Biotechnology Advances 31 (2013) 450–465

Contents lists available at SciVerse ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Recent advances in bioprocessing application of membrane chromatography

Valerie Orr, Luyang Zhong, Murray Moo-Young, C. Perry Chou *

Department of Chemical Engineering, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

ARTICLE INFO

Article history:
Received 6 November 2012
Accepted 20 January 2013
Available online 26 January 2013

Keywords:
Downstream bioprocessing
Liquid chromatography
Membrane adsorber
Membrane chromatography
Packed-bed column chromatography
Plasmid DNA purification
Protein purification
Resin
Virus purification

ABSTRACT

Compared to traditional chromatography using resins in packed-bed columns, membrane chromatography is a relatively new and immature bioseparation technology based on the integration of membrane filtration and liquid chromatography into a single-stage operation. Over the past decades, advances in membrane chemistry have yielded novel membrane devices with high binding capacities and improved mass transfer properties, significantly increasing the bioprocessing efficiency for purification of biomolecules. Due to the disposable nature, low buffer consumption, and reduced equipment costs, membrane chromatography can significantly reduce downstream bioprocessing costs. In this review, we discuss technological merits and disadvantages associated with membrane chromatography as well as recent bioseparation applications with a particular attention on purification of large biomolecules.

Contents

Abbreviations: *Ac*MNVP, *Autographa californica* multicapsid nucleopolyhedrovirus; AeDNV, *Aedes aegypti* densonucleosis virus; AGM, agmatine; BSA, bovine serum albumin; BUDGE, 1,4-butanediol diglycidyl ether; C, carboxyl weak cation-exchanger; CHO, Chinese hamster ovarian cells; CM, carboxyl methyl weak cation-exchanger; DBC, dynamic binding capacity; DEA, diethyl amine weak anion-exchanger; DEAE, diethylaminoethyl weak anion-exchanger; EEL, *Euonymus europaeus* lectin; EU, endotoxin unit; GFP, green fluorescent protein; GMA, glycidyl methacrylate; HA, hemoagglutinin; HAU, hemoagglutinin units; HCP, host cell protein; HMW, high molecular weight; IDA, iminodiacetic acid; IgG, immunoglobulin; L-DOPA, 3, 4-dihydroxy-phenylalanine; LRV, log reduction value; MAB, monoclonal antibody; MVM, minute viruses of mouse; NTA, nitrilotriacetic acid; PAA, polyallylamine; pDNA, plasmid DNA; PEI, polyethyleneimine; PES, polyethersulfone; PFU, plaque forming units; PHMB, polyhexamethylene biguanide; pI, isoelectric point; PP, polypropylene; PPV, parvovirus; PVDF, polyvinylidene fluoride; Q, quaternary amine strong anion-exchanger; RC, regenerated cellulose; RLP, rotavirus like particle; S, sulfonated strong cation-exchanger; SBC, static binding capacity; TAEA, tris-2-aminoethyl amine; $TCID_{50}$, 50% tissue culture infective dose; TFF, tangential flow filtration.

* Corresponding author at: Department of Chemical Engineering, University of Waterloo, 200 University Avenue West, Waterloo, Ontario, Canada N2L 3G1. Tel.: +1 519 888 4567x33310.
E-mail address: cpchou@uwaterloo.ca (C.P. Chou).

0734-9750/$ – see front matter
http://dx.doi.org/10.1016/j.biotechadv.2013.01.007

1. Introduction

Advances in the upstream stage of bioprocesses, in particular novel strain construction, have shifted the overall manufacturing costs towards downstream purification stage. On the other hand, technical requirements for high-throughput, single-use (i.e. disposable), and continuous operations have driven a recent reassessment of bioprocessing strategies. As a result, more effort has been devoted to the development of novel, consistent, and effective purification tools, among which liquid chromatography is common for purification of biomolecules. After decades of extensive applications, liquid chromatography is currently under scrutiny since conventional operations with chromatographic beads in packed-bed columns have several technical limitations and are progressively becoming a cost-limiting step for many bioprocesses.

Membrane chromatography has emerged as a cost-effective alternative to column chromatography and has captured growing attention, particularly for processing large volumes of dilute stream, polishing purposes in antibody and therapeutic protein production, and purifying large biomolecules such as viruses and plasmid DNA (Anspach and Petsch, 2000; Boi et al., 2007; Dimartino et al., 2011a; Liu et al., 2011). One of the most significant advantages for membrane chromatography is its reduced mass transfer resistance compared to column chromatography. This results in a fast binding behavior with a high linear velocity of the mobile phase. Thus, the flow rate becomes a critical determinant for cost-effective operation of this technology. Other benefits include reduced buffer usage due to a low void volume; lowered pressure drops, compression, and channeling which effectively simplify the operational facility; and high scalability for bioprocess development. Furthermore, membranes are simple to manufacture and, therefore, the cost of the stationary phase is reduced. Finally, while membrane adsorbers can be reused, they are often promoted as disposable, eliminating the need for lengthy cleaning and regeneration. These advantages indeed have made membrane chromatography common for industrial applications. Nevertheless, more revolutionary developments are still required due to several identified drawbacks, including poor binding capacities, ineffective device design, and irregular physical characteristics of the membrane such as pore size distribution, membrane thickness, and ligand density (Boi et al., 2007; Ghosh, 2002; Ghosh and Wong, 2006; Shi et al., 2008; Suen et al., 2003).

Several reviews have been published by focusing on specific aspects of membrane chromatography, such as immobilized metal affinity membranes (Suen et al., 2003), affinity membrane chromatography (Zou et al., 2001), membrane chromatography applications in monoclonal antibody purification (Boi, 2007; Zhou and Tressel, 2006) and protein purification (Ghosh, 2002), membrane chemistry development for plasmid purification (Sousa et al., 2012), or as a subsection of broader reviews of downstream processing or membrane-based separations (Avramescu et al., 2008; Cramer and Holstein, 2011; Gagnon, 2012; Gottschalk, 2008, 2010; Gutierrez et al., 2007; Thommes and Etzel, 2007; van Reis and Zydney, 2007). This article is meant to provide a more comprehensive review of recent technological advances in the bioprocessing application of membrane chromatography with a general discussion of the chemistry and mass transfer issues of chromatographic membranes. Note that much of industrial research and development on this subject is considered confidential and, thus, unavailable in literature.

2. Chromatographic stationary phases

Conventional liquid chromatography using micro-sized resins in a packed-bed column is a mature purification technique. However, these resins potentially suffer poor mass transfer and physical characteristics, resulting in low purification efficiency. Several other innovative stationary phases, including mixed-matrix membrane chromatography, monolithic columns, and membrane chromatography, have been developed as possible alternatives to resins and their general performance and characteristics are summarized in Table 1 and Fig. 1.

Table 1
Comparison of current chromatography stationary phases.

Characteristic	Resin	Mixed-matrix membrane	Monolith	Membrane
Flow rates	Low	High	High	High
Pressure drop	High	Low	Low–moderate	Low
Dominant transport	Diffusion	Diffusion	Convection	Convection
Binding capacities	High	Moderate	Moderate	Low
Resolution	High	Moderate–high	Moderate	Moderate
Hardware				
Cost	Moderate	Moderate	Inexpensive	Inexpensive
Foot print	Extensive	Small	Extensive	Small

Mixed-matrix membrane chromatography, also known as particle-loaded membrane chromatography, combines the advantages of high resolution and binding capacities of resins with the improved flow properties and reduced fouling, channeling, and pressure drops of microporous membranes through embedding resins within a membrane support. While the flow properties are improved in this type of system, dynamic binding capacities decrease with increasing flow rates, indicating that mass transfer limitation is still present (Avramescu et al., 2003). On the other hand, monoliths and membranes only differ in the longitudinal dimension and monolith columns are akin to a stacked disc membrane module. As a result, they share the same limitation of low binding capacities due to reduced surface area. Since the polymeric matrix forms within the column support, monoliths have a better axial uniformity than a stack of membranes which tend to show irregularities due to variance in individual membrane thickness. Based on the high flow rate with a reduced pressure drop in the column (associated with the decreased mass transfer resistance), monoliths are commonly employed for high-resolution analytical applications for which a high binding capacity is non-essential (Cramer and Holstein, 2011). In parallel, membrane chromatography is gaining popularity due to enhanced capacities to manufacture membranes in various non-conventional formats particularly for large-scale purification. Note that charged ultrafiltration membranes, primarily developed as a means to reduce fouling behavior upon concentration, are considered different from chromatographic membranes as they are not typically employed for purification.

3. Processing and modeling of membrane chromatography

Membrane chromatography potentially outperforms traditional column chromatography in various technical, operational, and economical aspects. The flow-independent behavior and lack of detrimental compression at high linear velocities of the mobile phase make the system easy to scale up. The low pressure drop relieves the requirement for pressure-resistant hardware and the smaller void volume reduces buffer usage, process space requirement, and equipment cost. Additionally, membrane chromatography can be operated in a disposable mode, eliminating the cleaning, regeneration, and validation of the process (Gottschalk, 2010). The advantages of membrane chromatography are prominently derived from the improved mass transfer. Mass transfer resistance within a membrane is significantly reduced due to the large pore size and the localization of the binding moieties on the pore surface. In membranes, transport of solute to the binding sites occurs primarily through convection and the diffusional path-length in membranes is significantly reduced, as compared to resins. Consequently, membrane adsorbers require a short residence time for exhibiting proper binding behavior. Based on this property, membrane adsorbers generally hold dynamic binding capacities independent of the flow rate over a broad range. Conversely, the majority of active binding sites are located inside the macropores/micropores of the resins, resulting in long intra-particle diffusional path-lengths in

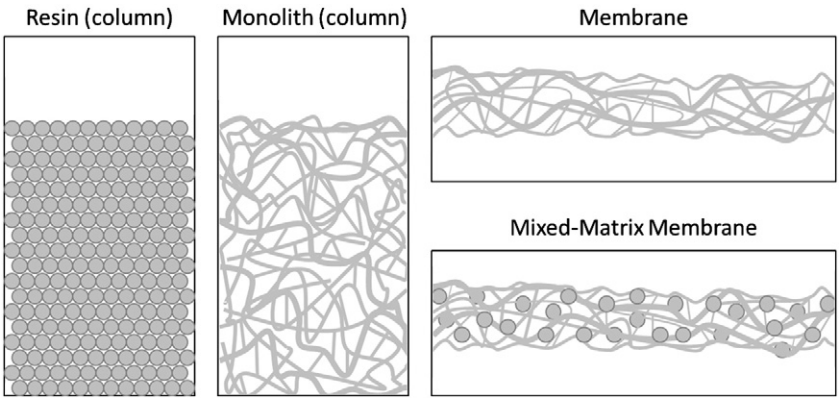

Fig. 1. Comparison of common chromatography stationary phases.
Adapted from Boi (2007) and Ghosh (2002).

addition to film diffusion. Also, these tiny pores can potentially result in the exclusion of large substrate molecules. Differences in dynamic binding behavior between resins and membranes are compared and illustrated in Fig. 2. Non-uniform membrane porosity, membrane thickness, and ligand grafting can potentially lead to variable flow resistance within the membrane frame and improper convective flow, resulting in early saturation of binding sites in large pores, incomplete utilization of binding sites in small or dead-end pores, and ultimately decrease in dynamic binding capacity of the membrane. This technical issue is similar to non-uniform flow resistance around and within resins that results in channeling and reduction of the overall binding capacity in a column. Thus, flow distribution remains as one of the major concerns for both resin (i.e. column) and membrane chromatography.

Through appropriate mathematic modeling and simulation, the operational behavior, performance, and limitations for membrane chromatography can be characterized and predicted. Like column

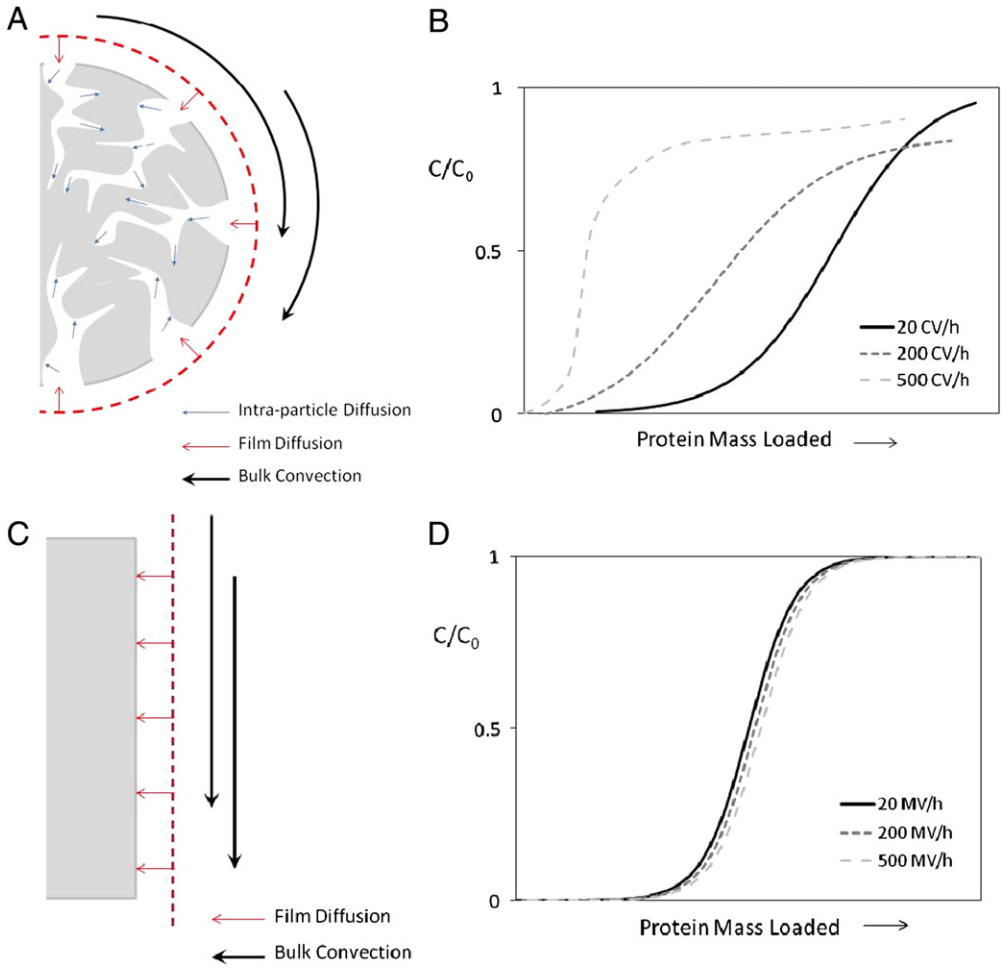

Fig. 2. Comparison of mass transport in (A) resin and (C) membrane adsorber. Effect of flow rate on dynamic binding capacity in (B) resin and (D) membrane adsorber. CV/h = column volume per hour, MV/h membrane volume per hour.
Adapted from Ghosh (2002) and Knudsen et al. (2001).

chromatography, simulation of membrane chromatography can be conducted based on coupling binding kinetics with mass transfer modeling. Typically, binding kinetics is represented using a single or multi-component form of the Langmuir isotherm though other sorption isotherms accounting for system non-idealities have been applied as well, including multi-site isotherm, random sequential adsorption isotherm, spreading isotherm, and steric mass action isotherm (Bower and Wickramasinghe, 2009; Dimartino et al., 2011a,b; Francis et al., 2011, 2012; Guerrero-German et al., 2011a; Riordan et al., 2010; Suen et al., 1993). Specific factors associated with separation chemistry, such salts and pH, are often unique to ligands and must be considered to accurately predict binding behavior (van Beijeren et al., 2012). Several models have been constructed for better design of membrane chromatography based on ion exchange (Bower and Wickramasinghe, 2009; Francis et al., 2011, 2012; Frerick et al., 2008; Labanda et al., 2011; van Beijeren et al., 2012), affinity (Boi et al., 2007; Dimartino et al., 2011a,b; Labanda et al., 2011; van Beijeren et al., 2008; Varadaraju et al., 2011), and hydrophobic interactions (Ghosh and Wong, 2006; Machado et al., 2007). In addition, the application of membrane chromatography modeling can be further extended for purification of large biomolecules (Guerrero-German et al., 2011a; Riordan et al., 2010), integration of downstream processing steps (Frerick et al., 2008; Varadaraju et al., 2011) and economic assessment of bioprocesses (Varadaraju et al., 2011).

Isothermal sorption of multiple solutes onto ligands is often used to describe membrane adsorption behavior while the bulk flow passing through the membrane is represented by a plug flow. Hence, a module of stacked flat membrane discs can be modeled with a plug flow reactor. When the binding capacity is unaffected by flow rate, the overall separation efficiency is limited by the adsorption kinetics between solutes and ligands (Guerrero-German et al., 2011a; Riordan et al., 2010; Suen and Etzel, 1992; Suen et al., 1993). This is true when the residence time exceeds the time needed for solute–ligand association to occur. In such cases, improvements in the mass transfer properties of the membrane will not significantly improve separation efficiency (Ghosh, 2002). However, this is often not the case for resin chromatography where the separation efficiency is dictated by the time required for diffusion of solutes to ligands (Suen et al., 1993). In addition to adsorption kinetics, the breakthrough behavior of solutes can be affected by other parameters, such as flow mal-distribution and dispersion (Bower and Wickramasinghe, 2009; Riordan et al., 2010). Mass transfer properties are significantly affected by several chemical and physical properties of the membrane adsorber and mobile phase. For example, increasing the ionic strength of the mobile phase resulted in a concurrent decrease in the flux of a hollow fiber strong cation membrane when operated in a cross-flow mode (Ventura et al., 2008). This effect could also be modulated by the degree of polymer cross-linking upon the synthesis of membrane, underlining the importance of membrane structure and chemical properties on the mass transfer for membrane systems. Another property of particular note is pore size, which plays a major role in the distinction of membrane chromatography from resin chromatography by determining the relative contribution of intra-pore diffusion to the bulk transport of solutes to the sites of adsorption (Francis et al., 2012). According to a developed model for affinity membrane chromatography, a 3% pore size variation would result in an 11% decrease in dynamic binding capacity and the variation in membrane thickness had a similar but lesser effect (Suen and Etzel, 1992). Additionally, changes in the chromatography medium during separation might result in changes in the volume and pore size of certain polymers under varying ionic strength and consequently the mass transfer properties of membrane (Tatarova et al., 2009). The contribution of internal radial and axial diffusion is also dependent on pore size and external diffusion within the membrane device module, demonstrating the importance of incorporating module design into modeling (Bower and Wickramasinghe, 2009; Francis et al., 2011; Frerick et al., 2008; Ghosh and Wong, 2006; Guerrero-German et al., 2011a; Liu and Fried, 1994; Suen and Etzel, 1992). The internal axial diffusion can be significant enough that its reduction would result in sharper breakthrough curves (Labanda et al., 2009, 2011). Recently, radial concentration gradients and external flow non-idealities caused by poor device design have been shown to contribute to elution band broadening in membrane separations and proper simulation of membrane adsorbers may offer invaluable solutions to the development of novel devices minimizing these effects (Dimartino et al., 2011a; Francis et al., 2011, 2012; Ghosh and Wong, 2006). Nevertheless, modeling of unconventional membrane operations, such as tangential or radial flow modules, and the adsorption of large biomolecules, such as nucleic acid, endotoxin, or viruses, are rare and will require more efforts (Guerrero-German et al., 2011a; Riordan et al., 2010). Development of simulations for membrane chromatography at an industrial level will be crucial and one of the few such models cannot accurately describe the breakthrough behavior associated with protein separation (Serafica et al., 1993).

4. Properties of chromatographic membranes

Despite numerous benefits arising from the improved mass transfer for membrane chromatography, wide-spread implementation of this technology is mainly impeded by poor binding capacity associated with the reduced surface area of membrane adsorbers. Major factors influencing the performance of membrane chromatography, such as flow properties and binding capacity, are largely dependent on membrane chemistry and structure. Unfortunately, several conventional approaches to increasing binding capacity of membranes, such as increasing ligand density via increasing grafting surface area, may decrease the average pore size and, consequently, deteriorate flow properties. Second-generation membrane adsorbers with enhanced binding capacity and desirable flow properties have emerged in response to this need. Generally, chromatographic membranes are synthesized by applying a support matrix onto a polymeric scaffold possessing desired physical and chemical properties. Table 2 lists a selection of commercial products based on physical and chemical properties, available module formats, and binding capacities, etc. It should be noted that accurate prediction of mass transfer effects (discussed in Section 3) associated with various membrane adsorbers greatly aids in the development of novel chromatographic membrane materials, housings, and even manufacturing processes.

4.1. Membrane supports

The support matrix of chromatographic membranes critically affects separation performance and, therefore, must possess certain characteristics for functional application. First, the pore size, structure, and distribution are typically designed for operations in the microfiltration range with high flow rates (Li and Chung, 2008; Wang et al., 2009; Yusof and Ulbricht, 2008). Pore size is particularly important for the separation of large biomolecules, such as DNA. The pore must be large enough to allow sufficient access of the ligands to large biomolecule with minimum exclusion at the pore entrance (Li and Chung, 2008; Yusof and Ulbricht, 2008). On the other hand, larger pores tend to increase the path length of target molecules in the bulk stream to adsorption sites and, consequently, diminish the range of operational flow rates. They may also result in the decrease in total surface area for ligand grafting or radial polarization of solute concentration, diminishing the total dynamic binding capacity (Wickramasinghe et al., 2006). The hydrophobicity of the membrane support can affect the water permeability of membrane as well as the degree of non-specific and/or irreversible interactions with the feed stream (Liu et al., 2010; Suen et al., 2003; Yusof and Ulbricht, 2008). In some cases, hydrophobic interaction may be selectively modulated by grafting hydrophilic polymers onto the hydrophobic supports (Mah and Ghosh, 2010). Membrane adsorbers must be capable of withstanding potential harsh

Table 2
Characteristics of commercially available chromatographic membranes.

Product name	Functionality	Pore size	Membrane material	Dynamic binding capacity (10%)
Ashai Kaseo Medical	*Available formats: hollow fiber*			
QyuSpeed D	Anion-exchange	n/a	GMA[a] grafted polyethylene	>40 mg/mL BSA
Millipore	*Available formats: stacked sheet modules*			
Chromasorb Membrane Adsorber	Anion-exchange	n/a	Ultra high molecular weight polypropylene	≥50 mg/mL BSA
Natrix Separations	*Available formats: spin columns, cut discs, syringe filters, spiral wound module, cross-flow cassettes*			
Natrix Q	Anion-exchange	0.45 μm	Hydrogel	>300 mg/mL BSA
Natrix S	Cation-exchange	0.45 μm	Hydrogel	>250 mg/mL lysozyme
Natrix C	Cation-exchange	0.45 μm	Hydrogel	>80 mg/mL hIGg
Natrix IMAC-Ni^{2+}	Immobilized metal affinity	0.45 μm	Hydrogel	>70 mg/mL GFP
Natrix Protein A	Affinity	n/a	Hydrogel	n/a
Natrix Aldehyde	Affinity coupling	n/a	Hydrogel	
Pall's Corporation	*Available formats: cut discs, syringe filters, stacked sheet modules, pleated sheets modules*			
Mustang Q	Anion-exchange	0.8 μm	Modified PES	56 mg/mL BSA
Mustang S	Cation-exchange	0.8 μm	Modified PES	47 mg/mL lysozyme 60 mg/mL hIgG
Mustang E	Anion-exchange	0.2 μm	Modified PES	4×10^6 MEU/mL endotoxin
Sartorius	*Available formats: spin columns, multi-well strips, 96 well plates, cut discs, syringe filters, stacked sheet modules, pleated sheets modules*			
Sartobind Q	Anion-exchange	>3 μm	RC	29 mg/mL BSA
Sartobind D	Anion-exchange	>3 μm	RC	21 mg/mL BSA
Sartobind STIC[a]	Anion-exchange	>3 μm	RC	50 mg/mL BSA in buffer with 150 mM NaCl
Sartobind S	Cation-exchange	>3 μm	RC	25 mg/mL lysozyme
Sartobind C	Cation-exchange	>3 μm	RC	21 mg/mL lysozyme
Sartobind Phenyl	Hydrophobic interaction	>3 μm	RC	15 mg/mL IgG
Sartobind IDA	Immobilized metal affinity	>3 μm	RC	3.6 mg/mL His-tagged protein
Sartobind Protein A	Affinity	0.45 μm	RC	5–7.5 mg/mL IgG
Sartobind epoxy	Affinity coupling	0.45 μm	RC	n/a
Sartobind aldehyde	Affinity coupling	0.45 μm	RC	n/a

[a] STIC—salt tolerant interaction chromatography; GMA—glycidyl methacrylate.

conditions (including a wide range of pH, temperature, and ionic strength, etc.) experienced during chromatographic operation, sterilization, and regeneration (Hu et al., 2009; Suen et al., 2003; Yusof and Ulbricht, 2008). Additionally, common impurities and additives, such as detergents, can cause potential deterioration of membrane (Suen et al., 2003). Thus, the chemical and physical stability of membrane support will critically affect the application and reusability of chromatographic membrane. Finally, the availability of functional groups on the basal material will affect the feasibility for membrane modulations, such as sufficient immobilization of ligands, further polymer grafting or other modifications to improve membrane stability (Chen et al., 2009; Hu et al., 2009; Suen et al., 2003; Yusof and Ulbricht, 2008).

Almost all commercial membranes employ organic support matrices, probably due to lower costs and higher resistance to non-specific binding of target molecules, particularly proteins (Chao, 2008; Che et al., 2011). Regenerated cellulose (RC) appears to be the most popular support material, particularly for ion-exchange membranes (Table 3). Regardless, the short lifetime and poor reusability limit its applicability (Che et al., 2011). Synthetic organic polymer supports, such as nylon, polyethersulfone (PES), polypropylene (PP), and polyvinylidene fluoride (PVDF), have better physical properties than natural polymers, but exhibit slightly higher non-specific adsorption of many biomolecules (Che et al., 2011; Chen et al., 2009). Hygroscopic hydrogel is a synthetic organic polymer based on a modified cross-linked polyacrylamide, which swells upon hydration with water (Zhong et al., 2011a, 2011b). While inorganic polymers, such as alumina and glass, have been explored due to their high degrees of uniformity, their application has been limited due to high levels of non-specific binding and prohibitive costs (Chang and Suen, 2006).

4.2. Membrane ligands

Chromatographic membranes are available in various standard interactions, including anion/cation exchange, affinity, and hydrophobic interaction. Affinity appears to be the most prevalent chemistry for experimental chromatographic membranes (Table 3). Common affinity ligands include protein A for purification of immunoglobulins (IgGs), immobilized metals for purification of his-tagged proteins, dye-affinity, and specialized ligands. Dye-affinity membranes vary in the type of interaction that dictates the separation behavior and Cibacron Blue F3GA specific to serum albumins is particularly common. Other ligands of interest are the mimetic ligands A2P monochloride, B14 monochloride, and Ligand 22/8 which are alternatives to protein A for immunoglobulin purification (Barroso et al., 2010; Boi et al., 2011). Customized affinity membranes can be fabricated through the coupling of ligands to aldehyde- or epoxy-functionalized membranes. Common ion-exchange ligands include quaternary amine (Q) as strong anion-exchangers, diethylaminoethyl (DEAE) as weak anion-exchangers, sulfonated (S) as strong cation-exchangers, and carboxyl (C) as weak cation-exchangers. A variety of alternative ion-exchange ligands are under development in an attempt to decrease their sensitivity to ionic strength and improve their accessibility to target molecules (Riordan et al., 2009b; Weaver et al., 2013a; Woo et al., 2011). Hydrophobic interaction is less common for membrane chromatography as higher resolutions can be obtained by resin chromatography. While the innate support matrix can have certain hydrophobicity, alkyl chains are often grafted for hydrophilic supports. Major applications include separation of aggregates and inactive product isoforms with hydrophobicity different from bioactive form and such separation often requires high resolutions that are difficult to achieve with other membrane adsorbers.

4.3. Membrane geometric and processing formats

Another major advantage of membrane chromatography is achieved through the employment of non-conventional geometric formats which are difficult to implement for resins. Common membrane devices based on three flow types, i.e. dead-end flow, cross (i.e. tangential) flow, and radial flow, have been developed primarily for microfiltration applications (Fig. 3). Dead-end flow devices, such as modules with multiple stacked membrane discs, are similar to traditional columns and, consequently, are interchangeable with most column chromatographic facilities. However, they are more suitable for scouting studies at the bench scale. In addition, membrane adsorbers in spin column or microplate format are available primarily for small-scale but high throughput operations (Harkensee et al., 2007; Kokpinar et al., 2006).

Table 3
Materials under development for improved chromatographic membranes.

Membrane materials	Ligands	Format	References
Affinity membrane chromatography			
Natural organic			
Chitosan & chitosan composites	Reactive Red 120 Reactive Brown 10 Reactive Blue 4 Reactive Green 19 Cibacron Blue F3GA p-Aminobenzoic acid Histidine Glutamic acid Tyrosine L-DOPA	Flat sheets	Chao (2008), Chen et al. (2009), Liu and Bai (2006), Nie and Zhu (2007), Nie et al. (2007), Peixoto et al. (2008), Zhang et al. (2010)
Cellulose & regenerated cellulose	IDA-Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, Cu^{2+}-ConA Cibacron Blue F3GA Protein A Ligand 22/8 Phenyl borate	Flat sheets 96 well plates Spin columns	Barroso et al. (2010), Ke et al. (2010), Ko et al. (2011), Ma and Ramakrishna (2008), Pereira et al. (2012)
Synthetic organic			
Nitrocellulose	Recombinant allophycocyanin	Flat sheets	Sun et al. (2008)
Nylon/Poly(2-(methacryloyloxy) ethyl succinate)	NTA-Cu^{2+}, Ni^{2+}	Flat sheets	Anuraj et al. (2012)
Poly(ethylene vinyl alcohol)	TAEA-Ni^{2+} IDA-Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}	Hollow fiber	de Aquino et al. (2006), Ribeiro et al. (2008)
Poly(2-hydoxyethylmethacrylate)	Procion Green H-4G Procion Brown MX-5BR Reactive green 5	Flat sheets	Bayramoglu et al. (2007)
Polysulfone	Red HE-3B Yellow HE-4R Cibacron Blue F3GA	Flat sheets	Ma et al. (2006a, 2006b)
Polyethersulfone	Cu^{2+}, Ni^{2+}, Zn^{2+} Cibacron Blue F3GA Heparin	Flat sheets Dual layer Hollow fiber	Li et al. (2008), Ma et al. (2009), Wang et al. (2011)
Poly(vinyl alcohol-co-ethylene)	Cibacron Blue F3GA	Flat sheets	Zhu et al. (2011)
Poly(acrylonitrile-co-hydroxyethyl methacrylate)	Glucose	Flat sheets	Che et al. (2011)
Polypropylene	Glucose	Flat sheets	Hu et al. (2009)
Inorganic			
Alumina and alumina composites	NTA-Cu^{2+}, Ni^{2+} Lysine	Flat sheets	Jain et al. (2007), Shi et al. (2010a)
Ceramic/chitosan	IDA-Cu^{2+}	Flat sheets	Nova et al. (2008)
Aluminum oxide silica/chitosan	Cu^{2+} Lysine	Flat sheets	Shi et al. (2008, 2010b)
Polyvinylidene fluoride	Tryptophan	Flat sheets	Yong et al. (2010)
Ion-exchange membrane chromatography			
Natural organic			
Chitosan & chitosan composites	Chitosan amino groups CM	Amphoteric flat sheets Flat sheets	Feng et al. (2008, 2009), Machado et al. (2006)
Cellulose & regenerated cellulose	Q S DEAE Q: poly((2-(methacryloyloxy) ethyl)-trimethylammonium chloride Polyacrylamide Agmatine Tris(2-aminoethyl)amine Polyhexamethylene biguanide Polystyrene sulfonate Polyethyleneimine Poly(diallyldimethyl ammonium chloride) Poly(2-(dimethylamino)ethyl methacrylate)	Spin columns Flat sheets	Bhut and Husson (2009); Bhut et al. (2010, 2011a,b, 2012), Liu et al. (2010), Menkhaus et al. (2010), Riordan et al. (2009a, 2009b), Singh et al. (2008), Wang et al. (2009), Zhang et al. (2008)
Synthetic organic			
Bromomethylated poly(2,6-dimethyl,-1,4-phenylene oxide)	DEA C	Amphoteric hollow fiber	Cheng et al. (2010b)
Nylon	Polystyrene sulfonate Polyethyleneimine Poly(diallyldimethyl ammonium chloride) Poly(allylamine) Poly(2-(methacryloyloxy)ethyl succinate	Flat sheets	Liu et al. (2010)
Polyethylene Polyethersulfone	2-Hyoxyethylamine	Hollow fiber	Hagiwara et al. (2005)

(continued on next page)

Table 3 (*continued*)

Membrane materials	Ligands	Format	References
	S	Flat sheets	Li and Chung (2008),
	Q	Dual layer	Liu et al. (2010)
	S: Polystyrene sulfonate	Hollow fiber	
	Polyethyleneimine		
	Poly(diallyldimethyl ammonium chloride)		
	Poly(allylamine) (PAA)		
Polyimide	Ethylene diamine	Flat sheets	Cheng et al. (2010a)
Polypropylene	Q: poly((2-(methacryloyloxy)	Flat sheets	He and Ulbricht (2008),
	ethyl)-trimethylammonium chloride		Yusof and Ulbricht (2008),
	Polyacrylamide		Zheng et al. (2010)
	DEA		
Polystyrene	S	Hollow fiber	Ventura et al. (2008)
Polyvinylidene fluoride	S: Polystyrene sulfonate	Flat sheets	Liu et al. (2010)
	Polyethyleneimine		
	Poly(diallyldimethyl ammonium chloride)		
	PAA		
Inorganic			
Glass fiber and modified glass	S	Flat sheets	Chang et al. (2008), Chiu et al.
	Diamine		(2007)
Carbon nanofibers	C	Flat sheets	Schneiderman et al. (2011)
Alumina and modified alumina	Diamine	Flat sheets	Chang et al. (2008)
Hydrophobic interactions membrane chromatography			
Natural organic			
Cellulose	Poly(N-vinylcaprolactam)	Flat sheets	Mah and Ghosh (2010)
Regenerated cellulose (Sartobind epoxy)	1,4-butanediol diglycidyl ether	Flat sheets	Pereira et al. (2012)
Synthetic organic			
Polyvinylidene fluoride	n/a	Flat sheets	Sun et al. (2009)
Inorganic			
Alumina	Octanoic acid	Flat sheets	Chang and Suen (2006)
	Octadecanoic acid		
Glass	Poly(N-isopropylacrylamide)		Chen et al. (2007), Meng et al.
	Octyltriethoxysilane		(2010)
	Butyltrichlorosilane		
	Octyldimethylchlorosilane		
	Butyldimethylchlorosilane		

Tangential or radial flow devices are adopted to reduce fouling for both industrial and small-scale applications (Cheng et al., 2010b; de Aquino et al., 2006; Hagiwara et al., 2005; Li and Chung, 2008; Li et al., 2008; Shirataki et al., 2011; Ventura et al., 2008). Cross-flow membrane chromatography devices are particularly amenable to scale up based on an effective separation capacity comparable to that associated with the use of stacked discs (Orr et al., 2012).

5. Bioprocessing application of membrane chromatography

Membrane chromatography has been increasingly applied for various bioprocessing purposes, including primary protein capture, purification of large biomolecules (e.g. nucleic acids, proteins, antibodies, and viruses), and polishing of bioprocess streams. Primary capture of biomolecules from dilute bioprocess streams often requires chromatographic media with a high binding capacity and high selectivity. On the other hand, polishing for the removal of diluted impurities, such as host cell proteins (HCPs), endotoxins, and viruses, in large-volume bioprocess streams values high flow rates for fast processing. Purification of large biomolecules requires an operating condition with a low shear stress and chromatographic media with a larger pore size to decrease rejection. Numerous reports for processing of industrial feedstocks, such as cell lysates, cell-free media or whey, have become available due to the increased accessibility of commercial membrane adsorbers, as is evident from Tables 4–7. In general, chromatographic membranes outperform resins particularly under high flow rate conditions. With the innate filtration capacity, the applicability of membrane chromatography can be further extended for simultaneous clarification and purification.

5.1. Protein purification

5.1.1. Protein capture and intermediate purification

The principal goal for protein capture is to selectively harvest the protein of interest by removing bulk impurities, typically contaminant proteins, whereas intermediate purification strives to remove the remaining protein impurities. Usually, the feedstock of protein mixture is a cell lysate or cell-free medium clarified with centrifugation or filtration. Affinity membrane chromatography is a common method for protein capture (Table 4). A representative example is using immobilized metal affinity ligands for the purification of his-tagged proteins. Another novel application has been explored for the purification of glycoproteins using lectin affinity membrane chromatography (Kokpinar et al., 2006). Over the past decades, the development of state-of-the-art membrane adsorbers with enhanced binding capacities has drastically widened the application of membrane chromatography in protein capture and purification. In a comparative study using weak anion exchangers, membrane adsorbers outperformed resins in terms of both binding capacity and purification performance (Bhut et al., 2010). Membrane chromatography can be further applied in protein capture and purification based on its generic feature for filtration, particularly with the implementation of tangential flow devices. In a study where crude and clarified *Escherichia coli* lysates containing heterologously expressed his-tagged proinsulin were processed with affinity membrane chromatography, no significant difference was observed in protein recovery between clarified and crude lysates when processed in cross-flow filtration mode using a hollow fiber, whereas processing of the crude (i.e. non-clarified) lysate was impossible in dead-end filtration mode (de Aquino et al., 2006). Simultaneous clarification of whole cell culture and

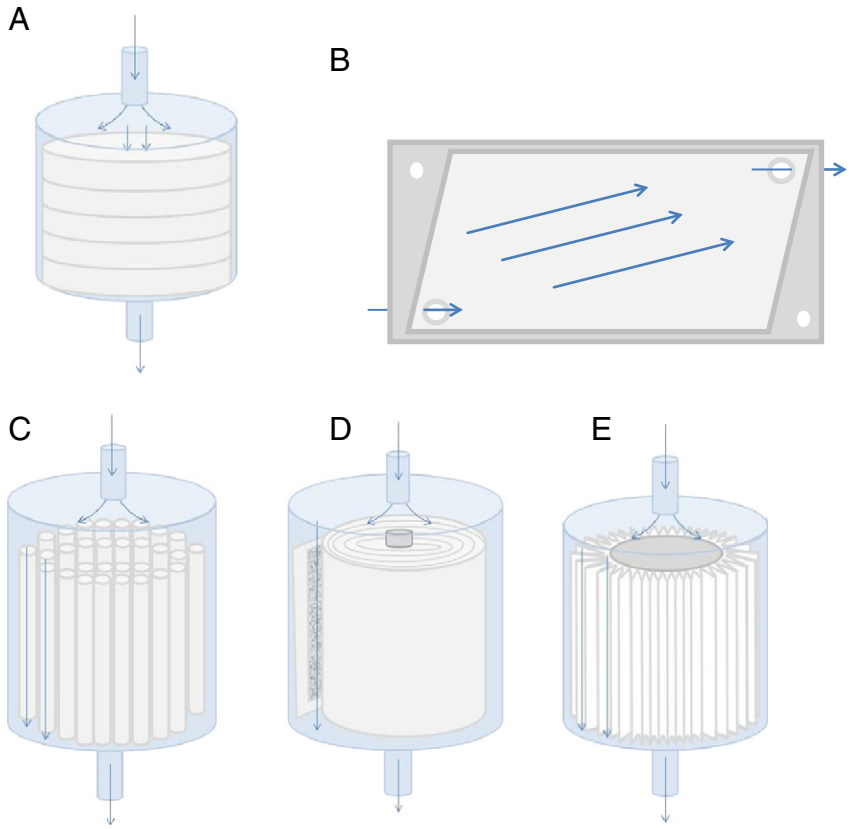

Fig. 3. Summary of commonly available membrane chromatography modules (Suen et al., 2003). (A) Stacked discs, (B) cross-flow flat sheet cassette, (C) hollow-fiber, (D) spiral wound, (E) pleated sheet.

purification of extracellularly released penicillin G acylase were effectively conducted by integrating anion-exchange membrane chromatography and tangential flow filtration (Orr et al., 2012). These studies have successfully demonstrated novel application of membrane chromatography in simultaneous clarification and purification, significantly reducing bioprocess complexity.

5.1.2. Polishing

Polishing is conducted for more extensive clearance of the remaining impurities, such as HCPs, endotoxins, nucleic acids, and viruses, after major capture or purification of target molecules. The relatively dilute and large-volume natures of bioprocess streams highlight the importance of fast flow rates for this step. Accordingly, membrane chromatography becomes particularly suitable for this operation. The performance of polishing can be evaluated by log reduction value (LRV), which is defined as the logarithm of the ratio of the impurity mass in the feed to that in the effluent (Woo et al., 2011). Membrane chromatography has a major advantage in clearance of large impurities, such as nucleic acids and viruses, as micron-sized pores on the membrane allow better access of large solutes to ligands with minimum fouling and pore exclusion which are commonly seen for resins (Riordan et al., 2009a; Shirataki et al., 2011). Polishing is particularly relevant to the production of therapeutic biologics based on its capacity for removal of high molecular weight (HMW) aggregates or separation of inactive isoforms. Various polishing applications based on the use of membrane adsorbers are summarized in Table 5. Based on different ligand chemistry, certain membranes (e.g. Mustang Q and Sartobind Phenyl) excel in clearance of HCPs (up to 80%) (Kuczewski et al., 2010; Liu et al., 2011), whereas others (e.g. QyuSpeed and Chromasorb) specialize in impurity clearance at high ionic strengths (Shirataki et al., 2011; Woo et al., 2011). Novel ligands can lead to economic advantages of bioprocesses. For example, the capacity for impurity clearance at high ionic strengths may eliminate the need for buffer exchange prior to the polishing step, dramatically reducing buffer costs and bioprocessing time (Riordan et al., 2009a, 2009b). The polishing capacity based on several ligands, including agmatine (AGM), tris-2-aminoethyl amine (TAEA), polyhexamethylene biguanide (PHMB), polyethyleneimine (PEI), 2-aminoethyl trimethylamine (Q), and the Mustang Q membrane was evaluated by challenging them with a biological mixture containing RNase A, phage ΦX174, HCP, and DNA under a high salt concentration (Riordan et al., 2009a, 2009b). While the Q membranes were only effective for clearance of highly charged DNA (LRV > 4.2), other ligands showed better clearance of both DNA and phage (LRV > 5.2). PHMB appears to have the highest tolerance to ionic strengths by being able to clear all impurities including HCPs (~95% clearance). These results demonstrate the feasibility of versatile ligands for polishing. Another common bioprocess impurity, particularly with the use of *E. coli* as the production host, is endotoxin which should be completely removed for the production of therapeutic or vaccine bioproducts. Several chromatographic membranes have shown excellent endotoxin clearance (Woo et al., 2011). For example, with the use of Q membranes, endotoxin was effectively cleared during the polishing step for the production of fibroblast growth factor (as an endotoxin-free therapeutic protein) (Chen et al., 2012) or plasmid DNA (Zhong et al., 2011b).

5.1.3. Monoclonal antibody purification

Manufacturing of therapeutic biologics, particularly monoclonal antibody (MAB), represents a growing area where membrane chromatography can make significant contributions. Currently, cultivation techniques can easily yield MAB titers up to 1–5 g/L, making the overall bioprocess expense be limited by downstream purification, particularly chromatographic steps (Boi, 2007). Nevertheless, the efficiency and economy of such bioprocesses heavily rely on proper synthesis of

Table 4
Summary of protein capture and purification applications of membrane chromatography.

Membrane material and ligand	Target	Capacity	Recovery/purity	Membrane geometry	Reference
Affinity membranes					
Chitosan aluminum oxide composite—Cu^{2+}	Hemoglobin in hemolysate	11–13 mg/mL DBC	91% recovery	Flat sheets	Shi et al. (2008)
Nylon/Poly(2-(methacryloyloxy) ethyl succinate)-NTA—Ni^{2+}	His-tagged myo-inositol -1-phosphate synthase in *E. coli* cell lysate	n/d	>90% recovery	Flat sheets	Anuraj et al. (2012)
Poly(ethylenevinyl alcohol)-IDA—Ni^{+2}	IgG in human plasma	40–44 mg/g DBC	78% recovery >98% purity	Hollow fiber	Ribeiro et al. (2008)
Poly(ethylenevinyl alcohol) IDA-Zn^{2+}	MAB from partially purified cell culture supernatant	3.2 mg/mL DBC	90% recovery 33% purity	Hollow fiber	Serpa et al. (2005)
Poly(ethylenevinyl alcohol)—IDA-Ni^{2+}	Hisproinsulin partially purified *E. coli* cell lysate	15–56 mg/g DBC	>80% recovery	Hollow fiber	de Aquino et al. (2006)
RC-IDA-Cu^{2+}	Penicillin G acylase (PGA) from *E. coli* cell lysate	1.04 mg/cm^2 lysozyme SBC	80% recovery 21 fold purification	Flat sheets	Ke et al. (2010)
RC (Sartobind)—IDA-Cu^{2+}, Ni^{2+}, Zn^{2+}, or Co^{2+}	HisGFP in *E. coli* lysate His-β-glucanase in *E. coli* culture supernatant	n/d	>90% purity for both	8-well strips	Kokpinar et al. (2006)
RC (Sartobind)—IDA-Cu^{+2}	Erythropoietin from CHO cell culture supernatant	n/d	75% purity	Flat sheets	Mellado et al. (2007)
RC (Sartobind)—IDA-Ni^{2+}	Recombinant his-tagged flavivirus domain III protein from *E. coli* lysate	n/d	95% clearance of host cell impurities	Flat sheets	Tan et al. (2010)
RC (Sartobind) Protein A	IgG1 from clarified transgenic tobacco extract	n/d	78% purity	Flat sheets	Yu et al. (2008b)
Nylon/chitosan—Reactive Red 120 Reactive Brown 10	Papain from papaya extracts	143.6 mg/g SBC for RR120 107.3 mg/g SBC for RB10	>80% recovery or RR120 50% recovery for RB-10	Flat sheets	Chen et al. (2009)
Poly(2-hydroxyethylmethacrylate)—Reactive Green 5	IgG in human serum	33.75 mg/mL SBC	81% purity, 67% recovery	Flat sheets	Bayramoglu et al. (2007)
Polysulfone/glycidyl methacrylate/dimethyl acrylamide—Red HE-3B dye	Lactoferrin from whey	111.0 mg/mL SBC	94% purity 91% recovery	Hollow fiber	Wolman et al. (2007)
Polysulfone/glycidyl methacrylate/dimethyl acrylamide—Yellow HE-4R	Protease from Flavourzyme fungal extract	24,220 U/mL for SBC	72% recovery 3.7 fold purification	Hollow fiber	Wolman et al. (2006)
RC (Sartobind)—Cibacron Blue F3GA	Erythropoietin from CHO cell culture supernatant	n/d	55% purity	Flat sheets	Mellado et al. (2007)
Chitosan-silica—p-aminobenzoic acid	Tyrosinase from crude *Agaricus bisporus* lysate	15 mg/g DBC	n/d	Flat sheets	Chao (2008)
Nitrocellulose-recombinant allophycocyanin (APC)	Polyclonal antibodies from rabbit serum	5.79 mg/g SBC	98% purity 82% recovery	Flat sheets	Sun et al. (2008)
RC (Sartobind epoxy)—B14 monochloride	IgG from cell culture supernatant	3.1 mg/mL SBC	>90% recovery	Flat sheets	Boi et al. (2011)
Polyethersulfone/polypyrrole—Heparin	Thrombin in partially fractionated bovine blood	2159–4042 U/g SBC	93% desorption	Flat sheets/electro-membranes	Wang et al. (2011)
Ion-exchange membranes					
Hydrogel (Natrix) Q	Penicillin G acylase from *E. coli* crude broth	n/d	72% of penicillin G acylase	TFF flat sheets	Orr et al. (2012)
RC (Sartobind) Q	Caseinomacropeptide (CMP) from skim milk	7.8 mg/mL for glycosylated CMP DBC	Separation of aglycosylated from glycosylated CMP	Flat sheets	Kreuss et al. (2008)
RC (Vivaspin) Q	β-Lactoglobulin from whey	n/d	87.6% purity	Spin column	Bhattacharjee et al. (2006)
RC (Sartobind) Q and S		n/d		Flat sheets	

Table 4 (*continued*)

Membrane material and ligand	Target	Capacity	Recovery/purity	Membrane geometry	Reference
	Multistep (Q & S) fraction of whey proteins		100% recovery BSA 100% recovery β-lactoglobulin 99% purity β-lactoglobulin >60% recovery lactoferrin 95% purity lactoperoxidase 88% purity α-lactoalbumin		Voswinkel and Kulozik (2011)
RC (Vivascience) Q or S	Human growth hormone in CHO cell culture supernatant	n/d	93% recovery on S membrane	96 well plate	Walter et al. (2007)
RC (Vivaspin) Q or S	Human growth hormone from CHO cell supernatant	n/d	100% purity 57% recovery	Spin column	Suck et al. (2006)
PES (Mustang) Q	Urease or lysozyme from corn or soya bean extracts	8.9 mg/mL for urease in soya extract DBC 6.7 mg/mL for urease in corn extract DBC	n/d	Flat sheets	Menkhaus and Roseland (2008)
RC (Sartobind) D & Q	β-Lactoglobulin from whey	1.2 mg/cm^2 DBC for both D & Q	>85% recovery		Goodall et al. (2008)
RC (Sartobind) S	IgG from clarified transgenic tobacco extract	n/d	100% recovery 11% purity	Flat sheets	Yu et al. (2008b)
RC (Sartobind) S	IgGs in tobacco extract	n/d	90% recovery, 130 fold purification	Flat sheets	Yu et al. (2008a)
RC (Sartobind) S	Lactoferrin and lactoperoxidase from cheese whey	0.7 mg/m^2 lactoferrin and lactoperoxidase SBC	90% recovery of lactoferrin 95% purity of lactoferrin 80% recovery for lactoperoxidase 85% purity for lactoperoxidase	Flat sheets	Plate et al. (2006)
RC (Sartobind) C	Fibroblast growth factor from *E. coli* clarified lysate	n/d	~80% recovery	Flat sheets	Chen et al. (2012)
RC (Sartobind) D	Anthrax protective antigen from *E. coli* cell lysate	55 mg/mL BSA SBC ~25 mg/mL BSA DBC	75% recovery, 65% purity of anthrax protective antigen	Flat sheets	Bhut et al. (2010)
PES diethyleneglycol dimethacrylate	Lysozyme from egg white	n/d	95% purity	Hollow fiber	Ventura et al. (2008)
HIC membranes					
Cellulose—Poly(N-vinylcaprolactam)	IgGs from simulated cell culture supernatant	12 mg/mL DBC	89% recovery 97% purity	Flat sheets	Mah and Ghosh (2010)
Modified PVDF-modulated with ammonium sulfate	IgG1 and MAB from simulated cell culture supernatant	49–64 mg/mL DBC for pure IgG1 31–35 mg/mL DBC for pure MAB	>94% purity and >97% recovery of IgG1 >91% purity and >98% purity of MAB	Flat sheets	Wang et al. (2006)
Modified PVDF-modulated with ammonium sulfate	IgGs in partially purified tobacco extract	n/d	85% purity and overall process recovery 77%	Flat sheets	Yu et al. (2008a)
RC (Sartobind)-Phenyl	IgGs in partially purified PER.C6 cell culture supernatant	20 mg/mL DBC	>90% recovery of IgGs 80% reduction in HCP	Flat sheets	Kuczewski et al. (2010)

SBC: static binding capacity; DBC: dynamic binding capacity at 10% breakthrough unless noted otherwise.

purification steps. For example, it appears that affinity membrane chromatography based on immobilized protein A cannot be used as a primary capture step for the purification of IgGs from tobacco extract due to membrane fouling and pressure drops (Yu et al., 2008b). However, the addition of a pretreating step based on the use of a cation-exchange membrane for filtration and polishing of the tobacco extract can address these issues with a high overall recovery of 89%. To date, membrane chromatography based on either ion exchange has been extensively explored as an intermediate polishing step in MAB production (Boi, 2007; Liu et al., 2011; Zhou and Tressel, 2006; Zhou et al., 2006). A cost–benefit analysis confirms the economic viability of membrane chromatography as a polishing step, even based on a disposable operation, in comparison to resin chromatography (Zhou and Tressel, 2006). Also, it has been estimated that a bioprocessing capacity of 3000 g/m^2 makes membrane chromatography competitive with resin chromatography (Shirataki et al., 2011; Zhou and Tressel, 2006) though membrane fouling due to aggregate formation represents a major technical issue (Lajmi et al., 2010). In an industrial study to combine several membrane chromatographic steps based on protein A, anion-, and cation-exchangers (Giovannoni et al., 2008), the developed bioprocess for MAB production was found to be extremely robust with excellent purification performance (up to 4 LRV of viruses,

Table 5
Summary of polishing applications of membrane chromatography.

Membrane material	Ligand	Adsorption capacity	Recovery	Membrane geometry	Reference
Chitosan	Innate amino groups	29,304 EU/mL	99% recovery of IgGs 96% endotoxin clearance	Flat sheets	Machado et al. (2006)
PES (Mustang)	S	22 mg/mL MAB at 5% DBC	>90% recovery of MABs 70–80% clearance of HCP	Flat sheets	Liu et al. (2011)
PES (Mustang)	Q	n/d	<1 LVR DNA >4 LVR endotoxin		Woo et al. (2011)
PES (Mustang)	Q		0 LRV clearance of phage ΦX174 and PR772 at 50–150 mM salt >6 LRV clearance of phage ΦX174 and PR772 at 0 mM salt >4.2 LRV for DNA	Flat sheets	Riordan et al. (2009b)
PES (Mustang)	Q	<0.6 u_{eq}/cm^2 minute viruses of mouse (MVM) SBC	>3.25 LRV of MVM at 14.4 mS/cm 2.7 LRV of MVM at 21.4 mS/cm with 5 g/L of MAB	Flat sheets	Weaver et al. (2013a,b)
Polyethylene (QyuSpeed)	DEA	57 mg/mL BSA DBC ~30 mg/mL DNA DBC >5 LRV for parvovirus (PPV)	90% clearance of HCP in simulated CHO supernatant	Hollow fiber	Shirataki et al. (2011)
Polypropylene (Chromasorb)	PAA	67–73 mg/mL BSA DBC	>4.5 LVR DNA >7 LVR endotoxin >3.5 LVR MVM from clarified 324 K cell lysate		Woo et al. (2011)
Polypropylene (Chromasorb)	PAA	<6.5 u_{eq}/cm^2 MVM SBC	>4.03 LRV of MVM at 22.0 mS/cm 1.2 LRV of MVM at 22.0 mS/cm with 5 g/L of MAB	Flat sheets	Weaver et al. (2013a,b)
RC (Sartobind)	Q	n/d	>99% clearance of endotoxin >98% recovery of fibroblast growth factor 2 from *E. coli* cell lysate	Flat sheets	Chen et al. (2012)
RC (Sartobind)	Q	n/d	>4.5 LVR DNA >4.5 LVR endotoxin >5 LVR MVM from clarified 324 K cell lysate		Woo et al. (2011)
RC (Sartobind)	Phenyl	17 mg/mL 10% DBC for IgG 28 mg/mL 10% DBC for ovalbumin 31 mg/mL 10% DBC for lysozyme 20 mg/mL 10% DBC for IgG1 in culture supernatant	>90% recovery IgGs 80% reduction in HCP	Flat sheets	Kuczewski et al. (2010)
RC (Sartobind)	Q	<1.5 u_{eq}/cm^2 MVM SBC	>3.47 LRV of MVM at 21.4 mS/cm 0.5 LRV of MVM at 21.4 mS/cm with 5 g/L of MAB	Flat sheets	Weaver et al. (2013a,b)
RC	Agmatine (AGM) TAEA[a] PHMB[a] PEI[a]		>5 LRV of phage ΦX174 and PR772 at 50–150 mM salt 1.3–1.5 LRV of HCP under low salt conditions >4.2 LRV DNA	Flat sheets	Riordan et al. (2009b)
RC/Poly([2-(methacryloyloxy)ethyl] trimethylammonium chloride)	Q	32 mg/mL genomic DNA DBC	>4.5 LRV for Minute Virus of Mice (MVM)	Flat sheets	Bhut et al. (2011b)

[a] Tris-2-aminoethyl amine (TAEA); polyhexamethylene biguanide (PHMB), and polyethyleneimine (PEI).

DNAs, and HCPs), recovery (82–99%), and purity (>99%). Additionally, the capacity for endotoxin removal for membrane adsorbers can be as high as 2.3×10^5 endotoxin unit (EU)/mL. Removal of viral particles is also critical for the manufacturing of therapeutic biologics. Ion-exchange membranes have been employed as an inline pre-filtration method for improving parvovirus filtration during MAB purification by removing charged foulants (Brown et al., 2010). The importance of such pre-filtration on bioprocess efficiency and scalability has been well recognized. A study based on the use of cation-exchange membranes and resins was conducted to compare their purification performance upon MAB production (Liu et al., 2011). While no significant differences were detected between resin and membrane in clearance of HCPs, the membranes were superior in resolving HMW aggregates from monomeric species. Interestingly, DNA was found to bind the negatively charged cation exchange membranes under acidic conditions possibly through other interactions. Hydrophobic interaction membrane chromatography was also useful for the separation of MAB aggregates (Wang and Ghosh, 2008). Note that, in this case, modified hydrophilic PDVF membranes were applied for the separation of MAB aggregates, including monomer, dimer, trimer, tetramer, and pentamer. Additionally, these hydrophilic PDVF membranes were also capable of resolving IgG1 and IgG2 subclasses (Wang and Ghosh, 2010).

5.2. Large biomolecules purification

Purification of large biomolecules has challenged traditional resin chromatography, which is developed primarily for purification of smaller molecules (Hanslip et al., 2006; Peixoto et al., 2008). The

Table 6
Summary of plasmid purification applications using membrane chromatography.

Membrane material	Ligand	Target	Adsorption capacity	Recovery	Membrane geometry	Reference
RC (Sartobind)	D	pDNA in *E. coli* lysate	n/d	70%	Flat sheets	Limonta et al. (2010)
RC (Sartobind)	D	7.6 kb pDNA in *E. coli* lysate	4 mg/mL DBC	n/d	Flat sheets	Syren et al. (2007)
Alumina composite	Diamine	6.0 kb pDNA in *E. coli* lysate	2.0 μg/cm^2 SBC	n/d	Flat sheets	Chang et al. (2008)
Glass fiber composite	Diamine	6.0 kb pDNA in *E. coli* lysate	n/d	>97%	Flat sheets	Chang et al. (2008)
Hydrogel (Natrix)	Q	6.4 kb pDNA in *E. coli* lysate	>12.4 mg/mL SBC	60%	Flat sheets	Zhong et al. (2011a, 2011b)
PES (Mustang)	Q	6.0 kb pDNA in *E. coli* lysate	n/d	>70%	Flat sheets	Guerrero-German et al. (2009, 2011b)
RC (Sartobind)	Phenyl borate	6.0 kb pDNA in *E. coli* lysate	n/d	n/d	Flat sheets	Pereira et al. (2012)
RC (Sartobind)	BUDGE[a]	6.0 kb pDNA in *E. coli* lysate	32.5 mg/mL SBC	73%	Flat sheets	Pereira et al. (2010, 2012)

[a] 1,4-Butanediol diglycidyl ether (BUDGE).

size of micropores within the interior of most resin beads is inadequately small for mass transfer of large biomolecules, such as nucleic acids and viruses (>0.2 μm) (Diogo et al., 2005), resulting in low binding capacity and poor purification performance. Generally, plasmid DNA (pDNA) represents the major target product for nucleic acid purification with membrane chromatography though there are also reports on the recovery of genomic DNA (Bhut et al., 2011a,b). Accordingly, membrane adsorbers with minimum exclusion have been developed for purification of large biomolecules (Riordan et al., 2010; Tatarova et al., 2009; Wang et al., 2009; Wickramasinghe et al., 2006). Unlike other chromatographic applications, recovery of the intact and bioactive form of large biomolecules is necessary since the bioactivity and proper molecular structure of these products can be critical for their applications. Since shear stress during downstream processing can potentially disrupt the complex structure of large biomolecules, operation facilities and processing conditions that generate the least shear stress should be adopted for optimizing their recovery (Levy et al., 2000; Morenweiser, 2005). Super-sized porosity associated with most membrane adsorbers (Teeters et al., 2004; Zhong et al., 2011a) has promoted gentle handling of pDNA and viruses for more effective recovery of these molecules in a bioactive form (Morenweiser, 2005).

Table 7
Summary of virus purification applications of membrane chromatography.

Membrane material	Ligand	Targets	Adsorption capacity	Recovery	Membrane geometry	Reference
RC (Sartobind)	D	Recombinant baculovirus	n/d	28–59%	Flat sheets	Vicente et al. (2011)
RC (Sartobind)	D	Recombinant baculovirus	n/d	65%	Flat sheets	Vicente et al. (2009)
RC (Sartobind)	Q	Recombinant baculovirus	2.28×10^{15} pfu/m^3 DBC	>100%	Flat sheets	Grein et al. (2012)
PES (Mustang)	Q	Recombinant baculovirus	1.81×10^{15} pfu/m^3 DBC	89%	Flat sheets	Grein et al. (2012)
Polypropylene (Chromasorb)	Q	Recombinant baculovirus	2.31×10^{11} pfu/m^3 DBC	<0.01%	Flat sheets	Grein et al. (2012)
RC (Sartobind)	S	Recombinant baculovirus	n/d	20%	Flat sheets	Wu et al. (2007)
PES (Mustang)	S	Recombinant baculovirus	n/d	78%	Flat sheets	Wu et al. (2007)
RC (Sartobind)	IDA-Zn^{2+}	Influenza virus A/ Puerto Rico/8/34	1152 kHAU/75 cm^2 DBC	64–75%	Flat sheets	Opitz et al. (2009a)
RC (Sartobind)	Q	Influenza A virus	5.2 kHAU/cm^2 DBC	86%	Flat sheets	Kalbfuss et al. (2007)
RC (Sartobind)	D	Influenza A virus	3.1 kHAU/cm^2 DBC	38%	Flat sheets	Kalbfuss et al. (2007)
RC (Sartobind Epoxy)	EEL Lectin[a]	Influenza Virus A/ Puerto Rico/8/34	9 kHAU/cm^2 DBC	93.7%	Flat sheets	Opitz et al. (2007)
RC	S	Influenza Virus A/ Puerto Rico/8/34	18 kHAU/cm^2 DBC = 14 μg/cm^2 of HA	81.6%	Flat sheets	Opitz et al. (2009b)
RC (Sartobind)	S	Influenza Virus A/ Puerto Rico/8/34	n/d	75.6%	Flat sheets	Opitz et al. (2009b)
RC (Sartobind)	C	Influenza Virus A/ Puerto Rico/8/34	n/d	63%	Flat sheets	Opitz et al. (2009b)
PES (Mustang)	Q	Adeno-associated virus serotype 1 and serotype 8 vectors	n/d	90%	Flat sheets	Okada et al. (2009)
Sartobind anion Direct	Q	Adenovirus	n/d	62%	Flat sheets	Peixoto et al. (2008)
RC (Sartobind)	IDA-Zn^{2+}	Adenovirus	n/d	87%	Flat sheets	Lee et al. (2009)
RC (Sartobind)	S	Modified Vaccinia Ankara	$TCID_{50}$[a] 1.2×10^8 DBC	65%	Flat sheets	Wolff et al. (2009)
RC (Sartobind)	C	Modified Vaccinia Ankara	$TCID_{50}$ 1.3×10^8 DBC	21%	Flat sheets	Wolff et al. (2009)
RC (Sartobind)	Q	Modified Vaccinia Ankara	$TCID_{50}$ $>9.3\times10^8$ DBC	77%	Flat sheets	Wolff et al. (2009)
RC (Sartobind)	D	Modified Vaccinia Ankara	$TCID_{50}$ $>9.3\times10^8$ DBC	72%	Flat sheets	Wolff et al. (2009)
Cellulose	Sulfonated	Modified Vaccinia Ankara	$TCID_{50}$ $>9.3\times10^8$ DBC	65%	Flat sheets	Wolff et al. (2009)
RC (Sartobind)	Heparin	Modified Vaccinia Ankara	$TCID_{50}$ 2.8×10^8 DBC	56%	Flat sheets	Wolff et al. (2009)
RC (Sartobind)	S	*Aedes aegypti* densonucleosis virus (*Ae*DNV)	95.1 ng/mL DBC = 1.35×10^{10} particles	n/d	Flat sheets	Wickramasinghe et al. (2006)
RC (Sartobind)	Q	*Ae*DNV	1.3 ng/mL DBC = 1.90×10^8 particles	n/d	Flat sheets	Czermak et al. (2008), Wickramasinghe et al. (2006)
RC (Sartobind	D	*Ae*DNV	$>1.36\times10^{10}$ viruses SBC	n/d	Flat sheets	Czermak et al. (2008)
PES (Mustang)	Q	Lentiviral vector	n/d	76%	Flat sheets	Kutner et al. (2009)
RC (Vivapure) LentiSELECT	Q	Lentiviral vector	n/d	43%	Flat Sheets	Zimmermann et al. (2011)
RC (Sartobind)	D	Rotavirus-like particle (RLP)	n/d	55% Capture step 97.6% concentration step	Flat sheets	Vicente et al. (2008)

[a] $TCID_{50}$—50% tissue culture infective dose, EEL—*Euonymus europaeus* lectin.

5.2.1. Plasmid DNA purification

Purification of pDNA takes advantage of the interaction between negatively charged phosphate groups on the DNA backbone and positively charged ligands on chromatographic media (Diogo et al., 2005). Major examples of pDNA purification based on membrane chromatography are summarized in Table 6. Due to the similarity in size and chemical properties between pDNA and other nucleic acid impurities, particularly RNA, complete separation becomes difficult for most chromatographic methods. In a laboratory, this limitation can be circumvented through the use of RNase A, an enzyme for selectively degrading RNA contaminants (Eon-Duval, 2003; Zhong et al., 2011b). However, the approach is not feasible for large-scale production due to high costs and regulatory issues associated with RNase A. Recently, potential application of anion-exchange membrane chromatography towards developing an RNase-free downstream bioprocess for pDNA manufacturing has been demonstrated (Chang et al., 2008; Guerrero-German et al., 2009; Syren et al., 2007; Zhong et al., 2011b). Generally, protein contaminants are removed in the washing stage as most proteins can be eluted under low salt concentrations (below 200 mM NaCl) whereas nucleic acids are eluted under relatively high salt concentrations (above 500 mM) (Zhong et al., 2011b). However, elution of nucleic acids with high salt concentrations does not necessarily improve the separation of RNA from pDNA. In a case study, while the bulk (97%) of RNA was removed during the washing and elution steps under high salt concentrations, additional ammonium sulfate precipitation and hydrophobic interaction chromatographic steps were deemed necessary for such complete separation (Guerrero-German et al., 2011b). Adding a second anion-exchange membrane chromatography step was also explored with limited efficiency for RNA removal (Guerrero-German et al., 2009). Another approach with some success was to explore selective binding of pDNA, but not RNA, by including more salts in the loading step (Syren et al., 2007). Precipitation with salts, alcohols, or detergents prior to anion-exchange membrane chromatography has been proved effective, but often at an expense of pDNA yield. For example, clearance of RNA, protein, and endotoxin impurities from pDNA was effective but with a relatively low pDNA recovery of less than 60% in a process involving salt precipitation, alcohol precipitation, and anion-exchange membrane chromatography (Zhong et al., 2011b). The strong interaction between pDNA and anion-exchange ligands can impact not only pDNA yield but also membrane regeneration (Syren et al., 2007; Zhong et al., 2011a). Several other factors, including membrane pore architecture, pDNA size, and mobile phase conditions, can affect pDNA elution and consequently yield (Zhong et al., 2011a). In particular, the pH and ionic strength of mobile phase can have a significant effect on the hydrodynamic volume of pDNA and membrane. This scenario becomes even more complicated when the ionic strength of mobile phase changes during the elution, resulting in potential entrapment of large biomolecules (Tatarova et al., 2009). With a proper molecular structure of the ligand, weak anion-exchange membranes might have a better recovery of bound pDNA than strong anion-exchange membranes (Syren et al., 2007). Hydrophobic interaction membrane chromatography has also been explored for pDNA purification, but with mixed results (Pereira et al., 2010, 2012). Although good resolution was observed, certain RNA contaminants remained and the overall pDNA yield was low as compared to anion-exchange membrane chromatography.

5.2.2. Virus purification

Virus purification using membrane chromatography has been extensively explored over the past decade. Purified viruses can be used for the production of attenuated vaccines and novel applications in the realm of gene therapy. These biomedical practices have driven a significant increase in the quantity and quality demand for such virus products. The applicative feasibility of membrane chromatography for clinical-grade manufacturing bioprocess has now been well recognized (Opitz et al., 2009a; Vicente et al., 2008, 2009). Existing bioprocesses for virus purification often employ centrifugation or ultrafiltration operations and, therefore, are labor intensive, time consuming, and expensive. Additionally, removal of DNA contaminants requires high amounts of DNase which must be removed subsequently (Wolff et al., 2010). Virus purification using traditional resin chromatography faces many technical limitations similar to pDNA purification, such as limited accessibility of ligands within resins and attachment of multiple ligands by a single macromolecule, leading to low binding capacities (Wickramasinghe et al., 2006). Membrane chromatography has offer an attractive solution to these technical issues and recent applications of it for virus purification are summarized in Table 7. The effect of biomolecule size on the dynamic binding capacity of membrane adsorbers was illustrated using *Aedes aegypti* densonucleosis virus (*Ae*DNV, ~20 nm), thyroglobulin (MW 660 kDa, ~20 nm), bovine serum albumin (BSA, MW 67 kDa), and lysozyme (MW 14 kDa) (Riordan et al., 2010; Wickramasinghe et al., 2006). It was found that large biomolecules primarily bound to the pore entrance whereas small biomolecules like lysozyme were mainly located in the interior of membrane pores. This effect of molecule size on ligand accessibility within membrane pores can be also related with membrane ligand density. In a study using recombinant baculovirus as a target biomolecule, it was found that a high ligand density of membrane adsorbers does not necessarily lead to an increased viral binding capacity (Vicente et al., 2011). In addition, the purity of recovered virus can be improved by appropriately reducing the ligand density to reduce the number of ligands inaccessible to viral particles since these sites, though inaccessible to viral particles, are often accessible to small impurities, such as proteins (Vicente et al., 2011). These results suggest that both membrane chemistry and ligand composition have to be simultaneously considered to optimize the performance of membrane chromatography.

Several technical factors specific to viruses, such as affinity interaction and isoelectric point (pI) of virus surface proteins, can potentially determine the ligand type and capture and separation conditions associated with membrane chromatography. Changes during chromatographic operations that can potentially affect the bioactivity of õrecovered virus particles must be avoided. For example, decreasing the ionic strength or pH of mobile phase resulted in an instantaneous decrease in the infectivity of baculovirus, *Autographa californica* multicapsid nucleopolyhedrovirus (*Ac*MNVP), and such loss in bioactivity was thought to be caused by the structural disruption of virus particles associated with the change in osmotic strength and the aggregation of virus particles at low pH (Grein et al., 2012). Similar effects were observed for lentiviruses (Zimmermann et al., 2011). Chemical compounds used for the elution of virus particles from membrane adsorbers can change mobile phase conditions (e.g. salts increase ionic strength and imidazole causes a pH shift of the mobile phase) and, consequently, affect the bioactivity of recovered viruses (Opitz et al., 2009a). For the production of attenuated viral vaccines, proper preservation of virus structure can be as important as virus purification. On the other hand, for the production of clinical-grade viruses for gene therapy applications, it is of particular importance to ensure complete removal of not only contaminants but also defective virus particles if any. In a two-stage bioprocess for purification of recombinant adenovirus from HEK293 cell lysate, inactive virus particles were removed from the partially purified adenovirus fraction using an extra step for affinity membrane chromatography (Lee et al., 2009). Defective/inactive and bioactive virus particles can have distinctive chemical or physical properties based on which separation can be performed. For example, the isoelectric point of empty virion capsids was found to be significantly higher than infective adenoviruses and the two types of virus particles were resolved through a dual anion/cation exchange step to yield the product fraction containing highly purified infective virus particles (<0.8% empty capsids) (Okada et al., 2009). In addition to the primary capture of virus particles with affinity membrane chromatography, ion-exchange membrane chromatography can be also effective for virus concentration (Kutner et al., 2009; Vicente et al., 2008).

6. Conclusions

Technological advantages and potentials associated with membrane chromatography have been well received these days, resulting in various novel applications in bioprocessing. While low binding capacity continues to be a major limitation, the emergence of membrane adsorbers with an improved binding capacity has significantly widened research scopes and industrial application for membrane chromatography. To address the varying needs of biotech and bioprocessing industry, membrane chromatography will benefit from an increasing variety of membrane adsorber materials, ligands, and available pore sizes. Hence, future developments in membrane chromatography should focus more on membrane support materials, surface chemistry, pore size manipulability, and device design. In addition, developing theoretical models with high accuracy and predictability will improve better understanding of the underlying transport phenomenon to optimize the separation performance for membrane chromatography. With these issues being resolved, membrane chromatography has a potential to outperform traditional resin chromatography in terms of future application in bioindustry.

References

Anspach FB, Petsch D. Membrane adsorbers for selective endotoxin removal from protein solutions. Process Biochem 2000;35:1005–12.

Anuraj N, Bhattacharjee S, Geiger JH, Baker GL, Bruening ML. An all-aqueous route to polymer brush-modified membranes with remarkable permeabilities and protein capture rates. J Membr Sci 2012;389:117–25.

Avramescu ME, Borneman Z, Wessling M. Dynamic behavior of adsorber membranes for protein recovery. Biotechnol Bioeng 2003;84:564–72.

Avramescu ME, Borneman Z, Wessling M. Membrane chromatography. In: Rizvi SSH, Sastre Requena AM, Pabby AK, editors. Handbook of membrane separation: chemical, pharmaceutical, food, and biotechnological applications. CRC Press; 2008.

Barroso T, Temtem M, Hussain A, Aguiar-Ricardo A, Roque ACA. Preparation and characterization of a cellulose affinity membrane for human immunoglobulin G (IgG) purification. J Membr Sci 2010;348:224–30.

Bayramoglu G, Oktem HA, Arica MY. A dye–ligand immobilized poly(2-hydroxyethylmethacrylate) membrane used for adsorption and isolation of immunoglobulin G. Biochem Eng J 2007;34:147–55.

Bhattacharjee S, Bhattacharjee C, Datta S. Studies on the fractionation of β-lactoglobulin from casein whey using ultrafiltration and ion-exchange membrane chromatography. J Membr Sci 2006;275:141–50.

Bhut BV, Husson SM. Dramatic performance improvement of weak anion-exchange membranes for chromatographic bioseparations. J Membr Sci 2009;337:215–23.

Bhut BV, Christensen KA, Husson SM. Membrane chromatography: protein purification from *E. coli* lysate using newly designed and commercial anion-exchange stationary phases. J Chromatogr A 2010;1217:4946–57.

Bhut BV, Weaver J, Carter AR, Wickramasinghe SR, Husson SM. The role of polymer nanolayer architecture on the separation performance of anion-exchange membrane adsorbers: I. Protein separations. Biotechnol Bioeng 2011a;108:2645–53.

Bhut BV, Weaver J, Carter AR, Wickramasinghe SR, Husson SM. The role of polymer nanolayer architecture on the separation performance of anion-exchange membrane adsorbers: Part II. DNA and virus separations. Biotechnol Bioeng 2011b;108:2654–60.

Bhut BV, Conrad KA, Husson SM. Preparation of high-performance membrane adsorbers by surface-initiated AGET ATRP in the presence of dissolved oxygen and low catalyst concentration. J Membr Sci 2012;390–391:43–7.

Boi C. Membrane adsorbers as purification tools for monoclonal antibody purification. J Chromatogr B 2007;848:19–27.

Boi C, Dimartino S, Sarti GC. Modelling and simulation of affinity membrane adsorption. J Chromatogr A 2007;1162:24–33.

Boi C, Dimartino S, Hofer S, Horak J, Williams S, Sarti GC, et al. Influence of different spacer arms on Mimetic Ligand A2P and B14 membranes for human IgG purification. J Chromatogr B 2011;879:1633–40.

Bower SE, Wickramasinghe SR. Elimination of non-uniform, extra-device flow effects in membrane adsorbers. J Membr Sci 2009;330:379–87.

Brown A, Bechtel C, Bill J, Liu H, Liu J, McDonald D, et al. Increasing parvovirus filter throughput of monoclonal antibodies using ion exchange membrane adsorptive pre-filtration. Biotechnol Bioeng 2010;106:627–37.

Chang C, Suen SY. Modification of porous alumina membranes with n-alkanoic acids and their application in protein adsorption. J Membr Sci 2006;275:70–81.

Chang CS, Ni HS, Suen SY, Tseng WC, Chiu HC, Chou CP. Preparation of inorganic–organic anion-exchange membranes and their application in plasmid DNA and RNA separation. J Membr Sci 2008;311:336–48.

Chao A. Preparation of porous chitosan/GPTMS hybrid membrane and its application in affinity sorption for tyrosinase purification with *Agaricus bisporus*. J Membr Sci 2008;311:306–18.

Che A, Huang X, Xu Z. Polyacrylonitrile-based nanofibrous membrane with glycosylated surface for lectin affinity adsorption. J Membr Sci 2011;366:272–7.

Chen YS, Chang CS, Suen SY. Protein adsorption separation using glass fiber membranes modified with short-chain organosilicon derivatives. J Membr Sci 2007;305:125–35.

Chen TX, Nie HL, Li SB, Branford-White C, Su SN, Zhu LM. Comparison: adsorption of papain using immobilized dye ligands on affinity membranes. Colloids Surf B Biointerfaces 2009;72:25–31.

Chen R, John J, Lavrentieva A, Müller S, Tomala M, Zhao Y, et al. Cytokine production using membrane adsorbers: human basic fibroblast growth factor produced by *Escherichia coli*. Eng Life Sci 2012;12:29–38.

Cheng JH, Xiao YC, Wu C, Chung TS. Chemical modification of P84 polyimide as anion-exchange membranes in a free-flow isoelectric focusing system for protein separation. Chem Eng J 2010a;160:340–50.

Cheng Z, Wu C, Yang W, Xu T. Bromomethylated poly(2,6-dimethyl-1,4-phenylene oxide) (BPPO)-based amphoteric hollow fiber membranes: preparation and lysozyme adsorption. Ind Eng Chem Res 2010b;2010:8741–8.

Chiu HC, Lin CW, Suen SY. Isolation of lysozyme from hen egg albumen using glass fiber-based cation-exchange membranes. J Membr Sci 2007;290:259–66.

Cramer SM, Holstein MA. Downstream bioprocessing: recent advances and future promise. Curr Opin Chem Eng 2011;1:27–37.

Czermak P, Grzenia DL, Wolf A, Carlson JO, Specht R, Han B, et al. Purification of the densonucleosis virus by tangential flow ultrafiltration and by ion exchange membranes. Desalination 2008;224:23–7.

de Aquino LC, de Sousa HR, Miranda EA, Vilela L, Bueno SM. Evaluation of IDA-PEVA hollow fiber membrane metal ion affinity chromatography for purification of a histidine-tagged human proinsulin. J Chromatogr B 2006;834:68–76.

Dimartino S, Boi C, Sarti GC. Influence of protein adsorption kinetics on breakthrough broadening in membrane affinity chromatography. J Chromatogr A 2011a;1218:3966–72.

Dimartino S, Boi C, Sarti GC. A validated model for the simulation of protein purification through affinity membrane chromatography. J Chromatogr A 2011b;1218:1677–90.

Diogo MM, Queiroz JA, Prazeres DMF. Chromatography of plasmid DNA. J Chromatogr A 2005;1069:3-22.

Eon-Duval A. Large-scale manufacturing of plasmid DNA for gene therapy and DNA vaccination part 2: toward an RNase-free downstream process—a review. Biopharm Int-Appl Technol Biopharm Dev 2003;16:26-+.

Feng Z, Shao Z, Yao J, Chen X. Protein adsorption and separation on amphoteric chitosan/carboxymethylcellulose membranes. J Biomed Mater Res A 2008;86:694–700.

Feng Z, Shao Z, Yao J, Huang Y, Chen X. Protein adsorption and separation with chitosan-based amphoteric membranes. Polymer 2009;50:1257–63.

Francis P, von Lieres E, Haynes CA. Zonal rate model for stacked membrane chromatography. I: characterizing solute dispersion under flow-through conditions. J Chromatogr A 2011;1218:5071–8.

Francis P, von Lieres E, Haynes C. Zonal rate model for stacked membrane chromatography part II: characterizing ion-exchange membrane chromatography under protein retention conditions. Biotechnol Bioeng 2012;109:615–29.

Frerick C, Kreis P, Gorak A, Tappe A, Melzner D. Simulation of a human serum albumin downstream process incorporating ion-exchange membrane adsorbers. Chem Eng Process 2008;47:1128–38.

Gagnon P. Technology trends in antibody purification. J Chromatogr A 2012;1221:57–70.

Ghosh R. Protein separation using membrane chromatography: opportunities and challenges. J Chromatogr A 2002;952:13–27.

Ghosh R, Wong T. Effect of module design on the efficiency of membrane chromatographic separation processes. J Membr Sci 2006;281:532–40.

Giovannoni L, Ventani M, Gottschalk U. Antibody purification using membrane adsorbers. Biopharm Int 2008:21.

Goodall S, Grandison AS, Jauregi PJ, Price J. Selective separation of the major whey proteins using ion exchange membranes. J Dairy Sci 2008;91:1-10.

Gottschalk U. Bioseparation in antibody manufacturing: the good, the bad and the ugly. Biotechnol Prog 2008;24:496–503.

Gottschalk U. Disposables in downstream processing. Adv Biochem Eng Biotechnol 2010;115:171–83.

Grein TA, Michalsky R, Lopez MV, Czermak P. Purification of a recombinant baculovirus of *Autographa californica* M nucleopolyhedrovirus by ion-exchange membrane chromatography. J Virol Methods 2012. http://dx.doi.org/10.1016/j.jviromet.2012.03.031.

Guerrero-German P, Prazeres DMF, Guzman R, Montesinos-Cisneros RM, Tejeda-Mansir A. Purification of plasmid DNA using tangential flow filtration and tandem anion-exchange membrane chromatography. Bioprocess Biosyst Eng 2009;32:615–23.

Guerrero-German P, Montesinos-Cisneros RM, Guzman R, Tejeda-Mansir A. Modelling and simulation of plasmid DNA adsorption on ion-exchange membrane columns. Can J Chem Eng 2011a;89:536–44.

Guerrero-German P, Montesinos-Cisneros RM, Prazeres DMF, Tejeda-Mansir A. Purification of plasmid DNA from *Escherichia coli* ferments using anion-exchange membrane chromatography and hydrophobic chromatography. Biotechnol Appl Biochem 2011b;58:68–74.

Gutierrez R, Martin del Valle EM, Galan MA. Immobilized metal-ion affinity chromatography: status and trends. Sep Purif Rev 2007;36:71-111.

Hagiwara K, Yonedu S, Saito K, Shiraishi T, Sugo T, Tojyo T, et al. High-performance purification of gelsolin from plasma using anion-exchange porous hollow-fiber membrane. J Chromatogr B 2005;821:153–8.

Hanslip SJ, Zaccai NR, Middelberg APJ, Falconer RJ. Assembly of human papillomavirus type-16 virus-like particles: multifactorial study of assembly and competing aggregation. Biotechnol Prog 2006;22:554–60.

Harkensee D, Kokpinar O, Walter J, Kasper C, Beutel S, Reif OW, et al. Fast screening for the purification of proteins using membrane adsorber technology. Eng Life Sci 2007;7:388–94.

He D, Ulbricht M. Preparation and characterization of porous anion-exchange membrane adsorbers with high protein-binding capacity. J Membr Sci 2008;315:155–63.
Hu MX, Wan LS, Xu ZK. Multilayer adsorption of lectins on glycosylated microporous polypropylene membranes. J Membr Sci 2009;335:111–7.
Jain P, Sun L, Dai J, Baker GL, Bruening ML. High-capacity purification of his-tagged proteins by affinity membranes containing functionalized polymer brushes. Biomacromolecules 2007;8:3102–7.
Kalbfuss B, Wolff M, Geisler L, Tappe A, Wickramasinghe R, Thom V, et al. Direct capture of influenza A virus from cell culture supernatant with Sartobind anion-exchange membrane adsorbers. J Membr Sci 2007;299:251–60.
Ke YM, Chen CI, Kao PM, Chen HB, Huang HC, Yao CJ, et al. Preparation of the immobilized metal affinity membrane with high amount of metal ions and protein adsorption efficiencies. Process Biochem 2010;45:500–6.
Knudsen HL, Fahrner RL, Xu Y, Norling LA, Blank GS. Membrane ion-exchange chromatography for process-scale antibody purification. J Chromatogr A 2001;907:145–54.
Ko YM, Chen CI, Chang HC, Chen HM, Shieh CJ, Syu YJ, et al. Exploring the complex effects of metal ions on d-hydantoinase purification with an immobilized metal affinity membrane. J Taiwan Inst Chem Eng 2011;42:735–40.
Kokpinar O, Harkensee D, Kasper C, Scheper T, Zeidler R, Reif O, et al. Innovative modular membrane adsorber system for high-throughput downstream screening for protein purification. Biotechnol Prog 2006;22:1215–9.
Kreuss M, Krause I, Kulozik U. Separation of a glycosylated and non-glycosylated fraction of caseinomacropeptide using different anion-exchange stationary phases. J Chromatogr A 2008;1208:126–32.
Kuczewski M, Fraud N, Faber R, Zarbis-Papastoitsis G. Development of a polishing step using a hydrophobic interaction membrane adsorber with a PER.C6-derived recombinant antibody. Biotechnol Bioeng 2010;105:296–305.
Kutner RH, Puthli S, Marino MP, Reiser J. Simplified production and concentration of HIV-1-based lentiviral vectors using HYPERFlask vessels and anion exchange membrane chromatography. BMC Biotechnol 2009;9:10.
Labanda J, Sabate J, Llorens J. Modeling of the dynamic adsorption of an anionic dye through ion-exchange membrane adsorber. J Membr Sci 2009;340:234–40.
Labanda J, Sabate J, Llorens J. Experimental and modeling study of the adsorption of single and binary dye solutions with an ion-exchange membrane adsorber. Chem Eng J 2011;166:536–43.
Lajmi AR, Nochumson S, Berges A. Impact of antibody aggregation on a flowthrough anion-exchange membrane process. Biotechnol Prog 2010;26:1654–61.
Lee DS, Kim BM, Seol DW. Improved purification of recombinant adenoviral vector by metal affinity membrane chromatography. Biochem Biophys Res Commun 2009;378:640–4.
Levy MS, O'Kennedy RD, Ayazi-Shamlou P, Dunnill P. Biochemical engineering approaches to the challenges of producing pure plasmid DNA. Trends Biotechnol 2000;18:296–305.
Li Y, Chung TS. Exploration of highly sulfonated polyethersulfone (SPES) as a membrane material with the aid of dual-layer hollow fiber fabrication technology for protein separation. J Membr Sci 2008;309:45–55.
Li Y, Chung TS, Chan SY. High-affinity sulfonated materials with transition metal counterions for enhanced protein separation in dual-layer hollow fiber membrane chromatography. J Chromatogr A 2008;1187:285–8.
Limonta M, Marquez G, Pupo M, Ruiz O. The purification of plasmid DNA for clinical trials using membrane chromatography. Biopharm Int 2010:23.
Liu C, Bai R. Preparing highly porous chitosan/cellulose acetate blend hollow fibers as adsorptive membranes: effect of polymer concentrations and coagulant compositions. J Membr Sci 2006;279:336–46.
Liu H, Fried JR. Breakthrough of lysozyme through an affinity membrane of cellulose-Cibacron Blue. AIChE J 1994;40:40–9.
Liu G, Dotzauer DM, Bruening ML. Ion-exchange membranes prepared using layer-by-layer polyelectrolyte deposition. J Membr Sci 2010;354:198–205.
Liu HF, McCooey B, Duarte T, Myers DE, Hudson T, Amanullah A, et al. Exploration of overloaded cation exchange chromatography for monoclonal antibody purification. J Chromatogr A 2011;1218:6943–52.
Ma Z, Ramakrishna S. Electrospun regenerated cellulose nanofiber affinity membrane functionalized with protein A/G for IgG purification. J Membr Sci 2008;319:23–8.
Ma Z, Kotaki M, Ramakrishna S. Surface modified nonwoven polysulphone (PSU) fiber mesh by electrospinning: a novel affinity membrane. J Membr Sci 2006a;272:179–87.
Ma Z, Masaya K, Ramakrishna S. Immobilization of Cibacron Blue F3GA on electrospun polysulphone ultra-fine fiber surfaces towards developing an affinity membrane for albumin adsorption. J Membr Sci 2006b;282:237–44.
Ma Z, Lan Z, Matsuura T, Ramakrishna S. Electrospun polyethersulfone affinity membrane: membrane preparation and performance evaluation. J Chromatogr B 2009;877:3686–94.
Machado RL, de Arruda EJ, Santana CC, Bueno SMA. Evaluation of a chitosan membrane for removal of endotoxin from human IgG solutions. Process Biochem 2006;41:2252–7.
Machado RL, Figueredo A, Carneiro DGP, Castilho LR, Medronho RA. CFD-aided design of hollow fibre modules for integrated mammalian cell retention and product purification. In: Smith R, editor. Cell technology for cell products. Springer; 2007. p. 757–60.
Mah KZ, Ghosh R. Paper-based composite lyotropic salt-responsive membranes for chromatographic separation of proteins. J Membr Sci 2010;360:149–54.
Mellado MCM, Curbelo D, Nobrega R, Castilho LR. Erythropoietin purification employing affinity membrane adsorbers. In: Smith R, editor. Cell technology for cell products. Springer; 2007.
Meng T, Xie R, Chen YC, Cheng CJ, Li PF, Ju XJ, et al. A thermo-responsive affinity membrane with nano-structured pores and grafted poly(N-isopropylacrylamide) surface layer for hydrophobic adsorption. J Membr Sci 2010;349:258–67.
Menkhaus T, Roseland J. Recovery of proteins from corn and soybean extracts by membrane adsorption. Biotechnol Prog 2008;24:1075–84.
Menkhaus TJ, Varadaraju H, Zhang L, Schneiderman S, Bjustrom S, Liu L, et al. Electrospun nanofiber membranes surface functionalized with 3-dimensional nanolayers as an innovative adsorption medium with ultra-high capacity and throughput. Chem Commun (Camb) 2010;46:3720–2.
Morenweiser R. Downstream processing of viral vectors and vaccines. Gene Ther 2005:S103–10.
Nie HL, Zhu LM. Adsorption of papain with Cibacron Blue F3GA carrying chitosan-coated nylon affinity membranes. Int J Biol Macromol 2007;40:261–7.
Nie HL, Chen TX, Zhu LM. Adsorption of papain on dye affinity membranes: isotherm, kinetic, and thermodynamic analysis. Sep Purif Technol 2007;57:121–5.
Nova CJM, Paolucci-Jeanjean D, Belleville M, Barboiu M, Rivallin M, Rios G. Elaboration, characterization and study of a new hybrid chitosan/ceramic membrane for affinity membrane chromatography. J Membr Sci 2008;321:81–9.
Okada T, Nonaka-Sarukawa M, Uchibori R, Kinoshita K, Hayashita-Kinoh H, Nitahara-Kasahara Y, et al. Scalable purification of adeno-associated virus serotype 1 (AAV1) and AAV8 vectors, using dual ion-exchange adsorptive membranes. Hum Gene Ther 2009;20:1013–21.
Opitz L, Lehmann S, Zimmermann A, Reichl U, Wolff MW. Impact of adsorbents selection on capture efficiency of cell culture derived human influenza viruses. J Biotechnol 2007;131:309–17.
Opitz L, Hohlweg J, Reichl U, Wolff MW. Purification of cell culture-derived influenza virus A/Puerto Rico/8/34 by membrane-based immobilized metal affinity chromatography. J Virol Methods 2009a;161:312–6.
Opitz L, Lehmann S, Reichl U, Wolff MW. Sulfated membrane adsorbers for economic pseudo-affinity capture of influenza virus particles. Biotechnol Bioeng 2009b;103:1144–54.
Orr V, Scharer J, Moo-Young M, Honeyman CH, Fenner D, Crossley L, et al. Simultaneous clarification of *Escherichia coli* culture and purification of extracellularly produced penicillin G acylase using tangential flow filtration and anion-exchange membrane chromatography (TFF-AEMC). J Chromatogr B 2012;900:71–8.
Peixoto C, Ferreira TB, Sousa MFQ, Carrondo MJT, Alves PM. Towards purification of adenoviral vectors based on membrane technology. Biotechnol Prog 2008;24:1290–6.
Pereira LR, Prazeres DM, Mateus M. Hydrophobic interaction membrane chromatography for plasmid DNA purification: design and optimization. J Sep Sci 2010;33:1175–84.
Pereira LR, Carapeto AP, Botelho do Rego AM, Mateus M. Grafting hydrophobic and affinity interaction ligands on membrane adsorbers: a close-up "view" by X-ray photoelectron spectroscopy. Sep Purif Technol 2012;93:75–82.
Plate K, Beutel S, Buchholz H, Demmer W, Fischer-Fruhholz S, Reif O, et al. Isolation of bovine lactoferrin, lactoperoxidase and enzymatically prepared lactoferricin from proteolytic digestion of bovine lactoferrin using adsorptive membrane chromatography. J Chromatogr A 2006;1117:81–6.
Ribeiro MB, Vijayalakshmi M, Todorova-Balvay D, Bueno SM. Effect of IDA and TREN chelating agents and buffer systems on the purification of human IgG with immobilized nickel affinity membranes. J Chromatogr B 2008;861:64–73.
Riordan W, Heilmann S, Brorson K, Seshadri K, He Y, Etzel M. Design of salt-tolerant membrane adsorbers for viral clearance. Biotechnol Bioeng 2009a;103:920–9.
Riordan WT, Heilmann SM, Brorson K, Seshadri K, Etzel MR. Salt tolerant membrane adsorbers for robust impurity clearance. Biotechnol Prog 2009b;25:1695–702.
Riordan W, Brorson K, Lute S, Etzel M. Examination of the adsorption of large biological molecules to anion exchange surfaces using surface plasmon resonance. Sep Sci Technol 2010;45:1-10.
Schneiderman S, Zhang L, Fong H, Menkhaus TJ. Surface-functionalized electrospun carbon nanofiber mats as an innovative type of protein adsorption/purification medium with high capacity and high throughput. J Chromatogr A 2011;1218:8989–95.
Serafica GC, Pimbley J, Belfort G. Protein fractionation using fast flow immobilized metal chelate affinity membranes. Biotechnol Bioeng 1993;43:21–36.
Serpa G, Augusto EF, Tamashiro WM, Ribeiro MB, Miranda EA, Bueno SM. Evaluation of immobilized metal membrane affinity chromatography for purification of an immunoglobulin G1 monoclonal antibody. J Chromatogr B 2005;816:259–68.
Shi W, Shen Y, Ge D, Xue M, Cao H, Huang S, et al. Functionalized anodic aluminum oxide (AAO) membranes for affinity protein separation. J Membr Sci 2008;325:801–8.
Shi W, Cao H, Song C, Jiang H, Wang J, Jiang S, et al. Poly(pyrrole-3-carboxylic acid)-alumina composite membrane for affinity adsorption of bilirubin. J Membr Sci 2010a;353:151–8.
Shi W, Shen Y, Jiang H, Song C, Ma Y, Mu J, et al. Lysine-attached anodic aluminum oxide (AAO)–silica affinity membrane for bilirubin removal. J Membr Sci 2010b;349:333–40.
Shirataki H, Sudoh C, Eshima T, Yokoyama Y, Okuyama K. Evaluation of an anion-exchange hollow-fiber membrane adsorber containing gamma-ray grafted glycidyl methacrylate chains. J Chromatogr A 2011;1218:2381–8.
Singh N, Wang J, Ulbricht M, Wickramasinghe SR, Husson SM. Surface-initiated atom transfer radical polymerization: a new method for preparation of polymeric membrane adsorbers. J Membr Sci 2008;309:64–72.
Sousa A, Sousa F, Queiroz JA. Advances in chromatographic supports for pharmaceutical-grade plasmid DNA purification. J Sep Sci 2012;35:3046–58.
Suck K, Walter J, Menzel F, Tappe A, Kasper C, Naumann C, et al. Fast and efficient protein purification using membrane adsorber systems. J Biotechnol 2006;121:361–7.
Suen SY, Etzel MR. A mathematical analysis of affinity membrane bioseparations. Chem Eng Sci 1992;47:1355–64.
Suen SY, Caracotsios M, Eztel MR. Sorption kinetics and axial diffusion in binary-solute affinity membrane bioseparations. Chem Eng Sci 1993;48:1801–12.

Suen SY, Liu YC, Chang CS. Exploiting immobilized metal affinity membranes for the isolation or purification of therapeutically relevant species. J Chromatogr B 2003;797:305–19.
Sun H, Ge B, Liu S, Chen H. Preparation of nitrocellulose (NC) immuno-affinity membrane for purification of rAPC antibody. J Sep Sci 2008;31:1201–6.
Sun X, Yu D, Ghosh R. Study of hydrophobic interaction based binding of immunoglobulin G on synthetic membranes. J Membr Sci 2009;344:165–71.
Syren P-O, Rozkov A, Schmidt SR, Stromberg P. Milligram scale parallel purification of plasmid DNA using anion-exchange membrane capsules and a multi-channel peristaltic pump. J Chromatogr B 2007;856:68–74.
Tan LC, Chua AJ, Goh LS, Pua SM, Cheong YK, Ng ML. Rapid purification of recombinant dengue and West Nile virus envelope Domain III proteins by metal affinity membrane chromatography. Protein Expr Purif 2010;74:129–37.
Tatarova I, Faber R, Denoyel R, Polakovic M. Characterization of pore structure of a strong anion-exchange membrane adsorbent under different buffer and salt concentration conditions. J Chromatogr A 2009;1216:941–7.
Teeters MA, Root TW, Lightfoot EN. Adsorption and desorption behavior of plasmid DNA on ion-exchange membranes: effect of salt valence and compaction agents. J Chromatogr A 2004;1036:73–8.
Thommes J, Etzel MR. Alternatives to chromatographic separations. Biotechnol Prog 2007;23:42–5.
van Beijeren P, Kreis P, Hoffmann A, Mutter M, Sommerfeld S, Backer W, et al. Computer-aided process design of affinity membrane adsorbers: a case study on antibodies capturing. Chem Pap 2008;62:458–63.
van Beijeren P, Kreis P, Zeiner T. Ion exchange membrane adsorption of bovine serum albumin—impact of operating and buffer conditions on breakthrough curves. J Membr Sci 2012;415–416:568–76.
van Reis R, Zydney A. Bioprocess membrane technology. J Membr Sci 2007;297:16–50.
Varadaraju H, Schneiderman S, Zhang L, Fong H, Menkhaus TJ. Process and economic evaluation for monoclonal antibody purification using a membrane-only process. Biotechnol Prog 2011;27:1297–305.
Ventura AM, Lahore HMF, Smolko EE, Grasselli M. High-speed protein purification by adsorptive cation-exchange hollow-fiber cartridges. J Membr Sci 2008;321:350–5.
Vicente T, Sousa MFQ, Peixoto C, Mota JPB, Alves PM, Carrondo MJT. Anion-exchange membrane chromatography for purification of rotavirus-like particles. J Membr Sci 2008;311:270–83.
Vicente T, Peixoto C, Carrondo MJT, Alves PM. Purification of recombinant baculoviruses for gene therapy using membrane processes. Gene Ther 2009;16:766–75.
Vicente T, Faber R, Alves PM, Carrondo MJ, Mota JP. Impact of ligand density on the optimization of ion-exchange membrane chromatography for viral vector purification. Biotechnol Bioeng 2011;108:1347–59.
Voswinkel L, Kulozik U. Fractionation of whey proteins by means of membrane adsorption chromatography. Procedia Food Sci 2011;1:900–7.
Walter J, Tappe A, Kasper C, Zeidler R, Reif O, Scheper T. Human growth hormone (HGH) purification from CHO-cell culture supernatant utilizing macroporous chromatographic media. In: Smith R, editor. Cell Technology for Cell Products. Springer; 2007. p. 635–41.
Wang L, Ghosh R. Fractionation of monoclonal antibody aggregates using membrane chromatography. J Membr Sci 2008;318:311–6.
Wang J, Ghosh R. Feasibility study for the fractionation of the major human immunoglobulin G subclasses using hydrophobic interaction membrane chromatography. Anal Chem 2010;82:452–5.
Wang L, Kanani DM, Ghosh R. Purification of humanized monoclonal antibodies by membrane-based hybrid bioseparation technique. J Immunol Methods 2006;314:1–8.
Wang J, Faber R, Ulbricht M. Influence of pore structure and architecture of photografted functional layers on separation performance of cellulose-based macroporous membrane adsorbers. J Chromatogr A 2009;1216:6490–501.
Wang J, Shi W, Jiang H, Wu G, Ruan C, Ge D. Heparin-doped affinity electromembranes for thrombin purification. J Membr Sci 2011;373:89–97.
Weaver J, Husson SM, Murphy L, Wickramasinghe SR. Anion exchange membrane adsorbers for flow-through polishing steps: Part I. Clearance of minute virus of mice. Biotechnol Bioeng 2013a;110:491–9.
Weaver J, Husson SM, Murphy L, Wickramasinghe SR. Anion exchange membrane adsorbers for flow-through polishing steps: Part II. Virus, host cell protein, DNA clearance and antibody recovery. Biotechnol Bioeng 2013b;110:500–10.
Wickramasinghe SR, Carlson JO, Teske C, Hubbuch J, Ulbricht M. Characterizing solute binding to macroporous ion exchange membrane adsorbers using confocal laser scanning microscopy. J Membr Sci 2006;281:609–18.
Wolff M, Siewert C, Lehmann S, Hansen SP, Djurup R, Faber R, et al. Capturing of cell culture-derived modified vaccinia ankara virus by ion exchange and pseudo affinity membrane adsorbers. Biotechnol Bioeng 2009;105:761–9.
Wolff MW, Siewert C, Lehmann S, Hansen SP, Djurup R, Faber R, et al. Capturing of cell culture-derived modified Vaccinia Ankara virus by ion exchange and pseudo-affinity membrane adsorbers. Biotechnol Bioeng 2010;105:761–9.
Wolman FJ, Grasselli M, Cascone O. Rapid neutral protease purification by dye-affinity membrane chromatography. Process Biochem 2006;41:356–61.
Wolman FJ, Maglio DG, Grasselli M, Cascone O. One-step lactoferrin purification from bovine whey and colostrum by affinity membrane chromatography. J Membr Sci 2007;288:132–8.
Woo M, Khan NZ, Royce J, Mehta U, Gagnon B, Ramaswamy S, et al. A novel primary amine-based anion exchange membrane adsorber. J Chromatogr A 2011;1218:5386–92.
Wu C, Soh KY, Wang S. Ion-exchange membrane chromatography method for rapid and efficient purification of recombinant baculovirus and baculovirus gp64 protein. Hum Gene Ther 2007;18:665–72.
Yong JK, Xiao Y, Chung TS. The facile synthesis of an aldehyde-containing graft copolymer membrane for covalent protein capture with retention of protein functionality. J Chromatogr A 2010;1217:1904–11.
Yu D, McLean M, Hall J, Ghosh R. Purification of monoclonal antibody from tobacco extract using membrane-based bioseparation techniques. J Membr Sci 2008a;323:159–66.
Yu D, McLean MD, Hall JC, Ghosh R. Purification of a human immunoglobulin G1 monoclonal antibody from transgenic tobacco using membrane chromatographic processes. J Chromatogr A 2008b;1187:128–37.
Yusof AHM, Ulbricht M. Polypropylene-based membrane adsorbers via photo-initiated graft copolymerization: optimizing separation performance by preparation conditions. J Membr Sci 2008;311:294–305.
Zhang L, Menkhaus T, Fong H. Fabrication and bioseparation studies of adsorptive membranes/felts made from electrospun cellulose acetate nanofibers. J Membr Sci 2008;319:176–84.
Zhang H, Nie H, Yu D, Wu C, Zhang Y, Branford-White CJ, et al. Surface modification of electrospun polyacrylonitrile nanofiber towards developing an affinity membrane for bromelain adsorption. Desalination 2010;256:141–7.
Zheng Y, Liu H, Gurgel PV, Carbonell RG. Polypropylene nonwoven fabrics with conformal grafting of poly(glycidyl methacrylate) for bioseparations. J Membr Sci 2010;364:362–71.
Zhong LY, Scharer J, Moo-Young M, Fenner D, Crossley L, Honeyman CH, et al. Potential application of hydrogel-based strong anion-exchange membrane for plasmid DNA purification. J Chromatogr B 2011a;879:564–72.
Zhong LY, Srirangan K, Scharer J, Moo-Young M, Fenner D, Crossley L, et al. Developing an RNase-free bioprocess to produce pharmaceutical-grade plasmid DNA using selective precipitation and membrane chromatography. Sep Purif Technol 2011b;83:121–9.
Zhou JX, Tressel T. Basic concepts in Q membrane chromatography for large scale antibody production. Biotechnol Prog 2006;22:341–9.
Zhou JX, Tressel T, Gottschalk U, Solamo F, Pastor A, Dermawan S, et al. New Q membrane scale-down model for process-scale antibody purification. J Chromatogr A 2006;1134:66–73.
Zhu J, Yang J, Sun G. Cibacron Blue F3GA functionalized poly(vinyl alcohol-co-ethylene) (PVA-co-PE) nanofibrous membranes as high efficient affinity adsorption materials. J Membr Sci 2011;385–386:269–76.
Zimmermann K, Scheibe O, Kocourek A, Muelich J, Jurkiewicz E, Pfeifer A. Highly efficient concentration of lenti- and retroviral vector preparations by membrane adsorbers and ultrafiltration. BMC Biotechnol 2011;11:55.
Zou H, Luo Q, Zhou D. Affinity membrane chromatography for the analysis and purification of proteins. J Biochem Biophys Methods 2001;49:199–240.