

Purification of Antibodies Using Novel *Convecdiff* Membranes Part 2: Robust Performance at Comparable Product Quality

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ABSTRACT

Convecdiff membranes, a new type of chromatographic material that combines high protein-binding capacity with high productivity while providing scalability, are introduced. The *convecdiff* membrane prototypes presented in this study reach a dynamic binding capacity of around 40 g/L at residence times in the seconds range while pressure drop is below 2.5 bar at a bed height of several millimeters. The robustness of the new material is proven in two cycling studies with up to 200 cycles. The material performance regarding yield, impurity reduction, etc., observed during cycling is comparable to the performance of established materials, and no performance decline occurred during cycling. Viral clearance by the new membrane was found on par with existing materials. With all these characteristics, the material is well suited for protein bind-and-elute applications ranging from laboratory to production scale.

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In part I of this article (1), the novel *convecdiff* Protein A membrane and its characteristics were introduced. The performance of the material was compared to commercially available chromatography materials, such as resins and fibers, demonstrating that the new membrane is capable to provide high binding capacity at residence times of seconds with significantly low back pressure. Early prototypes have been tested in a first cycling study, showing robust performance of the *convecdiff* Protein A (ProtA) membrane over 200 cycles resulting in consistent critical quality attributes.

A next generation of the *convecdiff* membrane was tested at AGC Biologics. AGC is a global contract and manufacturing organization, providing development and manufacturing services of therapeutic proteins.

CONVECDIFF MEMBRANE PERFORMANCE: 100 CYCLES

This second cycling study was performed over the course of 100 cycles using clarified harvest of a recombinant monoclonal antibody (mAb) produced at AGC. The feed solution was sterile filtered prior to the cycle study, relieving the need for an inline feed filtration strategy. The *convecdiff* membrane prototype device used in the cycling study had a membrane volume of 1 mL and a bed height of 4 mm. It was operated using a chromatography system (Äkta Avant 25, Cytiva) with elution fractions collected by the system's fraction collector (threshold 50 mAU). The applied protocol for cycling is specified in **Table I**.

The protocol contains equilibration, loading, wash, intermediate wash, and elution. Equilibration and wash buffer were phosphate-buffered saline (PBS) solutions. An

intermediate wash step with high salt was implemented, which is a standard procedure during production of mAbs to dissolve non-specific ionic interactions of contaminants with ProtA matrix or with mAb. The elution was initiated by pH reduction using acetic acid buffer. In total, one cycle without clean-in-place (CIP) lasted 9.6 minutes. CIP was done after every 10 cycles using 0.5 M sodium hydroxide (NaOH) in an upflow direction to ensure that possible fouling substances were flushed out, rather than pushed through the device. Until cycle 50, CIP was performed with a volume of 15 MV and a residence time of 20 s (3 MV/min). For all later CIP cycles, the contact time was increased by tripling the volume of NaOH. By this adaption of the protocol, fouling, which is more likely in the later cycles, is avoided.

Before cycling, the dynamic binding capacity (DBC) of the *convecdiff* membrane prototype device was determined by using purified mAb to determine a suitable load volume. To run cycling without loss of product, the load volume was defined as 60% of the previously determined DBC10%. This results in a load volume of 8 MV for the given feed concentration, which was 1.9 L. As mentioned in the previous section, this load volume may be increased by the operator as the membrane's capability is not limited to the given value.

The resulting signals of ultraviolet (UV) and delta pressure of every 10th cycle of the cycling test are shown in **Figure 1**. Curve lines are shaded from first cycle (light grey) to cycle 100 (black).

The overlay of the UV signals shows constant shapes of the breakthrough curve and elution peaks, proving the robustness of the membrane-based purification process over the course of the 100 cycles. The elution peaks have a width of 7.5 mL, which corresponds to 7.5 MV. This peak width is larger than that observed in the first internal cycling study (see Figure 4 in part 1 of this article) (1) due to the lower threshold value

Table 1. Purification protocol applied for cycling study with *convecdiff* membrane at AGC Biologics. PBS is phosphate buffered saline. HAc is hydrogen acetate (acetic acid). NaCl is sodium chloride. CIP is clean in place. NaOH is sodium hydroxide.

Step	Fluid/buffer	Volume [MV]	Flow [MV/min]	Time of process step [min]
Equilibration	1 x PBS pH 7.4, 16 mS/cm	10	10	1
Load	Clarified harvest with concentration of 1.9 g/L	8	5	1.6
Wash	1 x PBS pH 7.4, 16 mS/cm	10	10	1
Intermediate salt Wash	1 x PBS pH 7.4, 1M NaCl	10	10	1
Wash	1 x PBS pH 7.4, 16 mS/cm	10	10	1
Elution	0.1 M HAc pH 2.9 with 150 mM NaCl, 16 mS/cm peak fractionation with threshold 50 mAU	15	5	3
Reequilibration after elution	1 x PBS pH 7.4, 16 mS/cm	10	10	1
CIP (after every 10 cycles, upflow)	0.5 M NaOH	15 (45 after cycle 60)	3	5 (15 after cycle 60)
Wash after CIP (upflow)	1 x PBS pH 7.4, 16 mS/cm	25	10	2.5

(50 mAU instead of 100 mAU). By increasing this threshold, the peak width would decrease and would approach the peak width observed in the first cycling study. In terms of pressure drop, a gradual increase during cycling is observable. To counteract this trend, the CIP procedure was adapted from cycle 60 onwards (compare **Table 1**). **Figure 2** shows the pressure drop during cycling in more detail. Shown are the pressure drops during the load and intermediate salt wash of each cycle during the cycling study.

The pressure drop increases notably until cycle 60. From this cycle onwards, the CIP procedure was altered by tripling the CIP volume and time while maintaining its frequency (every 10 cycles). Obviously, this recipe modification was a suitable method to stop the increase of the pressure drop and even reverse the previous trend. After 100 cycles, the pressure drop stabilizes in a range from 0.1 to 0.2 MPa, depending on the flow rate of the respective process step (intermediate salt wash 10 MV/min, load 5.0 MV/min).

PRODUCT QUALITY ATTRIBUTES: 100 CYCLES

During 100 cycles, elution fractions were collected, with a peak collection threshold of 50 mAU, and analyzed to determine antibody concentration, yield, and contaminant content. Yield and concentration were determined using the load and elution volumes tracked by the ÄKTA and the concentrations of feed and eluate fractions. The eluate concentrations were measured with a NanoDrop One (Thermo Fisher Scientific), whereas the feed concentration was measured with an Octet RED 96e (FortéBio) using Protein A Dip and Read Biosensors (part number 18-5010 from FortéBio). **Figure 3** shows the determined yields and concentration during the cycling study.

The elution peaks have an average volume of 7.5 mL (at 50 mAU threshold) and an average concentration of 2.25 mg/mL over the course of 100 cycles. In cycles 40, 50, and 60, the fractionation threshold was decreased to collect the complete elution step to use it in a later viral clearance study (see viral clearance

Figure 1. Ultraviolet (UV) signals (solid line) and observed pressure drop (dashed line) during the purification of monoclonal antibody-containing supernatant over 100 bind-and-elute cycles with a *convectdiff* membrane prototype device.

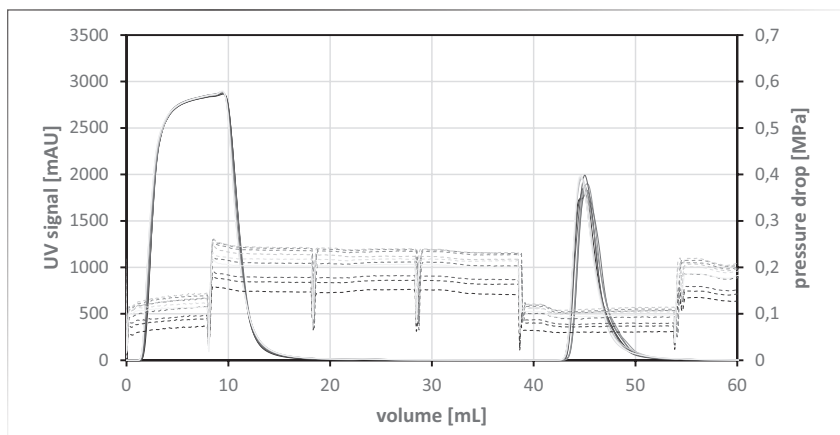
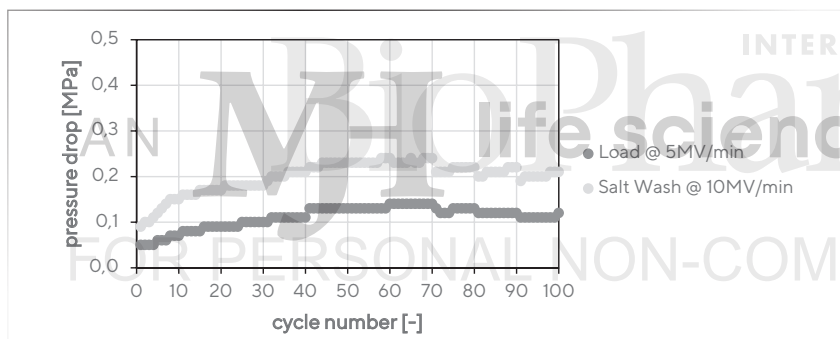


Figure 2. Observed pressure drop during load (5 MV/min) and intermediate salt wash (10 MV/min) during 100 bind-and-elute cycles with a *convectdiff* membrane prototype device.



study section in this article). Due to this decrease in the fractionation threshold, the corresponding eluate concentrations are only around 1.0 mg/mL. Cycle 81 exhibited a higher eluate concentration due to an error in setting the UV baseline for that run, resulting in a sharper eluate peak being collected.

The yields observed were around 100%. Yields above 100% are a measurement artifact of the two different methods used (NanoDrop and Octet) for determining product concentration in purified eluate and clarified harvest fluid. The measurement data from cycles 1 and 10 were corrupted and therefore not shown in **Figure 3**.

In comparison to the first internal cycling study in part 1, the yields observed here are higher, whereas the

eluate concentrations are lower. This discrepancy is a result of the lower threshold applied for elution peak fractionation (50 mAU [AGC] instead of 100 mAU [internal]).

The host cell protein (HCP) contaminants were measured with an Octet RED 96e (FortéBio) using Anti-CHO HCP Dip and Read Biosensors with Cygnus detection antibodies (part number 18-5081 from FortéBio). **Figure 4** shows the HCP concentrations and log reduction values (LRVs). The HCP concentration and LRVs were constant over the course of the 100 cycles performed. Moreover, no significant difference in HCP concentrations or LRVs before and after CIP is observable. The determined LRVs are between 2.5 to 3.0 and are thereby in the same range

as for the other prototype device. These LRVs are comparable to those achieved with resin materials (2).

Moreover, Protein A leachables were measured with an Octet RED 96e (FortéBio) using Residual Protein A Dip and Read Biosensors (part number 18-5128 from FortéBio). **Figure 5** gives an overview on the leached Protein A at up to 100 cycles. The measured concentration is below 4 µg/g. This low concentration may be interpreted as minor leaching compared to resin materials (3). However, the leached Protein A concentration also depends on the material's ligand density, which is unknown for most resin materials. Therefore, it is hardly possible to compare the concentration of leached Protein A.

In conclusion, the cycling study with this prototype including the *convectdiff* membrane showed a robust performance of the novel membrane at up to 100 bind-and-elute cycles. The observed chromatograms did not show any deviations or trends, and the measured quality attributes (yield, concentration, HCP concentration, and leachables) remained constant throughout all cycles. The pressure drop showed an increase during cycling, but this limitation was overcome by adapting the CIP procedure to the requirements of this feed stream.

VIRAL CLEARANCE CAPABILITY

The viral clearance capability of used and unused *convectdiff* membranes was tested at Sartorius Stedim BioOutsource Limited (Newhouse, United Kingdom). Two model viruses, minute virus of mice (MVM) and murine leukemia virus (MuLV), were cleared separately in duplicate runs on both used and unused *convectdiff* membrane prototype devices. Properties of these viruses can be seen in **Table II**.

The viral clearance study was performed using the same lot of clarified harvest with the mAb that was used in the cycling study performed at AGC Biologics. The *convectdiff* membrane prototype devices for the viral clearance

study were of the same type as the one used for the cycling study (4.0 mm bed height and 1.0 mL membrane volume). Four unused *convecdiff* membrane prototype devices were used for the viral clearance runs requiring an unused device (one for each run), and the device used in the cycling study at up to 100 cycles was used for runs requiring a used device. To avoid potential carryover, an extended CIP was included after each run of the used device, and runs spiked with MuLV were performed before those of MVM, which is more resistant to physico-chemical stress. The whole viral clearance study was carried out on a chromatography system (ÄKTA Avant 150, Cytiva). Spiked load material was prepared by spiking virus stock solutions into 0.22- μ m-filtered clarified harvest material at a 2% spike ratio. Titer of virus used for spiking was approximately 10^8 TCID₅₀/mL for MVM and 10^7 TCID₅₀/mL for MuLV. The applied protocol for operation is specified in **Table III**.

The purification protocol (Äkta method) contains pre-use flush, pre-use CIP, equilibration, loading, wash, intermediate wash, and elution. For pre-use flush, equilibration, and wash phosphate buffered saline (PBS) buffer was used. The equilibration step was performed until pH stabilized at pH 7.4. An intermediate wash step was implemented with PBS buffer containing a high concentration of sodium chloride (NaCl). Elution was done with acetic acid buffer containing NaCl. A CIP step was done before every viral clearance run for every device. For the device used in the cycling study ("used" device in the viral clearance study) CIP was also performed after every viral clearance run to re-use it for the next viral clearance run. In all cases, CIP was performed in the upflow direction to ensure that possible fouling substances are flushed out, rather than through the device. CIP was done with 15 MV at 3.0 MV/min, with an additional 60-minute hold for CIP of the cycled device.

Figure 3. Yield and concentration of monoclonal antibody in the eluate fractions collected during 100 bind-and-elute cycles with *convecdiff* membrane prototype device.

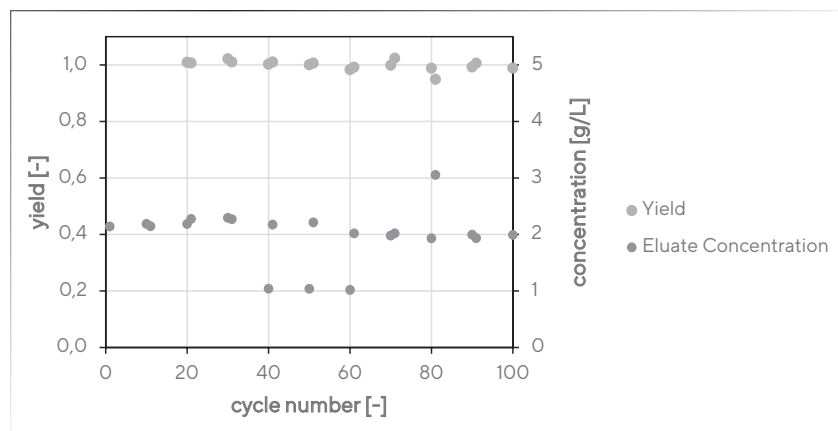


Figure 4. Concentration and logarithmic reduction values (LRVs) of host cell protein (HCP) impurities in selected eluates during 100 bind-and-elute cycles with *convecdiff* membrane prototype device.

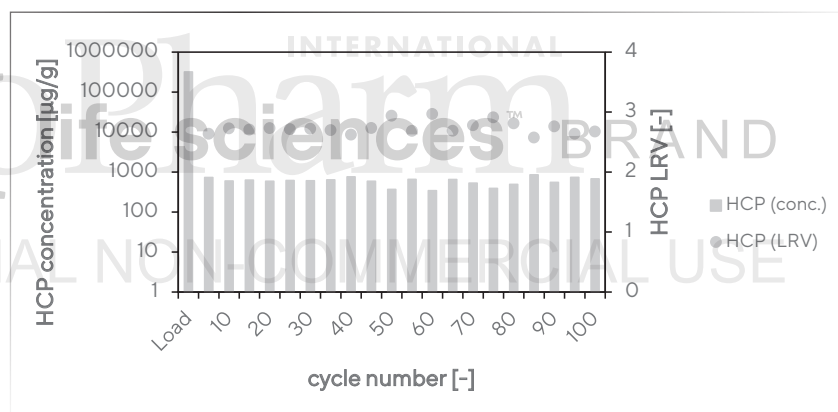


Figure 5. Residual Protein A concentration in selected eluate fractions collected during 100 bind-and-elute cycles with *convecdiff* membrane prototype device.

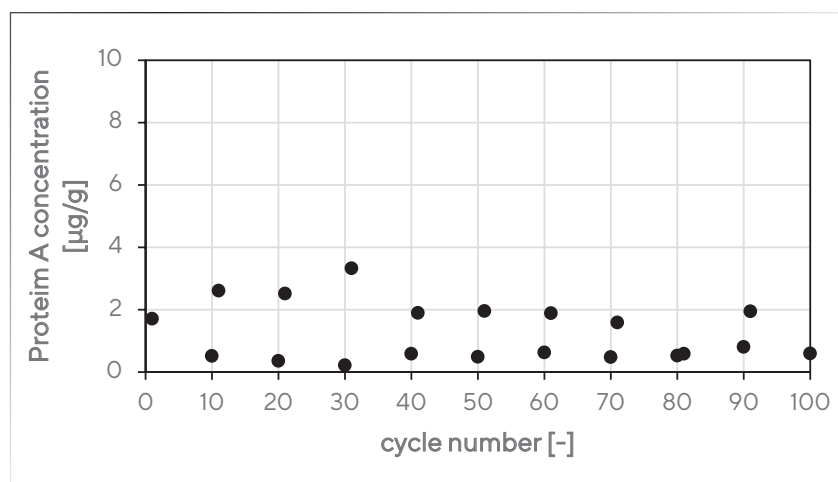


Table II. Properties of viruses used in the viral clearance study. MuLV is murine leukemia virus. MVM is minute virus of mice.

Virus	Family	Structure/genome	Size [nm]	Physico-chemical resistance	Characterization
MuLV	Retroviridae	Enveloped, single stranded RNA	80–110	Low	Represents a non-defective C type retrovirus. Typical model virus for endogenous C-type retrovirus-like particles produced in Chinese hamster ovary (CHO) cell lines. Mandatory for biological products derived from CHO cell lines and monoclonal antibody products.
MVM	Parvoviridae	Non-enveloped, single stranded DNA	18–24	High	Model for <i>Human parvovirus B19</i> , representing a severe test of the downstream process. Parvoviruses are known contaminants of CHO cell fermenters and are also potential contaminants of rodent derived biopharmaceuticals.

Table III. Protocol for viral clearance study with *convectdiff* membrane devices. PBS is phosphate buffered saline. CIP is clean in place. NaOH is sodium hydroxide. NaCl is sodium chloride. HAc is hydrogen acetate (acetic acid).

Step	Fluid/buffer	Volume [MV]	Flow [MV/min]	Time of process step [min]
Pre-use flush	1 x PBS pH 7.4, 16 mS/cm	10	5	2
Pre-use CIP (upflow)	0.5 M NaOH	15	3	5
Equilibration	1 x PBS pH 7.4, 16 mS/cm	~30	10	1
Load	Clarified harvest with concentration of 1.9 g/L	10.43	5	1.6
Wash	1 x PBS pH 7.4, 16 mS/cm	10	10	1
Intermediate salt wash	1 x PBS pH 7.4, 1M NaCl, 16 mS/cm	10	10	1
Wash	1 x PBS pH 7.4, 16 mS/cm	10	10	1
Elution	0.1 M HAc, 150 mM NaCl, pH 2.9, 16 mS/cm peak fractionation threshold 50 mAU	15	5	3
Reequilibration	1 x PBS pH 7.4, 16 mS/cm	10	10	1
CIP for cycled Device (upflow)	0.5 M NaOH	15 (followed by 60-minute hold)	3	65
Wash after CIP (upflow)	1 x PBS pH 7.4, 16 mS/cm	5	10	2.5

Table IV. Summary of viral clearance behavior with *convectdiff* membrane prototype devices. LRV is logarithmic reduction value. RTqPCR is reverse transcription-quantitative polymerase chain reaction. MuLV is murine leukemia virus. MVM is minute virus of mice.

Membrane/device	Virus	LRV by TCID ₅₀ assay [-]	LRV by RTqPCR [-]
Used	MuLV	>4.73	2.92
Used	MuLV	>4.48	3.39
Unused	MuLV	>5.34	2.07
Unused	MuLV	>4.72	1.97
Used	MVM	3.08	N/A
Used	MVM	3.09	
Unused	MVM	2.04	
Unused	MVM	2.22	

A summary of the results of the viral clearance runs is presented in **Table IV**. The TCID₅₀ assay is an endpoint assay defined as the dilution or extrapolated dilution of virus required to theoretically infect 50% of the inoculated cultures. To distinguish between physical removal versus inactivation of virus due to low pH of elution, real time quantitative poly-

merase chain reaction (RTqPCR) was also performed for MuLV because of its low physiochemical resistance.

For both used and unused *convectdiff* membrane devices, high LRVs (> 4) are achieved as determined by TCID₅₀ assay for MuLV clearance. No difference in LRV is observable between used and unused membrane adsorbers. With RTqPCR assay, only

mediocre LRVs ranging mostly from 2.0 to 3.0 were achieved for MuLV in both used and unused devices. Interestingly, used membrane adsorber devices featured a LRV of about 1.0 log₁₀ higher than unused membrane adsorber devices, indicating that MuLV was removed to a greater extent in the used membrane. For MVM, moderate LRV of 2.0–3.0 were achieved as determined by TCID₅₀ assay for both used and unused *convectdiff* membrane devices. Similar to MuLV removal, the used membrane adsorber device produced LRV of about 1.0 log₁₀ greater than the unused membrane adsorber, indicating that MVM was removed to a greater extent by the used membrane.

Both used and unused membrane adsorbers exhibited noteworthy clearance of MuLV and MVM virus, with a distinct trend of increased virus removal by used membranes. The log virus

reduction achieved by both unused and used *convecdiff* membranes in this study is on par with most Protein A resin processes, which typically achieve 2.0–4.0 log₁₀ reduction of MuLV and 1.0–3.0 log₁₀ reduction of MVM (2).

CONCLUSION

The newly developed *convecdiff* Protein A membrane was introduced in this study and its performance regarding mAb capture in bind-and-elute mode was successfully demonstrated.

Different membrane prototype devices were tested in two independent studies at up to 200 cycles (part 1) and 100 cycles (part 2). In both cases, the analyzed critical quality attributes, yield, and LRV of different contaminants are constant over all the cycles performed. No loss in performance was observed. The performance indicators and the viral clearance abilities of the material are comparable to state-of-the-art materials. These aspects underline the robustness and applicability of the *convecdiff* material.

Due to its characteristics and robustness, the *convecdiff* membrane is suited for rapid cycling chromatography. This approach purifies large amounts of target component by a multitude of successive bind-and-elute cycles. By rapid cycling chromatography, high process productivity is reached and lifetime capacity utilization of the membrane adsorber is possible during purification of one batch. Lifetime capacity utilization during one batch is key to overcoming a multitude of drawbacks associated with the current state-of-the-art industry practice, namely packed bed chromatography. First, clinical material can be produced with the rapid cycling approach at lower cost because Protein A resin is typically underutilized, and bulk resin purchase is avoided. Second, non-value adding activities related to the actual purification process (e.g., column packing, cleaning validation of columns, storage) are reduced to a minimum. Third and

most important, bioburden risks associated with packed bed column chromatography are eliminated because column storage between batches and re-use of the packed bed over multiple batches are no longer required.

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