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Review

Membrane adsorbers as purification tools for monoclonal antibody purification☆

Cristiana Boi*

Dipartimento di Ingegneria Chimica, Mineraria e delle Tecnologie Ambientali, Università degli Studi di Bologna, Viale Risorgimento 2, 40136 Bologna, Italy

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Abstract

Downstream purification processes for monoclonal antibody production typically involve multiple steps; some of them are conventionally performed by bead-based column chromatography. Affinity chromatography with Protein A is the most selective method for protein purification and is conventionally used for the initial capturing step to facilitate rapid volume reduction as well as separation of the antibody. However, conventional affinity chromatography has some limitations that are inherent with the method, it exhibits slow intraparticle diffusion and high pressure drop within the column. Membrane-based separation processes can be used in order to overcome these mass transfer limitations. The ligand is immobilized in the membrane pores and the convective flow brings the solute molecules very close to the ligand and hence minimizes the diffusional limitations associated with the beads. Nonetheless, the adoption of this technology has been slow because membrane chromatography has been limited by a lower binding capacity than that of conventional columns, even though the high flux advantages provided by membrane adsorbers would lead to higher productivity. This review considers the use of membrane adsorbers as an alternative technology for capture and polishing steps for the purification of monoclonal antibodies. Promising industrial applications as well as new trends in research will be addressed.

Keywords: Membrane adsorbers; Antibodies; Immunoglobulins; Affinity; Ion exchange

Contents

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* Tel.: +39 051 2090410; fax: +39 051 6347788.
E-mail address: cristiana.boi@mail.ing.unibo.it.

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1. Introduction

Antibodies and antibody derivatives constitute a significant percentage of biopharmaceutical products and many new therapeutic products based on MAbs are in the pipeline for approval. The production of MAbs is not limited by fermentation in which batches with capacities up to 100 kg and titers up to 4 g/L of antibody are foreseeable for the near future, but by the purification of these molecules from the complex media in which they are produced. Chromatographic separations play a dominant role in the downstream processing of monoclonal antibodies, recombinant proteins and therapeutic plasmids. Chromatography is widely used at all stages of the purification process ranging from capture from cell free harvests to the polishing step needed to remove trace levels of products and process related impurities. Its typical drawbacks are associated to the inability of affinity columns to handle high volumetric flow rates at reasonable ligand utilisation efficiencies. Ideally affinity processes should be deployed as far upstream as possible in order to best utilise their inherent specificity and to minimize the risk of product contamination due to ligand leaching.

Although many different separation technologies are available, downstream processing of MAbs, that typically involve different unit operations, is still based on bead-based chromatography with an affinity capturing steps and one or more ion exchange polishing steps [1–2]. However, limitations with regard to both equilibrium loading and mass transfer kinetics indicate that the overall throughput of bioprocesses may sooner or later be restricted by a chromatographic bottleneck. The gel structure of agarose chromatography beads demands the protein to diffuse into the porous bead due to a concentration gradient until protein saturation occurs. This results in a diffusion limited separation and, as a consequence, long process times are required [3–5].

Membrane-based separation processes can be used to overcome these mass transfer limitations. The ligand is immobilized in the membrane pores and convective flow brings the solute molecules very close to the active binding site and hence minimizes the diffusional limitations associated with the use of beads [6]. The presence of convective transport reduces mass transfer resistance and thus binding kinetics dominates the adsorption process as illustrated in Fig. 1.

Conventional chromatography is typically characterised by high pressure drops for small beads, compaction for soft gels at high flow rates. Conversely, membrane chromatography shows lower pressure drop, higher flow rates and higher productivity; in addition, a more rapid process decreases the probability of inactivation of the biomolecules [7,8].

For large proteins, with MW > 250 kDa, the surface area available for binding is larger for membranes than that of chromatographic media, because these molecules cannot enter the small bead pores and bind to their external surface area [4,9,10]. For smaller proteins, the static binding capacity of membranes per unit volume is lower than the static binding capacity of conventional chromatographic resins and the choice of the appropriate separation process is strongly dependent on the target biomolecule.

Although bead-based chromatography is still predominant and effective for the capturing step, it has several inherent disadvantages for trace-impurity removal or polishing applications, where binding capacity is not the process limit and often chromatography column are oversized. But while affinity membranes

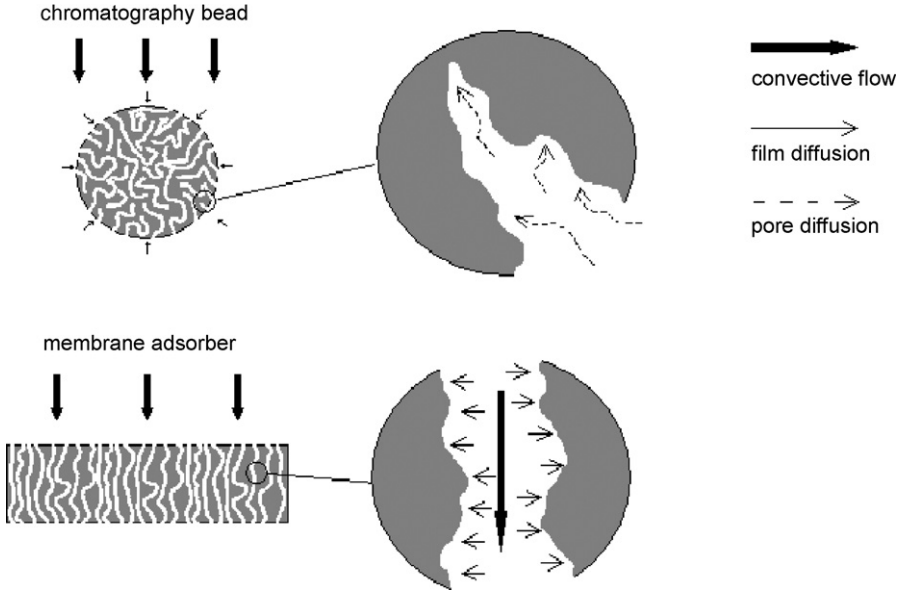

Fig. 1. Transport phenomena involved in chromatographic beads and membrane adsorbers.

are mainly used in research applications and no industrial case studies have been found in the literature, the use of membrane adsorbers for the polishing step is considered with increased interest by the biotech industry. Since membrane-based systems have a distinct flow advantage and sufficient capacity for binding trace level of impurities and contaminants, membrane adsorbers are ideally suited for this application. In addition, when membrane adsorbers are utilised for the polishing step they have excellent performance in terms of virus clearance [11–13]. Another advantage of membrane adsorbers is that they can be disposable, thus they can provide several cost savings and benefits including a reduction in the number and volume of buffers used due to the elimination of resin storage, cleaning, sanitization, and re-validation as well as the elimination of the need for column hardware and packing.

Purification sequences of MAbs based only on ion-exchange membranes have been studied [14] and are currently investigated; the purification scheme considers cation-exchange membranes for the capturing step followed by an anion-exchange membrane adsorber and by high performance tangential flow filtration (HPTFF) [15]. With the proposed process, purity and recovery of MAbs similar to these obtained with the classical affinity chromatographic purification scheme can be achieved, but the success of this technique will be dependent on the p*I* and biochemical properties of the target antibody [16].

Previous review articles have been focussed on the development of affinity membranes, with specific interest towards ligand immobilisation and coupling chemistries [7,8,17] and on process development for the application of adsorptive membranes for protein separations [4,5,8,18].

This review gives an overview of the current trends of the application of membrane adsorbers for the purification of monoclonal antibodies, with particular regard to the development of novel affinity membranes with synthetic ligands and to the illustration of mathematical models used to describe and predict the dynamic behaviour of these systems.

2. Membrane adsorbers: materials and properties

Adsorptive processes are based on the interaction existing between a solid support and a molecule present in the fluid phase that is in contact with the support itself. In the case of membrane adsorbers the support is a microporous membrane that needs to be properly functionalised in order to bind a target molecule. The preparation of a membrane adsorber is a three step process: first a suitable membrane support needs to be selected, then the support needs to be activated for the immobilisation of a ligand appropriate for the target molecule, finally the ligand is coupled to the activated membrane matrix.

2.1. Membranes

The membranes are generally selected among the class of commercially available microporous membranes with a pore size on the microfiltration range. The materials most frequently used are regenerated cellulose, polyethersulfone, and polyvinylidene fluoride. They can be modified by chemical activation, coating or grafting. As possible alternatives, they can be prepared by copolymerisation of two functional monomers or by other methods like cellulose derivative membranes, poly (ether–urethane–urea) membranes, PVC and PTFE microporous composite sheets, macroporous chitin and chitosan membranes [7].

In all cases the matrices needed for membrane chromatography must ideally possess several characteristics that are often physically contradictory [3]. They should have high internal surface to obtain an elevate ligand density and to maximize interactions with the target molecules during the binding step. They should have high mechanical resistance to avoid compaction at high pressure and to withstand process conditions. They should have a good chemical–physical resistance to the solvents used for activation and coupling reactions and to withstand the harsh conditions used for elution of the bound protein and for the sanitization step. They should have hydrophilic surfaces to avoid non-specific adsorption of undesired proteins, therefore these materials should not participate in van der Waals, or hydrophobic, interactions since such adsorption leads to non-specific retention of proteins. Even though inertness is one of the requirements, the support should have substituents that can be easily activated for subsequent ligand coupling. However, these groups and their derivatives should not produce charged sites that might bind proteins non-specifically. Very few materials meet these requirements; quite often the compromise which is less deleterious for the application of interest is selected [3].

2.2. Membrane activation

Activation of membrane supports has been adapted from protocols derived for chromatographic beads, the most common activating reagents are carbonyl diimidazole (CDI), 2-fluoro-3-methylpyridinium tosylate (FMP), cyanuric chloride (trichloro-s-triazine), *N*-hydroxy succinimide esters (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and epoxy, [3,7,8]:

- CDI, bisoxiranes, cyanuric acid, NHS, FMP are used to activate hydroxyl groups,
- CDI, EDC useful for the activation of carboxyl groups,
- epichlorohydrine, glutaraldeide, epoxide used to activate amino groups.

Further details on matrix activation and coupling chemistry are beyond the scope of this review and can be found in the literature [3,7,17,19].

2.3. Ligands

Membrane adsorbers used in the purification of monoclonal antibodies involve affinity and ion exchange adsorption mechanisms. Both unit operations are part of the antibody production process; affinity is generally used in the initial capturing step, whilst ion exchange is used for the final polishing step. Affinity interactions are characterised by a highly specific interaction between the ligand and the target molecule, whereas for an ion-exchange process the immobilised ligand and the target molecule should have opposite charge. Since this characteris-

tic is not critical, it will not be further discussed in this review, while the different affinity ligands used for the purification of immunoglobulins will be described in this section.

The choice of a ligand is strongly dependent on the target molecule characteristics and on its concentration in the feed solution. Due to the particular structure of immunoglobulins two different type of ligand are in use: one that takes advantage of the specificity of antigen binding, another that targets the Fc fragment of the antibody [1]. The use of an antigen for the purification of an antibody is generally called immunoaffinity purification, examples of this kind of purification with membranes have been reported in the literature [20–22], but are mainly targeted at diagnostics [23].

There are very different ligands targeting the constant Fc fragment of antibodies: Protein A from *Staphylococcus aureus* and Protein G from *Streptococcus* fall in this category. Protein A chromatography is by far the most widespread method for MAb capture from crude extracts.

Protein A binds the Fc part of many different antibodies with the exception of human IgG3 and mouse IgG1 [1]. Protein G binds a greater variety of antibodies than Protein A; it binds to all four subclasses of human IgG, to bovine polyclonal IgG and to most subclasses of mouse and rat monoclonal IgG [3]. Protein G has a stronger affinity for immunoglobulins than Protein A, while this is in favour of an efficient binding it complicates the ligate recovery from the stationary phase. Protein A–IgG complexes can be broken and the IgG recovered by eluting the support with a solution at pH up to 4, while Protein G–IgG complexes typically need pH values below 3.

Due to the high cost of these affinity ligands for the industrial production of therapeutic antibodies and to the unavoidable ligand leaching, new synthetic ligands, mainly developed through screening of combinatorial peptide libraries have become subject of experimental investigations by many research groups [24]. The introduction of these ligands can lead to more efficient, less expensive, and safer procedures for antibody purification at manufacturing scales. Among those protein A mimetics like A2P [25–28], D-PAM [29–32], hexamer peptide ligands [33] and CaptureSelect® [34] have been the subject of intense investigation.

MAbsorbent® A2P (Prometic BioSciences, UK) is composed of a di-substituted phenolic derivative of tri-chlorotriazine; this ligand, unlike many affinity ligands that show affinity for specific IgG subclasses, binds all subclasses of IgG including IgG3 that does not bind to Protein A. MAbsorbent® A2P has been developed to mimic the structure of two amino acid side chains of Protein A, Phe 132 and Thy 133 that play an important role in the formation of the complex between Protein A and the Fc fragment of IgG. This ligand has been immobilised to chromatographic beads and is commercially available coupled to cross-linked agarose beads, (Purabead®). Purabead® beads have been successfully used to purify polyclonal antibodies from ovine serum [35]. MAbsorbent® A2P has been immobilised onto regenerated cellulose membranes and tested with pure polyclonal human IgG [36].

D-PAM Protein A Mimetic, TG19320 (Xeptagen SpA, Italy) is a tetrameric polypeptide able to bind specifically and reversibly the constant portion of immunoglobulins [37]; it has a much broader selectivity in comparison with Protein A, since it is able to bind not only IgG but also IgM, IgA, IgE and IgY [38]. For this reason, it can be used as an alternative to traditional ligands to purify immunoglobulins that Protein A-supports cannot isolate.

This ligand has been immobilised onto pre-activated polyether sulphone membranes [39] and onto pre-activated regenerated cellulose membranes [36] and tested for the purification of antibodies.

2.4. Membrane formats and configurations available

Flat sheet is the format preferred by adsorptive membrane producers; in small downscale units membrane layers are stacked inside a circular holder. Scale-up of this kind of cartridges is difficult because increasing the number of membrane layers increases the pressure drop, while increasing the membrane diameter makes the fluid distribution a critical issue. For these reasons scale-up is generally realized by wrapping membrane layers around a porous core [40] and modules with area up to 8 m^2 are commercially available [41]. Further scale-up can be accomplished by combination of these units in series or in parallel depending on process needs.

Hollow fiber membranes have the ideal fiber geometry that allows the construction of modules with a high surface area inside a relatively small module, thus creating high capacity membrane units. Scale-up is accomplished by increasing matrix volume (i.e. number and length of fibers). Fluid residence times are kept constant; breakthrough behaviour, transmembrane pressure and volumetric productivity remain unchanged as the device size is increased. Cycle times are short and are preserved with scale-up. Hollow fiber membrane adsorbers have been prepared and used by different research groups [6,21,42–45], but despite their favourable characteristics membrane adsorbers of this format are not commercially available.

3. Application of membrane adsorbers for the purification of antibodies

3.1. Affinity membranes

The use of affinity membranes for the capture of immunoglobulins was introduced more than fifteen years ago and membrane matrices with immobilised Protein A [6,46,47] were commercially available [48,49]. However, the industrial application of this technology has never taken off due to the lower binding capacity of this new technology compared to affinity chromatography.

Recent development of therapeutics, based on monoclonal antibodies, has lead the biotech industry to look at alternatives to chromatography in view of increased production needs and affinity membrane adsorbers are among the processes considered [50].

Malakian et al., [48], prepared affinity matrices from MAC affinity membrane discs coupled with Protein A and Protein G for the purification of IgG1 and IgG2 from ascites fluids and from

human and rabbit sera. Ligand leaching, flow characteristics, effect of flow rate were investigated. The purity of the eluted samples was demonstrated with SDS PAGE electrophoresis and the increase of flow rates up to 150 mL/min did not decrease the yield of IgG1. The main advantage was the process speed: 1 L of sample at 150 mL/min was processed in 10 min.

Finger et al., [51], coupled thiophilic ligands onto regenerated cellulose membranes for the purification of monoclonal antibodies. The membranes were tested in two different modes: dead-end and cross-flow. Binding capacities for IgG1 and IgG2 were around 17 $\mu g/cm^2$ in dead-end mode, while a surprising value of 29 $\mu g/cm^2$ was found in cross-flow filtration. However, the capacity decreased after the first run, BSA fragments were irreversibly adsorbed in the membrane pores and the water flux could not be restored. It was proved that BSA was damaged inside the membrane pores and not by shear caused by the pump. Repeated runs gave better performances for the cross-flow mode of operation with respect to the dead-end mode.

Arica et al. used a membrane prepared by copolymerisation of 2-hydroxyethylmethacrilate (HEMA) monomer with an amino acid ligand introduced co-monomer 2-methacryloly-amino-histidine (MAAH). The membranes were characterised with adsorption of pure IgG and IgG from human serum, in batch and in continuous flow system configuration. Effects of membrane volume, ligand density, pH, ionic strength and temperature were investigated. Adsorption isotherms based on Langmuir or Freundlich models fitted the experimental data well. Kinetic studies with first order and second order kinetics were performed. In continuous experiments the IgG purity in the eluted fraction was 93% with 58% recovery; these values dropped to 87% and 54%, respectively, for batch experiments [52].

Castilho et al. coated nylon membranes before Protein A immobilisation to reduce non-specific adsorption. The use of hydrophilic coatings on hydrophobic membranes is a technique used to reduce fouling caused by protein adhesion on hydrophobic surfaces [3,7,21,42]. Different coatings were tested: bisoxirane–dextran, formaldehyde–dextran and PVA. PVA was the most efficient coating for the reduction of non-specific adsorption. The ligand density was measured with the BCA protein assay, [53]. The adsorption isotherms were fitted with the Langmuir equation for values of the equilibrium concentration up to 0.6 mg/mL; for higher concentrations, the multilayer Langmuir isotherm gave better results, indicating the existence of IgG–IgG interactions. In a subsequent paper different membranes and ligands were tested for immunoglobulin purification [39]. Protein A membranes showed good selectivity towards IgG and good stability during repeated cycles. Polyethersulfone membranes with immobilised TG19318, also known as PAM [29], showed low selectivity towards human IgG and higher selectivity towards human IgM and murine IgG. This behaviour has been observed also by Boi et al., they coupled D-PAM onto regenerated cellulose membranes obtaining good binding capacity for murine IgG and low non-specific binding, with different selectivities depending on the chemistry used to activate the matrix. They tested also membranes with immobilised A2P, with good results in terms of binding capacity towards human IgG, but high non-specific binding for human serum albumin [36].

A list of applications of affinity membranes for the purification of antibodies is reported in Table 1.

3.2. Ion exchange membrane adsorbers

The use of ion exchange membrane adsorbers for the purification of monoclonal antibodies has been proposed not only as possible substitution of bead-based columns for trace-impurity

Table 1
Applications of affinity membranes for antibody purification

Target protein	Ligands	Membranes	Reference
IgG	Protein A	Surface modified hollow fibers	[6]
Bovine γ-globulin	IDA	PE/grafted copolymer hollow fiber	[46]
IgG2 from hybridoma supernatant	Protein A	Regenerated cellulose	[14]
IgG	Protein A	Poly(ether–urethane–urea)	[47]
MAbs and polyclonal IgG	Protein A, protein G	Cellulose acetate	[48]
Antibodies	Protein A, protein G	Plastic sheets with silica particles	[49]
Human IgG	Protein A	PS with cellulose coating	[42]
IgE	Anti-rat IgE	Cross-linked regenerated cellulose	[22]
MAbs from cell culture media	Thiophilic	Regenerated cellulose	[51]
IgG	Protein A	Modified PES-poly(ethilene oxide)	[21]
IgG	Histidine	Poly(ethylene-co-vinyl alcohol)	[45]
IgG	Histidine	Poly(ethylene-co-vinyl alcohol)	[54]
IgG	Non-proteinogenous	Nylon	[55]
Bovine IgG	Protein G	Regenerated cellulose	[56]
IgG	Protein G	Nylon	[57]
IgG	DEAE	Nylon	[43]
Human IgG	Protein A	Nylon 66 with different coatings	[53]
Immunoglobulins	Protein A, TG19318, histidine, metal chelates, thiophilic	BD2, FD and PVA coated membranes, PES, regenerated cellulose	[39]
Human IgG, IgG from human serum	MAAH	Copolymerisation HEMA–MAAH	[52]
Human IgG, murine IgG	A2P, D-PAM	Regenerated cellulose	[36]

removal, but also in non-affinity purification schemes, in which the capture and polishing steps are made with a sequence of ion exchange membranes [11,14].

Langlotz and Kroner were able to purify IgG from a cell culture supernatant with a sulfonic acid membrane, however the eluted fractions contained traces of BSA that were not present in the eluted fraction of a Protein A affinity membrane used as a comparison. The S-membrane required a further polishing step that is generally present in purification processes for trace contaminants removal. This purification step can be easily performed by anion-exchange membranes, in this case the lower binding capacity of membranes is not a limitation and the high flux advantages offered by membranes would lead to higher productivity.

Conventional chromatographic column used for trace-impurity removal are designed on the basis of the required flow rate and throughputs, therefore large columns are used which have an available binding capacity 3–4 orders of magnitude higher than required [12]. The use of small, disposable membrane units with minimal buffer requirements has been proved to be well suited for this particular application [11–13,57,58]. However, it is still unclear whether the use of membrane adsorbers is economical when compared to bead-based chromatography, the price of membranes for single use units is higher than the cost of resins [11], but a more comprehensive economic evaluation is required.

Warner and Nochumson presented a case study for DNA removal based on 2100 L batch size; they compared the costs for Q membranes and anion-exchange column. In this study medium and equipment costs were evaluated together with buffer and labour costs, it was shown that the membrane process is economical when compared with beads and the higher saving was related to buffer consumption. In total, with the membrane process $ 10,000 (USD) were saved for each batch processed. In addition, cleaning and cleaning validation costs were not evaluated; these costs are sensibly higher for bead-based chromatography compared to disposable membrane cartridges and the overall costs are in favour to the membrane-based process [59].

4. Mathematical model of membrane adsorption chromatography

4.1. System dispersion

A good model of a membrane adsorber has to consider the entire flow system and not only the membrane unit. The effects of flow non idealities such as mixing, channeling and dead volumes, a term that comprises the membrane void volume, the volume of flow distributors, of the detector flow cell and of the pump head, should be included since their influence on the breakthrough curves is quite relevant. This is particularly true in the case of small downscale units in which the layered stack of membranes has often a volume that is smaller than the total volume of circuit, pump and detector. All these effects give rise to what is generally called system dispersion [60].

System dispersion has been accurately described by a combination of a continuous stirred tank reactor, CSTR, and an ideal plug flow reactor, PFR as illustrated in Fig. 2. The CSTR takes into account the effect of flow mixing and non idealities, while the PFR considers the effects of time shifts and dead volumes [61]. The overall system volume can be expressed as the sum of the two contributions:

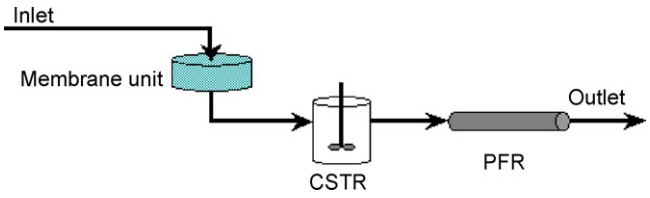

Fig. 2. Flowsheet used for the system dispersion model.

$$V_{sys} = V_{CSTR} + V_{PFR} \tag{1}$$

The CSTR dynamics is given by:

$$\frac{dc_{out}^{CSTR}}{dt} = \frac{Q}{V_{CSTR}}(c_{in}^{CSTR} - c_{out}^{CSTR}) \tag{2}$$

where c_{in}^{CSTR} and c_{out}^{CSTR} denote the inlet and outlet concentrations, respectively, and Q is the volumetric flow-rate. The initial condition is:

$$c_{out}^{CSTR} = 0, \quad \text{at } t = 0 \tag{3}$$

The PFR represents a pure time delay, t_d, since in a PFR all the fluid elements have the same residence time and there is no mixing nor axial diffusion. The PFR model can be written as:

$$c_{out}^{PFR}(t) = \begin{cases} 0; & t < t_d \\ c_{in}^{PFR}; & t \geq t_d \end{cases} \tag{4}$$

where c_{in}^{PFR} and c_{out}^{PFR} denote the inlet and outlet concentrations. The breakthrough curve of a non-binding solute is also called system dispersion curve; it characterizes the dispersive flow behaviour of the system independently of the binding mechanism between ligand and ligate.

Experimental system dispersion curves can be obtained in the same way as the adsorption breakthrough curves, but in non-binding conditions, for instance by using protein solution dissolved in elution buffer as a feed solution. Comparison of experimental and calculated dispersion curves for different systems are in good agreement indicating that this schematization gives a good description of the system [56,62]. V_{CSTR} and V_{PFR} can be determined by minimizing the difference between the experimental and simulated dispersion curves.

The response of the breakthrough curve was used as the influent concentration for the CSTR model. The CSTR response was used as the influent concentration for the PFR model. The PFR outlet, then, represents the system response.

4.2. Mathematical model of membrane adsorption

To model membrane chromatographic processes an accurate description of the hydrodynamics and of the nature of the binding process should be given. Indeed, the flow of the liquid phase along the activated matrix significantly influences

the interaction of the solute with the ligand. The transport of solute to the ligand includes a convective part, responsible for pumping the liquid through the membrane stack, and a diffusive part that accounts for the transport resistance to and within the membrane; this term can be further divided in two parts, pore diffusion and film diffusion. The binding process is usually specified by a kinetic equation that represents ligand–ligate interactions.

The general approach used to model membrane chromatography separations is derived from frontal analysis of chromatographic columns; the assumptions of cylindrical membrane pores, of uniform radial concentration profile and uniform velocity profile are used in order to schematise the problem with a mass balance equation coupled with a kinetic equation that describes the protein–ligand interactions. The equations maybe written in slightly different way, depending on the geometry of the system under investigation.

Briefs and Kula considered a flat sheet membrane system, but specified the continuity equation for a single cylindrical pore and extended the solution for the whole membrane stack. The kinetic equation considered was the usual second order equation that at equilibrium gives the Langmuir isotherm, however the system of equations was solved analytically for the simplified case of a linear kinetic equation [63]. A more general approach was the one of Suen and Etzel who considered the isothermal sorption of a single protein during laminar flow through a flat sheet porous affinity membrane [64]. Protein mass balance equation was written over the membrane stack, illustrated in Fig. 3, as follows:

$$\varepsilon\frac{\partial c}{\partial t} + \varepsilon v\frac{\partial c}{\partial z} = \varepsilon D\frac{\partial^2 c}{\partial z^2} + (1-\varepsilon)\frac{\partial c_s}{\partial t} \tag{5}$$

in which c represents the protein concentration in solution, c_s the concentration of the protein-ligand complex in the solid phase, D is the diffusion coefficient, v is the velocity and ε is the membrane porosity. In the absence of pore diffusion, as in membrane chromatography, the diffusive term can be substituted by introducing a term for axial dispersion.

Binding between protein P and vacant immobilized ligand L is of the form:

$$\mathrm{P} + \mathrm{L} \underset{k_d}{\overset{k_a}{\longleftrightarrow}} \mathrm{PL} \tag{6}$$

where PL represents the protein–ligand complex. Eq. (6) is associated to a reversible second-order rate expression that at equilibrium reduces to the Langmuir isotherm, and can be written as:

$$\frac{\partial c_s}{\partial t} = k_1 c(c_l - c_s) - k_2 c_s \tag{7}$$

where k_1 and k_2 are the association and dissociation rate constants, respectively.

The usual initial conditions indicate that at the beginning of the process the membrane is free of adsorbed protein:

$$c = 0, \quad \text{at } z \geq 0, \quad t = 0 \tag{8}$$

$$c_s = 0, \quad \text{at } z \geq 0, \quad t = 0 \tag{9}$$

Danckwerts' boundary conditions for frontal analysis were used in order to include axial diffusion at the inlet of the membrane, and mixing at the exit of the membrane:

$$\varepsilon v c - \varepsilon D\frac{\partial c}{\partial z} = \varepsilon v c_0, \quad \text{at } z = 0, \quad t > 0 \tag{10}$$

$$\frac{\partial c}{\partial z} = 0, \quad \text{at } z = L, \quad t > 0 \tag{11}$$

The problem has been written in dimensionless form and solved numerically with a software package that utilises the finite-difference method [64].

An analytical solution is possible for the case of $\mathrm{Pe} \to \infty$, that is for negligible axial diffusion; this problem has been solved by Thomas and has been generally used to describe protein adsorption in affinity chromatography [65]. The Thomas solution can be also used as a tool to verify, for the special case of negligible axial diffusion, the accurateness of a numerical solution.

Serafica et al. generalised Suen and Etzel model for both flat sheet and hollow fiber membranes. The problem was written in cylindrical coordinates, from which it was also possible to obtain a solution for the flat sheet configuration as a limiting case of the hollow fiber with an infinite radius of curvature. The model results were compared to experimental breakthrough curves obtained for different proteins with metal chelate affinity membranes. The model well agrees to the experimental data at the initial stages of breakthrough, whereas significant variations were observed at high concentration values [44].

In subsequent papers, Suen et al. extended the basic model to binary-solute bioseparations and showed that, with affinity membranes, it is possible to obtain separation of two solutes based on different sorption kinetics. This is a distinct advantage of membrane adsorbers with respect to packed bead columns, because depending on the system under investigation, two proteins can be separated either on the basis of different binding capacities or on the basis of different sorption kinetics [66].

In bead chromatography, with pore diffusion as the limiting step, the fast kinetics of the chromatographic binding reaction leads to equilibrium conditions and proteins with similar sorption isotherms cannot be separated. In membrane chromatography the lack of pore diffusion limitations allows a separation due to differences in binding kinetics.

The binary-solute model was validated with experiments for the system pepsin and chymosin over pepstatin A affinity membranes [60]: sorption isotherms and kinetic parameters

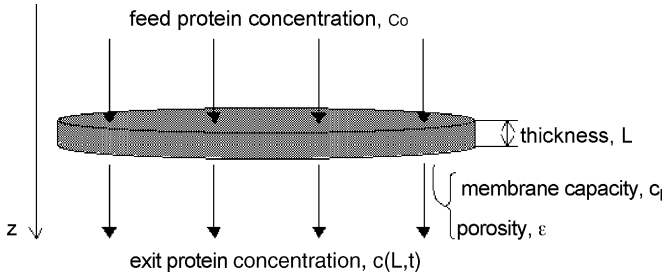

Fig. 3. Schematic drawing of a flat sheet affinity membrane stack.

for the two proteins were measured in batch experiments and the obtained values were used as input parameters for the mathematical model. Model predictions obtained using local equilibrium theory and the affinity membrane model were then compared to experimental breakthrough curves. The affinity membrane model was in good agreement with the experimental data, whereas the local equilibrium model predictions did not match with the data indicating that slow sorption kinetics was the dominant cause of broad breakthrough curves.

In general, ion exchange membranes (1–100 μm) have bigger pore sizes than affinity membranes (0.1–10 μm) and concentration gradients in the radial direction cannot always be neglected as often as in affinity membrane adsorption. Suen and Etzel adapted the correlation of Athalye for packed beds to porous membranes and derived the limiting condition required to neglect mass transfer in the radial direction, that is:

$$\frac{d_p^2}{4D} \ll \frac{L}{v} \tag{12}$$

where d_p is the membrane pore diameter, D is the diffusivity, L is the membrane thickness and v is the velocity. When this condition is not verified an additional term that considers mass transfer in the liquid film needs to be included.

Sarfert and Etzel that used ion-exchange membranes of 150 μm average pore size have reported an example of this situation. They obtained an analytical solution based on the assumption of complete saturation in the membrane pores. The effect of flow non idealities and system dead volumes was taken into account considering a system dispersion model and comparing the model with experimental dispersion curves obtained under non-adsorptive conditions. The model was a valuable tool to understand the process, but agreement with experiments was only qualitative indicating that complete saturation in the membrane pores was probably an assumption too strong for the problem under investigation [67].

A more rigorous numerical solution of the problem was obtained by Yang et al., in which mass transfer of solute was described using overall mass transfer coefficients. The simulated curves were able to fit the general trend of the experimental data in the case of a solid film, with linear driving force; however the curves were similar to those obtained with the analytical solution of Sarfert and Etzel, not justifying the major effort needed for a numerical solution. The experimental breakthrough curves did broadened as membranes approached saturation, following a trend which is not uncommon and has been observed by many research groups for different experimental systems [44,56,62,68].

A possible explanation can be found in the inaccurate expression used for the adsorption rate equation as highlighted by Serafica et al.: they suggested the use of an adsorption kinetic constant that decreases as the surface coverage increases. This corresponds to the steric hindrance model, known also as the "car parking" model [69], that is based on a modification of Eq. (7) where the adsorption rate constant is multiplied by a coefficient that decreases with increasing surface coverage [70].

Another theory, that has been used to explain the asymmetry of the breakthrough curves considers the possibility that a protein molecule, when attached to a surface, can change conformation and occupies more than one active binding site [71]. Yang and Etzel have implemented these kinetic expressions in the membrane adsorber model. Their results showed that the steric hindrance model gives a substantial improvement with respect to the classical Langmuir model represented by Eq. (7), however only with the spreading model they obtained a point by point fit of the experimental data even at later stages of breakthrough [72]. The two fitting parameters needed for the Langmuir kinetic equation correspond to the maximum binding capacity and to the equilibrium dissociation constant, and they have a simple and precise physical meaning for the adsorption process. Since the steric hindrance model is a modified Langmuir model it uses the two Langmuir parameters plus a third parameter, which represents the fraction of the total number of binding sites that are inaccessible. The spreading model requires five fitting parameters, derived from statistics, which do not have a simple physical meaning associated to the adsorption process. However, even if the spreading model gives a very good agreement of the experimental data, the use of five fitting parameters requires more experimental data for model validation.

5. Conclusions

Membrane adsorbers are attracting increasing attention for their peculiar properties. The polishing step based on ion exchange membrane units is already widely accepted as very convenient and appropriate. The application of affinity membrane adsorbers for the capturing step is facing so far the well developed technology based on column chromatography, which can offer the advantages associated to a more mature and consolidated technique and of a higher capacity per unit volume.

On the other hand we are witnessing a very important increase in the monoclonal antibody production worldwide, with several new production facilities already planned for the next coming years. In parallel also the research activity leading to improved membranes and membrane adsorbers is experiencing a significant increase with great appreciation of the potentiality of the membrane-based technique. Clear advantages, which have been recognized, are associated to the much shorter process time as well as to the significant reduction in buffer consumption for given batch volumes. Both these aspects become more and more relevant as the production volumes increase. Membrane adsorbers do not encounter any problem associated to column set-up and are scalable in a straightforward way. Indeed the operation is not yet optimised, as far as materials and module design are concerned, nonetheless the simulation techniques available allow to expect significant improvements in the near future.

Nomenclature

c	protein concentration in the liquid solution (mg/mL)
c_l	membrane capacity (mg/mL)
c_s	protein concentration in the solid phase (mg/cm)

d_p	pore diameter (μm)
D	diffusion coefficient (cm^2/s)
k_1	association rate constant (mL/mg min)
k_2	dissociation rate constant (min^{-1})
K_d	equilibrium dissociation constant (mg/mL)
L	membrane thickness (μm)
t	time (min)
v	superficial velocity, (cm/min)
V	volume (mL)
ε	membrane porosity

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