High Throughput Purification Scaling Up Study of Polyclonal Antibody Using MEP HyperCel™ Chromatography Sorbent

Chromatography Sorbent Screening Step

- **Protein A** was tested for different elution pHs
- **MEP HyperCel** being a mixed-mode sorbent, elution was tested at several pHs
- **CEX1 and CEX2** sorbents: Screening of binding conditions (pH variation), elution at 250, 500 and 1000 mM NaCl

Transfer on 5 mL Column

Optimization Step on MEP HyperCel Sorbent

- **Optimisation #1**: pH and conductivity adjustment to optimize binding conditions
- **Optimisation #2**: Increase of residence time

The modification of binding conditions (pH, conductivity, increase of residence time) allowed a 30% increase of pAb binding to the sorbent.

Transfer to 100 mL and 1 L Packed Columns

Synthesis of MEP HyperCel Sorbent Results on Columns From 5 mL to 1 L Scale

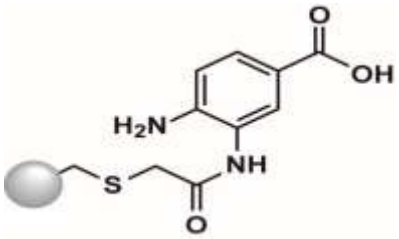
	Initial data	5 mL (Optimis. #2)	100 mL	1 L scale
Purity (Caliper GXII or SDS-PAGE)	~53-70%	99%	90-95%	96%
Aggregation by SEC	~6-9%	3.3 %	3.4%	NM
Yield of MEP HyperCel sorbent step	-	96%	~100%	97%

- Efficient process with MEP HyperCel sorbent in bind/elute mode: a single chromatography step post caprylic acid pretreatment allowed up to 97% pure monomers
- High yield of capture step with MEP HyperCel sorbent (>97%)
- Best performance (DBC, pAb purity and aggregate removal) with MEP HyperCel sorbent
- Mixed-mode chromatography with MEP HyperCel sorbent: A powerful tool to address future challenges in purification of antibodies and emerging biomolecules while reducing cost

Mixed-Mode Sorbent Family:

CMM HyperCel & MEP, HEA and PPA HyperCel™

MAbs and recombinant proteins, Alternatives to HIC, No-salt or Low-salt

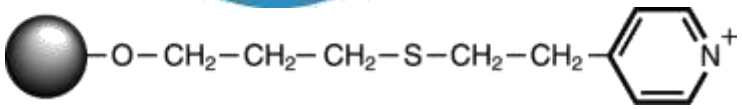


CMM HyperCel™

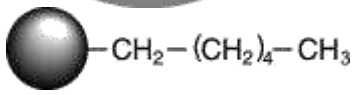
- Negatively charged at working pH
- Hydrophobic

- Neutral or positively charged at working pH Hydrophobic

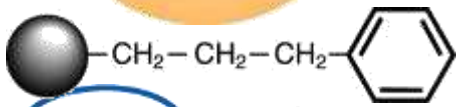
MEP HyperCel™



HEA HyperCel



PPA HyperCel



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CMM HyperCel - Main Properties

Main Properties

Particle size range	50-80 µm
Ligand description	Aminobenzoic acid
Ligand density	Av. 70 µeq/mL
Dynamic binding capacity – BSA(1) – IgG(2)	 >50 mg/mL at pH 4.5, 15 mS/cm >60 to 100 mg/mL at pH 4.0 to 5.0, 4 to 12 mS/cm
Working conditions – Binding – Elution	 pH ~ 4 to 6; conductivity up to 50 mS/cm(3) pH ~ 4 to 9; conductivity up to 50 mS/cm(3)
Working pressure at 1,000 cm/hr(4)	~ 1.0 bar g
Working pH	2 to 13
Cleaning pH	1 to 14
Cleaning in place	1 M NaOH - 1 hour contact time - 5 CV

(1) 4 g/L BSA in 50 mM Na acetate complemented with NaCl, 7 minutes residence time.

(2) Conductivity adjustment with NaCl (~ 0 - 0.5M)

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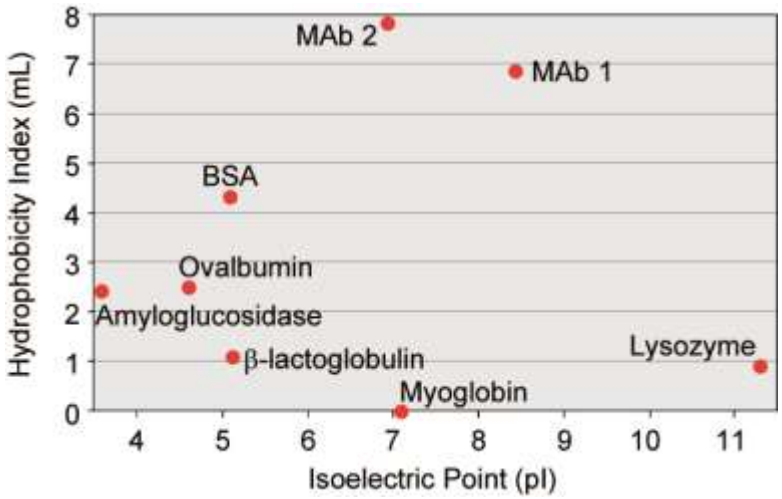
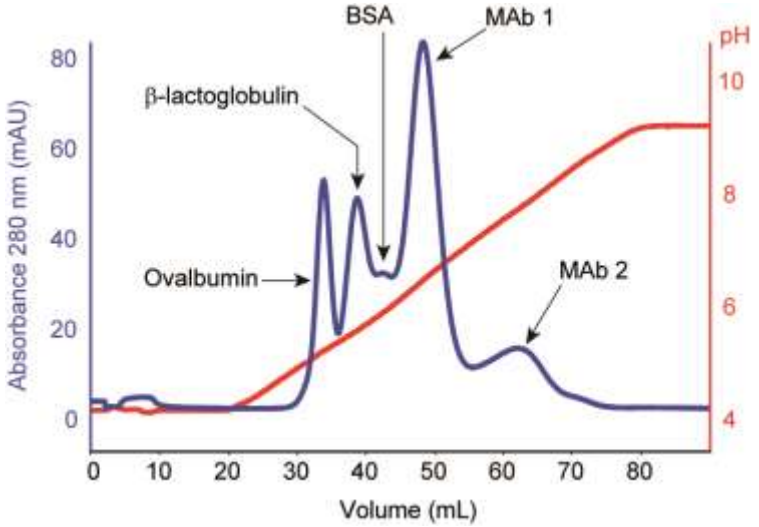
CMM HyperCel - Selectivity





- Load at 15 mS/cm
- pH Gradient from pH 4.5 to 10.0, 20 CV
- Column: 0.5 x 5 cm (1 mL)
- Flow rate: 1 mL/min

High selectivity leads to protein separation based on isoelectric point and hydrophobicity of each protein

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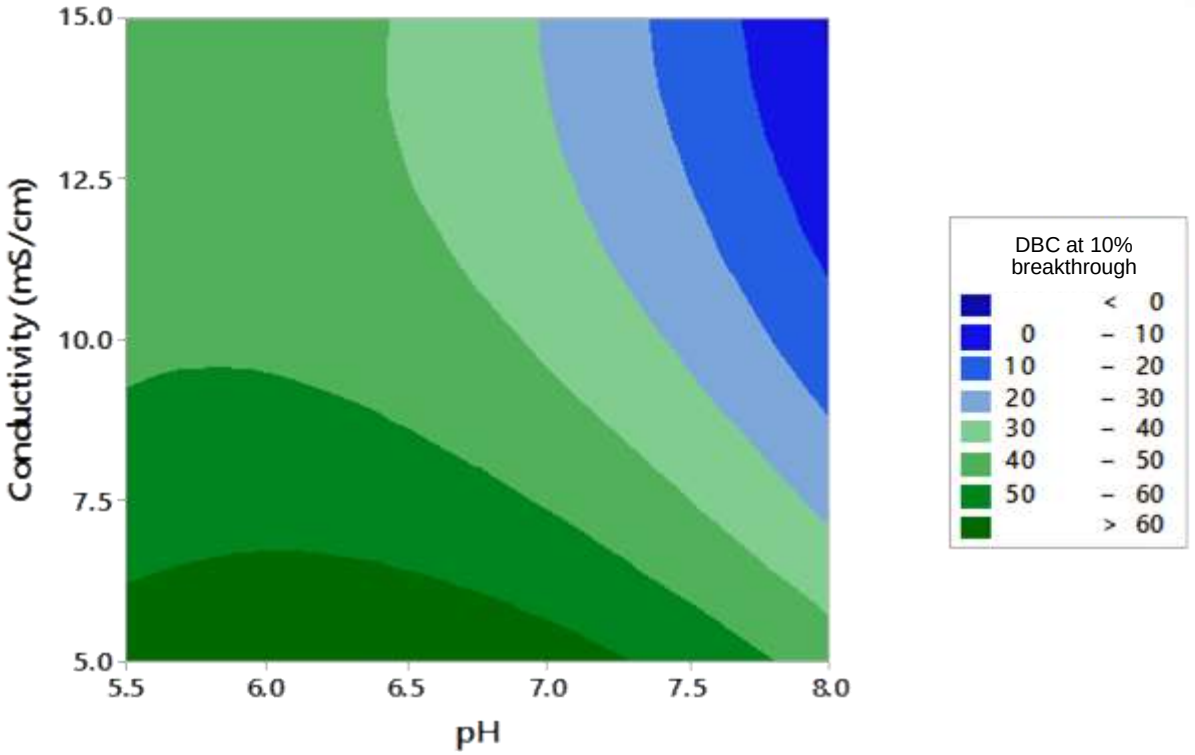
CMM HyperCel capacity: 10% mAbs breakthrough curve



- High capacity binding available at high pH, vs salt tolerant at low pH

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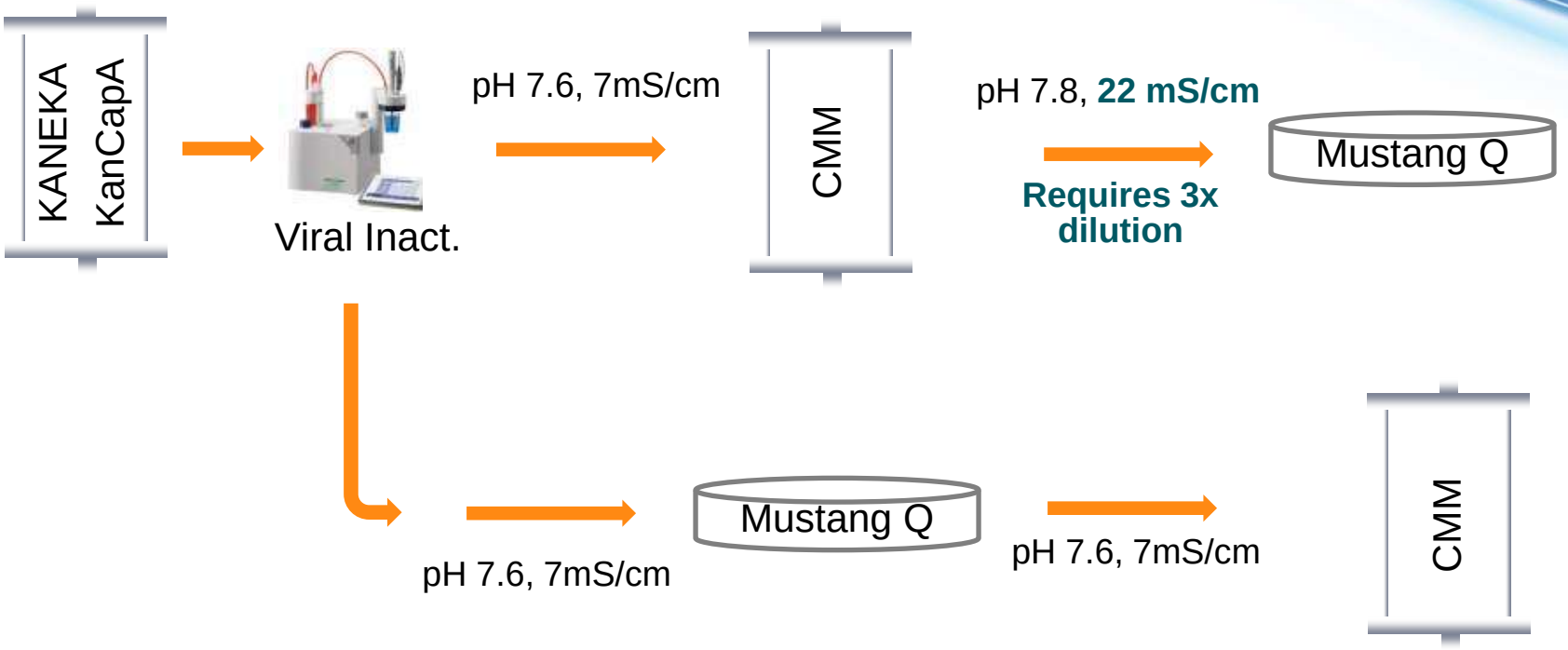
mAbs platform



Order of unit operations	HCP log reduction	% Aggregates
KANEKA KanCapA – CMM HyperCel – Mustang Q	4.1	1.1
KANEKA KanCapA – Mustang Q – CMM HyperCel	3.9	1.5

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Questions??