Purification of Staphylococcus aureus bacteriophages VDX-10 using CIM® monolithic columns

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INTRODUCTION

In recent years bacteriophages were identified as a useful potential tool for different applications such as alternative to antibiotics, detection of pathogenic bacteria, delivery vehicles for protein and DNA vaccines and as gene therapy delivery vehicles. For all listed fields of use it is important that phages are highly purified with preserved biological activity. Phage and other virus purification have traditionally been carried out by $CsCl_2$ density gradient ultracentrifugation, which is however difficult to be scaled-up. An alternative is chromatography, which already proved to be efficient for separation and purification of certain virus types. Methacrylate monoliths (CIM Convective Interaction Media® monolithic columns) were designed for purification of bionanoparticles and they already proved to be very efficient for concentration and purification of several plant and human viruses (influenza A, influenza B, adenovirus type 5, hepatitis A and others).

Our aim was to investigate whether CIM® methacrylate monolithic columns can be implemented for purification of phages. *Staphylococcus aureus* phage VDX-10 was selected. Chromatographic support chemistry and buffer screening led to development of purification method on strong anion exchanger. Optimised single step purification method developed for *S. aureus* VDX-10 phage on CIM® QA monolithic column resulted in efficient removal of host cell DNA and proteins with high recovery of viable phage.

MATERIALS AND METHODS

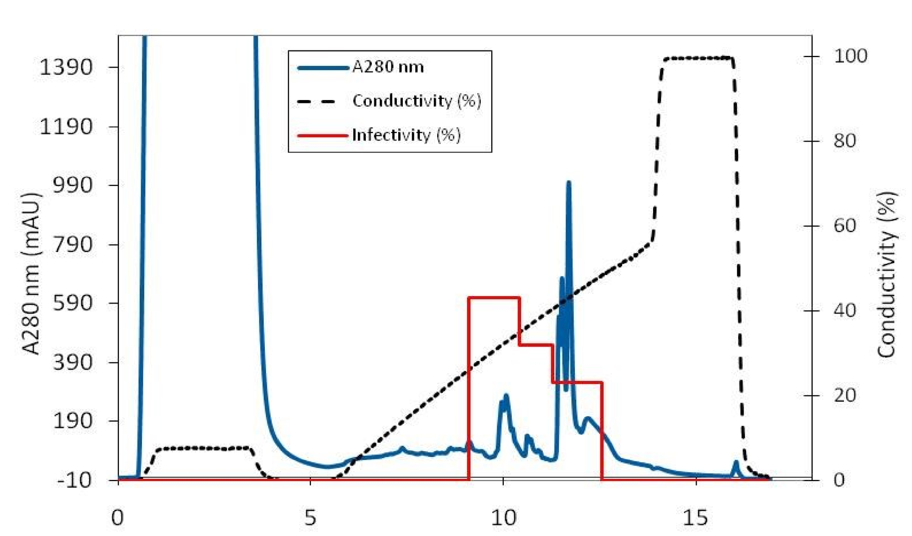
Phages: Phage VDX-10 was propagated in *S. aureus.* Before loading on the column phage suspension was filtered through 0.45 µm filter.

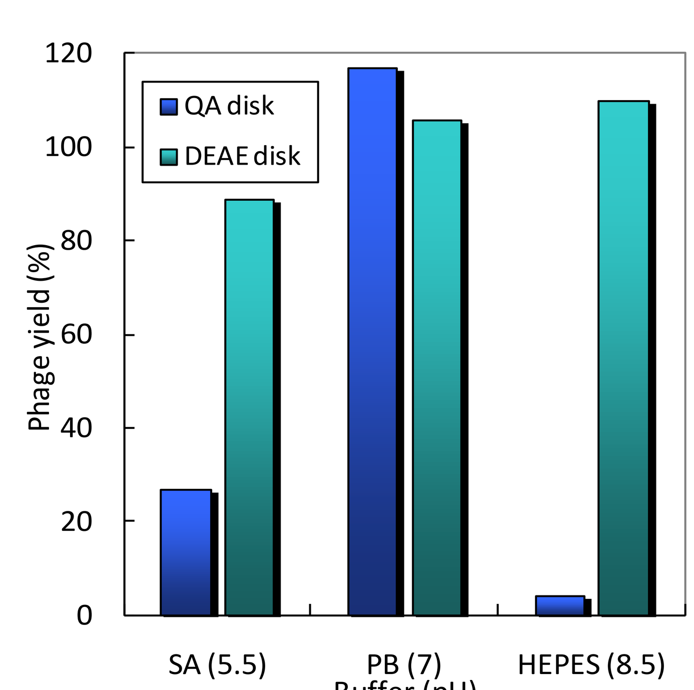
Chromatography on CIM® Monolithic Columns: Two different CIM® Monolithic Column chemistries were used (bed volume: 0.34 mL): strong anion exchanger – CIM® QA, weak anion exchanger – CIM® DEAE. For scale up of *S. aureus* phage VDX-10 CIM® QA 8 mL column was used.

Analytics: Phage quantification (plaque assay method), host cell DNA (qPCR), proteins (BradfordUltra).

S. aureus phage VDX-10: Buffer and chemistry screening

Anion exchanger – CIM® QA Monolithic Column

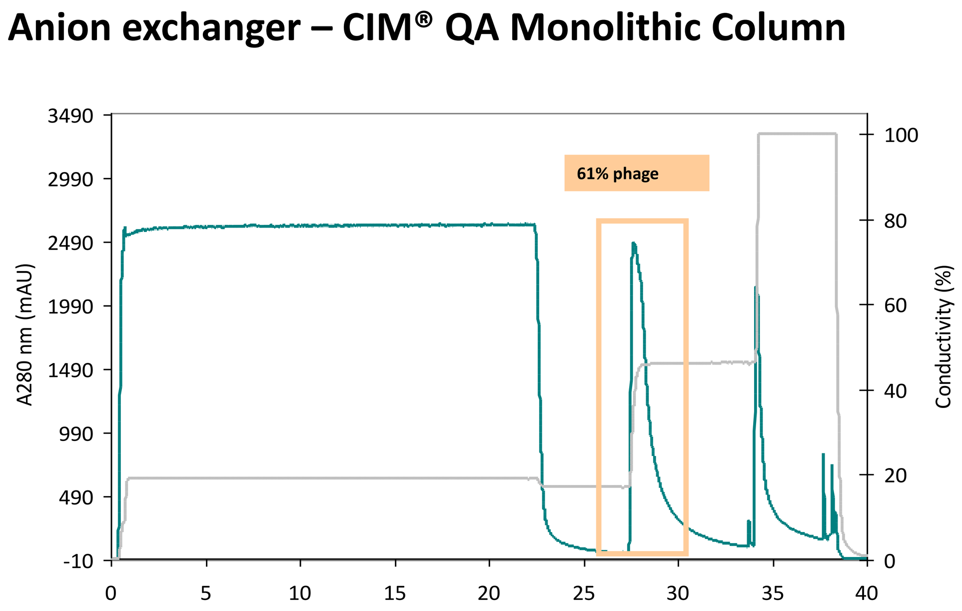




Phage separation on CIM® QA Monolithic Column. Sample: bacterial lysate-4x diluted in buffer A. Conditions: buffer A: 100 mM phosphate buffer pH 7, buffer B: 2.0 M NaCl in buffer A, pH 7.5, flow rate: 4 mL/min.

S. aureus phage VDX-10: Dynamic Binding Capacity

Anion exchanger – CIM® QA Monolithic Column

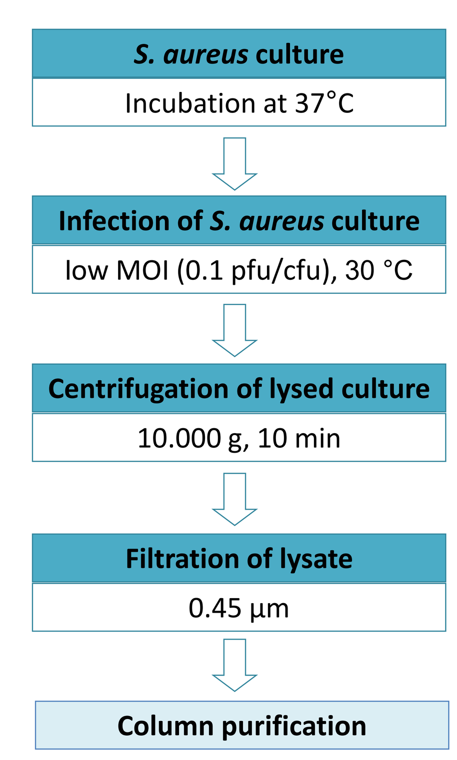


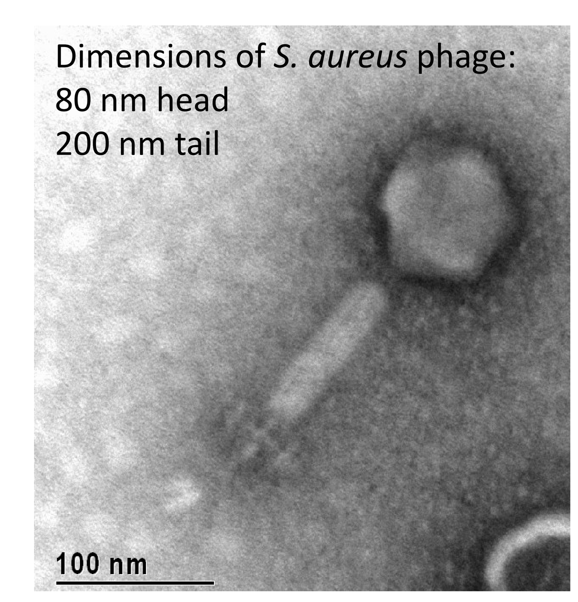
Monolithic Column	Max. load (pfu)
CIM® QA Disk	3.7E+9
CIM® QA 8 mL Tube	8.8E+10
CIM® QA 80 mL Tube	8.8E+11
CIM® QA 800 mL Tube	8.8E+12

DBC for *S. aureus* VDX-10 phage: $1.1x10^{10}$ pfu/mL

Dynamic Binding Capacity of *S. aureus* phage VDX-10 on CIM® QA Monolithic Column using salt step gradient. Sample: bacterial lysate-2x diluted in buffer A. Conditions: buffer A: 100 mM phosphate buffer pH 7, buffer B: 2.0 M NaCl in buffer A, pH 7.5, flow rate: 4 mL/min (210 cm/h), UV detection at 280 nm.

S. aureus phage VDX-10: Upstream



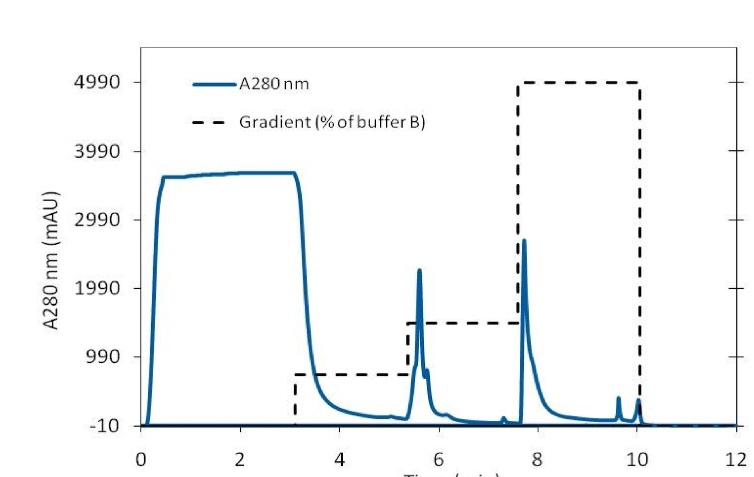


TEM by Magda Tušek Žnidaršič, NIB

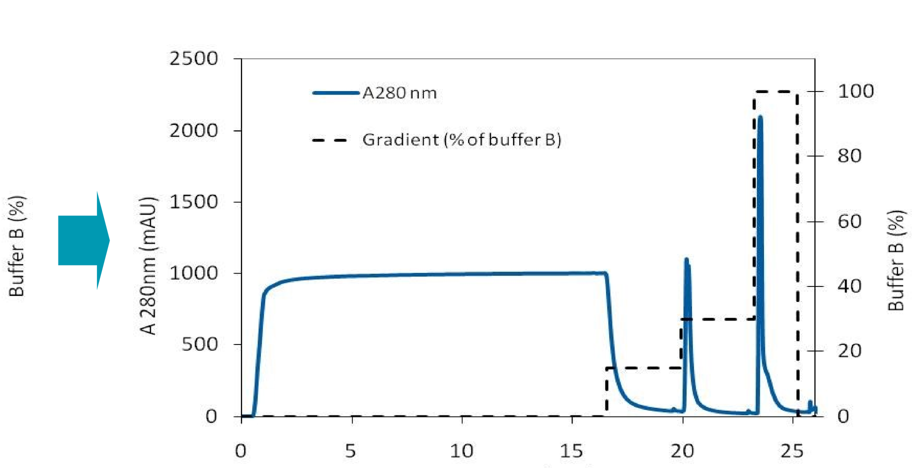
Upstream of *S. aureus* VDX-10. VDX-10 phages produced in host strain *S. aureus* ATCC 19685. A 1% inoculum of the bacteria was incubated with shaking at 37 ◦C to mid-log phase, infected with the phages at low multiplicity of infection (0.1 phage/colony forming unit), and the incubation continued with shaking at 30 ◦C until lysis was observed. The lysate was centrifuged (10,500×g for 10 min at 4 ◦C) and the supernatant was passed through a 0.45m filter and then stored at 4 ◦C. This cleared and filtered bacterial lysate was the starting material for purification of phages VDX-10 by CIM®.

S. aureus phage VDX-10: Scale-up

CIM® QA Monolithic Column (bed V: 0.34 mL)



CIM® QA 8 ml Column (bed V: 8 mL)



Phage separation on CIM® QA Monolithic Column. Sample: bacterial lysate-2x diluted in buffer A. Conditions: buffer A: 100 mM phosphate buffer pH 7, buffer B: 2.0 M NaCl in buffer A, pH 7.5, flow rate: 4 mL/min (210 cm/h).

Phage separation on CIM® QA 8 mL Column. Sample: bacterial lysate-2x diluted in buffer A. Conditions: buffer A: 100 mM phosphate buffer pH 7, buffer B: 2.0 M NaCl in buffer A, pH 7.5, flow rate: 30 mL/min (210 cm/h).

Monolithic Column	Phage recovery	Protein removal	Host cell DNA removal
CIM® QA Disk	54%	90%	>99%
CIM® QA 8 mL Tube	65%	91%	>99%

CONCLUSIONS

- Efficient purification method for phage was developed on CIM® ion exchangers: up to 65% recovery of viable phage.
- High dynamic binding capacity (1.1E+10 pfu/mL) and speed of CIM® supports enables high productivity.
- Scale-up of purification method from lab scale CIM monolithic column to 8 ml CIM® monolithic column.

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