A Comparison of Microparticulate, Membrane, and Monolithic Anion Exchangers for Polishing Applications in the Purification of Monoclonal Antibodies.

Pete Gagnon, Richard Richieri[1], Simin Zaidi[1], Francis Aolin[1]

Validated Biosystems, 240 Avenida Vista Montana, Suite 7F San Clemente, California 92672; Avid Bioservices Inc[1] 14282 Franklin Avenue, Tustin, California 92780

Introduction

Membrane based anion exchangers are being used increasingly for purification of monoclonal antibodies. The transition from particle-based anion exchangers is driven partly by the convenience of membranes and partly by the cost saving associated with their disposability, however the feature that makes them functionally superior is more effective mass transport.

Mass transport is a major contributor to anion exchange binding efficiency, especially for large contaminants such as DNA, endotoxins, and viral particles. Fluid flows preferentially through the spaces between particles—the void volume — in traditional packed beds, while binding depends on diffusion of solutes into and out of dead-end pores as the mobile phase passes down the column. The larger the contaminants, the slower their diffusion constants and the slower the flow rate must be to allow them to come in contact with binding sites inside the pores. DNA in particular has a very low diffusion constant, making it a good model for anion exchange efficiency (Table 1, Figure 1). Pore accessibility is another limitation with particle based media. So-called wide-pore media generally have average pore diameters of about 1000 Å, roughly the same as a 100 nm viral particle. Anything larger has access to only the particle surface, which represents a small fraction of the total ion exchange surface.

Convective mass transport operates independently of diffusion and is consequently independent of solute size. It is also independent of flow rate. This allows anion exchange membranes to achieve good capacity at high flow rates, however their mass transport efficiency is offset by the fact that each membrane represents only a single chromatographic plate. Space must be left between layers because the pore distribution between layers is discontinuous. Chromatographic efficiency declines further from turbulent mixing between membrane layers and elsewhere within the housing.

Monoliths are characterized by a network of highly interconnected channels, with diameters ranging from 1-5 μm. This architecture permits convective mass transport, endowing monoliths with the ability to capture large solutes with high efficiency at high flow rates. In addition, monoliths exhibit plate efficiencies rivaling the best microparticulate packings, and they lack the void volume that plagues both membranes and microparticles. [1,2] This last feature is important because turbulent mixing in the void volume (eddy dispersion) is a primary cause of band spreading in chromatographic separations. This combination of attributes suggests that monoliths should offer higher efficiency than either membranes or porous particles. This study challenges that hypothesis with two large, clinically significant contaminants: endotoxin and DNA.

Materials and methods

All experiments were conducted on an AKTA™ Explorer 100 (GE Healthcare). DNA, endotoxin, bovine serum albumin (BSA), buffers, and salts were obtained from Sigma. Q Sepharose™ Fast Flow in 1 mL HiTrap™ columns was obtained from GE Healthcare. Sartobind™ Q nano (1 mL) membranes were obtained from Sartorius. CIM® QA monoliths, 0.34 mL (axial) and 2.5 mL (radial flow), were obtained from BIA Separations.

DNA and endotoxin binding capacities were determined by conducting dynamic breakthrough studies with 0.1mg/mL DNA or endotoxin in 0.05 M Hepes pH 7.0. Solutions were membrane filtered to 0.22μm before chromatography. Q Fast Flow HiTraps were run at 1 mL/minute. Sartobind Q and CIM QA (axial flow) anion exchangers were run at 4 mL/min. Three CIM disks were combined in a single housing to give a 1 mL volume. Fresh media (all types) was used for each experiment.

To confirm the ability of monoliths to remove DNA from IgG solutions, 350 mL of 0.1 mg/mL DNA mixed with 1.0 mg/mL protein A-purified monoclonal IgG_1 chimera was applied to a 2.5 mL radial flow QA monolith at 6.0 mL/min. Samples were taken at 10 mL intervals. DNA levels were measured by picogreen testing, conducted by Southern Research Institute, Birmingham, AL USA (www.southernresearch.org).

Results and Discussion

Breakthrough curves for DNA are shown in Figure 2. Dynamic binding capacities for endotoxin and DNA are given in Table 2. DNA capacities are plotted in Figure 3. Consistent with the combination of low diffusion constants and narrow pore diameters, capacities for both DNA and endoxin were lowest on the particle based anion exchanger. At 1% breakthrough, endotoxin capacity per mL of media was more than 4 times higher on the membrane and more than 13 times higher on the monolith, even though both the latter were operated at a 4-fold higher flow rate. A similar but more dramatic pattern was observed with DNA capacity, which was nearly 20 times higher on the membrane and almost 50 times higher on the monolith.

Among convective anion exchangers, monoliths offer not only higher capacity than membranes but also higher binding efficiency. This is illustrated in Figure 4, where the profiles are scaled to 10% breakthrough. The earlier breakthrough and shallower slope of the membrane curve are consistent with lower binding efficiency. The percent differential from the point where breakthrough was visually detectable to the 10% breakthrough value was calculated. The "no-breakthrough" portion of the monolith curve was 93% of the 10% breakthrough value, compared to only 63% for the membrane. The presence of IgG did not impair DNA removal by the monolith. DNA levels in all fractions were beneath the detection level of the assay, about 1 ng/mL, indicating at least 5 logs of DNA removal across the entire sample application.

Table 1. Selected Diffusion Constants

Solute	Size	K_{diff}
BSA	66 kDa	6.7 x10^{-7}
IgG	150 kDa	4.9 x10^{-7}
Urease	480 kDa	3.5 x10^{-7}
IgM	960 kDa	2.6 x10^{-7}
Endotoxin	2 MDa	2.1 x10^{-7}
CMV	5 MDa	1.2 x10^{-7}
TMV	40 MDa	5.0 x10^{-8}
DNA_1	4.4 kbp	1.9 x10^{-8}
DNA_2	33.0 kbp	4.0 x10^{-9}

CMV: Cucumber mosaic virus
TMV: Tobacco mosaic virus

Table 2. Dynamic Capacities

Exchanger	Endotoxin	DNA
QFF		
1% bt, mgs	8.5	0.3
5% bt	12.1	0.4
10% bt	14.5	0.5
Q nano		
1% bt, mgs	35.5	5.9
5% bt	40.4	6.9
10% bt	42.9	7.6
CIM QA		
1% bt, mgs	114.7	14.6
5% bt	137.1	15.1
10% bt	147.2	15.4

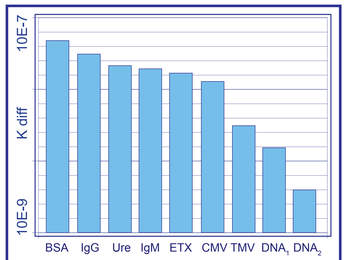

Figure 1. Selected diffusion constants.
See Table 1 for more precise values.

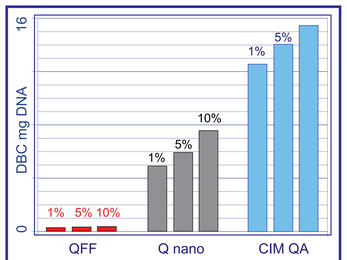

Figure 3. Dynamic capacities for DNA.
See Table 2 for more precise values.

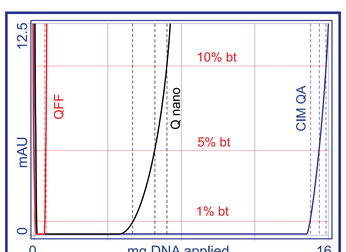

Figure 2. Breakthrough curves.
See Materials and Methods for details.

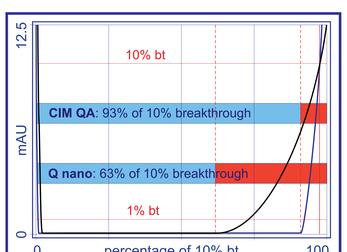

Figure 4. Binding efficiency.
Refer to discussion for explanation.

Conclusions

This study has important implications for manufacture of therapeutic antibodies. Although diffusive particle anion exchangers have proven adequate for reducing DNA and viral contamination to clinically acceptable levels, it is clear that they have done so in spite of their fundamental inappropriateness for the task. The higher capacity and efficiency of convective anion exchangers promise not only better process economics but, more importantly, lower patient risk in the clinic. According to the results of this study, a monolith with a bed volume 10% the size of a conventional anion exchanger could remove 5 times as much DNA in about the same amount of time. A monolith 20% the size of a conventional exchanger could remove 10 times as much DNA in half the time. Given their large size and slow diffusion constants, viral particles should be expected to behave similarly to DNA. Additional studies are required to confirm this, and to characterize the behavior of aggregates, leached protein A, and host cell proteins. This will be of special interest with the weak-partitioning conditions employed in 2-step (protein A /anion exchange) IgG purification procedures, where the low dispersion characteristics of monoliths should enhance contaminant discrimination.

Literature cited

1. Strancar et al, 2002, *Adv. Biochem. Eng. Biotechnol.,* **76** *50*
2. Hahn et al, 2002, *Sep. Sci. Technol.,* ***37****(7) 154*

Acknowledgements

Thanks to BIA Separations GmbH Austria for provding monolithic anion exchangers to conduct this study. This poster was originally presented at the IBC World Conference and Exposition, Boston, Massachusetts, USA. October 1-4, 2007. Copies of this poster can be downloaded at **www.validated.com**.