TOSOH

Application Note

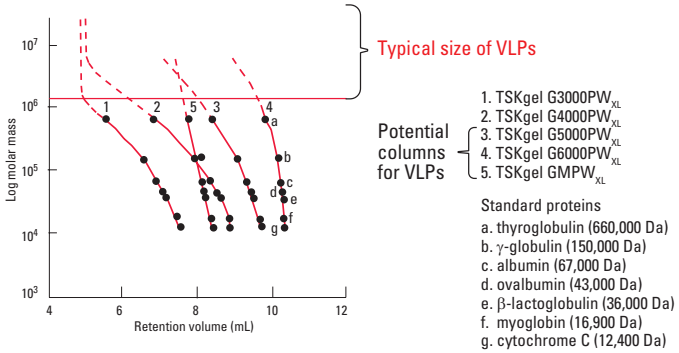
Molecular Weight Determination of VLPs Using LenS3 Multi-Angle Light Scattering Detector

Viruses and virus like particles (VLPs) are multimeric protein structures that mimic native viruses but are non-infectious. VLPs are subjects of interest, as their potential continues to grow as candidates in new vaccines and gene therapy products. For example, commercially available VLP-based vaccines are available for Hepatitis B and human papillomavirus. Robust analytical techniques are needed to not only ensure quality of final products but provide data for informed decision-making during the development process.

Size exclusion chromatography (SEC) is an analytical technique that provides results on the size and purity of macromolecules. When coupled with multi-angle light scattering (MALS), it offers both molecular weight (MW) and radius of gyration (R_g or size). Importantly, AU_{280} detection is only concentration dependent, whereas MALS corresponds to both concentration and molecular weight. Thus, the large molecular weight characteristic of VLPs inherently provides MALS with a strong scattered light response and enables VLP detection even in a dilute solution that is well below AU_{280} detection limit.

The primary challenge in the analysis of very large macromolecules by SEC is the selection of the appropriate analytical column. Here we explore TSKgel® PWXL series of SEC columns, which include a wide range of different pore sizes on a polymethacrylate stationary phase, for their utility in the analysis of large macromolecules such as VLPs. The protein calibration curves *(Figure 1)* show the separation range of TSKgel PWXL columns. The majority of VLPs have a molecular weight of >1 mega Daltons, which make the TSKgel G5000PWXL (100 nm pore size), TSKgel G6000PWXL (>100 nm pore size) and TSKgel GMPWXL (mixed bed) ideal columns of choice for analysis of VLPs.

Figure 1. Protein calibration curves on TSKgel PWXL columns



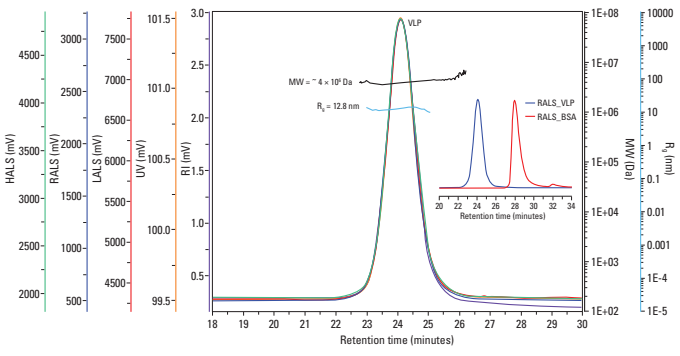
Material and Methods

Columns:	TSKgel GMPWXL, 13 µm, 7.8 mm ID × 30 cm TSKgel G5000PWXL, 10 µm, 7.8 mm ID × 30 cm
Instrument:	Thermo Scientific UltiMate® 3000
Mobile phase:	0.145 mol/L NaCl, 0.01 mol/L HEPES, 0.05% sodium azide, pH 7.4 (refractive index, 1.333)
Flow rate:	0.3 mL/min or as indicated
Detection:	UV: UltiMate 3000 multiple wavelength detector RI: Shodex RI-504 semi-micro RI detector MALS: LenS3 MALS detector
Sample:	Parvovirus VLP (MVM-MVP) (Cygnus Technologies), stock 1 × 10^{12} particles/mL (10-15 µL injection), (dn/dc = 0.19, dA/dc = N/A)
MALS calibrant:	BSA, 5 mg/mL (dn/dc = 0.185, dA/dc 0.66)

In this application, parvovirus VLP was separately analyzed on both a TSKgel GMPWXL and TSKgel G5000PWXL SEC column coupled with the LenS3 MALS detector. Either RI or UV can function as the concentration detector. RI was used with the right angle light scattering signal (RALS) to measure MW. Extreme low angle (LALS), right angle, and extreme high angle (HALS) signals were used to plot angular dissymmetry and to determine R_g. The MALS detector was calibrated with BSA prior to sample analysis and all data were processed and analyzed using SECview® software.

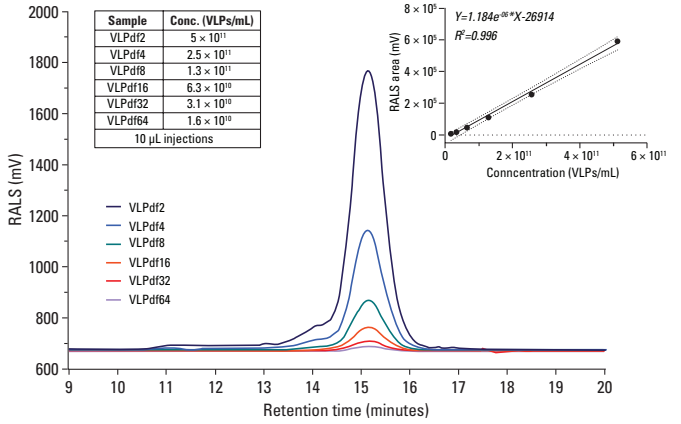
Analysis of parvovirus VLP by SEC-MALS using the TSKgel GMPWXL column revealed a MW of ~4 mega Daltons and R_g of 12.8 nm *(Figure 2)*. These results closely align with reported values for this VLP (*Biotech. Prog.* 34, 1213-1220, 2018).

Figure 2. Analysis of parvovirus VLP and BSA on TSKgel GMPWXL mixed bed pore size SEC column



As seen in *Figure 3*, parvovirus VLP was diluted up to 64-fold and injected at 10 µL onto a TSKgel G5000PWXL column. Approximately 3×10^{10} particles per mL can still be detected using the RALS signal from the LenS3 MALS detector, which allows for analysis of materials with low concentration or when working with limited sample.

Figure 3. Limit of detection by RALS using TSKgel G5000PWXL at 0.5 mL/min



Conclusion

Mass spectrometry is the most common method previously used for VLP size determination, but this technique is costly and impractical for frequent analysis. Inclusion of SEC-MALS as an analytical technique to determine the MW and R_g is a preferred alternative and allows for both routine analysis and process monitoring. The wide range in pore sizes and separation ranges of TSKgel PWXL SEC columns overcome challenges in analytical SEC where separations of large macromolecules require a larger pore sized stationary phase. When these SEC columns are then combined with the greatly enhanced sensitivity of Tosoh Bioscience's LenS3 MALS detector, fast and easy analysis of MW and R_g with an improved level of detection (LOD) is provided.