Qualified Cleaning and Sanitization of OPUS® Columns

Introduction

OPUS® (Open Platform User Specified) Pre-packed Disposable Chromatography Columns are designed to deliver the industry required flexibility of multi-use applications in downstream processing. Constructed from a medical grade polypropylene homopolymer, OPUS® Columns are configurable for any industry standard size, chromatography resin, and bioprocessing application. As such, OPUS® Columns must be compatible with accepted cleaning and sanitization protocols to be suitable for use in a typical downstream processing campaign for monoclonal antibodies, recombinant proteins, or vaccines. In this Tech Note, cleaning and sanitization strategies for Pre-packed Disposable OPUS® Columns are investigated and qualified through quantitative methods.

Experimental Procedures and Results

Cleanability and sanitization of a 20 cm internal diameter (ID) OPUS® column was assessed for small molecules, endotoxins, and large particles like bacteria. The OPUS® 20 cm design is representative of Repligen's Pre-Packed Disposable Columns with internal diameters ranging from 10 – 30 cm. All columns in this size range have consistent design parameters, packing procedures, and performance characteristics (visit www.repligen.com/opus for more information).

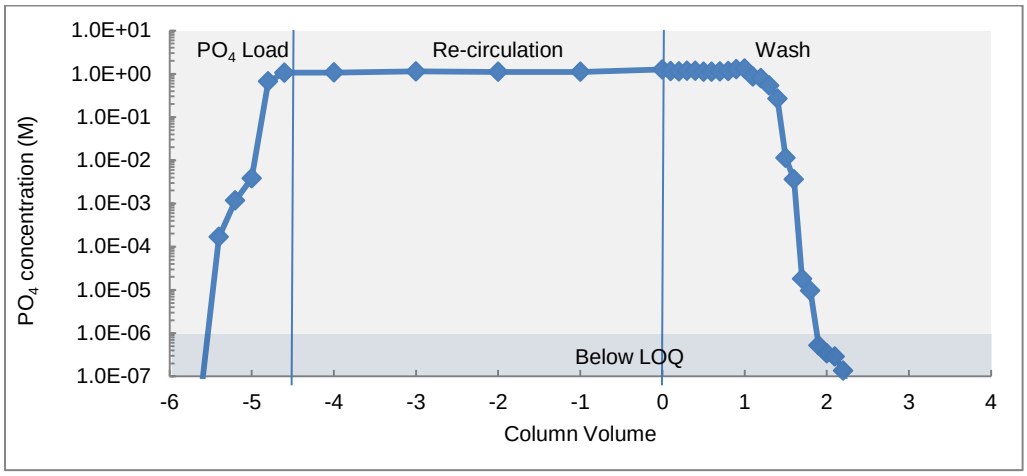
1. Cleanability: Assessment of Small Molecule Clearance in a Pre-Packed OPUS® Column

Method:

Inorganic phosphate was used as a small molecule tracer. A 20 x 20 cm OPUS® column packed with Sepharose® 6FF was loaded with 1 column volume of 1M sodium phosphate at a flow rate of 100 cm/h. The phosphate was re-circulated for a total of 4.5 column volumes to ensure saturation. The column was then washed with deionized water for 10 column volumes to remove any traces of phosphate. Samples were collected during load, recirculation, and wash, and then assayed for phosphate. A sensitive colorimetric method was performed capable of detecting phosphate to µM levels.[1]

Results:

Figure 1



[1] Chen PS, Toribara TY, Warner H (1956). Microdetermination of phosphorus. Anal Chem 28: 1756–1758.

Figure 1 shows a small molecule can easily be removed from an OPUS® column as a result of the well-engineered column design and packing procedures. A reduction of 6 logs is achieved in less than 2 column volumes of wash, and undetectable levels of phosphate are achieved in less than 2.5 column volumes.

2. Removal of Endotoxin and Bioburden from a Pre-Packed OPUS® Column

OPUS® columns are used for purification of biological products which have specific regulatory requirements for bioburden and endotoxin levels. Therefore, a quantitative cleaning investigation was performed to demonstrate the effectiveness of sanitization using sodium hydroxide as a cleaning agent.[2]

Method:

A 20 x 20 cm OPUS® column packed with Sepharose 6FF was loaded with 1 column volume of *E. coli* bacteria at a concentration of 0.5 OD (optical density) at 600 nm with a flow rate of 100 cm/h. The column was left to sit at ambient temperature for approximately 15 hours, and then flushed with reverse osmosis deionized (RODI) water for 2 column volumes in down-flow.
Sanitization procedure:

- Flush with 1 M sodium hydroxide in up-flow at 100 cm/h for 30 minutes
- Flush with 1 M sodium hydroxide in down-flow at 100 cm/h for 30 minutes
- Recirculation of 1 M sodium hydroxide for 2 hours in up-flow at 100 cm/h
- Incubation of the column in 1 M sodium hydroxide for 1 hour (static sanitization for compete removal of endotoxins)
- Flush with RODI water at 100 cm/h until neutral pH is achieved

Samples of pre and post inoculation and sanitization were collected and assayed for microbial colony forming units (CFU) and endotoxin.

Microbial testing was performed by filtering 1 mL of the sample through a 0.2 µm filter unit, washing the filter with 100 mL of 0.1% peptone water, removing the filter from the unit, and placing it on a Tryptic Soy Agar (TSA) plate. The flow-through after the overnight incubation was diluted $1:10^6$ prior to filtration, while the post-sanitization water rinse was filtered without dilution. The TSA plate was placed in an incubator at 32°C for 4 days, and colonies are counted at day 2 and day 4.

Endotoxin testing was performed using gel-clotting limulus amebocyte lysate (LAL) test with a sensitivity of 0.25 EU/mL.

Results:

Results for bioburden and endotoxin levels from the microbial challenge are outlined in Table 1, which shows the sanitization procedure completely removed bioburden from millions of CFU to zero CFU in the post-sanitization water rinse. In addition, endotoxin levels were brought below the limit of detection (0.25 EU/mL) for the assay.

[2] A sanitization procedure should always be compatible with the stationary phase.

Table 1: Bioburden and Endotoxin Removal Results

Sample	CFU/mL @ 2 days	CFU/mL @ 4 days	Endotoxin (EU/mL)
Pre-inoculation water rinse	0	0	<0.25
Flow-through after overnight incubation	$9x10^6$	$9x10^6$	> 0.25
Post-sanitization water rinse	0	0	<0.25

Conclusions

Through the phosphate removal experiments, the innovative design of the 10 – 30 cm ID OPUS® columns has been qualified for cleaning applications required in downstream processing. The results of the cleaning experiments demonstrate the absence of significant dead-spaces in the column design and the ease of cleaning a pre-packed OPUS® column. OPUS® columns are therefore suitable for use in standard downstream processing applications and can withstand the cleaning protocols required in today's downstream processing applications.

In order to test effectiveness of sanitization on an OPUS® column, a worst case scenario was devised where the column was loaded with an excess of *E. coli* culture (a gram-negative, endotoxin producing bacteria). The results of the sanitization protocol demonstrate the effective removal of bioburden and endotoxin contamination.