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A direct comparison between membrane adsorber and packed column chromatography performance

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Abstract

The purpose of this work was to compare side by side the performance of packed bed and membrane chromatography adsorption processes for protein purification. The comparison was performed using anion exchange media with the same ligand immobilized on the adsorbing surface, namely the strong Q quaternary ammonium group, R-CH_2-N^+-$(CH_3)_3$, and bovine serum albumin (BSA) as a model protein. In addition, the stationary phase volume was held constant for each geometry (3 mL) and runs were executed using the same mobile phase superficial velocity. As expected, the packed bed column showed higher equilibrium binding of BSA at 66.9 mg/mL versus 43.04 mg/mL for the membrane adsorber. Dynamic binding capacities were also higher in the packed bed; for example, at 97.5 cm/h, a capacity of 62.8 mg/mL was measured for the packed bed versus 20.7 mg/mL for the membrane adsorber. The higher equilibrium and dynamic capacities of the packed bed are likely due to the higher surface area per unit volume of the resin. However, the maximum productivity for the membrane adsorber was 111 mg/(mL h) a value that was 3.3 times higher than the one of the packed column. The bed utilization – defined as the ratio of the dynamic binding capacity at 10% breakthrough to the saturation binding capacity - was also higher for the packed column at long residence times and lower at short residence times confirming the better performance of membrane chromatography at high flow rates.

Keywords

Chromatography; Membrane adsorber; Packed column; Binding capacity; Productivity.

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1. Introduction

Packed bed chromatography is by far the most common technique used for high resolution separations of proteins, both as an analytical tool and as a process unit operation [1]. In a conventional process the column is operated in a capture mode, using highly porous resins with different functionalities according to the mode of operation. Among all chromatographic techniques, ion exchange has been the most widely employed method for protein purification since the development of cellulosic ion exchangers in the 1950s [2].

Membrane chromatography is a relatively new technique developed with the purpose of operating at higher flow rates and at reduced process time relative to bead-based column chromatography. Higher flow rates are possible with membranes because the resulting pressure drop is significantly lower than in traditional packed-column chromatography processes, and because solute transport in membrane devices is primarily by convection to the membrane internal surface, rather than the much slower intraparticle diffusion that is rate controlling in chromatography beads [1, 3-4]. In addition, since membrane adsorbers are generally less costly to manufacture, they can potentially be used as single-use devices that eliminate the difficulties and costs often associated with packing a chromatographic column, and the cleaning and validation steps required of multi-use columns [5].

One significant drawback associated with the use of membrane adsorbers in a capture mode is that their binding product capacities are generally lower than those of column chromatography resins. As a result, the industrial application of membrane adsorbers has been largely limited to flowthrough polishing applications in biopharmaceutical production processes in which small concentrations of impurities bind to the chromatography media, while product flows through [3-16]. Indeed, with the development of novel membrane materials with higher binding capacities the use of membrane adsorbers for product capture is of increasing interest [17-21]. Among those, cation exchange membranes made by porous polyacrylate hydrogels on a polypropylene support have been used for monoclonal antibody capture obtaining IgG dynamic binding capacities higher than 60 mg/mL at 10% breakthrough [6-7]. Comparable binding capacities were reported for high capacity multimodal cation exchanger membranes prepared by immobilizing poly(glycidyl methacrylate) tentacles on cellulosic membranes [8]. More recent work reports novel membranes with improved material structure: the use of polymer brushes and membrane based on electrospun nanofibers and nonwovens are the strategies that have been mostly employed [22-24]. These improved structures have resulted in high binding capacities. In addition, recent work has looked at the behavior of membrane adsorbers in different flow configurations [25-26] and has focused on developing interesting novel module designs that have resulted in improved performance [27-28].

The comparison of performance for different types of chromatographic supports has been considered by several authors, but most papers have reported results of experiments targeted to a specific application with columns of different volumes or experiments in which the operating conditions were not chosen with the aim of direct comparison. Ribero *et al.* investigated the performance of different chromatographic supports to be used for purification of recombinant Factor IX. They tested two different anion exchange resins, a membrane adsorber and a monolithic column and chose to continue with the monolith due to its higher binding capacity for the target molecule [29], based on their goal of finding the best material

for the purification of Factor IX. The higher value of the dynamic binding capacity was the main criteria of choice among different stationary phases together with the resolution and selectivity. However, the comparison did not keep column volume and superficial velocity constant among options.

Liu *et al.* tested commercial cation exchanger membranes and compared them to monoliths and packed columns for antibody capture with interesting results. Ultimately, they chose to operate with the protein A column for capture, despite the fact that the cation exchanger membrane adsorber, operated in overloaded isocratic mode, was more effective in the removal of host cell proteins and high molecular weight impurities [30]. Although Bhut *et al.* considered stationary phases of the same volume and the same weak anion exchange functionality, they presented newly designed membranes that were not commercially available with high binding capacity and compared them with standard materials: a regenerated cellulose membrane and an agarose resin [31]. However, superficial velocities between devices were not held constant. Similarly, in a pioneering work, Kubota *et al.* performed a very thoughtful comparison between hollow fiber membranes and a packed bed column of the same volume and the similar ion-exchange functionality; however, they did not operate the two supports at the same superficial velocity, which is an aspect that is often disregarded [32].

Comparisons between packed bed and membrane chromatography are rare in the literature and they are often undertaken to highlight the high throughput of novel membrane materials. The majority of the works that report such comparisons generally consider binding capacity as the major performance parameter for evaluation of the different options without looking at other chromatographic performance parameters such as resolution, productivity, or bed utilization.

Because of the numerous advantages that membrane chromatography potentially offers, this study aims to more directly compare properties and performance of the membrane and more traditional column geometries. The work presented in this paper considers porosity, binding capacity, mass transfer rate, bed utilization and productivity between the two geometries more fully than has been done in previous studies [29-32]. To do this, a side-by-side comparison of each geometry, using a membrane adsorber and chromatography resin that are commercially available, have the same strong anion exchange functionality, are readily scalable, and have identical bed volumes, 3 mL, was performed. Bovine serum albumin (BSA) is used as a model protein and the columns were operated in non-competitive conditions at the same superficial velocity to properly evaluate the transport phenomena limitations of the chromatographic supports. Comparison was also performed by looking at performance parameters with respect to residence time, even though due to the different bed geometries and packing characteristics it was not possible to operate the packed column at the same low residence times, i.e. high flow rates, of the membrane adsorbers.

2. Experimental

2.1 Materials

The membrane adsorber used during the experiments was an anion exchange Sartobind® Q Nano 3 mL, produced by Sartorius Stedim Biotech GmbH. This is a radial flow module consisting

of 30 membrane layers. The volume of the membrane bed is 3 mL and the membrane surface area available for flow is 110 cm^2. Liquid flows around the bed, then radially through the membrane layers into a central channel and finally through the outlet channel. The membranes present in the module have a base matrix of stabilized and reinforced cellulose with an immobilized quaternary ammonium group. This adsorber is a strong anion exchanger and has a long-term pH stability range from 2-12. The average membrane pore diameter is 3-5 µm. The module can be operated at a maximum pressure of 4 bar, the recommended flow rate is 10-15 mL/min, and the BSA binding capacity reported by the vendor is 29 mg/mL (conditions unspecified).

Q Sepharose™ Fast Flow resin, produced by GE Healthcare Life Sciences, was used as adsorbent for column experiments. This anion exchange resin has a matrix comprised of Sepharose™ (6% cross-linked agarose) and, like the Sartobind® Q Nano, the strong quaternary ammonium ligand. Beads are spherical with a diameter ranging from 45 to 165 µm and an average size of 90 µm as reported by the vendor. They can be used at superficial velocities up to 700 cm/h. The exclusion limit for globular proteins is 4 MDa, but the size distribution of intraparticle and interparticle pores is unknown. Beads are stable from pH 2 to 12 and they are resistant to all commonly used aqueous buffers (e.g. 1 M NaOH, 8 M urea, 8 M guanidine hydrochloride). A summary of the properties for each of the chromatographic media is given in Table 1.

All solution components, NaCl (USP/FCC/EP/BP grades), NaOH (NF/FCC grades), Tris (molecular biology grade), and HCl (reagent grade) for pH adjustment for Tris, used in these experiments were purchased from ThermoFisher Scientific. Lysozyme, molecular weight (MW) of 14388 Da and isoelectric point (pI) of 11.0 [33] was purchased from ThermoFisher Scientific [34]. Bovine Serum Albumin (BSA), MW 66430 and pI 4.7, was purchased from Sigma-Aldrich with a purity ≥ 96% [35]. BSA and lysozyme solutions were filtered with 0.21 µm cellulose nitrate membrane filters (Whatman) before experiments. The filter material was hydrophilic as to minimize non-specific protein adsorption.

2.2 Equipment

The characterizations of the chromatographic devices tested was performed on an ÄKTAexplorer 100, manufactured by GE Healthcare Life Sciences and controlled by UNICORN™ software. Experimental runs were carried out in parallel with both the Sartobind® Q Nano 3 mL module and with a column packed with Q Sepharose™ Fast Flow resin with a total bed volume of 3 mL.

For experiments that required a packed column, Q Sepharose™ Fast Flow resin was flow packed in an Econoline® Column from Sorbent Technologies Inc. The external tube is made of borosilicate glass with an inner diameter of 1 cm and an adjustable bed height from 0 to 12.5 cm. The pressure limit for the column is 80 bar.

2.3 Methods

2.3.1 Column packing

Resin was packed under flow with high purity water at a flow rate of 5 mL/min. To calculate the amount of resin slurry required for a bed volume of 3 mL, equal to the volume of the membrane unit, a packing factor of 1.1 was used. The resulting bed had a height of 3.8 cm, corresponding to a volume of 2.98 mL.

To assess the quality of packing, a pulse injection of a 2 M NaCl solution was made on the column equilibrated with 0.5 M NaCl to calculate the height equivalent of a theoretical plate (HETP) and the asymmetry factor of the packed column. Following the pulse injection, additional 0.5 M NaCl was fed to the column at 2 mL/min, which corresponds to a superficial velocity of 153 cm/h, to move the 2 M NaCl pulse through the column. Analysis of the conductivity peak from the column resulted in a reduced HETP value (=HETP/d_p, where d_p is the resin diameter) of 3.31 and an asymmetry value of 1.68.

2.3.2 Porosity determination

The porosities of the chromatographic media were determined by statistical moment analysis on experimental data obtained using lysozyme as a tracer [36-37]. Lysozyme has an isoelectric point of 11 [33] and therefore at pH 8 does not bind to the positively charged groups of the chromatographic supports; in addition, 0.25 M NaCl was added to the lysozyme solution to further prevent binding to the media. After equilibrating the membrane adsorber or the packed column with 50 mM Tris, 0.25 M NaCl, pH 8, a solution of 2.5 mg/mL lysozyme in equilibration buffer was injected using a 100 µL loop. Lysozyme in the column effluent was monitored by UV absorbance at 280 nm. For the membrane adsorber, injections were performed both with and without the membrane adsorber attached to the AKTAexplorer system. For the packed column, injections were performed both with a packed column attached to the AKTA system and an unpacked column with the plungers set to 0 mL.

Lysozyme injections were performed for each system setup (with membrane adsorber, without membrane adsorber, with packed column, and with unpacked column) at five different superficial flow velocities; lysozyme retention volume values were calculated as the averages of the results at each of the five superficial velocities. The superficial velocities were held constant between the experiments with the membrane capsule and those with the packed column. The experiments with the membrane unit were performed at 5, 7.5, 10, 12.5 and 15 mL/min, the latter being the operational flow rate recommended by the manufacturer. The superficial velocity corresponding to each of these flow rates was determined as an integral average of the radial velocity from the outer radius to the inner radius of the membrane bed, and the resulting formula is reported in eq. (1):

$$u_{MA} = \frac{F}{2\pi L(R_{ext}-R_{int})} \ln\left(\frac{R_{ext}}{R_{int}}\right) \quad (1)$$

where F is the volumetric flow rate, L is the length of the membrane bed, and R_{ext} and R_{int} are the outer and inner radii of the membrane bed, respectively. The resulting superficial velocity values were also used for the packed column experiments, but in this case, the corresponding volumetric flow rate was calculated from the definition of superficial velocity in a packed column:

$$u_{PB} = \frac{F}{\pi R_{col}^2} \quad (2)$$

where R_{col} is the inner radius of the column. Volumetric flow rates and superficial velocity values used in the porosity measurements are summarized in Table 2.

After each experiment, the chromatographic media, membrane adsorber or packed column, was regenerated with 2 M NaCl and sanitized with 1 M NaOH. During these steps, no UV absorbance peaks were observed, demonstrating that lysozyme in fact did not bind to the chromatographic media.

Once retention volumes for lysozyme were measured, the porosity of the membrane adsorber (designated MA in the equations below) was determined as follows. The total void volume of the membrane adsorber, including both membrane pores and other membrane volume contributions, was calculated by subtracting the retention volume of lysozyme with no module connected from the retention volume of lysozyme with a module connected:

$$V_{\text{total voids, MA}} = V_{\text{sys with MA}} - V_{\text{sys without MA}} \quad (3)$$

For the membrane adsorbers, the resulting void volume not only includes the membrane pores, but also includes the voids related to the various flow channels within the membrane module housing and the liquid distributors in the module. To determine the membrane module dead volume, the membrane adsorber was broken open and its internal dimensions were measured with a gauge. The volume of these additional contributions was then subtracted from the total void volume calculated with Eq. (3) to obtain the volume of the membrane pores.

$$V_{\text{pores}} = V_{\text{total voids, MA}} - V_{\text{module}} \quad (4)$$

The porosity is then calculated as the ratio of V_{pores} to the reported volume of the membrane adsorber, 3 mL.

The procedure is similar for the packed bed, with column voids – both interparticle and intraparticle - calculated as:

$$V_{\text{total voids, PB}} = V_{\text{sys with packed column}} - V_{\text{sys with empty column}} \quad (5)$$

And again, the porosity is calculated as the ratio of $V_{\text{total voids, PB}}$ to the total volume of the packed bed, 3 mL.

2.3.3 Adsorption isotherm determination

Adsorption isotherms for BSA on the solids supports were measured with two different procedures, in batch mode for Q Sepharose™ Fast Flow resin and with the AKTAexplorer system for the Sartobind® Q Nano membrane adsorber. Batch measurements were not possible for the membranes because at the time of this study, they were only available as part of a module and not as loose membrane sheets.

Adsorption isotherms for BSA in 50 mM Tris, pH 8.0 on Q Sepharose™ Fast Flow resin were measured in batch experiments at room temperature. First, a kinetic study was performed to determine the time necessary for the BSA in solution to reach the equilibrium with the adsorbent. Based on this study, a time of 180 minutes was shown to be sufficient. The isotherm was obtained using 12 mL Poly-Prep (Biorad) columns as the adsorption vessels, filled with 140 µL of resin slurry. BSA concentration in the feed ranged from 0.008 to 9.21 mg/mL. Fresh resin was loaded to the vessels and washed four times with 50 mM Tris at pH 8; during each wash,

the containers were placed for 10 min on an orbital shaker. Then buffer supernatants were removed. Successively, BSA solutions were loaded to the vessels, and they were incubated on the orbital shaker for 180 min at room temperature. BSA concentration was measured with UV absorbance readings at 280 nm and the mass of BSA adsorbed on the resin was calculated as the difference in the mass of BSA in the loading solution and the mass of BSA measured in the supernatant sample. The value was divided by the volume (mL) of solid resin to determine the value of the binding capacity, q, the mass (in mg) of BSA bound per unit volume (in mL) of resin. q is plotted against the final equilibrium concentration of BSA in Tris buffer.
The adsorption isotherm for BSA on Sartobind® Q Nano membranes was measured in breakthrough experiments performed using BSA, at different concentration values, in 50 mM Tris, pH 9.0. Specifically, BSA solutions with concentrations ranging from 0.023 to 2.01 mg/mL were fed to the membrane adsorber at a fixed flow rate of 10 mL/min. Feeding continued until saturation was achieved, as determined by a UV trace that asymptotically flattened out. Following the breakthrough run, BSA was eluted from the membrane adsorber using 2M NaCl. The amount of BSA recovered in the eluate was quantified by UV absorbance. This value was divided by the volume of the membrane adsorber to determine the BSA binding capacity. Upon completion of elution with 2M NaCl, the membrane was re-equilibrated and used again for another concentration of BSA.

2.3.4. Dynamic characterization

Breakthrough studies were conducted on the membrane adsorber and packed bed column to determine the dynamic binding capacity at 10% breakthrough ($DBC_{10\%}$) using solutions of 1 mg/mL BSA in 50 mM Tris, pH 8. These solutions were loaded to the column until, when the concentration of BSA, based on UV absorbance at 280 nm, plateaud. Experiments for each geometry were performed at 5 different flow rates in order to study the influence of the superficial velocity on dynamic binding capacity. The superficial velocities used are the same for both the membrane adsorber and packed bed and are equal to those used for porosity measurements (see Table 2). Upon completion of each breakthrough run, BSA was eluted from the relevant stationary phase using 0.5 M NaCl; the chromatographic media was then regenerated with 1 M NaOH for membranes and 2 M NaCl for the packed bed column, and then equilibrated with 50 mM Tris, pH 8 prior to the next experiment.
The $DBC_{10\%}$ value was determined by first calculating, from a mass balance, the amount of BSA that was bound to the media at 10% breakthrough:

$$m_{ads,10\%} = c_o V_{loaded,10\%} - c_o V_{sys} \quad (6)$$

where $m_{ads,10\%}$ is the mass of BSA adsorbed, c_o is the concentration of BSA in the feed, $V_{loaded,10\%}$ is the volume of BSA solution loaded at 10% breakthrough, and V_{sys} is the total system dead volume that was determined in a separate tracer experiment [38]. The last term of the equation introduces an approximation, since the concentration of biomolecule is considered uniform over the entire system and equal to the feed concentration, while, downstream of the column and, in particular, between the column outlet and the UV detector where the concentration is measured, it is lower. However, an estimate of the volume downstream of the

chromatographic column shows that the volume between the outlet and UV detector is only approximately 3% of the total dead volume; therefore the assumption that the concentration of BSA over the entire dead volume is equal to the concentration of BSA in the feed does not give rise to a significant error.

$DBC_{10\%}$ was then calculated by dividing $m_{ads,10\%}$ by the volume of the stationary phase.

For a more exhaustive comparison, process parameters such as bed utilization and productivity of the two different chromatographic processes have been evaluated. Bed utilization is defined as the amount of protein adsorbed at a given breakthrough point, with respect to the amount of protein adsorbed at complete saturation [39]; in particular, bed utilization at 10% breakthrough can be written as:

$$Bed\ Utilization = \frac{DBC_{10\%}}{DBC_{100\%}} \quad (7)$$

Productivity, P, is defined as the mass of the target biomolecule recovered in the elution step per unit volume of media per unit time of the complete chromatographic cycle; that is the sum of the equilibration, adsorption (load), washing, elution and regeneration times. In symbols:

$$P = \frac{m_{eluted\ product}}{V_{media}\ t_{chromatographic\ cycle}} \quad (8)$$

3. Results and Discussion

3.1 Porosity

For the Sartobind® Q Nano module, the porosity determined using the lysozyme tracer procedure previously described was 58%. For the Econoline® column packed with Q Sepharose™ Fast Flow resin, the resulting porosity was 64%. This value includes both intra-particle and inter-particle pores. As expected, the porosity of the packed bed is greater than that of the membrane adsorber given its higher specific surface area.

In addition to estimating porosity using lysozyme as a tracer, a similar procedure using 2 M NaCl as a tracer in a packed column equilibrated with 0.5 M NaCl was executed. Interestingly, the resulting packed column porosity determined with NaCl was 80%, significantly higher than the porosity measured by the lysozyme method. If it is assumed that the interparticle porosity of the bed is 0.36, the theoretical value for a bed of randomly packed hard spheres, the resulting intraparticle porosity values using lysozyme and NaCl as a tracer are 0.69 and 0.44, respectively. The value calculated using NaCl is in agreement with what has been reported previously for Sepharose FF resins [40-41], while the value for lysozyme is significantly lower. This suggests that a portion of resin pores is not accessible to lysozyme. The difference in porosity values using lysozyme and NaCl was not expected, given that the exclusion limit for Q Sepharose FF

resin is 4 MDa for globular proteins, while the molecular weight of lysozyme is only 14.4 kDa. However, the pore size distribution of the resin is unknown, therefore it is likely that this distribution is sufficiently broad to determine the difference observed. In addition, it is possible that the NaCl method for porosity determination overestimates porosity due to some binding interactions of NaCl to the anion exchange resin.

3.2 Adsorption isotherms

The equilibrium binding capacity of BSA on both stationary phases can be well represented by the Langmuir isotherm model:

$$q_{eq} = \frac{c_{eq}\ q_{max}}{c_{eq}+K_d} \tag{9}$$

In which the symbols q_{eq} and c_{eq} indicate the equilibrium values of the protein concentration on the surface and in the liquid solution, respectively, q_{max} indicates the maximum adsorption capacity, and K_d is the dissociation constant.

The equilibrium isotherm for BSA adsorption onto Q Sepharose™ Fast Flow resin is reported in Figure 1 as a function of the protein concentration in solution at equilibrium. The experimental results have been fitted using the Langmuir adsorption isotherm expression in Equation (9), which gives a maximum equilibrium binding capacity q_{max}, of 66.9 mg/mL and a dissociation constant, K_d, of 0.0836 mg/mL.

As discussed in the Experimental section, §2.3.3, the adsorption isotherm for the membrane was obtained in breakthrough experiments since the only format available for membranes was the Sartobind® Q Nano commercial unit. The data are shown in Figure 2, and are fitted to the Langmuir isotherm with a maximum binding capacity of 43.04 mg/mL and a dissociation constant, K_d, of 0.011 mg/mL.

Although the two supports are functionalized with a quaternary ammonium ligand, the dissociation constants differ by a factor of 7.6. This could be ascribed to the different base materials, the different immobilization chemistry, the possible use of spacers or brushes in the membrane adsorbers, and differences in the chemistry of the ligand itself. Similar or even higher differences have been observed by Hahn *et al.* in a comparison of different commercial protein A resins [42], suggesting that even chromatography media with the "same" chemistry might have significantly different K_d values. For both supports, the membrane and resin, the dissociation constant is extremely low, comparable to those observed in affinity chromatography processes [42-45]; a K_d of the same order of magnitude was reported by Tao *et al.* for BSA dissolved in 50 mM Tris pH 8.2 on DEAE-Sephadex ion exchange resin [46]. This means that BSA dissolved in Tris buffer at a pH close to 8 has a very high affinity for adsorbents that contain positively charged amine groups.

As expected, the maximum binding capacity of BSA onto the Q Sepharose™ FF resin is higher than on the Sartobind® Q Nano membrane, despite the fact that the isotherm data for the membrane were measured at a slightly higher pH (pH = 9) than the isotherm data for the resin (pH = 8). It is also worth noting that the ratio of the maximum binding capacity of the resin (66.9 mg/mL) with respect to the membrane (43.04 mg/mL) equals 1.55 and this value is lower than the ratio of the average ligand density of the two supports that equals 1.68 (0.21

mmol/mL vs. 0.13 mmol/mL), which indicates a slightly better level of ligand accessibility for the membrane adsorbers.

3.3 Dynamic characterization

The effect of flow rate on dynamic binding capacity was investigated for the two stationary phases in experiments performed by feeding 1 mg/mL BSA solutions. For the membrane adsorber, the breakthrough curves at different flow rates overlap very well from the onset to complete breakthrough as can be observed in Figure 3. This suggests that the dynamic binding capacity is independent of flow rate, thus confirming the dominant convective mass transport in the membrane adsorber [1, 3-4]. Conversely, for the packed column, breakthrough curves at different flow rates deviate significantly from one another; specifically, as the flow rate increases the curves broaden and the steepest curve corresponds to the lowest flow rate as illustrated in Figure 3. This is the behavior expected when intraparticle diffusion limits solute mass transfer as is the case with the packed bed.

It is worth noting that the breakthrough curves for the membrane device show a consistent "kink" starting at a volume of just more than 100 mL. In the Sartobind® Q Nano capsules used in this study, the membrane is wound to form a cylinder, with flow from the exterior of the cylinder through the layers of membrane, to the interior. The kink in the breakthrough curve shows up as the membrane adsorber nears complete saturation with BSA. Notably, breakthrough curves for the packed bed do not show similar behavior. A possible explanation is that as the BSA front moves to the interior of the membrane capsule, it may encounter an irregularity in the winding of the membrane that causes dispersion of the front and results in the kink.

The behavior of the dynamic binding capacity with respect to flowrate is better illustrated by plotting the dynamic binding capacity at 10% breakthrough as a function of the superficial velocity and of residence time for both supports. These plots are shown in Figure 4. As expected, the $DBC_{10\%}$ of the membrane adsorber remains constant, and it is almost 70% of the binding capacity obtained at saturation (data not shown), which corresponds to the bed utilization as defined in Eq. 7. Conversely, the $DBC_{10\%}$ of the packed column decreases at increasing flow rate as the breakthrough curves broaden. This result confirms that, in the range of flow rates tested, the membrane adsorber is not affected by solute mass transfer and kinetic limitations, thus this device can be used up to 300 cm/h without any decrease in binding capacity. Similar conclusions can be drawn from the behavior of $DBC_{10\%}$ as a function of residence time shown in Figure 4b. In this plot the $DBC_{10\%}$ of the two media– both actual values and values that have been extrapolated to the residence times that were out of the operating range of the device tested – are shown. The values for the membrane adsorbers were obtained by averaging the $DBC_{10\%}$ values at all flow rates ($DBC_{10\%}$=20.619 ± 0.177), whereas the data for the packed column were fitted with an exponential trendline using the function implemented in Microsoft Excel 2010 with a value of R^2 = 0.9971. It is interesting to note that at residence times lower than 0.5 minutes the $DBC_{10\%}$ of the membrane adsorber is larger than that of the packed column. Mass transfer limitations in membrane adsorbers are much less important than in conventional chromatographic columns where the loss in $DBC_{10\%}$ under the range of flow rates considered in this study is nearly 50%; this is in agreement with data from previous studies that measured the effect of flow rate on the DBC of antibodies on protein A resins [42,47].

Although values of dynamic binding capacity are higher for the bead-based chromatographic process, these values taken alone do not represent a complete evaluation of the process performance, which also requires consideration of buffer consumption, number of cycles for resin and/or membrane adsorber replacement, bed utilization and productivity. Even though a complete process evaluation was not the purpose of this work, data from the breakthrough studies was used to evaluate membrane and packed column bed utilization and productivity as simple tools to properly compare the two supports.

Since chromatographic processes are often operated at 10% breakthrough, bed utilization is an interesting parameter to compare the two different stationary phases. The results obtained for our experimental systems confirm that at low values of the residence time, i.e. at higher flow rates, bed utilization is higher for the membrane adsorber, as shown in Figure 5. In this figure bed utilization was plotted as a function of the residence time. Indeed, at low residence times the convective media outperforms the packed bed column, while at higher residence times the column binding capacity can be fully exploited and bed utilization becomes higher for the resin (Figure 5).

Since the two stationary phases were characterized in complete bind and elute studies, it is worth to compare the elution peaks obtained at different flow rates for both configurations. From the elution data reported in Figure 6, it can be observed that the profiles obtained with the membrane adsorber do not depend on flow rate, while the dependence on flow rate for the elution from the packed column shows only a small amount of tailing . However, the packed column peaks are not completely defined due to the high concentration of BSA recovered that was above the detection limit of the UV detector of the FPLC as can be noticed from their profiles (Figure 6).

Finally, the data from the breakthrough studies was used to estimate membrane and packed column productivity. Following each breakthrough run, BSA was eluted from the membrane adsorber and packed column. The amount of BSA eluted was divided by the cycle time, which was the sum of the time needed for all the chromatographic steps, namely equilibration, load, wash and elution, and a bed volume of 3 mL for each geometry. The results are plotted for the two stationary phases as a function of linear velocity as reported in Figure 7. The productivity of the membrane adsorber shows a linear dependence on the superficial velocity, since the dynamic binding capacity at 10% breakthrough is independent of flow rate in the range inspected. The productivity with the packed column slightly increases with superficial velocity, indicating that when the flow rate increases, the advantage related to the reduction of cycle duration overcomes the disadvantage related to $DBC_{10\%}$ decrease, but it is always lower than the productivity achieved with the membrane adsorber. The difference between the two technologies is greater at higher superficial velocities. The Sartobind® Q Nano capsule achieves a higher productivity than the column even if its binding capacity is lower because the membrane bed has a bigger cross section than the resin bed, thus the volumetric flow rate at a given superficial velocity with the module is higher and the cycle duration is lower. The maximum productivity with the membrane adsorber is 3.3 times higher than the maximum productivity obtained with the packed column. It is necessary to point out that the experimental protocols with the packed column were not optimized for the washing and elution steps and cycle duration can be reduced. However, even if the duration of the washing and elution steps in the cycles with the column were considered equal to that in the cycles with

the membrane module, the maximum productivity with the Sartobind® membrane module would be 3 times higher than the maximum productivity with the column.

4. Conclusions

Despite the development of new membranes with improved binding capacity, packed bed chromatography in bind-and-elute mode continues to be the dominant mode of purification, with the use of membrane adsorbers for bioprocessing relegated to flow through mode for polishing steps. While membrane chromatography, in which solute mass transfer is dominated by convection, is a fast process, packed bed chromatography has a higher binding capacity. In this work a direct comparison between the two chromatographic geometries was executed using an integrated approach that combines theory and experiments.

The performance of membrane adsorbers and packed bed columns has been experimentally studied in detail using the same bench-scale chromatography system. The results obtained using BSA as a model protein have been used to compare the two geometries in terms of binding capacity – both equilibrium and dynamic, productivity and bed utilization. As expected the maximum equilibrium binding capacity of the packed column is higher than that of the membrane adsorber, reflecting the greater surface area per unit volume in the packed bed. Likewise, the packed bed showed higher dynamic binding capacity values at 10% breakthrough over all superficial velocities studied. However, the percent difference in $DBC_{10\%}$ between the packed bed and membrane was significantly reduced at the higher superficial velocities. This results because the $DBC_{10\%}$ values for the membrane were independent of superficial velocity due to convective solute transport, while $DBC_{10\%}$ for the packed bed decreased significantly due to intraparticle mass transfer limitations.

The advantage of not having intraparticle transport limitations in the membrane device become more apparent when performance parameters like bed utilization and productivity are calculated. Bed utilization is significantly higher for the membrane device, even when solute fluid residence times are lower. Further, the productivity of the membrane adsorber is at least 3 times higher and this represents the true advantage of membrane chromatography, which can be successfully operated at high flow rates. Indeed, the obtained results confirm the potential of membrane adsorbers in bind-and-elute mode and the methodology employed might be used as a guide for process characterization of novel chromatographic membranes and improved membrane adsorber modules.

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Figure Captions

Figure 1: Equilibrium adsorption isotherm of BSA on Q Sepharose Fast Flow resin.

Figure 2: Equilibrium adsorption isotherm of BSA on Sartobind® Q Nano membranes.

Figure 3: Effect of flow rate on breakthrough curves of BSA solutions on membrane adsorber (a) and packed column (b).

Figure 4: Effect of superficial velocity (a) and residence time (b) on dynamic binding capacity of BSA solutions for the two chromatographic media.

Figure 5: Comparison of bed utilization values between membrane adsorbers and packed column as a function of residence time.

Figure 6: Elution profiles at different flow rates obtained in experiments at 100% breakthrough for the membrane adsorber (a) and the packed column (b)

Figure 7: Productivity obtained in experiments at 10% breakthrough as a function of linear velocity: comparison between membrane adsorbers and packed column.