SARTORIUS

Application Note

May 20, 2019

Keywords or phrases:
ADC, Antibody-drug conjugate, Site specific conjugation, Single-use membrane chromatography, DAR, Drug to antibody ratio, Chemical compatibility, Tandem mode, Sartobind® S, CEX, Sartobind® Phenyl, HIC, Two-dimensional chromatography

Single-Use Membrane Chromatography in ADC Production

Development of an ADC Process with Single-Use Membrane Chromatography Sartobind® S and Sartobind® Phenyl

Dr. Jieyu Zhou[1], Dr. Juan Carlos Cordova[1], Jesse Novak[1], Dr. Sheng Sun[1], Dr. Jeffrey Bos1, Dr. Srinath Thirumalairajan[1], Dr. Sanjeevani Ghone[1], Dr. Miyako Hirai[2,*], Dr. Ricarda A. Busse[2,*], Julia S. v. der Hardt[2], Ian Schwartz[3,*]

1. Abzena, Bristol PA, 19007 USA. Jieyu.Zhou@Abzena.com
2. Sartorius Stedim Biotech GmbH, August-Spindler-Strasse 11, 37079 Goettingen, Germany
3. Sartorius Stedim North America, 5 Orville Drive, Suite 200. Bohemia, NY 11716, USA

* Correspondence
E-Mail: miyako.hirai@sartorius.com, ricarda.busse@sartorius.com, ian.schwartz@sartorius.com

Abstract

Membrane chromatography is routinely used to remove host cell proteins, viral particles, DNA, and aggregates during antibody downstream processing.[1, 2] However, the application of membrane chromatography to purify antibody-drug conjugates (ADCs) has only been described briefly in the literature at research scale as of today.[3] Here, we describe the application of Sartobind® S and Phenyl for ADC purification during process development at milligram and gram scales. We utilized the cation exchange and hydrophobic interaction characteristics of the membrane adsorbers, Sartobind® S and Phenyl respectively, to refine drug to antibody ratio (DAR), reduce the heterogeneity of the drug load bound to the antibody, remove aggregate and remove free payload in a single chromatographic run. Sartobind® S membrane was used for the removal of excess payload while Phenyl was used to polish the ADC by removing unwanted DAR species and aggregates. The Sartobind® S and Phenyl membranes were placed in tandem to simplify the process in a single chromatographic run. Thus, presenting a novel and efficient purification scheme that can be realized during ADC manufacturing.[4]

Find out more: www.sartorius.com

Introduction

A model ADC conjugation process for a site-specific, engineered cys-mAb is shown in figure 1. Briefly, to conjugate an engineered cys-mAb, first, the engineered cys is uncapped, and then the uncapped cys is conjugated to the linker-cytotoxic agent. Aggregates, unwanted DAR species and free payload can then be removed by chromatography and tangential flow filtration (TFF) is used to diafilter the conjugate into its basal formulation and remove free payload, where applicable.

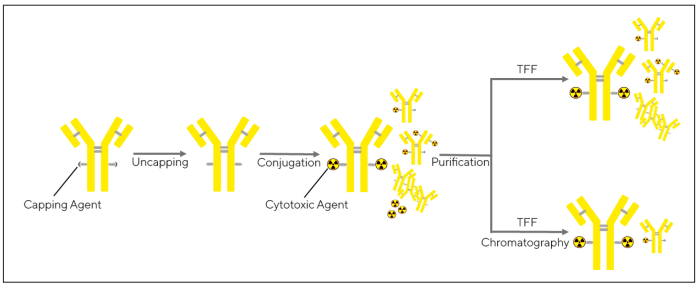

Figure 1: General method for manufacture of an engineered cysteine site specific ADC. Engineered cysteine antibody is diafiltered from the antibody formulation buffer into reduction reaction buffer, uncapped through reduction | oxidation steps, diafiltered into conjugation buffer, and conjugated to the cytotoxic agent, purified by chromatography (if necessary) and finally diafiltered into basal formulation buffer.

In this study, the development of a cation-exchange and hydrophobic interaction membrane chromatography purification process for site-specific ADC process is described using an engineered cysteine-mAb with pyrrolobenzodiazepine (PBD)-dimer and monomethyl auristatin E (MMAE) as model conjugation systems. Key process parameters such as product yield, efficiency of free PBD-dimer and aggregate removal are evaluated.

Materials and Methods

All cysteine engineered mAb (cys-mAb) used for conjugation experiments were manufactured by Abzena, San Diego California. All small molecule linker and payload were manufactured by Abzena, Bristol Pennsylvania. Conjugates were manufactured by first reducing the cys-mAb with 50 equivalents of dithiothreitol (DTT) at room temperature overnight. The cys-mAb was then diafiltered into a conjugation reaction buffer (50 mM phosphate, pH 7.0, 1 mM EDTA). The interchain disulfides were reformed by adding 12 equivalents of dehydroascorbic acid (DHAA) for 3 hours at room temperature. The uncapped cys-mAb was then conjugated to 3.5 equivalents of linker-payload (linker-PBD or linker-MMAE) with 20% (v/v) propylene glycol added as cosolvent (figure 1).

For process development, an ÄKTAexplorer was used for screening runs. The conjugates were purified using Sartobind® S and | or Phenyl membrane devices, both 3 mL, 8 mm, in bind and elute mode. ÄKTApilot system was used to assess scalability, conjugates were purified using Sartobind® S and | or Phenyl membrane devices, both 150 mL, 8 mm, in bind and elute mode. For all experiments, flow rates were 1 membrane volumes (MV)/minute. Further experimental details are provided in the results section of this application note.

Drug-to-antibody ratio (DAR) was evaluated by hydrophobic interaction chromatography (HIC) on a HPLC system (Agilent 1260 HPLC system, TSKgel Butyl-NPR column 4.6 ID × 3.5 cm, 2.5 µm). The HIC method used with 1.5 M ammonium sulfate in 25 mM potassium phosphate pH 7.0 (mobile phase A) and 25 mM potassium phosphate pH 7.0 containing 25% isopropanol (mobile phase B) run at a flow rate of 0.8 mL/min over a 12-minute linear gradient with UV monitoring at 254 and 280 nm.

To determine aggregation, conjugates were analyzed by size exclusion chromatography (column: Tosoh TSKGelSW3000xl 7.8 mm × 30 cm, 5 µm) using 0.2 M phosphate 0.2 M potassium chloride pH 6.5 with 15% (v/v) isopropyl alcohol as mobile phase.

Sample preparation for free payload species quantification with LC-MS/MS

The clearance of free linker-payload from Sartobind® S membrane was investigated utilizing a LC-MS/MS approach. The Sartobind® S membrane was first loaded with 16 µg/mL of linker-payload (PBD dimer), then washed up to 20 MV with either 20 mM MES buffer (pH 6.0) or the MES buffer with 10% propylene glycol. Finally, the membrane was washed with 3 MV of 20 mM MES, 350 mM sodium chloride buffer, pH 6.0. Each MV wash was collected separately for LC-MS/MS analysis.

An acetonitrile precipitation method was used before the LC-MS/MS analysis to extract the free payload species and remove salts or protein species. Specifically, 100 µL of sample from each MV fraction was mixed 1:9 with acetonitrile (ACN) prior to the centrifugation at 15000 g for 20 min. Then the supernatant was transferred into a new 1.5 mL Eppendorf tube. The solvent was completely removed by SpeedVac. The dried sample was dissolved in 20 µL of H2O/ACN (50:50 v/v) and applied for LC-MS/MS analysis using a SCIEX Triple Quad 6500 LC-MS/MS system.

Membrane chromatography purification process flow

Figure 2 shows an overview of the membrane chromatography process development experiments. Chromatograms from the experiments are shown in the results section below.

Membranes were used either stand-alone (figure 2, A and B) or in tandem (figure 2, C). Sartobind® S was used to remove free payload by binding the conjugate in the crude reaction mixture to the membrane, washing away free payload, and eluting the purified conjugate from the membrane (figure 2, A). Sartobind® Phenyl was used to remove undesirable DAR species and aggregates by binding the conjugate in the crude reaction mixture to the membrane, washing away less hydrophobic contaminants, and collecting the desired purified eluate fraction (figure 2, B). Sartobind® S and Phenyl in tandem were used to remove free payload, undesirable DAR species and aggregate in a single run (figure 2, C).

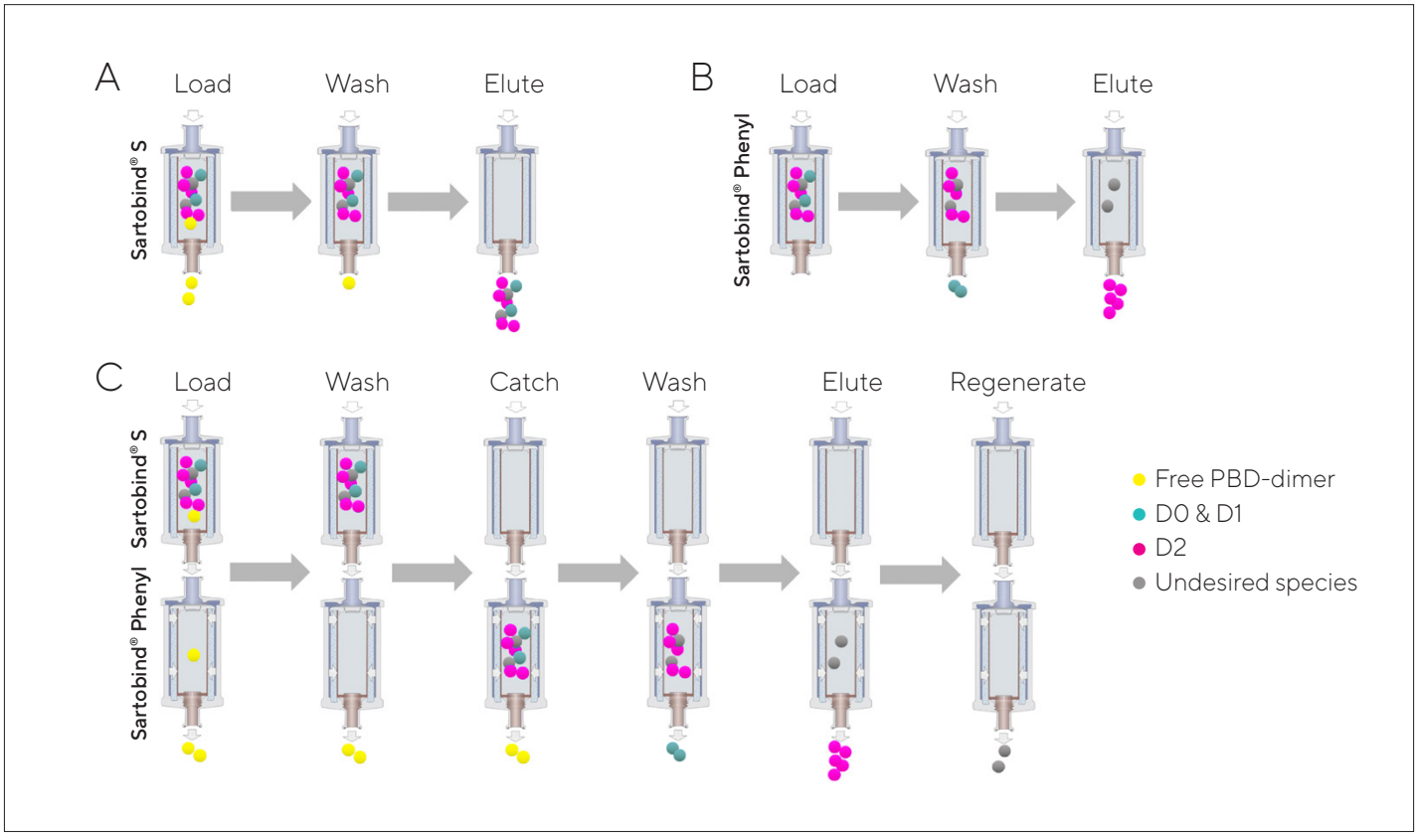

Figure 2: Sartobind® process overview. (A) Bind | Elute using Sartobind® S to remove residual payload, and (B) Bind | Elute using Sartobind® Phenyl to remove aggregate in stand-alone model; (C) Simplified process to purify target ADC with Sartobind® S and Phenyl in tandem model.

Results

1. Sartobind® S stand-alone experiments

a. Dynamic Binding Capacity

For the initial experiments, the purification steps for Sartobind® S and Phenyl were optimized separately. Cation exchange chromatography using Sartobind® S was used to remove free payload from the ADC product. Sartobind® S buffers were chosen for optimal dynamic binding capacity (DBC), yield and removal of free payload.

First, the DBC of Sartobind® S at 10% breakthrough was determined for three different loads: naked engineered cys-mAb (control) and cys-mAb conjugated to two different payloads, cys-mAb-MMAE and cys-mAb-PBD. Naked cys-mAb, cys-mAb-MMAE or cys-mAb-PBD in 50 mM sodium phosphate at 3.5 mg/mL was diluted to 1.0 mg/mL with 15 mM MES, pH 6.0 and loaded onto the membranes equilibrated 10 MV in 20 mM MES buffer pH 6.0 at 1 MV/min.

The breakthrough curves and the DBC values for each load are shown in table 1 and figure 3. The breakthrough curves for each load were visually similar and resulted in similar DBC at 10% breakthrough with values ranging from 32 – 37 mg load/mL membrane volume (mg/mL) (table 1).

Second, the eluate was analyzed for free payload levels. Figure 4 shows the chromatogram from a confirmatory run where 82 mg of cys-mAb-PBD was loaded onto a 3 mL membrane device (~80% of the 10% DBC value), washed to remove free payload and eluted with 15 mM MES, 350 mM NaCl, pH 6.0. A total of 83 mg were recovered for a 101% yield of purified conjugate. The A260 signal is higher than A280 during the load compared to the elution, illustrating free payload was removed prior to the elution.

Load	DBC, 10% Breakthrough
Cys-mAb	32 mg/mL
Cys-mAb-MMAE	37 mg/mL
Cys-mAb-PBD	34 mg/mL

Table 1: Dynamic binding capacity of model ADCs and control to Sartobind® S nano 3 mL

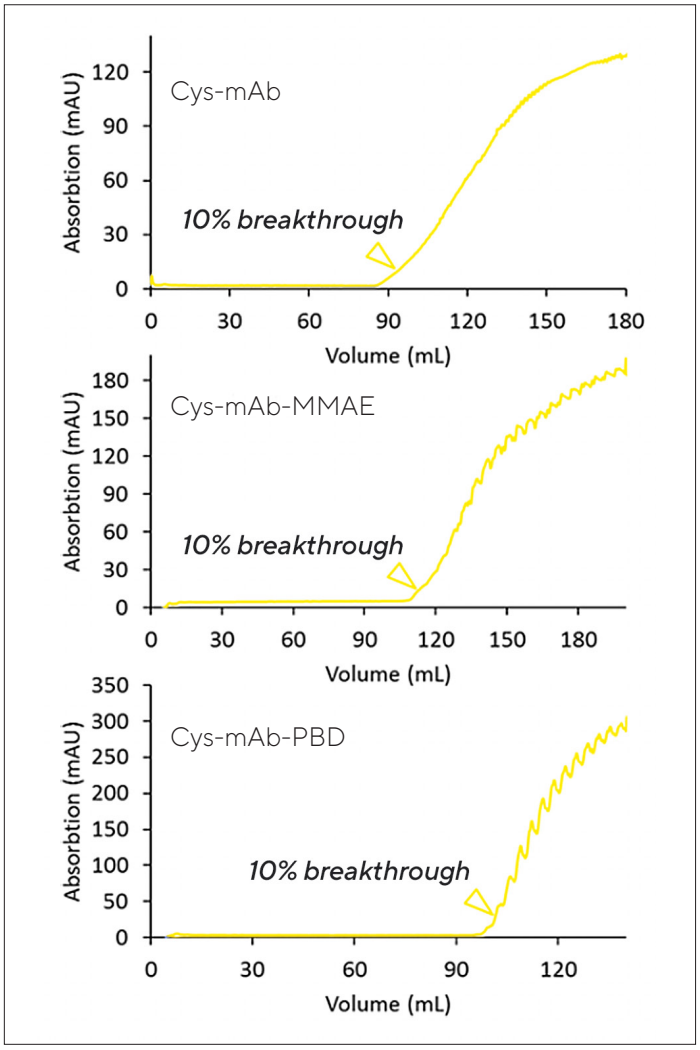

Figure 3: UV chromatograms at A280 for calculation of DBC at 10% breakthrough on Sartobind® S nano 3 mL.

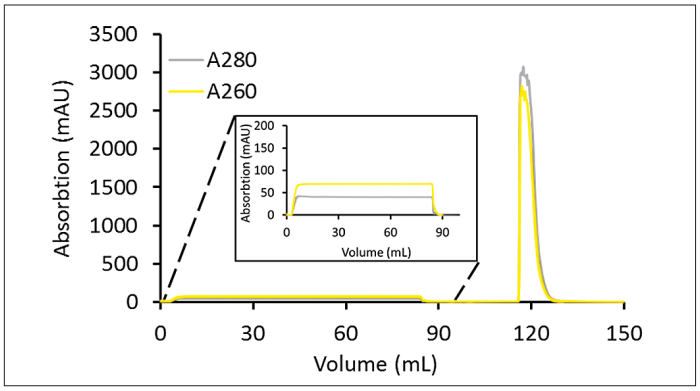

Figure 4: UV chromatogram at A280 for 82 mg load on Sartobind® S nano 3 mL.

b. Scale-up and determination of free payload removal

For the next experiment, the ability to scale-up the Sartobind® S membrane to remove free payload from the conjugate at gram scale was qualitatively visualized by HIC and SEC HPLC and the residue free linker-payload was quantitatively measured by LC-MS. Briefly, 1.7 g of cys-mAb-PBD in 50 mM phosphate buffer was diluted with 15 mM MES buffer pH 6.0 to a concentration of 1 g/L then loaded onto a 150 mL Sartobind® S, 8 mm bed height, equilibrated with 20 mM MES buffer pH 6.0, washed with 20 mM MES buffer pH 6.0 buffer and then eluted from the membrane with 20 mM MES 300 mM sodium chloride buffer pH 6.0 at the flow rate of 1 MV/min. Figure 5 shows the UV chromatogram from the run (figure 5, A). Afterwards the eluate was analyzed by HIC HPLC (figure 5, B), SEC HPLC (figure 5, C) and LC-MS (figure 5, D).

In conclusion, the results of the HPLC chromatograms and LC-MS data show that the Sartobind® S membrane could be scaled up to efficiently remove free payload.

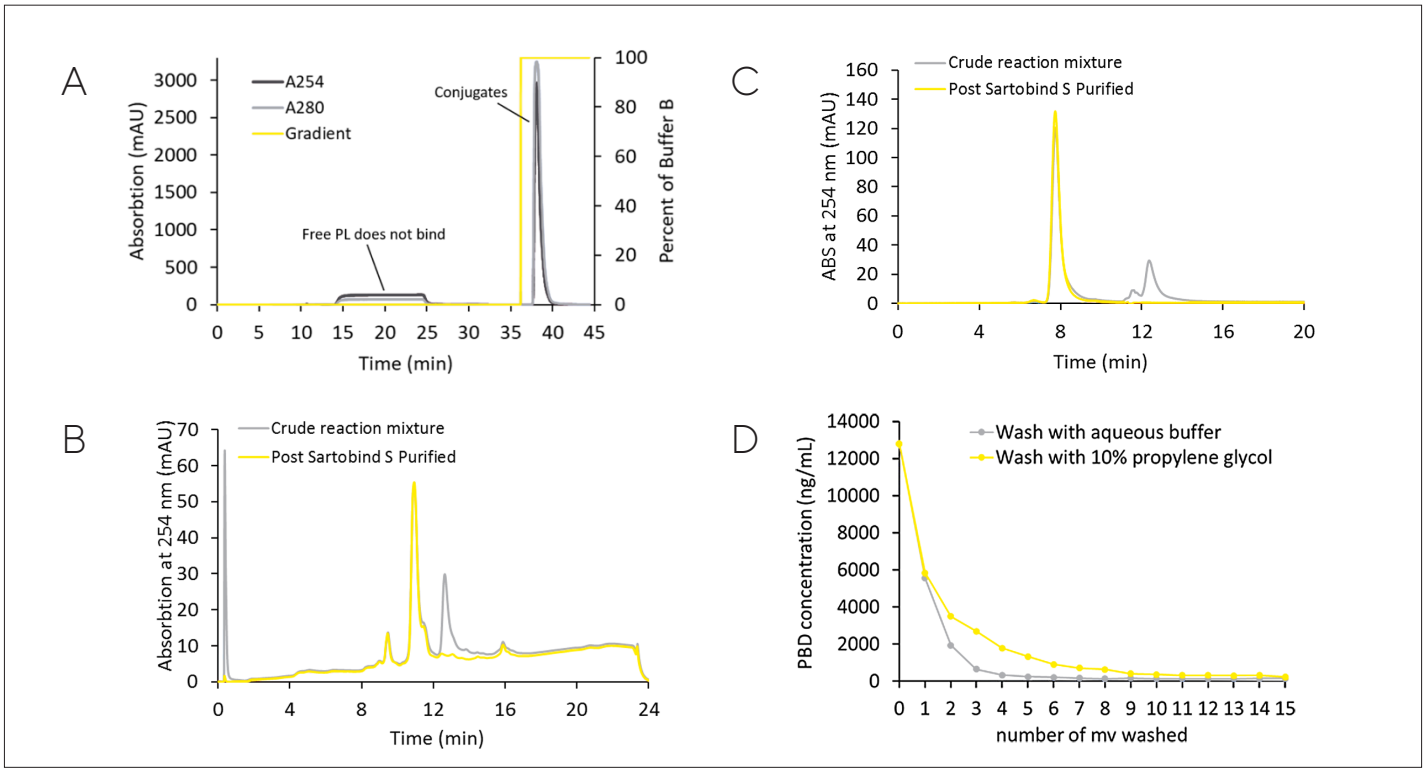

Figure 5: Removal of free payload by Sartobind® S at gram scale and analytical results (A). The quenched reaction mixture (gram scale) was loaded to a Sartobind® S 150 mL, then washed with 10 MV 20 mM MES pH 6.0 buffer prior to elution with 20 mM MES, 300 mM NaCl, pH 6.0 buffer. (B) HIC and (C) SEC profiles showing the removal of free PBD dimer. (D) Quantitative LC-MS data showing PBD dimer removal efficiency with Sartobind® S.

2. Sartobind® Phenyl stand-alone experiments

a. Dynamic Binding Capacity

The hydrophobic interaction based Sartobind® Phenyl membrane was used to refine DAR distribution and remove aggregate. First, the DBC of Phenyl at 10% breakthrough was determined for three different loads: naked cys-mab (control) and cys-mab conjugated to two different payloads, cys-mAb-MMAE and cys-mAb-PBD. Naked cys-mAb, cys-mAb-MMAE or cys-mAb-PBD in 50 mM sodium phosphate at 3.5 mg/mL was diluted to 0.8 mg/mL with HIC A buffer (25 mM sodium phosphate, 1 M ammonium sulfate, pH 7.0) and loaded onto the membranes equilibrated with HIC A buffer at 1 MV/min.

The breakthrough curves and the DBC values for each load are shown in figure 6. The breakthrough curves for each load were visually similar and resulted in similar DBC at 10% breakthrough with values ranging from 13–15 mg load/mL membrane volume (mg/mL) (see table 2 and figure 6).

Load	DBC, 10% Breakthrough
Cys-mAb	13 mg/mL
Cys-mAb-MMAE	14 mg/mL
Cys-mAb-PBD	14.9 mg/mL

Table 2: Dynamic binding capacity of control and model ADCs to Sartobind® Phenyl nano 3 mL

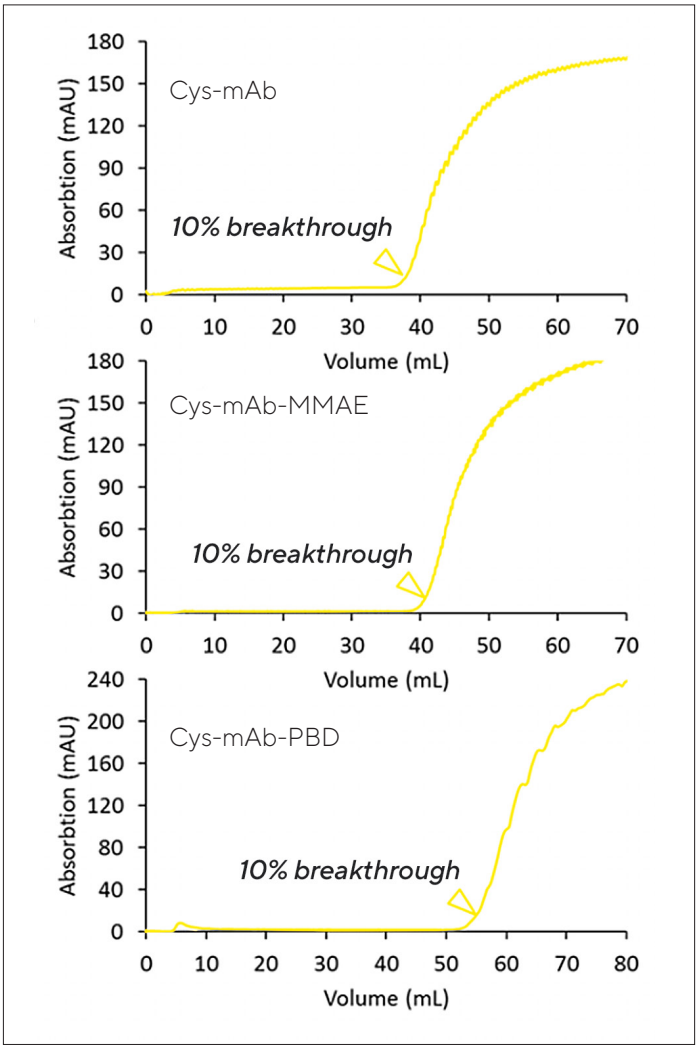

Figure 6: UV chromatograms at A280 for calculation of DBC at 10% breakthrough on Sartobind® Phenyl nano 3 mL.

b. Small scale DAR distribution refinement and aggregate removal

The ability to scale-up the Sartobind® Phenyl membrane and refine DAR distribution | removal of aggregates was assessed at small scale with cys-mAb-PBD. 20 mg of crude reaction mixture was loaded onto a Sartobind® Phenyl nano (3 mL) equilibrated with 25 mM sodium phosphate, 1 M ammonium sulfate, pH 7.0 buffer at 1 MV/min. The DAR of the crude reaction mixture was purposely made to have a high percentage of D0 and D1 species to create a worst case scenario to determine the resolving power of the Sartobind® Phenyl membrane to remove underconjugated species. The DAR of the load was 1.65 with 4.9% aggregate (only D0, D1, and D2 species were counted for DAR calculation; aggregates and higher order DAR species were excluded from DAR calculations). The purification was performed by loading the crude conjugate to the Sartobind® Phenyl membrane at 1 MV/min, washing with equilibration buffer, buffer A (25 mM sodium phosphate, 1 M ammonium sulfate, pH 7.0), washing with 34% buffer B (25 mM sodium phosphate, pH 7.0, with 20% (v/v) isopropyl alcohol), and eluting the target conjugate from the membrane with 70% buffer B, and finally stripping the membrane with 100% buffer B.

After the multi-step elution, the fraction containing the main peak was analyzed by HIC HPLC. The DAR of the conjugate increased from 1.65 to 1.92, denoting significant purification of the desired DAR 2 species and the aggregate decreased to less than 1%. Figure 7 below shows the UV chromatogram from the 20 mg scale run.

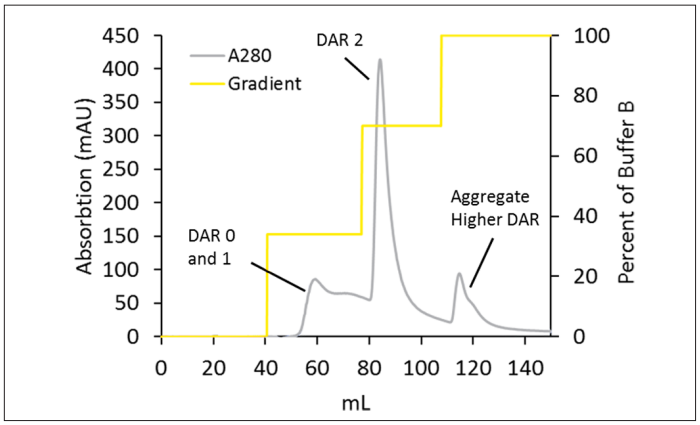

Figure 7: Small scale refinement of DAR distribution and aggregate removal. The crude reaction mixture was loaded to a Sartobind® Phenyl nano (3 mL), washed with 34% buffer B (25 mM sodium phosphate, pH 7.0, 20% (v/v) isopropyl alcohol), eluted using 70% buffer B, and finally stripped with 100% buffer B.

c. Scale-up DAR distribution refinement and aggregate removal

The ability to scale-up the Sartobind® Phenyl membrane and refine DAR distribution | removal of aggregates was assessed at the gram scale with cys-mAb-PBD. The gram scale Sartobind® S purified material (1.7 g) was loaded onto a Sartobind® Phenyl 150 mL equilibrated with 25 mM sodium phosphate, pH 7.0, 1 M ammonium sulfate buffer at 1 MV/min. The DAR of the Sartobind® S purified material (crude HIC load) was 1.82 with 5% higher order DAR species and aggregate (only D0, D1, and D2 species were counted for DAR calculation; aggregates and higher order DAR species were excluded from DAR calculations). The membrane was washed with 25 mM sodium phosphate, 1 M ammonium sulfate, pH 7.0 buffer A, eluted with 75% buffer B (25 mM sodium phosphate, pH 7.0, 20% (v/v) isopropyl alcohol), and stripped with 100% buffer B. After the one-step elution, 1.45 g of final purified ADC with a DAR of 1.82 and less than 1% aggregates was recovered with a yield of 85%.

Figure 8 shows the UV chromatogram from the run (A) and corresponding analytical HIC traces of the load (B), the single step elution (fraction 1) containing the purified ADC (C), and the strip (fraction 2) containing the aggregates and high DAR species (D). In conclusion, the results of the scale-up Sartobind® Phenyl membrane experiments demonstrate its ability to refine drug distribution and remove aggregates with a high recovery of purified ADC.

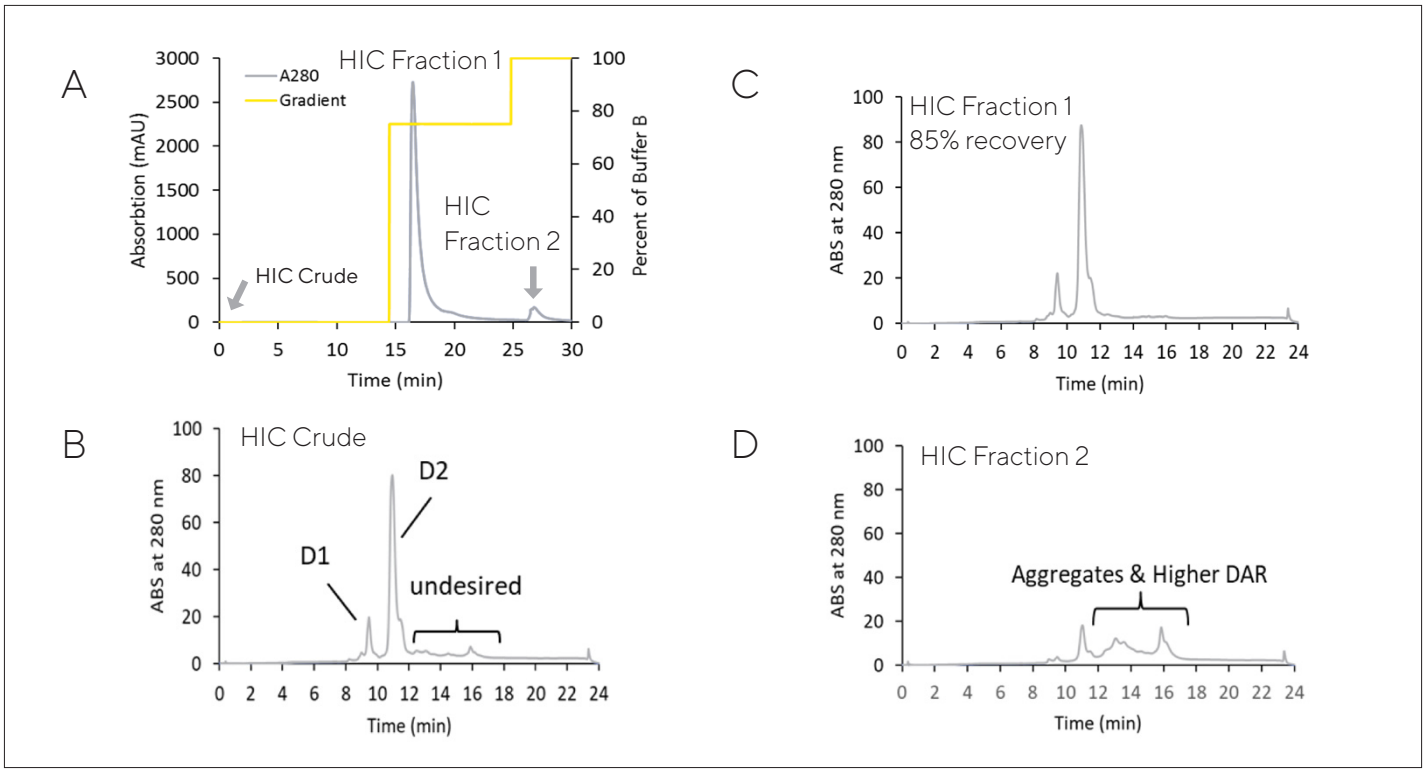

Figure 8: Gram scale refinement of DAR distribution and aggregate removal (A) Sartobind® S purified material (gram scale) was loaded to a Sartobind® Phenyl 150 mL, washed with 5 MV buffer A (25 mM sodium phosphate, 1 M ammonium sulfate, pH 7.0), eluted with 10 MV of 75% buffer B (25 mM sodium phosphate, pH 7.0, 20% (v/v) isopropyl alcohol), and stripped with 100% buffer B. (B) HIC HPLC chromatogram of the load (HIC Crude). (C) HIC HPLC chromatogram of the elution (Fraction 1) and (D) HIC HPLC chromatogram of the strip (Fraction 2).

3. Sartobind® S and Sartobind® Phenyl tandem (2-dimensional) chromatography for removal of free payload, removal of aggregate, and refinement of drug distribution profile

To save processing time and simplify production, the ability to remove free payload, remove aggregate and refine drug distribution profile of a cys-mAb-PBD in a single unit operation was assessed. For these experiments a 3 mL Sartobind® S and a 3 mL Phenyl membrane were used in tandem (series). The DAR of the crude reaction mixture was 1.68 with 5% aggregates. The purification was performed by diluting 30 mg crude reaction mixture with binding buffer (20 mM MES pH 6.0 (1:2)) before loading on the tandem Sartobind® S and Phenyl membranes. During the loading stage, all conjugate species were captured by Sartobind® S membrane, and the free linker-payload related species flowed through the membranes. After loading was complete, the conjugate species bound to the Sartobind® S membrane were then washed with binding buffer followed by elution with buffer A (25 mM sodium phosphate, 1 M ammonium sulfate, pH 7.0), and captured by Sartobind® Phenyl membrane. Finally, the Sartobind® Phenyl captured conjugate species were eluted with a series of isocratic eluent steps. As shown in the figure 9 A, C and D, the peak corresponding to the second isocratic elution was collected as the purified ADC fraction. Figure 9 shows the UV chromatogram from the run (A), and corresponding analytical HIC trace of the load (B), as well as the analytical HIC and SEC of the second isocratic elution corresponding to the purified ADC fraction (C and D). Results showed 16 mg of final purified ADC were purified with a recovery of 80% (D2 species) and a refined DAR of 1.94 containing less than 1% aggregates.

Overall Conclusions

- Membrane based process consumed less buffer, shortened process and hold times (less FTE days of GMP scientist) which reduces the cost and time of a cGMP manufacturing campaign.
- Membrane devices are scalable, single-use, closed systems that improve manufacturing safety, eliminate the need of packing, qualification, and cleaning validation studies associated with resin-based column chromatography.
- Removal of free payload, aggregates and refinement of drug distribution profile of an ADC by membrane chromatography presents a novel and efficient process that directly translates into improved efficiency both during process development and cGMP manufacturing.
  - Sartobind® S efficiently removes the free payload from crude ADC reaction mixture.
  - Sartobind® Phenyl was used successfully to refine DAR and to remove aggregates.
  - Sartobind® S and Phenyl in tandem efficiently removes free payload, refine DAR and remove aggregate in a single unit operation.

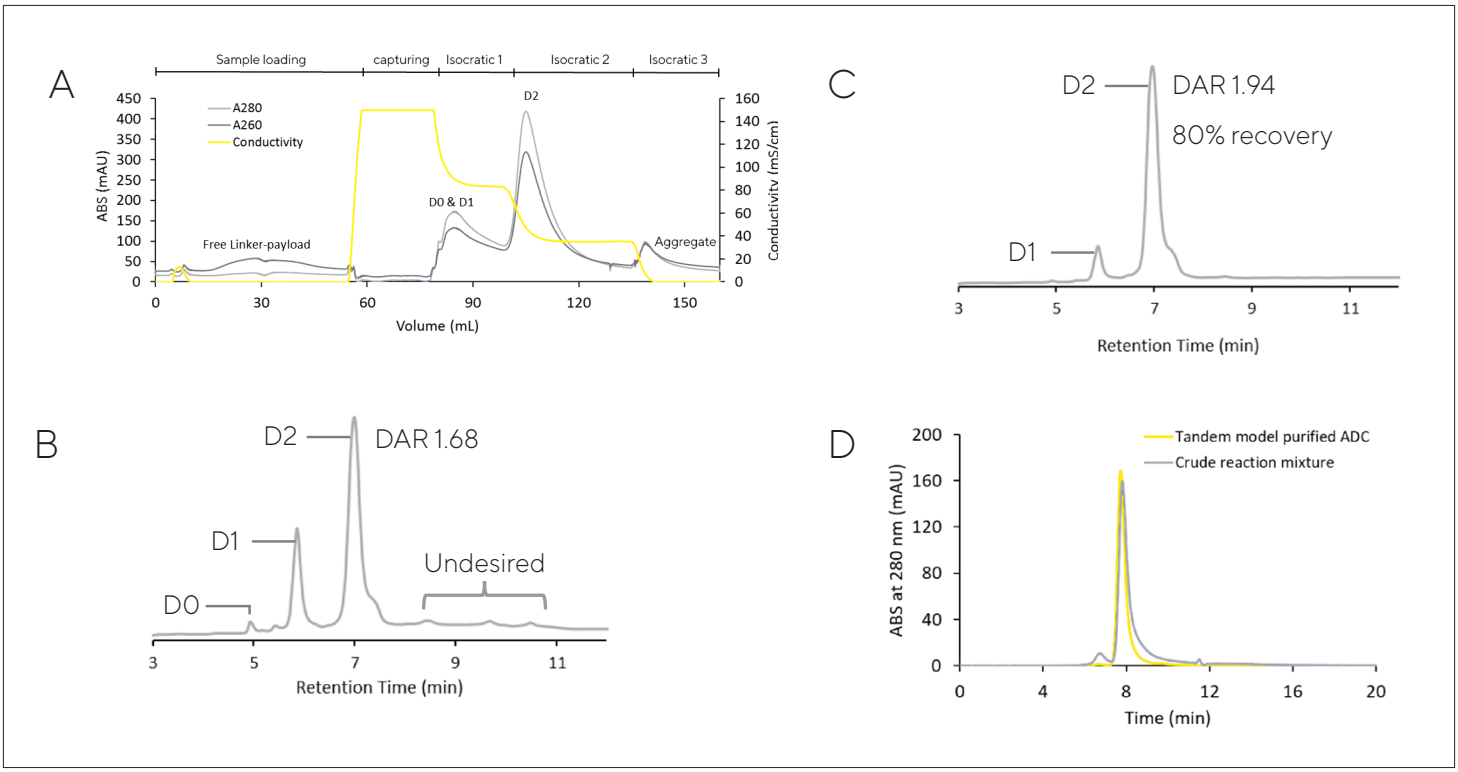

Figure 9: Free payload removal, aggregate removal and DAR distribution refinement in a single unit operation. (A) A total of 30 mg crude conjugate mixture was loaded on a 3 mL Sartobind® S and a 3 mL Phenyl membrane were used in tandem (series). (B) HIC HPLC chromatogram of load. (C) HIC HPLC chromatogram of the second isocratic elution. (D) SEC HPLC chromatogram of the second isocratic elution.

References

(1) Shukla AA, Gottschalk U, Trends in Biotechnology, 2013 31(3), 147-154

(2) Glynn J, Boyle D, Wealand J, Miller-Cary E, Chen B, Mensah P, 2010, BioPharm International, Supplement March 2

(3) McDonagh CF et al, mol can ther, 2008 Sep 7(9): 2913-23

(4) J. Zhou, J. Novak, J. C. Cordova, S. Sun, J. Bos, S. Thirumalairajan, L. N. Paul, S. Ghone, Development of an ADC Process with Single-Use Membrane Technology, Poster, World ADC Conference London, 5 – 6 March 2019

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For further contacts, visit
www.sartorius.com

Status: 07 | 2020