A newly developed hydrophilic polymer-based ion exchange chromatography column for separation of various biological molecules

Noriko SHOJI, Akiko MATSUI, Masakatsu OMOTE, Naohiro KURIYAMA

YMC Co., Ltd., Ishikawa, Japan

Introduction

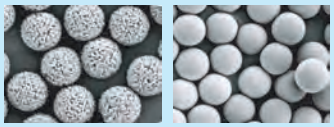
Ion exchange chromatography (IEC) is widely used for analysis and purification of biomolecules.

We have newly developed polymer-based IEC column, named YMC-BioPro, specially designed for separation of proteins, peptides and nucleic acids. YMC-BioPro IEC columns are based on 5 μm porous and non-porous hydrophilic polymer beads with low nonspecific adsorption, and they show higher binding capacity and higher recovery of biomolecules compared to conventional IEC columns.

The completely spherical and monodispersed beads, with optimal packing technology, provide high theoretical plate number and symmetrical peak shape. Excellent resolution is achieved from the high column efficiency coupled with the excellent selectivity of QA (quaternary ammonium) and SP (sulfopropyl) ion exchangers.

In this poster, we will show benefits of YMC-BioPro IEC columns and some example cases of superior separation of important biomolecules, such as monoclonal antibody and DNA.

SEM images of polymer beads of YMC-BioPro IEC columns



Porous polymer beads | Non-porous polymer beads

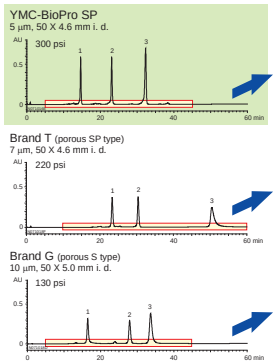
Main characteristics of YMC-BioPro IEC columns

	YMC-BioPro QA	YMC-BioPro SP	YMC-BioPro QA-F	YMC-BioPro SP-F
Matrix	hydrophilic polymer beads			
Particle size (μm)	5			
Pore size (Å)	1000		non-porous	
Charged group	$-CH_2N^+(CH_3)_3$	$-CH_2CH_2CH_2SO_3^-$	$-CH_2N^+(CH_3)_3$	$-CH_2CH_2CH_2SO_3^-$
Counter ion	Cl^-	Na^+	Cl^-	Na^+
Ion-exchange capacity (meq/ml-resin)	0.075-0.100	0.070-0.095	0.075-0.110	0.230-0.290
Dynamic binding capacity (mg/ml-resin)	110-150 (BSA)	70-100 (human-IgG)	12-20 (BSA)	10-18 (human-IgG)
Available pH range	2-12	2-12	2-12	2-12
Column size (i. d. X length (mm))	4.6 X 50		4.6 X 30 , 4.6 X 50, 4.6 X 100	

Features of porous polymer-based IEC columns
YMC-BioPro QA / YMC-BioPro SP

- Newly developed hydrophilic porous polymer with low nonspecific adsorption
- Excellent resolution, high binding capacity and high recovery of biomolecules
- Ideal for analysis and laboratory-scale purification

Comparison of protein separation on porous polymer cation-exchange columns



Eluent : A) 20 mM KH_2PO_4-K_2HPO_4 (pH 6.8)
B) 20 mM KH_2PO_4-K_2HPO_4 (pH 6.8) containing 0.5 M NaCl
Gradient : 0-100%B (0-60 min)
Flow rate : YMC-BioPro SP, Brand T 0.5 ml/min
Brand G 0.59 ml/min
Temperature : 25°C
Detection : UV at 220 nm
Injection : YMC-BioPro SP, Brand T 20 μl
Brand G 23.6 μl
Sample : 1. Ribonuclease A (0.5 mg/ml)
2. Cytochrome c (0.5 mg/ml)
3. Lysozyme (0.5 mg/ml)

The separation of standard protein mixtures is compared among YMC-BioPro SP and commercial porous polymer cation-exchange columns. Many impurities are resolved from the main peaks of proteins on YMC-BioPro SP with superior peak shapes.

Dynamic binding capacity and recovery of proteins

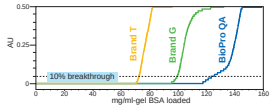
Comparison of dynamic binding capacity (DBC) and recovery for BSA

	Dynamic binding capacity (mg/ml-resin, 10% breakthrough)	Eluted amount (mg/ml-resin)	Recovery* (%)
YMC-BioPro QA	126	120	95
Brand G (porous Q type)	100	35	35
Brand T (porous Q type)	73	58	79

* Recovery : (Eluted amount / Dynamic binding capacity) X 100

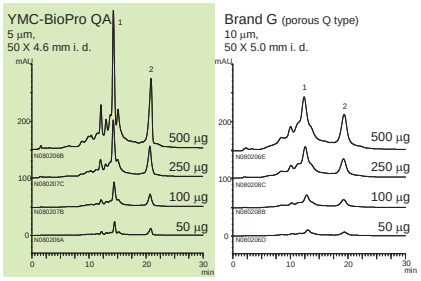
Compared with conventional porous-polymer anion-exchange columns, YMC-BioPro QA gives the superior DBC. Furthermore, the recovery is higher than conventional columns. The hydrophilic properties of the matrix polymer remarkably reduce nonspecific adsorption of proteins on YMC-BioPro columns.

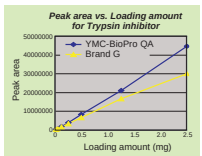
Comparison of breakthrough curves



Column : YMC-BioPro QA 50 X 4.6 mm i. d.
Brand G 50 X 5.0 mm i. d.
Brand T 50 X 5.0 mm i. d.
Linear velocity : 3.0 cm/min
Sample : 1.0 mg/ml Bovine serum albumin (BSA) in equilibration buffer
Equilibration buffer : 20 mM Tris-HCl (pH 8.6)
Elution buffer : 20 mM Tris-HCl (pH 8.6) containing 1.0 M NaCl
Detection : UV at 280 nm

Comparison of the effect of sample load on YMC-BioPro QA and commercial porous polymer Q type product



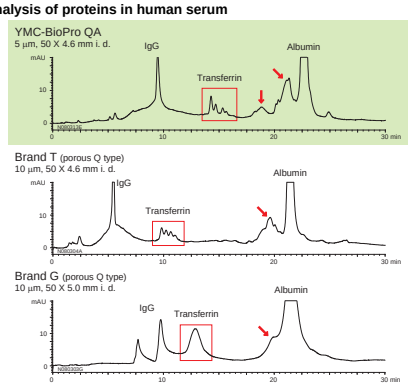


Eluent : A) 20 mM Tris-HCl (pH 8.1)
B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl
Gradient 10-80%B (0-30 min)
Flow rate : 0.5 mL/min
Temperature : 25°C
Detection : UV at 280 nm
Injection : 100 μL
Sample : 1. Ovalbumin
2. Trypsin inhibitor

YMC-BioPro QA shows the excellent peak shapes even when the loading amount increases. The column of Brand G cannot achieve acceptable peak shapes and resolution even in small amount of injection. The excellent linearity is observed between peak area and loading amount for Trypsin inhibitor on YMC-BioPro QA. These results indicate that YMC-BioPro QA would be suitable for laboratory-scale purification of proteins.

Application

Analysis of proteins in human serum



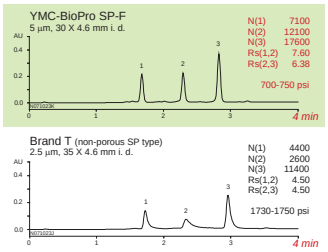
Eluent : A) 20 mM Tris-HCl (pH 8.6)
B) 20 mM Tris-HCl (pH 8.6) containing 0.5 M NaCl
Gradient 0-30%B (0-15 min), 30-100%B (15-30 min)
Flow rate : 0.5 mL/min
Temperature : 25°C
Detection : UV at 280 nm
Injection : 20 μL
Sample : Human serum (100 μl/ml)

The separation of the proteins in human serum is compared among YMC-BioPro QA and two commercial porous polymer anion-exchange columns. YMC-BioPro QA shows superior resolution in analysis of biological samples containing a large amount of impurities.

Features of non-porous polymer-based IEC columns
YMC-BioPro QA-F / YMC-BioPro SP-F

- Non-porous polymer beads with high chemical and mechanical stabilities
- 30 mm-length column for high-throughput analysis with low operating pressure
- 100 mm-length column for high-resolution analysis

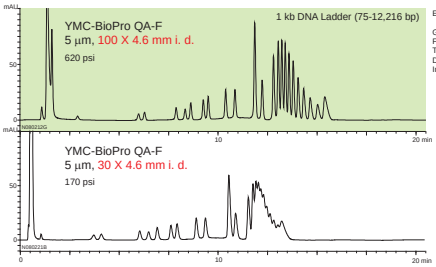
High-throughput analysis of proteins on non-porous polymer cation-exchange columns



Eluent : A) 20 mM KH_2PO_4-K_2HPO_4 (pH 6.8)
B) 20 mM KH_2PO_4-K_2HPO_4 (pH 6.8) containing 0.5 M NaCl
Gradient : YMC-BioPro SP-F 0-100%B (0-4 min)
Brand T 0-100%B (0-4.67 min)
Flow rate : 1.5 ml/min
Temperature : 25°C
Detection : UV at 220 nm
Injection : 20 μl
Sample : 1. Ribonuclease A (0.1 mg/ml)
2. Cytochrome c (0.1 mg/ml)
3. Lysozyme (0.1 mg/ml)

YMC-BioPro SP-F can elute the proteins sharply without peak-tailing rather than commercial non-porous SP column, Brand T. Furthermore, despite larger particle size, the theoretical plate number of SP-F is higher than that of Brand T.

High-resolution analysis of DNA fragments

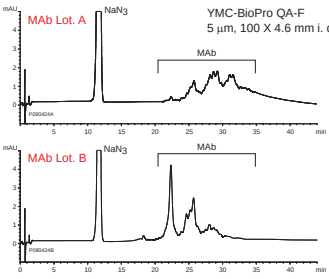


Eluent : A) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl
B) 20 mM Tris-HCl (pH 8.1) containing 1.0 M NaCl
Gradient : 40-100%B (0-30 min)
Flow rate : 0.5 ml/min
Temperature : 25°C
Detection : UV at 260 nm
Injection : 20 μl (0.25 mg/ml)

The separation of DNA fragments is compared between 100 mm-length and 30 mm-length of YMC-BioPro QA-F columns. The resolution of DNA fragments is dramatically improved by 100 mm column.
The combination of non-porous polymer beads and long column provides extremely high column

Application

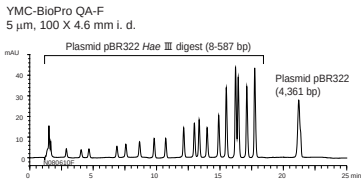
Analysis of monoclonal antibody (MAb) against human IgG4



Eluent : A) 20 mM Tris-HCl (pH 8.1)
B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl
Gradient 10-25%B (0-60 min)
Flow rate : 1.0 mL/min
Temperature : 25°C
Detection : UV at 220 nm
Injection : 14 μL
Sample : Monoclonal mouse IgG1 (0.1 mg/ml)
(Purified by DEAE chromatography, containing NaN3)

Two different lots of commercially available MAb, purified by DEAE chromatography, are separated with 100 mm-length of YMC-BioPro QA-F column.
The MAb is resolved into several peaks and the lot-to-lot variability is observed.
The 100 mm-length column of YMC-BioPro QA-F and SP-F has high efficiency and it is ideal for characterization or QC assessment of closely related proteins.

Analysis of plasmid pBR322 restriction fragments



Eluent : A) 20 mM Tris-HCl (pH 8.1)
B) 20 mM Tris-HCl (pH 8.1) containing 1.0 M NaCl
Gradient 70-85%B (0-20 min), 85%B (20-25 min)
Flow rate : 0.5 mL/min
Temperature : 35°C
Detection : UV at 260 nm
Injection : 10 μL

The extremely high-resolution of plasmid and its restriction fragments is achieved on 100 mm column of YMC-BioPro QA-F.

Conclusion

- The newly developed IEC columns based on highly hydrophilic polymer beads show significantly low non-specific adsorption of proteins.
- Porous types, QA and SP, show superior resolution, high binding capacity and high recovery for various biomolecules. They are useful for analysis and laboratory-scale purification of biological samples containing a large amount of impurities.
- Non-porous types, QA-F and SP-F, are useful for high-throughput analysis. Furthermore, 100 mm-length column is effective for high resolution analysis of complex mixtures, such as MAbs, DNA fragments and synthetic oligonucleotides.