Chapter 1

Introduction and Objectives

INTRODUCTION AND OBJECTIVES

1.1 Membranes: Classification and early developments

A membrane is defined as a phase that acts as a barrier to prevent mass movement but allows restricted and/or regulated passage of one or more species through it. The primary role of a membrane is to act as a selective barrier. It should permit passage of certain components and retain certain other components of a mixture. A membrane can be gaseous, liquid or solid or combinations of these. Membranes can also physically or chemically modify the permeating species or regulate the rate of permeation. Thus membrane may be either passive or reactive depending on the membrane's ability to alter the chemical nature of the permeating species. With an ideal semi permeable membrane, only water should permeate through the membrane.[1]

Membranes can be broadly classified as natural or synthetic based on their source. Based on their mechanism of action, membranes can be classified as adsorptive, diffusive, ion-exchange or osmotic membranes. Based on ultra structure, membranes may be classified as micro porous and asymmetric. Micro porous membranes usually have uniform pore size throughout the body of the membrane. Asymmetric membranes, on the other hand, are characterized by a thin "skin" on the surface of the membrane.[2]

First synthetic membrane made of nitrocellulose was developed by Fick in 1855. Pfeffer in 1877 reported the successful manufacture of membranes by precipitating copper ferrocyanide in the pores of porcelain.

The first quantitative measurements of diffusion phenomena and osmotic pressure were made using these early membranes. The period of 1870-1920 saw a rapid development of theories of thermodynamics of solutions, most notably those of van't Hoff and his theory of dilute solutions and Gibbs, whose work led to the primary relationship between osmotic pressure and other thermodynamic properties. Membrane filters were commercially available from 1927 onwards. Up until 1945, membrane filters were used primarily for removal of microorganisms and particles from liquid and gaseous streams. There was considerable interest in developing membranes for reverse osmosis applications, especially for desalination of seawater and purification of brackish water. In 1960, Loeb and Sourirajan prepared the first asymmetric membrane by modifying cellulose acetate membrane by heating and annealing process.[1] The asymmetric nature of the membrane is characterized by a thin "skin" on the surface of the membrane, while the main body of the membrane is sponge-like in nature with extremely porous voids.

A major milestone in membrane technology occurred in the late 1970s with the development of the second generation of membranes known as composite membranes. These are also generally referred to in the trade as thin-film composite, ultra thin, or thin-layer composite membranes. Considerable progress has been made in membrane science and manufacture since the mid-1980s. There continues to be a vast amount of research underway on membrane materials. The chemical nature of the membrane governs compatibility and physicochemical properties to a large extent, while the method of preparation primarily governs the physical structure of the membrane. A better understanding of

solute-solvent membrane interactions has enabled membranes to be used in an ever-increasing variety of applications, from processing to medicine and biotechnology. It is also possible to modify the surface of the membrane to change its properties.[1] General requirement of a good membrane is high flux and high separation capability. Either property can be combined by the preparation of so called composite membranes.[3]

1.2 Polymers used for membrane manufacture

Cellulose acetate, polysulfone, polyether sulfone, polyamides, polycarbonate, nylon, polyacrylonitrile, polyvinylidene fluoride, polystyrene etc. are polymers employed for membrane manufacture. However, despite the vast number of materials that have been studied as possible membrane materials, only a few of these have succeeded commercially. For ultrafiltration and microfiltration applications, polyether sulfone is practically in a class by itself.
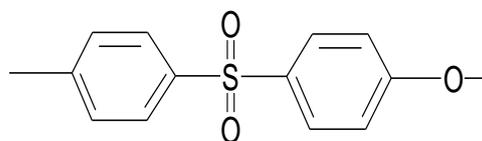

Figure1.1: Structure of polyether sulfone

The SO_2 group in the polymeric sulfone is quite stable because of electronic attraction of resonating electrons between adjacent aromatic groups. The oxygen molecules projecting from this group each have two pairs of unshared electrons to donate to strong hydrogen bonding of solute or solvent molecules. Both contribute to a high degree of molecular

immobility, producing high rigidity, strength, and creep resistance. Phenyl ether and phenyl sulfone groups have high thermal and oxidative stability, producing long-term, high temperature stability during use. The favorable characteristics of polyether sulfone membranes include:

1)   Wide temperature limits: polyether sulfone can be used up to 125^0C which is advantageous in fermentation and biotechnology where sterility is maintained by heat treatment at 121^0C.

2)   Wide pH tolerances: polyether sulfone can be exposed to pH from 1 to 13.

3)   Good chemical resistance to hydrocarbons, halogenated hydrocarbons, alcohols and acids.

4)   Easy to fabricate membranes in a wide variety of configurations and modules

1.3   Composite membranes

Primarily developed for reverse osmosis and nanofiltration applications, composite membranes have a thin dense polymer skin formed over a micro porous support film. By this definition they are direct descendants of the Sourirajan asymmetric membrane structure but differ from it in the manner in which the dense skin is formed.[1] While integrally skinned membranes are made in a one step procedure, composite membranes are made in two or more steps. The layers underneath the skin may consist of voids which serve to support the skin layer. Rejection occurs only at the surface and the retained macromolecules do not enter

the main body of the membrane. As a result, pore blocking rarely occurs with composite membranes. In the case of micro porous membranes, having uniform pore size throughout the body of the membrane, particles with approximately the same size as the pores may penetrate partially into the pores and block them. Composite membranes have the additional advantage that only a small amount of possibly expensive skin materials needs to be used.[4]

There are four general methods of forming composite membranes:

1)   Casting the ultra thin barrier layer separately, followed by lamination to the support film.

2)   Dip-coating of a solution of the polymer onto a microporous support and drying, or dip-coating a reactive monomer or pre-polymer solution followed by curing with heat or irradiation.

3)   Gas-phase deposition of the barrier layer from glow-discharge plasma.

4)   Interfacial polymerization of reactive monomers on the surface of the support film.[1]

Composite membranes are available in spiral, plate and tubular module configurations.

1.4   Membrane separation processes

Filtration is defined as the separation of two or more components from a fluid stream based primarily on size differences. In conventional usage, it usually refers to the separation of solid immiscible particles from liquid or gaseous streams. Membrane filtration extends this application

further to include the separation of dissolved solutes in liquid streams and for separation of gas mixtures.[5] The major membrane separation processes-reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF), microfiltration (MF), dialysis, electrodialysis and pervaporation- cover a wide range of particle/molecular sizes and applications. Among membrane separation processes, the distinction between the various processes is somewhat arbitrary. The characteristics of various membrane processes are given in table 1.1.

Table 1.1 Characteristics of membrane processes

Process	Driving Force	Retentate	Permeate
Osmosis	Chemical potential	Solutes, water	Water
Dialysis	Concentration difference	Large molecules, water	Small molecules, water
Microfiltration	Pressure	Suspended particles, water	Dissolved solutes, water
Ultrafiltration	Pressure	Large molecules, water	Small molecules, water
Nanofiltration	Pressure	Small molecules, divalent salts, dissociated acids, water	Monovalent ions, undissociated acids, water
Reverse osmosis	Pressure	All solutes, water	Water
Electrodialysis	Voltage/current	Nonionic solutes, water	Ionised solutes, water
Pervaporation	Pressure	Nonvolatile molecules, water	Volatile small solutes, water

Osmosis is the transport of solvent through a semi permeable membrane from the dilute solution side to the concentrated solution side of the membrane. It is driven by chemical potential differences between the water on either side of the membrane. The common laboratory technique of dialysis, on the other hand, is primarily a technique for purifying macromolecules, such as desalting of proteins, and the primary driving force is the difference in concentration of the permeable species between the solution in the dialysis bag and outside the bag. Electrodialysis relies primarily on voltage or electromotive force and ion-selective membranes to effect a separation between charged ionic species.

The real value of membrane separation is that they permit separation of dissolved molecules down to the ionic range, provided, the appropriate membrane is used. The advantage of membrane separations lies in their relatively low energy requirement as they do not involve phase transitions unlike conventional processes such as distillation, extraction and crystallization.

Membrane filtration is an attractive separation process, as it is usually performed under gentle conditions. Membrane processes have found wide application in especially the biochemical, food and beverage industry. Examples can be found in the treatment of waste water, desalination processes and concentration of protein solutions.[1-4] Separation of different components is achieved by a combination of sieving, hindered transport through the narrow membrane pores and other specific interactions between the components and the membrane material such as adsorption, electrical interactions etc.. The process can be driven

by different forces: a concentration difference on both sides of the membrane (dialysis), a pressure difference over the membrane (ultrafiltration, reverse osmosis) or by an externally applied electrical field (electrodialysis).

1.5 Pressure driven membrane filtration

The different pressure driven membrane processes are generally classified based upon the size of the solutes that the membrane filters reject. Various pressure driven membrane separation processes are listed in table 1.2.

Table 1.2 Characteristics of pressure driven membrane processes

Process	Applied pressure	Size of retentate	Applications
Microfiltration (MF)	below 20 psi	Particles in micron range (0.1μm-5 μm)	Separating suspended particles from dissolved substances e.g. starch, pollen, latex etc.
Ultrafiltration (UF)	20 - 70 psi	Retains macro-molecules of (10-200A^0) range	Purification, concentration and fractionation of macro-molecules
Nanofiltration (NF)	150 - 450 psi	5- 10A^0	Separation of ions, dissociated acids from undissociated one
Reverse osmosis (RO)	500-1500 psi	1-5A^0	Dewatering

1 atm = 1.01325 bar = 14.69 psi = 1.01325 x 10^5 Pa = 760 mm Hg

What distinguishes the more common pressure driven membrane processes-microfiltration, ultrafiltration, nanofiltration and reverse osmosis is the application of hydraulic pressure to speed up the transport process. In its definition, reverse osmosis retains all components other than the solvent (e.g., water) itself, while ultrafiltration retains only macromolecules or particles larger than about 10-200A^0. Microfiltration, on the other hand, is designed to retain particles in the micron range, that is, suspended particles in the range of 0.1 μm to about 5 μm. Thus, in its broader sense, reverse osmosis is essentially considered to be a dewatering technique, while ultrafiltration can be looked at as a method for simultaneously purifying, concentrating and fractionating macromolecules or fine colloidal suspensions.

Microfiltration is used mainly as a clarification technique, separating suspended particles from dissolved substances, provided the particles meet the size requirements of microfiltration membranes.

Nanofiltration (NF) is relatively a new process that uses charged membranes with pores that are larger than RO membranes, but too small to allow permeation of many organic compounds such as sugars. They also have a useful property in that they can separate dissociated forms of a compound from the undissociated form, e.g., organic acids such as lactic, citric, and acetic acids pass through NF membranes easily at low pH but are rejected at higher pH when in their salt forms. All these processes are driven by an externally applied pressure difference. However, during the process concentration differences and, in case of electrically charged components, an electrical field may develop. These can strongly influence the filtration process.

1.6 Ultrafiltration (UF)

Membrane ultrafiltration (UF) is a pressure-modified, convective process that uses semi permeable membranes to separate species in aqueous solutions by molecular size, shape and/or charge. Ultrafiltration involves pumping the feed solution under pressure over the surface of a membrane. The pressure gradient across the membrane would force solvent and smaller species through the pores of the membrane, while the larger molecules would be retained. Because UF deals with the separation of fairly large molecules, such as proteins, starch, gum, paints, pigments, latex particles etc., the osmotic pressure involved in UF processes are fairly low.

$$\text{Osmotic pressure, } \pi = iCRT/\,M$$

Where, i: number of ions for ionic solutes, C: concentration of solute, R: ideal gas constant, T: absolute temperature, M: molecular mass of solute.

From the above equation, it is clear that UF requires relatively low pressure compared to nanofiltration (NF) and reverse osmosis (RO).

1.6.1 Separation mechanisms in ultrafiltration

The rejection of solutes by the UF membrane is determined by different mechanisms:

o Distribution of components between liquid phase and membrane phase

o Interaction of solutes with the wall

o Interaction of solutes with other components in the solution

These mechanisms are briefly discussed below.

Distribution

J. D. Ferry showed how the distribution of solutes over liquid and membrane phase was determined by geometrical factors.[6] He considered the situation of a spherical solute rejected by a membrane with cylindrical pores. The situation under consideration is visualized in fig.1.2a. It is shown that a solute that is not much smaller than the size of the pores cannot be distributed evenly over the whole pore cross sectional area. Its centre cannot get closer to the pore wall than to a distance equal to the radius of the solute. So the actual space that is available to the large solute is bounded by the dashed line in fig.1.2a. If a solute approaches the pore with its centre being outside this area the solute collides against the outer membrane surface and will be rejected. Smaller molecules (e.g. the solvent) can get much closer and can thus occupy almost the whole pore cross section.
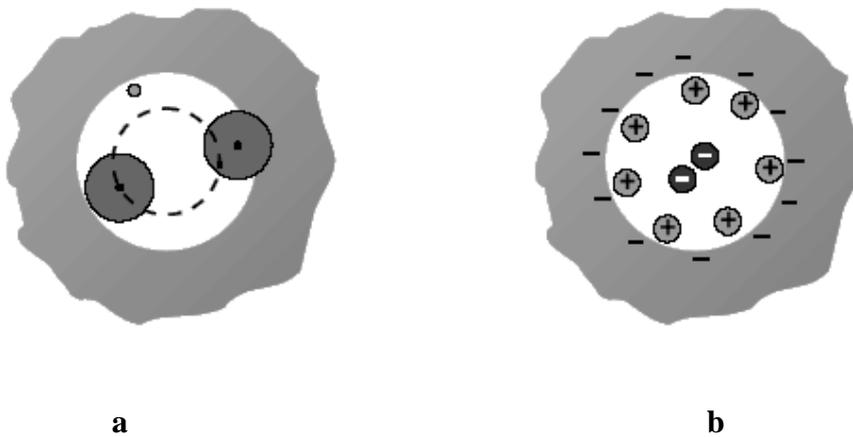

a   **b**

Figure 1.2: a) sterical exclusion; **b)** Donnan exclusion

So a jump in the concentration is observed going from outer liquid to the solution inside the pores. This concentration jump can be expressed in terms of the cross sectional area the solute can occupy compared to the total pore cross sectional area.[6] Specific interactions between solute and membrane material may also be very important. Examples are preferential adsorption of components on the pore surface or electrical interactions between a charged pore surface and ionic components. The latter has been described by Donnan[7] and is visualised in fig.1.2b. Membrane material is often weakly charged, due to specific adsorption of small ions onto the surface. If a membrane is negatively charged and a solution of ionic components is filtered, a concentration jump is induced due to electrical interaction with the pore wall. Positively charged ions are attracted by the membrane and will obtain a higher concentration inside the pore than in the outer liquid. Negatively charged ions are repelled however and will have a low concentration inside the pore. Besides the concentration jumps also a jump in electrical potential is expected. In the case of a negatively charged membrane this potential in the pore will be lower (or more negative) than in the outer liquid.

Interaction with wall

Inside the membrane, the separation process continues. Larger solutes will experience friction with the pore wall, more than smaller components. Where the smaller components pass relatively freely through the pores due to the pressure gradient, the larger components are retarded and separated from the fast moving smaller ones. The hindrance that components experience from the pore wall can be due to geometrical factors or to specific interactions such as adsorption and electrical effects.

Interaction with other solutes

Inside the pore, a velocity difference is induced between the retarded larger molecules and the fast moving smaller components. The latter, therefore, have to slip past the retarded components and will experience friction with them. Conversely, the larger molecules are dragged along by the smaller components. The rejection of the large solute is determined by the balance between these forces, in addition to the distributions at the entrance and exit of the membrane.

1.6.2 Common terms used in ultrafiltration

Pore size and pore size distribution: Pore size is usually expressed as pore diameter which can be determined by electron microscopy. In UF and MF membranes all the pore diameters may not be identical, but rather a distribution of pore sizes.

Pore density: Number of pores per unit membrane surface area.

Concentration: Enrichment of a solution by solvent removal.

Permeate: The solution passing through the membrane, containing solvents and solutes not retained by the membrane.

Retentate: That portion of the feed solution that is retained on the high pressure side of the membrane.

Rejection: The fraction of solute held back by the membrane. It is expressed in permeate concentration of the solute compared to the concentration in the feed.

$$\text{Rejection} = 1 - C_p/C_f$$

Where, C_p = concentration of permeate

C_f = concentration of feed

Yield: Amount of species recovered at the end of the process as a percentage of the amount present in the sample.

Flux: Amount of fluid passing through the membrane i.e., the volumetric rate of flow of permeate through the membrane. It is usually given in terms of volume/unit membrane area/unit time, e.g., litre/(m^2 hour) (LMH) or gallon/(ft^2 day) or m^3/(m^2 day). Pressure, feed concentration, temperature and turbulence in the feed channel are four major operating parameters that affect the flux.

Molecular weight cut off (MWCO): Molecular weight cut off (MWCO) is the molecular weight of a hypothetical globular solute (proteins) that will be 90% rejected by the membrane. MWCO is used to describe the potential separating capability of a UF membrane. It gives an idea about the size of a protein that would be almost completely retained by a particular UF membrane; a 10,000 MWCO membrane will normally reject 90% of molecules with a molecular weight of 10,000 Da. Since rejection is actually a function of physical size, shape, and electrical characteristics of a molecule, the MWCO is only a convenient indicator. Linear molecules like polysaccharides will tend to slip through a membrane that would reject globular molecules of the same molecular weight.

Concentration polarization: Ultrafiltration is driven by a pressure gradient. This induces convective transport of bulk liquid to the membrane interface. At this interface some of the solutes are (partly) rejected. Their concentrations at the interface build up until they get high enough to give a diffusive flux back to the bulk solution. This back diffusion is balanced by the convective transport of solutes to the interface and the flux of the solutes through the membrane. This build up of solutes at the membrane interface is called concentration polarization. Due to concentration polarization, solutes not passing through the membrane will accumulate on the membrane surface, causing both an increased resistance to the solvent transport or an increase in local osmotic pressure (either of which may decrease flux) and possibly affect the sieving characteristics of the membrane. Concentration polarization can be minimized by uniform stirring.

Fouling: The term fouling refers to the irreversible decline in membrane flux owing to deposition and accumulation of submicron particles within the pores of the membrane. The important factors affecting fouling are hydrophilicity, surface topography and pore size of the membrane. The more hydrophilic the membrane, it is less prone to fouling. If the membrane surface is smooth, the extent of fouling is less. Fouling can be minimized by pretreatment of membrane surface or passivating the membrane by blocking the active binding site of the membrane. Surface modification of membrane by cross linking, grafting etc. can also reduce membrane fouling considerably.

Ultrafiltration is a continuous molecular separation process that does not involve a phase change. This makes UF an excellent tool for food, pharmaceutical and biological processing. There is minimal change in the micro-environment during UF, i.e., no change in pH or ionic strength, a particular advantage when isolating and purifying proteins. In UF, the entire membrane-permeating species pass along with the solvent, independent of their concentration.

Several recent studies suggest that polyelectrolyte multilayer (PEM) films prepared by the alternate deposition of cationic and anionic polyelectrolytes on suitable substrates are promising candidates for "skin" layers in composite membranes for NF and RO processes.[8-12] NF is used for applications such as water softening, brackish water reclamation, and dye-salt separations. Layer-by-layer deposition of anionic and cationic polyelectrolytes readily converts polymeric ultrafiltration membranes into materials capable of nanofiltration.[13] Depending on the polyelectrolytes employed, PEM membranes can remove salt from sugar solutions, separate proteins, or allow size-selective passage of specific sugars. Before going into details of PEM, a brief discussion on polyelectrolytes and their properties are given below.

1.7 Polyelectrolytes

A polyelectrolyte is a macromolecular species which dissociates into a highly charged polymeric molecule in water or other ionizing solvents. These macromolecules carry covalently bound charged groups, (cationic or anionic) and low molecular counterions.[14-16] However, every neutral polymer can be transformed into a polyelectrolyte by covalently

attaching an appropriate number of ionic groups. Polyelectrolytes are mostly produced by free radical, ionic, stepwise polymerization methods as well as by chemical modification of neutral polymers. Many biological molecules are polyelectrolytes. For instance, polypeptides (thus all proteins) and DNA are polyelectrolytes.[17] Both natural and synthetic polyelectrolytes are used in a variety of industrial applications.

1.7.1 Classification of polyelectrolytes

Acids are classified as either weak or strong (and bases similarly may be either weak or strong). Similarly, polyelectrolytes can be divided into weak and strong types. A strong polyelectrolyte is one which dissociates completely in solution for most reasonable pH values. A weak polyelectrolyte, by contrast, has a dissociation constant (pk_a or pk_b) in the range of 2 to 10, meaning that it will be partially dissociated at intermediate pH. Thus, weak polyelectrolytes are not fully charged in solution, and moreover their fractional charge can be modified by changing the solution pH, counter ion concentration, or ionic strength. The physical properties of polyelectrolyte solutions are usually strongly affected by this degree of charging. Since the polyelectrolyte dissociation releases counter-ions, this necessarily affects the solution's ionic strength, and therefore the Debye length.[18] This in turn affects other properties, such as electrical conductivity.

Based on the charge carried by the polymer back bone, polyelctrolytes are classified as anionic and cationic. The back bone of anionic polyelectrolytes is negatively charged. Examples are poly(styrene sulfonic acid) sodium salt (PSS), poly(acrylic acid) sodium salt (PAA),

poly(methacrylic acid) sodium salt (PMA), etc. Cationic polyelectrolytes have positively charged back bone and some of the examples include poly(allylamine hydrochloride) (PAH), chitosan hydrochloride (CHI), poly(diallyl dimethylammonium chloride) (PDADMAC) etc. Polyelectrolytes are divided into linear polyelectrolytes and branched polyelectrolytes on the basis of their molecular architecture. For linear polyelectrolytes, the backbone is linear in a topological sense, but the actual shape of the polymers in solution is quite different.[19] Due to their simple architecture they are used as model molecules in many investigations.[20]

Branched polyelectrolytes are chains which are not linear in a topological sense. Various branched structures are known such as comb, stars (regular and irregular), H-shaped, super H-shaped polymers and dendrimers.[21,22] The dendrimers are macromolecules consisting of a polyfunctional central core covalently linked to layers of repeating units (generations) and a number of terminal groups. A special class of polyelectrolytes is called "polyampholytes", macromolecules which carry both anionic and cationic groups covalently bound to the polymer chain.[23]

1.7.2 Polyelectrolyte conformation

In water the polyelectrolyte is dissociated into macro ions (charged polymer) and small ions (counter ions). The amount of macro ions and counter ions has to be equal in order to satisfy electrical neutrality of the solution. The charges along the chain generate long range electrostatic interactions along and between polymer chains. The electrostatic interaction depends on the effective charge density per chain. The charge density (formal charge density) of polyelectrolytes is partially screened by

counter ions. The behavior of the counter ions in the vicinity of polyelectrolytes chains cannot be described satisfactorily by the Debye-Hückel theory because of the presence of strong electrostatic fields in the vicinity of the chains, even in dilute solution.[24]

Energetically, it is much more favorable that a fraction of the counter ions is condensed at chains. This so called counter ion condensation and has been explained by Manning.[25] The physical background of the counter ion condensation is related to the competition between the gain of energy in the electrostatic interaction and a loss of entropy in the free energy. The first theoretical model which describes conformation of polyelectrolyte was developed by Flory.[26] This model describes conformation of a single polyelectrolyte chain. The model is not a very realistic one because polyelectrolytes are hydrated in water, and the structure of the hydration shell as well as the interaction between polyelectrolyte and solvent depends on the local solvent structure. In practice it is difficult to measure the properties of one chain in solution since polyelectrolyte chains start to overlap at very low concentrations. The overlapping of chains and the electrostatic interactions between them cause the appearance of mesoscopic ordering (structuring) in solution.

A polyelectrolyte in low ionic strength solutions tends to be in extended and uncoiled form due to the intermolecular repulsion of the unscreened charges on each monomeric unit of the macromolecule. On the other hand, when the ionic strength of the solution is increased, a polyelectrolyte tends to become thicker and more coiled due to the screening effects of polymer charges by the excessive presence of smaller salt counter

ions in solution. The degree of charge screening of polyelectrolyte is an important factor in tuning the thickness,[27] uniformity,[28] stability,[29-31] swelling,[32-35] and permeability[36-43] properties of synthetically modified[44-48] as well as naturally occurring polyelectrolytes[49-52] and their corresponding layer-by-layer ultrathin film composites or assemblies.[53-62]

1.7.3 Polyelectrolytes: Applications

In addition to their essential functions in human physiology and cellular mechanisms in the form of proteins, polypeptides and nucleic acids, polyelectrolytes have found a number of important applications in the major fields of science and engineering such as chemistry, physics, biology, chemical and material engineering. Their applications in chemistry are mainly centered at the interface of polymer, materials, colloids, surface and analytical chemistry. Polyelectrolytes have been used in health and personal care industry as thickening agents, rheology modifiers,[63] viscosity enhancers for shampoos, conditioners, deodorants and body lotions. They have also been used in water treatment, waste treatment,[64] and the pulp and paper industry as retention aids as well as flocculating and coagulating agents for solid-liquid separations.[65] Recently, Shiratori and Sato have designed a polyelectrolyte thin film wrap made of chitosan and a polyelectrolyte containing a bamboo extracted enzyme which is effective in preventing decomposition and ripening of fresh fruits.[66] Above all, assembling these polyelectrolytes into ultrathin film composite membranes is getting significant attention and interest recently.

1.7.4 Polyelectrolyte complexes (PEC)

One of the remarkable properties of polyelectrolytes is their complexation capability when mixed with other polyelectrolytes of opposite charges. Polyelectrolyte complexes and polyelectrolyte multilayers share similar physical and chemical properties in terms of their internal structure, physical structure and morphology.

When two relatively high molecular weight polymers are mixed together in dilute aqueous solution, a precipitate is formed which contains almost exact stoichiometric proportions of the component polyions. The precipitate contains none of the counter ions initially associated with the individual polymers. This is referred to as an "intrinsically compensated" state in which all the charged units of the polymer chains are internally compensated by oppositely charged units from the other polyelectrolyte. In the presence of external salt solutions, salt ions tend to enter the bulk of the complex and transform it into an "extrinsically compensated" state. Similar properties are also shared by polyelectrolyte multilayer which are essentially a form of somewhat ordered, automated and reproducible deposition or build up of polyelectrolyte complexes on different substrates. Polyelectrolyte complexes, despite their unfavorable properties such as infusibility, insolubility in common solvents, high dielectric constant etc., find application in dialysis, ultrafiltration and fuel cell membranes. Because of the sequential adsorption approach, polyelectrolyte multilayer forms ultra thin, uniform, and smooth films.

1.7.5 Layer-by-layer assembly (LBL) of polyelectrolytes

The LBL assembly technique was first mentioned by Iler in 1966.[67] In 1991, however, Decher and Hong expanded this technique and brought it to the forefront of materials science and engineering.[16] The layer-by-layer (LBL) assembly technique involves alternate dipping of a substrate or a solid support in two oppositely charged polyelectrolytes along with water rinse steps in between to remove any extra material that is loosely bound to the surface. Thus, one can build as many layers as needed depending on the particular type or requirements of the experiment. Ultrathin films could be built offering high flux and selectivity for membrane separations, filtrations and purification applications. Thicker membranes could also be built offering hydrophobic coatings, hydrophilic coatings or anti-corrosive coatings. Multilayers reported in the literature had thickness that ranged between 10 nm and 10 μm.

A cleaned and functionalized substrate with net positive outer surface charge is immersed in the solution containing the anionic polyelectrolyte, and a monolayer of anionic polymer molecules with fixed negative functional groups along the polymer backbone are adsorbed (step 1). (If the surface charge of the substrate is negative, it is first dipped in cationic polyelectrolyte). The molecular order of such individual monolayers is nearly perfect, for the molecules automatically arrange themselves in a way so that the minimum total system energy is obtained. Since the adsorption is carried out at relatively high concentrations of polyelectrolyte, a number of ionic groups remain

exposed to the interface with the solution and thus the surface charge is reversed. After rinsing thoroughly in pure water to remove the loose molecules that may be held on the surface by weak van der Waals force (step 2), the substrate is immersed in the solution containing the cationic polyelectrolyte. Again a monolayer is adsorbed but now the original surface charge is restored (step 3). The surface is again rinsed with pure water to remove loosely bound molecules (step 4). By performing steps 1-4, one bilayer (bl) is formed.[16] By repeating the four steps in a cyclic fashion (1, 2, 3, 4, 1, 2, 3, 4…), alternating multilayer assemblies of both molecules are formed on the support. This process of multilayer formation is based on the attraction of opposite charges, and thus requires a minimum of two oppositely charged molecules.

Consequently, one is able to incorporate more than two molecules into the multilayer, simply by immersing the substrate in as many solutions of polyelectrolytes as desired, as long as the charge is reversed from layer to layer. In addition, individual nanoparticles may be incorporated into any or all of the monolayers, allowing wide design opportunities for thin films with specific or multifunctional properties.

Polyelectrolyte complexation is the driving force for multilayer formation. However, depending on the chemical nature of the polyions employed for deposition, electrostatic contribution should vary and other interactions such as van der Waals, hydrogen bonding or charge transfer may also be involved.

1.7.6 Advantage of LBL assembly technique

The LBL technique is superior, in many respects, to other deposition techniques that are reported in the literature such as vacuum deposition, solvent casting, spin coating or Langmuir-Blodgett (LB) deposition.[68] Vacuum deposition requires specialized expensive instrumentation and specific types of substrates. Solvent casting produces thick and non-uniform films. The earliest technique used to fabricate multilayer films was invented by Langmuir and Blodgett to make LB films. LB films are highly ordered and have uniform controlled thickness. However, they have several limitations. The requirements for substrates are stringent; they must be smooth, homogeneous and have regular shape.[68] Further more LB fabrication requires expensive equipment. LB multilayers have limited stability against solvents and thermal treatments. Also, it is a slow technique. On the other hand, the LBL technique is a very robust, reproducible, durable, cost effective and environmental friendly method. It is done at room temperature, so there is no need for any vacuum equipment or special instrumentation. It can be used to assemble various types of materials, polymers, composites, clay, proteins, dyes, carbon nanotubes or nanoparticles. Also, it can be coated on various kinds of substrates such as silicon, gold, platinum, plastics, glass, quartz, stainless steel, clay, nanoparticles, blood cells and colloidal particles. The whole process can be automated making the coating procedure less time consuming and applicable for commercial purposes where productivity and labour are major problems. Above all, this technique offers very precise control over thickness and unprecedented uniformity of the coating down to the sub nanometer scale.

The LBL procedure can be applied for the fabrication of multicomposite films ie, nanoscopic assembly of hundreds of different materials in a single device using environmental friendly, ultra-low cost techniques. The materials can be small organic molecules[69-73], inorganic compounds[74-77], macromolecules[78-95], biomacromolecules such as proteins or DNA[96-114], or even colloids (metallic or latex particles)[115,116]. The technique can be applied to solvent accessible surfaces of almost any kind and any shape; the more interesting ones are microcapsules, colloids or biological cells.[117-124]

Given the large set of materials which are easily incorporated into multilayer films, layer-by-layer deposition is a rather general approach for the fabrication of complex surface coatings. It is possible to coat almost any solvent-accessible surface starting with sub-micron objects up to the inside of tubing or even objects with a surface of several square meters. Like a chemical reaction, the precise structure of each layer depends on a set of control parameters such as concentration, adsorption times, ionic strength, pH, temperature, nature and concentration of added salt, rinsing time, humidity of the surrounding air, drying etc. While the LBL technique generally works very well due to the fact that the processing window is rather large, it is highly recommended to keep the deposition conditions as constant as possible in order to get highly reproducible results.

Recent experiments on polyelectrolyte multilayers formed by consecutive absorption of negatively charged and positively charged polyelectrolytes, have shown that, upon addition of a new layer, the number of charges carried by the incorporated polymer is large enough to

neutralize the charge of the previous layer and even to invert the sign of the zeta potential.[27,32] Neutron experiments also clearly indicate that the newly incorporated layer strongly interpenetrates the previous layer and even the one before.[60] But there is no complete mixing between the layers, each layer keeping its identity. Often it is said that polyelectrolyte multilayer films are independent of the underlying substrate. Since polyanion and polycation adsorption is repeated consecutively, after a few layers, the structure and properties of each layer are governed by the choice of polyanion/ polycation pair and by the deposition conditions and that the influence of the substrate is typically lost after a few deposition cycles.

1.7.7 Polyelectrolyte multilayers: Structure and composition

The adsorption of polyelectrolytes on to an oppositely charged surface can be considered to be an ion exchange phenomenon, where charged segments replace small (salt) ions compensating the surface charge as shown in the following equation.[27]

$$Pol^-M^+_{(m)} + Pol^+A^-_{(aq)} \rightarrow Pol^- \, Pol^+_{(m)} + M^+_{(aq)} + A^-_{(aq)}$$

Pol$^-$ and Pol$^+$ are charged polymer segments, Pol$^-$ Pol$^+$ is an ion pair, and M$^+$, A$^-$ are salt counter ions. The subscript 'm' refers to a region close to the surface.

The simplest structural model for subsequently deposited layers is a stack of well separated layered building blocks with defined interfaces between subsequent layers. There is evidence that the multilayer structure of the first layers differs from that of the last deposited layers.[84] This was described by different regions in a multilayer system, where a precursor

zone of about six layers is affected by the substrate properties, then in a core zone the growth is very regular and all charges are compensated.[84] Finally, in an outer zone the chains have a more loop-like conformation, and this is the zone in which the excess charge of the last layer is distributed. Experimental evidence for different zones was given by diffusion measurements of probe molecules through multilayers. An outer zone of about 10 nm, diffusion coefficients were enhanced by two orders of magnitude, which was attributed to a less dense complexation of the multilayers.[14] Inspired by their strong hydration and by their swelling properties, PEMs can be viewed as rather soft materials, i.e. as dense hydrogels or as cross-linked gels.[42] In such a picture, the cross-linking density, or the mesh size, is controlled by the charge distribution along the chain. Such models are very successful in explaining the permeabilities of different PEMs for small molecular probes. On the other hand, PEMs have been described as a glassy, quasi-frozen state, which suggests a rigid and compact layer structure.[125] In a phase diagram the glassy state of the layered complex is considered in equilibrium with a solution complex phase.[126] This phase diagram describes PEMs as a glassy state at low ion concentration, 'liquid-like' at higher ion concentration, and uncomplexed at very high ion concentration, making the assumption of thermodynamic equilibrium.

Multiple electrostatic bonds causing a strong attraction are generally discussed as being responsible for the formation and stability of PEMs. However, in order to explain the phenomenological behaviour of layer formation, not only the Coulomb attraction, but additional contributions to the free energy of complexation have to be considered.

These involve secondary interactions, such as hydrophobic attraction of the chains, and also entropic contributions, such as the entropy increase of counter ions as a consequence of their release. Furthermore, solvent molecules from the hydration shell can be released, further increasing the entropy on adsorption.

1.7.8 Kinetics of multilayer formation

The complexation of polyions occurs simultaneously with the adsorption of each layer, due to the interactions with the charges of the previously deposited layer. The extent of interdigitation is determined during the process of the adsorption of each layer, whereas internal chains in PEMs are generally assumed immobile. For controlling the inner structure, therefore, the adsorption process of each single layer is of great interest. One way of viewing the adsorption-complexation process is a time dependent process at a polymer concentration large compared to the saturation concentration. In kinetic studies of multilayer formation the timescales vary greatly.[58] A common feature is that the kinetics appears to be a two-step process with a fast adsorption occurring within seconds to minutes, and a much slower process, which can occur as slowly as over hours. The first kinetic step involves the transport of chains to the surface and a fast mass deposition.[27] The rate of deposition is determined by the diffusion of polyion coils. Then at the surface chain rearrangements take place and mass deposition continues at a slower rate, until saturation is reached. In this second kinetic step, slow chain rearrangements enable the diffusion of segments into the inner regions of the previously deposited layer. By mixing of positive and negative segments, the irreversible complexation of charges is finally achieved.[54]

The multilayer formation is apparently affected by both the kinetics and equilibrium properties. The transport of polyions to the surface takes place until a sufficiently repulsive ζ-potential is built up. Slower chain rearrangements allow for equilibration and the adsorption of additional chains. In this step, it is the flexibility of the last layers ensuring the interdigitation, and thus complexation. In conclusion, it is the repulsive interaction which limits further adsorption, while the efficiency of interdigitation and complexation determines the adsorbed amount in each layer. Layered complexes are not in thermodynamic equilibrium, since their formation never occurs spontaneously in solution, but only following a given preparation protocol.

Therefore, the structure of PEMs has to be considered as partly determined by the preparation history, and partly by equilibrium aspects. The outer part of a multilayer arrangement contains charged segments, which are compensated by counter-ions. This also has implications for the dynamics; while the inner layers are very stable and rigid due to the motional restriction of the multiple electrostatic interactions, the outer layer part is less dense and much more mobile.

1.7.9 Monitoring multilayer buildup

Ex-situ characterization

The easiest way to follow multilayer buildup is by UV-vis spectroscopy, which works for all coloured materials. Equivalent to measuring the optical absorbance, one can also determine the film thickness by ellipsometry and X-ray reflectometry.

In-situ characterization

Even though the characterization methods described above are straightforward and widely available, they require one to interrupt the deposition process to take the measurement. Not only are the measurements an interruption, they also have to be taken in the dry, which may not be desirable in some cases. Typical in-situ methods include quartz crystal microbalance(QCM)[43,127,128], surface plasmon spectroscopy[129,130] optical waveguide lightmode spectroscopy (OWLS)[61,88,131], scanning angle reflectometry (SAR)[85,107,132], ellipsometry[45,50,59,133], in-situ atomic force microscopy (AFM)[32,61], attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR)[111,134], X-ray and neutron reflectometry. [60,135,136]

1.7.10 Polyelectrolyte multilayer films for membrane applications

Ultrathin films assembled from alternate adsorption of polycations and polyanions onto a charged substrate has attracted much attention in recent years due to their facile fabrication, high versatility, and multifunctionality. The application of this technique has expanded into a number of areas, including biosensor surface modification,[137] gas permeation,[138] alcohol-water pervaporation,[139] nanofiltration,[140] and patterning.[141] Gold nanorods coated with multilayer polyelectrolyte are reported as a biocompatible optical probe with capability for dark-field imaging and for electron microscopy of cancer cells.[142]

Polyelectrolyte multilayers have also proven applications in several areas, including light emitting devices,[143] nonlinear optics,[144]

sensors,[116,145,146] enzyme active thin films,[147,148] conductive coatings,[90] and analytical separations.[149] PEMs have also been employed as selective membranes for the separation of gases[150] and dissolved species.[151-153] As a result of their ease of use and water compatibility, PEMs have been investigated as surface modifying agents for protein interactions.[110,113,154,155] Interactions of more biocomplex systems, such as cells, with PEMs, a topic of more recent interest, were studied, [113] and different designs for cytophilic/cytophobic PEMs have been proposed.[156]

The exceptional thinness of polyelectrolyte multilayer membranes (PEM) provides high flux for selective separations. PEMs are better suited for surface modification, where uniformity is at a premium and a minimal thickness of the film is advantageous. The first report of polyelectrolyte multilayer thin film assemblies as membranes for separations came in 1997. Levasalmi and McCarthy prepared asymmetric gas separation membrane by the deposition of PAH/PSS multilayers on surface-modified poly(4-methyl-1-pentene) film.[157] In 1998, Krasemann and Tieke reported the use of polyelectrolyte thin films as membranes for pervaporation applications as well as gas separations.[158] They observed that a 20 bilayer (bl) poly allylamine hydrochloride/polystyrene sulfonate (PAH/PSS) multilayer film on poly acrylonitrile/polyethylene terephthalate (PAN/PET) membranes were sufficient to reduce the argon flow to 7% of initial value. Bruening et al. discussed the preparation of high flux composite membranes by the deposition of polyelectrolyte multilayers on porous alumina supports. Selectivity values up to 310 for chloride over ferricyanide were obtained.[159]

Since 2000, a significant number of studies have been reported using the layer-by-layer assembly technique of polyelectrolytes to assemble thin film coatings for membrane separations.[160-170] Most works are concerned with separation of ions[7,160-164], gases [165-167] and small neutral molecules[48,168-170] under nanofiltration conditions. The high selectivities, water fluxes, and ion rejections of PSS/PAH membranes make them potentially attractive for applications in water and salt purification. It is reported that membranes composed of 4.5-5 bl PSS/PAH on porous alumina exhibits $MgSO_4$ rejections of 96% and a Cl^-/SO4$^-$ selectivity of 80.[7] Alternating electrostatic deposition of poly(amic acid) salts and poly (allylamine hydrochloride) (PAH) followed by heat-induced imidization yields ultrathin, high-flux, gas-selective polyimide membranes on porous alumina supports.[166] The transport of neutral molecules through multilayer polyelectrolyte films shows significant size-based discrimination among organic analytes. Simple 7 bl PSS/PAH films deposited on porous alumina exhibited a glucose/sucrose selectivity of 150 under nanofiltration conditions.[48] The high rejections of glucose and sucrose by PSS/PAH membranes along with a high water flux suggest that these materials may be applicable in salt/sugar separations or removal of organic pollutants from water.

1.8 Objectives

Polyelectrolyte multilayers (PEM) prepared by the alternate deposition of cationic and anionic polyelectrolytes on suitable substrates find several applications including surface modification, analytical separations, sensors etc. Several factors such as pH, ionic strength, number of bilayers and nature of polyelectrolyte have been shown to

affect the permeability and the selectivity of the assembled multilayer membranes. By varying these factors it is possible to fine tune the permeation and selectivity of multilayer membranes. Depending on the polyelectrolytes employed, PEM membranes can remove salt from sugar solution, allow size selective passage of specific sugars, amino acids and proteins.

There are only a few reports available on the surface modification of membranes by multilayer assembly.[11] One of the main objectives of the present study is to investigate whether the sieving characteristics of microfiltration membranes can be modified to render it ultrafiltration characteristics by forming a few polyelectrolyte multilayers on it. Different multilayer systems are prepared by varying pH, ionic strength and molecular weight of polyelectrolyte solutions.

Polyelectrolytes with low charge density are capable of forming less cross-linked multilayers, which in turn result in swollen membranes that are capable of separating larger molecules like proteins.[8,10] The transport of biomolecules like proteins and amino acids through multilayers under different conditions of pH and ionic strength is not well documented. The present work explores the possibility of protein separations and amino acid separations using polyelectrolyte multilayer membranes. Ultrafiltration finds applications particularly in protein separations. As chitosan/ polystyrene sulfonate (CHI/PSS) pair is less cross-linked and comparatively swollen, it was our interest to investigate whether CHI/PSS multilayer is suitable for protein separations under ultrafiltration conditions. The transport studies of three selected proteins,

bovine serum albumin (BSA), ovalbumin and lysozyme are conducted through these multilayer membranes by varying pH, ionic strength and concentration of the protein solutions. The pH dependent transport studies of selected amino acids are also studied. The possible fabrication of biofunctional membranes by immobilizing proteins into multilayers is explored. The optimum adsorption conditions have to be determined by varying factors like the number of bilayers, time of adsorption, the pH of protein solution and methods of adsorption.

Fluorescence quenching studies of lysozyme embedded membranes using known quenchers are expected to provide information on the adsorption pattern of the protein in the membrane. Hence a detailed fluorescence quenching studies of lysozyme immobilized multilayers have been carried out. Desorption studies are also conducted with a view to develop controlled delivery systems. *To sum up, the application of polyelectrolyte multilayer membranes in protein and amino acid separation are explored in the present work. The fabrication of biofunctional membranes by immobilizating proteins into multilayer membranes is also of current interest*.

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