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This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Boi C., Malavasi A., Carbonell R.G., Gilleskie G. (2020). A direct comparison between membrane adsorber and packed column chromatography performance. JOURNAL OF CHROMATOGRAPHY A, 1612, 1-9 [10.1016/j.chroma.2019.460629].

Availability: This version is available at: https://hdl.handle.net/11585/709091 since: 2019-12-18

Published:

DOI: http://doi.org/10.1016/j.chroma.2019.460629

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(Article begins on next page)

1 A direct comparison between membrane adsorber and packed column

- 2 chromatography performance
- 3
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- 8
- 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, $R-CH_2-N^+-(CH_3)_3$, 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. 28 29 30 **Keywords** 31 Chromatography; Membrane adsorber; Packed column; Binding capacity; Productivity. 32 33 34 35 * Corresponding author at: 36 Via Terracini 28, 40131 Bologna, Italy 37 Tel. +39 051 2090432 38 Email: cristiana.boi@unibo.it 39

40 **1. Introduction**

41

42 Packed bed chromatography is by far the most common technique used for high resolution

43 separations of proteins, both as an analytical tool and as a process unit operation [1]. In a

44 conventional process the column is operated in a capture mode, using highly porous resins with

45 different functionalities according to the mode of operation. Among all chromatographic

46 techniques, ion exchange has been the most widely employed method for protein purification

47 since the development of cellulosic ion exchangers in the 1950s [2].

- 48 Membrane chromatography is a relatively new technique developed with the purpose of
- 49 operating at higher flow rates and at reduced process time relative to bead-based column

50 chromatography. Higher flow rates are possible with membranes because the resulting

- 51 pressure drop is significantly lower than in traditional packed-column chromatography
- 52 processes, and because solute transport in membrane devices is primarily by convection to the
- 53 membrane internal surface, rather than the much slower intraparticle diffusion that is rate
- 54 controlling in chromatography beads [1, 3-4]. In addition, since membrane adsorbers are

55 generally less costly to manufacture, they can potentially be used as single-use devices that

56 eliminate the difficulties and costs often associated with packing a chromatographic column,

57 and the cleaning and validation steps required of multi-use columns [5].

58 One significant drawback associated with the use of membrane adsorbers in a capture mode is

59 that their binding product capacities are generally lower than those of column chromatography

60 resins. As a result, the industrial application of membrane adsorbers has been largely limited to

- 61 flowthrough polishing applications in biopharmaceutical production processes in which small
- 62 concentrations of impurities bind to the chromatography media, while product flows through
- 63 [3-16]. Indeed, with the development of novel membrane materials with higher binding

64 capacities the use of membrane adsorbers for product capture is of increasing interest [17-21].

65 Among those, cation exchange membranes made by porous polyacrylate hydrogels on a

66 polypropylene support have been used for monoclonal antibody capture obtaining IgG dynamic

67 binding capacities higher than 60 mg/mL at 10% breakthrough [6-7]. Comparable binding

68 capacities were reported for high capacity multimodal cation exchanger membranes prepared

- 69 by immobilizing poly(glycidyl methacrylate) tentacles on cellulosic membranes [8]. More recent
- 70 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

74 different flow configurations [25-26] and has focused on developing interesting novel module

75 designs that have resulted in improved performance [27-28].

76 The comparison of performance for different types of chromatographic supports has been

77 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

- 79 operating conditions were not chosen with the aim of direct comparison. Ribero *et al.*
- 80 investigated the performance of different chromatographic supports to be used for purification
- 81 of recombinant Factor IX. They tested two different anion exchange resins, a membrane
- 82 adsorber and a monolithic column and chose to continue with the monolith due to its higher
- 83 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
- capacity and compared them with standard materials: a regenerated cellulose membrane andan agarose resin [31]. However, superficial velocities between devices were not held constant.
- 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
- 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
- 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
- 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
- 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
- 110 it was not possible to operate the packed solumn at the same low residence times i.e. high
- 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 cm². 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 μ m 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
- 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.
- 146 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
- 148 were purchased from ThermoFisher Scientific. Lysozyme, molecular weight (MW) of 14388 Da
- and isoelectric point (pl) 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 μ m 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
- module and with a column packed with Q Sepharose[™] Fast Flow resin with a total bed volumeof 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
- 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

- The porosities of the chromatographic media were determined by statistical moment analysis 181
- 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

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

204

205 where F is the volumetric flow rate, L is the length of the membrane bed, and Rext and Rint 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

212

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

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.

(1)

215 216	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
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:
224	
225	$V_{\text{total voids, MA}} = V_{\text{sys with MA}} - V_{\text{sys without MA}} $ (3)
226	
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.
233	
234	$V_{\text{pores}} = V_{\text{total voids, MA}} - V_{\text{module}} \tag{4}$
235	
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:
240	
241	$V_{\text{total voids, PB}} = V_{\text{sys with packed column}} - V_{\text{sys with empty column}}$ (5)
242	And again, the perecity is calculated as the ratio of $V_{\rm eff}$ as to the total volume of the
243	nacked hed 3 ml
244	
245	2 3 3 Adsorption isotherm determination
240	Adsorption isotherms for BSA on the solids supports were measured with two different
248	procedures in batch mode for O Senharose™ Fast Flow resin and with the AKTAexplorer system
249	for the Sartobind [®] O 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

- 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.
- 266 g 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
- 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.
- 273 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 foranother concentration of BSA.

277

278 **2.3.4.** Dynamic characterization

279 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
- 281 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
- 283 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.
- 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
- $m_{ads,10\%} = c_0 V_{loaded,10\%} c_0 V_{sys}$
- 294
- 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
- 298 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
- 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

(6)

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

of protein adsorbed at complete saturation [39]; in particular, bed utilization at 10%

311 breakthrough can be written as:

312

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

314

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:

318

319 $P = \frac{m_{eluted \ product}}{V_{media} \ t_{chromatographic \ cycle}}$ (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

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

337 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

- 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

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

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

(9)

351

352 In which the symbols q_{eq} and c_{eq} indicate the equilibrium values of the protein concentration on

- 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),
- 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
- 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
- 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
- *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
- 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
- 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
- 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
- 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 in405 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
- 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
- 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 \pm 0.177), whereas the data for
- by averaging the DBC_{10%} values at all flow rates (DBC_{10%}=20.619 \pm 0.177), whereas the data for 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
- 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
- 440 column binding capacity can be fully exploited and bed utilization becomes higher for the resil 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
- 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
- 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
- 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
- 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
- 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
- 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
- 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.
- 473 474

475 **4. Conclusions**

476

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.

- 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
- 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
- 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.
- 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
- 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
- and improved membrane adsorber modules.
- 505

506 Acknowledgements

507

508 The Golden LEAF Biomanufacturing Training and Education Center (BTEC), NC State University 509 for supporting the experimental work. The Alma Mater Studiorum Università di Bologna for 510 supporting Andrea Malavasi.

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638	Figure Captions
639 640 641	Figure 1: Equilibrium adsorption isotherm of BSA on Q Sepharose Fast Flow resin.
642 643 644 645	Figure 2: Equilibrium adsorption isotherm of BSA on Sartobind [®] Q Nano membranes.
646 647 648 649	Figure 3: Effect of flow rate on breakthrough curves of BSA solutions on membrane adsorber (a) and packed column (b).
650 651 652 653	Figure 4: Effect of superficial velocity (a) and residence time (b) on dynamic binding capacity of BSA solutions for the two chromatographic media.
654 655 656 657	Figure 5: Comparison of bed utilization values between membrane adsorbers and packed column as a function of residence time.
658 659 660	Figure 6 : Elution profiles at different flow rates obtained in experiments at 100% breakthrough for the membrane adsorber (a) and the packed column (b)
661 662 663 664 665	Figure 7 : Productivity obtained in experiments at 10% breakthrough as a function of linear velocity: comparison between membrane adsorbers and packed column.