



TOYOPEARL® MX-Trp-650M SALT SELECTIVITY AND TOLERANCE

TOYOPEARL MX-Trp-650M, a new, mixed-mode chromatography resin combining a weak cation exchange and a hydrophobic interaction ligand for process scale chromatography applications is the latest addition to the TOYOPEARL product line. This resin, capable of being run much the same as a standard cation exchanger or in a more traditional HIC mode, truly lives up to the mixed-mode moniker.

INTRODUCTION

Chromatographic resins with high capacities, selectivities, and salt tolerances differing from those seen with traditional ion exchange media are now in demand. Mixed-mode chromatography media offers an alternative to traditional single-mode media. The polymethacrylic base bead (TOYOPEARL HW-65) is chemically modified with the amino acid tryptophan, which combines a weak cationic group with a hydrophobic functional group. The resulting resin exhibits dynamic binding capacities of approximately 90 mg/mL for human IgG. TOYOPEARL MX-Trp-650M offers chromatographers selectivity and salt tolerance combined with binding capacities that are similar to traditional cation exchange resins.

EXPERIMENTAL CONDITIONS

For selectivity and salt tolerance comparisons of TOYOPEARL MX-Trp-650M and a traditional strong cation exchange (TOYOPEARL GigaCap® S-650M) resin, various buffering salts at a set pH value were used. For the selectivity comparisons between different buffering salts at a single pH value, pH 6.0 was selected. Since there are multiple buffering salts with an effective range that includes this pH point; a total of four buffering salts

TOYOPEARL MX-Trp-650M MULTIPLE BUFFERING SALTS AT PH 6.0

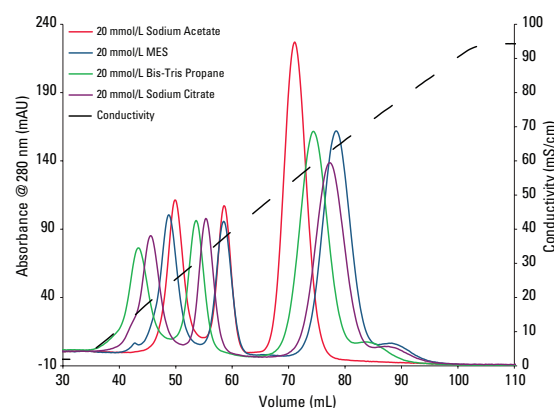


Figure 1

Resin: TOYOPEARL MX-Trp-650M
 Column size: 6.6 mm ID × 15.5 cm (5.30 mL)
 Buffer A (1): 20 mmol/L sodium acetate, Buffer A (2): 20 mmol/L MES, Buffer A (3): 20 mmol/L Bis-Tris Propane, Buffer A (4): 20 mmol/L sodium citrate, Buffer B: 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 Load: 5% CV (4.45 mg total protein)

were selected for these experiments: sodium acetate, MES, Bis-Tris Propane and sodium citrate. 6.6 mm ID × 15.5 ± 1.0 cm columns were packed with new resin. A three protein mixture (trypsinogen, cytochrome C, and lysozyme) was loaded onto the column and eluted with a linear salt gradient (Figures 1-2). Resolution between the peaks was measured and recorded for comparison (Tables 1-2).

TOYOPEARL MX-Trp-650M pH 6.0 MULTI BUFFER RETENTION AND RESOLUTION

	Trypsinogen		Cytochrome C		Trypsinogen/ Cytochrome C Resolution (Rs)	Lysozyme		
	Retention (mL)	Cond. (mS/cm)	Retention (mL)	Cond. (mS/cm)		Retention (mL)	Cond. (mS/cm)	Cytochrome C/ Lysozyme Resolution (Rs)
Sodium Acetate	50.01	25.37	58.45	37.40	0.89	69.87	52.98	1.04
MES	48.77	22.74	58.46	36.40	0.98	78.43	62.84	1.58
Bis-Tris Propane	43.43	16.38	53.64	30.58	0.78	74.36	59.08	1.41
Sodium Citrate	45.60	21.48	55.36	34.86	0.76	77.26	63.84	1.59

Table 1

TOYOPEARL GigaCap S-650M MULTIPLE BUFFERING SALTS AT pH 6.0

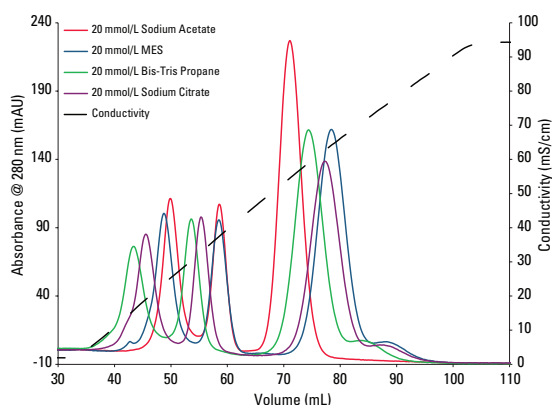


Figure 2

Resin: TOYOPEARL GigaCap S-650M
 Column size: 6.6 mm ID x 15.5 cm (5.30 mL)
 Buffer A (1): 20 mmol/L sodium acetate; Buffer A (2): 20 mmol/L MES;
 Buffer A (3): 20 mmol/L Bis-Tris Propane; Buffer A (4): 20 mmol/L sodium citrate; Buffer B: 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 Load: 5% CV (4.31 mg total protein)

The relative salt tolerance of the two resins tested in these experiments can be determined in part by peak conductivity for each of the proteins. Comparison of the conductivity at peak maximum (Table 3) as a function of the salt concentration required to desorb the proteins is indicative of the relative salt tolerance of the resins.

RESULTS

The order of elution for each of the chromatograms is as follows: trypsinogen, cytochrome C, and lysozyme. While the order of elution remained unchanged for all buffering salts used with TOYOPEARL MX-Trp-650M and TOYOPEARL GigaCap S-650M (Figure 1-2), the choice of buffer did have an effect on the resolution and the amount of NaCl needed to desorb each protein from the resin. The lysozyme was the most affected of the three proteins by the change in buffering salt (Table 1-2). Comparison of peak conductivities indicate that the TOYOPEARL MX-Trp-650M is more salt tolerant than TOYOPEARL GigaCap S-650M for all proteins with all of the buffers tested at pH 6.0 (Table 3).

TOYOPEARL GigaCap S-650M pH 6.0 MULTI BUFFER RETENTION AND RESOLUTION

	Trypsinogen		Cytochrome C			Lysozyme		
	Retention (mL)	Cond. (mS/cm)	Retention (mL)	Cond. (mS/cm)	Trypsinogen/Cytochrome C Resolution (Rs)	Retention (mL)	Cond. (mS/cm)	Cytochrome C/Lysozyme Resolution (Rs)
Sodium Acetate	43.44	17.17	52.46	30.04	1.16	57.20	36.92	0.75
MES	43.76	17.04	52.78	30.10	1.01	61.77	43.06	0.88
Bis-Tris Propane	37.16	8.00	48.70	25.46	1.31	59.09	40.51	0.95
Sodium Citrate	39.56	13.77	49.52	27.99	0.98	59.61	41.97	0.90

Table 2

TOYOPEARL MX-Trp-650M AND TOYOPEARL GigaCap S-650M SALT TOLERANCE

	TOYOPEARL MX-Trp-650M		
	Trypsinogen Peak Conductivity (mS/cm)	Cytochrome C Peak Conductivity (mS/cm)	Lysozyme Peak Conductivity (mS/cm)
Sodium Acetate	25.37	37.40	52.98
MES	22.74	36.40	62.84
Bis-Tris Propane	16.38	30.58	59.08
Sodium Citrate	21.48	34.86	63.84

	TOYOPEARL GigaCap S-650M		
	Trypsinogen Peak Conductivity (mS/cm)	Cytochrome C Peak Conductivity (mS/cm)	Lysozyme Peak Conductivity (mS/cm)
Sodium Acetate	17.17	30.04	36.92
MES	17.04	30.10	43.06
Bis-Tris Propane	8.00	25.46	40.51
Sodium Citrate	13.77	27.99	41.97

Table 3

CONCLUSIONS

TOYOPEARL MX-Trp-650M was able to separate all three test proteins in all four buffers tested; resin performance in different buffers did vary (Figure 1). MES, pH 6.0, produced the best results for the MX-Trp-650M, with the poorest results being the sodium acetate with respect to overall retentive properties and resolution (Table 1). TOYOPEARL GigaCap S-650M was also able to separate all three test proteins in the four buffers tested. Like the TOYOPEARL MX-Trp-650M, its performance in different buffers varied as well (Figure 2). Bis-Tris Propane, pH 6.0, produced the best results for the GigaCap S-650M with the poorest results being the sodium citrate with respect to overall retentive properties and resolution (Table 2). These results indicate that the TOYOPEARL MX-Trp-650M and TOYOPEARL GigaCap S-650M selectivities can vary depending on the buffer being used.

TOYOPEARL MX-Trp-650M is more salt tolerant than the traditional cation exchange resin tested in this experiment. For all four buffers tested at pH 6.0, TOYOPEARL MX-Trp-650M required higher concentrations of salt to desorb the trypsinogen, cytochrome C, and lysozyme than TOYOPEARL GigaCap S-650M (Table 3).