Establishing Resin Lifetime

Key Issues and Regulatory Positions

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Resins, chromatography media, gels . . . whatever you choose to call them, establishing their useful lifetime remains a critical issue for those producing biological and biotechnological therapeutic and diagnostic products. Several factors contribute to the importance of determining resin lifetime. One is the need to consistently produce intermediates and final products with defined quality and safety attributes. A second factor is process economics. Economical production may require repeated use of packed columns. Another factor is the inability of existing technologies to show us what is truly happening at the surface of materials that are used to produce high purity biotherapeutics and diagnostics. As a result, resin lifetime continues to be discussed at conferences and questioned by regulatory authorities for product licensure and during inspections.

Product Focus: Biotherapeutics and diagnostics, chromatography

Process Focus: Downstream processing, chromatography, resin lifetime

Who Should Read: Process scientists, regulatory affairs, quality assurance, validation

Keywords: Chromatography, resin lifetime

Level: Intermediate

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The very large surface area that enhances chromatographic separations also allows for multiple interactions with product and impurities. Proteins, nucleic acids, lipids, and other substances in the feedstream often become stabilized when immobilized at resin surfaces. Stabilized impurities and residual product and by-products can be washed off during cleaning and sanitization cycles, but some may remain and be slowly removed with extended resin storage time or eluted with product during the next production cycle. Detection methods have improved over the past decade or so, and techniques such as PCR may enhance our ability to understand what, if anything, remains bound to the resin surface. Cleaning, sanitization, and storage procedures that preserve resin function and integrity are essential for obtaining reasonable resin lifetimes.

In this article, current regulatory positions and citations related to resin lifetime lead to discussion of topics being addressed by industry and regulators. These topics include concurrent validation, measurable column attributes that might correlate with resin performance, viral clearance, and factors that affect resin lifetime. Although performing lifetime studies can be time-consuming and expensive, such studies provide confidence in the cost-effective production of pure and safe biotherapeutics and reliable diagnostics.

Current Regulatory Positions and Citations

"Number of times purification columns can be used not validated." This 483 was listed in a publication in April 2002 (1). But this type of 483 is not unusual. In January 2002, Andrew Chang of CBER presented a collection of the FDA's findings related to chromatography (2). In a review letter, a sponsor was told to

> Please provide validation data to demonstrate there is no negative impact of extended use of the . . . matrix to 150 production cycles on efficacy of cleaning and regeneration of the . . . column. Please provide data that show

> complete removal of viral contamination prior to reuse of the system.

In some postapproval inspections, comments included

> Storage times in between runs for all of the purification columns have not been validated for entire life cycle of column. Cleaning validation study was conducted only for up to five uses of the column, which could be used in the purification of up to 46 lots per laboratory scale study. Cleaning validation including LAL and bioburden studies of the . . . purification columns were only validated for up to five uses. In addition, the cleaning validation of these columns did not include removal of process-related impurities. However, columns can be used for following number of purification runs/years. . . . Storage in buffer not tested for attrition.

FDA's therapeutic compliance program guide, which serves as a guide for investigators, states that

> There should be an estimated life span for each column type, i.e., number of cycles. Laboratory studies are useful, even necessary, to establish life span of columns. There are situations where concurrent validation at the manufacturing scale may be more appropriate. Continued use may be based upon routine monitoring against predetermined criteria. (**3**)

At a PDA/FDA conference on process validation in 2000, Barry Cherney presented CBER's expectations on determining resin lifespan (**4**). He noted that the 1997 FDA *Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use* states that limits must be prospectively set (**5**). This PTC document reflects FDA's opinion not only for monoclonal antibodies, but for other biotherapeutics.

The ICH *Guideline on Viral Safety* states that "over time and after repeated use, the ability of chromatography columns and other devices used in the purification scheme to clear virus may vary" (**6**).

European Regulatory Authorities have expressed concern over consistency of chromatographic performance. One CPMP position statement noted that elimination of host cell proteins, in most cases, makes use of chromatographic columns for which the selectivity and yield of the procedures depend not only on the quality of the material, but also on storage conditions, sanitization, life span, and the way the columns are used and reused (**7**).

Current Issues in Lifetime Studies

Some current issues widely discussed in the biotechnology industry include the potential to use concurrent rather than prospective validation, correlation of resin performance with readily measurable attributes, correlation of performance with viral clearance, cleaning and sanitization of packed columns, and economic aspects of lifetime studies.

Concurrent Validation. Recently reported industrial experiences indicate that concurrent validation may be a useful strategy for some sponsors. However, this validation approach obviously requires considerably more in-process monitoring. If a go–no-go decision for a process step depends on data from a previously run chromatography step, waiting for the results of an assay may cause a delay. Validation of maximum allowable holding time with concomitant acceptance criteria such as product stability and bioburden control need to be performed. Test results will confirm that forward processing criteria have been met. However, in the event of an assay failure, critical economic decisions will have to be made regarding a sponsor's ability to continue processing at risk until the root cause of the out-of-specification result can be determined. Concurrent validation can potentially cause more failed batches unless

Definitions, Resources

483 (FDA-483) Form presented to a company by an FDA investigator at the end of an inspection indicating what the inspector believes may be violations of the law.

Concurrent Process Validation A subset of prospective validation, used only in special cases and with approval of the quality authority: Batches are released for distribution based on extensive testing and data generated during actual implementation of a process.

CPMP The EMEA *Committee for Proprietary Medicinal Products;* responsible for regulating human medical products in the European Union.

HSV *herpes simplex virus*

ICH The *International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use.*

LAL *Limulus* amebocyte lysate assay (from the blood of *Limulus* horseshoe crabs) for detecting pyrogenic endotoxins.

MuLV *Murine leukemia virus(es)*

PCR *Polymerase chain reaction,* a process that exponentially reproduces a short piece of DNA.

SV-40 Simian virus 40

TOC *Total organic carbon analysis,* a method used to test pure water and to validate cleaning procedures.

rapid, adequately sensitive, in-process monitoring tools are used.

Before deciding that concurrent validation is a viable strategy, it makes sense to determine what claims are made for each chromatographic step, what assay methods are available, and how long it takes to perform the assays. When viral clearance is claimed, concurrent validation — with rare exceptions — is not an option.

Column attributes. The biotechnology industry has seen recent reports from both sponsors and FDA on attempts to correlate performance attributes over time with key functions performed by packed chromatography columns. In 2001, Cindy Oliver of MedImmune presented observations on column failure and manufacturing surveillance (**8**). For immobilized Protein A columns, MedImmune found a decay of dynamic capacity with repeated use. For anion-exchange chromatography, a decrease in the removal of key impurities was observed. But with a cation-exchange column, no identifiable sources of column failure could be identified, even after extensive evaluation of cleaning, capacity, removal of impurities, yield, and purity. If no sources of column failure can be found, is it perhaps possible to use the column ad infinitum? It seems at least that if the process monitoring tools sufficiently ensure product quality, such continued use can be justified.

Genentech compared resin lifetime at small-scale and manufacturing scale for Protein A columns and for a cation exchanger (**9**). The small-scale cation exchange step was evaluated every 10 cycles for up to 51 cycles for yield, removal of host cell proteins, DNA, and removal of Protein A from the previous step. No significant changes were measured. The manufacturing runs also showed continued consistent product yield and removal of impurities up to 50 cycles. Likewise, the Protein A column showed consistency with repeated use. In fact, those columns were run more than 300 cycles at small scale and up to 225 cycles at manufacturing scale.

Correlation of performance attributes. In a recent poster presentation, Kurt Brorson of FDA's Division of Monoclonal Antibodies presented some findings related to resin reuse (**10**). He found that for an immobilized Protein A column, antibody step yield and breakthrough were performance quality attributes that decayed before decreases in monoclonal antibody eluate impurities, including retroviral particles. As a result, it is proposed that retrovirus removal validation studies need be performed only on new Protein A columns, but antibody step yield and breakthrough be monitored during Protein A unit operations. This study did not address nonenveloped adventitious virus clearance or virus carryover between cycles, but more reports may be forthcoming.

In most cases, viral clearance remains constant and even increases with extended resin reuse. This was noted by O'Leary et al. (**9**) and Darling (**11**). In the work described by Darling, multiple products were evaluated for approximately 100 runs each. Both anion and cation exchangers demonstrated consistent removal of polio virus over those runs.

However, a potential risk is associated with a concurrent validation strategy when the chromatographic step is used to remove potential adventitious viruses (**6**). Tom Smith of GSK presented a study in which hydrophobic interaction chromatography (HIC) was used following a Protein A column (**12**). The HIC column was shown to provide consistent product yields of 72% or better, aggregates of no more than 10%, and good Protein A removal (0.4 ppm) over 90 cycles. Although viral clearance remained consistent up to 91 cycles for two enveloped viruses (MuLV and HSV-1), the log reduction value for SV40, a nonenveloped virus, was decreased significantly in the 91st cycle.

Reduction in viral clearance with continued use of a column that appears by all other measured parameters to be performing according to specifications is uncommon — but it does happen. Extensive review of the database from the BioReliance contract viral clearance laboratory, which has performed thousands of these studies, shows that occasionally viral clearance decreases. Viral infectivity assays are generally lengthy and expensive. Performance of such assays for every cycle would not be a viable option. PCR would be a better choice, but noninfectious viral sequences may be detected, and a sponsor would have to define an acceptable limit for each potential virus and the assays performed after every cycle. This approach does not appear to be reasonable.

Factors that influence resin lifetime were addressed in a book chapter that also presented experimental approaches to determining and validating resin lifetimes (**13**). Those factors include the nature of the feedstream and raw materials used in its purification, the resistance of the resin to cleaning and sanitization agents, and proper storage conditions.

If mold spores contaminate a packed column, for example, it is highly unlikely that any cleaning or sanitizing agent can restore that column to its original function. Although some agents such as peracetic acid may inactivate mold spores, they are likely to degrade system components and are usually hazardous for the workers in the plant. The degradation not only destroys system capabilities, but

extractables that are toxic may be washed onto resins. Compatibility of the system components with potential cleaning and sanitization solutions should be investigated during development to ensure that breakdown products from those components do not degrade resin performance. In most cases, this investigation can be satisfied with data from suppliers and compliance with suppliers' specifications.

Storage affects performance in subsequent cycles. Contact time has a major impact on cleaning efficacy. For years, nucleotide-like and protein fragments have been found in the storage effluent of columns that appeared completely cleaned before storage. Even highly sensitive methods did not pick up carryover in blank runs performed after cleaning and sanitization, but residual carryover can sometimes be observed after storage. One published case study showed that storage in dilute alkali, followed by removal of solubilized material, enhanced cleaning and reduced carryover (**14**).

Carryover limits should be established and correlated with final product safety. Although it is optimal to have no carryover, some realistic limit may be acceptable for early purification steps. That limit will be based on the capability of current analytical methods. When no carryover is claimed and a more sensitive method is applied, some residual product degradants might be found. Setting realistic, achievable specifications will enable continued resin use.

Efficacy of cleaning is best assessed by a two-pronged approach. Using validated, small-scale models for development of the optimal cleaning/sanitization protocols saves both time and money. Production feedstream is used, and blank runs after repeated cycles of equilibration, sample application and elution, cleaning, and sanitization can be used to assess the adequacy of cleaning.

Evaluation by UV profile analysis, TOC, total protein analysis, electrophoresis, and product-specific assays can be useful for evaluating carryover. At this small scale, the media can be stressed with longer contact times and higher concentrations of cleaning/sanitization agents. Particularly problematic residuals, such as DNA on anion exchangers, should also be examined during these development studies.

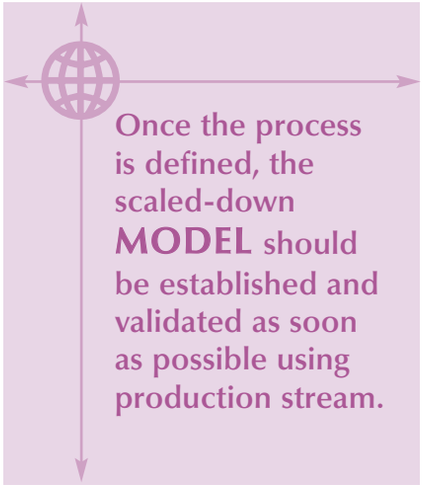
During production of the conformance batches, extensive analysis should be used for cleaning validation. During manufacturing, verification of cleaning and sanitization must be routinely performed to ensure continued resin reuse. In addition to protein carryover, endotoxin and bioburden analysis should be monitored. New rapid microbiological detection methods may prove to be very useful for downstream processing (**15**).

Single-use columns are preferred in some situations. If highly resistant hazardous materials are bound to the resin to remove them from the product, it may be more economical to use a less expensive resin and inactivate that agent, then dispose of the resin. In some cases, storage space is insufficient for columns not routinely used in production. In other cases, for resins used infrequently, the time and costs associated with validation of storage conditions and establishing acceptance criteria for resin reuse may exceed the cost of packing a new resin when it is needed.

On the other hand, large columns can be expensive to repack, requiring hoists to lift column lids and extensive testing for packing quality. This is particularly true for gel-filtration columns and other chromatographic modes that require high resolution to achieve the desired separation. Performance of an economic analysis will ensure the appropriate decision regarding the effort to be spent on demonstrating and validating resin lifetime.

Ensuring Success

For prospective resin lifetime studies, a scaled-down model that can be validated is essential. Inaccurate scale down has been a cause of regulatory citations and significant delays. Once the process is defined, the scaled-down model should be established and validated as soon as possible using production feedstream. This is best done in a location where extensive analytical



tools can be used to demonstrate that product purity and recovery and impurity profiles are the same as those found in manufacturing. If viral clearance is to be claimed, one should evaluate the effect of spiking by using mock spikes that contain everything but the virus.

Regulatory observations commonly cite manufacturing changes that invalidate the small scale studies — studies that no longer reflect current manufacturing conditions. In most cases, this is a disconnect between manufacturing and the development group who performed the small-scale clearance study. In one cited case, the manufacturing group increased the frequency of cleaning and sanitization between cycles, which invalidated an extensive lifetime study.

It is important to evaluate parameters that affect resin performance during development and make sure they are maintained. Cherney of the FDA noted that a resin is expected to continue to perform all its intended functions (**4**). The sponsor must determine

each chromatographic resin's intended function during development. These functions might include, for example, removal of defined amounts of host cell proteins, nucleic acid, or other impurities. Column integrity must be maintained, and cleaning, sanitization, storage, and regeneration procedures must remain effective according to predetermined specifications.

Future developments in analytical tools may enhance our understanding of what is occurring on the resin surface and also provide knowledge that will further our ability to use concurrent validation for resin lifetime. There is even talk of taking a generic approach to resin lifetime.

By now, we're all aware of the impending change from CBER to CDER for U.S. approvals of biotechnology therapeutic products. Will we see a change in the approach to lifetime studies? Only time will tell. However, the goal of producing safe and efficacious products consistently, lot after lot, is not going to change. Resin lifetime remains a key issue.

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