

Size Exclusion Chromatography

TOYOPEARL Resins for SEC

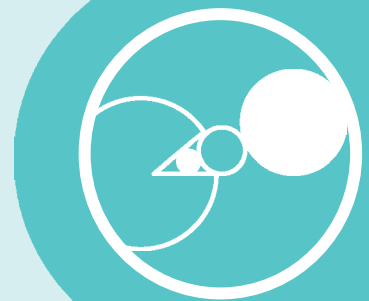
TOYOPEARL HW-40
TOYOPEARL HW-50
TOYOPEARL HW-55
TOYOPEARL HW-65
TOYOPEARL HW-75



TOSOH

TOSOH BIOSCIENCE

TOSOH BIOSCIENCE LLC
156 Keystone Drive
Montgomeryville, PA 18936-9637
Orders & Service: (800)366-4875
Fax: (215)283-5035
www.tosohbioscience.com
Email: info.sep.am@tosohbioscience.com



Size Exclusion Chromatography

TOYOPEARL Size Exclusion Chromatography

Size exclusion chromatography, also known as gel filtration, separates molecules in aqueous solution according to their size as they pass through a porous structure. Molecules with a diameter greater than the largest pores within the resin material are unable to enter the particle. Because they pass through the smallest accessible volume, they travel through the column and elute

first. Smaller molecules, which are able to access pores within the resin particles, pass through a larger accessible volume within the column depending on their apparent size and are eluted later, in order of decreasing molecular weight.

SEC is a mild, non-adsorption technique and is a non-destructive method for determining molecular weights. It is also used for







Features		Benefits
<ul style="list-style-type: none"> small particles available 		<ul style="list-style-type: none"> high resolution
<ul style="list-style-type: none"> hydrophilic porous polymer structure 		<ul style="list-style-type: none"> minimal non-specific adsorption effects
<ul style="list-style-type: none"> narrow particle size distribution 		<ul style="list-style-type: none"> high performance SEC
<ul style="list-style-type: none"> good mechanical stability 		<ul style="list-style-type: none"> excellent flow characteristics in large industrial size columns (up to 3 bar)
<ul style="list-style-type: none"> chemically stable (pH 2 - 14) 		<ul style="list-style-type: none"> constant packing volume over a wide range of salt concentrations compatible with organic solvents, can be cleaned in place with acid or base temperature stable polymer may be run at elevated temperature (4°- 60°C), autoclavable at 121°C
<ul style="list-style-type: none"> identical resin chemistry to TSK-GEL PW HPLC resins 		<ul style="list-style-type: none"> direct scale-up from TSK-GEL PW HPLC columns

Table I

Properties and molecular weight separation ranges for TOYOPEARL HW resins					
(HW = Hydrophilic, Water-compatible polymeric base resins)					
TOYOPEARL resin	Particle size (µm)	Pore size (Å)	Molecular weight of sample (Da)		
			Polyethylene glycols and oxides	Dextrans	Globular proteins
HW-40S	20 - 40	50	100 - 3,000	100 - 7,000	100-10,000
HW-40F	30 - 60				
HW-40C	50 - 100				
HW-50S	20 - 40	125	100 - 18,000	500 - 20,000	500 - 80,000
HW-50F	30 - 60				
HW-55S	20 - 40	500	100 - 150,000	1,000 - 200,000	1,000 - 700,000
HW-55F	30 - 60				
HW-65S	20 - 40	1000	500 - 1,000,000	10,000 - 1,000,000	40,000 - 5,000,000
HW-65F	30 - 60				
HW-75F	30 - 60	> 1000	4,000 - 5,000,000	100,000 - 10,000,000	500,000 - 50,000,000

process scale purification, often as a final stage polishing step to separate multimers, or molecular fractions or as a means of buffer exchange or desalting. Because it does not rely upon adsorption of the molecule to the resin, SEC is characterized by dilution of the loaded product.

Resins suited to SEC feature a predefined exclusion limit together with a fractionation range, governed respectively by the maximum pore size and pore size distribution. These important variables are controlled during manufacture.

Pore Size

The TOYOPEARL range includes 5 pore sizes covering 5 different fractionation ranges. The choice of resin depends on the molecular weight of the sample components; this information is shown for proteins, dextrans and PEG polymers in Table I and II. The TOYOPEARL HW resin range spans peptide and protein molecular weights between 100 - 50,000,000 Daltons. Each TOYOPEARL HW resin displays a typical calibration curve and exclusion limit for globular proteins (Figure 1).

Figure 1

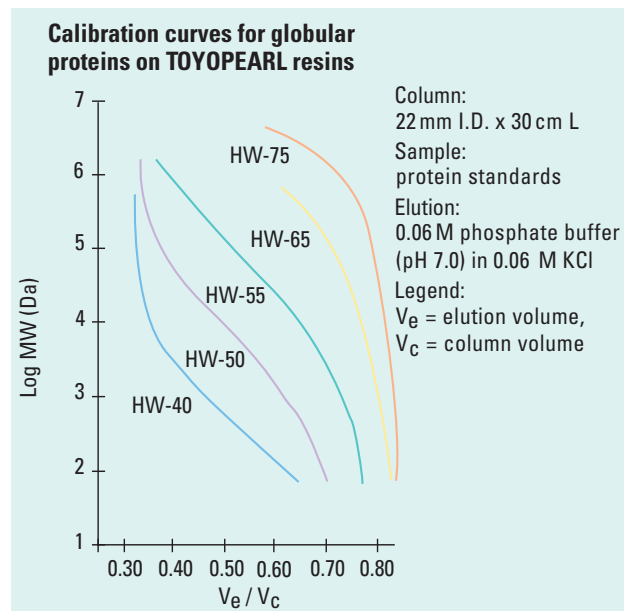


Table II

10 ²	10 ³	10 ⁴	10 ⁵	10 ⁶	10 ⁷	
						a) Globular proteins
<p>* and ** Base materials for IEC supports</p> <p>** Base material for HIC and AFC supports</p>						
						b) Dextran
						c) Polyethylene glycols
<p>Leucine</p> <p>Vitamin B12</p> <p>ACTH</p> <p>Insulin</p> <p>Cytochrome C</p> <p>Ribonuclease A</p> <p>α-Chymotrypsinogen</p> <p>Ovalbumin</p> <p>Albumin</p> <p>Human γ-Globulin</p> <p>Aldolase</p> <p>Catalase</p> <p>Urease</p> <p>Thyroglobulin</p> <p>Blue Dextran</p> <p>Hemocyanin</p> <p>Poliovirus RNA</p>						Calibration molecules

Size Exclusion Chromatography

Particle Size

Resolution is increased with decreasing particle size. Resin particle size is proportional to HETP and consequently inversely proportional to resolution of two peaks.

TOYOPEARL HW resins are available in three particle size ranges:

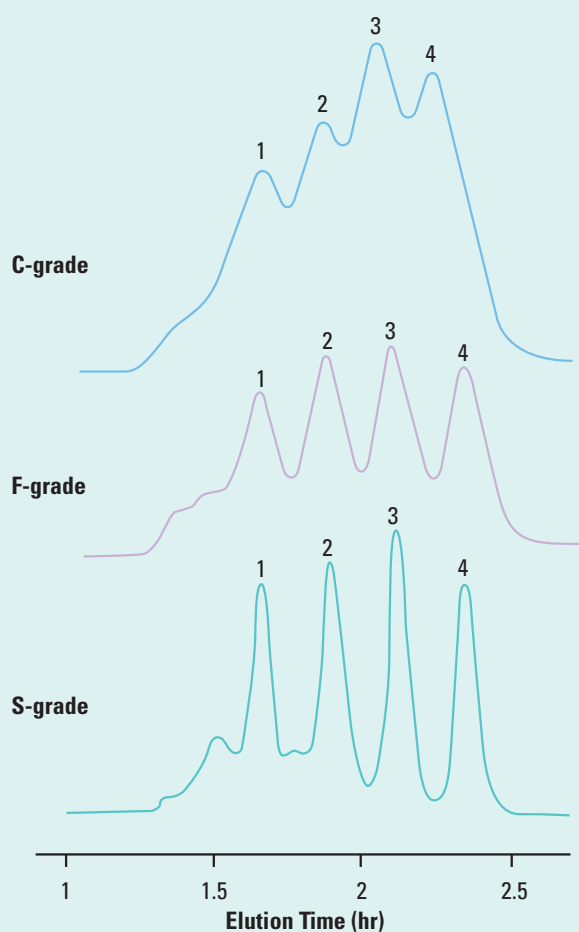
S-grade = 20 - 40 μm (superfine)

F-grade = 30 - 60 μm (fine)

C-grade = 50 - 100 μm (coarse)

Figure 2

Comparison of resolution on different particle sizes of TOYOPEARL HW-55



Column: TOYOPEARL HW-55, 26 mmID x 70cm
 Eluent: 1/30 M Phosphate buffer (pH 7.0), 0.2M NaCl
 Flow rate: 106mL/h (20cm/h)
 Temperature: 25°C
 Detector: UV@280 nm
 Sample: 1. Thyroglobulin (0.3%)
 2. γ -Globulin (0.3%)
 3. γ -Lactoglobulin (0.3%)
 4. Cytochrome C (0.1%)
 Inj. Volume: 1mL

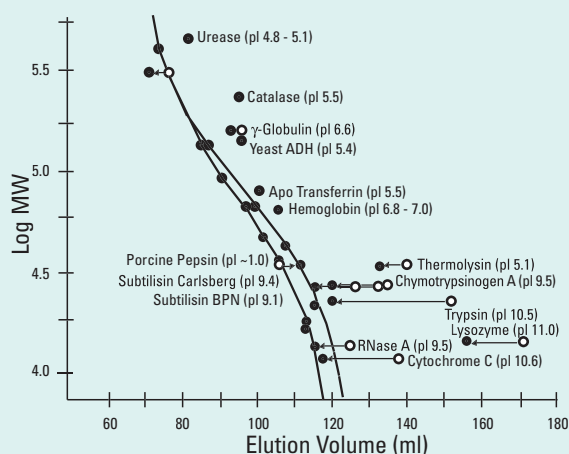
Mobile phase

Mobile phase components, such as neutral salts, can affect SEC separations. The presence or absence of sodium chloride influences the elution volume of proteins. This can be seen in Figure 3, in which a mixture of various proteins was separated on a column of TOYOPEARL HW-55F.

In most cases it is desirable to select a salt concentration which minimizes secondary interactions of the sample components with the resin. However, there are instances where secondary interactions, particularly hydrophobic interactions at higher salt concentrations, can be taken advantage of by increasing salt concentration in the mobile phase.

Figure 3

Comparison of the elution volumes of proteins in presence and absence of NaCl



Column: TOYOPEARL HW-55F, 22mm I.D. x 50cm
 Elution: 25 mM Tris-HCl with (●) or without (○) 0.5 M NaCl, (pH 7.5)
 Flow rate: 16 cm/h
 Temperature: 5-10°C
 Detection: UV @ 280nm, 420nm for heme proteins, 200nm for non-aromatic amino acids



Properties in aqueous solvent

✦ high mechanical stability

TOYOPEARL Resins have excellent mechanical properties, the gels can be operated at pressures up to 3 bar without deformation.

✦ minimum change in gel bed volume

Alterations of the bed volume when the eluent is changed, be it buffer with various pH values and/or different salt concentrations, are negligible. This is a great advantage particularly in elution with salt gradients. TOYOPEARL does not shrink or swell even in high concentrations of strong denaturing agents such as urea or guanidine hydrochloride.

✦ chemical stability

Conventional gels are usually sensitive to acid and alkaline hydrolysis. TOYOPEARL, however, is stable from pH 2-13, and tolerant to pH 0-14 for short periods. Substances which are only soluble at extreme pH values can be readily separated.

✦ sharp chromatographic peaks

Sample dilution after separation on TOYOPEARL is particularly small, due to the small uniform particle size and the narrow particle-size range (min. 90% – within declared limits). This property is particularly important when isolating high-value substances and in large-scale industrial separations.

✦ temperature stability

TOYOPEARL is thermally stable and does not degrade or denature even in boiling water. TOYOPEARL resins can be sterilized by autoclaving at 121°C.

✦ microorganism resistance

TOYOPEARL is an organosynthetic material, and is resistant to degradation by microorganisms. Growth of microbes is inhibited by storing the gels in 20% ethanol.

✦ suitability for affinity chromatography and enzyme immobilization

TOYOPEARL resins contain numerous hydroxyl groups on the external and internal surfaces of the resin. These, in combination with the chemical stability of the polymer, render the gels particularly suited to covalently bonding enzymes or other ligands. When fast flow supports are required, TOYOPEARL HW-65 is the base resin of choice.

Properties in organic solvent

✦ partition and adsorption effects

TOYOPEARL can be used in organic solvents or mixtures of organic solvents and water. Bed volumes swell or shrink relative to water depending on the solvent as shown in Figure 4. As seen in Table III, TOYOPEARL also swells in polar organic solvents. In general, however, the bed volume decreases slightly with decreasing solvent polarity. DMSO can be used for SEC of oligosaccharides and polyethylene glycols. The compatibility of DMF with TOYOPEARL also permits SEC separation of hydrophobic substances such as polystyrenes.

Figure 4

Swelling properties of TOYOPEARL HW-40 in various solvents

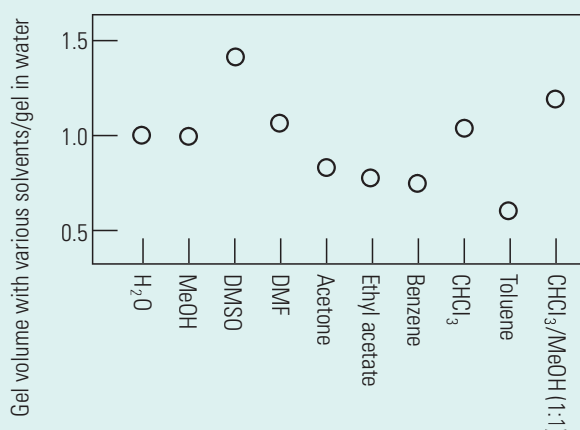


Table III

Swelling properties in various solvents							
TOYOPEARL	Water	KCl aq 0.2 M	MeOH	EtOH	DMF	Acetone	Toluene
HW-40	100	100	100	100	110	80	65
HW-50	100	100	100	100	110	80	70
HW-55	100	100	100	100	105	85	70
HW-65	100	100	100	100	105	90	75
HW-75	100	100	105	110	120	110	90

Ordering Information

TOYOPEARL LABPAK:

Part #	Product description	Container size (mL)	Particle size (μm)
19821	SECPAK LMW (HW-40F, HW-50F, HW-55F)	3 x 150	30 - 60
19819	SECPAK HMW (HW-55F, HW-65F, HW-75F)	3 x 150	30 - 60
19820	SECPAK HP (HW-40S, HW-50S, HW-55S, HW-65S)	4 x 150	20 - 40

TOYOPEARL SEC resins:

Part #	Product description	Container size (mL)	Particle size (μm)	Exclusion limit (Da)
19809	HW-40S	150	20 - 40	3×10^3
07451		250		
07447		500		
14681		1,000		
07967		5,000		
19808	HW-40F	150	30 - 60	3×10^3
07448		500		
14682		1,000		
07968		5,000		
19807	HW-40C	150	50 - 100	3×10^3
07449		500		
14683		1,000		
07969		5,000		
19811	HW-50S	150	20 - 40	1.8×10^4
07455		250		
07452		500		
14684		1,000		
08059		5,000		
19810	HW-50F	150	30 - 60	1.8×10^4
07453		500		
14685		1,000		
08060		5,000		
19813	HW-55S	150	20 - 40	1.5×10^5
07459		250		
07456		500		
14686		1,000		
08062		5,000		
19812	HW-55F	150	30 - 60	1.5×10^5
07457		500		
14687		1,000		
08063		5,000		
19815	HW-65S	150	20 - 40	1×10^6
07467		250		
07464		500		
14688		1,000		
08068		5,000		
19814	HW-65F	150	30 - 60	1×10^6
07465		500		
14689		1,000		
08069		5,000		
19816	HW-75F	150	30 - 60	5×10^7
07469		500		
14691		1,000		
08072		5,000		
07471	HW-75S	250	20 - 40	1×10^6
07468		500		
08071		5,000		

Conditions: Exclusion limits are +/- 30% and are determined using polyethylene glycol, polyethylene oxide, or dextran standards, as appropriate.