Purification of biomolecules using ion-exchange bulk media designed for high-throughput

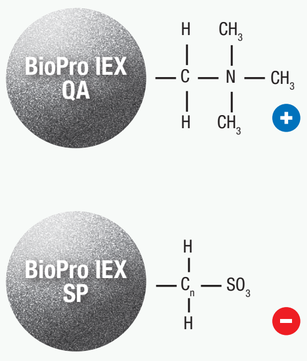
Abstract

Ion exchange media are widely used in the purification of biomolecules such as proteins, peptides and monoclonal antibodies. For instance, in industrial-scale protein production processes, IEX media are often used for initial product capture and intermediate purification stages as well as the final polishing steps. This separation technique offers several advantages compared to other chromatographic methods, e.g. high capacity and fast throughput.
Modern IEX resins for process scale based on hydrophilic polymer beads provide efficient purifications as they show low backpressures and can be used at high flow rates. Process resins offering a high dynamic binding capacity (DBC) further increase the efficiency of downstream processes as they provide higher sample loadings. If the IEX media are available in different particle sizes with full scalability, the flexibility in process development is increased leading to tailored solutions. Therefore, IEX resins that meet these demands can improve the economics and efficiency of downstream processes.

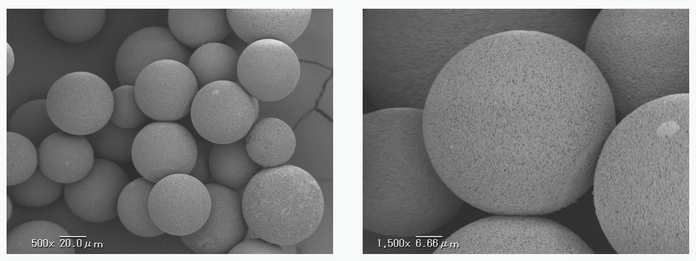
Resin characteristics

For the purification of biomolecules, high demands are required from the resin used. Modern IEX resins for process scale have to show highly stable performance under varying conditions.
Factors that are important for efficient purification processes include:

- **High DBC for high loadability**
- **Low back pressures**
- **Flexibility in process development**
- **CIP stability**



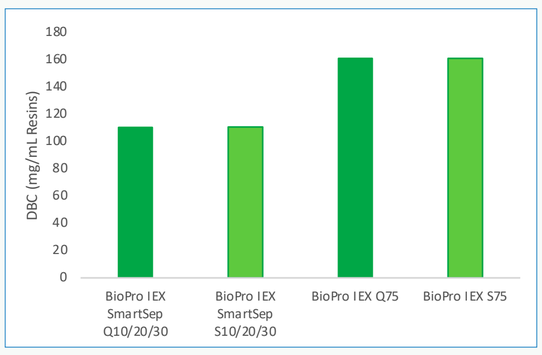
Hydrophilic polymethacrylate beads provide excellent mechanical stability and chemical resistance and lead to low back pressures for efficient purification processes.



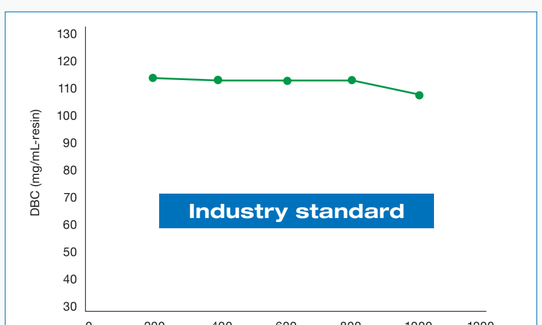
BioPro IEX S75 Particles

High DBC for high loadability

The loadability is determined by the dynamic binding capacity of an ion exchange resin. The higher the DBC of a resin, the higher is its productivity. All BioPro IEX resins show high DBC values for various target molecules.
The specified DBC for the resins is shown in the following table.



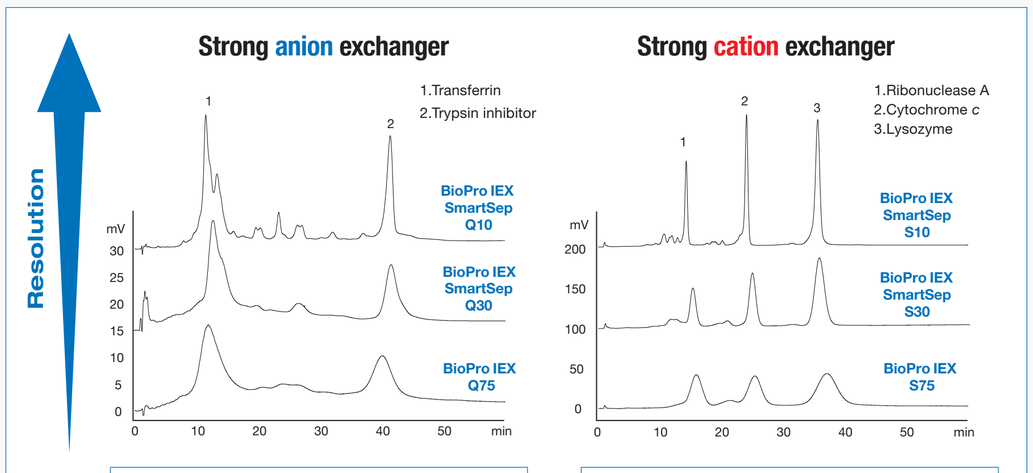
High sample loading at high flow rates increases the efficiency of purification processes. The dynamic binding capacity of BioPro IEX is excellent even at high flow rates.
When compared to the industry standard it consistently exhibits a higher dynamic binding capacity. This results in higher sample loading in preparative processes.



Column:	50 x 5.0 mm ID
Equilibration buffer:	20 mM Glycine-NaOH (pH 9.0)
Linear velocitiy:	200-1,000 cm/hr (0.66 mL/min-3.27 mL/min)
Sample:	1.5 mg/ml Lysozyme in equilibration buffer

High resolution separations

The BioPro IEX resins are available in different particle sizes (75 µm, 30 µm, 20 µm and 10 µm) which are fully scalable. The increased resolution of smaller particles results in the separation of closely eluted impurities. The identical chromatographic selectivity of BioPro series across different particle sizes enables easy method transfer between large and small particles.



Column:	50 x 4.6 mm ID
Elution buffer:	A) 20 mM Tris-HCl (pH 8.1) B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl
Gradient:	0–80%B (0–60 min)
Linear velocity:	180 cm/hr (0.5 ml/min)
Temperature:	30° C
Detection:	UV at 220 nm

Column:	50 X 4.6mm ID
Elution buffer:	A) 20 mM NaH_2PO_4-Na_2HPO_4(pH 6.8) B) 20 mM NaH_2PO_4-Na_2HPO_4(pH 6.8) containing 0.5 M NaCl
Gradient:	0–100%B (0–60 min)
Linear velocity:	180 cm/hr (0.5 ml/min)
Temperature:	30° C
Detection:	UV at 220 nm

Low backpressure and high flow rates

The pressure flow characteristics of IEX resins determine its productivity and are mainly based on the physical properties of the base beads used for the resin. Here, the pressure flow characteristics of BioPro IEX SmartSep S30 was experimentally investigated by measuring a pressure-flow curve.
Based on these results, the column packed with BioPro IEX SmartSep S30 can be operated at 3.000 cm/h considering the specified pressure maximum of the resin of 30 bar.

Study design

Preparation of the resin

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Packing of the resin into a 10 cm ID column
Consolidated bed height: 10.5 cm

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Stepwise increase of the flowrate
(100 cm/h steps)

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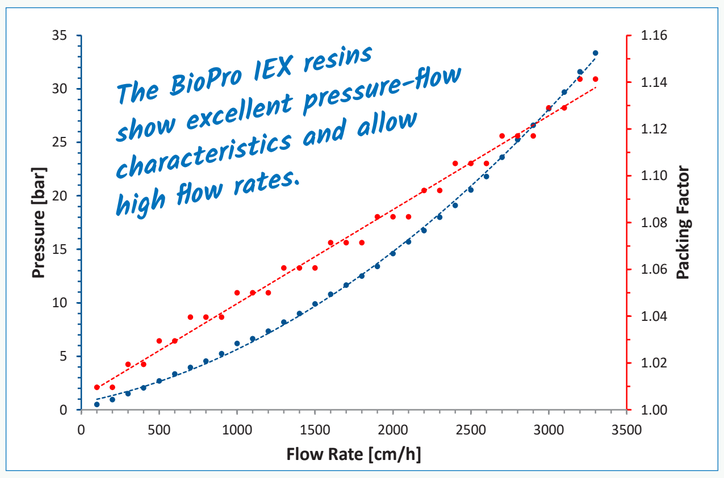
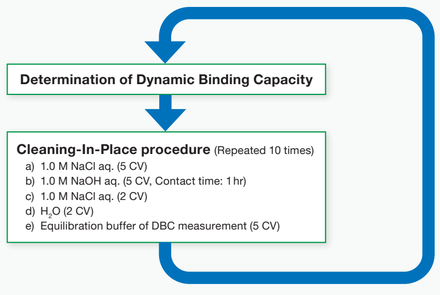
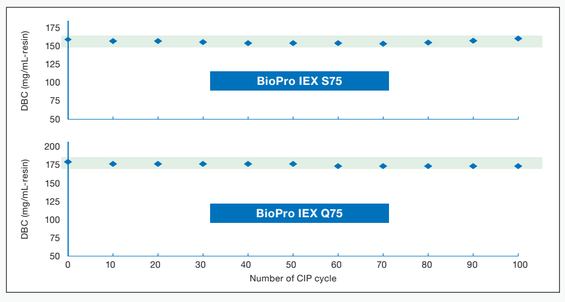
Monitoring of the resulting back pressure
and the degree of compresion
(packing factor)

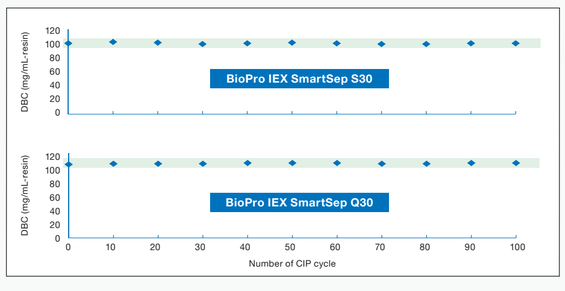


Stability towards Cleaning-in-Place Procedures

Cleaning-in-place (CIP) is essential for the economic use of packed chromatography columns. Efficient cleaning procedures increase the lifetime of the separation process and thereby contribute to the overall cost effectiveness. The BioPro IEX resins are fully compatible to typical CIP procedures as shown in this stability study.







DBC (IgG) (BioPro IEX SmartSep S30, BioPro IEX S75)			
Column:	50 × 5.0 mm ID	Temperature:	25°C
Equilibration buffer:	20 mM citric acid-NaOH (pH 5.3)	Detection:	UV at 280 nm
Elution buffer:	Equilibration buffer containing 0.5 M NaCl	Sample:	1.5 mg/mL human polyclonal IgG in equilibration buffer
Flow rate:	200 cm/hr (0.66 mL/min)		

DBC (BSA) (BioPro IEX SmartSep Q30, BioPro IEX Q75)			
Column:	50 × 5.0 mm ID	Temperature:	25°C
Equilibration buffer:	20 mM Tris-HCl (pH 8.6)	Detection:	UV at 280 nm
Elution buffer:	Equilibration buffer containing 0.5 M NaCl	Sample:	1.5 mg/mL BSA in equilibration buffer
Flow rate:	200 cm/hr (0.66 mL/min)		

CIP cycle	
Column:	50 × 5.0 mm ID
Flow rates:	200 cm/hr (1.0 M NaCl, H_2O, Buffer) 30 cm/hr (1.0 M NaOH)
Temperature:	25°C

Specifications

BioPro IEX Series	BioPro IEX Q75	BioPro IEX SmartSep Q30	BioPro IEX SmartSep Q20	BioPro IEX SmartSep Q10	BioPro IEX S75	BioPro IEX SmartSep S30	BioPro IEX SmartSep S20	BioPro IEX SmartSep S10
Ion exchange type	Strong anion exchanger				Strong cation exchanger			
Charged group	-R-N-$(CH_3)_3$				-R-SO_3^-			
Matrix	Hydrophilic polymer beads							
Pore Size	porous							
pH Range	2–12							
Particle size	75 µm	30 µm	20 µm	10 µm	75 µm	30 µm	20 µm	10 µm
Pressure resistance	0.3 MPa	2 MPa Max. 3 MPa		3 MPa Max. 4 MPa	0.3 MPa	2 MPa Max. 3 MPa		3 MPa Max. 4 MPa
Typical flow rate	200–1.000 cm/hr Max. 2.000 cm/hr							
Ion exchange capacity	0.10 meq/mL Resin	0.08 meq/mL Resin			0.10 meq/mL Resin	0.08 meq/mL Resin		
Dynamic binding capacity	Min. 160 mg/mL Resin (BSA)	Min. 100 mg/mL Resin (BSA)			Min. 160 mg/mL Resin (Lysozyme)	Min. 100 mg/mL Resin (Lysozyme)		

Regulatory support file available under non-disclosure agreement
Used in validated cGMP-manufacturing processes
Customised material available on request
DMF registered with FDA

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