

Natrix™

Single-Use High Performance Hydrophobic Interaction Chromatography (HIC):

A Study of the Intermediate Purification Step of a Monoclonal Antibody Process

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EXECUTIVE SUMMARY

INTRODUCTION: Typical downstream processing of monoclonal antibodies involves three orthogonal chromatographic steps, which can be either ion-exchange, affinity, hydrophobic interaction or mixed-mode.

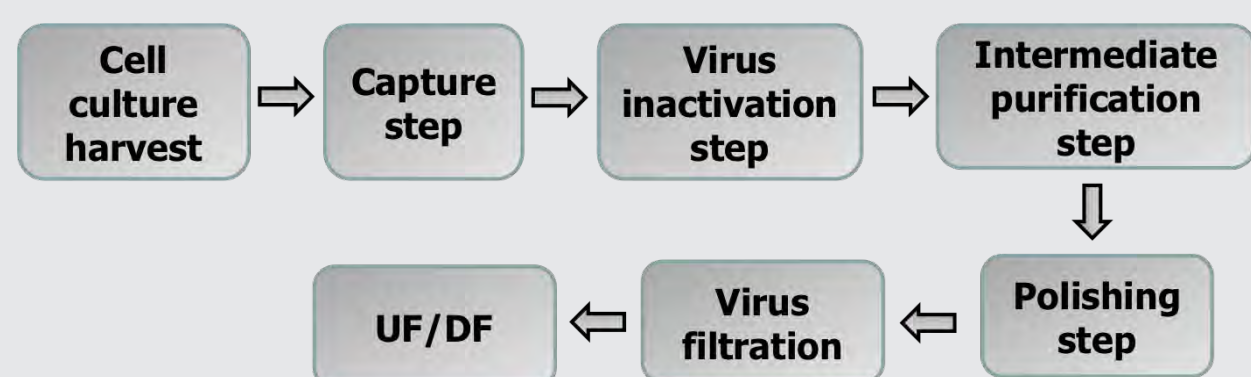


Figure 1: Three chromatographic steps based monoclonal antibody purification platform

Hydrophobic interaction chromatography

- Very well studied as a polishing step in downstream processing of monoclonal antibodies
- Provides good aggregate clearance in bind & elute as well as flow through mode
- Can potentially provide good HCP, DNA & endotoxin clearance as well

CONCLUSIONS: Natrix Separations has developed a single-use high performance hydrophobic interaction media that combines the high binding capacity, excellent resolution and high throughput for the purification of biotherapeutics such as monoclonal antibodies (mAb). This study reports the use of Natrix hydrogel platform-based HIC media for the purification of mAb from a representative feed stream. The data demonstrates versatile performance in both bind/elute and flow through modes, as summarized below:

Performance of Natrix HIC media in bind & elute mode

- 10% breakthrough binding capacity for mAb: **35 mg/mL**
- Yield: **>95%**
- Aggregate levels in elution pool: **<0.2%** (aggregate clearance: 13X)
- DNA clearance: **>99%**
- Cycle time: **15 minutes**
- Demonstration of **at least 10 cycles** without loss of performance

Performance of Natrix HIC media in flow through mode

- Load: **120 mg of mAb/mL of media**
- Yield: **>95%**
- Aggregate levels in product: **<0.2%**

ABOUT NATRIX SEPARATIONS

Natrix Separations is a leading supplier of **high performance, multi-cycle/single use disposable chromatography** products to the life science market.

Natrix has developed a unique patented technology that combines the high binding capacity and selectivity associated with resin-based chromatography with the high throughput and ease of use of traditional membrane products.

Natrix Separations' patented technology consists of a polymeric hydrogel formed within a flexible porous support matrix. The support matrix provides mechanical strength, while the hydrogel properties determine the separation chemistry of the product. The macroporous syntactic structure that is formed provides a large surface area for binding and facilitates rapid mass transfer via advective flow, supporting high flow rates while providing highly efficient capture of the target molecule. Natrix technology will enable highly selective chromatographic separations in many applications where no commercially viable separation process currently exists.

MAB PURIFICATION IN BIND AND ELUTE MODE

Equilibration buffer: 750 mM sodium sulfate in 20 mM citrate, pH 6.5

Sample: 1 g/L mAb1 in equilibration buffer

mAb1 sample was spiked with lambda DNA (Invitrogen) to 1 µg/mL concentration in order to test DNA clearance capability of HIC membrane

Wash buffer: Equilibration buffer

Elution buffer: 200 mM sodium sulfate in 20 mM citrate, pH 6.5

Load: 25 mg of mAb1/mL of membrane (~ 70% of 10% breakthrough binding capacity)

Membrane volume: 0.9 mL (two layers of membrane discs in 47 mm module)

TABLE 1: Performance of Natrix HIC media for mAb purification in bind & elute mode

	Flow rate	10 MV/min
Yield		>96%
Aggregate clearance		9X (from 1.3% to 0.1%)
DNA clearance		>99%
Cycle time (which consists of equilibration, load, wash, elution & cleaning steps)		15 minutes
Specific productivity		100 g/(L.h)

Faster processing means reduced risk of product loss since longer contact time on the hydrophobic surface in the presence of high concentration of lyotropic salt can increase the product denaturation

mAb Sample with Higher Levels of Aggregates

- Equilibration/wash buffer: 1 M Ammonium sulfate in 20 mM citrate, pH – 6.5
- Load: 28.9 mg of mAb1/mL of membrane
 - Aggregate levels in the feed sample: **2.7%**
- Elution buffer: 250 mM Ammonium sulfate in 20 mM citrate, pH – 6.5
- Flow rate: 10 MV/min
- Yield: 94%
- Aggregate levels in elution pool: **< 0.2%**
- Aggregate clearance: **13X**

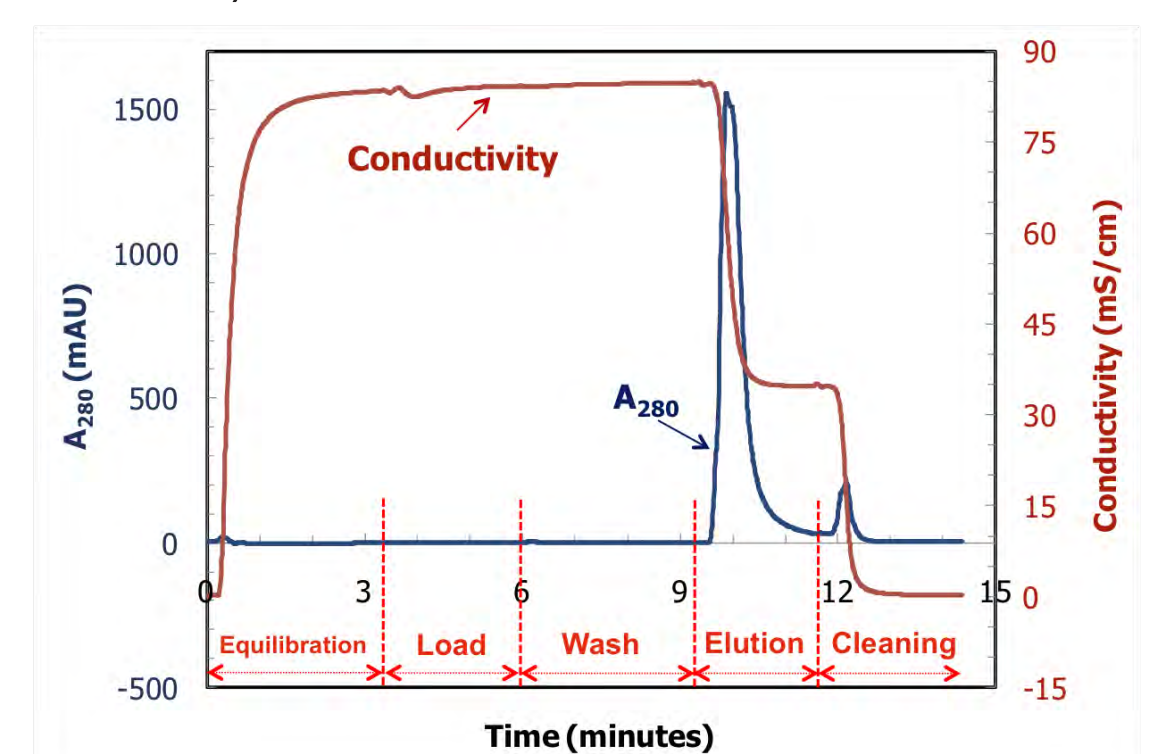
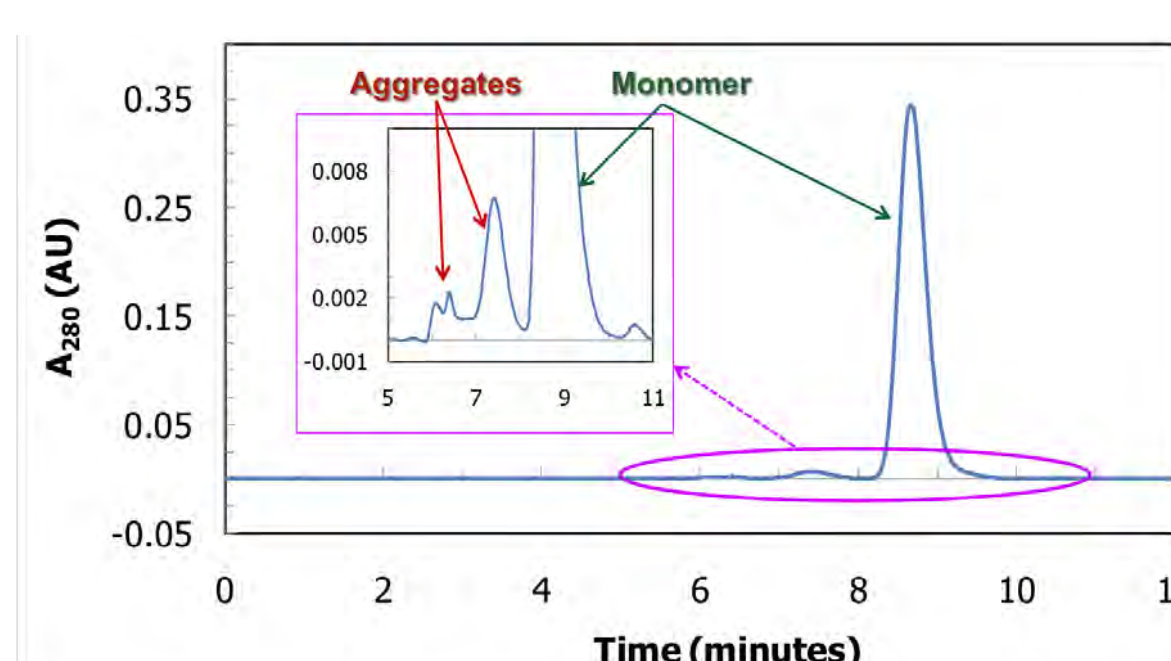


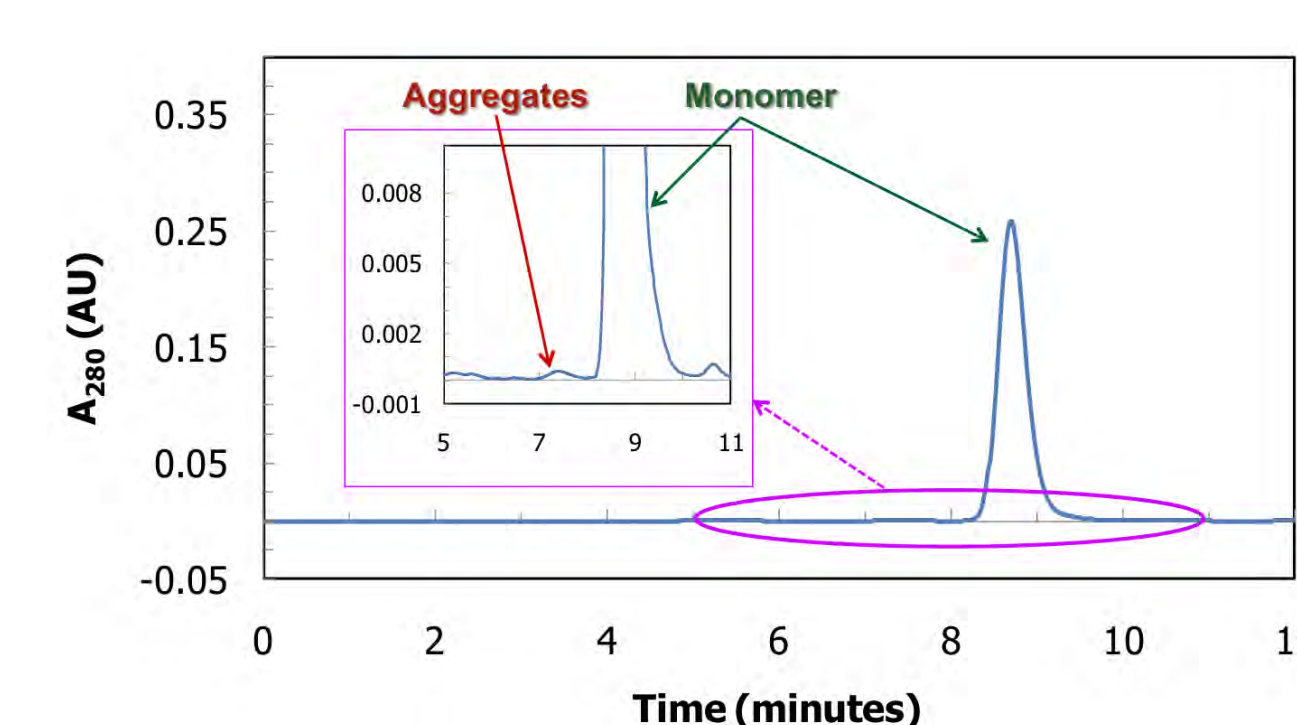
FIGURE 3: Chromatogram showing the complete cycle of mAb purification step with Natrix HIC media in bind and elute mode

SEC ANALYSIS OF FEED & ELUTION PEAK

Feed Chromatogram — Aggregates ~ 2.7%



Elution Peak Chromatogram — Aggregates <0.2%



METHODS & MATERIALS

EXPERIMENTAL

- Feed:** Protein A purified monoclonal antibody (mAb1)
 - IgG1 type of antibody which was recombinantly expressed in CHO cells
 - Molecular weight ~ 148 kDa; pI ~ 8.6
- Chromatographic separation experiments
 - Module/device: 25 or 47 mm stainless steel holders (Natrix Separations) containing either one or two layers of membrane discs
 - All the experiments were carried out on an AKTA Explorer 100 (GE Healthcare Life Sciences)

ANALYTICS

- Aggregate levels
 - Size exclusion chromatography (SEC) using a TSKgel G3000SWxl (Tosoh Bioscience) column
- DNA concentration
 - Quant-iT™ PicoGreen® dsDNA assay kit (Invitrogen) on NanoDrop 3300 (Thermo Scientific)
- mAb concentration
 - Spectrophotometer

FLOWRATE INDEPENDENT BINDING CAPACITY

- 10% breakthrough binding capacity ~ 35 mg/mL
- Binding buffer: 1 M ammonium sulfate in 20 mM citrate buffer, pH 6.5

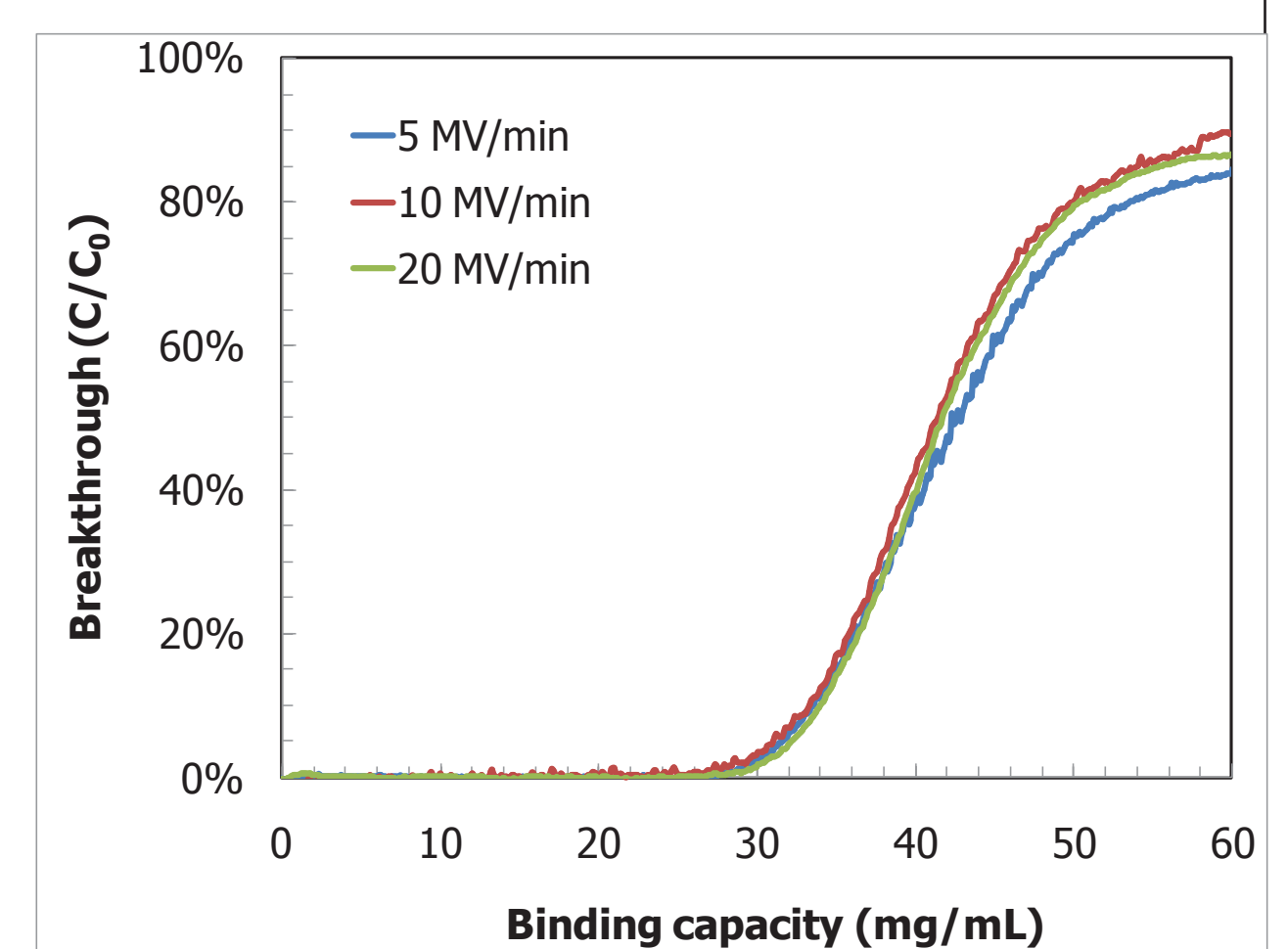


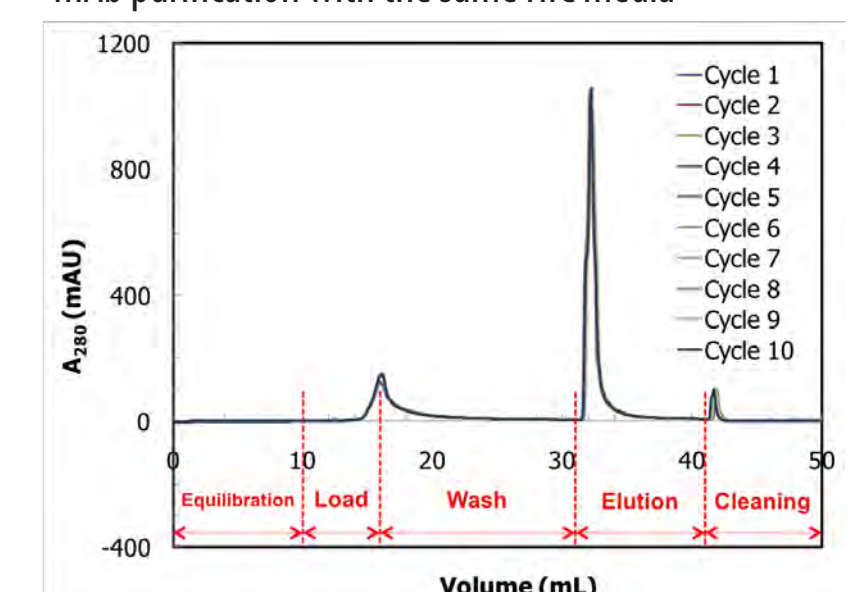
Figure 2: Binding capacity of mAb on Natrix HIC media at three different flow rates

SINGLE-USE MULTI-CYCLE PERFORMANCE

- Equilibration/wash buffer:** 1 M Ammonium sulfate in 20 mM citrate, pH 6.5
- Sample:** 1 g/L of mAb1 (aggregate concentration: 1.3%)
- Elution:** 250 mM Ammonium sulfate in 20 mM citrate, pH 6.5
- Cleaning:** 18% isopropanol
- Membrane volume:** 0.11 mL (one layer of membrane disc in 25 mm module)

	10% breakthrough binding capacity (mg/mL)	Elution peak	
		Recovery	Aggregate
1 st cycle	35	> 96%	0.17%
5 th cycle	35	> 96%	0.19%
10 th cycle	35	> 96%	0.19%

FIGURE 4: Overlaid chromatograms of 10 cycles of mAb purification with the same HIC media



MAB PURIFICATION IN FLOWTHROUGH MODE

- Equilibration/wash buffer:** 300 mM sodium sulfate in 20 mM Tris, pH – 8
- Sample:** 1 g/L of mAb1 in equilibration buffer
- Cleaning solution:** 18% isopropanol
- Membrane volume:** 0.23 mL (two layers of membrane discs in 25 mm module)

Table 2: Performance of Natrix HIC media for mAb purification in flowthrough mode

Total load	120 mg/mL
Yield	96%
Aggregate clearance	7X (1.3% to 0.2%)
Flow rate	10 MV/min

Figure 5: Chromatogram showing the complete cycle of mAb purification step with Natrix HIC media in flowthrough mode

