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Effect of geometry and scale for axial and radial flow membrane chromatography—Experimental study of bovin serum albumin adsorption

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ABSTRACT

During the last 10 years, membrane chromatography (MC) has been increasingly reported for biomolecule purification at both small and large scales. Although, several axial and radial flow MC devices are commercialized, the effect of the device dimensions on the adsorption performance has not been fully investigated. In this study, axial and radial flow anion ion-exchange MC devices were used for bovine serum albumin (BSA) adsorption. For both axial and radial flow, three devices at different scales were compared, two having similar diameter and two similar bed height. The pressure drop and the flow distribution using acetone as a non-binding solute were measured, as well as BSA breakthrough curves at different flow rates and BSA loading concentrations. For all devices, it was observed that the flow rate had no effect on the breakthrough curve, which confirms the advantage of MC to be used at high flow rates. In addition, the BSA binding capacity increased with increasing BSA concentration, which suggests that it could be preferable to work with concentrated solutions rather than with very dilute solutions, when using buffer at high phosphate concentration. For both axial and radial flow, the bed height had a negative impact on the binding capacity, as the lowest binding capacities per membrane volume were obtained with the devices having the highest bed height. Radial flow MC has potential at large-scale applications, as a short bed thickness can be combined with a large inlet surface area.

1. Introduction

Membrane chromatography (MC) was introduced in the late 1980s as a novel chromatographic technique based on the integration of membrane filtration and liquid chromatography into a single-step operation [1]. From the beginning of the 1990s, MC has been extensively designed and evaluated in different geometries such flat sheet systems and stacks of membranes, hollow fibers, radial flow cartridges, and different interaction modes including affinity interaction, ion exchange, hydrophobic interaction, reversed-phase and multistage chromatography [2–5]. Nowadays, MC is being employed for the purification and polishing of a large range of biomolecular species, including purification of monoclonal antibodies, DNA, and virus capture. MC devices are commercially available from several suppliers, ranging from laboratory scale to process scale.

The benefit of MC over conventional resin chromatography is mainly attributed to the shorter diffusion times, as the interactions between molecules and active sites in the membrane occur in convective through-pores rather than in stagnant fluid inside the pores of the adsorbent particles. Therefore, MC has the potential to maintain high efficiencies both at high flow rates and for use of large biomolecules with small diffusivities, reducing biomolecules degradation and denaturation. Low pressure drop associated with high flow rate, as compared to packed bed chromatography, reduced buffer usages due to low void volume and scalability for process development are other key advantages of MC. In addition, MC devices can be used as single-use units to eliminate the requirement for cleaning and regeneration and to reduce contamination risk. It has been estimated that single-use techniques can reduce by up to 40% the capital costs of production facilities in the biopharmaceutical manufacturing. Its advantages have made MC to have the highest market growth among all commercial disposable devices, such as mixing systems and bioreactors, with an annual growth rate of nearly 27% between 2006 and 2012 [6].

MC devices are often characterized by the shape of their breakthrough curves. The breakthrough curve shape is governed by

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adsorption kinetics within the functionalized membrane and by fluid hydrodynamics in the hold-up volumes of the MC devices. Commercial MC devices are optimized such as to obtain breakthrough curves that are as sharp as possible, in order to minimize buffer consumption and to maximize the utilized membrane capacity. Significant developments in MC devices have been obtained by considering advanced materials [7,8], polymer grafting of the surface of the pore walls [9,10], fluid flow distribution and collection within the MC device [11,12], as well as optimized geometry [13,14].

The scaling up of MC devices has been reported in several studies. For example, Briefs and Kula [15] increased the membrane diameter from 90 mm to 142 mm of a stack of 96 membranes without any change in the anion exchange membrane capacity. Using devices having two different diameters of 15 and 25 mm, a good resolution was obtained compared to DEAE-Sephacel gel for pyruvate decarboxylase purification. Huang et al. [16] investigated the radial flow MC devices made from modified cellulose for trypsin removal. The trypsin binding capacity was found linearly related to the bed volume for 250, 800 and 3200 ml devices. Puthirasigamany et al. [17] measured the binding capacity of two Nano Sartobind Q devices with different bed volume: 1 ml and 3 ml. For the 3 ml device, the dynamic binding capacity per unit of membrane volume was found around 20% lower than the one obtained with the 1 ml device, although this result was not discussed. Ghosh et al. [14] used two MC devices with a scale-up factor of 15,000: an axial flow Sartobind Pico MC capsule with 0.08 ml bed volume and a radial flow Sartobind 1.2 l MC capsule. A simulation based on CFD and on the spreading binding model was developed for analyzing MC at the very small scale, and transferring the identified binding mechanism and parameters for predicting the performance of the very large scale device. These authors underlined that the introduction of appropriate flow distribution and binding mechanism for each device was necessary to obtain a good fit between modeling and experimental values.

The effect of membrane diameter and bed height of membrane absorbers has also been investigated by some authors. For example, Josić et al. [18] used anion exchange MC devices made from poly(glycidyl methacrylate) membranes for separations of standard proteins. The thickness of the membrane layers was between 1 and 7 mm and the disc diameter between 10 and 50 mm. The results obtained showed that with increasing thickness better separation was achieved. The separation obtained with a 10 mm diameter disc could also be achieved with a 50 mm diameter disc. Knudsen et al. [13] determined the breakthrough capacities of Sartobind cation-exchange membranes as a function of layer number, from 1 to 60. The continued rise in breakthrough capacity was explained by the inefficient flow distribution within the MC devices and/or the housing and the experimental system.

The comparison between axial and radial flow has been little studied using MC devices. For example, Ghosh et al. [14] used two MC devices: an axial flow Sartobind Pico MC capsule and a radial flow Sartobind 1.2 l MC capsule. However, due to the very different scales, the comparison between the two devices was difficult. On the contrary, comparison of radial flow over axial flow chromatography using traditional resin columns has been largely investigated (e.g. Besselink et al. [19], Tharakan [20]). A radial flow column typically consists of two concentric cylinders between which the resin bed is packed. The liquid is directed from outside inwards or vice versa, resulting in horizontal, radial flow. In a recent study, Besselink et al. [19] compared axial and radial flow affinity chromatography using columns packed with affinity resin to adsorb BSA. No difference in performance between the two columns was observed. The authors concluded that for small-scale processes, axial flow chromatography may be preferable, for resin volumes of at least several tens of litres, radial flow chromatography is probably the best choice. Unlike radial flow chromatography, axial flow chromatography has significant limitation of scaling up because high volumes can be obtained by varying only the membrane diameter, while the bed height is maintained constant. Higher scalability is obtained using radial flow geometry by increasing both column height and diameter [20].

In this work, the effect of axial and radial flow, membrane area, membrane diameter, and bed height on the MC device performance is investigated. Commercialized strong anion ion-exchange MC is used for bovine serum albumin (BSA) adsorption. For both axial and radial flow, three devices with different membrane area are tested, two having similar diameter, and two similar bed height. The flow distribution is first observed under a non-binding condition loading an acetone solution. BSA breakthrough curves are then compared at different flow rates and BSA loading concentrations. The dynamic binding capacity at 10% breakthrough is calculated and compared for the various devices. Finally, the effect of flow configuration, dimensions of MC devices on flow distribution and binding capacity is discussed.

2. Materials and methods

2.1. Materials

BSA lyophilized powder (≥98.00% purity) was purchased from MP Biomedical (France). BSA was dissolved in a phosphate buffer prepared from 100 mM solution of K_2HPO_4 and KH_2PO_4, adjusted to pH 7.0. The elution buffer was phosphate buffer saline (PBS), prepared by adding 1 M NaCl to the above buffer, and adjusted at pH 7.0. The washing and regeneration buffers were 1 M NaOH. Except BSA, all chemical reagents used in this study were purchased from Sigma Aldrich (France). Ultra-pure water was obtained using a Milli-Q system (Millipore, France). Prior to use, all buffer solutions were filtered through a hydrophobic membrane filter with a 0.45 μm pore size (Millipore, France). A 0.22 μm polyethersulfone hydrophilic Millex-GP filter unit (Millipore, France) was set-up before the MC device to remove fine particles from solutions during the experiments.

The experiments were carried out on the Äktaprime Plus chromatography system (GE Healthcare Life Sciences, France), which includes a system pump, a fraction collector, a pressure sensor, and monitors for UV and conductivity. Valves for buffer selection, sample injection, gradient formation, and flow diversion are integrated into the system.

2.2. Strong anion ion exchange MC

All MC devices were obtained from Sartorius Stedim Biotech GmbH (Goettingen, Germany). They contain a stabilized reinforced cellulose membrane with thickness 275 μm and pore size around 3–5 μm. Functionalized quaternary ammonium (Q) groups are bound covalently to a grafted polymer layer.

The characteristics of the MC devices provided by the manufacturer are summarized in Table 1. For each radial flow devices, the outer diameter and the cylindrical height were obtained from the nuclear magnetic resonance (NMR) technique. For both axial and radial flow, three different devices were investigated. The flow configuration is shown in Fig. 1. Axial flow devices are composed of several stacked membrane sheets in capsules. The flow goes from top through the membrane bed to the outlet. Inside radial flow devices, the membrane is in the form of spiral wound or rolled around a cylindrical core. The flow pattern is from outside of the membrane cylinder through the membrane bed to the inside core of the membrane cylinder. The superficial velocity was determined as the flow rate divided by the cross-sectional area of the bed, that