Building Process Control into Chromatographic Purification of Viruses, Part 2

Purification As a Tool for Enhancing Process Control

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Purification represents a nexus in the manufacture of biopharmaceutical products: a point at which all manufacturing components and their individual variations converge. This challenges process developers to configure the components in ways that either cancel or minimize the potentially negative impacts of their respective variations. This is fully as important as achieving a specified level of purity because this is where it is established that a process will reproducibly yield high-quality product. One hallmark of chromatography methods is their ability to support this aspect of process development.

The more fundamental role of purification is to remove contaminants. Numerous chromatography methods have been reported for virus purification, including affinity (**1–4**), size exclusion (**4–8**), ion exchange (**6–12**), hydrophobic interaction (**7, 8**), and hydroxyapatite chromatography (**12**). Many studies comment that even single-step chromatography procedures achieve better purity than multistep density-gradient–based methods.

This two-part article focuses on chromatographic purification of viruses for human therapeutic applications. In Part 1 (*BioProcess International* 4(10) 2006: 22–30) we discussed qualification of raw materials. Now in Part 2 we discuss opportunities to enhance process control in purification process development, highlighting ways to maximize process control in chromatographic purification of viruses. Our suggestions are based on approaches that have been shown to have practical value in the manufacture of recombinant protein therapeutics and development of emerging viral products. Please refer to pertinent regulatory documents for more information (**13–20**).

PRODUCT FOCUS: ALL VIRAL PRODUCTS

PROCESS FOCUS: DOWNSTREAM PROCESSING

WHO SHOULD READ: PROCESS DEVELOPMENT AND MANUFACTURING

KEYWORDS: PROCESS OPTIMIZATION, CHROMATOGRAPHY, VALIDATION, QUALIFICATION, VACCINES, GENE THERAPIES

LEVEL: INTERMEDIATE

ORTHOGONAL PROCESS DESIGN

Orthogonal process design is the foundation of well-controlled purification procedures (**21**). The idea is that combining the steps with the greatest complementarity should provide the best overall purification. The strongest embodiment of the concept is usually achieved when respective steps are based on distinct separation mechanisms. For example, a two-step process that contains one fractionation step based on product size and another on product charge would be considered orthogonal; similarly, a process with one step based on product charge and another on hydrophobicity would also be orthogonal.

Artist's conception of a virus
RAFAL ZDEB (WWW.ISTOCKPHOTO.COM)

An important feature of orthogonal process design is that the purification capability of any one step is measurable only within the context of its potential partner(s) (**22**). This is illustrated in Figures 1 and 2. In Figure 1, each step is capable of achieving 90% purification from raw product, but a major contaminant coelutes with the product in both steps. In Figure 2, the first step is the same as in Figure 1. The second step achieves only 60% purification from raw sample, but when combined with

Figure 1: A two-dimensional plot of results from two different chromatographic methods. In spite of good fractionation by both methods, a common contaminant (black) coelutes with the viral product (blue). The plot shows that this combination of methods will not yield pure product. DNA is indicated in red. See text for discussion.

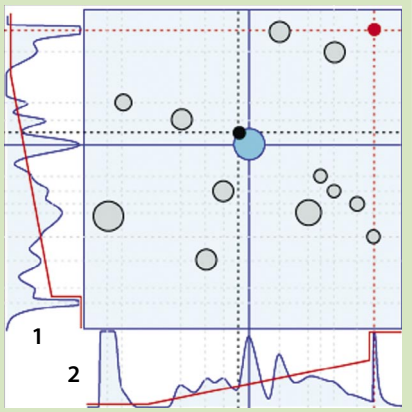


Figure 2: A two-dimensional plot of results from two different chromatographic methods. Method 1 is the same as in Figure 1. Method 2 is a different method, which by itself produces a relatively poor fractionation but yields pure product in combination with method 1. This is indicated by the lack of contaminants overlapping the product. DNA is indicated in red. See text for discussion.

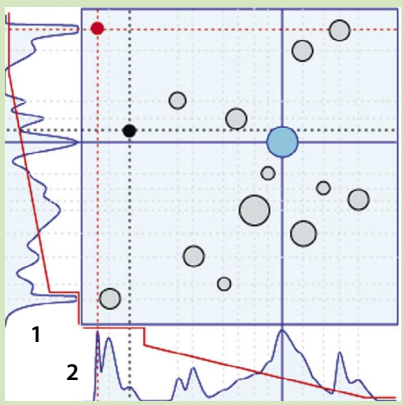
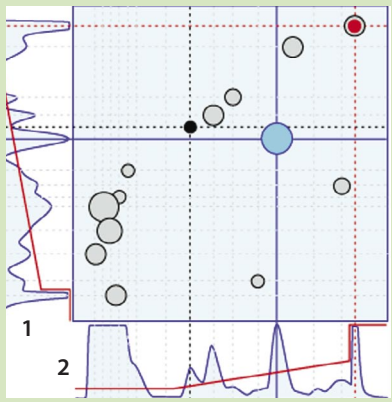


Figure 3: A two-dimensional plot of results from two different chromatographic methods. Method 1 is the same as in Figures 1 and 2. Method 2 is a different method than the previous. This combination of methods achieves the same purity as in Figure 2, but the larger open area around the product indicates that this combination should be more robust. DNA is indicated in red. See text for discussion.



step 1, the result is 100% purity because no major contaminants coelute between the two steps. This reveals the inherent fallacy in judging individual purification methods based solely on their purification factor. Context is paramount.

It is impossible to predict what combination of separation methods will work best for a given virus purification process, and how many steps will be required to achieve the degree of purification required to support a particular application. Development begins with screening potential candidates under various conditions, then evaluating the respective results for the highest degree of complementarity. It then proceeds to identify the smallest subset of methods that fulfills the application's needs. Purification procedures for protein-based therapeutics from cell culture commonly use three chromatographic steps but may use more depending on source material and application.

Figures 2 and 3 illustrate how maximizing complementarity among steps reduces the impact of variation and thereby increases process control. In Figure 3, the two steps provide the same purity as in Figure 2, but their complementarity is greater. None of the contaminants eluting near the product in the individual steps co-occur with the product in the combined process. This implies that the result will be more insulated from material or process variations. The implication needs to be proven, but the probabilities are more favorable than for a process that is already operating close to its tolerance limits under the best of circumstances.

Table 1: Relative retention of DNA and endotoxin by different chromatographic methods

	Contaminant	
Method	**DNA**	**Endotoxin**
Cation exchange	Unbound	Mostly unbound
Hydrophobic interaction	Unbound	Mostly unbound
Bioaffinity	Unbound	Mostly unbound
Anion exchange	Strongly bound	Strongly bound
Hydroxyapatite	Strongly bound	Variable, strongly bound

The other side of the complementarity coin is redundancy. *Redundancy* has a special meaning in chromatography: It refers not to repetition of the same chemical separation mechanism, but to the compound ability of complementary mechanisms to reduce levels of a specific contaminant or class of contaminants. This has particular value for process control because it relates to contaminants that must be reduced to extremely low levels (e.g. DNA, endotoxin, and contaminating virus) (**21**). From the perspective of DNA removal, all methods in Table 1 are redundant, even though they achieve redundancy through different mechanisms. This is also reflected in the distribution of DNA in Figures 1–3.

Linear Gradients, Step Gradients

The majority of chromatography-based industrial purification processes use step gradients. Most development and industrial chromatography systems can easily accommodate linear gradients, but such gradients tend to use larger volumes of buffer. Process water also has proven to be a major expense (**23**). Linear gradients may require collecting a larger number of fractions. This complicates plumbing at process scale and makes it necessary to perform more assays to determine which fractions to pool going into the next step.

Linear gradients have important advantages despite these issues, especially in terms of process control (**22**), and by providing real-time process monitoring. The evolving elution profile itself provides a continuous real-time index of process status and generally allows determination at a glance — whether or not a process is operating within specification. The finished elution profile provides hard-copy documentation that the process has been conducted within established specifications. Neither benefit is obtained with step gradients, which

produce a single peak per step without respect to the number of components eluting within that step.

The most important advantage of linear gradients is that they provide better process control. In the present context, this refers to their ability to absorb process variation, which in turn translates into better process consistency. Most process variables either weaken or strengthen chromatographic retention (**24**). The effect of such variables on a linear gradient is to shift the entire profile laterally but conserve the elution relationships of individual peaks (Figure 4). Figure 5 contrasts the effects of the same variation with a corresponding step-gradient elution of the same hypothetical sample. The leading contaminant elutes partially within the product peak, and the product elutes partially within the trailing contaminant peak. Product purity and recovery are both compromised.

The ability of linear gradients to endow better process control can be especially valuable at early stages of process development, when sources of process variation are not yet fully characterized. By the time a process is ready for licensure, sources of variation should be fully characterized and minimized. In parallel, step-gradient intervals should have been defined to ensure the ability of the purification process to reproducibly achieve the necessary fractionation.

Figure 4: The effect of external process variation on linear gradients. The blue component represents the viral product. The gray components represent contaminants. Profile A illustrates a gradient under ideal conditions. Profile B illustrates the difference when external variation is introduced. Variation like this might derive from process buffers, chromatography media, temperature, or other variables. As shown, the entire profile is shifted to the right but the separation is conserved, illustrating the ability of linear gradients to enhance process control. See text for discussion.

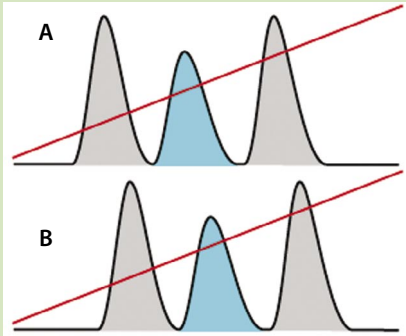
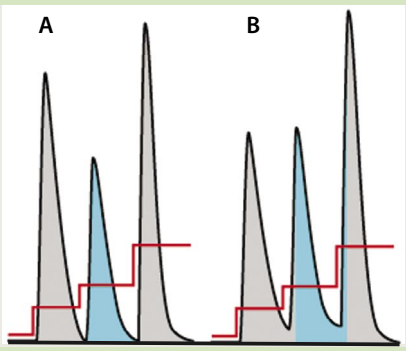


Figure 5: The effect of external process variation on step gradients. The blue component represents the viral product. The gray components represent contaminants. Profile A illustrates a gradient under ideal conditions. Profile B illustrates the difference when external variation is introduced. Product purity is compromised by partial elution of the leading contaminant in product peak. Recovery is compromised by loss of product in the trailing contaminant peak. See text for discussion.



Media Use (and Reuse)

The dominant material costs in bioprocessing are for chromatography media. It is therefore not surprising that many manufacturers seek to improve process economy by using a single lot of media for multiple process cycles (**25**). Some chromatography media have been validated to retain function for more than 1,000 manufacturing cycles, reducing the net cost per cycle to virtually nothing (**26**). This provides a powerful economic incentive for reuse. On the other hand, the single use of a chromatography column or other format has the potential to alter its function, making reuse a key process control issue (**16, 23, 26–29**).

The first step is development of cleaning, sanitization, and storage procedures that remove all contaminants and keep the media clean while restoring original function. The next is to demonstrate that no contaminant carryover occurs from one virus product lot to the next. That applies especially to microbial contaminants and endotoxins. In practice, personnel, process water, and the manufacturing environment are the most common sources of bioburden, but examples have been cited in which sanitizing reagents contained resistant, contaminating microbes (**30**). Lot-to-lot carryover of viral product is also a potential concern because it threatens lot integrity — and because product carryover also implies carryover of contaminants. Carryover can be measured after conducting a run without sample after a normal run (**26**).

The second major consideration in reuse is to demonstrate conservation of capacity and fractionation performance. Performance testing may include the same parameters used to evaluate incoming lots of the same media (see Part 1). Data from scale-down manufacturing simulations provide an additional dimension of characterization (**26, 29**). An increase in backpressure may indicate an accumulation of impurities, compression, or chemical breakdown of column media. Other tests may include comparison of elution profiles, comparison of product yield and purity, and clearance of specific impurities. Used chromatographic media are not expected to retain 100% of their original function, but at the end of their designated lifetimes they are expected to completely fulfill specifications for the processes in which they are used.

Reuse or Single-Use: Although reuse is driven by process economy, the expense of developing procedures for cleaning, sanitization, and storage — *and* the expense of developing the assays to validate their effectiveness, *and* the expense of materials and labor to conduct these procedures in a manufacturing setting — has created an interest in single-use products, especially in virus purification. Some chromatography media, such as ion-exchange filters, are marketed specifically for this application, but strictly speaking, any chromatography medium can be designated for single use. The choice represents a balance between capability and cost.

Fresh media can be used for preparation of early clinical lots, even if media are intended for multiple use in a final manufacturing process. Fresh media may be needed for use in early clinical trials, given that cleaning, sanitization, and storage procedures may not yet be validated. The purification process used for

preparation of phase 3 clinical material should follow the intended manufacturing process, including media restoration. In addition, comparability studies may be necessary if clinical data are to be used from viral products produced under different conditions (**20**). If a particular chromatography material is going to be used as a disposable, it should be designated accordingly before this point.

External Process Variation

Inadvertent microbial or endotoxin contamination can have a major impact on product safety. Cleanliness of the processing environment is therefore an important control issue. Chromatography can improve process controls by minimizing the potential impact of nonideal processing environments (**30**). Even the simplest chromatography systems can be configured to protect a product from external exposure before, during, and after separation, and such systems also can be sanitized to prevent internal exposure.

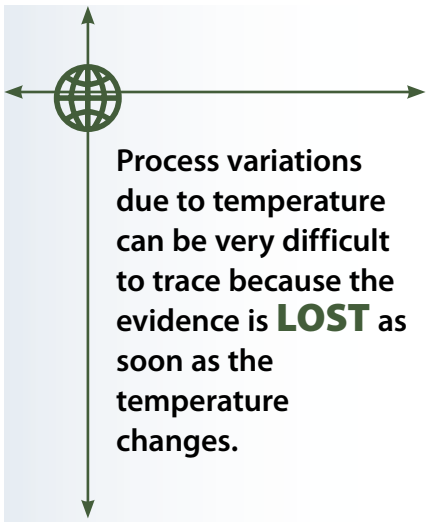
The skills and awareness of process operators are an important factor in keeping product free of microbial contamination. Skilled operators can achieve high levels of hygiene even in marginal environments. Operator influence can be minimized at later development stages by exploiting the automation capabilities of current chromatography skids, but well-trained operators will still help minimize process aberrations between process steps.

Temperature also can have a direct effect on chromatography mechanisms, especially those that have strong hydrophobic components such as hydrophobic-interaction chromatography and some affinity mechanisms (**31**). Temperature differences among processing environments can cause significant shifts in selectivity, and such differences can affect product quality. It is also important to consider that process variations due to temperature can be very difficult to trace because the evidence is lost as soon as the temperature changes. The best solution is to conduct all stages of process development as closely as possible to the temperature of the manufacturing environment in which the final purification process will be conducted.

Sources of Process Variation

For chromatographic methods, process variations can come from

- Sample composition
  - Product concentration
  - Concentration of key contaminants
- Buffer composition
  - pH
  - Conductivity
- Process temperature
- Flow rate
- Lot variations in media
- Variations across scale in chromatography instrumentation



Chromatographic equipment variations at different process scales can also contribute significantly to process variation. Mixing occurs between buffer inlet valves upstream of the pumps, through the pumps themselves, and through bubble traps leading to a column. That converts a programmed step in buffer composition to a gradient transition. The volume of the transition is characteristic for a given chromatograph, but the magnitude of the effect varies with the volume of the column (**24**). With a large ratio of column volume to transition volume, aberrations in buffer composition from the programmed values will be small. If the ratio is small, aberrations will be large. Those aberrations can affect column equilibration volume, the effectiveness of washes, and gradient precision. Transition volume and its effects are easy to characterize and accommodate, but it is important to address the issue proactively to ensure consistency of process control across scales.

Characterizing and Accommodating Variation

Variability occurs in all purification processes despite best efforts both to qualify manufacturing components and implement process controls that ameliorate their effects. This is not a problem so long as variation is well characterized, and each process is documented to reproducibly yield high-quality product within its range. The prevailing approach to characterizing variability and assessing its impact involves design of experiments (DOE) with subsequent statistical analysis to characterize individual contributions of each variable (**23, 32, 33**). This approach is commonly used for optimizing separation conditions, but it serves just as effectively for defining process-failure thresholds. DOE represents significant effort, but factorial designs enable dramatic reductions in the experimental work load. The "Sources of Process Variation" box identifies factors that may be included in DOE studies.

Good Communication Is Essential

Chromatography has proven its ability to support good process control and generate high-quality protein therapeutics, and it could do the same in the field of virus purification. The principles for developing well-controlled chromatographic procedures should apply equally to viruses, but viruses embody a different range of characteristics. Those can be expected to influence both the choice of purification tools and the conditions under which they are applied, with the result that each virus purification procedure will be unique. Establishing good communications with regulatory authorities early in the development process will help ensure that such procedures conform with current regulations.

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