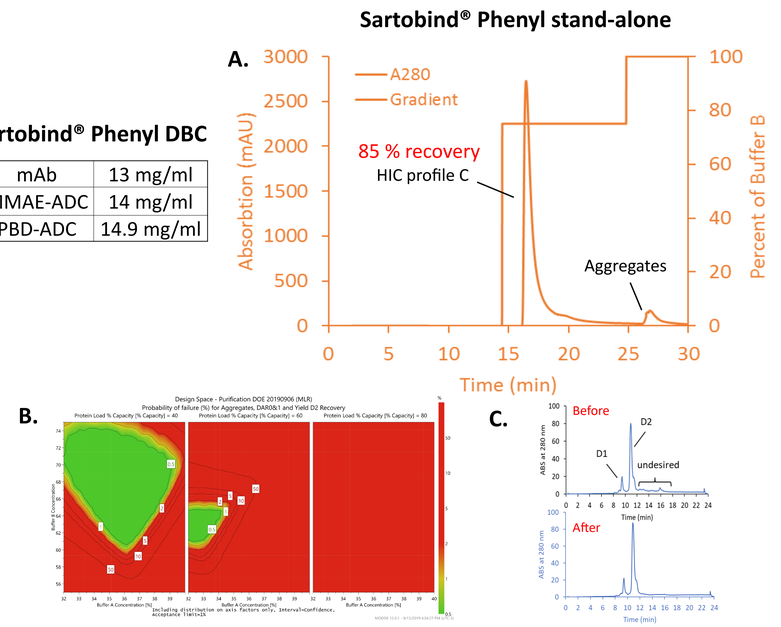

Figure 3. (A) Purification of engineered Cysteine-mAb-PBD ADC using Sartobind® Phenyl in stand-alone model. The Sartobind® S purified material (gram scale) was loaded to a 150 mL Sartobind® Phenyl membrane, then targeted and undesired ADC species were eluted sequentially. **(B)** DoE study to optimize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

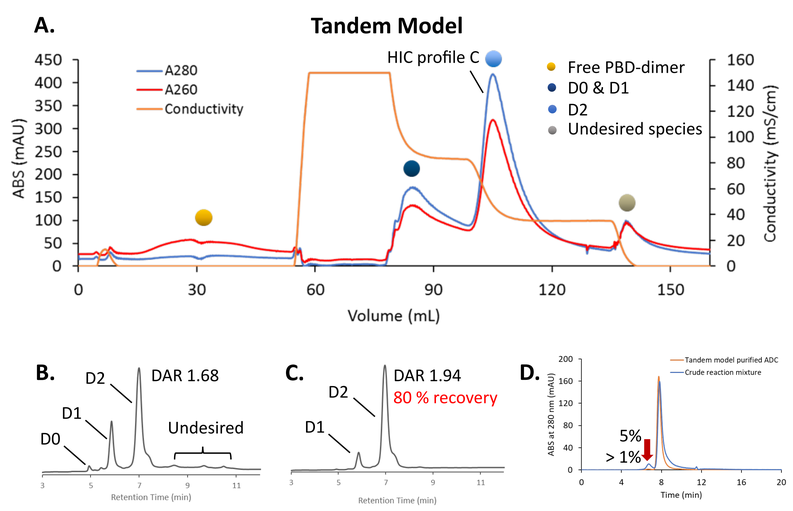

Figure 4. (A) Purification of engineered Cysteine-mAb-PBD ADC in tandem model. The quenched reaction mixture (gram scale) was loaded to Sartobind® S which was tandemly connected to Sartobind® Phenyl. The conjugation species were separated through loading, washing, and multiple elution steps. **(B)** 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.

Acknowledgements

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