WHITEPAPER

An Introduction to Monolith Chromatography

INTRODUCTION

Monoliths, as chromatographic stationary phases, were introduced approximately 35 years ago, independently by several researchers (ref 1). The main motivation was to improve separation efficiency of a variety of molecules and nanoparticles, especially in the field of biologics. The term "Monolith" refers to a wide group of convective-flow based stationary phases, defined as chromatography sorbents cast as a homogenous phase into chromatography columns as a single, continuous piece (ref 2). They can be prepared in any geometry or volume and can be used either for analytics or preparative isolation. They can have very different microstructure (Figure 1) and skeleton chemistry, defining their hydrodynamic properties, stability and performance. On the other hand, they share several common features, differentiating them from particle-based chromatographic phases, and making them attractive for analysis and purification of certain molecules and nanoparticles.

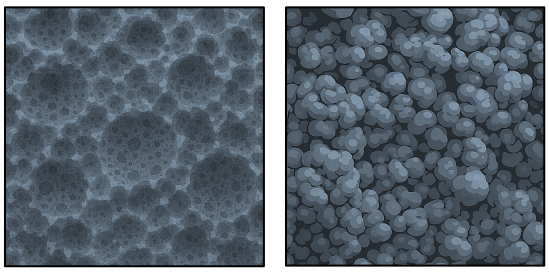

Figure 1: Cartoon illustrating polymethacrylate monoliths with varying microstructure achieved through different formulations.

MONOLITH FEATURES

All monoliths are highly porous, with a flow-through porosity above 0.5 and even up to 0.9. These values are substantially higher than the open porosity of particle-based chromatographic resins, exhibiting flow-through porosity of around 0.35. High flow-through porosity reflects in lower pressure drop to otherwise identical particle-based chromatographic media, as it can be concluded from the Blake-Kozeny equation (Kozeny–Carman equation - Wikipedia; ref 3). This allows application of high flowrates, while rigid monolith skeletons minimize monolith compression. In fact, the linear relation between pressure drop and flowrate is typically within the entire working flow range, confirming constant permeability.

Flow-through porosity consists of interconnected channels, without dead-end pores with stagnant liquid. Because of that, the entire mobile phase transport is governed by convection, carrying also sample molecules and nanoparticles, accelerating in this way their interaction with the surface. Because of that, chromatographic separation efficiency is no longer governed by pore diffusion, a mechanism typical for porous particle-based chromatographic resins. Since convective transport is much faster compared to diffusion transport, separation efficiency becomes practically independent of the mobile phase flow (Figure 2). Consequently, efficient separation of molecules can be performed in a short time, indicating their potential in fast analytics. Considering also their lower pressure drop, monoliths are a matrix of choice for in-process monitoring.

Besides flow-independent separation, convection governed transport also has significant impact on dynamic binding capacity. Maximal resin binding capacity depends primarily on the total surface area of the chromatographic resin. On the other hand, dynamic binding capacity depends also on time required for molecules to interact with the surface. While adsorption kinetics can be

considered instantaneous, overall binding kinetics are limited mainly by transport of the molecules to the active site. Clearly, due to much faster convective transport compared to diffusion transport typical for particle-based chromatographic resins, dynamic binding capacity of monoliths equals their maximal binding capacity, regardless of the applied flow rate. Flow-independent dynamic binding capacity has profound consequences on purification process efficiency, increasing productivity and robustness.

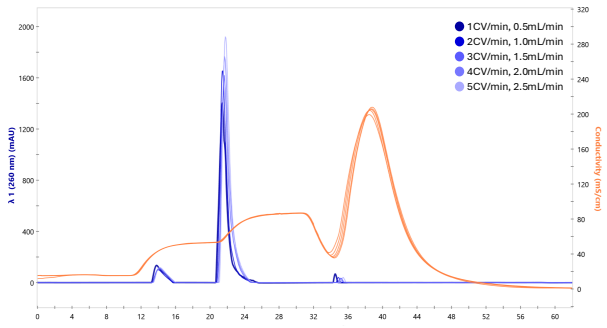

Figure 2: Overlay of chromatograms illustrating flow-independent separation of plasmid DNA (pDNA), maintained effectively across a range of flow rates (multiple column volume, CV/min).

For which type of molecules or nanoparticles are the features discussed above the most advantageous? Understandably, the slower the diffusion transport, the larger the benefit from convective transport. Since most biological molecules and nanoparticles are separated and purified from aqueous media, at room temperature and below, molecular diffusivity primarily depends on molecular size: the smaller the molecule, the higher its diffusion and vice-versa. Differences in diffusivity between various molecules and nanoparticles can vary up to three orders of magnitude. Just to illustrate practical consequences, a process that or a fast-diffusing molecule would last 1 minute would take for a very slow diffusing molecule up-to 1000 minutes, or 16 hours! It is therefore obvious that convection-based transport is most advantageous for very large biologic macromolecules such as plasmid DNA, or bionanoparticles, such as viruses, VLPs, exosomes, etc.

Another very important parameter for efficient purification is the high maximal binding capacity of the resin. Since in the case of biological macromolecules and bionanoparticles, monolayer adsorption occurs, maximal binding capacity is proportional to the accessible surface area of the resin. While the surface area of particle-based chromatographic resins is typically in the range of a few to several 100 m^2/ml, the surface area of the monoliths is commonly only several 10 m^2/ml or even lower. Based on these numbers, one can conclude that the maximal binding capacity of particle-based chromatographic resins is much higher than the binding capacity of the monolith. This is in fact the case for small and medium sized biomolecules, including medium sized proteins, but is not true for very large biologic macromolecules or nanoparticles. Small molecules can access the entire available surface area. However, most of the surface area in particle-based chromatographic resins is present within the dead-end pores, having typical size of several tens of nm. As the hydrodynamic diameter of very large biologic macromolecules and bionanoparticles is comparable or even larger than the pore size, they are not able to penetrate those pores, decreasing their maximal binding capacity dramatically, by an order of magnitude or more. For these kinds of molecules and nanoparticles, the binding capacity of monoliths surpasses the binding capacity of particle-based chromatographic resins. Monoliths are therefore the matrix of choice for purification of very large proteins like IgM, clotting factors, etc., and especially for plasmid DNA, viruses, virus-like particles (VLPs), exosomes, liposomes and others.

Due to their large sizes, these molecules and nanoparticles interact with the surface via several binding sites, making their interaction very strong. Therefore, elution from the surface is enabled via change of the mobile phase composition, typically via linear or even stepwise gradient. One very important consequence of such elution is that separation resolution is primarily determined by the slope of the linear gradient and not by the chromatographic column length. Because of that,

already very short chromatographic columns can provide excellent separation of large biologic macromolecules. This is important since pressure drop on the column is linearly proportional to the column length. Short column lengths therefore further decrease pressure drop, expanding the range of chromatographic equipment where such columns can operate at high flow rates. This explains why most of the monolithic columns are produced in a disk or annular format.

Another important consequence of multi-point interaction of large biologic macromolecules with the surface is peak spreading during elution. Without going into technical details, it was recently demonstrated theoretically and experimentally for convective based resins, that during gradient elution, the width of the elution peak decreases with increase in interaction sites (ref 4). From a practical perspective this means better separation and purification efficiency.

While any skeleton chemistry can be used, either inorganic or organic, silica-based monoliths, implemented predominantly for RP and affinity chromatography, and especially generally applied methacrylate monoliths, are the most extensively explored and commercialized. These types of chemistry were already extensively used previously for particle-based chromatographic resins, therefore their compatibility with various biologic materials as well as functionalization into desired interaction chemistry have been well investigated. In addition, they are both rigid, and methacrylate monoliths especially are also chemically stable and can routinely withstand sanitation with 1 M NaOH.

WHERE MONOLITHIC CHROMATOGRAPHIC COLUMNS SHOULD BE USED?

Based on their properties, monoliths can be used for any application when fast separation or isolation is required. However, flow-unaffected chromatographic properties, low pressure drop and high separation efficiency and dynamic binding capacity for very large macromolecules and bionanoparticles (ref 5, 6), make them the matrix of choice especially for:

- in-process analytics (monitoring) of various processes involving large macromolecules and bionanoparticles
- separation of different plasmid isoforms on either analytical or preparative scale
- separation of full and empty viral capsids on either analytical or preparative scale
- purification of larger quantities of plasmid DNA, especially in gene therapy
- purification of larger quantities of various viruses and virus-like particles
- purification of exosomes, liposomes, ribosomes and other biological nanoassemblies, when high purity is required.

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