TOSOH

TOYOPEARL® MX-Trp-650M

INTRODUCTION

Multimodal or mixed-mode chromatography expands the range of chromatographic modes applied in biopurification. Mixed-mode media combine ionic and hydrophobic interactions and offer new selectivities and a higher salt tolerance than traditional ion exchange media. Mixed-mode media can be used for direct processing of clarified feedstocks at physiological salt concentrations as well as for intermediate and polishing applications.

TOYOPEARL MX-Trp-650M is a multimodal cation exchange resin with unique selectivity and high recovery. It provides high protein binding capacities and tolerates high conductivity feedstocks. In addition to ionic groups its ligand also carries hydrophobic regions. Thus, the binding of target molecules is determined by electrostatic and hydrophobic contributions. TOYOPEARL MX-Trp-650M is especially suited for the purification of target molecules that are difficult to purify using common purification platforms.

RESIN STRUCTURE

TOYOPEARL MX-Trp-650M is based on the well proven methacrylic polymer backbone of TOYOPEARL media. The highly rigid base matrix provides excellent pressure/flow properties and allows high flow rates and low back pressure at large scale.

TOYOPEARL MX-Trp-650M uses tryptophan as the active ligand (Figure 1). This amino acid has both weak carboxyl cation exchange and indole hydrophobic functional groups. The selectivity of the resin can be adjusted through control of binding or elution pH, ionic strength, salt type and additives.

TOYOPEARL MX-Trp-650M STRUCTURE

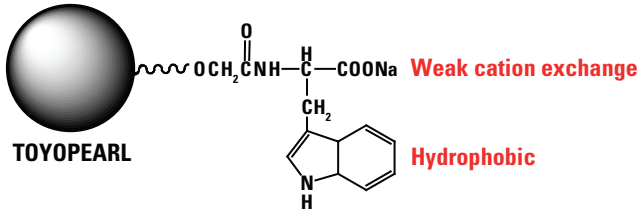

Figure 1

HIGHLIGHTS

- Multimodal cation exchange resin
- High binding capacity for IgG and other proteins
- Tolerates high conductivity feedstocks
- Sharp elution peaks with mild conditions
- Excellent pressure/flow characteristics

FEATURES

HIGH BINDING CAPACITY AT HIGH CONDUCTIVITIES
TOYOPEARL MX-Trp-650M exhibits dynamic binding capacities (DBC) for immunoglobulin G as high as 90-100 g/L at standard flow rates (Figure 2). At elevated flow rates/shorter residence times the binding capacity still remains high. Table 1 shows the DBC of the new resin at two feedstock conductivities: 12 mS/cm and 17 mS/cm. For comparison purposes, data for another agarose based multimodal cation exchanger (Brand M) is also shown. For both conductivity levels the new TOYOPEARL MX-Trp-650M resin shows much higher DBCs than the agarose based resin.

TOYOPEARL MX-Trp-650M DYNAMIC BINDING CAPACITY

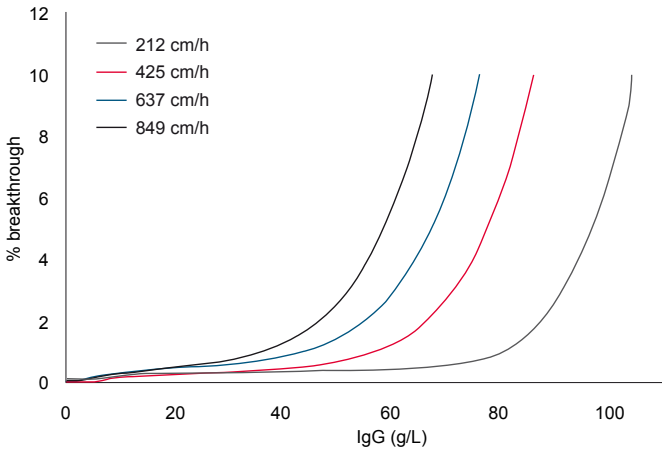

Figure 2

Column: TOYOPEARL MX-Trp-650M (6 mm ID x 4 cm); Sample: polyclonal human IgG (1 mg/mL) in 0.05 mol/L NaAc + 0.1 mol/L sodium chloride (pH 4.7); Linear velocity: 212, 425, 637, 849 cm/h; Detection: UV @ 280 nm

HIGH SALT TOLERANCE

Resin	Particle size (µm)	DBC (g/L)	Recovery %
TOYOPEARL MX-Trp-650M (12 mS/cm)	75	95	97
TOYOPEARL MX-Trp-650M (17 mS/cm)	75	48	96
Brand M Agarose (12 mS/cm)	75	14	86
Brand M Agarose (17 mS/cm)	75	11	85

Table 1

Resins: TOYOPEARL MX-Trp-650M, Brand M
Column size: 6 mm ID x 4 cm; Mobile phase: Buffer (12 mS/cm): 0.05 mol/L acetate (pH 4.3, 4.7, 5.0) + 0.10 mol/L NaCl, Buffer (17 mS/cm): 0.05 mol/L acetate (pH 4.3, 4.7, 5.0) + 0.15 mol/L NaCl; Flow rate: 1.0 mL/min (212 cm /h); Detection: UV @ 280 nm; Sample: polyclonal human IgG (1 mg/mL);
Dynamic binding capacity (DBC) calculated at 10 % breakthrough.

RIGID POLYMER MATRIX ALLOWS HIGH VELOCITIES

Applying high linear velocities can increase throughput when processing large volumes of feedstock in process scale operations. TOYOPEARL MX-Trp-650M is based on the well proven rigid polymethacrylate matrix used for all TOYOPEARL media. This matrix exhibits high mechanical stability and creates less than half the backpressure of agarose based media of the same particle size (Figure 3).

MASS TRANSFER PARAMETERS

The mass transfer properties of a resin influence the economics of loading and elution and the degree of resolution. In keeping with the exceptional target binding and elution properties of the TOYOPEARL GigaCap® resins, the new TOYOPEARL MX-Trp-650M also shows a narrow elution peak width to complement its higher capacity.

TOYOPEARL MX-Trp-650M PRESSURE / FLOW CURVE

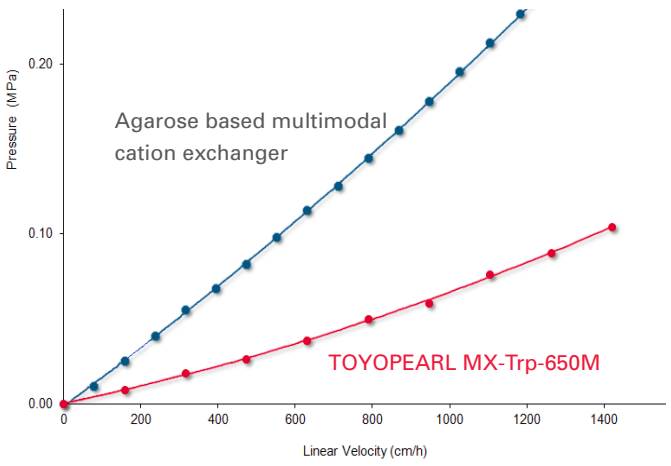

Figure 3

Column size: 22 mm ID x 20 cm; Eluent: distilled water

GOOD PEAK SHAPE AND HIGH RESOLUTION

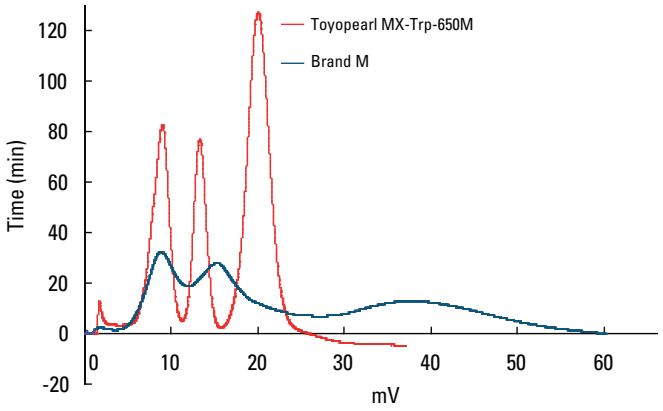

Figure 4

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: 1.0 mL/min; Detection: UV @ 280nm; Sample: trypsinogen (6.6 mg/mL) cytochrome C (3.6 mg/mL) lysozyme (6.6 mg/mL); Sample volume: 25 µL

The mass transfer properties also minimize peak broadening and contribute to the excellent peak shapes observed when comparing a separation of standard proteins on TOYOPEARL MX-Trp-650M versus the agarose based multimodal cation exchange material (Figure 4).

OPERATION

The ionic and hydrophobic properties of the multimodal ligand vary with salt concentration and pH. Thus optimization of the eluents for adsorption, wash steps and elution is crucial. To facilitate resin screening and method optimization the resin is also available in 1 mL and 5 mL ToyoScreen® columns and in 200 µL and 600 µL ToyoScreen RoboColumns® for automated processing.

INFLUENCE OF pH VALUE ON IgG DYNAMIC BINDING CAPACITY

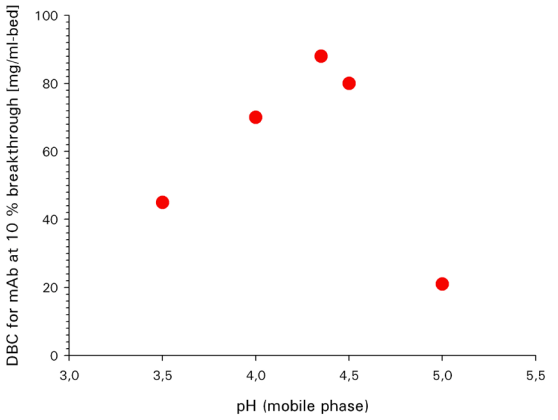

Figure 5

Column size: 6.6 mm ID x 2.2 cm; Binding buffer: 0.1 mol/L acetate buffer (pH 3.5 - 5.0) + 0.2 mol/L NaCl; Linear velocity: 150 cm/h
Detection: UV @ 280 nm; Sample: humanized monoclonal IgG
Dynamic binding capacity (DBC) calculated at 10 % breakthrough.

LOADING

The binding capacity of Toyopearl MX-Trp-650M greatly depends on pH (Figure 5). Buffer solutions with a pH approximately two pH units beneath the isoelectric point of the target molecule may serve as a first starting point for screening binding conditions. However it is not recommended to use a loading buffer pH below pH 3.0, as the capacity does not inversely correlate to pH but achieves a maximum at a specific pH, depending on the target protein. Further, very low pH values may accelerate oxidation of the resin.

Besides the pH, the applied salt concentration has a major impact on resin capacity. In a first approach, the overall salt concentrations may range from 0.1 mol/L to 0.3 mol/L. We suggest applying a concentration of 0.1 mol/L of the buffer salt with an addition of sodium chloride. However, the salt dependency of DBC is varying depending on the target molecule. For some proteins the salt dependency is less distinctive than for the mAb data presented in Figure 6.

RESOLUTION & ELUTION

The resin benefits from a selectivity, which is in many respects similar to hydrophobic interaction chromatography. Possible applications include mAb polishing, as the resin allows favorable resolution of closely related protein species, such as aggregates and monomers. This can be achieved by applying a pH gradient.

Antibodies captured by protein A affinity chromatography might be loaded at pH 4.5 and eluted with increased salt concentration and pH. Elution of various IgG monomers and their well separated aggregates was successful using an overall salt concentration of 0.5 mol/L and pH 6 (Figure 7). Monomer purities of up to 98.5 % can be achieved.

STORAGE & CLEANING

Cleaning-in-place (CIP) conditions depend on the type and composition of the feedstock. TOYOPEARL MX-Trp-650M withstands most of the standard CIP procedures.

INFLUENCE OF SALT CONCENTRATION ON IgG DBC

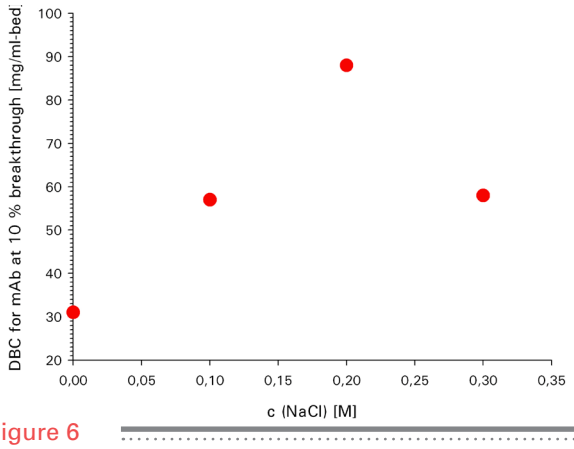

Figure 6

Column size: 6.6 mm ID x 2.2 cm;
Binding buffer: 0.1 mol/L acetate buffer (pH 4.25) + 0 - 0.30 mol/L NaCl; Linear velocity: 150 cm/h; Detection: UV @ 280 nm;
Sample: humanized monoclonal IgG
Dynamic binding capacity (DBC) calculated at 10 % breakthrough.

PURITY CHECK FOR mAb FRACTION

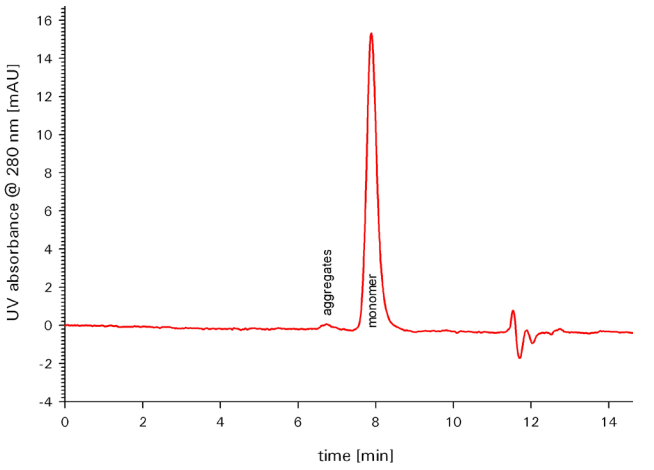

Figure 7

Column: TSKgel G3000SWXL (7.8 mm ID X 30 cm);
Mobile phase: 0.1 mol/L phosphate buffer (pH 6.7) + 0.1 mol/L Na_2SO_4; Flow rate: 1.0 mL/min; Detection: UV @ 280 nm
Sample: pooled monoclonal antibody fractions eluted from TOYOPEARL MX-Trp-650M @ 100 mM acetate buffer pH 5.5 + 0.4 mol/L NaCl; Injection Vol.: 20 µL

Figure 8 shows that the dynamic binding capacity for IgG is not changed even after 200 CIP cycles with 3 column volumes of 0.5 mol/L NaOH each.

Tryptophan the ligand of TOYOPEARL MX-Trp-650M is prone to oxidation by strong day light, UV light or oxidizing reagents. The resin is shipped in shaded containers. Avoid oxidizing reagents and exposure to UV light. It is recommended to wrap glass or plastic columns filled with the resin with UV tight material such as silver foil. Oxidation would lead to a discoloration to pale beige.

CIP STUDY WITH 0.5 M NaOH

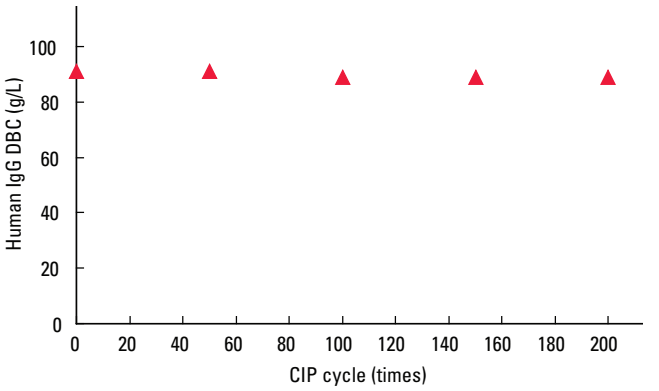

Figure 8

Alkaline cleaning conditions: 0.5 mol/L NaOH (3CV); 0.1 mol/L Tris-HCl pH 8.5 + 0.3 mol/L NaCl (5CV); Flow rate: 1 mL/min
DBC measurement: Column size: 6 mm ID x 4 cm;
Loading buffer: 50 mmol/L acetate (pH 4.7) + 0.1 mol/L NaCl;
Flow rate: 1 mL/min; Detection: UV@280 nm;
Sample: polyclonal human IgG; Sample Load: 1 mg/mL

Ordering Information

TOYOPEARL MX-Trp-650M

Part-No	Description	Resin volume	Pore size	Particle size
TOYOPEARL				
22817	MX-Trp-650M	25 mL	1000 Å	75 µm
22818	MX-Trp-650M	100 mL	1000 Å	75 µm
22819	MX-Trp-650M	1 L	1000 Å	75 µm
22820	MX-Trp-650M	5 L	1000 Å	75 µm
ToyoScreen				
22824	MX-Trp-650M	1 mL x 6	1000 Å	75 µm
22825	MX-Trp-650M	5 mL x 6	1000 Å	75 µm
45051	RoboColumn MX-Trp-650M	200 µL x 8	1000 Å	75 µm
45052	RoboColumn MX-Trp-650M	600 µL x 8	1000 Å	75 µm