Journal of Chromatography A, 1375 (2015) 92–100

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Particle size effects on protein and virus-like particle adsorption on perfusion chromatography media

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ARTICLE INFO

Article history:
Received 23 October 2014
Received in revised form
23 November 2014
Accepted 27 November 2014
Available online 4 December 2014

Keywords:
Perfusion chromatography
Mass transfer
Proteins
Virus-like-particles

ABSTRACT

The resin structure, chromatographic behavior, and adsorption kinetics of proteins and virus-like-particles (VLPs) are studied for POROS HS 20 and POROS HS 50 (23 and 52 μm mean diameter, respectively) to determine the effects of particle size on perfusion chromatography and to determine the predictive ability of available models. Transmission electron microscopy (TEM) and inverse size-exclusion chromatography (iSEC) show similar structures for the two resins, both containing 200–1000 nm pores that transect a network of much smaller pores. For non-binding conditions, trends of the height equivalent to a theoretical plate (HETP) as a function of reduced velocity are consistent with perfusion. The estimated intraparticle flow fractions for these conditions are 0.0018 and 0.00063 for POROS HS 20 and HS 50, respectively. For strong binding conditions, confocal laser scanning microscopy (CLSM) shows asymmetrical intraparticle concentrations profiles and enhanced rates of IgG adsorption on POROS HS 20 at 1000 cm/h. The corresponding effective diffusivity under flow is 2–3 times larger than for non-flow conditions and much larger than observed for POROS HS 50, consistent with available models. For VLPs, however, adsorption is confined to a thin layer near the particle surface for both resins, suggesting that the bound VLPs block the pores.

1. Introduction

The adsorption kinetics of proteins and other biomolecules in conventional stationary phases used for preparative and process scale applications is controlled predominantly by intraparticle diffusion. This is especially true for large biomolecules, whose transport is restricted by diffusional hindrance when the molecular size approaches the size of the matrix pores [1]. Increasing pore size alleviates the problem by reducing hindrance. However, even when the matrix pore size greatly exceeds the molecular size, transport is still slow since the molecular diffusion coefficients of these molecules are small [2]. Perfusion chromatography media were originally developed to overcome intraparticle diffusional limitations in chromatography columns through the use of adsorbent particles with a bimodal pore structure that allows convective, pressure-driven flow within larger pores transecting the particle while retaining significant binding capacity within the smaller, diffusive pores, which are connected to the convective through-pores [3].

Predicting the performance of perfusion chromatography rests on the ability to predict (1) the magnitude of intraparticle flow as a function of the particle structural characteristics, and (2) the effects of this intraparticle flow on chromatographic performance. A few models have been developed to predict the magnitude of intraparticle flow in such materials. With reference to Fig. 1, the following relationship has been proposed to estimate the ratio of intraparticle and extraparticle flows, F, assuming that the particles are an assemblage of microparticles of radius r_m and that the Carman-Kozeny equation describes both intraparticle and extraparticle permeabilities [4–8]:

$$F = \left(\frac{\varepsilon_M}{\varepsilon}\right)^3 \left(\frac{1-\varepsilon}{1-\varepsilon_M}\right)^2 \left(\frac{r_m}{r_p}\right)^2 \quad (1)$$

where ε_M and ε and are the intraparticle and extraparticle void fractions, respectively, and r_p is the particle radius. Assuming that the microparticle radius is equal to three times the pore radius leads to [6,7]:

$$F = 9\left(\frac{\varepsilon_M}{\varepsilon}\right)^3 \left(\frac{1-\varepsilon}{1-\varepsilon_M}\right)^2 \left(\frac{r_{pore,M}}{r_p}\right)^2 \quad (2)$$

where $r_{pore,M}$ is the macropores radius.

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http://dx.doi.org/10.1016/j.chroma.2014.11.083
0021-9673/

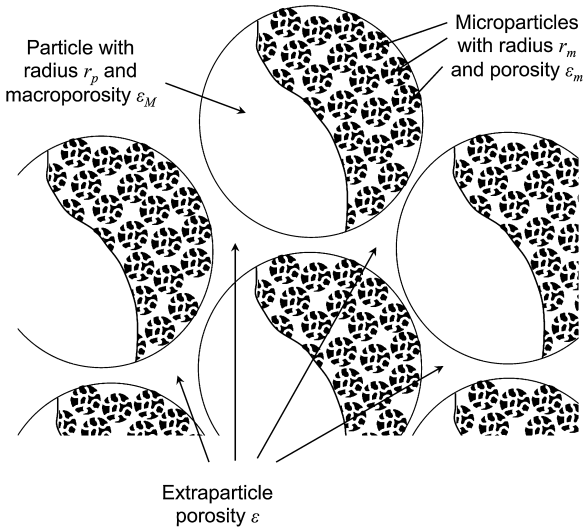

Fig. 1. Sketch of particles with a bi-modal pore size distribution defining extraparticle porosity, ε, macroporosity, ε_M, and microporosity, ε_m.

The model of Neale et al. [9] introduced by Carta et al. [10] to describe flow in perfusion chromatography media, uses the Happel free surface model [11] to describe flow in the extraparticle porosity of the chromatography column and the Brinkmann extension of Darcy's law [12] to describe intraparticle flow. In practice, since for actual perfusion media the intraparticle permeability is much smaller than the extraparticle permeability, the Neale et al. model predicts a nearly uniform intraparticle velocity whose magnitude agrees closely with that predicted by Eq. (1) [10]. However, direct experimental measurements of the intraparticle permeability of perfusion chromatography media made by Pfeiffer et al. [13] yielded values that are much higher than those estimated with Eq. (1) or (2), suggesting that the details of the actual particle structure may have to be taken into account in order to obtain reliable predictions.

Several studies have examined experimentally the chromatographic performance of columns packed with large-pore particles (e.g. [3,7,10,14,15]) and a few models have been advanced to predict the effects of intraparticle flow on chromatographic performance on that basis. Rodrigues et al. [16] introduced a model to predict the effects of intraparticle convection for slab-shaped particles based on the pulse response behavior of a chromatographic column under linear isotherm conditions. Carta et al. [10] and Carta and Rodrigues [6] developed a perfusion model for spherical particles. This model expresses the effect in terms of a convection-enhanced effective diffusivity $\tilde{D}_e$ given by:

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

where $Pe_{intra} = ur_pF/3D_e$ is an intraparticle Peclet number, D_e is the effective pore diffusivity in the absence of intraparticle convection, and u is the superficial velocity of the mobile phase in the chromatographic column. At high values of Pe_{intra}, Eq. (3) yields $\tilde{D}_e = ur_pF/9$ which corresponds to convection-controlled intraparticle transport. Similar relationships, also based on the assumption that the adsorption isotherm is linear, have been obtained by Rodrigues [17] based on a residence time distribution model and by Frey et al. [7] based on the Glueckauf linear driving force (LDF) approximation [18]. Liapis and McCoy [19] and Liapis et al. [20] obtained numerical solutions of the intraparticle convection-diffusion model of Carta et al. [10] for the Langmuir isotherm case for slab-shaped and spherical particles. For spherical particles under strong binding conditions, the calculations of Liapis et al. [20] show spherically asymmetrical intraparticle profiles that are increasingly skewed in the direction of flow at high Pe_{intra}. It should be noted that even though Eq. (3) was developed for the linear isotherm case, the same expression can also be used to predict breakthrough curves for a favorable isotherm using the LDF approximation by defining the rate coefficient as [21]:

$$k = \frac{15\tilde{D}_e}{r_p^2} \quad (4)$$

yielding results that are nearly coincident with the numerical results of Liapis and co-workers for the full model.

Regardless of the model used, it is evident that particle size has a critical effect. If intraparticle convection is absent, $\tilde{D}_e = D_e$, and Eq. (4) predicts that, as is well known, the mass transfer rate varies in inverse proportion to the square of the particle size. On the other hand, if intraparticle convection is dominant, combining Eqs. (1)–(3), yield $\tilde{D}_e \propto ur_pF \propto u/r_p$, which results in a mass transfer rate that is expected to vary with the cube of the particle size.

In our prior work [22], we studied the mass transfer kinetics of various proteins and virus like particles (VLPs) in POROS HS 50, a large pore cation exchanger with a 52 μm average particle diameter. Estimates of the intraparticle flow ratio were obtained for proteins and VLPs under non-binding conditions by comparing experimental van Deemter curves with the model of Carta and Rodrigues [6]. An average value of $F = 0.00063 \pm 0.0002$ was obtained for these conditions. Similar values of F were estimated for the same proteins based on the observation of intraparticle concentration profiles by confocal microscopy for strong binding conditions, suggesting that measurements under non-binding conditions can be extrapolated to predict the protein behavior under strong binding conditions. However, adsorbed VLPs (about 50 nm in radius), where found to block the pores so that their adsorption was limited to a thin layer near the outer particle surface.

The overarching goal of this paper is to determine experimentally the effects of particle size on perfusion chromatography. There are four specific objectives. The first is to compare the structure of POROS HS 20 (20 μm nominal particle size) with that of POROS HS 50 (50 μm nominal particle size). The second is to obtain the HETP as a function of flow rate for different proteins and VLPs under non-binding conditions and, thus, elucidate transport in the absence of binding. The third is to determine the intraparticle adsorbed concentration profiles during transient adsorption of proteins and VLPs on POROS HS 20 using CLSM and, thus, elucidate transport under binding conditions. The final objective is to compare the experimental results to available models to determine their ability to predict performance for actual systems.

2. Materials and methods

2.1. Materials

The resin used in this work, POROS HS 20, was obtained from Applied Biosystems (Life Technologies Corporation, Grand Island, NY, USA). According to the supplier, POROS HS 20 has the same structure as POROS HS 50, but smaller particle size. Both materials are based on a poly(styrene-divinylbenzene) backbone functionalized with sulfopropyl cation exchange groups. The particle size distribution was obtained from microphotographs and was found to span the range from 12 to 36 μm. The volume-average particle diameter is 23 μm, about one half the volume-average particle diameter of the POROS HS 50 sample used in our prior work [22].

The proteins and VLPs used are also the same as those used in our prior work [22] and are chicken egg white lysozyme (Lyo, $M_r \sim 15$ kDa, p$I \sim 11$), a monoclonal antibody (IgG, $M_r \sim 150$ kDa, p$I \sim 8.6$) available in our laboratory, and bovine thyroglobulin