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Endotoxin Removal by Ion Exchange with Sartobind® Lab Q and S Membrane Adsorbers

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Abstract

Endotoxins (lipopolysaccharides from membranes of gram negative bacteria) make up the majority of pyrogens which must be removed or reduced from pharmaceutical products, biologics for injection, cosmetics, and cell culture media. There are many factors to consider when designing a depyrogenation strategy for media or solutions containing proteins, peptides or other macromolecules: from the type, concentration, molecular weight and isoelectric point (pI) of the target molecule of interest, to the electrolyte concentration, pH and buffer system, and interactions such as interference or aggregation. This review presents examples where Sartobind® Lab membrane adsorbers have been used for the effective removal of pyrogens from various sample types.

Introduction

In solution, the molecular weight of endotoxins can range from 4 to 20 kDa but they can also readily form micelles and vesicles with diameters up to 0.1 µm (Figure 1). The presence of detergents, chelators and proteins promotes the formation of structures like micelles (300 – 1,000 kDa) and monomers (10 – 20 kDa), while bivalent ions promote the formation of larger structures like vesicles (>1,000 kDa).[1]

If the target substance in a solution to be depyrogenated has a low molecular weight (e.g. buffer, salt, nucleotides, amino acids, peptides, some carbohydrates etc.) the endotoxins can be separated from the target substance by ultrafiltration with an appropriate cut off centrifugal, pressure cell or crossflow filter, such as those available from the Vivaspin® and Vivaflow® product families. However, if the target molecule of interest is a macromolecule such as a protein, virus or nanoparticle, it is likely to be within a similar molecular weight or size range to endotoxins and therefore cannot be separated from these contaminants by ultrafiltration.

Due to the negatively charged phosphoryl and carboxyl groups in endotoxins, ion exchange chromatography is the most common depyrogenation method for macromolecules. However, this technique has several drawbacks which can limit its usefulness as a depyrogenation step. This includes handling and usage problems such as packing, channeling, low flow rates, susceptibility to fouling, long regeneration times, compressibility, high buffer consumption, and limited chemical stability. Taken together, the incorporation of chromatography resins for depyrogenation can be expensive and troublesome.

To overcome these challenges, Sartorius has developed a high capacity, scalable and ready-to-use ion exchange membrane technology, which provides excellent performance needed for depyrogenation in both laboratory and process scale workflows.

For the laboratory, two strategies are available for the removal of endotoxin from solutions with Sartobind® Lab devices. Using the strong basic ion exchanger type Q with a buffer system where the pH is lower than the pI of the target molecule, such that endotoxin will be bound and the molecule will pass through the membrane. Alternatively, using the strongly acidic ion exchanger type S and a buffer with pH lower than the molecule pI, the endotoxin will pass through the membrane while the target will bind and can be eluted in a subsequent step.

The following examples illustrate conditions and results for the removal of pure or native endotoxin from various solutions by Sartobind® Lab Q and S membrane adsorbers. All solutions were prefiltered through 0.2 or 0.45 µm cellulose acetate membranes prior to depyrogenation, to prevent Sartobind® membrane adsorber blocking. Endotoxin levels were assessed by LAL methods with a limit of detection of 0.06 EU/mL.

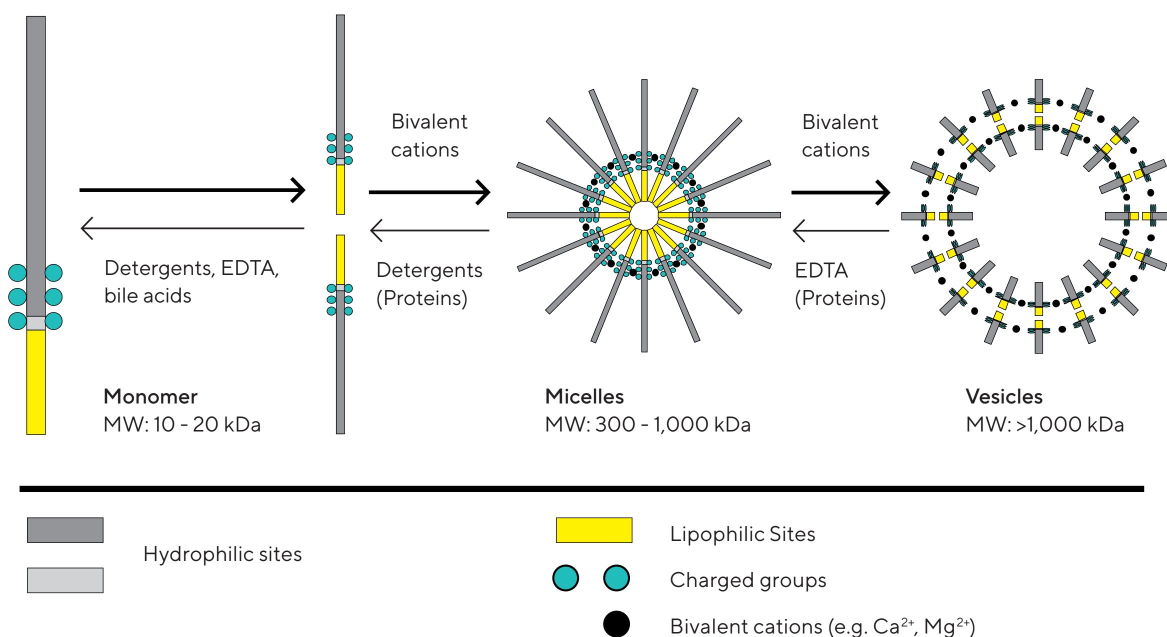

Figure 1: *Structure of Endotoxin Monomers and Aggregates in Aqueous Solutions of Varying Composition. Adapted From an Image Provided Courtesy of Prof. A. B. Anspach, Hamburg University of Applied Sciences, Department of Natural Science Technology.*

Endotoxin Clearance From an IgG Sample with Sartobind® Lab Q 75

Sample: Cytoglobin in 10 mM potassium phosphate, pH 6
Device: Sartobind® Lab Q 75

Immunoglobulins often have isoelectric points between 7.5 and 9.5. Therefore, they will not be bound at pH 6 by anion exchangers. Table 1 shows that endotoxin in a protein solution was effectively removed (99.4%) with Sartobind® Lab Q 75. In samples containing the target protein, some other protein impurities were bound by the membrane, leading to a reduced binding capacity for endotoxin. Therefore, the level of endotoxin clearance was not at the level (99.97%) observed for the control sample (protein free solution). Protein recovery after depyrogenation was in the range 84 – 86%.

Table 1: *Endotoxin Clearance From an IgG Sample*

	Sample 1	Sample 2	Control (Protein Free Solution)
Initial Endotoxin Concentration	1,000 EU/mL	1,000 EU/mL	1,000 EU/mL
Initial Sample Volume	10 mL	10 mL	10 mL
Total Endotoxin in Initial Sample	10,000 EU	10,000 EU	10,000 EU
Total Endotoxin in Flow Through	60 EU	60 EU	3 EU
LRV	2.2	2.2	3.5
Endotoxin Clearance	99.4%	99.4%	99.97%
Protein in Initial Sample	5 mg	5 mg	-
Protein in Flow Through	4.2 mg	4.3 mg	-
Protein Recovery	84%	86%	-
Bound Protein	16%	14%	-

Depyrogenation of Water and PBS with Sartobind® Lab Q 15

Sample: Water for injection (WFI) or PBS spiked with 1 µg/mL (10,000 EU/mL) endotoxin from *E. coli* (055:B5)
Device: Sartobind® Lab Q 15

For depyrogenation before use, 20 mL of 1 M NaOH was applied to each Sartobind® Lab Q 15 device and incubated for one hour. The devices were flushed with 100 mL pyrogen-free water and a control sample of the final drops of flow through was analyzed by LAL test to confirm they were pyrogen free.

Spiked WFI or PBS samples were passed through the pyrogen free membrane adsorbers at 50 mL/min (~125 MV/min). For endotoxin removal from WFI, 3x 500 mL samples were processed sequentially with a single device, with depyrogenation of the membrane adsorber between each cycle. Each flow through sample was collected in 5x 100 mL fractions for LAL testing. For endotoxin removal from PBS, 3x 100 mL samples were passed through separate Sartobind® Lab Q 15 devices, and 10x 10 mL filtrate fractions for LAL testing collected from each. Total process times were 10 minutes for WFI and 2 minutes for PBS. Results are presented in Table 2.

Table 2: *Depyrogenation of Endotoxin-Spiked Water & PBS*

		Sample 1	Sample 2	Sample 3
WFI	Initial Endotoxin	10,000 EU/mL	10,000 EU/mL	10,000 EU/mL
	Flow Through	<0.06 EU/mL	<0.06 EU/mL	<0.06 EU/mL
	LRV	>5.22	>5.22	>5.22
PBS	Initial Endotoxin	10,000 EU/mL	10,000 EU/mL	10,000 EU/mL
	Flow Through	0.96 EU/mL	0.96 EU/mL	0.96 EU/mL
	LRV	4	4	4

Due to the relatively high salt content of PBS (150 mM), the binding capacity of Sartobind® Lab Q was lower than with pure water. This is evident from the reduced endotoxin clearance observed for PBS samples (LRV = 4) when compared with water samples (LRV >5.22).

Depyrogenation of BSA with Sartobind® Lab Q 15 and 100

Sample: 1 mg/mL BSA (pI 4.7) in WFI, spiked with either 6 or 10 EU/mL endotoxin from *E. coli* (055:B5), pH 4.7
Device: Sartobind® Lab Q 15 and 100

For the effective removal of endotoxins from protein solutions, knowledge of the target characteristics such as pI, stability in diluted buffer, binding and purification conditions, interaction with other proteins and with endotoxin, or interference with the LAL test, can all be important for choosing the appropriate conditions.

Depyrogenation of Sartobind® Lab devices before use was performed as described above. 150 mL aliquots of the spiked BSA sample were each passed through different Q 15 or Q 100 devices at 5 – 20 mL/min. Fifteen filtrate fractions of 10 mL were collected from each Sartobind® Lab device for LAL testing. The total process time was 15 – 25 minutes and results are presented in Table 3.

Table 3: *Endotoxin Clearance From Spiked BSA Samples*

		Sample 1	Sample 2	Sample 3
Q 15	Initial Endotoxin	6 EU/mL	6 EU/mL	6 EU/mL
	Flow Through	<0.06 EU/mL	<0.06 EU/mL	<0.06 EU/mL
	LRV	>2	>2	>2
Q 100	Initial Endotoxin	9.9 EU/mL	9.9 EU/mL	9.9 EU/mL
	Flow Through	0.235 -to <0.06 EU/mL	0.235 -to <0.06 EU/mL	0.235 -to <0.06 EU/mL
	LRV	1.6 to >2.2	1.6 to >2.2	1.6 to >2.2

In this experiment, the buffer pH was not optimized. Improved endotoxin removal could be expected with a buffer pH <4.7. Under such conditions, BSA would still pass through Sartobind® Lab Q devices without adsorption, while endoxotin would continue to be bound by the membrane.

Depyrogenation of mAbs with Sartobind® Lab Q 15 or S 15

Sample: 0.5 mg/mL mAb (pI 7.4) at 85 – 95% purity, spiked with 100 EU/mL endotoxin from *E. coli* (055:B5)
Device: Sartobind® Lab Q 15 or S 15

Most mAbs have a pI in the range of 7 – 8. Therefore, either flow through or capture strategies may be employed for endotoxin removal, utilizing Sartobind® Lab Q or S membrane adsorbers, respectively. With the Q anion exchanger, depyrogenation can be accomplished using buffers with a pH from 5 to 8. Alternatively, with the S cation exchanger, a pH of 5 – 6.5 is appropriate.

Sartobind® Lab devices were depyrogenated before use. For anion exchange (Q 15), 200 mL of mAb in 20 mM Tris-HCl pH 7.5 or sodium phosphate buffer was applied through the membrane adsorber at 20 mL/min, and the mAb-containing flow through immediately re-buffered to avoid inactivation in a pH very close to its pI. For cation exchange (S 15), 200 mL of mAb in 20 mM MES pH 5.8 – 6.2, or sodium phosphate or acetate buffer (pH 5.5), was loaded at 20 mL/min, followed by washing twice with 20 mL of sample buffer, and elution with 6 mL of 1 M KCl.

Both methods provided a 3.2 LRV (Table 4) and protein recovery determined by BCA assay was >95%.

Table 4: *mAb Depyrogenation by Anion or Cation Exchange*

Q 15	Sample Buffer	Tris-HCl	Na-phosphate	
	Initial Endotoxin	100 EU/mL	100 EU/mL	
	Flow Through	<0.06 EU/mL	<0.06 EU/mL	
	LRV	>3.2	>3.2	
S 15	Sample Buffer	MES	Na-phosphate	Na-acetate
	Initial Endotoxin	100 EU/mL	100 EU/mL	100 EU/mL
	Flow Through	90 EU/mL	90 EU/mL	90 EU/mL
	Wash	10 EU/mL	10 EU/mL	10 EU/mL
	LRV	>3.2	>3.2	>3.2

Depyrogenation by Multiple Passes Through Sartobind® Lab Q 100

Sample: 1 – 1.7 mg/mL crude protein extract containing photolyase
Device: Sartobind® Lab Q 100

Whether a reduction or total removal of endotoxin is needed depends on the application. For instance, therapeutic proteins must be pyrogen-free, while proteins for cosmetic applications may only require a reduced pyrogen level.

A crude protein extract containing the target protein was pre-filtered (0.2 µm) and diluted 5-fold with PBS to a final volume of 150 mL. This would enable capture of pyrogens but not the photolyase. Initial endotoxin concentrations varied between 0.5 and 10 million EU/mL. A Sartobind® Lab Q 100 device was equilibrated with distilled water prior to loading 3x 50 mL aliquots of the sample at a flow rate of 15 mL/min (~5 MV/min). After each 150 mL pass, the membrane adsorber was regenerated with 50 mL of 1 M KCl, followed by 50 mL of 1 M NaOH and re-equilibrated with 50 mL distilled water. Flow through fractions were pooled and passed through the membrane adsorber again, and the process repeated until a total of three passes had been completed. Starting material and all filtrates were assayed for endotoxin, protein concentration and photolyase-specific activity (Table 5).

Table 5: *Depyrogenation of Crude Protein Extract (Average of 3 Runs)*

Sample	Endotoxin	Protein	Photolyase Activity
Initial	100%	100%	1%
1st Pass	10.5%	30.9%	8.4%
2nd Pass	0.35%	20%	18.4%
3rd Pass	0.09%	19.7%	19.2%

In the first pass, endotoxin and non-target protein were bound, as evidenced by total protein content reduction to 30.9% while photolyase-specific activity increased from 1 to 8.4%. In the second pass, due to the previous reduction in contaminant proteins competing for binding, a higher LRV of endotoxin was achieved. The third pass mostly bound only endotoxin.

In each case, endotoxin levels were reduced to only 50 EU/mL in just three passes, with the membrane adsorber exhibiting endotoxin binding capacities in excess of 2 million EU/cm². This process was successfully scaled to bioprocess Sartobind® devices for 15 L batches.[2]

Summary

Sartobind® Lab devices offer an effective means for rapid and convenient removal of endotoxins. When optimized conditions are chosen, protein purification and removal of contaminants can be carried out in a single step. Furthermore, in most cases, regeneration of a Sartobind® Lab device prior to re-applying the sample to be depyrogenated – as illustrated in the final example above – can be appropriate for reducing endotoxin levels even further. Alternatively, higher LRVs could also be achieved by using a larger Sartobind® Lab device or connecting multiple units in series.

The easy handling and high chemical compatibility of Sartobind® Lab membrane adsorbers also enable their regeneration with aggressive cleaning agents. This ensures efficient use of research budgets while avoiding cross contamination between batches.

References

1. Petsch, D. and Anspach, F.B. (2000). Endotoxin removal from protein solutions. J. Biotechnol. 76, 97-119.

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Note

Literature published up to c.2022 may reference the use of Sartobind® MA, which is a name previously used for the Sartobind® Lab membrane adsorbers. When these devices were renamed, there was no change made to fit, form or function. Therefore, results collected and methods established using Sartobind® MA devices remain valid also for Sartobind® Lab.

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