A New Mixed-Mode Resin for Large Scale Biomolecule Purification

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Abstract

Chromatographic resins with high capacities and selectivities differing from those seen with traditional hydrophobic interaction and ion exchange media are now in demand. Mixed-mode chromatography media is an alternative. Some mixed-mode resins combine both traditional hydrophobic interaction and ion exchange media. This poster introduces TOYOPEARL® MX-Trp-650M, a high-capacity weak cation mixed-mode resin for the purification of biomolecules.

The polymethacrylic base bead was chemically modified with the amino acid tryptophan which combines a weak cationic group with a hydrophobic functional group. The resulting resin exhibited high dynamic binding capacities (approximately 90 mg/mL for IgG). Separation of proteins from crude feedstocks with measured conductivities of 12 mS/cm was routinely possible. Increasing the salt concentration up to 200 mmol/L NaCl at pH values as low as 4.0 also had capacities usable in large scale biomolecule purifications.

Experiments were performed to compare TOYOPEARL MX-Trp-650M selectivity with two commercially available cation exchange resins and a commercially available mixed-mode resin. The selectivity was demonstrated by the elution of three common proteins at different pH values. Overall, TOYOPEARL MX-Trp-650M selectivity was different from the cation exchange media as well as the other commercially available mixed-mode resin.

Introduction

Experiments described in this poster focus on the operational capabilities of a mixed-mode chromatography media compared to standard cation exchange chromatography media. TOYOPEARL® MX-Trp-650M mixed-mode resin was tested against TOYOPEARL GigaCap® S-650M and TOYOPEARL GigaCap CM-650M cation exchange resins for selectivity over a range of neutral and low pH values.

Selectivity for this resin was demonstrated using two mixtures of three compounds:

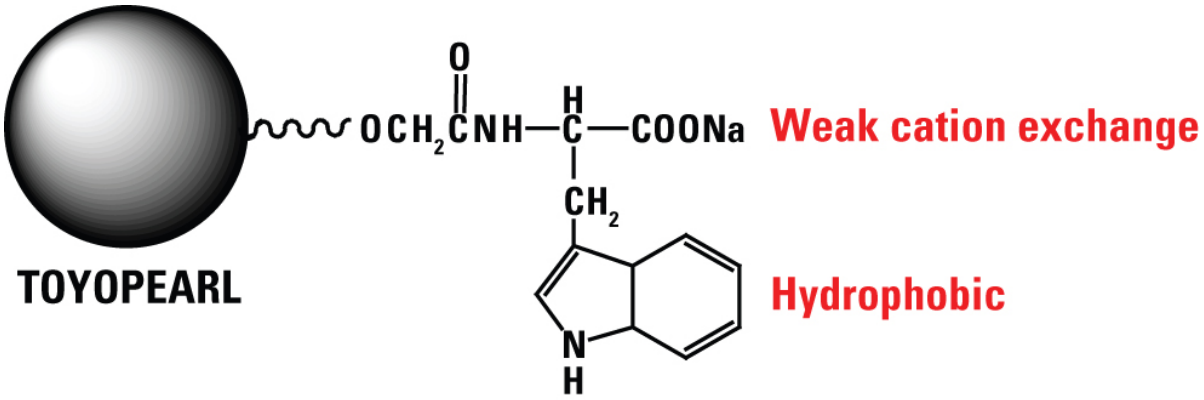
- Mixture 1 was trypsinogen (pI = 9.3), cytochrome c (pI = 10.0), and lysozyme (pI = 10.7).
- Mixture 2 was α-chymotrypsinogen (pI = 9.5), ribonuclease A (pI = 9.6), and lysozyme (pI = 10.7).

Using pH scouting at low protein loading conditions, the effect of pH on resolution between the proteins was observed.

The effect of multiple buffering salts at a single pH was also evaluated to determine how pronounced selectivity differences were.

Experiments were also performed using the TOYOPEARL MX-Trp-650M resin in both ion exchange and HIC modes and a selectivity comparison between the two modes was made.

TOYOPEARL MX-Trp-650M



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

Experimental Information

For all scouting experiments, TOYOPEARL mixed-mode and ion exchange resins (Tosoh Bioscience LLC) were packed in 6.6 mm ID x 15 ± 1.0 cm columns as described in the “Instruction Manual for TOYOPEARL and TSKgel® PW type Resins”. GE Healthcare Capto® MMC resin was packed similarly. The columns were performance tested and found to be acceptable for use in these experiments.

Lysozyme, trypsinogen, α-chymotrypsinogen, ribonuclease A and cytochrome c were purchased from Sigma-Aldrich Chemicals.

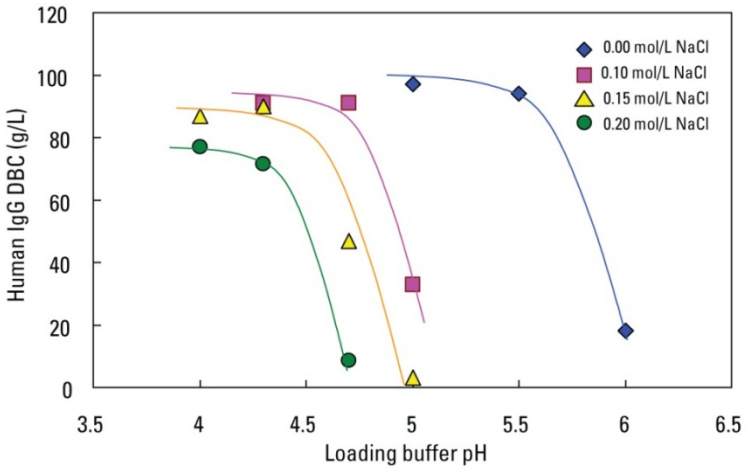
Separation and scouting conditions are listed with their respective chromatograms.

All experiments were carried out using an AKTA® Explorer 100 at ambient temperature.

Table 1: Properties of the Resins used in this Study

	TOYOPEARL MX-Trp-650M	TOYOPEARL GigaCap S-650M	TOYOPEARL GigaCap CM-650M
Particle size (µm)	75	75	75
Pore size (Å)	1000	1000	1000
Binding capacity (mg/mL resin)	> 75 static (γ-globulin)	80-120 dynamic (BSA)	> 110 static (γ-globulin) > 100 dynamic (γ-globulin)

Figure 1: 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:	1.0 mL/min (212 cm/hr)
Detection:	UV @ 280 nm
Sample:	human polyclonal IgG (1 mg/mL)

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

TOYOPEARL MX-Trp-650M was evaluated for dynamic binding capacities at different pH and salt concentrations. Even at 0.2 mol/L NaCl the resin exhibited a capacity of almost 80 g/L at pH 4.0.

Figure 2a: Effect of pH on TOYOPEARL MX-Trp-650M Selectivity

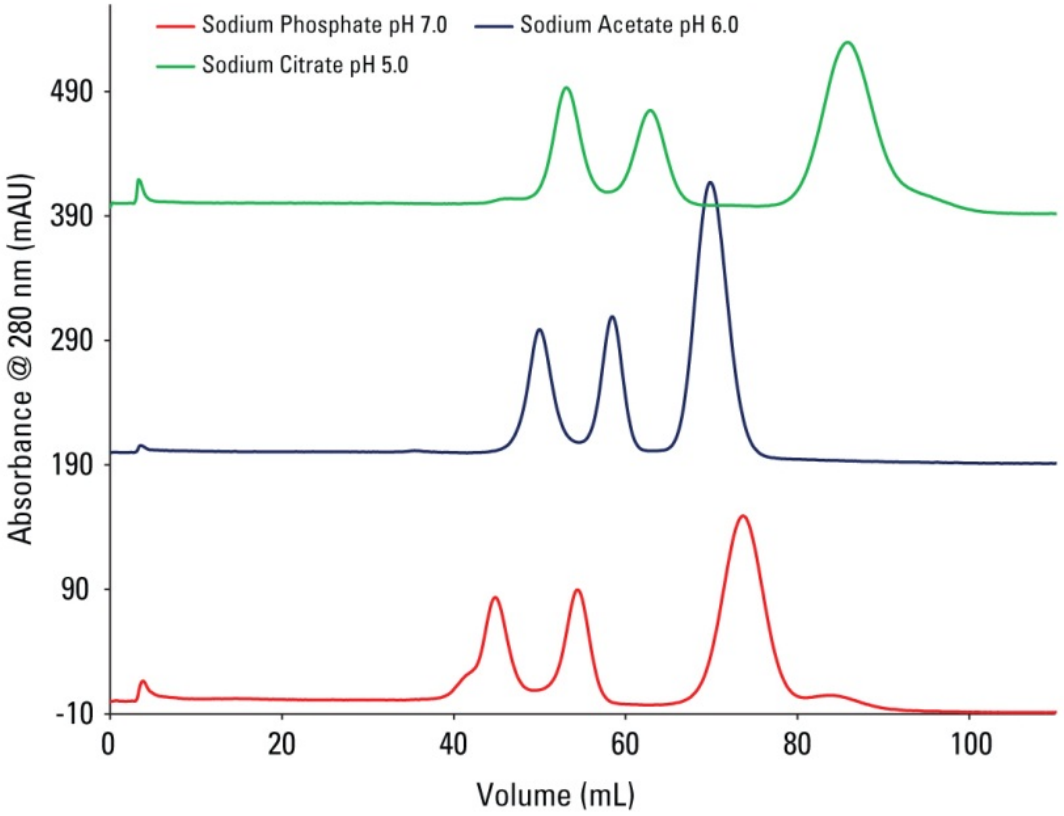


Figure 2b: Effect of pH on Capto MMC Selectivity

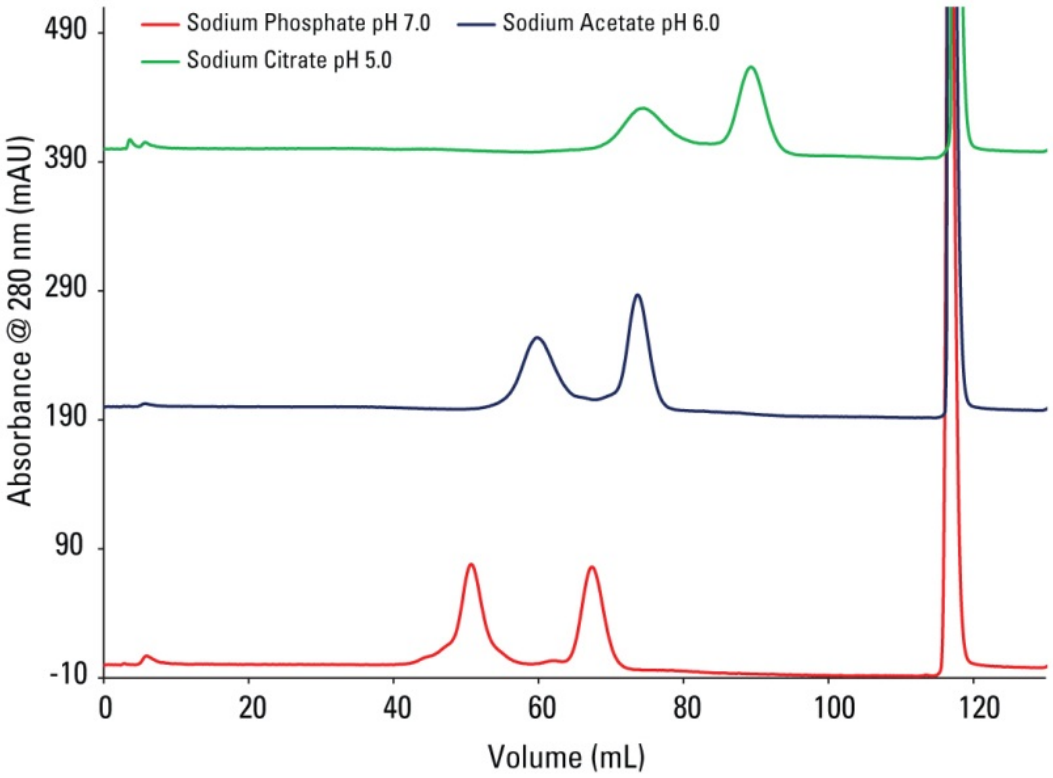


Figure 2c: Effect of pH on TOYOPEARL GigaCap S-650M Selectivity

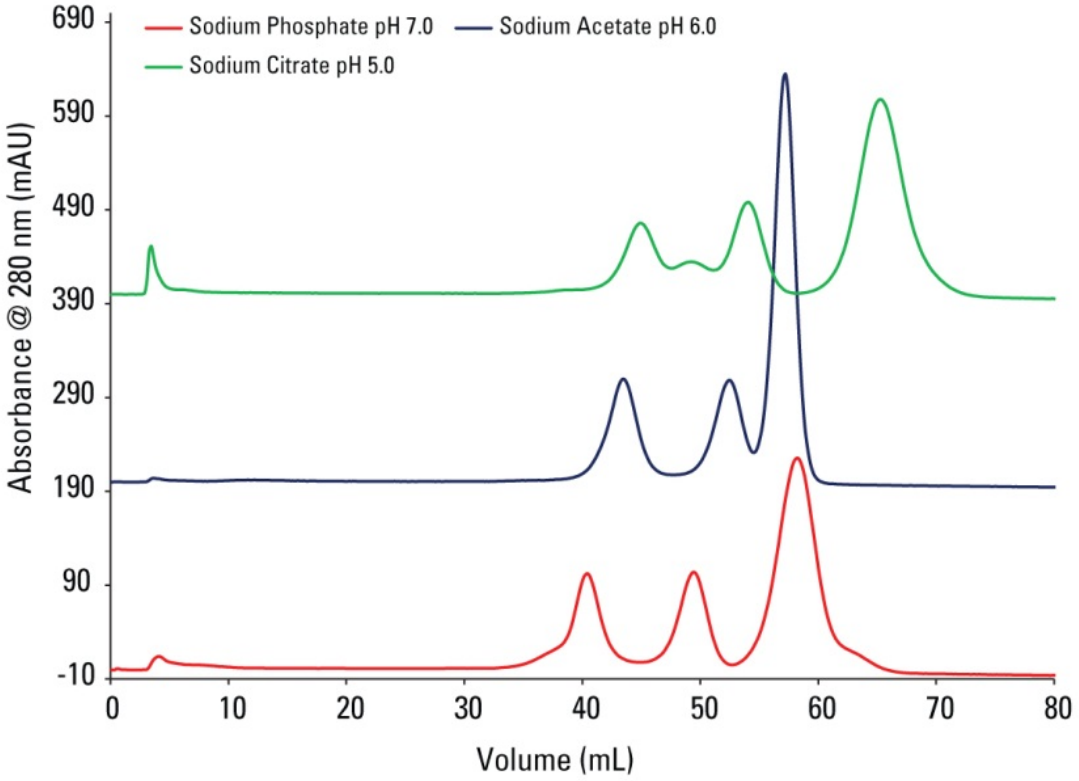
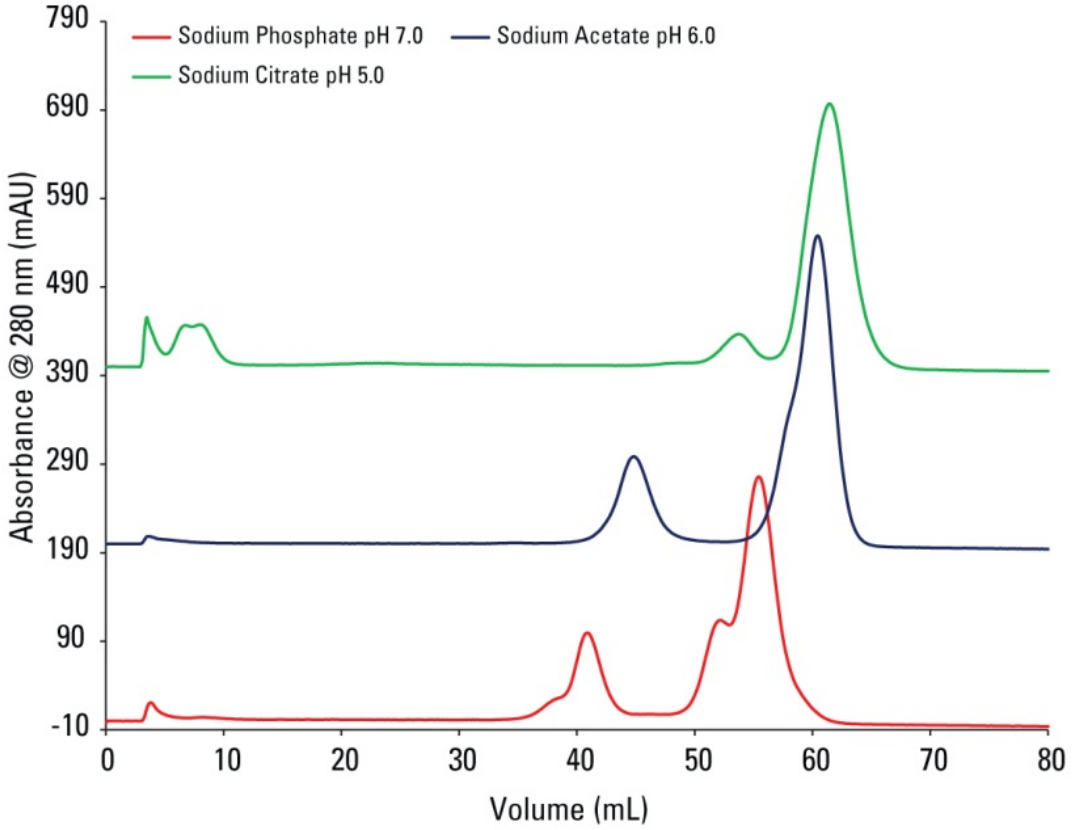


Figure 2d: Effect of pH on TOYOPEARL GigaCap CM-650M Selectivity



Experimental Conditions for Chromatograms in Figure 2

Resin:	As Indicated	Gradient:	60 minutes 0% B – 100% B
Column Size:	6.6 mm ID × 15.0 ± 1.0 cm	Flow Rate:	1.14 mL/min (200 cm/hr)
Buffer A (pH 7.0):	20 mmol/L sodium phosphate, pH 7.0	Detection:	UV @ 280 nm
Buffer B (pH 7.0):	Buffer A + 1.0 mol/L NaCl	Temperature:	ambient
Buffer A (pH 6.0):	20 mmol/L sodium acetate, pH 6.0	Sample:	1. trypsinogen (6.6 mg/mL),
Buffer B (pH 6.0):	Buffer A + 1.0 mol/L NaCl		2. cytochrome c (3.6 mg/mL)
Buffer A (pH 5.0):	20 mmol/L citrate, pH 5.0		3. lysozyme (6.6 mg/mL)
Buffer B (pH 5.0):	Buffer A + 1.0 mol/L NaCl	Sample Loaded:	5% CV (4.02 – 4.60 mg total protein)

The order of elution in each of the chromatograms is as follows: trypsinogen, cytochrome c and lysozyme. The mixed-mode resins and the GigaCap S-650M showed good selectivity between all three proteins. As the pH decreased, the resolution between the proteins increased slightly for the two mixed-mode resins and for the TOYOPEARL GigaCap S-650M (with the exception of the pH 6 acetate, see additional experiments in this poster). For the second cation exchange resin, TOYOPEARL GigaCap CM-650M, the resolution decreased. Interestingly, lysozyme did not elute from the Capto MMC resin until the resin was cleaned with NaOH. The elution volume of each peak also increased through peak broadening as the pH was lowered. The study also included runs performed at pH 4.0, and 3.5 where this effect was more pronounced (data not shown).

Figure 3a: Effect of Buffering Salt on Selectivity at pH 6.0

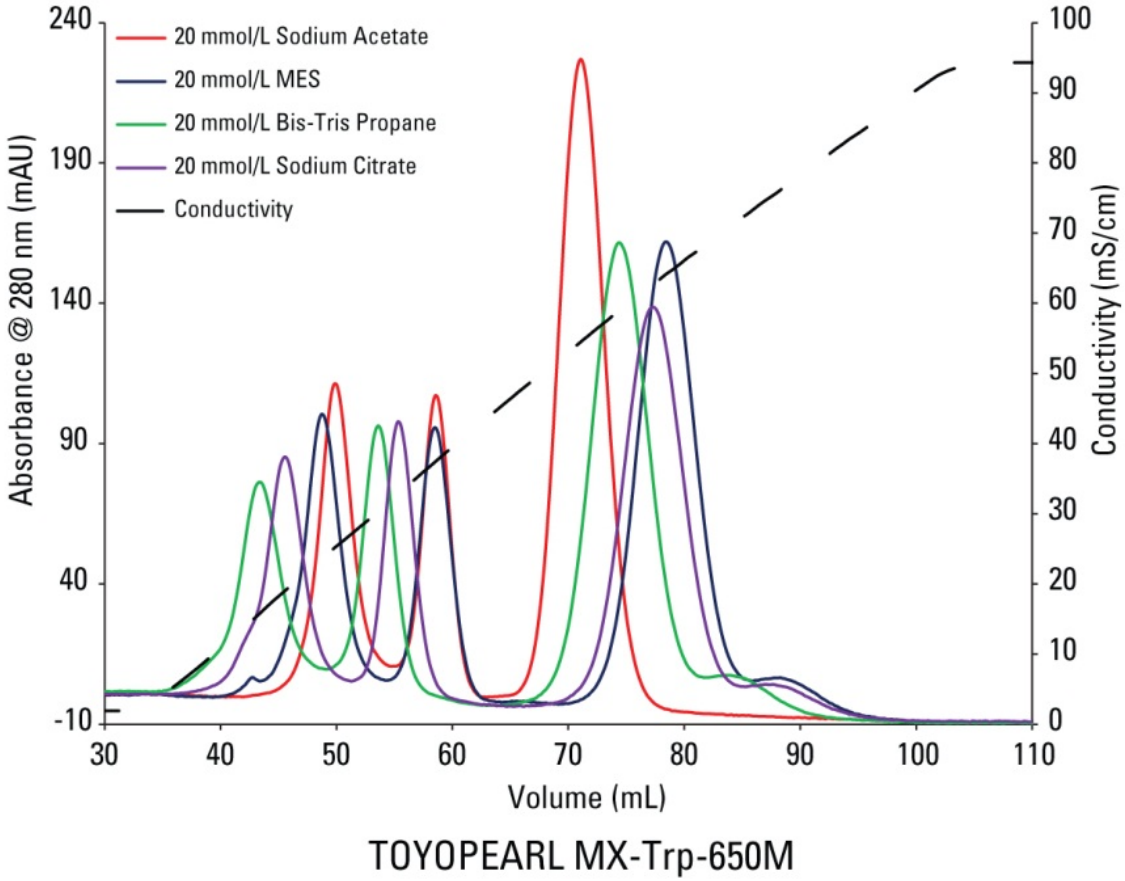
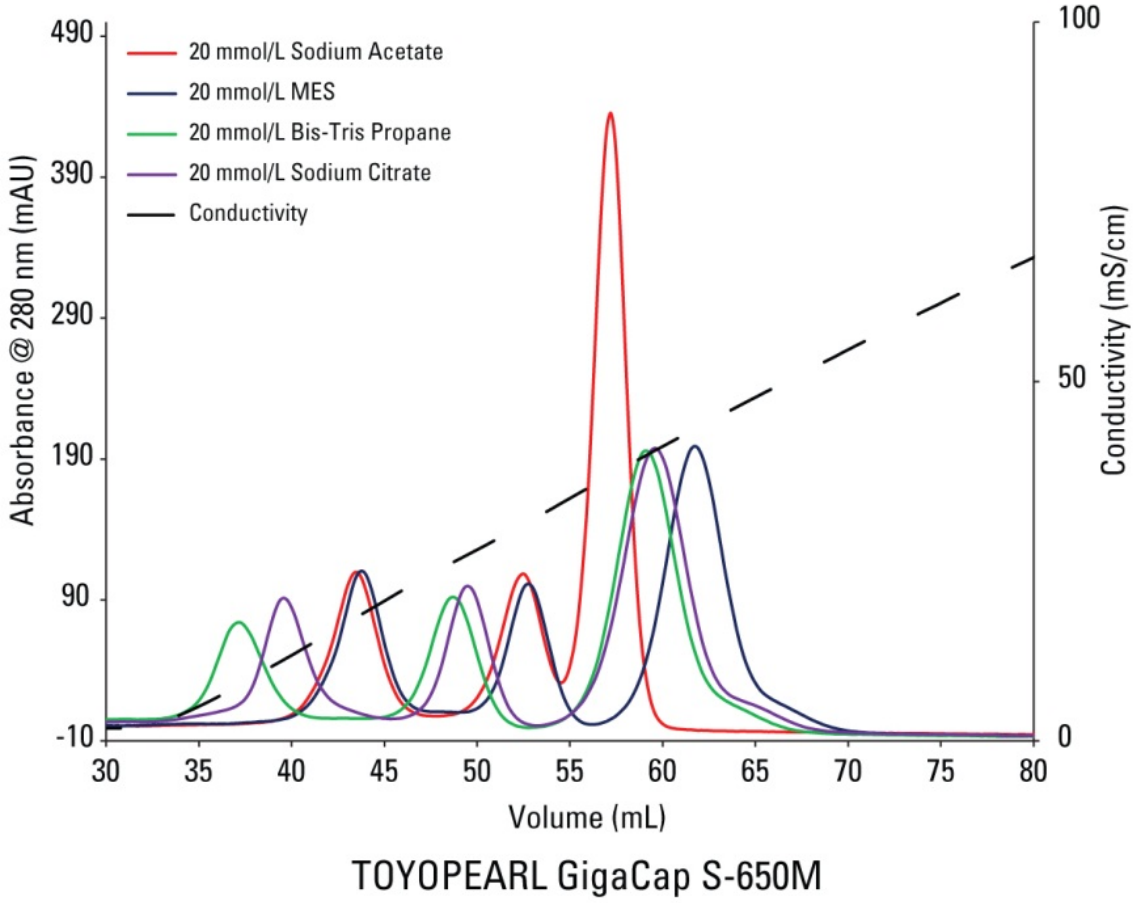


Figure 3b: Effect of Buffer on Selectivity at pH 6.0



Experimental Conditions for Chromatograms in Figure 3

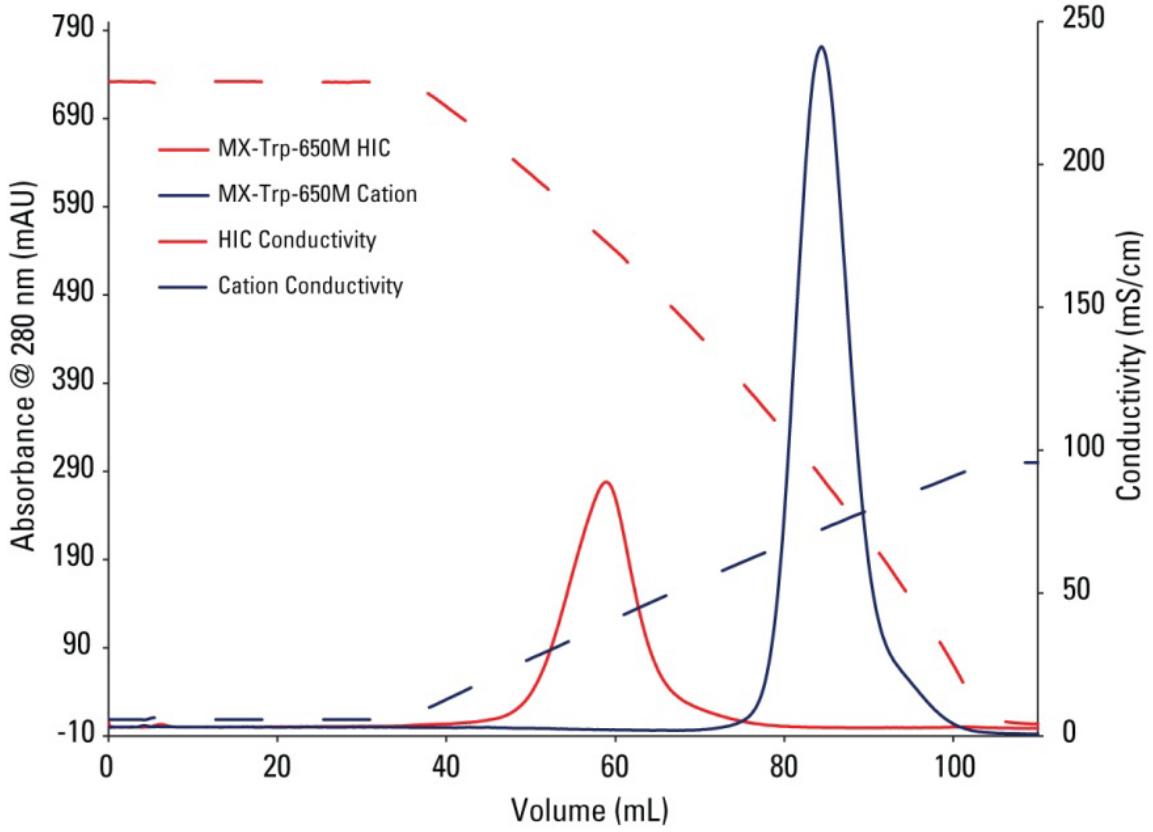
Resin:	As Indicated
Column Size:	6.6 mm ID × 15.0 ± 1.0 cm
Buffer A (Acetate):	20 mmol/L sodium acetate, pH 6.0
Buffer B (Acetate):	Buffer A + 1.0 mol/L NaCl
Buffer A (MES):	20 mmol/L MES, pH 6.0
Buffer B (MES):	Buffer A + 1.0 mol/L NaCl
Buffer A (BTP):	20 mmol/L Bis-Tris Propane, pH 6.0
Buffer B (BTP):	Buffer A + 1.0 mol/L NaCl
Buffer A (Citrate):	20 mmol/L sodium citrate, pH 6.0
Buffer B (Citrate):	Buffer A + 1.0 mol/L NaCl
Gradient:	60 minutes 0% B – 100% B
Flow Rate:	1.14 mL/min (200 cm/hr)
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 Loaded:	5% CV – (4.02 to 4.60 mg total protein)

Figure 3: Summary of Conductivities at Peak Maximum

	TOYOPEARL MX-Trp-650M			TOYOPEARL GigaCap S-650M		
	trypsinogen (mS/cm)	cytochrome C (mS/cm)	lysozyme (mS/cm)	trypsinogen (mS/cm)	cytochrome C (mS/cm)	lysozyme (mS/cm)
20 mmol/L sodium acetate	25.23	37.50	54.51	17.17	30.04	36.92
20 mmol/L MES	22.74	36.40	62.84	17.04	30.10	43.06
20 mmol/L Bis-Tris propane	16.38	30.85	59.08	8.00	25.46	40.51
20 mmol/L sodium citrate	21.48	34.86	63.84	13.77	28.00	41.97

The choice of buffering salt in the mobile phase has a more pronounced effect on selectivity for the mixed-mode resin than it does for the cation exchange resin.

Figure 4: Cation Exchange and HIC Comparison for TOYOPEARL MX-Trp-650M



Experimental Conditions for Chromatograms in Figure 4

Resin:	TOYOPEARL MX-Trp-650M
Column Size:	6.6 mm ID × 15.5 cm (5.30 mL)
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:	1.14 mL/min (200 cm/hr)
Detection:	UV @ 280 nm
Temperature:	ambient
Sample:	lysozyme (Cation – 10 mg/mL; HIC – 4 mg/mL)
Sample Loaded:	5% CV (1.06 and 2.65 mg total protein)

TOYOPEARL MX-Trp-650M is capable of operating as either a salt tolerant cation exchanger or as a more traditional hydrophobic interaction chromatography resin. Lysozyme will bind at low salt concentration and desorb as the conductivity is increased. It will also bind at high salt concentrations and desorb as the conductivity is decreased. These combined characteristics allow this resin to truly function as a mixed- and dual-mode media. Based on these results, it appears that there is a range of conductivities where the protein will not bind in either ion exchange or HIC modes.

Figure 5a: Cation Exchange Mode Separation for TOYOPEARL MX-Trp-650M

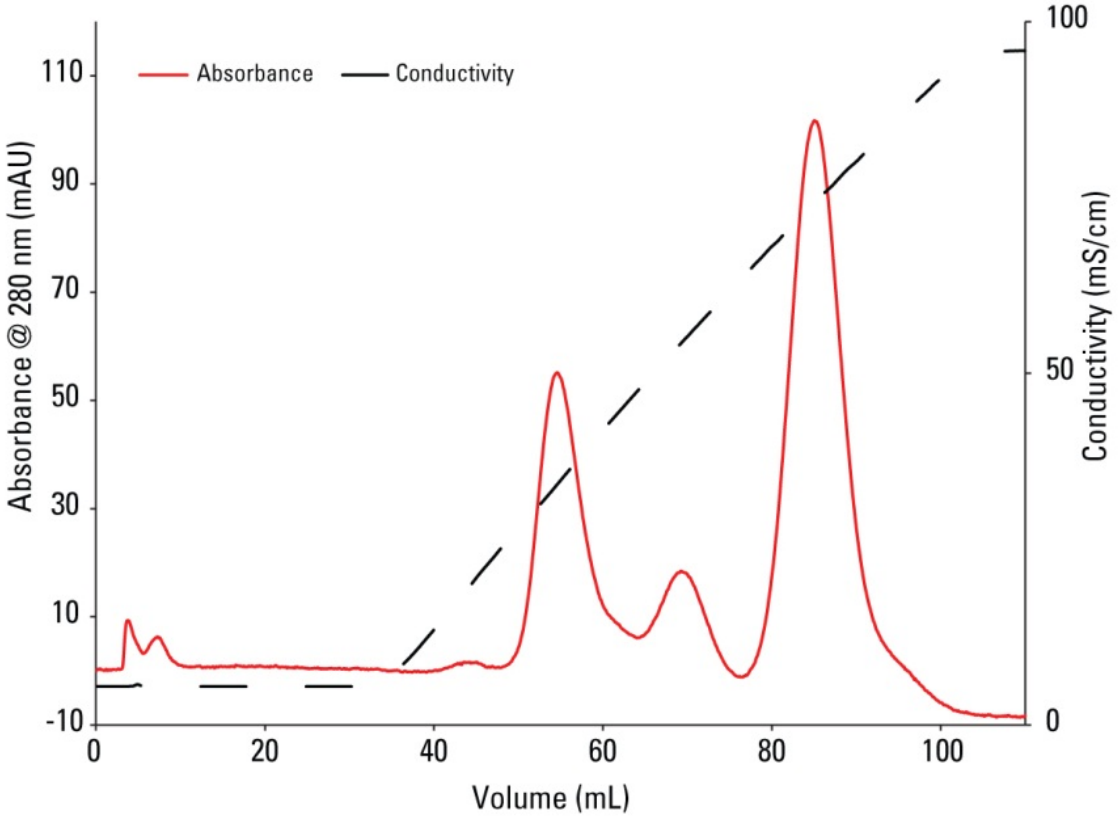
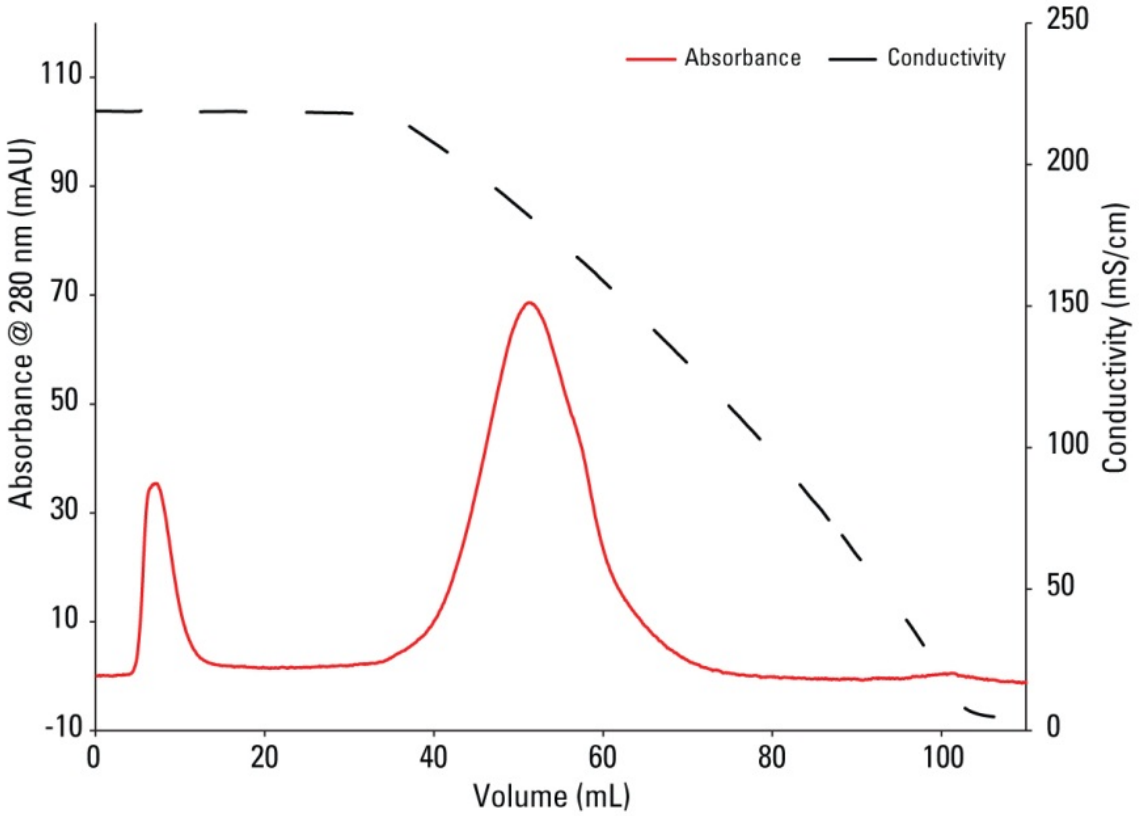


Figure 5b: HIC Mode Separation for TOYOPEARL MX-Trp-650M



Experimental Conditions for Chromatograms in Figure 5

Resin:	TOYOPEARL MX-Trp-650M
Column Size:	6.6 mm ID × 15.5 cm (5.30 mL)
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:	1.14 mL/min (200 cm/hr)
Detection:	UV @ 280 nm
Temperature:	ambient
Sample:	1. ribonuclease A (4.0 mg/mL), 2. α-chymotrypsinogen (5.0 mg/mL) 3. lysozyme (6.0 mg/mL)
Sample Loaded:	5% CV (3.98 mg total protein)

TOYOPEARL MX-Trp-650M exhibits different selectivity depending on the mode being used. When run as a cation exchanger, the resin is able to resolve all three proteins into individual peaks. When used in HIC mode, the resin is only able to resolve one of the proteins from the other two in the mixture under the conditions tested.

Conclusions:

- TOYOPEARL MX-Trp-650M is a new mixed-mode chromatography resin that can separate biomolecules under a variety of experimental conditions.
- TOYOPEARL MX-Trp-650M can bind approximately 80 g/L of human IgG in 200 mol/L NaCl at pH 4.0.
- Selectivity differences were more pronounced in the mixed-mode resin when compared to traditional ion exchange resins offering chromatographers more opportunities to optimize their separations.
- TOYOPEARL MX-Trp-650M was able to separate proteins using both traditional ion exchange and hydrophobic interaction, demonstrating the versatility of the resin. This dual-mode separation created a conductivity range where the protein did not bind. Further exploration of this range for other proteins at other pH values is underway.