TOYOPEARL MX-Trp-650M

The Role of Mixed-Mode Chromatography in Process Purification

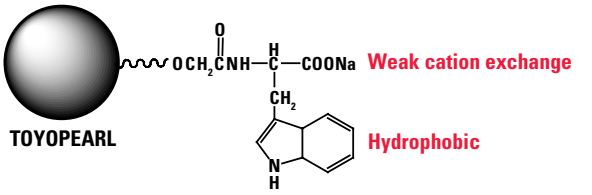
Multimodal or mixed-mode chromatography resins are based on media that have been functionalized with ligands inherently capable of several different types of interaction: ion exchange, affinity, size exclusion, and hydrophobic. The ability to merge and take advantage of these modes of protein separations can enhance overall selectivity in a purification process. This enhanced selectivity can be used to remove process impurities in a single column step that would otherwise require multiple processing steps to remove. Mixed-mode resins are in effect an amalgamation of complementary approaches to chromatographic separation on a single platform.

Unlike monomodal chromatographic methods where molecules are separated based on a single characteristic (activity, charge, hydrophobicity), with mixed-mode chromatography and mixed-mode ligands there is no known single specific interaction between the ligand and the molecule of interest. As such, screening mixed-mode resins becomes an exploration for sites on the target molecule that will deliver suitable selectivity and capacity. It is recommended that chromatographers screen for pH and conductivity as well as loading conditions when optimizing a purification process that incorporates mixed-mode resins. Protein-ligand interactions are not independent of one another on mixed-mode resins. For example, when using a mixed-mode resin having both hydrophobic interaction and ion exchange components, increasing conductivity will interrupt ionic bonds while at the same time enhancing any hydrophobic interactions. Because multiple dependent and independent variables are involved in using mixed-mode chromatography, the use of Design-of-Experiments (DoE) is recommended to characterize and optimize chromatographic conditions.

TOYOPEARL Mixed-Mode Chromatography Resin

TOYOPEARL MX-Trp-650M resin is a functionalized version of the TOYOPEARL HW size exclusion resin and is therefore based on a hydroxylated polymethacrylic polymer bead. Tosoh Bioscience offers one mixed-mode ligand, the amino acid tryptophan, which has both indole hydrophobic and weak carboxyl cationic functional groups (Figure 1).

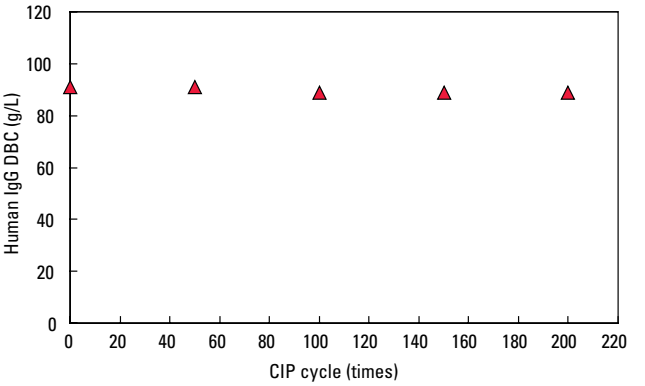
Figure 1: TOYOPEARL MX-Trp-650M structure



Product name: TOYOPEARL MX-Trp-650M
Particle size: 50-100 µm

The semi-rigid polymeric backbone of TOYOPEARL MX-Trp-650M permits high flow rates for maximum throughput and productivity. This mixed-mode resin may be operated at pressures up to 0.3 MPa and is chemically stable from pH 3-13. This allows a constant packing volume over a wide range of salt concentrations and cleaning in place (CIP) with acid or base. As shown in Figure 2, TOYOPEARL MX-Trp-650M has excellent stability to 0.5 mol/L NaOH and can be run for many CIP cycles without decreasing dynamic binding capacity (DBC).

Figure 2: Stability in 0.5 mol/L NaOH



Resin: **TOYOPEARL MX-Trp-650M**

Alkaline cleaning (CIP) conditions
- 3CV: 0.5 mol/L NaOH,
- 5CV: 0.1 mol/L Tris-HCl, pH 8.5 + 0.3 mol/L NaCl
- Flow rate: 212 cm/hr (1.0 mL/min)

DBC Measurement
- Column size: 6 mm ID × 4 cm
- Binding buffer: 0.05 mol/L acetate buffer, pH 4.7 + 0.1 mol/L NaCl
- Flow rate: 212 cm/hr (1 mL/min)
- Detection: UV @ 280 nm
- Sample: polyclonal human IgG
- Sample Load: 1 mg/mL

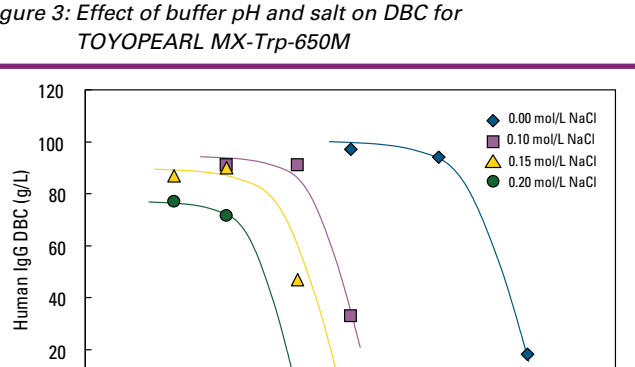
Table 1: Properties of TOYOPEARL MX-Trp-650M resin

TOYOPEARL resin	Functionality	Base bead	Pore size	Bead diameter	Ligand type	Ligand pKa ($-CO_2H$)*	DBC (g/L)	Pressure rating
MX-Trp-650M	cationic/HIC	HW-65	100 nm	75 µm	HIC/ weak cation	2.38	90 - 100	0.3 MPa

**Ligand pKa value is the pKa of the α-carboxyl group on the amino acid itself.*

TOYOPEARL MX-Trp-650M is a high capacity mixed-mode resin used for the purification of monoclonal antibodies and other proteins. The multimodal resin maintains DBC at elevated feedstock or buffer conductivities (Figure 3). Table 2 shows the DBC of TOYOPEARL MX-Trp-650M at two feedstock conductivities: 12 mS/cm and 17 mS/cm. For comparison purposes, data for an agarose based resin is also shown. For the 12 mS/cm and 17 mS/cm measurements, the TOYOPEARL MX-Trp-650M resin shows almost 7× higher and 4× higher DBC, respectively, than the agarose based resin. Superior product recovery over the agarose based resin is also demonstrated in Table 3.

Figure 3: Effect of buffer pH and salt on DBC for TOYOPEARL MX-Trp-650M



Resin: **TOYOPEARL MX-Trp-650M**
Column: 6 mm ID × 4 cm
Mobile phase: Buffer A: 0.05 mol/L acetate buffer, pH 4.0 - 6.0 + 0 - 0.2 mol/L NaCl
Buffer B: 0.1 mol/L Tris-HCl buffer, pH 8.5 + 0.3 mol/L NaCl
Flow rate: 212 cm/hr (1.0 mL/min)
Detection: UV @ 280 nm
Sample: human polyclonal IgG (1 mg/mL)

Dynamic binding capacity (DBC) calculated from 10% height of breakthrough curve

Table 2: Dynamic binding capacities at high conductivities

Resin	Particle size(µm)	Ion exchange capacity (meq)	DBC (g/L)	Recovery %
TOYOPEARL MX-Trp-650M (12 mS/cm)	50 - 100	0.12	95	97
TOYOPEARL MX-Trp-650M (17 mS/cm)	50 - 100	0.12	48	96
Brand M (Agarose 12 mS/cm)	75 (median)	0.24	14	86
Brand M (Agarose 17 mS/cm)	75 (median)	0.24	11	85

Resins: **TOYOPEARL MX-Trp-650M**
Brand M
Column size: 6 mm ID × 4 cm
Mobile phase: Buffer (12 mS/cm): 0.05 mol/L acetate buffer, pH 4.3, 4.7, 5.0 + 0.10 mol/L NaCl
Buffer (17 mS/cm): 0.05 mol/L acetate buffer, pH 4.3, 4.7, 5.0 + 0.15 mol/L NaCl
Flow rate: 212 cm/hr (1.0 mL/min)
Detection: UV @ 280 nm
Sample: human polyclonal IgG (1 mg/mL)

Dynamic binding capacity (DBC) calculated from 10% height of breakthrough curve.

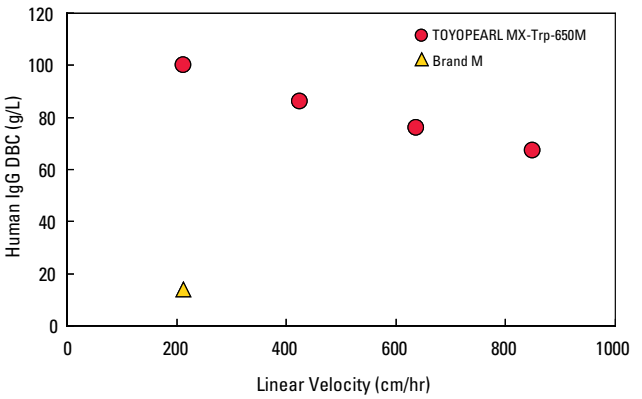
Table 3: Recovery comparison at conductivities of 12 and 17 mS/cm

Resin	IgG DBC 12 mS/cm	Recovery 12 mS/cm	IgG DBC 17 mS/cm	Recovery 17 mS/cm
TOYOPEARL MX-Trp-650M	95	97%	48	96%
Capto MMC	14	86%	11	85%

Resins: **TOYOPEARL MX-Trp-650M**
Capto MMC
Column size: 6 mm ID × 4 cm
Mobile phase: Buffer (12 mS/cm): 0.05 mol/L acetate buffer, pH 4.7 + 0.1 mol/L NaCl
Buffer (17 mS/cm): 0.05 mol/L acetate buffer, pH 4.7 + 0.15 mol/L NaCl
Flow rate: 212 cm/hr (1.0 mL/min)
Detection: UV @ 280 nm
Sample: polyclonal IgG

The mass transfer properties of a resin influence the economics of the loading and elution stages of a capture step and the degree of resolution for intermediate purification. Good mass transfer kinetics enables the resin to maintain its DBC at increased linear velocities (Figure 4). In keeping with the exceptional target binding and eluting properties of TOYOPEARL GigaCap ion exchange resins, TOYOPEARL MX-Trp-650M also shows a narrow elution peak width to complement its higher capacity (Figure 5). The mass transfer properties also contribute to minimal peak broadening. Figure 6 shows the excellent peak shape for TOYOPEARL MX-Trp-650M and the much broader tailing associated with the Brand M agarose material.

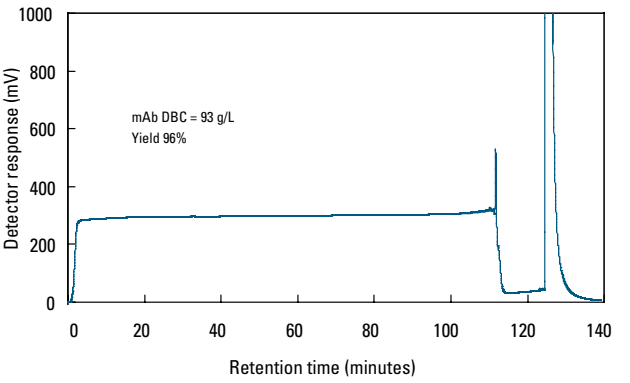
Figure 4: DBC at higher linear velocities



Resins:	**TOYOPEARL MX-Trp-650M**
	Brand M
Column size:	0.6 mm ID × 4.0 cm
Mobile phase:	Buffer A: 0.05 mol/L sodium acetate buffer, pH 4.7 + 0.1 mol/L sodium chloride
	Buffer B: 0.1 mol/L Tris-HCl buffer, pH 8.5 + 0.3 mol/L sodium chloride
Flow rates:	212, 425, 637, 849 cm/hr (1, 2, 3, 4 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Sample:	polyclonal human IgG (1 mg/mL)
Sample load:	1 mg/mL

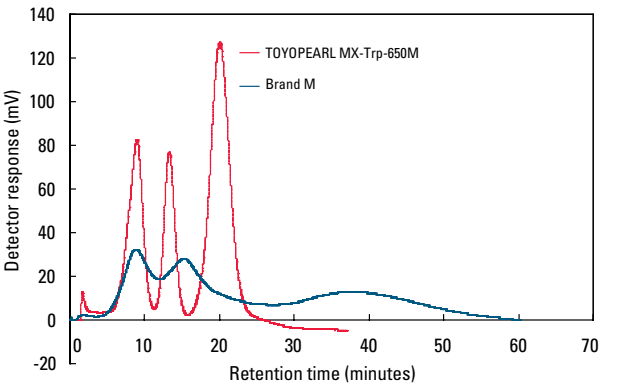
Dynamic binding capacities (DBC) were determined at 10% breakthrough

Figure 5: Narrow elution peak widths



Resin:	**TOYOPEARL MX-Trp-650M**
Column size:	6 mm ID × 4 cm
Mobile phase:	Buffer A: 0.05 mol/L acetate buffer, pH 4.7 + 0.1 mol/L NaCl (12 mS/cm)
	Buffer B: 0.1 mol/L Tris-HCl buffer, pH 8.5 + 0.3 mol/L NaCl
Flow rate:	A: 212 cm/hr (1.0 mL/min)
	B: 424 cm/hr (2.0 mL/min) started at 124 min
Detection:	UV @ 280 nm
Sample:	CHO cell culture media, monoclonal antibody (1 mg/mL) diluted with buffer A

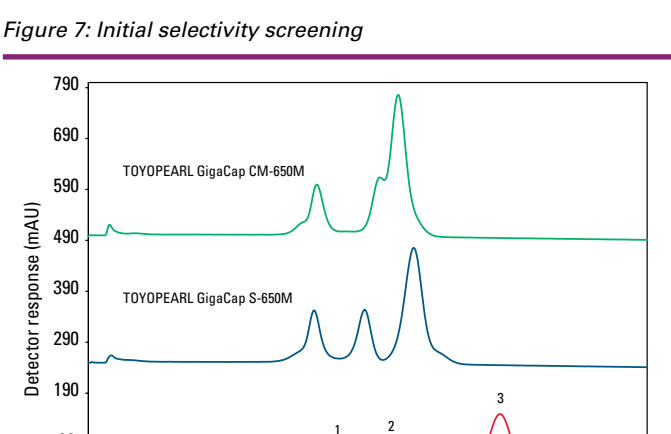
Figure 6: Good resolution for intermediate purification



Resins:	**TOYOPEARL MX-Trp-650M, Brand M**
Column size:	7.5 mm ID × 7.5 cm
Mobile phase:	Buffer A: 20 mmol/L phosphate, pH 7.0
	Buffer B: 20 mmol/L phosphate + 1.0 mol/L NaCl, pH 7.0
Gradient:	30 min. linear gradient from buffer A to buffer B
Flow rate:	136 cm/hr (1.0 mL/min)
Detection:	UV @ 280 nm
Sample:	trypsinogen (6.6 mg/mL)
	cytochrome C (3.6 mg/mL)
	lysozyme (6.6 mg/mL)
Load volume:	25 µL

Selectivity of TOYOPEARL MX-Trp-650M, when compared to a traditional weak cation exchange (TOYOPEARL GigaCap CM-650M) and a traditional strong cation exchange (TOYOPEARL GigaCap S-650M) resin, is noticeably different. A three protein mixture (trypsinogen, cytochrome C, and lysozyme) was loaded onto each resin in 20 mmol/L sodium phosphate buffer (pH 7.0) and eluted with a linear salt gradient (Figure 7). Resolution between the peaks was measured and recorded for comparison (Table 4). Further selectivity comparisons were done at decreasing pH levels for all three resins with the same protein mixture at pH 6.0 (20 mmol/L sodium acetate) and pH 5.0 (20 mmol/L sodium citrate) and were compared to the initial screening at pH 7.0 (Figures 8-10). Resolution between the peaks was likewise measured and recorded for comparison (Tables 5-7).

Figure 7: Initial selectivity screening

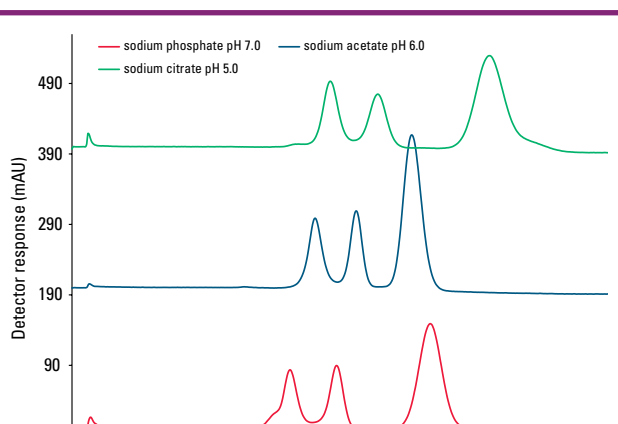


Resin:	**As Indicated**
Column size:	6.6 mm ID × 15.5 ± 1.0 cm
Mobile phase:	Buffer A: 20 mmol/L sodium phosphate, pH 7.0 Buffer B: buffer A + 1.0 mol/L NaCl
Gradient:	60 minutes 0% B - 100% B
Flow rate:	200 cm/hr (1.14 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Samples:	1. trypsinogen (6.6 mg/mL) 2. cytochrome C (3.6 mg/mL) 3. lysozyme (6.6 mg/mL)
Sample load:	5% CV (4.02 - 4.60 mg total protein)

Table 4: Initial selectivity screening peak resolutions

	Peak resolution	
Resin	**trypsinogen/ cytochrome C**	**cytochrome C/ lysozyme**
TOYOPEARL MX-Trp-650M	0.81	1.50
TOYOPEARL GigaCap S-650M	0.94	0.82
TOYOPEARL GigaCap CM-650M	1.40	0.43

Figure 8: TOYOPEARL MX-Trp-650M pH scouting

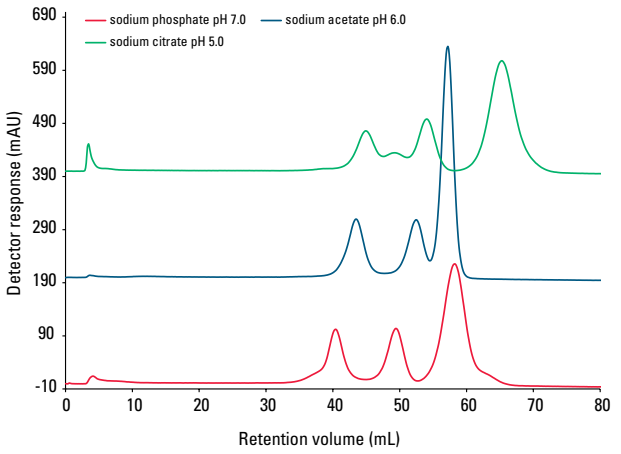


Resin:	**TOYOPEARL MX-Trp-650M**
Column size:	6.6 mm ID × 15.5 cm (5.30 mL)
Mobile phase:	Buffer A: 20 mmol/L sodium phosphate, pH 7.0 Buffer A: 20 mmol/L sodium acetate, pH 6.0 Buffer A: 20 mmol/L citrate, pH 5.0 Buffer B: buffer A + 1.0 mol/L NaCl
Gradient:	60 minutes 0% B - 100% B
Flow rate:	200 cm/hr (1.14 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Samples:	1. trypsinogen (6.6 mg/mL), 2. cytochrome C (3.6 mg/mL) 3. lysozyme (6.6 mg/mL)
Sample load:	5% CV (4.45 mg total protein)

Table 5: TOYOPEARL MX-Trp-650M pH scouting peak resolutions

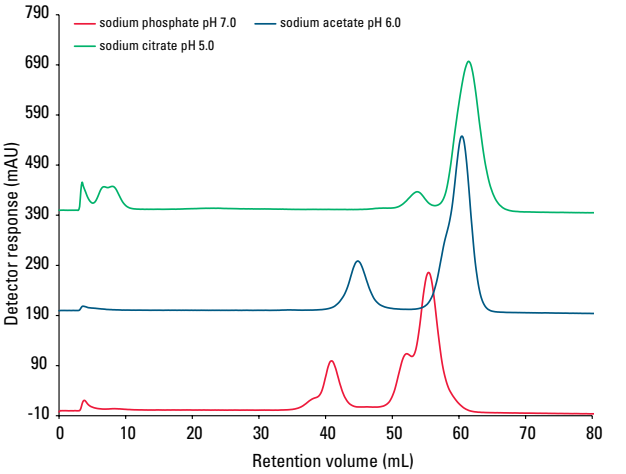
	Trypsinogen retention (mL)	Cytochrome C retention (mL)	Trypsinogen/ cytochrome C resolution (Rs)	Lysozyme retention (mL)	Cytochrome C/ lysozyme resolution (Rs)
Phosphate pH 7.0	44.88	54.36	0.81	73.63	1.50
Acetate pH 6.0	50.01	58.45	0.89	69.87	1.04
Citrate pH 5.0	53.08	62.94	1.07	85.97	1.57

Figure 9: TOYOPEARL GigaCap S-650M pH scouting



Resin:	**TOYOPEARL GigaCap S-650M**
Column size:	6.6 mm ID × 15.5 cm (5.30 mL)
Mobile phase:	Buffer A: 20 mmol/L sodium phosphate, pH 7.0 Buffer A: 20 mmol/L sodium acetate, pH 6.0 Buffer A: 20 mmol/L citrate, pH 5.0 Buffer B: buffer A + 1.0 mol/L NaCl
Gradient:	60 minutes 0% B - 100% B
Flow rate:	200 cm/hr (1.14 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Sample:	1. trypsinogen (6.6 mg/mL), 2. cytochrome C (3.6 mg/mL) 3. lysozyme (6.6 mg/mL)
Sample load:	5% CV (4.31 mg total protein)

Figure 10: TOYOPEARL GigaCap CM-650M pH scouting



Resin:	**TOYOPEARL GigaCap CM-650M**
Column size:	6.6 mm ID × 15.5 cm (5.30 mL)
Mobile phase:	Buffer A: 20 mmol/L sodium phosphate, pH 7.0 Buffer A: 20 mmol/L sodium acetate, pH 6.0 Buffer A: 20 mmol/L citrate, pH 5.0 Buffer B: buffer A + 1.0 mol/L NaCl
Gradient:	60 minutes 0% B - 100% B
Flow rate:	200 cm/hr (1.14 mL/min)
Detection:	UV @ 280 nm
Temperature:	ambient
Sample:	1. trypsinogen (6.6 mg/mL), 2. cytochrome C (3.6 mg/mL) 3. lysozyme (6.6 mg/mL)
Sample load:	5% CV (4.31 mg total protein)

Table 6: TOYOPEARL GigaCap S-650M pH scouting peak resolutions

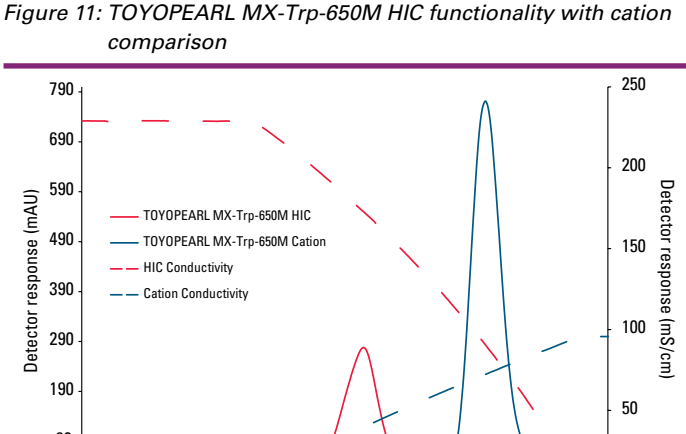
	Trypsinogen retention (mL)	Cytochrome C retention (mL)	Trypsinogen/ cytochrome C resolution (Rs)	Lysozyme retention (mL)	Cytochrome C/ lysozyme resolution (Rs)
Phosphate pH 7.0	40.38	49.46	0.94	58.27	0.82
Acetate pH 6.0	43.44	52.46	1.16	57.20	0.75
Citrate pH 5.0	44.96	54.05	1.23	65.29	1.00

Table 7: TOYOPEARL GigaCap CM-650M pH scouting peak resolutions

	Trypsinogen retention (mL)	Cytochrome C retention (mL)	Trypsinogen/ cytochrome C resolution (Rs)	Lysozyme retention (mL)	Cytochrome C/ lysozyme resolution (Rs)
Phosphate pH 7.0	40.89	52.20	1.40	55.45	0.43
Acetate pH 6.0	44.81	60.46	1.18	60.46	0
Citrate pH 5.0	53.71	61.46	0.84	61.46	0

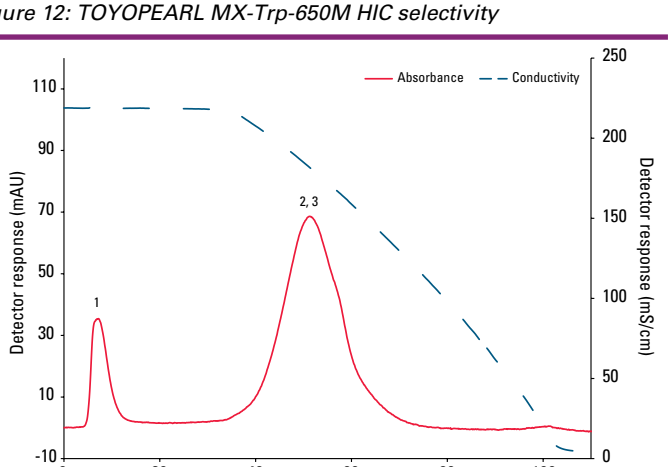
To examine the role the hydrophobic region of the tryptophan ligand can play in protein separations on TOYOPEARL MX-Trp-650M, the resin was tested to determine if it was possible to be used solely in HIC mode by loading lysozyme onto the column in 10 mmol/L sodium citrate, 1.8 mol/L ammonium sulfate, pH 5.0. The bound lysozyme was eluted with a decreasing linear gradient of 10 mmol/L sodium citrate, pH 5.0 (Figure 11). Comparison of resin selectivity in HIC mode and weak cation mode was done using a three protein mix (ribonuclease A, α α-chymotrypsinogen, and lysozyme) at pH 5.0 with sodium citrate as the mobile phase buffering salt (Figure 12 and 13). Further selectivity experiments with TOYOPEARL MX-Trp-650M can be found in *AN44: TOYOPEARL MX-Trp-650M Salt Selectivity and Tolerance.*

Figure 11: TOYOPEARL MX-Trp-650M HIC functionality with cation comparison



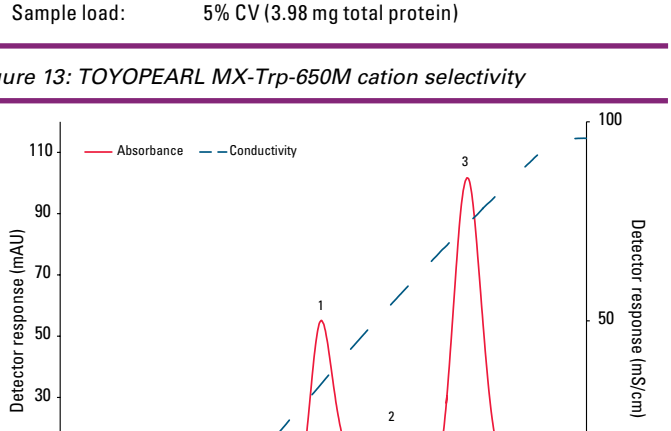
Resin:	**TOYOPEARL MX-Trp-650M**
Column size:	6.6 mm ID × 15.5 cm (5.30 mL)
Mobile phase:	Buffer A (HIC): 10 mmol/L sodium citrate, 1.8 mol/L ammonium sulfate, pH 5.0 Buffer B (HIC): 10 mmol/L sodium citrate, pH 5.0 Buffer A (cation): 20 mmol/L sodium citrate, pH 5.0 Buffer B (cation): buffer A + 1.0 mol/L NaCl
Gradient:	60 minutes 0% B - 100% B
Flow rate:	200 cm/hr (1.14 mL/min)
Detection:	UV @ 280 nm, conductivity (mS/cm)
Temperature:	ambient
Sample:	lysozyme (cation – 10 mg/mL; HIC – 4 mg/mL)
Sample load:	5% CV (1.06 and 2.65 mg total protein)

Figure 12: TOYOPEARL MX-Trp-650M HIC selectivity



Resin:	**TOYOPEARL MX-Trp-650M**
Column size:	6.6 mm ID × 15.5 cm (5.30 mL)
Mobile phase:	Buffer A (HIC): 10 mmol/L sodium citrate, 1.8 mol/L ammonium sulfate, pH 5.0 Buffer B (HIC): 10 mmol/L sodium citrate, pH 5.0
Gradient:	60 minutes 0% B - 100% B
Flow rate:	200 cm/hr (1.14 mL/min)
Detection:	UV @ 280 nm, conductivity (mS/cm)
Temperature:	ambient
Sample:	1. ribonuclease A (4.0 mg/mL), 2. α-chymotrypsinogen (5.0 mg/mL) 3. lysozyme (6.0 mg/mL)
Sample load:	5% CV (3.98 mg total protein)

Figure 13: TOYOPEARL MX-Trp-650M cation selectivity



Resin:	**TOYOPEARL MX-Trp-650M**
Column size:	6.6 mm ID × 15.5 cm (5.30 mL)
Mobile phase:	Buffer A (cation): 20 mmol/L sodium citrate, pH 5.0 Buffer B (cation): buffer A + 1.0 mol/L NaCl
Gradient:	60 minutes 0% B - 100% B
Flow rate:	200 cm/hr (1.14 mL/min)
Detection:	UV @ 280 nm, conductivity (mS/cm)
Temperature:	ambient
Sample:	1. ribonuclease A (4.0 mg/mL), 2. α-chymotrypsinogen (5.0 mg/mL) 3. lysozyme (6.0 mg/mL)
Sample load:	5% CV (3.98 mg total protein)

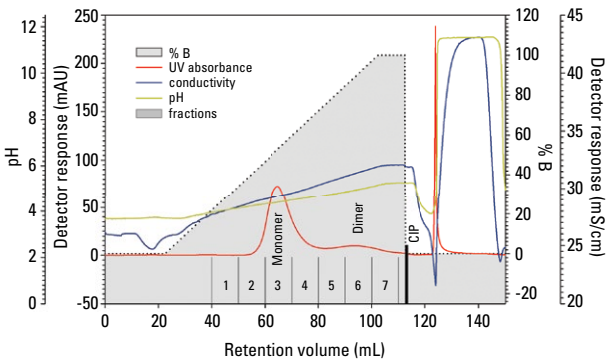
Parameters to Consider when Using TOYOPEARL MX-Trp-650M

Coordinating the hydrophobicity and charge of the therapeutic target to TOYOPEARL MX-Trp-650M is critical for the best overall purification performance. Operating at the extremes of hydrophobicity or charge for a given protein can result in drastically reduced performance of the resin or in some cases, a loss of biological activity. An optimum mixed-mode process step will balance high dynamic binding capacity, adequate selectivity, good mass recovery, and retention of biological activity. Execution of a DoE protocol during the screening process will enable developers to optimize protein separations by fine tuning mobile phase pH, conductivity and product load parameters.

Separation of Aggregates from mAbs

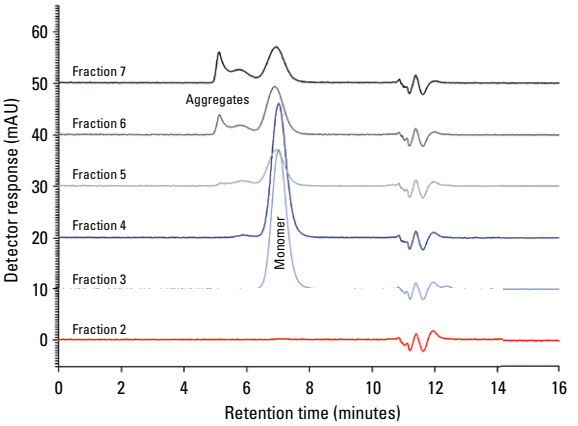
TOYOPEARL MX-Trp-650M successfully removes mAb aggregate from monomer using a narrow combination gradient of pH and conductivity (the pH and salt concentration range from pH 4.0 to 6.0 and 0.2 mol/L NaCl to 0.4 mol/L NaCl) respectively (Figure 14). The aggregate content in the monomer pool is below 1%, as shown in SEC chromatograms of the collected fractions analyzed in Figure 15. From these results it can be seen that TOYOPEARL MX-Trp-650M can be utilized as a highly efficient tool for aggregate removal of mAbs, as it offers capacities comparable to IEX, high recovery, and excellent selectivity.

Figure 14: Separation of mAb monomers and aggregates



Resin:	**TOYOPEARL MX-Trp-650M**
Column size:	6.6 mm ID × 2.0 cm
Mobile phase:	Buffer A: 0.1 mol/L acetate + 0.2 mol/L NaCl, pH 4.3 Buffer B: 0.1 mol/L acetate + 0.4 mol/L NaCl, pH 5.6
Flow rate:	150 cm/hr (0.86 mL/min)
Detection:	UV @ 280 nm, conductivity (mS/cm)
Sample:	10 mg mAb + mAb aggregates
Sample load:	1 g/L

Figure 15: SEC chromatograms of the collected fractions



Resin:	**TOYOPEARL MX-Trp-650M**
Column size:	6.6 mm ID × 2.0 cm
Mobile phase:	Buffer A: 0.1 mol/L acetate + 0.2 mol/L NaCl, pH 4.3 Buffer B: 0.1 mol/L acetate + 0.4 mol/L NaCl, pH 5.6
Flow rate:	150 cm/hr (0.86 mL/min)
Detection:	UV @ 280 nm
Sample:	10 mg mAb + mAb aggregates
Sample load:	1 g/L

A selection of screening tools are available for TOYOPEARL Mixed-Mode resin. See the Process Development Products section of this Product Guide for details.

Ordering Information

TOYOPEARL Mixed-mode resin:

Part #	Product description	Container size (mL)	Bead diameter (µm)	IgG capacity (g/L)
22817	TOYOPEARL MX-Trp-650M	25	50 - 100	90 - 100
22818	TOYOPEARL MX-Trp-650M	100	50 - 100	90 - 100
22819	TOYOPEARL MX-Trp-650M	1,000	50 - 100	90 - 100
22820	TOYOPEARL MX-Trp-650M	5,000	50 - 100	90 - 100


TOYOPEARL®
MX-Trp-650M
FOR IN VITRO USE ONLY / FOR PACKED-BED USE ONLY
Net Vol. 100 mL
Part No. 22818
Lot No.
TOSOH
Made in Japan
S1048, Rev. 0.0