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A direct comparison between membrane adsorber and packed column chromatography performance

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2 **chromatography performance**
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9

10 **Abstract**

11 The purpose of this work was to compare side by side the performance of packed bed and
12 membrane chromatography adsorption processes for protein purification. The comparison was
13 performed using anion exchange media with the same ligand immobilized on the adsorbing
14 surface, namely the strong Q quaternary ammonium group, $R\text{-}CH_2\text{-}N^+\text{-}(CH_3)_3$, and bovine serum
15 albumin (BSA) as a model protein. In addition, the stationary phase volume was held constant
16 for each geometry (3 mL) and runs were executed using the same mobile phase superficial
17 velocity. As expected, the packed bed column showed higher equilibrium binding of BSA at
18 66.9 mg/mL versus 43.04 mg/mL for the membrane adsorber. Dynamic binding capacities were
19 also higher in the packed bed; for example, at 97.5 cm/h, a capacity of 62.8 mg/mL was
20 measured for the packed bed versus 20.7 mg/mL for the membrane adsorber. The higher
21 equilibrium and dynamic capacities of the packed bed are likely due to the higher surface area
22 per unit volume of the resin. However, the maximum productivity for the membrane adsorber
23 was 111 mg/(mL h) a value that was 3.3 times higher than the one of the packed column. The
24 bed utilization – defined as the ratio of the dynamic binding capacity at 10% breakthrough to
25 the saturation binding capacity - was also higher for the packed column at long residence times
26 and lower at short residence times confirming the better performance of membrane
27 chromatography at high flow rates.

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30 **Keywords**
31 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

84   for the purification of Factor IX. The higher value of the dynamic binding capacity was the main
85   criteria of choice among different stationary phases together with the resolution and
86   selectivity. However, the comparison did not keep column volume and superficial velocity
87   constant among options.
88   Liu *et al.* tested commercial cation exchanger membranes and compared them to monoliths
89   and packed columns for antibody capture with interesting results. Ultimately, they chose to
90   operate with the protein A column for capture, despite the fact that the cation exchanger
91   membrane adsorber, operated in overloaded isocratic mode, was more effective in the removal
92   of host cell proteins and high molecular weight impurities [30]. Although Bhut *et al.* considered
93   stationary phases of the same volume and the same weak anion exchange functionality, they
94   presented newly designed membranes that were not commercially available with high binding
95   capacity and compared them with standard materials: a regenerated cellulose membrane and
96   an agarose resin [31]. However, superficial velocities between devices were not held constant.
97   Similarly, in a pioneering work, Kubota *et al.* performed a very thoughtful comparison between
98   hollow fiber membranes and a packed bed column of the same volume and the similar ion-
99   exchange functionality; however, they did not operate the two supports at the same superficial
100 velocity, which is an aspect that is often disregarded [32].
101 Comparisons between packed bed and membrane chromatography are rare in the literature
102 and they are often undertaken to highlight the high throughput of novel membrane materials.
103 The majority of the works that report such comparisons generally consider binding capacity as
104 the major performance parameter for evaluation of the different options without looking at
105 other chromatographic performance parameters such as resolution, productivity, or bed
106 utilization.
107 Because of the numerous advantages that membrane chromatography potentially offers, this
108 study aims to more directly compare properties and performance of the membrane and more
109 traditional column geometries. The work presented in this paper considers porosity, binding
110 capacity, mass transfer rate, bed utilization and productivity between the two geometries more
111 fully than has been done in previous studies [29-32]. To do this, a side-by-side comparison of
112 each geometry, using a membrane adsorber and chromatography resin that are commercially
113 available, have the same strong anion exchange functionality, are readily scalable, and have
114 identical bed volumes, 3 mL, was performed. Bovine serum albumin (BSA) is used as a model
115 protein and the columns were operated in non-competitive conditions at the same superficial
116 velocity to properly evaluate the transport phenomena limitations of the chromatographic
117 supports. Comparison was also performed by looking at performance parameters with respect
118 to residence time, even though due to the different bed geometries and packing characteristics
119 it was not possible to operate the packed column at the same low residence times, i.e. high
120 flow rates, of the membrane adsorbers.
121
122
123 **2. Experimental**
124
125 **2.1 Materials**
126 The membrane adsorber used during the experiments was an anion exchange Sartobind® Q
127 Nano 3 mL, produced by Sartorius Stedim Biotech GmbH. This is a radial flow module consisting

128 of 30 membrane layers. The volume of the membrane bed is 3 mL and the membrane surface
129 area available for flow is 110 cm^2. Liquid flows around the bed, then radially through the
130 membrane layers into a central channel and finally through the outlet channel. The membranes
131 present in the module have a base matrix of stabilized and reinforced cellulose with an
132 immobilized quaternary ammonium group. This adsorber is a strong anion exchanger and has a
133 long-term pH stability range from 2-12. The average membrane pore diameter is 3-5 µm. The
134 module can be operated at a maximum pressure of 4 bar, the recommended flow rate is 10-15
135 mL/min, and the BSA binding capacity reported by the vendor is 29 mg/mL (conditions
136 unspecified).
137 Q Sepharose™ Fast Flow resin, produced by GE Healthcare Life Sciences, was used as adsorbent
138 for column experiments. This anion exchange resin has a matrix comprised of Sepharose™ (6%
139 cross-linked agarose) and, like the Sartobind® Q Nano, the strong quaternary ammonium
140 ligand. Beads are spherical with a diameter ranging from 45 to 165 µm and an average size of
141 90 µm as reported by the vendor. They can be used at superficial velocities up to 700 cm/h. The
142 exclusion limit for globular proteins is 4 MDa, but the size distribution of intraparticle and
143 interparticle pores is unknown. Beads are stable from pH 2 to 12 and they are resistant to all
144 commonly used aqueous buffers (e.g. 1 M NaOH, 8 M urea, 8 M guanidine hydrochloride). A
145 summary of the properties for each of the chromatographic media is given in Table 1.
146 All solution components, NaCl (USP/FCC/EP/BP grades), NaOH (NF/FCC grades), Tris (molecular
147 biology grade), and HCl (reagent grade) for pH adjustment for Tris, used in these experiments
148 were purchased from ThermoFisher Scientific. Lysozyme, molecular weight (MW) of 14388 Da
149 and isoelectric point (pI) of 11.0 [33] was purchased from ThermoFisher Scientific [34]. Bovine
150 Serum Albumin (BSA), MW 66430 and pI 4.7, was purchased from Sigma-Aldrich with a purity ≥
151 96% [35]. BSA and lysozyme solutions were filtered with 0.21 µm cellulose nitrate membrane
152 filters (Whatman) before experiments. The filter material was hydrophilic as to minimize non-
153 specific protein adsorption.
154
155 **2.2 Equipment**
156 The characterizations of the chromatographic devices tested was performed on an
157 ÄKTAexplorer 100, manufactured by GE Healthcare Life Sciences and controlled by UNICORN™
158 software. Experimental runs were carried out in parallel with both the Sartobind® Q Nano 3 mL
159 module and with a column packed with Q Sepharose™ Fast Flow resin with a total bed volume
160 of 3 mL.
161 For experiments that required a packed column, Q Sepharose™ Fast Flow resin was flow packed
162 in an Econoline® Column from Sorbent Technologies Inc. The external tube is made of
163 borosilicate glass with an inner diameter of 1 cm and an adjustable bed height from 0 to 12.5
164 cm. The pressure limit for the column is 80 bar.
165
166 **2.3 Methods**
167 **2.3.1 Column packing**
168 Resin was packed under flow with high purity water at a flow rate of 5 mL/min. To calculate the
169 amount of resin slurry required for a bed volume of 3 mL, equal to the volume of the
170 membrane unit, a packing factor of 1.1 was used. The resulting bed had a height of 3.8 cm,
171 corresponding to a volume of 2.98 mL.

172   To assess the quality of packing, a pulse injection of a 2 M NaCl solution was made on the
173   column equilibrated with 0.5 M NaCl to calculate the height equivalent of a theoretical plate
174   (HETP) and the asymmetry factor of the packed column. Following the pulse injection,
175   additional 0.5 M NaCl was fed to the column at 2 mL/min, which corresponds to a superficial
176   velocity of 153 cm/h, to move the 2 M NaCl pulse through the column. Analysis of the
177   conductivity peak from the column resulted in a reduced HETP value (=HETP/d_p, where d_p is the
178   resin diameter) of 3.31 and an asymmetry value of 1.68.
179
180   **2.3.2 Porosity determination**
181   The porosities of the chromatographic media were determined by statistical moment analysis
182   on experimental data obtained using lysozyme as a tracer [36-37]. Lysozyme has an isoelectric
183   point of 11 [33] and therefore at pH 8 does not bind to the positively charged groups of the
184   chromatographic supports; in addition, 0.25 M NaCl was added to the lysozyme solution to
185   further prevent binding to the media. After equilibrating the membrane adsorber or the packed
186   column with 50 mM Tris, 0.25 M NaCl, pH 8, a solution of 2.5 mg/mL lysozyme in equilibration
187   buffer was injected using a 100 µL loop. Lysozyme in the column effluent was monitored by UV
188   absorbance at 280 nm. For the membrane adsorber, injections were performed both with and
189   without the membrane adsorber attached to the AKTAexplorer system. For the packed column,
190   injections were performed both with a packed column attached to the AKTA system and an
191   unpacked column with the plungers set to 0 mL.
192   Lysozyme injections were performed for each system setup (with membrane adsorber, without
193   membrane adsorber, with packed column, and with unpacked column) at five different
194   superficial flow velocities; lysozyme retention volume values were calculated as the averages of
195   the results at each of the five superficial velocities. The superficial velocities were held constant
196   between the experiments with the membrane capsule and those with the packed column. The
197   experiments with the membrane unit were performed at 5, 7.5, 10, 12.5 and 15 mL/min, the
198   latter being the operational flow rate recommended by the manufacturer. The superficial
199   velocity corresponding to each of these flow rates was determined as an integral average of the
200   radial velocity from the outer radius to the inner radius of the membrane bed, and the resulting
201   formula is reported in eq. (1):
202

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

204
205   where F is the volumetric flow rate, L is the length of the membrane bed, and R_{ext} and R_{int} are
206   the outer and inner radii of the membrane bed, respectively. The resulting superficial velocity
207   values were also used for the packed column experiments, but in this case, the corresponding
208   volumetric flow rate was calculated from the definition of superficial velocity in a packed
209   column:
210

211    $$u_{PB} = \frac{F}{\pi R_{col}^2} \tag{2}$$

212
213   where R_{col} is the inner radius of the column. Volumetric flow rates and superficial velocity
214   values used in the porosity measurements are summarized in Table 2.

215 After each experiment, the chromatographic media, membrane adsorber or packed column,
216 was regenerated with 2 M NaCl and sanitized with 1 M NaOH. During these steps, no UV
217 absorbance peaks were observed, demonstrating that lysozyme in fact did not bind to the
218 chromatographic media.
219 Once retention volumes for lysozyme were measured, the porosity of the membrane adsorber
220 (designated MA in the equations below) was determined as follows. The total void volume of
221 the membrane adsorber, including both membrane pores and other membrane volume
222 contributions, was calculated by subtracting the retention volume of lysozyme with no module
223 connected from the retention volume of lysozyme with a module connected:

225 $V_{total\ voids,\ MA} = V_{sys\ with\ MA} - V_{sys\ without\ MA}$ (3)

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

234 $V_{pores} = V_{total\ voids,\ MA} - V_{module}$ (4)

236 The porosity is then calculated as the ratio of V_{pores} to the reported volume of the membrane
237 adsorber, 3 mL.
238 The procedure is similar for the packed bed, with column voids – both interparticle and
239 intraparticle - calculated as:

241 $V_{total\ voids,\ PB} = V_{sys\ with\ packed\ column} - V_{sys\ with\ empty\ column}$ (5)

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

246 **2.3.3 Adsorption isotherm determination**
247 Adsorption isotherms for BSA on the solids supports were measured with two different
248 procedures, in batch mode for Q Sepharose™ Fast Flow resin and with the AKTAexplorer system
249 for the Sartobind® Q Nano membrane adsorber. Batch measurements were not possible for the
250 membranes because at the time of this study, they were only available as part of a module and
251 not as loose membrane sheets.
252 Adsorption isotherms for BSA in 50 mM Tris, pH 8.0 on Q Sepharose™ Fast Flow resin were
253 measured in batch experiments at room temperature. First, a kinetic study was performed to
254 determine the time necessary for the BSA in solution to reach the equilibrium with the
255 adsorbent. Based on this study, a time of 180 minutes was shown to be sufficient. The isotherm
256 was obtained using 12 mL Poly-Prep (Biorad) columns as the adsorption vessels, filled with 140
257 µL of resin slurry. BSA concentration in the feed ranged from 0.008 to 9.21 mg/mL. Fresh resin
258 was loaded to the vessels and washed four times with 50 mM Tris at pH 8; during each wash,

259 the containers were placed for 10 min on an orbital shaker. Then buffer supernatants were
260 removed. Successively, BSA solutions were loaded to the vessels, and they were incubated on
261 the orbital shaker for 180 min at room temperature. BSA concentration was measured with UV
262 absorbance readings at 280 nm and the mass of BSA adsorbed on the resin was calculated as
263 the difference in the mass of BSA in the loading solution and the mass of BSA measured in the
264 supernatant sample. The value was divided by the volume (mL) of solid resin to determine the
265 value of the binding capacity, q, the mass (in mg) of BSA bound per unit volume (in mL) of resin.
266 q is plotted against the final equilibrium concentration of BSA in Tris buffer.
267 The adsorption isotherm for BSA on Sartobind® Q Nano membranes was measured in
268 breakthrough experiments performed using BSA, at different concentration values, in 50 mM
269 Tris, pH 9.0. Specifically, BSA solutions with concentrations ranging from 0.023 to 2.01 mg/mL
270 were fed to the membrane adsorber at a fixed flow rate of 10 mL/min. Feeding continued until
271 saturation was achieved, as determined by a UV trace that asymptotically flattened out.
272 Following the breakthrough run, BSA was eluted from the membrane adsorber using 2M NaCl.
273 The amount of BSA recovered in the eluate was quantified by UV absorbance. This value was
274 divided by the volume of the membrane adsorber to determine the BSA binding capacity. Upon
275 completion of elution with 2M NaCl, the membrane was re-equilibrated and used again for
276 another concentration of BSA.
277
278 **2.3.4. Dynamic characterization**

279 Breakthrough studies were conducted on the membrane adsorber and packed bed column to
280 determine the dynamic binding capacity at 10% breakthrough ($DBC_{10\%}$) using solutions of 1
281 mg/mL BSA in 50 mM Tris, pH 8. These solutions were loaded to the column until, when the
282 concentration of BSA, based on UV absorbance at 280 nm, plateaud. Experiments for each
283 geometry were performed at 5 different flow rates in order to study the influence of the
284 superficial velocity on dynamic binding capacity. The superficial velocities used are the same for
285 both the membrane adsorber and packed bed and are equal to those used for porosity
286 measurements (see Table 2). Upon completion of each breakthrough run, BSA was eluted from
287 the relevant stationary phase using 0.5 M NaCl; the chromatographic media was then
288 regenerated with 1 M NaOH for membranes and 2 M NaCl for the packed bed column, and then
289 equilibrated with 50 mM Tris, pH 8 prior to the next experiment.
290 The $DBC_{10\%}$ value was determined by first calculating, from a mass balance, the amount of BSA
291 that was bound to the media at 10% breakthrough:
292
293     $m_{ads,10\%} = c_o V_{loaded,10\%} - c_o V_{sys}$ (6)
294
295 where $m_{ads,10\%}$ is the mass of BSA adsorbed, c_o is the concentration of BSA in the feed, $V_{loaded,10\%}$
296 is the volume of BSA solution loaded at 10% breakthrough, and V_{sys} is the total system dead
297 volume that was determined in a separate tracer experiment [38]. The last term of the
298 equation introduces an approximation, since the concentration of biomolecule is considered
299 uniform over the entire system and equal to the feed concentration, while, downstream of the
300 column and, in particular, between the column outlet and the UV detector where the
301 concentration is measured, it is lower. However, an estimate of the volume downstream of the

302 chromatographic column shows that the volume between the outlet and UV detector is only
303 approximately 3% of the total dead volume; therefore the assumption that the concentration of
304 BSA over the entire dead volume is equal to the concentration of BSA in the feed does not give
305 rise to a significant error.
306 $DBC_{10\%}$ was then calculated by dividing $m_{ads,10\%}$ by the volume of the stationary phase.
307 For a more exhaustive comparison, process parameters such as bed utilization and productivity
308 of the two different chromatographic processes have been evaluated. Bed utilization is defined
309 as the amount of protein adsorbed at a given breakthrough point, with respect to the amount
310 of protein adsorbed at complete saturation [39]; in particular, bed utilization at 10%
311 breakthrough can be written as:
312

313 $$Bed\ Utilization = \frac{DBC_{10\%}}{DBC_{100\%}} \hspace{6cm} (7)$$

314

315 Productivity, P, is defined as the mass of the target biomolecule recovered in the elution step
316 per unit volume of media per unit time of the complete chromatographic cycle; that is the sum
317 of the equilibration, adsorption (load), washing, elution and regeneration times. In symbols:
318

319 $$P = \frac{m_{eluted\ product}}{V_{media}\ t_{chromatographic\ cycle}} \hspace{4cm} (8)$$

320
321
322 **3. Results and Discussion**
323
324 **3.1 Porosity**
325 For the Sartobind® Q Nano module, the porosity determined using the lysozyme tracer
326 procedure previously described was 58%. For the Econoline® column packed with Q
327 Sepharose™ Fast Flow resin, the resulting porosity was 64%. This value includes both intra-
328 particle and inter-particle pores. As expected, the porosity of the packed bed is greater than
329 that of the membrane adsorber given its higher specific surface area.
330 In addition to estimating porosity using lysozyme as a tracer, a similar procedure using 2 M NaCl
331 as a tracer in a packed column equilibrated with 0.5 M NaCl was executed. Interestingly, the
332 resulting packed column porosity determined with NaCl was 80%, significantly higher than the
333 porosity measured by the lysozyme method. If it is assumed that the interparticle porosity of
334 the bed is 0.36, the theoretical value for a bed of randomly packed hard spheres, the resulting
335 intraparticle porosity values using lysozyme and NaCl as a tracer are 0.69 and 0.44, respectively.
336 The value calculated using NaCl is in agreement with what has been reported previously for
337 Sepharose FF resins [40-41], while the value for lysozyme is significantly lower. This suggests
338 that a portion of resin pores is not accessible to lysozyme. The difference in porosity values
339 using lysozyme and NaCl was not expected, given that the exclusion limit for Q Sepharose FF

340 resin is 4 MDa for globular proteins, while the molecular weight of lysozyme is only 14.4 kDa.
341 However, the pore size distribution of the resin is unknown, therefore it is likely that this
342 distribution is sufficiently broad to determine the difference observed. In addition, it is possible
343 that the NaCl method for porosity determination overestimates porosity due to some binding
344 interactions of NaCl to the anion exchange resin.
345
346 **3.2 Adsorption isotherms**
347 The equilibrium binding capacity of BSA on both stationary phases can be well represented by
348 the Langmuir isotherm model:
349
350 $$q_{eq} = \frac{c_{eq}\ q_{max}}{c_{eq}+K_d}$$ (9)

351
352 In which the symbols q_{eq} and c_{eq} indicate the equilibrium values of the protein concentration on
353 the surface and in the liquid solution, respectively, q_{max} indicates the maximum adsorption
354 capacity, and K_d is the dissociation constant.
355 The equilibrium isotherm for BSA adsorption onto Q Sepharose™ Fast Flow resin is reported in
356 Figure 1 as a function of the protein concentration in solution at equilibrium. The experimental
357 results have been fitted using the Langmuir adsorption isotherm expression in Equation (9),
358 which gives a maximum equilibrium binding capacity q_{max}, of 66.9 mg/mL and a dissociation
359 constant, K_d, of 0.0836 mg/mL.
360 As discussed in the Experimental section, §2.3.3, the adsorption isotherm for the membrane
361 was obtained in breakthrough experiments since the only format available for membranes was
362 the Sartobind® Q Nano commercial unit. The data are shown in Figure 2, and are fitted to the
363 Langmuir isotherm with a maximum binding capacity of 43.04 mg/mL and a dissociation
364 constant, K_d, of 0.011 mg/mL.
365 Although the two supports are functionalized with a quaternary ammonium ligand, the
366 dissociation constants differ by a factor of 7.6. This could be ascribed to the different base
367 materials, the different immobilization chemistry, the possible use of spacers or brushes in the
368 membrane adsorbers, and differences in the chemistry of the ligand itself. Similar or even
369 higher differences have been observed by Hahn *et al.* in a comparison of different commercial
370 protein A resins [42], suggesting that even chromatography media with the "same" chemistry
371 might have significantly different K_d values. For both supports, the membrane and resin, the
372 dissociation constant is extremely low, comparable to those observed in affinity
373 chromatography processes [42-45]; a K_d of the same order of magnitude was reported by Tao
374 *et al.* for BSA dissolved in 50 mM Tris pH 8.2 on DEAE-Sephadex ion exchange resin [46]. This
375 means that BSA dissolved in Tris buffer at a pH close to 8 has a very high affinity for adsorbents
376 that contain positively charged amine groups.
377 As expected, the maximum binding capacity of BSA onto the Q Sepharose™ FF resin is higher
378 than on the Sartobind® Q Nano membrane, despite the fact that the isotherm data for the
379 membrane were measured at a slightly higher pH (pH = 9) than the isotherm data for the resin
380 (pH = 8). It is also worth noting that the ratio of the maximum binding capacity of the resin
381 (66.9 mg/mL) with respect to the membrane (43.04 mg/mL) equals 1.55 and this value is lower
382 than the ratio of the average ligand density of the two supports that equals 1.68 (0.21

383 mmol/mL vs. 0.13 mmol/mL), which indicates a slightly better level of ligand accessibility for
384 the membrane adsorbers.
385
386 **3.3 Dynamic characterization**
387 The effect of flow rate on dynamic binding capacity was investigated for the two stationary
388 phases in experiments performed by feeding 1 mg/mL BSA solutions. For the membrane
389 adsorber, the breakthrough curves at different flow rates overlap very well from the onset to
390 complete breakthrough as can be observed in Figure 3. This suggests that the dynamic binding
391 capacity is independent of flow rate, thus confirming the dominant convective mass transport
392 in the membrane adsorber [1, 3-4]. Conversely, for the packed column, breakthrough curves at
393 different flow rates deviate significantly from one another; specifically, as the flow rate
394 increases the curves broaden and the steepest curve corresponds to the lowest flow rate as
395 illustrated in Figure 3. This is the behavior expected when intraparticle diffusion limits solute
396 mass transfer as is the case with the packed bed.
397 It is worth noting that the breakthrough curves for the membrane device show a consistent
398 "kink" starting at a volume of just more than 100 mL. In the Sartobind® Q Nano capsules used
399 in this study, the membrane is wound to form a cylinder, with flow from the exterior of the
400 cylinder through the layers of membrane, to the interior. The kink in the breakthrough curve
401 shows up as the membrane adsorber nears complete saturation with BSA. Notably,
402 breakthrough curves for the packed bed do not show similar behavior. A possible explanation
403 is that as the BSA front moves to the interior of the membrane capsule, it may encounter an
404 irregularity in the winding of the membrane that causes dispersion of the front and results in
405 the kink.
406 The behavior of the dynamic binding capacity with respect to flowrate is better illustrated by
407 plotting the dynamic binding capacity at 10% breakthrough as a function of the superficial
408 velocity and of residence time for both supports. These plots are shown in Figure 4. As
409 expected, the $DBC_{10\%}$ of the membrane adsorber remains constant, and it is almost 70% of the
410 binding capacity obtained at saturation (data not shown), which corresponds to the bed
411 utilization as defined in Eq. 7. Conversely, the $DBC_{10\%}$ of the packed column decreases at
412 increasing flow rate as the breakthrough curves broaden. This result confirms that, in the range
413 of flow rates tested, the membrane adsorber is not affected by solute mass transfer and kinetic
414 limitations, thus this device can be used up to 300 cm/h without any decrease in binding
415 capacity. Similar conclusions can be drawn from the behavior of $DBC_{10\%}$ as a function of
416 residence time shown in Figure 4b. In this plot the $DBC_{10\%}$ of the two media– both actual values
417 and values that have been extrapolated to the residence times that were out of the operating
418 range of the device tested – are shown. The values for the membrane adsorbers were obtained
419 by averaging the $DBC_{10\%}$ values at all flow rates ($DBC_{10\%}$=20.619 ± 0.177), whereas the data for
420 the packed column were fitted with an exponential trendline using the function implemented in
421 Microsoft Excel 2010 with a value of R^2 = 0.9971. It is interesting to note that at residence times
422 lower than 0.5 minutes the $DBC_{10\%}$ of the membrane adsorber is larger than that of the packed
423 column. Mass transfer limitations in membrane adsorbers are much less important than in
424 conventional chromatographic columns where the loss in $DBC_{10\%}$ under the range of flow rates
425 considered in this study is nearly 50%; this is in agreement with data from previous studies that
426 measured the effect of flow rate on the DBC of antibodies on protein A resins [42,47].

427   Although values of dynamic binding capacity are higher for the bead-based chromatographic
428   process, these values taken alone do not represent a complete evaluation of the process
429   performance, which also requires consideration of buffer consumption, number of cycles for
430   resin and/or membrane adsorber replacement, bed utilization and productivity. Even though a
431   complete process evaluation was not the purpose of this work, data from the breakthrough
432   studies was used to evaluate membrane and packed column bed utilization and productivity as
433   simple tools to properly compare the two supports.
434   Since chromatographic processes are often operated at 10% breakthrough, bed utilization is an
435   interesting parameter to compare the two different stationary phases. The results obtained for
436   our experimental systems confirm that at low values of the residence time, i.e. at higher flow
437   rates, bed utilization is higher for the membrane adsorber, as shown in Figure 5. In this figure
438   bed utilization was plotted as a function of the residence time. Indeed, at low residence times
439   the convective media outperforms the packed bed column, while at higher residence times the
440   column binding capacity can be fully exploited and bed utilization becomes higher for the resin
441   (Figure 5).
442   Since the two stationary phases were characterized in complete bind and elute studies, it is
443   worth to compare the elution peaks obtained at different flow rates for both configurations.
444   From the elution data reported in Figure 6, it can be observed that the profiles obtained with
445   the membrane adsorber do not depend on flow rate, while the dependence on flow rate for the
446   elution from the packed column shows only a small amount of tailing . However, the packed
447   column peaks are not completely defined due to the high concentration of BSA recovered that
448   was above the detection limit of the UV detector of the FPLC as can be noticed from their
449   profiles (Figure 6).
450   Finally, the data from the breakthrough studies was used to estimate membrane and packed
451   column productivity. Following each breakthrough run, BSA was eluted from the membrane
452   adsorber and packed column. The amount of BSA eluted was divided by the cycle time, which
453   was the sum of the time needed for all the chromatographic steps, namely equilibration, load,
454   wash and elution, and a bed volume of 3 mL for each geometry. The results are plotted for the
455   two stationary phases as a function of linear velocity as reported in Figure 7. The productivity of
456   the membrane adsorber shows a linear dependence on the superficial velocity, since the
457   dynamic binding capacity at 10% breakthrough is independent of flow rate in the range
458   inspected. The productivity with the packed column slightly increases with superficial velocity,
459   indicating that when the flow rate increases, the advantage related to the reduction of cycle
460   duration overcomes the disadvantage related to $DBC_{10\%}$ decrease, but it is always lower than
461   the productivity achieved with the membrane adsorber. The difference between the two
462   technologies is greater at higher superficial velocities. The Sartobind® Q Nano capsule achieves
463   a higher productivity than the column even if its binding capacity is lower because the
464   membrane bed has a bigger cross section than the resin bed, thus the volumetric flow rate at a
465   given superficial velocity with the module is higher and the cycle duration is lower. The
466   maximum productivity with the membrane adsorber is 3.3 times higher than the maximum
467   productivity obtained with the packed column. It is necessary to point out that the
468   experimental protocols with the packed column were not optimized for the washing and
469   elution steps and cycle duration can be reduced. However, even if the duration of the washing
470   and elution steps in the cycles with the column were considered equal to that in the cycles with

471 the membrane module, the maximum productivity with the Sartobind® membrane module
472 would be 3 times higher than the maximum productivity with the column.
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475 **4. Conclusions**
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477 Despite the development of new membranes with improved binding capacity, packed bed
478 chromatography in bind-and-elute mode continues to be the dominant mode of purification,
479 with the use of membrane adsorbers for bioprocessing relegated to flow through mode for
480 polishing steps. While membrane chromatography, in which solute mass transfer is dominated
481 by convection, is a fast process, packed bed chromatography has a higher binding capacity. In
482 this work a direct comparison between the two chromatographic geometries was executed
483 using an integrated approach that combines theory and experiments.
484 The performance of membrane adsorbers and packed bed columns has been experimentally
485 studied in detail using the same bench-scale chromatography system. The results obtained
486 using BSA as a model protein have been used to compare the two geometries in terms of
487 binding capacity – both equilibrium and dynamic, productivity and bed utilization. As expected
488 the maximum equilibrium binding capacity of the packed column is higher than that of the
489 membrane adsorber, reflecting the greater surface area per unit volume in the packed bed.
490 Likewise, the packed bed showed higher dynamic binding capacity values at 10% breakthrough
491 over all superficial velocities studied. However, the percent difference in $DBC_{10\%}$ between the
492 packed bed and membrane was significantly reduced at the higher superficial velocities. This
493 results because the $DBC_{10\%}$ values for the membrane were independent of superficial velocity
494 due to convective solute transport, while $DBC_{10\%}$ for the packed bed decreased significantly due
495 to intraparticle mass transfer limitations.
496 The advantage of not having intraparticle transport limitations in the membrane device become
497 more apparent when performance parameters like bed utilization and productivity are
498 calculated. Bed utilization is significantly higher for the membrane device, even when solute
499 fluid residence times are lower. Further, the productivity of the membrane adsorber is at least
500 3 times higher and this represents the true advantage of membrane chromatography, which
501 can be successfully operated at high flow rates. Indeed, the obtained results confirm the
502 potential of membrane adsorbers in bind-and-elute mode and the methodology employed
503 might be used as a guide for process characterization of novel chromatographic membranes
504 and improved membrane adsorber modules.
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506 **Acknowledgements**
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513 **References**
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Figure Captions

639
640 **Figure 1:** Equilibrium adsorption isotherm of BSA on Q Sepharose Fast Flow resin.
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643 **Figure 2:** Equilibrium adsorption isotherm of BSA on Sartobind® Q Nano membranes.
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646 **Figure 3:** Effect of flow rate on breakthrough curves of BSA solutions on membrane adsorber (a) and
647 packed column (b).
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650 **Figure 4:** Effect of superficial velocity (a) and residence time (b) on dynamic binding capacity of BSA
651 solutions for the two chromatographic media.
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654 **Figure 5:** Comparison of bed utilization values between membrane adsorbers and packed column as a
655 function of residence time.
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658 **Figure 6**: Elution profiles at different flow rates obtained in experiments at 100% breakthrough for the
659 membrane adsorber (a) and the packed column (b)
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661 **Figure 7**: Productivity obtained in experiments at 10% breakthrough as a function of linear velocity:
662 comparison between membrane adsorbers and packed column.
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