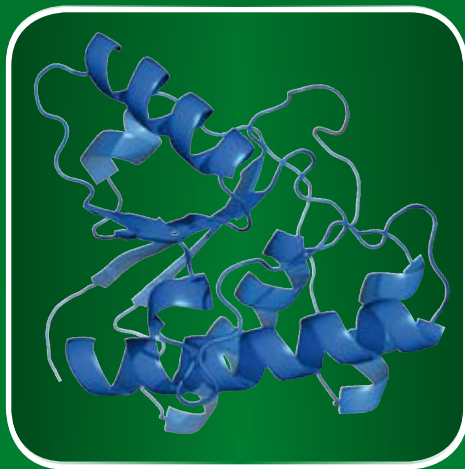


YMC-BioPro IEX materials



Bulk
media



YMC Phases for Biochromatography

Historically, small molecules have played the major role in diagnosis and therapy. However with the recent developments in the fields of genomics, proteomics and metabolomics, biological molecules have become an important tool for the treatment of diseases or help understanding biological processes.

YMC has always played an important role in the provision of materials for bioseparations. With the constant driving force of innovation, the focus has always been on column design and stationary phase manufacturing. As a consequence, YMC offers state of the art reversed phase, ion-exchange, size exclusion and normal phase/HILIC columns and bulk materials.

Contents

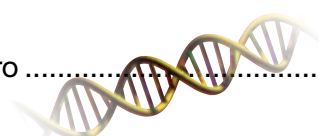
Dynamic binding capacity..... page 04-05

Stability of YMC-BioPro page 06-08

Separation properties..... page 09

Ordering Information page 09

Glas Columns page 10



Ion Exchange Resins for Capture & Purification of Biomolecules

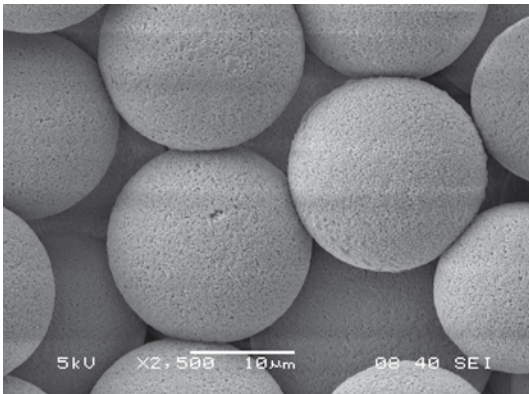


Figure 1: SEM picture of YMC BioPro 30µm resins (client)

Ion exchange chromatography (IEX) has been used for many years for analysis and purification of bio-molecules. It makes use of the simple concept of charge-induced reversible binding and has several important advantages over other bio-chromatographic methods. Two of these advantages are: binding is fast and media show a high capacity. YMC has its own high quality range of IEX material called YMC-BioPro. Media are based on a newly developed hydrophilic polymer matrix with a particle size of 30 or 75 µm and a pore size of 100 nm. The materials offer a high dynamic binding capacity (DBC), together with low non-specific adsorption and excellent recovery.

Dynamic binding capacity at increased linear velocities

The DBC is the capacity to bind the target molecule while the mobile phase is continually flowing through the IEX column. It is expressed in mg target molecule bound per ml of resin in the column (mg target/ml resin) and depends on the flow rate which is being applied. It is determined at 10% breakthrough and is different for every

combination of target molecule and resin. For YMC-BioPro S75, DBC was tested using BSA as the test substance. YMC-BioPro S75 shows very high DBC, typically above 220 mg BSA/ml resin. The results for the YMC material in figure 2 show higher DBCs than obtained for competitors' resins at all flow rates up to 1000 cm/h.

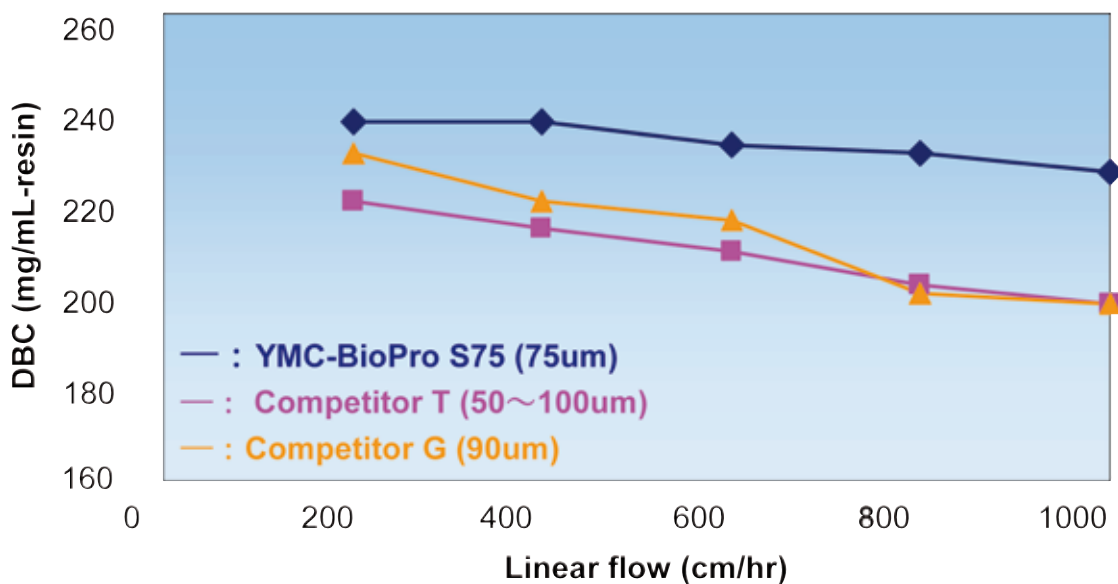


Figure 2: DBC of YMC BioPro and competitor materials up to a linear velocity of 1000 cm/h

Measurement of dynamic binding capacity and recovery

The steps performed in the determination of the DBC and recovery are shown in figure 3. At the start of the experiment, the column has to be equilibrated, if possible using the buffer used for making the protein solution. Then the protein solution of known concentration is loaded continuously onto the column, until the UV signal reaches a plateau (i.e. 100% value). The volume needed to reach 10% of the UV absorption multi-

plied by the concentration of the protein solution and divided by the volume of resin in the column gives the DBC (mg/ml resin). Next the column is washed with equilibration buffer to remove all unbound protein from the column. Finally the protein is eluted with a salt (step) gradient. Assaying the resulting eluate and comparing this value to the amount loaded gives the recovery.

Determination of DBC*

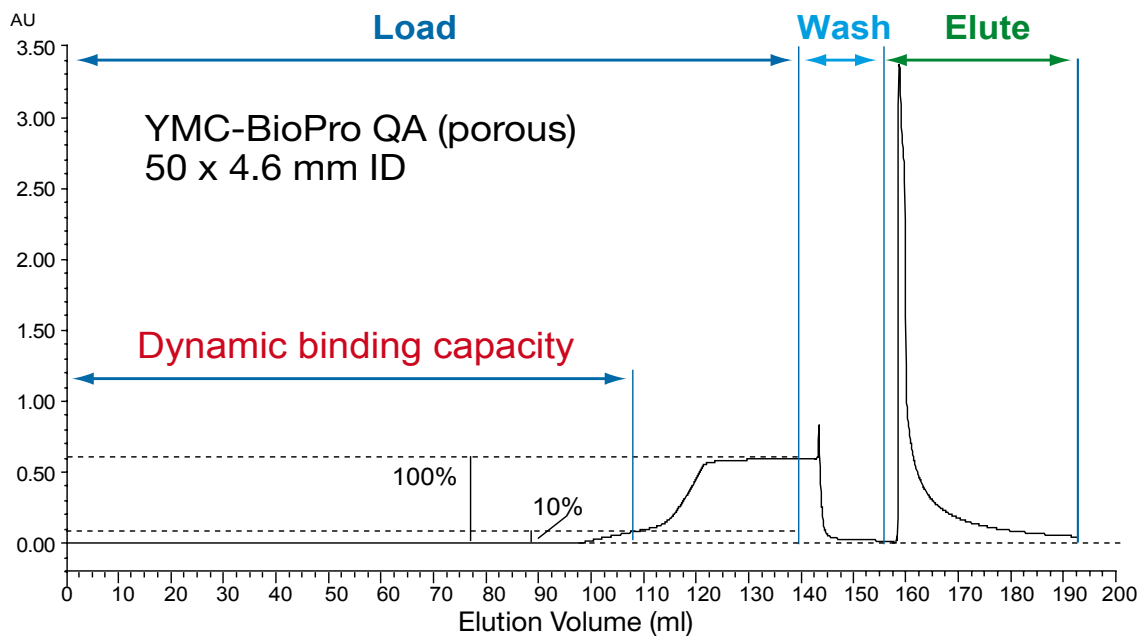


Figure 3: Schematic of an experiment to determine DBC and Recovery

Stability of BioPro IEX material under CIP conditions

Cleaning in place (CIP) is an important part of any pharmaceutical production process. Therefore the IEX materials YMC BioPro S75 and Q75 were tested with regards to their ability to withstand the CIP procedure. For the cation exchanger material (BioPro S75), a simulated production / CIP cycle has been used, whilst for the anion exchanger material (BioPro Q75) stability was measured after prolonged contact with a basic solution.

Figure 1 shows the schematic of the test cycle used. The first step in every cycle was to obtain a chromatogram for the separation of three standard proteins. This mixture was made up of ribonuclease A, cytochrome C and lysozyme. They were separated at a flow rate of 180 cm/h using a 20 mM potassium phosphate buffer, pH 6.8 as mobile phase A with a 20 mM potassium phosphate buffer, pH 6.8 containing 0.5 M NaCl as phase B.

The next step was to determine the DBC and recovery for lysozyme. The dynamic binding was determined at 10% breakthrough. For this, the column was loaded with lysozyme in 20 mM glycine-NaOH buffer, pH 9.0 at a linear flow of 800 cm/h. For the measurement of the recovery 0.5 M NaCl was added to the same buffer system (without lysozyme).

Finally the medium was cleaned in place by pumping with 1M NaOH for 5 column volumes (CV) at a linear flow of 400 cm/h.

Figure 2 shows the DBC and recoveries, plotted after each CIP step. For every cycle the DBC stays at a very good value, generally above 220 mg lysozyme per ml resin. The recovery remains constant at about 100%.

Finally, figure 3 shows that there is no difference in the ability to separate the protein mixture between the various runs.

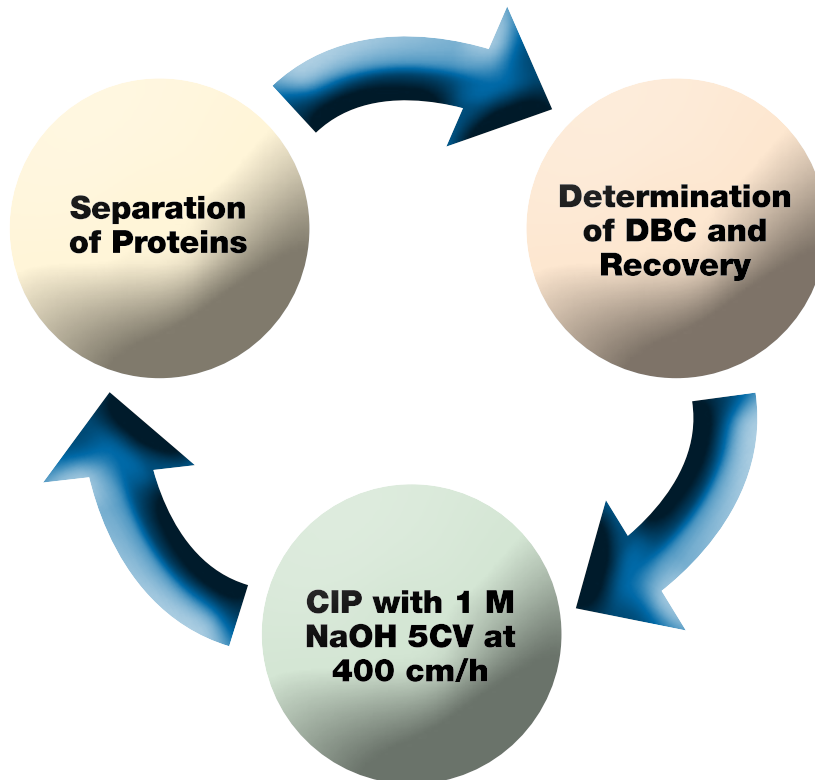


Figure 1: Schematic of the test cycle

DBC and recovery

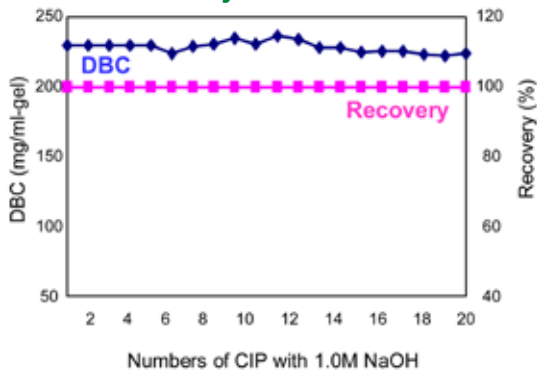


Figure 2: Values for DBC and recovery after each CIP cycle

This demonstrates that the use of 5 CV of 1M NaOH at a flow rate of 400 cm/h is a suitable CIP procedure for BioPro S. Furthermore, the experiment shows that the YMC BioPro S75 material withstands at least 20 such CIP cycles, whilst retaining its high DBC, recovery and separation power.

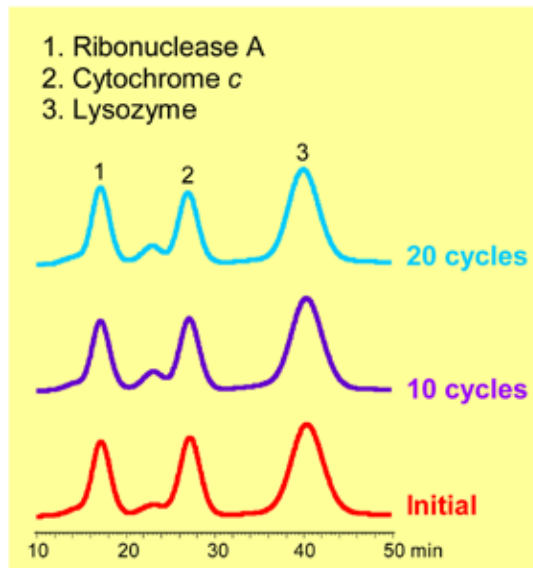


Figure 3: Chromatograms of the initial separation of the protein mixture and after 10 and 20 CIP cycles

Stability of YMC-BioPro Q75 after contact with 1M NaOH solution for at least 6 hours

For the BioPro Q75 anion exchanger medium, stability under basic conditions was assessed by two separate experiments. In the first, the material was simply kept in 1M NaOH solution for a total of 6 hours. In the second experiment, a column was packed and flushed with 1M NaOH for 6h at a linear flow of 200 cm/h. For both experiments, samples were taken at $t = 0h, 2h, 4h$ and $6h$ and the retention time for trypsin inhibitor was measured chromatographically. Additionally, for the first

experiment, the ion exchange capacity and for the second experiment, the DBC for BSA were determined, respectively. All experiments were performed at room temperature. The time period of 6 hours was assumed to be long enough for most CIP process; however the media is stable towards 1M NaOH for more than 6 hours.

The results obtained are summarized in figures 4 and 5 and tables 1 and 2.

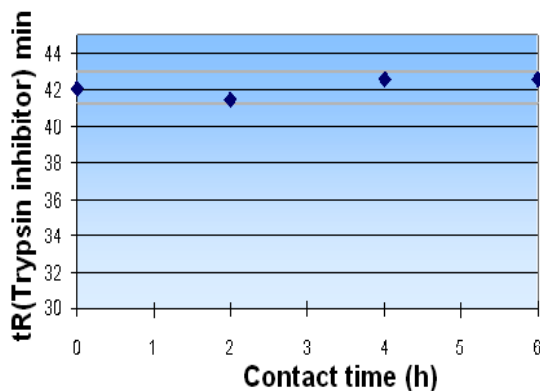


Figure 4: Retention times for trypsin inhibitor measured after 0h, 2h, 4h, and 6h contact time of the medium with 1M NaOH

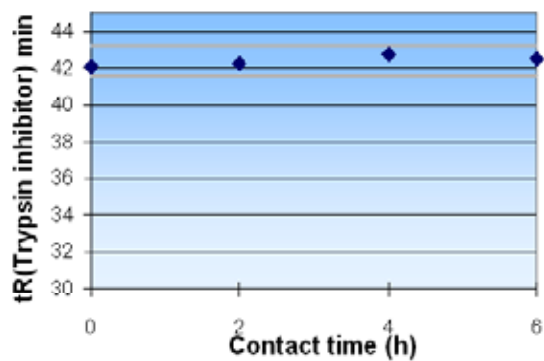


Figure 5: Retention times for trypsin inhibitor measured after 0h, 2h, 4h, and 6h of 1M NaOH solution flowing through the column

ION EXCHANGE MEDIA

Time [h]	Ion exchange capacity [eq/L]
0	0.16
2	0.16
4	0.16
6	0.16

Table 1: Values for ion exchange capacity following contact times of 0h, 2h, 4h and 6h with 1M NaOH

Time [h]	DBC [mg BSA/ml resin]
0	198.7
2	201.0
4	201.6
6	209.2

Table 2: Values for DBC of BSA following flow of 1M NaOH through the column for 0h, 2h, 4h and 6h

* the apparent increase of the DBC is fully explained by the experimental error associated with the experimental methods.

As shown above the retention times following treatment with 1M NaOH stay unchanged. In figures 4 and 5 the grey lines are drawn at values 2% below and above the average of the retention times. These 2% may be assumed to be the error associated to the measurement of the retention times. All values are within this 2% level of accuracy. Table 1 shows that the ion exchange capacity

is unchanged, whilst table 2 shows that the DBC does not change, remaining at the high values associated with YMC-BioPro Q75 material.

This means that all relevant values remain unchanged during the course of the experiment, proving that YMC-BioPro Q75 is stable towards 1M NaOH at room temperature for at least 6 hours.

Conclusion:

The results obtained show that both YMC-BioPro S75 and YMC-BioPro Q75 materials are stable towards 1M NaOH within the framework of a CIP procedure. Consequently, it can be said that YMC-BioPro IEX media comprehensively fulfil the requirements posed by industrial scale production processes of bio-pharmaceuticals. The resins are available as strong cation or strong anion exchangers with particle sizes of 30 or 75 µm and are produced in batch sizes of up to several hundred litres per lot.

Separation properties

Ion exchange is mostly used in capture and (intermediate) purification steps. In a capture step complete recovery of the target compound is the most important parameter, whilst in (intermediate) purification it is obviously the separation power that is most important. For YMC-BioPro the 75µm materi-

al already shows very good separation properties. Figure 6 below shows a separation of human serum, where Transferrin is well separated from the immunoglobulin and human serum albumin on a resin intended for capture steps.

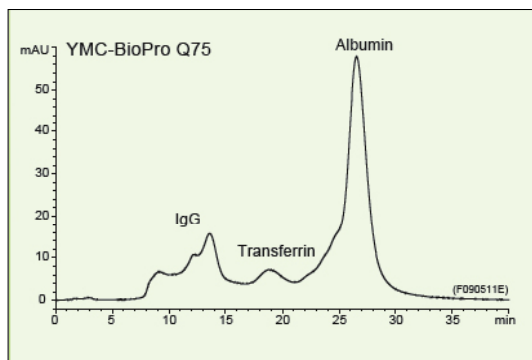


Figure 6: Separation of human serum

Column: YMC-BioPro Q75, 50 x 4.6 mm ID
 Eluent A) 20 mM Tris-HCl (pH 8.6)
 B) 1.0 M NaCl in 20 mM Tris-HCl (pH 8.6)
 Gradient 0% B (0-3 min), 0-15% B (3-18 min),
 15-50% B (18-33 min),
 50% B (33-40 min)
 Flow rate 0.5 mL/min (180 cm/hr)
 Temperature 25°C
 Detection UV at 280 nm
 Injection 20 mL (100 µL/mL)

Ordering Information

YMC-BioPro IEX media is available in QA and SP (strong anion and cation exchanger, respectively) with a pore size of 100 nm and particle sizes of

30µm or 75µm. All materials are suitable for use as process media and full regulatory support (incl. RSF and DMF) is given.

YMC-BioPro 30 µm, 100 nm material

Product	Particle Size	Code	Pack Sizes*				
			50 ml	250 ml	1 L	5 L	25 L
YMC-BioPro Q30	30 µm	QAA0S30	✓	✓	✓	✓	✓
YMC-BioPro S30	30 µm	SPA0S30	✓	✓	✓	✓	✓

YMC-BioPro 75 µm, 100 nm material

Product	Particle Size	Code	Pack Sizes*				
			50 ml	250 ml	1 L	5 L	25 L
YMC-BioPro Q75	75 µm	QAA0S75	✓	✓	✓	✓	✓
YMC-BioPro S75	75 µm	SPA0S75	✓	✓	✓	✓	✓

* Larger or customised pack sizes are available on request.

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www.ymc.de

YMC Co., Ltd.

YMC Karasuma-Gojo Bld. 284 Daigo-cho,
Karasuma Nisuiru Gojo-dori Shimogyo-ku,
Kyoto 600-8106 Japan
TEL. +81(0)75-342-4515, FAX +81(0)75-342-4550
www.ymc.co.jp

YMC America, Inc.

941 Marcon Boulevard Suite 301
Allentown, PA18109 USA
TEL. +1-610-266-8650, FAX +1-610-266-8652
www.ymcamerica.com

YMC India Ltd.

CX - 07, 3rd Floor, Lobe - 1,
Tower - A, The Corenthum, Plot No- A-41,
Sector - 62, Noida - 201301 (UP) India.
TEL. +91(0)120-4276020 - 25, FAX +91(0)120-4276026
www.ymcindia.com