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Membrane-based techniques for the separation and purification of proteins: An overview

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ABSTRACT

Membrane processes are increasingly reported for various applications in both upstream and downstream technology, such as microfiltration, ultrafiltration, emerging processes as membrane chromatography, high performance tangential flow filtration and electrophoretic membrane contactor. Membrane-based processes are playing critical role in the field of separation/purification of biotechnological products. Membranes became an integral part of biotechnology and improvements in membrane technology are now focused on high resolution of bioproduct. In bioseparation, applications of membrane technologies include protein production/purification, protein–virus separation. This manuscript provides an overview of recent developments and published literature in membrane technology, focusing on special characteristics of the membranes and membrane-based processes that are now used for the production and purification of proteins.

Contents

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1. Introduction

In simpler terms, a membrane can be described as an interphase, usually heterogeneous, acting as a barrier to the flow of molecular and ionic species present in the liquids and/or vapors contacting the two surfaces. During the last three decades, membranes attracted the attention of chemists, chemical and biotechnical engineers due to their unique separation principle, i.e. the selective transport and efficient separation in compare with other unit operations. Separations with membranes do not require additives, and they can be performed isothermally at low temperatures with less energy consumption compared to other thermal separation processes. Due to the unique properties of membranes, upscaling and downscaling of membrane separation processes as well as their integration into other separation or reaction processes are easy. The success attending any membrane-based unit operation, such as demineralization, desalination/purification of water, bioseparation of fermentation products, milk fractionation, decaidification of fruit juices, etc., depends on the availability of the suitable membranes. Recently, membrane-based processes gained importance in biotechnology due to their ability for size and/or charge based protein separation with high purity and throughput [1–6]. Most protein based products needs to be purified before they can be used. They need for purification is due to the following: (i) requirement of high purity; (ii) concentration enrichment; (iii) removal of specific impurities (e.g. toxins from therapeutic products); (iv) prevention of catalysis other than the type desired (as with enzymes); (v) prevention of catalysis poisoning (as with enzymes); (vi) recommended product specifications; (vii) enhancement protein stability; (viii) reduction of protein denaturation. Over the last two decades, new membrane modules and systems have been developed specially to meet the requirements of the biotechnology industries.

Membranes have traditionally been used for size-based separations with high-throughput but relatively low-resolution requirements. These uses include microfiltration (MF), clarification, sterile filtration and ultrafiltration (UF) for protein concentration and buffer exchange. Current research and development efforts are directed toward drastic improvements in selectivity while maintaining the inherent high-throughput characteristics of membranes. Although, essentially all membrane processes are used for bioseparation, but greatest interests have been shown in the pressure-driven technologies such as MF or UF (Fig. 1). Recently, electric or ultrasonic fields were imposed simultaneously to increase throughput and membrane selectivity as well as reducing membrane fouling which is a common phenomenon in pressure-driven membrane separation technologies. During last two decades, membrane technologies were frequently used for the size or charge based protein separation/fractionation. MF membranes were tailored to retain cells and cell debris while allowing proteins and smaller molecules to pass into filtrate. UF membranes were designed to provide high retention of proteins and other macromolecules. These membrane processes involve the filtration of biological solutions containing proteins, peptides, amino acids, salts and other compounds like organic acids, sugars, vitamins, etc. Some examples include concentration of whey proteins during the production of a variety of dairy products, filtration of wine or the purification of downstream solutions in biotechnology [7]. Nanofiltration (NF) was defined as a process that separates solvent, monovalent salts, small organics from divalent ions and larger species. Conventional UF is limited to separation of solutes that differ in 10 fold in size. High-performance tangential flow filtration (HPTFF) is a new technology for protein and peptide purification. HPTFF is a two-dimensional purification method that exploits differences in both size and charge characteristics of protein/biomolecules. Thus this methodology can be used to separate protein with same molecular weight. During recent years, electrophoretic membrane contactor process was also developed for size and charge based protein separations. Membrane chromatography is used as an alternative to conventional resin-based chromatography columns for a large range of chromatographic purification schemes, including ion-exchange, hydrophobic, reversed-phase, and affinity chromatography. Purification/separation procedures using membrane chromatography have been reported for a wide variety of biological compounds such as proteins, DNA and viruses.

The objective of this review is to provide an overview of recent development in membrane science and technologies, focusing on the special characteristics of membranes and processes that are now used in the production and purification of recombinant protein products.

2. Pressure-driven membrane technologies for separation/purification of proteins

Although essentially all membrane processes are used for protein separation/purification, the greatest interest has been in the application of the pressure-driven processes of MF, UF and NF. MF is now established unit operation in environmental engineering, biotechnology, life sciences and medicine as well as several other areas [2,8–12]. MF membranes are especially well suited for the separation of fine particles in the size range of 0.1–10.0 μm. While UF membranes with 1–100 nm pore size were designed to provide high retention of proteins and other macromolecules [4]. The UF process has become particularly important for concentrating proteinaceous solutions. Examples of commercial membrane processes involved the filtration

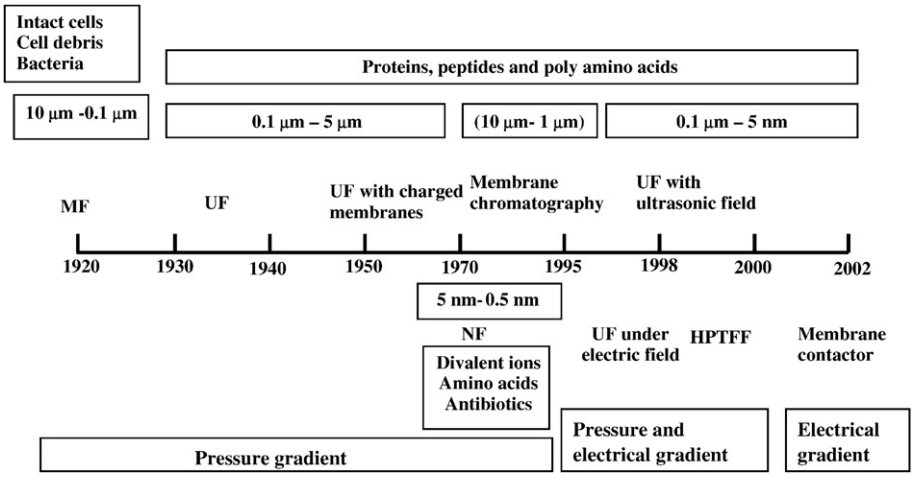

Fig. 1. Milestones in the development of membrane technologies for protein separation/purification.

of protein solutions in the presence of electrolytes, concentration of whey proteins in the dairy industry, protein recovery from blood plasma, protein concentration in downstream processing, etc. [13]. NF is another promising technology that separates solutes based on solute charge and size. Several research papers published on the application of NF for peptide fractionation in model systems of amino acids and peptides, which was based on a molecular sieve effect and/or on a charge effect depending on the membrane type and the feed phase composition [14–16].

2.1. Proteins separation/purification by MF

MF is widely used for the separation, purification and clarifying of protein-containing solutions, e.g. for the recovery of extracellular proteins produced via fermentation and for the removal of bacteria and viruses in the final formulation of therapeutic proteins. In all these processes the macromolecules and proteins involved are much smaller in size than the pores of the MF membrane and should not normally be retained by the membranes [17,18]. The basic operational concept of MF leads to a solute concentration that is higher and close to the membrane surface than it is in the bulk feed stream [19]. This is so-called concentration polarization, which causes due to diffusive flow of solute back to the bulk feed. That is the reason for the process permeates flux falling below the flux of clean fluid through the membrane. After a given period steady state conditions will be achieved. The effect of concentration polarization can be very served in MF applications because the fluxes are high and the mass-transfer coefficients are low as a result of the low diffusion coefficients of macromolecular solutes and of small particulates, colloids and emulsions. Module configuration of MF include hollow-fiber, tubular, flat plate, spiral-wound and rotating devices. The two standard modes of operation are dead-end and cross-flow configurations are shown in Fig. 2. In the cross-flow mode, the fluid to be filtered flows parallel to the membrane surface and permeates through the membrane due to pressure difference. The cross-flow reduces the formation of the filter cake to keep it at a low level.

Much effort is still being devoted to developing new membrane modules with improved mass-transfer characteristics for UF and MF processes. This includes rotating disk filters [20,21] cylindrical Taylor vortex devices [22], conically shaped rotors [23], and helical coiled Dean vortex systems [24,25]. Dean vortex devices have very high mass-transfer rates, owing to the presence of centrifugal flow instabilities. These devices show significant increases in protein transmission and capacity, although fouling remains a problem in many applications. An alternative approach was used for high frequency back pulsing to clean the membrane surface [26]. High frequency back pulsing was shown to improve flux, reduce fouling and increase protein transmission in the purification of conjugated vaccine products [27].

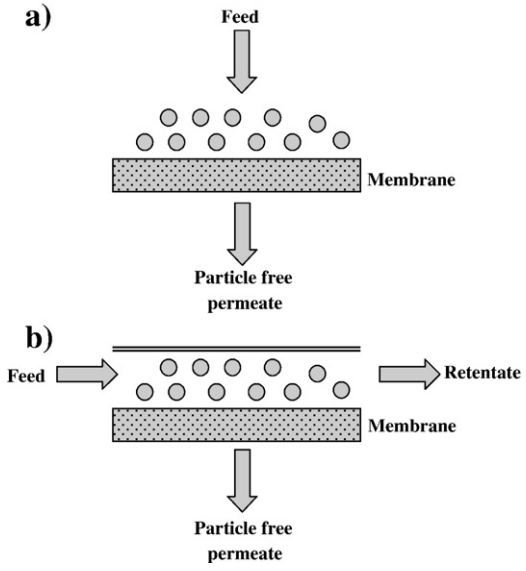

Fig. 2. Comparison between: (a) dead-end, (b) cross-flow configuration.

MF is commonly used to recover macromolecules and retain suspended colloidal particles, and are being integrated into both upstream and downstream processes [8,9]. A large range of MF applications is reported to pretreatment steps, removal of small molecules from bigger protein molecules, clarify suspensions for cell harvesting, and sterilize liquids to remove viruses and bacteria [4,28]. Other membrane processes include membrane bioreactors, where enzymes, microorganisms or antibodies were suspended in solutions and compartmentalized by a membrane in a reaction vessel or immobilized within the membrane matrix itself. The sterile filtration (bacterial removal) was achieved in dead-end configuration using 0.2 μm pore size membranes have been validated for the absolute removal of *Brevundimonas diminuta* [29]. However, such sterilizing filters can pass very small microorganisms under some process conditions. Therefore, some users employ 0.1 μm pore size membranes to provide enhanced sterility assurance in pharmaceutical process [30]. Membrane manufacturers are developing membrane filters with increasing resolution for virus and protein separation [10]. This was of significant importance to the biotechnology industry because incidents of parvovirus are particularly difficult to remove, as they are both small (about 20 nm diameters) and highly resistant to many thermal and chemical methods of inactivation. Continuous improvements in understanding the effects of solution environment on molecules, particles retention, and fouling [31] have also led to further enhancements in UF and MF performances. For example, numbers of recent studies have demonstrated that it is possible to control the rate of protein transport through membranes by adjusting the solution pH or the ionic strength [32–34]. The process has to be operated at the isoelectric point (p*I*) of the transmitted protein and far away from the p*I* of the retained protein. To enhance the separation, the ionic strength was kept low so that the thickness of the diffuse double layer of the charged solute was pronounced, leading to high retention, whereas the uncharged solute readily permeated the membrane.

Polyethersulfone (PES) becomes a kind of "standard" material for many membrane manufacturers, because it is reliably available in well-defined qualities and (almost) unlimited quantities [35,36]. PES based MF membranes were employed for biological applications like selective permeation, protein recognition/purification, controlled release, isolation of soya proteins etc. [37,38]. They have a wide range of applications in the food industry, including processed meat, nutritional beverages, infant formulas, and dairy product replacements. Critical functional properties necessary in protein ingredients include solubility, water and fat absorption, emulsion stabilization, whip ability, gelation, foaming and good organoleptic properties [39,40]. Many physical, chemical and enzymatic modifications have been used to expand the range of functional properties in soy proteins [41–43]. These modifications can be costly in terms of the process itself as well as losing some other properties at the expense of improving the targeted ones. Chemical modification also posed the problem of removing any unreacted reagents from the final product. Soy protein fractionation using chemical methods have been reported [44].

2.1.1. Advanced MF under electric field

Electrically enhanced membrane filtration (EMF) is an advanced technique, which consists in superimposing an electrical field to a conventional membrane filtration unit. In EMF, the electrical field acts as an additional driving force to the transmembrane pressure. Accordingly, differences in protein electrophoretic mobility are

coupled to the membrane sieving effect to enhance the selectivity of membrane fractionation in EMF. It has been mainly used as a strategy to improve protein solutions permeation flux by preventing concentration polarization and membrane fouling [45–48]. Furthermore, selectivity enhancement for biomolecules separation (amino acids and peptides) was recently obtained using EMF [49–52]. However, only few studies reported the effect of EMF on complex protein solutions separation selectivity [53].

The purification and separation of protein has been widely used in electrodialysis, UF and MF when comparing studies on the use of conventional membranes for different protein separation [54,55]. The newly developed techniques superimposed additional forces such as pressured hydraulic force. Recently dynamic filtration has represented a further possibility for reducing the surface layer on the membrane of rotating disc filtration [55,56]. However, the principle disadvantage of this technique is that it cannot be used in high concentrations of protein. Another superimposed force, that is, an electric field, was induced as a force on charged protein in order to reduce the surface layer of a membrane. Park et al. studied the purification of protein through membrane process under the influence of an electric field and explained how filtration time was reduced by the use of an electric field. In this case, concentration of protein in the membrane process in the presence of an electric field was reduced by over 300% in comparison with the membrane process without an electric field. For this investigation, polyvinylidene fluoride (PVDF) membrane and hemoglobin as a protein was used. It was observed that an electric field is a superimposed force which, induced as a force on charged protein to reduce the surface layer formation at the membrane interface and that led the development of EMF [56–59].

Pouliot et al. studied the effect of applying an external electrical field during lactoferrin (LF) and whey protein solutions by MF under influence of electrical field strength (0–3333 V m^{-1}) and polarity on the permeation flux and protein transmission through microfilter membrane with flat-sheet module [60]. In this case, electrical field had an important impact on protein transmission. Selectivity enhancements were obtained, particularly when the cathode was on the retentate side. In that configuration (3333 V m^{-1}), the separation factors obtained between LF and the two main whey proteins β-LG and α-LA were, respectively, 3.0 and 9.1. PVDF membrane with 0.5 μm of pore size diameters was used for this study.

2.2. Protein separation by UF

Protein fractionation is rapidly becoming more selective through improvements in membrane and module design. Compared to chromatographic methods, membrane separation techniques offer advantages of lower cost and ease to scale-up for commercial production. However, the lack of membrane selectivity and its fouling due to protein absorption during filtration has severely restricted UF applications [61]. Now, UF has been widely used as preferred method for protein concentration and buffer exchange, and replaced size-exclusion chromatography in these applications [62].

UF membranes, based on variety of synthetic polymers, have high thermal stability, chemical resistivity, and restricted the use of fairly harsh cleaning chemicals [4,63]. The choice of membrane was usually guided by its molecular weight cut-off (MWCO), which is defined as the equivalent molecular weight of the smallest protein that would exhibit above 90% rejection. Although this choice is arbitrary, but it has been adopted by most of the UF membrane user community. However, the experimental conditions and systems used to evaluate 90% MWCO have not been standardized [63]. Hollow fiber, flat-sheet cassettes, spiral-wound cartridges, tubular modules, and enhanced mass-transfer devices have been developed for UF. These modules provide physical separation of the retentate and filtrate streams, mechanical support for the membrane (if needed), high membrane packing densities (membrane area per device volume), easy access for cleaning and replacement, and good mass-transfer characteristics. Spiral-wound modules are sensitive for plugging/fouling, and are more difficult to clean. However, they have more limited range of scalability than hollow-fiber modules or flat-sheet cassettes. Rotating and Dean Vortex systems have been also developed for UF. These devices showed high mass-transfer coefficients but lower packing densities [64]. Additionally, their scale-up (or down) is difficult due to changes in hydrodynamic conditions [65].

PES is widely used UF membrane material, because of its high rigidity, creep resistance, good thermal and dimensional stabilities [66]. Ghosh et al. studied purification of lysozyme from chicken egg white using hollow-fiber PES UF membrane (30 kDa MWCO) [67]. UF of fermented cheese whey broth was also studied using a lab scale cross-flow membrane system with PES membranes (5, 20 kDa MWCO) [68]. Separation of β-LG from whey protein was achieved by its fractionation using two-stage UF with PES membrane (30 and 10 kDa MWCO) in stirred rotating disk module followed by ion-exchange membrane chromatography [69]. Other types of polymeric UF membranes such as polyacrylonitrile membrane [70,71], regenerated cellulose membrane [72], cellulose acetate membrane [73,74] and ceramic membranes [75] etc., were extensively studied for the separation of proteins. A schematic diagram of UF membrane set-up used for protein separation/purifications is shown in Fig. 3. Fractionation of dairy wastewater into lactose-enriched and protein-enriched streams using UF membrane technique was also studied. Three regenerated cellulose membranes of 3, 5 and 10 kDa MWCO were used to determine the efficiency of the process. The performance was determined under various processing conditions that include the operating temperature and transmembrane pressure across and the concentration of lactose in the feed solution [76].

2.3. Advanced UF techniques for proteins separations

Recently UF technique was modified for developing advanced technique with low membrane fouling, high selectivity and permeate flux in order the meet the industrial requirements for protein separations.

2.3.1. Protein separation using charged UF membranes

Charged UF membrane separation process involved both size and charge based exclusion rather than simply size-based separation of protein molecules, as in the case of UF. A positively or negatively charged UF membrane with definite pore structure and MWCO was generally used for selective protein separation because of high interactions between transporting species and membrane surface with extremely low fouling due to electrostatic repulsion between membrane surface and foulants. pH and ionic strength of the feed solution were adjusted to control the charge on the proteins and attenuate charge shielding from reduction of the double layer. Although protein concentration by UF has become a routine and successful operation in biotechnology, fractionation of proteins using

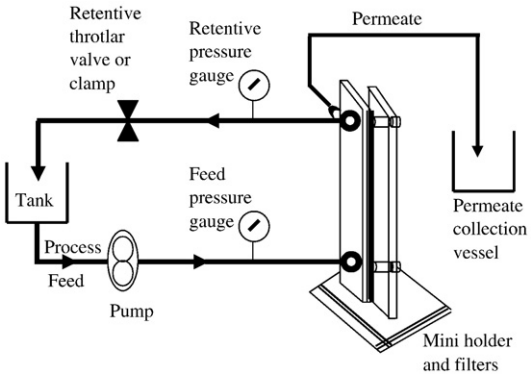

Fig. 3. A schematic diagram for the ultrafiltration membrane set-up [76].

UF is still a technological challenge and its effectiveness and efficiency are strongly dependent on operating parameters such as pH, salt concentration, permeate flux, and system hydrodynamics. Additionally, UF is size-based separation processes and it is difficult to achieve high selectivity with high throughput. Zydney and co-workers have studied electrostatic interactions between charged proteins and charged membranes [77–81] and demonstrated that pH values and ionic strengths have profound effects on protein separation. This is a promising technology for separation of proteins differing in molecular mass by at least a factor of 10, with high resolution by UF with charged membranes [82]. In this case, charged membranes were used for the fractionation of proteins at their isoelectric point (IEP) in binary or ternary solution. Nystrom et al. studied UF with charged membranes and separated enzymes from fermentation broth and myoglobin from BSA [83,84]. High selectivity was achieved for the smaller protein at its IEP. Zydney and co-workers also studied electrostatic interactions between charged proteins and charged membranes [77–81] and demonstrated that pH values and ionic strengths have profound effects on protein separation [80,81,85].

Fractionation of a protein mixture (myoglobin and cytochrom *C*) was also attempted with positively (sulphonated) and negatively charged (aminated with quaternary group) PES UF membranes near to isoelectric pH of one of the proteins. High transmission of the neutral protein and strong electrostatic repulsion of the charged protein with the membrane matrix was observed. This opened up exciting new opportunities for exploiting electrostatic interactions in the optimization of membrane systems for protein purification [86]. Cellulosic UF membranes with positive charges were also used to separate different monoclonal antibody (mAb) therapeutics from Chinese hamster ovary cell protein impurities [87]. Excellent work on developing a theoretical understanding of charged UF membrane separations was carried out by Zydney and collaborators [8,88]. More than 900-fold purification and 94% yield of BSA from an antigen-binding fragment (FAB) of recombinant DNA antibody using a negatively charged membrane to obtain very strong retention of the negatively charged BSA [89].

Suen et al. studied separation of BSA, lysozyme and γ-globulin using plate-and-frame modules with charged UF membranes made of with either cellulose phosphate or diethylaminoethyl cellulose [90]. In this single plate-and-frame module, operating conditions were greatly influenced the separation performance. At the elution stage, a better performance was achieved by using the cross-flow mode. Other operating conditions such as the number of membranes in a stack, the pH value at adsorption, and the mixed mode in the membrane stack also affected the separation performance. Cation-exchange membranes (cellulose phosphate, thickness 0.23 mm) and anion-exchange membranes (diethylaminoethyl cellulose, thickness 0.2 mm) were also found to be suitable for the separation of targeted protein with better recovery [90]. Fractionation of lysozyme and chicken egg white by UF were also studied using commercially available negatively charged membranes made of by regenerated cellulose or PES with 30 kDa MWCO. In optimized conditions, 99% lysozyme transmission with 2400 folds selectivity was obtained [91]. Effect of solution pH on the transmission of ovalbumin, myoglobin, and lysozyme in a rotating (Taylor vortex) module was also studied [92]. Very large reduction in protein transmission at pH<p*I* was attributed to the significant adsorption of the positively charged proteins on the negatively charged polyacrylonitrile (PAN) membrane under optimized conditions [70,71,92].

UF was traditionally performed using polymeric membranes such as polyethersulfone, polysulfone, cellulose acetate, and regenerated cellulose. However, these polymeric membranes are susceptible to chemical degradation by strong alkali or acid cleaning solutions leading to a significant reduction in membrane life. In addition, some polymeric membranes have limited mechanical stability, leading to a reduction in permeability at high pressures and possible membrane failure, particularly in systems employing rapid high-pressure back pulsing. These membranes are also incompatible with steam sterilization, potentially increasing the overall bioburden in subsequent processing steps [93,94]. These limitations have motivated the development of a variety of inorganic UF membranes with greatly enhanced chemical, thermal, and mechanical stability. Zydney et al. [93] synthesized nanoporous carbon UF membrane from a polymeric precursor mixture of poly (ethylene glycol) (PEG) and poly (furfural alcohol) (PFA). Performance characteristics for the nanoporous carbon were only slightly below those of commercial polymeric UF membranes. Additionally these membranes were stable even after prolonged exposure to 3 N NaOH solutions. Using this membrane about 0.62 sieving coefficient for BSA was achieved. UF separation of lysozyme and BSA was also studied using both zirconia membrane and zirconia modified with a positively charged polymer (polyvinyl imidazole) at different ionic strengths. Best selectivity (transmission of lysozyme/transmission of BSA) was obtained at low ionic strength with both membranes [95,96]. Chaufera et al. studied inorganic membranes chemically modified by a polyethyleneimine coating bearing positive charges for fractionation of negatively charged proteins at pH 7, α-LA (target protein) and β-LG (contaminant protein) from whey protein in order to reach a high selectivity of α-LA in the permeate [97]. Variation of retention of a single protein, either positively charged lysozyme or negatively charged BSA in a buffer solution at pH 7 was investigated by varying the ionic strength with unmodified or chemically modified zirconia UF membranes. Chemical modification was obtained by coating polyvinylimidazole, amine group reacted further with bis-epoxiranes in order to have, both, a partly quaternized amine group and a pH-stable network on the membrane surface [96].

2.3.2. UF in the presence of electric field (electro-ultrafiltration)

The use of electric field in UF goes back to the first study carried out by Bechhold by imposing electric field in UF and utilized a combination of electroosmosis and electrophoresis to purify colloids in an apparatus he called an 'electro-ultrafiltration' (EUF) [98]. EUF is an effective method to decrease gel layer formation on the membrane surface and to increase the filtration flux, owing to electrokinetic phenomena such as electrophoresis and electroosmosis [99,100–105]. Basic principle of EUF is presented in Fig. 4. This process aroused from a combination of a number of mechanisms, including ion association, ion adsorption or ion dissolution. The electrochemical properties of the membrane surface and the dispersed materials or solutes can have a significant influence on the nature and magnitude of the interactions

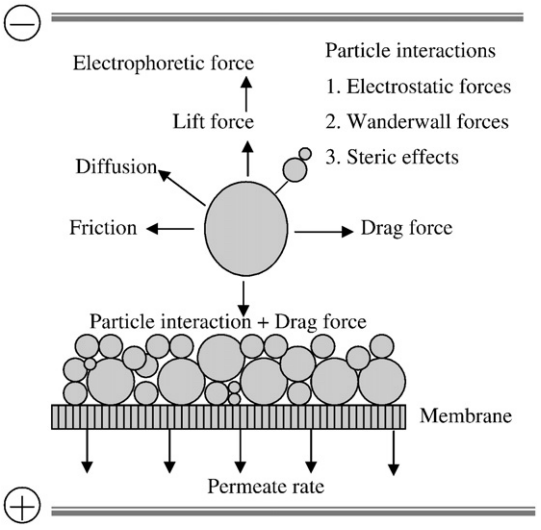

Fig. 4. Principle of electro-ultrafiltration [107].

between the membrane and the substances being used and their separation characteristics. The utilization of such properties by the application of external electric fields improved substantially the membrane performance. The conventional electrofiltration can also be the effective means of reducing both the concentration polarization and membrane deposition but it has some drawbacks which make this method uneconomical and difficult to handle for certain processes [106]. The electric field imposed an electrophoretic effect on the charged molecules dragging them away from the membrane surface. The concentration polarization layer is thereby reduced and the flux increases. The solvent flow through the membrane might also be enhanced by the electroosmotic effect; but this effect is considered secondary [107,108].

Generally, two electrodes were positioned, on either side parallel to the UF membrane, used to apply the electric gradient. The field vector perpendicular to the membrane provokes a displacement of colloid species towards the electrode with the opposite sign. This phenomenon is called electrophoresis. Several reports are available concerning to use EUF for separation or concentration of protenecious solutions [48,53,109–116]. In most cases, the electric field was applied by two parallel electrode plates. The simplest configuration is to insert the electrodes into the suspension and permeate channels. This construction has two major disadvantages: i. alteration of the product pH by electrolysis products; and ii. deposition of particles at the electrodes [107].

When an electric field is applied to the separator, permeate is either concentrated or diluted by differences in the charge of solute and the direction of the electric potential gradient. The accumulation of the solutes on the membrane surface is limited by the imposed electrophoretic force. In addition, the filtration rate through the filter cake is dramatically enhanced due to electroosmosis as a secondary electrokinetic phenomenon. This method is best suited for the separation of protein since its surface charge changes according to the solution pH [117–122]. As biological products like proteins and peptides are sensitive to shear stress and temperature, the coupled effects of electric field and pressure served as an additional driving force for the separation, which is an interesting way to improve the membrane permeates flux with out increasing the shear stress [99,123,124]. Since proteins carry a net electrical charge, an electrical field may be used to reduce the influence of polarized layer. By applying a suitable external dc electric field, the protein molecules were transmitted through the membrane due to the electrostatic attraction and concentration polarization was reduced [45]. EUF was also studied as a membrane cleaning procedure, in which electrical fields attracted the particles with opposite charge from the membrane surface, and initial permeate flux was restored by eliminating fouling up to large extent [125,126]. It was observed that as the voltage increased, the cake layers deposited on the membrane surface became thinner. Eventually when the critical voltage, at which the foulants were stationary, reached no particle deposition on the membrane surface was observed. Applying at the critical voltage has greatly mitigated membrane fouling problems and prolonged the frequency of its cleaning [109].

Bier et al. reported the membrane technique using an electric field to dewater colloidal suspensions [127]. In this case the electric field served as an additional driving force for the separation. Numerous research paper published, in which electrical fields have also been used to control membrane fouling and filter cake formation, enhance the filtration rate and increases the separation efficiency of the UF of proteins by altering the structure of the protein gel layer formed on the UF membrane surface [128–131]. The use of electric fields in the protein UF process has demonstrated that the initial permeate flux can be restored almost instantaneously by means of an exponential curve [132–136]. The permeability of protein was proportional to hydraulic pressure with an electric field, indicating that the protein quickly is oriented in the field direction through the membrane pores [137].

Effects of direct current electric field on the transient and dynamically balanced filtration rate in dead-end inclined and downward UF were explored under constant pressure using protein (BSA) solution. It was found that, in downward EUF, the dynamically balanced filtration rate was directly proportional to the electric field strength. Also, in inclined EUF, filtration rate increased (30–50%) with field strength above the critical electric field strength [45,47,138]. A three-fold flux increase was also reported by Oussedik et al. for BSA filtration by EUF [47]. EUF was used to improve the filtration rate and increase the selectivity while separating, e.g. amino acids and peptides [49]. During EUF the impact of operating parameters on fouling including flux, velocity, transmembrane pressure and electric field were also studied with BSA in the concentration range of 1–5 g/1 and demonstrated a 25–50% increase of the flux permeate compared to the case of without any electric field [139]. Kappler et al. used different UF membranes in a two-sided electro-filter apparatus with flushed electrodes brought significant enhancement of the protein fractionation process. Due to electrophoretic effects, the filtration velocity could be kept on a very high level for a long time, furthermore, the selectivity of a binary separation process carried out exemplarily for BSA and lysozyme could be greatly increased in the current case up to a value of more than 800. Thus, the new two-sided electro-UF technique achieves both high product purity and short separation times [140]. Saxena and Shahi prepared negatively charged UF membranes by different degree of sulfonation of PES [141]. These membranes showed different extent of charge density on the membrane matrix. Adsorption study at various pH of the protein solution was performed and it was observed that protein binding capacity of the membranes was strongly dependent on the pH of the protein solution as well extent of charge on the membrane matrix. Using these membrane transmission characteristics of BSA and LYS were investigated under single or coupled driving forces i.e. pressure difference and applied potential gradient. Schematic diagram of the experimental cell used for EUF has been shown in Fig. 5. It was found that membrane flux and observed protein transmission through the membrane increased under the simultaneous action of driving forces and pH of the protein solution (i.e. their isoelectric points), and extent of the charge on the membrane matrix have strong influence on the observed transmission of the protein. Schematic diagram for the mode of UF experiments were conducted without any applied potential gradient is presented in Fig. 6a. While Fig. 6b represents ultrafiltration experiment with applied potential gradient and electrical polarity. Polarity of the applied potential was fixed so that electrode in the feed side became anode and towards permeate side became cathode so

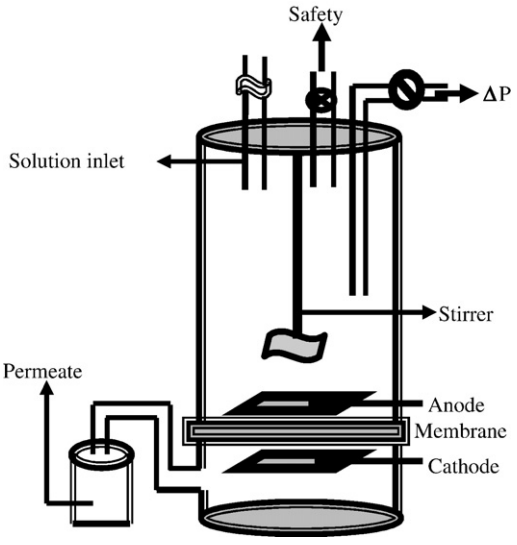

Fig. 5. Schematic diagram for the experimental cell used in EUF [141].

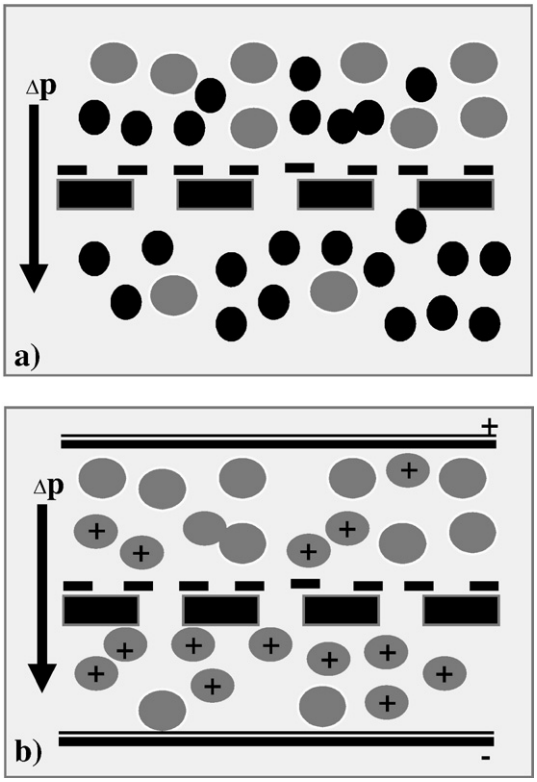

Fig. 6. Schematic presentation of: (a) ultrafiltration; and (b) ultrafiltration under applied potential gradient [141].

that only positively charged BSA (BSA^+) are facilitated to cross through the negatively charged membrane under the influence of coupled driving forces (pressure and potential difference). In other cases negatively charged BSA (BSA^-) or zwitterions BSA (BSA^0) are suppose to be exhibit very low transmission under given polarity and charged nature of the membrane. It was concluded that transmission of protein can be governed by varying nature of the charge on it (pH), nature and extent of the charge on the membrane matrix, polarity of the applied potential gradient with an ultrafilter membrane of given pore dimension. Coupling of the driving forces always results more membrane throughput with relatively high selectivity. Based upon these results, it is possible to achieve selective and efficient transport/separation of BSA-LYS at pH 4.8. Furthermore, this study established the basis for the development of new process and hydrophilic modified ultrafilter membrane for the fractionation of proteins depending on their isoelectric points. In this processes, charge on the protein, nature and extent of the charge on the membrane interfaces, polarity of the potential gradient all are governing the transport of given protein across the membrane, which will result high selectivity and membrane throughput. As spin off charged nature of the membrane matrix and bipolar electrical environment avoids the fouling of the membrane, which was the common phenomenon for the protein fractionation by UF membrane.

The conventional EUF can be effective means of reducing both the concentration polarization and membrane deposition but it has some drawbacks which make this method uneconomical and difficult to handle for certain processes. The disadvantages of this process are: (a) limitation of the process stream for relatively low conductivity of feed stream, (b) a high-energy requirement, (c) substantial heat production, and (d) changes in the process feed due to reaction at the electrode. For this reason, attention has been directed to the use of pulsed electric fields [133,134]. This process has the same mechanism at work in preventing fouling as conventional electrofiltration. The only difference is that in pulsed electric fields, the electric field can be applied at certain intervals, which can be adjusted to suit the process. In some cases, this process can enhance the flux better than the conventional electrofiltration [135]. Applying the electric pulse on dead-end filtration has also been reported to reduce the rate of fouling [142–145]. A pulsed electric field consumes less energy than a constant field, and for some systems a pulsed electric field results in an even higher flux compared to a constant field [47,146]. In order to minimize energy consumption, several researchers have proposed pulsed electric field rather than steady fashion [147,148]. Electrophoretic membrane cleaning was carried using electric pulses with process variables are the strength of the applied voltage, pulse interval and pulse duration. They observed it that the application of the electric pulse in the cleaning membrane surface was an effective means in reducing fouling and restoring high permeation rate. Application of electric pulses across the membrane resulted in an increase in permeation rate due to the removal of deposited materials on the membrane surface. It was identified from the data, the most suitable and effective conditions to give the highest average filtration flux can be attained by applying higher voltage, shorter pulse interval and longer pulse duration [149–151]. Side view of the dead-end EUF module is a shown in Fig. 7. Bowen et al. used electric pulsed to clean the cross-flow filtration; they found out that applying longer pulse duration might have changed the properties of filter cake [144]. Thus longer pulse duration seemed to produce an increase in resistance to fluid flow that caused the average flux to decrease [145].

2.3.3. UF in the presence of ultrasonic field

One of the critical issues in the performance of protein UF processes is the declination of permeates flux with respect to time that occurs as a result of both concentration polarization and membrane fouling. Concentration polarization occurs when a concentration gradient of the retained components is formed on or near the membrane surface. This phenomenon is predominantly a function of membrane hydrodynamics. Conversely, fouling is the result of accumulation of proteins drawn toward filtering surface by convective flow of filtrate through the membrane [152,153]. Various methods have been used to reduce the negative effects of concentration polarization and fouling for enhance the permeate flux and membrane separation efficiency. Ultrasonic physical effects and sonochemical effects are also used in membrane technology in order to clean surfaces to prevent formation of filter cake and to enhance filtration and separation rates [154,155]. Cleaning procedures are significantly enhanced by cavitation and acoustic streaming induced by ultrasonic waves. In the field of UF number of researchers have also demonstrated the effective use of ultrasound (US) to enhance the permeate flux [156–165]. Ultrasonic techniques provide an alternative and attractive method for membrane fouling control. Ultrasound generates acoustic streaming and cavitation bubbles in a liquid medium (e.g., water). Cavitation bubbles causes microstreaming, microstreamers, microjets, and shock waves [159]. Ultrasound is the sonic wave

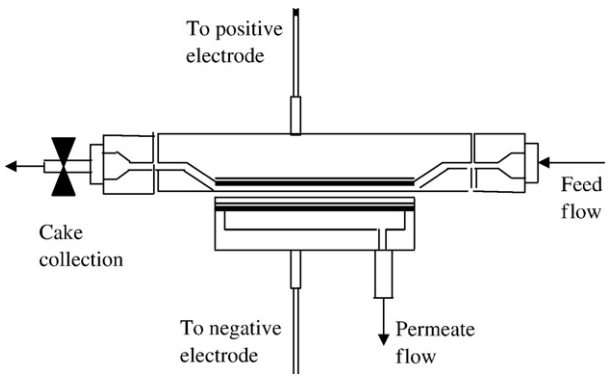

Fig. 7. Side view of the dead-end EUF module [228].

at frequencies ranging from 16 kHz to 1019 Hz. When the ultrasonic energy at high power is applied to a liquid, capitation takes place, which means the formation, growth, and sudden collapse of bubbles in liquids. The acoustic streaming and shear forces imposed by cavitations bubbles reduce the fouling on membrane surface. This leads to an increase of permeate flux [166].

Several mechanisms were proposed to particle release from a particle-fouled surface as a result of ultrasound illustrated in Fig. 8 [167–169]. Acoustic streaming does not require the collapse of cavitation bubbles and it was defined as the absorption of acoustic energy resulting in fluid flow [168]. This removal mechanism is expected to be important near surfaces with loosely attached particles or with readily dissolvable surfaces [170]. Higher frequency ultrasound tends to have higher energy absorption and thus greater acoustic streaming flow rates than lower frequencies for the same power intensity [167]. In addition, higher power intensities lead to greater acoustic streaming flow rates due to higher energy gradients in solution between acoustically and non-acoustically stimulated areas. This mechanism causes bulk water movement toward and away from the membrane cake layer, with velocity gradients near the cake layer that may scour particles from the surface.

Only few works has been done on the effect of ultrasonic wave on separation performance of protein mixture by UF. Tenga et al. studied the effect of ultrasound on the flux and solute rejection in cross-flow UF of BSA-lysozyme binary protein mixture using PES membrane (30 kDa MWCO). They observed that ultrasonic wave not only enhanced the UF flux but also increased the lysozyme rejection. In particular example, ultrasound wave (25 kHz and 240 W) resulted in an increase of UF flux by 135% and 120% with PES membrane at pH: 11 in the upward and downward modes, respectively, in contrast to the

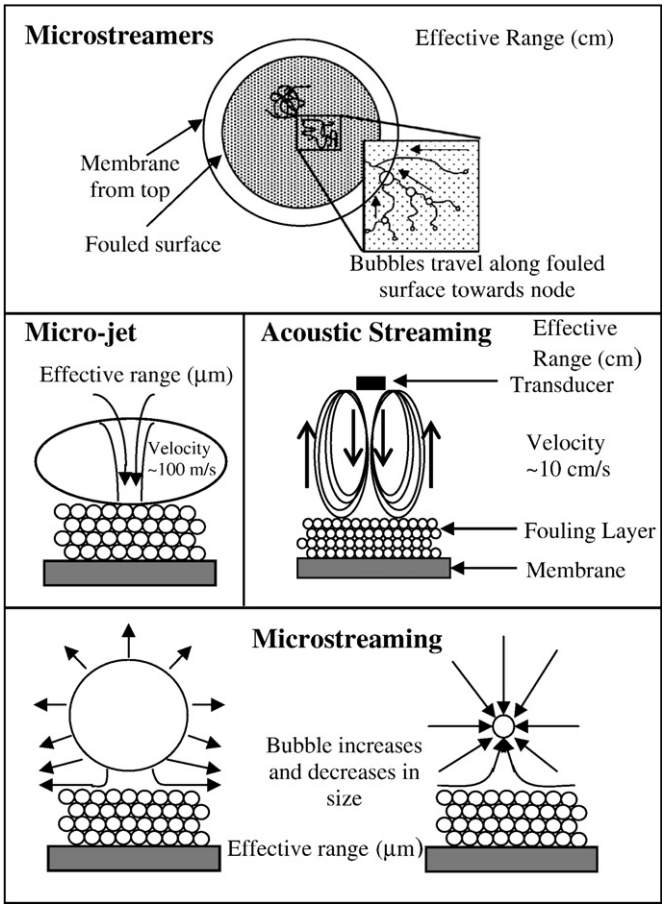

Fig. 8. Possible mechanisms for particle removal/detachment observed with ultrasonic cleaning [165].

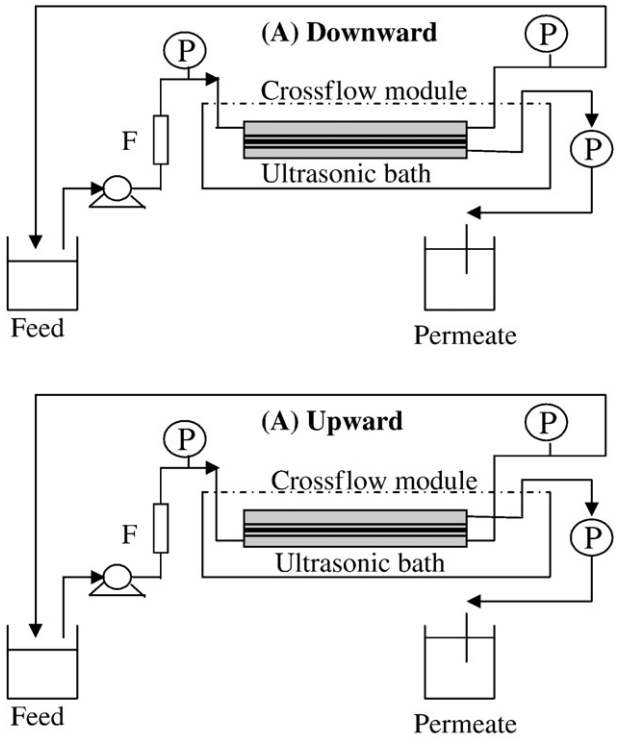

Fig. 9. Experimental set-up of cross-flow UF in the downward and upward modes [153].

case of without any ultrasound [171]. Effect of ultrasound on the flux and solute rejection in cross-flow UF in the (a) downward and (b) upward modes is shown in Fig. 9. Enhanced flux in continuous UF processes were suggested an interrupted ultrasound and more hydrophilic ultrafilter membranes in the upward operating mode for high performance [153]. It was noticed that the effectiveness of ultrasound in membrane filtration depends on many factors, such as orientation and position of ultrasonic field, ultrasonic frequency and power, ultrasonic radiation angle, position of ultrasonic vibration plate in the membrane module, membrane material, membrane housing, operating pressure and the fouling material [171]. It was widely believed that ultrasonic cavitation, acoustic streaming, ultrasonic-induced vibration of membrane and ultrasonic heating [172,173] was the main causes for the enhanced separation performance and permeate flux.

Muralidhara and Tarleton reported that both, electric and ultrasonic fields can reduce membrane fouling and in turn of enhanced flux. They observed a synergistic effect when both the fields were applied simultaneously [174–176]. In addition Wakeman and Williams also observed that filtrate flux could be markedly increased by the simultaneous addition of electric and ultrasound fields. Both electrical and ultrasonic fields reduced the fouling when applied individually, but the extent of improvement by the ultrasonic field could be minimal. The improvement by the electric field is invariably considerably greater than that due to the ultrasonic field, particularly when the particles are well dispersed (high zeta potential) [19].

2.3.4. High-performance tangential flow filtration

High-performance tangential flow filtration (HPTFF) is also an emerging technology that enables the separation of proteins with similar size. HPTFF technology has become possible by exploiting several new discoveries. It has been demonstrated that optimum selectivity and throughput were obtained in the pressure-dependent flux regime. Selectivity and throughput could also be enhanced through module design and process configurations that reduce the transmembrane pressure gradient. Optimizations of buffer pH and

ionic strength have a significant impact on the sieving behavior of proteins in membrane systems [65,88,89,177]. HPTFF is a two-dimensional unit operation that exploits both size and charge mechanisms. In addition, protein concentration, purification and buffer exchange can be accomplished in a single unit operation.

Conventional tangential flow filtration is limited to the separation of solutes that differ by ten-fold in size (e.g., cell–protein, virus–protein and protein–buffer). However, HPTFF is a two-dimensional purification method that exploited differences in both size and charge characteristics of biomolecules. Molecules that differ less than three-fold in size can be separated using highly selective charged membranes with careful optimization of buffer and fluid dynamics. Current protein separation processes often used ion-exchange chromatography, UF and size-exclusion chromatography (SEC) for concentration, purification and buffer exchange. HPTFF made it possible to perform all of these steps in a single unit operation by reducing production costs. It provided a high-resolution purification while maintaining the inherent high-throughput and high-yield characteristics of conventional UF.

The success of the HPTFF is based on several important factors such as operating pressure, flux and selectivity. Operating in pressure-dependent flux regime results in both the highest selectivity and mass throughput [177–179]. The best combination of selectivity and throughput was derived from a set of optimization equations using experimental flux and sieving data [180]. Optimizing buffer pH and ionic strength to increase flux difference between product and impurity further enhanced the resolution of the method [85]. Choice of automated process development systems depends on membrane selection, buffer choice and fluid dynamic optimization. Industrial-scale systems were based on the linear scale-up principles previously developed for UF [181,182]. Emerging trends for membrane development are being focused on enhanced pore size distribution and novel membrane chemistry. Despite significant enhancement in resolution, HPTFF has equivalent throughput to UF, measured in mass of product processed per unit membrane area and time. A limitation of the technology is the susceptibility to fouling by feed streams containing precipitates. In addition, high ionic strength feed streams have reduced selectivity and throughput.

The resolution of protein separation by HPTFF was further enhanced by the use of charged membranes. The sieving of positively charged proteins was dramatically lower with positively charged membranes due to like charge repulsion. These electrostatic repulsions prevented the proteins to enter in the membrane pores, which enhanced protein transmission and reduced membrane fouling. Selection of buffer pH, where the product protein was positively charged and the impurity proteins were neutral charged resulted in extremely low product flux with high flux of impurities [89]. This is in sharp contrast to conventional UF processes, in which at least 10-fold difference in size for effective separation is required. HPTFF has been used to separate monomers from oligomers based on their difference in size [88], protein variants differing at only a single amino acid residue [183], and an antigen-binding fragment from a similar size impurity [88]. In addition, HPTFF was potentially used for the purification process to remove specific impurities (e.g., proteins, DNA, or endotoxins) and/or eliminate protein oligomers or degradation products. In addition, it effected simultaneous purification, concentration, and buffer exchange, providing an opportunity to combine several different separation steps into a single scalable unit operation.

Commendable efforts were rendered to separate proteins by HPTFE. Van et al. [88,89] used Immunoglobulin–ovalbumin protein mixture as a model to understand the effect of membrane cut-off and variability on HPTFF protein separations. It has recently been shown that excellent HPTFF separations could be obtained even for molecules differing in size by less than two-fold. Although the ultimate range of HPTFF applications in bioprocessing (and related fields) remains to be determined, this membrane technology has potential to play a vital role in the development of new and improved separation processes for many macromolecular species. 99-fold purification of an antigen-binding fragment of a monoclonal antibody (FAB) from BSA by operating the membrane process near the isoelectric point of the BSA and using a positively charged membrane was obtained with high rejection of the positively charged FAB [89].

HPTFF exploited a number of different strategies to achieve high-resolution separations, including: (1) proper choice of pH and ionic strength to maximize differences in the hydrodynamic volume of the product and impurity, (2) use of electrically charged membranes to enhance the retention of like charged proteins, (3) operation in the pressure-dependent regime to maximize the selectivity, and (4) use of a dia-filtration mode to wash impurities through the membrane [184]. Since the selectivity in HPTFF is a function of the local filtrate flux, and thus the local transmembrane pressure, the selectivity can be further improved by maintaining a nearly uniform transmembrane pressure throughout the module. Conventional membrane modules typically have a large variation in transmembrane pressure drop due to the parasitic pressure losses associated with flow along the retentate channel. A simple approach for minimizing this transmembrane pressure variation is necessary to establish a co-current flow on the filtrate side of the membrane by using a recirculation pump to generate a pressure gradient in the filtrate channel that balances the gradient in the retentate. Comparison of flow and pressure profiles for conventional TFF module and co-flow arrangement to maintain a uniform transmembrane pressure throughout the module has shown in Fig. 10. Van et al. have proposed closed-loop cascade system for protein fractionation, based on HPTFF [88]. This system overcomes some of the problems associated with conventional MF/UF configurations and equipment and is reported to give high degree of protein fractionation [184].

2.4. Protein separation/purification by NF

NF is particularly useful for separation of peptides due to the suitable cut-off of the NF membranes and because of the electrochemical effects, which play an important role in the case of charged molecules. Negatively charged membranes have been applied to enrich cationic peptides with antibacterial properties from cheese whey [185,186]. A preliminary study on the desalting of peptide fractions from whey protein hydrolysate using NF membranes has revealed the possible occurrence of specific rejection phenomena involving

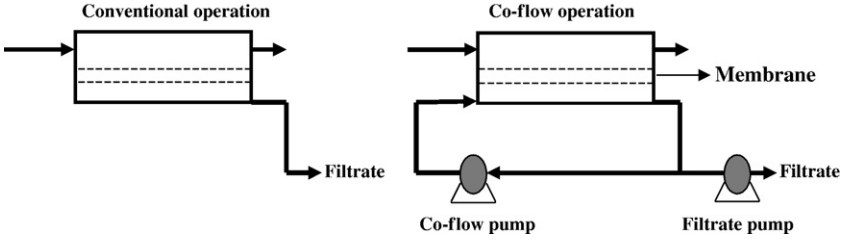

Fig. 10. Comparison of flow and pressure profiles for conventional TFF module and a module using co-flow arrangement to maintain a uniform transmembrane pressure throughout the module [4].

negatively charged peptides by NF membranes [187]. Pouliot et al. [188] has investigated the separation of peptides from tryptic hydrolysates of whey proteins with charged UF/NF membranes. Variation in pH and the ionic strength of the hydrolysate phase has proved charge effects on peptides separation performance [188].

2.5. Membrane fouling during protein separation by UF and MF

Fouling of MF/UF membrane during practical application for protein separation resulted from its adsorption on membrane surface significantly increases hydraulic resistance to flow, which reduced filtration flux rate and induced unfavorable effect on efficiency and economics of protein recovery processes [189,190]. Proteins are difficult foulants to deal because they readily adsorb onto membrane surfaces and pore walls. This leads to the formation of a secondary barrier that decreased permeate flux and changed solute selectivity. Therefore, to reduce fouling by rendering the membrane surface hydrophilic is one of the big challenges for achieving better membrane performance [191]. Membrane fouling referred to the irreversible alteration in membrane properties, caused by specific interactions feed stream components and membrane.

Fouling can occur by several forms in particular deposition of denatured or agglomerated proteins at the surface of the membrane, or adsorption of proteins inside the pore structure of the membranes. Many authors have studied the fundamental mechanisms involved in membrane fouling by protein suspensions, which may be grouped as follows.

(i) The formation of a gel layer due to concentration polarization [192].
(ii) Adsorption of species on the membrane surface and inside the pore structure [193].
(iii) Deposition and pore blocking after the formation of protein aggregates due to denaturation [194].

Modeling of the flux decline during filtration provided better understanding on membrane fouling and predictive tools for successful scale-up or scale-down of MF systems. Previous studies [195–197] have described the flux decline during protein filtration using the classical fouling models: complete pore blockage, intermediate pore blockage, pore constriction and cake filtration. Ho and Zydney [198] developed a combined membrane fouling model accounting for both, pore blockage and cake filtration to describe flux decline. Schematic diagram for all three mechanisms include pore narrowing, blockage, gel layer formation and pore plugging as shown in Fig. 11. The model shows a smooth transition from pore blockage to cake filtration, and is in good agreement with flux decline data obtained during bovine serum albumin filtration using polycarbonate track etched membranes. Although the combined pore blockage and cake filtration model described the flux decline during bovine serum albumin (BSA) filtration through the membrane pores, internal fouling was completely neglected. Internal fouling caused by protein adsorption in complicated membrane structure may be important for cases in which the feed stream has a relatively low concentration of foulant. For example, Taniguchi et al. reported some irreversible internal fouling by low molecular weight species. Internal fouling is also important in cases where fouling is not expected to occur by a size-based mechanism, such as clarification applications [199].

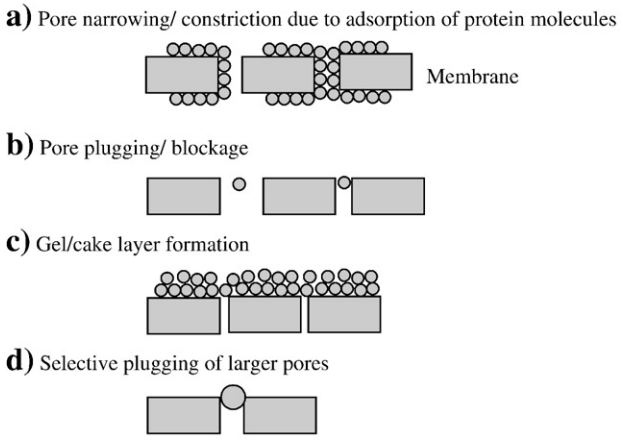

Fig. 11. Schematic diagram for all four mechanisms including pore narrowing, blockage, gel layer formation and pore plugging.

Two techniques are currently used to characterize membrane fouling. Measurements of the flux decline at constant pressure, and the measurement of the pressure increase in constant flow rate permeation. Most studies of fouling have been concerned with UF membranes but from a technological point of view, the fouling of MF membranes by protein adsorption can be more important [200,201]. Only few investigations have been devoted to protein interactions with MF membranes. Hlavacek and Bouchet studied dead-end filtration of BSA solution by MF membranes and quantified fouling by means of the constant filtration rate equation [202]. However, the different roles of adsorption, gel formation and the deposition of aggregates were not clear. Fouling of MF membranes were also investigated by measuring the pressure drops across two membranes fed in series by a constant rate pump, which enables a distinction to be made between surface fouling and internal fouling of the membrane. In the case of the MF of BSA solutions, the technique showed how the type of pump and the operating temperature influenced membrane fouling and how protein denaturation and adsorption increased different types of fouling [17]. Fouling of MF membranes with BSA and γ-globulin were studied to investigate how surface properties of membranes and proteins affect adsorption and deposition. Electrical properties of the solute and the membrane should be previously known in order to interpret fouling in terms of their mutual interaction [203,204].

In order to improve the properties of conventional MF membranes, microsieves were introduced about a decade ago [205,206]. Microsieves differ from conventional MF membranes by their well structured morphology and controlled porosity, which results in good separation behavior and high flow rates [207,208]. The main advantage of microsieves compared to conventional membranes is the larger permeate flux, which allows low-pressure operation and savings in the operational costs [209]. Another advantage of microsieves over MF membranes is their structural design, a very thin selective layer and perfectly shaped straight pores. These features would be a great advantage against fouling because of the smooth surfaces and the fact that proteins cannot be trapped inside the pore network such as normally occurs in polymeric membranes. However, for economical and sustainable industrial processes, cleaning should be performed at the lowest frequency possible. In pressure-driven processes, protein fouling of MF membranes is nowadays still an issue. Several studies [210–212] proved that, even with pore sizes much larger than the protein size, fouling under dynamic condition occurs due to aggregate formation [211,213], by hydrophobic and/or electrostatic interactions or van der Waals forces [212]. In the case of BSA, Kelly and Zydney [214] proposed fouling mechanism based on deposition and subsequent pore blockage due to protein aggregates on the surface. At longer filtration times, the flux decline is usually governed by a cake filtration mechanism. The initial fouling due to pore blockage caused by aggregates has been corroborated by several authors, and extensively studied and modeled for BSA [215–217] and other protein systems [218].

Surface modification has been widely used for the improvement of protein adsorption resistance and permeation property of hydrophobic membranes. It is well known that by increase in membrane

surface hydrophilicity can effectively minimize protein adsorption and prevent membrane fouling. Various methods, such as coating [219], surface graft polymerization [220,221], and chemical modification [222–225], have been reported in the literature to reduce the UF membrane fouling during protein separation. Nakao et al. studied that protein fouling during UF was entirely due to the formation of a secondary (gel) layer on the upper surface of the membrane. This layer provided an additional resistance to both, solute and solvent transport across the membrane [226]. Jiang et al. synthesized pegylated PES via a reaction of sulfonated PES with oligomeric poly (ethylene glycol) (PEG). The modified membranes showed superior resistance to BSA adsorption in compare with unmodified counterparts. Furthermore, UF experiments revealed that both pegylated PES and sulfonated PES could enhance the permeation properties of PES membranes, but the pegylated PES reduced BSA fouling in a wider pH range and endowed membranes with better permeation properties. The improved anti-fouling property rendered longer operation lifespan of modified membranes [66]. A model to predict the effect of pH and ionic strength on fouling in the cross-flow UF of protein solutions was also presented. The model was experimentally tested with 1 g/l BSA using Amicon H1P30-20 modules, for a range of pressures, ionic strengths and pH [227]. Reports on performance of the surface-modified PVDF membranes during UF of BSA and the enzyme solution suggested that the modified membranes exhibited non-fouling properties, whereas the PES membranes showed severe fouling problems [228]. The modified membranes showed increased hydrophilicity, which has translated into decrease in protein fouling relative to the unmodified PES membrane. Relatively higher filtration performances compared with commercial low protein adsorbing membranes was observed [229].

Efforts were also dedicated for the photo-modification of UF membranes for achieving low fouling during the filtration of biological solutions. Nystrom and Jarvinen, photochemically modified PES membranes (6 kDa MWCO) using monomers containing dextran and poly (ethylene oxide) groups to decrease membrane fouling during the filtration of BSA, lysozyme, and whey [230]. Yamagishi et al. described the grafting of wide varieties of hydrophilic vinyl monomers onto the surface of PES UF membranes to reduce protein fouling during the BSA filtration [231]. Effect of the deposited protein (BSA) layer on water (solvent) flux has been studied in detail by many researchers [232,233]. They determined that maximum BSA adsorption occurred at the isoelectric point (pH ~ 4.8), at which approximately 80 monolayers were surmise after 8 h use of PES membrane (30 kDa MWCO). The long-term flux reached a minimum at pH 5, at which protein has no net charge. Therefore, spacing between the packed protein molecules was less compared to the situation when protein molecules have a net charge and repel each other [233,234]. Thus, the deposited layer was porous at low BSA concentration (less than gel forming concentration). Zydney et al. also observed that BSA filtration and adsorption caused significant reductions in the diffusive, convective, and hydraulic transport characteristics of the fully retentive PES membranes [191].

3. Membrane chromatography

As a new technology membrane, affinity chromatography has proven its ability, efficiency and time stability for high-resolution separation and analysis of protein. This technology had already significant applications in the separation and purification of biomolecules.

Membrane chromatography is implemented by grafting specific ligands onto the pore surface in membranes and then adsorbing target biomolecules on these ligands during the convective flow through the membrane pores [235–240]. A larger pore size in membranes would allow much easier access of protein molecules to the binding sites on the pore wall, thus significantly reducing pressure drop and processing time. This technique are based on reversible biospecific interactions between the protein and a specific ligand that result in a change of protein properties such that they can be separated from complex biomolecules containing mixtures. The ligand is the molecule that binds reversibly to a specific molecule or group of molecules, enabling purification by affinity chromatography. Three basic requirements are fulfilled to successful operation of this technique. 1. A biospecific ligand must be available for target molecule to be separated or purified. 2. The ligand must have reactive chemical groups for its covalent attachment to a chromatographic matrix. 3. The membrane matrix should be easily derivatised (functional group should be easily available for the covalent attachment of one of the components of the binding pair).

The selection of the ligand for affinity chromatography is influenced by two factors: the ligand must exhibit specific and reversible binding affinity for the target substance(s) and it must have chemically modifiable groups that allow it to be attached to the matrix without destroying binding activity [241]. These ligand molecules are immobilized on the porous surface of the embedded particles and the mixture containing the protein of interest is passed through the affinity membrane (Fig. 12). A specific interaction takes place between ligand and ligate retains the desired protein within the matrix support, while the other feed components pass freely through the adsorbers. The protein is eluted in a specific buffer, either by pH and/or ionic strength shift or by competitively displacement elution [242,243]. Affinity chromatography allows for purification of biopolymers based on biological functions rather than individual physical or the chemical properties.

These processes are traditionally carried out using packed beds, which have several major limitations. The pressure drop across a packed bed is generally very high and tends to increase during a process due to the combined effects of bed consolidation (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 (Fig. 13). Since the transport of macromolecules by diffusion is slow and due to hindrance it increases the process time. Other major problems include channeling, i.e. the formation of flow passages due to cracking of the packed bed, radial and axial dispersion aroused from the use of conventional polydisperse media. These factors made packed bed chromatographic processes difficult for scale-up [1]. In all these processes, enhancement in transport phenomena is not likely to result in momentous improvement in process efficiency.

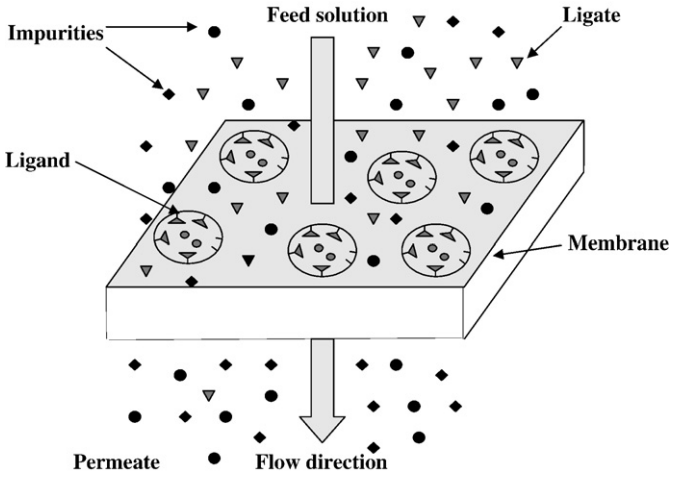

Fig. 12. Principle of membrane chromatography.

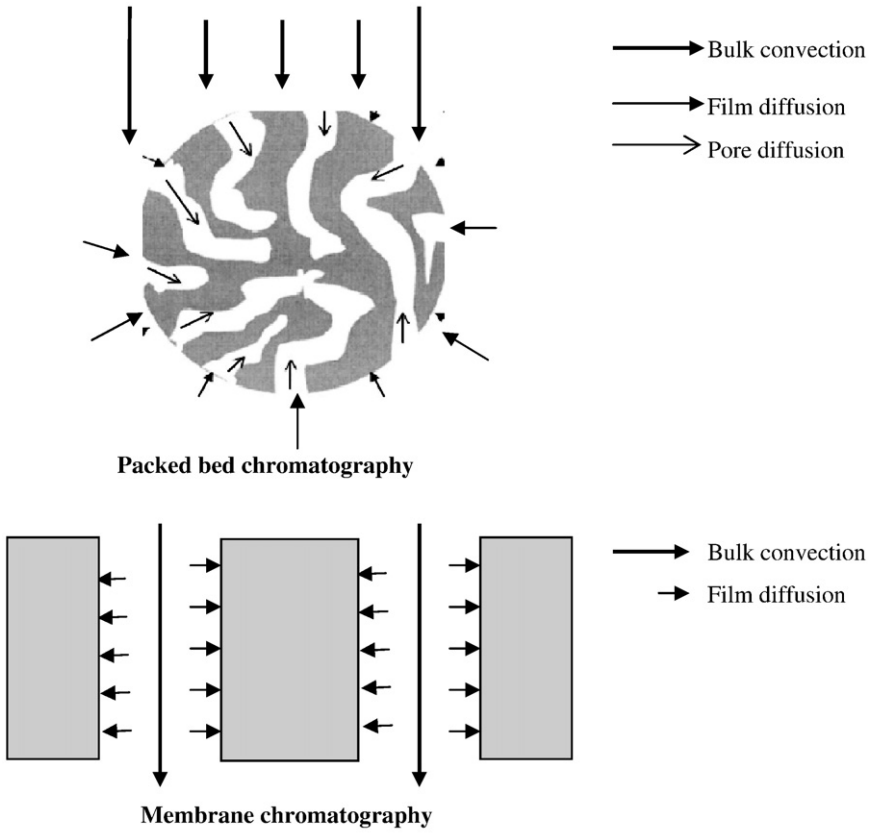

Fig. 13. Solute transport in packed bed chromatography and membrane chromatography [235].

Three types of membrane adsorbers were used for protein bio-separation: flat sheet, hollow fiber and radial flow. In flat-sheet membrane adsorbers, the liquid was usually introduced to the membrane surface. Stacks of several flat sheets were housed within membrane modules. A hollow-fiber membrane has a tubular geometry with the tubes typically ranging from 0.25 to 2.5 mm in diameter. A hollow-fiber membrane adsorber usually consists of a bundle of several hundred fibers potted together within a module in a shell and tube heat-exchanger-type configuration. In hollow-fiber membranes, the liquid initially flows parallel to the membrane surface. The liquid is then gradually directed towards and through the pores due to the hydrostatic pressure difference. Radial flow adsorbers were claimed to be suitable for large-scale applications. However, flow distribution in these devices is expected to be quite challenging. The membrane area also increased in a radially outward direction. This introduced complexities 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 [235]. In flat-sheet membrane absorbers, the liquid was usually introduced normal to the membrane surface Fig. 14. In hollow-fiber membranes the liquid initially flow parallel to the membrane surface. Then liquid is gradually directed towards and through the pores due to hydrostatic pressure difference. The main advantages of using a hollow-fiber configuration are the high membrane surface area to volume ratio, which reduced accumulation of particles near the pore entrance due to cross-flow. The liquid flow pattern in radial flow devices is also shown in Fig. 14, were claimed to be suitable for large-scale applications.

An approach to overcome the limitations associated with packed beds is to use synthetic microporous or macroporous membranes as a chromatographic media [235,244–246]. In membrane chromatographic processes the transport of solutes to their binding sites

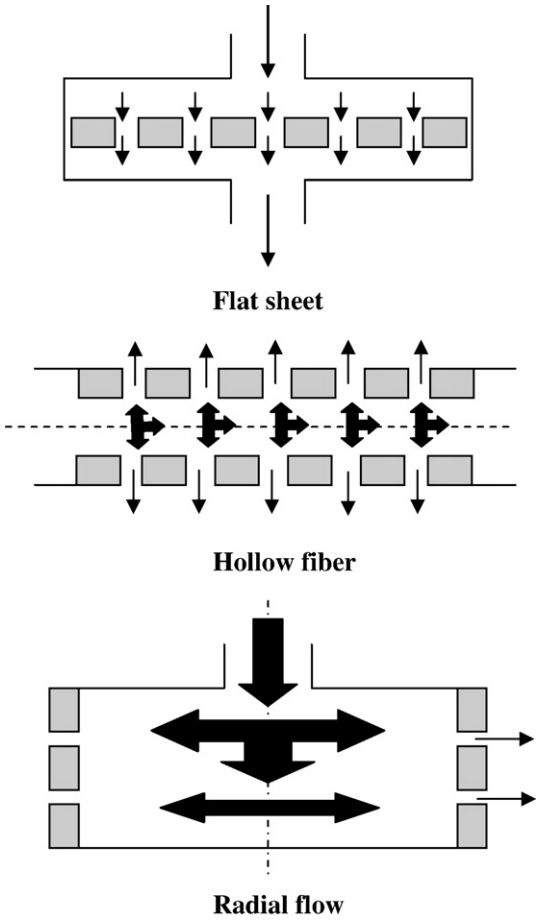

Fig. 14. Flow in membrane adsorbers [235].

takes place predominantly by convection (Fig. 13), thereby reducing both process time and recovery of 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. 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 utilized yet in the bioprocess industry. Membrane chromatography is particularly suitable for larger proteins (i.e. M_w> 250 kDa). Such proteins rarely enter pores present in particulate chromatographic media and only bind on the externally available surface area of such media. Therefore, the easy packing and scale-up, as well as the unlikely fouling/clogging, provided additional advantages [244–260].

3.1. Microporous materials for membrane chromatography

Microporous or macroporous membranes are used in chromatographic process. In this process, membranes are commonly made of natural or synthetic polymer. The base materials include cellulose (cellulose acetate, cellulose nitrate, cellulose ester, regenerated cellulose, etc.), aliphatic polyamides (nylon-6, nylon-66, etc.), aromatic copolymers (polycarbonate, polysulfone, polyethersulfone, etc.), hydrocarbon polymers (polyethylene, polypropylene, etc.), polyvinyl alcohol, glass hollow fiber, synthetic copolymer, etc. [261–266]. These materials can be modified by chemical activation, coating and grafting. They should have a good chemical–physical resistance to the solvents and harsh conditions used for elution of the bound protein and for the sanitization step. They should have hydrophilic surfaces to avoid non-specific adsorption of undesired proteins, therefore these materials should not participate in van der Waals, or hydrophobic, interactions since such adsorption leads to non-specific retention of proteins. Even though inertness is one of the requirements, the support should have substituents that can be easily activated for subsequent ligand coupling. However, these groups and their derivatives should not produce charged sites that might bind proteins non-specifically [267].

3.2. Affinity ion-exchange materials for membrane chromatography

Ion-exchange membranes materials for membrane chromatography can be produced either by modification of commercially available MF membranes or by embedding of IEX-resins into a polymeric porous matrix. Modern ion-exchange materials are prepared from synthetic polymers such as styrene divinylbenzene copolymers, which have been sulfonated to form strongly acidic cation exchangers or aminated to form strongly basic anion exchangers. Weakly basic anion exchangers are similar to the strong base except for the choice of amines. Weakly acidic cation exchangers are usually prepared from cross-linked acrylic copolymers. Non-cross-linked polymers are used only rarely because of their tendency to change dimensions depending on the ions bonded. Anion-exchange membrane chromatography bearing mainly quaternary amino groups or diethylaminoethyl (DEAE) groups as ligands has been used for the separation of serum proteins, microbial proteins and enzymes, membrane proteins, cytokines or nucleic acids [268–273]. BSA and HSA, α-chimotrypsinogen, lysozyme, trypsine inhibitor, cytochrom *C*, ovalbumin, α-lactoalbumin, conalbumin, ferritin, myoglobin, chymotrypsin, are just a few of the compounds isolated by anion-exchange membrane chromatography. The implementation of a monolith-based convective interactive media with DEAE functionality into a large-scale plasmid DNA purification process was recently performed [274]. Cation-exchange membranes are not as widely investigated as the anion-exchange membranes they allow recovery of human recombinant antithrombin from cell culture supernatants, purification of monoclonal and polyclonal antibodies and isolation of immunofusion proteins produced extracellularly by *Escherichia coli* [275,276]. The separation of similar size proteins such as serum albumin and hemoglobin using adsorber membranes loaded both with cation or anion-exchange resins was recently reported [277]. Li et al. have used a cation-exchange monolith (prepared directly in a fused-silica tube by polymerization of an aqueous solution of monomers including the desired ligand), as chromatographic supports for separation of four standard proteins (cytochrom *C*, lysozyme, myoglobine from horse and whale) [278]. Saiful et al. embedded ion-exchange resins in EVAL (a random copolymer of ethylene and vinyl alcohol) membranes for enzyme recovery. By use of these mixed matrix materials they obtained high adsorption capacities (63 mg lysozyme/ml membrane) while maintaining the biological activity of the lysozyme [279].

Additionally, Muller first proposed multilayer binding of protein on the extending polymer chains attached on the surface of adsorber particles. This approach has successfully applied for obtaining higher binding capacity of membrane absorbers [280]. A multi-step sequence including surface-initiated controlled atom transfer radical polymerization (ATRP) had been used for the preparation of high-capacity affinity-membrane adsorbers [281]. With this Husson and co-workers have developed a method for surface-initiated ATRP for the functionalization of macroporous polyvinylidene fluoride (PVDF) membrane [282], and the grafted brushes were reported to enhance static and dynamic capacity for protein (lysozyme) adsorption [283]. Recently Chung et al. has prepared the immobilized metal affinity membrane (dual-layer hollow fiber) from high-affinity sulfonated PES material with transition metal counter ions ($Cu^{2+,}$ Ni^{2+} and Zn^{2+}) via the sulfonation reaction and ion-exchange treatment. He observed fiber in Cu^{2+} form exhibits the highest protein separation performance due to the strongest affinity between Cu^{2+} and Hb molecules, thus achieving BSA with a purity of >90% (w/w) at the permeate side of membranes and high-purity Hb (>99%, w/w) in the elution medium [284].

3.3. Application of membrane chromatography for protein separation

The uses of ion-exchange and affinity interactions are more widely reported, but only small work has been done on hydrophobic interaction and reversed-phase based membrane chromatography of proteins. The fact that affinity interactions are widely used is 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: i—immunoaffinity ligands, ii—protein, iii—low-molecular-mass ligands, iv—other ligands. Lysozyme separation from egg white was achieved efficiently using macroporous chitin membrane [285], with >98% purity and >54,000 units/mg specific activity. These results indicate that macroporous chitin membranes are promising and economical matrixes for lysozyme separation. A mixture containing lysozyme and ovalbumin was also used to investigate the selectivity of the chitin membranes. Wheat germ agglutinin (WGA) is an important and very expensive lectin useful in medical studies. Macroporous chitin membranes with large pore sizes (average 18 μm) and high adsorption surface were used to separate wheat germ agglutinin from a wheat germ extract [286]. To obtain a high-purity wheat germ agglutinin, a two-step elution was employed. A purification factor of 5.5 and an activity yield of 40% were obtained. About 25 mg of pure wheat germ agglutinin was obtained from 50 g of wheat germ.

The large diversity in binding modalities in bead chromatography has found its way into membrane chromatography research. However, the current commercial availability is limited to ion exchangers. In fact, affinity based membranes appear to be the single largest binding modality in research [287]. These membranes are logical for classes of molecules such as antibodies [288–290] and fusion proteins [291] but the same limitations described above for bind–elute industrial applications. By contrast, the area of extracorporeal devices may be more amenable to these kinds of membranes [292]. Another intriguing area of research that is perhaps a long way off for biomolecules

is molecular imprinted membranes [293,294]. Purification procedures using chromatographic membranes have been reported for a wide variety of compounds, such as proteins (monoclonal antibody, serum antibody, serum albumin, enzymes, etc.), DNA and viruses. Examples of applications are:

(i) thiophilic membranes for the purification of monoclonal antibodies from cell culture media [295];
(ii) immobilized L-histidine in hollow-fiber membranes for the separation of immunoglobulin G from human serum [296];
(iii) affinity membranes for the separation of MBP fusion proteins [297];
(iv) ion-exchange membranes for the isolation of antibacterial peptides from lactoferrin [298];
(v) cation-exchange membranes for the purification of alpha viruses [299];
(vi) anion-exchange membranes for the adsorption of DNA [300];
(vii) strong anion-exchange membranes for reduction of endotoxin in a protein mixture.

Brandt et al. published the first research paper on membrane chromatography [301]. A hollow-fiber device was proposed for purification of fibronectin from blood plasma and immunoglobulin (IgG) from protein A. The high-throughput rate and the efficient ligand used in this device permitted rapid bind–elute cycle times. Because the volume of a typical affinity system was 100–1000 times that of the affinity-membrane device, the membrane device required only about 0.1% as much ligand to handle the same throughput at the same mass-transfer efficiency. The scale-up of strong anion-exchange membrane adsorbers that remove endotoxin from bacterial extracts while preserving enzyme activity in the protein mixture was demonstrated. The endotoxin removal procedure was directly adapted from the small-scale Q-100 MA cartridge (Sartorius Corporation, 100 cm^2 working surface area) to the large-scale Q-550 MA sheets (5500 cm^2 working surface area). The characteristics of endotoxin removal, protein absorption, and photolyase purification were similar in the two systems.

One of the major limitations of membrane chromatography is non-uniform flow distribution across the membrane, due to the large diameter-to-length ratio of the modules. This limitation was also pointed out by process mathematical modeling, which had to take into account the system dispersion curve to obtain an exact comparison between calculated and experimental concentrations. This represented a significant problem in many cases, reducing the membrane efficiencies to the level of packed beds. However, proper design of flow distributors was shown to reduce this problem. The 'membrane chromatography technique' has certainly not obtained the expected success (i.e., membrane discs for chromatography are no longer provided by Millipore Inc.). The reason is probably due to the reticence of potential users for this new technology. In addition, membranes for chromatography are particularly attractive for preparative chromatography, as initially developed by 'Sepracor Inc' [301], to purify large amounts of molecules. In this regard, hollow fibers are particularly well suited, more than flat-sheet membrane modules. Finally, membranes for analytical chromatography present less advantage over classical chromatographic supports than those obtained for preparative chromatography.

4. Electrophoretic membrane contactor for the separation of proteins

'Electrophoresis' is an electrically driven operation that constitutes a purification step used at the later stage of the downstream process because of their high resolution at the analytical scale. Different studies were devoted to find out the operating modes for scale-up of electrophoretic separations. One of them is continuous flow electrophoresis (CFE), which was carried in a flowing buffer film. Different experimental and theoretical studies were carried out to understand the transport phenomena involved in this process [302–304]. From these works, the limitations of CFE were pointed out and their origin was identified [305]. Proper resolution/separation was achieved under well optimized conditions [304]. The limitations were in terms of production capacity or productivity, demonstrated and it had a strong relationship between resolution and productivity [303]. Finally, it was found that the productivity could not be increased over a certain limit, typically about few milligrams per hour.

To overcome these limitations, electro-membrane operations offered the possibility to increase the productivity without compromising from separation efficiency. The most common electro-membrane operation is 'electrodialysis' (ED) in which ion-exchange membranes are used. The use of porous membranes in the place of ion-exchange membranes were investigated to extend the field of electrodialysis applications for biological molecules like polyamino acids, peptides and proteins. In that case, the porous membrane acts as a contactor and the separation is achieved with respect to the difference between the mass flow rates of the species. According to the membrane and solute properties, this difference can have various origins, like different electrophoretic mobility, sieving effects or a coupling of both [306–310]. The scalable preparative electrophoresis technique was used as a 'Gradiflow' apparatus [306]. It has been used for several applications like the separation of proteins from plasma, algal extracts [306] and egg white [307,308]. The influence of the voltage, pH, solute concentration and membrane has been studied over this process. However, no comprehensive work and no theoretical approach were devoted to understanding various transport phenomena involved in the process. Electrophoretic membrane contactor was developed for the separation of binary mixture of proteins by using ion-exchange and ultrafilter membranes.

The electrophoretic membrane contactor is schematically presented in Fig. 15(a and b). The separation chamber itself is composed of two compartments delimited by a porous membrane, acting as a contactor between the two streams in which the mass transfer takes place. The purified buffered solution is continuously fed into the chamber. The only driving force is a voltage, applied in a perpendicular direction to the feed flow. Two electrodes are located in compartments, which are separated from the separation chamber itself by ion-exchange membranes. As soon as a voltage is applied, the charged components contained in the feed would migrate from one compartment toward the other through the porous membrane. The mass flow of solute depends on its electrophoretic mobility, which is fixed by the pH of the buffered solution. Then, solutes having distinct electrophoretic mobilities were carried through the membrane at different rates. Two outlet streams with different compositions are thus obtained. The compartments in which the outlet concentration of the target solute are respectively lower and higher than the inlet, called "dilute" and "concentrate" respectively.

An electrodialysis process could be operated in two different operating modes. The first one depicted in Fig. 15a, where the same solution, containing the species to be separated, was fed on both sides of the membrane. This configuration would be further called "separation mode". The second mode of operation, illustrated for the purification of a negatively charged component was depicted in Fig. 15b. In that case, the solution to be purified was fed in one compartment. The other compartment, which is the elution compartment, was fed with the buffer. The solute A, which migrates through the membrane, is then collected in that compartment. That configuration would be later called the "elution mode". These two modes of operation could be used to achieve different objectives to favor quantitative or qualitative aspects. Indeed, as far as the production is concerned, the separating mode would be preferable. On the contrary, for achieving higher purification, the eluting mode would be used.

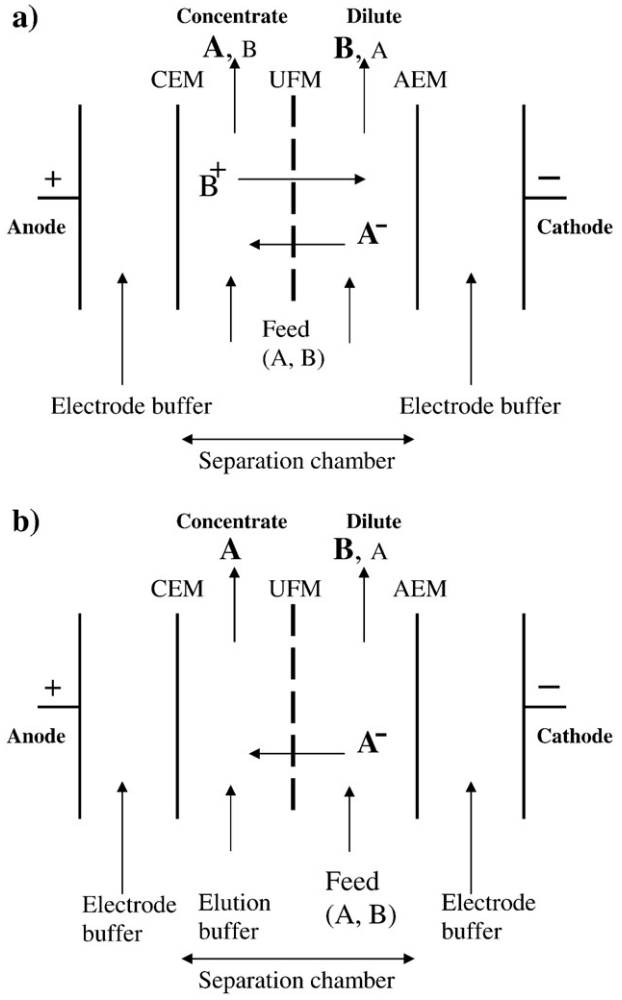

Fig. 15. (a) Schematic drawing of the electrophoretic membrane contactor: separation mode; UFM: ultrafiltration membrane; AEM: anion-exchange membrane; CEM: cation-exchange membrane. (b) Schematic drawing of the electrophoretic membrane contactor: elution mode [303].

Galier studied the purification of α-LA from a mixed solution containing α-LA and bovine hemoglobin using electrophoretic membrane contactor [311]. Three parameters were chosen to characterize the process performances, i.e. the productivity, purity and the product yield. It was confirmed that the productivity could be enhanced, by a factor of 5 by increasing the inlet concentration. It was further demonstrated that the increase of productivity was achieved without reducing the purity and the product yield. β-LG, one of the major whey components, can release by enzymatic hydrolysis of different bioactive peptidic sequences according to the enzyme used. However, the protein hydrolysates have to be fractionated to obtain peptides in a more purified form. Poulin [312] studied the feasibility of separating peptides from a β-LG hydrolysate using electrophoretic membrane contactor and studied the effect of pH on the migration of basic/cationic and acid/anionic peptides. It was concluded that electrophoretic membrane contactor appeared to be a selective method of separation since amongst a total of 40 peptides in the raw hydrolysate, only 13 were recovered. Amongst these 13 migrating peptides, three acid/anionic peptides migrated in one compartment, while three basic/cationic peptides migrated to another. Thus electrophoretic membrane contactor is an interesting way to separate bioactive peptides and other charged molecules of interest from complex feedstocks in the food, pharmaceutical, fine chemical and fermentation industries.

5. Integrated membrane technologies for protein separation

Over the past decade, many traditional chemical industries have been undergoing a change in their orientation from conventional chemicals to life-science products. Despite discussion about what a life sciences company should consist of, the required technology for the future will not change [313]. Recent developments in biotechnology, together with the recognition of the need for renewable resources have accelerated biotech research and development in academia, industries and government-funded laboratories. The success of biotechnology for bulk product manufacturing will heavily depend on engineering solutions in the downstream process in which separation and purification have a crucial role with respect to commercial development. Development of efficient bioseparation methods is important for a broad range of business areas including pharmaceuticals, nutrition and health products, biobased materials and crop production chemicals. Depending on the value of the end product and the scale of production, the processing required varies significantly. Key factors that have an impact on the choice of separation strategy include process throughput, particle size of the product, impurities and desired concentration of product.

Membrane systems are extensively used throughout the various biological and chemical industries to control variety of products. These membrane processes are successful because they are effective and economically implemented at the large stage, which is required for industrial applications. A combination of different membrane processes gives interesting benefits, which cannot be achieve by single membrane operation. The possibility of redesigning overall industrial production by the integration of various already developed membrane operations is becoming of particular interest. Because of the synergy effects are reach better [314]. Integration membrane technology also shows simplicity of the units and possibility of advanced levels of autoimmunization. Membrane hybrid technology works best when developed as one concept. This clearly lies in the influence that process have on each other The rationalization of industrial production by use of these technologies permits low environmental impacts, low energy consumption, and higher quality of final products [315].

Various membrane operations (MF, UF, NF, etc.) have introduced in industrial production lines as an alternative of existing units for fractionation of protenecious products. The categories are as follows of different field of integrated membrane separation processes for purification and fractionation of protein products [316].

A lot of work has performed to recover valuable protenecious components from dairy waste streams [317–319]. Dairy proteins are valuable products and, used as high-value food additives, nutraceuticals and therapeutics. Recently a number of papers have published to recover the protein using MF, UF and NF processes [320,321]. The largest membrane area was installed in the dairy industry. It has been estimated 2,000,000 m^2 membrane area were installed for the fractionation of milk and whey. About 2/3 of the membrane area installed in the dairy industry is used for the treatment of whey and about 1/3 for milk [322].

5.1. Membrane process for food and dairy industry

Membrane processes have become major tools in the food processing industry over the last 25 years. The food industry represents 20 to 30% of the current 250 million turnovers of membranes used in the manufacturing industry worldwide. The growth in this market is around 7.5% per year. Several hundreds of thousand square meters of membrane (UF: 400,000; NF: 300,000; RO: 100,000; MF: 50,000) are currently operating. The main applications of membranes are in the dairy industry (close to 40%, of which over 10% are used for milk protein standardization), followed by beverages (wine, beer, fruit juices, etc.) and egg products (2%). Other emerging fields are fruit and vegetable juices and concentrates, waste streams,

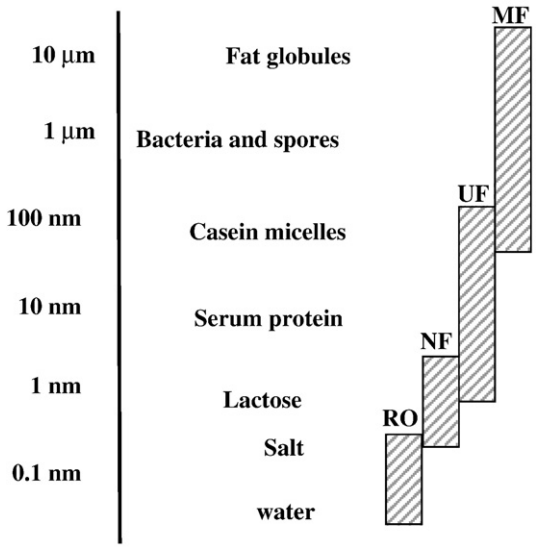

Fig. 16. Components in milk: size indication and membrane processes. MF: microfiltration, UF: ultrafiltration, NF: nanofiltration, RO: reverse osmosis [325].

co-products (recovery and recycling of blood plasma in abattoirs) and technical fluids (brines, cleaning-in-place solutions). Among the very numerous industrial applications, a few outstanding processes represent the very latest advances in food processing. They have been selected to show the trends that are emerging in the treatment and transformation of raw products from agriculture to safe food products or beverages well accepted or required by the consumer. The objective of the present paper is to complete and update the overall information reported recently by Cheryan and Daufin [323–325].

The main features of membrane separations adopted by the food industry are:

- In the preparation of traditional food products, improvement of process performance, and food quality.
- To design novel foods and intermediate food products by manufacturing fractions and co-fractions from initial products, this must comply with the requirements of the consumer: Safety + Novelty + Diversity + Quality.
- With respect to the environment, membrane separations are regarded as clean processes: a substitute for the use of polluting materials.
- Compared to competitive concentration (thermal processes) and separation operations (decantation, filtration, centrifugation, chromatography, etc.) membrane processes are attractive to industry, wherever they can be used, since they are easy to implement, have great flexibility (module systems), are compact (more or less depending on the type of module: spiral-wound, hollow-fiber, plate-and-frame, tubular), good automation.

Membrane processes give the food industry three advantages: food safety, competitiveness, environmental friendliness.

5.1.1. Milk and dairy industry

Most of the industrial developments of membrane technologies in the food industry originate from the dairy industry. They have been more or less tightly linked to the progress in membrane operations: asymmetric organic membranes by the late sixties; composite inorganic membranes in the early eighties; porous ceramic membranes with multi-channel configuration in the early nineties, which has enabled industrial application of the concept of uniform transmembrane pressure. UF is the most widely used process in the world dairy industry. The membrane area of RO has stabilized around 60,000 m^2, mainly for whey concentration. MF is developing due to its capability to retain, partly or totally, particles (microorganisms, casein micelles, fat globules), and NF is given a large field of applications due to its intermediate selectivity (200–1000 Da) between UF and RO (demineralization, de-ionization, purification) in particular for whey protein valorization. The integration of membranes has been implemented throughout the milk and dairy processing chains-milk reception, cheese making, whey protein concentration, fractionation of protein hydrolysates, waste stream purification and effluents recycling and treatment.

Milk considered as an emulsion of fat globules in an aqueous phase. The aqueous phase consists of suspended and dissolved components, such as casein micelles, serum proteins, lactose and salts. Given the relatively high concentrations and broad particle size distribution, milk is a challenging product for membrane fractionation. Besides the major components fat, casein and lactose, milk contains valuable minor components that can be interesting for specific isolation. Membrane separation technology seems a logical choice for the fractionation of milk, because many milk components can be separate on size (Fig. 16) [326]. Membranes are already well established in the processing of whey and are gaining popularity in other dairy applications [325]. However, full membrane fractionation of milk is hardly described in literature; most papers focus on a single stage, such as the separation and fractionation of fat globules for cream, the reduction of bacteria and spores in skim milk, the concentration of casein micelles as pretreatment in cheese manufacturing, and the purification of serum proteins for physico-chemical or nutritional purposes. This simple classification highlights the resourcefulness of membrane processes in the field of dairy industry (Fig. 17) [327].

Current membrane processes for milk have a rather low capacity due to strong flux decline by fouling or processes are energy demanding because of the high cross-flow velocity that is required to control fouling. Additionally, methods to control fouling have increased the complexity in equipment and operation. Besides fouling, selectivity is an important issue for membrane fractionation of milk. A number of factors are important in achieving a good combination of retention and transmission of components in the feed. This has led to the development of the uniform transmembrane pressure concept, and iso-flux and gradient porosity (GP) membranes [328]. The

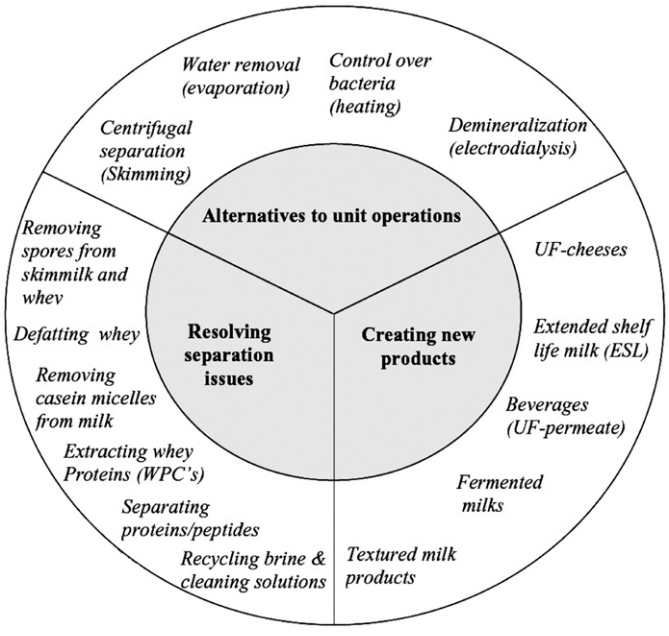

Fig. 17. Membrane processes in the dairy industry [327].

introduction of ceramic membranes and the use of the UTP concept enabled the commercial application of membranes for the reduction of bacteria and spores. There is an ongoing search for new types of membranes with better properties, and for improved process conditions. The development of membranes with narrow pore size distribution, such as asymmetric ceramic membranes, track etched membranes [329], silicon microsieves [330] and metal microfilters [331] in combination with optimized process conditions, could lead to full fractionation of milk on production scale.

5.1.1.1. Removal of bacteria and spores from skim milk (cold pasteurization). MF reduces the amount of bacteria, spores without affecting the taste of milk, and provides longer shelf life than pasteurization. Decimal reduction factors for MF are higher than for bactofugation, which is the reduction of bacteria and spores by centrifugation [332]. Besides the production of consumption milk with extended shelf life, this method can be used as pretreatment of skim milk for the production of raw milk cheeses [333] and the reduction of spores in acid cheese milk. The size distribution of bacteria in milk is 0.4–2.0 μm and there with partly overlapping with the fat globules.

A recent development is the microsieves, which was made with micro-machining technology. Microsieves have a narrow pore size distribution and a smooth inert silicon nitride surface. Further, their hydrodynamic resistance is very low, allowing extremely low transmembrane pressures. Van Rijn and Kromkamp described the use of microsieves to reduce the amount of bacteria in milk. With microsieves, a high reduction of bacteria can be achieved at low transmembrane pressure, since these membranes have very high permeabilities [334]. Hence, the use of microsieves seems very promising.

5.1.1.2. Recovery of serum proteins and cheese production. In industry, serum proteins are generally concentrated from whey. Therefore, they are often called whey proteins. Whey is a nutritious protein source, but application in food or feed products without demineralization is limited. The dairy industry has been one of the pioneers in the development of UF equipment and techniques based on the experience gained from its application in the dairy field. Various polymeric UF membranes are also used for protein recovery. UF has found a major application in the production of cheese. During cheese production, the milk is coagulated by precipitation of the milk proteins. That solid form sent to the cheese fermentation plant. The supernatant liquor (whey) represents a disposal problem [335]. Recently used UF process for cheese production process is shown in Fig. 18. Polymeric ultrafilter membranes are fully retentive for whey protein to remove lactose and minerals. Whey contains most of the dissolved salts and sugars present in the original milk and about 25% of the original protein. Initially whey was discharged to the sewer because its high salt and lactose content makes direct use as a food supplement difficult. Now, whey can be processed for obtain additional food values through newer process using UF membrane. The objective of these membrane processes is to increase the fraction of milk proteins used as cheese or some other useful products and reduce the waste disposal problem represented by the whey. The objective of the UF step is to concentrate the protein as much as possible to minimize evaporator-drying cost and simultaneously remove the lactose [336].

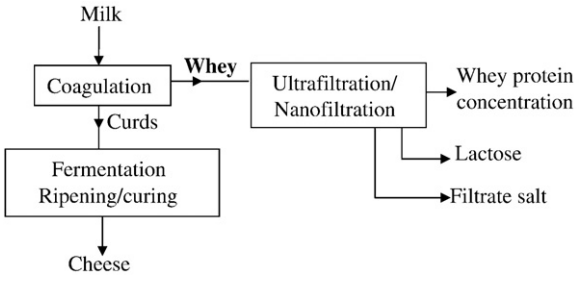

Fig. 18. Simplified flow schematic shows the cheese production method using ultrafiltration to increase the recycle of useful product.

5.1.1.3. Fractionation of buttermilk. Buttermilk is a by-product from butter manufacture that finds applications in various food products. Buttermilk contained comparable amounts of phospholipids compared to regular buttermilk but its protein content was lower due to the absence of caseins [337]. However, in most applications, buttermilk is used because of its typical flavor such as in baked goods [338]. Growing interest is showing on that particular byproduct because of its unique composition [339–342]. Various attempts have been made to fractionate buttermilk in order to create an enriched fraction in milk fat globules membrane (MFGM) components.

Surel and Famelart were the first to report the use of MF to fractionate buttermilk. The major issue they reported for the use of MF to fractionate buttermilk was the similar size of the casein micelles and the MFGM components present in buttermilk [343]. In order to overcome this problem Sachdeva and Buchheim used renetting and acid coagulation of buttermilk to remove caseins prior to fractionation by a combination of MF and UF [344]. The authors reported a recovery of 70–77% of the total phospholipids of buttermilk using this process. However, these results were highly dependent of various coagulation factors. Corredig et al. have reported the use of citrate to disrupt the casein micelles followed by either UF or MF on polysulfone membranes. Their results showed that this approach seems to be effective to concentrate MFGM proteins in the retentate [345–347].

An approach using whey cream as a starting material in order to concentrate MFGM components could also be considered. Whey cream is obtained by separation of fat from cheese whey by centrifugation. The whey cream obtained is mainly used to standardize milk fat prior to cheese making but can also be used to produce whey butter [348] and thus, a by-product, whey buttermilk. Because of objective of the cheese maker is to maximize casines recovery in the cheese, virtually no caseins can be found in cheese whey, therefore the buttermilk recovered from churning of whey cream is expected to contain very low amount of caseins. This is likely to enhance the membrane separation of MFGM components from the other milk solids.

5.1.2. Food and beverage industries

In the whole food and beverage industries, membrane processes have contributed to the revision of traditional flow sheets in the processing of fluids with the major issues of simplification, improvement of competitiveness, process or product novelty and environmental friendliness. Recent industrial applications have been developed for fruit, vegetable and sugar juices and beverages (based on vegetable proteins, beer, and wine). Further integration of membrane operations were designed in such a way that at each processing step, end products, co-products and wastes are given even attention. The safety and quality of the products manufactured must be ensured with regard to microbiological, functionality, texture, flavour and taste aspects.

5.1.2.1. Fruit and vegetables juices. In the field of fruit juices, the major application of membranes concerns the clarification by MF or UF and deacidification by electrodialysis. The apple juice industry has taken advantage membranes separation processes due to their simplicity and productivity that justifies long-term investment. For citrus (orange, lemon, grapefruit, tangerine) and pulpy fruits, membrane filtration allows pulp (retentate) and serum (permeate) to be separated. Fouling limit industrial UF for the clarification of orange juice [349]. On the other hand, industrial UF of lemon juice [350] meets an increasing need of limpid concentrated juices for use either as acidulous, bitter, or astringent agent in beverages, sauces or domestic cleaners.

Novel processes also combine membranes from MF to RO to allow the production of fruit juice concentrates in which the organoleptic quality after dilution is close to fresh fruit drink. MF and RO or osmotic evaporation combined with previous enzymatic treatment of raw material, can produce either clarified concentrates from turbid juices or pulps (lime, lemon) or stable pulps concentrates, with high sugar content (65%) and suspended solids (30%) from fruit pulps (mango) [351–353]. These membrane operations help with the creation of novel products and keep organoleptic properties of intermediate products reasonably good for export or of final products for domestic consumption in developing countries. MF and UF or RO processes were used for the clarification or concentration of exotic (pineapple, kiwi, etc.), red (strawberry, blackcurrant, and raspberry, cherry) fruits. The concentration of tomato pulp of high organoleptic quality by UF and by RO (4.5 to 9% DS) are the major industrial membrane processes applied to vegetable juices [353]. Other vegetables (cucumber, carrot, mushroom, celery, etc.) are clarified and concentrated by UF and RO and even demineralized by NF, which stabilizes the concentrate of red beetroot [354–356.].

5.1.2.2. Beer and wine. Beer clarification is probably one of the most important operations. Rough beer is filtered in order to eliminate yeast and colloidal particles responsible for haze; in addition, this operation should also ensure the biological stability of the beer. The use of MF is to provide an alternative to the conventional dead-end filtration with filter-aids such as diatomaceous earth. However, this operation should satisfy the same economic and qualitative criteria, such as: (i) to produce a clear and bright beer; (ii) to perform a separation in a single-step without additives; (iii) to operate at low temperature (0 °C); (iv) and to achieve economic flux [357–360]. Among the potential applications of cross-flow MF, clarification of rough beer represents a large potential market (approximately 200,000 m^2 membrane areas). Clarification of rough beer should be considered as an emerging market for membrane applications in the food industry. Industrial applications membrane technologies however, encountered two main problems: (i) the control of fouling mechanisms, (ii) the enhancement of permeates quality. Recent scientific and industrial studies are concerned with (i) fouling mechanisms [360–363]; (ii) relation between quantitative and qualitative performances [363]; (iii) industrial applications [364,365]. MF suffers from a low permeate flux in comparison to the conventional dead-end filtration with filter-aids such as diatomaceous earth (usual flux ranges from 100 to 500 l h^{-1} m^{-2}). However, the first industrial MF plant of rough beer with a capacity of 10,000 l/h [366] is running at Heineken. The plant contains 10 hollow-fiber modules (pore size: 0.45 mm, length: 1 m, inner diameter: 1.5 mm, filter area: 9.3 m^2). The key of this process is a specific cleaning procedure combining an enzymatic step, an alkaline step and a strongly oxidative step, which has been successful in maintaining run times of between 7 and 10 h for about 50 runs. The flux is maintained at 100 l h^{-1} m^{-2} and clarified beer respects the European Brewery Convention (EBC) standard in terms of turbidity (close to 0.6 EBC unit), bitterness, total extract, color, and protein content. The cost of membrane filtration for bright beer is about 0.68US$ per hectolitre.

Wines are made using a range of procedures with the objective of marketing clear and stable products. These procedures generally aim at limiting the presence of microorganisms, yeast and bacteria in wine and reducing the risks of tartar crystals and precipitates of coloring matter forming in the bottle. New technology substituted traditional methods with the objective of simplifying operations and/or making them more selective or economic; it can also be the impetus for new products [367]. MF can ensure microbiological limpidity and stability in a single operation. This has no effect on the quality of the wine treated and meets user's requirements in that it yields products with low turbidity (less than 1 NTU), low in germs (0.2 mm average pore diameter) and it maintains the wines organoleptic qualities. Many applications are, henceforth, available where wine makers can treat their wine after fermentation. Electrodialysis (ED) is used to avoid the tartaric salts deposits at the bottom of bottles that are not appreciated by the consumer. ED consists of extracting certain ions from wines, notably potassium, calcium and tartaric acid ions, which contribute to reducing the level of over saturation of tartaric acid salts. ED has been re-examined as a truly efficient solution for facing the current constraints. The reliability of electrodialysis of wines for preventing tartaric precipitation to occur, is based on a stability test integrated to an active control system, which determined for each wine both the treatment opportunity and the level of treatment. Compared to classical treatments, the electrodialysis option yields savings in filtering additives, seeding tartars or metatartaric acid, according to the technologies used.

5.1.2.3. Fisheries and gelatin industries. The wastewaters generated in fishmeal production contain a large amount of potentially valuable proteins. These proteins can be concentrated by means of UF and recycled into the fishmeal process, improving its quality and the economic benefits from the raw material, whereas the treated water can be discharged into the sea or reused in the plant [368].

Several authors have studied the recovery of proteins from fisheries effluents by membrane separation processes [368–372]. Lin et al. pointed out that the wastewater from the first washing in surimi production contains the highest concentrations of protein, non-protein nitrogen, fat, and ash, besides a strong fishy odor, although it constitutes only 1.5% of total wastewater [372]. Jaouen and Quemeneur studied surimi wastewaters treatment, using different types of UF membranes, namely, cellulose, polysulphone, and zirconium oxide (10 kDa<MWCO<100 kDa) [373]. They analyzed the contribution of proteins adsorption upon membrane fouling and membrane performance (permeation flux decline and regeneration after cleaning) against the operating conditions (transmembrane pressure and cross-flow velocity). Huang and Morrisey investigated the development of membrane fouling during MF of surimi wash water with the aim of recovering suspended myofibrillar proteins [374]. Technical and economical feasibility of proteins recovery from fish meal effluents using cross-flow membrane technologies (UF and NF) were also assessed. It was concluded that UF is a promising separation process for the recovery and concentration of proteins from fish meal effluents. A suitable treatment for fish meal effluents consisted of a MF pretreatment (5 mm pore size), followed by UF (membrane Carbosep M2, 15 kDa MWCO), operating at 4 bar and 4 m/s, which yielded a permeation flux of 28 l/(m^2 h) and proteins rejection of 62% for a volume reduction factor of 2.3 [375].

Gelatin is a very important fibrous protenecious material having numerous applications, particularly in the pharmaceutics and food industries due to its unique chemical and physical properties among which the ability to form thermally reversible gels. So, far most of the available gelatin has been manufactured from mammalian resources. Gelatins used in the food industry are required to be of a high purity. Traditionally, after acid or alkaline extraction from the raw material, the gelatin solution (≈4%) is being filtered and then passed through ion-exchange columns. The use of UF in the gelatin industry has been successfully tested [376–380], which has advantages of lower dewatering costs, low energy consumption, less thermal degradation of the gelatin and higher purity level. In addition, UF can provide a further product improvement through the removal of salts from the gelatin liquors by operating in the dia-filtration mode [380–383].

5.1.3. Biotechnology industry

Uses of membrane technologies in biotechnology or bioseparation sector recently became an essential tool. In biotechnological downstream processing product is obtained at low concentration in a complex liquid. The product has to be separated from a mixture of a cell mass, substrate components, additive and by-products. Since the

products are often labile, the separation has to be done rapidly and under gentle condition. The process has to be carried out in a closed system when working with pathogenic organism. Downstream treatment is an essential step in the fermentation process. Different module configurations and pre-treatments are common in this production step. The choice of the process depends on the sensitivity of the product and the viscosity. The optimization of a process also includes the adjusting of the membrane to the fermentation conditions [384,385]. Numerous applications of membrane technologies for the recovery of valuable bioproduct from fermentation broth have been reported in the literature. Lactic acid is one such value-added product that was produced from processing cheese whey or sugar cane molasses by fermentation. The processes of lactic acid production include two key stages, which are (a) fermentation and (b) product recovery. The biggest challenge in lactic acid production lies in the recovery and not in the fermentation step. Usually, most of the separation of microorganisms from fermentation broth was performed by centrifugation. Recently, cross-flow MF has been used to separate cells in continuous fermentation processes [386]. A successful lactic acid recovery approach has been that of continuous fermentation in a cell-recycled reactor where the cells are separated by a filtration unit and returned to the fermentor while the product is removed in the permeate [387,388]. The long-term performance of membrane units at high cell densities was affected by the fouling of filtration membranes, which require extensive cleaning protocols [389,390].

Other typical examples of membrane technologies in the biotech or pharmaceutical sector were recently reported. This include recovery of heterogeneous immunoglobulin (IgG) from transgenic goat milk by MF [391,392], concentration and purification of recombinant Brain-Derived Neurotrophic Factor (rBDNF) inclusion bodies from *E. coli* cell suspensions by cross-flow MF and dia-filtration [393], recovery of naturally glycosylated therapeutic proteins produced from animal cell cultures by MF [394], recovery and purification of yeast alcohol dehydrogenease (ADH) from bakers' yeast as typical of downstream processing for the extraction of an intracellular enzyme product [395]. The work of on the optimization of monoclonal antibody recovery from transgenic goat milk by MF is an interesting example. The optimization involved varying pH, transmembrane pressure, milk feed concentration, membrane module type, and axial velocity. Operation in the pressure-dependent regime at low uniform transmembrane pressures using permeate circulation in co-flow, at the p*I* of the protein is shown to increase IgG recovery from less than 1% to over 95%. Such methodology is generally applicable to the recovery of target proteins found in other complex suspensions of biological origin. [392]. Much effort is still being devoted to developing new membrane modules with improved mass-transfer characteristics for UF and MF processes. This includes rotating disk filters [393,394], cylindrical Taylor vortex devices [395], conically shaped rotors[392], and helical coiled 'Dean Vortex systems'. Dean vortex devices have very high mass-transfer rates, owing to the presence of centrifugal flow instabilities. These devices show significant increases in protein transmission and capacity, although fouling remains a problem in many applications. An alternative approach is to use high frequency back pulsing to continually clean the membrane surface. High frequency back pulsing was shown to improve flux, reduce fouling and increase protein transmission in the purification of conjugated vaccine products [395].

6. Conclusions

Membranes have been traditionally used to separate species of different size such as proteins from cells, fermentation broths, cell debris and separation of low molecular weight components from proteins. Since long it has been an integral part of biotechnology processes, the well known examples are MF and UF, which have become routine methods for protein separation/fractionation. The development of membrane chromatography, HPTFF and electrophoretic membrane contactor enable for the complete purification/separation of proteins using membrane systems. Although not implemented in any commercial processes, small-scale studies using this process show comparable yield, purification, and product quality with a conventional process. Continued efforts to develop improved membrane materials, modules, and process designs should enable membrane systems to play an important role in the next generation of biotechnology processes.

New applications of membrane processes continue to emerge, such as membrane biosensors and molecularly imprinted polymeric membranes for separation of molecules. Their industrial success will depend on their advantages over the existing technologies. Thus, deep understanding of physical and chemical phenomena across the membrane interfaces under the operating conditions will help to improve their performance in the biotechnology-based industries. Future trends of membranes in biotechnology will be driven by higher selectivity, lower cost of production, and enhanced membrane throughput. There is also trend towards increased use of disposable systems (bioreactors, ultrafilter membranes, and buffer bags), which are attractive for production scale manufacturing, eliminating the need for the development and validation of cleaning cycles. Future developments will determine whether such a membrane-based process can provide the required product quality, purity, yield and throughput with low cost for biotechnology industry.

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