Capto™ MMC

Capto MMC is a multimodal cation exhanger in the Capto family of BioProcess™ media for fast, efficient and cost-effective protein purification. Capto MMC combines agarose base matrix developments with innovative ligand chemistry. The adsorption is salt tolerant, meaning that binding of proteins can be performed at the conductivity of the feed material. The medium is based on a highly rigid agarose base matrix that allows high flow rates and low back pressure at large scale.

Capto MMC gives increased productivity and reduced cost with:

- high dynamic binding capacity at high conductivity
- high volume throughput
- new selectivity
- smaller unit operations

Fig 1. Capto MMC allows high-flow processing and binding of proteins at high conductivity. It increases throughput and productivity in large-scale bioprocessing operations.

Multimodal chromatography

In multimodal chromatography, the ligand interacts with the target molecule through multiple types of interactions. Ionic interactions are commonly involved, but hydrogen bonding and hydrophobic interactions can be significant. The strength of these individual interactions often depends on the target molecule and on the overall process conditions.

Multimodal chromatography media are characterized by selectivities that are different from those of "traditional" ligands, thereby opening up new opportunities for solving challenging purification problems. At the same time, the higher complexity of multimodal media normally requires process optimization studies in order to take full advantage of the outstanding potential of this technology. Having efficient, high-throughput process development tools and methodology facilitates this optimization work.

The multimodal ligand structure for Capto MMC is shown in Figure 2. It contains a carboxylic group and thus its features

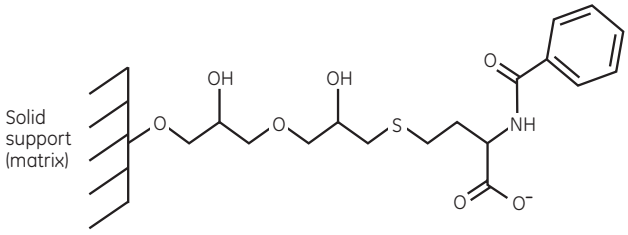

Fig 2. The multimodal ligand of Capto MMC.

partly resemble those of a weak cation exchanger. However, in addition to the ionic interactions, several other types of interactions are involved, including hydrogen bonding and hydrophobic interaction. The main characteristics of Capto MMC are summarized in Table 1.

Table 1. Characteristics of Capto MMC

Matrix	highly cross-linked agarose
Functional group	multimodal weak cation exchanger
Total ionic capacity	0.07-0.09 mmol H^+/mL medium
Particle size†	75 µm (d_{50v})
Flow velocity	at least 600 cm/h in a 1 m diameter column with 20 cm bed height at 20°C using process buffers with the same viscosity as water at < 3 bar (0.3 MPa).
Dynamic binding capacity‡	> 45 mg BSA/mL medium at 30 mS/cm
pH stability¶	
short term	2 – 14
long term	2 – 12
Working temperature	+4°C to +30°C
Chemical stability	all commonly used aqueous buffers, 1 M acetic acid, 1 M sodium hydroxide§, 8 M urea, 6 M guanidine hydrochloride, and 70% ethanol.
Avoid	oxidizing agents, cationic detergents

† d_{50v} is the median particle size of the cumulative volume distribution.

‡ Dynamic binding capacity at 10% breakthrough as measured at a residence time of 2 min, 300 cm/h in a Tricorn™ 5/100 column with 10 cm bed height in 50 mM Na-acetate, pH 4.75, 250 mM NaCl.

¶ Short term pH: pH interval where the medium can be subjected to, for cleaning- or sanitization-in-place (accumulated 90–300 h at room temperature) without significant change in function.

Long term pH: pH interval where the medium can be operated without significant change in function.

§ No significant change in ionic binding capacity after 1 week storage in 1 M NaOH at 40°C.

New selectivity and high salt tolerance

The multimodal functionality offers a selectivity different from traditional ion exchangers which includes binding of proteins at high salt concentration (Fig 3). The medium can be used for direct load of clarified feed stocks, without prior dilution to reduce the conductivity of the starting material. The new selectivity (Fig 4) can also be used to solve specific purification problems, at high or at low conductivity.

High salt tolerance

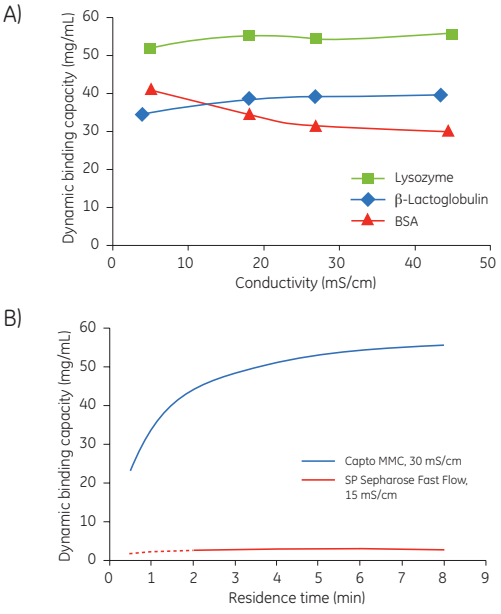

Fig 3. A) Dynamic binding capacity of Capto MMC at 1 min residence time for three different proteins at different conductivities. B) Dynamic binding capacity of bovine serum albumin (BSA) as a function of residence time. Capto MMC was run at 30 mS/cm, while SP Sepharose™ Fast Flow was run at 15 mS/cm.

New selectivity

Column: Tricorn 5/100
Medium: A) Capto MMC; B) SP Sepharose Fast Flow
Sample: human blood plasma diluted 5 times, 10 CV
Buffer A: 100 mM acetic acid, 50 mM Na-phosphate, 20 mM Na-succinate, pH 5.0.
Buffer B: 100 mM acetic acid, 50 mM Na-phosphate, 20 mM Na-succinate, pH 8.0 with 1 M NH_4Cl
Flow: 150 cm/h
Gradient: linear gradient 0% – 100% B over 10 column volumes (CV)
System: ÄKTAexplorer™ 10

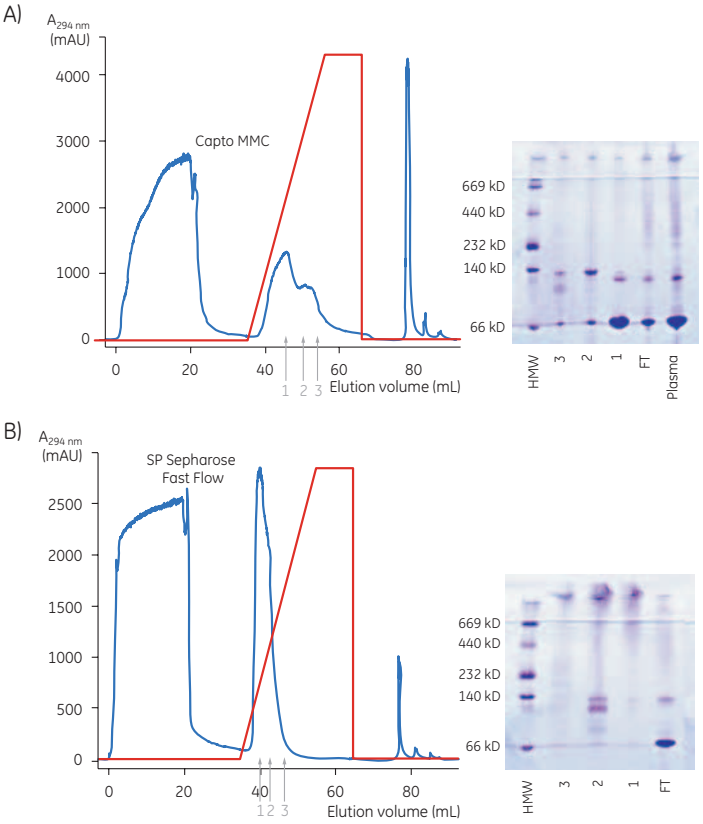

Fig 4. The selectivity of A) Capto MMC and B) SP Sepharose Fast Flow was investigated using human blood plasma. The sample was diluted 5 times to give a conductivity of 6 mS/cm and a pH of approximately 6. Fractions (indicated with arrows) and the flow-through pool (FT) were analyzed on native PhastGel™ gradient 8%-25% and Coomassie™ stained. High molecular weight marker (HMW, GE Healthcare) and unfractionated plasma sample were also applied to the gels. The elution profile on SP Sepharose Fast Flow revealed one peak whereas the elution profile on Capto MMC showed two, possibly three peaks. Native gel-electrophoresis also showed that the separation patterns differ between the media.

High flow rates and low backpressure in large scale

High flow velocities increase the productivity of large-scale bioprocessing operations and allow large volumes to be processed in one working shift. Capto MMC is characterized by high mechanical stability and low backpressure to allow columns to be operated at high flow velocities with a wide range of bed heights at large scale (Fig 5). Typical flow velocities for Capto MMC in a 1 m diameter column with 20 cm bed height are at least 600 cm/h, with a backpressure below 3 bar.

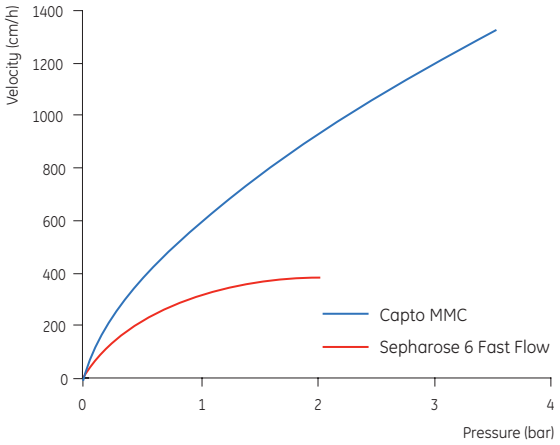

Fig 5. Pressure/flow properties of Capto MMC compared to Sepharose 6 Fast Flow. Running conditions: BPG™ 300 (30 cm i.d.), open bed at settled bed height equal to 20 cm, with water at 20°C.

Additional flexibility in process design

In addition to the flexibility in flow rates, bed heights and sample viscosities that all Capto media share, Capto MMC also gives flexibility in terms of conductivity of the start material. This increases productivity and enables a straight-forward process design, as the feed material can be loaded without the need for dilution (Fig 6).

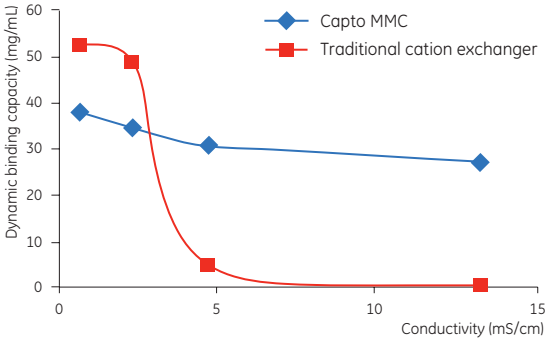

Fig 6. Capto MMC allows a much larger operating range (area below the curves) in terms of conductivity of the starting material than traditional cation exchangers.

Application

Optimization of binding and elution conditions

In contrast to traditional ion exchangers, Capto MMC may bind proteins above the isoelectric point of the target protein as illustrated in Fig 7. Therefore if pH is used for elution, higher pH is required with Capto MMC than with traditional ion exchangers. Since Capto MMC allows binding at high conductivity, it may not be necessary to screen for optimal loading conductivity with respect to binding capacity. However, binding selectivity may still be affected by the loading conductivity.

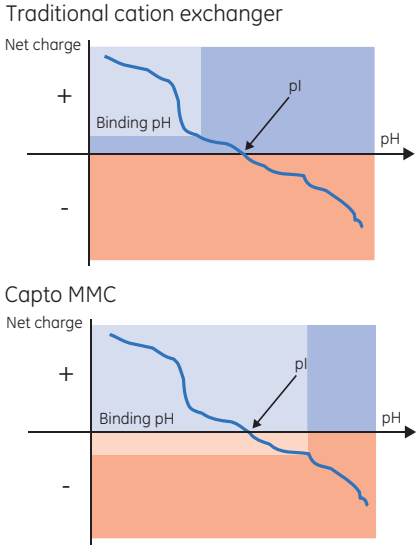

Fig 7. Typical protein titration curves with net charge on the y axis and pH on the x axis. For traditional ion exchangers, binding would be done at a pH below the isoelectric point and if then used for elution, the pH would be increased above the isoelectric point. In contrast, Capto MMC may bind proteins above the isoelectric point – as illustrated in the lower panel.

The fact that Capto MMC allows efficient capture of proteins at high conductivity in many cases limits the use of increasing salt concentrations as an efficient way of eluting proteins. Optimal elution is often achieved by a combination of changes in pH, buffer concentration and eluting salt. Design of Experiments (DoE) and high-throughput screening formats are effective tools for investigation of the effect of several parameters on protein recovery in order to establish the optimal elution protocol (Fig 8). An example of the DoE approach is described in application note 11-0035-48. Alternatively, a step-wise elution optimization protocol may be applied. An example is given in the instruction manual.

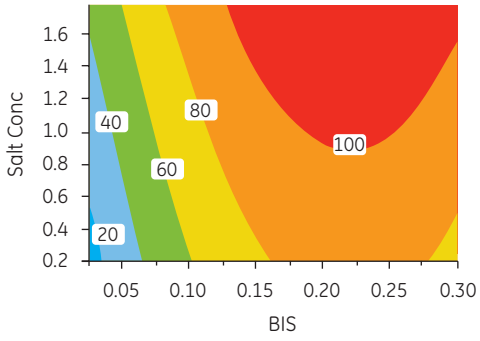

Fig 8. Response surface plots for the recovery of BSA. Recovery is plotted as a function of salt concentration and buffer ionic strength (BIS). Labels correspond to recovery in percent. For more information see application note 11-0035-48.

Improved productivity with Capto MMC

Capto MMC, SP Sepharose Fast Flow, and SP Sepharose XL were used to capture recombinant human serum albumin (rHSA, pI 5.5) from cell culture supernatant (CCS) of *Pichia pastoris*. The CCS was clarified by centrifugation and used directly as a feed material (conductivity 15 mS/cm) to measure dynamic binding capacities of the three media. In parallel experiments the feed was diluted to a conductivity of 3 mS/cm in order to test dynamic binding capacities of SP media. Figure 9 shows straightforward scale-up to pilot scale on Capto MMC.

Column:	A) Tricorn 5/100, 10 cm bed height (CV 2 mL) B) AxiChrom™ 50, 10 cm bed height (CV 208 mL)
Medium:	Capto MMC
Sample:	rHSA in *P. pastoris* CCS
Buffer A:	25 mM sodium acetate, pH 4.5
Buffer B:	50 mM sodium phosphate, pH 7.2 + 1 M NH_4Cl
Flow rate:	600 cm/h
Gradient:	100% B, 10 CV
System:	A) ÄKTAexplorer 100; B) ÄKTApilot™

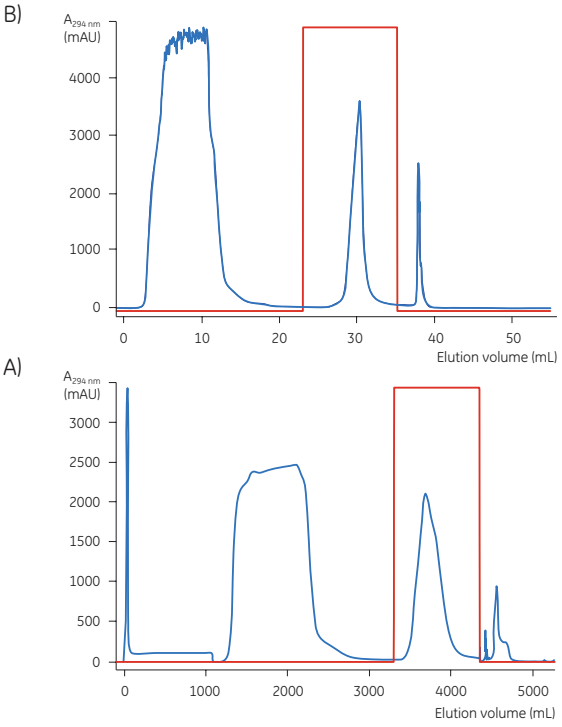

Fig 9. Straightforward scale-up from A) Tricorn 5/100 to B) AxiChrom 50 (100 times). In both cases the purification factor was 4 and the recovery was 93%.

Dynamic binding capacities at 10% breakthrough for all media and different dilutions of the feed were determined at different loading step residence times. The results obtained at residence times attainable in large diameter columns at 20 cm bed height were used for productivity calculations. The calculated results (Table 2) show that Capto MMC gives a productivity of 19 kg/m^3/h which is approximately 3 times higher than that obtained with SP Sepharose Fast Flow and SP Sepharose XL when the diluted feed is used and more than 13 times higher than when the undiluted feed is used. The latter result is expected since the SP ligand is not salt tolerant.

This example shows how high dynamic binding capacity at high conductivity combined with high flow velocities can improve the overall productivity of a capture step.

Operation

High-througput process development

High-throughput process development (HTPD) shortens development time at the same time as it increases the amount of information available during early process development. In multimodal chromatography, the availability of high throughput process tools are particularly valuable as the experimental conditions should be carefully screened in order to fully exploit the potential of the multimodal chromatography media. Capto MMC is available in PreDictor™ 96-well filter plate and PreDictor RoboColumn™ formats, as well as in HiScreen™ and HiTrap™ columns. PreDictor plates and Assist software are suitable for early chromatographic screening experiments. They can be used for the initial screening of process conditions, or for a more thorough investigation of a defined space as a basis for detailed process understanding.

After scouting and screening with PreDictor plates, verification and fine-tuning are still performed with ÄKTA™ design systems such as ÄKTA avant - with Design of Experiments (DoE) functionality. Prepacked columns such as HiScreen columns are recommended, or HiTrap columns if sample volumes are low. The UNICORN™ software on ÄKTA design systems makes it simple to transfer the optimized method to a production scale process system.

Table 2. Productivity calculations for purification of rHSA from *P. pastoris* CCS. Flow rates and residence times are based on the restrictions of the respective media in a large scale column at 20 cm bed height. In all cases, 93% recovery and 70% loading safety factor were used.

	Dilution factor	Capacity (g/L)	Residence time (min)	max flow rate (cm/h)	Productivity (kg/m^3/h)	Productivity (kg/24 h)[†]
Capto MMC 15 mS/cm	no dil	44	2	600	19	102
SP Sepharose XL 3 mS/cm	6.4	195	6	200	6.4	35
SP Sepharose XL 15 mS/cm	no dil	0	6	200	0	0
SP Sepharose Fast Flow 3 mS/cm	6.4	135	6	200	6.0	33
SP Sepharose Fast Flow 15 mS/cm	no dil	6	6	200	1.4	7.4

[†] Assuming column dimensions of 120 cm diameter, 20 cm bed height (CV ≈ 225 L)

Fully scalable

Capto MMC belongs to the BioProcess range of media that are developed and supported for production-scale chromatography. This includes validated manufacturing methods, secure supply and Regulatory Support Files to assist process validation and submission to regulatory authorities.

Scale-up is typically done by keeping bed height and flow velocity constant, while increasing column bed diameter and flow rate. However, since optimization is preferentially done with small column volumes, to save sample and buffer, some parameters such as the dynamic binding capacity may be optimized using shorter bed heights than those being used in the final scale. As long as the residence time† is constant, the binding capacity for the target molecule remains the same. Other factors, like clearance of critical impurities, may change when column bed height is changed and should be validated using the final bed height.

† The residence time is calculated by dividing the bed height (cm) by the flow velocity (cm/h) applied during sample loading.

Cleaning and sanitization

Cleaning-in-place (CIP) is a cleaning procedure that removes contaminants such as lipids, precipitates, or denatured proteins that may remain in the packed column after regeneration. Regular CIP also prevents the build-up of these contaminants in the media bed and helps to maintain the capacity, flow properties and general performance of Capto MMC.

A specific CIP protocol should be designed for each process according to the type of contaminants present. The frequency of CIP depends of the nature and the condition of the starting material, but one CIP cycle is generally recommended every 1–5 separation cycles. Capto MMC withstands all standard CIP procedures (e.g., 1 M NaOH, 2 M NaCl or 70% ethanol).

Equipment

Capto MMC can be used together with most equipment available for chromatography from lab scale to production scale. At process scale, the preferred packing technique for Capto media is axial compression. The optimal approach is to use AxiChrom columns with Intelligent Packing and pre-set packing methods for all Capto media. Appropriate columns from GE Healthcare are shown in Table 4.

All Capto media are also available within the ReadyToProcess™ platform, with pre-packed, pre-qualified and pre-sanitized ReadyToProcess columns ranging in size from 1-20 L.

Table 4. Appropriate columns

Column family range	Inner diameter (mm)
Lab scale:	
Tricorn	5, 10
HiScale™	16, 26, 50
Pilot and production scale:	
AxiChrom	50 – 1000
BPG	100 – 300†
Chromaflow	400 – 800‡

† The pressure rating of BPG 450 is too low to use it with Capto media.
‡ Larger pack stations might be required at larger diameters.

Storage

Store unused media and prepacked columns at +4°C to +30°C in 20% ethanol.

Ordering information

Product	Pack size	Code no
Capto MMC	25 mL	17-5317-10
Capto MMC	100 mL	17-5317-02
Capto MMC	5 L	17-5317-04
Capto MMC	10 L	17-5317-05
Capto MMC	60 L	17-5317-60
HiTrap Capto MMC	5 × 1 mL	11-0032-73
HiTrap Capto MMC	5 × 5 mL	11-0032-75
PreDictor CIEX screening plate, 20 µL	4 × 96 well filter plate	28-9432-91
PreDictor CIEX screening plate, 2 µL/6 µL	4 × 96-well filter plates	28-9432-90
PreDictor Capto MMC 50 µL	4 × 96-well filter plates	28-9258-16
PreDictor Capto MMC 20 µL	4 × 96-well filter plates	28-9258-15
PreDictor Capto MMC 6 µL	4 × 96-well filter plates	28-9258-14
PreDictor Capto MMC Isotherm	4 × 96-well filter plates	28-9432-81
PreDictor RoboColumn Capto MMC, 200 µL	one row of eight columns	28-9860-84
PreDictor RoboColumn Capto MMC, 600 µL	one row of eight columns	28-9861-78
HiScreen Capto MMC	1 × 4.7 mL	28-9269-80
ReadyToProcess Capto MMC	1 L	28-9511-18
ReadyToProcess Capto MMC	2.5 L	28-9291-20
ReadyToProcess Capto MMC	10 L	28-9291-21
ReadyToProcess Capto MMC	20 L	28-9291-22

All bulk media and prepacked column products are supplied in 20% ethanol. For more information, contact your local GE Healthcare representative.

Datafiles

PreDictor 96-well filter plates and Assist software	28-9258-39
PreDictor RoboColumn	28-9886-34
HiScreen prepacked columns	28-9305-81
ReadyToProcess columns	28-9159-87
AxiChrom columns	28-9290-41
HiScale columns	28-9755-23

Application Notes

Optimizing elution conditions on Capto MMC using Design of Experiments	11-0035-48
High-throughput screening for elution conditions on Capto MMC using PreDictor plates	28-9277-90
Methods for packing Capto MMC in production scale columns	28-9259-33

Handbooks

Ion Exchange Chromatography & Chromatofocusing: Principles and Methods	11-0004-21
High-throughput process development with PreDictor plates	28-9403-58

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