

Facing the challenges in bio-pharmaceutical production: newly developed polymer-based ion exchange chromatography media and their application to the purification for Immunoglobulin – from egg yolk

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Introduction

Much attention has been given to ion exchange (IEX) media as a means to improve productivity as a result of increasing demand for higher efficiency on the downstream process. Until recently, strain optimisation for high productivity and upstream purification were the bottlenecks for most bio-processes. However, with the progress made in recent years, titers in fermentation processes have increased significantly. Obviously, this increased volumetric productivity will help reducing the cost of goods, but it also has an impact on the downstream processing. This means that improved downstream processing media are required to handle the increased product load in the same timeframe.

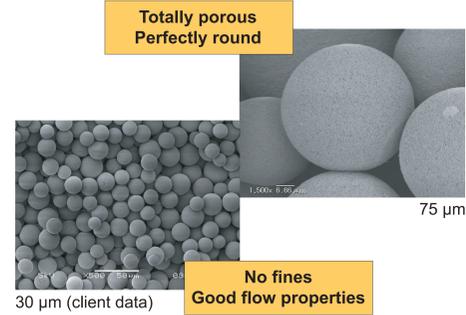
YMC has recently developed several polymer-based IEX media, known as YMC-BioPro Q and S (with quaternary ammonium [QA] and sulfobutyl [SB] functional groups, respectively). YMC-BioPro IEX media are based on the same porous hydrophilic polymer beads with 30 or 75 µm diameters, modified with the respective surface ligands. This new combination of matrix and surface ligand provides higher binding capacity and higher recovery for bio-molecules compared to conventional IEX media. The spherical beads, with optimal surface modification, provide high dynamic binding capacities (DBC) even at high flow rates up to 1000 cm/h. Excellent selectivity is achieved by bonding QA and SB functional groups to the ligand. Both factors impact on process economy, as high flows will allow fast separations whilst excellent selectivity will increase purity.

We show benefits of YMC-BioPro materials for superior downstream processing and separation of important bio-molecules, such as monoclonal antibodies and Immunoglobulin Y (IgY) from egg yolk.

Features of new ion exchange media for separation of biomolecules

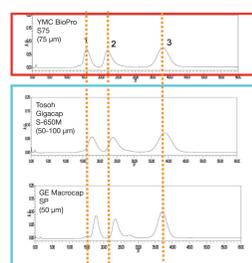
- Newly developed hydrophilic porous polymer with low nonspecific adsorption
- Porous polymer beads with high binding capacity and high recovery of biomolecules
- 30, 75 µm porous bulk materials for capture and intermediate purification
- Very economic prices

Property	analytical	preparative
Matrix:	Hydrophilic polymer-resin	Hydrophilic polymer-resin
Pore size	100 nm / 1000 Å or non porous	100 nm / 1000 Å
Particle size	5 µm	75 µm, 30 µm (10 µm under development)
Ion exchange Properties	S-Type: strong anion exchanger Q-Type: strong cation exchanger	S-Type: strong anion exchanger Q-Type: strong cation exchanger
pH-stability (long term)	pH 2-12	pH 2-12
pH-stability (for CIP)	n/a	1M NaOH up to 6h at RT



Cationexchange: Evaluation with Protein

BioPro S75 vs. Competitors



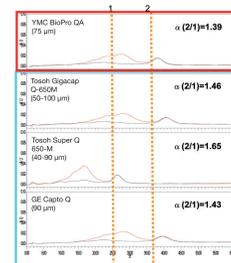
Testmethod:
 Sample : 20 mM Glycine-NaOH (pH 9.0) containing 1.5 mg/ml Lysozyme
 Equilibration buffer : 20 mM Glycine-NaOH (pH 9.0) containing 0.5 M NaCl
 Elution buffer : 20 mM Glycine-NaOH (pH 9.0) containing 0.5 M NaCl
 Flow : 180 cm/h
 Sample : 1. Ribonuclease A
 2. Cytochrome C
 3. Lysozyme

Results:
 DBC and recovery for YMC-BioPro S75 are the highest of all materials.
 Highly efficient processes possible with high end materials

	BioPro S75 (purification)	Gigacap S-650M (Tosoh)	Macrocap SP (GE)
DBC [mg/ml-gel]	186	182	81
Protein: Lysozyme 1.5 mg/ml			
Recovery (%)	109	108	108
Remarks	repeatable	repeatable	repeatable

Anionexchange: Evaluation with Protein

BioPro Q75 vs. Competitor



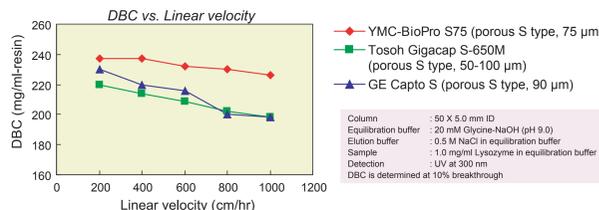
Testmethod:
 Sample : 20 mM Tris-HCl (pH 8.6) containing 1.5 mg/ml BSA
 Equilibration buffer : 20 mM Tris-HCl (pH 8.6) containing 0.5 M NaCl
 Elution buffer : 20 mM Tris-HCl (pH 8.6) containing 0.5 M NaCl
 Flow : 180 cm/h
 Sample : 1. Ovalbumin
 2. Trypsin inhibitor

Results:
 Highest DBC in this test for YMC-BioPro QA.
 Strong influence of particle size on peak shape

	BioPro QA (75 µm)	Gigacap Q-650M (Tosoh)	Super Q 650-M (Tosoh)	Capto Q (GE)
DBC [mgBSA/ml-gel] (10%)	187	147	149	102
Recovery (%)	100	93	32	127
Remarks	repeatable	repeatable		

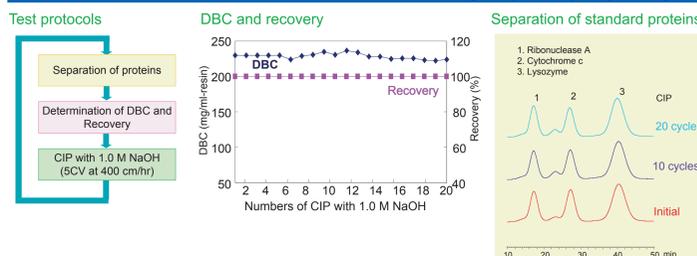
Elution is the same for all IEX media tested. The influence of particle size can clearly be seen. However, there is always a trade-off between particle size and flow/pressure. In the experiments, binding capacity varies widely and for anion exchange resins recoveries show drastic differences. YMC-BioPro IEX materials consistently give the highest DBC's at close to 100% recovery. The material gives good separation with low backpressure. All this results in YMC-BioPro being a high class material at a very economic price.

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 will provide increased productivity and reduced cost in biopharmaceutical production.

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



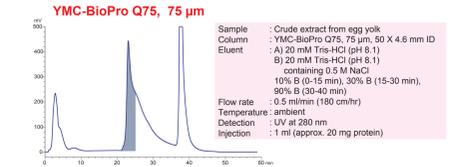
The DBC and the selectivity of protein separation are unaffected following 20 cycles of CIP with 1.0 M NaOH. The high chemical stability of BioPro resins allow effective cleaning with alkaline solution. The recovery is maintained at a constant value around 100%. This shows the hydrophilic properties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

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

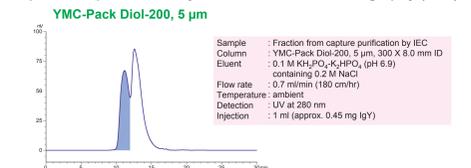
Crude extract from egg yolk containing IgY

Crude extract is the supernatant after precipitation of the lipids and lipoproteins from egg yolk.

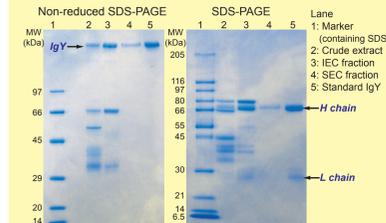
Step 1: Capture purification by ion-exchange chromatography (IEC)



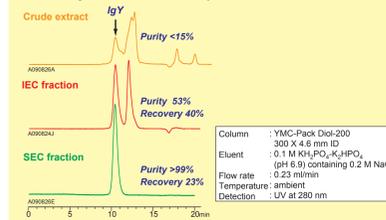
Step 2: Final purification by size-exclusion chromatography (SEC)



SDS-PAGE analysis of crude and purified fraction



SEC 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.

The application was optimised for purity and the purification protocol can isolate IgY with high purity of more than 99% in only two steps.

Conclusions

- Using the optimal IEX material for a specific application can result in a significant decrease in the costs of the biopharmaceutical production. Excellent flow properties, high dynamic binding capacities at high flow rates, coupled with generally 100% recovery can have a substantial impact on process economy. High flow rates will shorten process times, 100% recovery will reduce losses. The economic price of YMC-BioPro Material will further improve process economy.
- 30 µm and 75 µm bulk materials of porous polymer are useful for high capacity capture and high efficiency intermediate purification steps.

YMC will never knowingly change any product that is under use in a validated production procedure or validated analytical method.