Ultrafiltration and Diafiltration

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“Biotech Processes” discusses fundamental information about biotechnology manufacturing useful to practitioners in validation and compliance. Reader comments, questions, and suggestions are needed to make this column a useful resource for daily work applications. The key objective for this column: useful information.

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KEY POINTS

The following key points are discussed in this article:

• Ultrafiltration (UF) is a commonly used biotech process for concentrating a dilute product stream. UF separates molecules in solution based on the membrane pore size or molecular weight cutoff.
• Diafiltration (DF) is most often used to exchange product into a desired buffer (e.g., from an elution buffer into a final formulation buffer).
• UF and DF typically use tangential flow filtration, where feed flows parallel to the membrane surface rather than perpendicular to the surface.
• Materials of construction for UF/DF membranes include cellulose acetate, polyvinylidene fluoride (PVDF), and polyethersulfone (PES).
• The most commonly used UF/DF membrane configuration is one or more flat plate membranes that are stacked together.
• A series of defined steps are employed for most UF/DF processes including sanitization and pre-use testing, equilibration, concentration, diafiltration, product recovery, cleaning and post-use testing, and storage.
• Cleaning effectiveness is determined using the membrane permeability or normalized water permeability (NWP) test.
• System integrity is confirmed using a diffusion test.

INTRODUCTION

Ultrafiltration (UF) and diafiltration (DF) are commonly used as downstream processing steps for product concentration and/or buffer exchange. The basis of both UF and DF processing is filtration with a membrane that retains product and allows non-target molecules (e.g., water, salts, residuals, etc.) to flow through the membrane to waste. UF is most commonly used for concentrating a dilute product stream while DF is most often used to exchange a product into a desired buffer (e.g., from an elution buffer into a final formulation buffer). This article discusses the basic functions and mechanisms of UF and DF, the most common type of membrane that is used in the biotech industry, and the supporting validation studies that are typically performed for this unit operation.

ULTRAFILTRATION

During UF, molecules in solution are separated based on size using membranes (filters) of different pore sizes. The different pore sizes are notated by their respective molecular weight cutoff (MWCO). Membranes are available in a wide range of pore sizes (5–1000 kD are typical) and surface areas. Membrane pore size is selected based on the size of the target molecule. In general, particles larger than the MWCO will be retained by the membrane, while particles smaller than the MWCO will pass through. For example, if a membrane has an MWCO of 300 kD, particles larger than 300 kD will be retained by the membrane, while particles smaller than...
300 kD will pass through. A manufacturer with a 500 kD protein may select a 200 kD MWCO membrane to ensure that the protein is unable to pass through the membrane to waste while water, salts, and/or residual chemicals pass easily through the membrane.

During a typical filtration (i.e., dead-end filtration) step, such as sterile filtration of a buffer or product solution, material flow is always perpendicular to the membrane surface. Particles of larger size (e.g., target product, bioburden, etc.) are retained on the filter membrane surface. These larger particles can build up on the membrane surface and clog the filter membrane. To prevent this from happening during ultrafiltration, tangential flow is used. Instead of flowing perpendicular to the filter membrane, the feed stream flows parallel to the membrane surface. This is why UF is also referred to as tangential flow filtration (TFF).

Membrane surface area is selected based on the amount of product that is being processed. Even though product is flowing across the membrane surface during UF/DF operations, there is still a protein layer that builds up on the surface of the membrane. Too little total membrane surface area will result in membrane clogging due to protein build-up. However, if the membrane surface area is too high, the hold-up volume, processing time, and cost of membranes all increase. In addition, there is always some product loss in the protein gel layer on the surface of the membrane, so product recovery decreases if the membrane surface area is too large. Therefore, it is important to use an appropriate membrane surface area to achieve optimal conditions.

Figure 1 demonstrates the flow of material during UF/DF. The larger circles represent the target molecules, which flow parallel to the membrane and exit as retained product (“Retentate”). The smaller circles represent unwanted molecules (e.g., water or buffer components), which pass through the membrane as waste (“Permeate”).

As described in a previous article in this series (1), this type of filtration system can be compared to a storm drain. Water run-off containing sticks and leaves (i.e., the feed stream) is filtered by a drain grate. The water flows parallel to the drain grate and large sticks are retained, while water and leaves (i.e., smaller particles) pass through the grate and into the sewage system (i.e., permeate). The flow across the surface of the drain grate maintains a clean surface for “filtration” to continue. In this example, the driving force for getting water and leaves into the drain is gravity. In a UF/DF operation, this driving force is called transmembrane pressure (TMP), but works similarly. With no TMP, all molecules would be prevented from passing through the membrane and filtration would not occur.

During UF, the feed stream is continuously re-circulated across the membranes with the primary objective of removing excess water and buffer from the feed stream. The simplest UF setup consists of a vessel to hold the retentate and a pump to recirculate the product over the membranes (see Figure 2). Notice that in the UF example, there is no buffer being added to the system, so the product is being concentrated at whatever rate the permeate is being removed from the system. In recent years, UF/DF skids have become popular and are widely available. Instrumentation, such as pressure indicators, pH/conductivity probes, UV meters, flow meters, etc. can be added to the system. All skids are made up of the same main components shown in Figure 2.

**DIAFILTRATION**

In general, diafiltration is used to change the chemical properties of the retained solution under constant volume. Unwanted particles pass through a membrane while the make-up of the feed stream is changed to a more desirable state through the addition of a replacement solution.

In biotechnology, diafiltration is commonly used to prepare the product stream for a chromatography step (2, 3), to exchange drug product into the final formulation buffer, or other situations where higher or lower conductivity/pH levels are required.

The setup for diafiltration is identical to the setup for ultrafiltration shown in Figure 2, except the diafiltration buffer is fed to the retentate vessel at the same rate that permeate is leaving the system (see Figure 3). Because the solution is entering the system at the same rate as it is exiting the system, the retentate volume stays relatively constant. Diafiltration continues until the desired conditions (e.g., pH, conductivity, residual concentration, etc.) in the retentate vessel are achieved.

Development studies are routinely performed to determine the amount of diafiltration solution required to attain the desired endpoint. This amount is commonly communicated in terms of the volume of retentate present during diafiltration, which is called a diavolume. For example, if a development study showed it took 5 diavolumes to reach the desired endpoint, and the retentate level was 10 L throughout diafiltration, the step would continue until 50 L of diafiltration solution was added (and 50 L of permeate was collected).
**MEMBRANE TYPES**

UF and DF membranes are available in a variety of different materials of construction, which include cellulose acetate, polyvinylidene fluoride (PVDF), and polyethersulfone (PES). PES membranes, the most commonly used membrane type, are low protein binding membranes. PES membranes are used in many biotechnology processes because they can withstand high temperatures and a wide pH range.

The most common UF/DF membrane configuration is the flat plate cassette. Flat plate cassettes (see Figure 4) contain multiple flat sheet membranes that are stacked together in a sandwich arrangement. Flat plate modules are typically used for processing of biological products due to their low hold-up volume. However, these membranes tend to be difficult to clean. Therefore, it is important when using this type of membrane that appropriate surface area is used so that protein build-up is kept to a minimum. The membrane surface area is increased by stacking multiple membranes of the same type on top of each other. This helps prevent membrane clogging.

**ULTRAFILTRATION AND DIAFILTRATION MEMBRANE PROCESSES**

Similar to chromatography operations (2), a series of defined steps are employed for most UF/DF processes. These steps include sanitization and pre-use testing, equilibration, concentration, diafiltration, product recovery, cleaning and post-use testing, and storage.

Sanitization of the membranes is performed to remove any microbial contamination from the system prior to contact with the product. A sanitizing solution, compatible with the membrane materials of construction, is re-circulated through the membranes for a defined contact time.

The membranes and system are flushed with water, and the normalized water permeability (NWP) of the membranes is measured prior to the initial use to obtain flux and pressure baselines. The filtrate flux is the rate the

![Figure 1: Tangential flow filtration.](image-url)
liquid passes through the membranes, and may also be called the permeate flow rate. Therefore, the NWP is the amount of water that will flow through the membranes at a specific driving force (TMP). If the membranes were not cleaned appropriately, and the pores were partially clogged, this number would decrease.

The pre-use flux value is used to compare with the post-use value to determine how well the membranes have been cleaned. The NWP is measured prior to each subsequent use to confirm that the membrane was not damaged during storage and/or sanitization. An NWP value that is vastly different than the initial NWP would indicate damage.

A system integrity test is then performed to ensure the proper seating of the membranes and gaskets and the overall integrity of the system and membranes. This is basically a gross setup failure check. The membranes are wetted with water and subjected to air pressure on the retentate side of the membranes. The amount of air passing into the permeate is measured and is ensured to comply with the manufacturer’s recommended maximum allowable levels.

The membranes are equilibrated using a buffer solution that is appropriate for the product, the product is introduced into the system, and ultrafiltration and diafiltration are performed as previously described. The concentrated and diafiltered product is then recovered from the system. A flush of the membranes using diafiltration buffer may also be performed to recover any residual product held up by the membranes and by the UF/DF equipment.

UF and DF membranes are typically reused for multiple product batches (i.e., cycles) due to cost. Therefore, post-use membrane cleaning and testing to evaluate the cleaning effectiveness is important to ensure the membranes are acceptable for continued use. Often, the same procedure and solutions that are used for the pre-use sanitization step are also used for the post-use cleaning step.

The NWP of the membranes is measured again after each processing batch, following the post-use membrane cleaning step. The post-use NWP value is then compared to the original NWP to determine how effectively the membranes were cleaned. Ideally, the membranes are returned close to their original state, and there is little difference between the NWP values. In reality, the NWP may drop with repeated use of the membranes and should be monitored to determine when new membranes should be installed.

A post-use system integrity test is performed again after use to ensure the system and membranes were not compromised during processing. If another processing batch does not immediately follow, the membranes are stored in a solution that will keep the membranes from

Figure 2: Basic ultrafiltration setup.
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**Figure 3:** Basic diafiltration setup.

**Figure 4:** Flat-plate membrane.
drying out and will ensure microbial control during the storage period.

VALIDATION
As with other unit operations, supporting validation studies are performed on the UF/DF system and membranes. Studies that may be performed include membrane lifetime studies, sanitization, and storage studies (4). Membrane lifetime studies are performed to define the number of times a membrane can be reused without affecting the performance. Membrane reuse is important because of the cost of the membranes and the processing time in between batches. The lifetime studies are performed along with sanitization and storage challenge studies, which evaluate the effectiveness of the procedures used to sanitize and store the membranes. Proper sanitization of the membranes reduces the amount of microbial carryover from one batch to another, which is important when the membranes are reused.

CONCLUSION
UF/DF is implemented during downstream processing to concentrate product and/or to exchange product into a new buffer. UF and DF go hand-in-hand with column chromatography and other unit operations by assisting in preparing the product for the subsequent step. The type, size, area, and materials of construction of the membrane used during UF/DF vary based on the molecule of interest and the processing conditions. Therefore, it is important to choose the most compatible membrane for overall effectiveness.

REFERENCES

RECOMMENDED RESOURCES
Cheryan, Munir, Ultrafiltration and Microfiltration Handbook
Millipore Technical Brief, Protein Concentration and Diafiltration by Tangential Flow Filtration.
Wang, William K., Membrane Separations in Biotechnology,

GLOSSARY
Downstream processing. The recovery and purification steps of the manufacturing process.
Hydrophilic. Having an affinity for water.
Permeate. Particles that pass through the filter membrane.
Retentate. Particles that are retained by the filter membrane.

ARTICLE ACRONYM LISTING
DF Diafiltration
MWCO Molecular Weight Cutoff
NWP Normalized Water Permeability
PES Polyethersulfone
PVDF Polyvinylidene Fluoride
TFF Tangential Flow Filtration
TMP Transmembrane Pressure
UF Ultrafiltration