⟨1228.3⟩ DEPYROGENATION BY FILTRATION

1. INTRODUCTION

Depyrogenation by filtration involves the physical removal of endotoxins from pharmaceutical solutions by adsorption and size exclusion. Endotoxins are responsible for making up the majority of pyrogens, which must be removed from pharmaceutical products including injectable biologics. There are many factors to be considered when designing a depyrogenation filtration process for solutions containing proteins and peptides: type of target protein and its concentration, electrolyte concentration, pH and buffer system, protein molecular weight and isoelectric point (pI), filtration parameters (e.g., flow rate), and interactions with other components causing aggregation. In general, a combination of these factors determines the most effective depyrogenation method.

Depyrogenation of liquids may be accomplished by means of filtration through various types of filter media including microporous membranes, reverse osmosis (RO) membranes, ultrafilters, charge-modified depth filters, activated carbon, and membrane absorbers. Depyrogenation filtration processes are not intended to remove microorganisms from a process stream; however, by their nature, filters selected for use in depyrogenation processes may also be capable of retaining many types of microorganisms.

2. TECHNOLOGIES USED FOR DEPYROGENATION BY FILTRATION

2.1 Microporous Membrane Filtration

Microporous membranes (typically with pore size or retention ratings between 1.0 and 0.1 µm) can be very effective in removing intact bacteria via size exclusion and adsorption within flow pathways. The use of microporous membranes on a freshly prepared solution to be filtered can effectively prevent bacterial proliferation in the solution, along with any potential subsequent endotoxin formation. Endotoxin, however, is composed of fragments of bacterial cell wall, often <0.025 µm (*1*) that may easily penetrate most bacteria-retentive membrane filters. These negatively charged particles with endotoxin activity can be removed via adsorption by positively charged membranes (*2*). Adsorption of endotoxin has also been shown by hydrophobic membranes, where it is thought that a hydrophobic interaction occurs between the Lipid A core and hydrophobic sites on the membrane flow path surfaces (*3*). Reduction or removal of endotoxin activity by adsorption to microporous membranes can be dependent on flow rate, pH, concentration, and fluid and membrane surface properties. Once the effective binding capacity of the membrane approaches saturation under applied conditions, remaining endotoxin will pass through the membrane.

2.2 Reverse Osmosis

RO membranes are the tightest membranes in size separation. They can separate dissolved salts and sugars from water. Pyrogens, and essentially everything else, are removed from water via size exclusion. RO systems are operated most efficiently at high pressure (200–1000 psi) to overcome osmotic pressure. RO membrane rating or tightness is measured and expressed with retention or rejection of marker salts such as sodium chloride or magnesium sulfate.

RO membranes may be composites (thin film coated on top of ultrafiltration membranes) or cast as a single layer (cellulose acetate type). Configuration of RO membrane modules can be flat sheet, tubular, or hollow fiber. All commercially available RO membranes are polymeric, and most are of a spiral-wound, flat-sheet format.

RO systems are not intended to remove all bacteria, and because they are run at ambient temperatures, microbiological contamination is a concern. Ultraviolet (UV) light may be used in the system downstream from the RO units to control microbiological contamination.

2.3 Ultrafiltration

Ultrafiltration (UF) is a process whereby a fluid is passed through membranes with pore sizes nominally between about 1 and 100 nm under pressure. The filters are usually not rated by the pore size but by the molecular weight cut-off (MWCO). The methods to determine the MWCO vary by the manufacturer and usually involve measuring passage of molecules of a certain size, such as a solution of mixed dextrans, polyethylene glycol, or proteins to assign a numerical rating (*4*).

UF membranes are usually polymeric porous structures, manufactured from a range of materials, most commonly regenerated cellulose or polyether sulfone, but also ceramics. UF membranes may be produced as flat sheet, hollow fibers, or ceramic tubes.

UF is generally operated in tangential/cross flow mode, which separates the starting (feed) solution into two components: permeate (the portion of solution going through the membrane) and retentate (the concentrated solution that is passed over the membrane). UF membranes need to be encased in a suitable integral device to enable practical operation. Heat sealing, over-molding, and resin-potting are all used to assemble membrane devices and ensure integral flow paths. Ceramic tubes are sealed by gaskets within tubular cylinders.

It is generally assumed that the basic subunit of lipopolysaccharide (LPS) is about 10–20 kDa (*5*). Membranes of 6–10 kDa cut-off are often used for depyrogenation by size exclusion. However, monomeric LPSs are rarely found in solution because of their poor solubility in water. LPS is usually present in aggregated forms, such as vesicles ranging in molecular weight from 300 to 1000 kDa. Thus, endotoxin can be successfully removed by higher flux membranes, with MWCOs of 30–100 kDa (*6*).

Adsorption, in addition to size exclusion, also can be a mechanism of endotoxin removal by UF. Several hollow-fiber membrane materials have been evaluated, and the best removal was obtained with more hydrophobic membranes. Endotoxin removal was correlated to the degree of endotoxin adsorption on the membranes in an equilibrium experiment (*7*).

UF has been used successfully to depyrogenate small molecule drugs, buffers, electrolytes, antibiotics, and antifungal agents (*8*). UF is generally not recommended for endotoxin removal from solutions containing larger molecules such as proteins.

2.4 Charge-Modified Depth Filters

Depth filters exhibit two primary clarification mechanisms because of their structural and chemical composition: size exclusion, either through sieving or entrapment; and adsorption, either through electrokinetic (positive zeta potential) or hydrophobic interactions.

Size exclusion of particles is a function of the tortuous flow path through the media as well as the depth or length of the flow path in relation to the size distribution of the contaminate loading, e.g., cellular debris, including LPS from cell walls and hard particles. Depth filtration efficiency depends on many factors, including the filter media characteristics, materials of construction (e.g., cellulose, filter aids, binding resins), the fluid characteristics (e.g., viscosity, dirt load, cell debris, temperature), as well as the particle characteristics (e.g., solid/hard, pleomorphic, proteinaceous, colloidal). Electrokinetic adsorption is attributed to the resin binders and filter aids that impart a net-positive charge, positive zeta potential, to the filter medium. Adsorption is a complex mechanism that will vary based on a combination of parameters including positive zeta potential, hydrophobic adsorption, particle surface charge, pH, and ionic strength of process fluids. This positive zeta potential can remove negatively charged particles smaller than the nominal rating of the depth filter medium. The adsorptive mechanism results in high removal efficiencies for fine particles, colloidal and cellular materials, e.g., bacterial endotoxins, nucleic acids, and removal of negatively charged trace contaminants, whereas the depth medium porosity influences operating parameters such as pressure differentials, flow rates, dirt load capacity, and throughput.

Most cellulose-based depth filters contain a filter aid to enhance particle retention and flow characteristics. Filter aids are available in various particle sizes and levels of purity. Common filter aids include diatomaceous earth, perlite (volcanic origin), carbon (natural sources), and silica- and/or metallic-based materials.

The cartridges and capsule configurations are constructed of primarily polypropylene and other common elastomers and polymers, e.g., nylon, polycarbonate, polysulfone. Depth filters are available in standard filter cartridge/capsule configurations; lab-scale discs (47 mm/90 mm), flat stock sheets, lenticular cartridges (stacked discs), and capsules.

In general, the charge-modified depth filters showed lower endotoxin breakthrough levels at charge exhaustion as compared to charge-modified membrane filters.

Membranes demonstrated total endotoxin breakthrough once the charge capacity of the membrane is saturated. Generally, charge-modified depth filter media demonstrate lower endotoxin unit (EU) levels that increase slowly at the point of first endotoxin detection as compared to membranes that exhibit complete breakthrough.

Benefits of charge-modified depth filters include removal of bacterial endotoxin [4–5 log reduction value (LRV)] (*9–11*), DNA fragments, host cell protein, reduction of viruses, and economical throughput with low extractable levels. System flow rate determinations are necessary to optimize residence time to maximize adsorptive capture. This parameter is especially important for the removal of colloids and endotoxins.

Cellulosic depth filters commonly contain extractable *Limulus* amebocyte lysate (LAL)-reactive materials that are often determined to be β-1,3-glucans. β-1,3-Glucans activate an alternative LAL pathway, Factor G. The activation of Factor G by β-1,3-glucans will induce the proclotting enzyme, causing a non-endotoxin-positive LAL result (or enhanced result). To reduce the risk of β-1,3-glucan extractables from cellulosic depth filters, it is important to follow the recommended rinse conditions of the specific depth filter. An alternative to reduce the effects of β-1,3-glucans is to select LAL reagents tolerant of β-glucans or to add a β-glucan blocking buffer to LAL samples.

To some users, the most important attribute of charge-modified depth filters is their effectiveness as prefilters. In more difficult filtrations, such as those containing colloids, bacteria, or endotoxins, the user can realize substantial cost savings.

2.5 Activated Carbon Depth Filters

Depth filtration, using activated carbon as a filter aid adsorbent, removes color, odor, and bacterial endotoxins and nucleic acids. Activated carbon is derived from organic materials, e.g., peat, wood, coconut, bone, lignite coal. The microstructure of the carbon contains millions of pores that create a highly adsorptive material with a vast internal effective surface area as compared to polymeric microporous structures. These carbon filter aids are typically activated by steam or chemical treatment such as acid. Although highly effective in reducing endotoxin (4–5 log reduction) and other undesirable contaminants, active carbon may, because of this highly adsorptive characteristic, remove other process components and target molecules due to this nonspecific adsorption property. The high loading capacity and strong adsorptive attributes make activated carbon depth

filtration an attractive alternative to conventional filtration methods or addition of bulk carbon, where care must be taken to remove fine carbon particulates in the effluent.

2.6 Membrane Adsorbers

When the target protein in the solution is in the same molecular weight range as that of the endotoxins (10–20 kDa for endotoxin monomers), the target proteins cannot be separated by UF. Ion exchange chromatography is the most common depyrogenation method for proteins; however, it has some drawbacks, which limit its usefulness as a depyrogenation step. This includes handling and usage problems such as packing, channeling, low flow rates, long regeneration times, compressibility, and limited chemical stability. Charge-modified membrane adsorbers with ion exchange ligands functionalized on the membrane surface can provide the required performance needed for depyrogenation from the laboratory up to process scale. Generally, two strategies can be used for removal of endotoxin from solutions with such membrane adsorber devices. Using the strong basic anion exchanger of quaternary amine (Q) type in a buffer with pH lower than the pI of the protein, endotoxin will bind to the charged membrane substrate, and protein will pass through the membrane (negative chromatography). Alternatively, a strong acidic ion exchanger type S also can be used with a buffer pH lower than the pI of the protein. In this case, the endotoxin will pass through, and the protein will be bound to the charged membrane substrate, which can be subsequently eluted using appropriate buffers in the next step.

Such membrane adsorbers have been used as validated endotoxin clearance steps in downstream processing of monoclonal antibody (mAb) or recombinant protein manufacturing. Typical log reduction values (LRVs) reported are >4 (*12*) based on lab scale testing.

Another solution is to use mixed-mode membrane adsorbers exhibiting both anionic and hydrophobic chemistries. Endotoxins (hydrophobic and negatively charged) tightly bind onto the membrane surfaces. By adjusting the concentration of salt or pH appropriately, proteins flow through the mixed-mode membrane adsorber by charge repulsion, while endotoxins remain bound. Mixed-mode membrane adsorbers allow the depyrogenation of protein solution or buffers with higher concentrations of salt (e.g., 100–500 mM) than with the Q adsorber. Such membrane adsorbers have been used as validated endotoxin clearance steps in downstream processing of mAb or recombinant protein manufacturing. Typical LRVs reported are in the 3–4 range based on lab scale testing.

3. VALIDATION

See *Depyrogenation* ⟨1228⟩ for a comprehensive discussion of depyrogenation process validation and the use of endotoxin standards.

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APPENDIX

Additional References

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