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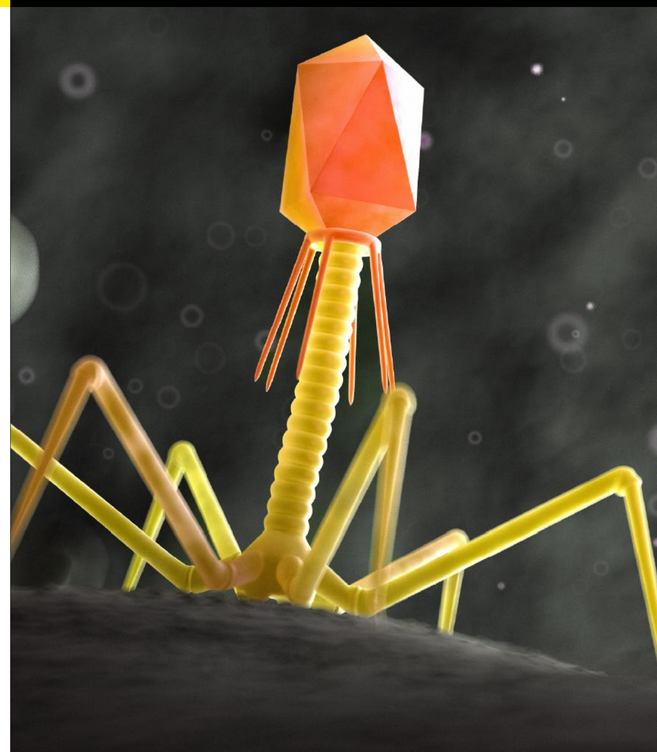
Case Study

# How to Overcome the Challenges in Producing Therapeutic-Grade Bacteriophages?

Bacteriophages (phages) hold significant potential as therapeutic agents, offering exciting possibilities for human therapy, especially to target bacteria either as a standalone alternative to antibiotics or as a supplementary treatment to antibiotics. Phages are considered safe for humans, but their unique properties present challenges in production and purification. In this article, we will explore the key considerations in phage purification and the innovative techniques used to overcome these challenges.

Producing therapeutic-grade phages presents several challenges, particularly in achieving efficient purification methods. Traditional packed-bed columns can be prone to clogging and reduced flow rates when dealing with larger particles. One of the main goals is to achieve excellent purity and recovery rates for phages, while maintaining their functional viability, which is essential for both research and therapeutic applications.

A significant challenge in the downstream process is the removal of impurities, such as host cell residuals and upstream media components. Major contaminants include host cell proteins (HCP), host cell DNA (hcDNA), and endotoxins from Gram-negative bacteria.



**For more information, visit** sartorius.com/products/process-chromatography/chromatography-consumables/monolithic-chromatography

# CIMmultus® Monolithic Chromatography: An Effective Solution

CIMmultus monolithic chromatography has been proven to successfully address many of these challenges, offering distinct advantages in terms of efficiency, scalability, and versatility. These columns can process large biomolecules like phages rapidly and efficiently, allowing for high flow rates and low backpressure. The process time can thus be significantly reduced, with a 2-liter lysate batch purification being completed in just a few hours, thereby saving valuable time without compromising the quality of the final drug product.

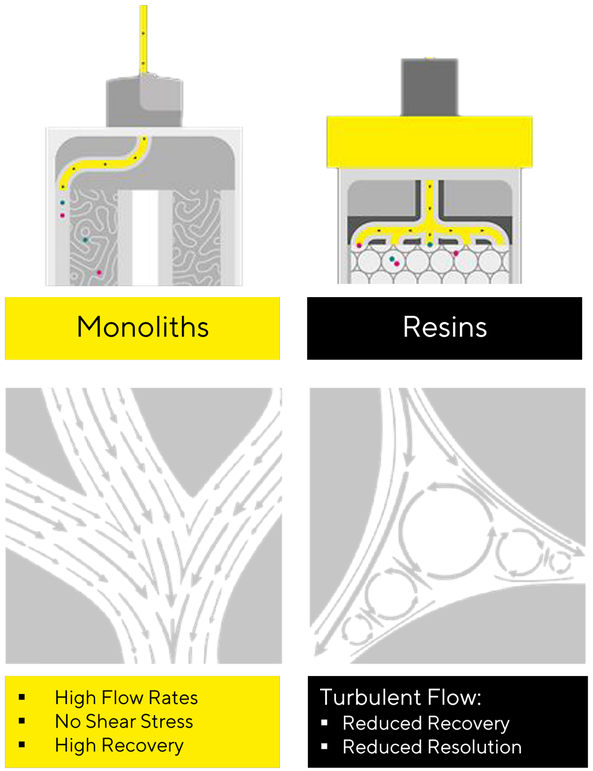



*Figure 1: Comparison of monolithic and resin-based chromatography.*

In addition, unique structure of the monolithic columns minimizes the risk of shear forces that can damage delicate viral particles, ensuring the integrity of the phages throughout the purification process. These columns are also highly reusable, capable of supporting multiple purification runs without significant performance loss. This reusability substantially reduces the cost per purification run, making the overall cost-effectiveness significantly better compared to traditional purification strategies.

The versatility of monolithic columns is a significant advantage, especially in the purification of phages for various types of applications, where purity requirements can differ greatly. For example, in the case of phages used in agriculture, the purity standards are generally less stringent compared to those required in medicine.

## Why You Need Monoliths to Purify Your Biomolecules

Watch the quick video to learn how convective chromatography supports robust biomolecule purification.

Watch Video

## Vesatility

### Same Monolith. Multiple Formats



**CIM® Octa** and **CIM® Monolithic Well Plates**




**CIMmultus®**

### Best Fit for Your Sample with Multiple Ligand Options.

**Capture:**

**CIMmultus® OH** (HIC)

**Three Polishing Options:**

**CIMmultus PrimaS®** (Mixed-Mode)

**CIMmultus PrimaT** (Mixed-Mode)

**CIMmultus® QA** (AEX)

# The Process

1. Bacterial Lysis
2. Clarification
3. Capture Step
   CIMmultus® OH
4. Polishing Step
   CIMmultus® PrimaS
   CIMmultus® PrimaT
   CIMmultus® QA
5. Phage

## Meeting Rigorous Purity Standards for Human Therapy

When phage products are intended for treating bacterial infections in humans, the purity requirements are far more rigorous. If phages originate from a Gram-positive bacterial production system, filtration followed by a single chromatography step with a hydroxy (OH) ligand is typically sufficient to achieve industry-standard purity levels. This is because Gram-positive bacteria lack endotoxins, which are usually the most challenging impurities to remove from phage samples.

To reach stringent standards for human therapy if phages originate from a gram-negative bacteria, a two-step chromatography process is recommended (Figure 2).

In the first step, a monolithic column with a hydroxy (OH) ligand is used to remove the majority of host cell impurities, achieving significant reductions in host cell proteins and DNA. The gentle interaction between the phages and the OH ligand ensures high recovery rates, typically maintaining 80-100% infectivity.

Subsequently, endotoxin removal can be performed with high efficiency using a monolithic column with a multimodal ligand (PrimaS), achieving substantial reductions in endotoxins regardless of the type of phage.

To simplify the formulation step, the buffer used for phage elution can be similar to the formulation buffer. This allows the final formulation conditions to be achieved through simple dilution with the formulation buffer, eliminating the need for a TFF step to prepare the phages for the final drug product.

## The Future of Phage Purification

In conclusion, CIMmultus monolithic columns represent a significant advancement in the field of phage purification, combining efficiency, scalability, and versatility. While the initial investment and optimization process may present challenges, the long-term benefits of using monolithic columns, particularly in terms of throughput and product integrity, make them a valuable and cost-efficient tool for both research and industrial applications.

## Looking Forward to Working With You

Exploring new processes can be challenging for various reasons. This is why our Cornerstone® Process Development team, is ready to develop purification process using CIMmultus monolithic columns with your phage of choice on a small scale and then transfer the process to your site.

Additionally, our tech support team (email: help.bia@sartorius.com) is available to provide remote support, offering detailed guidelines, suggestions for process improvements, and updating you on our latest publications. We’re here to support you at every step, helping you navigate potential challenges and celebrating your success. Don’t hesitate to reach out—we’re always happy to help.

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## Further Reading

**CIM Monolithic Chromatography as a Useful Tool for Endotoxin Reduction and Purification of Bacteriophage Particles Supported With PAT Analytics |** L. Rebula, A. Raspor, M. Bavčar, et al. | J. Chromatogr. B (2023) | DOI: 10.1016/j.jchromb.2023.123606

**CIM® Monolithic Anion-exchange Chromatography as a Useful Alternative to CsCl Gradient Purification of Bacteriophage Particles |** E.M. Adriaenssens, S.M. Lehman, K. Vandersteegen, et al. | Virology (2012) | DOI: 10.1016/j.virol.2012.09.018

**Characterisation of Methacrylate Monoliths for Bacteriophage Purification |** F. Smrekar, M. Ciringer, A. Štrancar, and A. Podgornik | Journal: J. Chromatogr. A (2011) | DOI: 10.1016/j.chroma.2010.12.083

**Monolithic Ion Exchange Chromatographic Methods for Virus Purification |** H.M. Oksanen, A. Domanska, and D.H. Bamford | Virology (2012) | DOI: 10.1016/j.virol.2012.09.019

**Potential of Bacteriophage Therapy in Managing Staphylococcus Aureus Infections During Chemotherapy for Lung Cancer Patients |** J. Li, H. Zheng, and S.S.Y. Leung | Sci Rep (2023) | DOI: 10.1038/s41598-023-36749-2

**Purification and Concentration of Bacteriophage T4 Using Monolithic Chromatographic Supports |** F. Smrekar, M. Ciringer, M. Peterka, et al. | J. Chromatogr. B (2008) | DOI: 10.1016/j.jchromb.2007.05.048

**Purification and Concentration of Mycobacteriophage D29 Using Monolithic Chromatographic Columns |** K. Liu, Z. Wen, N. Li, et al. | J. Virol. Methods (2012) | DOI: 10.1016/j.jviromet.2012.07.016

**Purification of Bacteriophages Using Anion-Exchange Chromatography |** D. Vandenheuvel, S. Rombouts, and E.M. Adriaenssens | (2018) | DOI: 10.1007/978-1-4939-7343-9_5

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