Simplifying Progress

Effective Endotoxin Reduction in Bacteriophage Samples Using CIMmultus® Monolithic Technology

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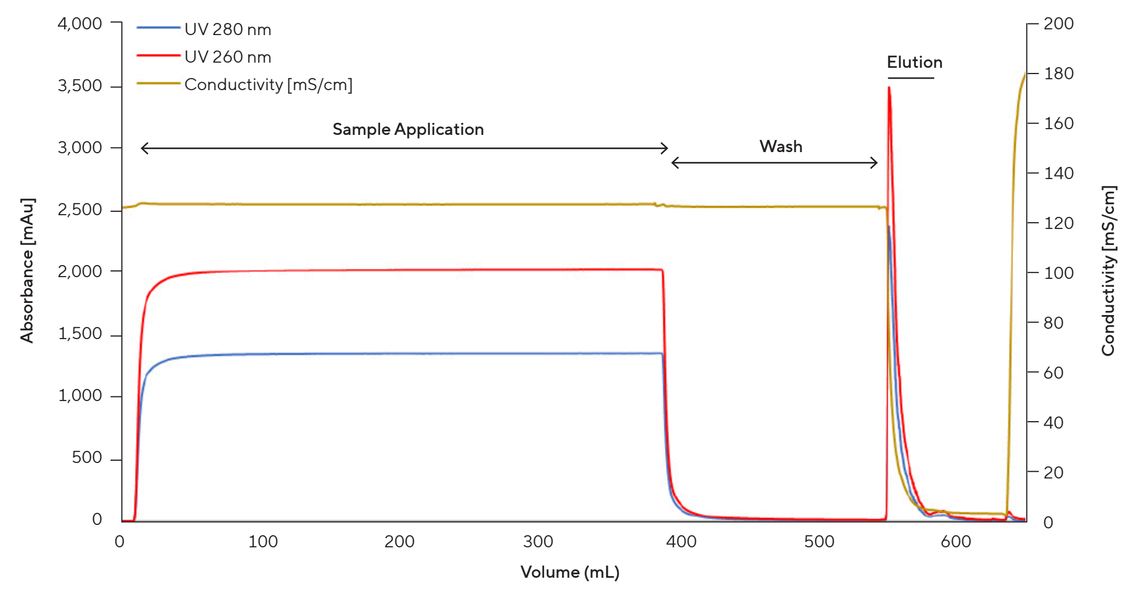
Introduction

Endotoxins are robust and persistent impurities that are native to the majority of phage substrates. Two anion exchangers, CIMmultus PrimaS® and CIMmultus® H-Bond, were tested for their capacity for endotoxin removal compared to a well known strong anion exchanger, CIMmultus® QA.

Capture of Phage on CIMmultus OH Column

PP-01 phage lysate was first clarified through 0.22 µm filter, and then diluted with 3M potassium phosphate buffer (pH 7.0), to reach 1.5 M potassium phosphate concentration in load. The sample was then applied to the CIMmultus® OH 8 mL (6 µm) column, and eluted in step to 100% Buffer B (Figure 1). The CIMmultus® OH capture step removed 99% of DNA and 98% of proteins from the sample. Phage recovery was approximately 100%.

Figure 1: *Preferential Exclusion Chromatography of Clarified Phage Lysate on CIMmultus® Oh 8 ML (6 MM) Column on ÄKTA Pure FPLC System.*

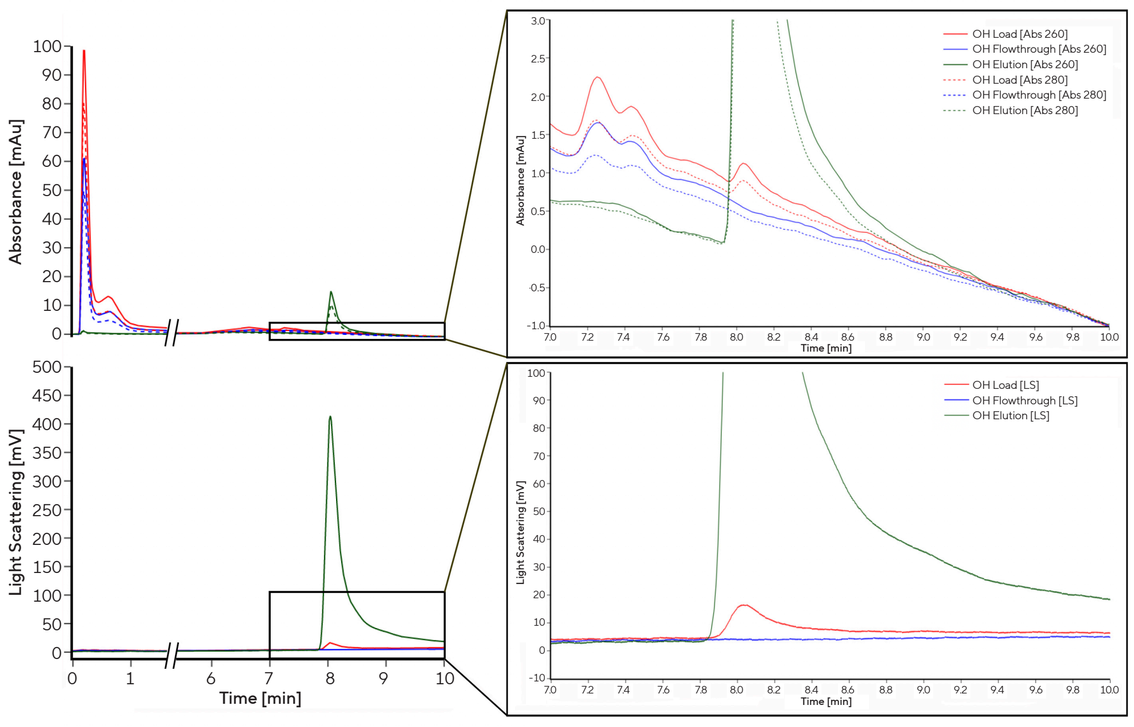


Note. Sample: Clarified phage lysate, diluted to 1,5 $MKHPO_4$ (pH 7.0), MPA: 1.5 $MKHPO_4$, (pH 7.0)MPB: 50 mM HEPES, 20 mM NaCl (pH 7.0), MPC: 1M NaOH. Flow rate: 8 mL/min. Method: direct inject, 20 column volume (CV) MPA wash, 10 CV step to 100% MFB, 10 CVMPC. Detection: absorbance (260 nm, 280 nm).

PATfix System Analytics of CIMmultus® OH Fractions

Fractions from CIMmultus® OH capture were analyzed with PATfix® Analytical System using the 0.1 mL CIMac™ Adeno monolithic column (2 µm). Bacteriophage is concentrated in main elution, and none is detectable in the flowthrough (FT) fraction (Figure 2). Impurities visible in UV absorption signals in the load are removed in the FT fraction and are not detectable in the main elution.

Figure 2: *Chromatograms of Bacteriophage CIMmultus® Oh Fractions Diluted 20x With 50 mM Tris (PH 7.5) and Analyzed With PATfix® Using a CIMac™ Adeno Monolithic Column.*

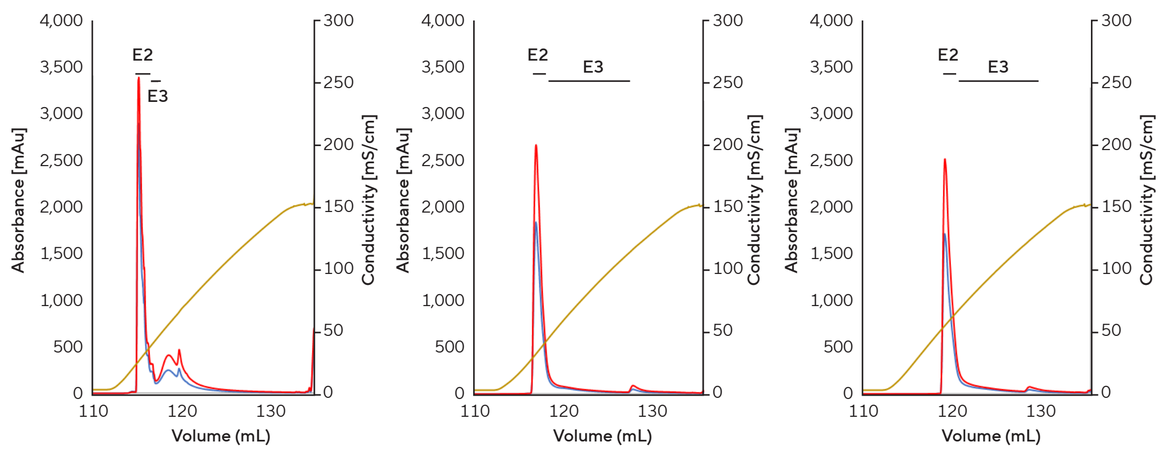


Note. MPA: 50 mM Tris (pH 7.5), MPB: 50 mM Tris, 1 M NaCl, (pH 7.5), MPC: 1 M NaOH, 2 M NaCl. Method: 50 CV of 100 % MPA, linear gradient 0-50 % MPB in 50 CV, 100 % MPC for 20 CV, regeneration with 30 CV of 100 %MPB and 150 CV of MPA. Only the FT and gradient parts of the chromatogram are shown.

Phage Polishing on Multimodal and Anion Exchange CIMmultus Columns

The CIMmultus® OH elution was diluted with 50 mM HEPES (pH 7.0), to lower the conductivity and applied to three different anion exchange columns: CIMmultus® QA, CIMmultus PrimaS®, CIMmultus® H-Bond. Phages were eluted from the column using a salt gradient (Figure 3).

Figure 3: *Elution Profiles on CIMmultus® Polishing Columns.*



Note. A) CIMmultus® QA; B) CIMmultus® H-Bond. C) CIMmultus PrimaS®. BufferA: 50 mM HEPES, 20 mM NaCl (pH 7.0), Buffer B: 50 mM HEPES, 2 M NaCl pH 7.0. Method: sample application, wash 20 CV (data not shown), linear gradient 0-100% Bin 20 CV. Sample: elution phage suspension from capture step mixed with buffer A toreduce conductivity. Detection: UV at 260 (red) and 280 nm (blue), conductivity (brown), fraction collection (grey).

Phage Recovery and Reduction of Impurities

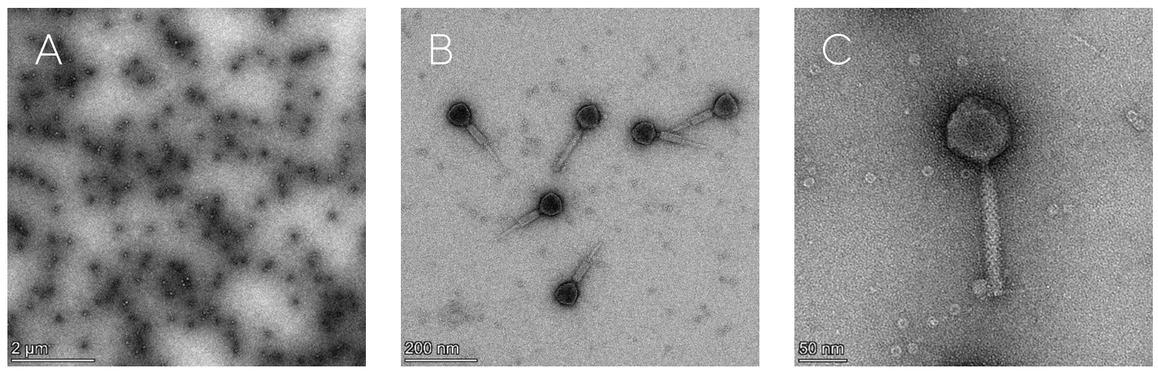
Analysis were performed on the main elution fractions to estimate phage recovery (Plaque spot assay) and depletion of the main impurities (DNA - PicoGreen; Protein – BCA; Endotoxin – Endosafe) achieved by purification (Table 1).

Table 1: *Summary of Phage Titer and Removal of Protein, DNA, and Endotoxins Following a Two-Step Purification of PP-01 on CIM® Monolithic Columns.*

	Total Titer (Spot Assay)		Total Proteins (BCA assay)		Total DNA (Picogreen Assay)		Total Endotoxins (Endosafe)	
	PFU	Recovery (%)	µg	Depletion (%)	ng	Depletion (%)	EU	Reduction (log_{10})
Phage Lysate	1.24E+12		1.15E+5		1.01E+6		4.23E+6	
Capture - CIMmultus® OH	1.58E+12	>100	2,367	98	6,335	99	5.29E+6	/
Polish - CIMmultus® QA E2	1.80E+12	>100	1,035	99	9,841	99	1600	4
Polish - CIMmultus® QA E3	1.10E+11	9	129	99	2,089	99	N.A.	4
Polish - CIMmultus H-Bond® E2	7.53E+11	61	636	99	1.05E+4	98	0.24	7
Polish - CIMmultus H-Bond® E3	3.34E+11	27	427	99	7,247	98	N.A.	7
Polish - CIMmultus PrimaS® E2	1.04E+12	84	267	>99	1.46E+4	98	0.80	7
Polish - CIMmultus PrimaS® E3	2.25E+11	18	<0.1	>99	7,541	98	N.A.	7

Transmission Electron Microscopy Results

Figure 3: *Negative Staining Pictures of Bacteriophage PP-01 Purified by CIMmultus PrimaS® Polishing Column Taken Using Transmission Electron Microscopy.*



Note. A) High concentration of bacteriophage and an even distribution on the grid; B and C) bacteriophage head (70x70), and contractile tail (130 x 16 nm). The bacteriophages have myovirus morphotype.

Acknowledgement

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