

Ion Exchange Chromatography

Toyopearl Resins for IEC

650 series for most proteins

Anion Exchange

Toyopearl DEAE-650

Toyopearl SuperQ-650

Cation Exchange

Toyopearl CM-650

Toyopearl SP-650

550 series for smaller proteins

Anion Exchange

Toyopearl QAE-550

Cation Exchange

Toyopearl SP-550

Toyopearl MegaCap™ SP-550

TSK-GEL High Performance Resins for IEC

5PW series for all proteins

Anion Exchange

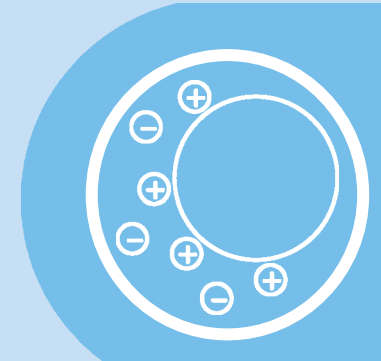
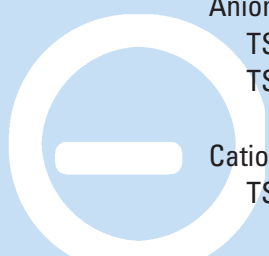
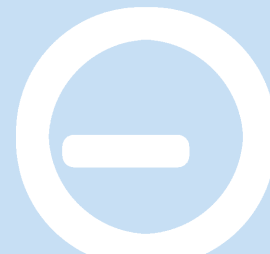
TSK-GEL SuperQ-5PW

TSK-GEL DEAE-5PW

Cation Exchange

TSK-GEL SP-5PW

Toyoscreen Process Development Columns for IEC



Toyopearl Ion Exchange Chromatograph resins

Ion Exchange Chromatography (IEC) is the most common liquid chromatographic method used in manufacturing therapeutic proteins. Due to the high dynamic binding capacities of ion exchange resins relative to those of the other chromatographic modes (*Table 1*), it is the technique selected by many developers for the capture or concentration chromatographic step.

Tosoh Bioscience offers a broad range of products for ion exchange applications.

How does IEC work?

IEC is based on the binding of proteins to positively or negatively charged groups which are immobilized on a stationary phase and which are in equilibrium with free counter ions in the mobile phase. In the process of adsorption, the mobile phase counter ions are exchanged by the protein solute. The binding of proteins to the ion exchange matrix predominantly occurs via charged amino acid residues located at the surface of the protein molecule.

The development of optimum chromatographic system conditions requires knowledge of both the protein's pI and the pKa of the ion exchange media. An eluent buffer pH is selected between the pI of the target and the ion exchanger's pKa (*Figure 1*). This ensures that the protein is in the opposite charge state of the ion exchange media. When possible, the pH is also optimized to effect the highest solubility of the target protein. Higher protein solubilities make more efficient use of the overall ion exchange capacity of the resin.

A salt is selected as the source of counter ions in the mobile phase and elution occurs as the salt strength is increased to a higher concentration than the target's binding salt conditions

Ion exchange groups available

Toyopearl and TSK-GEL IEC resins are available with 5 different ion exchange groups as shown in *Table II*:

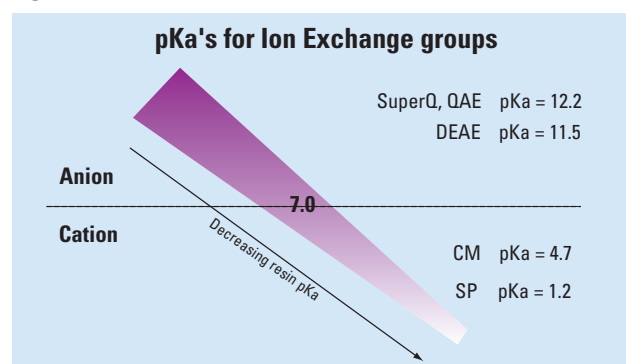
- 3 for anion exchange – SuperQ, QAE, DEAE
- 2 for cation exchange – SP, CM

The pKa's for these ion exchange groups are listed in (*Figure 1*).

Table I

Separation Mode	Binding capacity for standard proteins (mg/mL)	Binding capacity in production processes (mg/mL)
Ion Exchange	200 - 300	50 - 100
Hydrophobic Interaction	40 - 60	10 - 30
Affinity (group specific ligands)	40 - 100	20 - 60
Reversed Phase (polymeric media)	60 - 100	30 - 50

Figure 1









Pore sizes offered

Tosoh Bioscience offers a broad range of base bead pore sizes (*Figure 2*). There are currently 2 different mean resin pore diameters used for the ion exchangers: 1000Å and 500Å

- Toyopearl SuperQ-650, DEAE-650, SP-650, and CM-650; use the 1000Å mean pore diameter size exclusion chromatography (SEC) resin Toyopearl HW-65 as the base bead.
- TSK-GEL SuperQ-5PW, SP-5PW, and DEAE-5PW use the 1000Å mean pore diameter TSKgel 5000PW SEC resin as the support.
- Toyopearl QAE-550C, SP-550C, and MegaCap SP-550EC use Toyopearl HW-55 as the base resin, with a 500Å mean pore diameter.

Features		Benefits
<ul style="list-style-type: none"> porous, hydrophilic polymer based resin 		<ul style="list-style-type: none"> suitable for laboratory scale and process chromatography
<ul style="list-style-type: none"> chemical stability 		<ul style="list-style-type: none"> autoclavable at 121°C temperature range 4°-60°C pH range 2-13, can be regenerated with acid or base compatible with organic solvents
<ul style="list-style-type: none"> column bed stability 		<ul style="list-style-type: none"> constant packing volume over a wide range of salt concentrations
<ul style="list-style-type: none"> mechanical stability 		<ul style="list-style-type: none"> excellent flow characterization in large industrial columns (up to 3 bar)
<ul style="list-style-type: none"> continuous selectivity 		<ul style="list-style-type: none"> easy scale up from TSK-GEL IEC columns high yields of biologically active proteins

Table II

Structure of Toyopearl ion exchange resins		
Toyopearl resin	pore size	Functional group
DEAE-650S DEAE-650M DEAE-650C	1000Å	 -O-CH ₂ -CH ₂ -HN ⁺ -(C ₂ H ₅) ₂ anion exchanger
SuperQ-650S SuperQ-650M SuperQ-650C	1000Å	 -O-R'-N ⁺ -(CH ₃) ₃ strong anion exchanger
QAE-550C	500Å	 -O-CH ₂ -CH ₂ -N ⁺ -(CH ₃) ₃ strong anion exchanger
CM-650S CM-650M CM-650C	1000Å	 -O-CH ₂ -COO ⁻ weak cation exchanger
SP-650S SP-650M SP-650C	1000Å	 -O-R'-O-CH ₂ -CH ₂ -CH ₂ -SO ₃ ⁻ strong cation exchanger
SP-550C MegaCap SP-550EC	500Å	 -O-R'-O-CH ₂ -CH ₂ -CH ₂ -SO ₃ ⁻ strong cation exchanger

Note: R' = proprietary polymer

Figure 2

Tosoh Methacrylic base beads available for IEC							
Pore size(Å)	50	125	400-500	750	1000	>1000	>1700
Product name							
Toyopearl HW:	40	50	55	60	65	75	80
TSK-GEL PW:	1000	2000	4000		5000	6000	

← Increasing pore surface area

Higher surface area = more capacity

A bead with a small pore size has more surface area than the same size bead with a larger pore. If a protein will fit into the smaller pore, and specifically the effective pore which may be smaller because of the attachment process of the functional ion exchange groups *see* (Figure 3). It will typically have a higher dynamic binding capacity than the larger pore version, (see cation exchanger lysozyme breakthrough curves in (Figure 13). For a comparison of the dynamic binding capacities of all Toyopearl and TSK-GEL anion exchangers, *see* (Figure 12).

Multiple particle sizes simplify scaling up or down

Because Toyopearl HW-65 and TSK-GEL 5000PW products have the same resin backbone chemistry and selectivity, scale-up or scale-down for a selected ion exchanger is simple. Practically speaking, the chromatographic conditions that work for one particle size will work for a different particle size. The elution order of the feedstock components will remain the same with increasing resolution as the particle size gets smaller. (Figure 4)

Figure 3

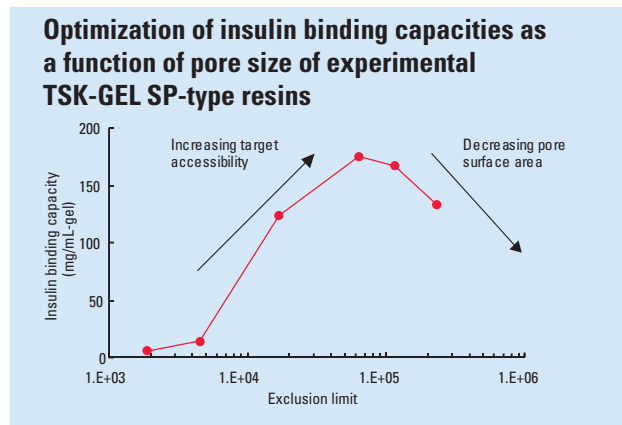
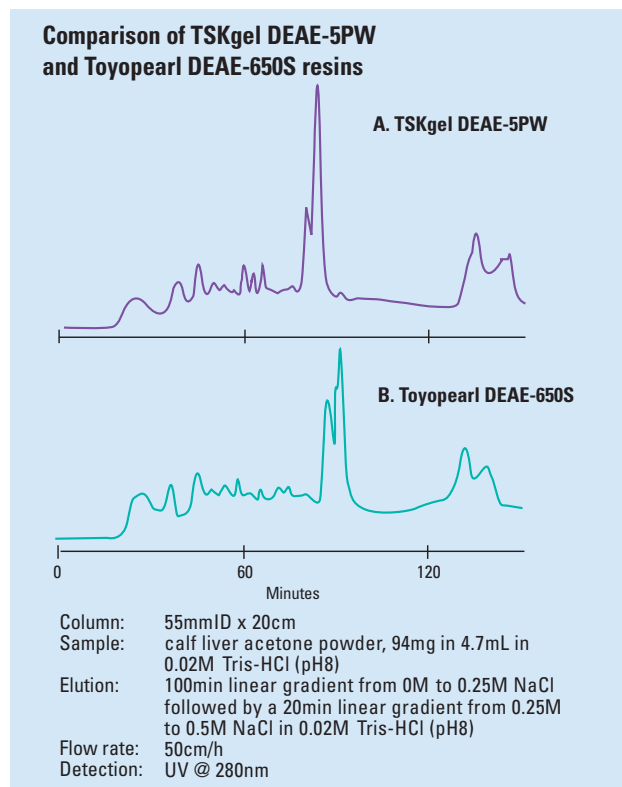


Figure 4



(Figure 5) lists the complete range of ion exchange products, particle sizes and suggests how they are typically placed into a manufacturing scheme. Please note that the specific particles shown are in mean diameter sizes of: 200 (EC), 100 (C), 65 (M), 35 (S), 30, and 20 microns.

IEC Resins are available in 4 particle diameter ranges:





	S	20 - 40 µm (Superfine)
	M	40 - 90 µm (Medium)
	C	90 - 120 µm (Coarse)
	EC	100 - 300 µm (Extra Coarse)

Figure 5

Process step	Bead size	Process media	
		Anion	Cation
Capture	200µm		Toyopearl MegaCap SP-550EC
	100µm	Toyopearl SuperQ-650C Toyopearl DEAE-650C Toyopearl QAE-550C	Toyopearl SP-650C Toyopearl SP-550C Toyopearl CM-650C
	65µm	Toyopearl SuperQ-650M Toyopearl DEAE-650M	Toyopearl SP-650M Toyopearl CM-650M
Intermediate Purification	35µm	Toyopearl SuperQ-650S Toyopearl DEAE-650S	Toyopearl SP-650S Toyopearl CM-650S
	30µm	TSKgel SuperQ-5PW (30) TSKgel DEAE-5PW (30)	TSKgel SP-5PW (30)
Polishing	20µm	TSKgel SuperQ-5PW (20) TSKgel DEAE-5PW (20)	TSKgel SP-5PW (20)
	10µm	TSKgel SuperQ-5PW 7.5mmIDx7.5cm TSKgel DEAE-5PW 7.5mmIDx7.5cm	TSKgel SP-5PW 7.5mmIDx7.5cm TSKgel CM-5PW 7.5mmIDx7.5cm
QC			

Same selectivity HPLC columns are available for most process media

Also, a number of products are available as prepacked TSK-GEL analytical columns which use 5 and 10 micron versions of the beads. Please refer to our Laboratory Catalog or our website for more information on these items.

Mechanical stability

If recommended packing procedures are followed, Toyopearl and TSK-GEL IEC resins maintain stable bed volumes during the pH and ionic strength changes that occur during normal ion exchange chromatography (Consult our Toyopearl and TSK-GEL 5PW Packing Guide for the recommended packing conditions for each ion exchanger). Multi-cycle gradient operation and re-equilibration are accomplished without volume changes in the Toyopearl column bed. Notice in (Figure 6) that the bed volume of competitive anion exchangers may change several percent during the course of a salt or pH gradient.

The mechanical stability of Toyopearl resins allows the use of longer column beds with more efficiency or higher operational flow rates. Typical linear velocities for Toyopearl SP-550C (100µm particle) packed in a 1.4m ID process column are shown in (Figure 7). The pressure/flow relationship remains linear up to 600cm/h. The recommended operational backpressure for Toyopearl resins is a maximum of 3 bar (45 psi).

TSK-GEL IEC resins have a smaller particle size and a corresponding higher intrinsic backpressure than the Toyopearl products. Since the TSK-GEL type resins have the same backbone methacrylic polymer chemistry as their larger particle Toyopearl complementary products, their degree of crosslinking is slightly higher allowing them to withstand operational backpressures up to 20 bar (300 psi).

Scale up from a 1.4cm ID to a 60cm ID column

A 5000-fold scale-up of the β-galactosidase enzyme purification was accomplished using Toyopearl DEAE-650M. The chromatograms in (Figure 8) demonstrate the excellent scale up characteristics of Toyopearl ion exchange media. Gradient slope and particle diameter remained unchanged in the scale up. Linear velocity was reduced by 15% in the largest scale separation, and resolution actually improved relative to the smallest scale separation. This may be partly attributed to increased bed height and the slower linear velocity. Although the column volume was increased in part by increasing the bed height, the principal change in column volume was a result of the greater column diameter (1.4cm to 60cm). This example illustrates how Toyopearl media can be conveniently scaled up from laboratory to production scale applications using the same particle size if desired.

Figure 6

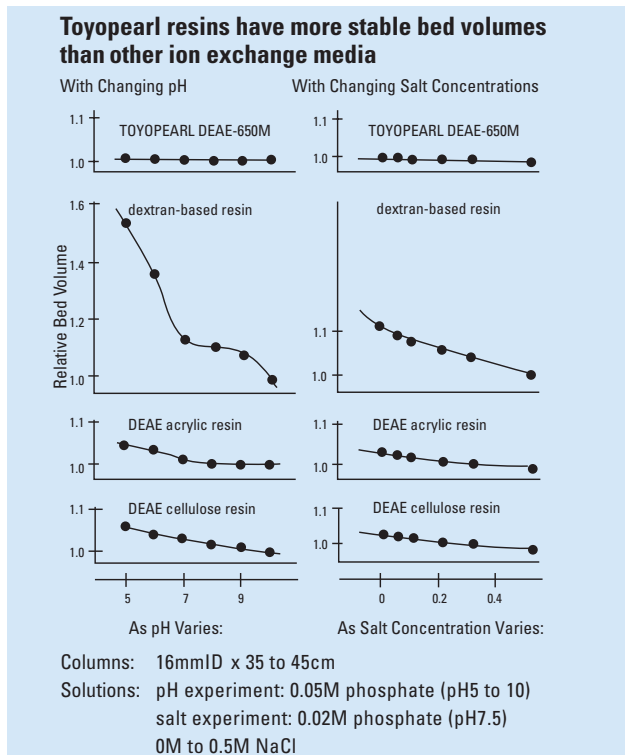
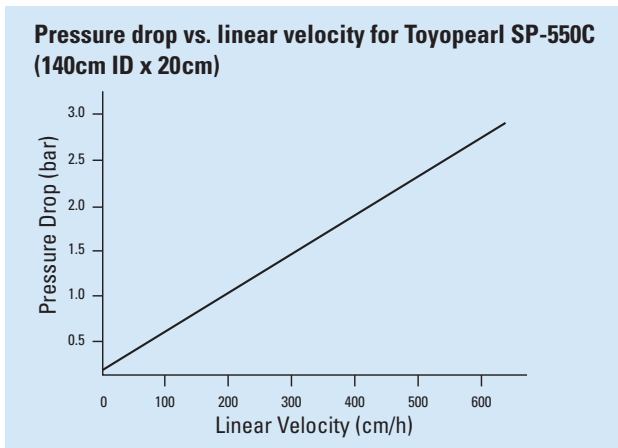


Figure 7



Chemical stability and routine cleaning

The polymeric base resins of all Toyopearl and TSK-GEL ion exchangers are chemically and thermally stable. Caustic or acidic solutions may be used for cleaning, sanitization and depyrogenation (Figure 9). Although ten days of exposure to strong base (pH = 12) decreases the small ion capacity of Toyopearl SuperQ-650M, the bovine serum albumin adsorption capacity remains constant after 28 days of exposure. Overnight cleaning or sterilization procedures with strong acid or base are therefore possible with Toyopearl and TSK-GEL ion exchange resins. These resins can also be autoclaved at 121°C. (Figure 10)

Figure 8

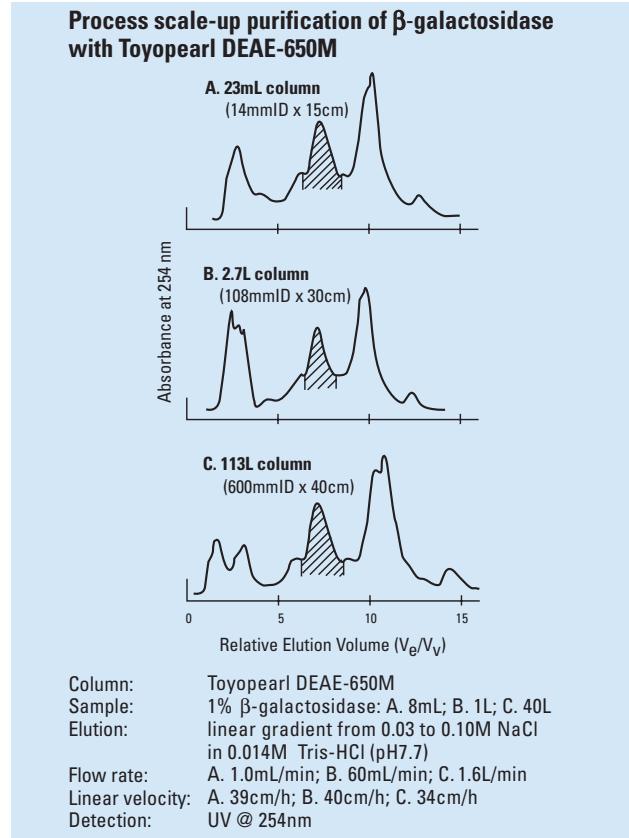


Figure 9

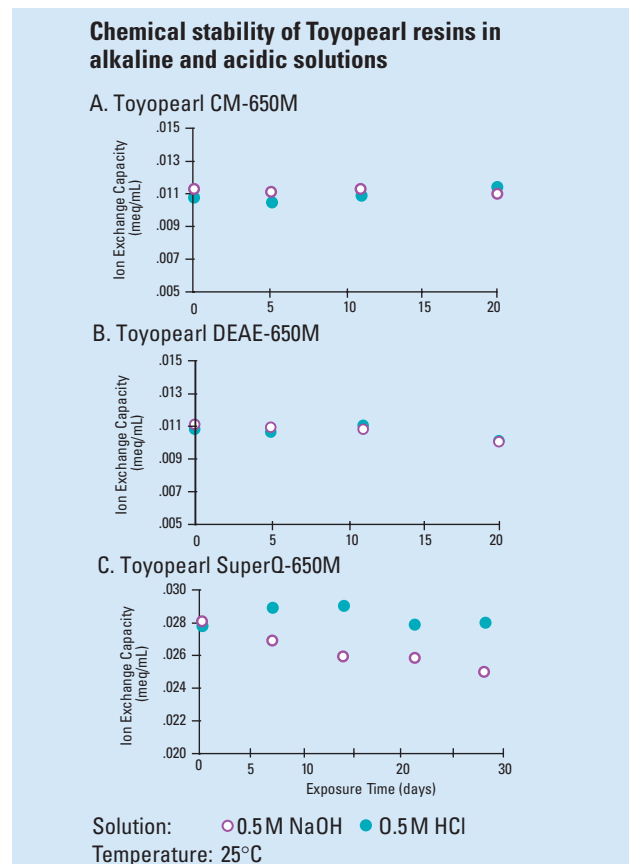


Figure 10

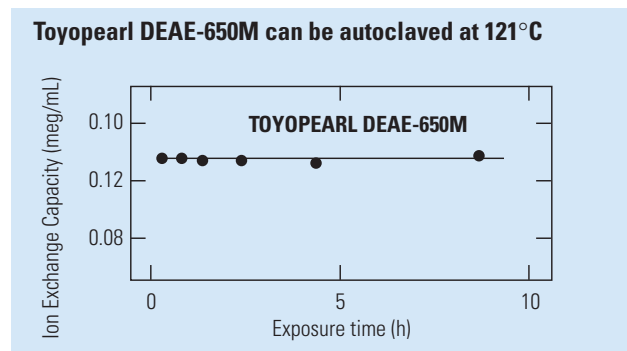


Table III

Recovery of enzymatic activity on Toyopearl CM-650M

Protein	Da	% Activity recovery
Phospholipase D	~56,000	87
Lipid transfer protein	69,000	91
Purine nucleotide phosphorylase	68,000	99

Table IV

Recovery of enzymatic activity on Toyopearl DEAE-650M

Protein	Da	% Activity recovery
Phospholipase D	~56,000	92
Prolyl endopeptidase	79,000	96
Alanine dehydrogenase	240,000	79
Phenylalanine dehydrogenase	310,000	95
Serine acetyltransferase	650,000	95

Protein recovery

Toyopearl and TSK-GEL ion exchange resins deliver exceptional protein mass recovery, as shown in *Table III and IV*. The mass recovery percentage of each protein was determined spectrophotometrically from the recovered fractions. Retention of activity indicates that protein-resin interactions do not disrupt the native conformation of the product. Nonspecific protein/resin interactions, which can lead to protein inactivation or irreversible binding, are minimized with Toyopearl resins.

The capture step

Ion exchange chromatography is well known for its high binding capacities of charged biomolecules. These high capacities allow the adsorption of significant quantities of target from a dilute feedstock and then the subsequent elution of the same molecule into a more concentrated fraction.

This is one of the principal reasons ion exchange is most often chosen as the initial capture step in a process. It is also the reason that ion exchange may be used after a diluting unit operation such as size exclusion chromatography.

Feedstock clarity and particle size selection

There are many considerations in the choice of the right particle size for an ion exchange capture step. If the feedstock has a high level of particulate, is viscous, etc. then a traditional adsorbent step using a very large particle, such as a 600 micron bead, is utilized. However, large particle diameters are detrimental to adsorption, because of their poor binding kinetics. This can lead to increased load times, longer elution times and low dynamic binding capacities.

On the other hand, if the feedstock has been clarified, such as through a 0.2 micron filter, then smaller particle sizes can be used and the bead size selection is more influenced by the pressure and flow specifications of the pumps and column hardware.

Because the smaller bead has faster adsorption kinetics, its effective dynamic binding capacity is significantly higher than that of the very large particles. The improved small particle kinetics also allows the loading of feedstock at a higher linear velocity if desirable.

A column packed with a smaller particle will also have more theoretical plates than one of the same dimensions packed with larger particles, thus increasing separation efficiency. Higher efficiency translates to higher resolution which can enhance the purity of the eluted product.

Tosoh Bioscience offers a wide range of particle sizes to address the needs of most process steps as seen in *Figure 5 (page 16)*.

The importance of target recovery

Product recovery is of critical importance whether dealing with expensive feedstocks such as mammalian cells or scarce resources such as the isolation of proteins from blood. A 3-step purification train with a 90% product recovery at each step will yield 73% of the original target feedstock load. If an 80% recovery is achieved for each step the process will only have a 51% yield. This means that twice as much feedstock is required to produce a given quantity of target. Also of importance is the retention of the molecules bioactivity throughout the process.

The high dynamic binding capacity of a given resin must be coupled with its bioactivity yield to compare with other resins. In many cases lower dynamic binding capacity resins with 100% mass and bioactivity recovery are a better choice than a higher capacity resin with compromised mass yield and bioactivity.

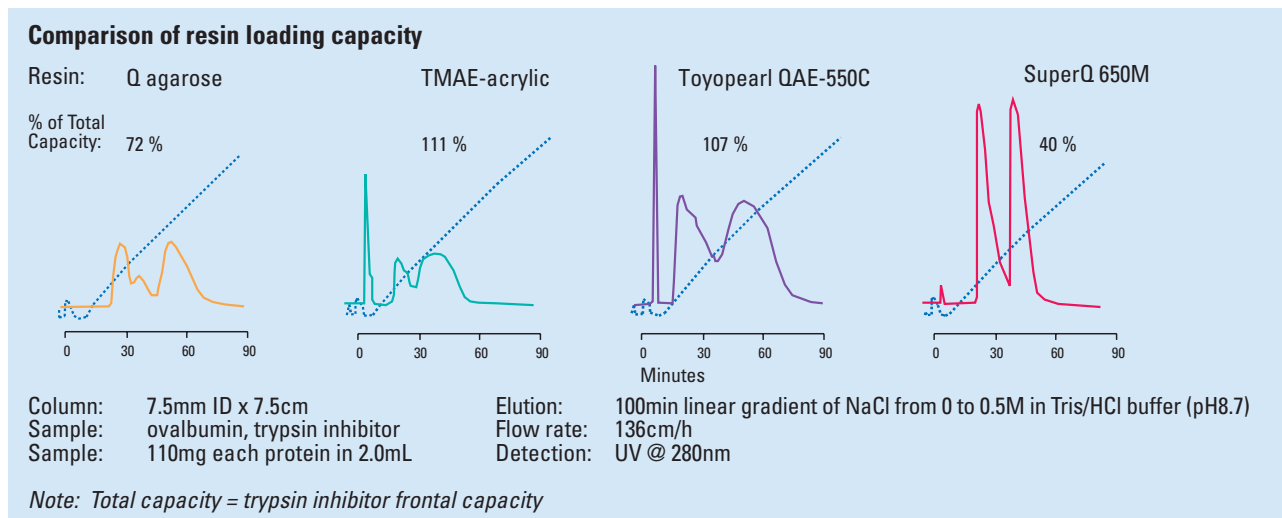
Toyopearl and TSK-GEL polymeric ion exchangers are excellent choices when high bioactivity recovery is paramount.

Downstream steps

IEC steps also may have high resolving power. This can be influenced by a number of variables such as: particle size, functional group, salt concentration, salt type, pH, and specific immobilization chemistry.

For this reason ion exchange chromatography is also a very useful technique for intermediate and polishing applications where separation factor plays as important a role as binding capacity.

Figure 11



Resolution as a function of load

The relationship of resolution to the amount of material loaded onto the resin is also an important consideration in developing a chromatography capture step. In *Figure 11*, four competitive resins are each loaded with 33mg of protein per mL of resin bed. Although the mass loading and bed volume are identical for all four resins, Toyopearl SuperQ-650M provides the best separation because it is loaded at a lower percentage of its potential capacity.

Sequential processing

Charge interactions between resin and target molecules are fundamental to the selective power of IEC. It may be complemented with other non-charge mediated chromatographic techniques in the design of an efficient downstream purification process. IEC is often used in conjunction with hydrophobic interaction (HIC), size exclusion (SEC), or reversed phase (RPC) techniques in a logical and effective sequence. In some cases it is used downstream from an affinity purification step. By careful selection of sequence, salt concentrations between steps can be matched, avoiding unnecessary addition or removal of salt which is time consuming and potentially costly.

Applications:

Proteins, antibodies, plasma proteins, peptides, tryptic digests, nucleotides, oligonucleotides, viruses, antibiotics, glycoproteins
 Please check the database on our website for numerous applications. (www.tosohbioscience.com)

Toyoscreen prepacked columns for process development

Toyoscreen columns packed with the full range of our Toyopearl IEC products are available in 1mL and 5mL resin volumes. They provide a convenient way to do early resin screening for both target retention and recovery. Multiple columns can be connected in series for additional separation. Please see the ordering information at the end of this section or contact us for more information on these products.

LabPak

For scientists wishing to develop a better physical understanding of the packing properties of Toyopearl and TSK-GEL ion exchange resins, we offer Toyopearl LabPaks of small quantities of the bulk resins. Please see the ordering information at the end of this section or contact us for more information on these products.



Figure 12

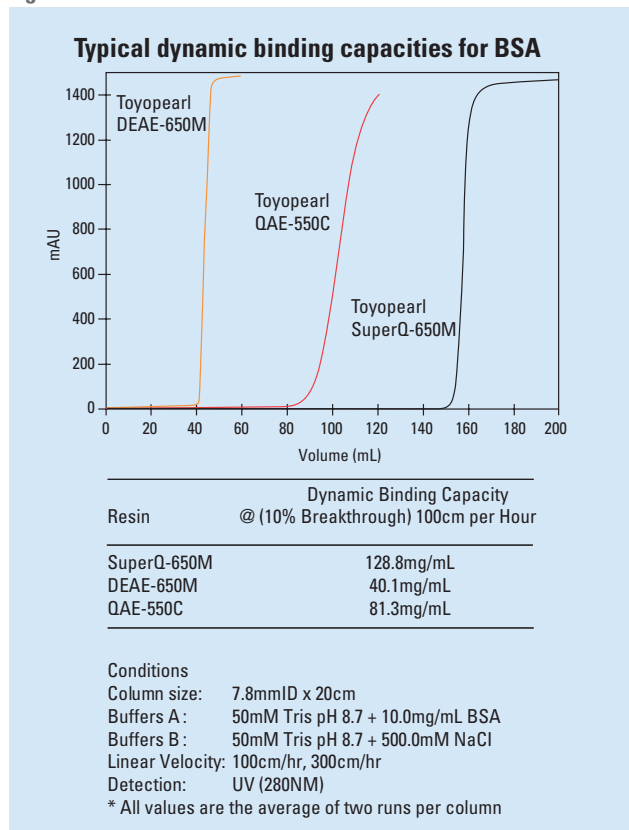
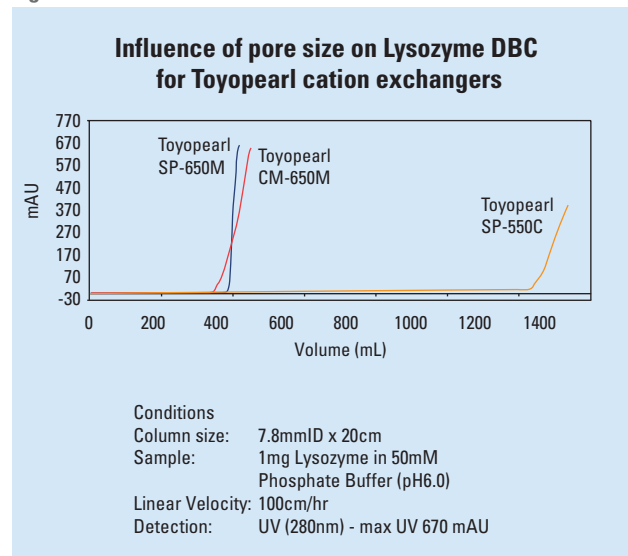


Figure 13



Ordering Information

ToyoScreen process development columns for IEC:

Part #	Product description	Package
21360	ToyoScreen DEAE-650M, 1mL	1mL x 6 each
21361	ToyoScreen DEAE-650M, 5mL	5mL x 6 each
21362	ToyoScreen SuperQ-650M, 1mL	1mL x 6 each
21363	ToyoScreen SuperQ-650M, 5mL	5mL x 6 each
21364	ToyoScreen QAE-550C, 1mL	1mL x 6 each
21365	ToyoScreen QAE-550C, 5mL	5mL x 6 each
21366	ToyoScreen CM-650M, 1mL	1mL x 6 each
21367	ToyoScreen CM-650M, 5mL	5mL x 6 each
21368	ToyoScreen SP-650M, 1mL	1mL x 6 each
21369	ToyoScreen SP-650M, 5mL	5mL x 6 each
21370	ToyoScreen SP-550C, 1mL	1mL x 6 each
21371	ToyoScreen SP-550C, 5mL	5mL x 6 each
21392	ToyoScreen IEC Anion Mix Pack, 1mL	1mL x 3 Grades x 2 each
21393	ToyoScreen IEC Anion Mix Pack, 5mL	5mL x 3 Grades x 2 each
21394	ToyoScreen IEC Cation Mix Pack, 1mL	1mL x 3 Grades x 2 each
21395	ToyoScreen IEC Cation Mix Pack, 5mL	5mL x 3 Grades x 2 each
21396	ToyoScreen IEC Mix Pack, 1mL	1mL x 6 Grades x 1 each
21397	ToyoScreen IEC Mix Pack, 5mL	5mL x 6 Grades x 1 each

ToyoScreen column accessories

Part #	Product description	Comment
21400	ToyoScreen Column Holder	
42194	ToyoScreen Holder w/ Fittings	Includes 21400, 42195 and 42196 (qty. 2)
42195	Column to Column Connector	
42196	Adaptor, M6 int to 10-32 ext, PEEK	
42197	Adapter, 1/4-28 int to 10-32 ext, PEEK	

TSK-GEL LabPak

Part #	Product description	Container size (mL)	Particle size (µm)
43380	IEXPAK PW (20) (SP-5PW, DEAE-5PW, SuperQ-5PW)	3 x 25mL	15-25
43280	IEXPAK PW (30) (SP-5PW, DEAE-5PW, SuperQ-5PW)	3 x 25mL	20-40

Toyopearl LabPak

Part #	Product description	Container size (mL)	Particle size (µm)
19817	IEXPAK HP (CM-650S, SP-650S, DEAE-650S, SuperQ-650S)	4 x 25mL	20-50
43210	AIEXPAK (DEAE-650M, SuperQ-650M, QAE-550C)	3 x 100mL	40-90 and 50-150
43220	CIEXPAK (CM-650M, SP-650M, SP-550C)	3 x 100mL	40-90 and 50-150

Ordering Information

Anion exchange resins:

TSK-GEL bulk media

Part #	Product description	Container size (mL)	Particle size (µm)	Ion exchange capacity (meq/mL resin)	Adsorption capacity (mg/mL resin) (BSA)
43383	SuperQ-5PW (20)	25	15-25	0.12-0.18	52-88
18535		250			
18546		1,000			
18547		5,000			
43283	SuperQ-5PW (30)	25	20-40	0.12-0.18	52-88
18536		250			
18548		1,000			
18549		5,000			
43381	DEAE-5PW (20)	25	15-25	0.05-0.11	25-45
14710		250			
14711		1,000			
18436		5,000			
43281	DEAE-5PW (30)	25	20-40	0.05-0.11	20-40
14712		250			
14713		1,000			
18370		5,000			

Toyopearl bulk media

43271	QAE-550C	100	50-150	0.28-0.38	60-80
14026		250			
14704		1,000			
14027		5,000			
19823	SuperQ-650S	25	20-50	0.20-0.30	105-155
17223		250			
17224		1,000			
17225		5,000			
43205	SuperQ-650M	100	40-90	0.20-0.30	105-155
17227		250			
17228		1,000			
17229		5,000			
43275	SuperQ-650C	100	50-150	0.20-0.30	105-155
17231		250			
17232		1,000			
17233		5,000			
19804	DEAE-650S	25	20-50	0.08-0.12	25-35
07472		250			
14692		1,000			
07973		5,000			
43201	DEAE-650M	100	40-90	0.08-0.12	25-35
07473		250			
14693		1,000			
07974		5,000			
07988	DEAE-650C	250	50-150	0.05-0.11	25-35
14694		1,000			
07989		5,000			

Ordering Information

Cation exchange resins:

TSK-GEL bulk media

Part #	Product description	Container size (mL)	Particle size (µm)	Ion exchange capacity (meq/mL resin)	Adsorption capacity (mg/mL resin) (lysozyme)
43382	SP-5PW (20)	25	15-25	0.06-0.12	20-40
14714		250			
14715		1,000			
18435		5,000			
43282	SP-5PW (30)	25	20-40	0.06-0.12	20-40
14716		250			
14717		1,000			
18384		5,000			

Toyopearl bulk media

43272	SP-550C	100	50-150	0.14-0.18	80-120
14028		250			
14705		1,000			
14029		5,000			
19822	SP-650S	25	20-50	0.13-0.17	40-60
08437		250			
14698		1,000			
08438		5,000			
43202	SP-650M	100	40-90	0.13-0.17	40-60
07997		250			
14699		1,000			
07998		5,000			
07994	SP-650C	250	50-150	0.12-0.18	35-55
14700		1,000			
07995		5,000			
19803	CM-650S	25	20-50	0.08-0.12	30-50
07474		250			
14695		1,000			
07971		5,000			
43203	CM-650M	100	40-90	0.08-0.12	30-50
07475		250			
14696		1,000			
07972		5,000			
07991	CM-650C	250	50-150	0.05-0.11	25-45
14697		1,000			
07992		5,000			
19828	MegaCap SP-550EC*	100	100-300	0.14-0.18	60-90*
19829		250			
19830		1,000			
19831		5,000			

* Adsorption capacity for insulin: 90-120 mg/mL resin

NEW! ToyoScreen Process
Development Columns

