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Purification of Norwalk Virus-Like Particles and Their SEC/MALS Analysis

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Introduction

In the fields of new modality, such as gene therapy drugs, virus vector, new vaccine, and drug delivery etc., applied researches on “bionanoparticles” are rapidly expanding and receiving the greatest interest these days. Due to their complex structures and distinct sizes, production and quality evaluation of their biological processes require integrated and multifaceted analytical techniques, even more than that of conventional biopharmaceutical compounds.

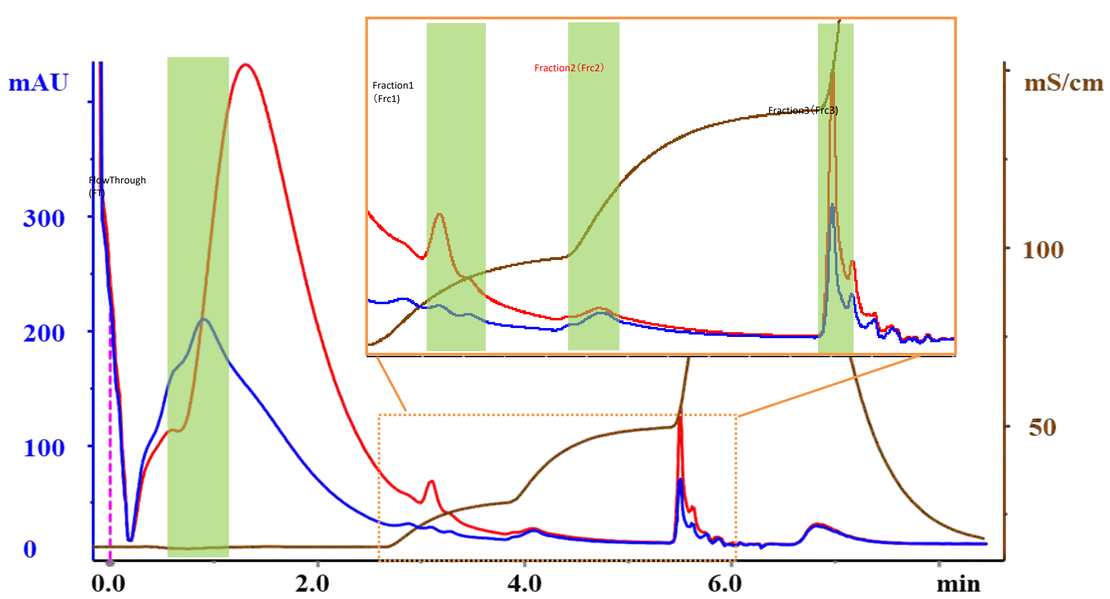
Virus-like particles (VLPs) are typical bionanoparticles. Since they are not infectious nor proliferated, but they can reproduce their original sizes and forms, they effectively elicit immune responses when used for vaccine. R&D for its commercialization is in progress as it would be an excellent platform.

This work presents an effective purification profiling using the Shodex OHpak SB-805 HQ, a Size Exclusion Chromatography (SEC) column, with different analytical devices. As a proof of concept, method development for a chromatographic purification of norovirus cell surface layer binding protein originated VLP (NVLP) is discussed.

Purification of NVLP by An Anion Exchange Column

We aimed to develop a simple and fast one-step purification method for a NVLP extract by using CIM™ monolith anion exchange column, which can be used at low pressure and at high flow velocity. The size of norovirus is 35 nm and it causes gastroenteritis. We obtained the NVLP extract from silkworms which were expressed with norovirus cell surface layer protein.

< UV chromatograms of NVLP crude extract >



< Analytical Condition>

Column	: CIMmultus QA-1 (1 mL) (BIA separations d.o.o.)
Eluent A	: 10 mM Sodium phosphate buffer (pH 7.5)
Eluent B	: A + 2M NaCl
Step gradient	: 7 %B/5 CV → 14 %B/5 CV → 26 %B/5 CV → 100 %B/5 CV *CV = Column Volume
Flow rate	: 5 mL/min
Detector	: UV（—280 nm, — 260 nm）
Column temp.	: Room temp.
Sample	: NVLP crude extract 2 mL (50 mg/mL)

< Sample Preparation>

1. Expressed with norovirus GII-4 cell surface layer VP1 protein using silkworm-baculovirus expression system
2. Grinding
3. Ultrasonication
4. Centrifuging
5. Use the filtrate as an NVLP crude extract sample

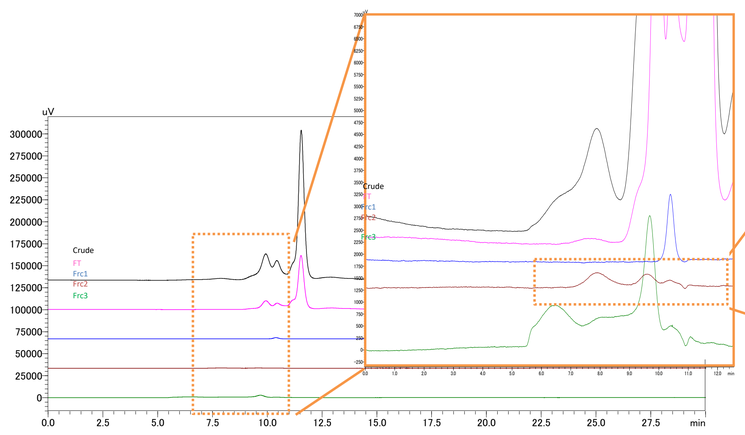
Profiling of Purified Fractions by SEC-MALS and Other Methods

The Shodex™ OHpak™ SB-805 HQ, a column for aqueous SEC, has low adsorption due to its highly hydrophilic design and has a pore size suitable for the nanoparticle analyses.

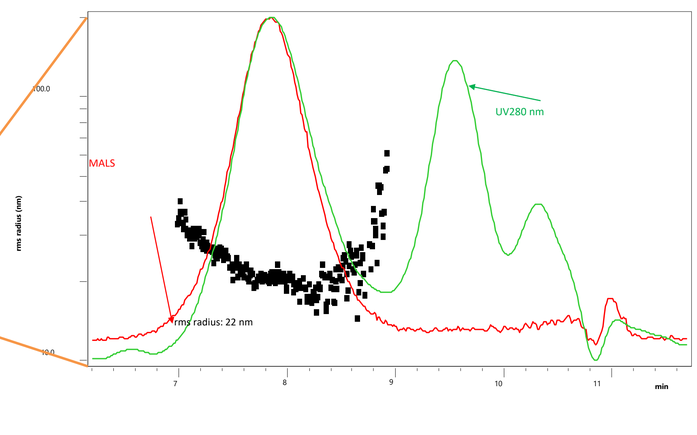
The scattered light detected by MALS does not only provide the targets’ size information, but also shows a significantly stronger response to nanoparticle-class-size diameter targets than low-molecular-weight impurities.

The profiles of each purified fraction were successfully monitored by SEC-MALS, SDS-PAGE, and TEM.

< UV chromatograms of Purified Fractions >



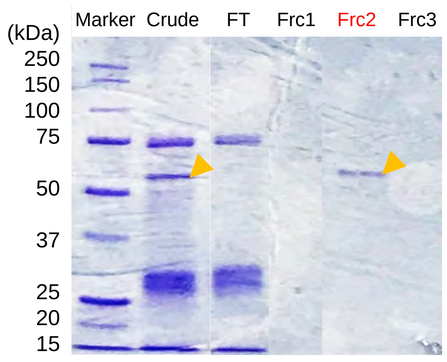
< UV and MALS chromatograms of Frc 2>



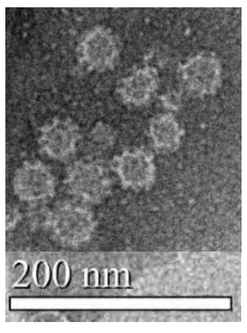
< Analytical Condition>

Column	: Shodex OHpak SB-805 HQ (8.0 mm I.D. x 300 mm)
Eluent	: PBS(-) (pH 7.4)
Flow rate	: 1 mL/min
Detector	: UV (280 nm) (small cell volume), MALS
Column temp.	: 25 °C
Sample	: NVLP purification Fraction 50 µL each

< SDS-PAGE Image of NVLP Crude Extract >



< TEM Image of Frc 2 >



Conclusions

- The targeted nanoparticles, which are generally difficult to be detected by a UV detector alone, was easily monitored by SEC-MALS with Shodex™ OHPak™ SB-805 HQ in a short time.
- The targeted profiles of each purified fraction were successfully monitored by combining the SEC with SDS-PAGE, MALS, and TEM.
- The multifaceted evaluation also helps providing important indicators for purification efficiencies, estimation of impurities, and improvement of purification method etc. in each purification step.