Next Generation Zirconia-Based Antibody Purification Media

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Outline

- Supports for Bio-Chromatography
- Modification of zirconia for bio-chromatography
- Small scale Mab purification
- Fast Mab purifcation
- Comparison to Protein G
- Binding Capacity Studies
- Conclusions

Current Supports for Bio-Chromatography

Soft-Gels:

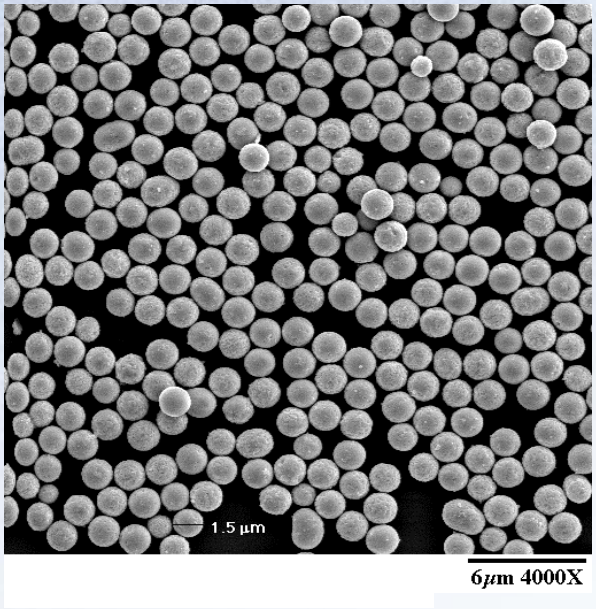
1. Cellulose
2. Agarose
3. Sepharose
4. Polyacrylamide
5. Polyamide
6. Polystyrene

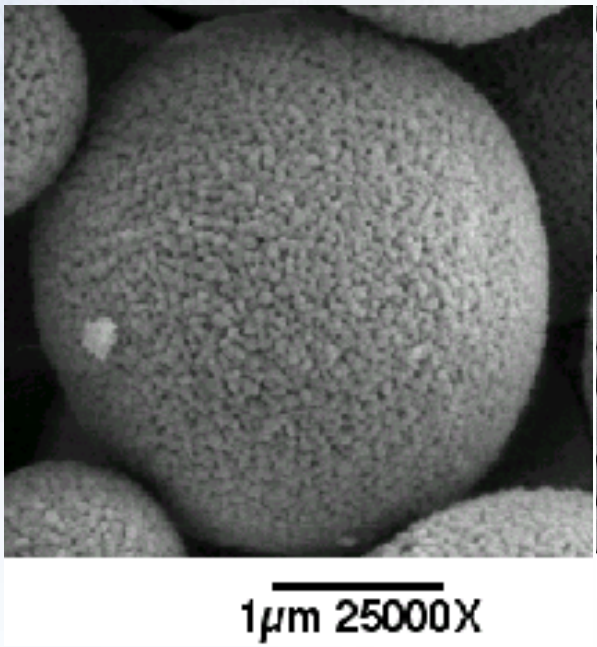
Hard-Gels:

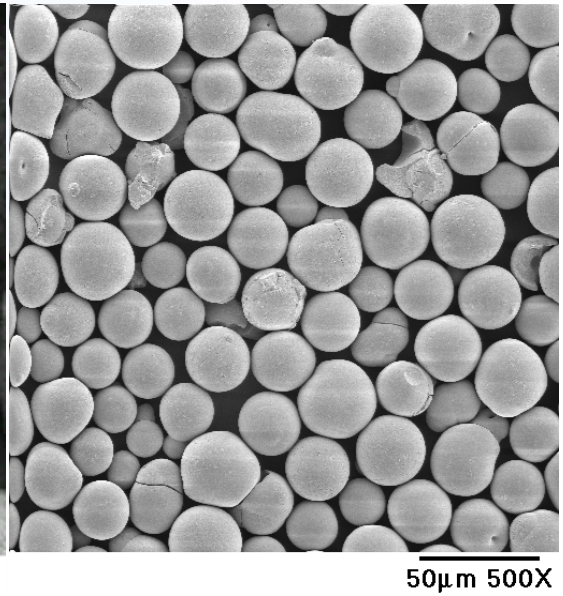
7. Silica
8. Hydroxy-apatite
9. Zirconia

Particle Sizes Available Data for Rhinophase®-AB

Both nonporous and porous analytical (3, 5 micron) and preparative (10, 25 micron) particles are available.

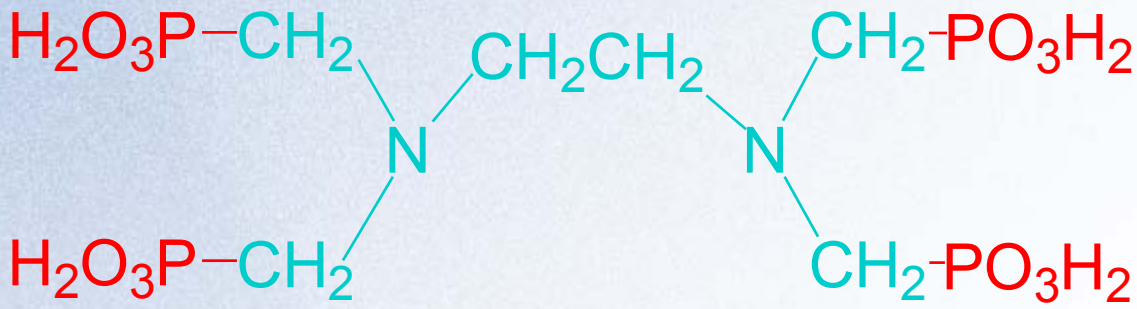






1.7 micron NPZ **3 micron** **25 micron**

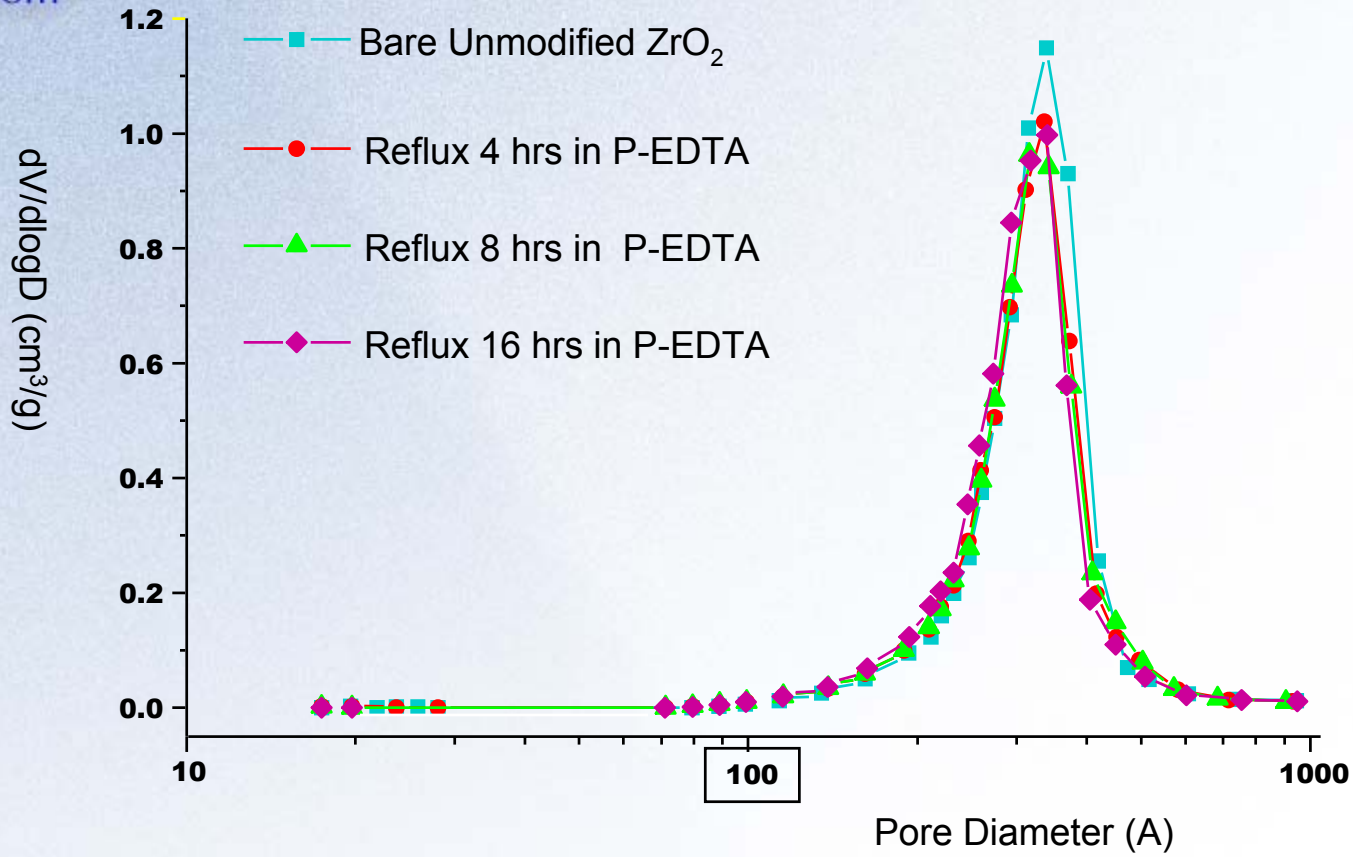
Modification: Chelating Ligand



Ethylenediamine-N,N'-tetra(methylenephosphonic) acid = "**EDTPA**"

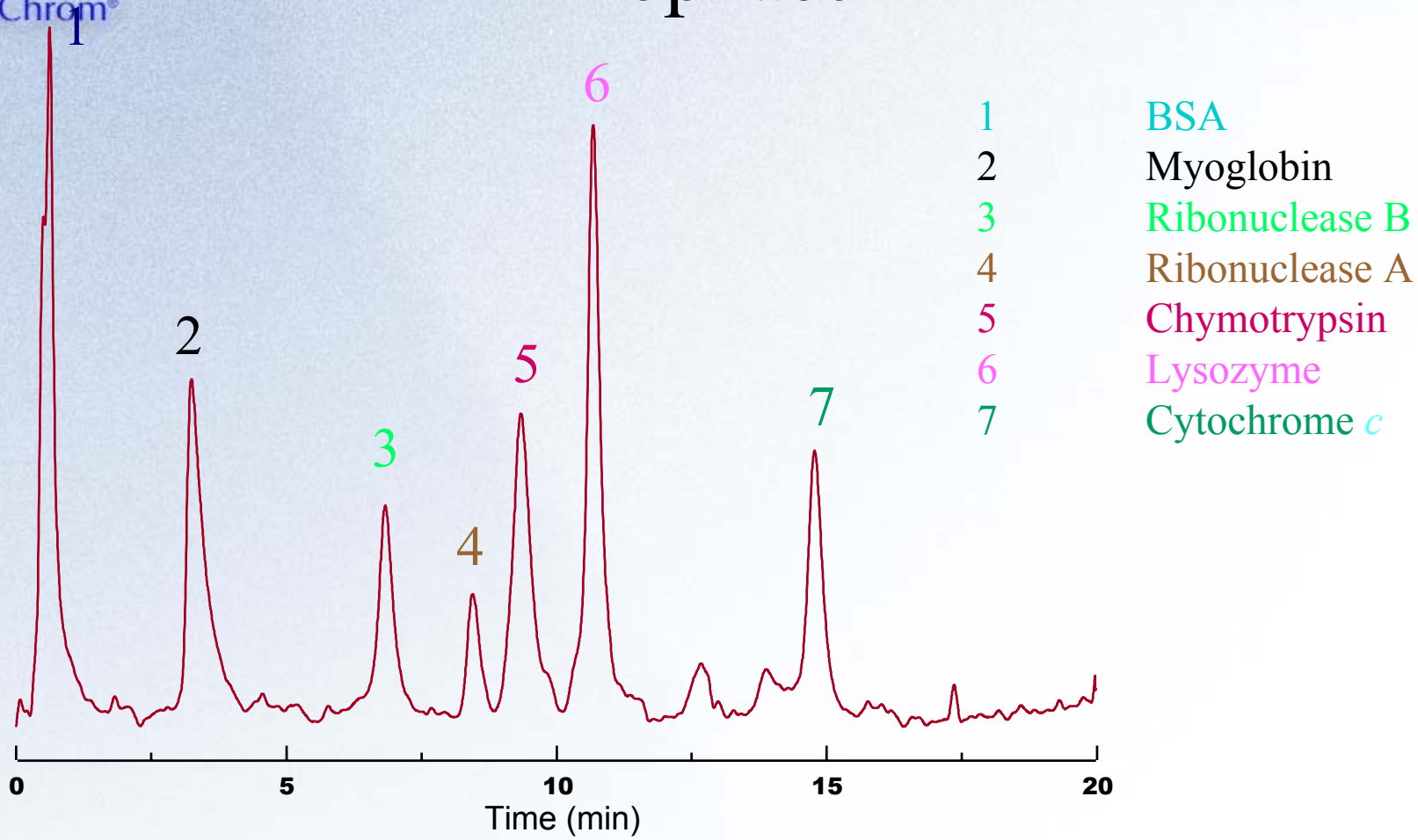
- Reflux particles in EDTPA solution
  - Forms multiple bonds
  - Blocks Lewis acid/base interactions

Nitrogen Porosimetry of P-EDTA Modified ZrO_2



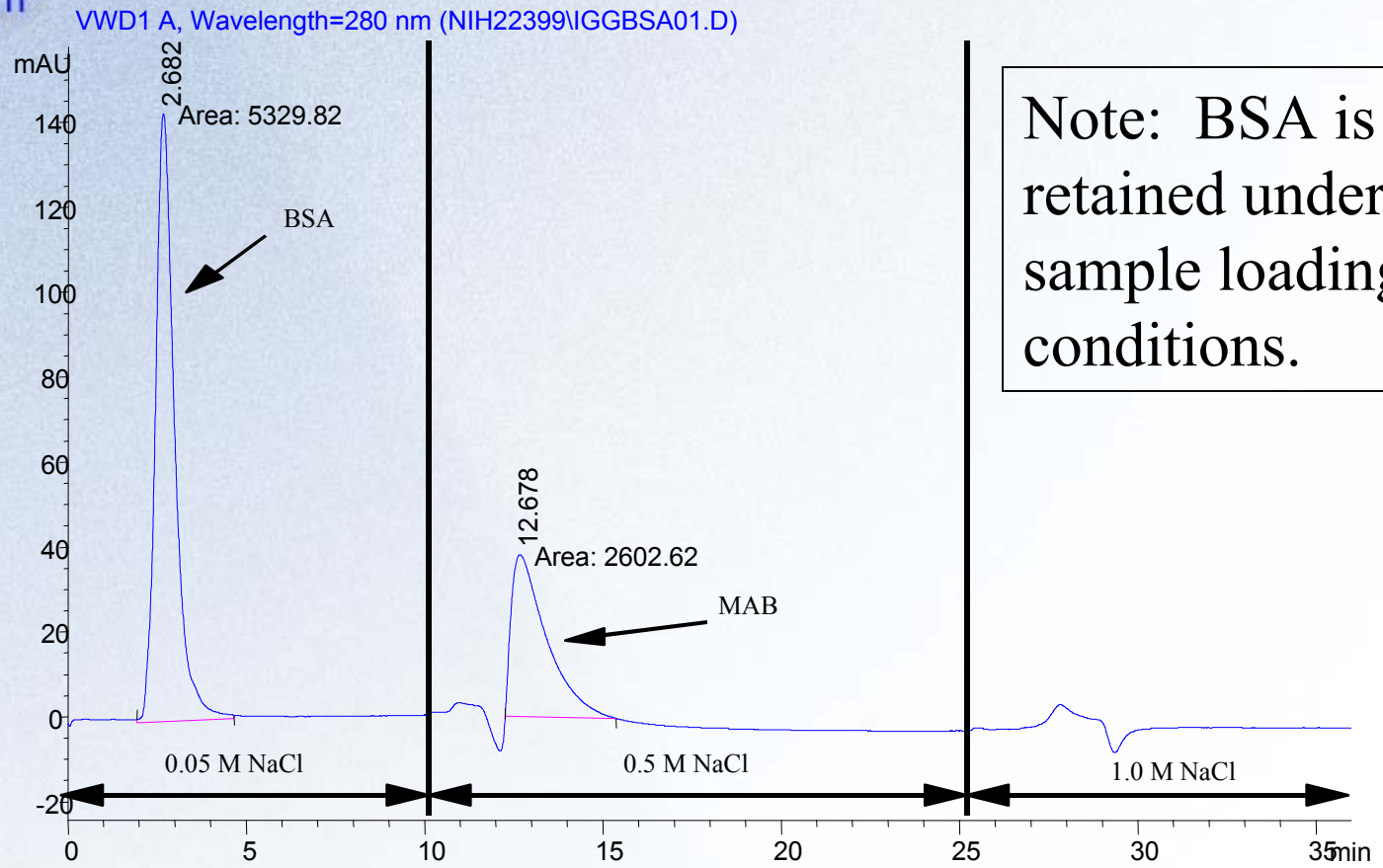
- No pore blockage
- No loss of surface area

Gradient Chromatography on Rhinophase®-AB



Protein mixture. Mobile phase: 4 mM P-EDTA, 20 mM MES, pH 5.5; linear gradient from 0 to 1 M Na_2SO_4 in 30 min. Flow Rate 1 mL/min. UV detection at 280 nm.

Small-Scale Purification of Mab IgG_{2a} Contaminated with BSA



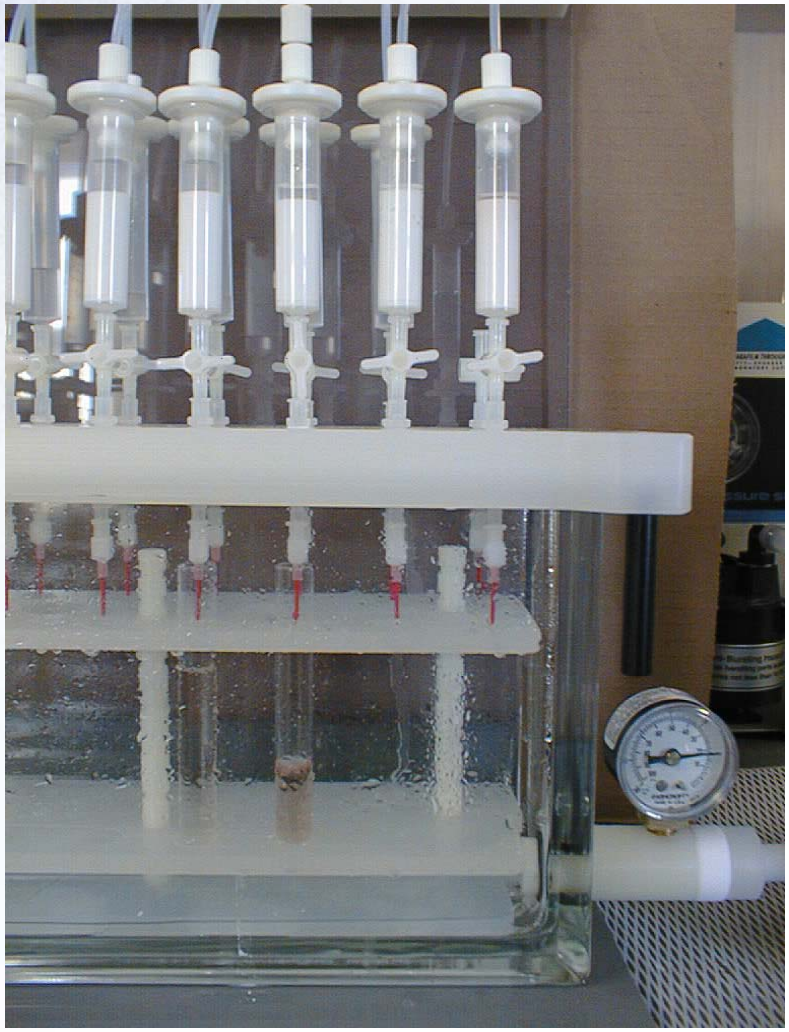
Note: BSA is not retained under sample loading conditions.

LC Conditions: 100 *u*l injection of BSA (6.0 mg/ml) contaminated MAB (1.0 mg/ml) eluted by salt step gradient. Mobile phase: 20 mM MES, 4 mM EDTPA, 0.05 M-to-1.0 M NaCl pH=5.5. Flow rate: 2.0 ml/min. Temperature: 30°C. Detection: 280 nm.

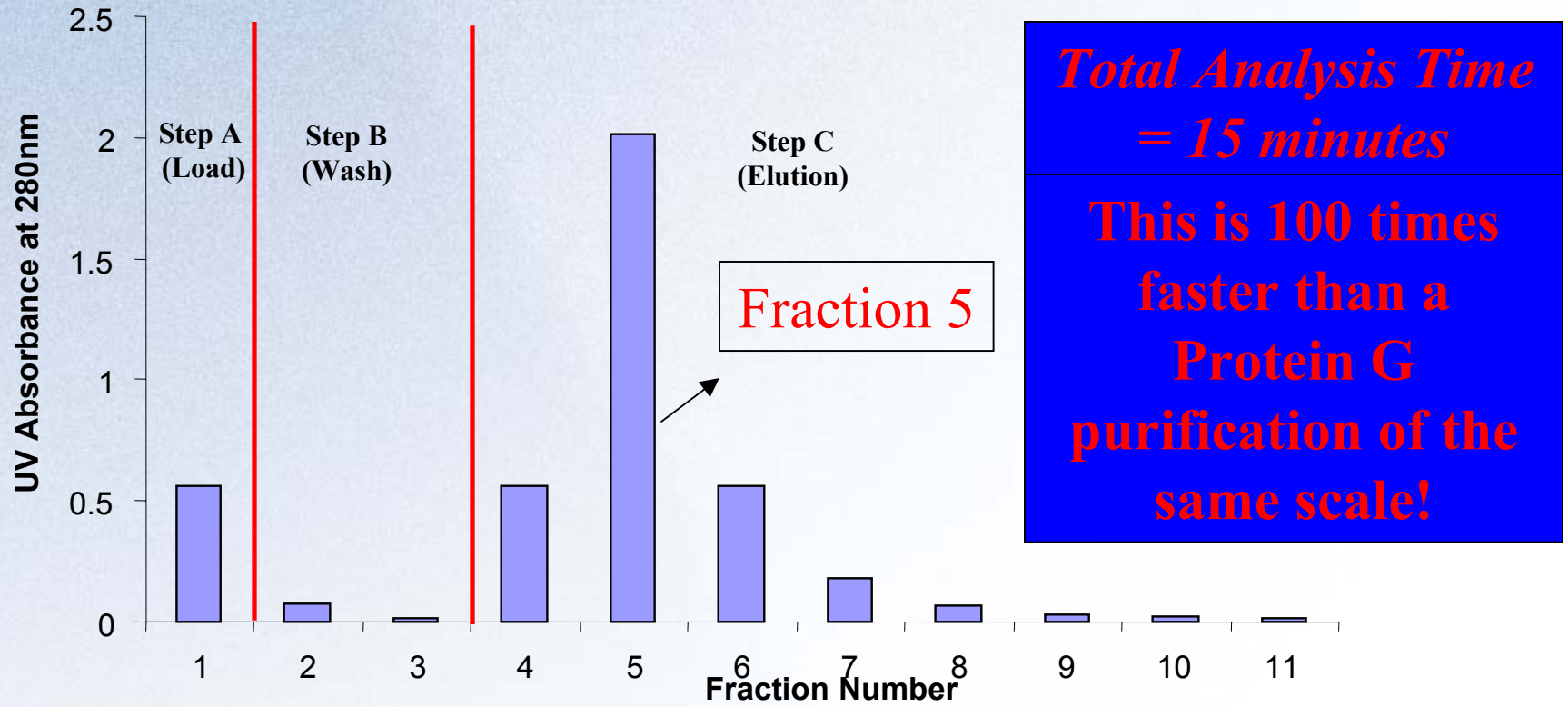
A New Ultrafast Preparative Purification Method Using Rhinophase®-AB

ZirChrom®

Due to zirconia's very high mechanical strength, Mab purifications can be performed at high mobile phase linear velocities. A simple vacuum filtration apparatus can be use to achieve very high flow rates through a packed bed (90 mL/min). This approach is not possible using soft affinity gels such as Protein A and Protein G media.



Ultrafast Preparative Purification of IgG_1 Using Rhinophase®-AB



Step A = 20 mM MES buffer, 4 mM EDTPA, 50 mM NaCl @ pH 4.0, Step B = 20 mM MES buffer, 4 mM EDTPA, 50 mM NaCl @ pH 4.0, Step C = 20 mM MES buffer, 4 mM EDTPA, 2.0 M NaCl @ pH 4.0. Flow Rate = 60 mL/min, Injection size = 31.6 mL serum-free cell culture supernatant diluted 4-times with loading buffer, (3.98 mg of Mab), Amount of Rhinophase®-AB in tube = 10 grams.

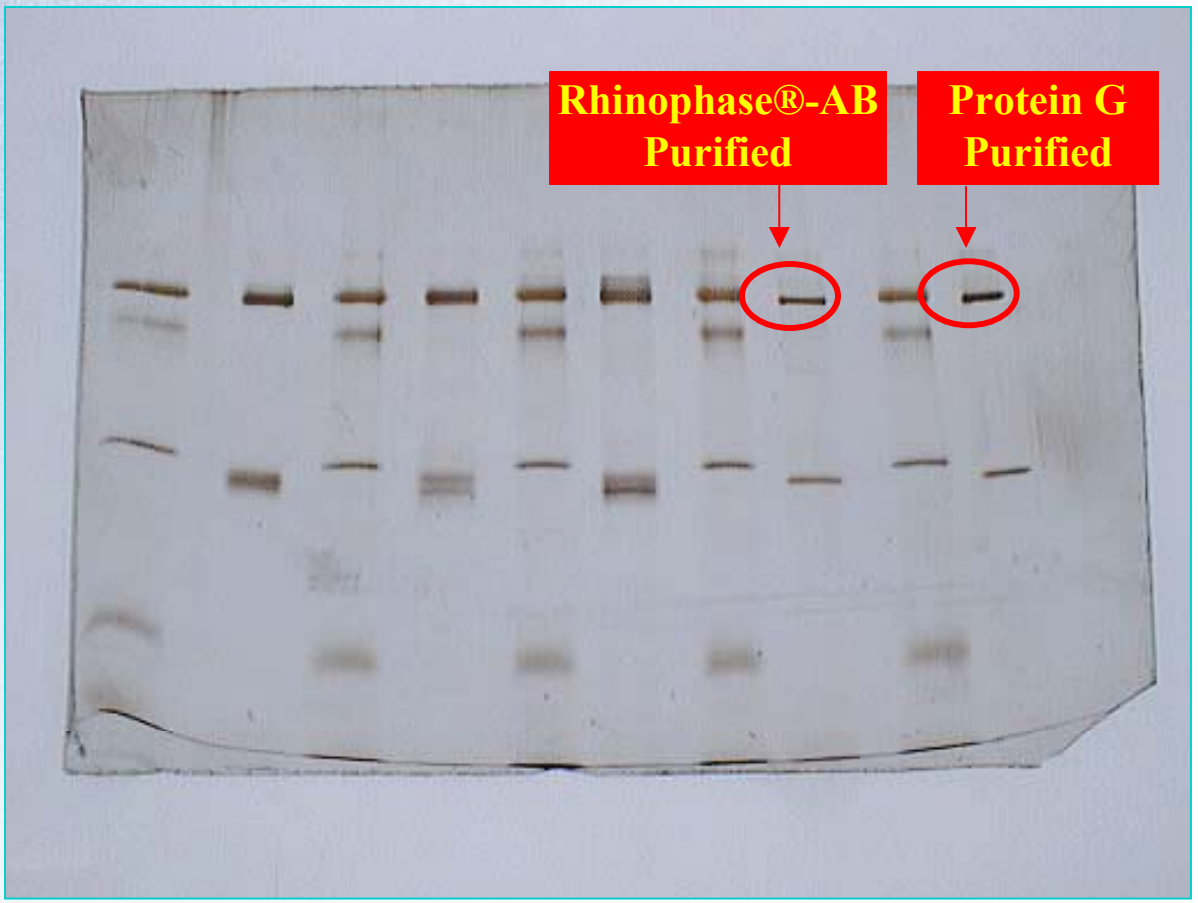
ELISA Plate Comparison of Protein G and the Ultrafast Purification on Rhinophase®-AB

	OD 280 nm		
Protein Offered	Protein G Purified IgG	Rhinophase®-AB Fraction 5	Fraction 5
15.6	0.0531	0.0502	94.5%
31.3	0.0946	0.0992	104.9%
62.5	0.1676	0.1892	112.9%
125	0.3176	0.3632	114.4%
250	0.5596	0.6362	113.7%
500	1.0166	1.1507	113.2%
1000	1.8151	1.8632	102.6%
		Average %	108.0%

An ELISA plate analysis using the same amount of Mab from Protein G and Rhinophase®-AB purifications showed an increased signal for the Rhinophase®-AB purified Mab.

Mab Purity Comparison from Semi-Preparative Run

Purified Mab was equally pure using Protein G and Rhinophase®-AB.

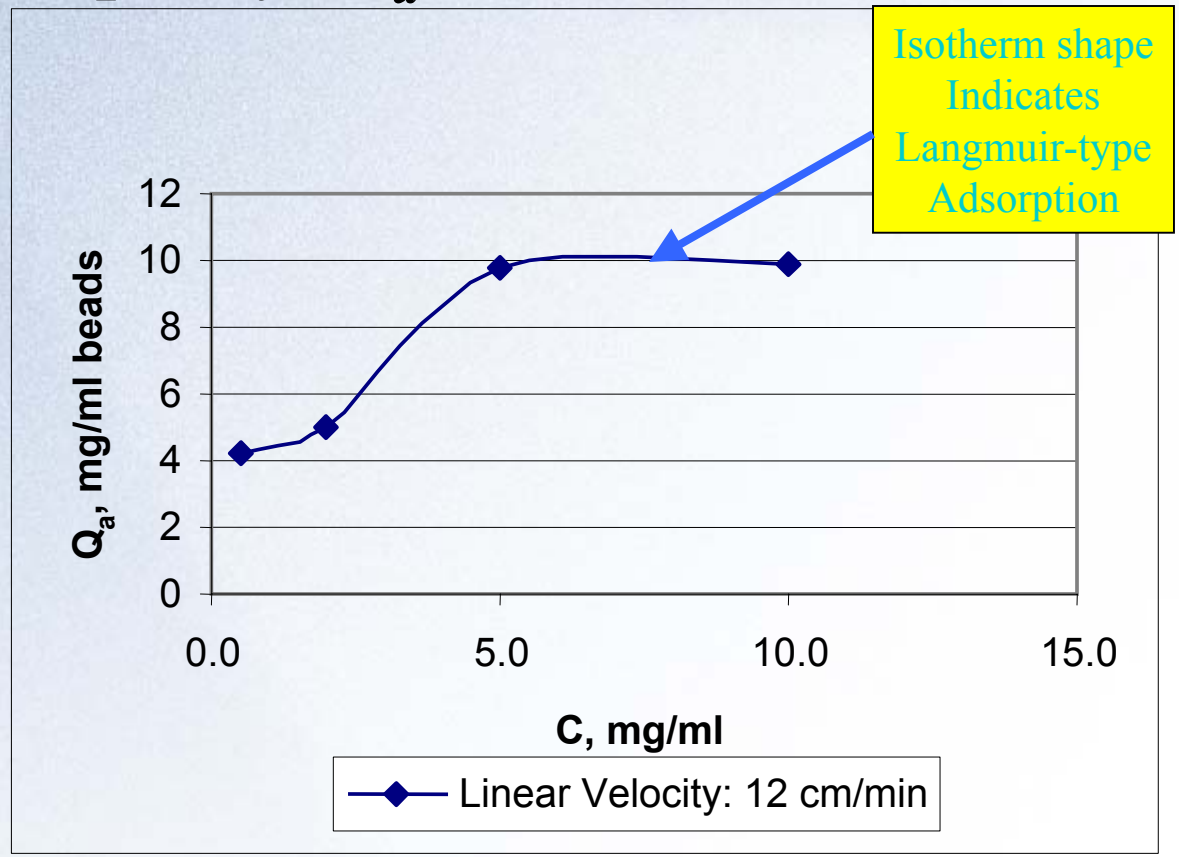


Silver-stained, SDS-PAGE gel Comparing IgG_1 purified by Protein G (row 1 from right) and Rhinophase®-AB (row 3 from right). Electrophoresis was run under reducing conditions. Sample loading at 1 *u*g per lane. All other lanes are standards.

Effect of Mab (hIgG) Concentration on Dynamic Bead Capacity (Q_a) of ZirChrom Mab Media

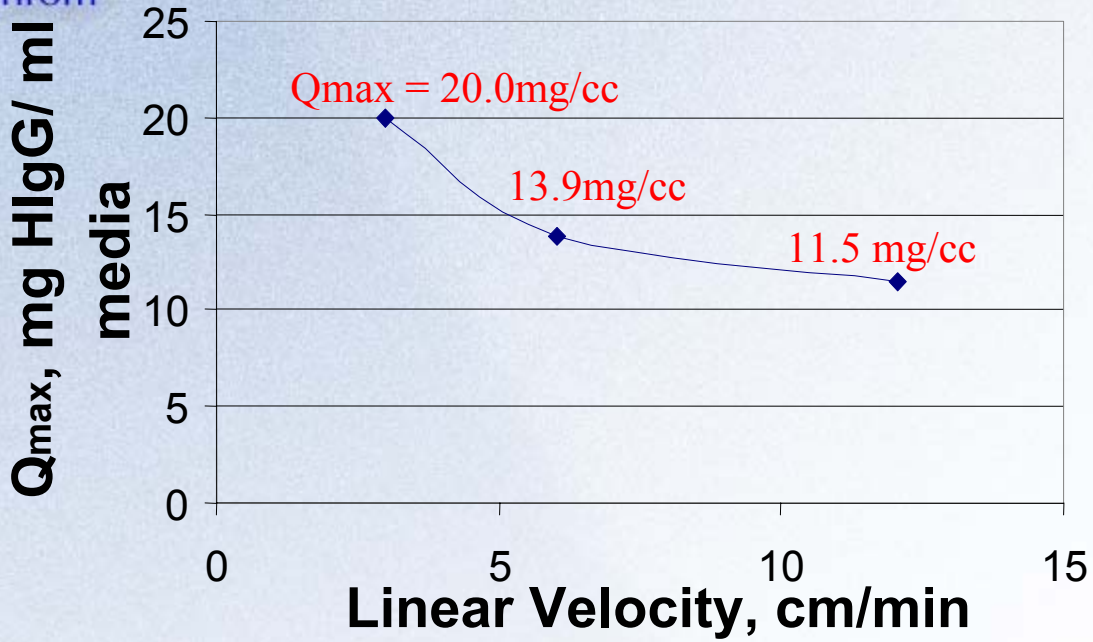
ZirChrom®

The Dynamic Capacity of ZirChrom Mab Media was Determined as the Amount of hIgG captured per mL of Media.



Conditions: Pure hIgG in Loading Buffer (20mM MES, 4mM EDTPA, 50mM NaCl, pH5.5) was loaded on a 5x4.6mm i.d. ZirChrom Mab Media column at a linear velocity of 12.0cm/min and detected at 280nm. The injection was continued until the absorbance of the effluent reached 80% of the inlet concentration. The column was then washed with Loading Buffer until it had reached baseline. The Adsorbed hIgG was then eluted with Elution Buffer (20mM MES, 4mM EDTPA, 1.0M NaCl, pH5.5).

Effect of Linear Velocity on the Dynamic Q_{max} of ZirChrom Mab Media



Typical value on Protein A = 2-4 mg/mL

Conditions: Pure hIgG in Loading Buffer (20mM MES, 4mM EDTPA, 50mM NaCl, pH5.5) was loaded on a 5x4.6mm i.d. ZirChrom Mab Media column at a linear velocities of 3.0, 6.0, and 12.0 cc/min and detected at 280nm. The injection was continued until the absorbance of the effluent reached 80% of the inlet concentration. The column was then washed with Loading Buffer until it had reached baseline. The adsorbed hIgG was then eluted with Elution Buffer (20mM MES, 4mM EDTPA, 1.0M NaCl, pH5.5).

Conclusions

- Rhinophase®-AB provides a widely applicable alternative to currently used Protein A and Protein G antibody purification media.
- Typical yields of Mab purifications greater than 95%, with purity levels equal to or greater than affinity gel-type media.
- Due to Rhinophase®-AB's excellent mechanical stability, purifications can be performed 100-fold faster with equivalent results.
- Rhinophase®-AB is chemically durable over the entire pH range, which allows for cleaning and depyrogenation.
- Rhinophase®-AB has affinity for a wide range of immunoprotein classes and subclasses including monoclonal and polyclonal IgG, IgA, and IgM.
- Rhinophase®-AB can be packed into large preparative scale columns and operated at high mobile phase linear velocities for fast large scale purifications of immunoproteins.
- **Acknowledgement: National Institutes of Health**