SARTORIUS

Method Guide

Chromatographic Purification of LNP-based Formulations with CIMmultus® OH

Introduction

Lipid nanoparticles (LNPs) are emerging as a key approach in nucleic acid delivery, however, producing them at larger scale demands purification methods that preserve functionality while ensuring uniformity. Standard purification techniques, such as tangential flow filtration (TFF) and dialysis, have been widely used, and although effective, often have limitations in processing speed, scalability, and disturbing the integrity of particles.

To address these challenges, we have developed a chromatography-based purification of LNPs using CIM® convective interaction media monolithic columns, which are specifically designed for the purification of large, shear-sensitive molecules. CIMmultus columns, part of the preparative line of monoliths, are designed with wide and highly interconnected channels that facilitate fast flow rates and minimize shear stress through laminar flow, making them ideal for the purification of delicate lipid nanoparticles (LNPs).

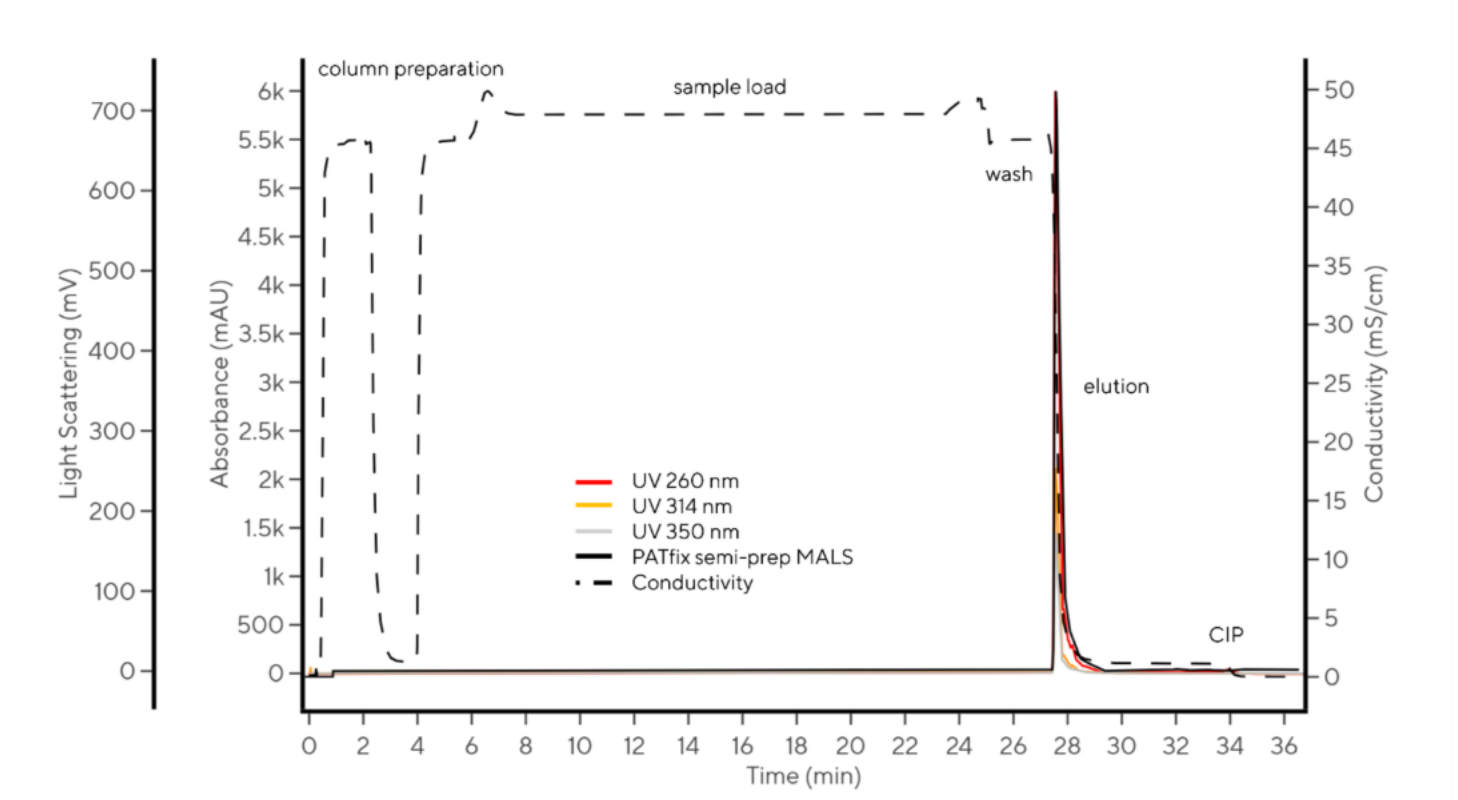
LNPs efficiently bind on CIMmultus OH during loading in a kosmotropic salt buffer and are eluted with low salt buffers. Meanwhile, ethanol (EtOH) and formulation buffer are removed from the sample, resulting in highly concentrated particles. This method achieves the same goal as TFF, but at a much faster and more scalable pace, while additionally, effectively removing non-encapsulated nucleic acids and poorly performing LNPs.

This purification method combines multiple processing steps: buffer exchange, EtOH removal, and LNP concentration into a single run. In contrast to TFF or dialysis, this method offers significantly higher recoveries, enhanced LNP stability, a more uniform size distribution, and can be completed up to 16 times faster. Additionally, the columns can be reused multiple times, further reducing process costs.

For more information, contact
help.BIA@sartorius.com

During the purification process (Figure 1), multiple UV wavelengths (260 nm, 314 nm, 350 nm) are tracked in combination with light scattering, for which the PATfix® semiprep MALS detector is used.

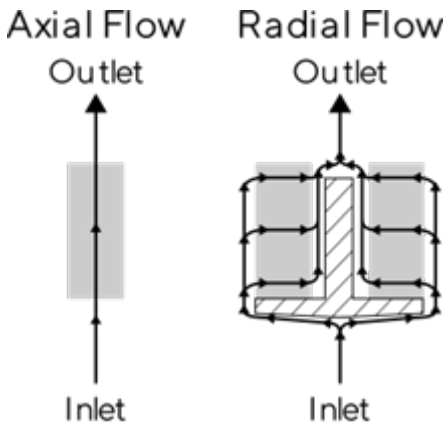
Figure 1: *Typical chromatogram of LNP purification using CIMmultus OH (6 µm channels), step gradient elution.*



Getting Started

To access Instructions for Use document for this column, scan the QR code on the right or use the following link: Product Documentation - Sartorius BIA Separations. CIMmultus® columns utilize a radial flow distribution (Figure 2) inside the housing, requiring the column to be connected to the chromatographic system with the correct flow direction. Note that some chromatographic systems have default reverse-flow functions built into their software that can cause the flow direction to be reversed without warning. Make sure this function is disabled before conducting any experiments.

Figure 2: *Comparison of axial flow and radial flow distribution within stationary phase of the chromatographic column.*



It is highly advised to perform an integrity test to check the monolith's integrity before the first run. Instructions are available by scanning the QR code on the right or by clicking here: [Column Integrity Test for CIM Monoliths - Sartorius BIA Separations](#).

How to Purify LNPs With CIMmultus® OH (6 µm channels)

CIMmultus® OH 6 µm is delivered in 20% EtOH. It is recommended to sanitize and regenerate the column as described below, prior to use.

Capacity and resolution on CIMmultus® columns are independent of flow rate, enabling fast processes at high flow rates. The recommended flow rate is 5-10 CV/min, depending on the ability and backpressure of the system.

Detectors: UV detector at 260 nm, 314 nm and 350 nm, PATfix® semi-prep MALS 90° (optional).

Sample preparation: After LNP formulation, it is recommended to dilute the sample to reduce EtOH to under 3% and raise pH above 7.2. Both can be achieved by 10x dilution with 50 mM TRIS, pH 7.4.

Buffers:

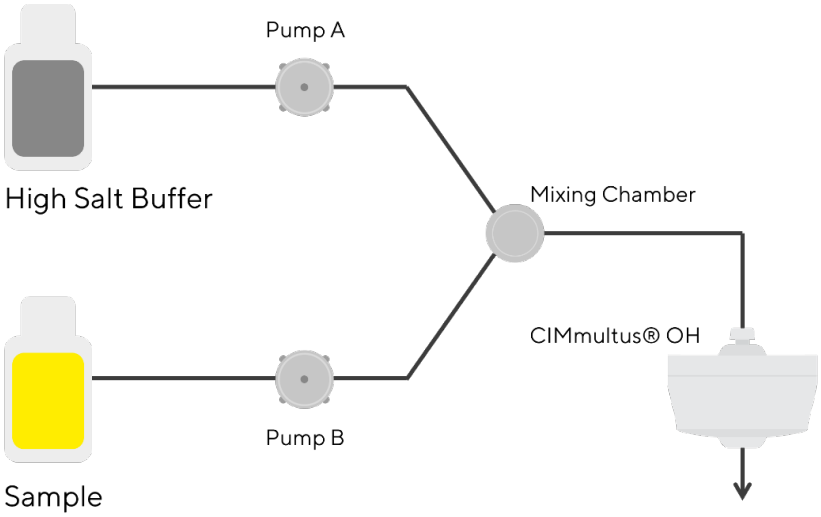
- **Sample dilution**: 50 mM TRIS; pH 7.4
- **Buffer A (loading)**: 15 mM TRIS, 1 M Na-Citrate, 200 mM sucrose; pH 7.4
- **Buffer B (elution)**: 15 mM TRIS, 200 mM sucrose; pH 7.4
- **Buffer C (strip)**: 50% EtOH
- **Buffer D**: deionized water (ddH_2O)
- **Buffer E (sanitization)**: 1 M NaOH
- **Buffer F (neutralization)**: concentrated buffer with neutral pH and high salt concentration (e.g., 0.5 M TRIS, 1 M NaCl)

Equilibrating the column: To achieve loading conditions of 15 mM Tris, 500 mM Na-citrate, and 200 mM sucrose, mix buffer A and buffer B in a 1:1 ratio, either in-line or offline. Begin by washing the column with 10 column volumes (CV) of the AB buffer mixture. Follow with a wash using 10 CV of buffer B. Finally, perform another wash with 10 CV of the AB buffer mixture to complete the conditioning process. pH and conductivity detectors at the column outlet should match target specification.

Loading the sample: Adjust the prepared sample to binding conditions (15 mM Tris, 500 mM Na-Citrate, 200 mM sucrose). This can be achieved by diluting the post-neutralization sample 1:1 with Buffer A either offline or inline. Inline dilution is recommended due to observed improved performance.

Inline dilution can be used for sample loading or column equilibration (Figure 3). One of the pumps is connected to a stock solution of buffer with high salt, and the second pump is pumping the sample (or low salt buffer in case of equilibration). Both combine in the appropriate ratio (set by the % pump B) just before the column.

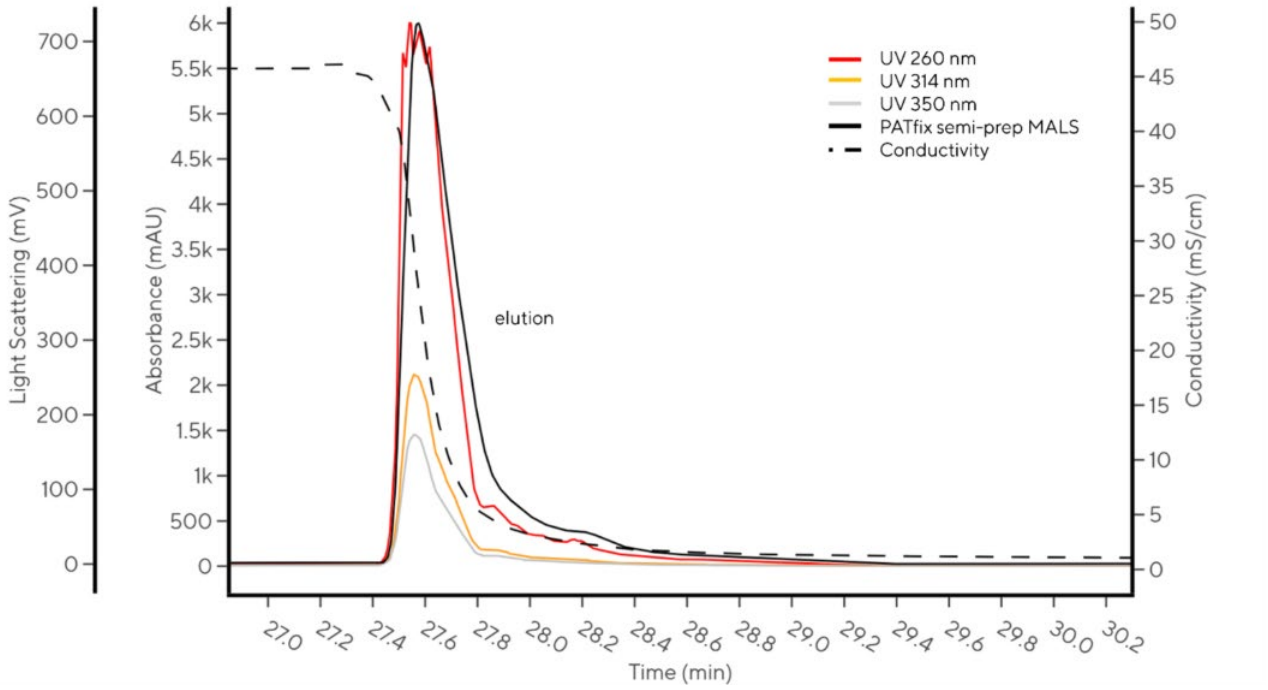
Figure 3: *Schematic representation of inline dilution.*



Wash step: After loading is complete, 20 CV wash is suggested using wash buffer or combination of buffers to achieve similar conductivity as the sample (50% buffer A, 50% buffer B).

Elution with buffer B: A step gradient to buffer B is recommended for high concentration of the particles (see chapter Optimization and Troubleshooting for more information). Perform 20 CV isocratic elution in buffer B and collect the fraction with high signals (typically 3 CV) (Figure 4).

Figure 4: *Zoom in of step elution using CIMmultus® OH (6 µm channels).*



Clean with buffer C: Wash the column with 20 CV of 50% EtOH to remove any LNPs that may still be bound to the column, followed by 10 CV water.
Note: Ensure the column has no Na-Citrate (e.g., wash with Buffer B or ddH_2O) before applying buffer C, as Na-Citrate will precipitate if in contact with EtOH.

Sanitize with buffer E: Wash the column with 10 CV ddH_2O to remove any EtOH present in the column after ethanol strip. Flushing with at least 10 CV of 1 M NaOH is recommended after every run to remove any sample that remains bound to the column. The sanitization step may be collected and neutralized upon elution for further analysis.

Note: Maintaining a minimal flow rate during sanitization tends to produce better results compared to holding NaOH in the column without flow, as it continuously washes foulants out of the column rather than merely hydrolysing them in place.

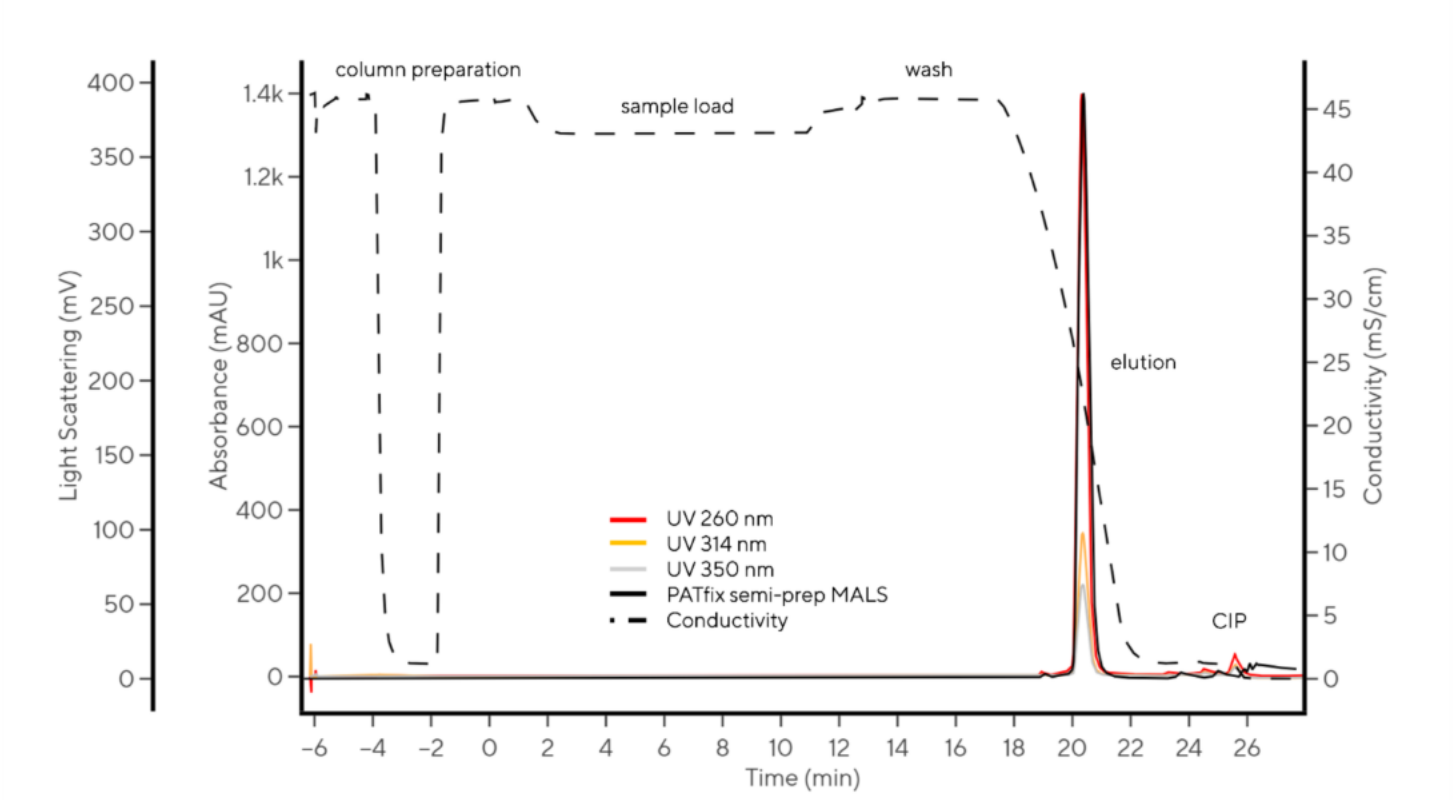
Neutralize and regenerate the column: Remove the 1 M NaOH from the column by flushing with 10 CV of ddH_2O, followed by washing with 10 CV of buffer F to neutralize the column environment.
To proceed with another run, wash with ddH_2O and continue with equilibration. Otherwise, store the column as recommended below.

Storage: After sanitization and neutralization, wash the column with 10 CV of ddH_2O and store in 20% EtOH.

Optimization and Troubleshooting

Comparison of gradient and step elution: Linear and step gradients are both effective options for elution, each providing unique benefits. However, if the main objective is concentrating the LNPs, a step gradient is recommended. To compare the benefits, a linear gradient can be applied (Figure 5).

Figure 5: Typical chromatogram of LNP purification using CIMmultus® OH (6 µm channels), linear gradient elution, from 50% buffer B and 50% buffer A to 100% buffer B in 20 CV followed by 10 CV of 100% B.



Testing different binding buffers: Instead of citrate, other kosmotropic salts can be tested, including anions such as sulphate, phosphate, chloride, and others, as well as cations like, ammonium, potassium, magnesium, and others. Before starting initial chromatographic experiments, it is advisable to test different salt concentrations (1.5 M, 1 M, 0.5 M etc.) using small sample volumes to determine the concentration at which the sample precipitates. For loading, select the buffer composition with the highest salt concentration that does not cause sample precipitation while achieving the best column performance.

Addition of additives in buffers: Different additives can be added to the buffers to improve recovery or increase LNP stability.

Cleaning: Inadequate cleaning may be indicated by a gradual increase of operating pressure over a series of runs, increased tailing during the post-load washing step, a change in the shape of the elution peak, increased contamination of the eluted sample, and/or the appearance of ghost peaks, where peaks appear during elution despite no sample having been injected, and reduced recovery. If such issues are observed, prolonged cleaning with 50% EtOH or 30% isopropanol may be performed.

Ordering Information

Cat No.	Product Name
311.8140-6	CIMmultus® OH 1 mL Monolithic Column (Hydroxyl) (6 µm channels)
BIA-414.8140-6	CIMmultus® OH 4 mL Monolithic Column (Hydroxyl) (6 µm channels)
411.8140-6	CIMmultus® OH 8 mL Monolithic Column (Hydroxyl) (6 µm channels)
614.8140-6	CIMmultus® OH 40 mL Monolithic Column (Hydroxyl) (6 µm channels)
611.8140-6	CIMmultus® OH 80 mL Monolithic Column (Hydroxyl) (6 µm channels)
814.8140-6	CIMmultus® OH 400 mL Monolithic Column (Hydroxyl) (6 µm channels)
BIA-811.8140-6	CIMmultus® OH 800 mL Monolithic Column (Hydroxyl) (6 µm channels)
BIA-1014.8140-6	CIMmultus® OH 4000 mL Monolithic Column (Hydroxyl) (6 µm channels)
BIA-1011.8140-6	CIMmultus® OH 8000 mL Monolithic Column (Hydroxyl) (6 µm channels)

For cGMP compliant columns and 40 L column, please visit www.biaseparations.com or contact sales@biaseparations.com.

FAQ

What is the typical dynamic binding capacity of CIMmultus® OH column?
Typical dynamic binding capacity of CIMmultus OH for LNPs is ~3.7 mg encapsulated mRNA/mL of monolith. Capacity may vary depending on the chromatographic conditions, sample properties, and degree of method optimizations.

Is the column reusable?
Yes, the column is reusable if appropriate cleaning after each run is performed. Column lifetime will be affected by sample properties, sample preparations, and column maintenance. The general Cleaning in Place (CIP) procedure is described in the Instructions for Use, available here: Product Documentation - Sartorius BIA Separations.

How to store the column? Can the column be stored in a different solution than EtOH 20%?
Recommended storage solution is 20% EtOH, but the column can also be stored in low salt working buffers or water overnight.

What operating flow rate do you recommend for each column size?
The minimum and maximum flow rate for each column is defined in the Instructions for Use: Product Documentation - Sartorius BIA Separations.

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
656 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

Slovenia
Sartorius BIA Separations
Mirce 21
5270 Ajdovscina
Phone +386 596 995 00

For more information, visit
www.sartorius.com

www.biaseparations.com