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Sartobind® Rapid A: Proven, Scalable New Membrane Platform for mAb Capture

Mario Gruenberg, Kim B. Kuchemueller, Ricarda A. Busse*
Sartorius Stedim Biotech GmbH, August-Spindler-Strasse 11, 37079 Goettingen

* Correspondence
Email: ricarda.busse@sartorius.com

Abstract

This application note introduces the convecdiff Sartobind® Rapid A membrane, a new type of chromatographic material that combines high protein binding capacity with high productivity while providing scalability. The convecdiff Sartobind® Rapid A membrane devices presented reach a dynamic binding capacity of around 40 g/L at residence times measured in seconds.

The new membrane was tested in three different device sizes featuring different membrane volumes, to demonstrate scalability. The different prototypes ran for a minimum of 40 cycles each. Results show that the performance of Sartobind® Rapid A, as measured by yield, impurity reduction and other factors, is comparable to the performance of established materials; no performance decline occurred during cycling. The material is well-suited for protein bind-and-elute applications ranging from laboratory to production scale with all these characteristics.

Introduction

Monoclonal antibodies (mAbs) are among the most successful biotherapeutics and are known for a wide range of medical applications, including cancer treatment. In terms of mAb-production, protein A based affinity chromatography is the established product capture step in downstream processing. This type of purification relies on the specific affinity interaction between the antibody's Fc-region and an immobilized protein A (ProtA) ligand on the surface of a chromatographic material.

Today, a variety of chromatographic materials are available on the market: resins, membranes, fibers, and monoliths. Most manufacturers choose resins because of their high binding capacities and robustness, and because they can draw upon years of experience working with the material. Unfortunately, resins have a high residence time due to long diffusive pathways, leading to low productivity. By contrast, purely convective materials — e.g., membranes, fiber-beds or monoliths — offer increased productivity as they do not suffer from diffusive mass transport limitations.

To provide the necessary specific surface area for acceptable levels of binding, however, these materials exhibit relatively small convective pores (~0.3 µm), which causes them to have low permeability and high propensity for fouling — issues that limit their usefulness in large-scale commercial production over many cycles.

In this application note, we present performance data of the newly developed Sartobind® Rapid A membrane, described as having convecdiff structure. This membrane combines high antibody binding capacity with high productivity while maintaining high permeability and low fouling propensity. It is fully integrated into the Sartobind® device family, which provides a scalable membrane adsorber portfolio from lab to production scale (1 mL to 100 L, depending on device and bed height), enabling the operator to simply scale up from lab to production [1].

Materials and Methods

Buffers, Reagents and Monoclonal Antibodies

Chemicals used for buffer preparation were purchased from Carl Roth (Karlsruhe, Germany), with buffer constitutions listed in Table 1. Buffers and recipes used in this study are subject of internal platform approach.

Table 1: *Buffers Utilized for the Chromatographic Experiments.*

Buffer	Phase	Ingedients	pH
PBS	(Re-) Equilibration, Wash, HPLC Mobile Phase	1× PBS	7.4 ± 0.2
Elution-buffer	Elution	0.1 M acetic acid, 150 mM NaCl	2.9 ± 0.1
Reg-buffer	Regeneration | Cleaning	0.2 M NaOH	> 12.5

Elution pH mAbs 1

The recombinant human monoclonal antibody was expressed in Chinese hamster ovary (CHO) cells using standard cell culture techniques (stirred bioreactor). The cultivations were done in Sartorius 5 L Biostat® reactors in batch mode for 14 days. Cell clarification was performed in a two-step depth filtration using Sartorius Sartoclear® DL20 and DL60 with subsequent sterile filtration using Sartorius Sartopore® 2 XLG. Table 2 contains the antibody properties used in this work.

Table 2: *Monoclonal Antibody Properties.*

Molecule	Class	pI	MW [kDa]
mAb1	Antibody IgG1	8.36	145.41

Protein A Chromatography Devices

Protein A chromatographic devices used were novel Sartorius Sartobind® Rapid A membrane devices with membrane volumes (MV) of 1.2 mL, 10 mL and 70 mL.

Protein Concentration and Monomer Determination by Size Exclusion HPLC

Protein concentrations and monomer | aggregate levels of HCCF and purified samples were determined by analytical high-performance size exclusion chromatography (SEC-HPLC) using a TSKgel® G3000SWXL-column (30 mm ID × 7.8 cm) from Tosoh (Griesheim, Germany) with an UltiMate™ 3000 HPLC System from Thermo Fisher Scientific (Dreieich, Germany). The HPLC system was operated at 1 mL/min with PBS as mobile phase applying 10 µL of sample. The elution profile was monitored at λ = 280 nm using the system's spectrophotometer. Elution peak area was converted to protein concentration using a standard curve generated with purified material. Aggregate levels were determined as a ratio of peak areas of the early-eluting aggregate peak(s), late-eluting fragment peak(s), and the monomer peak.

Dynamic Binding Capacity Measurements

DBC is defined as maximum amount of target protein that can be loaded onto a stationary phase without causing unnecessary loss, measured under realistic experimental conditions. Dynamic binding capacity (g of mAb per L of membrane) was determined for chromatographic devices using an ÄKTA™ Avant 150.

For the cycling studies, the DBC was determined with HCCF. The device was equilibrated and then loaded with the HCCF containing the mAbs until visible overloading appeared (at 12 seconds residence time). The flowthrough was fractionated in 1 mL portions to identify the volume when the stationary phase is fully saturated and mAb breakthrough occurs. A DBC (total bound mAb) was calculated for the amount of HCCF loaded where no monomer was measurable in the breakthrough. The exact protein concentration of the feed and the breakthrough-fractions were determined with SE-HPLC.

$$(1)\ DBC = \frac{V_{0\%} \times C_0}{V_{membrane}}$$

Where $V_{0\%}$= volume where no mAb can be measured in the flowthrough fraction (L), C0 is the mAb concentration (g/L) and $V_{membrane}$ is the volume of the membrane in the chromatographic devices.

Determination of DBC with HCCF reflects the real process due to physicochemical mAb interactions with impurities, as well as competition and hindering of accessibility to the ligand.

Determination of Productivity

The productivity of the utilized chromatography devices was calculated according to:

$$(2)\ PR = \frac{m_{mAb}}{V_{membrane} \times t_c}$$

where PR [g/L×h] is the productivity, m_{mAb} [g] is the average eluted mass of monoclonal antibody, $V_{membrane}$ [L] is the volume of the resin/membrane in the chromatographic devices and t_c [h] is the average cycle time over the whole process.

Host Cell Protein, hcDNA and Leached Protein A Measurements

Host cell protein (HCP) concentrations were measured using the CHO HCP ELISA Kit3G F550-1 Kit from Cygnus Technologies (Southport, USA). Host cell DNA concentrations have been measured using the Quant-iT PicoGreen dsDNA Assay Kit from Thermo Fisher Scientific (Dreieich, Germany). The log-reduction-value (LRV) of both impurities has been determined by means of the decadic logarithm of the quotient of impurity concentration in the feed and the impurity concentration in the elution fraction. Leached protein A has been quantified using the Protein A ELISA Kit (9000-1) from Repligen (Waltham, USA). The values listed refer to ng protein A per mg mAb. All assays have been performed according to the manufacturer's instructions and analyzed in an Infinite M Nano+ plate reader from Tecan (Maennedorf, Switzerland). Every 10th elution fraction was collected and analyzed regarding different CQAs (critical quality attributes) and CPPs.

Results

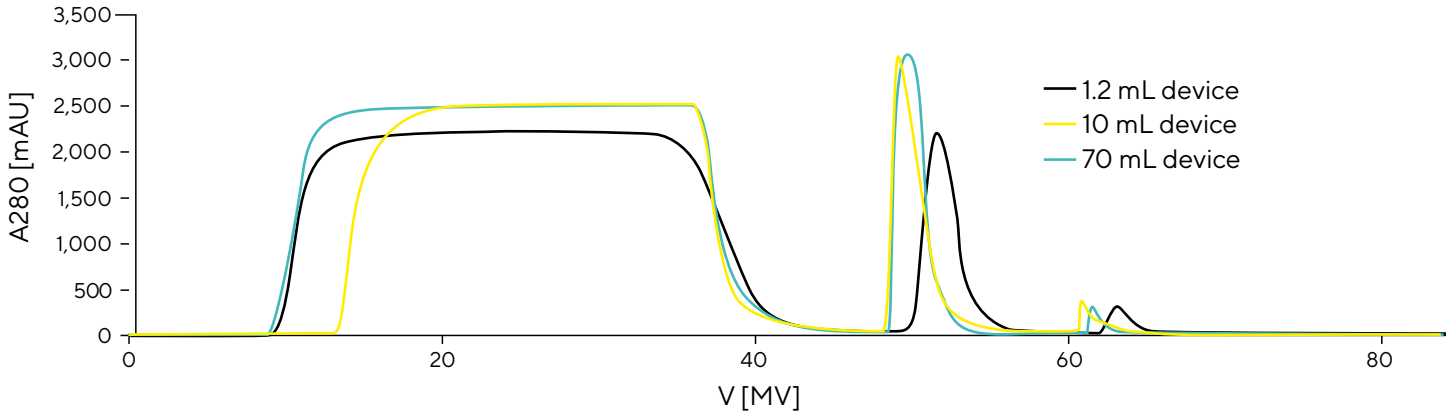
1. Scalability of Convecdiff Membrane Implemented in Different Device Sizes

To demonstrate the scalability of the novel convecdiff Sartobind® Rapid A membrane, the membrane was implemented in standard membrane capsules with a bed height of 4 mm for all scales. Scalability was investigated using three different membrane adsorber sizes with 1.2 mL, 10 mL and 70 mL membrane volumes. The purification protocol from Table 3 was applied, and HCCF containing mAb was implemented onto the membrane. The available HCCF originated from different cultivations and therefore contained slightly different mAb concentrations. The impurity and aggregate levels were in a similar range.

Table 3: *Overview of Process-Related Parameters and Product Quality Attributes.*

Buffer	1.2 mL device	10 mL device	70 mL device
Load [g/L]	25.0	25.0	25.0
Yield [%]	98.0 ± 2.5	98.8 ± 0.3	99.4 ± 1.9
HCP reduction [LRV]	2.2 ± 0.2	-	2.3 ± 0.1
hcDNA reduction [LRV]	2.8 ± 0.2	-	2.8 ± 0.1
Elution volume [MV]	4.4	4.1	5.1
Avg. Productivity [g/L×h]	141.4	142.5	143.4

Figure 1: *Overlays of UV Traces From Exemplarily Bind and Elute Cycles of mAb Implemented Into Three Different Device Sizes.*



As shown in Figure 1, the chromatograms displayed very similar peak shapes. Slight variations resulted from the different HCCF batches. The mAb concentration of the feed for the 10 mL device was slightly higher compared to the other two tested sizes and therefore the load phase was shorter, resulting in a shorter UV curve. The 70 mL adsorber required a pilot scale chromatography skid. In comparison to the benchtop system used for the 1.2 mL and 10 mL device, the larger skid had different void volumes, a less sensitive UV sensor and other signal smoothing factors. As a result, the UV signal for the 70 mL device during load and wash phases, as well as the elution peak, appears slightly later and with a lower intensity. However, this is a common challenge during scale-up.

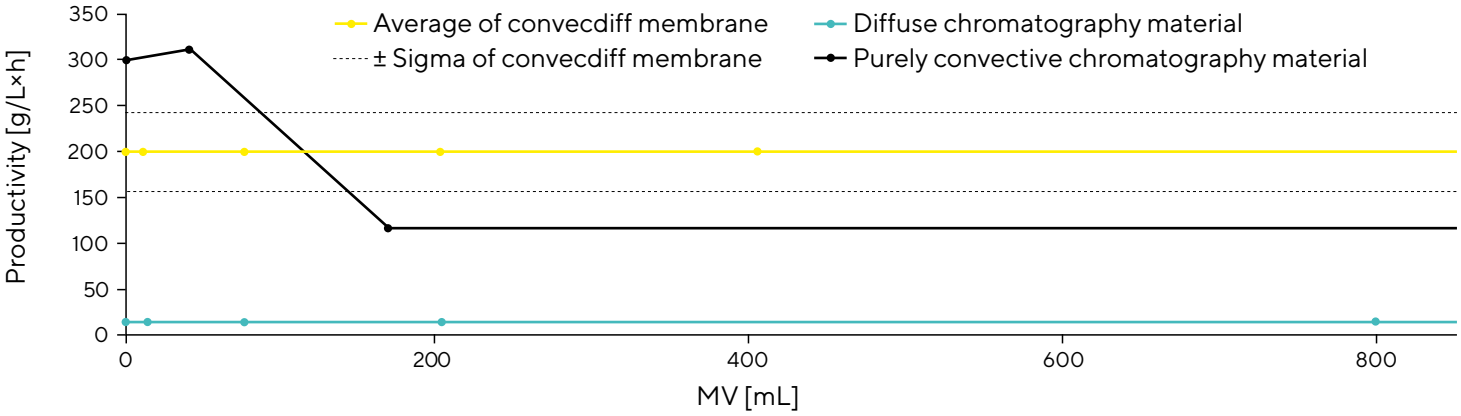
The analyzed CPP and CQA values, as summarized in Table 3, show very high comparability. Elution concentrations of the mAbs range from 4.1 to 5.0 g/L, elution volumes from 4.4 to 5.1 MV and yields from 98.0 to 99.4%. This data supports high scalability of the membrane when implemented into ready-to-use devices at a fixed bed height of 4 mm.

2. Productivity During Scale-up

Scalability of productivity is important during scale-up, as optimization of productivity at small scale is only valuable if kept constant over different process scales.

Figure 2 demonstrates that productivity can be influenced during scale-up. Only diffusion-limited chromatography materials and the novel convecdiff Sartobind® Rapid A membrane showed a constant productivity during scale-up. The purely convective protein A fiber material showed a decrease of productivity over different device scales (data analyzed from [2]). This lack of scalability might result from a small pore size, which requires adaptation of the bed height to accommodate increasing pressure drops when MV and device size are increased.

Figure 2: *Productivity as a Function of Membrane | Column Volume for Different Categories of Chromatographic Materials.*



Note. For the Convecdiff Membrane, an Average Value From This Study Is Shown, as Well as the Range of the Obtained Productivities.

Discussion

The data in this application note proves the scalability of the novel convecdiff Sartobind® Rapid A membrane implemented into different ready-to-use device sizes. Results consider both CQAs and CPPs with respect to 1.2 mL, 10 mL and 70 mL membrane volumes (and 200 mL MV, data not shown). The range of sizes supports process development in small scale, as well as its scale-up or process validation (scale-down).

In addition, we demonstrated a constant productivity over different device sizes at a fixed bed height, which enables easy scalability.

Conclusion

Due to its characteristics and robustness, the convecdiff Sartobind® Rapid A membrane readily supports rapid cycling chromatography. This technique purifies large amounts of target components by a series of successive bind-and-elute cycles. Rapid cycling leads to high process productivity and makes it possible to utilize the lifetime capacity of the membrane adsorber during the purification of one batch. Complete utilization during one batch is key to overcoming a multitude of drawbacks associated with the current state-of-the-art industry practice — packed bed chromatography. First, it eliminates the need for bulk resin purchases to accommodate protein A resin underutilization, which means clinical material can be produced at a lower cost. Second, it reduces non-productive purification activities (e.g., column packing, cleaning validation of columns, storage) to a minimum. Third, and most important, rapid cycling mitigates bioburden risks associated with packed bed column chromatography because it eliminates column storage between batches and reuse of the packed bed over multiple batches.

References

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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 more information, visit
www.sartorius.com

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