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Comparison of perfusion media and monoliths for protein and virus-like particle chromatography

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ABSTRACT

Structural and performance characteristics of perfusion chromatography media (POROS HS 20 and 50) and those of a polymethacrylate monolith (CIM SO3-1 tube monolith column) are compared for protein and virus-like particle chromatography using 1 mL columns. Axial flow columns are used for POROS while the monolith has a radial flow configuration, which provides comparable operating pressures. The POROS beads contain a bimodal distribution of pore sizes, some as large as 0.5 μm, which allow a small fraction of the mobile phase to flow within the particles, while the monolith contains 1–2 μm flow channels. For proteins (lysozyme and IgG), the dynamic binding capacity of the POROS columns is more than twice that of the monolith at longer residence times. While the DBC of the POROS HS 50 column decreases at shorter residence times, the DBC of the POROS HS 20 column for IgG remains nearly twice that of the monolith at residence times at least as low as 0.2 min as a result of intraparticle convection. Protein recoveries are comparable for all three columns. For VLPs, however, the eluted peaks are broader and recovery is lower for the monolith than for the POROS columns and is dependent on the direction of flow in the monolith, which is attributed to denser layer observed by SEM at the inlet surface of the monolith that appears to trap VLPs when loading in the normal flow direction.

1. Introduction

The slow diffusion of proteins and other large biomolecules such as plasmids, virus, and virus-like particles (VLPs) in aqueous solution adversely affects the performance of downstream purification processes that depend on diffusional mass transfer [1,2]. This is especially true for conventional porous stationary phases that are typically used in process-scale chromatographic columns for the purification of biopharmaceuticals. Since these columns are normally operated at low pressures, relatively large particle sizes (e.g. 50–100 μm) are typically used resulting, in turn, in long diffusion times and, thus, chromatographic performance that degrades rapidly as a function of flow rate [3]. Monoliths have been introduced during the last 10–20 years as an alternative to traditional diffusion-limited stationary phases. Monoliths are typically cast as a continuous matrix, which is intercalated with a network of relatively large flow channels where flow of the mobile phase and solute binding occurs virtually eliminating diffusional mass transfer limitations [4–8]. Smaller pores where convection does not occur can also exist in monolith matrices providing higher surface areas and, thus higher binding capacity (e.g. Ref. [9]). The chromatographic properties of monoliths have been experimentally investigated for both proteins, plasmids, and virus by several authors (e.g. Refs. [12–19]). In general, monoliths have shown flow-rate independent resolution and dynamic binding capacity for proteins demonstrating that diffusional mass transfer effects are absent. A drawback of monoliths is, however, that since the size of the flow channels is relatively small, typically 1–2 μm, their specific hydraulic permeability is usually low [5,10,11,20]. Thus, in order to achieve operating pressures that are acceptable in a manufacturing scale process, thin-disk or radial flow configurations are needed.

Perfusion chromatography media were developed to overcome intraparticle diffusional limitations in chromatography columns through the use of adsorbent particles with a bimodal pore size distribution. These matrices contain a network of small pores that provide surface area and, thus, binding capacity, which is transected by large through pores that allow convective transport within the particle [21–26]. Perfusion chromatography media can thus be viewed as intermediate between traditional porous

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particles and monoliths with respect to where convective flow occurs; in traditional porous particles, essentially none of the mobile phase flows within the structure where binding occurs (i.e. the particles); in monoliths, all of mobile phase flows within the structure where binding occurs (i.e. in the flow channels); and in perfusion matrices only a fraction of the mobile phase flows within the intraparticle through pores. Previous studies [3,23,26,27] have shown that, in practice, the fraction of mobile phase that flows within typical perfusion chromatography particles is small, typically much less than 1%, and is highly dependent of particle diameter, porosity, and pore size. Whether such a small fraction of flow can lead to a significant enhancement of intraparticle transport depends on the value of the intraparticle Peclet number [22–26]:

$$Pe_{\text{intra}} = \frac{ur_pF}{3D_e} \tag{1}$$

where u is the superficial velocity of the mobile phase in the chromatographic column, r_p is the particle radius, F is the fraction of the mobile phase that flows within the particles, and D_e is the effective pore diffusivity in the absence of intraparticle flow. In turn, Pe_{intra} can be related to a convection-enhanced diffusivity $\tilde{D}_e$ given by:

$$\frac{\tilde{D}_e}{D_e} = \frac{Pe_{\text{intra}}}{3}\left[\frac{1}{\tanh(Pe_{\text{intra}})} - \frac{1}{Pe_{\text{intra}}}\right]^{-1} \tag{2}$$

This ratio represents the enhancement of intraparticle transport due to the perfusive flow, relative to diffusion alone [23,24,28,29]. Eq. (2) predicts that when$Pe_{\text{intra}} > 5$ intraparticle transport becomes convection limited. For these conditions a maximum value of $\tilde{D}_e = ur_pF/9$ is obtained.

The behavior of columns packed with perfusion chromatography media has also been investigated experimentally by several authors for proteins, plasmids, and VLPs (e.g. Refs. [30–40]). Recently, we studied the mass transfer kinetics of various proteins and virus like particles (VLPs) in POROS HS 20 and POROS HS 50, two large-pore matrices designed for perfusion chromatography that differ principally in particle size (~20 and 50 μm, respectively) [41,42]. Estimated values of the intraparticle flow ratio, obtained for proteins and VLPs under non-binding conditions, were $F < 0.06\%$ for POROS HS 50 and $F \sim 0.2\%$ for POROS HS 20 [41,42]. The first of these values was shown to be too low to give any significant effect for typical proteins, while the second resulted in substantial enhancement. Similar values of F were also estimated for the same proteins based on the observation of intraparticle concentration profiles by confocal microscopy for strong binding condition. However, adsorbed VLPs (about 50 nm in radius) were found to block the pores so that their adsorption was limited to a thin layer near the outer particle surface. A similar result was also found by Trilisky et al. [11]. In their study comparing protein and virus adsorption on large pore particles and monoliths, the authors concluded that the large-pore particles studied (PL-SAX 4000A, 10 μm in diameter) were superior to monoliths for proteins at least as large as BSA, due to their larger binding capacity. However, the extent to which perfusion or intraparticle convection contributed to the experimental result is unclear from their study. For virus, these authors predicted, but did not demonstrate experimentally, that very large binding capacities could be obtained in monoliths

The overall goal of this work is a side-by-side comparison of monolith and perfusion chromatography media for identical conditions for both protein and VLP chromatography. We have three main specific objectives. The first is to compare the structure of a typical monolith with that of POROS perfusion chromatography media. The second is to compare their chromatographic performance for both proteins and VLPs for conditions for which the results can be directly compared. For the proteins, emphasis is on the dynamic binding capacity while for the VLPs emphasis is on the shape of the elution peak and on the corresponding recovery yield obtained with a salt gradient. The third objective is to establish a model taking into account intraparticle convection to predict the POROS column behavior and, thus, (a) determine the actual contribution of perfusion to chromatographic performance and (b) provide the means of extending the comparison beyond the range of experimental conditions studied.

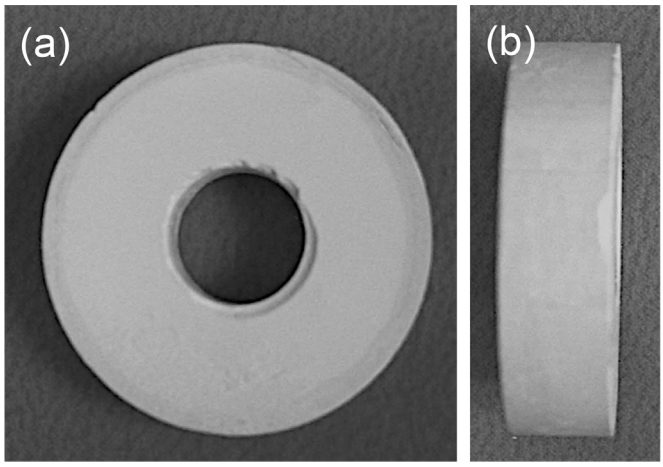

Fig. 1. Photograph of the 1 mL radial flow monolith used in this work removed from the housing. (a) top view. (b) side view. Normal flow direction is inward.

2. Materials and methods

2.1. Materials

The resins used in this work, POROS HS 20 and POROS HS 50, were obtained from ThermoFisher Scientific (Life Technologies Corporation, Grand Island, NY, USA). As shown in our prior work [42], the two resins have similar internal structure and differ principally in particle diameter (average diameter $d_p = 23$ μm for POROS HS 20 and 52 μm for POROS HS 50). Both resins are based on a poly(styrene-divinylbenzene) backbone functionalized with sulfopropyl cation exchange groups. However, based on previous data, POROS HS 20 has a smaller charge density than POROS HS 50 resulting in a smaller equilibrium binding capacity for typical proteins [42]. Both resins were flow-packed in 0.5-cm diameter, 5.0-cm long Tricorn columns from GE Healthcare (Piscatawy, NJ, USA) with a packed bed volume of about 1 mL each.

A CIM SO3-1 tube monolith column based on a sulfonyl-functionalized polymethacrylate backbone was obtained from BIA Separations (Ljubljana, Slovenia). The monolith is shaped as a hollow cylinder and contained in a plastic housing which allows the mobile phase to run in a radial direction, from the outer to the inner surface. Photographs of the monolith removed from the housing are shown in Fig. 1. The outer and inner diameters are $D_o = 1.86$ cm and $D_i = 0.67$ cm, respectively, and the height is $H = 0.42$ cm corresponding to a volume of 1.0 mL, exactly the same as the POROS columns used in this work. According to the manufacturer, the monolith porosity is $\varepsilon = 0.63$. The void volume associated with the monolith housing (i.e. the "dead volume") was estimated to be 0.7 mL by subtracting the monolith void volume (0.63 mL) from the chromatographic retention volume of a pulse injection of glucose to the assembled device.

The proteins and VLPs used here are the same as those used in our prior work [41,42] and are chicken egg white lysozyme ($M_r \sim 15$ kDa, pI ~ 11), obtained from Sigma Chemical Co. (St. Louis, MO, USA), a monoclonal antibody (IgG, $M_r \sim 150$ kDa, pI ~ 8.6), and VLPs of recombinant human papillomavirus (HPV) Type 11 capsid protein L1, provided by Merck & Co., Inc. (West Point, NJ, USA). All