TOSOH BIOSCIENCE

Toyopearl® HW Media for Scale-up Size Exclusion Chromatography of Nucleic Acids and Proteins

Chemistry of Toyopearl resins

Toyopearl resins are hydrophilic macroporous packings for bioprocessing chromatography. These resins are semi-rigid, spherical beads synthesized by a copolymerization of ethylene glycol and methacrylate-type polymers. They are highly resistant to chemical attack and are not degraded by microbes. Toyopearl packings are available in the size ranges preferred for production chromatography and can be packed easily into columns up to the largest industrial size.

Properties of Toyopearl HW media

Toyopearl HW media were developed for size exclusion (gel filtration) chromatography. There are five different products having increasing pore sizes and exclusion limits for proteins and water soluble polymers, as shown in **Table I**. **Table II** lists the features and benefits of Toyopearl HW resins.

Table I - Properties and Molecular Weight Separation Ranges for Toyopearl HW Resins

Toyopearl Resin	Particle Size (µm)	Pore Size (Å)	Molecular Weight of Sample		
			Globular Proteins	Dextrans	Polyethylene Glycols & Oxides
HW-40S HW-40F HW-40C	20 - 40 30 - 60 50 - 100	50	100 - 10,000	100 - 7,000	100 - 3,000
HW-50S HW-50F	20 - 40 30 - 60	125	500 - 80,000	500 - 20,000	100 - 18,000
HW-55S HW-55F	20 - 40 30 - 60	500	1,000 - 700,000	1,000 - 200,000	100 - 150,000
HW-65S HW-65F	20 - 40 30 - 60	1000	40,000 - 5,000,000	10,000 - 1,000,000	500 - 1,000,000
HW-75F	30 - 60	>1000	500,000 - 50,000,000	100,000 - 10,000,000	4,000 - 5,000,000

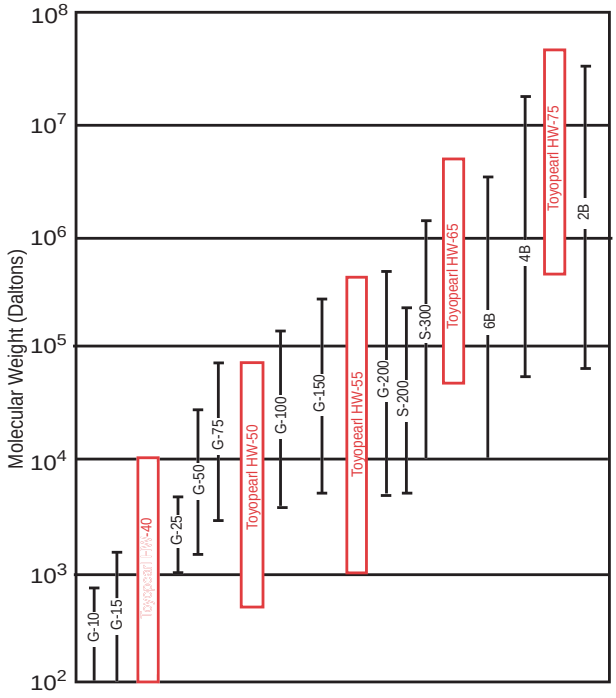
Table II - Features and Benefits of TSK-GEL HW Resins

- Constant packing volume over a wide range of salt concentrations
- High capacity for water soluble proteins
- High yield recovery using various aqueous eluents
- Stable from pH 2 to 12 for routine operation
  Stable from pH 2 to 13 for cleaning
- Stable up to 3 bars of pressure
- Stable in eluents containing surfactants and organic solvents
- Can be autoclaved repeatedly in clean water at 120˚C
- Bed stability
- Low cost chromatography
- Little loss or fouling
- Can be regenerated with base or acid
- Excellent flow rates, high linear velocities, can be scaled to very large columns
- Can be used for very hydrophobic proteins
- Easy to sanitize

Fractionation range

Figure 1 shows the fractionation range of the Toyopearl HW media. It can be seen that the product line covers the full range of normally encountered water soluble proteins. Also given is an indication of how Toyopearl HW resins compare with Sephadex® and Sephacryl® gels sold by Pharmacia.

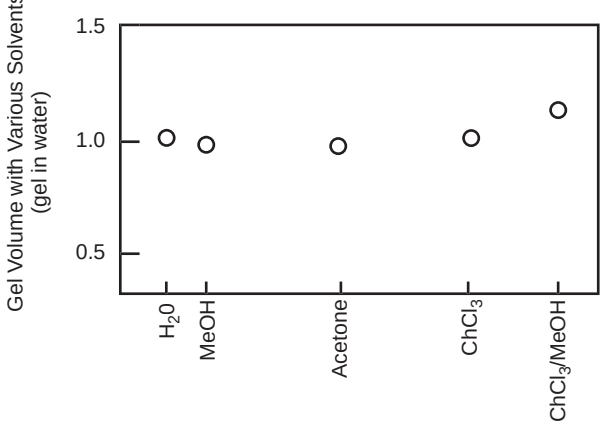
Figure I
Fractionation Range for Globular Proteins: Toyopearl HW Resins Compared with Pharmacia Gels



Bed stability in various solvents

Toyopearl Resins, due to their high degree of cross-linking, are quite resistant to changes in eluents as can be seen in **Figure 2**.

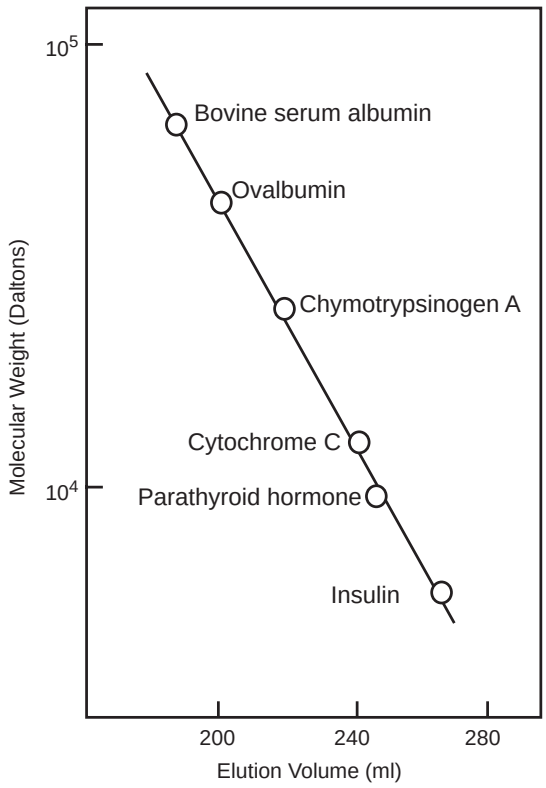
Figure 2
Swelling Properties of Toyopearl HW-40S in Various Eluents



Size sorting of proteins

The molecular weight of proteins can be estimated using size exclusion chromatography. **Figure 3** shows an example of this process using Toyopearl HW 55F in a chaotropic buffer.

Figure 3
Size Exclusion Chromatography of Proteins in 6M Guanidine Hydrochloride

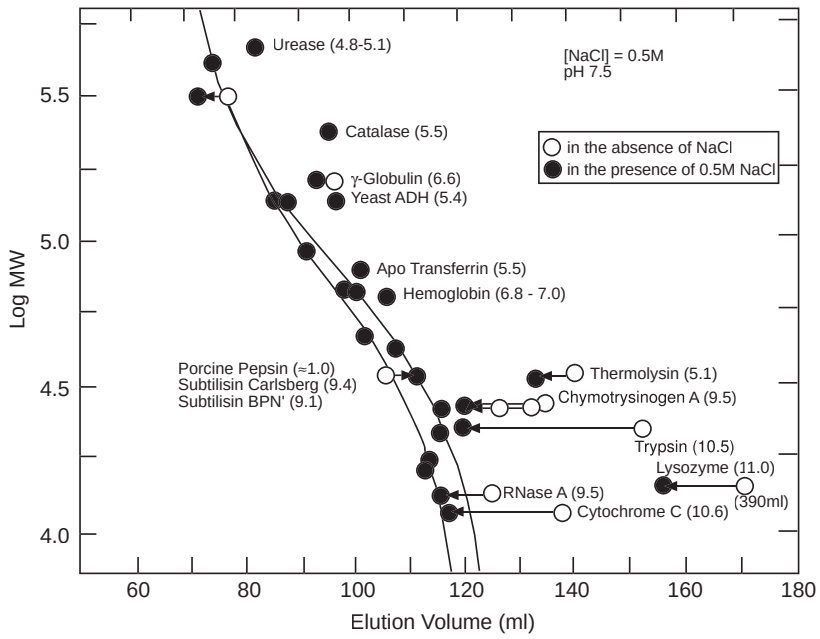


Packing:	Toyopearl HW-55F
Column:	2.2cm ID x 100cm
Eluent:	6M guanidine HCl
Flow rate:	12ml/min
Temperature:	20˚C
Detector:	UV @ 280nm

Effect of Salt on Protein Retention

Protein retention on HW resins can change depending on the pI of the protein and the pH of the mobile phase. These effects can be minimized by the addition of a salt to the mobile phase as shown in **Figure 4**.

Figure 4
Effect Of Salt on Protein Retention

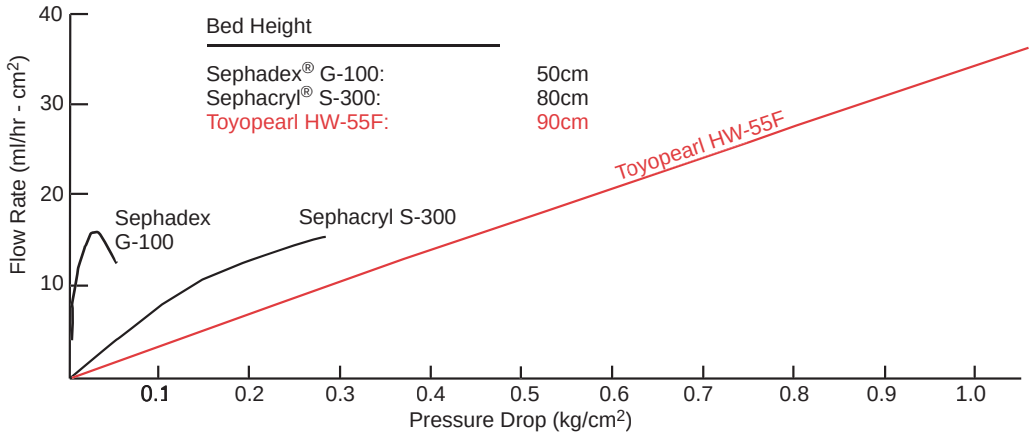


Chromatography was done on a Toyopearl HW-55F column (2.5cm x 50cm) with 25mM TrisHCl buffer, pH 7.5 (standard buffer). The number in the parenthesis is the value of the isoelectric point (pI) of each protein.

Pressure/Flow Characteristics

The superior rigidity of the Toyopearl Resin allows much higher flow rates than conventional gel products as illustrated in **Figure 5**.

Figure 5
Flow Rate as a Function of Pressure Drop On Toyopearl HW-55F

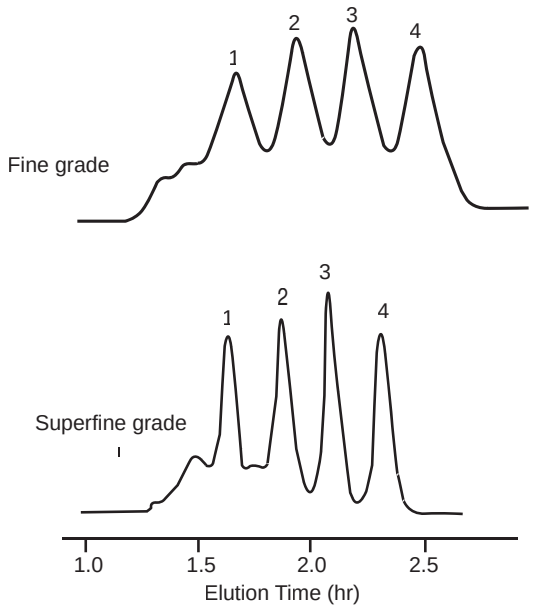


Column: 2cm I.D. x 70cm
Packing pressure: 1.5 to 2.5 kg/cm^2, using a peristaltic pump
Flow rate: 1 to 1.5ml/min

Effect of particle size on resolution

In **Figure 6**, two different grades of Toyopearl HW-55 are compared --- an exercise often done in scale-up research and development. This figure shows that resolution is best with the S-grade (20 to 50µm), while the F-grade provides adequate performance. With these options, the most economical solution for scale-up chromatography can be chosen.

Figure 6
Comparison of Resolution on Different Particle Sizes of Toyopearl HW-55

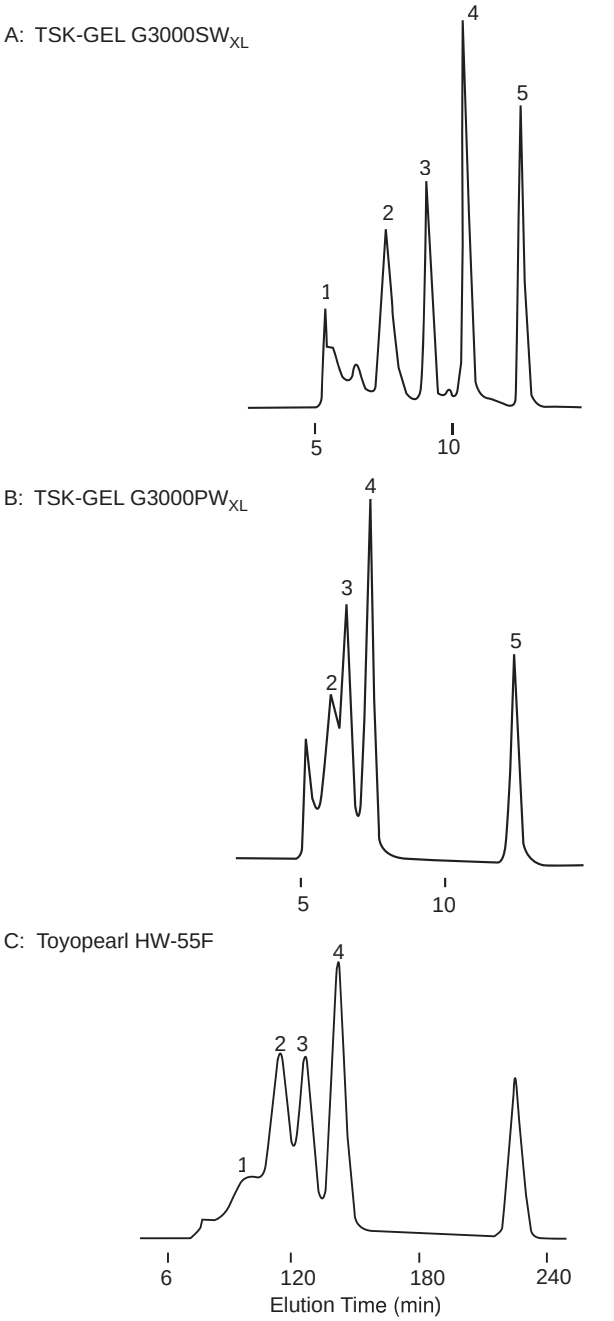


Column:	Toyopearl HW-55, 2.6cm ID x 70cm
Sample:	1ml of 1. Thyroglobulin (0.3%); 2. γ-Globulin (0.3%); 3. β-Lactoglobulin (0.3%); 4. Cytochrome C (0.1%)
Eluent:	33mM Phosphate buffer (pH 7.0), 0.2M NaCl
Flow rate:	106ml/hr (20cm/hr)
Temperature:	25˚C
Detector:	UV (280nm)

Scaling up protein separations for HPLC

Due to surface and pore characteristics which are almost identical, Toyopearl HW resins behave very much as TSK-GEL HPLC columns as shown in **Figure 7**.

Figure 7
Separation of a Protein Mixture on TSK-GEL G3000SW$_{XL}$, TSK-GEL G3000PW$_{XL}$ (5µm) HPLC Columns and Toyopearl HW-55F



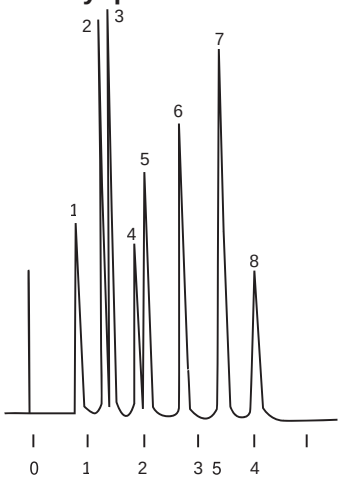
Columns:	A. TSK-GEL G3000SW$_{XL}$ (7.8mm ID x 300mm) B. TSK-GEL G3000PW$_{XL}$ (7.8mm ID x 300mm) C. Toyopearl HW-55F (22mm ID x 600mm)
Sample:	1. Thyroglobulin; 2. γ-globulin; 3. Ovalbumin 4. Ribonuclease A; 5. p-aminobenzoic acid
Eluent:	50mM phosphate buffer (pH 7.0) containing 0.3M NaCl
Flow rate:	1ml/min
Temperature:	25˚C
Detector:	UV (280nm)

Adsorption chromatography examples

Separation of nucleic acid-related compounds

Figure 8 shows a chromatogram of various nucleic acid related compounds obtained with Toyopearl HW-40S. This product is widely used for this kind of adsorption purification as well as for the separation of oligosaccharides.

Figure 8
Separation of Nucleic Acid Related Compounds by Adsorption Chromatography on Toyopearl HW-40S



Column:	2.6cm ID x 29cm
Sample:	1. Blue dextran; 2. ATP; 3. ADP; 4. 2'-AMP; 5. 3'-AMP; 6. 3', 5'-cAMP; 7. Adenosine; 8. Adenine
Eluent:	0.1M ammonium acetate (pH 4.6)
Flow rate:	80ml/hr
Temperature:	25˚C
Detector:	UV (280nm)

Chromatography in organic solvents

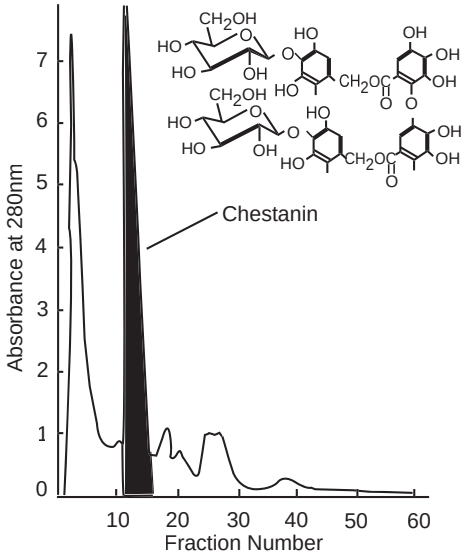
Figure 9 shows an example of purification of a plant-origin polyphenol in 50% methanol. Toyopearl HW-40 is being used in ways similar to ODS reverse phase packings or Sephadex LH-20 for the separation of polyphenolic substances.

Enzyme purification examples

Purification of glycoproteins

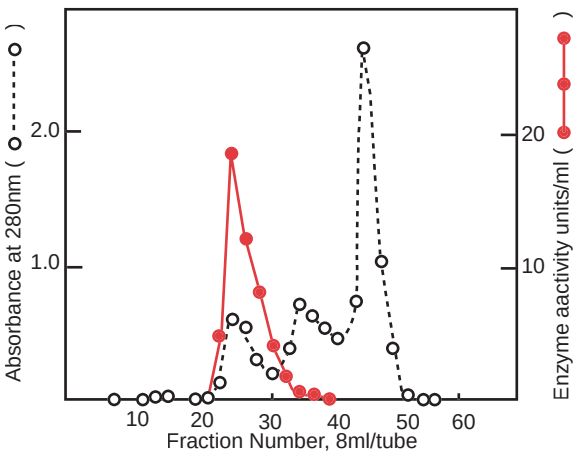
Membrane-bound enzymes, most of which are glycoproteins, tend to lose activity during conventional slow gel filtration. This process is due to excessive elution times as well as non-specific interaction between the glycoproteins and polysaccharide gel. High-speed elution with Toyopearl HW-55F solved this problem in the example shown in **Figure 10**. Recovery of enzymatic activity was up to 80%. Specific activity was increased as well, while chromatography with dextran gels destroyed 90% of the activity.

Figure 9
Elution Pattern of Polyphenolic Substances Obtained from Chestnut Galls



Column:	Toyopearl HW-40F, 0.9cm ID x 16cm
Eluent:	50% (v/v) methanol
Flow rate:	3.5ml/hr
Sample:	0.1ml
Detector:	280nm
Fraction size:	4g/tube

Figure 10
Chromatogram of Post-Proline Dipeptidyl Aminopeptidase from Pig Kidney

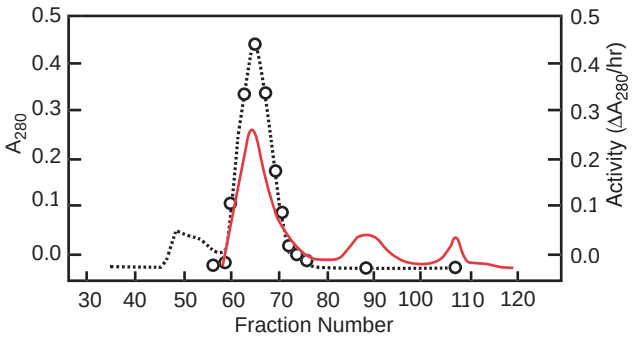


Column:	Toyopearl HW-55F, 2.5cm ID x 144cm
Eluent:	20mM tris-HCl buffer containing 0.2M KCl (pH 7)
Flow rate:	30ml/hr
Temperature:	4˚C
Sample load:	5ml

OTHER EXAMPLES OF ENZYME PURIFICATION

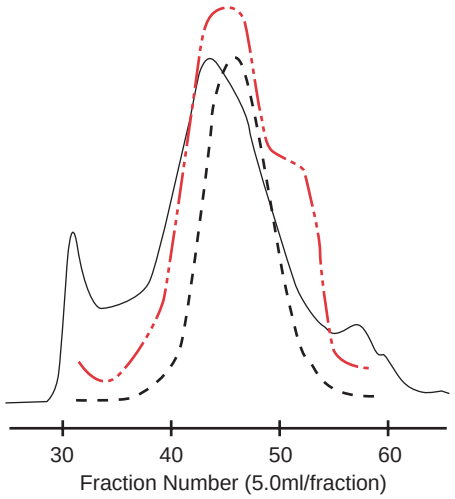
Figures 11 and **12** give two further examples of the use of Toyopearl HW resins for the recovery and purification of enzymes, one from a culture supernatant and the other from animal tissue.

Figure 11
Chromatogram of Extracellular Protease from Thermus Caldophilus GK24



Column:	Toyopearl HW-55F, 1.8cm ID x 91cm
Eluent:	100mM Tris-HCl buffer (pH 7.4) containing 1mM $CaCl_2$
Flow rate:	about 10ml/hr (3.9ml/hr - cm^2)
Temperature:	4˚C
Fraction size:	2ml/tube
Sample volume:	7ml
Detector:	UV @ 280nm (———)
	Enzyme activity (···o···o···o···)

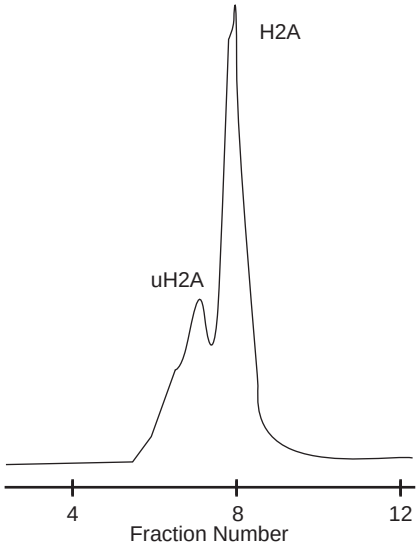
Figure 12
Purification of mRNA on Toyopearl HW-65F



Column:	Toyopearl HW-65F, 25mm x 90cm
Sample:	40mg of poly (A) RNA/5ml of formamide, sample from silkworm
Elution:	20mM sodium citrate, 5mM EDTA, 0.5% SDS, 6M urea, pH 3.5
Flow rate:	24ml/hr
Detection:	UV @ 254, total mRNA activity (- - -), mRNA activity for major plasma proteins (——·——)

Toyopearl HW-50S resin has been used to help isolate the ubiquitin-histone conjugate uH2A from the unicellular ciliated protozoan *Tertrahymena pyriformis.* **Figure 13** shows the separation of uH2A from the histone, H2A. The sole difference between these two components is a small polypeptide, ubiquitin (ca. 8,500Da).

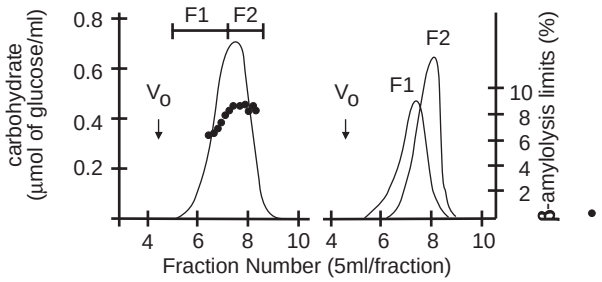
Figure 13
Isolation of a Complex Protein Conjugate on Toyopearl HW-50S



Column:	22mm x 83cm
Sample:	fraction from crude *Tetrahymena* H2A containing the ubiquitin-histone conjugate uH2A
Elution:	10mM HCl
Flow rate:	0.1ml/min
Detection:	UV @ 230nm

Gel filtration chromatography on a Toyopearl HW-75F column is also a sensitive and useful method for determining the purity of amylose specimens, and for demonstrating heterogeneity in molecular weight and branched structure. **Figure 14** shows the elution profiles of amylose subfractions isolated from potato. Such substances as wheat and tapioca have also been purified by gel filtration.

Figure 14
Isolation of Potato Amylose Subfractions on Toyopearl HW-75F



Column:	Toyopearl HW-75F, 26mm x 100cm
Sample:	potato amylose, 5mg/ml
Elution:	0.05M NaCl
Flow rate:	0.67ml/min
Temperature:	45˚C
Detection:	total carbohydrate: anthrone-sulfuric acid assay
	β-amylolysis limits: hydrolysis w. β-amylase

TOYOPEARL SEC RESINS:

Part #	Container size (ml)	Product description	Particle size (µm)	Exclusion limit (Da)
19809	150	HW-40S	20-40	3×10^3
07451	250			
14681	1000			
07967	5000			
19808	150	HW-40F	30-60	3×10^3
07448	500			
14682	1000			
07968	5000			
19807	150	HW-40C	50-100	3×10^3
07449	500			
14683	1000			
07969	5000			
19811	150	HW-50S	20-40	1.8×10^4
07455	250			
14684	1000			
08059	5000			
19810	150	HW-50F	30-60	1.8×10^4
07453	500			
14685	1000			
08060	5000			
19813	150	HW-55S	20-40	1.5×10^5
07459	250			
14686	1000			
08062	5000			
19812	150	HW-55F	30-60	1.5×10^5
07457	500			
14687	1000			
08063	5000			
19815	150	HW-65S	20-40	1×10^6
07467	250			
14688	1000			
08068	5000			
19814	150	HW-65F	30-60	1×10^6
07465	500			
14689	1000			
08069	5000			
19816	150	HW-75F	30-60	5×10^7
07469	500			
14691	1000			
08072	5000			

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

TOYOPEARL LABPAKS:

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

TOSOH BIOSCIENCE

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