Cleaning and Cleaning Validation in Process Chromatography

Current Industry Practices and Future Prospects

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During regulatory inspections, manufacturers of biopharmaceuticals and biological products often find attention directed to cleaning and cleaning validation of chromatography resins and multiuse purification systems. Chromatographic resins must either be disposed of or sufficiently cleaned to ensure reproducibility in subsequent cycles. The decision to recycle or dispose of resins is typically driven by cost. The economics depend on unit operation scale, resin cost and compatibility with cleaning agents, feedstream quality, position of the resin in the purification scheme, and stage of product development.

If columns are to be recycled, resin lifetime studies must include assessment of the cleaning on performance after continued use. The cost of validating reuse has been discussed (**1**). Once a decision is made to recycle, and after a certain number of cycles, replacing resin is often less costly than extending a lifetime study. The cost of the resin and the quantity needed should be taken into account. For example, a large protein A column typically warrants repeated use; whereas a small ion-exchange column may not. Consider, too, the ease of packing. If you need a production-scale exclusion column, you are unlikely to want to repack it until absolutely necessary. Another important factor is the resistance of the resin to adequately harsh cleaning agents. The use of a fragile ligand, such as a lectin, that does not allow adequate cleaning could introduce a patient safety risk. In addition to carryover, such a resin might present ligand leakage issues during cleaning, thereby reducing capacity with repeated use. Fortunately, today's resin manufacturers design and produce process resins that minimize this type of problem.

The nature of the feedstream and the position of the resin in the purification sequence also contribute to the decision to recycle. Resins used in early capture steps are almost always more difficult to clean. This is especially true when product is not secreted during production. On the other hand, although final polishing steps have cleaner feedstreams, at this late stage in manufacturing the risk of carryover becomes more significant in terms of final product quality. Finally,

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PROCESS FOCUS: DOWNSTREAM PROCESSING (CHROMATOGRAPHY)

WHO SHOULD READ: PROCESS DEVELOPMENT AND MANUFACTURING

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LEVEL: INTRODUCTORY

the stage of development should be taken into account. In early clinical development, manufacturing conditions are not finalized. Spending an inordinate amount of time on designing and evaluating cleaning protocols is usually not warranted. The economic gain of getting to market sooner typically outweighs the financial benefit of resin reuse.

In this article we first address basic principles of cleaning recycled chromatography resins and multipurpose equipment. We then discuss cleaning and cleaning validation for development and manufacturing. Specific issues in the final section include the potential of PAT to ensure consistent cleaning and recycled resins used for virus clearance.

Basic Principles

Here are some standard questions to address when considering cleaning for any unit operation: How will you measure cleanliness? What are you trying to remove? What is the best cleaning method? How clean is *clean*?

How Will You Measure Cleanliness?

Deciding which assays to use for cleaning protocol development and cleaning validation has been problematic in downstream processing, especially for resins and multiuse equipment. For years, the expectation was that product-specific assays would be used and a risk assessment of any detectable carryover performed to determine how that carryover might affect safety and potency of the final product. Many argued that product-specific assays would not detect anything after a column had been cleaned with harsh agents; whereas others found that there was, in fact, some cross-reacting material in ELISAs (**2, 3**).

However, in May 2005, the FDA acknowledged that total organic carbon (TOC) can be an acceptable method for evaluating cleaning effectiveness. Since the publication of the inspection guide on cleaning validation in 1993, a number of studies have demonstrated the adequacy of TOC in measuring contaminant residues:

> TOC or TC can be an acceptable method for monitoring residues routinely and for cleaning validation. In order for TOC to be functionally suitable, it should first be established that a substantial amount of the contaminating material(s) is organic and contains carbon that can be oxidized under TOC test conditions. This is an important exercise because some organic compounds cannot be reliably detected using TOC. (**4**)

Carryover might be overestimated when carbon-containing buffers are used because the background may be too high to use TOC. In this case, many companies use other assays such as HPLC and/or SDS-PAGE and include routine monitoring by UV, pH, and conductivity. The ability to use TOC and other nonspecific assays in downstream processing has been accepted by worldwide regulatory agencies. ICH guidelines indicate that the absence of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures (**5**). But it is always wise to discuss cleaning strategies in advance with the appropriate regulatory body.

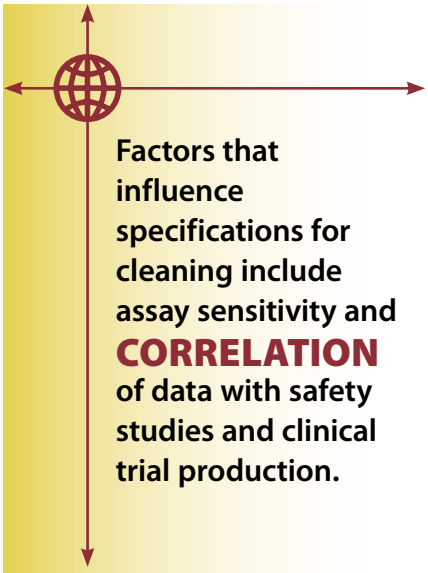
Visual inspection is always needed, but it is a fact that chromatography resins do change appearance with some feedstreams, notably those derived from *E. coli* fermentation, plasma, and plants. If discoloration occurs, it is important to evaluate where that color comes off. If the color is removed during column use, where that happens is crucial, and discoloration during product elution may represent a risk factor. However, many studies have been performed to extract discoloration from resins, and the results usually demonstrate that nothing takes off the color, including cleaning agents that destroy the resin. Still, if discoloration occurs, you should investigate using additional assays to demonstrate absence of risk from this phenomenon.

Figure 1: Sampling a column for cleaning evaluation using TOC; 1 (inlet valve), 2 (column gasket), 3 (base gasket), 4 (outlet valve) (WWW.GEHEALTHCARE.COM)

On-line TOC units are used to monitor and validate cleaning of chromatography skids and column hardware used for more than one product. Figure 1 illustrates sampling for TOC analysis of a column for cleaning validation of a manual direct (swab) sampling process. The sampling is performed at the sites that are most difficult to clean or classified as worst-case. Sampling those sites can be difficult at times and exposes equipment to potential contaminants. More and more bioprocess manufacturing facilities are implementing on-line TOC analysis with supporting in-line monitoring methods including UV, pH, and conductivity. Some TOC instruments are specifically designed to handle fluctuations in operating parameters such as pH, conductivity, flow, and temperature. Instruments such as the Sievers 900 and 500 RL on-line TOC analyzers have a selective membrane-conductometric detection system for determining true TOC content within a sample. The patented technology is not subject to interfering compounds that contain nitrogen, sulfur, or halogens such as chlorine that may be present during a cleaning process.

What Are You Trying to Remove?

Despite the acceptance of nonspecific assays for evaluating cleaning in downstream processing, to design a cleaning protocol you have to understand the basic chemistry of what you are trying to remove and the nature of the surface from which you want to remove it. Chromatography resins have large surface areas and present opportunities for multiple types of interactions. For example, unfolded hydrophilic proteins are likely to have exposed hydrophobic binding sites that



potentially interact with a resin cross-linking structure. Equipment surfaces also affect cleaning efficacy. One study demonstrated that an early feedstream with multiple components spiked onto glass resulted in poor recovery on the order of 55%. On the other hand, removal of a relatively pure protein from stainless steel led to a recovery of about 89% (**6**).

It is important to evaluate cleaning methods continually as feedstream changes are made in development. Addition of new components upstream — notably lipids — is likely to lead to problematic cleaning downstream.

What Is the Best Cleaning Method? In addition to understanding what you are trying to remove from which surface, designing a suitable cleaning protocol for a specific chromatographic step requires evaluating the compatibility of the resin and associated equipment wetted surfaces with available and affordable cleaning agents, the capability to use a cleaning agent in production environments, and ability of the cleaning protocol to deliver a resin meeting its acceptance criteria.

Vendors typically provide data on chemical resistance of resins and equipment wetted surfaces. This information may be provided in a regulatory support file or a drug master file. Regardless of the format in which such information is supplied, it is essential that the company using the resin or equipment has the information on hand during regulatory inspections. Companies have been cited for using cleaning agents known to degrade chromatography resins and not performing their own studies to demonstrate that no harmful effect decreased performance or resulted in leachables. Using data provided by the vendor is a good start and can reduce the need for chemical compatibility studies, but every company must demonstrate that the cleaning agent is appropriate for its application. Over the years, various cleaning agents have been proposed. When evaluating a new cleaning agent for a resin, make sure it is compatible with system components as well. Destruction of O-rings and other column components by a cleaning agent is a risk to a process and may even be a patient safety risk.

For resins, the most commonly used cleaning agents are sodium hydroxide and sodium chloride. Various wash sequences should be tested in an early evaluation: for example, low salt, followed by high salt, followed by NaOH to strip and clean an ion-exchange column. You should be aware in the protocol design that high salt may increase hydrophobic interactions, thereby exacerbating protein precipitation on a column, even one that is intended for hydrophilic feedstreams. Once an optimal sequence, concentrations, and volume are established, it is often economically prudent to combine strip and wash steps to reduce downtime.

Some situations require additional cleaning agents. When selecting a cleaning agent, make sure you can detect it to demonstrate its removal before reuse of resins and equipment. For example, when an anion-exchange column is loaded with a high level of DNA during protein purification, it can be very difficult to remove that DNA. Some companies have used DNase to clean the column. In such a situation, you might compare the cost of disposing the resin to the cost of the cleaning agent and the validation of its removal. Alcohols are sometimes used for cleaning, and their removal must also be validated.

A safety margin should be factored into the cleaning protocol. This margin can be determined on a small scale by increasing the concentration of cleaning reagents and/or extending contact time — which is probably the most important factor for cleaning resins. It is quite common to see a further increase in cleaning after a cleaned, cycled column is stored. Routines for flushing a packed column after storage should be established. Many companies use TOC to establish the baseline before reequilibrating columns for the next batch.

During cleaning protocol development, consider the capabilities of manufacturing, especially worker safety. At large scale, the volume and concentration of alcohols may require working in an explosion-proof environment. Some reports indicate that heated sodium hydroxide is an excellent cleaning agent. Even without the heating, high concentrations of sodium hydroxide require facility and equipment evaluation and use of safety equipment to protect workers.

How Clean Is *Clean*? This is one of the most problematic areas in cleaning. If you don't detect anything, does it mean that a chromatographic skid and packed column are clean? Factors that influence specifications setting for cleaning include assay sensitivity and correlation of data with safety studies and clinical trial production. No general rules for biopharmaceutical products exist such as those published some years ago for multiproduct drug manufacturing (**7**). Without other information, you can always take those numbers as a starting point. But a risk assessment is essential to developing acceptance criteria. According to the ICH document Q9 on risk management, the risk to quality should be based on scientific knowledge and linked to patient safety (**8**). Patient population, product indication, and dose should be factored into the assessment. When you use TOC to set carryover limits, assume that the total level of carbon measured is derived from the highest risk factors. The analytical method measures native protein (if it survives the cleaning process) and degraded protein, as well as other organic components such as buffers and potential impurities.

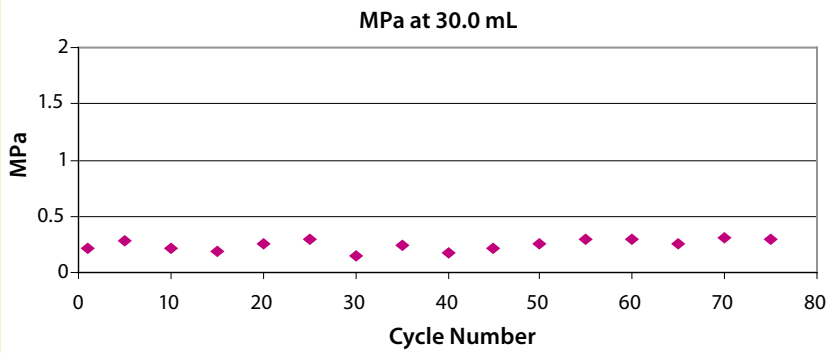
Adequately cleaned columns should repeatedly provide an intermediate product that possesses its critical quality attributes. This means, of course, that you must understand the product and the impurities that are removed in every purification step. An example is provided here for a capture step for an *E. coli* homogenate. The Capto Q resin was cleaned for four hours with 1 M NaOH after each cycle. Column packing consistency (plates/m), dynamic binding capacity (QB 10%), carryover of a specific impurity (endotoxin), backpressure, ionic capacity, and appearance were evaluated up to 79 cycles (Table 1 and Figure 2). No discoloration of the resin was observed after repeated use. The ionic capacity changed from 0.19 mmol Cl–/mL in the unused resin to 0.17 mmol Cl–/mL after the last cycle. The dynamic binding capacity decreased by 2.6% after 39 cycles and by 10% after cycle 79. All together, the total cleaning time with 1 M NaOH was 316 hours.

Table 1: Performance and carryover measurements after cycling; each cycle included cleaning for four hours with 1 M NaOH

Cycle Number	QB 10% (mg/mL Capto Q)	Endotoxin in Blank Runs (EU/mL*)	Column Packing Efficiency (plates/meter)
0	116	21	2,986
1	120	9.6	3,134
11	116	90	3,146
20	115	36	3,186
29	116	20	3,155
39	113	6.5	3,139
50	108	72	3,126
59	109	20	3,328
69	105	307**	3,180
79	104	11	2,986

* Clarified *E. coli* homogenate: ≈2.5 x 10^6 EU/mL ** Thought to be due to contamination

Figure 2: Backpressure over 80 cycles; an increase in backpressure indicates that a column has been inadequately cleaned.



Cleaning in Development and Production

A robust process cannot be designed on dirty columns and equipment. Appropriate cleaning methods must be used in early research and development stages. One of the more problematic issues related to cleaning during development is the continual modification of feedstreams due to optimization of upstream processing. When additives such as antifoams are added upstream, they can adhere to columns and present a new cleaning challenge. Communication between upstream and downstream groups is essential. Still, this means that a cleaning protocol is unlikely to be finalized until a process is finalized.

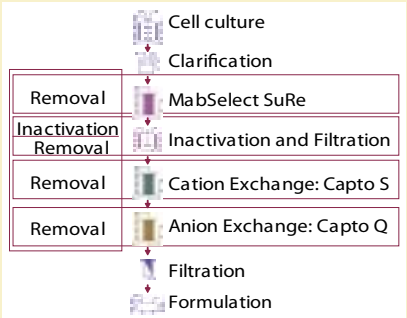
Keep in mind that all therapeutics used in clinical trials must be made according to CGMPs. At this stage, however, incomplete knowledge about feedstream consistency and components might stress a given cleaning protocol. The use of multiple, orthogonal, in-process assays provides confidence that cleaning is sufficient to ensure production of safe products. Blank runs can be very informative. At these early stages, measuring UV, conductivity, pH, and total carbon can routinely assess cleanliness.

As soon as a production feedstream is available, it should be used to reevaluate cleaning protocols and assays for assessing cleanliness. Those assays should be validatable, but at development stages (e.g., phases 1 and 2), qualifying them through system suitability testing is usually sufficient. Once a process is finalized, cleaning validation can begin. For chromatography resins, cleaning validation is best carried out by a combination of small-scale and production-scale studies. The small-scale studies must represent production scale, and they require a validated small-scale model. That model can be used for evaluating repetitive cycles that include cleaning and also factor in storage conditions (lifespan studies).

Clearly, such studies require documented design and implementation criteria so that their results will be acceptable to regulatory authorities. During manufacturing of conformance batches, cleaning validation is performed for packed columns at scale. Routine monitoring after those batches is essential. Today, periodic blank runs are expected to be performed in manufacturing to assess continued cleaning efficacy. Blank runs are typically performed every five to 10 cycles, but it is up to each company to decide what is appropriate based on existing knowledge.

Once production begins, the cleaning protocol must not be changed without evaluating safety and regulatory ramifications. At manufacturing scale, discoloration may become more apparent. Manufacturing personnel have, in the

Figure 3: A platform technology for MAb production (using GE Healthcare products); virus clearance is achieved by removal and inactivation.



past, decided to change cleaning contact times to enhance continued use of a packed resin. But this change can put those batches made with the new cleaning protocol at risk. Changing cleaning contact time will invalidate the small-scale prospective lifetime studies. It is particularly problematic and costly when virus clearance studies have been performed on aged resins whose integrity has not been validated under the extended cleaning contact time.

At manufacturing scale, it is also possible to unpack columns and reslurry them to get a better cleaning effect by increasing resin exposure to cleaning agents. However, this practice must be written into standard protocols. In addition, because handling large quantities can lead to resin attrition, consider how replenishment will be made. Will you have back-up and qualified resin? Can it be a different lot? How much resin loss can be tolerated while still ensuring consistent performance?

Specific Issues and Opportunities: PAT and Virus Clearance

Process analytical technologies (PAT) are intended to assure acceptable end-product quality at the completion of a process (**9**). The ability to use in-process controls and feedback for cleaning is an excellent opportunity for applying PAT to biotechnology. If during a cleaning cycle an analytical method indicates that the cleaning is insufficient, perhaps the contact time can be extended and resin quality also measured using the PAT concept. The future of applying this concept in cleaning chromatography columns depends on both assay sensitivity and appropriate feedback mechanisms. It is conceivable that blank runs in manufacturing could be eliminated if sufficiently sensitive analytical methods could eliminate potential masking of impurities by the feedstream. This would enable continual control of cleaning and, at the same time, potentially reduce production costs incurred by downtime during blank runs.

Virus clearance is achieved by both inactivation and removal. Chromatography enhances overall viral safety by removal and is used in conjunction with both virus filters and inactivation techniques in most platform technologies (Figure 3). With increased focus on better science and risk management, it seems appropriate to consider the need for virus clearance studies on used resins. The risk may be considered high for plasma-derived products, but it is low for mammalian cell culture of commonly used, low-risk cells such as CHO. CHO cell lines have been used for decades now, and no infectious retroviral particles have been detected. If such a cell line is used, unprocessed bulk is tested for virus infectivity, basic principles of GMP are put in place to control adventitious viruses, and chromatography is used to enhance overall virus safety, might the industry and regulatory agencies reconsider the need for used-resin viral-clearance studies?

Several studies have, in fact, been performed in a collaborative effort of the FDA and biotechnology companies. For example, one publication demonstrated that if anion-exchange columns operated in flow-through mode are cleaned with solutions that do not degrade the resin, and if accumulating backpressure, band spreading, and DNA clearance are measured, resin deterioration would be detected long before virus clearance decreased (**10**).

There has always been concern that viruses would be carried over on chromatography columns. As the biotechnology industry gains more experience, especially with platform technologies such as those used for monoclonal antibodies, it appears that a new approach to lifetime studies that (as always) include cleaning cycles can be considered. If performance, in this case virus removal, is the determining factor for continued reuse of a resin for enhanced viral safety, then in-process surrogate markers such as DNA (**9**) may provide scientific justification for eliminating end-of-resin-lifetime virus clearance studies.

Advancing Technology

There is a great deal of available information on cleaning and cleaning validation. Basic principles are described in several publications, but much of the information is outdated (**11–13**). Those publications provide general information but do not address the specifics of cleaning chromatography resins. In process chromatography, the first decision to make is whether to recycle or dispose of resins. Once you decide to recycle, it is essential to design a robust cleaning protocol that is eventually tested with production feedstream and validated, typically by a combination of small-scale and production runs. A critical element is selection of practical analytical tools to monitor cleaning performance. Setting specifications for cleanliness is a challenge and requires a risk assessment. Implementation of scientific and technological advances will enable more in-process measurements and feedback controls for cleaning of packed columns and multiproduct systems.

Acknowledgments

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