Characterization of POROS® MabCapture A™ Chromatography Media #15

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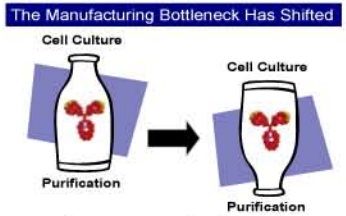
ABSTRACT

Applied Biosystems has proven manufacturing and quality systems capable of producing POROS® chromatography media that enable chromatography operations from development through commercial manufacturing scales. The optimized pore structure of POROS medias results in high dynamic binding capacities being maintained over a large flow rate range and a range of bed heights, thereby improving operational throughput and process flexibility. The base bead composition and proprietary coupling chemistries yield medias that have low leaching qualities, that are easy to handle and pack and that are robust to cleaning and sanitization solutions.
Performance benchmarking of the NEW POROS MabCapture A™ recombinant protein A affinity chromatography media is highlighted. The features and benefits of POROS chromatography medias as they relate to improving downstream purification process productivity are discussed.

INTRODUCTION

As cell culture technology improvements continue to result in monoclonal antibody expression level increases, the protein manufacturing throughput bottleneck has shifted to downstream purification. The neck of the funnel exists at the capture chromatography step, where it can take days to process a single reactor harvest due to flow rate and capacity limitations of existing rPA affinity medias. Chromatography medias with high dynamic binding capacity and high throughput capability are required to minimize this process bottleneck.

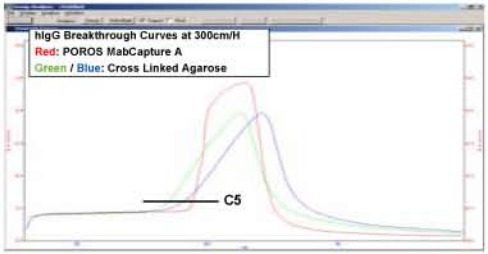


MATERIALS AND METHODS

Column format was 4.6mmD x 200mmL, stainless steel unless otherwise noted (Isolation Technologies, Hopedale, MA). All columns were packed within the pressure and flow limitations specified by the media vendor. Capacity determinations were made using purified human immunoglobulin (IgG, Sigma G-4386) at 5mg/mL in 20mM sodium phosphate, 150mM sodium chloride, pH 7.5 at the linear flow rate specified.
The leached recombinant Protein A concentration was measured using a recombinant protein A ELISA kit (Repligen, 10-9000-01).

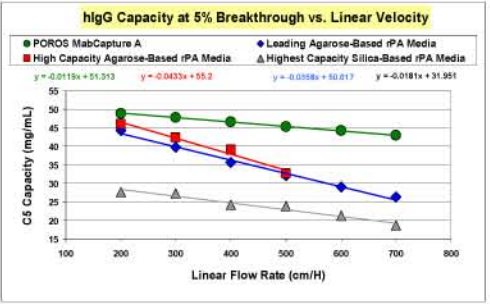
RESULTS

Figure 1. Purified Human IgG Breakthrough Curves of POROS MabCapture A and Cross-Linked Agarose Medias



POROS MabCapture A media demonstrates higher breakthrough capacities for purified IgG as compared to a cross-linked agarose protein A affinity medias. Binding or capture of the IgG is steep and efficient, demonstrating the superb mass transfer capabilities of the media.

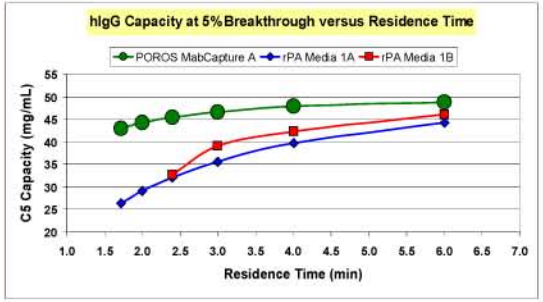
Figure 2. 5% Breakthrough Capacities of rPA Medias vs. Linear Velocity



The high dynamic binding capacity of POROS MabCapture A is maintained as linear flow rate increases over a 3 fold range. Only a 6mg/mL or 12% decay is realized as compared to agarose medias that realize a 21mg/mL or 45% decay in DBC over a more limited flow rate range.

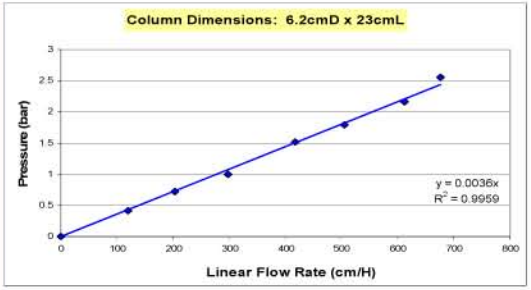
RESULTS CONTINUED

Figure 3. 5% Breakthrough Capacities as a Function of Residence Time



POROS MabCapture A media demonstrates higher dynamic binding capacity mostly independent of the residence time attained. Operational flow rate and column bed heights do not need to be optimized to allow for diffusion of the IgG to the rPA coated surface of the chromatography bead. Capture can be efficient and robust.

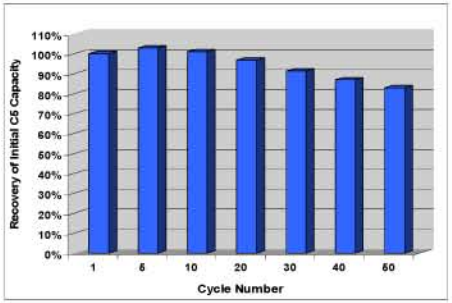
Figure 4. POROS MabCapture A Permeability Profile



POROS MabCapture A exhibits a linear pressure response to increased flow rate. The media can be operated at high linear flow rates within common commercial system pressure limitations of 3 bar.

RESULTS CONTINUED

Figure 5. Stability of POROS MabCapture A in 0.1N Sodium Hydroxide



The capacity of the media is only slightly decreased after exposure to 0.1N NaOH, for 30 minutes per cycle at 500cm/H, for 50 sanitization cycles.

CONCLUSIONS

POROS MabCapture A chromatography media presents with high IgG dynamic binding capacities at high linear flow rates and short residence times. Operating the media at high flow rates without compromising dynamic binding capacity provides Process Development scientists with an alternative to agarose based materials for the efficient capture chromatography of high volume, high titer feedstreams.
Using POROS MabCapture A imparts process design flexibility: columns may be operated at higher flow rates, with reduced residence times and with shorter bed heights.
POROS media is mechanically and chemically stable. The mechanical stability results in easy handling and packing and stable bed heights after packing and with column re-use. The chemical stability provides the capability for aggressive cleaning and therefore robust re-use performance. When using MabCapture A, sodium hydroxide can be incorporated into chromatography media cleaning steps.

TRADEMARKS/LICENSING

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