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Application Note

June 28, 2022

Keywords or phrases
Protein A, affinity chromatography, mAb capture, monoclonal antibodies, bioprocessing, process intensification, productivity, dynamic binding capacity, residence time, rapid cycling chromatography

Sartobind® Rapid A: High Binding Capacities at Short Residence Times

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Abstract

Sartobind® Rapid A, operated in Rapid Cycling Chromatography mode, provides high binding capacity at residence times of seconds. This leads to high productivity, making Sartobind® Rapid A a suitable tool to resolve the bottleneck in downstream processing, namely the mAb capture step.

Introduction

Membrane chromatography is a well-established technology in bioprocessing. It is routinely used in the capture of large particles, such as viruses and viral vectors, as well as in polishing steps for the removal of DNA, HCPs, and virus. One hallmark of membrane chromatography is high productivity. Because of this, the technology is currently attracting renewed attention to address possible bottlenecks in intensified processes.

In the past, process intensification efforts focused mainly on the upstream side, which put increased pressure on downstream processing (DSP). As a result, DSP scientists paid particular attention to the monoclonal antibody (mAb) capture step, one of the most expensive unit operations due to the need for specific and effective protein A resins, which result in > 95% of purity of the mAb. However, utilization of these resins requires slow flow rates, resulting in low productivity.

Limitations in productivity frequently lead to mAb capture by protein A resins to be described as a bottleneck in downstream processing. Recent developments, such as simulated moving bed or multi-column chromatography, drive the industry toward complex solutions with enhanced risks but only limited productivity gains.

Rapid Cycling Chromatography (RCC), in combination with Sartobind® Rapid A, will be a game-changer in downstream processing. With > 10-fold higher productivity, it's possible to choose between faster processing and membrane write-off per batch. RCC eliminates bioburden risk caused by resin reuse and opens the way for truly disposable, column-free chromatography.

This study investigates the performance of Sartobind® Rapid A and its newly developed base membrane, which combines convective and diffusive mass transport. The first part of the study examines the dynamic binding capacity dependent on residence time, then it measures dynamic binding capacity (DBC) of various Fc-containing molecules that bind to protein A. Starting from this data, we calculate cycle time and productivity. Further, we describe how feed concentration and residence time impact productivity.

This data shows the potential of Sartobind® Rapid A to solve the bottleneck in DSP by increasing productivity more than 10-fold compared to state-of-the-art resins.

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.

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

Buffer	Phase	Ingredients	pH
PBS	(Re-)Equilibration, Wash	1 × PBS	7.4 ± 0.2
Elution-buffer	Elution	0.1 M acetic acid, 150 mM NaCl	3.5
Reg-buffer	Regeneration | Cleaning	0.2 M NaOH	> 12.5

Protein A Chromatography Devices

Protein A chromatographic devices used were novel Sartobind® Rapid A devices with a membrane volume (MV) of 1.2 mL.

Dynamic Binding Capacity (DBC) 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. DBC (g of mAb per L of membrane) was determined for chromatographic devices (see previous chapter: Protein A Chromatography Devices) using an ÄKTA™ Avant 150 chromatography system from Cytiva (Uppsala, Sweden).

$DBC_{10\%}$, is defined as the amount of protein loaded at 10% breakthrough (g of mAb per L of membrane). Purified protein load was adjusted to pH 7.0 ± 0.2. The device was equilibrated and then loaded with protein (feed concentration: C_{Feed} ~1.0 g/L) until the stationary phase was saturated. The exact protein concentration of the feed was determined by offline A280 measurement using the Unchained Labs Little Lunatic (Pleasanton, USA).

$$(1)\ DBC_{10\%} = \frac{V_{10\%} - V_0 \times C_0}{V_{membrane}}$$

Where $V_{10\%}$ is the volume at which 10% breakthrough was observed, V_0 = system void volume (L), C_0 is the mAb concentration (g/L) and $V_{membrane}$ is the volume of the membrane in the chromatographic devices. The breakthrough was determined at a residence time of 12 seconds for Sartobind® Rapid A.

Determination of Productivity

The productivity of the utilized chromatography devices for the purification of the different mAbs 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 mAb, $V_{membrane}$ [L] is the volume of the membrane in the chromatographic devices and t_c [h] is the average cycle time over the whole process.

Protein A Capture Chromatography From Harvested Cell Culture Fluid

Capture of monoclonal antibodies from HCCF was conducted with Sartobind® Rapid A with different mAbs using an ÄKTA™ Avant 150 chromatography system. Chromatography was performed with buffers and chromatography recipes mentioned in Table 1 and Table 2. Buffers and recipes used for data shown in Figure 2 can deviate.

Table 2: *Chromatography Recipes for mAb Capture With Sartobind® Rapid A.*

	Sartobind® Rapid A	
Phase	**V [MV]**	**Flowrate [MV/min]**
Equilibration	5	10
Load [g/L]	34.4	5
Wash	12	10
Elution	12[1]	5
Regeneration	9 – 10[2]	5
Re-Equilibration	15 – 16[3]	10
Avg. Cycle Time [min]	–	9.6

[1] Fractionation of elution peak from 100 - 100 mAU at λ= 280 nm
[2] Hold until pH ≥ 12.3, then additional 4 MV
[3] Hold until pH ≤ 7.5, then cycle ends

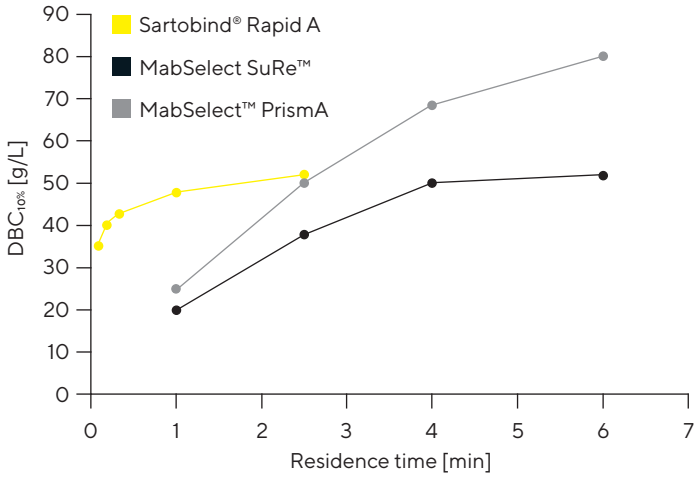
Results

1. $DBC_{10\%}$ in dependence of residence time compared to standard resins

First, the DBC at 10% breakthrough was investigated for Sartobind® Rapid A and its dependence on the different flow rates, which results in different residence time for the mAb to bind to protein A ligand. Residence times were varied from 12 seconds to 2 minutes by applying different flow rates (see Figure 1). This was compared with $DBC_{10\%}$ data for two state-of-the-art protein A resins [1, 2]. As shown in Figure 1, Sartobind® Rapid A shows an increase in $DBC_{10\%}$ when residence time is increased, similar to diffusion-limited resins. A $DBC_{10\%}$ of 50.1 g/L at 2 min residence time and 35.2 g/L at 0.1 min residence time respectively was measured. At about 1–2 min residence time, the plateau of max. $DBC_{10\%}$ is achieved, while resins reach the plateau around 4–6 min of residence time. This highlights that high binding capacity can be achieved with much less residence time due to the huge convective pores of Sartobind® Rapid A.

Sartobind® Rapid A, like protein A resins, also has diffusive regions and therefore exhibits a residence-time-dependent binding capacity. This is caused by a mass transport based on diffusion, a slow process. Due to the unique nature of the new membrane platform used in Sartobind® Rapid A, the $DBC_{10\%}$-residence time curve is shifted left compared to protein A resins.

Figure 1: *$DBC_{10\%}$ As a Function of Residence Time for Two Different Commercially Available Protein A Resins and Sartobind® Rapid A.*



Note. Due to the unique nature of the new membrane platform used in Sartobind® Rapid A, the DBC10%-residence time curve is shifted left compared to protein A resins. Resin data was adapted from vendor data [1, 2].

2. Overview of $DBC_{10\%}$ at 12 sec residence time for different mAbs

Next, a variety of antibodies were tested for their DBC at 10% breakthrough (see Figure 2). Here, different IgG subclasses (IgG1, 2 and 4), as well as IgE and Fc fusion molecules, were tested. The different $DBC_{10\%}$ values range between 30 g/L and 58 g/L (see Table 3). This distribution of $DBC_{10\%}$ for different molecules can also be observed with resins. An average of 43.6 g/L of $DBC_{10\%}$ at 12 sec residence time (5 MV/min) has been detected. These $DBC_{10\%}$ values are comparable to standard resins but are achieved within a residence time of 12 sec (0.2 min), while resins achieve those between 4–6 min residence time, as shown in Figure 1.

Figure 2: *DBC at 10% Breakthrough for Different mAbs at 12 sec Residence Time*

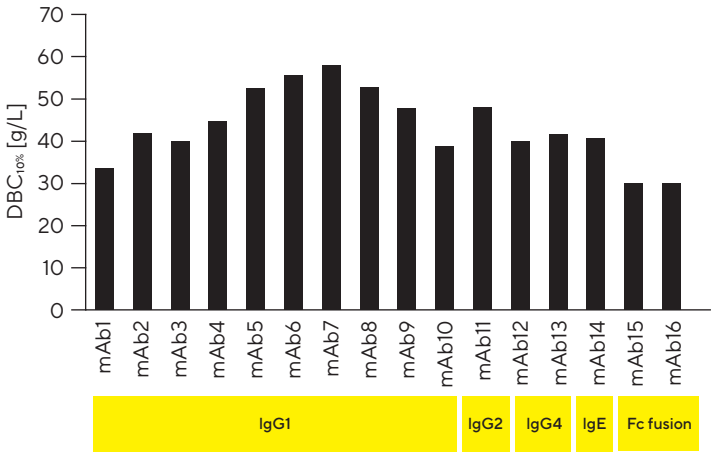


Table 3: *Overview on the $DBC_{10\%}$ Performance of Sartobind® Rapid A*

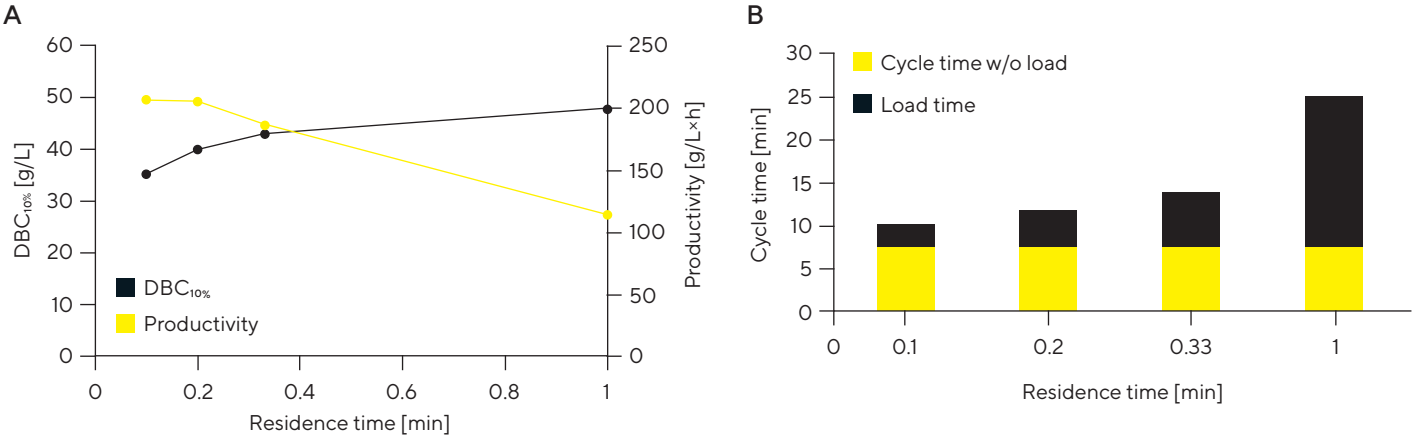
Sartobind® Rapid A	$DBC_{10\%}$ at 12 sec RT
Average $DBC_{10\%}$	43.6 g/L
Min. $DBC_{10\%}$	30 g/L
Max. $DBC_{10\%}$	58 g/L

3. Influence of Residence Time on Productivity and Overall Cycle Time

Based on the determined $DBC_{10\%}$ at different residence time for Sartobind® Rapid A from Figure 1, the corresponding productivity was calculated. Figure 3A shows the influence of residence time changes on productivity. Obviously, short residence time results in the highest productivity, while the productivity decreases at longer residence time.

The increase in residence time results in longer overall cycle times, as shown in Figure 3B. This means that fewer cycles can be performed in a certain time, which reduces the productivity. However, as illustrated in the next section, this does not automatically mean that the shortest cycle times should be favored.

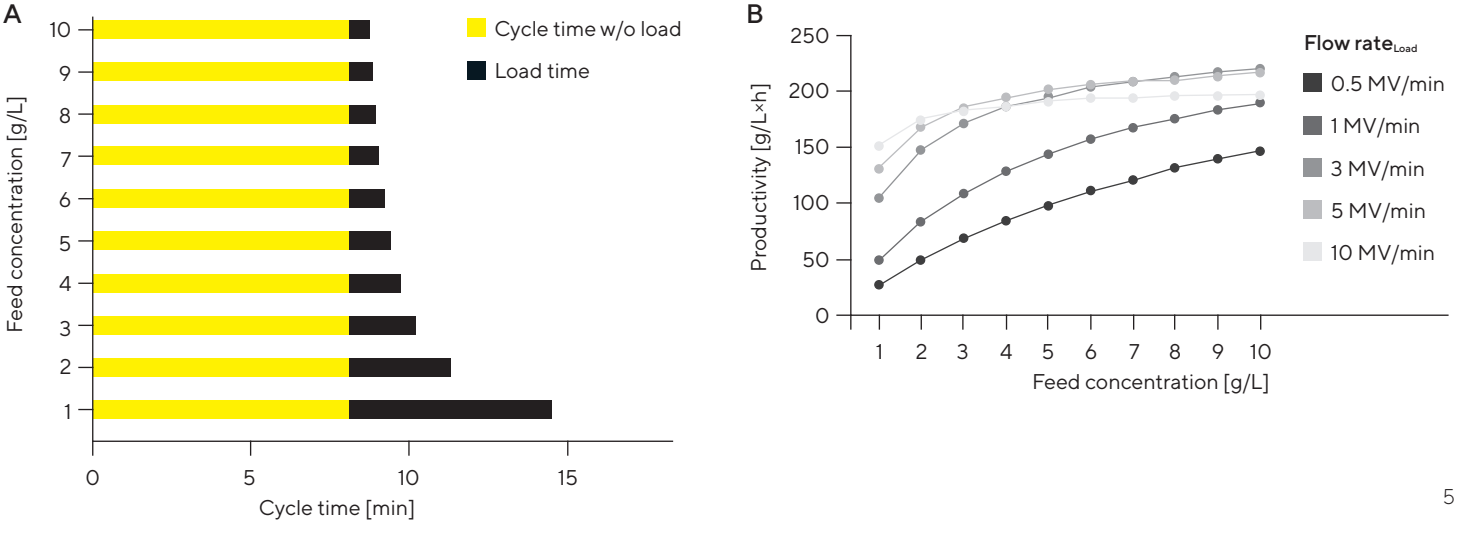
Figure 3: *(A) $DBC_{10\%}$ As a Function of Residence Time and Corresponding Productivity for Sartobind® Rapid A. (B) Influence of Residence Time During the Load Phase on Overall Cycle Time.*



4. Influence of Feed Titer on Productivity and Overall Cycle Time

Another factor influencing productivity is the feed titer. As shown in Figure 4A, cycle time decreases when feed concentrations increase. This is caused by shorter load phases for higher titers compared to lower titers, as $DBC_{10\%}$ is reached faster. To determine the optimal productivity, both titer and residence time have to be considered, as both factors influence cycle time. Based on the relationship between residence time and $DBC_{10\%}$, the productivity was calculated as a function of the flow rate as well as product titer (see Figure 4B). The product mass, and therefore the feed volume required to reach the $DBC_{10\%}$, was calculated according to the standard recipe recommended for Sartobind® Rapid A, including a regeneration step after each cycle.

Figure 4: *(A) Cycle Time Relative to Feed Concentration. (B) Productivity as a Function of Feed Concentration at Different Flow Rates During the Load Phase.*



Discussion

Figure 4B shows that for low titer feed streams (< 3 g/L) a flow rate of 10 MV/min is recommended to optimize productivity. For a feed concentration of ≥ 3 g/L, the flow rate should be decreased from 10 MV/min to 5 MV/min to maximize productivity. A further reduction of the flow rate to 3 MV/min is recommended for feed concentration of ≥ 8 g/L. Due to a reduction of flow rate, the $DBC_{10\%}$ is increased, which has a higher effect on productivity as the load phase for high feed concentrations is very short (see Figure 4A). This indicates that if high feed concentrations are available, the focus should be on optimizing the load time to better utilize the binding capacity of the membrane.

Taken together, highest $DBC_{10\%}$ are obtained at high residence times and low flow rates, but this combination does not automatically lead to the highest productivity, as shown in Figure 5. The highest productivity with 219.4 g/L×h for a feed concentration of 10 g/L was achieved at 3 MV/min (see Table 4). In general, with decreasing titer, the optimum was achieved by increasing flow rate. The findings are summarized in Table 4.

Figure 5: *Productivity as a Function of Product Titer and Flow Rate During Load for Sartobind® Rapid A. Data Points Are Based on Internal Investigations and Calculations.*

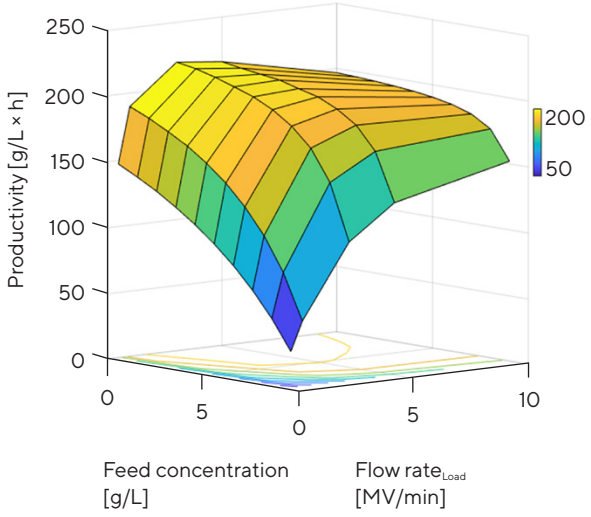


Table 4: *Highest Productivity Achieved Relative to Titer, Flow Rate During Load, and* ***DBC$_{10\%}$***

Productivity [g/L_{MV}×h]		
Titer = 1 g/L [Load= 10 MV/min, $DBC_{10\%}$ = 35.2 g/L]	**Titer = 5 g/L [Load= 5 MV/min, $DBC_{10\%}$ = 40.1 g/L]**	**Titer= 10 g/L [Load= 3 MV/min, $DBC_{10\%}$ = 43.1 g/L]**
152.1	201.5	219.4

Sartobind® Rapid A utilizes the newly developed convecdiff membrane platform, which combines high binding capacities typical for resins (diffusion-limited mass transport) with high flow rates of purely convective membranes. This leads to the advantage of achieving high dynamic binding capacities at much shorter residence times compared to resins. The new convecdiff membrane can achieve $DBC_{10\%}$ values above 40 g/L within 12 seconds versus state-of-the-art resins, which typically achieve similar $DBC_{10\%}$ values at 4 – 6 min residence time. The $DBC_{10\%}$ is dependent on the target molecule and ranges between 30 g/L and 58 g/L at 12 sec residence time. The average $DBC_{10\%}$ for Sartobind® Rapid A is 43 g/L. A range of $DBC_{10\%}$ values is also observed for resins, as binding capacity depends on the availability of binding sites, size of the target molecule and other factors.

The dependency of $DBC_{10\%}$ to residence time also affects productivity. However, this provides an opportunity to optimize the achievable productivity using Sartobind® Rapid A.

Several factors influence productivity: cycle time, feed concentration, and DBC. Providing that the novel convecdiff membrane used for Sartobind® Rapid A also shows a dependency of DBC on residence time, it follows that determining the best combination of those three influencing factors can maximize productivity. A modulation of productivity for the convecdiff membrane revealed that low feed titers exhibit the highest productivities at high flow rates as cycle time had the strongest influence on productivity. For feed titers larger than 3 g/L flow rates in the range of 5 MV/min revealed the highest productivities. High feed titers (beyond 8 g/L) cause a very short load phase; therefore a reduction of flow rate by improving $DBC_{10\%}$ at the same time is preferable to maximize productivity.

We also demonstrated that reducing the regeneration frequency would support further productivity increase (data not shown). However, a reduction of regeneration frequency is highly dependent on the feed conditions and contaminant levels. This impacts the membrane's fouling and might reduce its lifetime. The first indication of membrane fouling is an increase in pressure. The large convective pores in the membrane and the material's low propensity for unspecific binding allow for a reduction of the regeneration regime in specific cases e.g., HCCFs with low contaminant levels.

Conclusion

Optimizing productivity for each feed is recommended to best utilize the convecdiff membrane when implemented into processes. With high productivity, best utilization of membrane capacity and time is achieved.

In summary, this novel convecdiff membrane technology will solve the bottleneck occurring at the capture step in downstream processes of monoclonal antibodies (mAbs). Provided in ready-to-use devices, it also reduces hands-on time preparing packed bed columns, and the short cycle times enable lifetime utilization of the membrane within one batch.

Due to its characteristics and robustness, Sartobind® Rapid A provides a solution for rapid cycling chromatography (RCC). This approach purifies large amounts of target components by a series of successive bind-and-elute cycles. With RCC, the membrane adsorber delivers high process productivity and lifetime capacity utilization during the purification of one batch. Lifetime capacity utilization during one batch is key to overcoming many drawbacks associated with the current state-of-the-art industry practice, namely packed bed chromatography. First, rapid cycling reduces issues with underutilization of protein A resin, decreasing bulk resin purchases and allowing for production of clinical material at lower cost. Second, the use of Sartobind® Rapid A significantly reduces non-productive activities related to the actual purification process (e.g., column packing, cleaning validation of columns, storage, etc.). Finally, and perhaps most importantly, Sartobind® Rapid A minimizes bioburden risks associated with packed bed column chromatography because it does not require column storage between batches or reuse of the packed bed over multiple batches.

References

[1] Cytiva, data sheet CY553-17Sep20-DF “MabSelect™ PrismA” 2020
[2] Cytiva, data sheet CY7145-08Apr20-DF “HiTrap™ Fibro PrismA and HiScreen™ Fibro PrismA units” 2020

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Status 06 | 24 | 2022