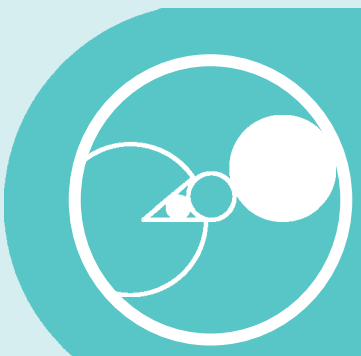
Size Exclusion Chromatography

The Physical Properties of the Base Beads for all Toyopearl Products

Toyopearl Resins for SEC

Toyopearl HW-40
Toyopearl HW-50
Toyopearl HW-55
Toyopearl HW-65
Toyopearl HW-75



The role of Size Exclusion Chromatography in process purification

Size exclusion chromatography, also known as gel filtration, separates molecules in an aqueous mobile phase according to their physical size in solution 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 are excluded from the pores they travel quickly through the column and elute first. Smaller molecules, which are able to access pores within the resin particles, permeate a larger accessible volume within the column and are eluted later, in order of decreasing molecular weight.

Because SEC has no adsorption capacity and its separation mechanism dilutes the the sample upon elution, it is not normally used in the capture or intermediate steps of manufacturing processes. It is sometimes used as a final polishing step where a target protein is being separated from its aggregates or other significantly different molecular weight impurities. Another application area is the desalting of the purified target protein.

Tosoh Bioscience offers a number of Toyopearl HW-type products for size exclusion chromatography *(Table I)*. These same SEC Toyopearl HW-type products are chemically modified with ion exchange groups, hydrophobic interaction or affinity ligands, and made into the Toyopearl products shown in the later sections of this catalog.

Much of the information in this catalog section pertains to the available pore sizes, fractionation ranges, and particle sizes of the Toyopearl HW-type products, along with their physical and chemical properties. This data not only aids in the evaluation of the best resin for a SEC process step, but also lends insight into understanding the physical nature and the selection process of all Toyopearl resins.

Resin chemistry

Toyopearl size exclusion resins are highly hydroxylated polymethacrylic polymer beads *(Figure 1)*. Their surface hydroxyl groups render them very hydrophilic and useful for protein separations. Toyopearl products including the functionalized materials seen in later catalog sections, have the least non-specific binding of any chromatographic resin. This is of particular note for separations such as blood factors where backbone interactions with the feedstock may result in decreased recovery of the targets.

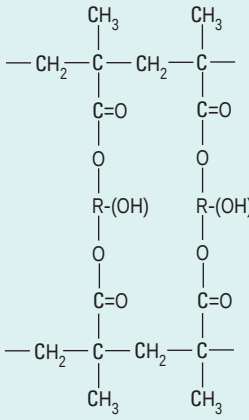
Their semi-rigid polymeric nature also gives them better pressure-flow than softer materials such as agarose.

Pore size

Commercial Toyopearl HW-type size exclusion materials are available in 5 pore sizes covering 5 different fractionation ranges. The choice of Toyopearl HW products depends on the molecular weight of the feedstock components. *Tables I and II* show this information for proteins, dextrans and PEG polymers.

Figure 1

Resin Chemistry of Toyopearl SEC resins (Hydroxylated Acrylic)



Features of Toyopearl SEC Resins	Benefits
small particles available	• high resolution
hydrophilic porous polymer structure	• minimal non-specific adsorption effects
narrow particle size distribution	• high performance SEC – more efficient separations better pressure-flow characteristics
good mechanical stability	• excellent flow characteristics in large industrial size columns (up to 3 bar)
chemically stable (pH 2 - 14)	• constant packing volume over a wide range of salt concentrations • compatible with organic solvents, can be cleaned in place (CIP) with acid or base • stable polymer may be run at elevated temperature (4°- 60°C), autoclavable at 121°C
identical resin chemistry to TSK-GEL HPLC resins	• direct scale-up from TSK-GEL HPLC columns

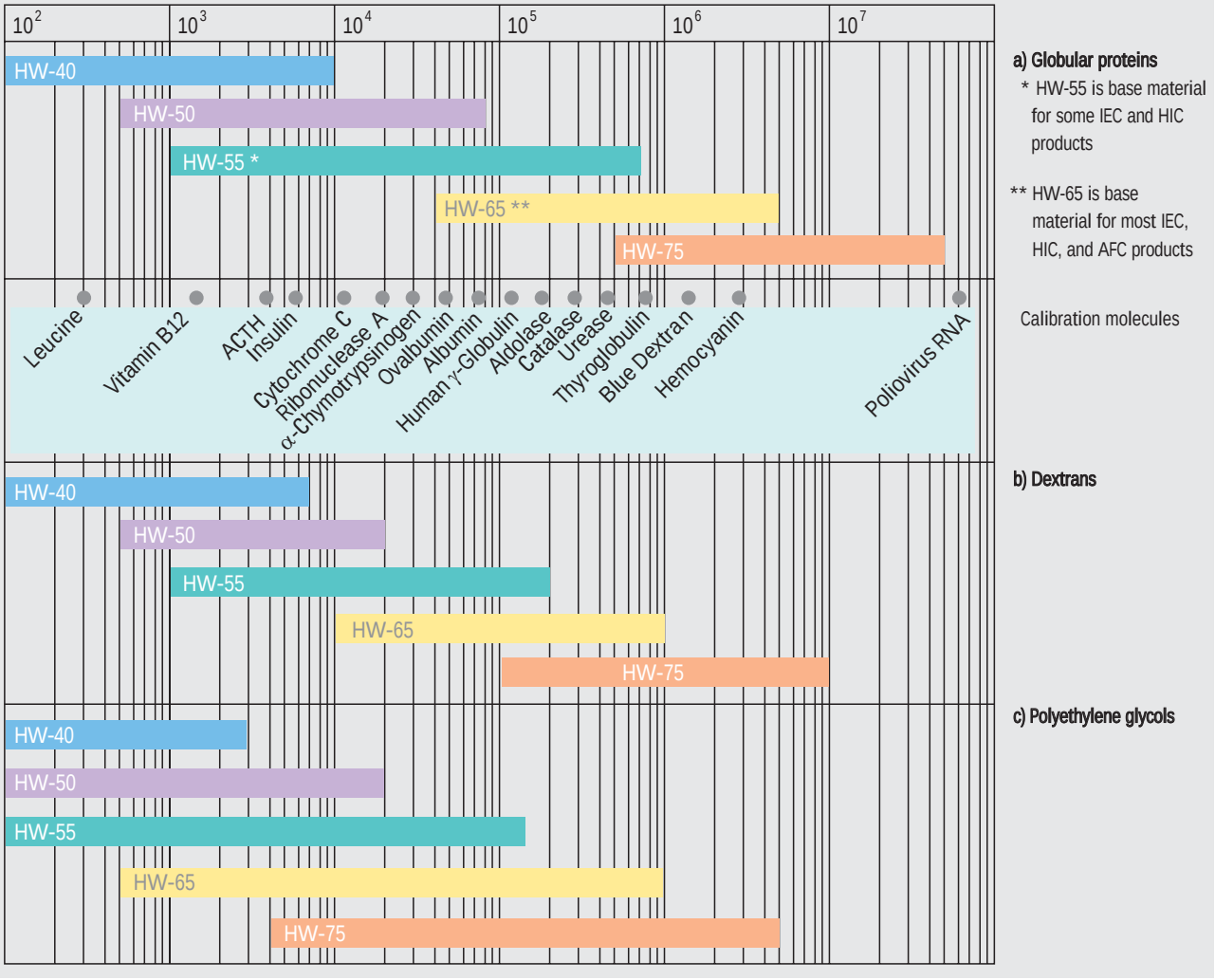
Table I

Properties and molecular weight separation ranges for Toyopearl HW-type resins

(**HW** = **H**ydrophilic, **W**ater-compatible polymeric base resins)

			Molecular weight of sample (Da)		
Toyopearl resin	*Particle size (µm)*	*Pore size (Å)*	*Polyethylene glycols and oxides*	*Dextrans*	*Globular proteins*
HW-40S HW-40F HW-40C	20 - 40 30 - 60 50 - 100	50	100 - 3,000	100 - 7,000	100–10,000
HW-50S HW-50F	20 - 40 30 - 60	125	100 - 18,000	500 - 20,000	500 - 80,000
HW-55S HW-55F	20 - 40 30 - 60	500	100 - 150,000	1,000 - 200,000	1,000 - 700,000
HW-65S HW-65F	20 - 40 30 - 60	1000	500 - 1,000,000	10,000 - 1,000,000	40,000 - 5,000,000
HW-75F HW-75S	30 - 60	> 1000	4,000 - 5,000,000	100,000 - 10,000,000	500,000 - 50,000,000

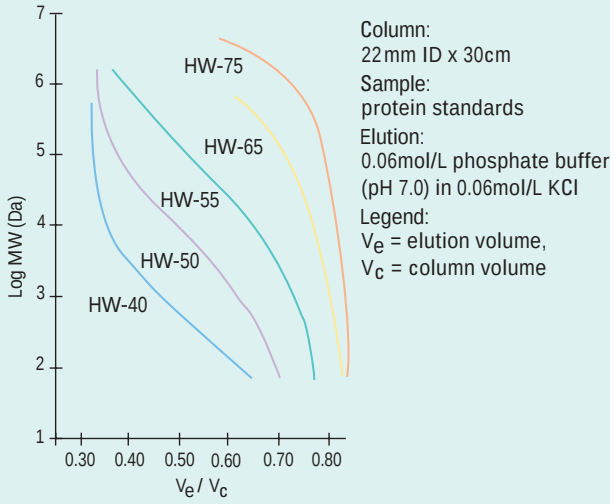
Table II



The Toyopearl HW-type resin range spans peptide and protein molecular weights between 100 - 50,000,000 Daltons. Each Toyopearl HW-type resin displays a typical calibration curve and exclusion limit for globular proteins *(Figure 2)*.

Figure 2

Calibration curves for globular proteins on Toyopearl resins



Toyopearl HW-65 and HW-55 are the base beads for many Toyopearl products listed in the later sections of this catalog. A product having "-650" in its name uses the Toyopearl HW-65 for the base bead. Products having "-550" are derived from the Toyopearl HW-55 bead. The impact of pore size and its relationship to dynamic binding capacity and resin selectivity for a particular protein application is discussed in subsequent catalog sections.

See the hydrophobic interaction chromatography section of this catalog for Toyopearl PPG-600, Toyopearl Phenyl-600 (new) and Toyopearl Butyl-600 (new) resins which are functionalized on the Toyopearl HW-60 bead (not available for use in SEC).

Particle size

Resolution increases with decreasing particle size *(Figure 3)*. Resin particle size is proportional to HETP and inversely proportional to the column efficiency and resolution of two peaks.

Most Toyopearl HW-type 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)

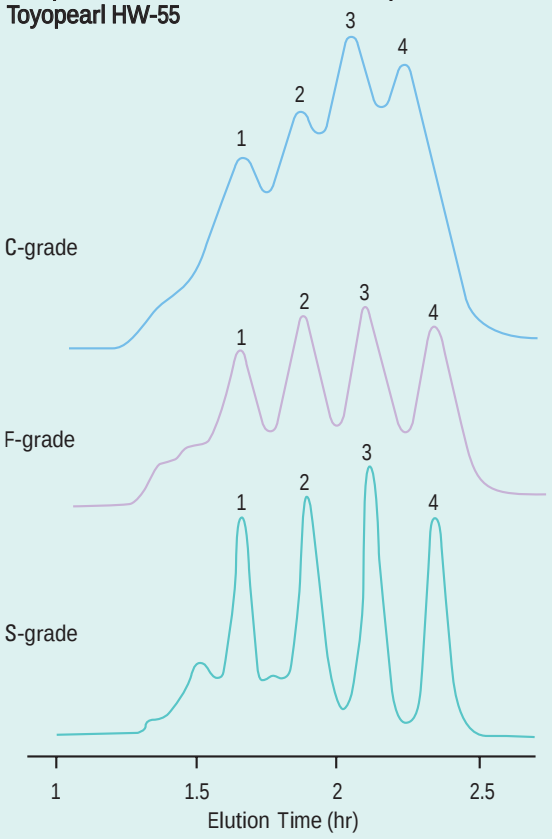
When the highest resolution is needed, the smaller S and F grade beads are preferred for process SEC. For desalting, where the resin is used in a filtration mode to remove the target from a buffer, the C grade is primarily employed because of its better flow dynamics at lower operating pressures.

Some Toyopearl HW-type products are also manufactured in "M-grade" (40-90µm) and "EC-grade" (100-300µm) to be used as the base beads for functionalized materials. These special grades are not commercially offered as SEC products.

Please note that for the functionalized base beads in later catalog sections a "C-grade" is specified as a 50-150µm bead and is not the SEC "C-grade" range of 50-100µm.

Figure 3

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



Column:	Toyopearl HW-55, 26mm ID x 70cm
Eluent:	33.3mmol/L phosphate buffer (pH 7.0), 0.2mol/L NaCl
Flow rate:	106mL/h (20cm/h)
Temperature:	25°C
Detector:	UV @ 280nm
Sample:	1. Thyroglobulin (0.3%)
	2. γ-Globulin (0.3%)
	3. β-Lactoglobulin (0.3%)
	4. Cytochrome C (0.1%)
Inj. Volume:	1mL

Mobile phase

Mobile phase components, such as salts, can affect SEC separations. The presence or absence of sodium chloride influences the elution volume of proteins. This is demonstrated in *Figure 4*, in which a mixture of various proteins was separated on a column packed with Toyopearl HW-55F. Salt concentrations can change the hydrodynamic radius of proteins and either increase or decrease their molecular size as a function of salt strength.

Ideally, in SEC sample components do not interact with the packing material. In practice it is often necessary 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 exploited.

It is important to note that relatively minor changes in protein structure may affect protein solubility and encourage secondary hydrophobic interactions causing similarly sized proteins or analogs to elute at different times. In those cases it may be necessary to modify the mobile phase composition to regain a separation based on molecular size alone.

Properties of Toyopearl SEC resins in aqueous eluents

high mechanical stability
Toyopearl resins can be operated at pressures up to 3 bar without deformation.

minimum change in gel bed volume
Changes in the column bed volume under operational salt conditions are negligible. Toyopearl does not shrink or swell even in high concentrations of strong denaturing agents such as urea or guanidine hydrochloride.

chemical stability
Toyopearl is stable from pH 2-13, and tolerant to pH 0-14 for short periods. Biomolecules which are only soluble at extreme pH values can be readily separated.

sharp chromatographic peaks
Toyopearl's narrow particle size distribution (min. 80% – within declared limits) results in better peak shapes and higher elution target concentrations than other SEC materials.

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.

suitability for enzyme immobilization
Toyopearl resins contain numerous hydroxyl groups on the external and internal bead surfaces . These, in combination with the chemical stability of the polymer, make the resin well suited for the covalent bonding of enzymes or other ligands. (Please see the affinity chromatography section for more information.)

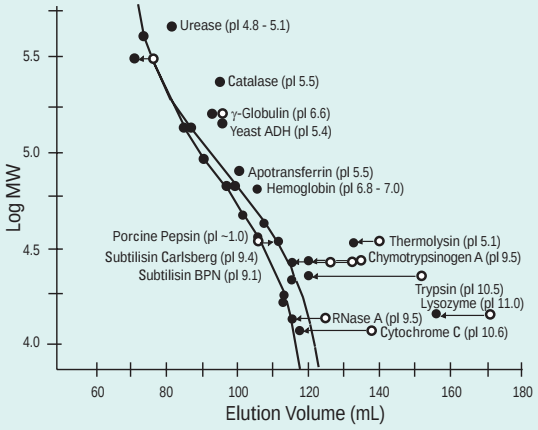
Properties in organic eluents

Toyopearl can be used in organic solvents or mixtures of organic solvents and water. Bed volumes may swell or shrink relative to water depending on the solvent as shown in *Tables III and IV*. 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.

All of the physical and chemical properties listed above for the Toyopearl HW-type series of SEC products makes them an excellent choice for use as the base beads for the ion exchange, hydrophobic interaction, and affinity chromatographic resins discussed in the later sections of this catalog.

Figure 4

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



Column: Toyopearl HW-55F, 22mm ID x 50cm
Elution: 25mmol/L Tris-HCl with (●) or without (○) 0.5mol/L NaCl, (pH 7.5)
Flow rate: 16 cm/h
Temperature: 5-10 °C
Detection: UV @ 280 nm, 420 nm for heme proteins, 200 nm for proteins without aromatic amino acid

Other applications:

The Toyopearl HW-type resins are commonly used in size exclusion chromatography and desalting applications. Some other important uses of these materials are:

- Removal of surfactants such as Triton® X-100 from biological solutions by an adsorption mechanism
- Use in hydrophobic interaction chromatography (HIC) for the separation of very hydrophobic molecules
- Use in HIC separations as a guard column for hydrophobic impurities
- Possible use as a stationary phase for either normal or reversed phase separations depending on solvent system selected

Table III

Swelling properties in various solvents

Toyopearl	*HW-40*	*HW-50*	*HW-55*	*HW-65*	*HW-75*
Water	100	100	100	100	100
0.2mol/L KCl	100	100	100	100	100
MeOH	100	100	100	100	105
EtOH	100	100	100	100	110
DMF	110	110	105	105	120
Acetone	80	80	85	90	110
Toluene	65	70	70	75	90

Table IV

Additional swelling data for Toyopearl HW-40

Toyopearl	DMSO	Ethyl Acetate	Benzene	$CHCl_3$	$CHCl_3$/ MeOH (1:1)
HW-40	140	80	70	105	120

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:

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

Part #	*Product description*	*Container size (mL)*	*Particle size (µm)*	*Exclusion limit (Da)*
19809	Toyopearl HW-40S	150	20 - 40	3×10^3
07451		250		
07447		500		
14681		1,000		
07967		5,000		
19808	Toyopearl HW-40F	150	30 - 60	3×10^3
07448		500		
14682		1,000		
07968		5,000		
19807	Toyopearl HW-40C	150	50 - 100	3×10^3
07449		500		
14683		1,000		
07969		5,000		
21484		50,000		
19811	Toyopearl HW-50S	150	20 - 40	1.8×10^4
07455		250		
07452		500		
14684		1,000		
08059		5,000		
19810	Toyopearl HW-50F	150	30 - 60	1.8×10^4
07453		500		
14685		1,000		
08060		5,000		
18368		50,000		
19813	Toyopearl HW-55S	150	20 - 40	1.5×10^5
07459		250		
07456		500		
14686		1,000		
08062		5,000		
19812	Toyopearl HW-55F	150	30 - 60	1.5×10^5
07457		500		
14687		1,000		
08063		5,000		
21918		50,000		
19815	Toyopearl HW-65S	150	20 - 40	1×10^6
07467		250		
07464		500		
14688		1,000		
08068		5,000		
19814	Toyopearl HW-65F	150	30 - 60	1×10^6
07465		500		
14689		1,000		
08069		5,000		
14690	Toyopearl HW-65C	1,000	50 - 100	1×10^6
08070		5,000		
21482		50,000		
07471	Toyopearl HW-75S	250	20 - 40	8.25×10^6
07468		500		
08071		5,000		
19816	Toyopearl HW-75F	150	30 - 60	8.25×10^6
07469		500		
14691		1,000		
08072		5,000		