The Characteristics of Novel Semi-Preparative Ion-Exchange Columns

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Abstract

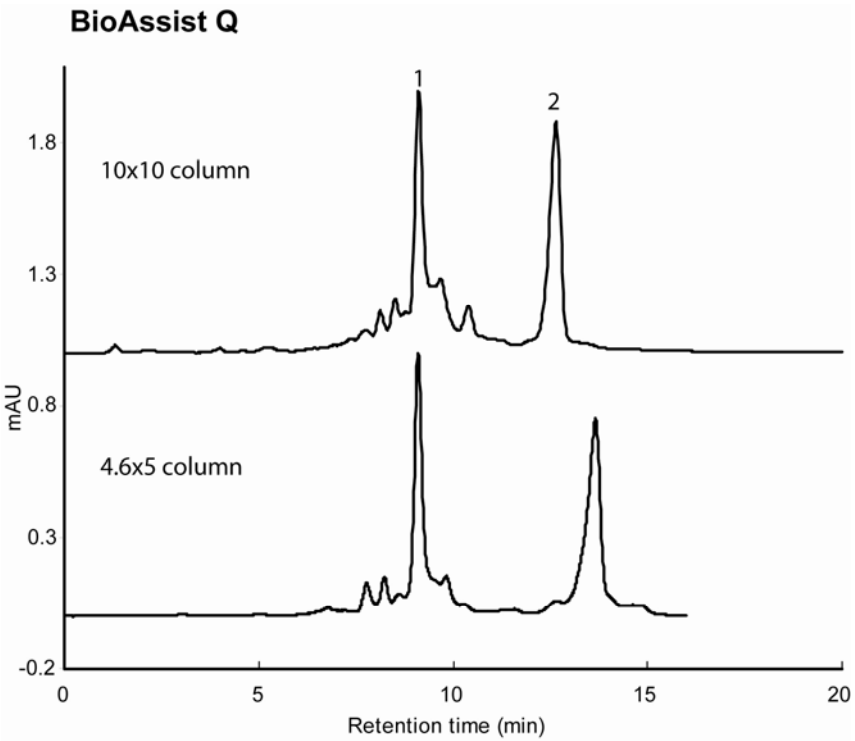
The analysis of monoclonal antibodies (mAb) has become more important for the treatment and diagnosis of disease. Due to the large size of such molecules, Tosoh has developed megaporous stationary phases specifically for the ion exchange chromatographic analysis (IEX) of mAb's such as IgG and IgM. The megaporous materials, commercialized under the name TSKgel BioAssist S and Q, initially were offered in 7 and 10 micron particle sizes respectively in the analytical format of 4.6mmID x 50mmL PEEK columns. The analytical format was limited to approx 5mg of sample load/column. Recently, Tosoh has developed a 13 micron particle with a similar megaporous structure in a 10mmID x 100mmL PEEK housing for semi-preparative mAb analysis. The increase in particle size allows for loading capacities over 100mg/column while the extra column length compensates for the decrease in surface area to provide equivalent resolution.

The current data highlights the comparison between the analytical TSKgel BioAssist IEX columns and the new 13 micron semi-preparative TSKgel BioAssist IEX columns. Performance comparisons with regard to scalability, sample loading and flow rate are shared along with application data from various sources of IgG molecules.

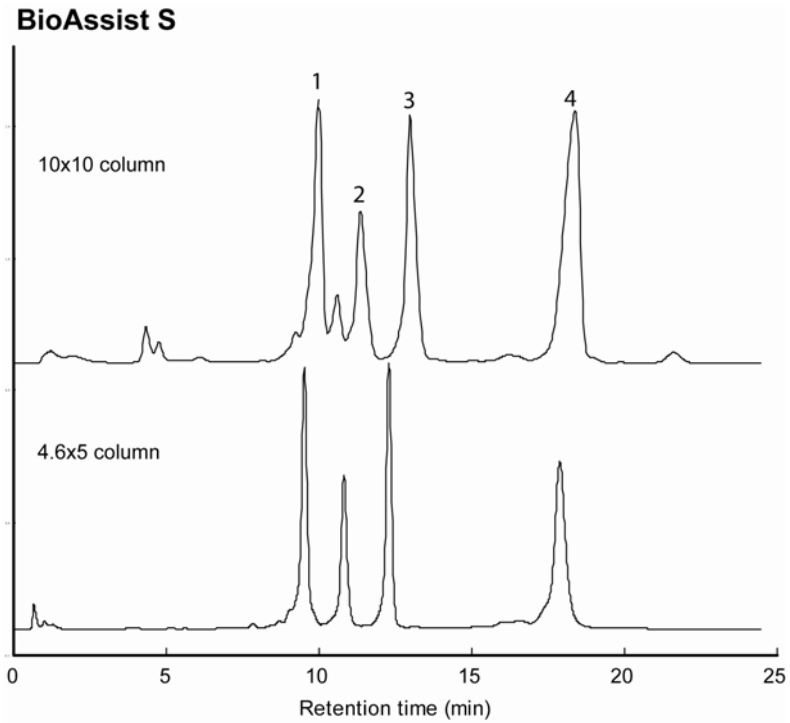
Abstract

	TSKgel BioAssist Q	TSKgel BioAssist S
Base Matrix	hydrophilic resin porous	hydrophilic resin porous
Particle size		
4.6mmID column	10µm	7µm
10mmID column	13µm	13µm
Pore size (Å)	ca. 4000	ca.1300
Ion exchange	ca. 0.1meq/L	ca. 0.1 meq/L
Ion site	Polyamine	Sulfapropyl
Column size	50mm x 4.6mmID 10cm x 10mmID PEEK	50mm x 4.6mmID 10cm x 10mmID PEEK
Optimal flow rate		
4.6mmID column	10.mL/min	0.8mL/min
10mmID column	5.0mL/min (10)	5.0mL/min (10)
Loading volume	<10mg	<100mg

Scaleability



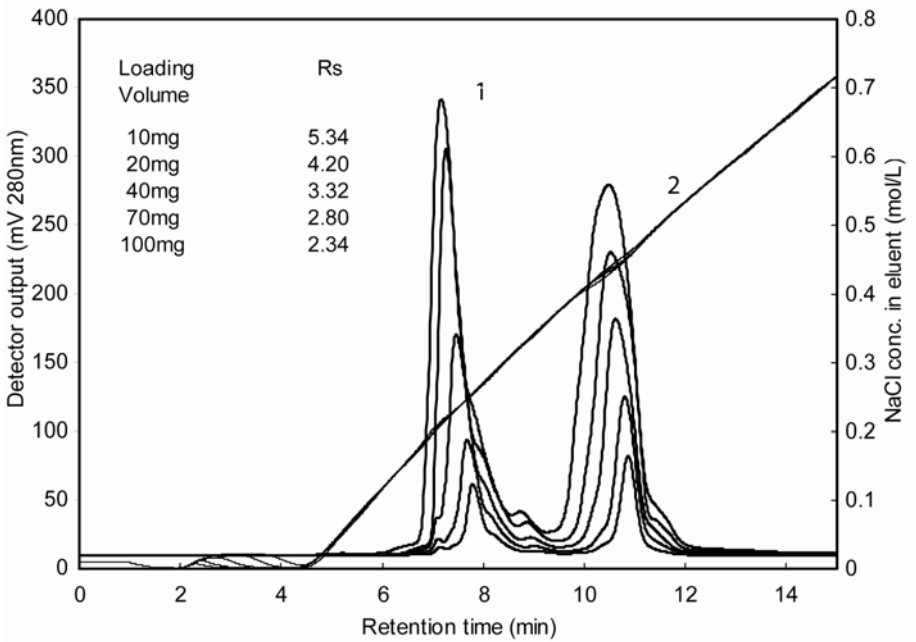
Eluent: 20mmol/L Tris-HCl buffer pH8.0
Gradient: 0 to 1mol/L NaCl 30min Linear.
Flow-rate: 1.0mL/min (4.6mmIDx5cm); 5.0mL/min (10mmIDx10cm)
Sample: 1) Ovalbumin; 2) Soybean trypsin inhibitor



Eluent: 20mmol/L Sodium phosphate buffer pH6.5
Gradient: 0 to 1mol/L NaCl 30min Linear.
Flow-rate: 0.8mL/min, Sample 2.5mg protein applied (4.6mmIDx5cm);
5mL/min, Sample 21mg Protein applied (10mmIDx10cm)
Sample: 1) a-Chymotrypsinogen A; 2) RNase A; 3) Cytochrome C
4) Lysozyme

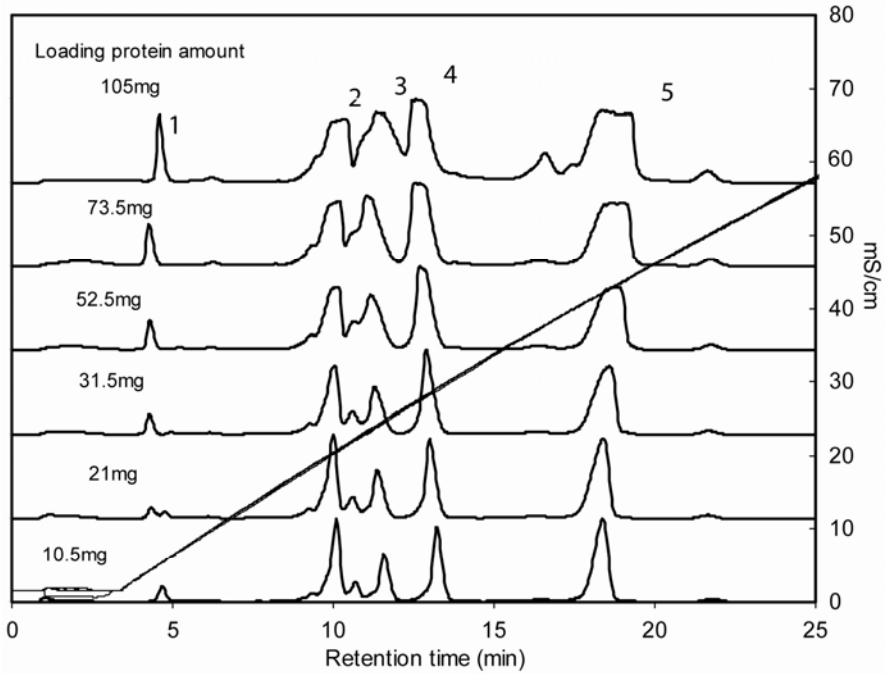
Loading

BioAssist Q



Column: TSKgel BioAssist Q 10cm x 10mmI.D.
Eluent: 20mM Tris-HCl buffer pH8.0
Gradient: 0 to 1M NaCl 15min Linear
Flow-rate: 3mL/min
Sample: 1. Ovalbumin 10mg/mL
2. Soybean Trypsin inhibitor 10mg/mL in eluent
Loading volume: 10mg-100mg proteins

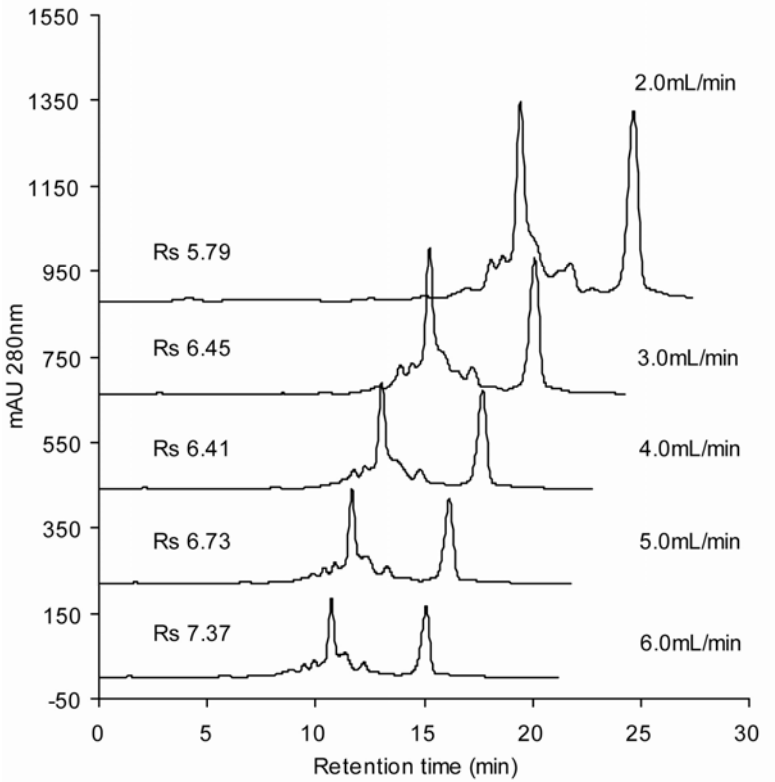
BioAssist S



Colum: BioAssist S 10x10
Eluent: 20mM Sodium Phosphate buffer pH6.5,
Gradient: 0 to 1M NaCl 20CV Linear, Flow-rate: 5mL/min
Sample: 1. Myoglobin(0.5mg/mL), 2. a-Chymotrypsinogen A(2),
3. RNase A(4), 4. Cytochrome C(2), 5. Lysozyme (2)

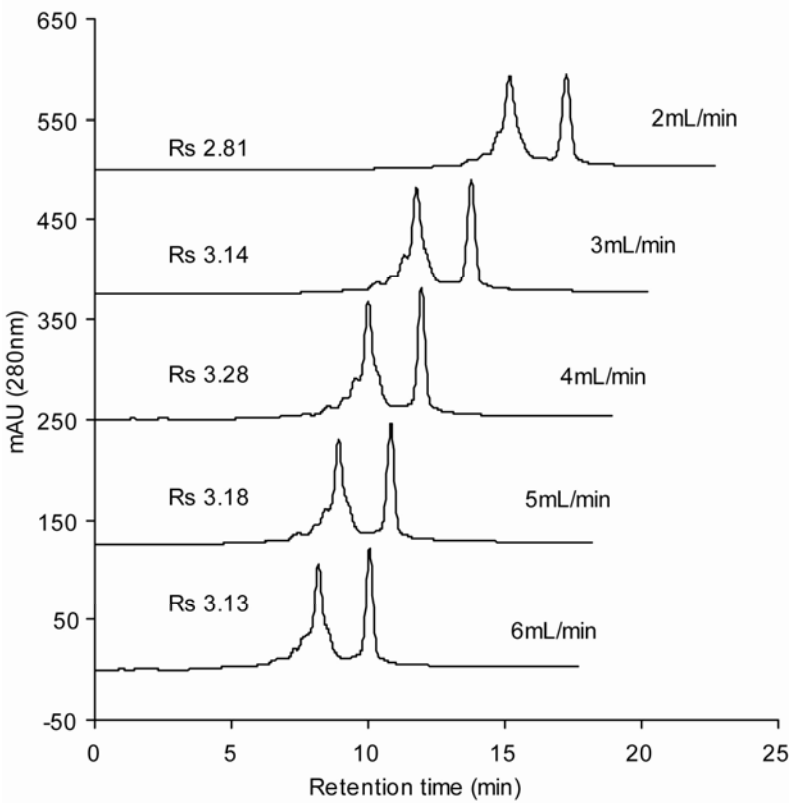
Flow Rate

BioAssist Q 10x10



Eluent: 20mmol/L Tris-HCl buffer pH8.0
Gradient: 0 to 1mol/L NaCl 30min
Sample: Ovalbumin, Trypsin inhibitor

Competitor Q-type 6mL



Eluent: 20mmol/L Tris-HCl buffer pH8.0
Gradient: 0 to 1mol/L NaCl 30min
Sample: Ovalbumin, Trypsin inhibitor

BioAssist Q

Separation of mAb in supernatant of cell culture medium on BioAssist Q 10x10 column (I)

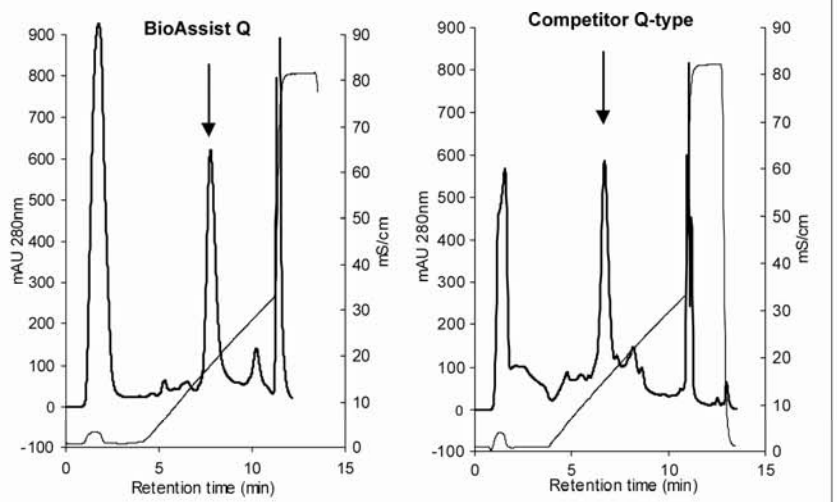
Supernatant of media

↓

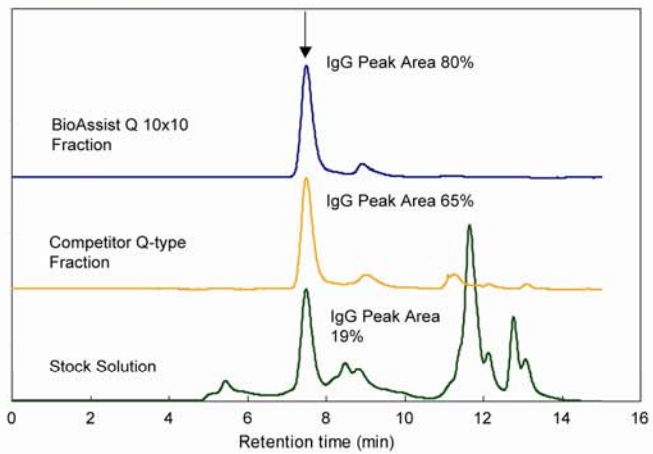
diluted 5 times with 20mmol/L Tris-HCl buffer pH8.0

↓

Ion-exchange chromatography
Column: BioAssist Q, Competitor Q-type
Eluent A: 20mmol/L Tris-HCl buffer, pH8.0
Eluent B: 20mmol/L Tris-HCl buffer containing 1mol/L NaCl, pH8.0
Gradient: 0-36% B/ 2.88-10.10min (50mmol NaCl/min linear gradient) 100% B/10.10min
Flow rate: 5.0mL/min
Sample: Anti-TSH, 5mL injection (approx. 1mg mAb)



Size exclusion chromatography
Column: TSKgel BioAssist G3SWXL
Eluent: 0.3mol/L NaCl in 0.1mol/L sodium phosphate buffer pH6.7
Flow rate: 1.0mL/min
Sample: 100μL each fraction



BioAssist Q

Separation of mAb supernatant of cell culture medium on BioAssist Q 10x10 column (II)

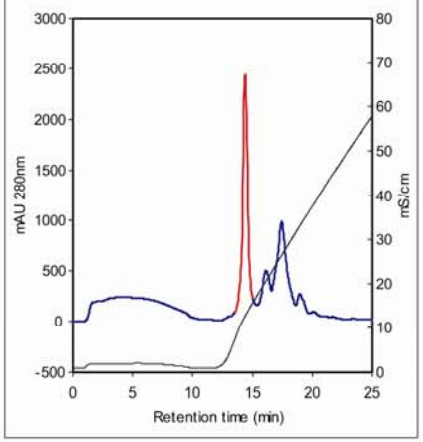
Supernatant of media

↓

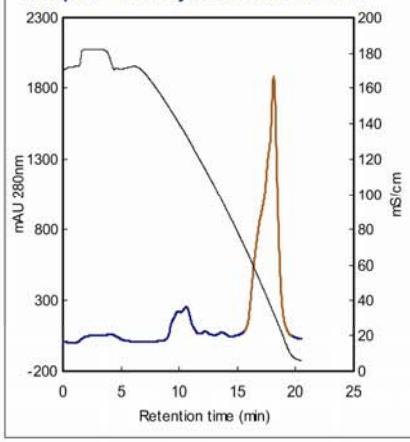
6mL supernatant diluted with 44mL of 20mmol/L Tris-HCl buffer pH8.0

↓

Ion-exchange chromatography
Column: BioAssist Q 10x10
Eluent A: 20mmol/L Tris-HCl buffer, pH8.0
Eluent B: 20mmol/L Tris-HCl buffer containing 1mol/L NaCl, pH8.0
Gradient: 0-91% B; 10.33-28.67min (50mmol NaCl/min linear gradient) 100%B; 28.67min
Flow rate: 6.0mL/min
Sample: Anti-TSH, 50mL injection

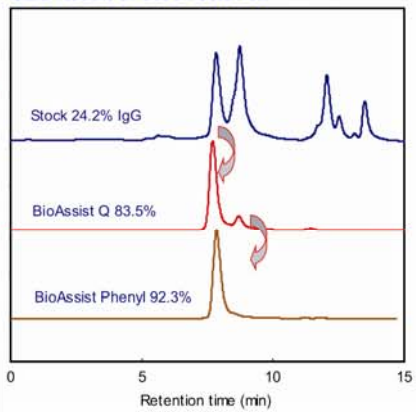


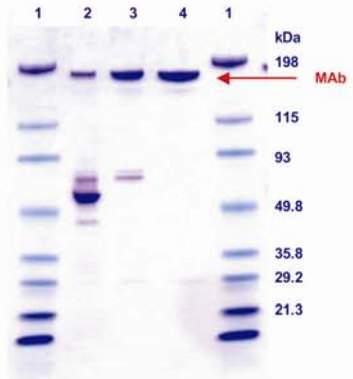
Hydrophobic chromatography
Column: BioAssist Phenyl 7.8mmIDx5cm
Eluent A: 50mmol/L Phosphate buffer containing 1.5mol/L (NH4)2SO4, pH6.8
Eluent B: 50mmol/L Phosphate buffer, pH6.8
Gradient: 0-100% B; 4.63-17.63min (115.3mmol (NH4)2SO4/min linear gradient) 100% B; 17.63min
Flow rate: 1.5mL/min
Sample: 5mL injection MAb fraction



Size exclusion chromatography
Column: TSKgel BioAssist G3SWXL
Eluent: 0.3mol/L NaCl in 0.1mol/L sodium phosphate buffer pH6.7
Flow-rate: 1.0mL/min
Sample: 100μL each fraction

SEC Result of Stock solution





1. Marker,
2. Stock,
3. Fraction (BioAssist Q),
4. Fraction (BioAssist Phenyl)

Conclusion

The new 13 micron semi-preparative TSKgel BioAssist IEX columns provide a 5 fold increase in sample loading to 100mg/column in loading capacities relative to the analytical format. The separation performance is maintained with BioAssist Q while the BioAssist showed a slight loss in resolution. However, the megaporous pore structure allows for improved separations of IgG samples relative to conventional competitive IEX chemistries.