Ion Exchange Chromatography

TOYOPEARL Resins for IEC

Anion Exchange
- TOYOPEARL DEAE-650
- TOYOPEARL SuperQ-650
- TOYOPEARL QAE-550

Cation Exchange
- TOYOPEARL CM-650
- TOYOPEARL SP-650
- TOYOPEARL SP-550
- TOYOPEARL MegaCap™ SP-550EC

TSK-GEL-5PW Bulk Resins for IEC

Anion Exchange
- TSK-GEL SuperQ-5PW
- TSK-GEL DEAE-5PW

Cation Exchange
- TSK-GEL SP-5PW

Method Development Glass Columns (MD-G)

- TOYOPEARL MD-G SuperQ-650S
- TOYOPEARL MD-G DEAE-650S
- TOYOPEARL MD-G SP-650S
- TOYOPEARL MD-G CM-650S

- TSK-GEL MD-G Q-5PW (20)
- TSK-GEL MD-G DEAE-5PW (20)
- TSK-GEL MD-G SP-5PW (20)

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TOYOPEARL Ion Exchange Chromatographic Resin

IEC is probably the most widely used liquid chromatographic method for the separation of proteins. It 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 counterions in the mobile phase. In the process of adsorption, the mobile ions are exchanged by the solute, the protein. The binding of proteins to the ion exchange matrix predominantly occurs via charged amino acid residues located at the surface of the protein molecule.

Capture

Ion exchange chromatography is well known for its high capacity for charged biomolecules. It is one of the principal reasons why it is often chosen as the initial capture step in a process containing a series of chromatography steps..

Sample Concentration

A major feature of ion exchange is its power to concentrate a bound component. It is often used to capture and concentrate very dilute feedstream components early in a process sequence.In addition, it is used as a concentration step following SEC. In both cases, the main aim of the step is to reduce sample volume and increase sample concentration.

Resolution and Selectivity

IEC also exhibits high resolving power, both binding and elution selectivity being controllable by a number of variables, such as salt concentration, salt type, pH, in addition to resin mode selection and specific chemistry. It is also extensively used for both intermediate and final stages of purification.

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. 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.

Resin Selection and Process Economics

The choice of an ion exchange resin will have a significant impact on the economics of a process. It is therefore important for the process designer to make an informed choice based on a knowledge both of the techniques available and the many ways of using the alternative resins.

Tosoh Bioscience offers an extended range of product options in both TOYOPEARL and TSK-GEL-5PW polymeric IEC media. The essential choices fall into the following:

- Ion exchange mode (anion or cation exchange)
- Performance attributes (TOYOPEARL for routine use, TSK-GEL 5PW for methods requiring higher resolution)
- Particle Size (selectable on basis of productivity, or throughput)

These resins may be easily used in scale up from analytical TSK-GEL-5PW and methods development (MD-G) columns, satisfying the analytical needs for resolution as well as manufacturing needs for productivity. Within each resin chemistry, method transfer between products is highly predictable. The entire range offers true seamless scalability, based on conserved selectivity.

Specific product features of Tosoh Bioscience IEC resins are also listed in this section.

Features	Benefits
porous, hydrophilic polymer based resin	• suitable for laboratory scale and process chromatography
chemical stability	• autoclavable at 121°C • temperature range 4°-60°C • pH range 2-13, can be regenerated with acid or base • compatible with organic solvents
column bed stability	• constant packing volume over a wide range of salt concentrations
mechanical stability	• excellent flow characterization also in large industrial columns (up to 3 bar)
continuous selectivity	• easy scale up from TSK-GEL-5PW IEC columns • high yields of biologically active proteins

TOYOPEARL ion exchange resins

The current TOYOPEARL group of ion exchangers offers four particle size ranges and five functional groups (Table I) to meet chromatographic needs across a broad spectrum of applications.

The TOYOPEARL-650 ion exchange series is designed on the HW-65, 1000 Å pore size, base resin. They provide outstanding performance in all stages of purification. High dynamic capacity for large and medium sized molecules, fast mass transfer kinetics, good selectivity and resolution are all important features of the product line.

The TOYOPEARL-550 series features the HW-55, 500 Å pore, base resin offering higher internal surface area and consquently higher capacity for most proteins in early downstream capture situations. The more highly cross-linked structure imparts higher rigidity, which together with a coarse grade particle permits faster flow for high throughput capture.

A high capacity SP Cation Exchange capture resin is available as an extra course grade (EC) product. Other resins can also be ordered as EC grade upon request.

Applications:

- Proteins, antibodies, plasma proteins, peptides, tryptic digests, nucleotides, oligonucleotides, viruses, antibiotics, glycoproteins

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)

Table I

Structure of TOYOPEARL ion exchange resins

TOYOPEARL resin	*pore size*	*Functional group*
DEAE-650S DEAE-650M DEAE-650C	1000 Å	HW-65 -O-CH_2-CH_2-HN^+-$(C_2H_5)_2$ anion exchanger
SuperQ-650S SuperQ-650M SuperQ-650C	1000 Å	HW-65 -O-R'-N^+-$(CH_3)_3$ strong anion exchanger
QAE-550C	500 Å	HW-55 -O-CH_2-CH_2-N^+-$(C_2H_5)_3$ strong anion exchanger
CM-650S CM-650M CM-650C	1000 Å	HW-65 -O-CH_2-COO^- weak cation exchanger
SP-650S SP-650M SP-650C	1000 Å	HW-65 -O-R-O-CH_2-CH_2-CH_2-SO_3^- strong cation exchanger
SP-550C MegaCap SP-550EC	500 Å	HW-55 -O-R-O-CH_2-CH_2-CH_2-SO_3^- strong cation exchanger

Note: R' = proprietary polymer

Please check the database on our website for numerous applications.

Chemical stability and routine cleaning

Cyclic cleaning is often accomplished by simply using a high salt wash, however the polymeric base resins of all TSK-GEL and TOYOPEARL ion exchangers are chemically and thermally stable. Caustic or acidic solutions may be used for cleaning, sanitization and depyrogenation, since the ion exchange capacity of our resins remain relatively unchanged for at least 20 days at extreme pH values (Figure 2). Although ten days of exposure to strong base (pH 12) decreases the small ion capacity of TOYOPEARL DEAE-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.

Resin storage

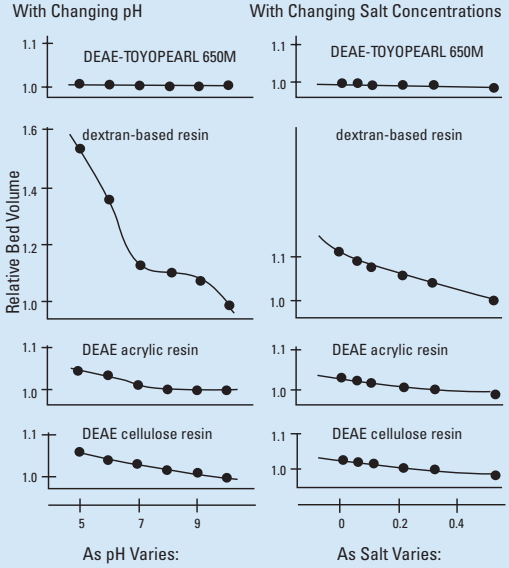
The recommended storage solution is 20% ethanol. Long-term storage in strong base (0.5 M NaOH) conditions, however, is not advised.

Sanitization and alternative cleaning options

All TOYOPEARL ion exchange resins retain full capacity after 28 day storage in 1% sodium hypochlorite, another effective sanitizing agent. Concentrated urea, guanidine, and detergent solutions could be used to clean these resins. Alcohols may be used to remove lipid residues and the resins can be subjected to repeated autoclave cycles. Figure 4 shows the relatively unchanged capacity after eight hours at 121°C.

Figure 1

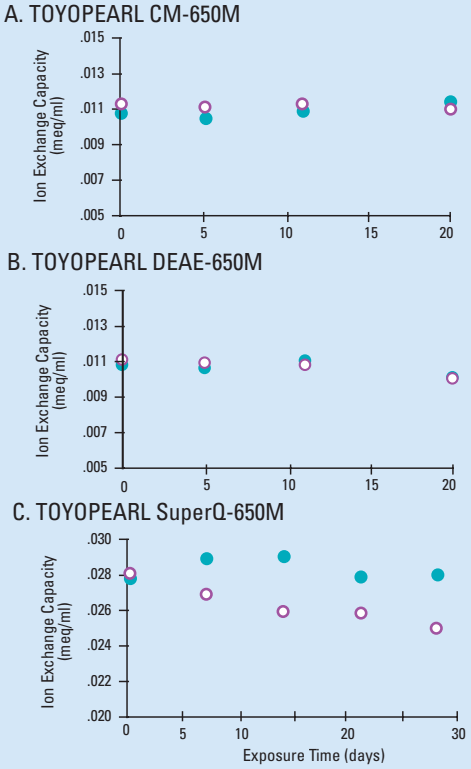
TOYOPEARL resins have more stable bed volumes than other ion exchange media



Columns: 16 mm I.D. x 35 to 45 cm L
Solutions: pH experiment: 0.05 M phosphate (pH 5 to 10)
salt experiment: 0.02 M phosphate (pH 7.5)
0M to 0.5 M NaCl

Figure 2

Chemical stability of TOYOPEARL resins in alkaline and acidic solutions



Solution: ○ 0.5 M NaOH ● 0.5 M HCl
Temperature: 25°C

Figure 3

Negligible volume change due to ionic strength TOYOPEARL DEAE- 650M

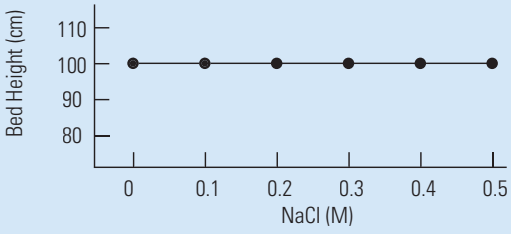
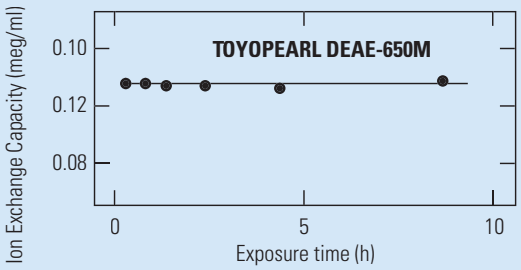


Figure 4

TOYOPEARL DEAE-650M can be autoclaved at 121°C



Mechanical stability

TOYOPEARL IEC resins maintain stable bed volumes during the pH and ionic strength changes that occur during IEC chromatography. Notice in Figure 1 that the bed volume of other anion exchangers may change several percent during the course of a salt or pH gradient. Multi-cycle gradient operation and re-equilibration is accomplished without volume changes in the TOYOPEARL resin. The mechanical stability of TOYOPEARL resins allows the use of longer column beds and/or higher flow rates for fast, efficient column washing and regeneration. Typical linear velocities for TOYOPEARL SP-550C packed in a 1.4 m I.D. process column are shown in Figure 5. The pressure/flow relationship remains linear up to 600 cm/h.

Column packing

It is best to pack TOYOPEARL ion exchange resins by the application of gentle pressure from 0.5 - 3 bar. We do not recommend packing TOYOPEARL resin by gravitational settling. TOYOPEARL resins should not be packed under high pressure (> 7 bar). Typical packing mobile phases include phosphate, Tris, or acetate buffer containing inorganic salt at the highest ionic strength to which the column will be exposed during operation (including any clean-in-place). Typical packing and operational velocities for TOYOPEARL ion exchange resins are shown in Table III.

For detailed packing instructions please refer to our website for downloads.

Recovery of mass and activity

TOYOPEARL and TSK-GEL ion exchange resins deliver exceptional protein mass recovery, as shown in Table II 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 bioactive conformation of the product. Nonspecific protein/resin interactions, which can lead to protein inactivation or irreversible binding, are minimized with TOYOPEARL resins. Examples of recovered protein activity listed in Table II and IV show that hydrophilic TOYOPEARL ion exchange resins have the essential characteristics for high activity recovery of bioactive species, including large proteins.

Table II

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

Figure 5

Pressure drop vs. linear velocity for TOYOPEARL SP-550C (1.4 m I.D. x 20 cm L)

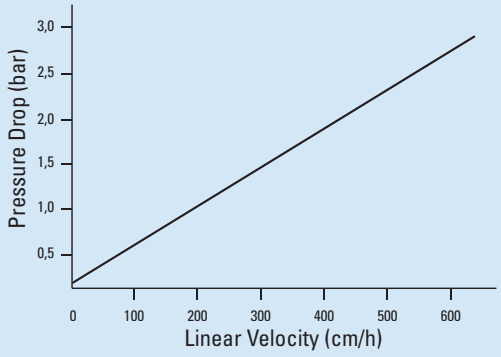


Table III

Optimum packing and operational velocities for TOYOPEARL ion exchange resins

Column size (I.D. x L)	*Grade*	*Packing flow rate (cm/h)*	*Operating flow rate (cm/h)*
TOYOPEARL DEAE-650, SuperQ-650, CM-650, SP-650			
22 mm x 20 cm	S	400 - 600	50 - 65 cm/h
	M	800 - 1,000	80 - 130 cm/h
	C	800 - 1,200	80 - 650 cm/h
TOYOPEARL QAE-550, SP-550			
22 mm x 20 cm	C	700 - 1,000	80 - 240 cm/h

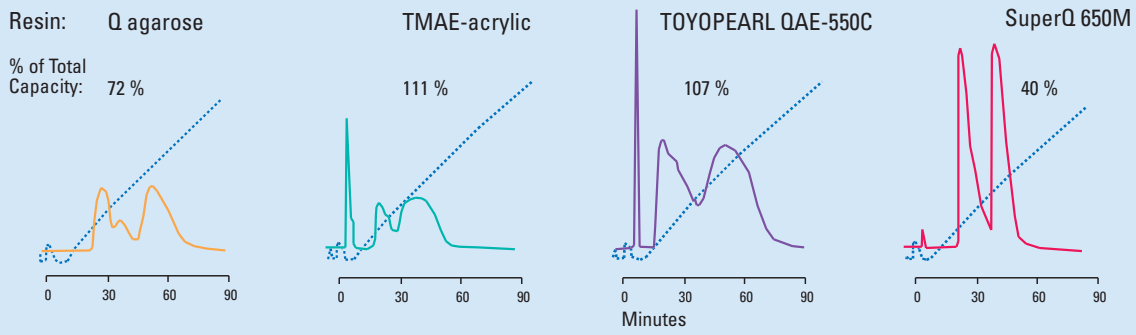
Table IV

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

Figure 6

Comparison of resin loading capacity



Column: 7.5 mm I.D. x 7.5 cm L
Sample: ovalbumin, trypsin inhibitor
Sample: 110 mg each protein in 2.0 ml
Elution: 100 min linear gradient of NaCl from 0 to 0.5 M in Tris/HCl buffer (pH 8.7)
Flow rate: 136 cm/h
Detection: UV @ 280 nm

Note: Total capacity = trypsin inhibitor frontal capacity

Load

The ability to load large amounts of material on a column is important when developing a chromatography capture step. This is shown in Figure 6, as 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 to a lower percentage of its potential capacity. The same load is bound in a narrower, more concentrated zone within the column.

Scale-up

Ion exchange chromatography is used at all scales, from research and development through manufacturing. The hallmark of a successful scale up is the ability to maintain resolution as the chromatographic bed volume is increased in direct proportion to the volume of feedstock. The focus of production scale is to increase the throughput of the separation system to handle the increase in sample mass. Factors such as mobile phase composition and sample load (mg sample/ml bed volume) are optimized at small scale during methods development. Thus column volume (CV (initial): CV (final)) is increased in direct proportion to the increase in sample mass (M (initial): M (final)), while the sample load or concentration (mg/ml) remains constant as in the equation below:

$$M_{(i)} / M_{(f)} = CV_{(i)} / CV_{(f)}$$

Scale-up of column volume is typically achieved by increasing the cross-sectional area of the column, while maintaining the bed height (z). This assumes that the efficiency of the large scale chromatographic bed equals that of the small scale bed. Such performance has been demonstrated in column efficiency studies with TOYOPEARL size exclusion resins. In practice, however, large scale chromatography columns sometimes provide less efficient flow distribution than laboratory or pilot-scale columns. Column hardware design and wall effects with some resins do not always permit direct scale up. To restore column efficiency, bed height (z) is increased and column diameter decreased to obtain a target mass throughput with the same calculated final column volume, V (f). Resolution (R_S) in gradient elution ion exchange chromatography can be described by the equation:

$$R_S = z^{1/2}/[d_p(u)]^{1/2}[GS]^{1/2}$$

particle size ($_{dp}$); linear velocity (u); normalized gradient slope (GS); bed height (z)

It is possible to preserve chromatographic resolution during scale up by keeping constant the terms in the equation. Linear velocity (u) and particle size ($_{dp}$) are optimized at small scale, and are used in the large-scale process.

The equation above may also be used to select an appropriate particle size grade of resin for an application.

TOYOPEARL resins are available in three particle size grades to allow efficient process optimization.

The gradient term in the equation, GS, refers to normalized gradient slope, and is dependent on resin volume. Further discussions on the gradient separation of proteins and the calculation of GS may be found in S. Yamamoto, K. Nakanishi and R. Matsuno, Ion Exchange Chromatography of Proteins, Marcel Dekker, New York, 1988.

A 5000-fold scale-up of the β-galactosidase enzyme purification was accomplished on TOYOPEARL DEAE-650M with the strategy described in the previous paragraph. The chromatograms in Figure 7 demonstrate the excellent scale-up characteristics of TOYOPEARL ion exchange media. The operational parameters for the scale up are listed in Figure 8. Gradient slope (GH) and particle diameter (dp) remained unchanged. Linear velocity (u) 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. Although column volume was increased in part by increasing the bed height (z), 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 columns.

TSK-GEL 5PW bulk resins

It is frequently necessary to rapidly develop gram scale quantities of a product for preclinical tests or Phase I trials. Direct scale-up from an analytical method is the most convenient solution to this challenge. Tosoh Bioscience offers 20 and 30µm particle size versions of TSKgel DEAE-5PW, TSKgel SuperQ-5PW, and TSKgel SP-5PW resins for those researchers who need direct scale-up from analytical resins of the same type and who need to pack their own columns. TSK-GEL 5PW preparative resins are made from the same polymer, (G5000PW), which is used in the prepacked TSK-GEL 5PW analytical ion exchange columns, and provide identical selectivity.

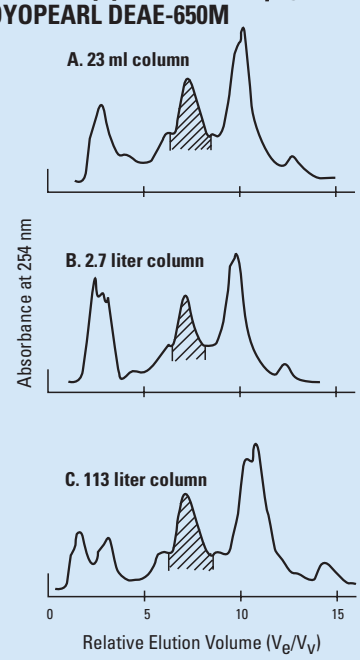
Designed for high performance medium scale separations, or for final stage product polishing, TSK-GEL 5PW resins have been used in columns up to 30 cm in diameter. The enhanced mechanical stability and excellent permeability of TSK-GEL 5PW resins permit high linear flow rates. For example, bed compression was not observed at the highest flow rate studied, 540 cm/h (20 bar or 300 psi), with a 55 mm I.D. column. TSK-GEL 5PW preparative LC resins allow process development groups to directly scale up methods developed on TSK-GEL analytical ion exchange columns with a pressure maximum around 20 bar for the resin.

Transfer of methods between TOYOPEARL and TSK-GEL resins

For larger scale manufacturing purposes, Tosoh Bioscience provides the TOYOPEARL line of process scale resins in 35, 65, and 100µm particle sizes. Due to the similarity of both base resin chemistry and surface functionalization, TSK-GEL (pressures up to 20 bar) and TOYOPEARL (pressures up to 3 bar) offer similar selectivity. Methods can be transferred across series with a high degree of predictability. This is shown clearly in Figure 8.

Figure 7

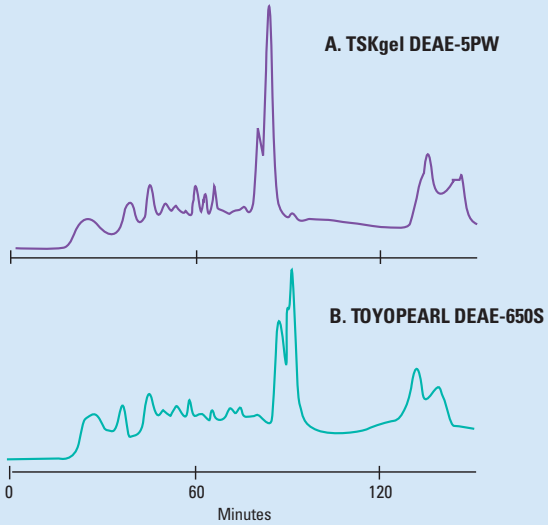
Process scale-up purification of β-galactosidase with TOYOPEARL DEAE-650M



Column:	TOYOPEARL DEAE-650M A. 14 mm I.D. x 15 cm L B. 108 mm I.D. x 30 cm L C. 600 mm I.D. x 40 cm L
Sample:	1 % β-galactosidase: A. 8 ml; B. 1 liter; C. 40 liters
Elution:	linear gradient from 0 to 0.03 M NaCl in 0.014 M Tris-HCl (pH 7.7)
Flow rate:	A. 1.0 ml/min; B. 60 ml/min; C. 1,600 ml/min
Linear velocity:	A. 39 cm/h; B. 40 cm/h; C. 34 cm/h
Detection:	UV @ 254 nm

Figure 8

Comparison of TSKgel DEAE-5PW and TOYOPEARL DEAE-650S resins



Column:	55 mm I.D. x 20 cm L
Sample:	calf liver acetone powder, 94 mg in 4.7 ml in 0.02 M Tris-HCl (pH 8)
Elution:	100 min linear gradient from 0 M to 0.25 M NaCl followed by a 20 min linear gradient from 0.25 M to 0.5 M NaCl in 0.02 M Tris-HCl (pH 8)
Flow rate:	50 cm/h
Detection:	UV @ 280 nm

For ordering information, see page 26.

TOYOPEARL SuperQ, highest capacity from a unique chemistry

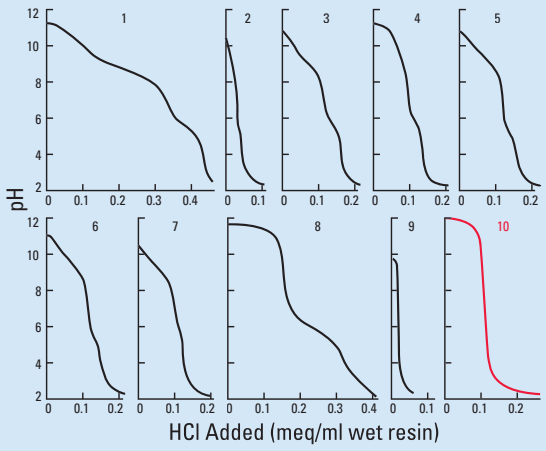
The Tosoh Bioscience anion exchange resin TOYOPEARL SuperQ features a quaternary amine functionalized polymer attached to TOYOPEARL HW-65. This polymeric technology provides significantly higher protein binding capacity, as shown in Table V. For example, the capacity of TOYOPEARL SuperQ for bovine serum albumin is more than double the capacity of a commonly used Q agarose-based material. TOYOPEARL SuperQ is available in three particle sizes to enable productivity optimization consistent with high capacity.

TOYOPEARL DEAE-650, a resin with a difference

TOYOPEARL DEAE-650 is the resin of choice when a high resolution anion exchanger is needed. The titration curve of TOYOPEARL DEAE-650, shown in Figure 9, indicates that it is a true monofunctional diethyl amino ethyl group with a single pKa value of 11.5, a value notably higher than those of other DEAE-type ion exchangers. This resin remains fully protonated within the pH range required for the purification of most biomolecules, and can be utilized at pH values up to 10.5 for basic proteins. The resulting rapid re-equilibration kinetics allow TOYOPEARL DEAE-650 to work as efficiently as a quaternary anion exchanger. The homogeneous functionality also contributes to enhanced resolution capabilities displayed by this particular DEAE resin.

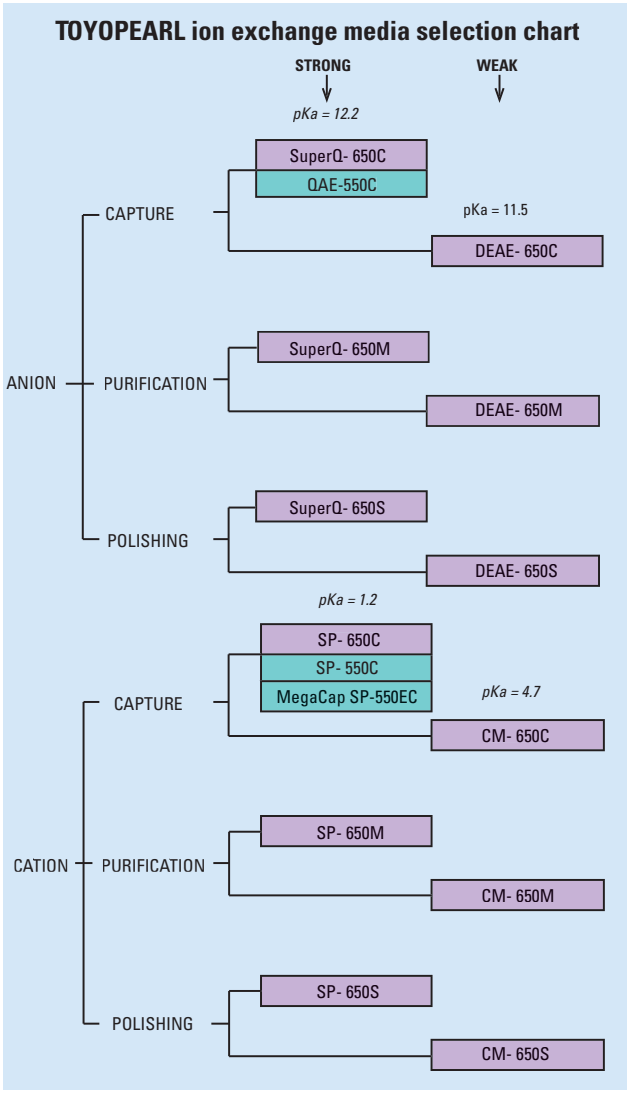
Figure 9

Titration curves of commercial anion exchangers



Titration curves of commercial anion exchangers in 0.5 M sodium chloride with 0.5 M hydrochloric acid. Curves:
1 = DEAE-Sephadex A-25; **2** = DEAE-Sephadex A-50;
3 = DEAE-Sepharose CL-6B; **4** = DEAE-Sephacel;
5 = DEAE-Cellulose DE-52; **6** = DEAE-Cellulose DE-23;
7 = DEAE-Cellulose; **8** = DEAE-Trisaeryl M; **9** = DEAE-Bio-Gel A; **10** = TOYOPEARL DEAE-650M

Figure 10



Different selectivities also available for cation exchange

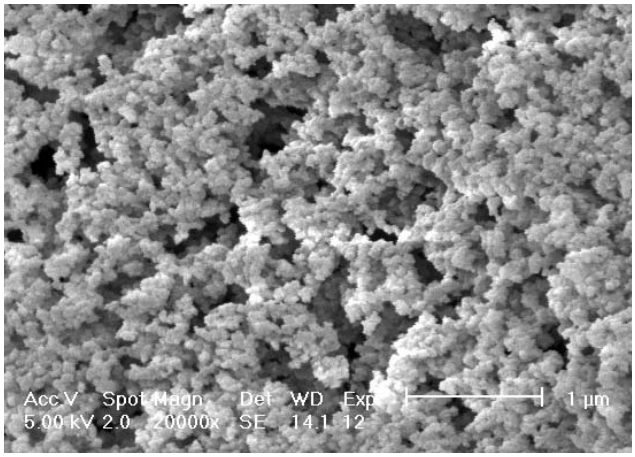
Two types of TOYOPEARL cation exchangers, with alternative selectivities, are available. TOYOPEARL SP-650 resins are sulfonated with strongly acidic groups and can be used at ph values as low as 2-4. For users preferring a weakly acidic resin, the carboxymethyl functional groups of TOYOPEARL CM-650 resins provide a pKa of ~ 4.7.

High capacity for capture with three special resins

Tosoh Bioscience also offers three high capacity capture resins: TOYOPEARL QAE-550C, TOYOPEARL SP-550C and TOYOPEARL MegaCap™ SP-550EC.

TOYOPEARL QAE-550C is a quaternary amine strong anion exchange resin and TOYOPEARL SP-550C is a strong cation exchange material. The higher surface area of the HW-55 base resin result in approximately twice the protein binding capacity of the HW-65 derived resins, as shown in Tables V and VI. These coarse grade materials provide high linear velocities required for maximum throughput. They are the resins of choice for initial column or batch capture processes for peptides and small proteins.

TOYOPEARL MegaCap SP-550EC extra coarse grade resin is available with a particularly high capacity for smaller protein molecules (e.g. insulin).



TOYOPEARL SP-650M – 20,000 magnification

Table V

Equilibrium protein adsorption capacities and properties of commercial ANION exchange resins

	BSA (67 kDa)	*Ferritin (444 kDa)*	*Thyroglobulin (660 kDa)*	*Particle size (µm)*	*Pore diameter (Å)*
Strong anion exchange resins					
TOYOPEARL SuperQ-650M	143 mg/ml	7.0 mg/ml	9.1 mg/ml	40 - 90	1,000
TOYOPEARL QAE-550C	70 mg/ml	25 mg/ml	9 mg/ml	50 - 150	500
Q agarose-based material#	56 mg/ml	NA	3 mg/ml *	45 - 165	400
Weak anion exchange resins					
TOYOPEARL DEAE-650M	30 mg/ml	15 mg/ml	12 mg/ml	40 - 90	1,000
DEAE agarose-based material#	97 mg/ml	4.3 mg/ml	2 mg/ml *	45 - 165	500

*Notes: Adsorption capacities listed are batch adsorption capacities except where noted by *, indicating results reported by the manufacturer;*
 # This material is not a Tosoh Bioscience product.

Table VI

Equilibrium protein adsorption capacities and properties of commercial CATION exchange resins

	Lysozyme (14.4 kDa)	*Hemoglobin (64 kDa)*	*IgG (155 kDa)*	*Particle size (µm)*	*Pore diameter (Å)*
Strong cation exchange resins					
TOYOPEARL SP-650M	50 mg/ml	42 mg/ml	ND	40 - 90	1,000
TOYOPEARL SP-550C	110 mg/ml	111 mg/ml	48 mg/ml	50 - 150	500
SP agarose-based material#	101 mg/ml	50 mg/ml *	50 mg/ml *	45 - 165	400
Weak cation exchange resins					
TOYOPEARL CM-650M	38 mg/ml	50 mg/ml	ND	40 - 90	1,000
CM agarose-based material#	94 mg/ml	30 mg/ml *	15 mg/ml *	45 - 165	400
CM agarose-based material#	97 mg/ml	4.3 mg/ml	2 mg/ml *	45 - 165	500

Notes: Adsorption capacities listed are Equilibrium adsorption capacities except where noted by (reported by the manufacturer).*
 # This material is not a Tosoh Bioscience product. ND=not determined.

TOYOPEARL MegaCap™ SP-550EC

TOYOPEARL MegaCap SP-550EC is designed as a capture resin for peptides and small proteins such as insulin. The excellent flow properties of this resin combine with enhanced separation efficiency and capacity to provide a highly cost effective initial step. Low flow-induced pressures within the packed bed and the large particle size permit the passage of crude biological feed materials.

Figure 11

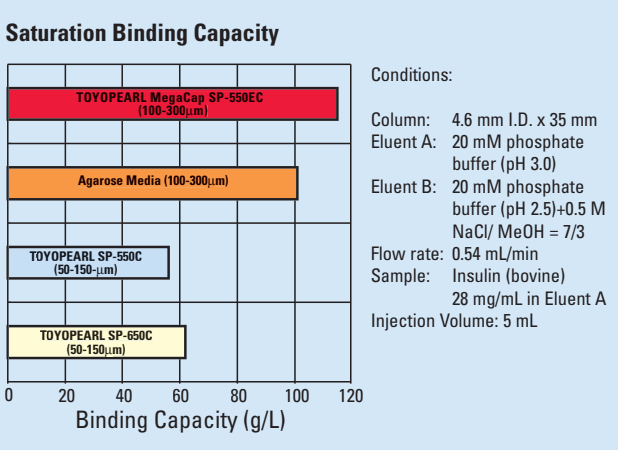


Figure 12

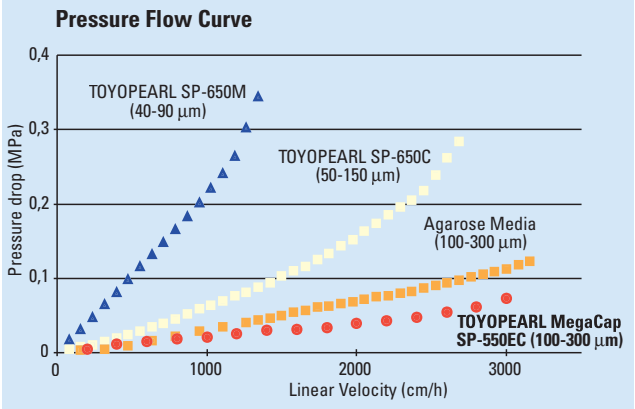
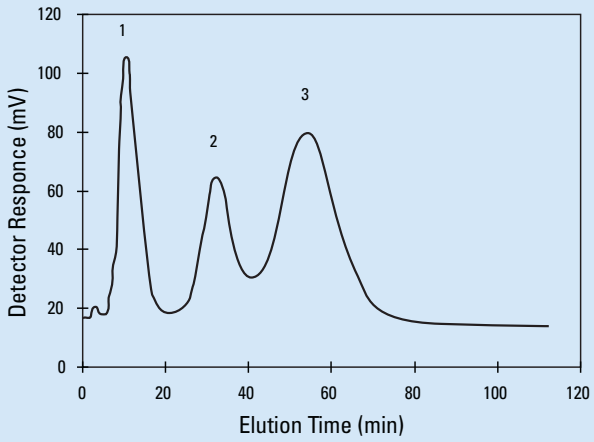


Figure 13



Column: TOYOPEARL MegaCap SP-550EC (7.5 mm I.D. x 75 mm)
Eluent: A. 20 mM Phosphate buffer + 1 M Na Cl (pH 6.0)
B. 20 mM Phosphate buffer + 1 M NaCl (pH 6.0)
gradient: a) to b) 180 min linear
Flow rate: 1 mL/min
Detection: UV @ 280 nm
Samples: 1. Insulin, 2. Cytochrome C, 3. Lysozyme (1 mg/mL)
Injection: 500 µL

Table VII

Equilibrium adsorption capacity for analytes of different molecular weight

	Carnosine[1]	*GGTA* [2]	*Bradykinin* [3]	*Insulin*	*Lysozyme*	*α-ChymotrypsinogenA*
Molecular weight (Da)	226	451	1,060	5,800	14,000	25,000
Absorption capacity	4	38	52	102	72	54

[1] Carnosine: β-Alanyl-L-Histidine; [2] GGTA: Gly-Gly-Tyr-Arg; [3] Bradykinin: Arg-Pro-Pro-Gly Phe-Ser-Pro-Phe-Arg · 2 AcOH · 3H$_2$O

Equilibrium adsorption capacity vs pore size and particle size

Resin	*Particle size (µm)*	*Pore Size (Å)*	*Static Binding Capacity (g/L)* *Insulin*	*Lysozyme*
TOYOPEARL MegaCap SP-550EC	100 - 300	300	95 - 120	60 - 90
TOYOPEARL SP-550C	50 - 150	500	120	80 - 120
TOYOPEARL SP-650C	50 - 150	1,000	47	35 - 55
Agarose based media	100 - 300	300	105	80

TOYOPEARL MD-G Series Columns

The Tosoh Bioscience Method Development Glass (MD-G) series is a new generation of preparative columns designed for methods development of downstream chromatographic processes. Packed with either 20 or 35µm ion exchange resin, MD-G columns are used to develop purification protocols for scale up to any level. The MD-G column geometry and design is tailored for method optimization, and bed volume is sufficient to provide predictable information about production scale separations.

Successful purification strategies can be developed with identical chemistry and selectivity throughout the TOYOPEARL and TSK-GEL product range.

This scale-up strategy is shown in Figure 14 where a separation method for standard proteins was optimized on a TOYOPEARL SP-650S MD-P column, and then scaled more than 200-fold in a Merck Superformance® pilot-scale column.

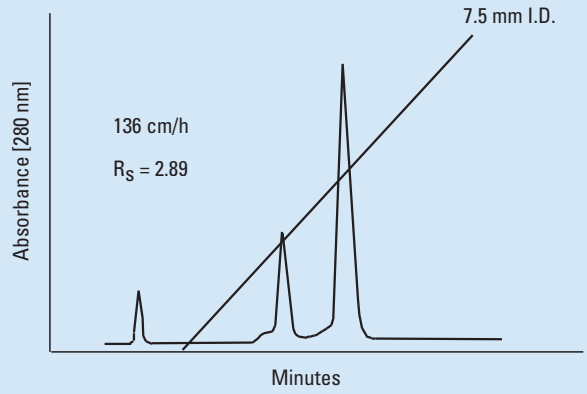
TOYOPEARL and TSK-GEL MD-G columns (10 mm I.D. x 6.8 cm L, glass) feature the small particle versions of commercial 1,000 Å pore resins. These resins exhibit the same properties as those described previously within this section.

MD-G columns are resilient to the mechanical and chemical conditions imposed by the rigors of biological feedstreams and the specific demands of the process environment. They are compatible with HPLC, FPLC®, ÄKTA™, BioCAD®, BioSYS™ and ProSYS™ systems.

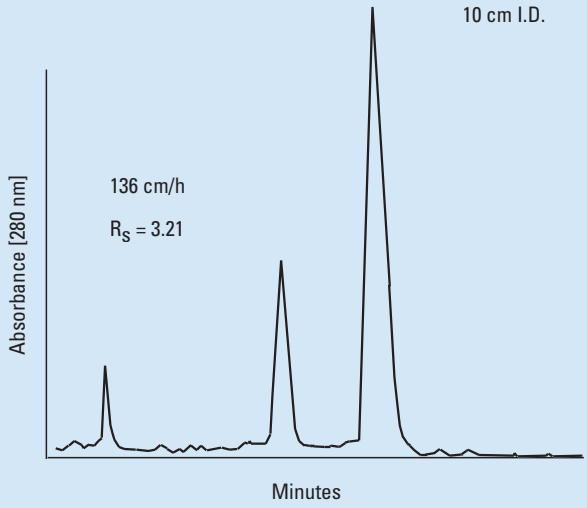
Figure 14

Comparison of MD-P separation and scale-up separation on a pilot-scale column

A. TOYOPEARL SP-650S MD-P column



B. TOYOPEARL SP-650S preparative column



Column:	A. MD-P SP-650S, 7.5 mm I.D. x 8.5 cm L B. Merck Superformance column packed with Toyopearl SP-650S (10 cm I.D. x 10 cm L)
Sample:	A. 0.5mL of 5 mg/mL each of cytochrome C and ribonuclease A B. 20mL of 25 mg/mL each of cytochrome C and ribonuclease A
Elution:	A. 0.02 M phosphate (pH 6.8) B. 0.02 M phosphate (pH 6.8 with 0.5M NaCl)
Flow rate:	136cm/h linear velocity
Gradient:	0 to 100% B in ten column volumes
Detection:	UV @ 280 nm

Note: *The MD-P column is an earlier version of the MD-G columns. The same 35 µm resin now packed in glass columns was formerly packed in 7.5 mm I.D x 8.5 cm PEEK® columns.*

Ordering Information

TSK-GEL LABPAK:

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

TOYOPEARL LABPAK:

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

TOYOPEARL MD-G series columns:

Part #	*Product description*	*Dimensions (ID x L)*	*Particle size (µm)*
22231	TOYOPEARL MD-G SuperQ-650S	10mm x 6.8cm	35
22232	TOYOPEARL MD-G DEAE-650S	10mm x 6.8cm	35
22233	TOYOPEARL MD-G SP-650S	10mm x 6.8cm	35
22234	TOYOPEARL MD-G CM-650S	10mm x 6.8cm	35

TSK-GEL MD-G series columns:

22241	TOYOPEARL MD-G Q-5PW (20)	10mm x 6.8cm	20
22242	TOYOPEARL MD-G DEAE-5PW (20)	10mm x 6.8cm	20
22243	TOYOPEARL MD-G SP-5PW (20)	10mm x 6.8cm	20

Anion exchange resins:

Part #	*Product description*	*Container size (mL)*	*Particle size (µm)*	*Ion exchange capacity (meq/mL resin)*	*Adsorption capacity (mg/mL resin)*
TSK-GEL bulk media					
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			

Anion exchange resins:

Part #	*Product description*	*Container size (mL)*	*Particle size (µm)*	*Ion exchange capacity (meq/mL resin)*	*Adsorption capacity (mg/mL resin)*
TOYOPEARL					
43271	QAE-550C	100	50-150	0.28-0.38	60-80
14026		250			
14704		1,000			
14027		5,000			
18365		50,000			
19823	SuperQ-650S	25	20-50	0.20-0.30	105-155
17223		250			
17224		1,000			
17225		5,000			
19679		50,000			
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			
18367		50,000			
07988	DEAE-650C	250	50-150	0.05-0.11	25-35
14694		1,000			
07989		5,000			

Conditions: samples for adsorption capacity are: SuperQ-650, QAE-550C, DEAE-650, SuperQ-5PW, and DEAE-PW, bovine serum albumin.

Cation exchange resins:

Part #	*Product description*	*Container size (mL)*	*Particle size (µm)*	*Ion exchange capacity (meq/mL resin)*	*Adsorption capacity (mg/mL resin)*
TSK-GEL bulk media					
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			

Cation exchange resins:

Part #	*Product description*	*Container size (mL)*	*Particle size (µm)*	*Ion exchange capacity (meq/mL resin)*	*Adsorption capacity (mg/mL resin)*
TOYOPEARL					
43272	SP-550C	100	50-150	0.14-0.18	80-120
14028		250			
14705		1,000			
14029		5,000			
18366		50,000			
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			
18369		50,000			
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			
19832		50,000			

** Adsorption capacity for insulin: 90-120 mg/mL resin*
Conditions: samples for adsorption capacity are: SP-650, SP-550C, MegaCap SP-550EC, CM-650, and SP-5PW, Lysozyme