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## Virus Filtration

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# PDA TECHNICAL REPORT NO. 41

## Virus Filtration

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## 1 Introduction

Scope. This PDA Technical Report addresses virus removal filters that retain viruses by a size exclusion mechanism. It explains how they work, their selection, characterization, testing, and validation. **This document should be considered as a guide; it is not intended to establish any mandatory or implied standard.**

Biotechnological and biological therapeutic products are often manufactured using materials of animal or human origin, including cultured primary or transformed cells, milk or other components from transgenic animals, natural extracts, and human or animal blood plasma components. These products are usually proteins that are manufactured by complex manufacturing processes. Although approved recombinant biotherapeutics have an excellent safety record, the risk of contamination by known or unknown pathogens exists (e.g., 1–10).

Virus filtration is performed as part of a manufacturer's overarching virus safety strategy. In this context, virus filtration (size-based removal) is a complement to virus inactivation, both of which contribute to virus clearance (e.g., 11–20). Implementation of virus clearance complements additional measures such as control over raw materials and testing of cell culture or plasma feedstocks. Collectively, these measures form the framework of a virus safety strategy.

The risk of transmission of certain infectious pathogens cannot be completely mitigated by donor screening, vaccination of patients, a single virus inactivation step, or, in the case of cell-based products, virus testing of cell banks and raw materials. It is desirable to introduce additional robust steps in biotherapeutic purification processes to help reduce or eliminate viruses without compromising the molecular integrity of the products. Virus removal filters (often incorrectly termed “nanofilters”) are specifically designed to remove viruses and other biomolecules from the product (protein) solution through a size exclusion mechanism.

Commercially available virus removal filters have different membrane characteristics particular to each filter or filter type. These characteristics include membrane material, membrane surface graft characteristics, pore structure and pore size distribution, virus retention capacity, and protein transmission and adsorption properties. Virus filtration is conducted by either direct (also termed dead-end or normal) flow filtration (DFF) or tangential flow (also termed cross-flow) filtration (TFF). Selection of the mode of filtration (DFF or TFF) is usually

based on the characteristics of the solution to be filtered or process issues such as ease of use, ease of validation, and economics.

## 2 General Concepts

Virus filters are cast polymeric membranes with a complex internal porous structure. Solutions pass through this pore network while particles carried along by the fluid may be retained on the membrane surface or penetrate some distance into the membrane structure. Retention that occurs because particles are too large to pass through a pore is referred to as sieving or size exclusion. Traditional asymmetric surface “skinned” ultrafiltration membranes that contain pinhole defects, while still suitable for protein concentration, may not provide consistent high clearance for large viruses (21).

Smaller particles capable of passing through a pore may also be captured by adsorption to membrane pore surfaces. Retention by adsorption may involve a variety of different forces at different adsorption sites within the membrane structure. Adsorption of viruses, when it occurs, is a secondary phenomenon, while size-based exclusion is considered to be the main mechanism of clearance by filtration.

### Terminology

Minimum Exposure Time: The shortest period for which a treatment step will be maintained.

Nonspecific Model Virus: A virus used for characterization of viral clearance of the process when the purpose is to characterize the capacity of the manufacturing process to remove and/or inactivate viruses in general, i.e., to characterize the robustness of the purification process.

Process Characterization of Viral Clearance: Viral clearance studies in which nonspecific model viruses are used to assess the robustness of the manufacturing process to remove and/or inactivate viruses.

Process Evaluation Studies of Viral Clearance: Viral clearance studies in which relevant and/or specific “model” viruses are used to determine the ability of the manufacturing process to remove and/or inactivate these viruses.

Relevant Virus: Virus used in process evaluation studies that either is the identified virus, or of the same species as the virus, known to or likely to contaminate the cell substrate or any other reagents or materials used in the production process.

**Specific Model Virus:** Virus that is closely related to the known or suspected virus (same genus or family), having similar physical and chemical properties as those of the observed or suspected virus.

**Viral Clearance:** Elimination of target virus by removal of viral particles or inactivation of viral infectivity.

**Viral Inactivation:** Reduction of virus infectivity caused by chemical or physical modification.

**Virus Removal:** Physical separation of virus particles from the intended product.

*From ICH Q5A Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin*

A typical virus filter provides consistent, high virus clearance for viruses larger than the filter rating. For viruses with sizes below the filter rating, clearance may be dependent on fluid and process conditions, especially for small viruses significantly below the filter's rating.

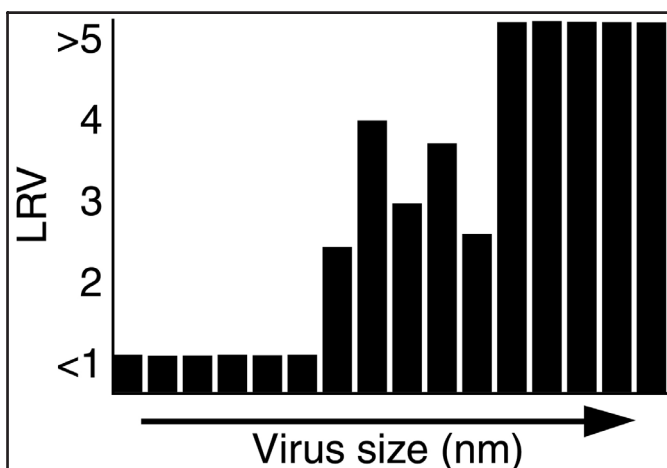


Figure 1. Depiction of the log reduction factor (LRV) as a function of virus size. Especially for filters designed to retain larger viruses, retention will be high and reproducible for larger viruses, case-specific and less predictable for intermediate sized viruses, and negligible for the smallest of viruses.

The lack of sensitivity of virus retention by a membrane to changes in the virus type (aside from size), feed solution, and operating conditions is referred to as the robustness of filter retention. Designing a reliable process requires the identification of the significant parameters of the process that can provide consistent assurance of virus retention. Parameters that can influence retention include virus size, membrane thickness, number of filter layers, and filter pore size distribution. Other factors, such as feedstream pH and ionic strength, can also influence retention, but to a much lesser degree (22).

Virus filter retention properties may change during the course of filtration. Protein aggregates may deposit on the surface of the filter during the filtration process. For TFF, protein adsorption can add an additional layer on the surface of the membrane and reduce the effective pore size of the filter. The degree of protein polarization acting as a secondary virus separation layer can vary during startup and under the changing conditions of concentration and diafiltration processing. For DFF, protein aggregates depositing within the depth of a virus filter can also change the flow patterns within the membrane structure, potentially increasing the effective pore size distribution by blocking the smaller pores. In both cases, process fluid flow relative to the initial or H<sub>2</sub>O flux is lower.

Spiking studies are commonly used to characterize the ability of a virus filter to retain a particular virus under specified conditions. In these studies, scaled-down virus filter systems are used, and the feedstream is spiked with high titer viruses.

## 2.1 Size Exclusion

As described above, viruses are carried along by the fluid as it approaches and passes through the porous membrane structure. These viruses may encounter pore openings that are too small to penetrate. As a result, the virus is retained through size exclusion, a robust mechanism that is generally insensitive to feed solution and operating conditions. However, dependence of retention on filtrate volume per unit of filter area has been recognized (22–24). With DFF, titer reduction may decline with extended throughputs. This phenomenon has been speculated to be due to partial plugging by particulates of the finer end of the membrane pore size distribution, diverting more flow through larger pores leading to lower viral titer reductions. With TFF, the protein gel layer that forms on the membrane surface can act as a secondary, tighter separation layer, leading to changes in titer reduction as a function of process variations that may affect the gel layer. To be useful, virus retention capability must be determined under model “worst case” process conditions.

Membranes with very fine pore surfaces having a narrow pore size distribution can provide size exclusion retention on the membrane surface. This has also been referred to as surface screening. For more open symmetrical membranes or asymmetric membrane structure where the pores get narrower as fluid passes through the membrane, size exclusion based retention can occur within the depth of the membrane structure. This has also been referred to as entrapment or depth size exclusion.

## 2.2 Other Retention Mechanisms

Viruses that are smaller than pore openings may be retained on the membrane surface or on surfaces within the porous structure through an adsorption mechanism. Adsorptive sites may hold the virus through a variety of forces such as charge, van der Waals forces, hydrogen bonding, steric entrapment, etc. These forces may act alone or in a complementary manner. Adsorptive retention depends on virus diffusion and virus size relative to the effective membrane pore size to bring the virus into contact with the membrane pore surfaces, referred to as an interception or impaction process (22).

Similar adsorptive forces can lead to viral aggregation within fluids with other viruses or proteins or other contaminants. Such aggregation may be non-specific or specific, as in the formation of immune complexes with antibodies in immunoglobulin products. In either case, high viral clearance, even for small viruses, can be achieved by the combination of adsorption to form larger aggregates followed by size exclusion of the aggregates.

For the case of TFF, a region of high protein concentration exists above the membrane surface called the boundary layer. This layer can also play a role in obstructing viruses from reaching the membrane surface, thereby contributing to virus removal.

All of these retention mechanisms depend on fluid properties (e.g., pH, salinity, presence of surfactants, etc.) and operating conditions. Thus, they are not nearly as robust as the size exclusion properties of filters and probably should be a lesser consideration in filter selection.

## 2.3 Virus Retention Probability of Reduction Factor (RF)

The fraction of total viruses passing through the filter can be considered as the probability that any individual virus will pass through. Spiking studies at artificially high titers can be used to project the results of low titer challenges representative of industrial operation. The retention probability, the reduction factor (RF)—also termed the (log) titer reduction (LTR) or log reduction value (LRV)—of the filter remains constant, while for virus/filter combinations demonstrating detectable penetration, the filtrate virus titer will change proportionally with any changes in feed virus titer.

## 2.4 Virus Size/Retention Rating

Retention ratings should assist the filter user in selecting the appropriate virus filter for the application. However, the bases for retention ratings for virus filters vary considerably among filter manufacturers. In some cases,

manufacturers have assigned a rating associated with a particular type of virus (e.g., parvovirus or retrovirus). In other cases, a virus size rating has been assigned based on either the retention of a particular model (e.g., bacteriophage) at a given RF, or an average pore size rating is established from a mathematical model for the permeability. In yet other cases, a molecular weight retention rating has been assigned based on a particular surrogate molecular model (e.g., dextran) by analogy with ultrafiltration membrane ratings, or a molecular weight passage rating has been assigned based on recovery of particular proteins. Even using a common basis to describe retention (mammalian virus, bacteriophage, dextran), the level of retention (the RF) can vary depending on how a measurement is made.

As described above, virus filters can provide consistent, high virus clearance for viruses larger than the filter rating, with variable clearance for viruses below the rating. What is ultimately important in selecting a virus filter is the retention performance with mammalian viruses under actual drug product and process conditions. Virus filter ratings should be used only as nominal guides, and users should not overly emphasize slight differences in rating values. On a nominal basis, virus filters can be considered as retaining either large viruses (retroviruses, >80–120 nm diameter), medium sized viruses (hepatitis B virus, 35 nm; SV40 virus, 40 nm), and/or small viruses (parvovirus, >18 nm).

The PDA has organized an effort to develop a common nomenclature system for virus filters. Because of technical issues, the effort began by addressing large virus filters. A committee composed of filter manufacturers, end users and regulatory authorities reached an agreement to define a nomenclature standard for large virus filters (Appendix A). The standard is based on a  $>6 \log_{10}$  retention of a 64–82-nm bacteriophage, PR772 (25, 26), and 95% passage of a model process stream, IVIG, under defined test conditions (see Appendix B). Some flexibility was built into the method to accommodate supplier-specific operating conditions, for example, flux, operating pressure. After agreement on the test method, filters from three manufacturers were tested and found to conform to the new standard (Brorson et al., PDA Journal 2005 (32)). In the future, the PDA anticipates developing nomenclature standards for filters capable of removing smaller viruses as well.

## 2.5 Protein Size/Sieving/Passage Rating

Protein active pharmaceutical ingredient (API) passage is a critical element for consideration during virus filter selection and process development. The ratio of the fil-

trate protein concentration to the feed protein concentration is variously described as the passage, sieving coefficient, or transmission. The small pores in virus filters that enable them to retain viruses or protein aggregates can also retain large proteins and decrease yield. Some TFF virus filters have an assigned passage rating based on globular protein molecular weights. Filter manufacturers may provide some guidance for other filters in validation guides or product literature.

The level of protein passage can vary depending on how a measurement is made and the conditions under which the membrane is operated, including protein concentration, flow rates, and pressures. Buffer conditions can also play a strong role in protein passage insofar as they affect protein size through dimerization and double layer formation (27). Protein passage ratings published by filter manufacturers should be used only as rough guides, and slight differences in rating values should not be over-emphasized. As a nominal guide, virus filters can be considered as passing either medium-sized proteins (160 kDa) or small proteins (30–60 kDa). Careful process-specific analysis is key for virus filter selection and favorable process economics.

Further insights into the principles of filtration are included in PDA Technical Report No. 26 and other publications (28, 29, ).

### 3 Virus Filter Selection and Characterization

Selecting a virus filter requires consideration of many of the same issues that play a role in any filtration process, such as materials of construction and their compatibility with the product. Important parameters in the selection of virus filters are the virus clearance and protein transmission requirements, sterilization requirements, availability of a sensitive in-process integrity test applicable to a production environment, and ability to validate virus clearance on a small scale. The selection also should consider the desired processing characteristics, including the batch size, filter sizing and/or capacity, flow rates, pressure differentials, temperature, and the chemical characteristics of the product.

The selection of the filtration mode (DFF or TFF) is governed by product and process-specific considerations. Product attributes to be considered include the size of the target molecules; the product's concentration, pH or other attributes that may affect chemical compatibility, impurity, or particulate loads in the feedstream; and characteristics of potential viral contaminants. Process considerations include process requirements such as batch vol-

ume, pressure, temperature and time limitations, sterilization options, ease, logistics, and economics of operation. The following sections consider important topics in determining the best filter for a given application

#### 3.1 Filter Construction

Current available virus filter membranes include, but are not limited to, polymers such as polyamide, polyethersulfone and surface-modified polyethersulfone, polysulfone, surface-modified polyvinylidene fluoride, and (cuprammonium-) regenerated cellulose. Additional materials of construction that constitute the filter hardware may include, but are not limited to, polymers such as polycarbonate, polyester, polyetherimide, polypropylene, urethane, and polyvinylidene fluoride. Additional components may include surfactants or humectants that may be added to the filter to enhance wettability, surface modifications and/or additives used to render the filter membrane hydrophilic, or preservatives to prevent microbial growth during storage.

Despite the common misnomer “nanofilters”, virus filter membranes may range from fine microporous membranes with <0.1-micron pore ratings to coarse ultrafiltration membranes with nominal molecular weight cut-off (NMWCO) ratings >100,000 kDa. Microporous membranes used in virus filters may be symmetric (or isotropic) in that the pore size distribution is more or less uniform through the thickness of the membrane. In asymmetric (or anisotropic) membranes, the pore size distribution is not uniform throughout the thickness of the membrane. When used in DFF mode, asymmetric microporous membranes generally have a wider pore structure at the upstream membrane surface that allows for good flow properties and contaminant (e.g., colloidal particles or protein aggregates) capacity, leading progressively to a more restrictive “retentive” region that is largely responsible for the virus removal properties. Ultrafiltration membranes are also generally asymmetric, albeit in the reverse orientation, consisting of a very thin “skin” upstream layer, which is responsible for the retentive properties of the membrane, and a highly porous (downstream) sub-layer that essentially serves to support the skin layer without overly restricting the flow properties.

#### 3.2 Filter System Configurations

During filter evaluation, it is best to consider all processing variables so the appropriate filter surface area and configuration can be selected. Based on filtration mode, there are two major types of filter systems generally in use for virus filters. In DFF, also referred to a “dead-end” or “normal” flow filtration, the fluid flow path is perpendicular to the filter membrane surface. The primary ad-

vantage of DFF is its ease of use, essentially similar to the familiar operation of bacterial sterilizing filters. Because there is no recirculation of the product on the upstream side of the filter, there is no “retentate”, resulting in low shear and high levels of product recovery. DFF generally requires clean process fluids, as particles can penetrate into and be retained throughout the pore structure, thereby clogging the filter. For this and other reasons, DFF systems are designed for single-use applications, eliminating the need for cleaning and cleaning validation.

The filter membranes used in the DFF systems can range from microporous to ultrafiltration membranes. Similar to the “sterilizing grade” filters, DFF virus filters are available in a variety of cartridge or module formats and filter areas. They range from small-area membrane discs (13-mm to 47-mm diameter) and hollow fiber modules typically used for small-scale filter performance evaluation and viral removal validation studies, to multiple filter cartridge or module assemblies (with filtration areas of tens to hundreds of square feet) used in large-scale bioprocesses. Unencapsulated filter discs and cartridges are installed in reusable stainless steel holders and housings that should be cleaned, sanitized, or sterilized between uses. While flat stock membranes have been used for many years for small-scale filtrations, there are advantages to working with small modules or self-contained capsules, which are more practical and easier to handle than flat stock membranes. Most DFF filters are now available as fully disposable units with encapsulated plastic holders or housings that eliminate cleaning and cleaning validation of filter housings.

While in the past only small-area filters, typically under 1500 cm<sup>2</sup>, were available as disposable filter capsules, there has been a growing trend in the biopharmaceutical industry towards so-called “disposable” or “single use” manufacturing. This has resulted in the recent availability of large-scale disposable filter capsules incorporating elements with up to 6 m<sup>2</sup> of filter area. These large capsules can also be manifolded in groups for even greater membrane area. It is important to note that most disposable filter capsules or elements cannot be sterilized by steaming-in-place (SIP), although there are some exceptions. Most DFF filter capsules are autoclavable and may be available pre-sterilized, either by autoclaving or by gamma irradiation.

In TFF, the bulk of the fluid to be filtered is recirculated on the upstream side of the filter, so that the primary flow occurs tangential (or parallel) to the membrane surface. This “crossflow” constantly sweeps the surface of the

membrane, keeping it clear of debris (such as particulates and agglomerates) and reducing the rate and extent of clogging. TFF filters are in general thus capable of handling higher particulate loads potentially present in more upstream intermediates than DFF filters. The portion of the fluid that passes through the membrane and is collected as the “permeate”. As the filtration process progresses, an increasing volume of the initial fluid is drawn off into the permeate, and the volume of the recirculating retentate continually drops. To maximize step yield, product can be flushed into the permeate by continuous buffer addition to the retentate side during diafiltration. As a result, viable and nonviable contaminants that are retained by the filter are concentrated in the retentate.

The filter membranes used in TFF filters can be asymmetric or symmetrical ultraporous or microporous filtration membranes. TFF filters are available either as flat sheet cassettes or as hollow fiber modules. TFF virus filter modules/cassettes are available in areas ranging from d”100 cm<sup>2</sup> for use in laboratory-scale evaluations to high-area modules for large process volume applications. Cassettes or modules are usually installed in reusable housings, and can be stacked or manifolded together to provide the filtration area needed for large-scale applications. Hollow fiber cartridges are available in areas ranging from 10 cm<sup>2</sup> to up to 6 m<sup>2</sup>, and can be used in either the DFF or TFF mode.

The appropriate filter surface area for a particular filtration application can be estimated from laboratory experiments utilizing the identical filter membrane. Flow rate and particulate removal studies performed on small-area filter discs or elements usually may be extrapolated to larger configurations, but the effects of filter configuration and the process stream should be considered. Filter sizing is discussed in more detail in Section 4.

### 3.3 Particulates and Extractables

Filters can potentially release particulates or extractables (e.g., soluble chemical components of the filter) into bioprocess streams. Potential sources of extractables from virus filters include surfactant and wetting agents, materials and oligomers of materials of construction, surface modifications and/or additives used to render the filter membrane hydrophilic, preservatives added to prevent microbial growth during storage, additives used in the polymeric component manufacture, and manufacturing debris.

Virus filters, unlike sterile filters, are generally never the last step in bioprocessing, but are incorporated somewhere

near the end of the biopharmaceutical drug substance manufacture scheme. As such, there may or may not be additional processing steps such as diafiltration or chromatography capable of removing extractables and/or particulates from the API. USP Chapter <788> (Particulate Matter in Injections) specifies limits on the levels and sizes of particulates allowable in small-volume and large-volume injectable final products. Thus, even though there may be manufacturing steps that can remove extractables, it is warranted as a best practice to minimize introduction of filter extractables and particulates into bioprocess intermediates.

Several levels of control for particulates and extractables are generally applied during virus filtration. First, virus filters rated for pharmaceutical service are constructed specifically of materials that leach small, if any, levels of chemicals or fibers into aqueous solutions. Filter manufacturers generally lot-release-test them for particulate cleanliness of the model effluents and can provide appropriate data to end users on filter extractables, in terms of quantitative levels and qualitative composition, in standard solvents. In almost all instances, water-based buffers, which generally do not solubilize plastics or other filter materials, are the appropriate solvent for virus filtration of biopharmaceuticals, although buffer attributes such as pH or detergent content should be considered during a process-specific assessment of potential extractables. Filter manufacturers generally perform USP Biological Toxicity <87> (Biological Reactivity Tests, In Vitro) or <88> (Biological Reactivity Tests, In Vivo) on potential filter extractables and choose materials of construction to minimize toxicity.

During bioprocessing, end users typically employ filter flushing procedures that are designed to ensure removal of preservatives/humectants or storage solutions, as well as particulates and extractables, prior to use. It is the user's responsibility to obtain extractable data for the bioprocess intermediate or buffer formulation and assess whether a specific product can contain objectionable levels of extractables from the filter. This analysis can be based on filter manufacturer or in-house data, depending on the application and type of buffer used in bioprocessing. Test filter(s) used in extractable studies should be identical in construction to the filter device used in the process. The extractables study/evaluation should address the applicability of the extraction test data to the actual process. This should include an assessment of impact of process time and volume on extractable levels, as well as the maximal concentration in the product after additional processing. Processing conditions, including process volume, duration, temperature, filter sterilization, and pre-

flushing, should be considered when performing extraction studies or evaluating the impact of filter extractables.

Suitable analytical techniques for measuring extractable levels and types include "bulk" analytical methods, such as non-volatile residue (NVR) and Fourier transform infra-red spectroscopy (FTIR), applied to an extract without fractionation, to avoid possible loss of unknown analytes in workup. Analytical techniques suitable for measuring and identifying extractables include gas chromatography (GC), high pressure liquid chromatography (HPLC), capillary electrophoresis (HPCE) and gas chromatography mass spectrometry (GC-MS). Where applicable, standardized and/or compendial methods should be followed, for example, ASTM, USP.

### 3.4 Filter Compatibility

Compatibility between the filter and the product fluid also covers the effect of the product and/or process on the chemical and physical attributes and thereby the removal performance of the filter. In most instances, biopharmaceuticals are aqueous protein solutions at neutral pH. The most likely product effect on the filter is either the formation of a gelation layer on the surface of the membrane or pore plugging. It should be noted that both of these effects could affect the RF.

Numerous chemical interaction possibilities exist in a filter system, and they should be considered during filter selection. It is important to consider all of the filter system components. In addition to the membrane, these include support materials for the membrane; filter structural components such as core, cage, end caps, and screens; cartridge/module/cassette shell and housing material; encapsulants; potting materials; and O-rings used to seal the module/cartridge and housing. A more subtle contributor to compatibility issues is the possible presence of filter component treatments, for example, surface modification of filter membranes.

Assessment of filter compatibility should start with a careful review of whether any process condition, product attribute, or buffer constituent has a potential to exhibit incompatibility with any material of construction of the filter. In the case of biopharmaceuticals, water is the most relevant solvent, but impacts of potential high salt or extreme pH conditions should be part of this assessment. Pore plugging by aggregates or other impurities in the process stream should also be considered. This review can be based on past experience and/or standard references, for example, chemical resistance charts. However, a simple chemical compatibility chart will often not provide enough information for predicting filter system

compatibility, thereby requiring actual physical testing. If this compatibility review indicates any potential incompatibility, physical testing should focus on aspects of product exposure identified as potentially affecting the physical strength of the filter or filter components.

The goal of the physical compatibility test is to physically challenge the process filter (not just the filter membrane) under actual process conditions. The test filter(s) used in the compatibility study should be identical in construction to the filter device used in the process. The use of a filter device with smaller membrane area is permissible only if all other components and materials of construction are identical to that used in the process filter. The test filter(s) used in the study should be sterilized or sanitized under process conditions. The test filter(s) should be exposed to the product or surrogate model solvent under process conditions for at least the maximum process time (twice the maximum process time is a common safety factor). This is accomplished by re-circulating the product through a suitable test filter under “worst case” process conditions, including differential pressure, flow rate, and temperature. Instantaneous flux rates should be monitored during the process simulation; it is only meaningful to assess flow decay using actual product intermediates, not model solvents.

The test filter(s) should be visually examined and integrity-tested using the standard method for the filter type both before and after process simulation. Integrity testing is key to detect any significant changes in membrane porosity or pore size distribution. Bacteriophage retention by the test filter before, during, and after process simulation can also measure the impact of processing on size-based sieving performance of the filter.

### 3.5 Protein Recovery (Adsorption/Retention/Biocompatibility)

Biopharmaceuticals and biologicals are typically protein solutions at high concentrations (e.g., >1 mg/mL) in aqueous buffers at neutral pH. In most cases, process economics dictates high recovery of the protein API, at least 95%. In theory, protein recovery may be adversely affected by either of the following two phenomena: protein adsorption, which is caused by the binding of the protein to filter components, and protein retention due to either the small effective pore size of virus filters or the so-called gel polarization effects.

Generally, the impact of protein adsorption in DFF (including viral filtration) is maximal at the start of the filtration process, when the rate of adsorption is at its highest and the protein concentration in the initial effluent the

lowest. As filtration continues, the filter surface starts to saturate, and the rate of adsorption decreases, resulting in an increase in the effluent protein concentration. This could be just the opposite for TFF, where the filter surface takes some time to polarize. Once the filter surface is saturated, no further net adsorption can take place and the effluent protein concentration approaches that of the inlet feed concentration.

In practice, true protein adsorption is rarely a problem in virus filtration of biotechnological/pharmaceutical products. Almost all commercial virus filtration steps operate under high protein load conditions where the saturable binding of the protein API to the filter is a miniscule fraction of the total amount of processed by the step. Two cases could be:

- when small batches of expensive and relatively dilute protein solutions need to be filtered for maximum product recovery and
- in direct filling operations with such solutions where it is important to maintain constant concentration levels in each vial and when pre-flushing is not feasible because of economic and/or time constraints.

The vast majority of marketed biotech products do not fit into either of these categories. It is conceivable that custom-made products for individual patients or micro-batch products made in research labs could fall into one of these categories. In these instances, early consultation with filter manufacturers and/or regulatory authorities is warranted.

Protein recovery, on the other hand, can be a key consideration in virus filter selection. Because virus filters are designed to remove virus particles as small as 20 nm, passage of large protein molecules almost this size (e.g., monoclonal antibodies have a molecular weight (MW) of 150,000 kDa and a hydrodynamic diameter of about 10–15 nm) can become problematic under poorly chosen process conditions. Physical retention of the target protein in viral filtration can occur by sieving or size exclusion due to the small effective pore size and pore size distribution of virus filters relative to the target protein size.

In instances of significant product retention, DFF unit operation would terminate early because of excessive backpressure and/or near complete flow decay. TFF operations may not necessarily terminate early, but the efficiency of protein passage can be monitored on-line by conventional methods, for example,  $A_{280}$  absorbance. Key decisions during early development, such as proper se-