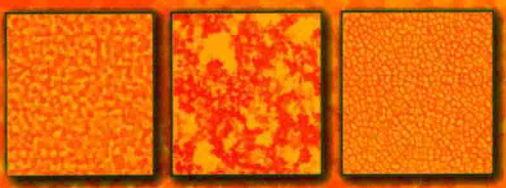
MEMBRANE PROCESSES IN BIOTECHNOLOGY AND PHARMACEUTICS



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Membrane Processes in Biotechnology and Pharmaceutics

To my parents,
To Jean-Philippe, Laurane and Sébastian

Preface

One of the growing applications of membrane processes is the downstream processing and purification of pharmaceuticals, enzymes, antibiotics and therapeutic proteins. The purpose of this book is to provide a general overview of membrane processes used in the biotechnology and pharmaceutical industry. Ultrafiltration, microfiltration, virus filtration, membrane chromatography and membrane emulsification are examined in detail. Other processes include liquid membranes, membrane reactors, solvent-resistant nanofiltration and membrane crystallization. For each process, membrane material and devices, process parameters, control of fouling and general rules on modelling are discussed. A broad range of applications is presented for each membrane process.

Chapter 1 deals with general aspects of membrane processes which will be discussed in the following chapters. Chapter 1 examines some general rules on membrane materials, including organic and inorganic membranes. The device is also a key point of a membrane process, developed for both cross-flow and dead-end configurations. Membrane characterization techniques include chemical structure characterization, permeability measurement, measurement of solute rejection, liquid displacement techniques, as well as microscopic methods. Membrane fouling is an essential and complex process because of the enormous range of inorganic, organic and biological components that can interact with the membrane and the different mechanisms by which these interactions can occur. As a consequence, membrane cleaning is an essential component of almost all membrane processes including physical and chemical cleaning.

Chapter 2 provides a general overview of ultrafiltration (UF), a pressure-driven separation process which first came into use in the 1960s. UF is extensively used for the concentration, diafiltration and separation of biological solutions, both for the final product formulation and as conditioning of feed streams prior to other separation processes. UF membrane materials, especially developments to improve membrane properties such as selectivity and permeability and reduce fouling, are examined. Various methods have been proposed to limit the negative effects of fouling and concentration polarization by improving the hydrodynamics of the cross-flow over the membrane surface such as dynamic filtration which uses a rotating disk, or by rotating or vibrating the membrane, backflushing and backpulsing, Dean vortices, gas sparging, corrugated surfaces and electro-UF. UF can be operated in various modes including diafiltration, high-performance tangential flow filtration (HPTFF) and affinity UF. Some applications of UF are discussed including processing of antibiotic

broths, DNA purification, monoclonal antibodies purification, whey fractionation and lysozyme purification from chicken egg white. In the last section, theoretical backgrounds of UF are considered.

Chapter 3 focuses on various aspects of microfiltration (MF) for biotechnologies and pharmaceutical applications. MF is an older process than UF which traces its roots back to Germany in the beginning of the twentieth century. MF is a pressure-driven separation process, which is widely used in cell recycle and harvesting, separation of recombinant proteins from cell debris and purification of process streams. In this chapter, MF membrane materials are briefly examined, such as developments to improve permeability and reduce fouling. Like for UF, hydrodynamic methods have been proposed to reduce the negative effects of fouling and concentration polarization. The techniques include dynamic filtration, vortex systems, backflushing and backpulsing, and electric field. Other techniques are proposed such as constant filtrate flux operation, flocculation, gas sparging, secondary membranes and turbulence promoters. Common applications of MF are discussed, including bacterial removal (sterile filtration), concentration and washing of cultures of single-cell organisms, recovery of intracellular molecules produced from fermentation broth including proteins, antibiotics, lactic acid and polysaccharides, and purification of nanoparticle suspensions. Finally, some results on MF modelling are examined.

Chapter 4 deals with virus filtration, whose principles are very similar to those of UF and MF. Virus capture is needed in the biotechnology industry for two main goals: viral clearance and purification of viral vectors and vaccines for gene therapy applications. Virus clearance is an essential component in the production of biopharmaceutical products derived from human or animal origin, as manufacturers are required to validate virus clearance before regulatory authorities approve the product. Commercial membranes are specifically designed for virus clearance. Purification methods of viral vectors and vaccines are aimed at eliminating contaminants originating from host cells or culture media and producing large volumes of concentrated, biologically active viruses. The main features of virus filtration are presented including membranes and configurations, fouling, and applications related to virus clearance of plasma products and monoclonal antibodies, and purification of viral vectors and vaccines.

Chapter 5 provides an overview of membrane chromatography, which dates back to the 1980s with a major paper published by Brandt et al. in 1988 who reported the use of Protein A hollow fibre membranes for the purification of fibronectin from blood plasma and purification of IgG. Membrane chromatography is now a competitive technique which has the potential to maintain high efficiencies both at high flow-rates and for use of large biomolecules with small diffusivities, reducing biomolecules degradation and denaturation. Interaction mode include affinity interaction, ion exchange, hydrophobic interaction, reversed-phase and multistage chromatography. Chapter 5 highlights the principles, membrane materials and devices, membrane activation and interaction modes, operational parameters and some general modelling aspects of the purification process. The large range of applications is examined,

including purification of monoclonal antibodies and DNA, and viral clearance. A specific section is also devoted to monolith chromatography.

Membrane emulsification, which is discussed in Chapter 6, was introduced more than 20 years ago by Nakashima et al. at the annual Meeting of the Society of Chemical Engineers in Japan in 1988. Its use has been increasingly reported these last 10 years. In membrane emulsification, the dispersed phase is pressed through the pores of a microporous membrane, while the continuous phase flows along the membrane surface. Droplets grow at pore openings until they detach. The resulting droplet size is controlled primarily by the choice of the membrane and not by the generation of turbulent droplet break-up. Thus, emulsions with narrow droplet size distributions are produced and shear-sensitive ingredients can be used. Chapter 6 provides a general overview of membrane emulsification focusing on membrane materials and devices, process parameters, optimization and some general aspects of modelling. Many applications are reported in the literature which may be of use in the pharmaceutical and biotechnological industries, such as simple emulsions, multiple emulsions, polymeric and lipid nano- and microparticles, and liposomes.

In Chapter 7, some other membrane processes related to biotechnologies and pharmaceutical industry are considered. Liquids that are immiscible with the source (feed) and receiving (product) phases can serve as a liquid membrane. Liquid membranes are reported for concentration and separation of biochemical compounds like amino acids, organic acids and antibiotics. Another field of application of liquid membranes is chiral resolution. In the first section of this chapter, the principles, configurations, applications and some theoretical backgrounds of liquid membranes are examined. In the second section, membrane reactors are presented. Membrane bioreactors are alternative approaches to classical methods of immobilizing enzymes, microorganisms and antibodies. The biocatalysts are suspended in solution and compartmentalized by a membrane in a reaction vessel or immobilized within the membrane matrix itself. In the last section, membrane bioreactors are considered including membranes and configurations, applications such as chemical synthesis and protein hydrolysis, and general rules on modelling.

Chapter 8 provides an overview on two recent membrane processes. The first section of the chapter focuses on solvent-resistant nanofiltration (SRN). Nanofiltration has recently emerged from its traditional application area with the development of SRN. The extraction of drugs and natural compounds become possible using these new membranes. Membranes and devices, governing phenomena, configurations and applications are summarized. The second section describes a recent membrane process which uses membranes to improve crystallization efficiency of various pharmaceutical compounds. Several configurations are discussed such as osmosis and reverse osmosis, evaporative membrane crystallization and membrane contactor. For each configuration, membranes, devices, governing phenomena and applications are presented.

Contents

1 Principles on membrane and membrane processes

Chapter Outline

Membrane Processes in Biotechnology and Pharmaceutics. DOI: 10.1016/B978-0-444-56334-7.00001-0

1.1 Membrane materials

1.1.1 General characteristics

Depending on their internal structure, membranes can be classified as symmetrical or asymmetrical. Symmetrical membranes show uniform pore sizes in cross section. The pores of asymmetric membranes are usually smaller on the membrane surface. Composite membranes combine two different structures into the same membrane. The different layers can be either symmetrical or asymmetrical, with a distinct pore-size distribution, aspect ratio (ratio of pore sizes on the two faces of the membrane) and thickness. Multi-layer membranes have different membranes layered together, each of which is cast separately with the desired pore size and surface characteristics. The first layer is typically used as a pre-filter while the pore size of the second layer depends on the application.

From their morphological point of view, membranes can be divided into two large categories: dense and porous. Membranes are considered to be dense when the transport of components involves a stage of dissolution and diffusion across the material constituting the membrane. A membrane is denominated as porous when permeate transport occurs preferentially in the continuous fluid phase which fills the membrane pores.

Membranes are usually classified accordingly to their average pore sizes (Figure 1.1). Microfiltration (MF) membranes typically have pore sizes on the order of 0.1–10 μm. Ultrafiltration (UF) membranes have pore sizes in the range of 0.001–0.1 μm and are capable of retaining species in the molecular weight range of 300–10,00,000 Da. Membranes designed specifically for virus filtration fall between these limits. Reverse osmosis (RO) membranes retain solutes, such as salts and amino acids, with molar mass below 1000 Da. Nanofiltration (NF) membranes retain solutes, such as small polypeptides, in the range of molar mass between 1000 and 3000 Da.

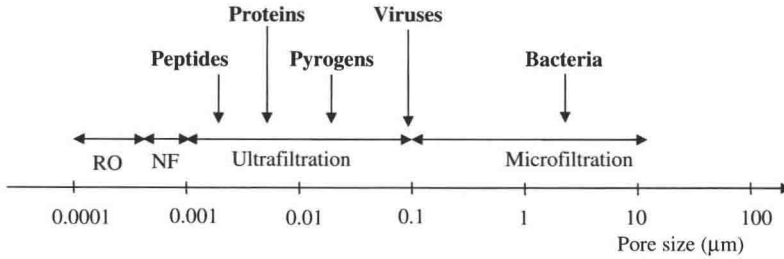

Figure 1.1 Approximate pore size ranges of different types of membranes, compared to dimensions of some components separated by membrane processes [1].
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1.1.2 Organic membranes

Common polymers used in commercial applications such as UF, MF, membrane chromatography and virus filtration are listed in Table 1.1. In addition, many polymers are grafted, custom-tailored, blended or used in the form of copolymers, to improve membrane properties, such as lower protein adsorption, higher flux, higher flux recovery ratio and lower membrane fouling. Common polymeric membranes are obtained by casting technologies, including air, immersion and melt casting. Other common techniques include track-etched membranes, membranes made by controlled stretching of films, composite membranes and nanofibrous membranes.

Table 1.1 Common polymers used in commercial membrane manufacture

Polymer	UF	MF	MC	VF
Cellulose diacetate and triacetate (CA, CTA)	×	×		
Cellulose nitrate (CN)	×			
CA/CN blends	×			
Cellulose and regenerated cellulose	×	×	×	×
Polyacrylonitrile (PAN)			×	
Polyamide (aromatic and aliphatic)	×	×		
Polysulfone (PS)	×	×		
Polyethersulfone (PES)	×	×	×	×
Polycarbonate (track-etched)	×	×		
Polyethylene terephthalate (PET) (track-etched)	×	×		
Polyimide		×		
Polyethylene (PE)	×			
Polypropylene (PP)	×			
Polytetrafluoroethylene (PTFE)	×			
Polyvinylidene fluoride (PVDF)	×	×		×
Polyvinylchloride (PVC)	×	×		

UF: ultrafiltration, MF: microfiltration, MC: membrane chromatography, VF: virus filtration

They are briefly described below. Details on these various techniques are given in various references [2,3].

Casting technologies

Polymeric membranes are usually manufactured by a phase-inversion process. This technique involves preparing a casting solution consisting of one or more polymers in an appropriate solvent or solvent blend and possibly one or more non-solvents, surfactants and other additives such as inorganic salts. The polymeric membrane is prepared by causing this casting solution to undergo phase inversion, which involves the transformation of a homogeneous solution in which the polymer molecules are dispersed in the mixture of solvent(s) and non-solvent(s) into a porous membrane in which the polymer forms an interconnected matrix. The phase-inversion process is obtained (1) by removing solvent from a casting solution that contains non-solvent (wet casting); (2) by simultaneously removing solvent while adding non-solvent (dry casting) or (3) by cooling a casting solution that contains a latent solvent displaying only a limited ability to dissolve the polymer (thermally induced phase-separation [TIPS]) [4].

The wet casting process involves immersing the casting solution into a non-solvent bath that causes simultaneous loss of solvent and gain of non-solvent. The dry casting process entails evaporating sufficient solvent from a casting solution that initially contains some non-solvent. The TIPS process necessitates cooling a casting solution containing a solvent that can dissolve the polymer only at elevated temperatures. Combinations of these three fundamental phase inversion methods lead to hybrid processes. For example, wet casting may involve a precursor evaporation step that predisposes the interface of the casting solution to gel or vitrify. The thermally assisted evaporative phase separation process combines dry- and TIPS-casting, in which phase separation is caused by evaporative solvent loss coupled with temperature control. The vapour induced phase-separation process combines wet and dry casting whereby the casting solution is initially contacted with humid air (i.e. water is a non-solvent) followed by immersion into a non-solvent bath. Membranes from polymers with excellent chemical and thermal resistance can thus be produced, such as polyolefins, polyfluorocarbons and poly(ether ether ketone).

In recent years, several polymer blends have been used for the development of novel membranes with improved properties [5–7]. In addition, organic or inorganic additives as the third component to the blend polymers have been used to control the morphology and performance of membranes.

Studies were conducted by adding additives such as polyethylene glycol (PEG), hyperbranched polyglycerol (HPG) and polyvinylpyrrolidone (PVP) in the casting [8,9]. Su et al. [8] prepared a series of amphiphilic poly(ethylene glycol)-*graft*-polyacrylonitrile (PEG-*g*-PAN) UF membranes with various molecular weights of PEGs by wet phase precipitation copolymerization using ceric(IV) ammonium nitrate as an initiator. All prepared PEG-*g*-PAN UF membranes have lower BSA adsorption, higher flux for protein solution, higher flux recovery ratio and lower membrane fouling during protein UF in comparison with the control PAN membrane. The authors concluded that these improved properties endow PEG-*g*-PAN membranes

with potential applications in protein separation and purification. Sivakumar et al. [9] investigated the effects of PVP on CA/PS blend UF membranes and showed that an increase in the concentration of PVP in casting solution resulted in improved performance. Arthanareeswaran et al. [10] introduced sulfonated poly(ether ether ketone) (SPEEK) to modify a cellulose acetate (CA) membrane in order to obtain a CA/SPEEK blend UF membrane with improved performance. The same authors prepared PS and SPEEK blend membranes and characterized their UF performance [11]. Susanto and Ulbricht [12] prepared polyethersulfone (PES) UF membranes by a non-solvent-induced phase separation method using different macromolecular additives: PVP, poly(ethylene glycol) (PEG) and poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) (Pluronic®). Pluronic showed the best results in water flux and rejection of BSA.

Track-etched membranes

Track-etched membranes are made from thin polymeric films of polycarbonate, poly(ethylene terephthalate [PET]) or polyimide. Tracking is produced by bombardment of the film (10–20 μm thick) with a beam of high energy nuclear particles generated in a nuclear reactor. The interaction of the high energy beams leads to an array of linear, perpendicular tracks. The subsequent etching operation in a bath containing typically a hot NaOH solution, or H_2O_2, enlarges the tracks into cylindrical pores. The range of commercially available pore diameters is approximately 0.05–20 μm. UF and MF membranes of this type are manufactured by several companies, such as Millipore, Osmonics and Whatman.

Stretching

The controlled stretching technique is based on a controlled uniaxial or biaxial stretching of the film made of a homogeneous semi-crystalline polymer such as poly(tetrafluoroethylene) (PTFE) or polyolefins. This technology produces openings in the film in the form of slits or fissures in the size range of 0.02–20 μm. The resultant filter structures often consist of nodes of solid polymer connected by filaments. Membranes made by controlled stretching of films are commercialized by companies such as Millipore, Osmonics and Sartorius.

Composite membranes

The method of producing composite membranes is based on a very thin selective layer deposited on top of a highly permeable substrate that provides the necessary mechanical support. Such methods are reported to include (1) casting of the selective layer separately, followed by lamination to the substrate; (2) dip coating of the substrate with a precursor solution, followed by curing with heat or radiation; (3) gas-phase deposition of the selective layer from a glow-discharge plasma and (4) interfacial polymerization of reactive monomers on the surface of the substrate. The defect-free characteristics of the selective layer allowed, for example, the development of virus retentive membranes (Viresolve membranes, Millipore).

Nanofibrous membranes

Electrospinning is a simple and versatile method for producing fibres with diameters ranging from several micrometres to tens of nanometres from a variety of materials [13,14]. In electrospinning, an electrostatic field is applied between a nozzle and a collector. The polymer solution is ejected from the nozzle towards the collector due to electric force. The solvent evaporates from the polymer jet, but the jet also undergoes instabilities, which are mainly responsible for the nano-sized fibre diameters. The diameter of electrospun nanofibre depends on a number of parameters: (1) properties of the polymer solution (molecular weight, concentration, surface tension, viscosity, conductivity, etc.) and the environment (temperature, humidity, pressure, etc.); (2) operational parameters including applied voltage, flow rate and distance between the nozzle and the collector. The resultant fibre diameter determines the properties of the electrospun fibre membrane, such as mechanical, electrical and optical properties.

The electrospun nanofibre membranes have found applications in filtration, drug delivery carrier, tissue engineering, wound dressing, nano-sensors and enzyme immobilization due to their features such as high bulk porosity (up to 80%) with fully interconnected pore structures, and high surface area-to-volume ratio. When used as supports for enzyme immobilization, nanofibrous membranes offer many attractive features, such as the large surface area for the attachment of enzymes, and macroporous structure for improvement of the mass transfer rate of substrate [15,16].

Morphology

The morphology of polymeric membranes is complex, diverse and irregular. Typical scanning electron micrographs of polymeric membrane surfaces are shown in Figure 1.2. Membranes prepared by casting technologies show usually one of these three morphologies: opened-cellular (a percolating foam), lacy (a fibrous network) or nodular (a layer of packed nodules) [2]. The opened-cell morphology is typical for many MF membranes, prepared by any of the three main casting technologies (air, immersion and melt casting). The pore space is formed by coalesced or communicating quasi-spherical hollow domains (Figure 1.2a). Only one side of a membrane may exhibit the opened-cellular morphology, while the opposite side has a lacy structure. Any of the three main casting technologies can generate lacy structured membranes (Figure 1.2b and c). The nodular morphology is typical for surface layers of UF membranes. With crystalline polymers, membrane structures can be obtained that consist entirely of large (5–30 μm) packed or impinged nodules. Any of the three main casting technologies can produce nodular membranes. In addition, membranes prepared by a track-etched process show uniform and cylindrical pores (Figure 1.2d).

1.1.3 Inorganic membranes

Inorganic membranes are of great interest in membrane technology because of their higher chemical, thermal and mechanical stability compared to most polymeric membranes. With inorganic membranes, processing at high temperature (up to

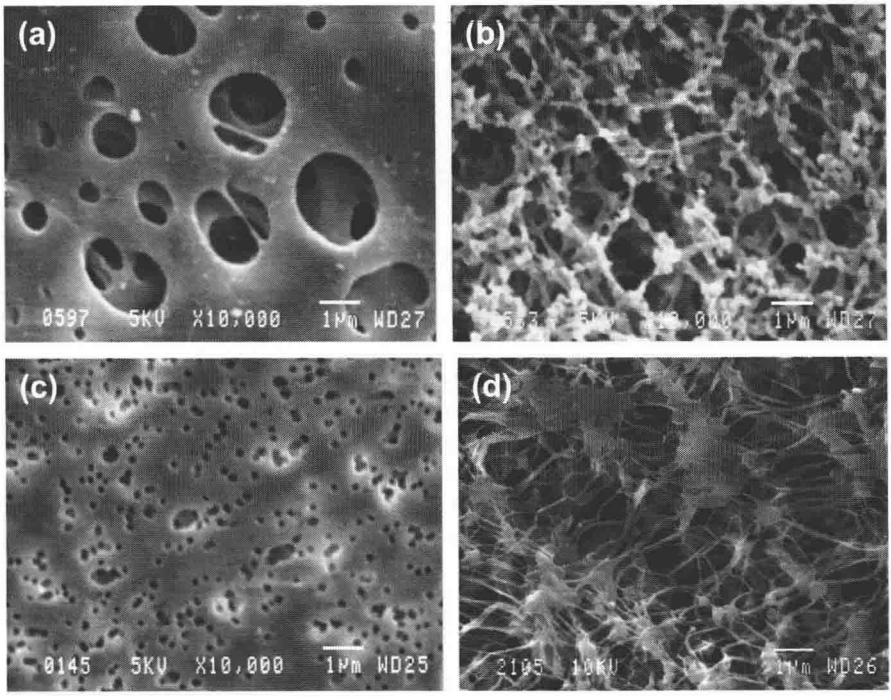

Figure 1.2 Scanning electron micrographs of the surface: (a) 0.16-μm polyethersulfone (PES) (Filtron, MA) membrane, (b) 0.22-μm mixed cellulose esters membrane (Millipore, MA), (c) 0.2-μm cellulose acetate (Sartorius, NY), and (d) 0.2-μm polycarbonate (PCTE) (Osmonics, CA) [29].
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500 °C) and extreme pH-value (pH 1–14) is possible. In addition, inorganic membranes can be cleaned with aggressive chemicals, organic solvents or hot water stream.

Inorganic membranes can be classified into (1) porous and amorphous membranes, (2) porous and crystalline membranes and (3) dense membranes. Dense inorganic membranes are usually manufactured from metals such as palladium, nickel, silver, zirconium and their alloys. Porous inorganic membranes are produced from metallic oxides (alumina, titania, zirconia), carbon, silica (glass), metals and zeolite. Ceramic membranes with a wide range of pores sizes can be obtained by using different preparation techniques including slip casting, tape casting, pressing, extrusion, sol–gel process, dip coating, chemical vapour deposition and preparation of hollow fibre ceramic membranes. In addition, anodized aluminium membranes share common features with both the track-etched films (showing arrays of straight parallel pores of uniform size) and inorganic membranes (due to the nature of membrane material). The specific techniques of sol–gel, chemical vapour deposition and