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Review

Protein separation using membrane chromatography: opportunities and challenges

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Abstract

Some of the problems associated with packed bed chromatography can be overcome by using synthetic macroporous and microporous membranes as chromatographic media. This paper reviews the current state of development in the area of membrane chromatographic separation of proteins. The transport phenomenon of membrane chromatography is briefly discussed and work done in this area is reviewed. The various separation chemistries which have been utilised for protein separation, along with different applications, are also reviewed. The technical challenges facing membrane chromatography are highlighted and the scope for future work is discussed.

Keywords: Reviews; Membrane chromatography; Stationary phases, LC; Proteins

Contents

1. Introduction

Chromatography is by far the most widely used technique for high-resolution separation and analysis of proteins. These processes are traditionally carried out using packed beds, which have several major limitations. The pressure drop across a packed bed is generally high and tends to increase during a process due to the combined effects of bed consolidation

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(caused by media deformation), and column blinding caused by accumulated colloidal material. Another major limitation with conventional chromatographic bioseparation processes, particularly those employing soft chromatographic media, is the dependence on intra-particle diffusion for the transport of solute molecules to their binding sites within the pores of such media (see Fig. 1). This increases the process time since transport of macromolecules by diffusion is slow, and particularly so when it is hindered. Consequently, the recovery liquid volume (needed for elution) also increases. Channelling, i.e. the formation of flow passages due to cracking of the packed bed, is a major problem. This results in short-circuiting of material flow, leading to poor bed utilisation. Other problems include radial and axial dispersion limitations arising from the use of conventional polydisperse media. Some of these factors and the fact that the transport phenomenon is complicated make scale-up of packed bed chromatographic processes difficult.

Some of the limitations of packed bed chromatog-

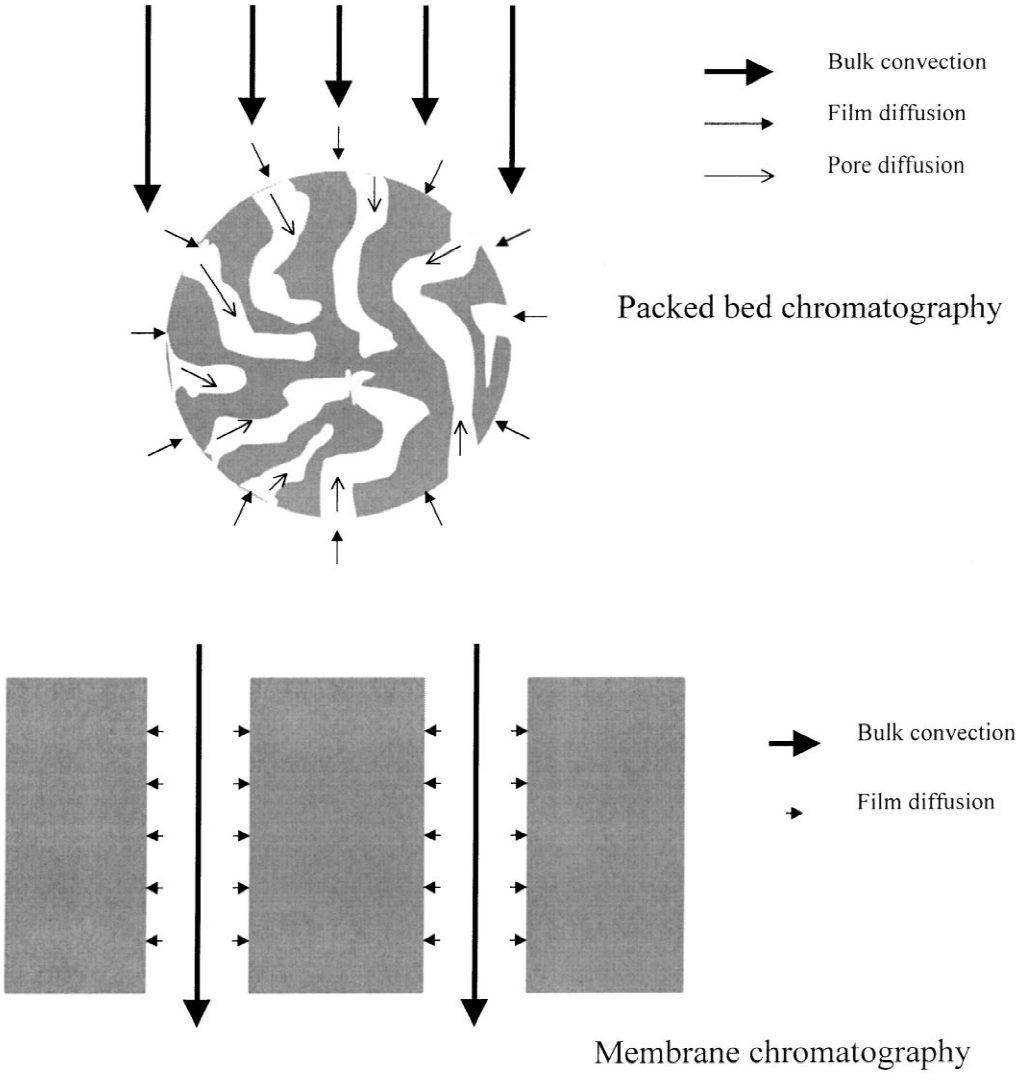

Fig. 1. Solute transport in packed bed chromatography and membrane chromatography.

raphy have been overcome by using newly developed monodisperse, non-porous, rigid chromatographic media (e.g. Refs. [1,2]). However, these media are generally expensive and the solute binding capacity is greatly reduced since binding can now only take place on the external surfaces. Also with these materials, the problem of high-pressure drop still persists.

A radically different approach to overcome the limitations associated with packed beds is to use synthetic microporous or macroporous membranes as chromatographic media (e.g. Refs. [3–6]). In membrane chromatographic processes the transport of solutes to their binding sites takes place predominantly by convection (see Fig. 1), thereby reducing both process time and recovery liquid volume. The binding efficiency is generally independent of the feed flow-rate over a wide range and therefore very high flow-rates may be used. The pressure drop is also significantly lower than with packed beds. Another major advantage of membrane adsorbers is the relative ease of scale-up when compared with packed beds. However, this potential has not been fully utilised as yet in the bioprocess industry. Membrane chromatography is particularly suitable for larger proteins (i.e. $M_r > 250\,000$). Such proteins rarely enter pores present in particulate chromatographic media and only bind on the externally available surface area of such media. Therefore, for larger proteins, the surface area available for binding is significantly greater with membranes. The binding capacity of membrane adsorbers for smaller proteins is generally lower than with conventional gel-based media, but significantly higher than with monodisperse, non-porous, rigid media.

Membrane chromatographic devices are generally easier and cheaper to mass-produce. This makes it possible to have disposable membrane adsorbers. These devices can be used until the desirable properties (i.e. hydraulic permeability, binding capacity, selectivity and resolving power) are maintained. Once they cease to function properly these devices can be replaced. This type of flexibility eliminates the requirement for cleaning and equipment revalidation.

Different separation chemistries are utilised in membrane chromatography of proteins. Some membranes already in use for other types of membrane processes (e.g. microfiltration) have been found to be suitable as chromatographic media. However, in most cases these available membranes have been modified to make them more suitable for use as membrane adsorbers. Novel synthetic membranes have also been developed. Another alternative to packed bed chromatography, which has certain similarities with membrane chromatography, is based on the use of monolith columns. These columns are prepared using rod-shaped porous structures through which convective flow of mobile phase can take place. The main advantages of monolith columns are similar to those for membrane chromatography. However, monoliths differ from membranes in terms of material of construction and morphology. While a membrane by definition is a barrier in which the lateral dimension far exceeds the longitudinal dimension, the converse is probably true with monoliths. Monoliths are perhaps more similar to packed bed chromatographic columns than to membranes.

In this review article, the current state of development in the area of membrane chromatography is discussed. Published literature in the area of membrane chromatography of proteins is reviewed (e.g. Refs. [3–109]). Potential limitations of membrane chromatography are also highlighted. The wider acceptance of this technology depends largely on finding solutions to these limitations.

2. Transport phenomena of membrane chromatography

The advantage of membrane chromatography lies in the predominance of convective material transport. However, as evident from Fig. 1, diffusive transport is not totally absent. The predominance of convection alone does not necessarily guarantee efficiency. Convective flow of inappropriate type can be a serious disadvantage. Flow distribution is a major concern in chromatographic and indeed most types of separation processes. Rational design of the membrane chromatographic process and equipment is possible only when the transport phenomena involved are properly understood. However, it may be worth mentioning that, in many chromatographic processes, particularly those relying on affinity-type interactions, the binding kinetics may be limiting. In

such processes, improvement in transport phenomena is not likely to result in significant improvement in process efficiency.

Generally speaking, three types of membrane adsorbers are used for protein bioseparation: flat sheet, hollow fibre and radial flow. Single flat sheets are rarely used. More often, stacks of several flat sheets are housed within membrane modules. In addition to providing more adsorbent volume, the use of membrane stacks has certain other benefits which are discussed below. A hollow fibre membrane has a tubular geometry with the tubes typically ranging from 0.25 to 2.5 mm in diameter. A hollow fibre membrane adsorber usually consists of a bundle of several hundred fibres potted together within a module in a shell and tube heat-exchanger-type configuration. Radial flow adsorbers are prepared by spirally winding a flat sheet membrane over a porous cylindrical core. Fig. 2 summarises the relative reported usage of the three major types of membrane adsorbers (based on the papers reviewed in this article). Flat sheet membranes are by far most widely used. Hollow fibres, even though advantageous in other types of membrane based technologies (e.g. microfiltration, ultrafiltration, and dialysis), are perhaps not so well suited for membrane chromatography. The reasons for this are explained in the next paragraph. Most of the reports on the use of hollow fibres are from research groups actively engaged in development of hollow fibre membranes for different uses. The use of radial flow devices is also not that widely reported in the published literature even though several adsorbers of this type are available on the market. Table 1 lists some of the commercially available membrane adsorbers. The fact that there are relatively few manufacturers of membrane adsorbers indicates the *newness* of the technology.

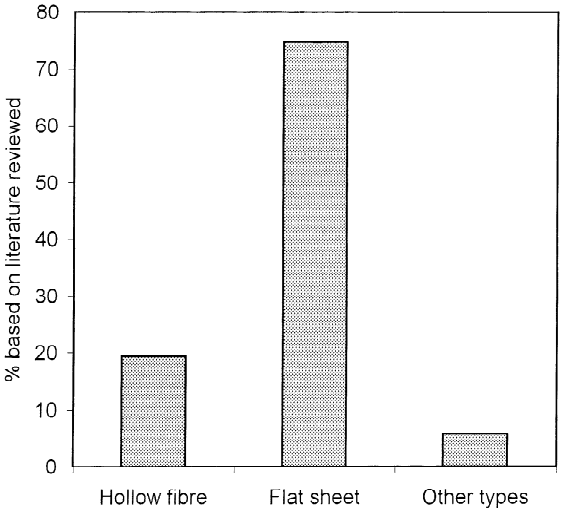

Fig. 2. Membrane adsorber types (geometry).

In flat sheet membrane adsorbers, the liquid is usually introduced normal to the membrane surface (see Fig. 3). In hollow fibre membranes the liquid initially flows parallel to the membrane surface (see Fig. 3). The liquid is then gradually directed towards and through the pores due to the hydrostatic pressure difference. The main advantage of using a hollow fibre configuration is the high membrane surface area to volume ratio it provides. Another advantage of using hollow fibres is the reduction in accumulation of particles near the pore entrance due to cross-flow. Observation of the liquid flow patterns in hollow fibres suggests that this type of adsorber cannot be used for pulse chromatography which relies on sample injection in the form of a pulse, the duration of which is insignificant when compared with the overall processing time. Even in the bind and elute mode, the breakthrough is expected to be broadened, leading to poor adsorber utilization. The liquid flow pattern in a radial flow device is shown in Fig. 3.

Radial flow adsorbers are claimed to be suitable for large-scale applications. However, flow distribution in these devices is expected to be quite challenging. The membrane area also increases in a radially outward direction. This is bound to introduce complexities resulting from the drop in superficial velocity of the liquid stream during its flow through the membrane. The radial flow adsorber is clearly not suitable for pulse chromatography. It is likely to be more suitable for use in the bind and elute mode. However, the binding and elution processes might be difficult to model and predict. In spite of such perceived disadvantages, radial flow adsorbers are popular with users in the industry. Several successful products have been commercialised by companies such as Sartorius and Pall. In designing these products, considerable effort has been put into enhancing flow distribution. Certain researchers are of the opinion that the advantages gained by stacking of

Table 1
Commercially available membrane adsorbers

Product name	Membrane material/type	Configuration	Manufacturer
Sartobind MA5, MA15 and MA100	Reinforced stabilised cellulose, strong cation exchange (S type), strong anion exchange (Q type), weak cation exchange (C type), weak anion exchange (D type)	Flat sheet, ready to use adsorbers	Sartorius
Sartobind MA120, MA550, MA600X5 and MA5500X10	Reinforced stabilised cellulose, strong cation exchange (S type), strong anion exchange (Q type), weak cation exchange (C type), weak anion exchange (D type)	Flat sheet, discs	Sartorius
Sartobind Factor-two family	Reinforced stabilised cellulose, strong cation exchange (S type), strong anion exchange (Q type), weak cation exchange (C type), weak anion exchange (D type)	Radial flow cartridge	Sartorius
Sartobind C5F, C15X and C100X	Reinforced stabilised cellulose, weak cation exchange	Flat sheet	Sartorius
Vivapure	Strong and weak, anion and cation exchange	Flat sheet, centrifugal	Vivascience
Mustang Q	Hydrophilic polyethersulfone, anion exchange	Radial flow capsule and cartridge	Pall

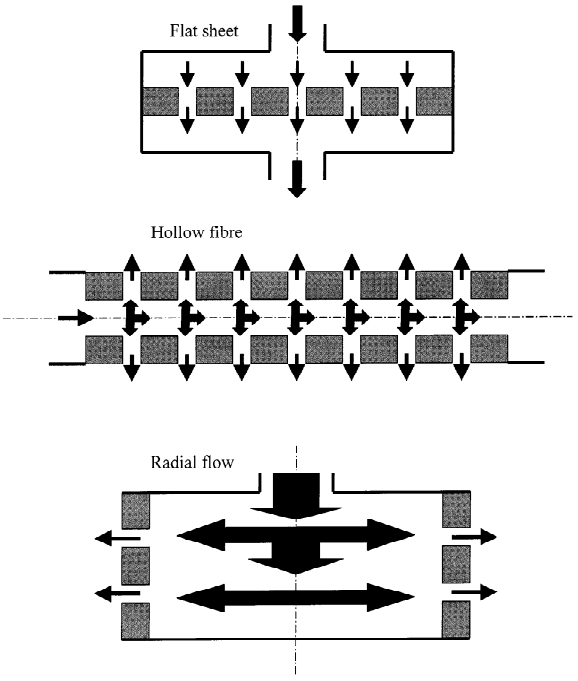

Fig. 3. Flow in membrane adsorbers.

membranes in radial flow devices far outweigh the disadvantage resulting from poor flow distribution.

Once the feed enters the membrane it flows through the pores, which are generally assumed to be aligned normal with respect to the membrane surface. The overall flow of the liquid through the membrane takes place in a normal direction. However, due to the tortuous nature of the pores in most microporous membranes, the localised flow is not necessarily always so. The liquid flow regime is usually laminar. The axial transport of the protein molecules within cylindrical pores is largely convective while the radial transport is largely diffusive. Axial transport can potentially be influenced by Taylor dispersion. However, this effect is expected to be small.

Several researchers have investigated the transport phenomena of membrane chromatography (e.g. Refs. [5,21,48,49,55,58,82,108,109]). Most of these papers are based on flat sheet membranes as the model system. One of the earliest papers dealing with transport phenomena in membrane chromatography

was by Briefs and Kula [5]. A mathematical formulation for an idealised membrane adsorber based on stacks of flat sheets was presented and solved to predict breakthrough and elution profiles. The trends predicted by the mathematical formulation were experimentally verified by dynamic adsorption and elution studies using the enzymes formate dehydrogenase and pyruvate decarboxylase. Suen and Etzel [48] presented a mathematical model, which took into consideration convection, diffusion and a Langmuir-type adsorption isotherm. This model was also based on a flat sheet-type membrane adsorber. In this paper it was predicted that, with thin membranes, the upper limit for the flow-rate could be restricted by the ligand–protein association kinetics. The use of stacks of several thin membranes was recommended to overcome this limitation. The limitations brought about by variation in membrane porosity and thickness were also highlighted. The mass transport implications of this mathematical model were explored using experimental studies (involving monoclonal antibodies) in a subsequent paper [49].

Tennikova and Svec [21] examined the mass transport phenomena of membrane chromatography (also flat sheet) primarily based on operating parameters. The observed effects of mass transport influencing operating parameters (e.g. superficial velocity, pore diameter, membrane thickness, protein diffusivity) on process efficiency were discussed. They reported that the process efficiency of the systems examined by them were not limited by flow-rate. This was thought to be due to the enhancement of diffusive protein transport due to increase in flow velocity (i.e. increase in mass transfer coefficient). The diffusivity of the proteins within the pores was reported to be nearly four orders of magnitude higher than their respective free solution diffusivities.

Liu and Fried [82] discussed a model system based on the adsorption of lysozyme on a flat sheet affinity membrane system. The mass transfer implications were discussed in significant detail. The importance of radial and axial diffusion and the effects of pore size distribution and variation of membrane thickness were considered. Increasing the pore size distribution and variation in membrane thickness were found to significantly broaden the breakthrough curve. It was suggested that using stacks of large numbers of membranes to ''average out'' the flow dispersion would increase the sharpness of the breakthrough curves and therefore increase the binding efficiency.

Roper and Lightfoot [109], in their review article, qualitatively discussed the mass transfer phenomena of different types of membrane adsorbers. More recently, Sarfert and Etzel [55], Yang et al. [58] and Tejeda et al. [108] have discussed the implications of mass transfer in the design of membrane adsorbers.

3. Review of separation chemistries

Earlier review articles have tended to concentrate heavily of the separation chemistries utilised in membrane chromatography (e.g. Refs. [17,30,32, 65]). These include ion-exchange (IEX), affinity, hydrophobic interaction (HI) and reversed-phase (RP) based separations (see Fig. 4). The uses of ion-exchange and affinity interactions are more widely reported. There has been significantly less work done on hydrophobic interaction and reversed-phase based membrane chromatography of proteins.

Based on the literature reviewed, affinity separation seems to constitute the single largest segment. This is probably due to the relative ease with which different ligands can be attached onto membranes. The fact that affinity interactions are widely used is clearly evident from the indiscriminate use of the term ''affinity membranes'' by some researchers to denote all types of membranes with binding properties. The ligands used for affinity membrane chromatography can be broadly classified into four types:

1. Immunoaffinity ligands;
2. Protein A or G;
3. Low-molecular-mass ligands;
4. Other ligands.

Table 2 lists the various reported uses of immunoaffinity ligands. Immunoaffinity chromatography depends on utilisation of biospecific antigen–antibody recognition and binding. The use of antibody as the ligand is perhaps more widespread. However, in theory an antigen might equally well be used as ligand to purify antibody specific towards it. The use of both polyclonal and monoclonal antibodies as immunoaffinity ligand has been reported. However, the binding efficiency and selectivity are expected to be significantly higher with monoclonal antibody.

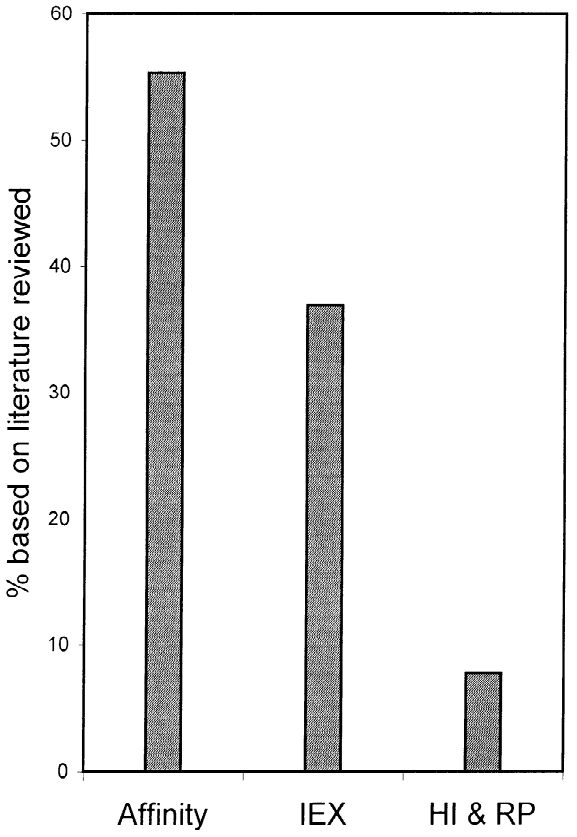

Fig. 4. Relative usage of different separation chemistries in membrane chromatography.

The chemistry involved in attachment of antibody to the membranes largely follows from that developed for immobilisation of enzymes on membranes and other polymeric surfaces. While carrying out these attachment procedures care must be taken to ensure that the bound antibody retains its biospecific antigen-binding capacity.

Protein A and protein G are substances that can specifically bind immunoglobulin G (IgG) via the Fc region of the antibody. This type of recognition and binding is not an example of antigen–antibody binding and is therefore classified as a separate category. Table 3 lists the various reported uses of protein A and protein G in membrane chromatography. As with antibody attachment, the chemistry involved in the attachment of proteins A and G to membranes largely follows from that developed for enzyme immobilisation.

Table 4 lists the various reported uses of low-molecular-mass (LMM) ligands. Synthetic dyes are the most widely used LMM ligands. This is largely a legacy of earlier developments in the field of packed bed affinity chromatography. Other LMM ligands include amino acids, sugars and substrate analogues. Table 5 lists other types of ligands, including peptides, polymeric substances and immobilised metal ions. Different membrane forming materials (e.g. chitin) have intrinsic affinity binding capacity for different proteins.

Table 2
Immunoaffinity ligand based membrane chromatography

Ligand	Membrane	Target protein/s	Adsorber geometry	Ref.
Anti IgE antibody	Regenerated cellulose	IgE	Flat sheet	[77]
Anti BSA antibody	Regenerated cellulose	BSA	Hollow fibre	[26]
Human IgG	GMA–EDMA	Protein G	Flat sheet	[23]
Monoclonal antibody	Hydrazide	Interleukin-2 receptor	Hollow fibre	[73]
Monoclonal antibody	Hydrazide	Interleukin-2	Hollow fibre	[74]
Anti BSA monoclonal antibody	Regenerated cellulose	BSA	Flat sheet	[85]
Anti hSAP antibody	Cellulose	Human serum amyloid P (hSAP)	Flat sheet	[78]
Anti rINF-α2A monoclonal antibody	Hydrazide	Recombinant interferon-α2A (rINF-α2A)	Hollow fibre	[73]
IgG	(GMA–EDMA) co-polymer	Recombinant protein G	Flat sheet	[25]
IgG	Microporous membrane	Human low-density lipoprotein	Flat sheet	[69]
IgG	Microporous membrane	Human low-density lipoprotein	Flat sheet	[57]

Table 3
Protein A and G based affinity membrane chromatography

Ligand	Membrane	Target protein/s	Adsorber geometry	Ref.
Protein A	Hydroxyethyl cellulose treated blend of polyethersulfone and polyethylene oxide	IgG	Hollow fibre	[31]
Protein A/G	Methyl methacrylate based copolymer	IgG	Flat sheet	[34]
Recombinant protein G	Regenerated cellulose	IgG	Flat sheet	[53]
Protein A	Nylon based	Human IgG	Flat sheet	[70]
Protein A	Poly(ether–urethane–urea)	Human IgG	Flat sheet	[86]
Protein A	Composite membrane	Human IgG	Hollow fibre	[3]
Recombinant protein A	Polyethersulfone	Human IgG	Hollow fibre	[27]
Recombinant protein A	Polysulfone	Human IgG	Hollow fibre	[28]
Recombinant protein A	Composite cellulosic membrane	Human IgG	Radial flow	[87]
Recombinant protein A/G	Poly-caprolactam	Human IgG	Flat sheet	[29]
Protein A	Epoxy	Mouse monoclonal antibody (IgG)	Flat sheet	[88]
Protein G	Nylon based	Human IgG	Flat sheet	[103]
Protein A	Poly(vinylidene difluoride)	Human IgG	Flat sheet	[100]
Protein A	Poly(GMA–EDMA)	Human IgG	Flat sheet	[64]
Recombinant protein G	Immobilon AV	Human IgG sub-classes	Flat sheet	[59]

Table 4
Low-molecular-mass ligand based affinity membrane chromatography

Ligand	Membrane	Target protein/s	Adsorber geometry	Ref.
Cibacron Blue F3-GA	Nylon based	BSA	Flat sheet	[5]
Procion Yellow HE-4R	Nylon based	Pyruvate decarboxylase	Flat sheet	[5]
Procion Red HE-3R	Nylon based	Formate dehydrogenase	Flat sheet	[5]
Cibacron Blue 3GA	Cellulose	Lysozyme	Flat sheet	[82]
Maltose	Cellulose	Concanavalin A	Flat sheet	[18]
Phenylalanine	Polyethylene based	IgG	Hollow fibre	[35]
Tryptophan	Polyethylene based	IgG	Hollow fibre	[36]
Histidine	Polyethylenevinyl alcohol	Human IgG	Hollow fibre	[89]
Cibacron Blue F3-GA	Nylon based	Adenylate kinase	Flat sheet	[4]
Cibacron Blue F3-GA	Modified cellulose	Alkaline phosphatase	Flat sheet	[76]
Cibacron Blue F3-GA	Acrylic copolymer	BSA	Tubular	[71]
Cibacron Blue F3-GA	Poly(hydroxyethyl methacrylate)	Bovine catalase	Flat sheet	[90]
Cibacron Blue F3-GA	Sartobind Blue 2	Yeast glucose-6-phosphate dehydrogenase	Flat sheet	[6]
Cibacron Blue F3-GA	Supported chitosan	Human serum albumin	Flat sheet	[11]
p-Aminomethylbenzoyl sulphonamide	Poly(glycidyl methacrylate) based	Carbonic anhydrase	Flat sheet	[61]
Aspartate	Regenerated cellulose	Aspartase	Flat sheet	[105]
p-Aminobenzamidine	Chitosan membrane	Trypsin	Flat sheet	[14]
Cibacron Blue F3-GA	Chitosan membrane	Human serum albumin	Flat sheet	[15]
Cibacron Blue F3-GA	Nylon based	Alanine dehydrogenase	Flat sheet	[102]
Procion Blue MX-R	Modified polyethylene	Creatine phosphokinase	Hollow fibre	[99]

Table 5
Other types of affinity membrane chromatography

Ligand	Membrane	Target protein/s	Adsorber geometry	Ref.
Peptide ligand				
Pentadecapeptide	GMA–EDMA	IgG	Flat sheet	[24]
Hexadecapeptide	GMA–EDMA	IgG	Flat sheet	[24]
Other polymeric ligands				
Chitin based	Macroporous chitin membrane	Lysozyme	Flat sheet	[12]
Collagen	Epoxy	Annexins	Flat sheet	[62]
Trypsin	Modified polysulfone	Soybean trypsin inhibitor	Flat sheet	[75]
Chitin based	Macroporous chitin membrane	Wheat germ agglutinin	Flat sheet	[16]
Soybean trypsin inhibitor	Modified polyethylene	Trypsin	Hollow fibre	[46]
Thiophilic ligand		Monoclonal antibody	Spiral wound	[8]
Immobilised metal ion ligands				
Cu^{2+}	Sartobind IDA membrane	Cytochrome *c*, lysozyme and chymotrypsinogen	Flat sheet	[67]
Cu^{2+}	Glass membrane	Cytochrome *c*, lysozyme, chymotrypsinogen A and ribonuclease A	Tubular	[91]
Cu^{2+}	Nylon based	Lysozyme, ovalbumin and concanavalin A	Flat sheet	[92]

Ion-exchange membranes represent another major segment of media used in membrane chromatography. A large number of membranes used for microfiltration are known to have ion-exchange properties. In many applications this was considered to be a major disadvantage. However, this property proved to be potentially useful for carrying out chromatographic separations. Some of these membranes were modified to enhance their ion-exchange capacity. Different charged groups such as sulfonic acid (S), sulfopropyl (SP), diethylaminoethyl (DEAE) and quaternary ammonium (Q) were introduced to obtain high protein binding membranes. Table 6 lists the various reported uses of ion-exchange membrane chromatography for protein separation. In terms of chromatographic membranes commercially available, ion-exchange membranes constitute the largest segment.

Table 7 lists the various reported uses of reversed-phase and hydrophobic interaction based separation in membrane chromatography. Most available synthetic membranes are incompatible with organic solvents. This probably explains why there are few reports on reversed-phase membrane chromatography. Hydrophobic interaction is known to have several advantages over other separation chemistries, particularly from the point of view of protein stability. It is therefore surprising to note the scarcity of reports on its utilization for membrane chromatographic protein separation. The general approach in hydrophobic interaction membrane chromatography has been to attach hydrophobic ligands (usually hydrocarbon chains or rings) to various membranes. Perhaps the intrinsic property of the various available membranes for carrying out hydrophobic interaction based separations also needs to be investigated [79].

The different types of membranes used for chromatographic separation have been reviewed in earlier review papers (e.g. Refs. [17,30]). The use of cellulose based membranes is by far most widely reported.

4. Review of applications

As is evident from Tables 2–7, membrane chromatography has been used for a wide variety of protein separations. Most of the literature reviewed deals with separation of binary or multi-protein mixtures. There are also several reports dealing with binding and breakthrough studies based on a single protein. Applications may be categorised based on

Table 6
Ion-exchange membrane chromatography

Type	Membrane	Target protein/s	Adsorber geometry	Ref.
Cation exchange	Sartobind S (sulfonic acid type)	Hemoglobin, lysozyme	Radial flow	[81]
Anion exchange	DEA containing GMA–EDMA copolymer	Myoglobin, conalbumin, ovalbumin and soyabean trypsin inhibitor	Flat sheet	[21]
Cation exchange	Sartobind S (sulfonic acid type)	Lysozyme, ovalbumin	Flat sheet	[83]
Anion exchange	Sartobind Q (quaternary ammonium type)	BSA, IgM	Flat sheet	[83]
Cation exchange	Sartobind S (sulfonic acid type)	Plasma proteins	Flat sheet	[9]
Anion exchange	Sartobind Q (quaternary ammonium type)	Plasma proteins	Flat sheet	[9]
Cation exchange	Sartobind S (sulfonic acid type)	Monoclonal antibody (IgG)	Flat sheet	[84]
Anion exchange	Sartobind Q (quaternary ammonium type)	Monoclonal antibody (IgG)	Flat sheet	[84]
Anion exchange	Macroporous chitosan membrane	Cytochrome *c*, lysozyme, ovalbumin, human serum albumin, soybean trypsin inhibitor	Flat sheet	[13]
Cation exchange	S-type cellulosic membrane	BSA	Flat sheet	[55]
Cation exchange	Sartobind S (sulfonic acid type)	Lysozyme, chymotrypsinogen A and soybean trypsin inhibitor	Flat sheet	[66]
Anion exchange	Sartobind Q (quaternary ammonium type)	Lysozyme, chymotrypsinogen A and soybean trypsin inhibitor	Flat sheet	[66]
Anion exchange	Quick Disk Q membrane	Human tumour necrosis factor	Flat sheet	[63]
Cation exchange	SP polyethylene	Lysozyme	Hollow fibre	[37]
Cation exchange	Sartobind S (sulfonic acid type)	Mouse monoclonal antibody (IgG)	Flat sheet	[88]
Cation/anion	Sartobind S and Sartobind Q	Mouse monoclonal antibody (IgG)	Flat sheet	[93]
Anion exchange	DEAE MemSep 100	Ovalbumin and myoglobin	Flat sheet	[94]
Anion exchange	DEAE MemSep	Phosphodiesterase	Flat sheet	[95]
Cation/anion	S and Q cellulose membranes	Whey proteins	Flat sheet	[52]
Cation/anion	S and Q cellulose membranes	Whey proteins	Flat sheet	[54]
Anion exchange	Poly(glycidyl methacrylate) based	Ovalbumin, conalbumin, myoglobin, soybean trypsin inhibitor	Flat sheet	[40]
Cation exchange	Sartobind S (sulfonic acid type)	Lysozyme, BSA	Flat sheet	[10]
Cation/anion	S, DEA and EA presenting membranes	BSA	Hollow fibre	[42]
Cation/anion	S, DEA and EA presenting membranes	BSA	Hollow fibre	[43]
Cation/anion	S and DEA presenting copolymer and cellulose membranes	Milk proteins	Flat sheet	[68]
Cation/anion	DEAE and SP Zetaprep 100	Human albumin	Radial flow	[96]
Cation exchange	SP MemSep 1010	Lactalbumin, BSA	Flat sheet	[50]
Cation/anion	CM and DEAE MemSep 1010	Lysozyme, cytochrome *c*, chymotrypsinogen, lactalbumin, conalbumin and ovalbumin	Flat sheet	[97]
Cation/anion	S and Q cellulose membranes	Lactoglobulin, lysozyme, conalbumin, cytochrome *c* and chymotrypsinogen	Flat sheet	[69]
Anion exchange	QAE-Cellulose/acrylic	β-Galactosidase	Flat sheet	[104]
Cation exchange	SP-Modified polyethylene	Lysozyme	Flat sheet	[38]
Anion exchange	DEAE-poly(GMA–EDMA)	Soybean trypsin inhibitor, myoglobin and conalbumin	Flat sheet	[62]
Anion exchange	Modified cellulose–poly(vinyl chloride) composite	BSA	Flat sheet	[101]
Cation exchange	*S*-Poly(glycidyl methacrylate)	Lysozyme	Hollow fibre	[47]
Cation exchange	CM MemSep 1010	Immunotoxin, monoclonal antibody	Flat sheet	[106]
Anion exchange	DEAE MemSep 1000	Amino terminal domain of formyltetrahydrofolate dehydrogenase	Flat sheet	[107]
Anion exchange	DEAE-polymer	BSA	Hollow fibre	[41]
Cation exchange	Sulfonic acid type membrane	Lactoferrin and lactoperoxidase	Flat sheet	[56]

Table 7
Reversed-phase and hydrophobic interaction membrane chromatography

Membrane/ligand	Target protein/s	Adsorber geometry	Ref.
Styrene–divinylbenzene	Ovalbumin, human serum albumin	Flat sheet	[21]
Phenyl grafted polyethylene	BSA	Hollow fibre	[44]
Dodecyl methacrylate containing GMA–EDMA	Myoglobin, ribonuclease A, lysozyme and chymotrypsinogen A	Flat sheet	[21]
Quick Disk C4	Human tumour necrosis factor	Flat sheet	[63]
Dodecyl methacrylate containing GMA–EDMA	Myoglobin, ribonuclease A, lysozyme and chymotrypsinogen A	Flat sheet	[21]
Hydrophilised poly(vinylidene difluoride)	Mouse monoclonal antibody (IgG)	Flat sheet	[79]
Decanol supporting poly(vinylidene difluoride) membrane	Humanised monoclonal antibody (IgG)	Flat sheet	[80]
Modified polyethylene	BSA	Hollow fibre	[45]

the type of proteins separated. In Fig. 5, proteins separated by membrane chromatography (based on the literature reviewed) have been classified into five broad categories.

Fig. 5 is perhaps not truly representative of the potential applications of membrane chromatography. This is due to the diverse interests of the researchers in this area, who can be broadly divided into three categories: (a) biotechnologists keen to use membrane chromatography for biological mixtures of interest, (b) membrane researchers keen to find applications for new membranes, and (c) process engineers keen to model membrane chromatography. There are certainly those who belong to more than one of these categories. Application based research primarily deals with process development for separation of specific proteins from complex natural mixtures (e.g. purification of monoclonal antibody from cell culture supernatant). The separation of simulated mixtures of proteins, which co-occur naturally (e.g. serum albumin and immunoglobulins), may also be broadly classified as application based research. However, separation of proteins which are not simultaneously present in natural mixtures [e.g. bovine serum albumin (BSA) and lysozyme] probably does not fall into this category. Simulated mixtures that have been separated are based on well-characterised and easily available proteins such as BSA, lysozyme, myoglobin, ovalbumin, conalbumin, cytochrome *c* and chymotrypsinogen. These proteins, in addition to being well characterised, are available in very pure forms and represent a broad range of physicochemical properties (i.e. isoelectric point, molecular mass). These are therefore ideally suited as model proteins, which are primarily used to demonstrate the performance and suitability of new membranes, and for theoretical studies.

In Fig. 6 an attempt has been made to represent the potential application of membrane chromatog-

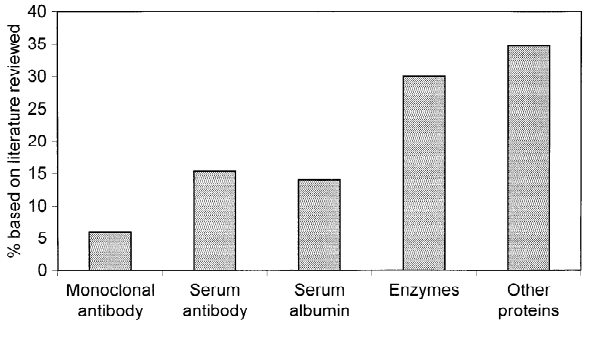

Fig. 5. Proteins separated by membrane chromatography.

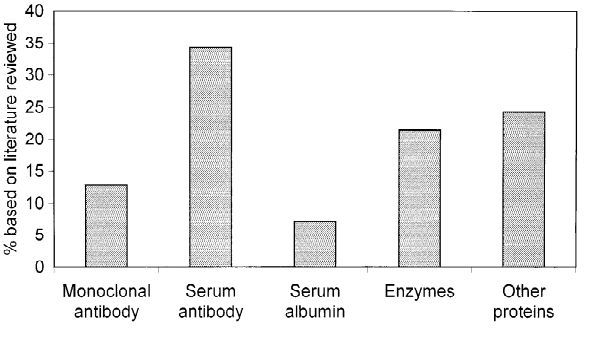

Fig. 6. Application of membrane chromatography for protein separation.

raphy for protein purification. This is based on literature dealing with separation of real protein mixtures or simulated mixtures of naturally co-occurring proteins. Serum antibody represents the single largest application segment. Enzymes, monoclonal antibodies and serum albumin follow in that order. However, it must be noted that the enzyme category consists of dissimilar molecular entities.

Critical analysis of the literature reviewed seems to suggest the suitability of membrane chromatography for purification of proteins which are not the major components of their respective naturally occurring mixtures. For example, antibodies occur at a much lower concentration than albumin in serum or plasma. Similarly, the concentration of monoclonal antibodies in cell culture supernatant is generally much lower than the major contaminating protein, i.e. BSA. This points towards two things: firstly, the suitability of membrane adsorbers for processing large volumes of liquid, and, secondly, the generally lower protein binding capacity. Membrane chromatography is therefore likely to be better suited for a purification process in which a large volume of liquid containing low concentration of target protein is to be processed. When the target protein is the major constituent in the feed, the use of membrane chromatography is likely to be largely restricted to the removal of small amounts of a specific impurity [110].

5. Challenges: the driving force for further research and development

Membrane chromatography has several clear advantages over packed bed chromatography. However, there are some limitations too, which need to be overcome. Some of the major limitations, or shall we say challenges, with membrane chromatography are:

1. inlet flow distribution;
2. membrane pore size distribution;
3. uneven membrane thickness;
4. lower binding capacity.

Flow distribution problems are not unique to membrane adsorbers alone. Indeed, Yuan et al. [111] and Lightfoot et al. [112] have discussed the problems associated with flow distribution in chromatographic processes in general. However, this problem can be particularly acute in membrane chromatography due to the presence of a large frontal area with respect to the bed height. The inlet into a membrane adsorber is generally in the form of a circular channel entering a larger circular cross-section. In a flat sheet membrane adsorber, the feed is usually distributed radially over the entire leading membrane surface using a suitable arrangement. During the introduction of the feed into the membrane adsorber, the solute (i.e. protein) front should ideally hit all points of the leading membrane simultaneously. This is quite difficult to achieve due to a variety of reasons. A certain degree of distortion of the solute front takes place within the tubing itself, due to the developed velocity profile. However, this is a minor contributory factor. Inefficient flow distribution within the adsorber itself further distorts the solute front, thus broadening the shape of the breakthrough curve leading to reduction in efficiency of adsorbent utilisation. Improvement of inlet flow distribution is one area where a significant amount of work needs to be done if membrane chromatography is to be competitive. The use of more membrane sheets in the stacks can also minimise this problem. However, the perceived disadvantages resulting from poor flow distribution are more likely to affect pulse chromatography. With frontal (or capture) chromatography, disadvantages due to poor flow distribution are not likely to be that critical.

The pores present in microporous and macroporous membranes are generally not all of the same diameter. There is usually a pore size distribution, which in most cases is mono-modal. In certain cases there could be a bimodal distribution. The problem of having a wide pore size distribution is that the flow of feed will preferentially take place through the larger ‘‘flow-pores’’ and very little material will be carried through the smaller pores. As a result, the efficiency of adsorbent utilisation will be greatly reduced. Isoporous membranes, which have all pores of nearly the same diameter, are now available. However, the problem with these new types of membranes is that they are expensive to manufacture and generally have very low porosity. Therefore, most of the membranes that are currently used for membrane chromatography are non-isoporous. Suen and Etzel [48] have recommended the use of stacks

of large numbers of sheet membranes to even out the pore size distribution related dispersion effect.

Uneven membrane thickness presents a problem similar to that with large pore size distributions. Flow is encouraged where the thickness is smaller due to the lower pressure drop. However, this problem is not normally associated with most commercially available membranes, which have a remarkably uniform thickness. Suen and Etzel [48] have recommended the use of stacks of large numbers of sheet membranes to even out this membrane unevenness related dispersion effect.

The lower binding capacity of membrane adsorbers can be attributed to lower surface to bed volume ratio as well as to flow distribution problems. The most direct approach to address the former is to develop membranes having a high specific surface area. This is not quite so easy to achieve. Increasing the specific surface area without compromising other membrane properties such as mechanical strength, hydraulic permeability and pore size distribution may prove to be tricky. With low-molecular-mass ligands, low protein binding may sometimes be attributed to steric hindrance. Adding a spacer arm between the ligand and the support can solve this problem. Another method by which membrane binding capacity can be increased is by coating the pores with a porous polymer. This technology has been commercialised by different companies. The objective is to create a three-dimensional coating that would lead to a significant increase in the binding surface.

6. Conclusion and scope for future work

Membrane chromatography has obvious advantages over packed bed chromatography. However, there are major challenges which need to be overcome if all these advantages are to be capitalised. The predominance of convective solute transport is the main reason for the advantages of membrane chromatography. Researchers have highlighted the complexities of the transport phenomena in porous membranes. However, this is considerably less complex when compared with packed beds. Therefore, scale-up is expected to pose less of a challenge. Affinity and ion-exchange based separations are commonly used for membrane chromatography of proteins. Significantly less work has been done on reversed-phase and hydrophobic interaction based separations. Serum antibodies represent the largest applications segment. Other major protein types separated include enzymes, monoclonal antibodies and serum albumins.

Future work on membrane chromatography is likely to be concentrated in the following areas.

6.1. Improved process and equipment design

Work in this area is likely to be focused around solving inlet flow distribution problems. Yuan et al. [111] and Lightfoot et al. [112] have discussed new approaches for investigating and solving these problems.

6.2. Development of new membranes

New membranes having improved binding and operational properties need to be developed. A lot of this development is likely to be targeted at specific applications now that the feasibility of using membrane chromatography for protein purification has already been demonstrated.

6.3. Screening of binding properties of existing membranes

Different microporous membranes used for other types of separation processes could potentially be useful in membrane chromatography. The binding properties of the different membranes available need to be systematically screened. The main attraction of screening commercially available membranes is that these membranes have already been developed to possess high thermal and chemical stability along with good mechanical strength and durability. These properties are also desirable in membrane chromatography. Many of the newly developed membranes lack these properties and turn out to be unsuitable for real applications.

6.4. Proper system selection

The application of membrane chromatography for protein purification is likely to be in very specific niche areas only. Membrane chromatography is

probably better suited for purification processes in which large volumes of liquid containing low concentrations of target protein are to be processed.

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