Application Brief

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Agilent Bio SEC-5 for Analysis of Virus-Like Particles (VLP)

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Introduction

Immunotherapy represented by vaccines is an important means of disease prevention and treatment. The development of vaccines is a critical advancement in modern medicine. Virus-like particles (VLPs) are highly structured protein particles that are self-assembled from one or more structural proteins, typically 20 to 150 nm in diameter. VLPs maintain the natural composition of viral antigen proteins and thus have the function of stimulating the host's immune response without containing the virus genome or replication machinery required to be infectious. Based on this feature, VLPs have been a good platform for vaccine development and application for many years.[1,2]

Size exclusion chromatography (SEC) is an effective method for analyzing high-molecular-weight aggregates and related low-molecular-weight proteins. Based on the larger dynamic diameter of the VLP molecule, an SEC column with a large pore size should be used to achieve better resolution. Current commercially available SEC columns with larger pore diameters are relatively limited in choice, and the separation available suboptimal. The Agilent Bio SEC-5 column family provides a range of super-large pore sizes to facilitate method development and optimization with high column efficiency to provide ideal separation results. In this application, Bio SEC-5 chromatographic columns with different pore diameters were used to perform SEC analysis on a VLP, and the results compared.

Virus-like particle analysis

To make a buffer, 0.71 g of sodium dihydrogen phosphate, 15.8 g of disodium hydrogen phosphate dodecahydrate, and 23.4 g of sodium chloride were accurately weighed and dissolved in 1 L of de-ionized water. The buffer was mixed well and used as mobile phase for these experiments.

Chromatography analysis uses the Agilent 1260 Infinity II LC System with an Agilent 1260 Infinity II quaternary pump and a variable wavelength detector. The chromatography conditions used are shown in Table 1, and the results of chromatography separation are shown in Figure 1.

Because the concentration of VLP samples is relatively low (<1 mg/mL), 220 nm was selected as the detection wavelength to obtain a higher signal response than 280 nm at the same sample input, providing a balance between signal response and baseline noise.

This study compared the results of using Bio SEC-5, 2,000 Å and Bio SEC-5, 1,000 Å columns (see Figure 1). When comparing the resulting chromatograms of VLP, a better separation was achieved using the Bio SEC-5, 2,000 Å column. Considering the relatively large dynamic diameter of the VLP in solution, selection of an SEC column with an appropriate pore size is crucial for achieving efficient separation.

Table 1. Liquid chromatography analysis conditions.

Parameter	Value
Column	Agilent Bio SEC-5, 7.8 × 300 mm, 5 µm, 2000 Å (p/n 5190-2541) Agilent Bio SEC-5, 7.8 × 300 mm, 5 µm, 1000 Å (p/n 5190-2536)
Flow	0.6 mL/min
Mobile Phase	50 mM phosphate buffer (pH 7.4) with 400 mM sodium chloride
Column Temperature	Room temperature
Sample Volume	5 µL
Detection Wavelength	220 nm
Run Time	30 min
HPLC System	Agilent 1260 Infinity II LC System with Quaternary Pump

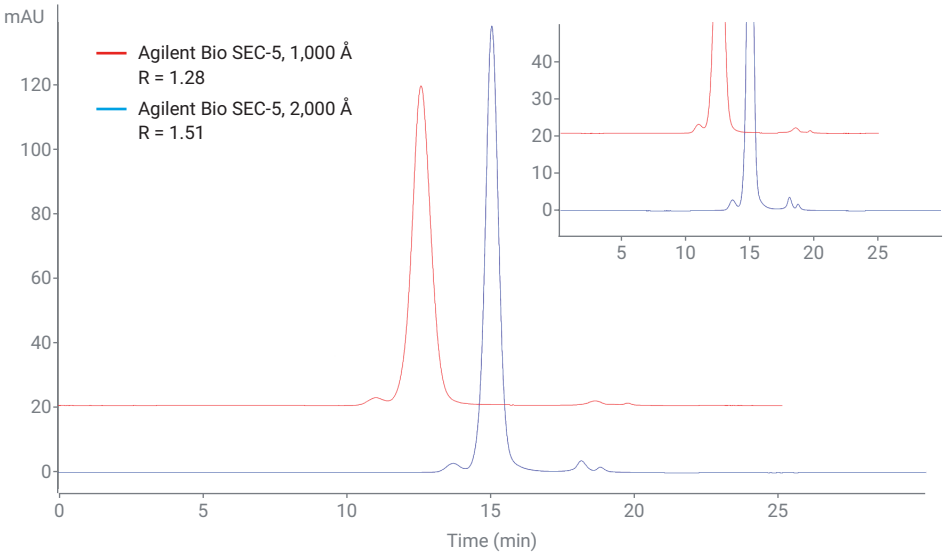

Figure 1. The results of the same virus-like particle were analyzed using Agilent Bio SEC-5, 2,000 Å and Bio SEC-5, 1,000 Å columns.

References

1. Rob, N; Polly, R. Virus-Like Particles as Immunogens. *Trends Microbiol.* **2003**, *11(9)*, 438–444.
2. Grgacic, E. V. L.; Anderson, D. A. Virus-Like Particles: Passport to Immune Recognition. *Methods* **2006**, *40(1)*, 60–65.

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