

Developments of Hydrophilic Polymer-based Ion Exchange Media for Analysis and Purification of Various Biological Molecules

Noriko Shoji, Akiko Matsui, Saoko Nozawa, Taeko Nakajima, Masakatsu Omote and Naohiro Kuriyama
YMC Co., Ltd., Ishikawa, Japan

Introduction

The recent development of bio-pharmaceutical industry has been remarkable, and shortening the development time and reducing the cost become increasingly important. The development of efficient, economical and selective separation method is required for successful commercialization of bio-pharmaceutical products. To meet these demands, we have developed new polymeric packing materials named YMC-BioPro series, which are specially designed for ion exchange (IEX) separation and purification of proteins, peptides and nucleic acids. YMC-BioPro series includes the packed columns with 5 micron porous/non-porous polymer for analysis and laboratory scale purification, and the bulk materials of 30, 75 micron porous polymer for capture and intermediate purification. The all materials are based on the same hydrophilic polymer beads with low nonspecific adsorption. Compared to conventional materials available in the market, the BioPro series shows higher binding capacity and higher recovery of biomolecules.

As for the analytical BioPro columns, 5 micron 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. The bulk materials of 30, 75 micron porous polymer resin, which have increased binding capacity and low pressure drop, are ideal for capture and intermediate purification steps. The bulk materials have similar retention selectivity to 5 micron porous type BioPro columns and it allows predictable scale-up from analytical to preparative separation in bio-processes.

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

Features of new ion exchange media for separation of biomolecules

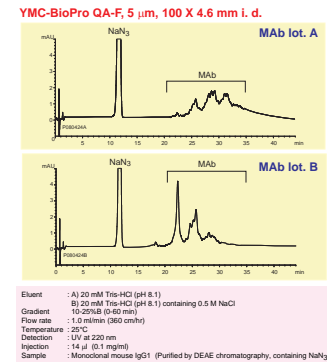
- Newly developed hydrophilic polymer with low nonspecific adsorption
- Porous polymer beads with high binding capacity and high recovery of biomolecules
- Non-porous polymer beads with high chemical and mechanical stabilities
- 5 μm packed column for high-resolution and high-throughput analysis, or laboratory-scale purification
- 30, 75 μm porous bulk materials for capture and intermediate purification

Pre-packed columns Bulk materials

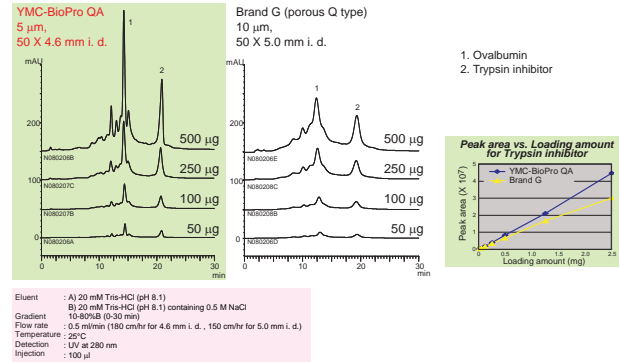
	YMC-BioPro QA-F/SP-F	YMC-BioPro QA/SP	YMC-BioPro Q30/S30	YMC-BioPro Q75/S75
Particle size (μm)	5		30	75
Matrix	non-porous polymer beads	porous polymer beads	porous polymer beads	
Charged group	QA-F/QA: $-\text{CH}_2\text{N}^+(\text{CH}_3)_3$ SP-F/SP: $-(\text{CH}_2)_2\text{SO}_3^-$	QA: > 110 (BSA) SP: > 70 (IgG)	Q30/Q75: $-\text{CH}_2\text{N}^+(\text{CH}_3)_3$ S30/S75: $-(\text{CH}_2)_2\text{SO}_3^-$	
Dynamic binding capacity (mg/ml-resin)	QA-F: > 12 (BSA) SP-F: > 10 (IgG)		Q30/Q75: > 160 (BSA) S30/S75: > 160 (Lysozyme)	
Available pH range	2-12		2-12	

High-resolution analyses of proteins on 5 μm non-porous/porous polymer packed columns

Analysis of monoclonal antibody (MAb) against human IgG4



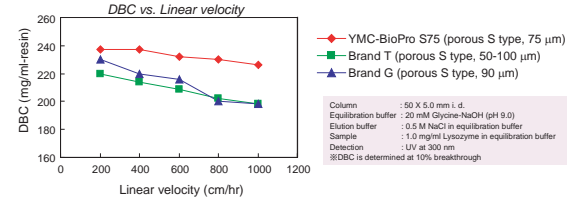
Comparison of the effect of loading amount on resolution and peak area



Two different lots of commercially available MAb, purified by DEAE chromatography, are separated on 100 mm length-column packed with non-porous polymer beads. 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.

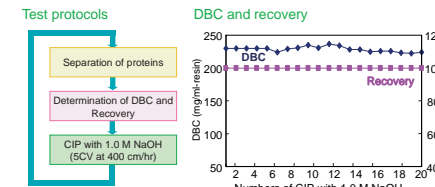
The effect of loading amount is evaluated by changing the loading protein from 50 μg to 2.5 mg on porous polymer anion-exchange columns. YMC-BioPro QA shows the excellent peak shapes even when the loading amount increases. The column of Brand G cannot achieve acceptable peak shapes and resolution even 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.

Comparison of Dynamic binding capacity (DBC) at different flow rate on IEX-resins for capture and intermediate purification

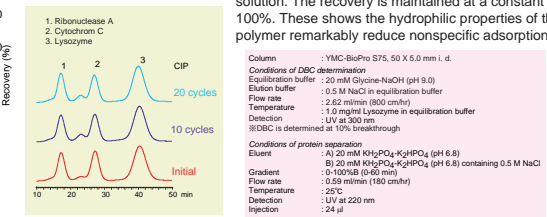


The dependency of DBC to linear velocity is compared on various commercially available S type resins for capture and intermediate purification. YMC-BioPro S75 shows the highest DBC over a wide range of linear velocity, and the difference of DBC is only less than 5% between 200 cm/hr and 1000 cm/hr on YMC-BioPro S75. This shows 75 μm BioPro resin would give increased productivity and reduced cost in biopharmaceutical production.

Cleaning-in-place (CIP) study of BioPro S75

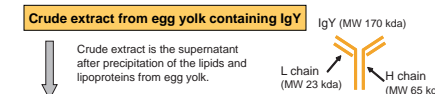


Separation of standard proteins

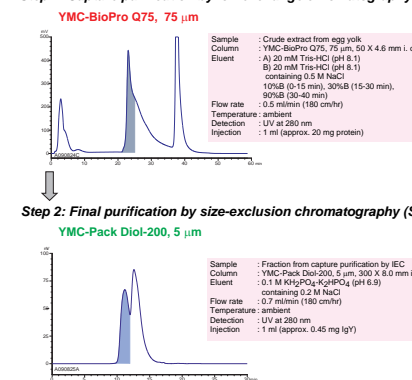


The DBC and the selectivity of protein separation are unaffected following 20 cycles of CIP with 1.0 M NaOH. The high chemical stability of BioPro resins allow effective cleaning with alkaline solution. The recovery is maintained at a constant value around 100%. These shows the hydrophilic properties of the matrix polymer remarkably reduce nonspecific adsorption of proteins.

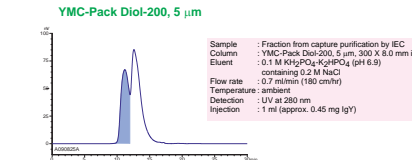
Application - Two step purification of IgY from crude egg yolk extract -



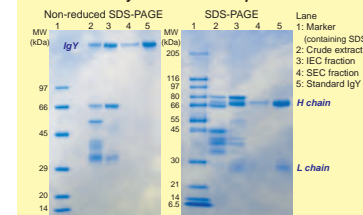
Step 1: Capture purification by ion-exchange chromatography (IEC)



Step 2: Final purification by size-exclusion chromatography (SEC)

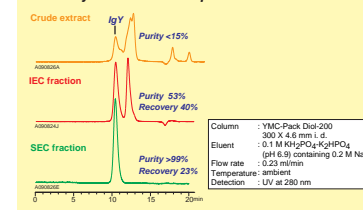


SDS-PAGE analysis of crude and purified fraction



Egg yolk antibody, IgY, is purified by two chromatographic steps using IEC as a capture purification and SEC as a final purification. Purity of the IgY is checked with SDS-PAGE and SEC analysis shown below. This purification protocol can isolate IgY with high purity more than 99%.

SEC analysis of crude and purified fraction



Conclusions

- Using the optimal IEX material for a specific application can result in a significant decrease in the costs of the biopharmaceutical production. YMC-BioPro series, which based on the same hydrophilic polymer with low non-specific adsorption, is scalable from analytical to large-scale preparative separation.
- 5 μm pre-packed columns with optimal packing technology provide superior resolution. Non-porous type BioPro QA-F/SP-F columns are effective for high resolution analysis or QC assessment of complex mixtures, such as MAbs. Porous type BioPro QA/SP columns are useful for analysis and laboratory-scale purification of biological samples.
- 30 μm and 75 μm bulk materials of porous polymer are useful for high capacity capture and high efficiency intermediate purification steps.