

Purification of Monoclonal Antibodies with BioPro SmartSep

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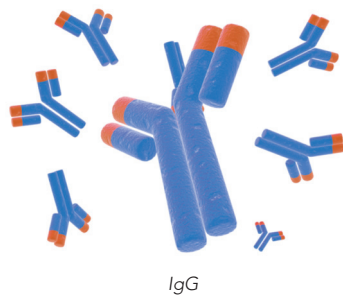
Ion exchange (IEX) is widely used for the analysis and purification of bio-molecules such as proteins, peptides and monoclonal antibodies. In industrial-scale production, IEX is used for initial capture, intermediate purifications or final product polishing.

Challenges During mAb Purification

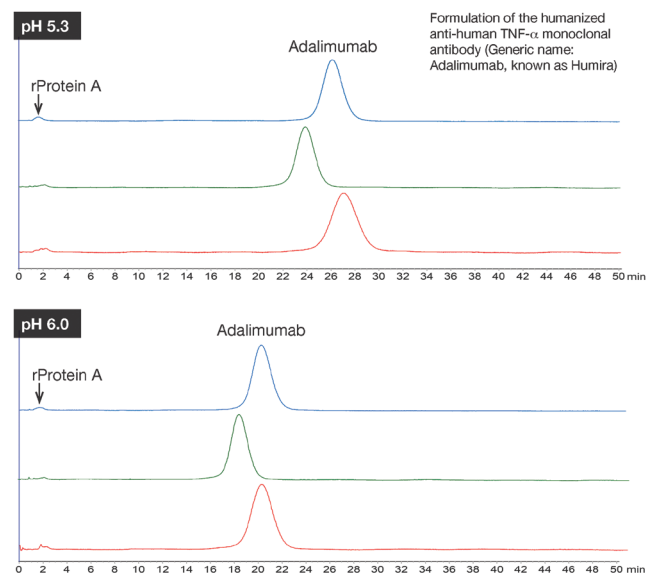
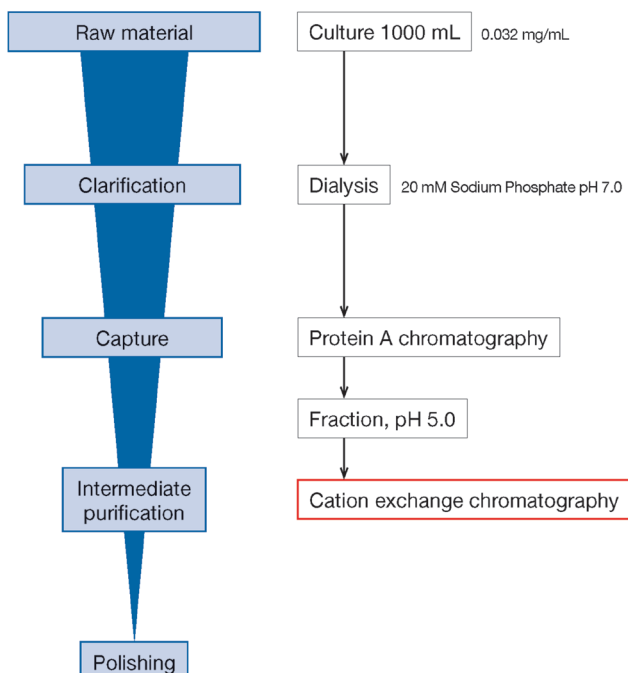
For purification of monoclonal antibodies, high demands are required from the separating material. Factors influencing the binding characteristics of IgG are pH, linear velocity and/or salt concentration (conductivity) at the time the sample is loaded onto the column.

Therefore, a material with highly stable performance is required with regard to all those factors. In order to demonstrate the performance of YMC-BioPro materials, several studies have been performed.

- Purification of Adalimumab
- Influence of pH
- Influence of linear velocity
- Influence salt concentration



Purification Scheme for Adalimumab



Column:	50 x 5.0 mm ID	BioPro Smart Sep S30
Eluent:	A) 20 mM citric acid-NaOH (pH 5.3) B) Eluent A containing 0.5 M NaCl	GE Source 30S
Gradient:	0-100% B (0-30 CV)	Tosoh TSKgel SP-3PW
Flow rate:	180 cm/hr (0.59 mL/min)	
Temperature:	ambient	
Detection:	UV at 280 nm	
Sample:	Anti-hTNF α (after affinity chromatography)	
IgG Load:	0.1 mg	
Injection:	0.25 mL	

In order to demonstrate the behaviour of BioPro SmartSep under different elution conditions, experiments with different values of pH, linear velocity and salt concentration were performed and the dynamic binding capacity (DBC) recorded.

The parameters changed were:

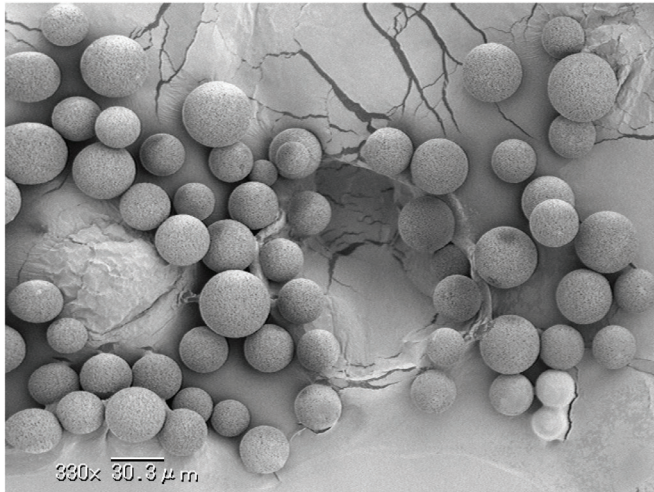
Experimental Conditions

pH: 6.0 vs. 5.3

Linear velocity: 200 - 800 cm/hr

Salt concentration: 0 - 50 mM NaCl

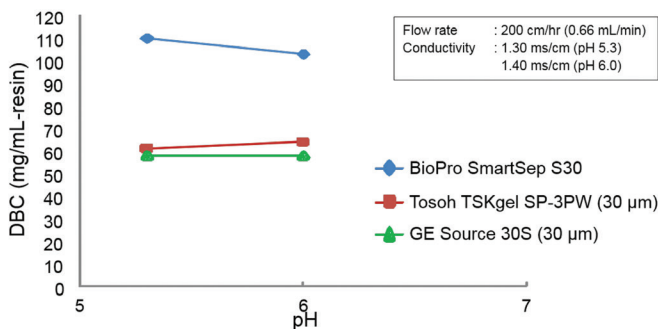
Column: 50 x 5.0 mm ID
 Equilibration buffer: 20 mM citric acid-NaOH buffer (pH 5.3 or 6.0)
 Elution buffer: Equilibration buffer containing 0.5 M NaCl
 Flow rate: 200 - 800 cm/hr (0.66-2.62 mL/min)
 Temperature: ambient (25°C)
 Detection: UV at 280nm
 Sample: 1.5 mg/mL human polyclonal Adalimumab in equilibration buffer



BioPro SmartSep S30 particles

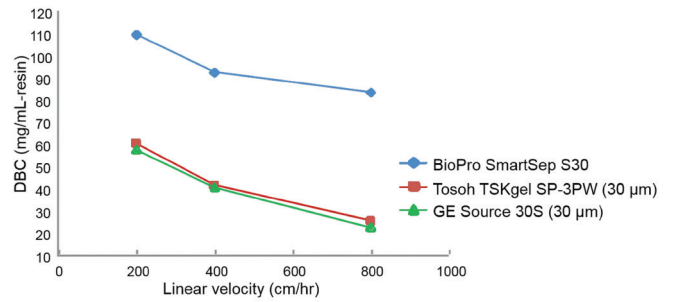
Influence of pH

pH	DBC (mg/mL-resin, 10% breakthrough)	
	pH 5.3	pH 6.0
BioPro SmartSep S30	110	103
Tosoh TSKgel SP-3PW (30 μm)	61	64
GE Source 30S (30 μm)	58	58



High binding capacities are achieved regardless of elution of pH. Therefore, milder eluting conditions for Adalimumab can be selected.

Linear velocity	DBC (mg/mL-resin, 10% breakthrough)		
	200 cm/hr	400 cm/hr	800 cm/hr
BioPro SmartSep S30	110	93	84
Tosoh TSKgel SP-3PW (30 μm)	61	42	26
GE Source 30S (30 μm)	58	41	23

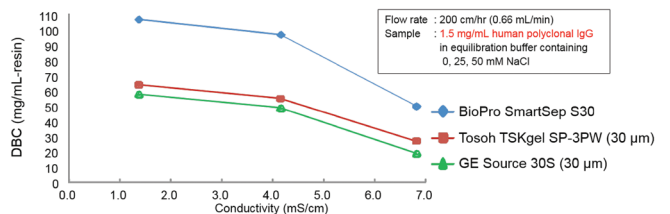


Influence of Linear Velocity

BioPro SmartSep maintains higher binding capacity values over a wider range of linear velocity. This will increase product throughput for the purification work without loss of efficiency.

Influence of Salt Concentration

	DBC (mg/mL-resin, 10% breakthrough)		
pH	5.3		
NaCl concentration	0 mM	25 mM	50 mM
Conductivity	1.36 mS/cm	4.14 mS/cm	6.8 mS/cm
BioPro SmartSep S30	107	97	50
Tosoh TSKgel SP-3PW (30 μm)	64	55	27
GE Source 30S (30 μm)	58	49	19



BioPro SmartSep has higher salt concentration tolerance. This simplifies the desalting process after Protein A chromatography and will help to shorten the production process.

Conclusions

BioPro SmartSep materials meet the highest demands for the purification of monoclonal antibodies. High binding capacity is achieved regardless of elution of pH, linear velocity or salt concentration. This allows purification processes to be carried out more efficiently.

- Milder eluting conditions can be selected
- Higher throughput at stable efficiencies
- Simplification of desalting processes for shorter processes