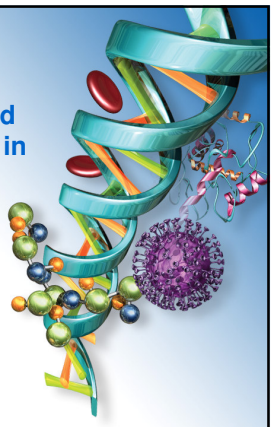
Ion Exchange and Mixed Mode Chromatography in Process Separations

SCI 2012 Technical Training
Cambridge, September 2012

Dr. Sylvio Bengio



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Outline

- Mixed Mode Chromatography : principles
- Purification of Monoclonal Antibody using a Two-Step Scheme without Protein-A
- Orthogonal separations: mixed mode and ion exchange chromatography, two case studies

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Mixed-Mode Chromatography

- Exploits multiple, distinct protein-ligand interactions to adsorb target proteins or impurities.
- Offers new solutions where traditional chromatographic methods are not effective.
  - *Where feedstream conductivity is too high for efficient capture on traditional ion exchange resins*
  - *Alternative/complement to conventional Hydrophobic Interaction (HIC) or hydroxyapatite*
  - *Separations where affinity ligands are too expensive*

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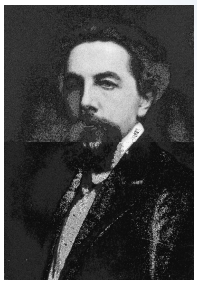
Mikhail Semenovich Tswett (1872-1919) the « *inventor* » of chromatography



" Like light rays in the spectrum, the different components of a pigment mixture, obeying a law, are resolved on the calcium carbonate column and then can be qualitatively and quantitatively determined. I call such a preparation a chromatogram, and the corresponding method the chromatographic method."

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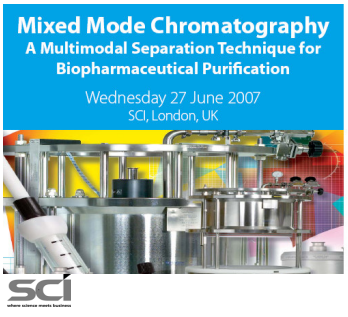
Mixed-Mode Chromatography



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Commercial Mixed Mode or Multi-Mode Ligands

- Hydroxyapatite (calcium phosphate)
- Trichlorotriazine dyes
- Multi-modal cation exchange (N-Benzyl-homocysteine)
- Multi-modal anion exchange (N-Benzyl-N methyl ethanolamine)
- 4-MEP (4-Mercapto Ethyl Pyridine)
- HEA (Hexylamine)
- PPA (Phenyl Propylamine)

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Key Sorbent design Parameters

Ligand density:	Cooperativity between ligands for protein adsorption in physiological conditions dictates high densities.
Hydrophobicity:	Since this parameter mediates the adsorption, the nature of the linker or of the activating agent is critical.
pK of the ligand:	This parameter influences the pH of elution of the antibody. To have mild desorption conditions this value has to be between 4 and 9.

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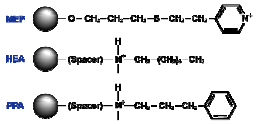
MEP, HEA, PPA HyperCel™

- **Binding:** dominant mode is hydrophobic interaction.
  - Binding typically achieved without addition of binding-promoting salt, or at significantly lower salt concentration than for conventional HIC.
- **Desorption:** driven by electrostatic charge repulsion.
  - Target pool typically recovered in dilute or relatively low conductivity buffer.



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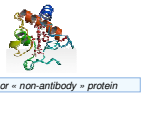
Interaction of 4-MEP Ligand with Antibody or Protein

Adsorption at near-neutral pH, physiological conditions

Hydrophobic interaction

pKa = 4.8



or « non-antibody » protein

Desorption at pH 4.0 - 5.8

Electrostatic repulsion

pH	% in (+) form
4.8	50%
5.8	10%

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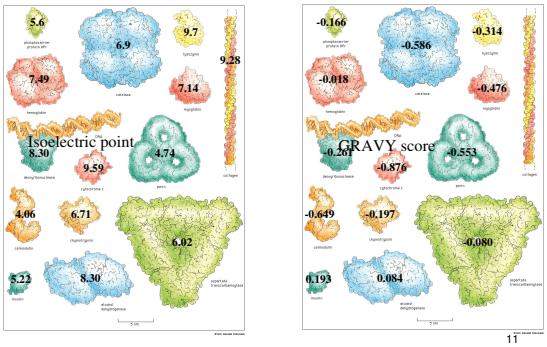
Proteins have different charges and hydrophobicities



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Optimization of Elution-pH on Mixed-Mode Sorbents

- pH 5.5
- pH 5.2
- pH 4.9
- pH 4.6
- pH 4.3
- pH 4.0
- pH 3.0

Elution of relatively basic proteins.

Elution of relatively acidic proteins.

Elution of relatively hydrophilic proteins.

Elution of relatively hydrophobic proteins.

Elution of basic / hydrophilic impurities

Elution of target antibody or other target protein

Elution of acidic / hydrophobic impurities

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Final Three Step Elution Sequence

- pH 5.5
- pH 4.6
- pH 3.0

Elution of relatively basic proteins.

Elution of relatively acidic proteins.

Elution of relatively hydrophilic proteins.

Elution of relatively hydrophobic proteins.

Elution of basic / hydrophilic impurities.

Elution of target antibody or other target protein

Elution of acidic / hydrophobic impurities.

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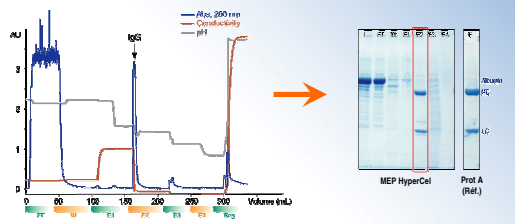
Purification of Monoclonal IgG1 on MEP HyperCel™ Sorbent



Elution: E1: 100 mM Na Ac pH 5.5, NaCl 0.5 M
E2: 50 mM Na Ac pH 5.0
E3; E4: 50 mM Na Ac pH 4.0; 3.0

Optimized elution step at pH 5.0 leads to IgG purity of 97% and yield of 93% (albumin eluted at pH 5.5 in the presence of 0.5 M NaCl)

Courtesy of S.Allen, ABBOTT Diagnostics Division, IL, USA and S. Chaudoreille, Pall R&D Applications Group, Cergy (F)

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Mixed-Mode Chromatography for Post-Capture Purification of Monoclonal Antibodies

- Capture on Protein A sorbent as a standard method ; Cation exchange in some cases
- Post-capture typically done by IEX or HIC
- Mixed-mode sorbents of growing interest because
  - Orthogonal to other methods (cation exchange, hydroxyapatite)
  - Optimization allows selective desorption of MAb from contaminants (HCPs, aggregates).
  - Contributes to decrease purification costs.

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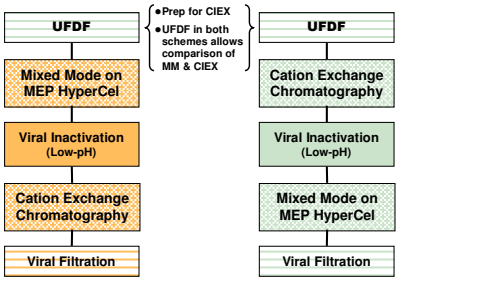
Scale-Up of Mixed Mode Chromatography

Scale-up on MEP HyperCel sorbent, presented by Dr. Richard Francis, SCI Mixed Mode Conference, London, June 2007

Industry Case Studies

- 1. Two-Column Processes to Purify Monoclonal Antibodies Without use of Protein-A Sorbent
- 2. Combination of mixed mode chromatography and membrane adsorbers

Two-Column Processes to Purify Monoclonal Antibodies Without use of Protein-A Sorbent



A Two-Column Process to Purify Antibodies Without Protein-A, G.M. Ferreira, J. Dembecki, A. Patel, A. Arunakumari, BioPharm International, May 1, 2007. See Figure-3.

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Purification of Monoclonal Antibody using a Two-Step Scheme without Protein-A

Mode	In Eluate or Load	HuMAb-1	HuMAb-2
Mixed Mode on MEP HyperCel	HCP in load (ng/mg)	323,279.	157,069.
	HCP in eluate (ng/mg)	3,470.	1,810.
	DNA in load (pg/mg)	138,091.	783.
	DNA in eluate (pg/mg)	4.12	0.19
	Purity (% monomer in eluate)	99.91	98.82
	Step Recovery (%)	83.	84.
Cation Exchange	HCP in eluate (ng/mg)	68.	24.
	DNA in eluate (pg/mg)	0.4	0.2
	Purity (% monomer in eluate)	100.	99.82
	Step Recovery (%)	85.	84.
Overall Recovery (%)		71.	71.

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Purification of MAbs using a Two-Step Scheme without Protein-A

Mode	In Eluate or Load	HuMAb-6	HuMAb-7
Cation Exchange	Column Volume (L)	5.3	5.0
	HCP in load (ng/mg)	54,502.	251,467.
	HCP in eluate (ng/mg)	1,047.	379.
	DNA in load (pg/mg)	nd	1,140,000.
	DNA in eluate (pg/mg)	nd	20
	Purity (% monomer in eluate)	98.47	99.87
	Step Recovery (%)	84.	88.
Mixed Mode on MEP HyperCel	Column Volume (L)	10.6	4.7
	HCP in load (ng/mg)	1047.	366.
	HCP in eluate (ng/mg)	39.	11.
	DNA in load (pg/mg)	nd	20.
	DNA in eluate (pg/mg)	< 6	< 1
	Purity (% monomer in eluate)	98.85	99.94
	Step Recovery (%)	95.	92.
Overall Recovery (%)		80.	81.

"A Two-Column Process to Purify Antibodies Without Protein-A", G.M. Ferreira, J. Dembecki, A. Patel, A. Arunakumari, BioPharm International, May 1, 2007. See Table-2.

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Viral Clearance Studies using A-MuLV

	HuMAb-6 Log_{10} PFU Reduction	HuMAb-7 Log_{10} PFU Reduction
Viral Challenge	• 2000 mg dose • 9.87 logs infectious & non-infectious viral particles	• 1000 mg dose • 9.33 logs infectious & non-infectious viral particles
Cation Exchange Chromatography on Fractogel SE Hicap	2.23	2.95
Low pH Treatment	5.04	>5.69
Mixed Mode Chromatography on MEP HyperCel	5.15	>4.59
Viral Filtration	>4.92	>5.95
TOTAL REDUCTION	>17.34	>19.18

A Two-Column Process to Purify Antibodies Without Protein-A, G.M. Ferreira, J. Dembecki, A. Patel, A. Arunakumari, BioPharm International, May 1, 2007. See Table-3.

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The Protein'eXpert group

PX'Therapeutics Presentation

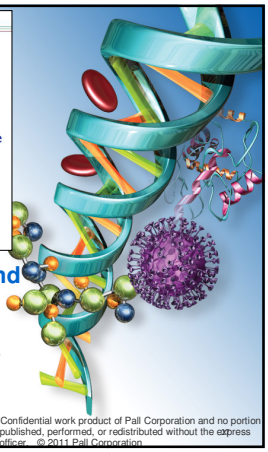
Case Study:

Optimisation of mixed-mode intermediate purification on *E. coli* protein and subsequent membrane polishing step

N. Maguet

Presented at SBCN 2010, october, 19th

Ecole Centrale de Lyon, Ecully, France



Featuring MEP HyperCel™ and Mustang® Q

Courtesy of Nicolas Maguet, PX'Therapeutics France

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The Protein'eXpert group

Manufacturing process: Flowchart

Capture step is performed on cation exchange to maintain a pH below 7 and to elute with a low salt concentration.

Intermediate purification step has been tested on different HIC and multimodal media: the protein of interest is not binding on HIC.

Multimodal (mixed-mode) medium allow the binding and purification of the protein of interest.

Polishing step on ion exchange capsule to reduce DNA and endotoxin levels.

1. *E. Coli* Culture
2. Cell harvest
3. Cell disruption
4. Clarification with depth filtration
5. Capture on Cation exchange
6. Intermediate purification on MEP HyperCel
7. Concentration and diafiltration by TFF
8. Polishing on Mustang Q
9. Formulation

➢ **Objective: determine and improve the intermediate purification step (MEP HyperCel)**

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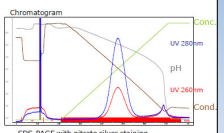
MEP HyperCel: pH gradient elution (MEP01)

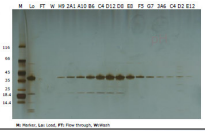
➢ **Separation parameters**

- Resin: 16mL of MEP HyperCel™ (Pall Life Sciences)
- Starting material: pooled fractions from cation exchange
- Loading buffer: 10 mM sodium phosphate, 100 mM NaCl pH 6.0
- Residence time: 8 min
- Elution buffer: 5 mM citric acid pH 3.0
- Elution gradient on 25 CV from 0% to 100% with elution buffer and step on 5 CV of 100% elution buffer

Chromatogram



SDS-PAGE with nitrate silver staining



➢ **Results**

- **Capture of the protein of interest onto the MEP resin**
- **Recovery: 61%**
- **The elution pH of the protein of interest is at 5.3**

«MEP01» Loading at pH6, pH gradient down to 3 SEE ABOVE

«MEP02» Loading at pH6, steps pH 5 and pH 3

«MEP03» Loading at pH6, steps pH 5,75; pH 5,3 and pH 3 NEXT SLIDE, BEST RESULTS

«MEP04» Loading at pH6, steps pH 5,8; pH 5,45 and pH 3

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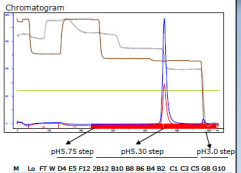
The Protein'eXpert group

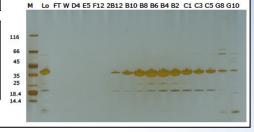
MEP HyperCel: step elution (MEP03)

➢ **Separation parameters**

- Resin: 16mL of MEP HyperCel™ (Pall Life Sciences)
- Starting material: pooled fractions from Cation exchange
- Loading buffer: 10 mM sodium phosphate, 100 mM NaCl pH 6.0
- Residence time: 8 min
- Elution buffers and steps:
  1/ 10 CV of 20 mM sodium acetate **pH5.75**
  2/ 10 CV of 20 mM sodium acetate **pH5.3**
  3/ 5 CV of 5mM citric acid **pH3.0**

Chromatogram





➢ **Results**

- **Full capture of the protein of interest onto the MEP resin**
- **Recovery: 98%**
- **Improvement of the purity grade**

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MEP HyperCel: Scale-up X180

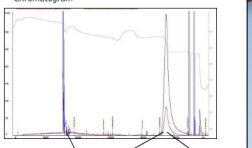
➢ Separation parameters

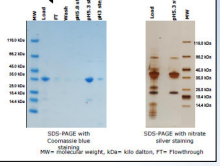
- Resin: **2.9 L** of MEP HyperCel™ (Pall Life Sciences),
- Column BPG™ 140/500 (GE Healthcare)
bed size= **ID 14 cm x H 18.5 cm**
- Starting material: pooled fractions from cation exchange
- Loading buffer: 10 mM sodium phosphate, 100 mM NaCl pH 6.0
- Residence time: 6 min
- Elution buffers and steps:
1/ 10 CV of 20 mM sodium acetate pH5.8
2/ 10 CV of 20 mM sodium acetate pH5.3
3/ 5 CV of 5 mM citric acid pH3.0

➢ Results

- **Little loss of the protein of interest in the flow through and the wash step**
- **Recovery: 93%**
- **Purity: >95%**

Chromatogram





SDS-PAGE with Coomassie blue staining

SDS-PAGE with nitrate silver staining

MW= molecular weight, kDa= kilo dalton, FT= Flowthrough

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The Protein'eXpert group

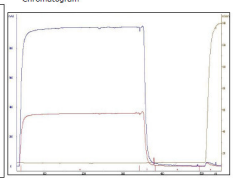
Polishing step on Mustang Q cartridge

Objectives: Endotoxins and DNA reduction by polishing step on Mustang Q capsule

➢ Separation parameters

- Medium: Mustang® Q XT5 capsule 5 mL (Pall Life Sciences)
- Starting material: pooled fractions from MEP HyperCel purification step after buffer exchange by diafiltration **(endotoxins level 0.08 UI/mg and residual DNA concentration 2 ng/mL)**
- Loading buffer: 20 mM sodium phosphate and 0.1 M NaCl, pH6.0
- Elution buffer (in case of capsule re-use):
1/ 20 mM sodium phosphate and 0.1 M NaCl, pH6.0
2/ 20 mM sodium phosphate and 2 M NaCl, pH6.0

Chromatogram



➢ Results:

- **Endotoxins level < 0.08 UI/mg protein of interest.**
- **The residual DNA concentration in the protein of interest solution is < 1 ng/mL.**

Mustang Q XT5 capsule, 5ml of membrane

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General Conclusions

- Mixed-mode chromatography can be easily combined to ion exchange for orthogonal separations.
- In these and other studies, MEP, HEA & PPA HyperCel sorbents have been efficient during capture & post-capture purification of MAb and non-MAb proteins, contributing to clearance of HCPs, aggregates, endotoxin and residual DNA.
- Selectivity is dependent on ligand structure, so high throughput screening of mixed mode sorbents is advised.
- Use of HTS in 96 well filter plates and Design of Experiments (DoE) can hasten optimization.

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Thank You !

sylvio_bengio@europe.pall.com