

Development of Monodispersed Silica Particles in Three Micron Size and with Ultra Wide Pores for BioSEC separation of Virus Like Particles



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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 emerging nanoscale protein assemblies applied as prophylactic vaccines and in development as therapeutic vaccines or cargo delivery systems. VLPs are highly structured protein particles, 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.

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 an ultra wide pore size (>1000Å) should be used to achieve better resolution. There is a delicate balance of large pore size, large pore volume, and mechanical strength. Current commercially available SEC columns with larger pore diameters are relatively limited in choice, typically in 5 µm or bigger, and with broad particle size distribution and pore volume below 1 ml/g in order to maintain mechanical strength.

In order to achieve ultra high resolution of VLPs, we have developed 3 µm monodispersed silica particles with ultra wide pores (500Å, 1000Å, 1250Å, and 1500Å), UniSil HP series, for BioSEC purpose. We evaluated effect of pore volume vs. mechanical strength. The unique pore structure and monodispersed particle size allow the particle pore volume larger than 1.0 ml/g while particles still maintain reasonable mechanical strength. The particles properties such as pore size, pore volume, and mechanical strength, are fully characterized. The applications of these particles using protein standards, adenovirus associated virus (AAVs), and IgM antibody will be demonstrated for their high resolution separations.

Particle synthesis:

UniSil particles are monodispersed particles using a patented process from NanoMicro Technology. UniSil particles covers sub 2 micron to 50 micron for analytical to process chromatography. Based on monodispersed UniSil series of 3 µm, 500Å/800Å/1000Å/1500Å particles, which have pore volume of 0.70-0.75 ml/g and are suitable for reversed phase chromatography, a new UniSil HP series of 3 µm, 500Å/1000Å/1250Å/1500Å particles with larger pore volume of 1.0 ml/g were developed for BioSEC purpose.

Particle characterization:

The physical properties is listed in Table 1. SEM of the particles and inside pores were taken as shown in Figure 1 and 2. Hg intrusion was used to measure the particle physical properties in Figure 3. The final particle size is measured by Coulter Multisizer 3.

The particle strength test is done as following:

The particles were packed into a 2.1 x 50 mm column. The column was put on a HPLC system. The pressure was recorded as the flow rate gradually increased. The pressure increased linearly to flow rate increase. If the particles break, the pressure will jump dramatically.

Chromatographic tests:

The particles are bonded with a hydrophilic silane, and then packed into a 4.6 x 150 mm column. The BioSEC chromatography was performed to check the particle performance. Temperature: 30 °C; Injection: 1 µl; Detection: UV 280 nm; Instrument: Thermo UltiMate 3000.

Table 1: SEM Physical properties of UniSil and UniSil HP

	Unisil series				Unisil HP series			
	3-500	3-800	3-1000	3-1500	HP 3-500	HP 3-1000	HP 3-1250	HP 3-1500
Particle size (µm)	3	3	3	3	3	3	3	3
Surface Area (m ² /g)	65	50	35	25	70	35	30	25
Pore Volume (ml/g)	0.75	0.75	0.70	0.70	1.0	1.0	1.0	1.0
Pore Size (Å)	500	800	1000	1300	500	1000	1250	1500

Figure 1: SEM of UniSil and UniSil HP 3-1500 particles

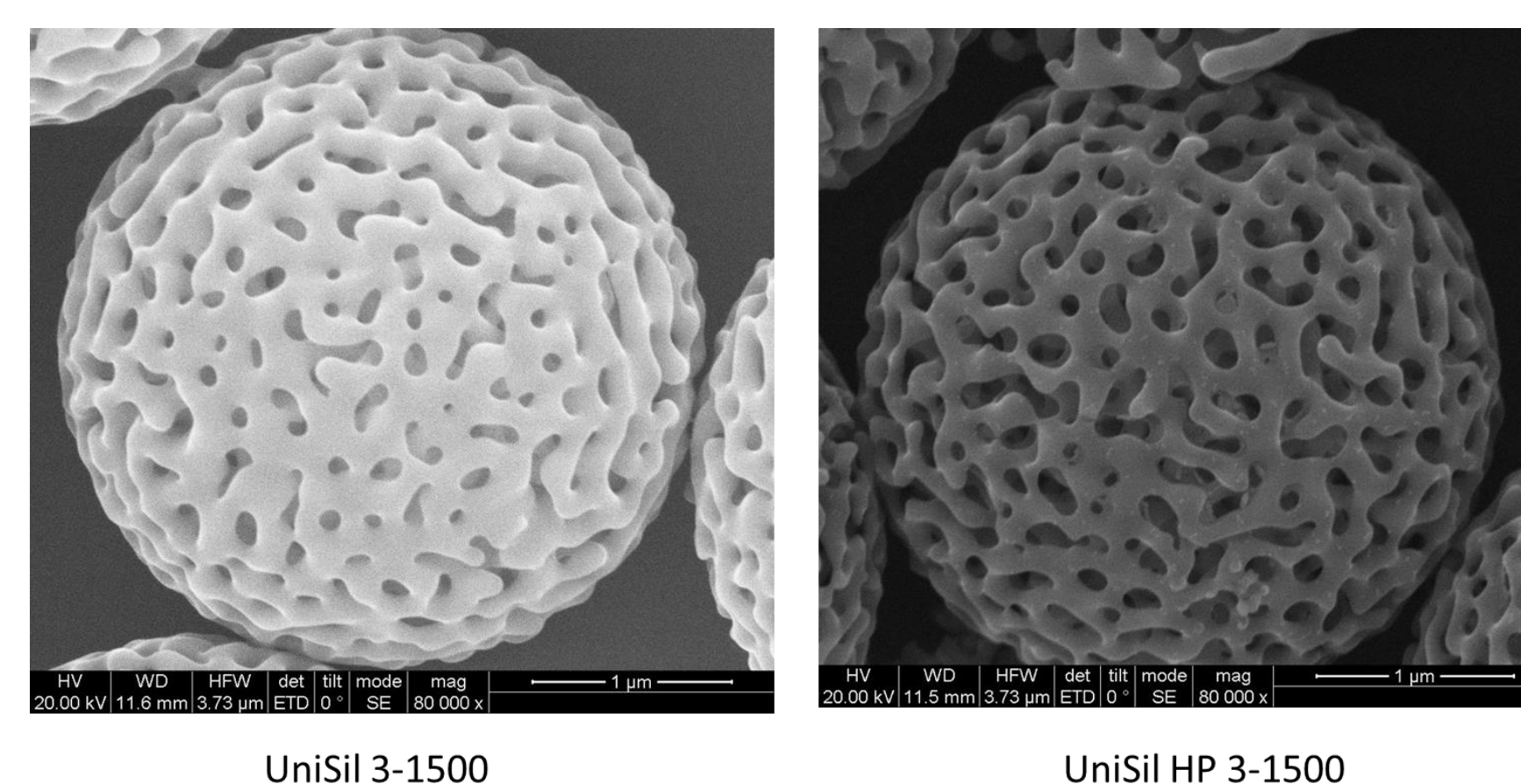


Figure 2: SEM of UniSil HP 3-1500 particles

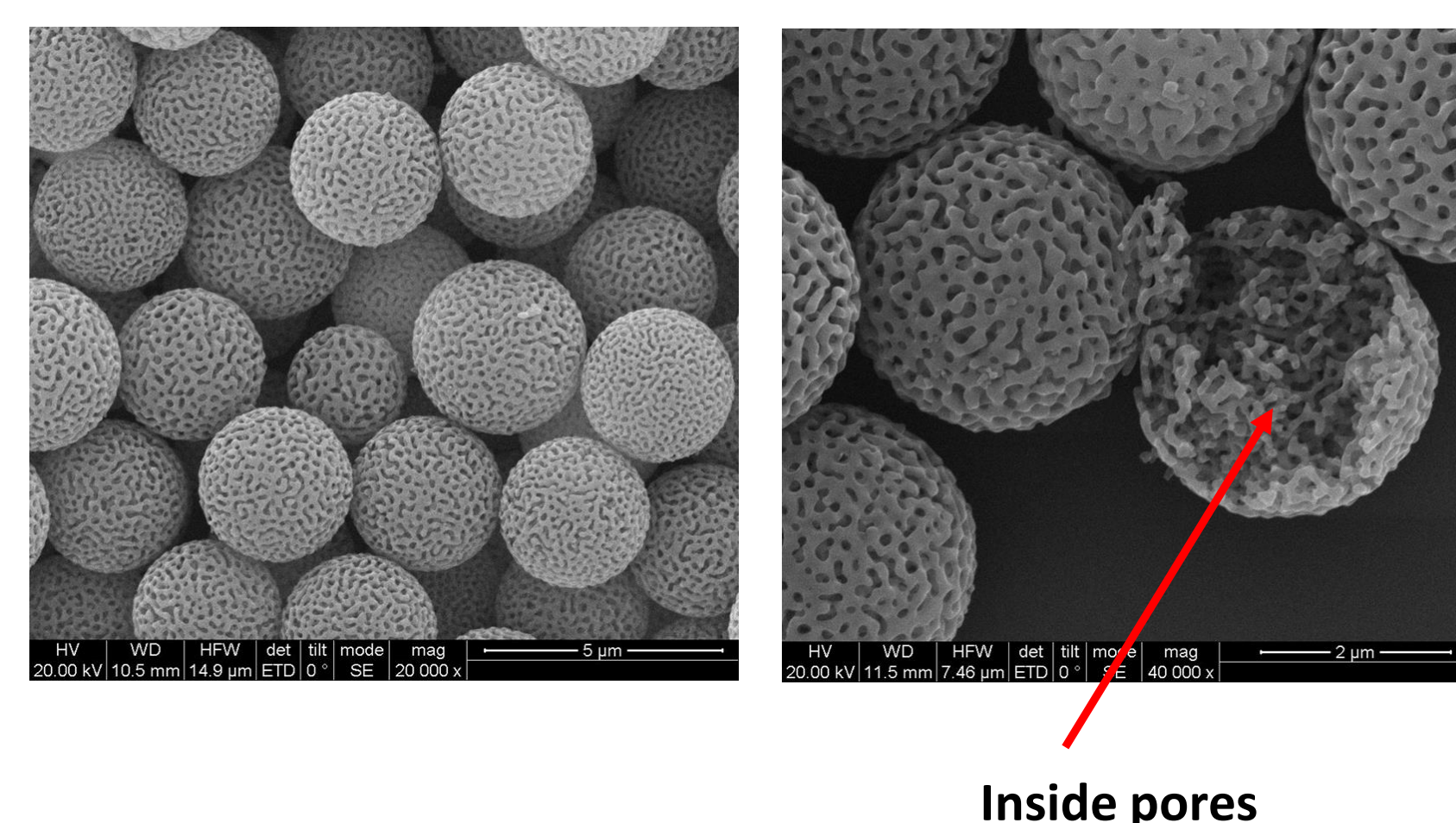
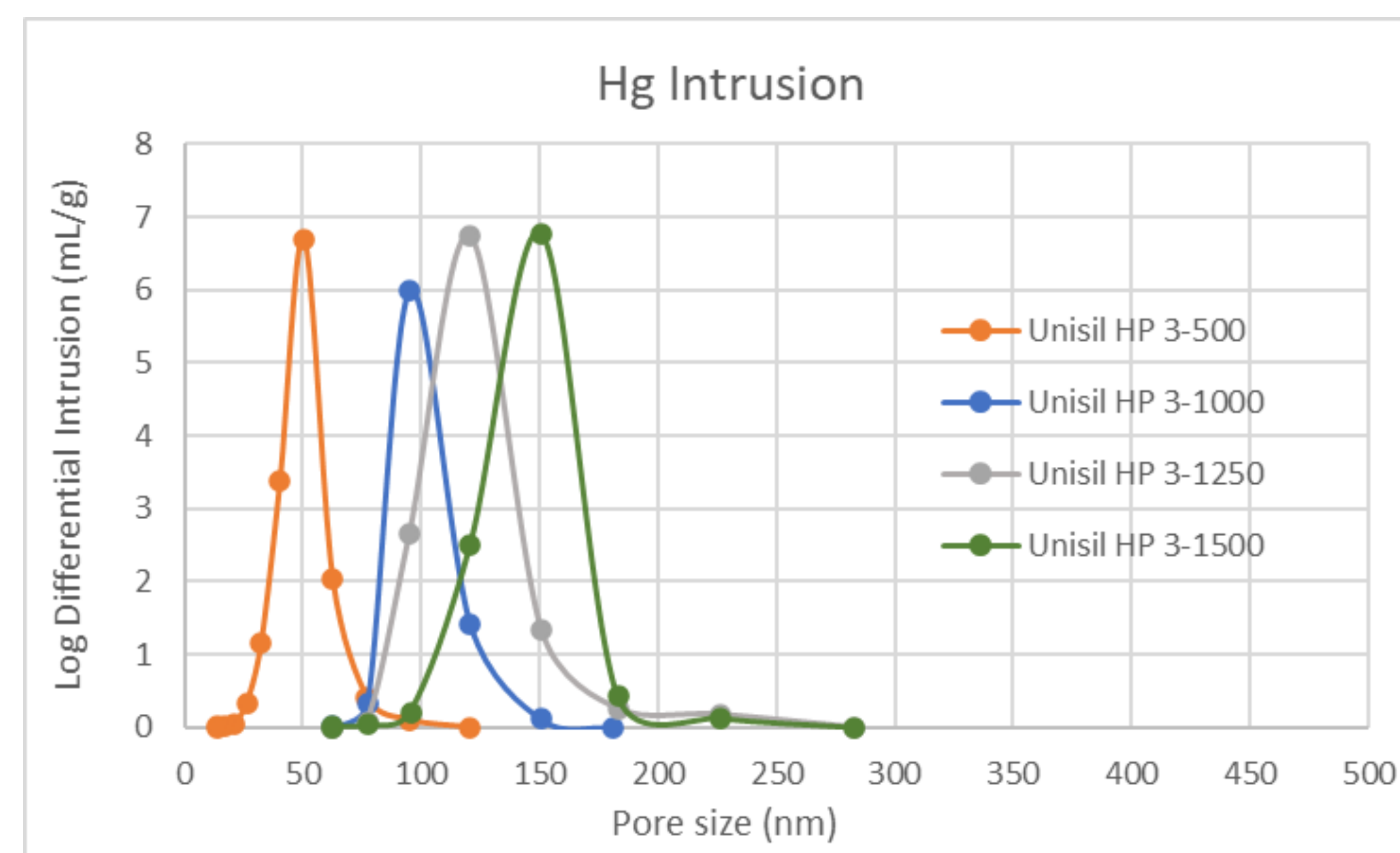
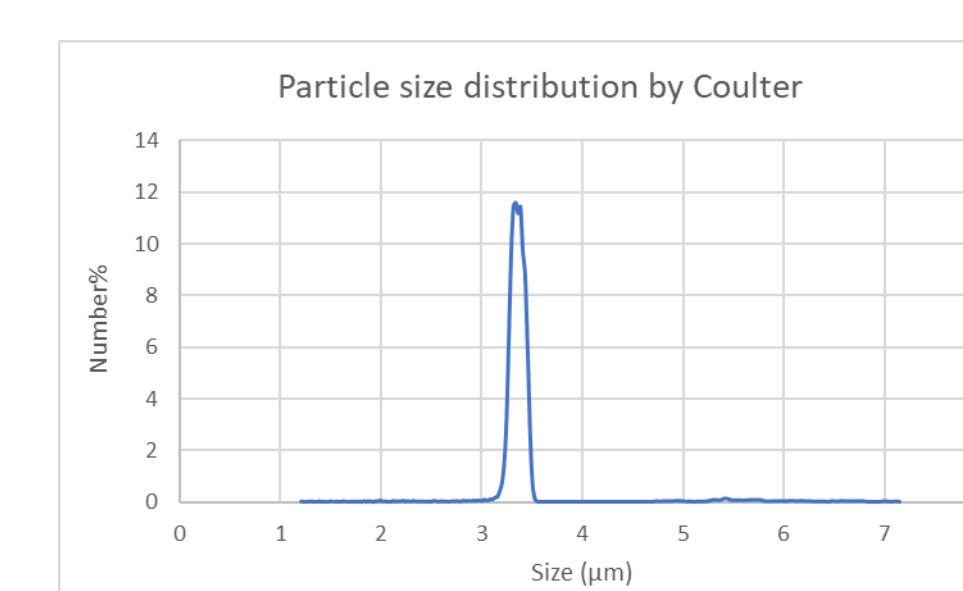


Figure 3: Pore size distribution



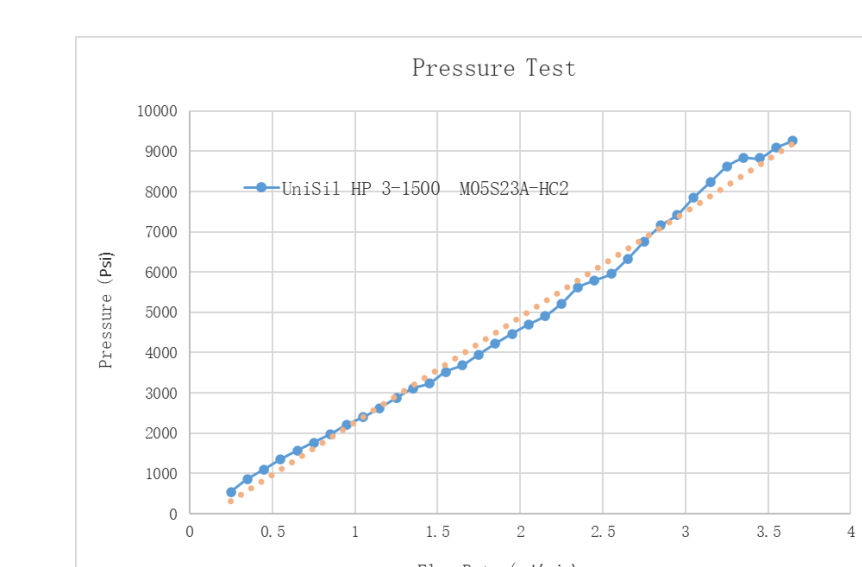
The UniSil HP 3-500/1000/1250/1500 particles have throughput pores with narrow pore size distribution.

Figure 4: Particle size distribution



UniSil HP particles have monodispersed particle size.

Figure 5: Particle strength test result:



The UniSil HP 3-1500 particles can stand pressure up to 600 bar (9000 psi).

Figure 6: BioSEC chromatography evaluation

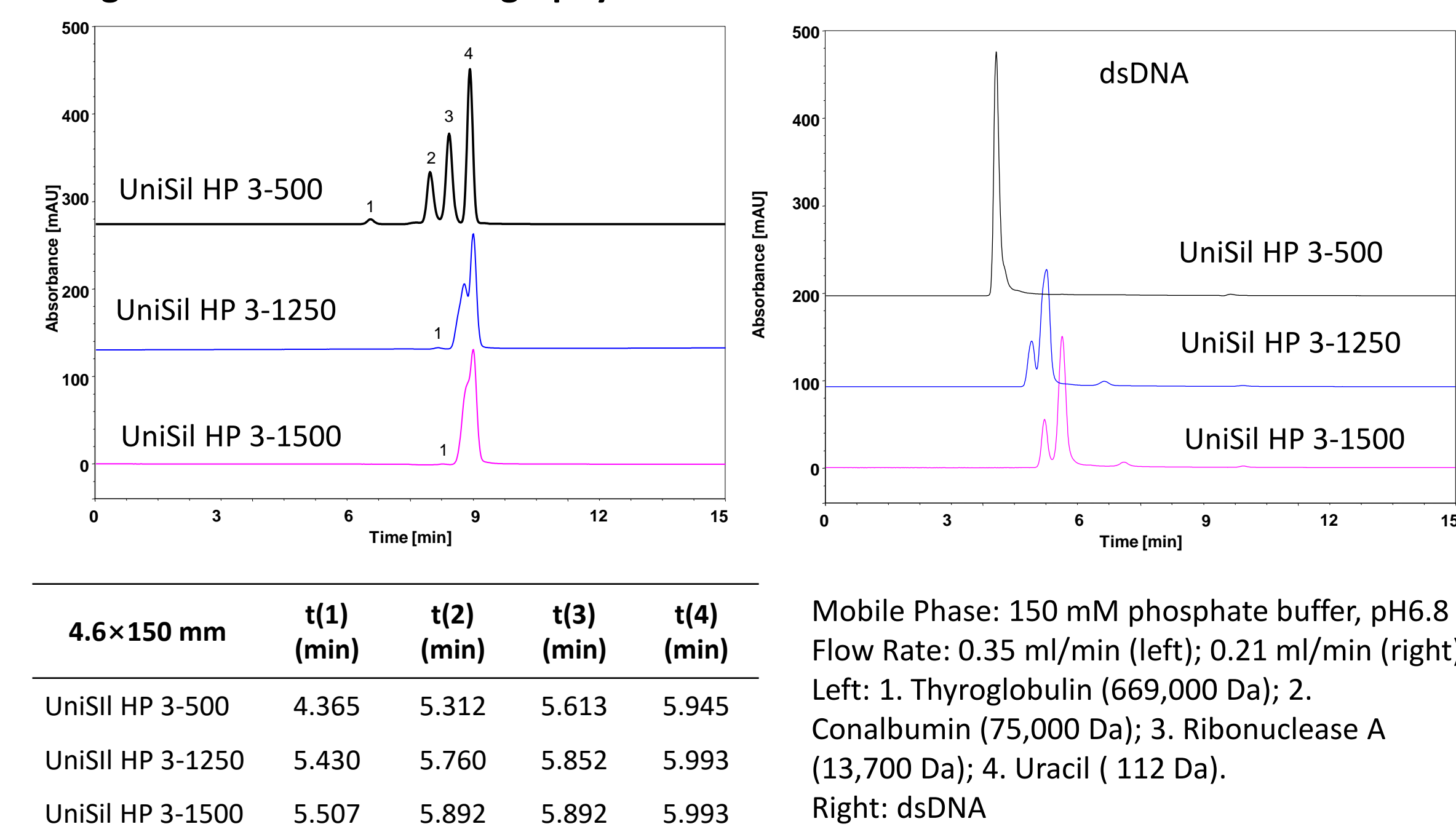
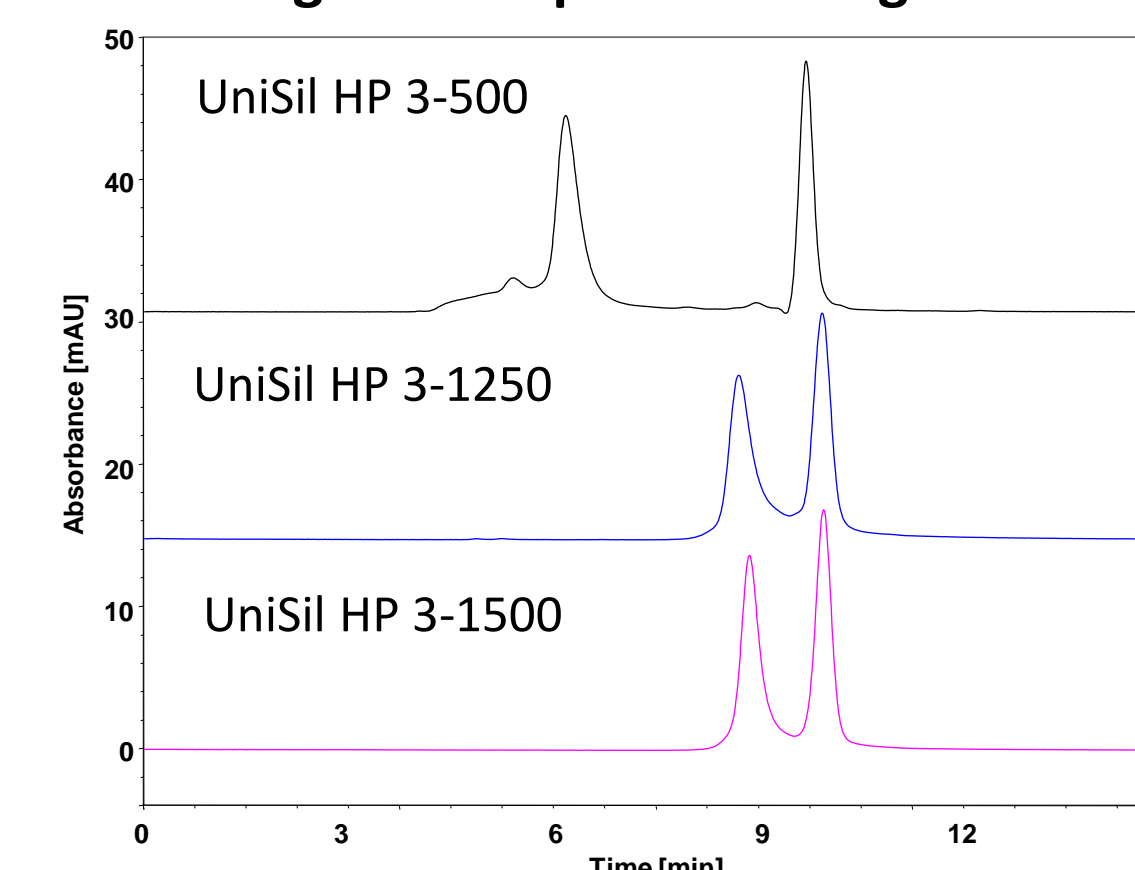
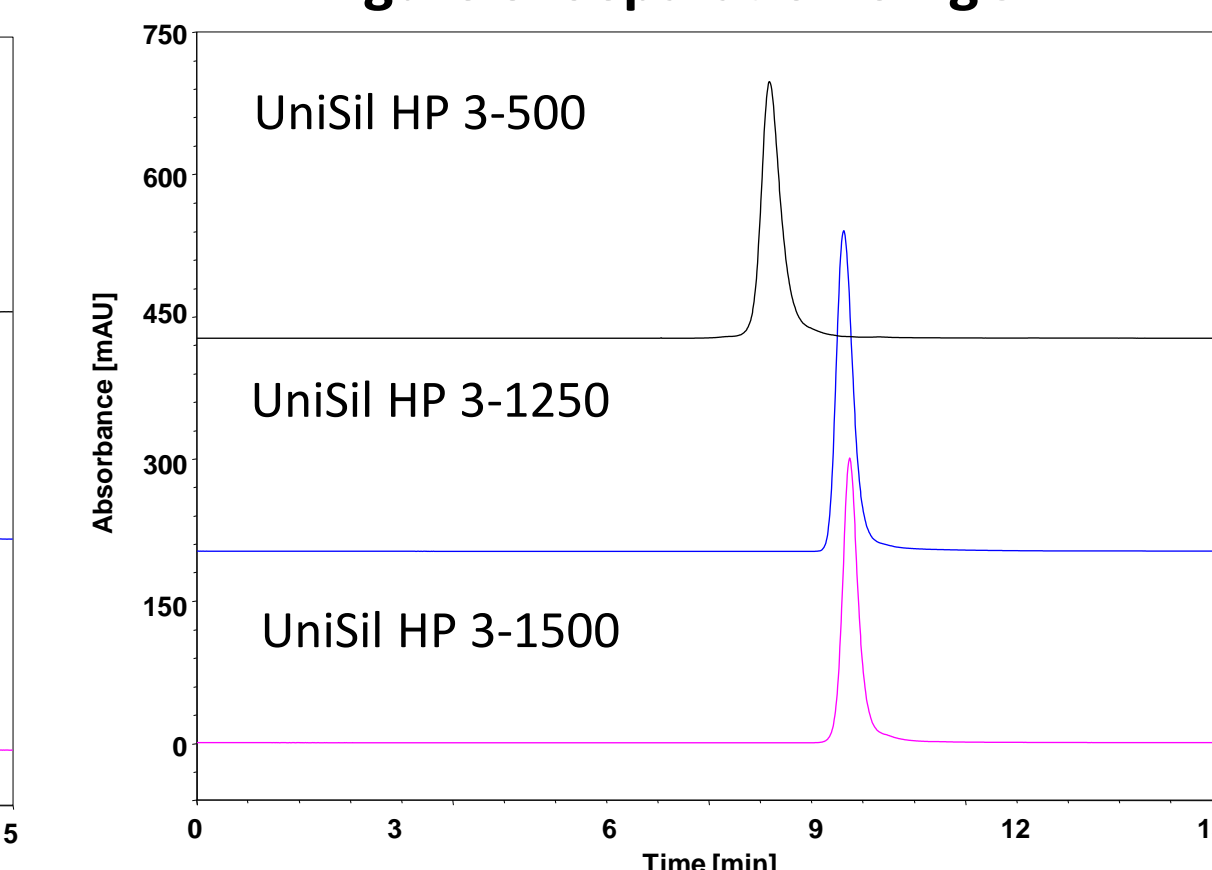


Figure 7: Separation of IgM



Mobile Phase: 10/90 (v/v) ACN/300 mM NaCl in 50 mM phosphate buffer, pH6.8; Flow Rate: 0.21 mL/min;

Figure 8: Separation of IgG



- The new UniSil HP 3-500/1000/1250/1500 particles have monodispersed particle size, uniform pore size, and large pore volume of 1.0 ml/g, and still maintain mechanical strength up to 600 bar.
- BioSEC application of large bio molecules such as IgG and IgM of these particles have been demonstrated.
- For more information of UniSil particles, please visit en.nanomicrotech.com

