Efficient cleaning-in-place methods for protein-based antibody affinity chromatography resins

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In a chromatographic process, cleaning is the procedure where impurities, both product-related (fragments, aggregates, etc.) as well as process-related (host cell proteins, DNA, etc.), are removed from the resin to minimize carryover to the next process cycle. Cleaning is most often performed without disassembly of the equipment and is thus referred to as cleaning-in-place (CIP). This whitepaper discusses cleaning of affinity resins intended for use in the purification of monoclonal antibodies and antibody fragments, for example, Fab fragments. Various cleaning strategies and the impact of different cleaning agents and concentrations on the resin lifetime are presented. As the use of sodium hydroxide (NaOH) is considered standard in the bioprocessing industry, the impact of treatment of affinity resins with various concentrations of this cleaning agent is demonstrated.

Introduction

Chromatography resins used for production of biopharmaceuticals need to be cleaned efficiently to ensure safety and efficacy of the bioproduct. Impurities that are not removed can build up on the chromatography column and start to leach into the process material in subsequent cycles. An efficient cleaning of the column in each cycle will prevent fouling and minimize risk of carry over between cycles and batches. Column fouling can also result in a capacity decrease over time with a subsequent decrease in the resin lifetime.

The increased resin utilization by column reuse for an appropriate number of cycles will positively impact on process economy (Fig 1). This is especially true for protein-based affinity resins, like protein A resins, which have a higher price tag due to more costly development and production of the ligand compared with resins based on low-molecular weight ligands such as ion exchange chromatography (IEC) or hydrophobic interaction chromatography (HIC) resins. Harsh cleaning conditions will result in a more efficient cleaning, but can at the same time destroy a susceptible resin. Therefore, it is essential to balance the harshness of the cleaning procedure with the compatibility (stability) of the resin. Although process economy is of utmost importance, patient safety will always be the overarching objective.

Here, we exclusively discuss CIP of protein-based affinity resins for purification of monoclonal antibodies and antibody fragments, whereas sanitization and bioburden control is addressed elsewhere (1). Both CIP and sanitization are thoroughly reviewed in "Biopharmaceutical processing: development, design, and implementation of manufacturing processes" (2).

Nature of impurities and impact on resin performance

The impurities can be related to both the product and the process. Examples of product-related impurities are degraded/modified or aggregated variants of the target protein, whereas process-related impurities comprise host cell proteins (HCP), host cell DNA (hcDNA), and lipids, as well as cell debris that are released during cell culture and harvest. Additionally, bacterial hosts can produce heat-stable toxins that can contaminate the process material. Additives supplemented during the upstream or downstream process can also pose challenges to cleaning of the chromatography resin.

The type and amount of impurities that come in contact with the column will depend on the position of the column in the purification train as well as on the chromatographic mode in which the column is run: bind-elute (B/E) or flow-through (FT) mode. An affinity resin used as the first capture step will be exposed to complex harvest loads, whereas the material loaded onto a polishing resin will be of higher purity. A polishing column used in B/E mode, however, might be challenged to a higher degree compared with a column used in FT mode, where only trace amounts of impurities are bound (3).

Column fouling can cause changes in the re-equilibration profile (pH, conductivity) due to the buffering capacity of impurities bound to the resin. Bound impurities can also cause changes in the elution profile, resulting in peak broadening. A pressure buildup over the column over time can be an indication of column fouling that will require a more stringent CIP procedure or replacement of the resin. Column fouling can also reduce yield, caused by a decrease in the resin capacity, as well as reduce purity over cycles. It is of interest to investigate whether a capacity decrease is caused by fouling due to an inefficient CIP or by degradation (hydrolysis) of the resin ligand due to the harsh CIP conditions (4). The cause will probably differ between resins and process materials, and in some cases, especially with the more sensitive affinity resins, it will be a combination of both.

Column maintenance program

CIP is part of the complete maintenance program, which also includes column regeneration, strip, sanitization, and storage. Column regeneration and strip are performed to remove tightly bound product after elution to prevent proteins to precipitate and severely foul the column during cleaning with high concentrations of NaOH. After elution of the target product, the affinity column is most often stripped using a buffer with an even more acidic pH than of the elution buffer. For an IEC column, the strip can be conducted with a buffer of high conductivity, at high and low pH, to prevent electrostatic interactions between the target protein and the ligand. For HIC columns, on the other hand, low-conductivity buffers or solvents can be used to break hydrophobic interactions.

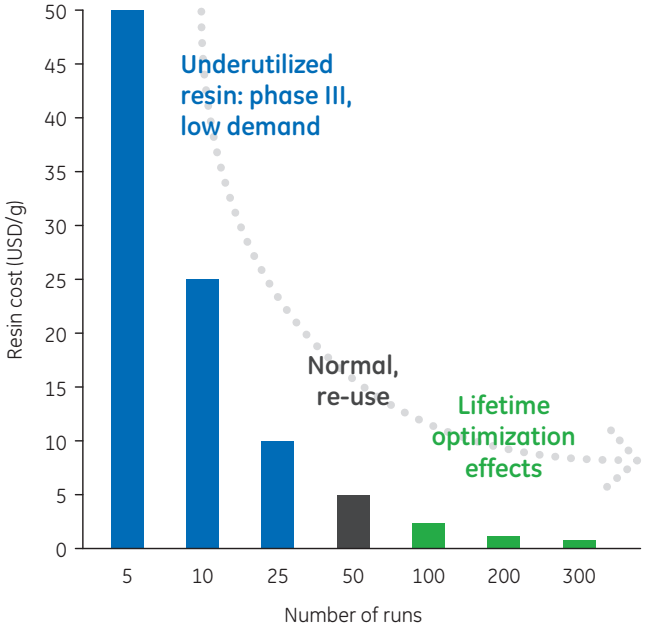


Fig 1. Process economy effect of resin reuse. The calculation is based on a 300 L protein A column, 10 kUSD/L, 40 g/L capacity. Adapted from "Biopharmaceutical processing: development, design, and implementation of manufacturing processes" with kind permission of Elsevier, Amsterdam, NL. (2).

Resin lifetime studies

The functional lifetime is the number of cycles that the resin can be used to consistently produce intermediate and final product that meet the criteria for quality, safety, and yield. The useful lifetime is depending on the need of the biopharmaceutical producer and is determined by factors such as cost of the chromatography resin, number of batches produced each year, column storage, labor to pack columns, and similar. Using a resin to the end of its functional lifetime can be beneficial if the resin is expensive or the number of cycles in its functional lifetime is low (3, 5).

Functional lifetime studies in which columns are evaluated over hundreds of cycles are time consuming, especially if the initial cleaning protocol fails and there is a need to restart the study with an improved CIP protocol. Development of CIP protocols should be part of process development and is typically conducted using a small-scale model in which the column lifetime is estimated (Table 1). The CIP protocols are later confirmed in manufacturing scale during the validation period (6). The most common approach, referred to as prospective validation, includes small-scale studies for guidance followed by full-scale confirmation and validation, as opposed to concurrent validation that is entirely conducted in manufacturing scale (3, 5).

Table 1. Example of a lifetime study setup in small scale (100 cycles with harvest material)

Cycle	Analyses
	Frontal analysis (FA) using purified target protein or harvest material for determination of DBC
1–10	Analysis of all elution pools, for recovery and purity
11–20	Analysis of elution pool from cycle 20 for recovery and purity
	Blank run with mock eluate for analysis of carryover
	FA for determination of DBC
21–30	Analysis of elution pool from cycle 30 for recovery and purity
31–40	Analysis of elution pool from cycle 40 for recovery and purity
	Blank run with mock eluate for analysis of carryover
	FA for determination of DBC
41–50	Analysis of elution pool from cycle 50 for recovery and purity
51–60	Analysis of elution pool from cycle 60 for recovery and purity
	Blank run with mock eluate for analysis of carryover
	FA for determination of DBC
61–70	Analysis of elution pool from cycle 70 for recovery and purity
71–80	Analysis of elution pool from cycle 80 for recovery and purity
	Blank run with mock eluate for analysis of carryover
	FA for determination of DBC
81–90	Analysis of elution pool from cycle 90 for recovery and purity
91–100	Analysis of elution pool from cycle 100 for recovery and purity
	Blank run with mock eluate for analysis of carryover
	FA for determination of DBC

As the impurity proportion and profile might vary at different production stages and scales, it is recommended to use production feed stream to obtain the most relevant data (3). Temperature should also be the same as used in manufacturing.

As column fouling can lead to reduced resin capacity as well as decreased product yield and purity, eluates should be collected for analyses of recovery and purity (HCP, leached ligand, aggregates, fragments, etc.) to ensure consistency over the resin lifetime. Frontal analysis should be performed regularly to determine dynamic binding capacity (DBC) at a defined breakthrough of the target molecule. Frontal analysis could either be performed using the purified target protein and measuring the UV signal during breakthrough or by using unpurified material and collect fractions during breakthrough for off-line analysis of concentration of the target protein. By including frontal analysis every 20th cycle, it will be possible to monitor remaining DBC over the resin lifetime to enable detection of capacity loss not necessarily reflected in a reduced recovery.

In a blank (or mock) run, all phases of the chromatography step are performed, except for the protein loading phase, and the mock eluate is collected for analysis of carryover of target product and other impurities. Analyses can be performed by monitoring the UV signal, SDS-PAGE, a total protein assay, a product-specific assay (e.g., ELISA), and/or determination of total organic carbon (TOC)/total nitrogen (TN). Worth remembering is, however, that many of the buffers used for purification of biomolecules, such as acetate and citrate buffers, contain carbon and will contribute to a high background in a TOC analysis. To avoid a high carbon background, a phosphate buffer with the same pH as the elution buffer used in the process can instead be used for the mock elution. Samples from column effluents should be analyzed in the same manner.

Monitoring of process performance can also be conducted by transition analysis of UV, conductivity, and pH traces. The use of chemometrics for analysis of a set of chromatography profiles during column recycling can predict underperformance of a column, even before an event of backpressure buildup or improper impurity clearance occurs (7, 8). Pulsed-input testing for column qualification measurements such as height equivalent to a theoretical plate (HETP) and asymmetry factor (A_s) can also be used for monitoring of the quality of the packed bed. Pressure tracking over time and cycles as well as visual inspection of the packed bed (discoloration, channeling, dry areas in bed, etc.) can also reveal column fouling.

Cleaning agents and conditions

Cleaning agents should be selected based on the impurities that need to be removed. In addition to efficient removal of impurities, the cleaning agent should have little or no impact on the performance and lifetime of the resin. The agent should also have good manufacturability attributes, that is, be compatible with wetted materials of the column and equipment and have good safety profile for operators and the environment. The agents should also be cost-efficient and easy to remove and dispose. Both concentration of the cleaning agent and the duration of the cleaning (contact time) are important for cleaning efficiency and chromatography resin compatibility (4). Temperature will also play an important role, at least for resin stability. Protein A resin degradation rate has shown to be faster at room temperature than at lower temperatures (4). Resin compatibility studies conducted by vendors are sometimes forced and performed at elevated temperature. Resin compatibility at room temperature is thereafter approximated using the Arrhenius equation. Cleaning efficiency might not be affected by temperature to the same extent as resin stability (4).

Sometimes CIP fails to restore a packed bed that is often more heavily fouled at the top than in the rest of the column. In such a case, the column can be unpacked and the resin cleaned out-of-place during stirring (3). Automated column packing facilitates this procedure (Fig 2). A fouled column can also benefit from being run in up-flow direction during CIP.

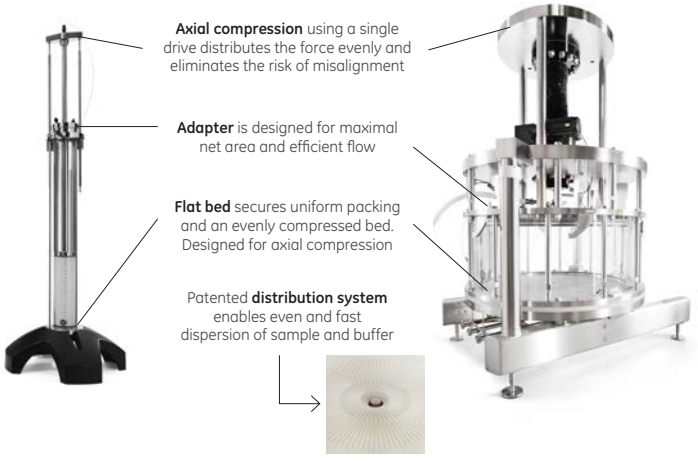


Fig 2. The AxiChrom™ column design allows for automated packing and unpacking of the column. The column design also enables cleaning with the adaptor raised, by flowing the cleaning liquid upwards through a liquified bed in a closed manner (9). The fluidized bed can thereafter be repacked without opening the column.

Sodium hydroxide, the universal cleaning agent

NaOH is widely accepted and used for cleaning and sanitization of hardware and chromatography resins (10). NaOH is effective in dissolving and removing proteins, solubilizing lipids by alkaline hydrolysis of lipids constituting esters of alcohols and fatty acids (saponification), removing nucleic acid, and inactivating viruses, fungi, as well as bacteria and their endotoxins. In addition to having a low cost, NaOH is also easy to detect, remove, and dispose. Considering its benefits, NaOH is the standard CIP agent in bioproduction and should be applied for cleaning whenever the stability of the resin allows for such a treatment. For chromatography resins with low-molecular weight ligands other than protein, NaOH is often used at concentration of 0.5 to 1 M. If a too high concentration is applied on protein-based affinity resins, the NaOH solution will damage the ligand and cause a significant decrease in capacity and overall performance over time. However, moderate NaOH concentrations can be applied on many modern protein-based affinity resins to ensure consistent performance over hundreds of cycles (Fig 3). However, there will be a tradeoff between the number of cycles and the harshness of the cleaning. Safety margins regarding resin capacity utilization can be incorporated, as incomplete utilization of the capacity will allow for an increase in CIP stringency. The possibility to use higher concentration of NaOH can enable reducing the contact time, which can be of specific importance for continuous processes.

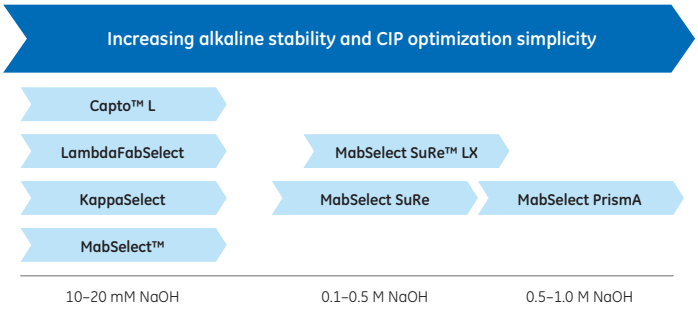


Fig 3. Relative alkaline stability of affinity chromatography resins for purification of mAbs and antibody fragments. While resins intended for purification of antibody fragments (Capto L, LambdaFabSelect, KappaSelect) as well as early ptotein A affinity resins (MabSelect) withstand NaOH concentrations in the reange of 10–20 mM, the first generation of alkaline-stabilized protein A resins (MabSelect SuRe, MabSelect SuRe LX) enable the use of 0.1 to 0.5 M NaOH for cleaning. The latest-generation protein A resin (MabSelect PrismA) can withstand 0.5 to 1.0 M NaOH, implying that the same cleaning conditions can be used for both capture and polishing steps, offering greater simplicity, robustness, and a more manufacturing-friendly CIP strategies.

Figure 4 shows the efficiency of 0.1 M NaOH in removing fouled proteins from a protein A resin, and as shown in Figure 5, the cleaning efficacy will increase with increasing NaOH concentration from 0.1 to 0.5. It is also important to be aware of that additives supplemented with the intention of protecting the affinity ligand during CIP can impair the cleaning efficiency. For example, a mixture of 0.1 M NaOH and 1.0 M NaCl significantly decreases the cleaning efficiency compared with cleaning with 0.1 M NaOH alone (Fig 5), as the presence of NaCl in the NaOH cleaning solution not only stabilizes the recombinant protein A ligand, but also the fouled proteins on the resin. Additives, such as sucrose, ethylene glycol, or propylene glycol, have also been proposed to stabilize the protein A ligand in alkaline solutions (4). When including such stabilizing agents in the cleaning solution, it is of importance to verify that the cleaning efficiency is not impaired by the additives.

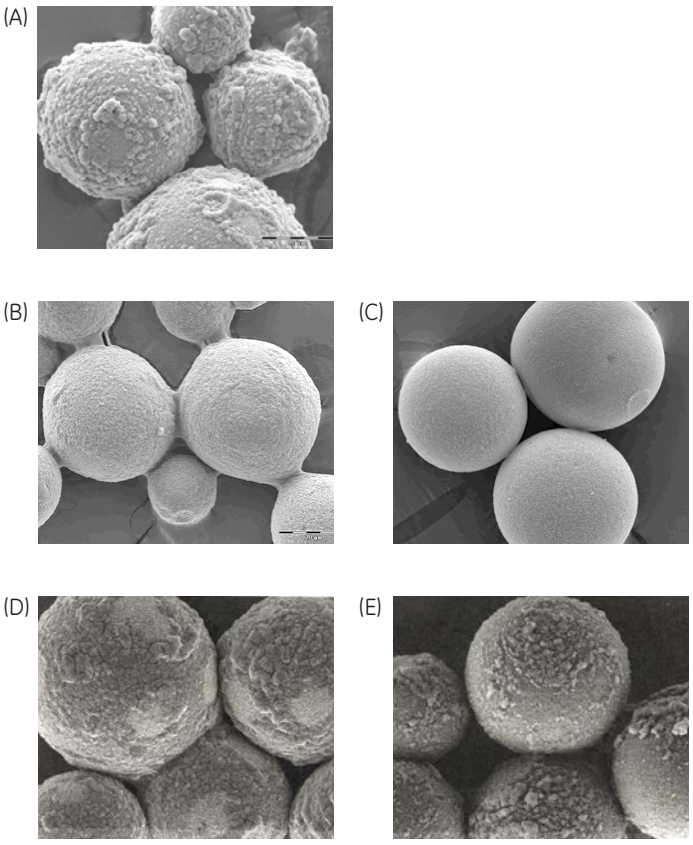
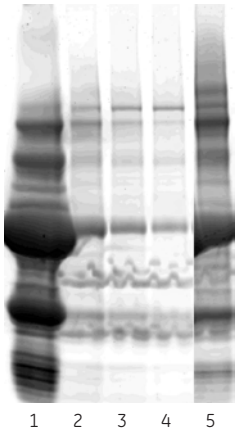


Fig 4. (A) Fouling of MabSelect particles due to inefficient cleaning with 0.1 M phosphoric acid for 300 cycles in a mAb process. Cleaning with 0.1 M NaOH for (B) 40 min and (C) up to 3 h removed the fouled proteins. Incubation of the fouled particles in 6 M guanidine hydrochloride for (D) 40 min or (E) 3 h did not offer the same cleaning effect as 0.1 M NaOH. Reprinted with kind permission from Genentech, CA, USA.



Lanes
1. PBS (control)
2. 0.1 M NaOH
3. 0.2 M NaOH
4. 0.5 M NaOH
5. 0.1 M NaOH and 1 M NaCl

Fig 5. Protein impurities extracted from MabSelect SuRe resin after cleaning with various cleaning agents. The use of increasing concentration of NaOH from 0.1 to 0.5 M offers improved cleaning (lanes 2 to 4). A mixture of 0.1 M NaOH and 1.0 M NaCl (lane 5) significantly decreases the cleaning efficiency compared with cleaning with 0.1 M NaOH alone (lane 2). The extraction from a resin that has not been subjected to cleaning, only washed with PBS (lane 1, control), contains the highest impurity levels.

Guanidine hydrochloride is a strong chaotropic and denaturing agent. It is also effective for dissolving precipitated/denatured proteins because of its ability to disrupt hydrophobic interactions and hydrogen bonds. For earlier generations of protein A resins, cleaning with guanidine hydrochloride has been the method of choice, as these resins are susceptible to harsh treatment with high concentrations of NaOH. As shown in Figure 4, however, 6 M guanidine hydrochloride removes fouled proteins but is less effective compared with 0.1 M NaOH. Urea works in a similar fashion as guanidine hydrochloride. However, both urea and guanidine hydrochloride are more expensive to both purchase and dispose than NaOH. Waste disposal quantities of nitrogen-containing compounds is for most regions limited by national and/or local regulations. Organic solvents can be a solution if lipid fouling is a major issue. The most frequently applied protocol is 30%–40% isopropanol in combination with NaOH. In this case, the NaOH concentration will depend on the alkaline stability of the resin.

Improved stability of the protein A affinity ligand allows for more stringent CIP protocols

Benefits and challenges of affinity chromatography resins

The predominant affinity ligands used in purification of monoclonal antibodies and antibody fragments, such as protein A (11) and protein L (12) originate from nature (Fig 6). Evolution over millions of years has resulted in a very specific binding with high affinity of these proteins to immunoglobulins. Protein A binds to the Fc-region of mAbs and Fc-fusion proteins, whereas protein L interacts with antibody fragments at the variable part of the kappa light chain. Vendors of chromatography resins have taken advantages of the properties of these natural affinity ligands, and via development, refined them further through an accelerated evolution to meet the demands of biopharmaceutical producers. The specific binding of affinity ligands to target proteins makes the purification very efficient, with high purity and yield as the result. Most often, generic conditions can be used when directly capturing recombinant proteins from clarified host cell supernatant or cell homogenates. Typically, proteins elute within the pH range 2.5 to 3.5. As a result, limited process development is required and a platform approach for antibody capture can be employed, with one or two additional polishing steps to remove remaining impurities.

Although the ligands of protein A and protein L resins are often considered to be the weakest link of the chain, the base matrix composition, coupling chemistry, and the spacer with which the ligand is connected to the backbone are also important. Highly crosslinked agarose is one example of a matrix with good stability under both high and low pH conditions. Other matrices can be less stable under extreme pH conditions. Silica or controlled pore glass matrices, for example, display limited stability under alkaline conditions. Here, we focus on describing cleaning conditions for chromatography resins with chemically stable high-flow agarose back bones, where the ligand is the most sensitive part of the resin.

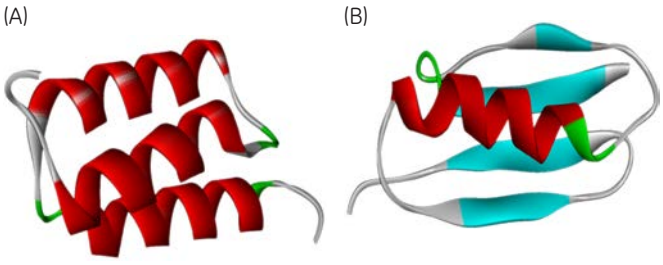


Fig 6. Ligands of (A) MabSelect SuRe and (B) Capto L resins.

For MabSelect and Capto L resins, the initial DBC is reduced to below 80% already after 20 to 30 cycles using 0.1 M NaOH (Fig 7). This low utilization can be acceptable in early phases with low demand, but might need to be optimized for manufacturing applications, where more than 100 cycles would be required for a good process economy. By applying a load safety margin, not utilizing the full capacity of the resin, would also allow for the use of a more stringent CIP. For a remaining capacity above 80% after 172 CIP cycles, NaOH concentration should be below 20 mM at a contact time of 15 min in each cycle (Fig 8).

Development of alkaline-stable Protein A ligands for improved cleaning with NaOH

As NaOH is recognized as standard for cleaning of chromatography resins, efforts have been made by suppliers over the last two decades to improve the alkaline stability of protein A-based ligands used for mAb purification. Exchange of amino acids sensitive to deamidation and other mutation to strengthen the stucture of the protein have been made to improve alkaline stability of the protein A ligand. In 2005, the MabSelect SuRe protein A resin, the first mAb affinity resin based on an engineered alkaline-stable ligand, was launched. The MabSelect SuRe ligand is a tetramer of the alkaline-stabilized B-domain of protein A. The resin can withstand cleaning with 0.1 M NaOH at a contact time of 15 min, with a remaining DBC above 80% after as many as 200 cycles (Fig 9). Longer contact time with 0.1 M NaOH, or higher NaOH concentrations of up to 0.5 M, is recommended for more extensive cleaning or sanitization. MabSelect SuRe LX, launched 2011, is based on the same alkaline-stable ligand as MabSelect SuRe and was developed for increased capacity at longer residence times. Compared with MabSelect SuRe resin, the higher ligand density of MabSelect SuRe LX provides an increased resin lifetime when using 0.5 M NaOH for cleaning (Fig 10).

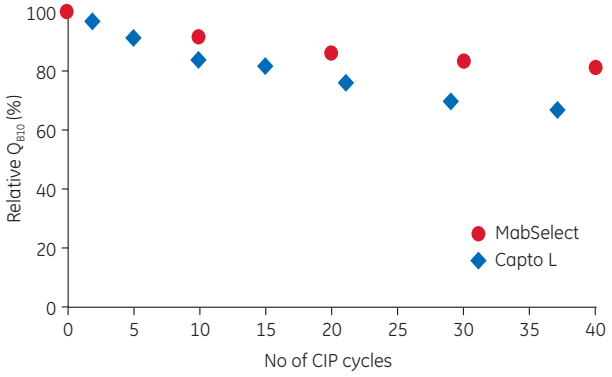


Fig 7. Remaining DBC at 10% breakthrough (Q_{B10}) of MabSelect and Capto L resins. The stability of Capto L under caustic conditions was investigated and compared with that of MabSelect resin. The resins were both exposed to repeated CIP cycles using 0.1 M NaOH at a contact time of 15 min.

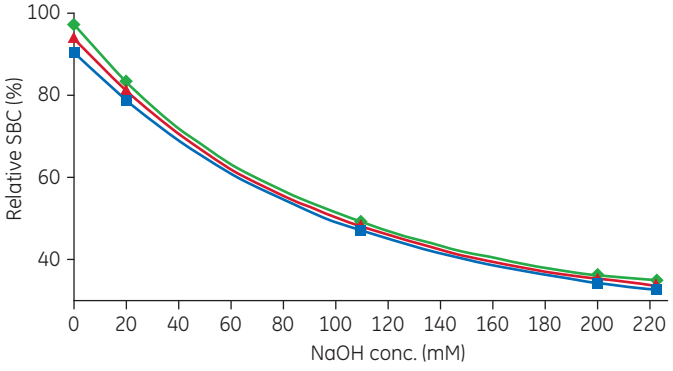


Fig 8. PreDictor plates were used for screening of resin compatibility in NaOH. Remaining static binding capacity (SBC) of MabSelect resin after storage in 0–220 mM NaOH for 43 h, corresponding to 172 CIP cycles at 15 min contact time. The remaining capacity decreases with increasing NaOH concentration. For a remaining capacity of > 80%, NaOH concentration needs to be below 20 mM.

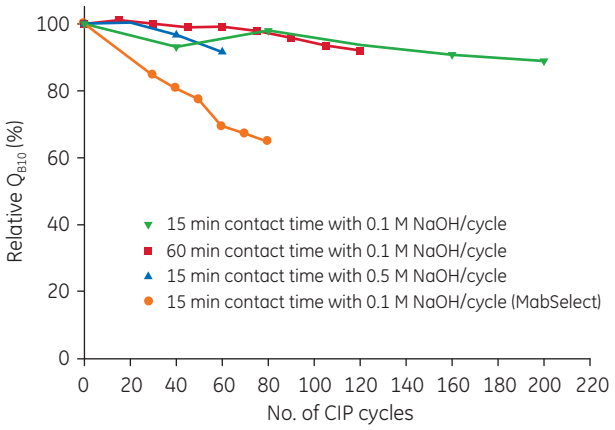


Fig 9. Remaining DBC at 10% breakthrough (Q_{B10}) of MabSelect SuRe resin for human polyclonal IgG using different cleaning protocols.

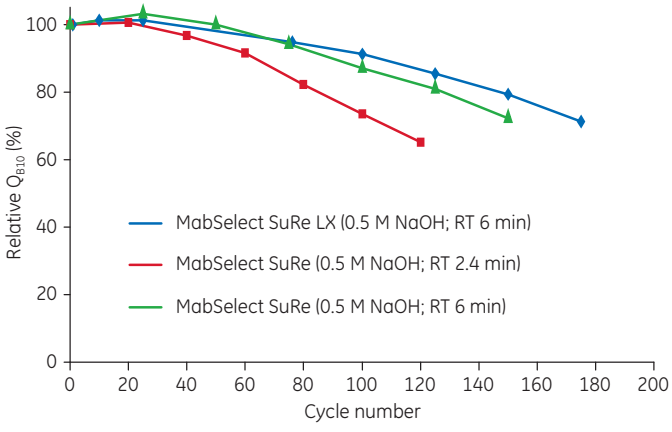


Fig 10. Remaining DBC at 10% breakthrough (Q_{B10}) of MabSelect SuRe and MabSelect SuRe LX resins after CIP with 0.5 M NaOH for 0–175 cycles. DBC was tested using polyclonal IgG and residence times of 2.4 and 6 min.

With MabSelect SuRe LX, 0.5 M NaOH have been used for cleaning in up to 100 cycles with maintained yield, consistent clearance of HCP and hcDNA, and low ligand leaching in a mAb purification process (Fig 11). Studies show that resin fouling due to inefficient cleaning (too short time of exposure to 0.1 M NaOH) might, under certain conditions, have a larger negative impact on resin capacity and lifetime than the effect of NaOH on ligand degradation (hydrolysis) (4).

Since the launch of the MabSelect SuRe resins, continuous development of the protein A-derived ligand has been conducted to further improve its alkaline stability. The resulting MabSelect PrismA resin is based on an improved high-flow agarose base matrix and a genetically engineered protein A-derived ligand that together provide an enhanced capacity compared with MabSelect SuRe and MabSelect SuRe LX (Fig 12). In comparison with its predecessors, MabSelect PrismA exhibits an enhanced alkaline stability and can tolerate NaOH concentration of up to 1.0 M NaOH. Compared with MabSelect SuRe LX, for which 50% of the initial DBC remains after 150 cleaning cycles using 1.0 M NaOH, more than 90% of the initial capacity of MabSelect PrismA remains after the same treatment, enabling more efficient cleaning for better bioburden control and lower risk of carryover between cycles (Fig 13) (1).

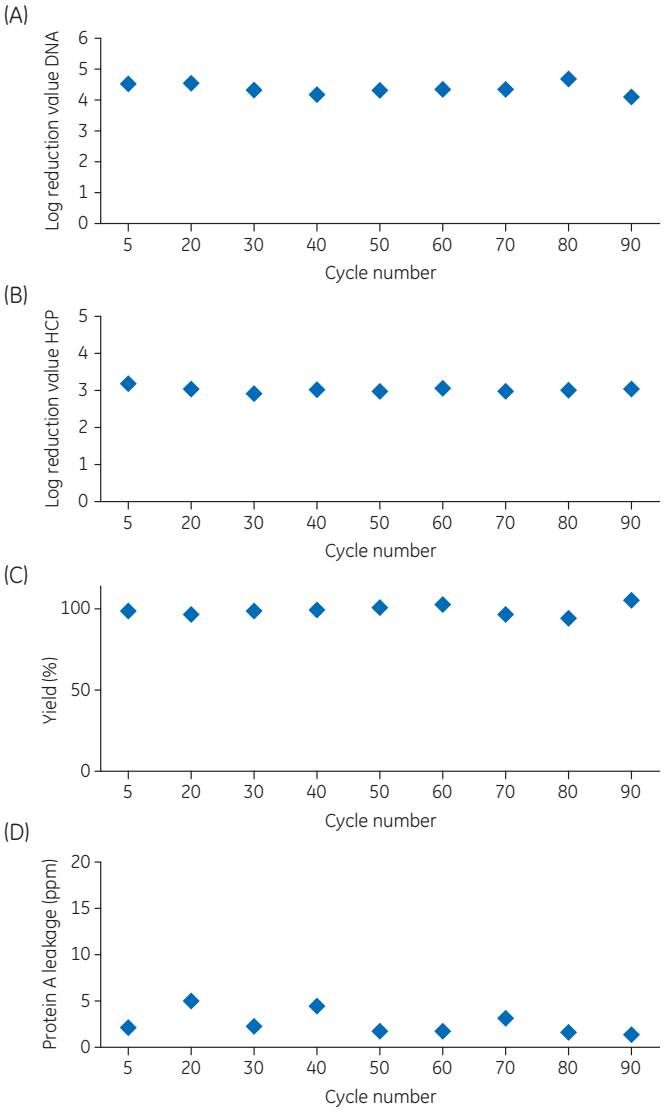


Fig 11. MabSelect SuRe LX exhibits stable (A) hcDNA clearance, (B) HCP clearance, and (C) mAb yield as well as (D) low ligand leaching using 0.5 M NaOH for cleaning at a contact time of 15 min over 90 cycles.

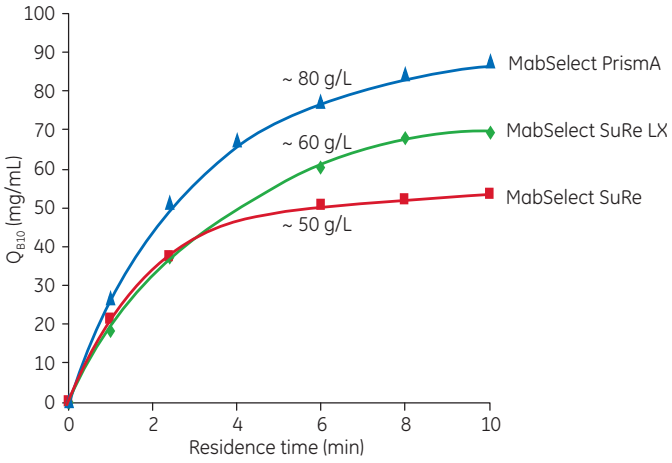


Fig 12. Compared with MabSelect SuRe LX, the optimized base matrix of MabSelect PrismA offers an up to 40% increase in DBC at a residence time of 2.4 min or a 30% increase in DBC at 4 min residence time. At 6 min residence time, a binding capacity of 80 mg human IgG/mL resin was observed for MabSelect PrismA, which is more than 25% higher than for MabSelect SuRe LX.

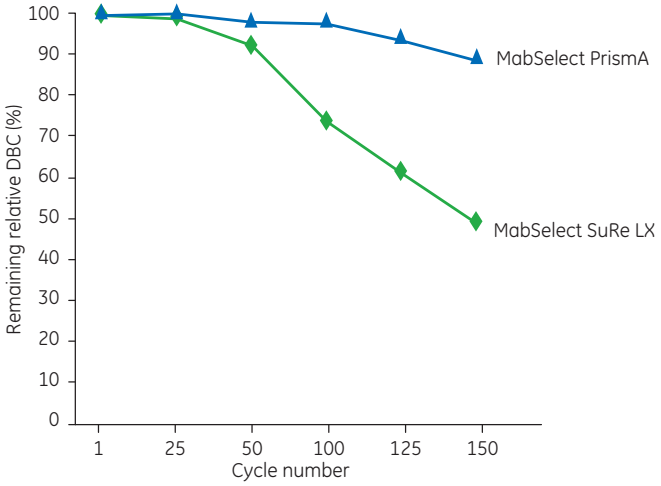


Fig 13. Cycling of MabSelect PrismA and MabSelect SuRe LX using pure buffers and 1.0 M NaOH for cleaning in each cycle shows that MabSelect PrismA maintains > 90% of its initial capacity after 150 cleaning cycles.

Optimization of cleaning conditions for affinity resins sensitive to NaOH

Determination of cleaning efficiency

A high-throughput method was developed for rapid screening of cleaning conditions to obtain a better understanding of the cleaning efficiency of various cleaning agents at different concentrations, of sequences of cleaning steps, and the effect of additives on the cleaning efficiency (13, 14). The developed method is particularly useful for sensitive affinity resins, for which lower NaOH concentrations and possibly also a sequence of different cleaning steps might need to be employed. The screening methodology consists of three parts. First, resin fouling is generated by repeated loading and elution of the resin (prefilled in a PreDictor™ 96-well filter plate), using clarified cell culture supernatant without cleaning included in the cycles. Secondly, different cleaning agents at various concentrations are added to the fouled resin. After incubation for a defined time, the cleaning agents are removed. Finally, residual impurities remaining on the resin after cleaning are analyzed by capillary electrophoresis for high-throughput or by traditional SDS-PAGE.

PreDictor plates are useful for screening of cleaning conditions and finding relative values, enabling ranking of different cleaning conditions according to cleaning efficiency. Using PreDictor plates, however, it is not possible to predict whether the cleaning efficiency will be sufficient over a certain number of cycles. The resin in the filter plate is not packed, but rather in a slurry where the cleaning during agitation of the resin can be more efficient compared with that of a packed bed. Furthermore, fouling is more homogeneous in the filter plate compared with in a column, where the top of the packed bed often is more fouled than the rest of the column. Thus, identified CIP agent candidates need to be further investigated in column lifetime studies. Typically, this is done in a sequential manner in bench scale, which is both material and labor intensive. If instead conducted using mini-columns on a robotic system, several cleaning regimens can be evaluated in parallel (15).

Cleaning protocols for alkaline-sensitive affinity resins

For mAb affinity resins based on native or recombinant protein A ligands, and thus more alkaline sensitive, moderate NaOH in the range of 10 to 15 mM can be used (Fig 14A). If the NaOH concentration is not sufficient for cleaning, a two-step protocol using a reducing agent such as 1-thioglycerol followed by 10 to 15 mM NaOH can be evaluated (Fig 14B). Aggregated proteins such as target mAb molecules fouled on the resin will be fragmented by the reducing agent and more easily solubilized by the subsequent contact with NaOH. Note that a mixture of reducing agent and NaOH is not an option. Instead, the cleaning agents should be applied one after the other in a sequential manner, and the order is critical. The two-step cleaning sequence for the MabSelect resin developed in PreDictor plate experiments was later verified in a lifetime study (16). An 8 M urea solution of pH 2.5 can also be included occasionally to regain capacity that has been lost due to fouling.

Former recommendations for cleaning of the MabSelect resin, comprised 50 mM NaOH + 1 M NaCl, as salt worked as a stabilizer of the recombinant protein A ligand in the caustic solution. However, further investigations showed that the presence of NaCl in the NaOH cleaning solution impaired the cleaning efficiency (Fig 14A).

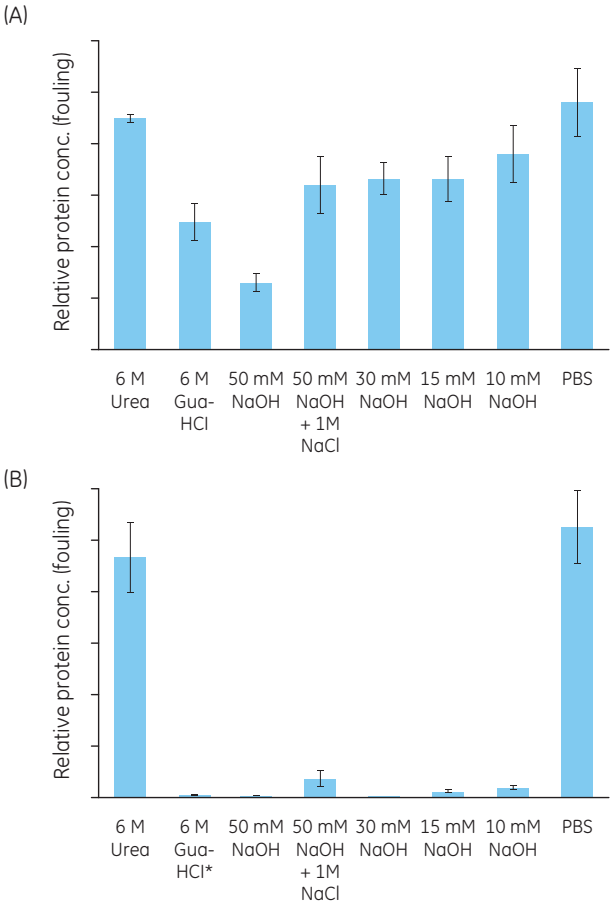


Fig 14. Screening of one- and two-step CIP protocols for MabSelect resin in a mAb process. The bar graphs show the relative concentration of proteins extracted from MabSelect subjected to a mAb cell culture supernatant and cleaned with various agents. (A) Cleaning efficiency of 6 M Urea, 6M guanidine hydrochloride and different concentrations of NaOH from 10 to 50 mM. The highest NaOH concentration without NaCl gives the most efficient cleaning, whereas 50 mM NaOH + 1 M NaCl results in a less efficient cleaning comparable with 15–30 mM NaOH without NaCl. (B) Cleaning efficiency is greatly improved by using a two-step sequence protocol with 100 mM 1-thioglycerol (reducing agent) followed by low concentrations of NaOH or a two-step sequence with 1-thioglycerol followed by 6 M guanidine hydrochloride (*17).

The protein L ligand exhibits a similar, or somewhat lower, alkaline stability than the MabSelect protein A ligand. The recommended CIP protocol for Capto L is 15 mM NaOH at a contact time of 15 min included in each cycle. This CIP protocol has been verified in column experiments using Fab-containing *E. coli* homogenate. Higher NaOH concentrations can be used, but will reduce the lifetime of the resin. In case of a reduced performance, the cleaning procedure can be complemented with 8 M urea/0.05 M citric acid, pH 2.5 to remove strongly bound impurities (Fig 15). This cleaning solution can be used at regular intervals or when considered necessary, depending on the nature of the harvest material. Another alternative is using a two-step sequence protocol including a reducing agent (100 mM 1-thioglycerol), followed by 15 mM NaOH (Fig 16). Using reducing agent as a pre-NaOH step has shown to be a successful cleaning strategy. Also for Capto L, addition of salt to the caustic CIP solution decreases the cleaning efficiency. In contrast to what has been observed for the protein A ligand, the protein L ligand is less alkaline stable in the presence of salt.

The ligands of KappaSelect and LambdaFabSelect affinity resins are based on single-chain antibody fragments that are screened for human immunoglobulin kappa and lambda light chains, respectively. KappaSelect is stable in 10 mM NaOH for 96 h, whereas LambdaFabSelect is stable in up to 50 mM NaOH for 72 h. Similarly to the MabSelect resin, these resins can be cleaned using a mild alkaline solution as well as using 8 M urea or 6 M guanidine hydrochloride. The ligands of KappaSelect and LambdaFabSelect are not considered compatible with reducing agents.

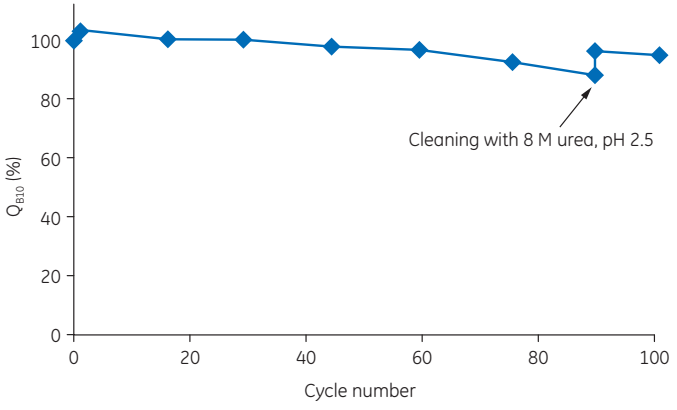


Fig 15. The Capto L resin retains more than 90% of its initial binding capacity over 80 cycles with the recommended 15 mM NaOH CIP solution. At cycle 89, the Capto L column was cleaned with 8 M urea at pH 2.5 with positive effects on binding capacity.

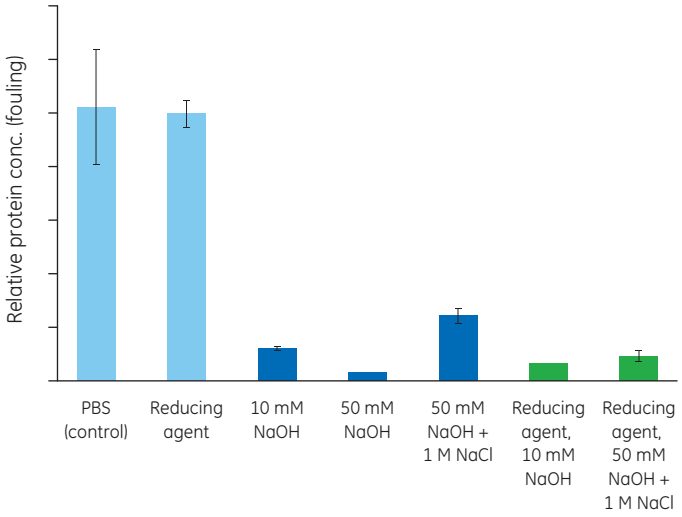


Fig 16. Residual impurities on Capto L after cleaning with various cleaning agents. The control (PBS) corresponds to the impurity level of uncleaned resin. Applying a single cleaning step, using 100 mM 1-thioglycerol, will not result in any improved cleaning compared with the control. A 10–50 mM NaOH solution offers a relatively good cleaning. Using a mixture of 50 mM NaOH and 1 M NaCl, the cleaning is less efficient. A two-step sequence with reducing agent followed by NaOH will improve the cleaning efficiency compared with a single cleaning step suing NaOH alone.

Conclusions

Efficient cleaning of chromatography resins used in biopharmaceutical production will prevent buildup of impurities in the chromatography column that eventually can leach into the process material in subsequent process cycles and challenge the safety and efficacy of the biopharmaceutical. For efficient resin cleaning, the cleaning agent should be selected based on the type of impurities that are to be removed. The selected cleaning agent should exhibit efficient impurity removal, without affecting resin performance or lifetime.

For good process economy and bioburden control, 1 M NaOH is typically used in cleaning of resins based on low-molecular weight, non-protein ligands. However, such a high concentration of NaOH will damage many affinity resins based on protein ligands. Thus, there needs to be a balance between the harshness of the cleaning protocol (concentration of the cleaning agent and the contact time) and the compatibility (stability) of the resin. There will also be a tradeoff between the number of cycles that can be conducted with the resin and the harshness of the cleaning protocol. However, modern mAb affinity resins such as MabSelect PrismA are developed to withstand the same harsh cleaning conditions as used with resins based on non-protein ligands, enabling the use of the same cleaning protocol throughout the purification train.

Disclaimer

The information contained herein is not representative of any specific claims or any relevant environment, health, and safety laws and regulations, including use authorization, product registration or application licensing, or similar legal requirements.

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Related literature

Data files	Product code
MabSelect	18114994
MabSelect SuRe	18001165
MabSelect SuRe LX	28987062
MabSelect PrismA	KA553200917DF
Capto L	29010008
KappaSelect LambdaFabSelect	28944822
PreDictor 96-well filter plates	28925839

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

gelifesciences.com/bioprocess

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