PERFORMANCE CHARACTERISTICS OF CHARGED ULTRAFILTRATION MEMBRANES:
FUNDAMENTAL STUDIES AND APPLICATIONS

A Thesis in
Chemical Engineering
by
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ABSTRACT

Ultrafiltration is used extensively in the biotechnology and food industries for protein concentration and buffer exchange. Although several recent studies have demonstrated the importance of electrostatic interactions in ultrafiltration, there is little data available on the role of the nature and the number of charge groups on the overall membrane performance characteristics. The overall objective of this thesis was to develop a more quantitative understanding of the effects of the membrane surface charge density, the nature of the functional charge groups, and the spacer arm length on protein transport and fluid flow during ultrafiltration.

Electrically-charged ultrafiltration membranes were generated by chemical modification of commercially-available composite regenerated cellulose membranes using a variety of chemistries. A new framework was developed to analyze the performance characteristics of the different ultrafiltration membranes, analogous to the “Robeson Plot” used to analyze the performance of gas separation membranes. This framework captures the inherent trade-off between hydraulic permeability and membrane selectivity, providing a quantitative method for evaluating the performance of ultrafiltration membranes.

The effects of membrane surface charge density on protein transport, hydraulic permeability, and separation of binary protein mixtures was studied using a series of surface-modified cellulose membranes, with the charge density controlled by varying the extent of addition of a quaternary amine functionality. The transmission of positively charged cytochrome c decreased by a factor of 100 as the membrane
zeta potential was increased from 0.3 to 6.6 mV due to the strong electrostatic exclusion of the like-charged protein. The protein sieving data were in good agreement with a partitioning model accounting for electrostatic effects, while the hydraulic permeability data were consistent with a flow model accounting for the effects of counter-electroosmosis.

These electrically-charged membranes also provided very high resolution for the separation of myoglobin and lysozyme. High selectivity could be achieved with moderately charged membranes by using very low ionic strength solutions, while the more heavily charged membranes provided high selectivity up to salt concentrations of 40 mM. These results clearly demonstrate the potential of using membrane charge to design effective protein purification processes. Limited data obtained with a commercially relevant feedstock showed that these charged membranes could be used to significantly reduce the concentration of host cell proteins in the purification of a monoclonal antibody product.

A variety of novel ultrafiltration membranes were synthesized with multiple charge groups along the spacer arm. These membranes provide very high performance characteristics. In addition, the results provide important insights into the affects of the spacer arm length and the nature of the functional group on protein transmission and fluid flow. These results not only demonstrate the capabilities of electrically-charged ultrafiltration membranes for bioprocessing applications, they also suggest a variety of strategies for the development of new classes of charged membranes with greatly enhanced performance.
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Chapter 1

INTRODUCTION

1.1 Introduction

Biotechnology typically refers to any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use (Convention on Biological Diversity, 2006).

The use of biotechnology can be traced to as early as 8000 BC based on evidence that Mesopotamian people used selective breeding or artificial selection to improve the characteristics of their livestock. Over the last several thousand years, biotechnology has been employed for making fermentation products like alcohol, bread (using yeast), and yoghurt and cheese (using lactic acid producing bacteria). However, the rapid advances in molecular and cell biology and genetic engineering, in the past quarter century have led to a dramatic increase in academic and industrial interest in research and applications of biotechnology, particularly for the development of new and innovative biologically-derived pharmaceuticals.

In the United States alone, research and development investments in the year 2000 in the area of biotechnology were approximately $8 billion. Out of more than 1000 drugs that are currently in development, 350 of those are biotechnology related products or biotherapeutics (PhRMA Annual Report, 2001). These biotherapeutics are designed for the treatment of a range of autoimmune diseases, cancers, cardiovascular disease, transplant rejection, etc. (Carter et al., 1992; Baselga et al.,
1996; Anderson et al., 1997). Table 1.1 provides a partial list of the protein-based biotherapeutics that are currently available clinically.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood clotting factors</td>
<td>Hemophilia and related blood disorder</td>
</tr>
<tr>
<td>Colony stimulating factors</td>
<td>Cancer, low blood cell count</td>
</tr>
<tr>
<td>Epidermal growth factors</td>
<td>Cancer, skin ulcers</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Anemia</td>
</tr>
<tr>
<td>Human growth hormone</td>
<td>Cancer, AIDS and growth deficiency in children</td>
</tr>
<tr>
<td>Interferons (α, β, and γ)</td>
<td>Cancer, asthma, arthritis and infectious diseases</td>
</tr>
<tr>
<td>Interleukins</td>
<td>Cancer, AIDS and bone marrow failure</td>
</tr>
<tr>
<td>Monoclonal antibodies</td>
<td>Cancer and rheumatoid arthritis</td>
</tr>
<tr>
<td>Recombinant insulin</td>
<td>Diabetes</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>Prevention of oxygen toxicity</td>
</tr>
<tr>
<td>Tissue plasminogen activator</td>
<td>Stroke and heart attack</td>
</tr>
<tr>
<td>Vaccines</td>
<td>Vaccination against hepatitis B, malaria, herpes, meningococcal, streptococcal infections</td>
</tr>
</tbody>
</table>

Table 1.1: Protein-based biopharmaceutical products. Adapted from Walsh and Headon (1994)
Protein-based pharmaceuticals can be manufactured in transgenic plants, animals, microorganisms, or cell cultures. However, independent of the source of production, the desired protein is provided as part of a highly complex mixture that typically contains thousands of distinct impurities. These impurities can be host cell related (intact cells, residual substrates, host cell proteins, DNA, viruses, and endotoxins) or product-related (variants of the product produced by oxidation, deamidation, acetylation, dimerization, incorrect glycosylation, etc) (Lightfoot and Moscariello, 2004). The product related variants often differ in biological activity and immunogenicity from the desired protein and may be produced during cell culture or downstream processing or they may be due to some type of posttranslational modification. For example, the use of a low pH viral inactivation can cause deamidation of asparagine and glutamine residues. Posttranslational modifications like phosphorylation of serine, acylation of alanine, and isomerization of asparate to isoasparate are well known in recombinant proteins produced using mammalian cells (Aswad et al., 2000). In addition to the host cell and product-related impurities, contaminants can enter the product stream during processing including antifoams, antibiotics, leachables from membranes and chromatographic resins, adventitious viruses, and bacteria.

Safety is of paramount importance for biopharmaceuticals since they are typically administered intravenously or subcutaneously. The Food and Drug Administration (FDA) and World Health Organization (WHO) have set stringent purification requirements for all biopharmaceutical products (Fahrner et al., 2001).
For example, the target concentration of host cell proteins (HCP) in the final product should be less than 10 ppm (10 ng HCP/ mg of product). Virus and DNA levels are currently-targeted at less than one virus per million doses and 10 ng of DNA per dose. In addition, the product must be absolutely sterile (free of bacteria and other microorganisms). These stringent purification requirements require the use of a very specific purification train, with individual steps designed to remove specific impurities and contaminants.

In addition to the very high safety / purity standards, there are several other factors that are fairly unique to the processing of biological products. For example, all proteins are relatively labile and require fairly mild processing conditions, including operation over a limited temperature range. The initial feed streams are typically very dilute, requiring the processing of large volumes of material to produce only a small quantity of purified product. Blanch and Clark (1997) reported that the cost of downstream purification for recombinant DNA fermentation products can amount to as much of 80 – 90% of the total manufacturing costs. Other studies have estimated protein purifications costs at 50 – 60% of the total production costs (Ladisch, 2001).

Currently, the downstream process for the purification of therapeutic proteins relies heavily on column chromatography, with the product (or impurities) preferentially binding to the surface of the resin. Ion-exchange chromatography uses charged resins to separate proteins on the basis of their electrical charge. Reverse phase chromatography uses hydrophobic materials to separate proteins based on their
hydrophobicity. Affinity chromatography involves highly selective binding interactions to a specific ligand immobilized on or within an affinity matrix or gel. Although column chromatography provides high-resolution protein separations, it is also very expensive, it is an inherently batch process, and it has significant limitations on the overall product throughput. Moreover, the rising demand for several biotherapeutics, in combination with the growing market competition and ever-increasing pressures on the cost of healthcare, has created a significant need to reduce the overall cost and increase the throughput of downstream purification process.

There is thus considerable commercial interest in developing alternative technologies and novel purification schemes that can improve process economics in the biotechnology industry. This includes the development of technologies that reduce the number of steps in the purification train, that increase process throughput, that use less expensive raw materials, and that decrease the reliance on column chromatography. Some examples currently under investigation include high performance tangential flow filtration (van Reis et al., 1999), aqueous two-phase extraction (Li and Peeples, 2004), three-phase partitioning (Jain et al., 2004), precipitation (Kumar et al., 2003), crystallization (Lee and Kim, 2003), monolithic stationary phases (Branovic et al., 2003) and membrane chromatography (Ghosh, 2002).
1.2 Protein Purification using Membranes

Membranes are very well suited for protein purification since they can be easily operated under mild conditions, they are athermal, and they involve no phase changes or chemical additives. Moreover, membrane separations are very robust, they provide high throughput, and they offer several cost advantages over conventional chromatography systems. In addition, membrane systems are much easier to scale up than chromatographic processes, reducing the time and risk in scale-up to commercial manufacturing.

A number of membrane processes are currently used to meet critical downstream purification requirements in the biotechnology industry. This includes the use of microfiltration, ultrafiltration, virus filtration, nanofiltration, and reverse osmosis. Microfiltration membranes typically have a pore size between 0.1 and 10 µm and are used for initial cell harvesting, clarification, and sterile filtration. Ultrafiltration membranes have a pore size between about 1 and 100 nm, corresponding to a molecular weight cut off between 3 and 1000 kD. These membranes can retain proteins and other macromolecules and are thus used for protein concentration (water removal) and buffer exchange. Size based virus filters are used to remove viruses from the smaller-sized proteins. Nanofiltration and reverse osmosis membranes have pores less than 1 nm in size that can retain small molecules (salts, organics, and antibiotics) and are used in the preparation of water for injection (WFI). All of these current applications involve relatively low-resolution separations in which the species of interest are significantly different in
size (with the exception of virus filtration).

There is, however, increasing evidence in the literature that ultrafiltration can be used as a highly selective tool for protein purification. A number of recent reports have conclusively demonstrated that it is possible to exploit electrostatic interactions between proteins and membranes for high-resolution protein purification. For example, Saksena and Zydney (1994) demonstrated that the selectivity for ultrafiltration of bovine serum albumin (BSA) and immunoglobulin G (IgG) could be increased from a value of only two at pH 7 and high salt concentrations to more than 30 simply by adjusting the pH to 4.7 and lowering the solution ionic strength. van Eijndhoven et al. (1995) separated BSA (69 kDa) and hemoglobin (67 kDa) using ultrafiltration with a selectivity of 70 by proper choice of solution pH and the ionic strength. Optimal performance was achieved at pH 7 where the negatively charged BSA was electrostatically excluded from the membrane pores while the neutral hemoglobin passed easily through the membrane. van Reis et al. (1999) used high performance tangential flow filtration (HPTFF) to separate bovine serum albumin from an antigen binding fragment of a recombinant DNA antibody. The authors reported 94% yield of the desired product and approximately 1000-fold purification.

These recent reports indicate that membranes may be an attractive alternative to conventional chromatography systems for high-resolution protein purification. However, the successful implementation of these membrane systems at a commercial scale requires a more detailed understanding of the various factors that govern the performance of these systems, including the electrostatic interactions between
membranes and proteins. Figure 1.1 shows a schematic of the selective electrostatic exclusion of a charged protein from a like charged membrane pore. The charged protein and membrane are surrounded by a diffuse electrical double layer consisting of a greater concentration of counter-ions, which tend to balance the electrical charge on the surface. The electrostatic interactions between the charged protein and membrane generate an energetic barrier for the charged protein, significantly reducing its ability to partition into the pores of the like charged membrane. In contrast, proteins that are electrically neutral are able to enter the charged pore much more readily, thus providing a basis for high selectivity separations even for proteins with nearly identical physical size.

Figure 1.1: Schematic showing enhanced selectivity using electrically charged membranes
Most studies of electrostatic interactions in membrane systems have focused on the effects of solution pH or ionic strength, which are the two variables that are most easily manipulated in a given experimental system. However, these studies have provided little insight into the effects of membrane properties on the overall separation performance. This includes the effects of the membrane surface charge density, the nature of the chemical groups used to provide the membrane charge, and the details of the coupling chemistry used to attach the charged ligand to the surface of the membrane.

1.3 Thesis Program

The overall objective of this thesis was to develop a more fundamental understanding of the role of electrostatic interactions on the transport and separation characteristics of charged proteins during ultrafiltration through electrically-charged membranes. This included: (1) analysis of the effects of pore size distribution and membrane charge on the trade-off between the membrane hydraulic permeability and selectivity, (2) experimental and theoretical evaluation of the effects of membrane charge density on fluid and protein transport through charged ultrafiltration membranes, (3) theoretical analysis of the effects of membrane charge density on the separation of binary protein mixtures, and (4) examination of different functional charged groups and different coupling chemistries on the performance of charged ultrafiltration membranes.

Chapter 2 provides a brief review of the theoretical analysis of solute and solvent transport through membranes with a particular emphasis on the effects of
electrostatic interactions on solvent flow (counter-electroosmosis) and on solute partitioning into charged pores.

Chapter 3 describes the experimental apparatus, materials, and methods used in the majority of the experimental studies described in this thesis. Specific details on some of the experimental procedures and membrane modification techniques are provided in the relevant chapters.

Chapter 4 examines the trade-off between the hydraulic permeability and selectivity of commercially available neutral ultrafiltration membranes using literature data obtained with bovine serum albumin (BSA) and lysozyme. The data were compared with model calculations accounting for the effects of the membrane pore size distribution on both the permeability and selectivity. This analysis provides a quantitative framework for comparing the performance characteristics of different ultrafiltration membranes for protein concentration. The effectiveness of this approach was demonstrated using limited data for prototype negatively- and positively-charged membranes.

Chapter 5 presents a quantitative evaluation of the effects of membrane charge density on protein transport and solvent permeability. Experimental data on protein and fluid transport were obtained with a series of charge modified ultrafiltration membranes. The membrane surface charge density was controlled by varying the extent of addition of a quaternary amine functionality, with the actual charge evaluated from streaming potential measurements. The protein sieving data were compared with a partitioning model accounting for electrostatic effects, while the
hydraulic permeability data were compared with a flow model accounting for the effects of counter-electroosmosis.

In Chapter 6, experimental results are presented for the effects of membrane charge density on the separation of a model binary protein mixture of lysozyme (14.1 kDa) and myoglobin (17 kDa). Experiments were performed at pH 6.2 and over a range of solution ionic strength using a series of positively charged 100 kD membranes.

Chapter 7 examines the application of charged membranes in High Performance Tangential Flow Filtration (HPTFF) for the removal of Chinese Hamster Ovary cell proteins (CHOP) from an industrial scale feedstream containing a monoclonal antibody produced using recombinant DNA technology. Diafiltration and protein sieving experiments were performed using positively charged membranes with different nominal molecular weight cut offs. The effects of solution ionic strength and filtrate flux on monoclonal antibody retention and CHOP clearance were also examined.

Chapter 8 examines the effects of the surface modification chemistry on the performance of the ultrafiltration membranes. Membranes were modified using several different chemistries to vary the spacer arm length and the nature of the functional group (using both weak and strong bases). In addition, novel ultrafiltration membranes with multiple charge groups along the spacer arm length were synthesized and their effect on ultrafiltration performance was investigated.

Chapter 9 summarizes the major findings of this thesis and makes several
recommendations for future work on the development of electrically charged membrane systems for protein purification.
Chapter 2

THEORETICAL DEVELOPMENT

2.1 Introduction

The overall rate of protein transport through a semipermeable membrane is determined by the combined effects of bulk and membrane transport. While bulk transport involves the movement of protein from the bulk solution to the membrane surface, membrane transport involves protein motion through the membrane pores. The rate of bulk transport to the membrane surface is primarily a function of the device hydrodynamics and the protein diffusion coefficient. Transport of protein through the membrane pores can occur by convection, diffusion, and/or electrophoretic motion. In each case, both thermodynamic and hydrodynamic factors influence the rate of protein transport. While thermodynamic factors determine the initial partitioning of solute into the pore, the hydrodynamic factors determine the rate of solute motion inside of the pores. The rate of solvent and solute transport is also affected by the inherent pore size distribution of the membrane. This chapter briefly reviews the general theoretical models used to describe bulk and membrane transport during protein filtration with an emphasis on the effects of electrical interactions on these transport processes.
2.2 Bulk Mass Transport

During membrane filtration, the convective flow towards the membrane causes an accumulation of retained protein adjacent to the upper surface of the membrane (Figure 2.1).

**Figure 2.1: Concentration polarization during membrane filtration**

This phenomenon, typically referred to as concentration polarization, is a complex function of the device hydrodynamics, solution conditions, and the retention characteristics of the membrane. Polarization causes the protein concentration to increase from the bulk concentration, $C_b$, to a much higher concentration at the membrane surface, $C_w$, over a distance equal to the concentration polarization boundary layer thickness, $\delta$. The high concentration of retained protein at the
upstream surface of the membrane can generate a significant osmotic pressure, thereby reducing the solvent flux compared to a system without retained protein under identical conditions. Moreover, concentration polarization alters the rate of protein transmission across the membrane due to the higher local protein concentration adjacent to the upstream surface of the membrane. In addition, the accumulated protein can cause irreversible changes in the physical properties of the membrane by pore plugging and/or by forming an insoluble gel layer on the surface of the membrane.

2.2.1 Stagnant Film Model

The stagnant film model has been widely used to describe the extent of concentration polarization in membrane systems. This model provides an approximate analysis of the concentration profile upstream of the membrane while eliminating the complexities involved in solving the full, coupled mass and momentum transport problem. The net protein flux towards the membrane in the stagnant film model has two contributions - one from convection towards the membrane and another from diffusion back into the bulk solution:

\[
N_s = -J_v C - D_\infty \frac{dC}{dz}
\]  

(2.1)

where \( C \) is the local solute concentration at position \( z \) above the membrane, \( D_\infty \) is the free solution diffusivity, and \( J_v \) is the filtrate flux, which is taken here to be a positive quantity even though the flow is actually in the negative \( z \)-direction (see Figure 2.1).
The net solute flux towards the membrane is assumed to be constant throughout the boundary layer with a value equal to the solute flux through the membrane:

\[ N_s = -J_v C_f \]  

(2.2)

where \( C_f \) is the protein concentration in the filtrate. Equation (2.1) is integrated across the concentration boundary layer (from \( z = 0 \) where \( C = C_w \) to \( z = \delta \) where \( C = C_b \)) to give:

\[ J_v = k \ln \left( \frac{C_w - C_f}{C_b - C_f} \right) \]  

(2.3)

where the ratio of the free solution diffusivity (\( D_\infty \)) to the boundary layer thickness (\( \delta \)) has been set equal to \( k \), the mass transfer coefficient in a particular membrane device. Equation (2.3) is the classical form of the stagnant film model. It relates the filtrate flux to the protein concentrations in the bulk solution (\( C_b \)), in the filtrate solution (\( C_f \)), and at the membrane surface (\( C_w \)). Although Equation (2.3) is approximate, it is widely used in the analysis of both solute and solvent transport in membrane systems. The validity of the stagnant film model has been discussed in some detail by Zydney (1997) and Zeman and Zydney (1996).

**2.2.2 Bulk Mass Transfer Coefficient**

The mass transfer coefficient (\( k \)) is a function of the solute diffusivity as well as the device hydrodynamics. In principal, the mass transfer coefficient can be
obtained by solving the relevant fluid mechanics and mass transfer equations for the appropriate system geometry. Since it is often difficult to develop complete solutions to the governing equations due to the complexities in the fluid flow within typical membrane devices, empirical or semi-empirical correlations are often used for the mass transfer coefficient based on analogies with the corresponding heat or momentum transfer problems.

Smith et al. (1968) developed a correlation for the average mass transfer coefficient in a stirred cell for laminar flow (Re < 32,000) using data for the rate of benzoic acid dissolution as:

\[ Sh = \chi (Re)^{0.567} (Sc)^{0.33} \]  

(2.4)

where \( Sh = kd/2D_\infty \) is the Sherwood number, \( Re = \omega d^2/4\nu \) is the Reynolds number, \( Sc = \nu/D_\infty \) is the Schmidt number, \( d \) is the stirred cell diameter, \( \omega \) is the stirring speed, and \( \nu \) is the kinematic viscosity. The coefficient, \( \chi \), is a function of the actual system geometry (Smith et al., 1968) and possibly the membrane porosity (Malone and Anderson, 1978). The value of \( \chi \) for the 25 mm diameter Amicon stirred cell that was used in this thesis was evaluated by Opong and Zydney (1991) as \( \chi = 0.23 \) by analyzing data for the filtrate flux of a BSA solution through fully retentive OMEGA polyethersulfone membranes as a function of the applied transmembrane pressure drop.

The protein diffusion coefficient can be estimated from the correlation developed by Young et al. (1980):
\[ D_\infty = 8.34 \times 10^{-8} \left( \frac{T}{\mu M^{1/3}} \right) \] (2.5)

where \( D_\infty \) is the bulk diffusivity in units of \( \text{cm}^2/\text{s} \), \( \mu \) is the solution viscosity in cP, \( T \) is the absolute temperature in Kelvin, and \( M \) is the protein molecular weight in g/mol.

Since Equation (2.5) neglects protein-protein interactions, it is strictly valid only at infinite dilution. Moreover, this correlation for \( D_\infty \) is independent of solution ionic strength, even though experimental data for \( D_\infty \) for some proteins show a strong dependence on ionic strength (e.g., Doherty and Benedek, 1974; Pujar, 1996). The development leading to the stagnant film model implicitly assumes that the solute diffusivity and solution viscosity are constant throughout the boundary layer. More advanced models that account for the concentration dependence of the solute diffusivity have been developed (Zydney, 1992; Saksena and Zydney, 1997), but these have not been widely used in the analysis of membrane filtration due to the lack of available data on the protein diffusion coefficient over the full range of concentrations from \( C_b \) to \( C_w \).

### 2.3 Membrane Transport

The transport of solute and solvent through semipermeable membranes has chiefly been described using three different approaches. The two earliest approaches, the Kedem-Katchalsky analysis (1958) and the Stefan-Maxwell multi-component
Diffusion analysis (Lightfoot, 1974; Robertson, 1989) are based directly on the principles of irreversible thermodynamics (de Groot and Mazur, 1962). These approaches lead to a set of phenomenological equations that relate the solute and solvent fluxes to the pressure and concentration gradients. The phenomenological coefficients in these equations generally have to be determined experimentally and there is no explicit dependence on the membrane or solute characteristics.

The third approach is based on hydrodynamic theory (Anderson and Quinn, 1974) and provides a mean to evaluate the solute and solvent fluxes for the transport of a well-defined solute through a well-defined pore based on fundamental information on the size, shape, and electric charge of the solute and pore. Most hydrodynamic analyses treat the membrane as a collection of long parallel cylindrical capillaries, while the solutes are treated as rigid spheres. Since these models also make the implicit assumption that the solvent is a continuum, they are strictly limited to physical situations in which both the pore radius \( r_p \) and solute radius \( r_s \) greatly exceed the radius of the solvent molecules. Hydrodynamic theories can also be easily extended to incorporate the effects of a pore size distribution by numerical integration of the appropriate fluxes over the distribution (Mochizuki, 1992).

### 2.3.1 Solvent Transport

The rate of solvent transport through a membrane is generally described in terms of the hydraulic permeability \( L_p \):
where $J_v$ is the solvent flux (volumetric solvent flow rate per unit membrane area), $\eta$ is the fluid viscosity, and $\Delta P$ is the applied transmembrane pressure difference. For laminar flow through a membrane composed of uncharged parallel uniform cylindrical pores of radius ($r_p$) and length ($\delta_m$), the hydraulic permeability is given by:

$$L_p = \frac{N\pi r_p^4}{8\delta_m} \quad (2.7)$$

where $N$ is the number of pores per unit area of the membrane. Equation (2.7) ignores end-effects, which is valid for most ultrafiltration membranes since $r_p/\delta_m$ is typically less than 0.01. The membrane permeability is often used as a measure of the extent of membrane fouling. Partial blockage or constriction of the membrane pores reduces the filtrate flux at a given transmembrane pressure difference, resulting in a corresponding decrease in the membrane permeability.

### 2.3.1.1 Solvent Transport through a Charged Cylindrical Pore

Although the rate of solvent transport is often assumed to be a unique function of the membrane pore size distribution, parameters like the membrane charge and the solution ionic strength can also significantly alter the solvent flux. The presence of a net surface charge on the pore wall causes an increase in the concentration of counterions in the region adjacent to the pore wall (the electrical double layer). The pressure driven fluid flow through this charged pore thus generates an unequal flux of
cations and anions. To maintain electro-neutrality, an induced (streaming) potential develops, which is just sufficient to compensate for the net convective current flow through the charged pore. Under these conditions, the conductive ion flux due to the streaming potential exactly balances the pressure driven convective ion flux. The streaming potential also alters the velocity profile within the pore because of the force on the solvent associated with the ion motion (electrical Maxwell stress terms in the governing momentum equation). The momentum equation for flow through a cylindrical pore is given as (Newman, 1991):

\[
\frac{\eta}{r} \frac{d}{dr} \left( r \frac{dv_z}{dr} \right) + \rho_e E_z - \frac{dP}{dz} = 0
\]  

(2.8)

where \(v_z\) is the axial velocity, \(\eta\) is the viscosity, \(\rho_e\) is the local charge density of the solution, \(E_z\) is the electrical (streaming) potential and \(\frac{dP}{dz}\) is the pressure gradient.

The electrical potential gradient in the axial direction, \(\frac{\partial \Phi}{\partial z}\), can be expressed in terms of electrical (streaming) potential (\(E_z\)). The electrical potential in the radial direction (\(\Phi\)) satisfies Poisson's equation in cylindrical coordinates:

\[
\frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial \Phi}{\partial r} \right) = -\frac{\rho_e}{\varepsilon_o \varepsilon_r} = -\frac{F}{\varepsilon_o \varepsilon_r} \sum z_i C_i
\]  

(2.9)

where \(F\) is Faraday's constant, \(\varepsilon_o\) is the permittivity of free space, \(\varepsilon_r\) is the dielectric constant of the medium, and \(z_i\) and \(C_i\) are the ion valence and concentration, respectively.
The velocity profile in a charged capillary is evaluated by integrating Equation (2.8) over the radial direction, with \( \rho_E \) given by Equation (2.9), using the boundary conditions that \( v_z = 0 \) at \( r = r_p \) and that \( v_z \) and \( \Phi \) are finite at \( r = 0 \) (Newman, 1991). This yields:

\[
v_z = E_z \frac{\varepsilon_0 \varepsilon_f}{\eta} (\Phi - \Phi_{r=r_p}) - \frac{dP}{dz} \frac{r_p^2 - r^2}{4\eta}
\]

where \( \Phi_{r=r_p} \) is the electrical potential at the pore surface. In order to evaluate the radial dependence of the electrical potential from Equation (2.9), an expression is needed for the concentration of ion \( i \) (\( C_i \)) in terms of the electrical potential. The required relationship is developed by recognizing that there is no net ion flux in the radial direction, i.e.:

\[
N_i = D_i \frac{\partial C_i}{\partial r} + u_i z_i F E_r = 0
\]

where \( E_r \) is the radial electric field (\( = -\frac{\partial \Phi}{\partial r} \)) and \( u_i \) is the ion mobility (\( = D_i R T \)).

Integration of Equation (2.11) over the pore radius gives the Boltzmann distribution:

\[
C_i = C_i^0 \exp\left[-\frac{z_i F}{RT} (\Phi - \Phi_{r=0})\right] = C_i^0 [1 - \frac{z_i F}{RT} (\Phi - \Phi_{r=0})]
\]

where the second expression is valid for low potentials (the Debye-Hückel approximation). The resulting potential field is then evaluated from Equation (2.9) assuming that the surface has a constant charge density (Newman, 1991):
\[(\Phi - \Phi_{r=0}) = \frac{q_p}{\varepsilon_0 \varepsilon_r \kappa} \left[ \frac{I_0(\kappa r) - 1}{I_1(\kappa r_p)} \right] \] (2.13)

where \(I_0\) and \(I_1\) are modified Bessel functions of the first kind, and \(q_p\) is the surface charge density of the pore. \(\kappa^{-1}\) is the Debye length and is given by:

\[\kappa^{-1} = \left[ \frac{\Phi^2}{\varepsilon_0 \varepsilon_r RT \sum_i (z_i^2 C_i^0)} \right]^{1/2} \] (2.14)

For an uncharged pore, the fluid velocity is maximum at the center of the pore where \(v_z = (dP/dz) r^2_m/(4\mu)\) and decreases parabolically to \(v_z = 0\) at the pore wall (see Equation 2.10). The radial dependence of the velocity in a charged cylindrical pore is more complex due to the counterelectroosmotic flow that acts in the opposite direction to the pressure driven flow. As \(E_z\) becomes increasingly large, the electroosmotic contribution (backflow) can exceed the pressure driven contribution at radial positions near the wall. The average solvent velocity through the pore is obtained by integrating Equation (2.10) over the pore radius yielding:

\[\langle V \rangle = \frac{\Delta P \ r_p^2}{\delta_m^2 \ 8\eta} - \frac{q_p I_2(\kappa r_p)}{\kappa \eta I_1(\kappa r_p)} \] (2.15)

where \(I_2\) is a modified Bessel function of the first kind. The counterelectroosmotic flow given by the second term in Equation (2.15) is always in the direction opposite to the pressure driven flow regardless of the polarity of the membrane surface charge. This backflow disappears when the pressure drop across the pore is zero.
The streaming potential is evaluated using the constraint that there is no net electric current flow through the pore. The net electric current through the pore is evaluated from the sum of the convective and conductive contributions (the diffusive contributions are zero since gradients in the axial direction are assumed to be negligible):

\[
I = 2\pi \int_{0}^{r_p} \int_{z_1}^{z_2} v_z \rho_k r \, dr \, dz + E_z \int_{0}^{r_p} \Lambda \, dr
\]

(2.16)

where \( \Lambda \) is the local conductivity:

\[
\Lambda = F^2 \sum_i z_i^2 u_i C_i
\]

(2.17)

The induced electric field \( (E_z) \) associated with the streaming potential is obtained by setting \( I \) equal to zero. \( E_z \) is evaluated from Equation (2.16) with \( \rho_k \) given by Equation (2.9), \( v_z \) given by Equation (2.10), and \( C_i \) given by Equation (2.12) yielding (Newman, 1991):

\[
E_z = \frac{\Delta \rho}{\Lambda_{\text{eff}}} \frac{q_p I_2(kr_p)}{\kappa \eta I_1(kr_p)}
\]

(2.18)

where \( I_2 \) is a modified Bessel function of the first kind and \( \Lambda_{\text{eff}} \) is the effective conductivity of the electrolyte inside the capillary arising from the natural ion mobility as well as the increased ion motion caused by electrophoretic flow.
\[ \Lambda_{\text{eff}} = \Lambda_{\text{avg}} + \frac{q_p^2}{\eta} \left[ 1 - \frac{I_0(\kappa r_p)I_2(\kappa r_p)}{I_1(\kappa r_p)} \right] \]  

(2.19)

with

\[ \Lambda_{\text{avg}} = \Lambda^0 \left\{ 1 - \frac{q_p F}{\varepsilon_0\varepsilon_r \kappa R T} \left[ \frac{2}{\kappa r_p} - \frac{1}{I_0(\kappa r_p)} \right] \sum_i z_i^2 u_i C_i^0 \right\} \]  

(2.20)

where \( \Lambda^0 \) is the conductivity at the centerline of the pore. The term in the bracket in Equation (2.18) accounts for the radial variation of the solution conductivity within the pore arising from the radial dependence of the ion concentrations. Since the Debye-Hückel approximation was used in the development of Equations (2.19) and (2.20), their applicability is limited to low values of the surface potential (< 25 mV).

Equation (2.13) was derived for the general case where the centerline concentrations of the ions are different from their bulk values. In the limit of thin double layers (i.e., at high salt concentrations), these concentrations will be equal to their bulk values and \( \Phi_{r=0} = 0 \). Under these conditions, Equation (2.9) can be integrated as before to give:

\[ \Phi = \frac{q_p}{\varepsilon_0 \varepsilon_r \kappa} \frac{I_0(\kappa r)}{I_1(\kappa r_p)} \]  

(2.21)

The electrical potential at the surface of shear is defined as the zeta-potential (\( \zeta \)), which is the value typically used to characterize the electrical properties of surfaces. The zeta-potential is approximately equal to the electrical potential at the
pore wall \((\zeta = \Phi_{r=r_p})\) and can be evaluated from the streaming potential by substituting Equation (2.21) into Equation (2.18). The resulting expression reduces to the classical Helmholtz-Smoluchowski equation \([\zeta = (\Lambda_{\text{eff}} \eta/\varepsilon_0 \varepsilon_r \delta_m)(E_z/\Delta P)]\) in the limit as \(\kappa r_p \to \infty\). This expression is discussed in more detail in Chapter 5.

A similar analysis can be easily performed for conditions where the pore surface potential is kept constant (rather than surface charge density). Rice and Whitehead (1965) analyzed this situation for capillaries with small surface potentials. The average solution velocity under these conditions is given as:

\[
\langle V \rangle = \frac{\Delta P}{\delta_m} \frac{r_p^2}{8 \eta_{\text{app}}} 
\tag{2.22}
\]

with the ratio of the apparent viscosity \((\eta_{\text{app}})\) to the actual solvent viscosity given as a complex function of the dimensionless pore radius \((\kappa r_p)\):

\[
\eta_{\text{app}} = \left(\kappa r_p\right)^2 \left\{1 - B \left[1 - \frac{2 I_1(\kappa r_p)}{\kappa r_p I_0(\kappa r_p)} \right] \right\}
\tag{2.23}
\]

The parameter \(B\) is given by:

\[
B = \left(\frac{\varepsilon_r \varepsilon_0 \Phi_p}{\eta}\right)^2 \frac{\eta \kappa^2}{c} 
\tag{2.24}
\]
where $\Phi_p$ is the surface potential and $c$ is the electrolyte conductivity (Rice and Whitehead, 1965). Levine et al. (1974) have extended this analysis to higher surface potentials.

### 2.3.2 Solute Transport

In general, theoretical analyses of solute transport in liquid filled pores are developed by equating the gradient in the chemical potential of the solute ($-\nabla \mu_s$) to the hydrodynamic drag force acting on the solute in the pore (Deen, 1987):

$$-\nabla \mu_s = f_\infty K(U - GV)$$

(2.25)

where $f_\infty$ is the friction coefficient of the solute in the bulk solution, and $U$ and $V$ are the solute and fluid velocity, respectively, both evaluated relative to a coordinate system fixed with respect to the pore walls. The lag coefficient ($G$) and the enhanced drag coefficient ($K$) account for the hydrodynamic interactions between the solute and pore walls and are complex functions of the solute and pore size, the solute position in the pore, and the presence of any long range (e.g., electrostatic) interactions.

The gradient of the chemical potential of the solute in the presence of a potential field can be written as (Evans, 1979):

$$\nabla \mu_s = RT \nabla \ln C_s + \nabla \psi$$

(2.26)
where $\psi$ is the potential energy of interaction and $C_s$ is the solute concentration in the pore at radial position $r$ and axial position $z$. Equation (2.26) implicitly assumes that solute-solute interactions are negligible, i.e. that the solute concentration in the pore is infinitely dilute. Equation (2.26) is substituted into Equation (2.25), which can then be solved explicitly for the solute flux ($N_s$) yielding:

$$N_s = UC_s = -K^{-1}D_\infty VC_s + GVC_s - K^{-1}D_\infty \frac{C_s}{RT} \nabla \psi$$

(2.27)

where $D_\infty = RT/f_\infty$ is the free solution diffusion coefficient at infinite dilution. The three terms in the Equation (2.27) represent the diffusive, convective, and electrophoretic contributions to the solute flux. The electrophoretic contribution to solute transport can arise from an applied external field or from an induced (streaming) potential caused by the flow of the electrolyte solution through the charged pore as discussed in the last section. Pujar and Zydney (1994) presented the first complete analysis of all three contributions to the overall rate of solute transport through a charged membrane.

Since the radial variation in the solute flux within the pore cannot be measured experimentally, most previous analyses have examined the radially averaged solute flux through the pore (Deen, 1987). The area-averaged flux for a rigid spherical solute in a cylindrical pore can be evaluated as:

$$\left\langle N_s \right\rangle = -2D_\infty \int_0^{1/\lambda} K^{-1} \frac{\partial C_s}{\partial z} \beta d\beta - 2D_\infty \int_0^{1/\lambda} K^{-1} C_s \frac{1}{RT} \frac{\partial \psi}{\partial z} \beta d\beta + 2 \int_0^{1/\lambda} GC_s \sqrt{\beta} d\beta$$

(2.28)
\[
\beta = r/r_p \quad \text{is the dimensionless radial position in the pore and} \quad \lambda = r_s/r_p \quad \text{is the ratio of solute to pore radii (} \lambda = r_s/r_p \). \quad \text{The upper limits for the integrals in Equation (2.28) are fixed at} \quad 1-\lambda \quad \text{due to the steric exclusion of the solute from the region within one solute radius of the pore wall.} \quad \text{The radial concentration profile within the pore can be described by a Boltzmann (equilibrium) distribution (analogous to Equation 2.12) as:}
\]
\[
C_s(\beta) = C_s(\beta = 0) \exp \left[ -\frac{\psi(\beta) - \psi(\beta = 0)}{RT} \right] \tag{2.29}
\]
\[
\text{where} \quad \psi(\beta = 0) \quad \text{and} \quad C_s(\beta = 0) \quad \text{are the potential energy of interaction and solute concentration at} \quad \beta = 0. \quad \text{Equation (2.29) can also be developed by integration of Equation (2.26) with} \quad V = 0 \quad \text{and} \quad N_s = 0 \quad \text{in the radial direction.} \quad \text{Deen (1987) provides an excellent discussion of the validity of the radial equilibrium approximation for membrane systems.}
\]

\text{Substituting Equation (2.29) into (2.28) yields:}
\]
\[
\langle N_s \rangle = K_c \langle V \rangle C_s - K_d D_s \frac{d\langle C_s \rangle}{dz} - K_e \langle C_s \rangle \frac{D_s}{RT} \left( \frac{\partial \psi}{\partial z} \right) \tag{2.30}
\]
\[
\text{where} \quad \langle V \rangle \quad \text{and} \quad \left( \frac{\partial \psi}{\partial z} \right) \quad \text{are the radially averaged velocity and potential energy gradient.} \quad \text{The coefficients} \quad K_c, \quad K_d, \quad \text{and} \quad K_e \quad \text{are given as:}
\]
\[
K_c = \frac{\int_{1-\lambda}^{0} G V \exp(-\psi(\beta)/RT) \beta d\beta}{\left[ \int_{0}^{1-\lambda} \exp(-\psi(\beta)/RT) \beta d\beta \right] \left[ 2 \int_{0}^{1} V \beta d\beta \right]} \tag{2.31}
\]
As mentioned earlier, the solute flux (Equation 2.30) has contributions from convection, diffusion, and electrophoretic motion (associated with the induced streaming potential); the magnitude of these contributions are reduced from their bulk solution values by an amount given by the coefficients $K_c$, $K_d$, and $K_e$.

A detailed expression for the potential energy of interaction $[\psi(\beta, z)]$ for a protein in a cylindrical pore in the presence of an axial electric field is currently unavailable. Thus, in order to proceed further with the analysis, it is assumed that the actual potential is given by the pair-wise summation of the potential energies arising from: $[\psi_1(\beta)]$, the interaction energy between the protein and pore in the absence of any flow and thus in the absence of a streaming potential, and $[\psi_2(z)]$, the interaction energy between the protein and the streaming potential in an unbounded system with the streaming potential assumed to be unaffected by the presence of the protein. This approximation neglects the possible effects of the streaming potential on the structure of the double layer surrounding the protein, as well as any effects of the protein on the streaming potential itself. Under these conditions, we can write:

\[
K_d = \frac{\int_0^{1-\lambda} K^{-1} \exp(-\psi(\beta)/RT) \beta d\beta}{\int_0^{1-\lambda} \exp(-\psi(\beta)/RT) \beta d\beta}
\]

\[
K_e = \frac{\int_0^{1-\lambda} \frac{\partial}{\partial z} [\psi(\beta)] \exp\left(-\frac{\psi(\beta)}{RT}\right) \beta d\beta}{\left[\int_0^{1-\lambda} \exp\left(-\frac{\psi(\beta)}{RT}\right) \beta d\beta\right] \left[2 \int_0^{1-\lambda} \frac{\partial}{\partial z} [\psi(\beta)] \beta d\beta\right]}
\]
\[ \psi(\beta, z) = \psi_1(\beta) + \psi_2(z) \]  
(2.34)

where \( \psi_1 \) is only a function of \( \beta \) and \( \psi_2 \) is only a function of \( z \). Smith and Deen (1980; 1983) have developed expressions for \( \psi_1(\beta) \), the electrostatic potential energy of interaction, under conditions of both constant surface charge and constant surface potential. These are discussed in more detail in Section 2.3.2.2.

The derivative of the potential energy of interaction associated with the streaming potential is assumed to be equal to that of an equivalent electric field acting on an isolated protein in an unbounded system:

\[ \frac{d\psi_2}{dz} = -\frac{u_E RT}{D_\infty} E_z = -\alpha(V) \frac{u_E RT}{D_\infty} \]  
(2.35)

where \( E_z \) is the electric field due to the streaming potential and \( u_E \) is the bulk electrophoretic mobility of the protein. The streaming potential in this system is proportional to the average solution velocity with the proportionality constant (\( \alpha \)) a function of the pore radius, the Debye length, and the solution conductivity. The parameter, \( \alpha \), can be evaluated from Equations (2.15) and (2.18) as:

\[ \alpha = \frac{\epsilon}{L_p \delta_m \Lambda_{\text{eff}}} \frac{q_p I_2(\kappa r_p)}{\kappa I_1(\kappa r_p)} \]  
(2.36)

Note that \( \frac{d\psi_2}{dz} \), as given by Equation (2.35), is a constant independent of axial position, so that at steady state (\( N_s = \text{constant} \)) Equation (2.30) becomes a linear first
order ordinary differential equation for $\langle C_s \rangle$ with constant coefficients. In addition, since $\psi_2$ is independent of $\beta$, $K_e$ must equal $K_d$ from Equations (2.32) and (2.33).

In order to relate the solute flux to the solute concentrations at the upstream ($C_w$) and downstream ($C_f$) surfaces of the membrane, Equation (2.30) is integrated across the membrane with boundary conditions at the upper ($z = 0$) and lower ($z = \delta_m$) surfaces developed by assuming that the solute concentrations across the interface are in equilibrium (Deen, 1987):

$$
\phi = \frac{\langle C_s \rangle_{z=0}}{C_w} = \frac{\langle C_s \rangle_{z=\delta_m}}{C_f} = 2 \int_0^{1-\lambda} \exp(-\psi_1/kT)\beta d\beta
$$

(2.37)

The results are conveniently expressed in terms of the actual sieving coefficient ($S_a$), which is defined as the ratio of the solute concentration in the filtrate ($C_f$) to that at the upper surface of the membrane ($C_w$) (Opong and Zydney, 1991):

$$
S_a = \frac{C_f}{C_w} = \frac{S_a(1+\omega) \exp[P_{e_m}(1+\omega)]}{S_a(1+\omega) + \exp[P_{e_m}(1+\omega)] - 1}
$$

(2.38)

where

$$
S_{\infty} = \phi K_c
$$

(2.39)

$$
P_{e_m} = \left( \frac{K_c}{K_d} \right) \left( \frac{V}{D_w} \delta_m \right) = \left( \frac{S_{\infty}}{\phi K_d} \right) \left( \frac{V}{D_w} \delta_m \right)
$$

(2.40)

$$
\omega = \frac{\phi K_c}{\phi K_c} \alpha u_E = \frac{\phi K_d}{\phi K_c} \alpha u_E
$$

(2.41)
Thus a complete description of solute transport (sieving) during membrane filtration requires the knowledge of three distinct parameters: the asymptotic sieving coefficient \( S_\infty \), which describes the convective contribution to the solute flux; the membrane Peclet number \( Pe_m \), which describes the relative importance of solute convection to diffusion inside the membrane; and the electrophoretic ratio \( \omega \), which describes the relative importance of electrophoretic transport to convection inside the membrane.

When the diffusive contribution to transport is negligible compared to the convective contribution, \( Pe_m \to \infty \) and Equation (2.38) can be simplified to:

\[
S_a = S_\infty (1 - \omega) = K_c \phi (1 - \omega)
\]

(2.42)

The coefficient \( K_c \) has been evaluated by Bungay and Brenner (1973) using matched asymptotic expansions for small and large values of \( \lambda \). Their results can be approximated by:

\[
K_c = \left(2 - (1 - \lambda)^3\right) \exp\left\{-0.7146\lambda^2\right\}
\]

(2.43)

with better than 2% accuracy compared to the full analytical expression (Zeman and Zydney, 1996). In cases where the electrophoretic contribution to transport is negligible, \( \omega \to 0 \) and Equation (2.42) reduces to \( S_a = S_\infty \).

The total solute flux at any given solvent flux can be evaluated from Equation (2.28) (Saksena, 1995):
\begin{equation}
\langle N_s \rangle = \frac{\phi K_c (1 + \omega) \langle V \rangle [C_w \exp(Pe_m (1 + \omega)) - C_f]}{\exp(Pe_m (1 + \omega)) - 1}
\end{equation}

In the limit of high fluxes and when \( \omega > -1 \), Equation (2.44) reduces to:

\begin{equation}
\langle N_s \rangle = \phi K_c \langle V \rangle (1 + \omega) C_w
\end{equation}

Under these conditions, the solute flux is linearly proportional to \( \langle V \rangle \) and depends only on the solute concentration at the upstream surface of the membrane (\( C_w \)) since there is no back transport of solute against the strong convective flow. In the limit of high solvent flow rates in the negative \( z \) direction (i.e., \( Pe_m \to -\infty \)), and under conditions where \( \omega > 0 \), Equation (2.44) reduces to:

\begin{equation}
\langle N_s \rangle = \phi K_c \langle V \rangle (1 + \omega) C_f
\end{equation}

Thus, the solute flux at large negative \( \langle V \rangle \) is determined entirely by the solute concentration in the filtrate solution. Equation (2.46) is also valid for \( Pe_m \to -\infty \) if \( \omega < -1 \), in which case the electrophoretic transport in the negative \( z \) direction dominates the fluid convection.

In the diffusion-controlled regime (i.e., at low solvent flow rates when convection is negligible), the radially averaged solute flux reduces to:

\begin{equation}
\langle N_s \rangle = \frac{\phi K_d D_\infty}{\delta_m} (C_w - C_f)
\end{equation}

Equation (2.47) is independent of the parameter \( \omega \) since the induced electric field is negligible at very low filtration velocities.
2.3.2.1 Transport Parameters: Hydrodynamic Contribution

The lag coefficient (G) and the enhanced drag coefficient (K) can be evaluated by solving the governing Navier-Stokes equation for the motion of an isolated sphere in a long cylindrical pore. Since detailed expressions for G and K\(^{-1}\) for arbitrary radial positions are currently unavailable, the above integrals are typically evaluated using the centerline approximation (Deen, 1987) in which the hindrance coefficients and electrostatic potentials are assumed to be constant at their centerline values.

Under these conditions Equations (2.31) - (2.33) reduce to:

\[
S_e = \phi K_e = G(\lambda, 0)\exp\left(-\frac{\psi(0)}{kT}\right)\xi \tag{2.48}
\]

\[
\phi K_d = \phi K_e = (1 - \lambda)^2 K^{-1}(\lambda, 0)\exp\left(-\frac{\psi(0)}{kT}\right) \tag{2.49}
\]

where

\[
\xi = \frac{\int_0^{1-\lambda} V\beta d\beta}{\int_0^1 V\beta d\beta} \tag{2.50}
\]

The velocity profile for an uncharged pore is parabolic which yields \(\xi = (1-\lambda)^2[2-(1-\lambda)^2]\) upon performing the required integration in Equation (2.50) (Deen, 1987).

The velocity profile for a charged membrane is distorted by the electroosmotic flow.

Under these conditions, the parameter \(\xi\) is evaluated from Equation (2.50) using the velocity profile given by Equations (2.10) and the axial electric field given by
Equation (2.18). Note that this analysis implicitly assumes that the velocity profile is unaffected by the presence of the protein, which is typically valid since the protein concentration in the pore is sufficiently low that it has no effect on the hydrodynamics or the streaming potential.

Bungay and Brenner (1973) have developed expressions for \( G(\lambda, 0) \) and \( K^{-1}(\lambda, 0) \) for an uncharged sphere in an uncharged cylindrical pore which are valid for all values of the ratio of solute to pore radius (\( \lambda \)) using matched asymptotic expansions for both small and close fitting spheres yielding:

\[
G(\lambda, 0) = \frac{K_s}{2K_t}
\]

\[
K^{-1}(\lambda, 0) = \frac{6\pi}{K_t}
\]

where the indices \((\lambda, 0)\) refer to the ratio of sphere to cylinder radii and the dimensionless radial position, \(\beta = 0\). The hydrodynamic functions \(K_s\) and \(K_t\) are expressed as expansions in \(\lambda\):

\[
\begin{bmatrix} K_t \\ K_s \end{bmatrix} = \frac{9}{4\pi^2\sqrt{2}}(1 - \lambda)^{-5/2} \left[ 1 + \sum_{n=1}^{2} \left( \begin{array}{c} a_n \\ b_n \end{array} \right) (1 - \lambda)^n \right] + \sum_{n=0}^{4} \left( \begin{array}{c} a_{n+3} \\ b_{n+3} \end{array} \right) \lambda^n
\]

(2.53)
The coefficients $a_n$ and $b_n$ are given in Table 2.1 below. Figure 2.2 shows a plot of the hydrodynamic parameters $G$ and $K^{-1}$ as a function of the ratio of the solute to pore. Both $G$ and $K^{-1}$ decrease as $\lambda$ increases due to the increased hydrodynamic interactions with the pore wall. As $\lambda \to 1$, $K^{-1} \to 0$ whereas $G \to 0.5$. This is because when $\lambda \to 1$ the sphere moves like a piston in response to the pressure-driven fluid flow, with its center traveling at the average velocity in the pore which is simply one-half the centerline velocity for an uncharged cylinder (Deen, 1987). Equations (2.47) and (2.48) are in good agreement with more rigorous analyses of hindered diffusion valid for $\lambda < 0.1$ (Brenner and Gaydos, 1977) and $\lambda > 0.9$ (Mavrovouniotis and Brenner, 1988) developed by explicitly averaging the local solute concentration in the pore over the radial direction.
Table 2.1: Expansion coefficients for hydrodynamic functions $K_t$ and $K_s$ in Equation (2.53)

<table>
<thead>
<tr>
<th>Subscript $n$</th>
<th>$a_n$</th>
<th>$b_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-73/60</td>
<td>7/60</td>
</tr>
<tr>
<td>2</td>
<td>77,293/50,400</td>
<td>-2,227/50,400</td>
</tr>
<tr>
<td>3</td>
<td>-22.5083</td>
<td>4.0180</td>
</tr>
<tr>
<td>4</td>
<td>-5.6117</td>
<td>-3.9788</td>
</tr>
<tr>
<td>5</td>
<td>-0.3363</td>
<td>-1.9215</td>
</tr>
<tr>
<td>6</td>
<td>-1.216</td>
<td>4.392</td>
</tr>
<tr>
<td>7</td>
<td>1.647</td>
<td>5.006</td>
</tr>
</tbody>
</table>

Figure 2.2: Hydrodynamic functions $G$ and $K^{-1}$
2.3.2.2 Transport Parameters: Thermodynamic Contribution

The thermodynamic contribution to the transport parameters arises through the solute partition coefficient \((\phi)\) given by Equation (2.37). For an uncharged sphere partitioning into an uncharged cylindrical pore, the potential energy of interaction \((\psi_1)\) is equal to zero and Equation (2.37) reduces to:

\[
\phi = (1-\lambda)^2
\]  

(2.54)

which reflects the excluded volume contribution to the partition coefficient in this geometry.

The electrostatic contribution to the equilibrium energy of interaction for a charged sphere in a charged cylindrical pore \((\psi_1)\) has been evaluated by Smith and Deen (1980, 1983). The potential energy of interaction was determined by solving the linearized Poisson-Boltzmann equation:

\[
\nabla^2 \psi = \tau^2 \psi
\]

(2.55)

where the dimensionless parameter \(\tau\) is equal to the ratio of the pore radius to the Debye length, \(\kappa^{-1}\):

\[
\tau = \kappa r_p
\]

(2.56)

Equation (2.55) was solved by Smith and Deen (1980) using a series representation in spherical coordinates and an integral solution in cylindrical coordinates. The boundary conditions for the particle and pore surfaces were applied to the solutions in spherical and cylindrical coordinates, respectively. The complete solution was then
obtained by matching the coefficients in the two coordinate systems using appropriate
cordinate transformations. The energy of interaction was evaluated by calculating
the change in the energy of the sphere and cylinder system as the particle is brought
into the pore following the procedure of Verwey and Overbeek (1948). The
dimensionless energy of interaction for a sphere situated at the center of the cylinder
(i.e. for the centerline approximation) for constant surface charge boundary
conditions is given as (Smith and Deen, 1980):

\[
V_\alpha = \frac{A_s \sigma_s^2 + A_{sp} \sigma_s \sigma_p + A_p \sigma_p^2}{\pi \tau \exp(-\tau \lambda) - M_0 h(\tau \lambda)}
\]  

(2.57)

where

\[
A_s = \frac{4 \pi \tau \lambda^4 e^{\tau \lambda} M_0}{1 + \tau \lambda}
\]  

(2.58)

\[
A_{sp} = \frac{4 \pi^2 \lambda^2}{I_1(\tau)}
\]  

(2.59)

\[
A_p = \frac{\tau^2 h(\tau \lambda)}{\tau^2 I_1^2(\tau)}
\]  

(2.60)

\[I_1\] is a Bessel function and \(\sigma_s\) and \(\sigma_p\) are the dimensionless surface charge densities of
the solute (sphere) and pore (cylinder), respectively:

\[
\sigma_s = \frac{F \lambda q_s}{\varepsilon_0 \varepsilon_n RT}
\]  

(2.61)
\[
\sigma_p = \frac{F r_p q_p}{\varepsilon_0 \varepsilon_r RT}
\]  

(2.62)

where \( q_s \) and \( q_p \) are the dimensional charge densities. The functions \( h(\tau \lambda) \) and \( M_0 \) are given by:

\[
h(\tau \lambda) = (1 + \tau \lambda) e^{-\tau \lambda} - (1 - \tau \lambda) e^{\tau \lambda}
\]  

(2.63)

\[
M_0 = \int_0^\infty \frac{K_1[(\tau^2 + \alpha^2)^{1/2}]}{I_1[(\tau^2 + \alpha^2)^{1/2}]} d\alpha
\]  

(2.64)

where \( K_1 \) and \( I_1 \) are again the appropriate Bessel functions. The integral in Equation (2.64) can be evaluated approximately for \( \tau \geq 3 \) as:

\[
M_0 = \frac{\pi}{2} \left( \frac{\pi}{\tau} \right)^{1/2} e^{-2\tau} \left[ \tau + \frac{15}{16} - \frac{39}{512\tau} + O(1/\tau^2) \right]
\]  

(2.65)

The dimensionless interaction energy \( V_\sigma \) is related to \( \psi_1(0) \) by:

\[
\psi_1(0) = r_p \varepsilon_0 \varepsilon_r \left[ \frac{RT \gamma^2}{F} \right] V_\sigma
\]  

(2.66)

Smith and Deen subsequently extended their analysis to solutes located at arbitrary radial positions within the pore. In this case the dimensionless energy of interaction for a sphere at a position \( \beta \) within the pore assuming constant surface charge boundary conditions is given as (Smith and Deen, 1983):
\[
V_0 = \frac{A_s \sigma_s^2 + A_{sp} \sigma_s \sigma_p + A_p \sigma_p^2}{\pi \tau e^{-\tau \lambda}} - \frac{2(e^{\tau \lambda} - e^{-\tau \lambda}) \tau \lambda L(\tau \lambda) \Lambda}{1 + \tau \lambda}
\] 
(2.67)

where

\[
A_s' = \left[ \frac{8 \pi \tau \lambda^4 e^{\tau \lambda}}{(1 + \tau \lambda)^2} \right] \Theta
\] 
(2.68)

\[
A_{sp} = \frac{4 \pi^2 \lambda^2 I_0(\tau \beta)}{(1 + \tau \lambda) I_1(\tau)}
\] 
(2.69)

\[
A_p = \left[ \frac{\pi I_0(\tau \beta)}{\tau I_1(\tau)} \right]^2 \left[ \frac{(e^{\tau \lambda} - e^{-\tau \lambda}) \tau \lambda L(\tau \lambda)}{1 + \tau \lambda} \right]
\] 
(2.70)

The functions \( L(\tau \lambda) \) and \( \Theta \) are given by:

\[
L(\tau \lambda) = \cosh(\tau \lambda) - \frac{1}{\tau \lambda}
\] 
(2.71)

\[
\Theta = \frac{\pi}{2} I_0(\tau \beta) \sum_{t=0}^{\infty} \frac{\beta_t (2t)!}{2^t t!} I_1(\tau \beta) \left[ \tau K_{t+1}(2 \tau) + \frac{3}{4} K_t(2 \tau) \right]
\] 
(2.72)

According to both Equations (2.57) and (2.67), \( \psi_1 \) has three distinct contributions:

(1) the increase in energy arising from the deformation of the electrical double layer surrounding the sphere (given by the term involving \( \sigma_s^2 \)), (2) the increase in free energy arising from the deformation of the electrical double layer associated with the
pore (given by the term involving $\sigma^2_p$) and (3) the change in free energy arising from direct charge-charge interactions (given by the term involving $\sigma_s \sigma_p$). The relative magnitudes of these contributions depend on the charges of the sphere and cylinder as well as the solution ionic strength ($\kappa^{-1}$) and the sphere and pore radii.

These three distinct contributions to the dimensionless energy of interaction are shown in Figure 2.3. The results were evaluated for the transport of a positively charged solute with a diameter of 15 Å and a surface charge density of $1.5 \times 10^{-22}$ C/m$^2$ through a positively charged membrane with a uniform pore size of 30 Å and a charge density of $3 \times 10^{-23}$ C/m$^2$. The solid and dashed curves represent the contributions to the total energy of interaction corresponding to the deformation of the double layer around the protein ($A_s \sigma^2_s$), the deformation of the double layer adjacent to the pore wall ($A_p \sigma^2_p$), and the direct charge-charge interactions ($A_{sp} \sigma_s \sigma_p$), with the total energy of interaction given by the sum of these three contributions. The $A_s \sigma^2_s$ and $A_p \sigma^2_p$ terms always act to increase the electrostatic repulsion due to the dependence on the square of the surface charge densities. The $A_{sp} \sigma_s \sigma_p$ term will be attractive (negative) when the protein and membrane are oppositely charged but will be repulsive when the protein and membrane have the same polarity. The $A_{sp} \sigma_s \sigma_p$ term is positive in Figure 2.3 as model calculations were performed with a positively charged protein and membrane. The dominant contribution to the energy is the direct charge-charge interactions, with the energy associated with the distortion of the
double layer around the protein also providing a significant contribution. The contribution associated with the deformation of the double layer adjacent to the pore wall is quite small since the calculations were performed using a surface charge density on the pore that is approximately an order of magnitude smaller than that on the protein.

Figure 2.3: Contributions to the dimensionless electrostatic energy of interaction for the transport of a positively charged spherical solute through a positively charged cylindrical pore as a function of solution ionic strength for \( r_s = 15 \, \text{Å}, \, r_p = 30 \, \text{Å}, \, q_s = 1.5 \times 10^{-22} \, \text{C/m}^2 \), and \( q_p = 3 \times 10^{-23} \, \text{C/m}^2 \).
2.3.3 Pore Size Distribution

All of the equations presented in the previous sections of this chapter are valid for either a single pore or for a membrane in which all of the pores have the same radius. Direct measurements using electron (Kim et al., 1990) or atomic force microscopy (Dietz et al., 1992) indicate that the pore size in a single membrane can vary by well over a factor of ten. Mochizuki and Zydney (1993) and Saksena and Zydney (1995) have examined the effects of an assumed pore size distribution on solute and solvent transport in the presence of both steric and electrostatic interactions. Most of these calculations were performed assuming that the membrane has a log-normal pore size distribution. The following sections present the mathematical form of the log-normal pore size distribution and briefly summarize the effects of a pore size distribution on both solvent and solute transport.

2.3.3.1 Log-Normal Pore Size Distribution

Although the actual pore size distribution of available ultrafiltration membranes is unknown, most previous workers have employed a log-normal pore size distribution to characterize and analyze membrane transport (Causserand et al., 1996). The log-normal pore size distribution has the distinct advantage of being defined only for positive values of the pore radii in contrast to the standard normal (Gaussian) distribution which includes pore radii from \(-\infty\) to \(+\infty\). A number of different forms have been used in the literature to represent the log-normal
distribution and these have been shown to be mathematically equivalent (Zydney et al., 1994). The distribution is most conveniently represented as:

\[
f(r_p) = \frac{n(r_p)}{n_0} = \frac{1}{r_p \sqrt{2\pi b}} \exp \left[ -\frac{1}{2b} \left( \ln \left( \frac{r_p}{\bar{r}} \right) + \frac{b}{2} \right)^2 \right]
\]  

(2.73)

where the parameter \( b \) is given by:

\[
b = \ln \left[ 1 + \left( \frac{\sigma}{\bar{r}} \right)^2 \right]
\]  

(2.74)

with \( \bar{r} \) the mean and \( \sigma \) the standard deviation of the distribution.

Representative plots of the pore-size distributions described by the log-normal density function are shown in Figure 2.4 for several values of the reduced standard deviation, \( \sigma/\bar{r} \). The top panel shows the actual probability density function \( f_R(r_p) \), while the bottom panel shows the cumulative probability function \( F_R(r_p) \), which is defined as the integral of \( f_R(r_p) \) and is thus equal to the fraction of pores in the distribution with radius \( r < r_p \). As shown in the upper panel, the most likely pore radius (the maximum in the probability density function) occurs at a value of \( r_p/\bar{r} < 1 \), with the difference between the most likely radius and the mean pore size increasing with an increase in the breadth of the distribution. This behavior arises from the asymmetry in the log-normal distribution, which has a relatively long tail at large \( r_p \), corresponding to a significant number of very large pores in the distribution. This
can be seen very clearly in the bottom panel for the curve with \( \sigma/\bar{r} = 1 \) in which case more than 10% of the pores have radii \( r_p > 2 \bar{r} \).
Figure 2.4: Representative plots of the log-normal probability density function (upper panel) and the cumulative probability function (lower panel) for several values of the reduced standard deviation. Adapted from Zeman and Zydney (1996).
2.3.3.2 Solvent Transport

The average solvent flux through the membrane is evaluated by integrating the expression for the velocity in a single pore \( \langle V \rangle \) over the pore size distribution (Saksena, 1995):

\[
\overline{V} = \frac{\int_0^\infty \langle V \rangle n(r_p) \pi r_p^2 dr_p}{\int_0^\infty n(r_p) \pi r_p^2 dr_p}
\]  

(2.75)

with \( \langle V \rangle \) given by Equation (2.15) for a cylindrical pore of radius \( r_p \). In this case the streaming potential must be evaluated from the constraint that the total electric current across the membrane is equal to zero:

\[
\int_0^\infty I \cdot n(r_p) \pi r_p^2 dr_p = 0
\]  

(2.76)

where I is the net current flux through a pore with radius \( r_p \). Thus, the magnitude of the induced streaming potential for the membrane is given by:

\[
E_z = \frac{\Delta P}{\delta_m \kappa \eta} B
\]  

(2.77)

where

\[
B = \frac{\int_0^\infty \frac{I_2(\kappa r_p)}{I_1(\kappa r_p)} n(r_p) \pi r_p^2 dr_p}{\int_0^\infty \Lambda_{eff} n(r_p) \pi r_p^2 dr_p}
\]  

(2.78)
2.3.3.3 Solute Transport

The average solute flux can be calculated in a similar manner by integrating the expression for the flux in a single pore $\langle N_s \rangle$ over the pore-size distribution (Saksena, 1995):

\[
\bar{N}_s = \frac{\int_0^r \langle N_s \rangle n(r_p) \pi r_p^2 \, dr_p}{\int_0^r n(r_p) \pi r_p^2 \, dr_p}
\quad (2.79)
\]

with $\langle N_s \rangle$ given by Equation (2.44). In order to perform the integration in Equation (2.79) one needs to account for the fact that the convective solvent flux in the smallest pores can actually be negative since the magnitude of the counter-electroosmotic flow can be greater than the pressure-driven flow in pores with very small radii due to the very different dependence of the pressure-drive and electrically-driven flow rates on the pore radius. The pore radius at which the average solute flux $\langle N_s \rangle$ is equal to 0 is defined as $r_p = r_p^*$ and can be evaluated using Equation (2.15) with $E_z$ evaluated using Equation (2.77). Pores with $r_p < r_p^*$ will have a negative solvent flow and thus a negative (convective) solute flux, while pores with $r_p > r_p^*$ will have a positive solvent and (convective) solute flux.

The average effective asymptotic sieving coefficient is defined experimentally as:

\[
\bar{N}_s = \overline{S}_{eff} \overline{VC_w}
\quad (2.80)
\]
The asymptotic solute flux in Equation (2.80) is evaluated by splitting the integral in the numerator of Equation (2.79) into two parts (Saksena, 1995):

\[
\overline{N}_s = \frac{C_f \int_{r_p^*}^{r_p} S_x (1 + \omega) \langle V \rangle n(r_p) \pi r_p^2 \, dr_p + C_w \int_0^\infty S_x (1 + \omega) \langle V \rangle n(r_p) \pi r_p^2 \, dr_p}{\int_0^\infty n(r_p) \pi r_p^2 \, dr_p}
\]  
(2.81)

An analogous expression for \(\overline{N}_s\) can be developed accounting for a positive solute flux in pores with \(r_p < r_p^*\) (given by Equation 2.45) and a negative flux in pores \(r_p > r_p^*\) as given by Equation (2.46). Equation (2.81) can be combined with Equations (2.80) and (2.75) to yield an expression for the average effective asymptotic sieving coefficient for \(\omega > -1\):

\[
\overline{S}_{\text{eff}} = \frac{\int_{r_p^*}^{r_p} S_x (1 + \omega) \langle V \rangle n(r_p) \pi r_p^2 \, dr_p}{\int_0^{r_p^*} \langle V \rangle n(r_p) \pi r_p^2 \, dr_p} + \frac{\int_0^\infty S_x (1 + \omega) \langle V \rangle n(r_p) \pi r_p^2 \, dr_p}{1 - \int_0^\infty \langle V \rangle n(r_p) \pi r_p^2 \, dr_p}
\]  
(2.82)

using the following relation:

\[
\overline{S}_{\text{eff}} = \frac{C_f}{C_w}
\]  
(2.83)

The integral involving the parameter \(S_x(1+\omega)\) in Equation (2.82) is calculated using Equations (2.48) and (2.41) to evaluate \(S_x\) and \(\omega\) as a function of \(r_p^*\), with \(\psi(0)\)
determined from Equation (2.66) (or the corresponding expression without the centerline approximation). The parameter $\alpha$ is now given by:

$$\alpha = \frac{E_z}{V} = \left( \frac{\kappa r_p^2 B}{8q_p \eta} - \left( \frac{q_p}{\kappa \eta} \right) \frac{I_z(\kappa r_p)}{I_1(\kappa r_p)} \right)^{-1} \quad (2.84)$$

The bulk electrophoretic mobility of the solute ($u_E$) can be calculated from the solute radius and surface charge density ($q_s$) using Henry’s expression (Heimenz and Rajagopalan, 1986).

$$u_E = \frac{2\varepsilon_0 \varepsilon_r \zeta_s}{3\eta} \left[ 1 + \frac{1}{16} (\kappa r_s)^2 - \frac{5}{48} (\kappa r_s)^4 + \frac{1}{96} (\kappa r_s)^6 \right] - \left[ \frac{1}{8} (\kappa r_s)^4 - \frac{1}{96} (\kappa r_s)^6 \right] e^{\kappa r_s} \int_{-\infty}^{\infty} e^{-\eta t} \frac{d}{dt} \quad (2.85)$$

where $q_s$ is related to the surface potential ($\zeta_s$) by:

$$q_s = \frac{\varepsilon_0 \varepsilon_r (1 + \kappa r_s) \zeta_s}{r_s} \quad (2.86)$$

The average effective diffusion coefficient ($\overline{\phi K_d}$) is defined experimentally using an expression for the solute flux in the diffusion-controlled regime (Equation 2.47):

$$\overline{N_s} = \frac{\overline{\phi K_d} D^\infty}{\delta_m} (C_w - C_f) \quad (2.87)$$

$\overline{\phi K_d}$ can thus be evaluated using Equations (2.47), (2.79), and (2.87) as:
\[
\phi_{K_d} = \frac{\int_0^\infty \phi_{K_d} n(r_p) r_p^2 \, dr_p}{\int_0^\infty n(r_p) r_p^2 \, dr_p}
\]  \hspace{1cm} (2.88)

where \( \phi_{K_d} \) is given by Equation (2.49) or (2.52) with \( \psi_1 \) determined from Equation (2.57) or (2.67) for solutions based on the centerline approximation or the full radial analysis, respectively.
Chapter 3

MATERIALS AND METHODS

3.1 Introduction

This chapter describes the apparatus, materials, and methods used for the experimental procedures that were common to most of the studies described in the thesis. Additional details on specific procedures are provided in the appropriate chapters.

3.2 Membranes

3.2.1 Membrane Properties

Asymmetric membranes are used in almost all commercial applications of ultrafiltration. These membranes are anisotropic and have a thin skin, which provides the membrane its functionality, and a much thicker and more porous support that provides the membrane its structural integrity. The small thickness of the skin allows much higher fluxes to be obtained compared to symmetric membranes with comparable selectivity.

Although a variety of polymers can be used to make asymmetric ultrafiltration membranes, the most widely used materials are polysulfone, polyethersulfone, and regenerated cellulose. Polysulfone and polyethersulfone are thermally very stable, having relatively high glass transition temperatures, and they are also chemically inert.
and resistant to most acid, base, and bleach solutions used for membrane cleaning and sterilization. Polyethersulfone is somewhat less hydrophobic than polysulfone (Figure 3.1) due to the increased proportion of the sulfone groups, although most commercial polysulfone/polyethersulfone membranes are surface modified to render them even more hydrophilic to reduce protein adsorption (Mochizuki and Zydney, 1992). The large numbers of hydroxyl groups on regenerated cellulose membranes render them extremely hydrophilic significantly reducing protein binding and fouling during use. The structural integrity of regenerated cellulose membranes is enhanced by casting the cellulose onto a microporous substrate (typically, polyethylene). The resultant composite regenerated cellulose (CRC) membranes have a uniform, robust structure, with high integrity and greater resistance to back pressure. Since regenerated cellulose membranes are not very stable to extreme acidic or basic conditions their application is limited to applications that do not require harsh chemicals for cleaning and sanitization. These membranes have become a major component of the downstream purification process for therapeutic protein products in the biotechnology industry.
Figure 3.1: Molecular structures of polysulfone, polyethersulfone, and cellulose
Most of the experiments carried out in this thesis were performed using composite regenerated cellulose (CRC) membranes with molecular weight cut-offs of 30 and 100 kDa. The molecular weight cut-off corresponds to the molecular weight of a solute that has a 90% rejection level as determined by the manufacturer. These membranes were generously provided by Millipore Corporation (Bedford, MA) in the form of large flat sheets, which were cut into appropriate size disks using a cutting device fabricated in our laboratory. All membranes were flushed with either deionized water or with saline to remove wetting agents using at least 100 L/m² of membrane area. Once flushed, the membranes were kept immersed in water or saline to prevent collapse of the pore structure by drying.

The composite regenerated cellulose membranes consist of (1) a regenerated cellulose skin approximately 0.5 µm thick, (2) a porous substructure (approximately 60 µm thick), and (3) a porous polyethylene support matrix. A scanning electron micrograph of the cross section of the CRC membrane is shown in Figure 3.2 (adapted from Christy et al., 2004).
3.2.2 Charge Modification

Most of the methods for surface modification of cellulose membranes are based on the activation and subsequent reaction of hydroxyl groups on the base cellulose (see Figure 3.1). Modification of hydroxyl moieties is typically carried out either by physical modification, like UV irradiation or by chemical attachment of different functional groups. Usually, these chemical modifications involve esterification or etherification reactions of the hydroxyl groups. Etherification of cellulose proceeds
under alkaline conditions, generally in aqueous NaOH solutions. Treatment of native cellulose with NaOH causes the cellulose to swell, which makes it more readily accessible to the modification reagent. Two types of reactions dominate cellulose etherification (Mondt, 1983):

1. **Williamson etherification:** An organic halide is used as the etherification reagent and reaction is carried out in presence of alkali.

2. **Alkali-catalyzed oxyalkylation:** In this reaction an epoxide is added to the swollen alkali cellulose. Only catalytic amounts of alkali are required, thus, in principle no alkali is consumed. The reaction may proceed further as new hydroxyl groups are also generated during this reaction.

Charge modification of CRC membranes for experimental studies in this thesis was performed using several different strategies. Positively- and negatively charged versions of the CRC membranes were prepared by chemical attachment of quaternary and sulfonic acid groups, respectively. Initial studies were performed using a proprietary chemistry developed at Genentech involving in situ chemical modification using an organic halide (Williamson etherification). The reaction employed 2M bromo-propyl-trimethyl-ammonium bromide and bromo-propyl-sulfonic acid in 0.1N sodium hydroxide (van Reis, 2001). Subsequent experiments used a proprietary chemistry provided by Millipore. An alternative approach developed at Penn State employed multiple epoxy and diamine reactions to generate positively charged membranes with different spacer arm length. This chemistry is described in more detail in Chapter 8.
3.2.3 Membrane Charge

The surface charge density of the charge-modified CRC membranes was evaluated using streaming potential measurements obtained with the device shown in Figure 3.3. The system consisted of two Plexiglas chambers, each 2 cm in diameter and approximately 2.4 cm in length. The ends of the chambers were threaded so that they could be screwed together to give a tight seal. Ag/AgCl electrodes were screwed tightly into the ends of the chambers to ensure reproducible placement relative to the membrane surface. The electrodes were placed approximately 1-2 mm away from the membrane surface. To prevent leakage, O-rings were used to seal the electrodes. The Ag/AgCl electrodes were first prepared by placing a 1 mm diameter silver wire (Sigma Chemical Co., St. Louis, MO) and a reducing electrode in a 1 M KCl solution. The wire and electrode were connected to a DC power source, and the current was maintained at 20 mA for approximately 20 minutes to deposit a uniform Ag/AgCl layer on the wire surface.
The feed and exit chambers of the streaming potential device were assembled after carefully sealing the membrane in place. The chambers were slowly filled with a buffered saline at the desired pH and ionic strength, taking care to remove any trapped air bubbles. A feed reservoir, containing buffered saline, was attached to the feed port, with the exit from the second chamber directed to the drain. The system was pressurized by adjusting the height of the feed reservoir or by air pressurization. The system was allowed to equilibrate for approximately 30 minutes after which the transmembrane voltage, also known as the streaming potential, $E_z$, was measured using a high impedance 8060A True RMS Multimeter (Fluke Corp., Everett, WA) connected to the two electrodes. Streaming potential measurements were obtained at
several distinct pressures up to 35 kPa (∼5 psi) with the system allowed to stabilize at each pressure. The membrane zeta potential was evaluated from the slope of a plot of the streaming potential as a function of the applied pressure. Wilbert et al. (1999) have shown that this approach gives more accurate and reproducible data than using a continuous pressure ramp as employed in most commercial zeta potential devices.

3.3 Model Proteins

Most of the protein sieving experiments were performed using cytochrome c (Sigma C-2506, St. Louis, MO) as a model protein. Cytochrome c is a small globular protein (see Figure 3.4) consisting of 104 amino acids, 12 of which are negatively charged at neutral pH (Glu, Asp) and 21 of which are positively charged at neutral pH (Lys, Arg). Cytochrome c has an isoelectric point of about 10.2, it has a molecular weight of approximately 12400 Da (g/mol), and an approximately spherical shape (axbxb = 15×17×17 Å³). The tertiary structure of cytochrome c is quite stable over a wide range of pH and ionic strength and its physico-chemical properties are very well characterized (Baglioni et al., 2004). Cytochrome c thus provides a very good model protein for these experimental studies. Limited experiments were also performed using bovine serum albumin (Sigma A-7906, St. Louis, MO). BSA has a molecular weight of approximately 67,000 Da and an isoelectric pH of 4.8. Experiments performed at Genentech (Chapter 7) used a feedstock containing a recombinant monoclonal antibody.
Figure 3.4: Tertiary structure of Horse heart cytochrome C (taken from RCSB Protein Data Bank http://www.rcsb.org/pdb/). Hydrophobic residues are highlighted in dark grey while hydrophilic residues are in light grey.

3.4 Solution Preparation

3.4.1 Salt Solutions

Salt solutions (KCl and phosphate buffered saline) were prepared by dissolving pre-weighed quantities of the appropriate salts in deionized water obtained from a Barnstead Nanopure ultrapure water system (Barnstead, Dubuque, IA) with resistivity of at least $18 \text{ M}\Omega\cdot\text{cm}$ (equivalent to a conductivity of less than $5.6 \times 10^{-5}$ mS/cm). All salts were of certified ACS grade and were obtained from Fischer Scientific (Pittsburgh, PA). The 0.15 M phosphate buffered saline (PBS) was made
by dissolving 8.04 gm Na$_2$HPO$_4$·7H$_2$O, 4.08 gm KH$_2$PO$_4$·H$_2$O and 0.67 gm NaOH in 1 liter of deionized water. Higher ionic strength PBS solutions were prepared using greater amounts of these salts (keeping the relative amounts constant). Lower ionic strength solutions were prepared by appropriately diluting the 0.15 M solution with deionized water. KCl solutions were prepared similarly by dissolving pre-weighed amounts of KCl (Sigma Chemicals, St. Louis, MO) in deionized distilled water. These solutions were buffered by adding approximately 20 mM of Bis-Tris buffer (Sigma Chemicals, St. Louis, MO). The solution pH was then adjusted using small amounts of 0.1 M KOH or 0.1 M HCl as required. The pH was measured to within 0.1 pH units using a Thermo Orion Model 420 pH meter, and the solution conductivity was measured using a Thermo Orion Model 105 conductivity meter. PBS solutions were typically used at pH 7.4 and Bis-Tris buffered solutions were used at pH 7. All solutions were prefiltered through 0.2-µm pore size Supor 200 membranes (Pall Life Sciences, Ann Arbor, MI) to remove any particles prior to use. The ionic strengths of the salt solutions were evaluated as:

$$I = \frac{1}{2} \sum z_i^2 C_i \tag{3.1}$$

where $z_i$ and $C_i$ are the net charge and total concentration of each ion.
3.4.2 Protein Solutions

Protein solutions were prepared by dissolving an appropriate amount of the protein powder in a buffered salt solution. The pH of the solution was then readjusted by adding small amounts of 0.1 M KOH or 0.1 M HCl as required, and the pH measured to within 0.1 pH units using a Thermo Orion Model 420 pH meter. All protein solutions were prefomed through 0.2-µm µ-Star nylon filters (8010, Costar Corporation) to remove large aggregates and any undissolved protein. The filtered protein solutions were stored at 4°C prior to use. Experiments performed at Genentech used feedstocks obtained directly from purification of the harvested cell culture fluid as described in Chapter 7.

3.5 Protein Diagnostics

Concentrations of cytochrome c and bovine serum albumin (BSA) were determined spectrophotometrically using a Shimadzu UV-Vis Mini 1240 spectrophotometer (Shimadzu, Columbia, MD). For cytochrome c, the absorbance was evaluated at 410 nm, which is the natural absorbance peak for the protein. Actual concentrations were evaluated by comparison of the measured absorbance with that of known cytochrome c standards using the calibration curve shown in Figure 3.5. Cytochrome c concentrations could be measured accurately down to concentrations of 0.001 g/L. BSA concentrations were evaluated using the measured absorbance at 280 nm, which is the natural absorbance of the aromatic groups. Actual concentrations were again determined by comparison to known BSA standards.
using the appropriate calibration curve (Figure 3.6). The recombinant monoclonal antibody was also analyzed using UV spectrophotometry. The concentration of host cell proteins was determined by enzyme linked immunosorbent assay (ELISA). These latter techniques are described in more detail in Chapter 7.

Figure 3.5: Calibration curve for Cytochrome c using absorbance at 410 nm
3.6 Filtration Cell and Operation

Most filtration experiments were performed in a 12 ml volume, 25 mm diameter stirred ultrafiltration cell (Amicon Model 8010, Millipore). A schematic diagram of the stirred cell set-up is shown in Figure 3.7. The membrane was placed on top of a microporous Tyvek support in a housing at the bottom of the cell with the skin side (typically, shiny surface) facing up. A rubber O-ring was placed between the membrane and the sleeve of the cell to form a leak-free seal. The entire apparatus was carefully filled with saline taking care to eliminate any entrapped air bubbles in the cell and associated tubing. Additional solution was fed to the stirred cell from a 1 or 2 liter solution reservoir. The transmembrane pressure drop was set by adjusting
the height of the solution reservoir (for pressures less than 1 psi) or by air pressurization. The pressure on the filtrate side was atmospheric under all conditions. The stirring speed was adjusted using a magnetic stirrer. The actual stirring speed was evaluated using a Strobotac Type 1531-AB phototachometer (General Radio Co., Concord, MA).

Figure 3.7: Schematic of the stirred cell apparatus
3.6.1 Hydraulic Permeability

The membrane hydraulic permeability was evaluated by measuring the flow rate of buffer solution through the membrane as a function of the transmembrane pressure drop. Filtrate flow rates were evaluated using timed collection with the filtrate mass determined using a Sartorius digital balance with accuracy of ± 1 mg (Model 1518 Sartorius, Westbury, NY). The applied transmembrane pressure drop was typically varied over the range from 1 - 5 psi to prevent any compression of the membrane at high pressures.

3.6.2 Protein Sieving

Protein sieving experiments were typically performed with membranes that were preadsorbed with protein. Protein adsorption was allowed to occur by soaking the flushed membrane (i.e. the membrane after removal of any wetting agents) in a protein solution for at least 12 hours at 4°C to ensure equilibrium adsorption. This long equilibration time was required because of diffusion limitations within the porous membrane (Robertson and Zydney, 1990). The membrane was then removed from the protein solution and gently rinsed with saline to remove any labile protein. The membrane was placed in the 12 ml Amicon 8010 stirred cell and the stirring speed was set to 600 rpm. The hydraulic permeability was evaluated before performing any sieving experiments.

After evaluating the hydraulic permeability, the ultrafiltration cell was filled with a protein solution at the desired protein concentration, solution pH, and ionic
strength. Filtration was performed either at constant transmembrane pressure or at a constant filtrate velocity. In both cases, data collection was begun after the system had attained stable operation, i.e. after filtration for a minimum of 2 minutes and after collection of a minimum of 500 µl of filtrate, with the latter required to wash out the dead volume downstream of the membrane in the stirred cell (approximately 200 µl).

A constant transmembrane pressure was set by air pressurization of the buffer reservoir connected to the stirred cell, while a constant filtration velocity was maintained using a microprocessor controlled, variable speed, 10-roller peristaltic pump (Rabbit-Plus, Rainin Instrument Co., Woburn, MA) connected to the filtrate line. The actual filtration velocity was evaluated by weighing samples obtained by timed collection. A schematic of the set-up used for the constant filtrate velocity experiments is shown in Figure 3.8. The protein concentration in the filtrate samples were determined spectrophotometrically as described previously. The filtrate port was then clamped and a small sample (approximately 100 µl) was taken directly from the stirred cell to evaluate the bulk protein concentration. The stirred cell was then re-filled with protein solution and the entire procedure was repeated either at a new value of the applied pressure or at a new filtrate velocity. Membranes were stored in buffered saline at 4 °C between experiments.
Figure 3.8: Schematic of stirred cell set-up for protein sieving experiments at constant filtrate flux. Most of the ultrafiltration experiments in this thesis were performed at a constant filtrate flux of 7 µm/sec.

3.7 Pore Size Characterization

The pore size characteristics of the ultrafiltration membranes were determined using polydisperse dextrans as neutral probe molecules following the basic approach described by Mochizuki and Zydney (1992).

3.7.1 Dextran Solution Properties

Dextrans are polysaccharides produced by a strain of the bacterium *Leuconostoc mesenteroides*. The naturally occurring polymer is reduced to a desired range of molecular weight by partial hydrolysis. Dextrans are polymers of glucose, joined by $\alpha$-1,6 linkages, with a small number of branches attached to the main chain by $\alpha$-1-3 links. The structure of a typical dextran molecule is shown in Figure 3.9.
Dextrans do not have any ionizable side groups making them a useful probe molecule for studying size-based interactions.

Figure 3.9: Structure of dextran (adapted from Pujar, 1996)
Granath (1958) measured the diffusion coefficient for different molecular weight dextrans using a diffusion cell. The dextrans used in this thesis were similar to the dextrans studied by Granath having 5% branch points. Granath correlated the dextran diffusion coefficient for molecular weights between 21,600 and 526,000 using a simple power law expression as:

\[
\log(D_{\infty}) = -8.1154 - 0.47752 \log(MW)
\]  

with \(D_{\infty}\) given in units of cm\(^2\)/s. Preston et al. (1982) presented a similar correlation for the diffusion coefficients of dextrans with MW of 3,000 - 200,000:

\[
\log(D_{\infty}) = -7.75 - 0.552 \log(MW)
\]  

The diffusion coefficients given by Equation (3.3) are slightly larger than those given by Equation (3.2) at low dextran molecular weights with the opposite behavior seen at high molecular weights. The maximum deviation is less than 10% over the entire range of molecular weight. The Stokes radii of the different dextrans were evaluated from Equation (3.2) as (Granath and Kvist, 1967):

\[
R_{SE} = 0.31 (MW)^{0.47}
\]  

with \(R_{SE}\) given in Å and the MW given in g/mol. Squire (1981) provided a slightly different correlation for \(R_{SE}\) based on sedimentation, viscosity, and light scattering measurements. These results are discussed in more detail by Mochizuki (1992).
3.7.2 Dextran Solution Preparation

Dextran solutions were prepared by dissolving pre-weighed quantities of the powdered dextran (Sigma Chemical Company, St. Louis, MO) in the desired buffered saline at appropriate pH and ionic strength. The pH was adjusted by adding 0.1 N HCl or 0.1 N KOH as required.

3.7.3 Dextran Analysis: Size Exclusion Chromatography

Size-exclusion chromatography (SEC) was used to analyze the size distribution of the dextran samples. A schematic of the SEC apparatus is shown in Figure 3.10.

Figure 3.10: Schematic of size exclusion chromatography system
The SEC column was first equilibrated using at least two column volumes of the eluent buffer at a flow rate of 0.8 ml/min. This also served to flush both the sample and reference cells in the refractive index detector. The analysis was started after ensuring a steady baseline. Dextran standards were obtained from American Polymer Standards (Mentor, OH) and run before each analysis, with the samples fed using an intelligent auto-sampler (AS-400, Hitachi Instrument Inc., Columbia, MD). The column was checked by verifying the linearity of the retention time as a function of log (MW) for the dextran standards. Solute detection was performed using an LC-30 RI (Refractive Index) detector. Data collection was performed using ChemStation software (Agilent Technologies, CA) installed on a Dell Pentium 4 computer.

3.7.4 Membrane Pore-Size Distribution

The membrane pore size distribution was determined from the dextran sieving profile. Polydisperse dextrans with an average molecular weight of 15 kDa and 75 kDa (Sigma Chemicals) were used to characterize the 30 and 100 kD CRC membranes, respectively. The dextrans were dissolved in a 0.5 M KCl solution buffered with 20 mM Bis-Tris at pH 7. The total dextran concentration was evaluated from the amount of dextran added to the salt solution. Dextran sieving data were obtained using an Amicon 8010 stirred cell with the stirrer speed set at 600 rpm. Permeate samples were collected after filtration of a minimum of 1 ml to wash out the dead volume beneath the stirred cell. The molecular weight distributions for the dextrans in the bulk and permeate solutions were determined by size exclusion
chromatography (Agilent 1100) with refractive index detection by running the samples through a TSK-Gel G 2000 SW silica resin column (Tosoh Biosciences, PA). Column calibration was performed using narrow molecular mass dextran standards (4.4, 9.9, 18.5, 22.5 and 27.5 kDa) obtained from American Polymer Standards (Mentor, OH). A typical calibration curve for the TSK Gel 2000 SW column is shown in Figure 3.11.

Figure 3.11: Dextran calibration curve for TSK Gel 2000SW column
The size exclusion chromatograms for the bulk and filtrate samples were sliced into segments covering small molecular weight fractions (approximately 1% of the dextran molecular weight) using ChemStation software (Agilent Technologies, CA). The observed sieving coefficient was then evaluated from the ratio of the filtrate to bulk concentration for a particular molecular weight dextran fraction. All calculations and data analysis were performed using the ChemStation Integrator program.
Chapter 4

PERMEABILITY-SELECTIVITY ANALYSIS FOR
ULTRAFILTRATION MEMBRANES

4.1 Introduction

Ultrafiltration is currently used for the concentration of a wide range of protein products, including recombinant therapeutics, industrial enzymes, and a variety of food and beverages (van Reis and Zydney, 1999; Wang, 2001). Since the development of ultrafiltration as a viable industrial process in the 1960’s, there have been literally thousands of different UF membranes sold commercially. For example, the chapter on Ultrafiltration in the 1992 Edition of the Membrane Handbook (Kulkarni et al., 1992) lists 26 different manufacturers of UF membranes, many of which produced several different series of membranes (e.g., polysulfone, cellulose acetate, and regenerated cellulose), with each series containing membranes having a range of pore size or molecular weight cut-off. The most recent edition of Munir Cheryan’s Ultrafiltration and Microfiltration Handbook (Cheryan, 1998) lists more than 90 companies providing membrane and/or module systems for ultrafiltration and microfiltration.

One of the major challenges facing end-users of ultrafiltration membranes is the enormous difficulty in comparing membrane products provided by different manufacturers and made from different polymeric or ceramic materials. Ultrafiltration membranes are normally rated by their nominal molecular weight cut-
off, which is typically defined as the molecular weight of a solute that has a rejection coefficient of 90%. However, there is no standardization in this 90% value, and different manufacturers measure the rejection using solutes with very different physical properties and under very different operating conditions. Dextrans are probably the most widely used test solutes (Muhlerkar and van Reis, 2004), but other polymers and different series of model proteins have also been considered. The net result is that two membranes rated as having the same nominal molecular weight cut-off can have very different pore size and performance characteristics. In addition, the molecular weight of a solute with 90% rejection provide no quantitative information on the molecular weight cut-off required to achieve the 99% or 99.9% retention currently targeted for applications of ultrafiltration in the purification of high value proteins (van Reis and Zydney, 1999; Mulherkar and van Reis, 2004).

In the area of gas separation membranes, Lloyd Robeson (1991) presented a very simple approach for comparing membranes made from different materials and different manufacturers. The separation factor, $\alpha$, defined as the ratio of the permeability of the more permeable gas to that of the less permeable species, was plotted as a function of the permeability of the more permeable gas on a log-log scale. A typical Robeson plot is shown in Figure 4.1 for the separation of nitrogen and hydrogen. Data for a large number of different membranes all clustered below a critical line, or upper bound, which is often referred to in the gas separations community as the “line of death” since there are few (if any) membranes that provide a combination of selectivity and permeability above this limit. The original
discussion of the “Robeson Plot” has become one of the most highly cited papers in the gas membrane separations literature with more than 250 citations as of early 2004.

Figure 4.1: A typical Robeson plot for binary gas mixtures. The separation factor, $\alpha$ is plotted against the more permeable gas (reprinted from Robeson, 1991)

The objectives of this chapter were: (1) to develop an analogous “Robeson Plot” suitable for examining the trade-offs between permeability and selectivity for different ultrafiltration membranes, (2) to examine the theoretical basis for the underlying relationship in this plot, and (3) to use this Robeson plot to examine the performance characteristics of recently developed charged ultrafiltration membranes.
4.2 Materials and Methods

Protein sieving experiments with charged ultrafiltration membranes were performed using bovine serum albumin (BSA) (Fraction V heat shock precipitated BSA, catalogue # A7906, Sigma Chemical, St. Louis, MO) and lysozyme (Catalogue # L6876, Sigma Chemical, St. Louis, MO). Both BSA and lysozyme solutions were prepared using a phosphate buffered saline, which was made by dissolving pre-weighed amounts of Na$_2$HPO$_4$	extcdot7H$_2$O and KH$_2$PO$_4$	extcdotH$_2$O in deionized distilled water. The ionic strength of the buffer solution was adjusted to 10 mM by diluting the 0.14 M PBS solution with an appropriate volume of deionized water.

Both buffer and protein solutions were prefiltered through 0.2 µm pore size Super-200 membranes (Pall Corp., Ann Arbor, MI) to remove particulates and any protein aggregates. The hydraulic permeability measurements and protein sieving experiments were performed using the stirred cell apparatus shown in Chapter 2. Protein concentration in the bulk and filtrate was determined spectrophotometrically with a UV-VIS spectrophotometer (UV mini 1240, Shimadzu, Kyoto, Japan) using the natural absorbance at 280 nm. Actual concentrations were evaluated by comparison of the measured absorbance with that of standard solutions of known concentrations.

All filtration experiments with BSA and lysozyme were performed with negatively- and positively-charged versions of the Composite Regenerated Cellulose (CRC) membranes (Millipore Corp., Bedford, MA), respectively. The negatively-charged membranes were prepared by the covalent attachment of sulphonic acid
groups to the surface of the membrane using the base-activated chemistry developed by van Reis (2001). The positively-charged membranes were prepared by covalent attachment of quaternary ammonium groups to the base cellulose matrix using a proprietary chemistry developed at Millipore. All membranes were thoroughly flushed with approximately 100 L/m$^2$ of 0.1 N NaOH followed by 100 L/m$^2$ of distilled deionized water to remove any residual chemicals.

4.3 Permeability-Selectivity Analysis

In its most basic form, ultrafiltration is a pressure-driven process designed to remove solvent (typically water) and small solutes (e.g., salts and sugars) from a large macromolecule (Figure 4.2). Since mass transport is dominated by convection, the rate of mass transport for both the product and the small impurities is proportional to the filtrate flux and the corresponding solute sieving coefficients ($S_i$), where $S_i$ is equal to the ratio of the solute concentration in the filtrate to that in the bulk (feed) solution. The sieving coefficient is simply equal to 1-R where R is the protein rejection coefficient.
Figure 4.2: Schematic of a typical ultrafiltration process

Since the filtration velocity is the same for all species, the separation factor would simply be equal to the ratio of the sieving coefficients for the small impurities to that of the protein product:

$$\alpha = \frac{S_{\text{small}}}{S_{\text{protein}}}$$  \hspace{1cm} (4.1)

Since very small impurities, e.g., most buffer components, pass freely through the membrane, $S_{\text{small}} \approx 1$ and the separation factor becomes equal to the reciprocal of the protein sieving coefficient.

The permeability of interest in an ultrafiltration process is that with respect to the solvent and is given as:

$$L_p = \frac{J_v}{\Delta P}$$  \hspace{1cm} (4.2)

where $J_v$ is the volumetric filtrate flux (volume flow rate per membrane area) and $\Delta P$ is the transmembrane pressure driving force. $L_p$ is often referred to as the hydraulic permeability since water is the typical solvent. The permeability is often normalized
by the solvent viscosity to account for the effects of temperature and/or different solvent compositions. The literature includes a wide range of different units for the permeability, with the filtrate flux given in m/s, L/m²/hr (LMH), or gal/ft²/day (gfd) and the pressure driving force given in Pa, mm Hg, psi, or atm (among others).

Figure 4.3 shows the permeability-selectivity trade-off using literature data for the ultrafiltration of bovine serum albumin (Fane et al., 1983; Opong and Zydney, 1991; Miller et al., 1992; Nakatsuka and Michaels, 1992; Pujar and Zydney, 1994; Shukla et al., 2000). Bovine serum albumin (BSA) was chosen as the protein of interest due to the large amount of available data on BSA ultrafiltration. The separation factor has been evaluated using Equation (4.1) based on the actual protein sieving coefficient \( S_a \) to account for differences in concentration polarization within the various modules used in these studies: stirred cells, hollow fibers, screened channel cassettes, and ceramic monoliths. The values thus provide a measure of the intrinsic separation factor or selectivity for the membrane. The actual sieving coefficients were evaluated from experimental data for the observed sieving coefficients \( S_o = C_{\text{filtrate}}/C_{\text{feed}} \) using the classical stagnant film model, which is described in detail in Chapter 3:

\[
S_a = \frac{S_o}{(1-S_o)\exp\left(\frac{J_v}{k}\right) + S_o}
\]

(4.3)
The mass transfer coefficients \( k \) in the different modules were calculated using appropriate experimental correlations, all of which can be conveniently expressed in the dimensionless form as (Zeman and Zydney, 1996):

\[
Sh = \beta \text{Re}^a \text{Sc}^b \left( \frac{d_h}{L} \right)^c
\]  

(4.4)

where

\[
Sh = \frac{kd_h}{D} = \text{the Sherwood number}
\]

\[
\text{Re} = \frac{\rho Ud_h}{\eta} = \text{the Reynolds number}
\]

\[
\text{Sc} = \frac{\eta}{\rho D} = \text{the Schmidt number}
\]

\[L = \text{channel length}\]

\[D = \text{solute diffusion coefficient}\]

The parameters \( \beta, a, b, \) and \( c \) are functions of the specific system geometry and flow conditions.

The hydraulic diameter, \( d_h \), for each membrane channel is defined as

\[
d_h = 4 \times \frac{\text{cross-sectional area available for flow}}{\text{wetted parameter of the channel}}
\]  

(4.5)

The hydraulic diameter, \( d_h \), is equal to the tube diameter, \( d \), for an open tube or hollow fiber and is equal to twice the channel spacing (height), \( 2h \) for a slit shaped channel formed between two parallel plates. Theoretical results for the parameters \( \beta, \)
a, b, and c for fully developed laminar flow, developing laminar flow, and turbulent flow are given in Table 4.1 along with experimental correlations for systems with spacers and in a stirred ultrafiltration cell (Zeman and Zydney, 1996).

### Table 4.1: Mass Transfer Coefficient Correlations (adapted from Zeman and Zydney, 1996)

<table>
<thead>
<tr>
<th>Conditions</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>( \beta )</th>
<th>Approach</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laminar tube</td>
<td>0.33</td>
<td>0.33</td>
<td>0.33</td>
<td>1.62</td>
<td>Theoretical</td>
<td>(Leveque, 1928)</td>
</tr>
<tr>
<td>Laminar slit</td>
<td>0.33</td>
<td>0.33</td>
<td>0.33</td>
<td>1.86</td>
<td>Theoretical</td>
<td>(Leveque, 1928)</td>
</tr>
<tr>
<td>Turbulent</td>
<td>0.875</td>
<td>0.25</td>
<td>__</td>
<td>0.023</td>
<td>Theoretical (eddy model)</td>
<td>(Deissler, 1961)</td>
</tr>
<tr>
<td>Stirred cell</td>
<td>0.567</td>
<td>0.33</td>
<td>__</td>
<td>0.23</td>
<td>Mass transport</td>
<td>(Smith et al., 1968)</td>
</tr>
</tbody>
</table>
Figure 4.3: Robeson plot for ultrafiltration membranes using BSA as the model protein. Solid curves represents model calculations using a log-normal pore size distribution with \( \sigma/\bar{r} = 0.2 \) and \( \varepsilon/\delta_m = 1 \mu m^{-1} \). Membrane porosity (\( \varepsilon \)) and skin thickness (\( \delta_m \)) were chosen as 0.5 and 0.5 \( \mu m \), respectively.

Literature results for the permeability-selectivity trade-off shown above (Figure 4.3) were taken under conditions where the actual sieving coefficient was approximately equal to the asymptotic sieving coefficient, and where \( J_v/k < 10 \) to avoid highly polarized conditions. Care was taken to use data obtained during the initial stages of the ultrafiltration where membrane fouling was likely to be low. The data obtained by Opong and Zydney (1991) were for membranes that had been pre-
adsorbed in the protein solution prior to the ultrafiltration experiment, with the hydraulic permeability and the sieving coefficients evaluated after this initial protein adsorption. Thus, the separation factor and permeability in these studies were both evaluated for membranes in the same physical “state”.

The filled circles in Figure 4.3 represent data for polysulfone and polyethersulfone membranes, the dominant materials used in ultrafiltration, while the filled squares represent results for cellulosics. Acrylic and acrylonitrile membranes are represented by filled triangles. Results for other materials, including ceramics (zirconium) and polycarbonate track-etched membranes, are shown with open symbols. These membranes have nominal molecular weight cut-offs (provided by the manufacturers) ranging from 30 kD to 1000 kD compared to the 69 kD molecular weight of BSA. The data for this wide range of membranes all tend to cluster along, and below, a single curve, which represents the upper limit (or upper bound) of current ultrafiltration membranes. All of the existing membranes display a similar trade-off between separation factor and permeability; membranes with high separation factors will have relatively low permeability while those with high permeabilities have low separation factors. The ideal UF membrane would have a very high separation factor (providing very high product retention and yield of the desired protein) and very high permeability (providing the potential for very high filtration rates). Such a membrane would be located in the upper right hand corner of the plot, a region that is currently inaccessible by existing UF membranes.
Although most of the membranes in Figure 4.3 cluster along the same curve, there are a small number of outliers that seem to have unusually low separation factors and/or permeabilities. The open squares at a permeability of $0.03 \times 10^{-9}$ m/s/Pa and $0.04 \times 10^{-9}$ m/s/Pa are for polycarbonate track-etch membranes, which are typically used only for laboratory studies. These membranes have very low porosity (typically around 5%) and a homogeneous pore morphology, with an effective thickness of 10 µm compared to the 0.5 µm skin thickness of commercial asymmetric membranes. This combination of low porosity and large thickness gives a very low permeability structure at the same separation factor. The open triangles in Figure 4.3 are for E-series membranes produced by Desalination Systems and available in spiral wound modules (Pradanos and Hernandez, 1995). It is unclear why these particular membranes, or the ceramic membranes studied by Millesime et al. (1996), have such low permeabilities.

4.4 Theoretical Analysis

In order to obtain additional insights into the trade-off between the separation factor and permeability, theoretical calculations were performed for an idealized UF membrane consisting of a parallel array of cylindrical pores having a distribution of pore radii. Fluid flow through each pore is described by the Hagen-Poiseuille equation, with the permeability for the membrane as a whole given as (Mochizuki and Zydney, 1993):
\[
\overline{L}_p = \frac{\varepsilon}{8\mu \delta_m} \frac{\int_0^\infty n(r_p) r_p^4 dr_p}{\int_0^\infty n(r_p) r_p^2 dr_p}
\]  \hspace{1cm} (4.6)

where \( \varepsilon \) is the membrane porosity, \( \delta_m \) is the membrane thickness, \( \mu \) is the solvent viscosity, and \( n(r_p) \) is the pore size distribution. The \( r^4 \) dependence in the numerator comes from the Hagen-Poiseuille equation, while the \( r^2 \) dependence in the denominator is from the cross-sectional area of the cylindrical pores.

The protein sieving coefficient can also be evaluated by integration over the pore size distribution:

\[
\overline{S_a} = \frac{\int_0^\infty S_a(r_p) n(r_p) r_p^4 dr_p}{\int_0^\infty n(r_p) r_p^2 dr_p}
\]  \hspace{1cm} (4.7)

\( S_a(r_p) \), the actual sieving coefficient in a pore with radius \( r \), was evaluated using the expression developed by Zeman and Wales (1981):

\[
S_a(r) = (1 - \lambda)^2 \left[ 2 - (1 - \lambda)^2 \right] \exp\left(-0.7146\lambda^2\right)
\]  \hspace{1cm} (4.8)

where \( \lambda = r_s/r_p \) with \( r_s \) being the protein radius. Note that Equation (4.8) is only valid if protein transport through the membrane pore is dominated by convection, which is a reasonable approximation for most ultrafiltration systems. Equation (4.8) gives values of \( S_a \) that are within 2% of more complex expressions over the full range of \( \lambda \). (Zeman and Zydney, 1996).
Theoretical calculations were performed using a log-normal pore size distribution (Zydney et al., 1994):

\[
n(r_p) = \frac{n_0}{r_p \sqrt{2\pi}} \left[ \ln \left( 1 + \left( \frac{\sigma}{\bar{r}} \right)^2 \right) \right]^{\frac{1}{2}} \exp \left\{ -\left( \frac{\ln \frac{r_p}{\bar{r}} - 1 + \left( \frac{\sigma}{\bar{r}} \right)^2}{2\ln \left[ 1 + \left( \frac{\sigma}{\bar{r}} \right)^2 \right]} \right)^2 \right\}
\] (4.9)

where \( \bar{r} \) is the mean and \( \sigma^2 \) the variance of the distribution, respectively. The log-normal density function has been used extensively in the past to describe membrane pore size distributions (Zydney et al., 1994) and is discussed in greater detail in Section 3.3.3.1. The assumption of a log-normal pore size distribution is particularly convenient for this type of analysis since it is only defined for positive values of the pore radius.

The solid curve in Figure 4.3 was developed using Equations (4.6) to (4.9) with the coefficient of variation kept at a constant value of \( \sigma/\bar{r} = 0.2 \). For these calculations, the radius of BSA was taken as \( r_s = 36.5 \, \text{Å} \) and the viscosity of water was taken as 0.001 Pa-s. The ratio of the membrane porosity to the membrane thickness was chosen as \( \varepsilon/\delta_m = 1 \, \mu \text{m}^{-1} \), which is consistent with a membrane having a porosity of 0.5 and a skin thickness of 0.5 microns. Calculations were performed by varying the mean pore size (keeping \( \sigma/\bar{r} \) and \( \varepsilon/\delta_m \) fixed), with the separation factor and the permeability for each value of \( \bar{r} \) plotted as the solid curve in Figure 4.3.

Membranes with large mean pore radius have high permeability but low separation factors (i.e., poor protein retention), while membranes with small mean pore radius have very high separation factors but low permeability. The model calculations are in
surprisingly good agreement with the upper bound for the experimental data, suggesting that the best membranes currently available have pore size distributions that are at least approximately described by a log-normal density function with coefficient of variation equal to about 0.2.

The effects of the membrane properties on the trade-off between the permeability and separation factor is examined in more detail in Figure 4.4. The upper panel shows simulations with different values of the coefficient of variation (with $\varepsilon/\delta_m = 1 \mu m^{-1}$), while the lower panel shows results for membranes with different values of $\varepsilon/\delta_m$ (with $\sigma/r = 0.2$). As the coefficient of variation is reduced from 0.5 to 0.1, the upper bound moves up and towards the right since the tighter pore size distribution reduces the number of very large pores that are permeable to the protein of interest. The solid curve (upper panel) represents the trade-off for a membrane with a uniform pore size and marks the maximum upper limit on the performance of an ultrafiltration membrane for purely size-based separations (assuming a constant membrane porosity and thickness). The simulations suggest that even such an idealized membrane would provide only a small improvement in performance compared to membranes that possess a pore size distribution with a coefficient of variation less than 0.2 (at least for systems in which 99% retention is sufficient).

The upper bound also moves up and to the right as $\varepsilon/\delta_m$ is increased, corresponding to an increase in the membrane porosity and/or a reduction in the skin thickness. The curve with $\varepsilon/\delta_m = 2$ would correspond to a membrane with
ε = 0.5 and δₘ = 0.25 μm, which is considerably thinner than existing ultrafiltration membranes. In addition, it is important to note that protein diffusion may become important with such thin membranes, which would cause an increase in protein transmission and thus a reduction in the separation factor relative to that predicted using Equation (4.8) (Opong and Zydney, 1991).
Figure 4.4: Influence of membrane properties on the trade-off between the separation factor and permeability: (A) effect of breadth of pore size distribution, (B) effect of porosity to thickness ratio. Filled circles represent data for prototype charged CRC membranes.
4.5 Electrically Charged Ultrafiltration Membranes

The filled circles in Figure 4.4 represent data for the ultrafiltration of BSA using prototype negatively charged 100 kD CRC membranes prepared by the chemical modification described earlier. This involved the covalent attachment of sulfonic acid groups to the base cellulose. Ultrafiltration experiments were performed at pH 7.4 in a 10 mM phosphate buffer solution, with this low ionic strength chosen to enhance the electrostatic repulsion between the negatively-charged BSA and the negatively-charged membrane. This electrostatic exclusion causes a dramatic increase in the separation factor, with relatively little change in the membrane permeability. Data obtained with the neutral CRC membrane, or with the negatively-charged membrane but at high salt concentrations, gave results that are in good agreement with the upper bound for conventional UF membranes (represented very well by the solid curve in Figure 4.3). These data clearly demonstrate that electrically-charged ultrafiltration membranes can provide a dramatic improvement in ultrafiltration performance; the permeability at a given separation factor is approximately twice as large as that of a comparable neutral membrane while the separation factor at a given permeability is improved by 15-fold. That level of improvement is also considerably better than what is predicted for a two-fold reduction in the coefficient of variation, and it is similar to what would be achieved with a two-fold reduction in membrane skin thickness (but without concerns about protein diffusion or loss of membrane structural integrity).
Figure 4.5: Robeson plot for ultrafiltration membranes using lysozyme as the model protein. Solid curves represent model calculations using a log-normal pore size distribution with $\sigma/F = 0.2$ and $\varepsilon/\delta_m = 0.3 \, \mu m^{-1}$. Membrane porosity ($\varepsilon$) and skin thickness ($\delta_m$) were chosen as 0.15 and 0.5 $\mu m$, respectively.

Figure 4.5 shows a similar plot of the permeability–selectivity tradeoff for lysozyme using literature data obtained with several ultrafiltration membranes (Fane et al., 1983; Balakrishnan and Agarwal, 1996; Millesime et al., 1996; Ghosh, 2000; Ghosh et al., 2000; 2002; Magueijo et al., 2002; Muller et al., 2003). The total number of data points on this plot is fairly limited since there are many fewer quantitative studies of lysozyme ultrafiltration (compared to that for BSA). The separation factors were again evaluated using Equations (4.1) and (4.3), with the mass transfer coefficients determined from the correlations given in Table 4.1 for the
various membrane modules. Literature data for several membranes like polysulfone, polyethersulfone, cellulosics, ceramics, etc. are represented by filled and open symbols.

The solid curve in Figure 4.5 represents the theoretical tradeoff between permeability and selectivity for lysozyme and was developed using Equations (4.6) to (4.9) as discussed earlier. For these calculations, the radius of lysozyme was taken as $r_s = 20.5$ Å (Wilkins et al., 1999) and the viscosity of water was taken as $0.001$ Pa·s. The coefficient of variation ($\sigma/r$) was kept at a constant value of 0.2 and the ratio of the porosity to the membrane skin thickness ($\varepsilon/\delta_m$) was chosen as $0.3 \mu m^{-1}$. This latter value is considerably smaller than the ratio chosen for BSA, reflecting the use of much lower molecular weight cut off membranes ($10$ kD – $100$ kD) in the experiments performed with the smaller lysozyme. Several researchers (Singh et al., 1998; Masselin et al., 2000, 2001) have reported that the porosity of low molecular weight cut off membranes typically lies in the range of 5 to 20% with the lower end representing polysulfones and track etch membranes and the upper end representing polyvinyl and acrylonitrile. This range of porosity is considerably smaller than that found in higher molecular weight cut-off membranes, justifying the use of a smaller value of $\varepsilon/\delta_m$ for the lysozyme calculations. Most of the data in Figure 4.5 tend to lie in the vicinity of the model calculation, consistent with the inherent tradeoff between the permeability and selectivity of ultrafiltration membranes. The outliers in Figure 4.5 are mostly polycarbonate track etch membranes (represented by open squares);
these membranes have very low permeabilities due to low porosity (≤ 5%) and the much greater skin thickness (10 µm).

The filled triangles in Figure 4.5 represent experimental data obtained as part of this thesis using prototype positively charged membranes synthesized by chemical attachment of quaternary ammonium groups to 30 and 100 kD CRC membranes (using the chemistry discussed in Section 3.2.2). Ultrafiltration experiments with these charged membranes were performed at pH 7 and 10 mM ionic strength to enhance electrostatic interactions between the positively charged lysozyme and the positively charged membranes. Consistent with the earlier results obtained with BSA using negatively charged membranes, these positively charged ultrafiltration membranes provide a much better combination of permeability and selectivity than corresponding neutral membranes due to the enhanced electrostatic exclusion of the like charged protein.

4.6 Discussion

The permeability-selectivity analysis, or “Robeson plot”, developed in this chapter provides a very convenient means for comparing the performance characteristics of different ultrafiltration membranes. In this case, there is an inherent trade-off between the separation factor, which is simply equal to the reciprocal of the protein sieving coefficient, and the membrane permeability. Data for a number of different ultrafiltration membranes tend to fall along, or below, an “upper bound” that reflects the current state-of-the-art in commercial ultrafiltration membranes. The
shape of this upper bound is also consistent with a theoretical analysis of solute and solvent transport through a membrane composed of a parallel array of cylindrical pores with the selectivity determined entirely by size-based interactions.

These results also provide a framework that can be used to analyze the performance of new ultrafiltration membranes. This was demonstrated using data for prototype ultrafiltration membranes developed by attaching either a sulfonic acid or quaternary amine moiety to existing composite regenerated cellulose membranes. These charge-modified membranes provide a much better combination of separation factor and permeability than existing ultrafiltration membranes due to strong electrostatic exclusion effects. The negatively-charged membrane thus showed excellent performance characteristics for ultrafiltration of the negatively-charged BSA while the positively-charged membrane showed excellent performance for the positively-charged lysozyme. The performance of these charge-modified membranes also exceeds what would be expected for membranes having a much tighter pore size distribution, suggesting that the addition of electrical charge may be much more effective at improving membrane performance than attempts to control the membrane pore size.

The data and analysis presented in this chapter were focused on the behavior of ultrafiltration membranes for protein concentration (or buffer exchange) using BSA and lysozyme. Similar plots could also be constructed for other proteins, allowing one to examine the behavior of membranes over an even broader range of nominal molecular weight cut-offs. In addition, a very similar approach could be
used to construct a Robeson plot for selective ultrafiltration membranes used for protein separations, with the separation factor now given by the ratio of the sieving coefficients for the two proteins of interest. This type of protein separation has now become feasible through the development of High Performance Tangential Flow Filtration, a process that is discussed in more detail in Chapter 7.
5.1 Introduction

Until fairly recently, ultrafiltration was viewed as a purely size-based separation, with the rate of solute transmission determined entirely by the relative size of the solute and the membrane pores. The limited experimental results presented in Chapter 4 showed that protein transport through a semipermeable membrane is also strongly affected by electrostatic interactions. A number of other investigators have also conclusively demonstrated the importance of electrostatic interactions in determining both the filtrate flux and solute retention during ultrafiltration. For example, Pujar and Zydney (1994) showed that a reduction in solution ionic strength from 0.15 M to 0.001 M caused more than a two order of magnitude reduction in the rate of protein transmission through a negatively-charged polyethersulfone membrane, with this behavior in good agreement with model calculations accounting for the effects of electrostatic interactions on the partitioning of a charged sphere into a charged cylindrical pore. Burns and Zydney (1999) examined the effects of solution pH on the transport of a series of proteins with different isoelectric points (the pH at which the net protein charge is zero) having molecular weights ranging from 12.2 kD to 185 kD. In each case, the maximum transmission was obtained near the protein isoelectric point, with sharp reductions in protein transmission observed at
pH both above and below the isoelectric point due to the energetic penalty associated with the deformation of the electrical double layer around the protein.

There have also been some limited studies specifically focused on the effects of membrane charge on protein transport. Nakao et al. (1988) performed experiments using surface modified polysulfone membranes with the greatest protein rejection seen under conditions when the protein and membrane had similar polarity. Miyama et al. (1988) obtained data for the filtration of BSA through both neutral and positively charged polyacrylonitrile membranes. Transmission of the negatively charged BSA was considerably greater with the positively-charged membrane, which the authors attributed to the electrostatic attraction under these conditions. Nyström et al. (1998) examined the fractionation of binary mixtures of model proteins, with the best fractionation obtained when one protein was at its isoelectric point while the other one was retained by a charged membrane with like polarity. Burns and Zydney (2001) studied the transport of ovotransferrin (MW = 80 kD, isoelectric point = 5.5) through both a standard (negative) polyethersulfone membrane and a positively-charged version of that membrane generated by covalent attachment of a quaternary amine functionality. The highest protein transmission was achieved with the positive membrane at low salt concentrations using a pH where the protein had a small negative charge, conditions that correspond to a weak attractive interaction between the oppositely charged protein and membrane. However, it was difficult to draw any direct conclusions about the effects of membrane charge due to the large increase in
the breadth of the pore size distribution caused by the chemical modification of the polyethersulfone membrane used in this study.

In addition to altering the rate of protein transmission, the membrane charge also affects the rate of fluid flow due to the generation of counter-electroosmosis, which is often referred to in the literature as the electroviscous effect (Hunter, 1981). As discussed in Chapter 2, fluid flow through the charged pores generates a voltage (streaming potential) across the membrane, which is needed to satisfy the condition of no net current flow due to the unequal convective transport of the co-ions and counter-ions. This streaming potential induces a back flow of fluid due to the electrical stresses in the Navier-Stokes equation. Pujar and Zydney (1994) showed that counter-electroosmosis reduced the permeability of a polyethersulfone membrane by approximately 15% at low salt concentrations (1 mM). Huisman et al. (1988) evaluated the electroviscous effect for both microfiltration (ceramic) and ultrafiltration (polysulfone, cellulose acetate) membranes, with the permeability at very low ionic strength (0.1 mM) reduced by 8% for the alumina microfiltration membranes and close to 15% for the 100 kDa polysulfone ultrafiltration membranes.

Although these studies provide some insights into the effects of membrane charge on protein ultrafiltration, they do not provide any direct measurements of the relationship between the fluid flow and protein transmission and the actual magnitude of the membrane surface charge. The primary objective of this chapter was to obtain quantitative data for both the protein transmission and filtrate flux for a series of charge-modified cellulose membranes produced by chemical modification with a
quaternary amine functionality for different reaction times. The surface charge
density of the resulting membranes was evaluated from streaming potential
measurements, with the fluid flow rate and the transmission of cytochrome c
determined over a range of solution ionic strength. The data provide the first
quantitative analysis of the effects of membrane charge density on the performance of
ultrafiltration membranes, and they demonstrate the potential of using these charge-
modified membranes to provide a better combination of flux and selectivity than
traditional ultrafiltration membranes.

5.2 Materials and Methods

KCl solutions were prepared by dissolving pre-weighed amounts of KCl
(Sigma Chemicals, St. Louis, MO) in deionized distilled water as described in Section
3.4.1. Solutions were buffered at the desired ionic strength by adding approximately
20 mM of Bis-Tris buffer (Sigma Chemicals, St. Louis, MO). The solution pH was
then adjusted to pH 7 using small amounts of 1 M KOH or 1 M HCl as required. All
solutions were prefILTERed through 0.2-µm pore size Supor 200 membranes (Pall Life
Sciences, Ann Arbor, MI) to remove any particles prior to use.

Protein solutions were prepared by dissolving appropriate amounts of horse
heart cytochrome c (C 2506, Sigma Chemical) in the buffer solution. All protein
solutions were prefILTERed through 0.2-µm µ-Star nylon filters (8010, Costar
Corporation) to remove large aggregates and any undissolved protein. The protein
concentration was determined spectrophotometrically using a Shimadzu UV-Vis Mini
1240 spectrophotometer (Shimadzu, Columbia, MD). The absorbance was evaluated at 410 nm, which is the natural absorbance peak for cytochrome c. Actual concentrations were evaluated by comparison of the absorbance with that of known protein standards using the calibration curve shown in Figure 3.5. Protein concentrations could be measured accurately down to concentrations of 0.001 g/l.

5.2.1 Membrane Preparation

A number of techniques have been described in the literature for synthesis of charged membranes. Ehsani et al. (1997) increased the magnitude of negative charge on polysulfone ultrafiltration membranes using Ultraviolet (UV) irradiation, which resulted in the formation of carboxylate and sulphonic acid groups. Su et al. (2004) used chloromethylation and aqueous trimethylamine solutions to add quaternary amine groups to nanofiltration membranes. Nakao et al. (1988) synthesized negatively- and positively-charged polymers by sulfonation and chloromethylation followed by quaternization, respectively, with the resulting polymers used to cast the corresponding charged ultrafiltration membranes.

The series of positively-charged membranes described in this chapter were generated by chemical attachment of a quaternary ammonium group to the free hydroxyls in the glucose rings of cellulose membranes using a proprietary solution chemistry developed by Millipore Corporation (Billerica, MA) as discussed in Section 3.2.2. Neutral composite regenerated cellulose (CRC) membranes with nominal molecular weight cutoff of 30 kD were obtained from Millipore Corporation.
The extent of charge modification was controlled by varying the reaction time from 10 to 180 min. X-ray Photoelectron Spectroscopy (XPS) was used to confirm the presence of the charge modification by tracking the peak corresponding to the amine nitrogen (which was completely absent from the base CRC membranes). All membranes were thoroughly flushed with 0.1 M NaOH followed by 1 M KCl buffered with 0.02 M Bis-Tris to remove any residual chemicals, using a minimum of 50 L/m² per membrane.

5.2.2 Permeability Measurements

The hydraulic permeability of the membranes at high salt concentration was evaluated before and after reaction to estimate the reduction in membrane pore size associated with the charge modification. The membranes were placed in a 25 mm diameter stirred cell (Model 8010, Amicon) on top of a microporous Tyvek support, and the device was filled with a 1 M KCl solution buffered with 20 mM Bis-Tris. This high salt concentration was used to minimize the effect of counter-electroosmosis on the membrane permeability. The filtrate flux was evaluated by timed collection over a range of transmembrane pressures from 14 to 34 kPa (corresponding to 2 – 5 psi). The hydraulic permeability \( L_p \) was evaluated directly from the slope of the flux versus pressure data as:

\[
L_p = \frac{J_v}{\Delta P}
\]  

(5.1)

where \( J_v \) is the volumetric filtrate flux (volume flow rate per membrane area) and \( \Delta P \) is the transmembrane pressure.
Additional hydraulic permeability measurements were obtained over a range of solution ionic strength to evaluate the extent of counter-electroosmosis. The filtrate flux was evaluated as a function of the applied pressure beginning with a 1 mM KCl solution (buffered with 20 mM Bis-Tris) followed by several other buffer solutions each with increasing KCl concentration. Repeat measurements were then performed with decreasing KCl concentration to insure that no fouling had occurred during the permeability measurements. The charge-modified membranes were allowed to equilibrate with the different ionic strength solutions by flushing approximately 100 L/m$^2$ of each solution through the membrane before evaluating the permeability.

5.2.3 X-Ray Photoelectron Spectroscopy

X-ray photoelectron spectroscopy (XPS) was used to examine the elemental composition of the charge-modified membranes. The membranes were first flushed with deionized water to remove any residual salt or contaminants. The membranes were cut into small pieces using a sharp razor blade and the final samples were mounted on conducting carbon tape using tweezers.

XPS analysis was performed using a Kratos Analytical Axis Ultra instrument (Kratos Analytical Inc., Chestnut Ridge, NY) available in the Materials Research Institute at The Pennsylvania State University. Data were obtained using monochromatic Al as the X-Ray source (1486.6 eV photons). The photoelectron take-off angle was set at 90$^\circ$ with respect to the sample plane. The analysis spot size

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was 110 µm with a sampling depth of approximately 25 Å. All binding energies were referenced to the C-1s hydrocarbon peak at 285.0 eV. The XPS data were analyzed by integrating the peak areas and applying the appropriate relative sensitivity factors to account for the x-ray cross-section and the transmission function of the spectrometer.

### 5.2.4 Membrane Characterization

#### 5.2.4.1 Membrane Pore Size

The pore size distribution for each membrane was determined from the sieving profile evaluated using polydisperse dextrans with an average molecular weight of 15 kDa (Sigma Chemicals). The dextrans were dissolved in a 0.5 M KCl solution buffered with 20 mM Bis-Tris at pH 7. Data were obtained using an Amicon 8010 stirred cell with the stirrer speed set at 600 rpm. Permeate samples were collected after filtration of a minimum of 2 mL to wash out the dead volume beneath the stirred cell. The molecular weight distributions for the dextrans in the bulk and permeate solutions were determined by size exclusion chromatography (Agilent 1100) with refractive index detection using a TSK-gel G 2000 SW silica resin (Tosoh Biosciences, PA) as described in Section 3.7.3. A log-normal pore density function (Equation 2.74) was assumed for the membranes used in this thesis; this type of distribution has been used extensively for characterizing the pore size distribution of ultrafiltration membranes (Zydney, 1994). The mean, $\bar{r}$, and standard deviation, $\sigma$, in the distribution were determined by minimizing the sum of the squared residuals.
between the experimental data and model calculations for the actual sieving coefficients for dextrans with molecular weights ranging from 5 kD to 25 kD. At the high salt concentration used in these experiments, any long-range (e.g., electrostatic) interactions are negligible and the actual dextran sieving coefficient is given as

\[
S_a = \frac{\int_0^\infty S_a(r_p)n(r_p)r_p^4 \, dr_p}{\int_0^\infty n(r_p)r_p^4 \, dr_p}
\]  

(5.2)

where \( S_a \) is the actual sieving coefficient in a pore of radius \( r_p \) and can be evaluated assuming purely steric interactions as:

\[
S_a = \phi K_c = (1 - \lambda)^2 \left[ 2 - (1 - \lambda)^2 \right] G(\lambda,0)
\]  

(5.3)

where \( G(\lambda,0) \) is the lag coefficient evaluated using Equation (2.51) as given by Bungay and Brenner and \( K_c \) is calculated from Equation (2.31). The radii of the different molecular weight dextrans were evaluated using the Stokes-Einstein equation (see Equation 5.17 in Section 5.3.1). All integrals were evaluated numerically using Mathematica 5.2 software.

### 5.2.4.2 Membrane Charge

Streaming potential measurements have been used widely in the literature for evaluating the membrane surface charge. For example, Nyström et al. (1989) used streaming potential measurements to characterize ultrafiltration membranes and to estimate the effects of small ions and charged polyelectrolytes on the apparent zeta potential. Kim et al. (1996) used streaming potential measurements and
electroosmosis to evaluate the surface charge characteristics of different polymeric membranes.

The surface charge densities of the positively charged membranes examined in this study were evaluated from streaming potential measurements following the procedure described in Chapter 3. The membrane was sealed between two Plexiglas chambers, which were then filled with a 10 mM KCl solution buffered with 1 mM Bis-Tris at pH 7. Ag/AgCl electrodes were inserted through the ends of the chambers and sealed using O-rings to prevent leakage. The electrodes were placed approximately 0.001 m from each surface of the membrane. The upstream chamber was pressurized with air, with the outflow collected from a port on the upper surface of the downstream chamber. The streaming potential was evaluated as a function of the transmembrane pressure using a Keithly 2000 Multimeter connected to Ag/AgCl electrodes. The membrane surface charge density and apparent zeta potential were evaluated from the slope of the measured voltage as a function of the transmembrane pressure. The membrane surface charge density \( q_p \) was evaluated from the streaming potential data using the analysis developed by Saksena and Zydney (1995) as discussed in Section 2.3.3.2:

\[
\frac{dE_z}{d\Delta P} = \left( \frac{q_p}{\kappa \eta} \right) B
\]

(5.4)

where \( E_z \) is the measured streaming potential, \( \kappa^{-1} \) is the double layer thickness, and \( \eta \) is the solution viscosity. The parameter \( B \) accounts for the pore size distribution and is given as (see Equation 2.78):
where $I_0$, $I_1$ and $I_2$ are Bessel function of zero, first, and second order, $\Lambda^0$ is the bulk solution conductivity, and $r_p$ is the pore radius. The membrane surface charge density was calculated iteratively using the experimentally measured values of $\frac{dE_z}{d\Delta P}$ and the pore-size distribution $n(r_p)$ determined from the dextran sieving data. Note that this analysis accounts for the increase in conductivity within the membrane pore caused by the presence of the surface charge, but it does not account for the possibility of an irregular pore morphology and/or hindrance to ion diffusion in the pores.

### 5.2.5 Protein Surface Charge Density

The net charge for cytochrome c at a given pH and solution ionic strength was calculated by summing over all the ionizable groups:

$$z_{\text{protein}} = z_{\text{max}}^+ - \sum_{i=1}^{N} r_i$$

(5.6)

where $z_{\text{max}}^+$ is the maximum possible positive charge on the protein (equal to +25 for the native unmodified cytochrome c based on the residues shown in Table 5.1), $N$ is the total number of residues, and $r_i$ is the number of dissociated groups. The dissociation equilibrium of a typical amino acid residue (for example, an
α-carboxylic acid) is described by the intrinsic dissociation constant of that ionizable group:

\[
K_{\text{int}}^i = \frac{[R - \text{COO}^-][H^+]}{[\text{RCOOH}]} \tag{5.7}
\]

Equation (5.7) is written in terms of the local H\(^+\) ion concentration at the protein surface, which is different than the bulk solution concentration due to electrostatic interactions between the charged protein and the charged hydrogen ion. The local H\(^+\) is related to the concentration in the bulk solution (H\(_b^+\)) by the Boltzmann distribution:

\[
H^+ = H_b^+ \exp \left( \frac{-e\psi_s}{k_bT} \right) \tag{5.8}
\]

where e is the electron charge (1.609\(\times\)10\(^{-19}\) C) and \(\psi_s\) is the electrostatic potential at the protein surface. The number of dissociated groups (\(r_i\)) can thus be calculated at a given pH from:

\[
pH = pK_{\text{int}}^i + \log \frac{r_i}{n_i - r_i} \tag{5.9}
\]

with \(n_i\) being the number of titratable groups of type i. The number (\(n_i\)) and \(pK_{\text{int}}^i = -\log(K_{\text{int}}^i)\) for the ionizable groups in cytochrome c (Horn and Heuck, 1983) are given in Table 5.1.
In addition to the dissociation of various acidic and basic groups summarized in Table 5.1, cytochrome c may also bind a variety of ions. The effect of ion binding on the net charge of cytochrome c was neglected in this study due to lack of information on the extent of binding of different ions to cytochrome c.

In order to evaluate the protein charge, one needs to develop a relationship between charge and potential. Such a relationship is developed by solving the Poisson-Boltzmann equation for the electrostatic potential around the protein molecule. To simplify the calculation, the protein molecule is approximated as an equivalent sphere of radius $r_s$, with the surface charge distributed uniformly over the
surface of the sphere. The spherically symmetric form of the Poisson equation is given by:

\[
\frac{1}{r^2} \frac{d}{dr} \left( r^2 \frac{d\psi}{dr} \right) = -\frac{\rho_E}{\varepsilon_0 \varepsilon_r} = -\sum \frac{z_i C_i}{\varepsilon_0 \varepsilon_r}
\]

(5.10)

where \(\rho_E\) is the local charge density and \(C_i\) is the local ion concentration. The concentrations of the ions are given by the Boltzmann distribution as described in Chapter 2. Even with this spherical approximation, the Poisson-Boltzmann equation can only be solved analytically under conditions of low surface potential (the Debye-Huckel approximation). Under these conditions, the linearized form of the Poisson-Boltzmann equation for a 1:1 electrolyte becomes:

\[
\frac{1}{r^2} \frac{d}{dr} \left( r^2 \frac{d\psi}{dr} \right) = \kappa^{-1}\psi
\]

(5.11)

where \(\kappa^{-1}\) is the double-layer thickness as defined previously in Chapter 2. Equation (5.11) can be solved subject to the boundary conditions that \(\psi = \psi_s\) at \(r = r_s\) and \(\psi \to 0\) as \(r \to \infty\) for the isolated protein. This gives:

\[
\psi = \psi_s \frac{r_s \exp \left[ -\kappa (r - r_s) \right]}{r}
\]

(5.12)

A direct relationship between the surface potential (\(\psi_s\)) and the net surface charge density (\(q_s\)) can be obtained using the overall electroneutrality condition for the system (Heimenz and Rajagopalan, 1997):

\[
q_s = -\varepsilon_0 \varepsilon_r \frac{d\psi}{dr} \bigg|_{r=r_s}
\]

(5.13)

which becomes:
where the derivative of the potential has been evaluated using Equation (5.12). The surface charge density, $q_s$, is directly related to the number of charged groups on the protein surface as:

\[ q_s = \frac{-\varepsilon_0 \varepsilon_r \psi_s (1 + \kappa r_s)}{r_s} \]  

(5.14)

where $q_s$ is the surface charge density, $\varepsilon_0 \varepsilon_r$ is the permittivity of the medium, $\psi_s$ is the potential, $r_s$ is the radius of the protein, and $\kappa$ is the reciprocal Debye length.

The model calculations for the net charge on cytochrome c in 10 and 150 mM KCl are shown in Figure 5.1 as an explicit function of solution pH. The net protein charge was evaluated by solving iteratively Equations (5.6), (5.8), (5.9) and (5.14) at given values of the solution pH and ionic strength. The calculated isoelectric point for the protein was approximately 10, which is in good agreement with the literature value of 10.2 reported by Horn and Heuck (1983). The protein charge decreases fairly rapidly at low pH due to the protonation of the carboxylic acid groups, but then remains fairly constant between pH 5 and 8 due to the absence of any significant number of ionizable residues with pK$_a$ between pH 5 and 10 (see Table 5.1). The difference in net cytochrome c charge at lower and higher salt concentrations can be attributed to charge regulation effects. At lower salt concentration (10 mM), the H$^+$ concentration at the protein surface is different than that in the bulk solution due to electrostatic interactions between the charged protein and the charged H$^+$ ions. For bulk pH less than the pI (i.e., when the protein is positively charged), the local H$^+$
concentration near the protein surface is lower at 10 mM ionic strength compared to 150 mM ionic strength due to the electrostatic repulsion between the positively charged protein and the positively charged H\(^+\) ions. This causes lower protonation of the carboxylic groups resulting in a lower net positive charge at 10 mM ionic strength. For bulk pH higher than the pI (i.e., when the protein is negatively charged), the localized pH near the protein molecule is lower at 10 mM ionic strength compared to 150 mM ionic strength due to the electrostatic attraction between the negatively charged protein and the positively charged H\(^+\) ions. This increases the protonation of amine groups resulting in a lower net negative charge at lower ionic strength.

Figure 5.1: Calculated net charge of cytochrome c as a function of solution pH at lower (10 mM) and higher (150 mM) ionic strengths
5.2.6 Protein Ultrafiltration

All ultrafiltration experiments were conducted in a 25 mm diameter Amicon stirred ultrafiltration cell as described in Chapter 3 (Section 3.4). The membranes (and Tyvek supports) that were to be used in the sieving experiments were soaked overnight in the protein solution to insure equilibrium adsorption. The membrane was then placed at the bottom of the stirred cell, directly on top of a Tyvek support, and the entire system was flushed with approximately 50 L/m$^2$ of a 0.5 M KCl solution buffered with 20 mM Bis-Tris at pH 7. The stirred cell was then filled with the cytochrome c solution, and the stirring speed was set to 600 rpm. The filtrate flux was maintained at 7×10^{-6} m/s (25 L/m$^2$/h) using a Rabbit Plus peristaltic pump (Rainin Instrument Corp.) connected directly to the filtrate exit port. Filtrate samples were obtained for subsequent protein analysis after collection of a minimum of 2 mL of filtrate to wash out the dead volume beneath the membrane in the stirred cell. Filtrate flow rates were evaluated using timed collection with the filtrate mass determined using a digital balance.
5.3 Results and Analysis

5.3.1 Membrane Characterization

Figure 5.2 shows typical experimental data for the streaming potential ($E_z$) as a function of the transmembrane pressure for a series of charge modified cellulose membranes each held in the reaction mixture for a different length of time. Repeat measurements of the slope of the streaming potential versus transmembrane pressure with the same membrane were highly reproducible, and in each case the data were highly linear with $r^2$ values greater than 0.99. The slope of the streaming potential versus pressure data, which provides a direct measure of the membrane surface charge, increases with increasing reaction time as expected.

![Figure 5.2: Streaming potential data for a series of charge modified CRC 30 kD membrane using a 10 mM KCl solution at pH 7](image)

Figure 5.2: Streaming potential data for a series of charge modified CRC 30 kD membrane using a 10 mM KCl solution at pH 7
Figure 5.3 shows experimental data for repeat measurements of slope of the streaming potential versus pressure data for a sample charge-modified membrane. Although the intercept varied during different experimental measurements, the slope, which is a direct measure of the membrane charge, remained constant. The variation in intercept is most likely due to small asymmetries in the Ag/AgCl electrodes. This displacement of intercept however had no effect on the slope or the calculated values of the membrane surface charge.

Figure 5.3: Repeat measurements of streaming potential data for a sample charge-modified membrane using a 10 mM KCl solution
The presence of the quaternary amine groups was also confirmed by X-ray Photoelectron Spectroscopy (XPS) through the presence of the nitrogen group in the quaternary ammonium functionality as shown in Figure 5.4; the nitrogen peak at a binding energy of approximately 400 eV was completely absent in the unmodified cellulose membrane. Quantitative analysis of the XPS data gave a nitrogen concentration of approximately 2% for the membrane modified for 180 min. This corresponds to the presence of approximately 1 quaternary amine group for every 6 glucose monomers in the cellulosic polymer.
The apparent zeta potential ($\zeta_{\text{app}}$) for each membrane was calculated from the Helmholtz-Smoluchowski equation as (Hunter, 1981):

$$
\zeta_{\text{app}} = \frac{\eta \lambda_0}{\varepsilon_0 \varepsilon_r} \left( \frac{dE_z}{dP} \right) \quad (5.16)
$$

where $\varepsilon_0$ is the permittivity of free space, $\varepsilon_r$ is the relative permittivity (or dielectric constant) of the solvent, $\eta$ is the solution viscosity, and $\lambda_0$ is the solution conductivity. Equation (5.16) would give the true zeta potential only under conditions where the double layer thickness is a small fraction of the pore radius (Hunter, 1981). The evaluation of the actual zeta potential (discussed subsequently) requires knowledge of the detailed pore size distribution of the membrane. The apparent zeta potential of the unmodified CRC membrane was $-0.2$ mV, with this small negative charge arising from the preferential adsorption of chloride ions onto the membrane surface, similar to the behavior observed by Burns and Zydney (2001) for a polyethersulfone membrane. The apparent zeta potential of the modified membranes increases from 0.3 mV after 10 min of reaction to 5.8 mV after 60 min and then to 8.7 mV after 180 min (Figure 5.5). Although the rate of modification slows down at long reaction times, there is no evidence of saturation of the reaction sites even after 180 min, which is consistent with the presence of a large number of unmodified glucose monomers. Data obtained at even longer reaction times (not shown) demonstrated further increases in zeta potential.
Figure 5.5: Variation of apparent membrane zeta potential ($\zeta_{\text{app}}$) with exposure to charging ligand

The dextran sieving profiles for several of the charge-modified membranes are shown in Figure 5.6. The sieving coefficients are plotted as a function of the Stokes-Einstein radius of each dextran

$$R_{SE} = \frac{k_B T}{6\pi \eta D_\infty}$$ (5.17)

with the dextran diffusion coefficient $D_\infty$ evaluated from the dextran molecular weight using the correlation presented by Granath and Kvist (1967) as:

$$\log D_\infty = -4.1154 - 0.47752 \log(MW)$$ (5.18)
The dextran sieving coefficients decrease with increasing reaction time, reflecting the reduction in the effective pore radius due to the attachment of the quaternary amine functionality. This effect is relatively small for the smaller dextrans, but becomes fairly significant for the larger dextrans. For example, the observed sieving coefficient for a 17.5 kD dextran having a radius of 34 Å decreases from a value of 0.28 after 10 min of reaction to 0.14 after 180 min.

Figure 5.6: Actual sieving coefficients for different molecular weight dextrans through charge-modified CRC membranes at a flux of 25 L/m²/hr. The solid curves are model fits to the actual sieving coefficient data using the best fit values of the model parameters shown in Table 5.2.
The solid curves in Figure 5.6 are model calculations for the dextran sieving coefficients developed by integrating over the membrane pore size distribution using Equations (5.2) and (5.3). The best fit values of the mean and standard deviation of the log-normal pore size distribution for each membrane were determined by minimizing the sum of the squared residuals between the measured and calculated values of the dextran sieving coefficients. The model calculations are in good agreement with the data over the full range of dextran size using the parameter values given in Table 5.2. The charge modification had no measurable effect on the breadth of the distribution, with the ratio of the standard deviation to the mean equal to 0.15 – 0.16 for all the membranes. There was, however, a significant reduction in the mean pore size, which decreased by more than 20%, from 4.2 nm to 3.3 nm, as the reaction time increased from 10 to 180 min.

Table 5.2: Membrane charge and pore size characteristics for different reaction times

<table>
<thead>
<tr>
<th>Charging Time (min)</th>
<th>Zeta Potential (mV)</th>
<th>Charge Density (C/m²)</th>
<th>Mean Pore Size, ( \bar{r} ) (nm)</th>
<th>Standard Deviation (( \sigma/\bar{r} ))</th>
<th>Reduction in permeability (1-( L_p/L_{po} ))</th>
<th>Reduction in permeability using ( \bar{r} ) and ( \sigma/\bar{r} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.3</td>
<td>( 2.3 \times 10^{-4} )</td>
<td>4.2</td>
<td>0.15</td>
<td>3.8 %</td>
<td>---</td>
</tr>
<tr>
<td>40</td>
<td>3.2</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>5.8 %</td>
<td>---</td>
</tr>
<tr>
<td>60</td>
<td>4.1</td>
<td>( 3.5 \times 10^{-3} )</td>
<td>3.7</td>
<td>0.16</td>
<td>6.3 %</td>
<td>21 %</td>
</tr>
<tr>
<td>120</td>
<td>5.8</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>13.9 %</td>
<td>---</td>
</tr>
<tr>
<td>180</td>
<td>6.6</td>
<td>( 7 \times 10^{-3} )</td>
<td>3.3</td>
<td>0.15</td>
<td>16.7 %</td>
<td>38 %</td>
</tr>
</tbody>
</table>
Membrane charge density (third column of Table 5.2) was determined from the streaming potential data using Equation (5.4). On a molecular level, while the charge density of $7 \times 10^{-3} \text{C/m}^2$ represents two quaternary amine groups separated by approximately 4.8 nm on the membrane surface, the charge density of $2.3 \times 10^{-4} \text{C/m}^2$ corresponds to two quaternary amine groups separated by approximately 25 nm. The second to last column of Table 5.2 shows the experimental values for the percent reduction in the membrane hydraulic permeability associated with the charge modification. In each case, the permeability was evaluated immediately before and after the charge modification using a 1 M KCl solution to minimize contributions from counter-electroosmosis. The reduction in permeability was less than 4% for the membrane modified for only 10 min, but this increased to more than 16% for the membrane that was modified for 180 min. This change in permeability was significantly less than that predicted based on the change in the mean pore size determined from the dextran sieving data (shown in last column in Table 5.2), which could reflect a more complex set of changes in the membrane pore morphology and/or to a non-uniform modification of the membrane pores. It is also important to note that the zeta potential values given in Table 5.2 were determined after the membranes were exposed to the protein solution for several days over the course of the filtration experiments. These values are all slightly smaller than those plotted in Figure 5.5, with the latter determined immediately after charging the membrane (and before exposure to any protein). This small discrepancy is likely due to the affects of protein adsorption and/or changes in ion adsorption. There was no evidence of any leaching
of the ligand based on experiments in which the membrane was stored in the buffer solution for periods of more than 14 days.

In order to develop a more complete understanding of the pore size characteristics of the membrane, a series of calculations were performed to examine the sensitivity of the model results to the specific values of the model parameters. Figure 5.7 shows calculations for the actual sieving coefficients for several values of the coefficient of variation ($\sigma/\bar{r}$) using a mean pore size of 3.3 nm. The filled circles represent the experimental data for the dextran sieving coefficients for the membrane charged for three hours. Increasing $\sigma/\bar{r}$ increases the actual sieving coefficient, with the magnitude of this effect being greatest for the higher molecular weight dextrans. For example, the actual sieving coefficient for the dextran with a Stoke’s radius of 40 Å increases approximately an order of magnitude from 0.002 to 0.02 as the coefficient of variation varies from 0.1 to 0.2 due to the increase in the number of pores with larger pore size as the value of $\sigma/\bar{r}$ is increased. The large variation in the predicted values of the sieving coefficient for the largest solutes provides a clear bound on the best fit values of the coefficient of variation, with the results in Figure 5.6 indicating that values smaller than 0.1 or larger than 0.2 are well outside of the range appropriate to describe the experimental data for the charge-modified membrane.
Figure 5.7: Predicted dextran sieving coefficients for a 30 kD membrane charged for three hours for several values of the coefficient of variation using a mean pore size of 3.3 nm. Filled circles represent experimental data for dextran sieving for membrane charged for 3 hours.

5.3.2 Counter-electroosmosis

All of the data in Figure 5.6 were obtained using very high ionic strength solutions to minimize the effects of electrostatic interactions on dextran transport and fluid flow. Figure 5.8 shows the effects of solution ionic strength on the measured permeability of the charge-modified membranes. The results are plotted as the ratio of the permeability at a given ionic strength to that evaluated using the 1 M KCl solution to eliminate the effects of any variability in the membrane skin thickness or porosity (neither of which can be measured directly). The membrane permeability decreases with decreasing salt concentration because of the greater magnitude of the back flow associated with counter-electroosmosis. This effect is very small for the
membrane that was charged for only 10 min, corresponding to a reduction in permeability of about 4% as the ionic strength was reduced from 1 M to 0.002 M. In contrast, the reduction in hydraulic permeability for the membrane that was charged for 180 min was more than 20% over the same range in salt concentration. The net result is that the permeability of the membrane charged for 180 min and evaluated using a 0.002 M KCl solution is more than 30% smaller than that for the membrane charged for 10 min, with this large difference arising from the combined effects of the greater pore constriction associated with the chemical modification and the greater extent of counter-electroosmosis in the low ionic strength solution.

The solid and dashed curves in Figure 5.8 are model calculations accounting for the effects of counter-electroosmosis on the fluid flow and were developed by integrating the theoretical expression for the average solution velocity \( \langle V \rangle \) in a single pore over the log normal pore size distribution using a constant surface charge density model. The average solution velocity (Equation 2.15) was obtained by solving the Navier-Stokes equation including the electrical stress term (Newman, 1973) and is given as:

\[
\langle V \rangle = \frac{J}{\varepsilon} = \frac{dP}{dz} \left( \frac{r_p^2}{8\eta} \right) + E_z \frac{q_p I_2 \left( \kappa r_p \right)}{\kappa \eta I_1 \left( \kappa r_p \right)}
\]

(5.19)

where \( \varepsilon \) is the membrane porosity, \( dP/dz \) is the pressure gradient, \( q_p \) is the surface charge density of the membrane, and \( \eta \) is the solvent viscosity. \( E_z \) is the average electric field, which is equal to the streaming potential divided by the membrane
thickness. The first term on the right hand side of Equation (5.19) represents the pressure driven (Poiseuille) flow while the second term describes the back flow associated with counter-electroosmosis arising from the induced streaming potential.

The membrane surface charge density \( q_p \) is related to the apparent zeta potential \( \zeta_{\text{app}} \) as:

\[
q_p = \frac{\zeta_{\text{app}} \kappa \varepsilon \varepsilon_r}{\lambda_o B}
\]  

(5.20)

where \( \kappa^{-1} \) represents the thickness of the electrical double layer, \( \lambda_o \) is the solution conductivity, and \( B \) is a function of the membrane pore size distribution given by Equation (5.5).

The effective hydraulic permeability of the membrane was evaluated theoretically by integrating Equation (5.19) over the log-normal pore size distribution giving:

\[
L_p = \frac{J \eta}{\Delta P} = \frac{\int_{0}^{\infty} \left( \varepsilon r_p^2 + \frac{E_z \varepsilon q_p I_2(\kappa r_p)}{\Delta P \kappa I_1(\kappa r_p)} \right) n(r_p) \pi r_p^2 dr_p}{\int_{0}^{\infty} n(r_p) \pi r_p^2 dr_p}
\]  

(5.21)

where \( \delta_m \) is the membrane thickness, \( \varepsilon \) is the membrane porosity, and \( \Delta P \) is the transmembrane pressure drop. The ratio of the voltage gradient to the applied pressure was evaluated from Equation (5.16) using the previously determined values of the apparent zeta potential. The mean and standard deviation in the log-normal pore size distribution were taken from Table 5.2, with these values determined from
the dextransieving data as described previously. The solid and dashed curves in Figure 5.8 represent the results assuming a constant surface charge density (evaluated using Equation 5.21 based on the measured streaming potential in the 10 mM KCl solution). The model calculations capture the general trend seen with the experimental data, but they significantly over-predict the effects of counter-electroosmosis at very low ionic strength. This is probably due to: (1) the breakdown of the Debye Huckel approximation associated with the high surface potentials (> 25 mV) that occur at low ionic strengths, (2) the inadequacy of the assumption of constant surface charge density, and/or (3) the effects of surface conduction.

Figure 5.8: Effect of counter-electroosmosis on the hydraulic permeability of charge-modified membranes. Data are normalized by the permeability evaluated using a 1 M KCl solution. The solid and dashed curves are model calculations assuming a constant surface charge density as described in the text.
In order to explore the assumption of a constant surface charge density in more detail, a separate set of calculations were performed assuming a constant surface potential (instead of constant surface charge) with the results shown in Figure 5.9. The effective hydraulic permeability of the membrane was determined by integrating the expression for the average solution velocity developed using the constant surface potential boundary condition (given by Equations 2.21 – 2.23) over the log-normal pore size distribution using the values of the mean and standard deviation given in Table 5.2. The results using a constant surface potential boundary condition are in somewhat better agreement with the data obtained with the 0.1 M solution but show a minimum in the permeability ratio at an ionic strength of approximately 0.05 M, which is not seen in any of the experimental results. This minimum in permeability occurs for values of $\kappa r_p$ approximately equal to 1 or 2 where $\kappa$ is the inverse Debye length and $r_p$ is the dimensionless pore radius. At smaller values of $\kappa r_p$, the double layer overlap is very extensive, with the electrostatic potential remaining nearly constant over the entire pore radius. Under these conditions, the back flow associated with counter electroosmosis becomes very small, leading to the minimum seen in Figure 5.9.
Figure 5.9: Effect of counter-electroosmosis on the hydraulic permeability of charge-modified membranes. Data are normalized by the permeability evaluated using a 1 M KCl solution. The solid and dashed curves are model calculations assuming a constant surface potential as described in the text.

Figure 5.10 shows the estimated values for surface charge and surface potential of the membrane that was charged for three hours ($\zeta_{app} = 6.6$ mV) as a function of solution ionic strength. The calculated values of the surface charge density and surface potential were obtained by fitting the experimental data for the hydraulic permeability at each ionic strength to constant surface charge and surface potential models, respectively. The calculations suggest that the surface potential remains nearly constant at the higher salt concentrations, with the surface charge
density remaining fairly constant at the lower salt concentrations. The use of this type of variable charge / potential boundary condition would provide better agreement with the experimental permeability data, although there was no independent data to justify the use of any specific functional dependence for the charge density or potential.

Figure 5.10: Model calculations for the surface potential and membrane charge density as a function of solution ionic strength based on the hydraulic permeability data
5.3.3 **Protein Transmission**

Experimental data for the observed sieving coefficient of cytochrome c through the charge-modified CRC membranes are shown in Figure 5.11 as a function of the solution ionic strength. The observed sieving coefficient ($S_o$) is equal to the ratio of the protein concentration in the filtrate solution ($C_f$) to that in the bulk solution ($C_b$) and is thus a measure of the fractional transmission of the protein through the membrane. The data were all obtained using 1 g/L solutions of cytochrome c at a constant flux of 25 L/m$^2$/hr and a stirring speed of 600 rpm. The membranes were allowed to equilibrate at each ionic strength by flushing with 50 L/m$^2$ of buffered saline solution before switching to a protein solution of the same ionic strength. Repeat measurements of the sieving coefficients at selected conditions were within 30%, indicating that there was negligible fouling over the course of the experiments. This was also confirmed by the hydraulic permeability measurements obtained using the 1 M KCl solution, with the permeability values before and after the protein filtration differing by less than 10%.
The observed sieving coefficients for cytochrome c increase with increasing ionic strength due to the increase in electrostatic shielding provided by the electrolyte, with the greatest dependence on ionic strength seen with the membrane having the greatest apparent zeta potential (i.e., the largest electrical charge). For example, the observed sieving coefficient for cytochrome c through the membrane with $\zeta_{\text{app}} = 6.6$ mV increases by more than a factor of 100 as the ionic strength is increased from 10 mM to 500 mM compared to only a 3-fold increase in $S_o$ for the membrane with $\zeta_{\text{app}} = 0.3$ mV.
The data in Figure 5.11 are re-plotted in Figure 5.12 as an explicit function of the apparent membrane zeta potential for four values of the solution ionic strength. The sieving coefficient in the 500 mM solution is nearly independent of the membrane zeta potential, with values ranging from 0.64 to 0.5, due to the insignificant electrostatic shielding at this high salt concentration. The sieving coefficients for the more highly charged membranes in the 500 mM salt solution were slightly smaller than those for the weakly charged membranes, which is primarily due to the reduction in the effective pore size associated with the greater extent of chemical modification (Table 5.2). In contrast, the observed sieving coefficient decreases by approximately two orders of magnitude as the apparent zeta potential increases from 0.3 mV to 6.6 mV in the lower ionic strength solutions (5 mM and 10 mM). This is a direct result of the strong electrostatic exclusion arising from the direct charge-charge interactions between the positively-charged membrane and the positively-charged cytochrome c.
Figure 5.12: Observed sieving coefficients for cytochrome c as a function of the apparent membrane zeta potential at several ionic strengths

In order to obtain additional insights into the sieving behavior, the experimental data for cytochrome c were compared with theoretical calculations using the model developed by Smith and Deen (1980) for the partitioning of a charged spherical solute into a charged cylindrical pore. The actual sieving coefficient \( S_a \) in a pore of radius \( r_p \) is expressed as

\[
S_a = (1 - \lambda)^2 K_c \exp \left( \frac{-\psi_E}{k_B T} \right)
\]

(5.22)

where \( \lambda \) is the ratio of solute to pore radius \( (r_s/r_p) \), \( K_c \) is the hindrance factor for convection, and \( \psi_E/k_B T \) is the dimensionless electrostatic energy of interaction. Equation (5.22) assumes that solute transport is dominated by convection, with no
significant contributions from either diffusion or electrophoretic protein transport (Pujar and Zydney, 1994). The first term on the right-hand-side accounts for the steric exclusion of the solute from the region within one solute radius of the pore walls. As discussed in Chapter 2, Smith and Deen (1980) evaluated the electrostatic energy of interaction by solving the linearized Poisson-Boltzmann equation, with the final result given by Equations (2.56) and (2.66) for the centerline approximation and for the full radial analysis, respectively. The generalized form of the final result is given as:

$$\frac{\Psi_E}{k_B T} = \frac{A_s \sigma_s^2 + A_p \sigma_p^2 + A_{sp} \sigma_s \sigma_p}{A_{den}}$$

where $A_s$, $A_p$, $A_{sp}$, and $A_{den}$ are all positive coefficients which depend on the solution ionic strength, pore radius, and solute radius. Detailed expressions for these coefficients and for $K_c$ are given in Chapter 2.

The actual sieving coefficients were evaluated by integrating Equation (5.23) over the pore size distribution using the mean and standard deviation in the log-normal density function determined from the dextran sieving data (values given in Table 5.2). The protein charge was calculated from the pK$_a$ values of the various amino acids accounting for the effects of charge regulation as described in Section 5.2.5. The hydrodynamic radius of cytochrome c was taken as 18 Å based on NMR measurements (Wilkins, 1999). The membrane charge was assumed to be constant, independent of solution ionic strength, using the values given in Table 5.2 (determined from the streaming potential measurements).
Figure 5.13: Actual sieving coefficients for cytochrome c as a function of the solution ionic strength. Solid and dashed curves are model calculations as described in the text.

Figure 5.13 shows a comparison between the model predictions and the experimental data for cytochrome c sieving through the charge-modified membranes. The data are plotted as the actual sieving coefficient, which was evaluated directly from the observed sieving coefficient data by accounting for the effects of concentration polarization in the stirred cell using the simple stagnant film model discussed in Chapter 2. The final result is given as:
\[ S_a = \frac{S_o}{(1-S_o) \exp(J_v/k) + S_o} \]  

(5.24)

The bulk mass transfer coefficient \( k \) was calculated from the correlation presented by Smith et al. (1968):

\[ \left( \frac{kr_c}{D_\infty} \right) = 0.27 \text{Re}^{0.567} \text{Sc}^{0.33} \]  

(5.25)

where \( \text{Re} = \Omega r_c^2/\nu \) is the Reynolds number, \( \text{Sc} = \nu/D_\infty \) is the Schmidt number, \( r_c \) is the stirred cell diameter, \( \Omega \) is the stirring speed in radians/sec, \( D_\infty \) is the bulk diffusivity of the solute, and \( \nu \) is the kinematic viscosity. The mass transfer coefficient, \( k \), was evaluated from Equation (5.25) as \( 6.7 \times 10^{-6} \) m/s using the bulk diffusion coefficient of cytochrome c, \( D_\infty = 10 \times 10^{-11} \) m\(^2\)/s (Lewus and Carta, 1999).

The mass transfer coefficient was assumed to be independent of the solution ionic strength since there was insufficient data on the variation of the bulk diffusivity with salt concentration.

The actual sieving coefficients are slightly smaller than the observed sieving coefficients due to the effects of concentration polarization, although this effect is relatively small at the low filtrate flux (25 L/m\(^2\)/hr) used in these experiments. The model predictions in Figure 5.13 are in good agreement with the experimental data, although there is a slight under-prediction of the actual sieving coefficients in the lowest ionic strength solutions. This is likely due at least in part to the assumption of a constant surface charge density on the membrane, with the results for the
counterelectroosmotic flow suggesting that there is some reduction in surface charge at low ionic strength.

Figure 5.14 shows the effects of the different contributions to the total electrostatic energy of interaction on the predicted values of the actual sieving coefficient for cytochrome c through the positively charged membrane with an apparent zeta potential of 6.6 mV. The model calculations were performed by integrating Equation (5.22) over the log-normal pore size distribution using the parameters given in Table 5.2 but only accounting for one of the three terms in the expression for the energy of interaction (Equation 5.23). For example, the simulations to evaluate the effects of \( \sigma_s \sigma_p \) term were performed using the full model but with \( A_s \) and \( A_p \) both set equal to zero. The filled circles represent the experimentally determined sieving data for cytochrome c. The dominant contribution to the electrostatic energy of interaction is due to the charge-charge interaction between cytochrome c and the membrane, with the calculations using just that term in very good agreement with the experimental results over the full range of ionic strength.
Figure 5.14: Calculated values of the actual sieving coefficients accounting for the different contributions to the total electrostatic energy of interaction for cytochrome c sieving through the positively-charged CRC membrane with an apparent zeta potential of 6.6 mV.

It is important to note that the model predictions shown in Figure 5.13 and 5.14 are true predictions since all of the required parameters were evaluated from independent experimental measurements: the membrane pore size distribution was determined from the dextran sieving measurements, the membrane surface charge was determined from the streaming potential measurements, and the protein charge was evaluated theoretically from the amino acid composition. The good agreement
between the model and data strongly supports the physical picture that cytochrome c rejection is determined primarily by electrostatic interactions between the charged protein and the charged membrane, with the dominant contribution arising from the direct charge-charge interactions (the final term in Equation 5.23). More accurate calculations would likely be obtained by accounting for the variation in membrane and protein charge due to charge regulation effects as described by Pujar and Zydney (1997). However, there was insufficient data on the surface charge characteristics to include this phenomenon in the theoretical analysis.

5.3.4 Sensitivity Analysis

In order to obtain additional insights into the phenomena governing protein sieving in this system, a series of calculations were performed to examine the effects of the different model parameters on the model calculations. Figure 5.15 shows the effects of the coefficient of variation ($\sigma/\bar{r}$) on the predicted sieving coefficient of cytochrome c through a membrane charged for three hours ($\zeta_{app} = 6.6$ mV). These model calculations were performed by varying the value of $\sigma/\bar{r}$ while keeping all other parameters fixed. The filled circles represent the experimental data for cytochrome c. The predicted value of the sieving coefficient increases with increasing $\sigma/\bar{r}$ due to the increase in the number of large (non-selective) pores associated with the greater breadth of the distribution, with the greatest effect seen at the lowest ionic strength where protein retention is largest. For example, the sieving coefficient of cytochrome c at an ionic strength of 20 mM increases by approximately
an order of magnitude as $\sigma/r$ increases from 0.1 to 0.2. The experimental data are in best agreement with the model calculations using a coefficient of variation between 0.15 and 0.1, which is only slightly smaller than the value determined from the dextran sieving measurements. The results also demonstrate that a small error in the determination of the coefficient of variation would not have caused any significant discrepancy between the model predictions and experimental data for cytochrome c sieving.

Figure 5.15: Effect of coefficient of variation on cytochrome c sieving through a positively charged membrane with an apparent zeta potential of 6.6 mV. The filled circles represent the experimental data for the actual sieving coefficient of cytochrome c.
Figure 5.16 shows the effects of the membrane surface charge density on the sieving coefficient of cytochrome c over a range of solution ionic strength. The filled circles represent the experimental data for cytochrome c sieving through the membrane charged for three hours. The predicted values of the actual sieving coefficient for the membrane with the largest charge density are uniformly smallest due to the greater electrostatic repulsion under these conditions. The impact of the membrane charge density was greatest at low ionic strength as expected. For example, at an ionic strength of 20 mM, the actual sieving coefficient increases by approximately an order of magnitude when the membrane charge density decreases from $7.0 \times 10^{-3} \text{ C/m}^2$ to $3.5 \times 10^{-3} \text{ C/m}^2$. The experimental data at high ionic strength are in best agreement with the model using a membrane charge density of $7 \times 10^{-3} \text{ C/m}^2$ while the results at lower ionic strength are in better agreement with the predictions using a membrane charge density of $3.5 \times 10^{-3} \text{ C/m}^2$. This reduction in membrane charge density at low ionic strength is consistent with the results for counter-electroosmosis.
Figure 5.16: Effect of membrane charge density on cytochrome c sieving over a range of ionic strength. The filled circles represent experimental data for the sieving coefficient using a membrane charged for 3 hours.

5.3.5 Permeability-Selectivity Analysis

As discussed previously, the charge modification reduces the membrane hydraulic permeability due to the combined effects associated with the steric constriction of the pores (due to the finite size of the quaternary amine ligand) and the development of a counter electro-osmotic flow, with the latter contribution being most significant at low ionic strength. In contrast, cytochrome c retention increases significantly for the more heavily charged membranes due to the greater electrostatic
exclusion of the charged protein from the highly charged pores. This trade-off between the membrane selectivity and the hydraulic permeability is examined in more detail in Table 5.3. The experimental values of the membrane permeability in the 10 mM KCl solution were used to evaluate an effective membrane pore size by assuming that the membrane is composed of a parallel array of uncharged cylindrical pores, i.e. assuming that counter-electroosmosis is negligible. The effective mean pore size was calculated using a log-normal pore size distribution with the coefficient of variation taken as \( \sigma / \bar{r} = 0.15 \) based on the results obtained with the dextrans. The effective pore size decreased from 4.1 to 3.4 nm as the apparent zeta potential increased from 0.3 to 6.6 mV. The predicted values of the actual sieving coefficient for cytochrome c were then calculated using Equation (5.2) assuming that the membrane is uncharged. The predicted sieving coefficients decrease from 0.51 to 0.38 as the apparent zeta potential increases from 0.3 to 6.6 mV, with this increase in retention arising solely from the change in membrane pore size since the membrane was assumed to be uncharged in the calculation of both the pore size (from the measured permeability) and the predicted sieving coefficients. The experimental data for the actual sieving coefficients show a much stronger dependence on the apparent zeta potential, decreasing from 0.12 to 0.001 over this range of apparent zeta potentials, clearly demonstrating the dramatic improvement in protein retention due to the electrostatic interactions between the charged protein and the charged pores.
Table 5.3: Permeability and protein retention in 10 mM KCl

<table>
<thead>
<tr>
<th>Apparent Zeta Potential (mV)</th>
<th>Permeability (LMH/psi)</th>
<th>Effective Pore Size, $\bar{r}$ (nm)</th>
<th>$S_{a,pred}$</th>
<th>$S_{a,exp}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>20.5</td>
<td>4.1</td>
<td>0.51</td>
<td>0.12</td>
</tr>
<tr>
<td>4.1</td>
<td>18.6</td>
<td>3.9</td>
<td>0.48</td>
<td>0.009</td>
</tr>
<tr>
<td>6.6</td>
<td>14.0</td>
<td>3.4</td>
<td>0.38</td>
<td>0.001</td>
</tr>
</tbody>
</table>

The effect of membrane charge on the hydraulic permeability and the selectivity of the charge-modified membranes is shown graphically in Figure 5.17 in the form of a Robeson plot (as discussed in Chapter 4). The solid curve represents the predicted trade-off between the permeability and selectivity for cytochrome c through an uncharged membrane and was developed theoretically using Equations (4.6) – (4.8) with the coefficient of variation ($\sigma/\bar{r}$) kept at a fixed value of 0.15. Calculations were performed by varying the mean pore size (keeping $\sigma/\bar{r}$ and $\varepsilon/\delta_m$ fixed), with the permeability and selectivity evaluated for over a range of $\bar{r}$. The dashed curves represent the predicted trade-off accounting for electrostatic interactions and were generated for different values of the membrane charge density with $\sigma/\bar{r}$ again fixed at a value of 0.15. In this case, the permeability and selectivity were evaluated for a 10 mM ionic strength solution by varying the mean pore size. The filled circles represent experimental data for cytochrome c sieving through the charge-modified membranes in the 0.5 M ionic strength solution where electrostatic
interactions are negligible, while the open symbols represent data obtained in the 10 mM ionic strength solution.

The results at high ionic strength fall right along the expected trade-off curve in the absence of electrostatic interactions, consistent with the data for BSA and lysozyme presented in Chapter 4. In contrast, the results for the charged membranes at lower ionic strength fall well above and to the right of the expected upper bound. For example, the selectivity for the membrane with a zeta potential of 5.8 mV is nearly 500-fold larger than the selectivity of a neutral membrane with the same hydraulic permeability. Alternatively, the charged membrane has a 10-fold higher permeability than the neutral membrane at a comparable value of the selectivity. The model calculations clearly show the importance of the membrane charge density on the predicted trade-off, particularly for membranes having small permeabilities (corresponding to small mean pore size). For example, at a permeability of 20 L/m²/hr/psi, the selectivity increased by an order of magnitude as the membrane charge density increased from $1 \times 10^{-4}$ C/m² to $1.5 \times 10^{-3}$ C/m². Another order of magnitude increase in the membrane charge density increased the selectivity by an additional two orders of magnitude. The model calculations also demonstrate that very highly charged membranes (surface charge density of $1 \times 10^{-2}$ C/m²) can provide nearly an order of magnitude higher permeability than corresponding neutral membranes at comparable selectivities.
Figure 5.17: Effect of membrane charge on permeability and selectivity of charge-modified CRC 30 kD membranes. The solid curve represents predicted trade-off for uncharged membranes while the dashed curves represent trade-offs for charged membranes. The open and filled symbols represent data for cytochrome c sieving at low and high ionic strength with charged membranes.

5.4 Conclusions

The results presented in this Chapter provide the first quantitative analysis of the effects of the membrane surface charge density on both solvent and solute transport in membrane systems. The membrane charge was controlled by varying the extent of reaction with a quaternary amine functionality. The data clearly demonstrate that the more highly charged membranes provide much greater retention of the positively-charged cytochrome c due to the strong electrostatic repulsion.
between the protein and membrane. This effect is quite dramatic, with the sieving coefficient in a 10 mM KCl solution decreasing by a factor of 100 as the apparent zeta potential increases from 0.3 to 6.6 mV. Even relatively small changes in the membrane charge had significant affects on the sieving coefficient, with $S_o$ in the 5 mM KCl solution decreasing from 0.008 to 0.003 as the apparent zeta potential increased from 3.2 to 4.2 mV.

The sieving data were in good agreement with model calculations developed from a theoretical expression for the partition coefficient of a charged sphere in a charged cylindrical pore accounting for the presence of a log-normal pore size distribution. The model calculations were performed with all of the key parameters determined from independent experimental measurements: the pore size distribution was determined from dextran sieving profiles, the membrane surface charge density was determined from streaming potential measurements, and the protein surface charge was evaluated from the amino acid composition using a charge regulation model. The good agreement between the model and the data indicates that electrostatic effects on protein partitioning are the primary factor governing the sieving behavior, with the direct charge-charge interactions providing the dominant contribution to the electrostatic repulsion.

The charge modification also reduces the membrane hydraulic permeability due to two separate effects. First, there is a small steric constriction of the pores associated with the finite size of the quaternary amine groups. Second, the fluid flow is reduced by counter-electroosmosis arising from the induced streaming potential.
Again, the experimental results were in fairly good agreement with model calculations accounting for the electrical stresses in the fluid arising from the induced streaming potential. There were some significant deviations between the model and data at the lowest ionic strength, which is likely due at least in part to the breakdown in the assumption of a constant surface charge density. Further studies are required to clarify the origin of this behavior.

The trade-off between the increase in protein retention and the reduction in hydraulic permeability indicates that there will typically be an optimum in the magnitude of the membrane charge for different applications. The results obtained in this study show that one could achieve a target protein retention of 99.9% using charged membranes with a higher permeability than the corresponding neutral membrane, similar to results reported previously by Mehta and Zydney (2005) as discussed in Chapter 4. Higher degrees of membrane modification would further increase the protein retention but potentially with an unacceptable (or at least unnecessary) reduction in permeability. In addition, it may be possible to use the model to obtain insights into the use of these charged membranes for protein separations, with calculations performed for the individual proteins based on the specific surface charge characteristics of the different molecules. This is discussed in more detail later in this thesis.
Chapter 6
EFFECT OF MEMBRANE CHARGE ON
SEPARATION OF BINARY PROTEIN MIXTURES

6.1 Introduction

The experimental data presented in Chapters 4 and 5 clearly demonstrate the importance of electrostatic interactions in enhancing the performance of ultrafiltration systems, particularly for protein concentration and buffer exchange. A number of recent reports however have also demonstrated that ultrafiltration membranes can potentially be used for high-resolution protein purification. For example, Saksena and Zydney (1994) showed that the selectivity for the separation of bovine serum albumin (BSA) and immunoglobulin G (IgG) by ultrafiltration could be increased from a value of only two at pH 7 and high salt concentrations to more than 30 simply by adjusting the pH to 4.7 and lowering the solution ionic strength. These conditions provided high retention of the positively-charged IgG while the albumin was able to pass relatively easily through the membrane. van Eijndhoven et al. (1995) were able to separate BSA (69 kDa) and hemoglobin (67 kDa) using ultrafiltration with a separation factor of 70, with this high selectivity again obtained by properly adjusting the pH and ionic strength. van Reis et al. (1999) explored the effects of solution conditions and membrane charge for the separation of BSA and an antigen binding fragment (45 kD) using high performance tangential flow filtration. Dramatic improvements in membrane selectivity were obtained by selecting the appropriate
solution conditions. The authors also showed that it was possible to achieve more than 990-fold purification and 90% yield of BSA in the retentate solution using a diafiltration process.

Almost all of these studies have focused on exploring the effects of solution conditions like pH and ionic strength. In contrast, there have been no detailed studies that have quantitatively examined the effects of membrane charge on the separation of protein mixtures. The primary objective of the work described in this chapter was to examine the effects of membrane charge density on the separation of a model binary protein system of lysozyme (14.1 kDa) and myoglobin (17 kDa). Data were obtained using a series of charge-modified cellulose membranes developed by chemical modification with a quaternary amine functionality as discussed in Chapter 5. The surface charge density of the membranes was controlled by varying the reaction time, with the effective charge evaluated from streaming potential measurements.

6.2 Materials and Methods

6.2.1 Solution Preparation

Myoglobin from horse heart (Sigma Chemical Co., St. Louis, MO, M1882) and lysozyme from chicken egg white (Sigma Chemical Co., St. Louis, MO, L6876) were used as model proteins. KCl solutions were prepared by dissolving pre-weighed amounts of KCl (Sigma Chemicals, St. Louis, MO) in deionized distilled water as described in Section 3.4.1. Solutions were buffered at the desired ionic strength by
adding approximately 10 mM of Bis-Tris buffer (Sigma Chemicals, St. Louis, MO). The solution pH was then adjusted to pH 6.2 using small amounts of 1 M HCl as required. All solutions were prefiltered through 0.2-µm pore size Supor 200 membranes (Pall Life Sciences, Ann Arbor, MI) to remove any particles prior to use. Protein solutions were prepared by dissolving 1 g/L of myoglobin and 3.5 g/L of lysozyme in the buffer solution. All protein solutions were prefiltered through 0.2-µm µ-Star nylon filters (8010, Costar Corporation) to remove large aggregates and any undissolved protein.

6.2.2 Protein Assays

The concentrations of lysozyme and myoglobin in the collected samples were determined spectrophotometrically using a technique similar to the one used by Ghosh (2000). The absorbance of the collected protein samples was measured at both 280 and 408 nm using a Shimadzu UV-Vis Mini 1240 spectrophotometer (Shimadzu, Columbia, MD). The absorbance at 280 nm provides a measure of both the lysozyme and myoglobin concentrations, while the absorbance at 408 nm is dominated by the myoglobin. The myoglobin concentration was evaluated assuming that the absorbance at 408 nm was only due to myoglobin, with the lysozyme concentration then determined from the total absorbance at 280 nm by subtracting off the contribution associated with the “known” concentration of myoglobin. Ghosh (2000) demonstrated that this approach could be used to accurately evaluate the concentrations of both myoglobin and lysozyme over a range of concentrations, with
the largest difference between the actual and measured concentrations of less than 2%.

6.2.3 Membrane Preparation

A series of positively-charged membranes was generated by chemical attachment of a quaternary ammonium group to the free hydroxyls in the glucose rings of the base cellulose using a proprietary solution chemistry developed by Millipore Corporation (Billerica, MA) as discussed in Sections (3.2.2) and (5.2.1). Neutral composite regenerated cellulose (CRC) membranes with nominal molecular weight cut-off of 100-kD were obtained from Millipore Corporation. The extent of charge modification was controlled by varying the reaction time from 1 to 9 hours. All membranes were thoroughly flushed with 0.1 M NaOH followed by 0.5 M KCl buffered with 10 mM Bis-Tris to remove any residual chemicals, using a minimum of 50 L/m² per membrane.

6.2.4 Permeability Measurements

The hydraulic permeability of the membranes was evaluated by placing them in a 25 mm diameter stirred cell (Model 8010, Amicon) on top of a microporous Tyvek support as discussed in Section 5.2.2. The cell was then filled with an appropriate KCl solution buffered with 10 mM Bis-Tris and connected to a reservoir containing the same buffer solution. The buffer reservoir was air pressurized and the
filtrate flux evaluated by timed collection over a range of transmembrane pressures from 14 to 34 kPa (corresponding to 2 – 5 psi).

6.2.5 Membrane Characterization

The surface charge characteristics of the charge-modified membranes were evaluated from streaming potential measurements following the procedure described in Chapter 3 (Section 3.2.3). The apparent zeta potential ($\zeta_{\text{app}}$) was evaluated from the slope of the measured voltage as a function of the transmembrane pressure using Equation (5.2).

6.2.6 Protein Filtration

All ultrafiltration experiments were performed with a binary mixture of 3.5 g/L lysozyme and 0.7 g/L myoglobin using a 25 mm diameter Amicon stirred ultrafiltration cell as described in Chapter 3 (Section 3.6). The stirring speed was set at 600 rpm and the filtrate flux was maintained at 25 L/m²/hr using a peristaltic pump connected directly to the filtrate exit port. Protein samples were collected from the filtrate exit and the bulk solution as described in Section 3.4.2. The protein concentration in the bulk and filtrate samples were determined spectrophotometrically using the method described in Section 6.2.2.
6.2.7 Diafiltration

A diafiltration process was used for actually separating a binary mixture of myoglobin and lysozyme. Diafiltration experiments were performed using a charged-modified 100-kD membrane in the Amicon 8010 stirred cell. The ultrafiltration cell was filled with 10 mL of the protein mixture and the stirrer speed was set to 600 rpm. A reservoir containing a buffer solution, having the same pH and ionic strength as the protein solution, was connected to the stirred cell. Diafiltration was performed at a constant filtrate flux of 25 L/m$^2$/hr using a peristaltic pump connected to the filtrate line. The buffer was added to the ultrafiltration cell at the same rate at which the filtrate was removed so that the volume in the stirred cell was maintained constant throughout the diafiltration process. Small samples of the retentate solution were collected at appropriate time intervals (from a sampling port in the stirred cell) for subsequent analysis of the protein concentrations.

6.2.8 Protein Surface Charge

The net charge of myoglobin and lysozyme at a given pH and solution ionic strength was calculated using the equilibrium dissociation constants of the different ionizable groups accounting for the effect of charge regulation as discussed in Chapter 5 (Section 5.2.5). The number ($n_i$) and the internal dissociation constants ($pK_{int}^i$) of different ionizable groups in myoglobin and lysozyme are given in Tables 6.1 and 6.2, respectively.
Table 6.1: Number ($n_i$) and $pK_{int}^i$ values of charged amino acids in myoglobin (Ebersold, 2004)

<table>
<thead>
<tr>
<th>Type</th>
<th>$pK_{int}^i$</th>
<th>Number ($n_i$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>4.3</td>
<td>8</td>
</tr>
<tr>
<td>Glu</td>
<td>4.3</td>
<td>13</td>
</tr>
<tr>
<td>His</td>
<td>6.6</td>
<td>7</td>
</tr>
<tr>
<td>Lys</td>
<td>10.3</td>
<td>19</td>
</tr>
<tr>
<td>Tyr</td>
<td>10.3</td>
<td>2</td>
</tr>
<tr>
<td>Arg</td>
<td>12.5</td>
<td>2</td>
</tr>
<tr>
<td>$\alpha$-amino</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>$\alpha$-carboxyl</td>
<td>2.19</td>
<td>1</td>
</tr>
<tr>
<td>Heme-carboxy</td>
<td>4.3</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 6.2: Number ($n_i$) and $pK_{int}^i$ values of charged amino acids in lysozyme (Sharma et al., 2003)

<table>
<thead>
<tr>
<th>Type</th>
<th>$pK_{int}^i$</th>
<th>Number ($n_i$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Glu</td>
<td>4.4</td>
<td>2</td>
</tr>
<tr>
<td>His</td>
<td>6.3</td>
<td>1</td>
</tr>
<tr>
<td>Lys</td>
<td>10.5</td>
<td>6</td>
</tr>
<tr>
<td>Tyr</td>
<td>9.6</td>
<td>3</td>
</tr>
<tr>
<td>Arg</td>
<td>12.5</td>
<td>11</td>
</tr>
<tr>
<td>$\alpha$-amino</td>
<td>7.5</td>
<td>1</td>
</tr>
<tr>
<td>$\alpha$-carboxyl</td>
<td>3.8</td>
<td>1</td>
</tr>
</tbody>
</table>
The net protein charge was evaluated by iterative solution of Equations (5.6), (5.8), (5.9) and (5.14), with typical results shown in Figure 6.1. The calculated isoelectric points for myoglobin and lysozyme were approximately 6.9 and 10.5, respectively, which are in very good agreement with the literature values of 6.8 and 10.6 (Balakrishnan and Agarwal, 1996). The protonation of the carboxylic acid groups at low pH results in a rapid reduction in the net protein charge at low pH. The net charge for lysozyme remains fairly constant between pH 5 and 8 due to the absence of any significant number of ionizable residues with pK_a between pH 5 and 10 (Table 6.1). On the other hand, the net charge for myoglobin varies substantially between pH 5 and 8 due to the significant number of histidine residues with pK_a equal to 6.6.
Figure 6.1: Estimated charge on myoglobin and lysozyme as a function of solution pH at 10 mM ionic strength

6.3 Data Analysis

The optimization of membrane separation processes involves a trade-off between product yield and purification. An increase in product yield results in a decrease in the product purity and vice versa. van Reis and Saksena (1997) developed a set of equations that describes this trade-off in terms of two dimensionless parameters: the selectivity, $\Psi$:

$$\Psi = \frac{S_{o,1}}{S_{o,2}}$$  \hfill (6.1)
and a dimensionless throughput parameter, $N\Delta S$:

$$N\Delta S = N(S_{o,1} - S_{o,2}) \quad (6.2)$$

where $S_{o,1}$ and $S_{o,2}$ are the observed sieving coefficients of the less and more retained solutes, respectively. The number of diavolumes, $N$, is simply equal to the total filtrate volume divided by the constant retentate volume:

$$N = \frac{JAt}{V} \quad (6.3)$$

where $J$ is the filtrate flux (assumed to be constant), $A$ is the membrane area, $t$ is the process time, and $V$ is the retentate volume.

When the product is retained by the membrane (solute 2 in this case), the overall yield, $Y_2$, after a constant volume diafiltration is simply the ratio of the final mass of protein in the retentate, $m_{2,f}$, to the initial mass, $m_{2,i}$:

$$Y_2 = \frac{m_{2,f}}{m_{2,i}} = \frac{C_{2,f}}{C_{2,i}} \quad (6.4)$$

where $C_{2,f}$ and $C_{2,i}$ are the final and initial protein concentrations in the retentate, respectively. The purification factor for a product in the retentate is defined as the ratio of its yield divided by the yield of the impurity (the more permeable solute):

$$P_2 = \frac{Y_2}{Y_1} = \left( \frac{C_{2,f}}{C_{2,i}} \right) \left( \frac{C_{1,i}}{C_{1,f}} \right) \quad (6.5)$$

van Reis and Saksena (1997) evaluated the yield and purification factor using simple mass balances with the final results given as:

$$Y_2 = P_2^{\gamma(1-\psi)} \quad (6.6)$$

$$P_2 = \exp(N\Delta S) \quad (6.7)$$
The corresponding expressions for a product collected in the filtrate solution are:

\[ P_1 = \frac{Y_1}{1 - (1 - Y_1)^{1/\psi}} \]  \hspace{1cm} (6.8)

\[ P_1 = \frac{Y_1}{1 + (Y_1 - 1)\exp(N\Delta S)} \]  \hspace{1cm} (6.9)

Figure 6.2: Optimization diagram when the desired product is in the retentate (adapted from van Reis and Saksena, 1997)

Figure 6.2 (adapted from van Reis and Saksena, 1997) shows an optimization diagram for a product in the retentate. The chart consists of two families of curves,
both plotted on yield \((Y_2)\) versus purification factor \((P_2)\) co-ordinates, with one evaluated for different values of the selectivity while the other is for different values of \(N\Delta S\). The diafiltration process begins in the upper left corner at \(Y_2 = 1, P_2 = 1\) since the product is initially contained in the retentate. The product yield decreases during the diafiltration due to the low-level of product transmission through the membrane, while the purification factor increases because of the more rapid clearance of the more permeable impurity. The solid curves in Figure 6.2 represent the trade-off between yield and purification for fixed values of the selectivity, \(\Psi\). The vertical lines represent the purification factor that can be attained for different values of \(N\Delta S\). For any targeted purification factor, as the value of \(\Delta S\) increases the number of diafiltrations needed to achieve that degree of purification decreases. While large values of selectivity ensure high product yield, a large value of \(\Delta S\) is important economically and practically as it significantly reduces buffer consumption, membrane area, and process time. High values of selectivity and \(\Delta S\) are thus desirable for obtaining high product yield and high purification factor during a diafiltration process.

van Reis and Saksena (1997) developed similar optimization diagrams for a product collected in the filtrate; with the best performance again obtained when both the selectivity and mass throughput are high. Whether the product is collected in the retentate or in the filtrate, the selectivity determines the specific trade-off between yield and purification factor during the diafiltration, while the value of \(N\Delta S\) (or \(\Delta S\))
has a large impact on the economics of the process (buffer consumption, membrane area, and process time).

6.4 Results and Analysis

6.4.1 Membrane Characterization

Figure 6.3 shows streaming potential data as a function of the transmembrane pressure using a 10 mM KCl solution for a series of charge modified cellulose membranes produced with the quaternary amine functionality but with different reaction times. The slope of the streaming potential versus transmembrane pressure data, which provides a direct measure of the membrane surface charge, increases with increasing reaction time as expected. The $R^2$ values for all measurements were greater than 0.98. Note that the best fit lines are typically displaced from the origin, which is a direct result of small asymmetries in the Ag/AgCl electrodes (Burns and Zydney, 2000). This displacement had no affect on the slope or the calculated values of the membrane surface charge.
Figure 6.3: Streaming potential data for a series of charge modified CRC 100 kD membrane using a 10 mM KCl solution. $R^2$ values are all greater than 0.98.

The apparent zeta potential ($\zeta_{app}$) for each membrane was evaluated directly from the slope of the data in Figure 6.3 using the Helmholtz - Smoluchowski equation (Equation 5.16), with the results summarized in Table 6.3. The apparent zeta potential increased from $1.6 \pm 0.1$ mV after 1 hour of reaction to $3.4 \pm 0.3$ mV after 3 hours and then to $8.9 \pm 0.3$ mV after 9 hours (Table 6.3). The actual zeta potential at the pore surface will be different than the apparent zeta potential due to the overlap of the electrical double layer within the pore and the effects of surface conduction, both of which are ignored in the Helmholtz-Smoluchowski equation.
Table 6.3: Apparent zeta potentials ($\zeta_{\text{app}}$) for 100-kD membranes modified with a quaternary amine functionality for different reaction times

<table>
<thead>
<tr>
<th>Charging Time (hours)</th>
<th>Zeta Potential, $\zeta_{\text{app}}$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$1.6 \pm 0.1$</td>
</tr>
<tr>
<td>3</td>
<td>$3.4 \pm 0.3$</td>
</tr>
<tr>
<td>6</td>
<td>$7.2 \pm 0.3$</td>
</tr>
<tr>
<td>9</td>
<td>$8.9 \pm 0.3$</td>
</tr>
</tbody>
</table>

6.4.2 Counter-electroosmosis

Figure 6.4 shows the effects of solution ionic strength on the measured permeability of the charge-modified membranes. The results are plotted as the ratio of the permeability at a given ionic strength to that evaluated using the 0.5 M KCl solution to ensure that any variability in the membrane skin thickness or porosity (neither of which could be measured directly) does not effect the analysis. As discussed in Chapter 5, the membrane permeability decreases with decreasing salt concentration because of the greater magnitude of the back flow associated with counter-electroosmosis. The membrane permeability decreased by approximately 15% for the membrane that was charged for 1 hour as the ionic strength was reduced from 1 M to 0.002 M. In contrast, the hydraulic permeability decreased by nearly 60% for the membrane that was charged for 9 hours for a similar change in the solution ionic strength. The greatest reduction in permeability was observed as the
salt concentration was reduced from 0.1 M to 0.01 M, with little change in permeability as the ionic strength was reduced from 0.01 M to 0.002 M. This could be due to some type of charge regulation at very low salt concentrations, or it might reflect a conformational change in the charged cellulose under these conditions.

Figure 6.4: Effect of counter-electroosmosis on the hydraulic permeability of charge-modified membranes. Data are normalized by the permeability evaluated using 0.5 M KCl solution (L_p).

6.4.3 Protein Transmission

Figures 6.5 and 6.6 show typical data for the observed sieving coefficients of myoglobin and lysozyme through the different charge-modified membranes as a
function of the solution ionic strength. The data were obtained at pH 6.2 and a constant filtrate flux of 25 L/m²/hr using a protein mixture containing 3.5 g/L of lysozyme and 0.7 g/L of myoglobin. Repeat measurements of the sieving coefficients with the same membrane at selected conditions were within 20%, indicating that there was negligible fouling over the course of the experiments. This was also confirmed by hydraulic permeability measurements obtained using the 0.5 M KCl solution, with the permeability values before and after the protein filtration differing by less than 5%.

The observed sieving coefficient for myoglobin increases with increasing ionic strength due to the increase in electrostatic shielding provided by the electrolyte. The dependence on ionic strength was much less dramatic than that observed for cytochrome c in Chapter 5 (Figure 5.8), reflecting the lower net positive charge on myoglobin (+2) compared to cytochrome c (+7) under the respective experimental conditions. The myoglobin sieving coefficient decreases with increasing membrane charge due to the combined effects of the greater electrostatic repulsion and the slight pore constriction associated with the increased ligand density. For example, at 10 mM ionic strength the observed sieving coefficient decreased by a factor of 3 as \( \zeta_{\text{app}} \) increased from 1.6 to 7.8 mV compared to only a 10% reduction in the 0.1 M ionic strength solution.
The observed sieving coefficient for lysozyme (Figure 6.6) showed a much stronger dependence on both the ionic strength and the membrane charge since lysozyme has a much larger positive charge than myoglobin at pH 6.2 (+8 versus +2 in the 10 mM solution). For example, the lysozyme sieving coefficient for the membrane with $\zeta_{app} = 8.9$ mV increased by a factor of 1000 as the solution ionic strength was increased from 10 mM to 100 mM, and there was a 100-fold reduction in the sieving coefficient in the 10 mM solution as the membrane charge was increased from 1.5 mV to 8.9 mV. The large error bars on the data in the 2 mM ionic
strength solution for membranes charged for 6 and 9 hours (corresponding to $\zeta_{\text{app}} = 7.3$ and 8.9 mV, respectively) represent the error associated with the detection of the very low lysozyme concentrations in the filtrate under these conditions. The error bars for the rest of the experimental results were typically smaller than the height of the symbols.

Figure 6.6: Observed sieving coefficients for lysozyme as a function of the solution ionic strength through a series of charge-modified 100-kD membranes

The experimental data shown in Figures 6.5 and 6.6 have been re-plotted in Figure 6.7 in terms of the selectivity, $\Psi$, which is simply the ratio of the observed
sieving coefficients for the more and less permeable proteins (myoglobin and lysozyme in this case). The selectivity is plotted as a function of the solution ionic strength for each of the charge-modified membranes. The selectivity increases dramatically with decreasing salt concentration, particularly for the more heavily charged membranes, primarily due to the increase in electrostatic exclusion of the positively charged lysozyme by the positively charged membrane. At the lowest ionic strength (2 mM), the selectivity was essentially independent of the membrane charge for membranes that were charged for more than three hours ($\zeta_{\text{app}} \geq 3.4$ mV).

In contrast, the more heavily charged membranes ($\zeta_{\text{app}} = 7.3$ and 8.9 mV) had significantly larger selectivity at moderate ionic strengths. For example, the selectivity in the 20 mM ionic strength solution increased nearly 10-fold as the membrane charge was increased from 1.5 mV to 7.3 mV with even further improvement seen as the surface charge increased to 8.9 mV.
The selectivity data shown in Figure 6.7 are re-plotted in Figure 6.8 as an explicit function of the apparent membrane zeta potential to highlight the affect of the membrane charge. The selectivity in the 100 mM solution was very low and nearly independent of the membrane charge due to the significant electrostatic shielding at high salt concentrations. In contrast, the selectivity increased by approximately two orders of magnitude as the $\zeta_{\text{app}}$ increased from 1.6 mV to 7.3 mV in the lower ionic strength solutions (2 mM and 10 mM). This was directly related to the strong electrostatic exclusion of lysozyme from the membrane pores. The selectivity in the
2 mM solution appears to go through a maximum at an apparent zeta potential of 7.3 mV, although this may simply reflect the relatively large error bars on the results at low salt concentrations associated with the very small values of the lysozyme concentration in the filtrate solution. However, the data clearly demonstrate that the benefits of using a more heavily charged membrane are most significant at moderate salt concentrations where the increase in electrostatic repulsion associated with the greater membrane charge can be effectively realized.

Figure 6.8: Selectivity for the separation of myoglobin and lysozyme with a series of positively-charged membranes as a function of membrane charge
The mass throughput, $J\Delta S$, defined as the product of the filtrate flux and the difference in the observed sieving coefficients between the more and less permeable species, is shown in Figure 6.9 for the same membranes examined in Figures 6.5 – 6.8 for data obtained at a filtrate flux of 25 L/m$^2$/hr. The mass throughput provides a measure of the practicality and economics of a given separation. The greater the mass throughput, the less resources (buffer consumption, membrane area, and/or process time) required to achieve the desired purification. At low ionic strength (2 and 10 mM), the mass throughput decreases with increasing membrane charge, primarily due to the significant reduction in myoglobin transmission associated with the enhanced electrostatic repulsion under these conditions. In contrast, $J\Delta S$ goes through a maximum at an intermediate value of the apparent zeta potential for experiments performed at 20 and 40 mM ionic strength. The increase in mass throughput at small $\zeta_{\text{app}}$ was due to the large reduction in lysozyme transmission (relative to myoglobin) as the membrane charge increased, while the reduction in mass throughput at high $\zeta_{\text{app}}$ was due to the decrease in myoglobin transmission when the membrane becomes very heavily charged.
Figure 6.9: Mass throughput values for myoglobin and lysozyme separation as a function of the apparent zeta potential at different ionic strength

The experimental values of the selectivity and mass throughput can be used to estimate the design of an effective diafiltration process for the separation of lysozyme and myoglobin. Sample calculations were performed for a system designed to provide a purified lysozyme product in the retentate solution with a purification factor of 100. The number of diavolumes required for the desired purification was evaluated using Equation (6.7) and the experimentally determined mass throughput, with the results shown in Figure 6.10. At high ionic strength, the number of diavolumes decreases with increasing membrane charge due to an increase in lysozyme retention by the more highly charged membranes. The opposite behavior is
seen in the low ionic strength solutions due to the reduction in the myoglobin transmission under these conditions.

Figure 6.10: Calculated values for the number of diavolumes required to obtain a 100-fold purification of lysozyme

Corresponding results for the product (lysozyme) yield are shown in Figure 6.11, with the yield evaluated using Equation (6.6) and the experimentally determined values of the selectivity. The product yield at a given membrane charge decreases with increasing solution conductivity, with this effect being more pronounced at lower values of the membrane charge. For example, the predicted lysozyme yield decreases from 92% to 18% as the solution ionic strength was increased from 2 to 20
mM at ζ_{app} = 3.4 mV, while the yield decreases from 98% to 85% for the same change in ionic strength at ζ_{app} = 8.9 mV. The dramatic reduction in product yield at low membrane charge is due to the loss of lysozyme retention associated with the electrostatic shielding at high electrolyte concentrations, an effect that could be significantly reduced by using more heavily charged membranes.

Figure 6.11: Estimated product yield for a diafiltration process targeting 100-fold purification of lysozyme
6.4.4 Diafiltration Results

The results in the previous section clearly demonstrate that it should be possible to separate lysozyme and myoglobin using a membrane diafiltration process by proper selection of the solution conditions and membrane charge. A sample diafiltration was performed using a feed solution containing 3.5 g/L lysozyme and 0.7 g/L myoglobin mixture using a positively-charged 100-kD membrane generated by reaction for 9 hours (giving $\zeta_{\text{app}} = 8.9$ mV). Data were obtained at pH 6.2 using a 10 mM ionic strength solution based on the results in Section 6.4.3. The diafiltration was performed at a constant filtrate flux of 25 L/m$^2$/hr with the feed volume maintained constant at 10 mL.

Figure 6.12 shows the normalized protein concentration in the stirred cell as a function of the number of diavolumes (N) during the diafiltration process. The normalized protein concentration is simply the ratio of the protein concentration in the stirred cell to that in the initial feed, while the number of diavolumes is equal to the cumulative filtrate volume divided by the constant retentate volume in the stirred cell. The solid curves are model calculations developed using a simple material balance (Zeman and Zydne, 1996):

$$\frac{C_i}{C_{i0}} = \exp\{-NS_{o,i}\}$$

(6.10)

where the observed sieving coefficient ($S_{o,i}$) was assumed to be constant throughout the diafiltration. The observed sieving coefficients were evaluated from data obtained in a short ultrafiltration experiment performed immediately prior to the diafiltration.
Figure 6.12: Normalized concentrations of myoglobin and lysozyme in the retentate solution as a function of number of diavolumes. Diafiltration was performed with 3.5 g/L of lysozyme and 0.7 g/L of myoglobin. Solid curves are model calculations as described in the text.

The concentration of myoglobin in the retentate solution was reduced by a factor of 60 by the end of the 20-diavolume process, while the concentration of lysozyme dropped by only 12%. The net result was a highly purified lysozyme product in the retentate, with a yield of 88% and a purification factor of nearly 50. The collected filtrate solution was highly enriched with myoglobin, with the myoglobin yield of greater than 98% with a purification factor of approximately 10. This diafiltration process was thus able to generate two relatively purified product streams, one in the retentate and one in the permeate, where the final yield and
purification factor can be adjusted by simply changing the total number of diavolumes used for the separation.

6.5 Conclusions

Although several recent studies have demonstrated the importance of solution conditions like pH and ionic strength on the separation of proteins using high performance tangential flow filtration, there has been no extensive study of the effects of the membrane charge on the separation characteristics. The data presented in this chapter provide the first quantitative results on the effects of membrane charge density for the design of an effective membrane purification process for the separation of a model protein mixture.

The selectivity increased dramatically as the salt concentration was reduced due to the strong electrostatic exclusion of the positively charged lysozyme by the positively charged membrane. For weakly charged membranes, it was necessary to go to very low ionic strength (approximately 2 mM) to obtain selectivities above 100-fold, conditions that may not be practical for commercial separations due to the reduction in protein stability and solubility at very low salt concentrations. In contrast, data obtained with more highly charged membranes (\(\zeta_{\text{app}} = 7.3\) and 8.9 mV) provided significant selectivity up to moderate ionic strengths. For example, the selectivity in a 20 mM ionic strength solution was 40 times larger using a membrane with an apparent zeta potential of 8.9 mV compared to that obtained with a membrane having an apparent zeta potential of only 1.5 mV. These results clearly demonstrate
that high performance membrane separations can be achieved at moderate ionic strengths, conditions that are much more amenable to large scale commercial processes, by using membranes with a greater electrical charge to compensate for the increased electrostatic shielding at higher salt concentrations.

Experimental results for the separation of myoglobin and lysozyme were analyzed using the process optimization equations developed by van Reis and Saksena (1997). The sieving coefficient data were used to evaluate the selectivity and mass throughput, with these values then used to determine the product yield and number of diavolumes required to obtain a targeted purification factor of 100. The results highlight the effects of both solution ionic strength and membrane charge on the separation characteristics. For example, the product yield increased from 40% to more than 90% as the membrane charge was increased from 1.6 mV to 8.9 mV at an ionic strength of 10 mM. In contrast, the product yield in the 2 mM solution went through a maximum for the membrane with an apparent zeta potential of 7.3 mV. The required number of diavolumes decreased with increasing membrane charge at an ionic strength of 40 mM, but it increased with increasing membrane charge at much lower ionic strength. A sample diafiltration run demonstrated the potential of this type of protein separation. In this case, a purified lysozyme product was recovered in the retentate solution while the myoglobin was recovered in the filtrate, with both proteins obtained with good purification factors and yields.

These results clearly demonstrate that the membrane charge density can be used to optimize the performance of membrane separation processes, providing an
additional degree of freedom along with pH, ionic strength, and filtrate flux. More work will be needed to fully explore the interplay between these parameters for particular separation problems, hopefully providing a framework to design high performance membrane systems to meet the growing challenges of the biotechnology industry.
Chapter 7
RECOMBINANT MONOCLONAL ANTIBODY PURIFICATION

7.1 Introduction

The experimental data presented in Chapters 4 to 6 clearly demonstrate the importance of electrostatic interactions in enhancing the performance of ultrafiltration systems for protein concentration, buffer exchange, and the separation of model protein mixtures. These results suggest that it should be possible to develop commercial membrane systems that can be used to purify the complex protein mixtures encountered in the biotechnology, food processing, and biomedical industries. This includes the purification of recombinant monoclonal antibodies produced by mammalian cell culture, the separation of whey proteins in cheese manufacturing, and the fractionation of blood plasma for both therapeutic and commercial applications. Membrane systems can be particularly attractive for these applications because of their high throughput, robustness, cost-effectiveness, and easy scalability.

HPTFF is an emerging membrane technology that can be used to separate proteins of similar size by exploiting a number of phenomena, including electrostatic interactions between the charged proteins and membrane (van Reis et al., 1997; 1999). For example, as discussed in previous Chapter, van Reis et al. (1999) showed that it was possible to obtain more than 990-fold purification and 90% yield for the separation of BSA (69 kD) from an antigen binding fragment (45 kD) derived from a
recombinant DNA antibody. There is also a considerable interest in the development of this technology for purification of therapeutic proteins produced using recombinant DNA technology (van Reis, 1996; Christy et al., 2002). These therapeutic proteins are commercially manufactured using a variety of recombinant cell lines including bacteria (e.g., *E. Coli*), yeast, and mammalian cells (e.g., Chinese hamster ovary cells). The desired protein must be purified to extremely high levels, with the concentration of host cell proteins and DNA reduced to the range of parts per million relative to the desired product, or lower. The final product must also be sterile (no viable bacteria) and virus-free, and it should meet the stringent purity requirements specified for therapeutic grade proteins (Table 7.1). This purification is typically accomplished using a combination of different chromatographic steps like ion exchange, affinity, hydrophobic interaction, etc. (Fahrner et al., 2001).

**Table 7.1: Purity requirements**

<table>
<thead>
<tr>
<th>Impurities</th>
<th>Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host cell proteins</td>
<td>typically &lt; 10 ppm</td>
</tr>
<tr>
<td>DNA (WHO)</td>
<td>&lt; 10 ng/dose levels</td>
</tr>
<tr>
<td>Product Variants (aggregates, etc.)</td>
<td>&lt; 5% in final bulk</td>
</tr>
<tr>
<td>Virus</td>
<td>1 virus per 10^6 doses</td>
</tr>
</tbody>
</table>

Even though current recovery processes are able to meet these stringent purification requirements, they are quite expensive and can be challenging to apply for very high volume production typical of most monoclonal antibodies. There is
thus considerable interest in developing alternative technologies to reduce the downstream purification costs, which can constitute as much as 20% to 80% of the total manufacturing costs (Blanch and Clark, 1997). HPTFF is particularly attractive as there is extensive experience in effective scale-up (and scale-down) of membrane processes. In addition, HPTFF has the ability to provide protein purification, protein concentration, and buffer exchange in a single processing step, which can significantly reduce capital costs while also improving product yield.

Although recent studies have demonstrated the feasibility of using HPTFF technology for protein purification, most of this work has been performed using model protein mixtures. There is a critical need for more detailed studies of the application of this technology to the purification of recombinant protein products from harvested cell culture fluids encountered in the biotechnology industry. The experimental studies described in this chapter were designed to investigate the potential of using HPTFF for purification of a recombinant monoclonal antibody (Mab) expressed in Chinese hamster ovary cells. The focus of this work was on the removal of host cell proteins (Chinese hamster ovary proteins, CHOP) while maintaining high yield of the recombinant monoclonal antibody. Filtration experiments were conducted with prototype, positively charged versions of composite regenerated cellulose (CRC) membranes with 100-kD and 150-kD nominal molecular weight cut-off (MWCO) that were provided by Millipore (Bedford, MA, USA). Data were obtained using an industrial scale feed stream containing recombinant monoclonal antibody (molecular weight of 140 kDa) and Chinese Hamster Ovary
(CHO) cell proteins (with molecular weights from 5 to 100 kDa). The isoelectric point (pI) of the antibody was approximately 9.3 whereas the pI of the CHOP population varied from 4 to 9.3 (Champion et al., 1999). All experiments were performed at Genentech, Inc., in South San Francisco, CA.

7.2 Experimental

7.2.1 Proteins and Buffer Solutions

The Harvested Cell Culture Fluid (HCCF) containing recombinant Mab and CHOP (at a level of approximately 500,000 ppm) was obtained from the cell culture department at Genentech, Inc. (South San Francisco, CA). HCCF was purified by first conditioning it to an appropriate pH and conductivity and loading it on a cation-exchanger in a bind and elute mode. The eluent was passed through an anion-exchange chromatography step in a flow through mode. The anion-exchange pool was concentrated and transferred into Histidine buffer by ultrafiltration / diafiltration using a 10-kD composite regenerated cellulose membrane in a Pellicon™ cassette (Millipore, Bedford, MA) operated using a fully automated scale-down skid (Millipore, Bedford, MA). The concentrated pool had a Mab concentration of approximately 67 g/L and a CHOP concentration of 60 ppm. Host cell DNA levels were less than detectable (< 2 pg/mL).

HPTFF experiments were performed at pH 6 and at different values of the solution conductivity, which was altered by adjusting the buffer (Histidine)
concentration. The pH and conductivity were measured using an Omega probe (Omega Engineering, Stamford, CT). All buffers were prefiltered through 0.22 µm Acropak capsule filters (Pall Biopharmaceuticals, Pensacola, FL) prior to use. HPTFF experiments were performed in total recycle mode and the feedstock was reused for several experiments. At the end of each experiment, the feedstock was recovered from the tank, cassette, retentate, and filtrate lines and was then filtered through a 0.22 µm Acropak capsule filter (Pall Biopharmaceuticals, Pensacola, FL) prior to reuse.

### 7.2.2 Instruments

Laboratory chromatography experiments were run on an AKTA Explorer 100 while pilot scale chromatography was done on an AKTA Pilot, both from GE Healthcare (Uppsala, Sweden). HPTFF experiments were conducted on a fully automated scale down skid. Instrumentation on the feed and filtrate lines included flow meters, conductivity meters, temperature probe, pressure transducers, and ultraviolet light detectors. All piping was constructed of stainless steel, and all valves were pneumatically actuated. Stainless steel Pellicon-2™ holders (Millipore Corporation, Bedford, MA) were used for assembling prototype positively-charged versions of the 100-kD and 150-kD membranes in the form of Pellicon™ mini cassettes. These membranes will be denoted as 100-kD (+) and 150-kD (+). Each mini-cassette had 0.1 m² of membrane area. Pressure transducers were placed close to the feed inlet, retentate outlet, co-flow inlet, and co-flow outlet for measuring the
transmembrane pressure.

A schematic of the filtration set-up used for Mab and CHOP separation is shown in Figure 7.1. HPTFF experiments were performed in total recycle mode (returning both retentate and filtrate to the feed tank). A co-current flow on the filtrate side of the membrane was used to maintain an essentially uniform transmembrane pressure throughout the membrane module as discussed by van Reis (1996).

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**Figure 7.1:** Schematic diagram of the HPTFF process with co-current filtrate flow
All membranes were cleaned with 0.1 N NaOH and appropriate buffer prior to use. Hydraulic permeability was then evaluated from the measured buffer flux at several different transmembrane pressures. After the permeability measurements, the feed tank was drained, keeping the filtrate and retentate lines and cassette filled with buffer. 600 ml of feedstock was then added to the feed tank and recirculated in a total recycle mode for 10 minutes while maintaining zero transmembrane pressure to ensure proper mixing in the retentate lines and feed tank. Since the hold-up volume in the retentate line was approximately 400 mL, the Mab and CHOP concentrations in the retentate after mixing were approximately 37 g/L and 60 ppm, respectively. The feed flow rate was then set to 323 L/m$^2$/hr and the filtrate flow rate was controlled using a three-roller peristaltic pump. The system was operated in a total recycle mode until the Mab concentration in the filtrate reached a stable value (typically 30 min). Samples of the retentate and filtrate were then collected for subsequent protein assays.

7.3 Protein Assays

7.3.1 Mab Concentration

Mab concentration in the HCCF was determined by HPLC using a protein-A affinity column. Mab was effectively captured on the resin due to its high affinity for protein A. The bound Mab was then eluted by changing the solution conditions, with the measured peak area used to evaluate the protein concentration by comparison to a
calibration constructed using Mab standards. Mab concentrations in the cation and anion-exchange pool were determined spectrophotometrically using an UV spectrophotometer. The absorbance was measured at 280 nm, which is the peak absorbance for the aromatic groups of different amino acids. Actual Mab concentrations were evaluated from the absorbance using the known extinction coefficient of the Mab. Mab concentrations could be measured accurately down to 0.006 g/l.

7.3.2 Host Cell Protein (HCP) Assay

CHOP concentration was determined by an enzyme-linked immunosorbent assay (ELISA) developed at Genentech, Inc. Polyclonal goat anti-CHO protein antibodies (i.e. anti-host cell protein antibodies) were immobilized in microtiter plate wells. Samples were diluted several-fold and then added to the wells and incubated to allow binding of HCP to the immobilized antibodies. This was followed by incubation with conjugated-peroxidase whole anti-CHO-protein antibody. Unbound conjugated antibody was removed by washing the wells, and horseradish peroxidase activity was quantified with the substrate o-phenylenediamine by reading the absorbance at 492 nm. Horseradish peroxidase activity was correlated to HCP concentration.
7.4 Results and Analysis

Typical data for HPTFF experiments performed using the 150-kD (+) membrane with 37 g/L Mab and approximately 60 ppm CHOP in Histidine buffer at pH 6.0 for different values of solution conductivity are shown in Figure 7.2. As mentioned earlier, all HPTFF experiments were performed in a total recycle mode on a fully automated scale-down system. The conductivity of the feedstock was adjusted by varying the concentration of the Histidine buffer. The Y-axis represents the observed sieving coefficient for the Mab, $S_{o,Mab}$, defined as the ratio of the Mab concentration in the filtrate to that in the retentate. Results are shown as a function of the filtrate flux (represented on the X-axis) from 30 to 70 L/m$^2$/hr. In each case, the membranes were allowed to equilibrate at each conductivity by flushing at least 60 L/m$^2$ of buffer through the system before switching to a feedstock of the same conductivity. The Mab concentration in the filtrate was constantly monitored and retentate and permeate samples were collected only after the Mab concentration in the filtrate had reached a stable value.
As seen in Figure 7.2, the Mab sieving coefficient increases with an increase in filtrate flux due to the greater extent of concentration polarization at high filtrate flux. $S_{\text{o,Mab}}$ increases dramatically with an increase in solution conductivity due to the increase in electrostatic shielding provided by the electrolyte. For example, $S_{\text{o,Mab}}$ increases by more than a factor of 30 as the solution conductivity was increased from 0.6 mS/cm to 2.5 mS/cm at a filtrate flux of 30 L/m$^2$/hr. In contrast, the Mab sieving coefficient increased only about 3-fold as the filtrate flux was increased from 30 to 70
L/m²/hr at any fixed value of the solution conductivity.

The experimental data in Figure 7.2 are re-plotted in Figure 7.3 as an explicit function of the solution conductivity for different values of the filtrate flux. The data clearly show that the Mab sieving coefficient is a relatively weak function of the filtrate flux in the range investigated compared to the very strong dependence on the solution conductivity.

![Graph](image_url)

**Figure 7.3:** Observed sieving coefficients for monoclonal antibody as a function of the solution conductivity through a 150-kD (+) membrane at different filtrate fluxes

Data for the observed sieving coefficient for CHOP, S₀,CHOP, evaluated with the same 150-kD (+) membrane, are shown in Figure 7.4. Even though there is some
scatter in the results due to the inherent limitations of the CHOP assay, the data clearly show that the CHOP sieving coefficient also increases with increasing filtrate flux and solution conductivity. However, the effect of conductivity is much smaller than that seen with the Mab; \( S_{o,CHOP} \) increases approximately 70% as the solution conductivity increases from 0.6 mS/cm to 2.5 mS/cm at 30 L/m²/hr and by only 10% at a flux of 70 L/m²/hr compared to the 30-fold increase seen in Figure 7.2 for the Mab.

![Figure 7.4: Observed sieving coefficients for CHOP, \( S_{o,CHOP} \), as a function of the filtrate flux through a charge modified 150 (+) kD membrane at different values of solution conductivity](image)
The calculated values for the selectivity between the Mab and CHOP are shown in Figure 7.5 as a function of filtrate flux for different values of the solution conductivity. The selectivity value is simply equal to the observed sieving coefficient for CHOP ($S_{o,CHOP}$) divided by that of the Mab ($S_{o,Mab}$) at each filtrate flux. The maximum selectivity of 600 is obtained at a filtrate flux of 30 L/m$^2$/hr at pH 6.0 and a conductivity of 0.6 mS/cm. A 2-fold decrease in selectivity is observed as the filtrate flux increases from 30 to 70 L/m$^2$/hr primarily due to the increase in Mab transmission (Figure 7.3). This is completely consistent with the higher degree of concentration polarization of the large Mab associated with the smaller bulk diffusion coefficient. In contrast, the selectivity decreases by almost 20-fold as the solution conductivity is increased from 0.6 to 2.5 mS/cm at any fixed value of the filtrate flux, largely due to a reduction in electrostatic exclusion of the positively charged Mab by the positively charged membrane due to the increase in electrostatic shielding provided by the electrolyte at higher conductivity.
The corresponding values of the mass throughput are shown in Figure 7.6. The mass throughput \( (J\Delta S) \) was evaluated by multiplying the filtrate flux by the difference in observed sieving coefficients of the more and less permeable species (CHOP and Mab in this case). The mass throughput increases with an increase in solution conductivity at a fixed value of filtrate flux due to the increase in transmission of the CHOP. Note that the dimensionless parameter \( N\Delta S \) (Equations 6.2 and 6.3) can be evaluated directly from the experimentally measured values of \( J\Delta S \) using an estimate of the system parameters: the membrane area, \( A \), process time, \( t \), and retentate volume, \( V \). For example, if the ratio \( At/V \) is chosen as 1 m\(^2\) hr/L.
(consistent with current commercial processes), \( \Delta S \) simply becomes equal to \( J \Delta S \) provided \( J \) is measured in the same units as \( \frac{V}{At} \). This simplifies the calculation of yield and purification factor as they can be easily calculated using the optimization equations presented by van Reis and Saksena (1997) using the observed sieving coefficients for the Mab and CHOP at different values of the filtrate flux.

Figure 7.6: Mass throughput values for Mab and CHOP separation as a function of the filtrate flux through a 150-kD (+) membrane for different values of the solution conductivity.
Based on the experimentally determined selectivity and mass throughput values shown in Figure 7.5 and 7.6, an effective diafiltration process can be designed for the purification of Mab and CHOP using HPTFF. Since the CHOP concentration in the feedstock is approximately 60 ppm, a purification factor of 60 would be required to reduce the CHOP levels to less than 1 ppm in the final product, assuming complete product retention. Theoretical calculations can be performed to evaluate the number of diavolumes required to achieve this 60-fold purification using Equation 6.6 with the values of mass throughput shown in Figure 7.6. The results are shown in Figure 7.7 as an explicit function of the filtrate flux for different values of the solution conductivity. The number of diavolumes decreases with an increase in the solution conductivity and filtrate flux, both of which enhance CHOP transmission through the positively charged membrane, thus enabling a more rapid clearance of the CHOP.
Figure 7.7: Calculated values for the number of diavolumes required to obtain a 60-fold purification of feedstock containing Mab and CHOP using a 150-kD positively charged membrane.

The corresponding calculations for the product yield are shown in Figure 7.8. The product yield was evaluated using Equation (6.7) and the experimentally determined values of the selectivity shown in Figure 7.5. The product yield decreases with an increase in solution conductivity at any fixed value of the filtrate flux, with this effect being more dramatic at the higher fluxes. For example, the Mab yield decreases from 99% to 85% as the solution conductivity is increased from 0.6 to 2.5 mS/cm at 30 L/m²/hr while the yield decreases from 98% to less than 60% for a
similar increase in conductivity at 70 L/m²/hr. This dramatic reduction in product yield at higher filtrate flux is due to the rapid decrease in selectivity (Figure 7.5) associated with the combined effects of the electrostatic shielding provided by the electrolyte and the high degree of concentration polarization.

Figure 7.8: Calculated values for the product yield for a diafiltration process targeting 60-fold purification of a feedstock containing Mab and CHOP
The calculations in Figures 7.7 and 7.8 clearly demonstrate the trade-off between product yield and number of diavolumes. A large number of diavolumes, N, typically increases the process cost by increasing buffer requirements, membrane area, and/or process time, while an increase in product yield provides a direct economic benefit to the overall process. It is thus necessary to optimize the solution conditions (conductivity, pH, etc.) and process conditions (filtrate flux) to maximize product yield while minimizing buffer composition, process time, and membrane area.

The theoretical calculations for product yield and number of diavolumes were all performed using the assumption that the observed sieving coefficients for the antibody and host cell proteins remain constant throughout the diafiltration. However, since CHOP represents a very diverse protein population, ranging in molecular weight from 10 to 100 kDa and in isoelectric point from 4.5 to 9.3, it is likely that the measured CHOP sieving coefficient will decrease with increasing numbers of diavolumes due to the initial preferential removal of certain CHOP species. The resulting variation in the CHOP sieving coefficient during diafiltration might limit the ability of HPTFF to reduce the CHOP levels in the feedstock below certain values.

HPTFF’s ability to clear CHOP to very low concentrations (< 10 ppm) was examined using a 100-kD (+) membrane. These experiments were performed with a slightly different feedstock (generated by an alternative purification process) that had a Mab concentration of 0.95 g/L and a CHOP level of approximately 20 ppm. This
feedstock was first concentrated to a Mab concentration of approximately 18 g/L by ultrafiltration. The overall Mab yield was 97% with the CHOP concentration reduced from 20 to 6 ppm at the end of the concentration step.

A diafiltration was then done with this new feedstock using a 20 mM Histidine buffer (1 mS/cm) at pH 6 and a filtrate flux of 100 L m$^{-2}$ h$^{-1}$. The measured CHOP levels are shown in Figure 7.9 as a function of the number of diavolumes. The final product had a CHOP concentration of 2 ppm, which is much lower than the purity requirements mentioned in Table 7.1 (< 10 ppm). An overall product yield of 95.8% was obtained after both the concentration and diafiltration steps.

![Figure 7.9: Reduction in CHOP concentration in the retentate during diafiltration](image-url)
The effects of membrane pore size on the antibody and CHOP sieving coefficients were examined using 100-kD (+) and 150-kD (+) MWCO membranes having similar surface charge density. The clean membrane permeabilities of the 100 and 150 kD (+) membranes measured with 10 mM Histidine buffer were 32 and 35 L/m²/hr/psi, respectively. Results are shown in Figure 7.10 over a range of filtrate flux for experiments performed at pH 6.0 and a conductivity of 0.6 mS/cm. The solid curves are spline fits to the experimental data, with the filled and open symbols denoting results obtained with the 100-kD (+) and 150-kD (+) MWCO membranes, respectively. The results at low filtrate flux are similar, with the Mab sieving coefficient through the 150-kD (+) membrane being about a factor of two larger than that for the 100-kD (+) membrane at the highest flux. The similarity in sieving coefficients observed at lower filtrate flux could simply be due to assay errors, although it might also reflect a greater contribution from Mab diffusion through the 100-kD (+) membrane at low flux.
Figure 7.10: Effect of membrane pore size on the Mab sieving coefficient. Solid curves are spline fits to the experimental data for the 100-kD (filled symbols) and 150-kD (open symbols) MWCO membranes.

Figure 7.11 shows experimental data for the selectivity, Ψ, and mass throughput, JΔS, for the 100 and 150-kD (+) MWCO membranes at pH 6 and 0.6 mS/cm. The solid and dashed curves are spline fits to the experimental data for the 100-kD (filled symbols) and 150-kD (open symbols) MWCO membranes. The mass throughput values obtained with the 150-kD (+) membrane were slightly larger than those for the 100-kD (+) membrane due to the higher CHOP sieving coefficients.
through the larger MWCO membrane. The selectivity decreases with an increase in filtrate flux for both membranes, with higher values of selectivity obtained for the 100-kD (+) membrane except at the very lowest flux.

Figure 7.11: Effect of membrane pore size on mass throughput and selectivity for the 100 kD (+) and 150 kD (+) membranes. The solid and dashed spline fits represent selectivity and mass throughput values, respectively.
All of the experimental results discussed so far were performed with a co-current flow on the filtrate side of the membrane to keep the transmembrane pressure essentially constant along the length of the membrane module. Figure 7.12 shows a schematic representation of the effect of this co-current filtrate flow on the transmembrane pressure along the module length.

**Figure 7.12:** A schematic representation of the pressure drop along the length of the TFF module when a co-current flow is employed on the filtrate side. Solid and dashed lines near the pressure indicator represent feed and filtrate pressure, respectively.

In the absence of a co-current filtrate flow a non-uniform transmembrane pressure ($\Delta P_{TM}$) is generated along the membrane length (Figure 7.13) due to the
significantly higher axial pressure drop on the retentate side compared to that on the permeate side. This is largely due to the significantly higher feed flow rate (323 L/m²/hr) compared to the filtrate flow rate of 30 L/m²/hr. This $\Delta P_{\text{TM}}$ gradient significantly alters the filtrate flux along the length of the membrane module, resulting in greater Mab sieving in regions of high filtrate flux due to the greater extent of concentration polarization and vice-versa. Even if the average filtrate flux (integrated along the entire length of the membrane module) is similar in the presence and absence of the co-current filtrate flow, the overall Mab sieving coefficient will likely be much higher in the absence of co-current filtrate flow due to the non-linear increase in Mab sieving with an increase in filtrate flux (Figure 7.2).

![Figure 7.13: A schematic representation of the non-uniform pressure drop along the length of the TFF module in the absence of a co-current flow on the filtrate side. Solid and dashed lines near the pressure gauge represent feed and filtrate pressure, respectively.](image-url)
While an increase in cross flow/feed flow rate lowers the antibody concentration near the membrane wall by enhancing the mass transfer of antibody molecules away from the membrane wall and back into the bulk solution, it also significantly increases the axial pressure drop on the retentate side of the membrane resulting in a steeper $\Delta P_{TM}$ gradient along the membrane length in the absence of co-current filtrate flow. This sharp increase in the $\Delta P_{TM}$ gradient along the membrane length could significantly increase the product sieving coefficient and thereby reduce the selectivity. This suggests that the design of an effective HPTFF process in the absence of co-current flow will be strongly affected by the cross flow rate due to the trade-off between the pressure gradient and the mass transfer coefficient both of which affect product sieving.

Experiments were designed to investigate the effects of cross flow rate on the observed sieving coefficient of Mab and CHOP with 150-kD (+) membrane in the absence of co-current flow on the filtrate side. The experiments were performed at pH 6 and 0.6 mS/cm by varying the cross flow rate from 90 to 323 L/m$^2$/hr using a feedstock containing approximately 37 g/L Mab and 100 ppm CHOP. The filtrate flux was set to 30 L/m$^2$/hr using a filtrate pump. The experimental results for the observed sieving coefficients of Mab and CHOP are shown in Figure 7.14.
Figure 7.14: Effect of feed flow rate on the observed sieving coefficient of Mab and CHOP in the absence of co-current flow on the filtrate side. Filled and open symbols represent the observed sieving coefficients for Mab and CHOP, respectively.

The observed sieving coefficient of Mab at a feed flux of 323 L/m$^2$/hr in the absence of co-current flow on the filtrate side was approximately an order of magnitude higher than that with co-current filtrate flow (Figure 7.2). This clearly demonstrates the dramatic effect of the non-uniform transmembrane pressure along the module length on ultrafiltration performance. The Mab sieving decreased significantly with decreasing feed flow rate and reached a minimum between 150 and 225 L/m$^2$/hr. This can be attributed to the decrease in axial pressure drop on the
retentate side, thus lowering the transmembrane pressure, $\Delta P_{\text{TM}}$, gradient along the module length. The observed sieving coefficient for Mab increased dramatically (more than 2-fold) as the feed flow rate was reduced from 150 to 90 L/m$^2$/hr. This is most likely due to a considerable decrease in the mass transfer coefficient of antibody molecules from the membrane wall to the bulk solution, resulting in a significant increase in Mab concentration near the membrane wall. Although a qualitatively similar trend is also seen with the CHOP sieving coefficient; the variation in $S_{0,\text{CHOP}}$ over the range of feed flux examined is not statistically significant. These results clearly demonstrate that the feed flow rate can significantly alter the performance of charged membranes during HPTFF in the absence of co-current filtrate flow.

Besides optimization of cross flow rate, ultrafiltration performance in the absence of a co-current filtrate flow can also be enhanced by replacing the filtrate channels in the current TFF modules with newer ones that provide approximately an order of magnitude higher pressure drop than those on the retentate side. These high resistance filtrate channels can give pressure drop on the filtrate side that is approximately similar to the one on the retentate side even at lower filtrate flow rates, resulting in essentially uniform transmembrane pressure along the module length.

7.5 Conclusions

Although a number of recent studies have demonstrated the importance of solution pH, conductivity, and filtrate flux on protein sieving coefficients during membrane ultrafiltration, there have been very few studies of these effects during
filtration of complex multicomponent feedstocks found in the biotechnology industry. The experimental results presented in this chapter investigated the effects of filtrate flux, solution conductivity, and membrane pore size on the purification of a recombinant monoclonal antibody focusing on the removal of the host cell (Chinese hamster ovary) proteins.

Positively charged 100-kD (+) and 150-kD (+) membranes were used to obtain very high retention of the positively charged Mab, with greater than >99.9% retention observed at low conductivities (1 mS/cm) at a filtrate flux of 30 L/m²/hr. The Mab retention decreased dramatically with an increase in solution conductivity due to the greater electrostatic shielding provided by the electrolyte. For example, Mab retention decreased from 99.96% at 0.6 mS/cm and 30 L/m²/hr to approximately 70% at 2.5 mS/cm and 70 L/m²/hr. The net result was that the maximum selectivity of 600 was obtained at a conductivity of 0.6 mS/cm and a filtrate flux of 30 L/m²/hr. In contrast, the mass throughput increased almost linearly with increasing filtrate flux for fluxes from 30 to 70 L/m²/hr.

The measured values of the selectivity and mass throughput were used to calculate the required number of dia volumes and the overall product yield for a diafiltration process targeting a 60-fold purification of feedstock containing 60 ppm CHOP. The results indicated that the CHOP level could be reduced to 1 ppm by diafiltration at 30 L/m²/hr and 0.6 mS/cm after 28 dia volumes with a final product yield of 99.9%; a similar CHOP clearance could be achieved with only 15 dia volumes at a flux of 50 L/m²/hr at 1.5 mS/cm although the Mab yield decreased slightly to
95% under these conditions. An actual diafiltration with a 100-kD (+) membrane using a different feedstock gave a 10-fold reduction in CHOP levels with a Mab yield of approximately 96%, demonstrating the feasibility of using HPTFF to significantly reduce CHOP levels in the purification of a monoclonal antibody from commercially-relevant feedstocks.
8.1 Introduction

A number of recent reports (Nakao et al., 1988; Pujar and Zydney, 1997; Burns and Zydney, 1999) have conclusively demonstrated that the performance of ultrafiltration membranes can be enhanced by exploiting electrostatic interactions between charged proteins and charged membranes. These charged membranes have the potential to dramatically improve various commercial scale bioseparation processes including protein concentration, buffer exchange, and protein fractionation (van Reis et al., 1999; van Reis, 2001; Christy et al., 2002).

The performance of charged ultrafiltration membranes depends on the electrical charge of the protein molecules and the membranes pores, both of which are functions of the solution pH and ionic strength. In addition to the number of charge groups, the membrane performance can also be affected by the detailed coupling chemistry used to attach the charged groups to the membrane, the nature of the functional groups themselves (e.g., the use of weak versus strong acid or base functionalities), the specific location of the charged groups, and the overall pore size and charge distribution. Several researchers (Miyama et al., 1988; Saksena and Zydney, 1994; Balakrishnan and Agarwal, 1996; Millesime et al., 1996; Yang and Tong, 1997) have investigated the effects of solution environment on protein and
solvent transport through charged membranes, while the studies in Chapter 5 examined the effects of the membrane surface charge density on both solvent flow and protein transport.

Although there have been no significant studies of the effects of different coupling chemistries, spacer arm length, or the nature of the functional group on the performance of ultrafiltration membranes, there is an extensive literature on these parameters in the field of chromatography. An optimal spacer arm ensures that the functional groups are placed a suitable distance from the surface of the solid support and are easily accessible to the macromolecules. Results from a variety of studies in ion exchange and dye-affinity chromatography suggest that the ideal spacer arm has (Zou et al., 2001): (1) proper length (at least three atoms); (2) no active center that could cause nonspecific adsorption; and (3) at least two functional groups, one to react with the base support and one to serve as the chromatographic ligand.

Hirota and Shimamura (1985) showed that the purification of fumarase from pig heart was dependent on the length of the methylene carbon spacer arm that was used to couple the affinity ligand to a Sepharose gel. Gels developed using a six-carbon spacer arm were found to have the highest adsorption capacity for the enzyme.

Lowe (1977) investigated the effect of the nature of the spacer arm (hydrophilic versus hydrophobic) on the binding of lactate and alanine dehydrogenase to chromatography gels using adenosine 5' monophosphate as the affinity ligand. The author observed that enzymes were bound more tightly to the affinity media produced using hydrophobic spacer arms with the strength of the interaction decreasing with
increasing hydrophilicity. DePhillips and Lenhoff (2001) investigated protein retention on cation exchange resins with different charge groups. Protein retention on strong cation-exchangers, typically constructed through the attachment of sulfonic acid groups, was found to be greater than that for the corresponding weak cation-exchangers (produced using carboxylic acids). Savina et al. (2005) studied the adsorption capacity of cryogels that were modified by grafting multiple acrylic acid groups onto long polymer chains. A dramatic increase in the lysozyme binding capacity was observed above a critical grafting limit. The authors hypothesized that this dramatic increase in binding capacity was due to (1) the presence of multiple functional groups along the polymer chains that provided multipoint interactions and (2) the flexibility of the polymer chains which allowed conformational changes that provided more efficient binding of lysozyme.

Results with membrane adsorbers (or affinity membranes) have also demonstrated the importance of the spacer arm to the overall performance characteristics. For example, Suen et al. (2000) showed that attaching 1,4-diaminobutane as a spacer arm to the crosslinking molecule (ethylene glycol diglycidyl ether) provided higher dye ligand density and lysozyme adsorption capacity compared to immobilized metal affinity membranes produced using only the ethylene glycol diglycidyl ether. Tsai et al. (2002) found that polyvinylidene fluoride affinity membranes with 1,8-diaminoctane as the spacer arm had the highest adsorption capacity for lysozyme, with the capacity decreasing for both shorter and longer spacers. The authors hypothesized that this spacer arm had the optimal length
in terms of functional group accessibility and number of binding sites. Tsuneda et al. (1995) synthesized novel microporous ion exchange membranes by grafting long polymer chains containing multiple diethylamino groups to the base membrane. These ion exchange membranes provided 11-fold higher adsorption capacity for BSA compared to the theoretical monolayer binding capacity of the membranes. The authors attributed the higher adsorption capacity to the three-dimensional packing space available between the long polymer chains.

These studies of chromatographic systems provide useful insights into the effects of the ligand attachment on protein binding, but it is impossible to use these results to understand the behavior of membrane systems in which the performance is determined by electrostatic exclusion effects instead of binding interactions. The objective of the studies described in this chapter was to examine the effects of spacer arm length, charge group functionality, and ligand design on the behavior of electrically charged ultrafiltration membranes. Membranes were made by attachment of the appropriate ligands to commercially-available composite regenerated cellulose (CRC) membranes. Charged CRC membranes were produced with both strong (quaternary amine) and weak (primary amine) groups. In addition, the behavior of novel ultrafiltration membranes with multiple charged groups along the spacer arm length was examined.
8.2 Experimental

8.2.1 Materials and Apparatus

Composite regenerated cellulose (CRC) membranes with nominal molecular weight cut offs of 30 and 100 kD were provided by Millipore Corporation (Bedford, MA). Epichlorohydrin (EPI) and a series of diamines with different alkyl chain length (1,2-diaaminoethane, 1,4-diaaminobutane, 1,6-diaminohexane, 1,8-diaaminoctane, and 1,10-diaaminodecane) were purchased from Sigma (St. Louis, MO). Diethylenetriamine, triethylenetetramine, pentaethylenehexamine, and polyethyleneimine (with average molecular weight of 423 Da) were also purchased from Sigma (St. Louis, MO). All reagents were of analytical grade.

Ultrafiltration experiments were performed in a 25 mm diameter Amicon stirred cell connected to a peristaltic pump (Rainin Instrument Corp.) on the filtrate line. Filtrate samples were analyzed using a UV-Vis spectrophotometer (Shimadzu, Columbia, MD). Additional details on the experimental apparatus and filtration procedures are provided in Chapter 3.

8.2.2 Membrane Preparation using Diamines

All experiments were performed with 25 mm diameter membrane disks cut from a large flat sheet of membrane (roll stock) provided by Millipore Corporation (Bedford, MA). The membrane disk was handled carefully with contact limited to the edges of the disk to avoid scratching the glossy surface (the membrane skin). Each disk was thoroughly rinsed to remove glycerin, which is used as a wetting / storage agent. The membranes were floated in a beaker of distilled water with the skin (glossy) side down.
for at least one hour, with the solution changed three times with fresh water. The CRC membranes were then stored overnight in a 0.1 M NaOH solution prior to surface modification.

The reaction scheme used to couple epichlorohydrin (EPI) and the diamines to the hydroxyl groups on the cellulose membrane is shown in Figure 8.1. The basic approach used for the surface modification was adapted from the work by Liu et al. (2005), but the specific process conditions (e.g., temperature and NaOH concentration) were modified to avoid degradation of the cellulose membrane. All reactions were carried out in a 25 mL capped plastic jar kept in a shaker that was agitated at approximately 150 rpm. The clean membrane disk was immersed in a solution containing 10 mL of 0.1 M NaOH and 5 mL of EPI, and the resulting reaction mixture was incubated at 45°C for 2 h. The membrane was then carefully removed, rinsed with deionized (DI) water, and then immersed in 20 mL of a 1 M diamine solution with the pH adjusted to 11.2 by addition of a small amount of 1 M HCl. The reaction was allowed to progress at 45°C for 12 hours, with the membrane then removed from the solution and rinsed thoroughly in DI water for a minimum of 60 min.

The diamines possess a secondary amine group that is located relatively close to the membrane surface and a primary amine group at the end of the alkyl chain (furthest from the membrane). The location of the terminal amino group was controlled by selection of the alkyl chain length in the original diamine. Membranes were constructed with diamines having between 2 and 10 carbon atoms, corresponding to alkyl chain lengths of approximately 0.15 to 1.35 nm.
**Figure 8.1:** Schematic of reactions used to couple EPI and different diamines to the composite regenerated cellulose membranes

### 8.2.3 Membrane Preparation using Multiple Charged Groups

Membranes with multiple charged groups along the spacer arm length were synthesized using two different reaction schemes. The first approach involved multiple EPI and diamine additions, while the second approach involved coupling of a polyethyleneamine to the membrane. In the first scheme, the membrane was initially treated using the reaction shown in Figure 8.1 to generate a modified membrane with a secondary and primary amine. The terminal primary amine was then reacted with epichlorohydrin (EPI) to form an epoxy group as shown in Figure 8.2. This terminal epoxy was then reacted with a second diamine to extend the overall chain. The resulting membranes had a series of secondary amine groups, with the spacing between the groups determined by the alkyl chain length in the diamine, with a primary amine at the terminal end of the molecule.
In the second approach, the hydroxyl groups on the membrane surface were reacted with EPI as shown in the first step of Figure 8.1. The membrane was then rinsed with DI water and placed in a 1 M solution of a polyethyleneamine at pH 11.2 for 12 hours at 45°C. The number of amine groups and the overall length of the final ligand were determined by the length of the polyethyleneamine used in the reaction. Table 8.1 shows the specific compounds employed in this study. The resulting membranes contained a series of secondary amines separated by two CH2 groups, with a primary amine at the terminal end of the molecule.

**Table 8.1: Chemical structure of polyethyleneamines used to generate CRC membranes with multiple charged groups**

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Chemical Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethylenetriamine</td>
<td>H₂N─NH─NH₂</td>
</tr>
<tr>
<td>Triethylenetetramine</td>
<td>H₂N─NH─NH─NH─NH₂</td>
</tr>
<tr>
<td>Pentaethylenehexamine</td>
<td>H₂N─NH─NH─NH─NH─NH─NH₂</td>
</tr>
<tr>
<td>Polyethyleneimine (Avg. MW – 423)</td>
<td>11 amine groups on average</td>
</tr>
</tbody>
</table>
8.2.4 Protein Filtration

All ultrafiltration experiments were performed with 1 – 2.5 g/L solutions of cytochrome c using a 25 mm diameter Amicon stirred ultrafiltration cell as described in Chapter 3 (Section 3.6). Cytochrome c solutions were prepared by dissolving cytochrome c powder in a Bis-Tris buffered KCl solution. The resulting solution was prefiltred through a 0.22 µm µ-Star nylon filter (8010, Costar Corporation) prior to use. The membrane permeability was then evaluated using a buffered saline solution at the same pH and ionic strength to be used for the protein filtration. The stirred cell was then emptied and refilled with a protein solution. The stirring speed was set at 600 rpm and the filtrate flux was maintained at 25 L/m²/h using a peristaltic pump connected directly to the filtrate exit port. Protein samples were collected from the filtrate exit and the bulk solution as described in Section 3.4.2. The stirred cell was then carefully emptied, rinsed with buffered saline, and refilled with a fresh protein solution at a new ionic strength. After completion of the protein filtration, the membrane hydraulic permeability was re-evaluated.

The protein concentrations in the filtrate and bulk samples were determined spectrophotometrically by evaluating the absorbance at 410 nm, which is the natural absorbance peak for cytochrome c. Actual concentrations were evaluated by comparison of the absorbance with that of known protein standards using the calibration curve shown in Figure 3.5.
8.2.5 Membrane Characterization

The pore size distributions for the charge-modified membranes were determined from dextran sieving measurements, with the dextran concentrations determined by size exclusion chromatography using a TSK-gel G2000 SW silica resin as described in Chapter 3 (Section 3.7.3). The mean, $\bar{r}$, and standard deviation, $\sigma$, in the assumed log normal distribution were determined by minimizing the sum of the squared residuals between the dextran sieving data and model calculations as discussed in Chapter 5 (Section 5.2.4.1). The surface charge characteristics of the charge-modified membranes were evaluated from streaming potential measurements following the procedure described in Chapter 3 (Section 3.2.3). The apparent zeta potential ($\zeta_{\text{app}}$) was evaluated from the slope of the measured voltage as a function of the transmembrane pressure using the Helmholtz-Smoluchowski equation (Hunter, 1981)

$$\zeta_{\text{app}} = \frac{\eta \lambda_0}{\varepsilon_0 \varepsilon_r} \left( \frac{dE_z}{dP} \right)$$

(8.1)

where $\varepsilon_0$ is the permittivity of free space, $\varepsilon_r$ is the relative permittivity (or dielectric constant) of the solvent, $\eta$ is the solution viscosity, and $\lambda_0$ is the solution conductivity.
8.3 Results and Discussion

8.3.1 Effects of Spacer Arm Length

A series of charge-modified membranes with different spacer arm length was generated by activation of the base cellulose membrane using epichlorohydrin (EPI) followed by reaction with different diamines. Figure 8.3 shows a plot of the measured streaming potential ($E_z$) as a function of the applied pressure for 30 kD membranes prepared with diamines having alkyl chain lengths of $n = 2$, 6, and 10. Repeat measurements of slope between $E_z$ and transmembrane pressure with the same membrane were highly reproducible, and in each case the data were highly linear with $r^2$ values greater than 0.98. The small displacement between the data for the different membranes is due to asymmetries in the Ag/AgCl electrodes (Burns and Zydney, 2000). The resulting non-zero intercept had no affect on the data analysis or interpretation. The slope of the streaming potential versus pressure data, which provides a direct measure of the membrane charge, increased slightly with an increase in alkyl chain length from $n = 2$ to 6 and then increased even further for $n = 10$.

The apparent zeta potential for membranes with different spacer arm length were calculated from the streaming potential data using Equation (8.1), with the values summarized in Table 8.3. The apparent zeta potential for the membranes with $n = 4$, 6, and 8 were very similar, with values ranging from 7.5 to 7.9 mV. The apparent zeta potential for the membrane with $n = 10$ was significantly larger ($\zeta_{\text{app}} = 9.5$ mV). This increase in membrane charge is likely in part due to a shift in the $pK_a$
values of the charge groups associated with electrostatic interactions between the two amine groups in each functional ligand (Figure 8.1). Albert and Serjeant (1962) evaluated the pK\(_a\) values and the net charge at pH 7 for diamines with different numbers of alkyl groups, with the results summarized in Table 8.2 (Albert and Serjeant, 1962). pK\(_a\)(1) refers to the protonation of the first amine group (the diamines are symmetric), with pK\(_a\)(2) describing the pH associated with the protonation of the second amine. The two pK\(_a\) values are within 1 pH unit when the amines are separated by 8 or more alkyl groups. However, the pK\(_a\) of the second amine is significantly reduced when the two amines are located in closer proximity. This effect is quite large when n = 2, resulting in a significant reduction in the net charge of the diamine at pH 7. These results cannot be directly applied to the charge-modified membranes examined in this thesis since the membrane modification converts one of the amine groups from a primary to a secondary amine. However, the larger net charge for diamines with greater numbers of alkyl groups (Table 8.2) is consistent with the observed increase in apparent zeta potential of the charge-modified membranes produced using the ligand with n = 10.
Figure 8.3: Streaming potential data for a series of charge modified CRC 30 kD membranes with different spacer arm length. $R^2$ values are all greater than 0.98.

Table 8.2: $pK_a$ values and net charge at pH 7 and pH 5 for diamines with different alkyl chain lengths

<table>
<thead>
<tr>
<th>Spacer Arm Length (n)</th>
<th>$pK_a$ (1)</th>
<th>$pK_a$ (2)</th>
<th>Net Charge at pH 7</th>
<th>Net Charge at pH 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>10.09</td>
<td>7</td>
<td>1.5</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>10.80</td>
<td>9.35</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>10.9</td>
<td>9.9</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>11</td>
<td>10.1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>11.1</td>
<td>10.5</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>
The results for the dextran sieving coefficients for membranes prepared with diamines having alkyl chain length of n = 2, 6, and 8 are shown in Figure 8.4. In each case, the dextran sieving data were obtained at a flux of 25 L/m²/hr, which was chosen to minimize the extent of concentration polarization while still insuring that the sieving coefficient was determined primarily by convection (and not diffusion). The sieving coefficients are plotted as a function of the Stokes-Einstein radius of each dextran, which was evaluated using Equations (5.17) and (5.18). The dextran sieving coefficients decreased slightly when the alkyl chain length of the diamine was increased from n = 2 to 8, which could be due to the greater pore constriction associated with ligands having longer spacer arms. The solid and dashed curves in Figure 8.4 are model calculations for the dextran sieving coefficients developed by integrating over the membrane pore size distribution using Equations (5.2) and (5.3). The best fit values of the mean and standard deviation of the log-normal distribution were determined by minimizing the sum of the squared residuals between the measured and calculated values of the dextran sieving coefficients. The model calculations are in good agreement with the data over the full range of dextran size using the parameter values given in Table 8.3.
Figure 8.4: Actual sieving coefficients for different molecular weight dextrans through CRC membranes having different spacer arm length. The solid curves are model fits to the actual sieving coefficient data using the best fit values of the model parameters shown in Table 8.3.

Table 8.3 summarizes the calculated values of the apparent zeta potential ($\zeta_{\text{app}}$) along with the mean and standard deviation in the pore size distribution for the charge-modified 30 and 100 kD membranes with different spacer arm length.
Table 8.3: Apparent zeta potential and pore size characteristics for charge-modified membranes with different spacer arm length

<table>
<thead>
<tr>
<th>Spacer Arm Length (n)</th>
<th>Native Membrane (kD)</th>
<th>Mean Pore Size, ( \bar{r} ) (nm)</th>
<th>Coeff. of Variation (( \sigma/\bar{r} ))</th>
<th>Apparent Zeta Potential (mV) at pH 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>30</td>
<td>2.9</td>
<td>0.21</td>
<td>6.0 ± 0.1</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>3.1</td>
<td>0.19</td>
<td>7.9 ± 0.4</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>3.1</td>
<td>0.21</td>
<td>7.5 ± 0.3</td>
</tr>
<tr>
<td>8</td>
<td>30</td>
<td>2.9</td>
<td>0.19</td>
<td>7.8 ± 0.3</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>---</td>
<td>---</td>
<td>9.5 ± 0.5</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>5.2</td>
<td>0.31</td>
<td>10.2 ± 0.6</td>
</tr>
<tr>
<td>8</td>
<td>100</td>
<td>5</td>
<td>0.30</td>
<td>10.2 ± 0.9</td>
</tr>
</tbody>
</table>

In contrast to the apparent zeta potential data, the best fit values of the mean pore size of the charge-modified 30 kD membranes were all approximately 3 nm, irrespective of the length of the spacer arm used to generate the charged membrane. Similarly, the coefficient of variation was also independent of the size of the diamine, with values of approximately 0.2 for all of the membranes. Re-analysis of the dextran sieving data with \( \sigma/\bar{r} \) fixed at 0.2 yielded the same conclusion, with the length of the spacer arm having no measurable effect on the best fit values of the mean pore size.

The last 2 rows of Table 8.3 show similar results obtained with the 100 kD membranes using \( n = 4 \) and \( n = 8 \). The mean pore size, standard deviation, and
apparent zeta potential for these two membranes were all essentially identical. The
100 kD membranes had a mean pore size of about 5 nm, and they also appear to have
a somewhat broader pore size distribution than the 30 kD membranes, with a
coefficient of variation around 0.3 compared to only 0.2 for the 30 kD membrane.
This increase in the coefficient of variation may be due differences in the casting
conditions used to generate the different molecular weight cut-off membranes. No
quantitative conclusions could be developed regarding this behavior since the specific
casting conditions are proprietary. The apparent zeta potential of the 100 kD
membrane is larger than that for any of the 30 kD membranes, although this effect is
likely due at least in part to the difference in mean pore size (5 nm versus 3 nm) and
the corresponding reduction in the extent of double-layer overlap in the 100 kD
membrane.

8.3.1.1 Effect of Spacer Arm on Membrane Permeability

Figure 8.5 shows the hydraulic permeability of the native uncharged
membrane and of the charge-modified membranes with different spacer arm lengths.
In each case, the permeability was evaluated with a 1 M KCl solution buffered with
20 mM Bis-Tris at pH 7 to minimize the effects of counter-electroosmosis on the
solvent flow. The flow rate data for all of the membranes were highly linear, with $r^2$
values greater than 0.98. The permeabilities were evaluated directly from the slope of
the flow rate versus pressure data using simple linear regression. The permeabilities
of the charge-modified membranes were all approximately 20% smaller than that of
the base (un-modified) CRC membrane, with no obvious correlation on the length of the alkyl chain. This behavior is completely consistent with the best fit values of the mean pore size determined from the dextran sieving data (Table 8.3), which were also independent of the spacer arm length.

Figure 8.5: Effect of spacer arm length on the hydraulic permeability of charge-modified 30 kD membranes evaluated with 1 M KCl solutions
8.3.1.2 Counter-electroosmosis

As discussed in Chapter 2, pressure driven (convective) flow through a charged membrane generates an electrical (streaming) potential across the membrane due to the unequal partitioning of the positive and negative ions into the pore. This streaming potential generates a flux of counterions opposed to the convective flow, with this counterion flux reducing the overall solvent flow rate compared to that expected for purely pressure-driven. This effect is often referred to as counter-electroosmosis or the electroviscous effect.

Figure 8.6 shows the effect of counter-electroosmosis on the measured permeability of the charge-modified membranes. The results are plotted as the ratio of the permeability at a given ionic strength to that evaluated using the 1 M KCl solution to properly normalize the results for small differences in the initial thickness or pore size of the membranes. The normalized permeability for the membrane made with the 1,2-diaaminoethane (n=2) decreased by 12% as the ionic strength was reduced from 1 to 0.005 M. The reduction in permeability was even greater for the membranes with larger spacer arms, with the permeability of the membrane with n=8 decreasing by 25% over the same range of ionic strength. This behavior is consistent with the larger apparent zeta potential for the membranes with larger spacer arms (Table 8.3). The results for the membranes with n = 4 - 8 were very similar, again consistent with the similar values of the apparent zeta potential. The extent of counter-electroosmosis for the membrane with n = 10 is very similar to that seen with the other membranes, even though this membrane had a significantly larger apparent
zeta potential (Table 8.3). The origin of this apparent discrepancy is unclear, but it may be related to the ability of the fluid to flow through the region between the terminal charge group and the pore surface.

Figure 8.6: Effect of counter-electroosmosis on the hydraulic permeability of the charge-modified membranes with different spacer arms. Data were normalized by the permeability evaluated using a 1 M KCl solution.
8.3.1.3 Protein Ultrafiltration

Protein sieving experiments were performed using 1 – 2.5 g/L solutions of cytochrome c at pH 7 over a range of ionic strength. Figure 8.7 shows the observed sieving coefficients as an explicit function of the solution ionic strength for the charge-modified membranes with different spacer arm lengths. In each case, the membranes were allowed to equilibrate with buffer by flushing at least 50 L/m² through the membrane before switching to a protein solution having the same ionic strength. Thus, the results represent data for 6 different membranes each with a given spacer arm.

![Figure 8.7: Observed sieving coefficients for cytochrome c as a function of solution ionic strength through a series of charge-modified CRC 30 kD membranes possessing different spacer arms](image-url)
The observed sieving coefficients decrease with decreasing ionic strength due to the increase in electrostatic exclusion of the positively-charged protein from the positively-charged membranes. The sieving coefficient for the membrane made with the 1,2-diaaminoethane (n=2) decreased by slightly more than an order of magnitude as the ionic strength was reduced from 1 to 0.005 M. A much greater reduction was seen with the membranes having longer spacer arms, with the sieving coefficients for the membranes with n = 4, 6, and 8 decreasing by approximately two orders of magnitude. This behavior is very consistent with the results for the apparent zeta potential and counter-electroosmosis, both of which showed a significant increase in effective charge as the chain length was increased from 2 to 4. The sieving coefficients for the membrane with n = 10 showed an even greater reduction in cytochrome c transmission, with the sieving coefficient in the 0.005 M solution dropping to 0.001.

Corresponding results for the hydraulic permeability and the cytochrome c transmission for a series of 100 kD membranes having different spacer arms are summarized in Table 8.4. The permeabilities were evaluated with 1 M KCl, i.e., in the absence of any significant counter-electroosmosis, while the observed sieving coefficients were evaluated using a 5 mM ionic strength solution to highlight the effects of electrostatic interactions.
Table 8.4: Permeability and observed sieving coefficients for cytochrome c for charge-modified 100 kD membranes with different spacer arm lengths

<table>
<thead>
<tr>
<th>Spacer Arm Length (n)</th>
<th>Hydraulic Permeability (LMH/Psi)</th>
<th>Observed Sieving Coefficient ($S_o$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>96</td>
<td>0.91 ± 0.04</td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>0.036 ± 0.01</td>
</tr>
<tr>
<td>6</td>
<td>75</td>
<td>0.027 ± 0.008</td>
</tr>
<tr>
<td>8</td>
<td>82</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>10</td>
<td>80</td>
<td>0.024 ± 0.012</td>
</tr>
</tbody>
</table>

The permeabilities of the charge-modified membranes were uniformly smaller than that for the native (unmodified) membrane. This effect was relatively small for the membrane made with 1,4-diaminobutane (n = 4), but increased to approximately 15% for the membranes with n = 6, 8, and 10. This difference may be related to the spacer arm length, although it is possible that this simply reflects the inherent variability in the permeability of the different membrane samples. There was no apparent correlation between the membrane permeability and the spacer arm length for the 100 kD membranes with n > 6, similar to the results obtained with the 30 kD membranes. The observed sieving coefficients for the charge-modified membranes were all approximately equal to 0.03, which is a factor of 30 smaller than that for the
unmodified membrane. The cytochrome c sieving coefficient for the membrane with $n = 4$ was slightly larger than that evaluated for the other charge-modified membranes, which is consistent with the slightly larger permeability for this particular sample.

Figure 8.8 examines the effect of solution pH on the observed sieving coefficient for cytochrome c through 30-kD membranes modified with the 1,2-diaminoethane and 1,4-diaminobutane. In this case, the sieving experiments were performed with 1 g/L cytochrome c solutions at 25 mM ionic strength – the somewhat higher ionic strength for these experiments was chosen to ensure that there was substantial transmission of cytochrome c at pH 5 where the protein has a higher net positive charge than that at pH 7. At pH 7, the membrane with the diaminoethane ligand had a significantly larger sieving coefficient, but this difference largely disappears for the data at pH 5. This difference reflects the large reduction in the cytochrome c sieving coefficient through the membrane with the diaminoethane ligand as the pH is lowered from 7 to 5 while the sieving coefficients for the membrane with the daminobutane ligand are almost independent of solution pH over this pH range.

The strong affect of pH on the sieving characteristics for these different membranes is likely due to the different degrees of protonation of the primary and secondary amine groups in the diamines as discussed previously (Table 8.2). If we assume that the $pK_a$ values of the two amines are unaffected by the attachment to the membrane, then the calculated net charge of the membrane with the diaminoethane
will be significantly smaller than that for the diaminobutane at pH 7 due to the very
different pK$_a$ values of the second amine, while the net charge on the two ligands will
be almost identical at pH 5 (Table 8.2). Although the assumption of no change in pK$_a$
upon attachment to the membrane is not likely to be valid, these calculations do
suggest that the different sieving behavior in Figure 8.8 is due to the different degrees
of protonation of the diamine ligands at the different pH.

Figure 8.8: Effect of pH on cytochrome c sieving through membranes with
different diamine ligands
Figure 8.9 shows a Robeson type analysis (discussed in Chapter 4) for charge-modified membranes with different spacer arms. The solid curve represents the predicted trade-off between the permeability and selectivity for cytochrome c through an uncharged membrane and was developed theoretically using Equations (4.6) – (4.8). Calculations were performed by varying the mean pore size (keeping $\sigma/\bar{r}$ and $\epsilon/\delta_m$ fixed at values of 0.2 and 0.35 $\mu m^{-1}$, respectively), with the permeability and selectivity evaluated over a range of $\bar{r}$. The filled squares represent experimental data for cytochrome c sieving through the charge-modified membranes in the 0.5 M ionic strength solution where electrostatic interactions are negligible, while the open symbols represent data obtained in the 5 mM ionic strength solution. The results at high ionic strength fall along the expected trade-off curve, consistent with the data for BSA and lysozyme presented in Chapter 4 and the data for cytochrome c shown in Chapter 5. In contrast, the results for the charged membranes at low ionic strength fall well above and to the right of the expected upper bound.

The dashed curve in Figure 8.9 represents the theoretical trade-off for a positively charged membrane with a charge density of $5.5\times10^{-3}$ C/m², with this value determined from the apparent zeta potential of the 100-kD membranes made with the diamines. The membrane surface charge density was calculated by solving Equations (5.4) and (5.5) iteratively using the experimentally measured values of $\frac{dE_z}{d\Delta P}$ and the pore-size distribution $n(r_p)$ determined from the dextran sieving data (Table 8.3). The
theoretical values of the permeability and selectivity were evaluated at 5 mM ionic strength by varying the mean pore size while keeping $\sigma/r$ fixed at 0.3 (Table 8.3). The permeability was evaluated using a model that accounts for the effects of counter-electroosmosis (Section 2.3.1.1) while the selectivity was evaluated using a partitioning model including electrostatic interactions (Section 2.3.2). The experimental data for the 100-kD membranes with diamines lie fairly close to the predicted trade-off, similar to the results in Chapter 5 for the membranes generated using a short quaternary amine functionality. The 30-kD membranes with diamines lie well below the predicted trade-off, which could in part be due to the lower apparent zeta potential compared to those of the 100-kD membranes made with the same ligands (Table 8.3).

Also shown in Figure 8.9 are data obtained with charge-modified membranes having quaternary ammonium (strong base) groups produced using the chemistry described in Chapter 3 (and having a very small spacer arm). The results for these membranes fall close to the predicted trade-off curve for the charged membranes, with behavior similar to the results seen using the 100 kD membranes but lying well above the results for the 30 kD membranes. For example, at a hydraulic permeability of 15 LMH/Psi, the selectivity for the membrane with attached quaternary ammonium groups was nearly 3-fold higher than that for the diamine membrane having a spacer arm length of 12 carbon atoms and it was 1500-fold larger than the selectivity of the neutral membrane. The superior ultrafiltration performance obtained with membranes having quaternary ammonium groups is most likely due to their strong
basic nature which provides a higher charge density than membranes made with either primary or secondary amines. This behavior is discussed in more detail later in this chapter in Section 8.3.4. The performance characteristics of the 30 kD membranes with the diamine modification were better with longer spacer arms, with the selectivity increasing from 80 to 2000 as n goes from 2 to 10 with essentially no change in the permeability. This increase in performance is again consistent with the increase in net charge arising from the reduction in the electrostatic interactions between the two amine groups in the diamine (Table 8.2).

Figure 8.9: Permeability-Selectivity analysis for membranes developed using different surface modification chemistries. The solid curve represents the predicted trade-off between the permeability and selectivity for cytochrome c through an uncharged membrane while the dashed curve represents the predicted trade-off for membranes with a charge density of $5.5 \times 10^{-3} \text{ C/m}^2$. 

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8.3.2 Membranes with Multiple Charge Groups

Membranes with multiple charged groups were synthesized using two different approaches: sequential additions of multiple diamines with repeated activation using epichlorohydrin (EPI) or attachment of a single polyethyleneamine following activation with EPI. Figure 8.10 shows the hydraulic permeability and observed sieving coefficients for 100-kD membranes with similar spacer arm length (n=12) but with different number of amine groups on the spacer arm. The spacer arm length, n, includes the carbon atoms on the diamine as well as those on EPI. The membrane with two amine groups was made by coupling 1,10-diaminodecane (n=10) following the activation with EPI, whereas the membrane with four amine groups was prepared by sequential addition of two 1,4 diaminobutane (n=4) ligands along with activation by EPI prior to each addition. Although, the spacer arm lengths for the two membranes were equal, the membrane with four-amine groups had approximately 25% lower permeability than the membrane with two amine groups. This change in permeability may reflect a difference in flexibility and/or orientation of the attached ligands, with the ligand having four amine groups extending further away from the polymer surface and into the pore interior.

In contrast to the relatively small difference in the permeability, the observed sieving coefficient for cytochrome c evaluated in a 5 mM KCl solution through the membrane with four amine groups was more than an order of magnitude smaller than that for the membrane with only two amine groups and was nearly three orders of magnitude smaller than that for the unmodified membrane. This large difference in
protein transmission is a direct result of the increased charge density of the membrane with four amine groups coupled with the greater pore constriction and possibly the different orientation of the charged groups.

Figure 8.10: Hydraulic permeability and observed sieving coefficients for 100-kD membranes with similar spacer arm length (n = 12) but different number of amine groups. Permeabilities were evaluated with 1 M KCl solution and observed sieving coefficients were evaluated at 5 mM ionic strength.
Figure 8.11 shows the Robeson plot for membranes with different numbers of amine groups on spacer arms having 12 carbon atoms. Filled circles denote 100-kD membranes having four amine groups on the spacer arm, while the open triangles denote 30 and 100-kD membranes with two amine groups on the spacer arm. The solid curve represents the predicted trade-off between the permeability and selectivity for cytochrome c through an uncharged membrane and was developed with the coefficient of variation ($\sigma/f$) kept at a fixed value of 0.2. As discussed earlier, the dashed curve represents the theoretical trade-off for a positively charged membrane with a charge density of $5.5 \times 10^{-3}$ C/m$^2$, with this value determined from the apparent zeta potential for the 100-kD membranes made with diamines. While the experimental data for the membranes with two amine groups lie fairly closer to the predicted trade-off accounting for the electrostatic interactions, the membranes with four amine groups lie well above the predicted trade-off. These novel membranes with four amine groups thus provide a much better combination of permeability and selectivity than the membranes generated with two amine groups. For example, the hydraulic permeability of membranes with four amine groups was 3-fold higher than those of 30-kD membranes with two amine groups at an equivalent selectivity of approximately 3000. Moreover, even though these novel membranes were developed with weak base groups (primary and secondary amines), their performance was similar to that for membranes having a quaternary amine functionality.
Figure 8.11: Robeson plot for membranes with different number of amine groups on the spacer arm. The solid curve represents the predicted trade-off between the permeability and selectivity for cytochrome c through an uncharged membrane. The dashed curve represents the theoretical trade-off for a charged membrane with a charge density of $5.5 \times 10^{-3} \text{C/m}^2$.

The behavior of membranes having multiple charged groups on a single ligand was also examined using a series of membranes synthesized by the attachment of different polyethyleneamine compounds (Table 8.1). This eliminated the need for multiple activation steps, significantly simplifying the membrane modification. In
addition, the resulting membranes had a significantly larger number of amine groups per chain length, with each amine separated by only two CH₂ groups.

Figure 8.12 shows the hydraulic permeability and observed sieving coefficients for a series of 30 kD membranes modified with polyethyleneamine compounds possessing different numbers of amine groups on the spacer arm. The data shown is an average of either two or three experiments. The permeability of these membranes evaluated using 1 M KCl was nearly independent of the number of amine groups, with values approximately 25% smaller than that for the unmodified CRC membrane, even though the membranes with more amine groups were generated using significantly longer ligands. In contrast, the cytochrome c transmission decreased dramatically as the number of amine groups was increased. For example, the observed sieving coefficient for the membrane with 3 amine groups was 0.0037 ± 0.0021, which is more than an order of magnitude smaller than the sieving coefficient through the membrane with only 2 amine groups. Increasing the number of amine groups to 6 caused an additional order of magnitude reduction in the sieving coefficient. The protein sieving coefficient was smallest for the membrane made using a polyethyleneimine compound with an average molecular weight of 423 g/mol, corresponding to approximately 11 amine groups, with a value of 0.0003 ± 0.00015, a factor of 2500 smaller than that through the unmodified membrane under identical conditions.
Figure 8.12: Effect of the number of amine groups on the hydraulic permeability and cytochrome c sieving coefficients through 30 kD CRC membranes modified with polyethyleneamines

Results for similar experiments performed with 100-kD membranes are shown in Figure 8.13. The permeability was largely independent of the number of amine groups, similar to the results obtained with the 30 kD membranes. The protein transmission decreased by approximately an order of magnitude when the number of amine groups on the spacer arm was increased from 2 to 6, but then remained nearly constant at a value of approximately 0.01 as the number of amine groups on the spacer arm was increased from 6 to 11. The lack of any significant difference in sieving coefficients for the membranes with 6 and 11 amine groups may reflect the
more branched structure of the polyethyleneamine ligand, although this could also simply be due to differences in the pore size characteristics of the initial membrane samples used in these experiments.

Figure 8.13: Effect of the number of amine groups on hydraulic permeability and observed sieving coefficient of cytochrome c through 100 kD CRC membranes. The amine groups on the spacer arm were varied using different polyethyleneamine compounds as described in the text.
In order to obtain additional insights into the effects of the number of amine groups on the performance characteristics of the charge-modified membranes, streaming potential measurements were used to evaluate the apparent zeta potential of the different membranes, with the results summarized in Table 8.6. Also shown for comparison is the calculated charge of the polyethyleneamine compounds evaluated using the published pK\textsubscript{a} values of the specific amine groups on the polyethyleneamine spacer arm (Table 8.5). pK\textsubscript{a}(1) refers to the protonation of the first amine group, pK\textsubscript{a}(2) describes the pH associated with the protonation of the second amine, and so on and so forth. The pK\textsubscript{a} values for the different amine groups depend on the intramolecular electrostatic interactions between amine groups and the induction effects due to the neighboring alkyl groups. The pK\textsubscript{a} values of the two terminal amine groups on a given polyethyleneamine are within 1 pH unit when 4 or more alkyl groups separate the amines. However, the pK\textsubscript{a} values of the other amine groups on the polyethylene spacer arm are significantly altered depending on the number of amines and alkyl groups.

The apparent zeta potential of both the 30 and 100-kD membranes increased with an increase in the number of amine groups on the polyethyleneamine ligand. For example, $\zeta$\textsubscript{app} for the 30-kD membrane increased from 3.8 mV to 5.1 mV as the number of amine groups was increased from 2 to 3. A further increase in $\zeta$\textsubscript{app} of 1 mV was observed when the number of amine groups was increased from 3 to 6. This behavior was consistent with the increase in the calculated charge on the
polyethyleneamine compounds (evaluated assuming that the pK$_a$ values for the amine groups on the attached ligands were the same as those for the ligands in free solution). There was no change in ζ$_{app}$ when the number of amine groups was increased from 6 to 11, similar to the lack of any significant difference in the sieving coefficients for cytochrome c between these membranes. As mentioned previously, this may be due to the branched structure of polyethyleneamine with 11 amine groups; all of the other ligands examined in Table 8.6 had linear conformations. The branched structure could alter the pK$_a$ values of the amine groups, and thus the net charge on the ligand, and it might also reduce the accessibility of the charged functional groups.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>pK$_a$ (1)</th>
<th>pK$_a$ (2)</th>
<th>pK$_a$ (3)</th>
<th>pK$_a$ (4)</th>
<th>pK$_a$ (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylenediamine (a)</td>
<td>10.09</td>
<td>7</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Diethylenetriamine (b)</td>
<td>9.94</td>
<td>9.23</td>
<td>4.78</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Triethylenetetramine (b)</td>
<td>9.99</td>
<td>9.36</td>
<td>7.01</td>
<td>3.89</td>
<td>_</td>
</tr>
<tr>
<td>Tetraethylenepentamine (b)</td>
<td>9.92</td>
<td>9.08</td>
<td>7.87</td>
<td>4.25</td>
<td>2.65</td>
</tr>
</tbody>
</table>
Table 8.6: Apparent zeta potential and estimated net charge for membranes made using different polyethyleneamine ligands

<table>
<thead>
<tr>
<th>Molecular weight cut off (kD)</th>
<th>Number of amine groups</th>
<th>Apparent zeta potential, $\zeta_{\text{app}}$ (mV)</th>
<th>Net charge on ligand at pH 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>2</td>
<td>3.8 ± 0.3</td>
<td>1.5</td>
</tr>
<tr>
<td>30</td>
<td>3</td>
<td>5.1 ± 0.3</td>
<td>2</td>
</tr>
<tr>
<td>30</td>
<td>6</td>
<td>6.1 ± 0.1</td>
<td>3</td>
</tr>
<tr>
<td>30</td>
<td>11</td>
<td>6.0 ± 0.2</td>
<td>uncertain</td>
</tr>
<tr>
<td>100</td>
<td>2</td>
<td>3.8 ± 0.1</td>
<td>1.5</td>
</tr>
<tr>
<td>100</td>
<td>6</td>
<td>6.4 ± 0.8</td>
<td>3</td>
</tr>
<tr>
<td>100</td>
<td>11</td>
<td>6.4 ± 0.9</td>
<td>uncertain</td>
</tr>
</tbody>
</table>

Although the membranes with multiple amine groups on the charged ligand had very high performance characteristics during their initial use, repeat measurements obtained with membranes that had either been used continuously for several days or were subjected to high salt concentrations occasionally showed a large increase in protein transmission. For example, data obtained with a 30 kD membrane having 6 amine groups showed a sieving coefficient for cytochrome c of only 0.0005 during its first use, but this increased to more than 0.04 after five uses (including exposure to a cytochrome c solution at 0.5 M ionic strength). In contrast, the hydraulic permeability of this membrane increased by less than 5%. It is possible that the large increase in protein transmission, and the corresponding decrease in performance characteristics, was due to the “collapse” of the spacer arms due to
hydrophobic interactions between the alkyl groups. This type of collapse could significantly reduce the accessibility of the charged functional groups, thereby decreasing the extent of electrostatic interactions with the cytochrome c. Although the regeneration of these membranes was not studied in any detail, limited experiments showed that the performance characteristics of at least some of these membranes could be largely restored by treating the membrane with 50% ethanol solution followed by a thorough rinsing in DI water.

8.3.3 Effect of the Location of Charge Groups

Chemical modification using the different diaminos gives a membrane that possesses two charged groups: a secondary amine located near the pore surface and a primary amine located at the far end of the spacer arm. In order to explore the role of this dual charge group structure on membrane performance, several different membranes were synthesized with different spacer arm chemistries as shown in Figure 8.14. Structures (a) and (b) were generated by coupling 1,6-diaminoehexane and 1-amine 6-hexanol, respectively, to the pore surface following an activation with EPI as discussed in Section 8.2.2. Structure (c) was synthesized by sequential additions of diamine and EPI as described in Section 8.2.3. Structure (d) was generated by first coupling EPI and a diamine followed by an additional EPI activation and then 1-amine 6-hexanol. The net result is that membranes (a) and (b) have identical structures except the terminal amine group in membrane (a) is replaced by a hydroxyl in membrane (b). The same is true for membranes (c) and (d),
although in this case both membranes have multiple amine groups.

(a) \[\text{NH} \quad \text{NH}_2\]

(b) \[\text{NH} \quad \text{OH}\]

(c) \[\text{NH} \quad \text{NH} \quad \text{NH} \quad \text{NH}_2\]

(d) \[\text{NH} \quad \text{NH} \quad \text{NH} \quad \text{OH}\]

**Figure 8.14:** Schematic showing different surface modifications of base CRC membranes

Experimental data for the hydraulic permeability and cytochrome c sieving coefficient for the membranes with these different ligand chemistries are summarized in Table 8.7. The first column denotes the spacer arm chemistry, while the second column gives the nominal molecular weight cut-off of the base CRC membrane. The hydraulic permeability data were obtained using both 1 M KCl (with minimal electrostatic interactions) and 5 mM KCl (significant counter-electroosmosis), while the observed sieving coefficients were evaluated using 1 g/L solutions of cytochrome c at pH 7.0 and 5 mM ionic strength.
Table 8.7: Hydraulic permeabilities and observed sieving coefficients for membranes with different ligand chemistries

<table>
<thead>
<tr>
<th>Spacer arm chemistry (Fig. 8.14)</th>
<th>Molecular weight cut off</th>
<th>Permeability (LMH/Psi) 1 M</th>
<th>Permeability (LMH/Psi) 5 mM</th>
<th>Observed sieving coefficient (S_o)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) 30 kD</td>
<td>15.5</td>
<td>11.6</td>
<td>0.0046</td>
<td></td>
</tr>
<tr>
<td>(b) 30 kD</td>
<td>17</td>
<td>13.6</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>(c) 100 kD</td>
<td>45.8</td>
<td>33.0</td>
<td>0.0014</td>
<td></td>
</tr>
<tr>
<td>(d) 100 kD</td>
<td>46.8</td>
<td>39.5</td>
<td>0.038</td>
<td></td>
</tr>
</tbody>
</table>

As expected, the hydraulic permeability evaluated at high ionic strength for membranes (a) and (b) were very similar, as were the permeabilities of membranes (c) and (d), demonstrating that the chemical nature of the terminal group (amine versus hydroxyl) had no effect on the solvent flow at high ionic strength. However, the permeability at 5 mM ionic strength was lower for the membranes having terminal amine groups. This was most likely due to the greater magnitude of the counter-electroosmotic flow associated with the extra charge due to the terminal amine group. The observed sieving coefficient for the 30-kD membrane with the terminal amine group (membrane a) was approximately 3-fold smaller than that for the same membrane having a terminal hydroxyl group due to the additional electrostatic repulsion provided by the extra amine group. On the other hand, the observed sieving coefficient for the 100-kD membrane with the terminal amine group (membrane c) was approximately 30-fold smaller compared to a similar membrane.
with a terminal hydroxyl group (membrane d). The significantly higher protein transmission observed with the 100-kD membrane having a terminal hydroxyl group was most likely due to a combination of the reduced electrostatic repulsion and the larger pore size.

8.3.4 Ligands with Weak versus Strong Base

The effect of the nature of the functional charge group on the ultrafiltration performance was examined by coupling strong bases (quaternary amine groups) and weak bases (primary and secondary amine groups) to the base CRC membrane. Quaternary amine ligands were attached using the proprietary chemistry developed at Millipore as described in Section 3.2.2. Primary and secondary amines were attached using 1,4-diaminobutane as described earlier in Section 8.2.2. The pore size characteristics of the membrane were evaluated from dextran sieving data, with the results summarized in Table 8.8. Also shown are the membrane hydraulic permeability (determined using 1 M KCl) and the apparent membrane zeta potential value (determined from the measured streaming potential in 10 mM KCl solutions). As seen in Table 8.8, the pore size, permeability, and zeta potential of the CRC membranes modified with the strong and weak bases were essentially identical.
Table 8.8: Pore size characteristics, hydraulic permeability, and apparent zeta potential values of 30-kD membranes with ligands having weak and strong bases

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Mean Pore Size, ( \bar{r} ) (nm)</th>
<th>Coefficient of Variation (( \sigma/\bar{r} ))</th>
<th>Hydraulic Permeability (LMH/Psi)</th>
<th>App. Zeta Potential, ( \zeta_{app} ) (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak Base</td>
<td>3.1</td>
<td>0.19</td>
<td>14.4</td>
<td>7.9</td>
</tr>
<tr>
<td>Strong Base</td>
<td>3.3</td>
<td>0.15</td>
<td>16.9</td>
<td>8.3</td>
</tr>
</tbody>
</table>

Figure 8.15 shows the effect of counter-electroosmosis on the permeability of these charge-modified membranes. Data are shown as the normalized permeability, evaluated as the ratio of the permeability at a given ionic strength to that evaluated using the 1 M KCl solution to account for any small differences in the membrane pore size distribution or thickness. The permeability data for ionic strengths greater than 0.01 M were essentially identical, while the membrane with the weak base groups had a lower permeability at the lowest ionic strength. This difference is most likely due to the presence of the two amine groups on the ligand with the weak base compared to only a single amine group on the quaternary amine ligand.
Figure 8.15: Effect of counter-electroosmosis on hydraulic permeability of 30–kD membranes with weak and strong bases

Figure 8.16 shows the observed sieving coefficients of cytochrome c through the membranes with strong and weak base groups. The sieving data were obtained with 1 g/L solutions of cytochrome c at pH 7.0 over a range of ionic strength. Although the effect of ionic strength on protein sieving was similar with the two membranes, protein transmission through the membrane having the weak base groups was consistently higher, with the greatest difference seen at the lowest ionic strength. This behavior is exactly opposite that seen for counter-electroosmosis in which the
electrostatic contributions were greater with the membrane having the weak base
groups on the charged ligand.

The higher protein transmission observed with membranes having weak bases
could be due to the effects of charge regulation. When the positively charged protein
enters into the membrane pore, the local pH inside the pore will tend to increase due
to the exclusion of the positively charged H\(^+\) ions from the space between the protein
and the pore wall. This increase in local pH can reduce the net charge on both the
membrane and the protein resulting in reduced electrostatic repulsion and thus higher
protein transmission. This charge regulation effect is likely to be much more
significant for membranes having weak base groups as the degree of protonation of
the primary and secondary amines is a strong function of solution pH. This type of
charge regulation effect will be much less significant for the membrane having the
quaternary amines since these groups remain completely dissociated over the entire
pH range. Note that this charge regulation effect would probably not be observed
during the counter-electroosmosis experiments since the exclusion of H\(^+\) ions is
directly associated with the partitioning of the positively-charged protein into the
membrane pore.
8.4 Conclusions

The experimental data presented in this Chapter provide the first ever analysis of the effects of different physical and chemical properties of charged ligands on the performance characteristics of ultrafiltration membranes. Charged ultrafiltration membranes were developed to examine the effects of: (1) different spacer arm length, (2) multiple charged groups, and (3) the nature of the charge group (weak versus strong base). In each case data were obtained for both the hydraulic permeability and the sieving coefficient of cytochrome c, which are the critical performance characteristics of these membranes during protein ultrafiltration.

The results for membranes having different spacer arm lengths clearly showed
that the observed sieving coefficients decreased with increasing spacer arm length. For example, the observed sieving coefficient of cytochrome c for the membrane made with the 1,2-diaaminoethane (n=2) was approximately two orders of magnitude higher than a similar membrane made using 1,10-diaminodecane (n=10) with essentially no change in the permeability at high ionic strength. This behavior was very consistent with the results for both the apparent zeta potential and the magnitude of counter-electroosmosis, both of which showed a significant increase as the chain length was increased from 2 to 10.

Novel ultrafiltration membranes having multiple charged groups were developed either by sequential addition of diamines followed by epichlorohydrin activation or by coupling of different polyethyleneamines to the pore surface. These membranes provided a much better combination of permeability and selectivity than those developed using single diamines as the functional ligand. For example, the observed sieving coefficient for cytochrome c through the membrane with four amine groups was more than an order of magnitude smaller than that for the membrane with only two amine groups even though the permeabilities of these membranes were quite similar. This significant increase in protein retention was a direct result of the increased charge density of the membrane associated with the multiple amine groups. The protein transmission decreased monotonically with an increase in the number of amine groups on the functional ligand. For example, the membrane with 6 amine groups provided approximately an order of magnitude higher protein retention than a membrane with 3 amine groups and was more than two orders of magnitude greater.
than a membrane having only 2 amine groups.

The nature of the functional group, in this case either a weak base (primary and secondary amine) or a strong base (a quaternary amine), also had a significant effect on membrane performance. Membranes modified with weak base groups had lower permeability than those with strong base groups at the lowest ionic strength due to the greater magnitude of counter-electroosmosis associated with the presence of the two weak amine groups compared to the single quaternary amine. In contrast, the membranes with weak base groups provided significantly less protein retention than the membrane with quaternary amine groups, particularly at very low ionic strength. This behavior was most likely due to the effects of charge regulation, with the presence of the positively-charged protein causing a shift in the local pH within the pore and thus a reduction in the net charge (degree of protonation) of the primary and secondary amines.

A permeability-selectivity analysis (Robeson Plot) showed that membranes with multiple charge groups along the spacer arm length provided the best combination of permeability and selectivity, with results significantly better than membranes with only 2 amines and slightly better than data for the membranes with quaternary amines. These results provide new insights into the effects of electrostatic interactions, and in particular the nature of the charged ligands, on the performance characteristics of ultrafiltration membranes, and they also suggest a variety of approaches that can be used to develop novel charged membranes with enhanced ultrafiltration behavior.
Chapter 9

CONCLUSIONS AND RECOMMENDATIONS

9.1 Conclusions

This thesis provides the first detailed study of the effects of membrane surface charge density, as well as the specific nature of the charged ligand used for generating the charged membranes, on protein and fluid transport during ultrafiltration. The experimental data were analyzed using a new framework that was developed to specifically account for the inherent trade-offs between membrane permeability and selectivity. The results were also compared with theoretical calculations of electrical interactions in membrane systems; protein transport was described using a model that accounts for the energetics associated with protein partitioning into a charged pore while the fluid flow was described using a model accounting for the affects of counter-electroosmosis. The following sections summarize the key experimental and theoretical findings for the different parts of this thesis.

9.1.1 Permeability-Selectivity Analysis

One of the challenges in selecting appropriate membranes for ultrafiltration, as well as in the development of new ultrafiltration membranes for specific applications, has been the lack of any quantitative framework for comparing the performance characteristics of different membranes. In Chapter 4 of this thesis, an appropriate framework was developed that captures the inherent trade-off between the
membrane selectivity, which is directly related to the extent of protein retention, and the membrane permeability, which is directly related to the filtrate flux. This permeability-selectivity analysis is analogous to the “Robeson plot” that has been used so successfully for the past 15 years to characterize and compare the performance characteristics of different gas separation membranes based on data for model binary systems (e.g., oxygen and nitrogen).

Literature data for model systems of BSA (actually the separation of BSA and water) and lysozyme transmission through a number of different ultrafiltration membranes fell along, or below, an “upper bound” that reflects the current state-of-the-art performance characteristics of commercial ultrafiltration membranes. The shape of this upper bound was shown to be consistent with theoretical predictions of solute and solvent transport through a membrane composed of a parallel array of cylindrical pores having a log-normal pore size distribution with the selectivity determined entirely by size-based interactions. Model calculations demonstrated that even significant reductions in the breadth of the pore size distribution would have relatively little impact on the permeability-selectivity trade-off, indicating that reducing the pore size distribution is unlikely to be an effective approach for the development of new ultrafiltration membranes with significantly improved performance.

In contrast, experimental data for prototype electrically-charged ultrafiltration membranes fell well above and to the right of the upper bound due to the strong electrostatic exclusion of like charged proteins by the charged membrane. These
charged membranes thus provide a significantly better combination of permeability and selectivity than corresponding neutral ultrafiltration membranes, with performance characteristics that are well above that predicted for membranes having a completely uniform pore size. These results clearly demonstrate that the addition of electrical charge can provide a much more effective method for developing new ultrafiltration membranes with significantly enhanced performance.

9.1.2 Effect of Membrane Charge Density on Protein and Fluid Transport

The experimental results presented in Chapter 5 provide the first quantitative analysis of the effects of the membrane surface charge density on both solvent and solute transport in membrane systems. The membrane charge was controlled by varying the extent of reaction with a quaternary amine functionality, with the surface charge density estimated using streaming potential measurements. The charge modification resulted in a reduction in the membrane hydraulic permeability due to two separate effects: (1) steric constriction of the pores associated with the finite size of the functional charge groups and (2) counter-electroosmosis, also known as the electroviscous effect, associated with the induced streaming potential. For example, the hydraulic permeability of an electrically-charged membrane with apparent zeta potential of 6.6 mV at 10 mM ionic strength was approximately 40% smaller than that of the unmodified membrane, with 15% of the reduction arising from pore constriction. The experimental results for counter-electroosmosis were in good qualitative agreement with model calculations accounting for the electrical stresses in
the fluid arising from the induced streaming potential, although there were significant quantitative differences, particularly at very low ionic strength where the affects of charge regulation and surface conduction may become important.

The transmission of a positively-charged protein (cytochrome c) decreased significantly with increasing positive charge on membrane due to the strong electrostatic repulsion between the protein and membrane. This effect was quite dramatic with relatively small changes in the membrane charge having a significant affect on the protein sieving coefficient at low ionic strength. The sieving data were in good agreement with theoretical calculations based on available models for the partitioning of a charged sphere into a charged cylindrical pore accounting for the presence of a log-normal pore size distribution. Experiments were designed to directly (and independently) measure all of the key model parameters, thus the model calculations provide clear evidence that the sieving behavior was primarily governed by the affects of electrostatic interactions on protein partitioning into the charged pores. There were some significant deviations between the model and data at the lowest ionic strength (below 10 mM), which was likely due to the breakdown in the assumptions of a constant surface charge density and the use of the linearized form of the Poisson-Boltzmann equation.

Membranes made with different surface charge densities show a distinct trade-off between the increase in protein retention and the reduction in hydraulic permeability, both of which are directly associated with the electrostatic interactions. These results suggest that there will typically be an optimal membrane surface charge
for different bioprocessing applications; membranes with very high charge densities will have strong retention but at unacceptably low permeabilities while membranes with very low charge density will provide higher permeabilities but with unacceptable protein retention.

9.1.3 Effect of Membrane Charge Density on Protein Separations

Several recent studies have demonstrated the importance of solution conditions like pH and ionic strength on the separation of proteins using high performance tangential flow filtration. The data presented in Chapter 6 provide the first quantitative results on the effects of membrane charge density on the design of an effective membrane process for the purification of a model protein mixture, the separation of myoglobin and lysozyme.

The selectivity between the two proteins was a strong function of membrane charge and solution ionic strength. Selectivities above 100-fold could be obtained with moderately charged membranes by operating at very low ionic strength, but these solution conditions may not be compatible with processing constraints in the biotechnology industry due to concerns about protein aggregation and precipitation. In contrast, data obtained with more highly charged membranes provided significant selectivity even at moderate ionic strengths. For example, the selectivity in a 20 mM ionic strength solution was 40 times larger using a membrane with an apparent zeta potential of 8.9 mV compared to that obtained with a membrane having an apparent zeta potential of only 1.5 mV. These results clearly demonstrate the feasibility of
obtaining high resolution membrane separations at moderate ionic strengths, conditions that are much more amenable to large scale commercial processes, by using highly charged membranes to compensate for the increased electrostatic shielding at higher salt concentrations.

The performance characteristics of the different charged membranes were analyzed using process optimization diagrams, which provide a framework to predict the product yield, purification factor, and required number of diavolumes based on the measured values of the selectivity and mass throughput. The results clearly demonstrate the importance of the solution ionic strength and membrane charge on the overall separation characteristics, with the membrane charge density providing an additional degree of freedom to optimize the system performance for given design criteria.

9.1.4 Effect of Physical and Chemical Properties of Functional Ligand

Almost all of the previous work with charged ultrafiltration membranes used simple quaternary amine or sulfonic acid moieties to generate the surface charge. The experimental results presented in Chapter 8 provide the first reported study of the effects of different physical and chemical properties of the charged ligands on the performance characteristics of the resulting ultrafiltration membranes. Charged ultrafiltration membranes were developed to examine the effects of: (1) different spacer arm length, (2) the presence of multiple charge groups, and (3) the nature of the charge group (weak versus strong base) on fluid and protein transport during
ultrafiltration.

The results for membranes having different spacer arm length clearly showed that protein transmission decreased as the spacer arm length increased, which is likely due to both the larger size of the ligand and the greater degree of protonation of the two amine groups on each ligand as the distance between the amine groups increases. The reduction in protein transmission was also consistent with results for the apparent zeta potential and counter-electroosmosis, both of which were greater for membranes with longer spacer arms. Novel ultrafiltration membranes having multiple charge groups along the spacer arm provided a much better combination of permeability and selectivity than a variety of other charged membranes developed in this thesis. This was due to the significant increase in protein retention associated with the greater electrostatic exclusion with these very highly charged membranes.

The nature of the functional group, in this case either a weak base (primary or secondary amine) or a strong base (a quaternary amine), also had a significant effect on membrane performance. Membranes with weak base groups provided significantly less protein retention than membranes modified with quaternary amine groups, particularly at very low ionic strength. This behavior was most likely due to the effects of charge regulation, with the partitioning of the positively-charged protein altering the local pH within the pore leading to a reduction in the net charge of the weak base. This effect was not seen in measurements of the apparent zeta potential or counter-electroosmosis, suggesting that the different behavior of the strong and weak bases is directly linked to differences in protein partitioning into the membrane.
These results provide new insights into the effects of electrostatic interactions, and in particular the nature of the charged ligands, on the performance characteristics of ultrafiltration membranes. These studies also suggest a variety of approaches that can be used in the future to develop novel charged membranes with enhanced ultrafiltration behavior.

9.1.5 Industrial Implications

Although ultrafiltration is used widely in the biotechnology and food processing industries, its application has generally been limited to low resolution separations between species with very different size like those involved in buffer exchange and protein concentration. Limited ultrafiltration experiments were performed in Chapter 7 using high performance tangential flow filtration (HPTFF) for the purification of a monoclonal antibody from a commercially relevant feedstock containing host cell proteins as impurities. These experiments were performed at Genentech. The highest selectivity of 600 was obtained using a positively-charged composite regenerated cellulose membrane at a conductivity of 0.6 mS/cm and a filtrate flux of 30 L/m²/hr. However, it was possible to obtain 100-fold selectivities even at moderate solution conductivities (1.4 mS/cm) using a filtrate flux of 50 L/m²/hr, conditions which are much more practical for large-scale bioprocessing applications.

The monoclonal antibody product and the host cell proteins were separated using a diafiltration process that allowed host cell proteins to be washed into the
filtrate while retaining the monoclonal antibody in the retentate solution. The host cell protein concentration in the feedstock was reduced to extremely low levels (2 ppm) while obtaining approximately 96% product yield. These results clearly demonstrate the feasibility of using HPTFF to significantly reduce host cell protein levels in the purification of a monoclonal antibody from commercially-relevant feedstocks.

9.2 Recommendations

The results presented in this thesis not only provide significant fundamental insights into the effects of electrostatic interactions on protein and fluid transport during ultrafiltration, they also suggest a number of novel ways to develop next generation charged ultrafiltration membranes. However, there are a number of important areas that could benefit from additional theoretical and experimental investigation.

The permeability-selectivity analysis presented in this thesis was focused on the behavior of ultrafiltration membranes for protein concentration or buffer exchange using the model proteins BSA and lysozyme, both of which have been examined extensively in the literature. It would be very desirable to develop similar plots for higher molecular weight recombinant proteins used in the biotechnology industry, e.g., monoclonal antibodies, due to the growing clinical and commercial importance of this class of proteins. In addition to providing information into the behavior of ultrafiltration membranes over a broader range of nominal molecular weight cut-offs,
This analysis might also provide insights into possible differences in the underlying pore size distributions of ultrafiltration membranes with small and large pore size.

It would also be worthwhile to develop similar “Robeson plots” for ultrafiltration membranes designed for use in high resolution protein separations. In this case the selectivity (or separation factor) would be defined by the ratio of the sieving coefficients for the two proteins of interest (instead of just the reciprocal of the sieving coefficient of the single protein) and the membrane permeability would be replaced by the mass throughput (the product of the filtrate flux and the difference in the sieving coefficients of the two proteins). The best membranes would have a combination of high selectivity and high mass throughput, lying in the upper right hand corner of the trade-off plot. This type of trade-off curve could also be used to examine the affects of the membrane pore size distribution and surface charge on high resolution protein separations, thereby providing a better understanding of the importance of these two underlying membrane properties on the separation characteristics.

Most of the experimental studies performed in this thesis used a simple stirred ultrafiltration cell, with the data obtained at relatively low filtration velocities to minimize the affects of concentration polarization. Although these conditions were very attractive for obtaining fundamental insights into the affects of membrane charge on protein retention and membrane permeability, it is difficult to directly extrapolate these results to commercial devices which are operated using tangential flow filtration and at significantly higher filtrate flux. Concentration polarization effects become
very significant in these devices, influencing both the protein retention and the filtrate flux. Future experimental studies should be performed using linearly scalable tangential flow filtration modules so that the results can be more directly extrapolated to predict the behavior of large-scale commercial membrane systems. These studies should also examine the possible affects of electrostatic interactions on bulk mass transfer phenomena, with the electrostatic repulsion between the like charged protein and membrane potentially enhancing the rate of protein transport away from the membrane and back into the bulk solution.

The theoretical analyses of fluid and protein transport through charged ultrafiltration membranes employed in this thesis implicitly assumed that the membrane had a constant surface charge density or constant surface potential. However, the actual surface charge density / potential will vary with the solution conditions and may also be influenced by the presence of the protein due to the affects of charge regulation. Theoretical calculations using a variable surface charge / potential boundary condition that accounts for the affects of charge regulation would provide additional insights into the fundamental factors governing the behavior of these membrane systems. This type of theoretical analysis could be based on the framework developed by Pujar and Zydney (1996), which requires a much more extensive characterization of the membrane charge characteristics over a broad range of solution conditions than provided by the experiments performed in this thesis.

The results in Chapter 8 clearly demonstrate the potential of developing a range of novel ultrafiltration membranes using different functional ligands containing
different numbers of charge groups. However, the behavior of these membranes was examined only over a fairly limited range of solution conditions. For example, the surface charge characteristics of the membranes with multiple primary and second amine groups will be a strong function of solution pH, a phenomenon that was not studied in this thesis.

The results obtained with novel ultrafiltration membranes having multiple charge groups along the functional ligand indicated that these membranes could provide a significantly better combination of permeability and selectivity than membranes having only one or two primary or secondary amines. Future studies are needed to fully identify the potential of these membranes for both protein ultrafiltration and high performance tangential flow filtration. This work should also include the development of membranes in which the multiple weakly basic groups (the primary and secondary amines) are replaced with multiple strongly basic groups (quaternary amines). More detailed experimental and theoretical studies are needed to identify the role of fluid flow through the functional ligands (between the surface of the polymer and the location of the outer charge group) on the overall performance characteristics of these charged structures. This additional fluid flow would clearly alter the membrane permeability and the extent of counter-electroosmosis, and it may also influence the rate of transport of both charged and neutral proteins.

All of the spacer arms used in this thesis were hydrophobic in nature due to the presence of multiple alkyl groups between the secondary amines; these hydrophobic interactions are potentially important in determining protein partitioning
into the pores, the extent of membrane fouling, and the long-term stability of the membrane structures. Limited experimental data indicated that the ligand structure could “collapse” within the pores, probably due to hydrophobic interactions between the alkyl groups on the spacer arm at high salt concentrations. These phenomena should be studied in significantly more detail, with a series of experiments designed to specifically examine the long-term stability of the membranes during operation, cleaning, and storage. It would also be very useful to develop a class of novel ultrafiltration membranes in which the spacer arms are more hydrophilic in nature, e.g., through the incorporation of hydroxyl groups or ethylene glycol moieties along the ligand backbone. These membranes might have greater long-term stability, and they may also have attractive separation characteristics due to the elimination of long-range hydrophobic interactions between the protein and the pore surface.

The data presented in Chapter 8 clearly indicate that the nature of the charged functional group has a strong affect on both fluid flow and protein transport during ultrafiltration. It would be interesting to extend these studies using ligands with much more controlled charge groups so that one could more accurately identify the different behavior of membranes with primary, secondary, tertiary, and quaternary amines. In addition, one could also alter the nature of the ligands attached to the nitrogen group, changing both the hydrophilicity as well as the size of any attached groups. The resulting structures might have unique properties for particular applications. This work should also be extended to membranes having negatively-charged surfaces, in this case using weakly acidic (carboxyl) and strongly acidic (sulfonic acid) functional
groups. These negatively-charged membranes would be useful for ultrafiltration applications in which the proteins of interest have relatively low isoelectric proteins and are thus negatively-charged under typical processing conditions.
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Appendix

COMPUTER CALCULATIONS

1 Evaluation of Protein Sieving Coefficient

As discussed in Chapter 2, the actual sieving coefficient for protein is dependent on both steric and electrostatic interactions between the protein and membrane pores. This section presents the Mathematica notebook (version 5.2) used to evaluate the protein sieving coefficients used in this study. The electrostatic energy of interaction for a protein partitioning into a charged pore was evaluated using the model of Smith and Deen as outlined in Section 2.3.2.

mul = 0;

Do[
  rs = 18; (*Cytochrome C radius, Å*)
  λ = rs / r;
  F = 96500; (*Faraday’s Constant*)
  z₁ = 1; (*Ion Charge*)
  z₂ = -1; (*Ion Charge*)
  C₁ = 5 * 10⁻³⁰; (*Ion Concentration, M/Å³*)
  C₂ = 5 * 10⁻³⁰; (*Ion Concentration, M/Å³*)
  U₁ = 7.6235 * 10²; (*Ion Mobility, C−Å/N−s*)
  U₂ = 7.913 * 10²; (*Ion Mobility, C−Å/N−s*)
]
\( \sigma_* = 22; \quad (\text{Standard Deviation}) \)
\( \epsilon = 0.3; \quad (\text{Membrane Porosity}) \)
\( q_p = 5.5 \times 10^{-23}; \quad (\text{Membrane Charge Density, C/Å}^2) \)
\( q_s = 2.93 \times 10^{-22}; \quad (\text{Protein Charge Density, C/Å}^2) \)
\( \delta_m = 8500; \quad (\text{Membrane Skin Thickness, Å}) \)
\( \eta = 0.001 \times 10^{-20}; \quad (\text{Viscosity, N·s/Å}) \)
\( R = 8.314 \times 10^{10}; \quad (\text{Gas Constant, N·Å/M·K}) \)
\( T = 298; \quad (\text{Temperature, K}) \)
\( \epsilon_0 = 8.854 \times 10^{-32}; \quad (\text{Permittivity, C}^2/\text{N·Å}^2) \)
\( \epsilon_r = 80; \quad (\text{Boltzmann Constant, N·Å·K}) \)
\( k = 1.3807 \times 10^{-13}; \quad (\text{Coefficient of Variation}) \)

\( a_1 = -73/60; \)
\( a_2 = 77293/50400; \)
\( a_3 = -22.5083; \)
\( a_4 = -5.6117; \)
\( a_5 = -0.3363; \)
\( a_6 = -1.216; \)
\( a_7 = 1.647; \)
\( b_1 = 7/60; \)
\( b_2 = -2277/50400; \)
\( b_3 = 4.0180; \)
\( b_4 = -3.9788; \)
\( b_5 = -1.9215; \)
\( b_6 = 4.392; \)
\( b_7 = 5.006; \)

\[ \kappa = \left( \frac{F^2 \cdot (\sum_{i=1}^{2} z_i^2 \cdot C_i)}{\epsilon_0 \cdot \epsilon_r \cdot R \cdot T} \right)^{0.5}; \quad (\text{Inverse of Debye Length}) \]

\[ \Lambda_0 = F \sum_{i=1}^{2} z_i^2 \cdot C_i \cdot U_i; \quad (\text{Bulk Solution Conductivity}) \]

\( \text{Ion} = 0.5 \times 10^{27} \sum_{i=1}^{2} z_i^2 \cdot C_i; \quad (\text{Ionic Strength}) \)
Effective Conductivity Inside the Pore

\[ K_e = \frac{9}{4} \pi^2 (2 \cdot 0.5) (1 - \lambda)^{-2.5} \left( 1 + \sum_{k=1}^{2} b_k (1 - \lambda)^k \right) + \sum_{k=3}^{7} (b_k) (\lambda)^{-k} ; \]

\[ K_t = \frac{9}{4} \pi^2 (2 \cdot 0.5) (1 - \lambda)^{-2.5} \left( 1 + \sum_{k=1}^{2} a_k (1 - \lambda)^k \right) + \sum_{k=3}^{7} (a_k) (\lambda)^{-k} ; \]

\[ a[r_] = \left\{ \frac{\pi \cdot r^2}{r \cdot (2 \cdot \pi)^{0.5}} (\log[1 + (z)^2])^{-0.5} \cdot \exp \left\{ \frac{-\left( \log \left[ \frac{\nu}{z^2} \right] \right)^2}{2 \cdot \log[1 + (z)^2]} \right\} \right\} ; \]

(*Log-normal Pore Size Distribution*)

\[ b[r_] = \left\{ \Lambda_0 \left\{ 1 + \frac{q_p \cdot F}{\epsilon \cdot e \cdot \kappa \cdot \rho \cdot T \left( \frac{2}{\kappa \cdot r} - \frac{1}{(\text{Bessel}[1, \kappa \cdot r])^2} \right) \right\} \cdot \frac{2 \cdot \sum_{i=1}^{2} \zeta_i}{\sum_{i=1}^{2} \zeta_i \cdot \xi_i \cdot \zeta_i} \right\} + \]

\[ \left\{ \frac{q_p^2}{\eta} \left( 1 - \frac{(\text{Bessel}[0, \kappa \cdot r]) \cdot (\text{Bessel}[2, \kappa \cdot r])}{(\text{Bessel}[1, \kappa \cdot r])^2} \right) \right\} ; \]

(*Effective Conductivity Inside the Pore*)

\[ c[r_] = \frac{\epsilon \cdot r^2}{8 \cdot \delta_m} - \frac{\epsilon \cdot q_p^2 \cdot (\text{Bessel}[2, \kappa \cdot r])^2}{\delta_m \cdot \kappa^2 \cdot \eta \cdot (\text{Bessel}[1, \kappa \cdot r])^2 \cdot b[r] ; \]

\[ \delta[r_] = \frac{dP \cdot r^2}{8 \cdot \delta_m \cdot \eta} - \frac{dP \cdot q_p^2 \cdot (\text{Bessel}[2, \kappa \cdot r])^2}{\delta_m \cdot \kappa^2 \cdot \eta^2 \cdot (\text{Bessel}[1, \kappa \cdot r])^2 \cdot b[r] ; \quad (*\text{Flux]*) \]

\[ c[r_] = \text{NIntegrate} \left\{ \frac{\text{Bessel}[1, ((\kappa \cdot r)^2 + x^2)^{0.5}]}{\text{Bessel}[1, ((\kappa \cdot r)^2 + x^2)^{0.5}]}, \{x, 0, 100\} \right\} ; \]

\[ e[r_] = (1 + \kappa \cdot r \cdot \lambda) \cdot e^{-\kappa \cdot r \cdot \lambda} - (1 - \kappa \cdot r \cdot \lambda) \cdot e^{\kappa \cdot r \cdot \lambda} ; \]

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\begin{align*}
\sigma_s &= \frac{F \cdot r \cdot q_s}{\varepsilon_0 \cdot \varepsilon_r \cdot R \cdot T}; \\
\sigma_p &= \frac{F \cdot r \cdot q_p}{\varepsilon_0 \cdot \varepsilon_r \cdot R \cdot T}; \\
A_s &= \frac{4 \cdot \pi \cdot \lambda^4 \cdot \varepsilon(-\varepsilon \cdot \varepsilon_r \cdot \lambda)}{(1 + \varepsilon_r \cdot \varepsilon_r \cdot \lambda)}; \\
A_p &= \frac{4 \cdot \pi^2 \cdot \lambda^2 \cdot \text{Bessel}[1, \varepsilon_r \cdot \lambda]}{(\varepsilon_r \cdot \lambda)^2 \cdot \text{Bessel}[1, \varepsilon_r \cdot \lambda]^2}; \\
A_{sp} &= \frac{\pi^2 \cdot \varepsilon[r]}{(\varepsilon_r \cdot \lambda)^2 \cdot \text{Bessel}[1, \varepsilon_r \cdot \lambda]^2}; \\
A_p &= \frac{\pi^2 \cdot \varepsilon[r]}{(\varepsilon_r \cdot \lambda)^2 \cdot \text{Bessel}[1, \varepsilon_r \cdot \lambda]^2}; \\
f[r] &= \frac{A_s \cdot \sigma_s^2 + A_{sp} \cdot \sigma_s \cdot \sigma_p + A_p \cdot \sigma_p^2}{(\pi \cdot \varepsilon_r \cdot \lambda)(1 + \varepsilon_r \cdot \varepsilon_r \cdot \lambda) \cdot \varepsilon(-\varepsilon \cdot \varepsilon_r \cdot \lambda) - d[r] \cdot c[r]}; \\
g[r] &= \left(\varepsilon[r] \cdot \varepsilon_0 \cdot \varepsilon_r \left(\frac{R \cdot T}{F}\right)^2 \cdot f[r]\right); \\
h[r] &= \left(\varepsilon[r] \cdot \varepsilon_0 \cdot \varepsilon_r \left(\frac{R \cdot T}{F}\right)^2 \cdot f[r]\right); \\
j[r] &= \frac{K_s \cdot \exp\left(-\frac{\varepsilon[r]}{k \cdot T}\right) \cdot \int_0^{1-\lambda} i[r] \cdot r_p \, d \cdot r_p}{2 \cdot K_t \cdot \int_0^{1} i[r] \cdot r_p \, d \cdot r_p}; \\
\alpha[r] &= \frac{\varepsilon \cdot q_p \cdot \text{Bessel}[2, \varepsilon_r \cdot \lambda]}{c[r] \cdot \delta_m \cdot (b[r]) \cdot \kappa \cdot \text{Bessel}[1, \varepsilon_r \cdot \lambda]}; \\
s[r] &= \left\{i[r] \cdot (j[r]) \cdot \left(1 + \left(\frac{h[r]}{j[r]}\right) \cdot \mu_c \cdot \alpha[r]\right)\right\};
\end{align*}
\texttt{mul1 = s[r] \ast a[r];}

\texttt{mul = mul1 + mul, \{r, 18.001, 500\};}

\texttt{Seive = \left( \frac{mul}{N\text{Integrate}[i[r] \ast a[r]]} \right);}

\texttt{Print["The ionic strength is ", Ion "M"]};

\texttt{Print["The Asymptotic Seiving coefficient is ", Seive];}
2 Evaluation of Hydraulic Permeability

This section presents the Mathematica notebook (version 5.2) used for evaluation of the fluid transport through charged membranes. The hydraulic permeability was evaluated using a model accounting for counter-electroosmosis effect as described in Section 2.3.1.1.

Table 1 = Table[

\[ F = 96500; \text{(*Faraday's Constant*)} \]
\[ z_1 = 1; \text{(*Ion Charge*)} \]
\[ z_2 = -1; \text{(*Ion Charge*)} \]
\[ C_1 = 5 \times 10^{-30}; \text{(*Ion Concentration, M/Å^3*)} \]
\[ C_2 = 5 \times 10^{-30}; \text{(*Ion Concentration, M/Å^3*)} \]
\[ U_1 = 7.6235 \times 10^2; \text{(*Ion Mobility, C−Å/N−s*)} \]
\[ U_2 = 7.913 \times 10^2; \text{(*Ion Mobility, C−Å/N−s*)} \]
\[ \epsilon = 0.3; \text{(*Membrane Porosity*)} \]
\[ q_p = 5.5 \times 10^{-23}; \text{(*Membrane Charge Density, C/Å^2*)} \]
\[ \delta_m = 8500; \text{(*Membrane Skin Thickness, Å*)} \]
\[ \eta = 0.001 \times 10^{-20}; \text{(*Viscosity, N−s/Å*)} \]
\[ R = 8.314 \times 10^{10}; \text{(*Gas Constant, N−Å/M−K*)} \]
\[ T = 298; \text{(*Temperature, K*)} \]
\[ \epsilon_0 = 8.854 \times 10^{-32}; \text{(*Permittivity, C^2/N−Å^2*)} \]
\[ \epsilon_r = 80; \text{(*Coefficient of Variation*)} \]
\[ z = 0.3; \text{(*Coefficient of Variation*)} \]
\[ \kappa = \left( \frac{F^2 \sum_{i=1}^{2} z_i^2 \cdot C_i}{\varepsilon_0 \cdot \varepsilon_r \cdot R \cdot T} \right)^{0.5}; \quad \text{(*Inverse of Debye Length*)} \]

\[ \Lambda_0 = \frac{F \cdot \sum_{i=1}^{2} z_i^2 \cdot C_i \cdot U_i}{\varepsilon_0 \cdot \varepsilon_r \cdot R \cdot T \cdot (2 \cdot \pi)^{0.5}}; \quad \text{(*Bulk Solution Conductivity*)} \]

\[ \text{Ion} = 0.5 \cdot 10^{-27} \cdot \sum_{i=1}^{2} z_i^2 \cdot C_i; \quad \text{(* Ionic Strength*)} \]

\[ a[r_] = \frac{\pi \cdot r^2}{r \cdot (2 \cdot \pi)^{0.5}} \cdot \left( \log(1 + (z)^2) \right)^{-0.5} \cdot \exp\left[ -\left( \log\left( \frac{r^2}{\pi} \right)^{0.5} \right)^2 \right]; \quad \text{(*Log–Normal Pore size Distribution*)} \]

\[ b[r_] = \left( \Lambda_0 \cdot \left(1 + \frac{q_p \cdot F}{\varepsilon_0 \cdot \varepsilon_r \cdot R \cdot T \cdot (2 \cdot \pi)^{0.5}} \cdot \frac{2}{\kappa \cdot r} - \frac{1}{(\text{BesselI}[1, r \cdot \kappa])^2} \cdot \frac{\sum_{i=1}^{2} z_i^3 \cdot U_i \cdot C_i}{\sum_{i=1}^{2} z_i^2 \cdot U_i \cdot C_i} \right) \right) + \]

\[ \frac{q_p^2}{\eta} \cdot \left(1 - \frac{\text{BesselI}[0, r \cdot \kappa]}{(\text{BesselI}[1, r \cdot \kappa])^2} \cdot \frac{\text{BesselI}[2, r \cdot \kappa]}{(\text{BesselI}[1, r \cdot \kappa])^2} \right); \quad \text{(*Effective Conductivity inside the Pore*)} \]

\[ c[r_] = \frac{\varepsilon \cdot r^2}{8 \cdot \delta_m} - \frac{\varepsilon \cdot q_p^2 \cdot (\text{BesselI}[2, r \cdot \kappa])^2}{\delta_m \cdot \kappa^2 \cdot \eta \cdot (\text{BesselI}[1, r \cdot \kappa])^2 \cdot (b[r])}; \quad \text{(*Permeability*)} \]

\[ i[r_] = -\frac{dP \cdot r^2}{8 \cdot \delta_m \cdot \eta} - \frac{dP \cdot q_p^2 \cdot (\text{BesselI}[2, r \cdot \kappa])^2}{\delta_m \cdot \kappa^2 \cdot \eta^2 \cdot (\text{BesselI}[1, r \cdot \kappa])^2 \cdot (b[r])}; \quad \text{(*Flux*)} \]

\[ \left( \frac{\text{NIntegrate}[c[r] \cdot a[r], \{r, 0.001, 200\}]}{\text{NIntegrate}[a[r], \{r, 0.001, 200\}]} \right) \cdot 10^{-10} \]

\[ \text{NIntegrate}[i[r] \cdot a[r], \{r, 0.001, 200\}]; \quad \{\sigma_*, 8, 24, 1\}; \]

TableForm[Table1]

Export["permcharged.csv", Table1]

Print["The ionic strength is ", Ion "M"];
VITA

Amit Mehta

Education

2002 to 2006 PENNSYLVANIA STATE UNIVERSITY University Park, PA
• Ph.D., Chemical Engineering GPA: 3.94/4.00
• Focus on protein separations using charged membranes

1997 to 2001 BANGALORE UNIVERSITY Bangalore, India
• B.E., Chemical Engineering Aggregate - 82.04 %
• Gold Medalist in Chemical Engineering, 4th Rank in the University

Honors
• Winner of “North American Membrane Society”, 2004 Travel Award
• Awarded additional fellowship from department endowment funds in recognition of undergraduate accomplishments
• Awarded “Gayatri Memorial Rolling Shield” for the best undergraduate student from 1997 – 2001
• Winner of National Level Technical Quiz organized by IICHE – Students Chapter, Tumkur, 2001

Employment Experience

June 06 – Present Engineer II, Genentech Inc. South San Francisco, CA
• Process and technology development for biotherapeutics

Aug 02 – May 06 Graduate Student, Penn State University University Park, PA
• Fundamental studies and development of charged membranes for protein purification

June 05 – Jan 06 Co-op Engineer, Genentech Inc. South San Francisco, CA
• Development of charged membranes for biotherapeutics purification

Key Publications: