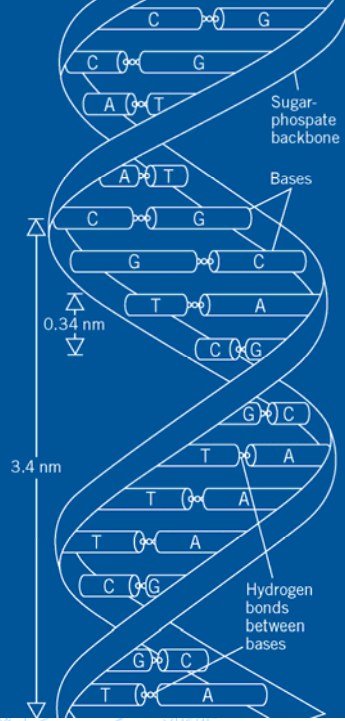

Radial Flow Chromatography Update

Genentech: Mark Garcia, Sushil Marwaha

Sepragen: Vinit Saxena, Salah Ahmed

12/10/2009

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Outline

Introduction
- **Technology Description**
- **Project Background and Goal**

Pilot Scale Evaluation
- **Packing Studies**
- **Comparability Runs**

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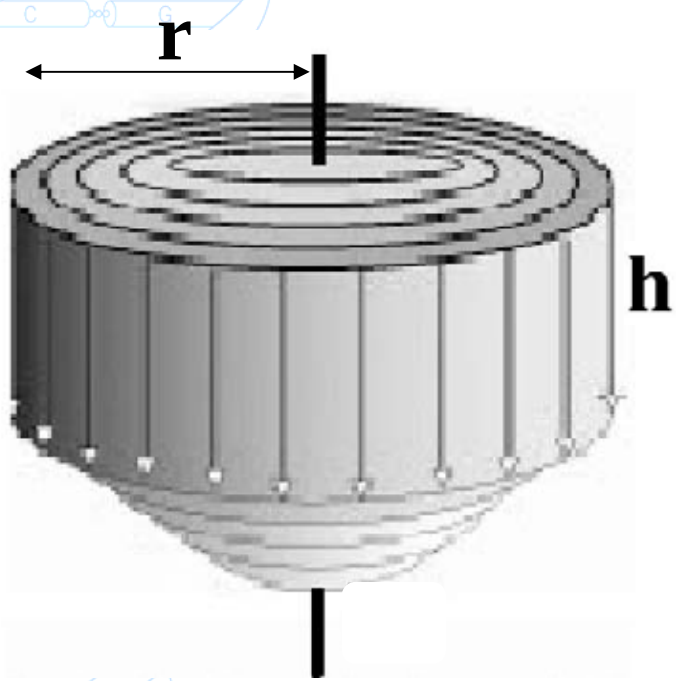
Conventional vs. Radial Flow Chromatography

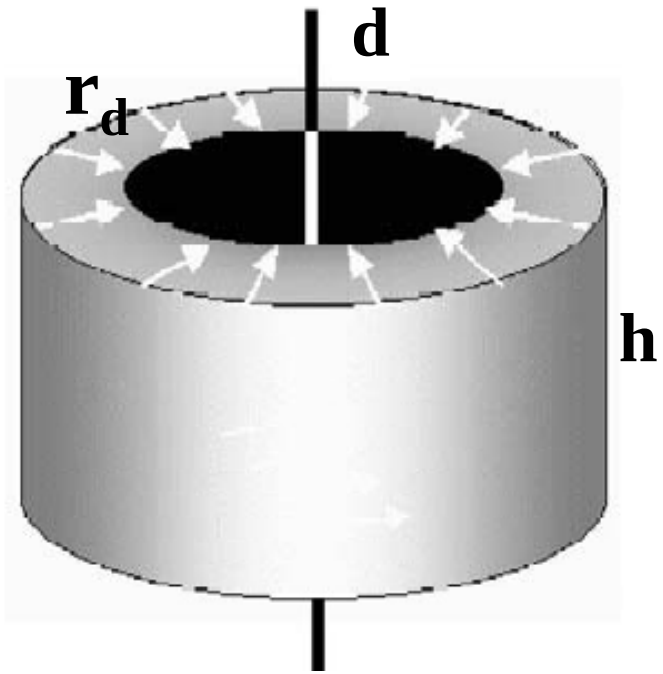
Axial Flow Chromatography (AFC)

- Effective cross-sectional area = $\pi \bullet r^2$
- Column back pressure as a function of bed height (h)
- Linear velocity (cm/h) affected by wall effects.



Radial Flow Chromatography (RFC)

- Effective cross-sectional area = height $\bullet \pi \bullet 2(r_d)$ where r_d = radius at a given bed depth
- Column back pressure as a function of bed depth (d)
- Linear velocity (cm/h) increases uniformly as approach center of column.



W. Pfeiffer / J. Chromatogr. A 1006 (2003) 149–170

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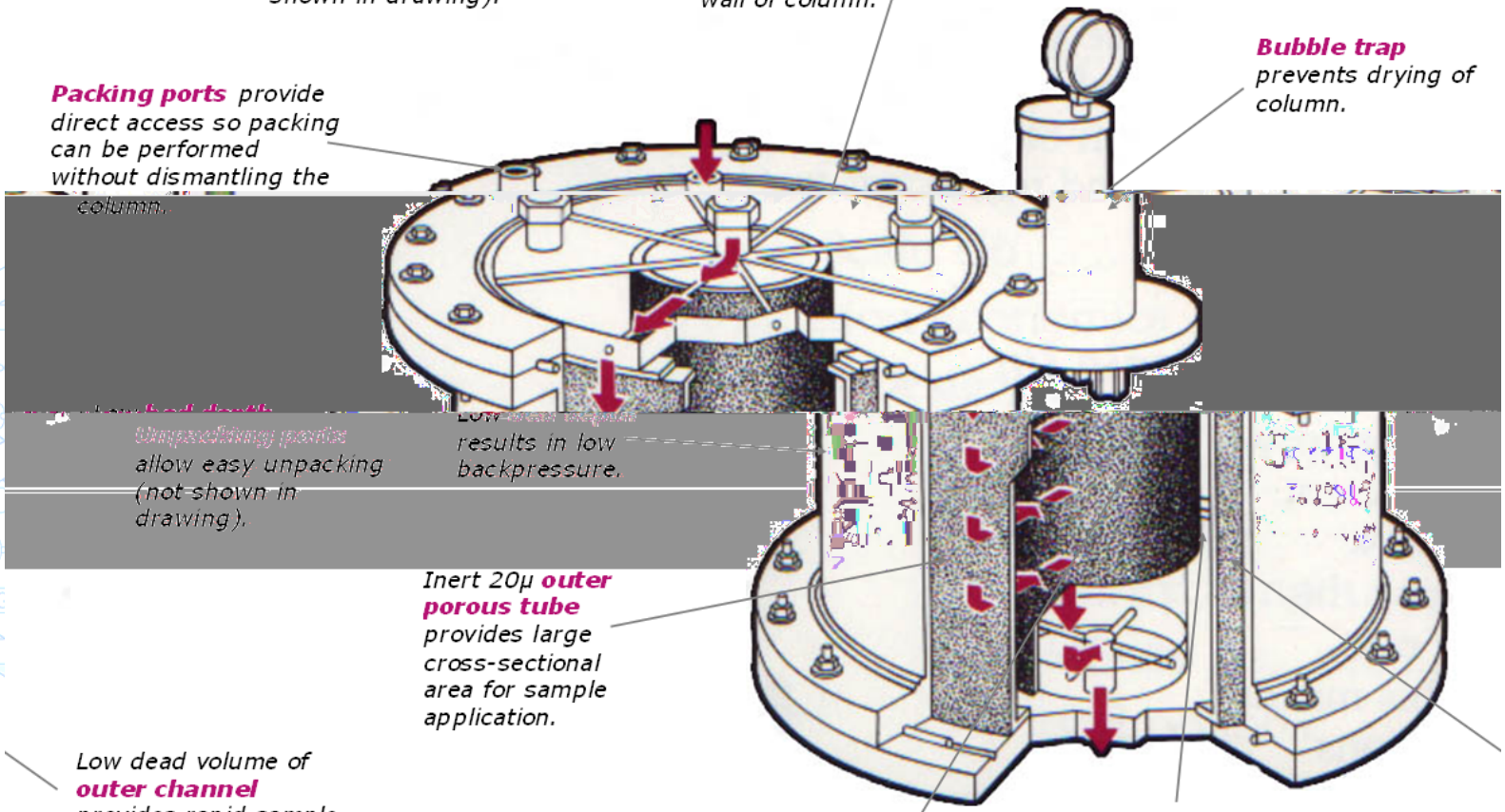
Original Column Design



Courtesy of Sepragen

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Project Goal

Project History

- Technology was first evaluated by Genentech in the 1990s
- Recently evaluated at lab scale for mAb A by Mark Smith (VV-MSAT)

Project Goal

- Evaluate scalability of radial flow chromatography using mAb A for a cation exchange application at pilot scale.

RFC Competitive Advantages

- Low back pressure compared to AFC
- Capable of performing at faster flow rates, leading to shorter processing times

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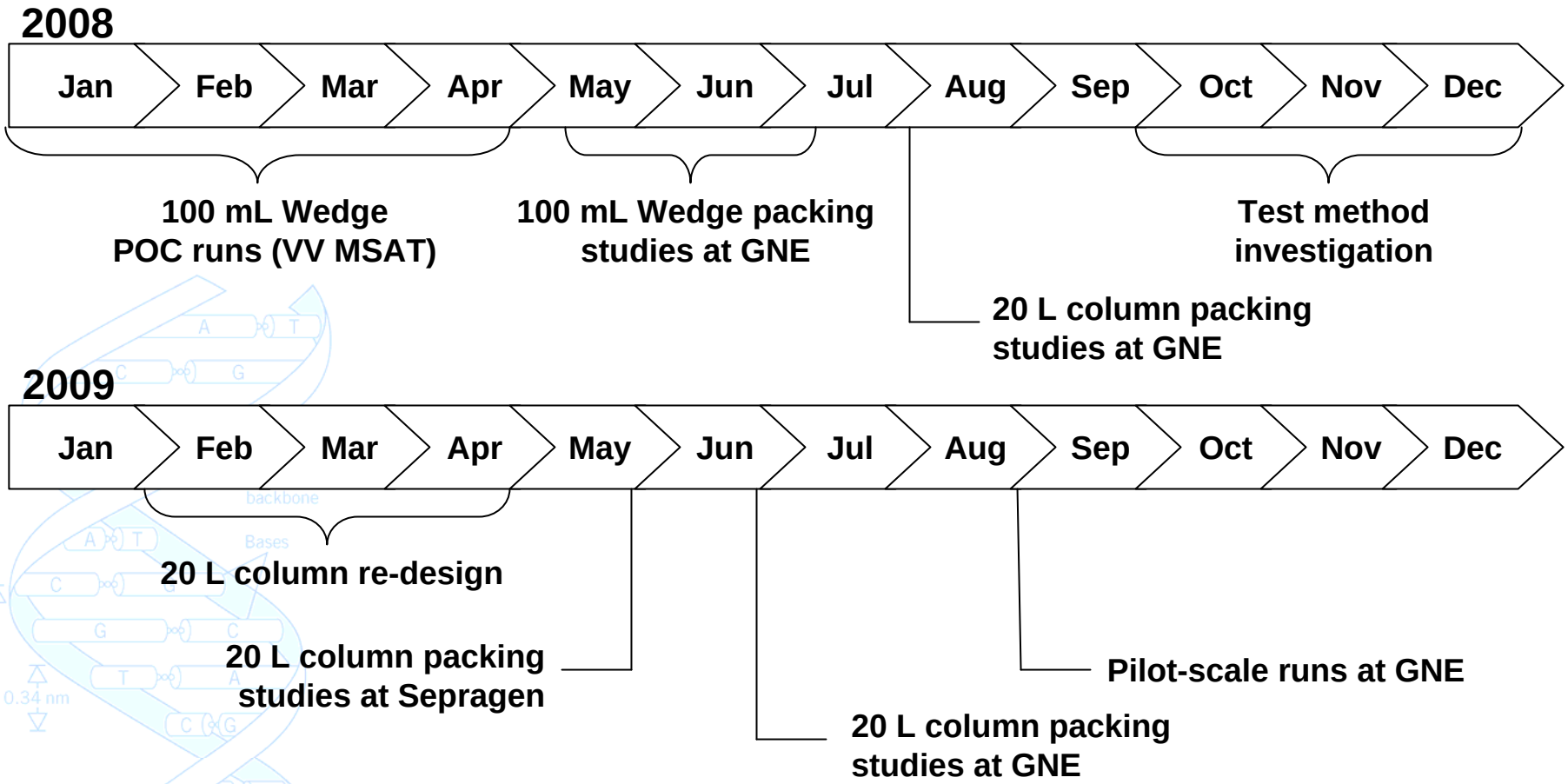
Project Timeline



Column re-design:

- Packing ports moved to improve packing efficiency
- Acrylic and stainless steel column prototypes were tested

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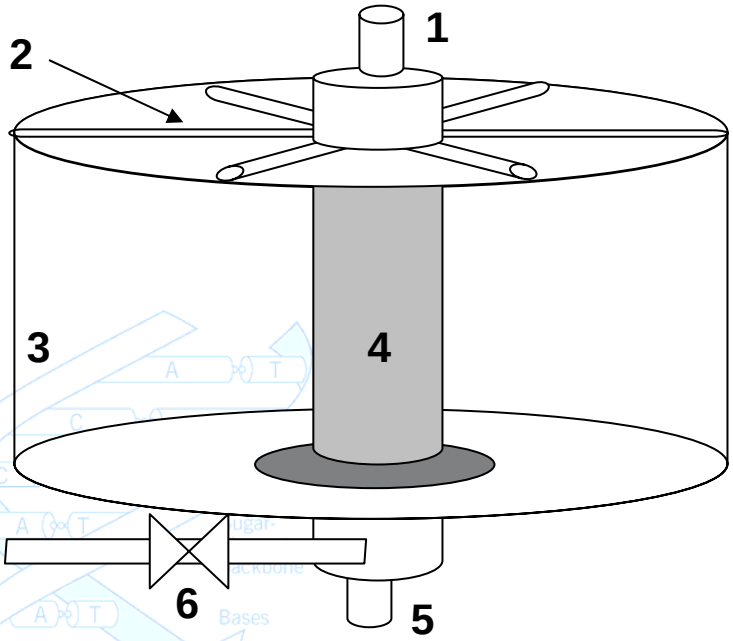
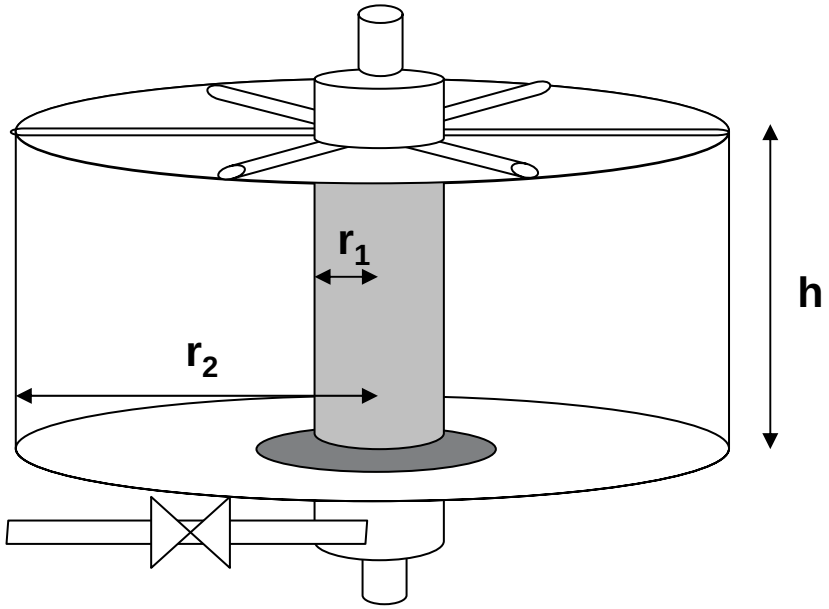
Prototype Column Design



1. Column inlet
2. Distribution channels
3. Column outer frit
4. Column inner frit
5. Column outlet
6. Packing port



$BV = (\pi \bullet r_2^2)\, h - (\pi \bullet r_1^2)\, h = 19.97\ L$

$r_1 = 8.1\ cm$
$r_2 = 19.7\ cm$
$h = 19.7\ cm$

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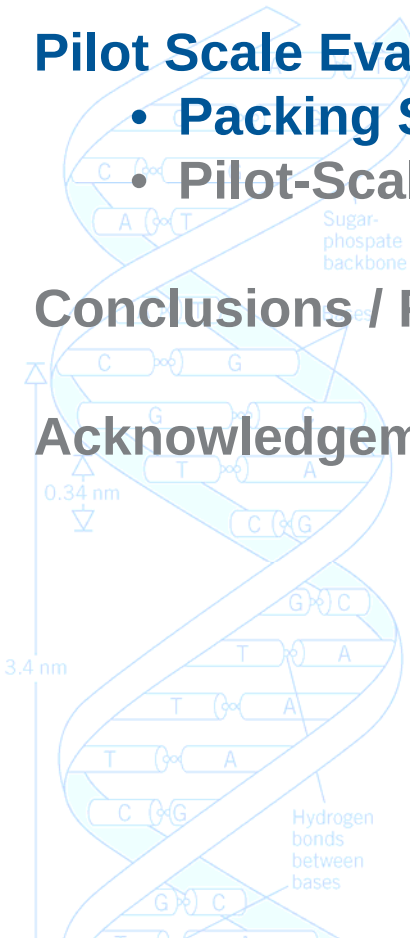

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Pilot-Scale Packing Evaluation

Goal: To define a procedure for packing of the 20 L RFC column.

	Axial Flow Column	Radial Flow Column
Packing Solution	**Water**	**Water**
Resin Slurry	**~ 50 %**	**20 %**
Flow Direction	**Forward** **Effluent flow to drain**	**Reverse** **Effluent flow recycled**
Flow Control	**Constant Flow or** **Constant Pressure**	**Constant Flow**
Flow Rate	**Dependent on operating parameters**	**16 – 18 L/min** **(48 – 54 BV/h)**
Transition Parameter	**When target bed height is achieved**	**When pressure increases to 25 psi**

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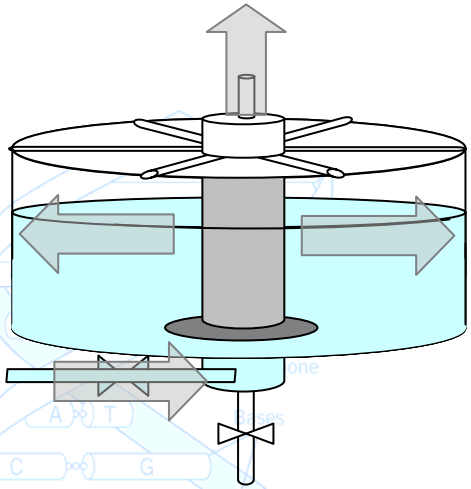
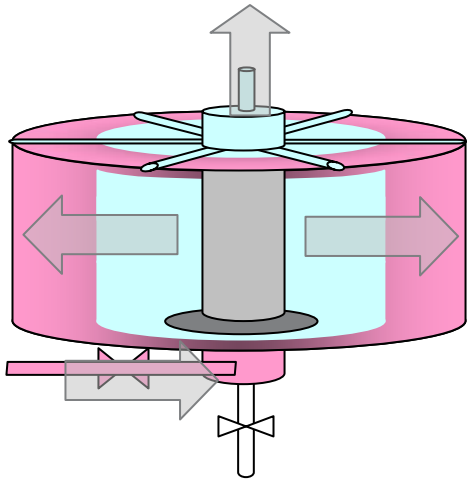
Flow Path – Column Packing

water | resin | flow direction

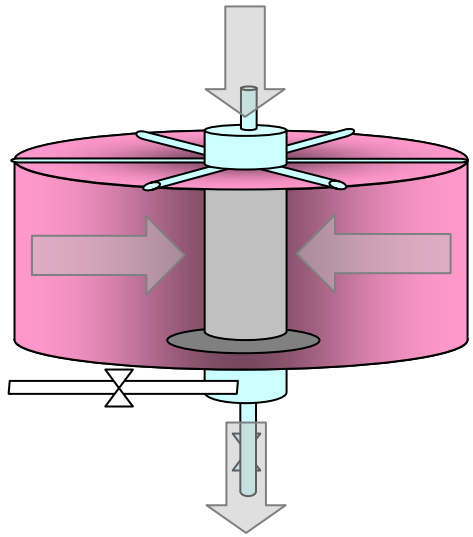
1. Prime the column body

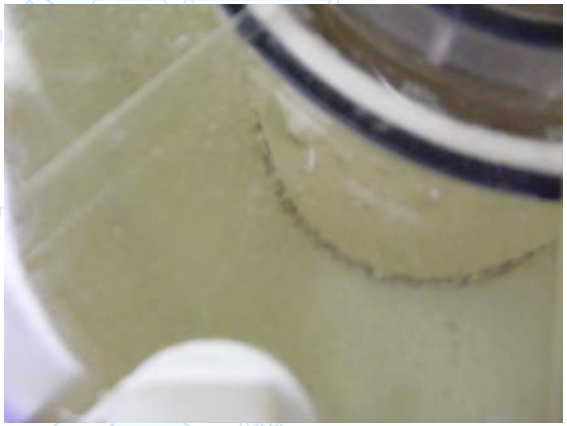


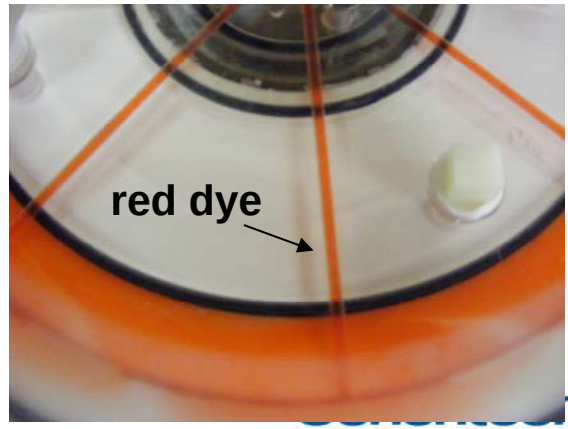
2. Pack the column with resin



3. Stabilize the resin bed.







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Bed Integrity Test Methods

	Sepragen		Genentech
Test Method	**S1**	**S2**	**G1**
Injection Pulse:	**0.1 M KCl**	**Vitamin B12[a]**	**0.5 M NaOAc**
Load Volume:	**~1% BV**	**~1% BV**	**2% BV**
Load Flow Rate:	**Paired with Elution**	**Paired with Elution**	**0.6 BV/h[b]**
Equil/Elution:	**Water**	**Water**	**0.05 M NaOAc**
Equil/Elution Flow Rate:	**12 – 40 BV/h**	**15 BV/h**	**Varied**

Expected Bed Integrity	**S1**	**S2**	**G1**
HETP (cm):	**> 0.003**		
Asymmetry Factor:	**0.7 – 1.5**		

[a] Cyanocobalamin form of B12 (red dye), food additive
[b] Equivalent to 5 cm/h on AFC

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Bed Integrity Test Results

Acrylic Column				Stainless Steel Column			
Column Pack #	Flow Rate (BV/h)	HETP (cm)	A_F	Column Pack #	Flow Rate (BV/h)	HETP (cm)	A_F
1	13.7	0.032	0.9	2	23.4	0.009	1.3
	21.6	0.007	1.0		39.6	0.006	1.0
	31.5	0.008	0.8		29.7	0.004	1.2
	35.1	0.004	0.9		15.0	0.025	1.5
	15.0[a]	0.016[a]	0.9[a]		12.1	0.101	1.6
3	21.6	0.005	0.9	4	21.6	0.029	1.1
	21.4	0.004	0.7		21.4	0.022	1.3
	15.3	0.005	0.8		15.3	0.030	1.4
				5	15.3	0.027	1.3
					21.4	0.029	1.0
					27.3	0.037	1.4

[a] Bed integrity tested using test method S2 (red dye).

Sepragen

Genentech

- **Tests at Sepragen used test method S1 (KCl), unless otherwise indicated.**
- **All tests at Genentech used test method G1 (NaOAc).**

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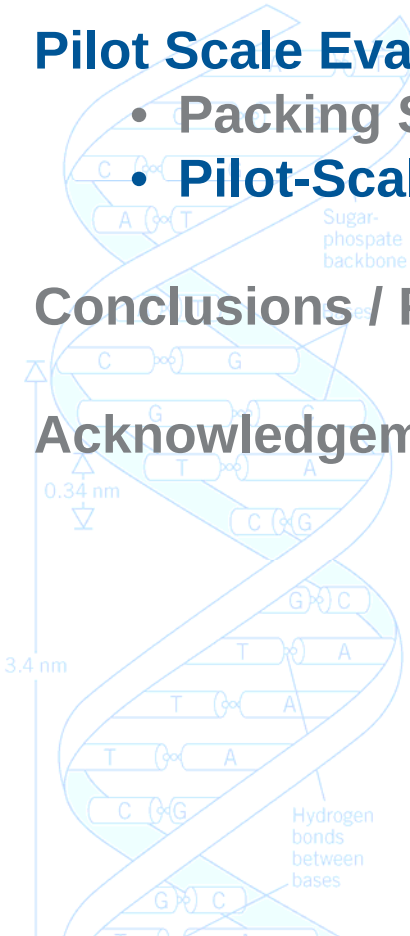

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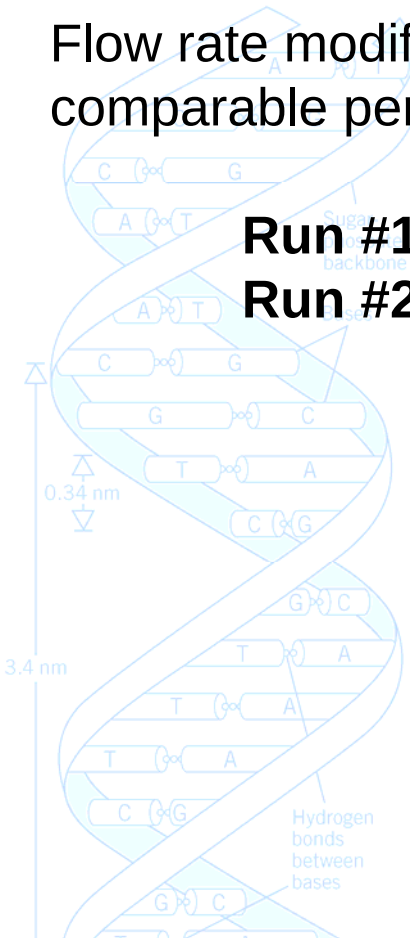
Pilot-Scale Runs with mAb A

Goal: To compare chromatographic performance of RFC column with that of traditional AFC column, using an established product-specific chromatographic method.

Flow rate modified beyond the traditional axial flow rate to demonstrate comparable performance with reduced processing times.

Run #1: 5.0 LPM (15.0 BV/h, 4.0 min residence time)
Run #2: 8.3 LPM (24.9 BV/h, 2.4 min residence time)



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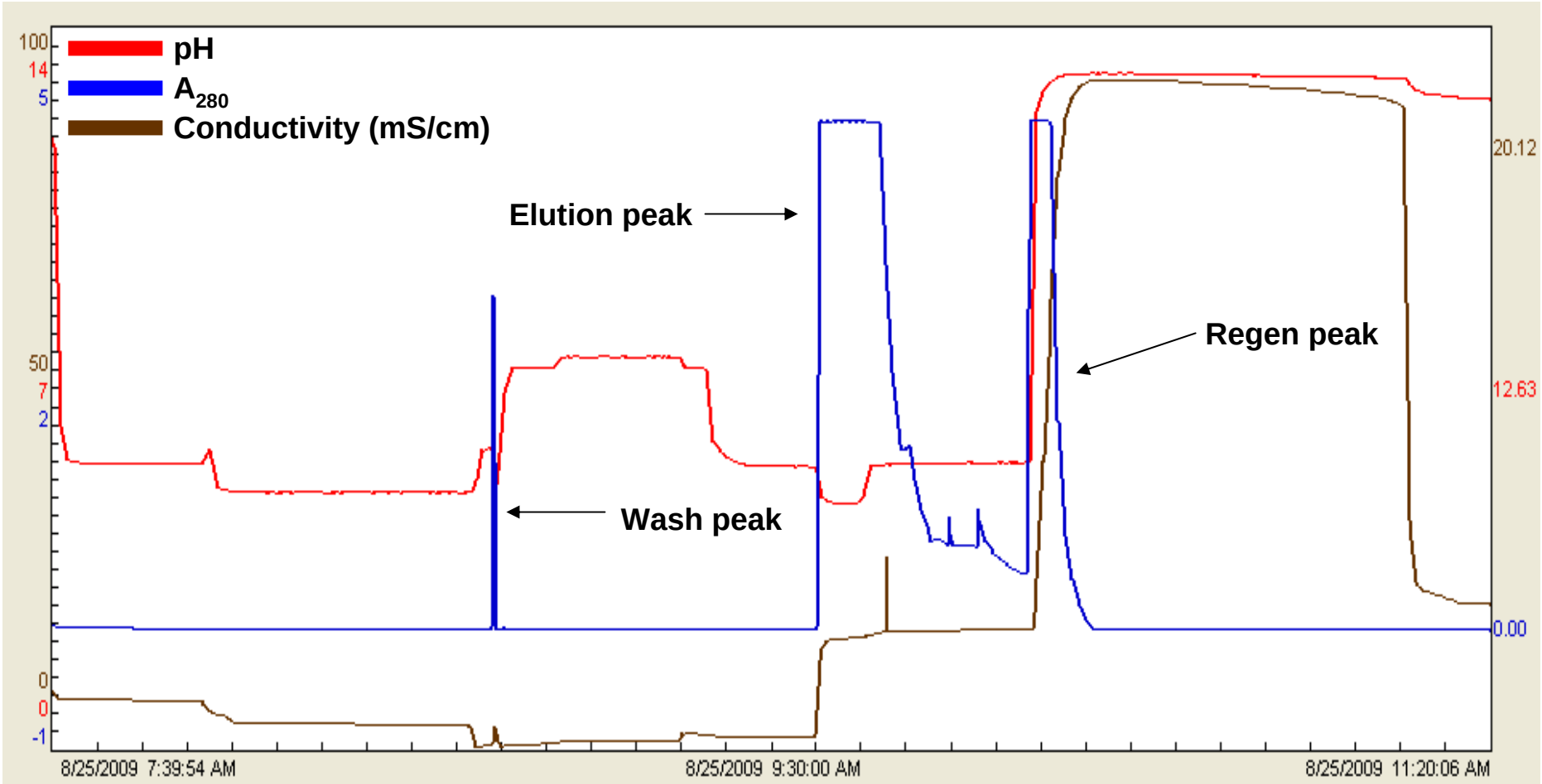
Chromatogram – Run #1



Total Run Time (hours)[a]	AFC	RFC
	8	3

[a] Includes Sanitization and Storage.

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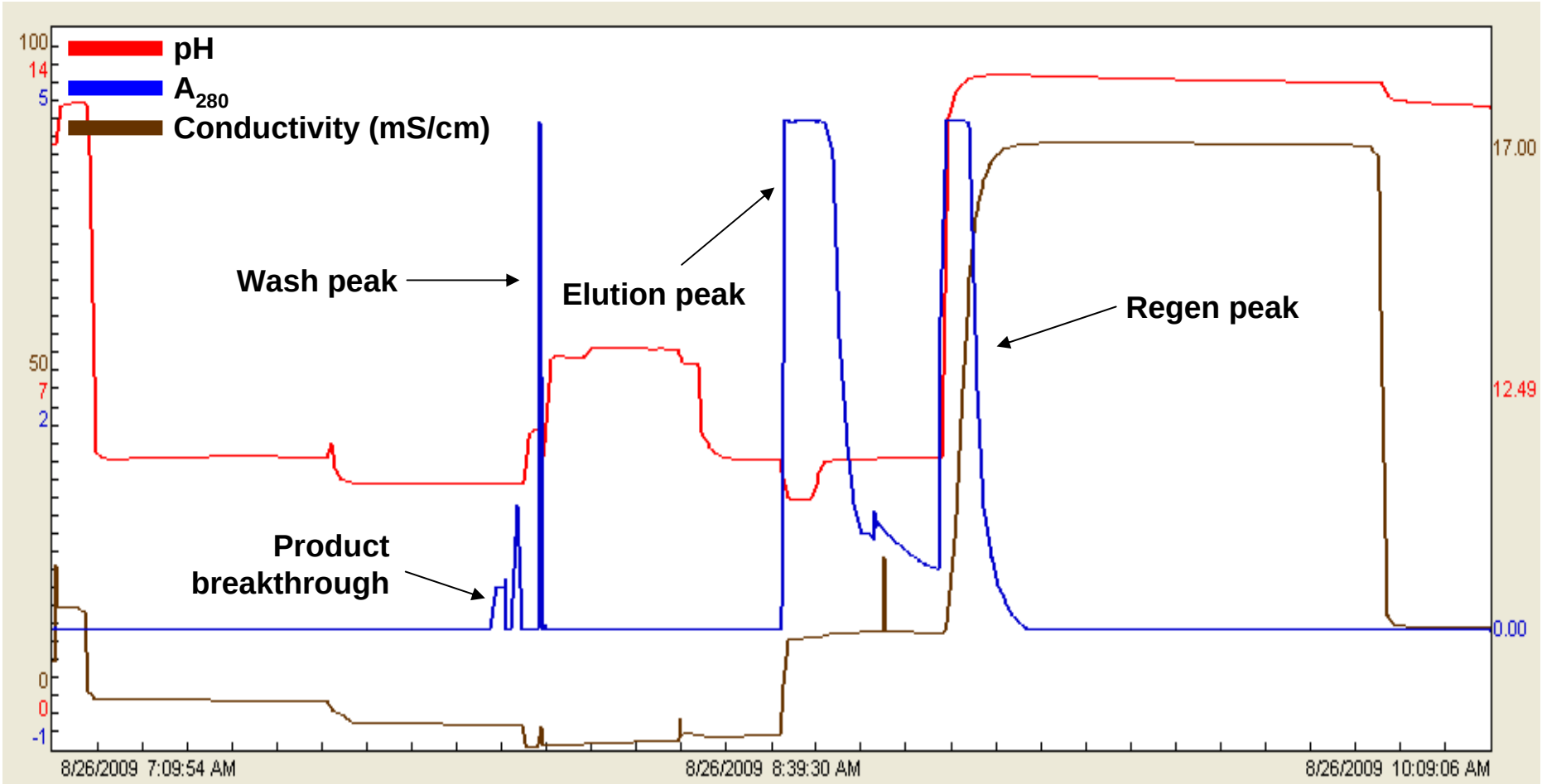
Chromatogram – Run #2



Total Run Time (hours)[a]	AFC	RFC
	8	2

[a] Includes Sanitization and Storage.

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Impurity Clearance

		Run 1	Run 2
Pool	Product Recovery (%)	✓	✓
	Monomer (%)	✓	✓
	HCP (ng/mg)	✓	✓
	DNA (ng/mg)	✓	✓
	Leached Protein A (ng/mg)	✓	✓

Chromatographic performance of RFC CEX column is acceptable.

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Conclusions

Pilot-scale column (new design) packed with acceptable bed integrity.

- Peak asymmetry and HETP depict high packing efficiency.
- Further studies required to establish bed integrity acceptance criteria.

Pilot-scale tests with mAb A CEX step show comparable results.
Chromatographic performance is similar to expected AFC performance.

- Chromatographic profile
- Product recovery
- Impurity clearance

Able to perform unit operation with 2 – 3 hour processing time

- 62-75% reduction

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APPENDIX

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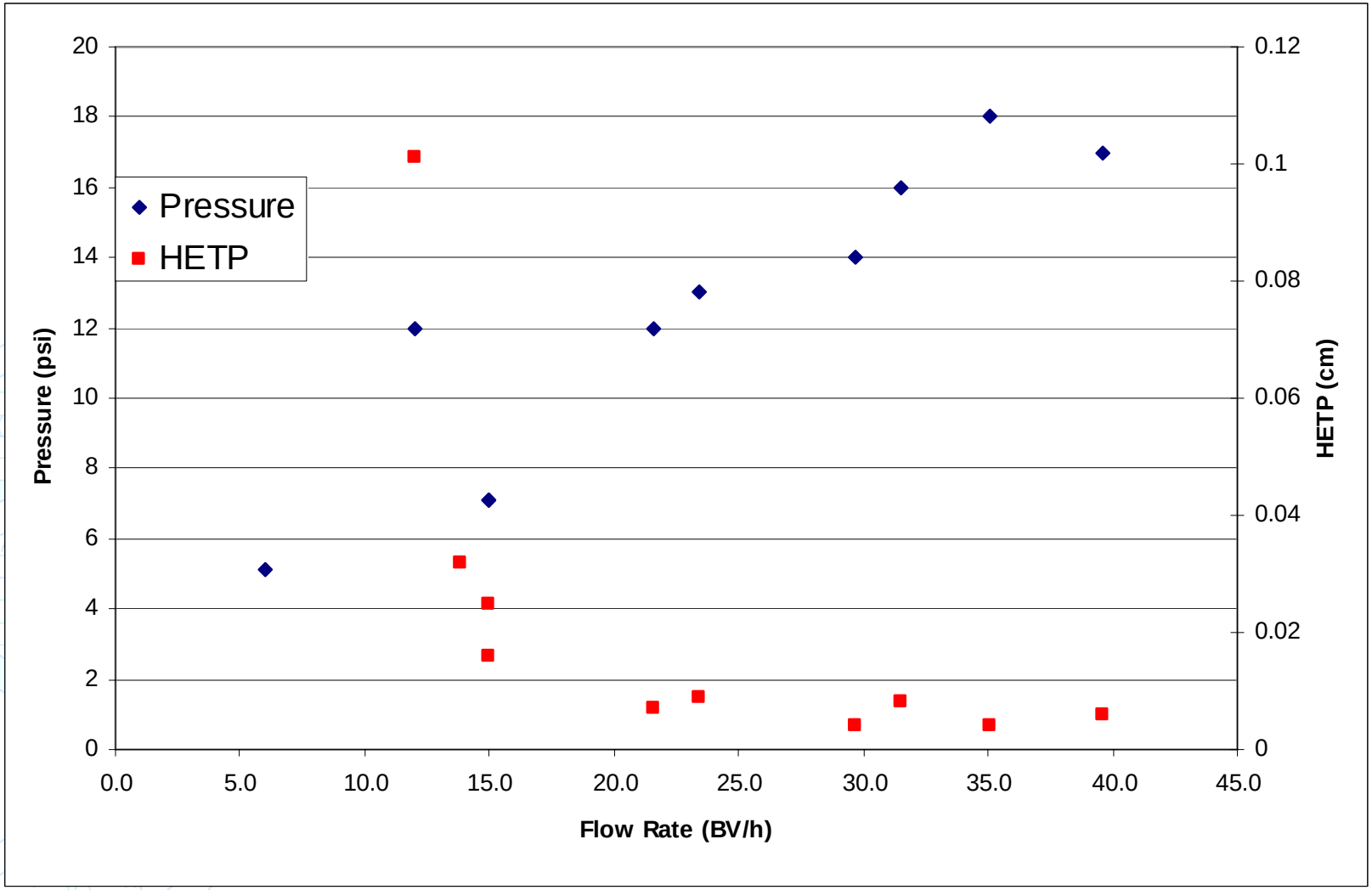
Flow Rate Impact on HETP and Pressure



Low pressure and better HETP measured at faster flow rates.

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