Journal of Chromatography A, 1374 (2014) 180–188

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Perfusive ion-exchange chromatographic materials with high capacity

Bertrand Coquebert de Neuville, Alexandros Lamprou, Massimo Morbidelli, Miroslav Soos*

Institute for Chemical and Bioengineering, Department of Chemistry and Applied Bioscience, ETH Zurich, 8093 Zürich, Switzerland

ARTICLE INFO

Article history:
Received 3 June 2014
Received in revised form 21 October 2014
Accepted 25 November 2014
Available online 2 December 2014

Keywords:
Macro-porous perfusive particle
Reactive gelation
Polymeric brush media
Protein separation
Dynamic binding capacity
Protein separation

ABSTRACT

In this work, novel macro-porous chromatographic stationary phases, combining low mass transfer resistance and high binding capacity, were thoroughly characterized in terms of porosity, HETP, resolution and binding capacity. These new stationary phases exhibited better performance compared to commercially available materials, i.e. Poros 50HS and Fractogel EMD SO_3 (M). With the technique of reactive gelation under shear, it is possible to produce particles with pores from 100 nm to several microns, in which part of the flow can go through. This way, the mass transport inside the particles is significantly increased with perfusive flow faction values between 0.02 and 0.01. Despite the low pore surface area resulting from the large pore size, high binding capacity is obtained by functionalizing the pore surface with charged polymeric brushes resulting in a binding capacity in the range from 25 to 140 mg/mL col. This, together with the high mass transfer, gives excellent resolution performance and dynamic binding capacity compared to other commercial materials even at high flow rates.

1. Introduction

A large fraction of the production cost of therapeutic proteins comes from downstream chromatographic purification [1]. Despite the efforts made to replace chromatography, this technology remains one of the most used in industrial processes for protein purification [2]. In preparative chromatography, porous particles are usually chosen for their high adsorption capacity. However, a trade-off has to be made between the column backpressure and the column separation performance. A column with small particles has low intraparticle transport resistance but a high backpressure per unit column length, whereas large particles offer higher bed permeability but longer intraparticle diffusion pathway, which in connection to low protein diffusion coefficients results in strong peak broadening.

Some alternatives to porous particles with small pores have been developed to achieve high performance at high flow rates, such as monolithic stationary phases, chromatographic membranes or macro-porous particles with enhanced mass transport [3]. For the three cases, the principle is to promote convection through the particle pores so as to shorten the intra-particle diffusion path and then reduce mass transport resistances. The continuous structure of the monolith and the chromatographic membranes indeed enables convective flow through the pores. However, monolith housing and production of long columns are often difficult [4], while for the membrane technology, the columns are limited to short stack of membranes due to low mechanical resistance. Therefore, these technologies are typically applied for flow-through purification or for the capture of diluted proteins [5], but due to their low binding capacity related to the length constraints, they are not suitable for capture step of concentrated supernatants. By combining the properties of porous particles and monoliths, macro-porous particles have been introduced [6]. For such macro-porous stationary phases, the large particle size ensures low pressure drop while the large pores enable a part of the flow to go through the particles (perfusion), resulting in a high permeability with enhanced mass transfer. These particles are commonly produced through the traditional suspension polymerization technique, resulting in two kinds of pores: large pores with a radius between 300 nm and 400 nm in which convective flow is possible and small pores of radius between 25 nm and 75 nm where the solute moves only by diffusion [7]. In this way, the diffusion path is shortened thus improving the performance of the column. Perfusion chromatography has been applied for protein analytics [8,9], as well as for protein purification using preparative chromatography [10,11]. A drawback of this method is the reduction of the surface area inside the particle due to the presence of large pores, with a consequently reduced binding capacity.

By combining elements from colloidal and polymer reaction engineering, a novel route towards macro-porous materials has

* Corresponding author. Tel.: +41 44 6334659; fax: +41 44 6321082.
E-mail address: miroslav.soos@chem.ethz.ch (M. Soos).

http://dx.doi.org/10.1016/j.chroma.2014.11.066
0021-9673/

been developed named "Reactive Gelation". This has been applied both under stagnant conditions to produce monoliths [4,12] as well as under shear to produce particles [13–15]. In both cases, the internal porous structure is characterized by a fractal geometry, which is more dense in the case of the particles. This results in pore size distributions with average size in the order of 1 μm. The pores are typically all accessible to proteins that have a size between 1 nm and 20 nm, e.g. monoclonal antibodies.

From such substrates, polyelectrolyte brushes have been grafted via flow-through Atom-Transfer Radical Polymerization (ATRP), so that electrostatic interactions become effective [14–16]. An advantage of this technique with respect to the commonly used functionalization methods [17], with which a single charged group is located directly on the pore surface, is that the pores are covered with brushes containing several charged groups, offering a higher binding capacity. Comparison between classical and perfusive particles was carried out more than a decade ago for the previous generation of macro-porous media produced by suspension polymerization [18]. Following a similar approach, this work aims at characterizing the new macro-porous particles so as to define their potential in protein large scale purification processes. For this, a comparison is shown with classical commercial materials: Poros 50HS, which are perfusive particles with no brushes and Fractogel EMD SO_3 (M) which are non perfusive particles containing brushes. The aim is to show the synergisms between brushes, to improve capacity, and perfusive characteristics, to improve transport characteristics, in determining the most suitable stationary phases for bio-molecule purification processes.

2. Theory

In the sequel, we briefly summarize the parameters that are used to evaluate the performance of the different stationary phases.

2.1. Column efficiency

The mass transfer resistance inside the particles induces broadening of the chromatographic peaks. This can affect the performance of the column by reducing its resolution power as well as its dynamic binding capacity. The mass transfer resistance can be conveniently quantified with the Height Equivalent to a Theoretical Plate (HETP), whose inverse expresses the separation efficiency of the column at a specific linear velocity. For porous particles, the variation of the HETP with the interstitial velocity ν can be described as follows [19,20]:

$$\mathrm{HETP} = A + \frac{B}{\nu} + C\nu \tag{1}$$

where the coefficient A is a function of the eddy diffusion in the column and the B term stands for the molecular diffusion along the column. Since the considered velocities are large, the B term is neglected in this work. The coefficient C is a measure of the mass transfer resistance in the particles and is inversely proportional to the effective pore diffusion coefficient, as derived for example from the general rate model in non-retaining conditions [20,21]:

$$C = \frac{1}{D_{\mathrm{eff}}}\frac{R_{\mathrm{p}}^2}{15}\frac{2\varphi\varepsilon_{\mathrm{p}}^2}{(1+\varphi\varepsilon_{\mathrm{p}})^2} \tag{2}$$

where D_{eff} is the effective diffusion coefficient in the pores, ε_{p} the accessible particle porosity for the solute, φ the column phase ratio equal to $(1-\varepsilon_{\mathrm{b}})/\varepsilon_{\mathrm{b}}$ and R_{p} the particle radius. Due to their size proteins typically exhibit rather large values of the coefficient C in comparison to small molecules. This leads to strong limitations on the velocity values at which protein chromatographic process can be operated with reasonable HETP values.

In order to overcome this problem, porous particles containing large pores through which the liquid can perfuse and thus enhance intra-particle mass transfer were prepared by Regnier [22–24] and thoroughly studied by Carta and Rodrigues [11,25,26,27,28]. Using Eq. (1) it is possible to relate the HETP value under perfusive conditions to the operating conditions by replacing the coefficient C with a new one, C_{perf} defined as follows [25,29]:

$$C_{\mathrm{perf}} = \frac{1}{D_{\mathrm{eff}}}\frac{R_{\mathrm{p}}^2}{15}\frac{2\varphi\varepsilon_{\mathrm{p}}^2}{(1+\varphi\varepsilon_{\mathrm{p}})^2}\frac{3}{(2/3)Pe_{\mathrm{perf}}} \times \left(\frac{1}{\tanh((2/3)Pe_{\mathrm{perf}})} - \frac{1}{(2/3Pe_{\mathrm{perf}})}\right) \tag{3}$$

where Pe_{perf} refers to the perfusive Peclet number, which describes the relative importance of convection through the pores with respect to intra-particle diffusion [25]:

$$Pe_{\mathrm{perf}} = \frac{FuR_{\mathrm{p}}}{2D_{\mathrm{eff}}} \tag{4}$$

with F being the fraction of the flow perfusing through the particle and u the superficial velocity. From the pressure drop equality on the column and the particle [29,30], F is calculated as a function of the particle radius, R_{p}, the pore radius, r_{pore}, the particle porosity, ε_{p}, and the bed porosity, ε_{b}, according to:

$$F = 9\left(\frac{\varepsilon_{\mathrm{p}}}{\varepsilon_{\mathrm{b}}}\right)^3\left(\frac{1-\varepsilon_{\mathrm{b}}}{1-\varepsilon_{\mathrm{p}}}\right)^2\left(\frac{r_{\mathrm{pore}}}{R_{\mathrm{p}}}\right)^2 \tag{5}$$

As it can be seen from Eq. (3), for low perfusive Peclet number Pe_{perf}, perfusion is negligible compared to diffusion, and Eq. (3) is then equivalent to Eq. (2). Therefore the HETP increases linearly with velocity. On the other hand, for large perfusion contributions and then large perfusive Peclet number, Pe_{perf}, the HETP increases first with velocity but then reaches a plateau value which then remains constant at higher velocity values.

2.2. Column resolution

Another important parameter to consider for column characterization is its resolution, which indicates the potential of the column to separate two different solutes. This is defined as the ratio of the difference of the solutes retention times t_{r} (i.e. column separation power) over the sum of the respective baseline widths W (i.e. column performance) as follows [19]:

$$R_s = 2\frac{|t_{\mathrm{r}3} - t_{\mathrm{r}1}|}{W_1 + W_2} \tag{6}$$

Two components are considered to be fully separated when the resolution is larger than one.

2.3. Column binding capacity

In order to increase productivity in preparative chromatography, it is important to use a column with a high dynamic binding capacity (DBC). This corresponds to the amount of protein that a column can adsorb until the solute starts to break through. It is strongly affected by the mass transfer properties of the medium, and therefore varies with the flow rate. On the other hand, the equilibrium binding capacity (EBC) indicates the total amount of protein that can be adsorbed in the column at equilibrium and its value is independent from the flow rate and the mass transfer resistance. Both these quantities have to be considered in evaluating the capabilities of a chromatographic column as the EBC gives information on the adsorption capacity at equilibrium conditions, while the DBC shows the influence of the mass transfer resistance in the loading process [19].