TWO STEP PURIFICATION PROCESS FOR BACTERIOPHAGES WITH CIM® MONOLITHS

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INTRODUCTION

Bacteriophages represent immense potential as therapeutic agents. Many of the most compelling applications of bacteriophages involve human therapy, some pertinent to gene therapy, others involving antibiotic replacement. In bacteriophage research and therapy, most applications ask for highly purified phage suspensions, as such it is crucial to reduce proteins, endotoxins, DNA and other contaminants. The most common technique for purification is ultracentrifugation using cesium chloride gradients. This technique is elaborate, cumbersome, expensive and difficult to scale-up. Alternative techniques for purification are usually time consuming and affect phage recovery and/or viability. In this study we present efficient two-step chromatographic purification method with binding phages to a stationary phase - Convective Interaction Media (CIM®) monoliths. The aim of the study was to develop robust, fast and effective virus purification platform that can be used for several types of bacteriophages for any application. In this work bacterial lysate with bacteriophage T4 (host *E.Coli)* was used.

RESULTS

Capture step

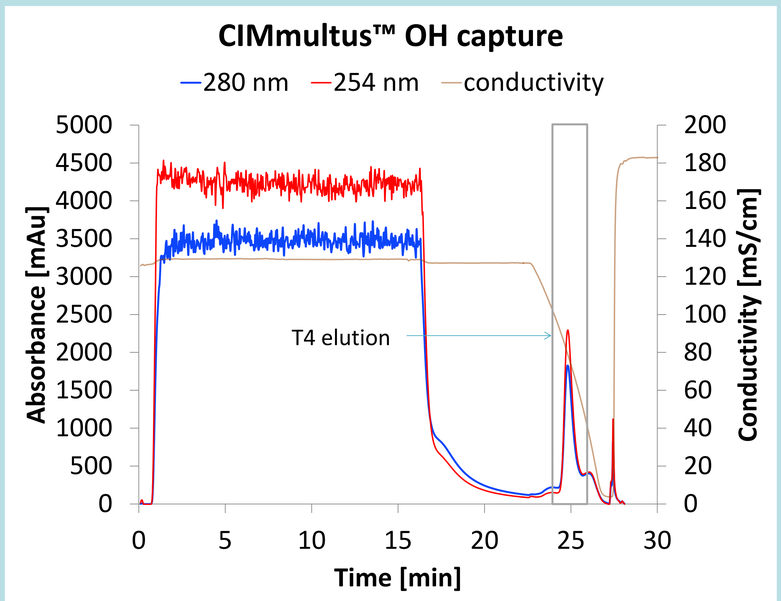


Figure 1: Chromatographic profiles of OH run, Column: CIMmultus™ OH-1 mL (6 µm). Sample preparation: T4 bacteriophage lysate 2x diluted with dilution buffer. Method: load, wash with buffer A, linear gradient 0-100 % buffer B in 20 CV. CIP: 1 M NaOH.

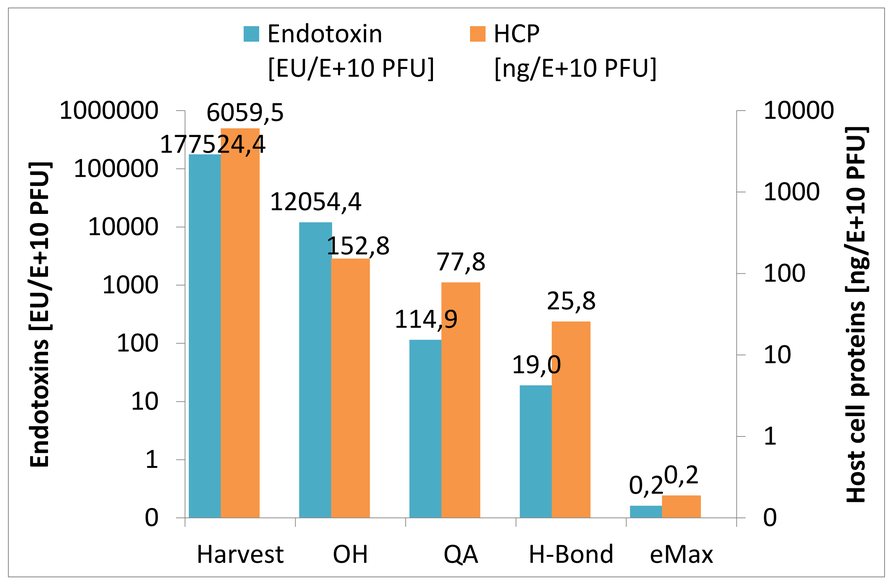
Chromatography summary:

Conditions capture:
- Buffer A: 1.5 M KH_2PO_4, K_2HPO_4 pH 7.0
- Buffer B: 20 mM KH_2PO_4, K_2HPO_4 pH 7.0
- Dilution buffer: 3 M KH_2PO_4, K_2HPO_4 pH 7.0
- Flow rate: 5 mL/min
- Detectors: UV 280 nm, 254 nm; conductivity

Conditions polishing:
- Buffer A: 20 mM KH_2PO_4, K_2HPO_4 pH 7.0
- Buffer B: 500 mM KH_2PO_4, K_2HPO_4 pH 7.0
- Flow rate: 1 or 5 mL/min
- Detectors: UV 280 nm, 254 nm; conductivity

Analytical assays:
- Titer - Plaque assay (PA)
- Host protein - *E. coli* HCP ELISA Kit
- Endotoxin - Endosafe® nexgen-PTS™

For capture and concentration of bacteriophages from bacterial lysate, CIMmultus™ OH- 1 mL (6 µm) column was used. In first step we reduce host cell proteins, DNA and endotoxins. For additional endotoxin and protein removal second purification step was developed on CIMmultus™ eMax- 1, CIMac™ QA and CIMac H-Bond™ ADC, to offer different strategies for various phage products and purity demands. With this two-step process phages can be efficiently purified to meet the regulations for various bacteriophage applications.



Polishing option 1

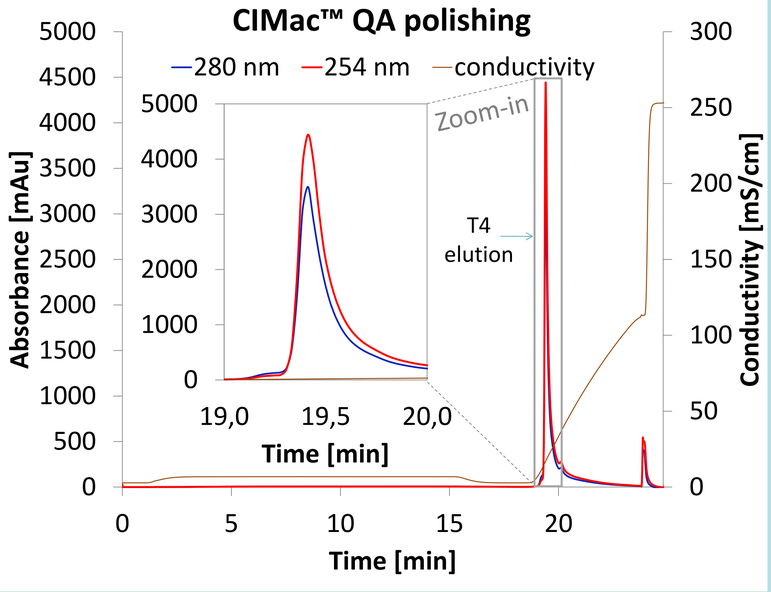


Figure 2: Chromatographic profiles of QA run (left: elution peak zoom in). Column: CIMac™ QA-0.1. Sample preparation: elution fraction from OH capture 20x diluted with buffer A. Method: load, wash with buffer A, linear gradient 0-100 % buffer B in 20 CV. CIP: 1 M NaOH, 2M NaCl.

Polishing option 2

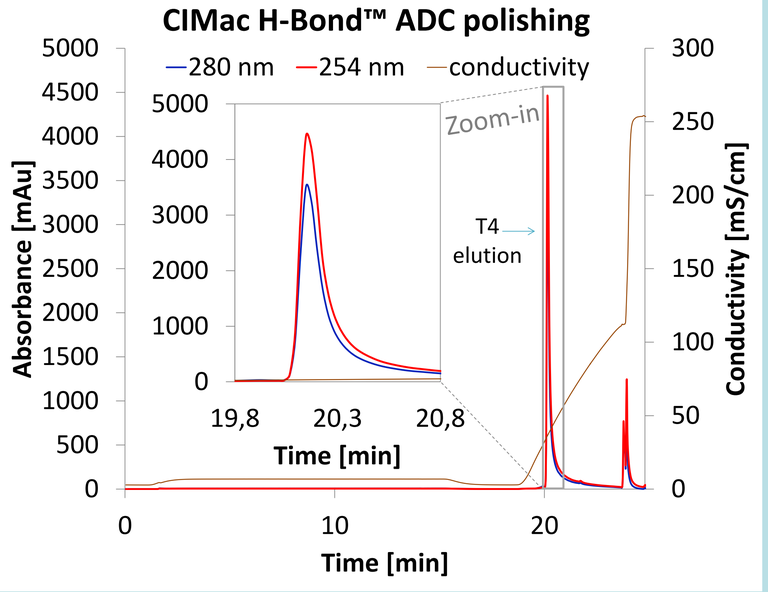


Figure 3: Chromatographic profiles of H-Bond run (left: elution peak zoom in). Column: CIMac H-Bond™ ADC 0.1 mL (2 µm). Sample preparation: elution fraction from OH capture 20x diluted with buffer A. Method: load, wash with buffer A, linear gradient 0-100 % buffer B in 20 CV. CIP: 1 M NaOH, 2M NaCl.

Polishing option 3

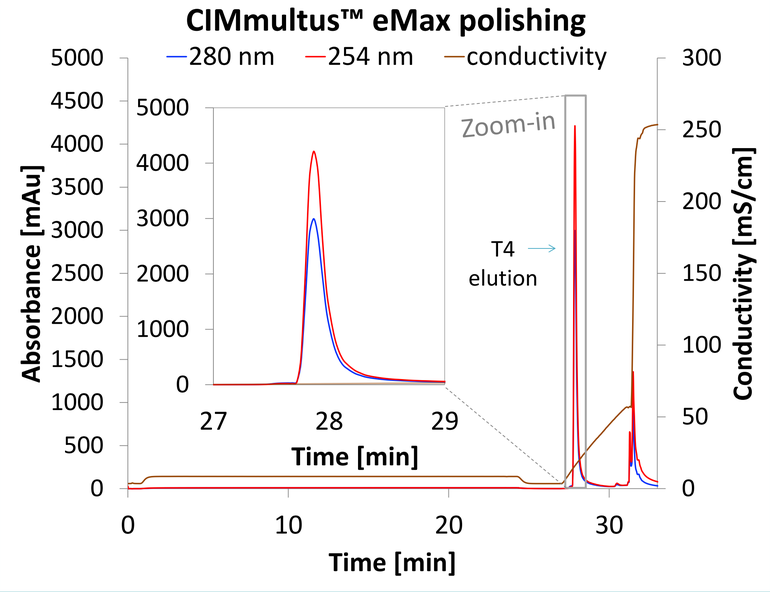


Figure 4: Chromatographic profiles of eMax run (left: elution peak zoom in). Column: CIMmultus™ eMax-1 mL (2 µm). Sample preparation: elution fraction from OH capture 20x diluted with buffer A Method: load, wash with buffer A, linear gradient 0-100 % buffer B in 20 CV. CIP: 1 M NaOH, 2M NaCl.

CONCLUSIONS

- From Harvest to purified sample in approximately one hour
- Simple scale up from analytical to industrial scale purification process
- CIMmultus™ OH-1 mL (6 µm) is efficient method to reduce main impurities from bacterial lysate like proteins and endotoxins with > 80 % recovery
- Capture step concentrates phage product from bacterial lysate 5 to 20-times
- 3 different polishing options enable polishing of various different phage types
- The traditional method of endotoxin reduction with QA achived more than 100-fold reduction, from 12054.4 to 114.9 EU/E+10 PFU
- H-Bond™ ADC achived more than 600-fold reduction from 12054.4 to 19 EU/E+10 PFU
- eMax achived more than 60 000-fold reduction from 12054.4 to 0.2 EU/E+10 PFU
- Host cell protein reduction tracked closelly with endotoxin reduction
- eMax* is the best polishing option for T4 (*Results with eMax were obtained with a pre-commercial prototype)

ACKNOWLEDGEMENTS

The authors would like to thank Centre of Excellence for Biosensors, Instrumentation and Process Control (COBIK), Ajdovscina, Slovenia for providing bacteriophage harvest.