A study of charge and hydrodynamic effects in protein ultrafiltration

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A STUDY OF CHARGE AND HYDRODYNAMIC EFFECTS IN PROTEIN ULTRAFILTRATION

by

Nils O. Becht

A Doctoral Thesis submitted in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy of Loughborough University

Advanced Separations Technologies Group
Department of Chemical Engineering

May 2008

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Abstract

This thesis is concerned with the study of different effects in protein ultrafiltration including device configuration, solution chemistry and membrane charge. In the recent and more established literature, membrane fouling remains a challenging problem that limits the wider application of ultrafiltration. Thus, investigations which can aid understanding and potentially reduce membrane fouling are of particular interest and in this study the problem has been addressed from several different angles.

Polyethersulfone membranes were studied at varying pH and two ionic strengths using bovine serum albumin and lysozyme as the model proteins. The study was conducted both in a stirred cell and a crossflow configuration in order to evaluate the influence of different system hydrodynamics on filtration. This work was further substantiated through the application of filtration models. An attempt was also made to modify the membrane surface by low-temperature plasma modification with the intention to preferentially alter the characteristics of the membrane surface. Both unmodified and plasma-modified polyethersulfone membranes were characterised using a range of analytical methods including flux data, streaming potential, contact angle and MWCO measurements to aid results interpretation.

The research showed that MWCO data quoted by manufacturers is mostly greater than that obtained during laboratory studies. The MWCO technique was also used to highlight differences between the unmodified and plasma-modified membranes demonstrating that the modification resulted in a membrane with tighter pores in the lower molecular weight region. Concentration polarisation effects were found to be reduced as a result of the plasma-modification. The study of protein filtration at different pH and ionic strengths demonstrated that ionic strength effects were more pronounced than pH effects. It was also shown that changes in the ionic strength can be used to alter the degree of protein rejection. For the given system concentration polarisation was found to be higher during crossflow filtration compared to stirred cell filtration. The thesis adds to existing knowledge in the area of ultrafiltration, emphasizing the importance of device configuration, solution chemistry as well as the potential of charged membranes.

Keywords: ultrafiltration, ionic strength, membrane modification, stirred cell, crossflow
Acknowledgements

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Abbreviations

AA       Acrylic acid
AAG      Acrylamidoglycolic acid monohydrate
ALY      Allylamine
BSA      Bovine serum albumin
CF       Crossflow
Cys      Cysteine
Da       Dalton
DACH     Diaminocyclohexane
DAD      Diode array detector
DLVO     Deryaguin-Landau-Verwey-Overbeek
EDL      Electrical double layer
FEGSEM   Field emission scanning electron microscopy
FR       Flux reduction
HMDSO    Hexamethyldisiloxane
HPLC     High-performance liquid chromatography
IPA      Isopropanol alcohol
LMH      Litres per metre squared per hour
MF       Microfiltration
MW       Molecular weight
MWCO     Molecular weight cut-off
nMWCO    Nominal molecular weight cut-off
NMWL     Nominal molecular weight limit
nRIU     nano Refractive Index Unit
NVP      n-vinyl-2-pyrrolidone
NWP      Normalised water permeability
PE       Polyethylene
PEG      Polyethylene glycol
PES      Polyethersulfone
pI       Iso-electric point (also IEP)
PSD      Pore size distribution
PSU      Polysulphone
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<td>PTFE</td>
<td>Polytetrafluoroethylene</td>
</tr>
<tr>
<td>RI</td>
<td>Refractive index</td>
</tr>
<tr>
<td>RMM</td>
<td>Relative molecular mass</td>
</tr>
<tr>
<td>RPM</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>SC</td>
<td>Sturred cell</td>
</tr>
<tr>
<td>SEC</td>
<td>Size exclusion chromatography</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>SMM</td>
<td>Surface modifying macromolecule</td>
</tr>
<tr>
<td>TFF</td>
<td>Tangential flow filtration</td>
</tr>
<tr>
<td>TMP</td>
<td>Transmembrane pressure</td>
</tr>
<tr>
<td>Trp</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>Tyr</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>UF</td>
<td>Ultrafiltration</td>
</tr>
<tr>
<td>UV/Vis</td>
<td>Ultraviolet/visible</td>
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Nomenclature

\( a \)  Channel width (m)
\( A \)  Absorbance (-)
\( A_c \)  Cross-sectional area (m\(^2\))
\( A_m \)  Membrane area (m\(^2\))
\( b \)  Channel height (m)
\( B \)  Constant (-)
\( c \)  Concentration (kg/m\(^3\) or g/L)
\( C_b \)  Concentration of solute in the bulk (kg/m\(^3\))
\( C_f \)  Concentration of solute in the feed (same as \( C_b \) (kg/m\(^3\))
\( C_G \)  Solute concentration at the gel layer surface (kg/m\(^3\))
\( c_i \)  Molarity concentration of the ion (mol/L)
\( c_m \)  Molar concentration (mol/L)
\( C_M \)  Solute concentration at the membrane surface (kg/m\(^3\))
\( C_{m} \)  Bulk electrolyte concentration (mol/m\(^3\))
\( C_p \)  Concentration of solute in the permeate (kg/m\(^3\))
\( C_w \)  Solute concentration at the membrane wall (kg/m\(^3\))
\( d \)  Diameter (m)
\( D \)  Diffusion coefficient or diffusivity (m\(^2\)/s or cm\(^2\)/s)
\( d_h \)  Hydraulic diameter (m)
\( D_{imp} \)  Impeller diameter (m or cm)
\( d_{mp} \)  Diameter of the membrane pore (nm)
\( d_t \)  Solute diameter (nm)
\( e \)  Elementary charge \(= 1.602 \times 10^{-19} \) (C)
\( E \)  Electrical potential (V)
\( E_i \)  Electrostatic energy of interaction (-)
\( F \)  Faraday constant \(= 96,485 \) (C/mol)
\( FR \)  Flux reduction (%) 
\( h \)  Blade height (cm)
\( H_F \)  Wall correction parameter (-)
\( I \)  Ionic strength (mol/L or mol/m\(^3\))
\( J \)  Membrane flux (L/m\(^2\)h)
\( J_P \)  Water permeate flux of the fouled membrane (L/m\(^2\)h)
\( J_s \)  Membrane solvent flux (m\(^3\)/m\(^2\)s)
\( J_W \)  Water flux (L/m\(^2\)h)
\( k \)  Constant (-)
\( K \)  Solution conductivity (S/m)
\( k_B \)  Boltzmann constant (J/K)
\( k_m \)  Mass transfer coefficient (m/s)
\( k_p \)  Permeability (m\(^2\))
\( l \)  Sample thickness or path length (cm)
\( L \)  Axial length of the membrane (m)
\( L_c \)  Membrane channel length (m)
\( L_e \)  Entry length (m)
\( L_m \)  Membrane thickness (m)
\( L_p \)  Permeance (L/m\(^2\)h.bar)
\( M \)  Molecular weight - corrected for polydispersity (g/mol)
\( M_l \)  Membrane length (mm)
\( M_n \)  Number average molecular mass (g/mol)
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<td>$M_w$</td>
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<td>Number of stirrer blades (-)</td>
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<tr>
<td>$P$</td>
<td>Hydrodynamic pressure (Pa)</td>
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<td>Radius (radial position along blade) (cm)</td>
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<td>Final (maximum) blade radius (cm)</td>
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<td>$W_{SC}$</td>
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<tr>
<td>$y$</td>
<td>Constant (-)</td>
</tr>
<tr>
<td>$z$</td>
<td>Distance from surface (m)</td>
</tr>
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<td>Number of effective charges on the molecule (-)</td>
</tr>
<tr>
<td>$z_i$</td>
<td>Charge number of the ion (-)</td>
</tr>
<tr>
<td>$X$</td>
<td>Constant (-)</td>
</tr>
<tr>
<td>$\Delta P$</td>
<td>Applied or transmembrane pressure drop (Pa)</td>
</tr>
<tr>
<td>$\Delta P_m$</td>
<td>Pressure drop across the membrane length (Pa)</td>
</tr>
<tr>
<td>$\Delta t$</td>
<td>Sampling time (h)</td>
</tr>
<tr>
<td>$\Delta W$</td>
<td>Solvation energy barrier/Born energy (J)</td>
</tr>
<tr>
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</tr>
<tr>
<td>$\zeta$</td>
<td>Zeta potential (mV)</td>
</tr>
<tr>
<td>$\Delta \pi_m$</td>
<td>Membrane osmotic pressure difference (-)</td>
</tr>
<tr>
<td>$\Pi$</td>
<td>Osmotic pressure (atm)</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Constant (-)</td>
</tr>
<tr>
<td>$\chi$</td>
<td>Constant (-)</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Membrane thickness (m)</td>
</tr>
<tr>
<td>$\delta_b$</td>
<td>Boundary layer thickness (m or mm)</td>
</tr>
<tr>
<td>$\varepsilon$</td>
<td>Molar extinction/absorption coefficient (mol/L·cm)</td>
</tr>
<tr>
<td>$\varepsilon_r$</td>
<td>Dielectric constant (-)</td>
</tr>
<tr>
<td>$\varepsilon_0$</td>
<td>Permittivity of free space (C$^2$/J·m)</td>
</tr>
<tr>
<td>$\phi$</td>
<td>Partitioning coefficient (-)</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Surface tension (N/m)</td>
</tr>
<tr>
<td>$\gamma_L$</td>
<td>Liquid-vapour interface surface tension (N/m)</td>
</tr>
<tr>
<td>$\gamma_{ns}$</td>
<td>Shear rate at the membrane surface (1/s)</td>
</tr>
<tr>
<td>$\gamma_S$</td>
<td>Solid-vapour interface surface tension (N/m)</td>
</tr>
<tr>
<td>$\gamma_{SL}$</td>
<td>Solid-liquid interface surface tension (N/m)</td>
</tr>
<tr>
<td>$\eta$</td>
<td>Solute diameter to pore diameter ratio (-)</td>
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<tr>
<td>$\lambda$</td>
<td>Ratio of solute to the pore radii (-)</td>
</tr>
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<td>Debye length (m)</td>
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<td>Solution viscosity (Pa·s, cP or P)</td>
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<td>Viscosity at 20 °C (Pa·s)</td>
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<td>Electrophoretic mobility (m$^2$/V·s)</td>
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<tr>
<td>$\mu_T$</td>
<td>Viscosity at temperature $T$ (Pa·s)</td>
</tr>
<tr>
<td>$\theta$</td>
<td>Contact angle (°)</td>
</tr>
<tr>
<td>$\rho$</td>
<td>Density (kg/m$^3$ or g/mL)</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>Reflection coefficient (-)</td>
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<tr>
<td>$\sigma_m$</td>
<td>Membrane surface charge density (C/m$^2$)</td>
</tr>
<tr>
<td>$\tau$</td>
<td>Shear stress (N/m$^2$)</td>
</tr>
<tr>
<td>$\omega$</td>
<td>Angular velocity (rad/s)</td>
</tr>
<tr>
<td>$\psi_0$</td>
<td>Electrical or double-layer potential (V)</td>
</tr>
<tr>
<td>$\psi_E$</td>
<td>Electrostatic energy of interaction (J)</td>
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Chapter 1 – Introduction

This thesis is concerned with the topic of ultrafiltration (UF). Membrane fouling is known to be a major limiting factor in terms of wider industrial application of this, and other, membrane filtration processes. Moreover, it has more recently been demonstrated both theoretically and experimentally that UF is not solely governed by separation based on size. Other factors such as hydrodynamic conditions and solution environment are known to influence separation performance. In the last decade it has become increasingly obvious that charge effects at the colloidal level may be exploited in order to selectively separate macromolecules. As a result, a range of techniques have emerged which may be used to place a charge onto the membrane surface.

It was desired to better understand the underlying principles that govern filtration using conventional, polymeric membranes when factors such as solution pH, ionic strength, operating pressure etc. are varied. Moreover, a range of characterisation methods such as molecular weight-cut off measurements using dextrans and scanning electron microscopy were employed to provide physical data on the asymmetric membranes employed. This work also studied the separation performance of plasma-modified membranes. Such modified membranes were produced in collaboration with Wrocław University of Technology, Poland, by modifying the surface of commercially available polyethersulphone\(^1\) (PES) membranes through the application of low-temperature plasma and subsequent dipping into acrylic acid solution. Charged groups can thereby potentially be placed on the membrane surface providing one way of utilising charge effects in membrane filtration.

In a laboratory environment membrane filtration studies are frequently carried out by employing stirred cell systems. However, it is well known that industrial applications are invariably conducted employing crossflow (module) systems therefore questioning the validity of some laboratory results in terms of their scalability. Hence, a crossflow apparatus was designed and a range of experiments were carried out with both a stirred cell and a crossflow cell.

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\(^1\) Polyethersulphone membranes were considered synonymous to polyethersulfone membranes, i.e. referring to the aromatic polymer composed of phenyl groups linked by ether and sulfone groups.
1.1 Background

Substantial commercial interest in protein separation using UF processes is in evidence over the last two decades. Advantages of UF over other processes include relative cost effectiveness, high selectivity, low energy requirements and the gentle concentration of proteins. Until recently UF was not considered as a fractionation method as protein fractionation could only be conducted successfully if the proteins to be separated had at least a ten-fold difference in molecular weight. Recent research has shown that viewing UF as a purely size-based separation process is not correct as a number of studies have shown that electrostatic interactions are crucial forces acting at the size range of interest (Burns and Zydney, 1999; Burns and Zydney, 2001; Irtran et al., 1997; Mehta and Zydney, 2006, Teng et al., 2006). It has been known for about a decade that solute separation of proteins or other macromolecules of similar molecular weight is possible by adequate control of the pH, ionic strength and applied pressure conditions (Saksena and Zydney, 1994).

The introduction of charged groups on the membrane surface has been found to aid not only in the separation of proteins of similar molecular weight but also in reducing the deposition of proteins at the membrane surface. This is one of a range of methodologies (changing of the feed pH, membrane pretreatment, use of spacers or turbulence promoters, application of back flushing etc) which may be employed in order to reduce the extent of membrane fouling.

1.2 Objectives

This research project was aimed at two aspects of protein UF. Firstly, it was desirable to study the separation behaviour of proteins in two different laboratory scale systems, namely the commonly used stirred cell system and a purpose-built crossflow system. Secondly, it was of interest to study the potential differences between protein filtration using conventional, unmodified membranes and polymenc UF membranes that were modified by low-temperature plasma.
The original objectives of the research included:

- Development of a crossflow apparatus for UF studies
- Protein filtration studies under varying pH and ionic strength conditions
- Comparative filtration studies using both a stirred cell and crossflow device; influence of hydrodynamic effects
- Modification of the membrane surface in order to obtain a novel membrane surface
- Characterisation studies of both unmodified and plasma-modified membranes
- Elucidating differences between UF using unmodified and plasma-modified membranes

1.3 Thesis outline

This thesis is divided into the following chapters:

- Chapter 2 – Literature Review
  The UF process and the various influences on membrane fouling are discussed in the light of published work by other researchers. Several techniques, in particular low-temperature plasma modification, are reviewed to demonstrate how they can be used to alter the surface and hence separation characteristics of a membrane.

- Chapter 3 – Modelling of the Ultrafiltration Process
  Early UF models are described and more recent models, which take into account additional factors not considered in the traditional models, are introduced. An overview of some of the more common models is also provided and the foundation is laid for their use in aiding the interpretation of the results presented in Chapters 6 and 7.
Chapter 4 – Materials, Methods and Experimental Apparatus
The experimental methodologies and the stirred cell and crossflow devices employed in the research are described. In addition, the low-temperature plasma surface modification apparatus is also introduced. This apparatus was employed for the modification of PES membranes with the intention to improve membrane filtration characteristics.

Chapter 5 – Membrane Characterisation
A range of characterisation methods were employed to aid the understanding of filtration behaviour for a given membrane. Important results obtained from the various techniques used are highlighted in order to help support the findings presented in Chapters 6 and 7.

Chapter 6 – Filtration with Unmodified Membranes
Experimental results of an investigation into the effects of pH and ionic strength on the filtration of proteins using the two different filtration devices are described. The chapter is completed by a discussion and interpretation of the experimental findings in relation to the literature and with the help of the filtration models outlined in Chapter 3.

Chapter 7 – Filtration with Plasma-Modified Membranes
The filtration of proteins using plasma-modified membranes is discussed. The results are interpreted with the aid of models in a similar manner to that employed in Chapter 6 but with the addition of a charge-based model.

Chapter 8 – Overall Conclusions and Recommendations
The conclusions of the research findings are presented together with possible areas for future research.

The Appendices include a list of publications, more detail of the crossflow apparatus design, detailed calculations and additional experimental data.
Chapter 2 – Literature Review

Typical effects that are important in the context of this thesis and which govern separation during ultrafiltration (UF) such as membrane fouling, membrane cleaning and the influence of electrostatic interactions are reviewed. The study of charge effects and hydrodynamic conditions on UF has found more interest in recent years and both are discussed. Furthermore, the modification of the surface of polymeric membranes is described, particularly where a low-temperature plasma modification technique is employed. Since it has been both theoretically and experimentally demonstrated that charge effects can play a vital role during UF, charge modification of the membrane surface is of great interest. Existing work in this area is highlighted and shortcomings are pointed out where appropriate.

2.1 Concepts of ultrafiltration

UF is a pressure-driven membrane process where the typical pore size ranges from approximately 2 to 100 nm and the molecular weight cut-off (MWCO) varies between 1 and 1000 kDa (Rautenbach and Albrecht, 1989; Schweitzer, 1997). Filtrate flux rates are commonly reported in L/m²h (LMH) and typically range from 25 to 250 LMH (Desai, 2000). UF processes are mostly operated at 0.2 to 4 bar(g) transmembrane pressure (TMP).

Macromolecular solutes such as proteins are separated from a solvent, typically water. At the industrial scale, factors such as the level of permeate flux and frequency of membrane cleaning is of particular importance in determining the processing cost (Waite et al., 1999). Permeate flux is determined by several factors including the membrane nominal pore size, the amount of fouling, the extent of concentration polarisation (solute build-up near the membrane surface) and charge interaction effects between the solute and the membrane as well as solute-solute interactions, all of which are described further below (see also, for instance, Almeida et al., 2007; Bhattacharjee et al., 1996; Blatt et al., 1970; Fane and Fell, 1987; Howell et al., 1999, Opong and Zydney, 1991; Tarleton and Wakeman, 1993; Wijmans et al., 1985; Zhang and Liu, 2003).
2.2 Membrane fouling

Membrane fouling has been identified by numerous authors as one of the key problems in pressure-driven membrane processes (Bartlett et al., 1995; Chan et al., 2002; Fane and Fell, 1987; Lindau and Jonsson, 1999; Marshall et al., 1993, Meireles et al., 1991; Nabe et al., 1997; Nyström, 1989; Steen et al., 2001). It is important to distinguish genuine membrane fouling from the phenomenon of membrane compaction, the densification of a polymeric, porous membrane structure due to a high feed pressure (Mosqueda-Jimenez et al., 2004b). One method to combat fouling is to modify the surface of the membrane in order to prevent non-specific adsorption of solute (e.g. proteins), a modification that can be performed in a number of different ways.

Fouling has been defined as the process resulting in loss of performance of a membrane due to deposition of suspended or dissolved substances on its external surfaces, at its pore openings, or within its pores (Koros et al., 1996). Concentration polarsation (Section 2.2.2) is a largely reversible phenomenon that must not be confused with true causes of membrane fouling (Schaefer et al., 2005). Many authors consider a membrane as irreversibly fouled only if the original process flux cannot be restored after flushing with water and/or chemical cleaning (Cheryan, 1998; Van der Bruggen et al., 2002). It is apparent that membrane fouling can be considered to be the main problem limiting more widespread commercial application of UF. Fouling problems and potential fouling control methods for UF membranes have been reviewed by Fane and Fell (1987) More recently an ultrasonic technique was developed enabling a more accurate analysis of protein fouling (Li et al., 2005).

Commonly, flux decline is rapid at the beginning of the UF process and thereafter becomes less pronounced emphasizing its time dependency. Flux decline may be more pronounced due to progressive blocking of membrane pores which, in some cases, may lead to entirely blocked pores (Figure 2.1).
The dramatic decline in permeate flux (also observed in other membrane processes) can be attributed to progressive solute accumulation at the membrane surface which tends to lessen once a near equilibrium filtration rate has been established. Fouling may be separated into two mechanisms where, firstly, rapid deposition of molecules at the membrane and some penetration takes place. Secondly, in some instances, a secondary layer of molecules (or particles in the case of microfiltration) is formed adjacent to the membrane surface which can then even become the layer dictating the filtration process; this layer is also termed the “dynamic membrane” (Tarleton and Wakeman, 1993)

Common approaches to limit fouling include (Schaefer et al, 2005):

a) Selection of a membrane material which minimises attractive interactions between the fouling substance and the membrane surface

b) Use of pretreatment methods to remove the main fouling components from the feed

c) Membrane module design and operation including the promotion of turbulent flow and lower pressure operation to reduce fouling effects.
The approach relevant to the current work is to introduce charged groups onto the surface of a polymeric membrane with the intention to make use of charge interaction effects and to potentially improve membrane characteristics (Section 2.5.2).

Fouling tends to be more extensive when a membrane has high pure water permeability or if the contact angle (Section 5.7) is large (Manttari et al., 2002). However, such observations are not always true for solvents other than water and the fouling tendency is more closely dependent on the relationship between the pore size and the size of the potential foulant. The observation concerning the contact angle is in agreement with the fact that a more hydrophilic membrane has a low contact angle. Moreover, studies of intermolecular forces between the protein lysozyme (LYZ) and a modified polysulphone (PSU) film confirm that a hydrophilic membrane can not only possess a low contact angle but also feature less fouling, reduced adhesion forces and less adsorbed material (Koehler et al., 2000).

2.2.1 Internal and external fouling

Membrane fouling can occur at different locations including the selective layer surface and the pore walls (Hilal et al., 2005). Internal fouling refers to the deposition and adsorption of small particles or molecules onto the pore entrances or the inside of a pore of the membrane whereas external fouling refers to the accumulation of rejected molecules upon the membrane surface (Choi et al., 2000). Especially in protein filtration, adsorption or deposition of macromolecules may lead to significant flux loss (Kim and Fane, 1995). Two main mechanisms of irreversible fouling are often stated during protein processing, namely pore narrowing due to protein adsorption and pore plugging (Lindau and Jonsson, 1999). Protein adsorption mostly refers to adsorption of macromolecules to the membrane surface or membrane pores without blocking off the entire pore. Pore plugging refers to blockage inside the pore where the macromolecule(s) actually completely block(s) off the trajectory path of the pore. The three different forms of fouling mentioned are schematically illustrated in Figure 2.2.
Many researchers have examined the influence of fouling on flux reduction. Less attention has been paid to the effect of fouling on the retention of typical macromolecules such as proteins by the membrane. Schafer et al. (2000) demonstrated that UF membrane rejection increases with fouling, mainly due to a reduction in the membrane pore size as a result of both, pore plugging and adsorption of macromolecules inside the pores. According to Boyd and Zydney (1998) fouling due to adsorption is also influenced by the hydrodynamic drag exerted during filtration. They found that the protein layer formed at the membrane surface was much more tightly packed during pressure filtration compared to static adsorption experiments.

2.2.2 Concentration polarisation

Concentration polarisation is the result of solute accumulation adjacent to the membrane surface, a phenomenon which has been reviewed by Song and Elimelech (1995). The concept is valid in both microfiltration (MF), typical size range of 0.1 to 20 μm (Tarleton and Wakeman, 2007), and UF, and it is often described as the formation of a ‘cake layer’ of molecules (or particles when referring to MF) at the membrane surface which causes an increase in the hydraulic resistance to filtrate flow. The process is essentially totally reversible and does not consider attractive interactions between deposited solutes and those in the bulk (Bacchin et al., 2002).

Polarisation of solute at the membrane surface causes a non-linear relationship between permeate flux and pressure (Porter, 1972). Concentration polarisation occurs with varying degrees of solute accumulation largely dependent on the pore size to solute size.
ratio in the absence of charge effects. In any case, concentration polarisation provides extra resistance to flow in addition to the membrane and boundary layer resistance (Cheryan, 1998). A schematic description of concentration polarisation, based on a schematic by Van den Berg (1988), helps to illustrate the phenomenon (Figure 2.3)

![Schematic diagram of concentration polarisation](image)

**Figure 2.3. Concentration polarisation schematic, adapted from Van den Berg (1988).**

The frequently used film model is based on Figure 2.3 above and discussed in more detail in Chapter 3. Bacchin et al. (2002) demonstrated schematically how concentration polarisation fits into other fouling mechanisms occurring at the colloidal size range (Figure 2.4). The diagram implies that as the driving force increases, molecule deposition or the formation of a tight gel layer becomes more likely, which typically occurs at a TMP beyond which the permeate flux does not notably increase with an increase in TMP.

Several methods exist to counteract the extent of polarisation including increasing shear stress at the wall, operating at a lower TMP and employing high solution velocities noting that the latter does not always lead to an improvement.
2.3 Membrane cleaning

Cleaning, despite its importance, is not as widely covered in the literature as other membrane-related issues. Cheryan (1998) points out that what is really a cleaning problem is often mistaken for a fouling problem. Cleaning is critical to membrane lifetime. Generally, three types of cleaning are distinguished: chemical, thermal and mechanical cleaning all of which are influenced by the cleaning time.

The stability of the membrane becomes important when harsh chemical cleaning agents with extremes of pH are used. In this context it should be pointed out that strong chemical cleaning agents may have undesirable effects on the membrane structure and care must be taken that no structural changes occur as a result of their use. Cleaning is thought to be most successful at low operating pressures because a high TMP may result in renewed deposition of unwanted material in the membrane pores. A decreasing cleaning performance with an increase in TMP was confirmed in the work by Bartlett et al. (1995). In fact, Nystrom and Zhu (1997) confirmed that if cleaning is performed under pressure adverse effects may be observed which they attributed to additional pore plugging and a hindered effect on diffusion of fouling material out of the pores. A combination of cleaning without application of pressure followed by the application of a low pressure may be advisable.
Adequate cleaning of fouled membranes is very important because of its impact on membrane lifetime. Interactions between the foulant and the cleaning agent were found to be dependent on membrane hydrophlicity, membrane charge and morphology (Weis et al., 2003). A comparison of relative flux decline due to fouling of a PES and a PSU membrane showed that the latter was more susceptible to fouling than the former which the authors attributed to a lower surface charge on the PSU membrane (Weis et al., 2003). A typical membrane filtration cycle and the effects of cleaning are illustrated in Figure 2.5.

![Typical flux decline and level of recovery due to cleaning](image)

**Figure 2.5.** Typical flux decline and level of recovery due to cleaning. Diagram adapted from Pieracci et al. (1999).

### 2.4 Solution chemistry

Proteins are polymers made up of different amino acids and it is the functional groups at the outer edges of the protein structure that dictate the charge of the protein and they can be altered by changing the solution environment. Hence, factors such as the solution pH and solution ionic strength become important. In addition, any charge on the membrane itself is dependent on the solution conditions.
2.4.1 Electrical double layer and streaming potential

Over the colloidal size range charge interactions between, for instance, proteins and the membrane surface and also protein-protein interactions are prominent. A like charge on the membrane surface and the protein, for example, will cause electrostatic repulsion between the two entities, which is one way that can help to reduce fouling. When charge interactions are of importance the concept of the electrical double layer (EDL) has to be taken into account. The EDL describes the interfacial region in a system where ions are present which can be regarded as two charged portions with an equal and opposite charge.

![Diagram of electrical double layer and zeta potential](image)

**Figure 2.6. Illustration of the electrical double layer and zeta potential.**

An EDL will also be present around a molecule in an electrolyte solution as graphically illustrated in Figure 2.6. Here, zeta potential is also shown which is related to the electrostatic potential caused by the charge difference between the colloid particle or molecule and any reference point in the bulk liquid. In essence, the zeta potential is the electrical potential measured at a distance from the interface between the Stern layer (the ion layer of opposite charge surrounding the molecule in a liquid) and the diffuse layer in liquid systems. Motion is required for the zeta potential to be measured and therefore the potential is not a measure of the true potential on the molecule but at the
shear plane, a small distance from the surface. In summary, there are two layers formed around a molecule in an electrolyte solution, the Stern layer and the diffuse layer which are referred to as the EDL.

The thickness of the EDL and its interaction with other molecules and a membrane surface has a noticeable effect upon permeate flux and membrane fouling. The EDL is an important part of the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory which concerns the forces acting between charged surfaces (e.g. molecules, the membrane or any other particle immersed in a polar liquid) in a liquid environment. Essentially the DLVO theory is concerned with the balance between electrostatic repulsive forces and van der Waal's attraction forces. Repulsion occurs as the electric fields of two charged spheres approach each other and an overlap of their outer layers occurs. The repulsive force creates an energy barrier which may be overcome if the van der Waal's force exceeds the repulsive force often coinciding with a decrease in distance between the two charged spheres. In other words, if intermolecular repulsive forces are sufficiently low, then aggregation occurs and attractive van der Waal's forces prevail. The magnitude of the energy barrier can be influenced by changing the solution ionic environment or the pH. The EDL itself is influenced by the ionic strength in that a high ion concentration causes the double-layer to compress. The important phenomena of this theory are best described in a diagram (Figure 2.7). In summary, the DLVO theory allows the determination of the net potential energy between two colloidal spheres by combination of the van der Waal's attractive force model and the electrostatic repulsion model.

![Diagram of Electrical Double-Layer and Ionic Strength Charge Interaction](image)

**Figure 2.7. Electrical double-layer and ionic strength charge interaction.**
The streaming potential, a measurement technique which can be used to determine the zeta potential of a colloidal particle/molecule or that of a surface such as a membrane plays an important role in interpreting any charge interactions. According to Shaw (1992) the streaming potential is defined as the “electric field which is created when liquid is made to flow along a stationary charged surface”. In contrast to other electrokinetic effects such as electrophoresis (an electric field is applied to move a charged surface relative to a liquid), or electro osmosis (an electric field is applied to move the liquid relative to a surface), a pressure gradient instead of an electric field is applied to move the liquid through the porous medium resulting in an electric potential that may be measured using a sensitive electrometer (Hunter, 1981). On a molecular level it is the charge in the mobile part of the electrical double layer which is transported towards the pore ends causing charge accumulation, thereby creating the electrical field (Chiu and James, 2006).

Nyström et al (1998) conducted streaming potential measurements on fouled membranes and converted these measurements into zeta potentials. Comparing the zeta potential of several fouled membranes allowed them to determine which protein caused the fouling. Another application of the streaming potential technique is to estimate the interaction between the membrane and a foulant and for general investigation of the surface characteristics of UF membranes (Lawrence et al., 2006). The technique is important because protein fouling can depend on the interaction of the protein charge with the charge on the membrane as determined by the zeta potential (Nyström et al., 1994). Both a streaming potential through the membrane pore and along the surface of the membrane can be measured. Lawrence et al. (2006) point out that most measurements are conducted through the pore, because most often the charge potential of the membrane and the identification of the isoelectric point (pI) of the membrane are of interest, whereas the other technique is used to observe any potential change with distance along the membrane surface. Moreover, any charge in the pore or the pore ends has been found to be more important in terms of its effect on filtration than the charge on the membrane surface (Pihlajamäki, 2007). At the isoelectric point (pI), sometimes also referred to as the IEP, the molecule/particle or charged surface in question has an overall net charge of zero. The pI is defined as the hydrogen ion concentration in solution at which dipolar ions are at a maximum (Walker, 1995). Generally, proteins tend to be least soluble (but not always) at their pI (Cooper, 2004).
Figure 2.8 shows the effect of pH on the interaction between proteins in aqueous solution. The zeta potential, $\zeta$, of a membrane is typically calculated by converting streaming potential data into zeta potentials using the Helmholtz-Smoluchowski equation (Hunter, 1981):

$$\zeta = \frac{\mu K \Delta E}{\varepsilon_0 \varepsilon_r \Delta P}$$  \hspace{1cm} (2.1)

where $\mu$ is the liquid viscosity, $K$ the solution conductivity, $\Delta E$ the streaming potential (as a result of the applied pressure), $\varepsilon_0$ the permittivity of free space, $\varepsilon_r$ the dielectric constant and $\Delta P$ the pressure difference across the channel. An example of a graphic depiction of a typical zeta potential graph obtained for a polymeric membrane through which a dilute electrolyte solution is passed is presented in Figure 2.9. The zeta potential can be used to determine the pI (4.2 in the example below) of a material and to quantify the charge potential of that material at a given solution pH (Martín et al., 2003).
2.4.2 pH effects

The pH of a solution is an important factor in determining any charge on the membrane and the solute. It has also been demonstrated that the influence of pressure on the permeate flux rate is pH dependent (Nyström et al., 1998) and it must be noted that many other parameters are also under the influence of pH. Therefore, a change in the solution pH has a direct effect on the transmission of proteins through a UF membrane. In this context, the concept of the pI is of importance, particularly for proteins as they are amphoteric molecules, i.e. they possess both acidic and basic functional groups and are positively charged below their pI and negatively charged above their pI (as shown in Figure 2.8).

Several studies have shown that protein transmission is generally highest at the pI (Burns and Zydney, 1997; Gan, 2001; Nyström et al., 1998). This may be explained in that electrostatic interactions between proteins and the membrane are thought to be minimal at the pI of the protein due to the neutral charge (Salgun, 2007). The same
author also points out in her study that bovine serum albumin (BSA) permeate flux was higher above the pI and at its lowest below the pI. It was concluded that both the membrane and the protein were negatively charged above pH 4.9 (the pI of BSA) resulting in lower fouling levels. During protein filtration with a commercial nanofiltration membrane (polyampholyte amide on a PSU microporous support) Teixeira et al. (2005) also found protein retention to be lowest at the pI and noted the highest permeate flux rates at this pH. The result stands in direct contrast to the work by Ghosh and Cui (1998) who found permeate flux to be at its lowest during BSA filtration with UF membranes. Teixeira et al. (2005) also studied several electrolyte solutions and measured the respective zeta potential along the surface and through the pores of the nanofiltration membrane. On the one hand it was found that the negative charge of the membrane increased with an increase in pH. On the other hand the electrolyte solution flux decreased with an increase in pH. The membrane pores were found to be less negatively charged than the membrane surface, albeit the pI remained the same.

The level of pH is known to be a parameter that, if correctly adjusted, can prolong membrane lifetime and reduce fouling as made evident in the work by Teixeira and Rosa (2003). It becomes apparent that solution pH can govern the separation process to some extent hence colloidal interactions have been studied over different pH ranges (e.g. Van der Meeren et al., 2004). These authors studied filtration of colloidal silica dispersions at different pH and electrolyte concentrations through semi-ceramic zirconia/PSU membranes and observed that strong interactions between the membrane surface and charged colloids occurred when the membrane and the colloid charge were opposite, resulting in serious flux decline. This study demonstrates that it is not always wise to assume that pH adjustments in a manner where repulsive forces between the solute and the membrane dominate lead to both improved flux and increased retention.

It has been mentioned earlier that the pI of a membrane can be found using streaming potential measurements (see Section 2.4.1) which are subsequently converted into zeta potentials. If the pI of a molecule/particle is of interest then electrophoretic mobility measurements in an electrolyte solution can be conducted using a zeta potential instrument.
2.4.3 Ionic strength

The ionic strength is defined as a measure of the intensity of the electric field existing in solution (Walker, 1995) and mathematically expressed as:

\[ I = \frac{1}{2} \sum z_i^2 c_i \]  

(2.2)

where \( z_i \) is the charge number of the ion and \( c_i \) the molarity concentration. The magnitude of the ionic strength can have a significant impact on permeate flux. Choi et al. (2003) demonstrated that during the filtration of carboxylated microspheres an increase in the ionic strength resulted in a steeper flux decline. The authors attributed this result to a compressed electrical double layer around the spheres which resulted in higher packing density and increased resistance to permeate flow. Similar findings were reported by Falbish et al. (1998) who explained the reduced permeate flux at a higher ionic strength with a decrease in the Debye screening length (Coulomb interactions become unimportant beyond this distance (as ionic strength increases), see Section 2.4.4 and equation (2.3)). Salgin (2007) studied electrostatic interaction effects during BSA separation from solution in a crossflow system at different pH and ionic strength. Her results are in direct contrast to the findings of Falbish et al. (1998) since the study showed permeate flux to increase with an increase in ionic strength (both groups used KCl and comparable ionic strengths). Waite et al. (1999) studied a hematite suspension in a batch cell system and found permeate flux decline to be less severe at high ionic strength. Again, this observation contrasts the findings by Choi et al. (2003) and Falbish et al. (1998). The discrepancy may be explained in the size difference of the molecules and particulates employed in comparison to the membrane cut-off. The studies by Salgin (2007) and Waite et al. (1999) considered solutes which were significantly larger than the typical pore size of the membrane hence 100% rejection could be expected at any solution chemical condition. Therefore, one can argue that the hydraulic resistance of any fouling layer on the membrane is lower when the combined effects of solutes unable to penetrate the membrane and the absence of electrostatic repulsion effects due to increased ionic strength are present. In the work by both Falbish et al. (1998) and Choi et al. (2003), however, the membrane nominal pore size was large enough to allow at least some solute transmission. It may be that the effect of ionic strength and any
fouling layer(s) formed under such conditions are different to the properties a fouling layer will have if the solutes are completely rejected by the membrane. Such a conclusion is supported by the study of Kim et al (1993) who found that for a polymeric membrane with a pore size large enough to transmit some of the solute (silver particles) a low ionic strength (i.e. non-aggregated state) resulted in significant flux decline due to pore blocking effects. In the aggregated state, at higher ionic strength, particles which previously were able to block the pore were now retained in the fouling layer(s) at the surface, hence limiting the severity of flux decline.

Zeta potential is dependent on ionic strength which becomes evident in the decreased potential of both the membrane and the protein at higher ionic strength; as measured by Salgín (2007). This finding supports the theory of gradual charge-shielding or compression of the electrical double layer as a result of an increase in ionic strength (Mulder, 1991; Rabiller-Baudry et al, 2001; Shaw, 1992).

2.4.4 Electrostatic interactions

Electrostatic interactions are the result of fixed charges and the magnitude of the electrostatic force is dependent on the distance between charges. Such interactions can arise from different forces acting between molecules, for instance, hydrogen bonds, van der Waals forces or salt bridges. Several studies have examined the importance of electrostatic interactions (Burns and Zydney, 2001; Ebersold and Zydney, 2004a; Fernández et al, 2005; Mehta and Zydney, 2006; Menon and Zydney, 1999, Pujar and Zydney, 1994; Pujar and Zydney, 1997, Rezwan et al, 2005; Saksena and Zydney, 1994; Salgín, 2007).

Kato et al (1995) conducted protein adsorption tests on polymer surfaces grafted with ionic polymer chains in order to investigate the interrelation of adsorption and membrane fouling. Their work showed that electrostatic interactions were prominent and attractive forces dominated when the protein and membrane surface charge were opposite, whilst repulsive forces dominated at like charge. Notably, adsorption tended to be prevented, or at least reduced, when the protein was of like charge to the ionic substance on the membrane surface. However, at opposite charge, accelerated protein adsorption was observed and linked to the ionic polymer chains at the membrane surface. In addition, the same authors grafted non-ionic polymer chains, such as
acrylamide, onto the membrane surface which resulted in low adsorption independent of the pI (see Section 2.4.2) of the protein which was ascribed to steric hindrance effects.

Falbush et al. (1998) investigated the effects of electrostatic interactions on permeate flux decline and cake layer formation using a crossflow filtration apparatus with a 20 nm pore size membrane. They studied silica colloidal suspensions of different sizes over a pH range from 6.1 to 10.0 and considered four different ionic strengths. The authors concluded that an increase in ionic strength leads to more severe flux decline and hence a steady-state flux was reached more quickly than at a lower ionic strength. Moreover, the authors showed that on inspection of the equation for the Debye screening length in an electrolyte ($\lambda_D$), as the ionic strength increases the repulsive force between molecules decreases such that

$$\lambda_D = \sqrt{\frac{\varepsilon_0 \varepsilon T}{2N_A e^2 I}} \tag{2.3}$$

where $k_B$ is the Boltzmann constant, $T$ the absolute temperature, $N_A$ the Avogadro's number, $e$ the elementary charge and $I$ the ionic strength of the electrolyte. The significance of the Debye screening length is a way to express the 'double-layer thickness' and if a diagram of electrostatic potential versus distance (of a charged sphere from a charged surface) is plotted one can clearly see the effect of electrolyte concentration on the electrostatic potential. Figure 2.10 shows that for a 1:1 electrolyte such as NaCl adjacent to a surface with 100 mV potential the decay is more rapid at higher salt concentrations. This implies that the higher the salt concentration the more rapid the decay, explaining why charged molecules can approach each other more closely in a high ionic strength environment. Falbush et al. (1998) also noted in their study that pH had little effect over the range studied. Ionic strength effects on filtration performance were more pronounced for smaller colloids of about 50 nm in size and below. Colloidal particles in excess of 310 nm seemed to be independent of ionic strength effects. Proteins, however, are molecules of the order of a few nanometres hence ionic strength effects are indeed important, for instance, in influencing the porosity/permeability of fouling layers.
The surface charge on a membrane is an important property in UF. In this context, it is noteworthy that the separation of proteins only differing by a single amino acid residue has been achieved using UF membranes, although the selectivity tends to be relatively low. Ebersold and Zydney (2004b) found that a narrower pore size and an increased membrane charge density promote electrostatic interactions, thereby increasing the selectivity of the process. Hence, if electrostatic interactions are desirable for a given process it is sensible to utilise a technique capable of placing a high surface charge density onto the membrane surface. In earlier studies, Burns and Zydney (2000) showed that the surface charge of the membrane varies with the presence of buffer-ions in the processing solution which may have a great effect on the actual surface charge. Their findings suggest that membrane storage in buffer solutions to prevent bacterial growth may have substantial effects on membrane fouling behaviour due to a potential shift in the membrane charge.

2.4.5 Electroviscous effect

The electroviscous force is related to the distortion of the EDL by the liquid flow (Chun and Ladd, 2004). In other words the apparent viscosity of the liquid can be influenced
by the surface charge of the membrane pore and the solution ionic strength, known as the electroviscous effect. Its potential relevance during membrane filtration of charged solutes has been widely recognised (Bowen and Jenner, 1995; Chun and Ladd, 2004; Huisman et al., 2000, Pujar and Zydne, 1994; Sbai et al., 2003). The streaming potential which develops between the pore ends is what causes the flow of counter-ions and water in the double layer region near the pore surface (Farbish et al., 1998).

Electroviscous effects are considered to be of minor importance if the membrane charge is low and may then be neglected (Bowen and Yousef, 2003) However, when the charge on the membrane is high an apparent increase in viscosity may be observed during molecule transport through charged pores.

In a study by Sbai et al. (2003) it was reported that the electroviscous effect has a maximum at intermediate ionic strength (~50 to 200 mM) but becomes negligible at low (<50 mM) and high ionic strengths (>200 mM). This is in agreement with Farbish et al. (1998) who reported the electroviscous effect to be least pronounced at high ionic strengths. According to Huisman et al. (2000) the electroviscous effect increases with increasing salt concentration in the lower ionic strength region, then reaches a maximum and eventually decreases with further increases in salt concentration.

2.5 Additional effects in membrane ultrafiltration

Sections 2.4.1 to 2.4.5 have shown that whilst the UF process is predominately a size-based separation, it is influenced by other factors including charge and solution chemistry. Additional factors such as molecule shape and membrane morphology can also influence filtration.

2.5.1 Molecule shape

Proteins are polyampholytes (a polyelectrolyte containing both acidic and basic functional groups) and their size, shape and interaction depend on the degree of ionisation of their charge groups in an ionic environment (Sudareva et al., 1992) If protein-protein interactions are sufficiently strong then the effective size of the molecule can change. Sudareva et al. (1992), for instance, demonstrated that in a mixture of cytochrome-c (pI = 10.6) and human serum albumin (pI = 4.9) at pH 5.5 (i.e. between
the isoelectric points of the two proteins) electrostatic attractions occur between the two
different proteins causing "dynamic" molecule formation which are molecules larger in
their effective size. It is noteworthy that the greater the difference in pI between the two
proteins in a mixture, the greater will be their effective size due to a larger "excess" net
charge.

McDonogh et al. (1989) briefly mentioned that the shape of a macromolecule such as
BSA may alter with a change in pH. However, the shape of macromolecules can also
change with variations in, for example, temperature, ion concentration or voltage.
Maruyama et al (2001) studied BSA fouling on PSU UF and polytetrafluoroethylene
(PTFE) MF membranes and used Fourier transform infrared spectroscopic analysis in
order to determine any conformational changes of BSA. Interestingly, adsorption of
BSA onto the somewhat hydrophilic PSU membrane resulted in almost no change in the
BSA structure, whereas conformational changes were observed with the MF membrane.
Oppenheim et al. (1996) investigated conformational changes of LYZ when interactng
with UF membranes using electron paramagnetic resonance spectroscopy. Their study
looked at cellulosic (hydrophilic) and PSU (hydrophobic) UF membranes. Whilst it was
possible for the authors to detect a change in LYZ during protein-membrane interaction
no differences between the two membrane types were observed which the authors
attributed to a shortcoming of their testing technique.

2.5.2 Hydrophilicity/hydrophobicity

If a material is hydrophilic it is attractive to water, i.e. in the case of a hydrophilic
membrane water will form hydrogen bonds with the membrane polymer and itself. This
is because hydrophilic membranes readily adsorb water and a pure water layer is formed
at the membrane surface because of the high attraction of water molecules to each other.
The thickness of the water layer is believed to increase with an increase in hydrophilic
character (Israelachvili, 1992). Hydrophobic materials have a low tendency to adsorb
water and exhibit contact angles greater than 90°. Once the membrane is fully wetted,
hydrophobic membranes, just like hydrophilic membranes, will also be covered by a
water layer but they are subject to secondary effects such as, e.g. they may be more
prone to internal fouling and pore blocking ultimately leading to lower permeate flux.
It is generally accepted that hydrophilic membranes are less prone to fouling than hydrophobic ones during the filtration of aqueous suspensions (Jonsson and Jonsson, 1995). If hydrophobic components are present in the feed they will tend to avoid attachment to hydrophilic surfaces which is why hydrophilic membranes often exhibit lower fouling levels than hydrophobic membranes. In addition, if the membrane contains charge groups these tend to form hydrogen bonds with the water thereby increasing the degree of membrane hydrophilicity.

### 2.6 Surface modification of polymeric membranes

A range of methods exist that are suited to the modification of polymeric membrane surfaces. The emphasis in this section is on membrane modification by low-temperature plasma, also known as a microwave plasma modification. Polysulphone (PSU) membrane modification and polyethersulphone (PES) membrane modification are discussed in more detail, because a lot of work has been carried out using the former membrane type and the latter is the base polymer employed for this research.

#### 2.6.1 Methods of modification

Kemmere and Keurentjes (2001) summarized methods which have recently been used in order to modify the surface of a previously formed porous membrane. The idea is to increase the hydrophilic character of the membrane and/or to allow functionalization of the inherent polymer segments. The methods include chemical oxidation, plasma treatment, classical organic reactions and polymer grafting.

Polymers containing double bonds, hydroxyl groups or benzene rings can be modified by the use of classical organic reactions to yield increased hydrophilicity. Grafting methods and the structural properties of grafting surfaces have been extensively reviewed by Kato et al. (2003). Polymer grafting can also be induced by exposure of the membrane to ultraviolet (UV) light. The use of homogeneous chemical reactions to graft molecules onto PSU membranes was studied by Nabe et al. (1997). More recently it became clear that surface modification of the polymer provides a cheaper and less troublesome alternative (Kilduff et al., 2000). Such methods include surface alterations by means of low-temperature plasma (Ulbricht and Belfort, 1996) and UV induced grafting (Yamagishi et al., 1995). It is important to note that grafting involves a change
in the polymer structure through the creation of additional branches upon the main backbone, thus affecting the membrane permeability (Rickles, 1967). A summary of the different modification methods available including pretreating the membrane with proteins, physical coating, blending, chemical and photochemical modification methods has been presented by Sun et al. (2003).

2.6.2 Non-plasma surface modification

A membrane surface can be modified by exposure to UV light; the process is commonly conducted by immersing the membrane in reverse osmosis-treated water during exposure (Ehsani et al., 1997). Gamma-ray irradiation of PEG onto hollow-fibre UF membranes was conducted by Mok et al. (1994) where the modified membrane was reported to be less prone to fouling. Hamza et al. (1997) and Mosqueda-Jimenez et al. (2004a) employed a casting method where surface modifying macromolecules (SMMs) were added to the surface during the polymerisation process. Membranes prior to and after three minutes of evaporation treatment with SMM-containing solvent with 12% and 18% of PES content, respectively, were compared by assessing their respective scanning electron micrograph (SEM) (Figure 2.11). Several other authors studied placing SMMs onto polymeric membrane surfaces including Rana et al. (2005) and Khayet (2004). Rana et al. (2005) found PES membranes polymerised with SMMs to perform better than unmodified PES membranes. However, this method required the modification to take place during the formation process of the polymer membrane which was not within the scope of the current research.
Another technique used to increase the hydrophilicity of PES and PSU membranes is UV-assisted graft polymerisation using different hydrophilic monomers, for example, 2-acrylamidoglycolic acid monohydrate (AAG) (Kaeselev et al., 2001; Kilduff et al., 2002; Pieracci et al., 2000; Taniguchi and Belfort, 2004; Taniguchi et al., 2003). Pieracci et al. (1999) used the same method and found PES membranes to be more sensitive to the irradiation than the PSU membranes.

Yet another modification technique is one-step dip-modification, which has been employed in order to improve the characteristics of, for instance, hollow-fibre (Bequet et al., 2002) and PES (Pieracci et al., 2002) membranes. In dip-modification the membrane is coated with solution and subjected to irradiation as opposed to immersion modification where the membrane is directly irradiated being immersed in a nitrogen-purged solution.

Recently, Saxena and Shahi (2007) produced sulfonated PES membranes by casting the membranes in their own laboratory which subsequently allowed them to conduct filtration studies with both BSA and LYZ. They were able to show that variations in pH had noticeable effects on protein transmission depending on the nature and magnitude of the membrane charge.
2.6.3 Plasma surface modification

Plasma treatment of membranes is conducted by exposing the membrane to a continuous electrical discharge in either an inert gas such as Ar or He or a reactive gas such as CO$_2$ or N$_2$. Kramer et al. (1989) pointed out that not only the plasma process characteristics such as gas flow rate, gas pressure and plasma duration play a role in defining the subsequent characteristics of the polymeric membrane but also the nature of the underlying substrate and its surface morphology. A simple schematic of a typical plasma treatment device is shown in Figure 2.12:

![Figure 2.12. Schematic of a plasma generator.](image)

The effects of plasma treatment on a porous membrane are generally divided into three categories (Kemmere and Keurentjes, 2001):

- crosslinking of the active layer and pore size reduction; the latter is achieved by careful selection of exposure time of the membrane surface to the plasma (Gancarz et al., 2002)
- introduction of functional groups to the surface; commonly, oxygen- or nitrogen-containing groups may be introduced on the surface depending on the gas employed (Gancarz et al., 1999)
• grafting and deposition of a thin selective layer onto the membrane; based on plasma induced graft polymerisation where the plasma is generated from gaseous organic monomers (Bryjak, 1994).

Surface modification of membranes by plasma is a non-destructive method which can result in increased hydrophilicity of the membrane. In addition, polymer segments can be chemically functionalised in order to exploit surface charge effects. The advantage of this technique lies in the active species generated by the plasma, which can “activate the upper molecular layers on the surface, thus improving wettability, adhesion and biocompatibility without affecting the bulk of the polymer” (Gancarz et al., 1999) In addition, the plasma treatment time is short, handling of the plasma generator is relatively easy and the method is efficient and environmentally friendly (Bryjak et al., 2004). Yasuda (1984) first introduced low-temperature plasma modification as a viable technique to improve the surface functionality of a polymeric membrane.

Plasma treatment can be applied to a range of membrane types but the interest of this work lies in the modification of PES membranes. The microwave plasma technique relies on the use of non-deposited gases such as air, oxygen, carbon dioxide, nitrogen, ammonia or argon. Even though in many circumstances the pore size seems to reduce during the plasma modification process it is dependent on the gas used. With carbon dioxide, for instance, the pores tend to become larger the longer the plasma action is applied (Bryjak et al., 2004). The modification using gas plasma has the drawback that the modified membrane surface can be subject to changes within several days after the initial treatment.

Plasma polymerisation can also result in partial or even total plugging of membrane pores depending on the gas reaction mixture used (e.g. n-butylamine on its own results in pore reduction). On the contrary, if argon is added to the reaction mixture a degradation of the polymer matrix occurs and hence larger pores are obtained (Pozniak et al., 2002). During plasma action substrate etching and polymer deposition seem to compete with each other where the former increases pore size and the latter decreases it.

One has to distinguish between non-polymerising and polymersing plasma. Non-polymerising plasma such as argon is more likely to penetrate deeper into the polymer
than a polymerising plasma gas such as butane. Furthermore, studies by Steen et al. (2001) suggest that the extent of penetration of the membrane structure also depends on the type of plasma system used. In contrast, earlier work by Yasuda et al. (1994) suggested that the type of plasma used is not important in terms of the resulting extent of the surface penetration. The successful modification of a membrane by plasma, particularly when a change beyond a certain depth of the membrane surface is desirable, strongly depends on polymer type. Johansson and Masuoka (1999) showed that polycarbonate membranes are successfully penetrated by the plasma gas whereas no penetration of the plasma-forming species is observed when using nylon- or poly(vinylidene fluoride)-based membranes.

2.6.4 Modification of polyethersulphone membranes

One of the first modifications of PES membranes by low-temperature plasma treatment was conducted by Chen and Belfort (1999) using helium plasma followed by the grafting of n-vinyl-2-pyrrolidone (NVP) onto the membrane surface. Helium plasma alone achieved a significant increase in the hydrophilic character of the membrane. The reduced susceptibility to fouling was compared to the virgin membrane and commercially available low-protein binding membranes. In both cases the plasma-modified membrane gave the best result. Steen et al. (2002) managed to modify PES membranes with a similar degree of permanent hydrophilicity. The claim for substantial improvement in hydrophilicity of the membrane surface was supported with contact angle data where a reduction in the contact angle after modification could be observed. Modification of polyethylene membranes, however, did not necessarily result in permanent hydrophilicity. This problem was previously noted when modifying polymers in order to increase their wettability (Gengenbach et al., 1994). The contact angle of the water/air interface became larger with storage time because of surface reactions with air. In other studies Wavhal and Fisher (2002 and 2003) employed both argon plasma alone and argon plasma treatment followed by polyacrylic acid grafting or acrylamide grafting, respectively. Only the combined plasma and grafting process resulted in permanent membrane hydrophilicity. Desirable benefits such as improved ease of cleaning, reduced protein fouling and an increase in water flux where all observed with plasma-modified membranes (Kull et al., 2005).
Low-temperature plasma modification using oxygen, acrylic acid (AA), acetylene, diaminocyclohexane (DACH) and hexamethyldisiloxane (HMDSO) as the plasma gas of PES membranes was conducted by Cho et al. (2004) Both acetylene and HMDSO were found to deteriorate the membrane performance and the membrane also became hydrophobic. Membranes treated with the other gases all became hydrophilic, exhibited improved fouling resistance and gave a higher process flux than unmodified PES membranes. A low initial flux was obtained with DACH-modified membranes, a high or low initial flux was obtained with AA-modified membranes (dependent on the plasma modification conditions), and a higher flux but a lower rejection were obtained with oxygen-treated membranes.

2.6.5 Modification of polysulphone membranes

Gancarz et al. (1999) pointed out that the modification of PSU (Figure 2.13) membranes in particular has been attempted with plasmas of various gases and one of the findings made was that the surface modification is strongly influenced by the nature of the feed gas as well as the modification conditions.

![Figure 2.13. The chemical structure of polysulphone (Kim et al., 2002).](image)

The surface modification of PSU membranes has been induced using plasma generated from various types of gases (Bryjak et al., 2002; Chen et al., 1997; Chen and Belfort, 1999, Gancarz et al., 2000, Gancarz et al., 2002; Hopkins and Badyal, 1996a; Hopkins and Badyal, 1996b; Hopkins and Badyal, 1996c; Johansson et al., 1998; Keil et al., 1998; Kim et al.; 2002; Lee et al.; 1996; Pozniak et al., 2002; Ulbricht and Belfort, 1996, Van Delden et al., 1997; Wavhal and Fisher, 1995) Among the various gases Argon is most frequently used and believed to be more efficient than, for instance, Oxygen or Carbon Dioxide (Gancarz et al., 1999) (see Figure 2.14)

Gancarz et al. (1999) also attempted the modification of PSU membranes by plasma grafting and plasma polymerisation of acrylic acid. In Section 2.6.1 it was mentioned that an advantageous change in the surface properties of a membrane induced by a non-
polymerising gas is not necessarily permanent. In fact, the hydrophilic groups introduced and the acidic character of the new, modified membrane may deteriorate with storage time (Gancarz et al., 1999).

![Figure 2.14. Effect of plasma distance and plasma gas employed on grafting yield, adapted from Gancarz et al. (1999).](image)

Subsequent immersion in water of plasma-treated membranes which were stored in air could not fully restore the wettability of the plasma treated surfaces, as observed by Terlington et al. (1995). These property changes with time are often referred to as “hydrophobic recovery”. In order to alleviate this problem the attachment of a polymer chain to the membrane surface by introducing functional groups was attempted (Gancarz et al., 1999). A low-temperature H₂O plasma treatment method developed by Steen et al. (2001) achieved permanent hydrophilicity of PSU membranes whilst modification of the entire membrane cross-section was achieved as a consequence of the plasma generator used. This result could be put into question, however, because it is generally accepted that a key limitation of the plasma-modification technique is that the modification only occurs at the membrane surface so that in the depth of the pores no charge is to be expected and fouling will still be an issue (Asatekin et al., 2007). Furthermore, tests with argon or helium-based plasmas did not result in permanent membrane hydrophilicity as was obtained with H₂O plasma suggesting that the choice
of gas is also crucial. Hydrophobic recovery effects can be clearly observed with nitrogen or ammonia modified membranes whose polarity decreased to about a third of the polarity achieved directly after treatment (Bryjak et al., 2002; Gancarz et al., 2000). However, the remaining polarity was permanent and due to stable residual functional groups (Bryjak et al., 2004).

Gancarz et al. (2002) also investigated the properties of PSU membranes modified by depositing amines on the membrane surface by use of the microwave plasma technique. Both, butylamine and allylamine (ALY) were employed but only the latter was found to improve membrane filtration performance. In addition, the use of both of these amines as the plasma medium was found to decrease the surface tension of PSU. Membranes modified with ALY plasma exhibit promising filtration behaviour in both acidic and basic environments. This becomes of interest in relation to proteins, because they can contain surface groups such as carboxylic and amine. Membranes produced with ALY plasma are particularly interesting because they also contain both amine and carboxyl groups on their surface, thus exhibiting amphoteric character, which may be exploited to promote electrostatic repulsion effects during protein filtration.

Oxygen plasma treatment of PSU membranes introduces hydroxyl, carbonyl and carboxyl groups onto the membrane surface where the hydroxyl concentration increases with plasma treatment time but the carbonyl and carboxyl group concentration remains stagnant (Kim et al., 2002). Another interesting observation by this group was the shift in the pI from pH 3 to pH 4.5 with the membrane modification during their filtration experiments with gelatine solution, indicating a change in the charge structure of the membrane surface. In addition, a better cleaning efficiency was observed for the oxygen plasma-modified membrane (60% of the original water flux was restored) compared to the unmodified one (50% of the original water flux was restored).

Carbon dioxide plasma treatment has been reported to result in PSU membranes with a water contact angle of zero, which was maintained for several months (Wavhal and Fisher, 2005). An increase in plasma treatment time seemed to correlate with a decrease in contact angle and protein adsorption could be reduced by up to 75% with the plasma-modified membrane compared to the unmodified one. Earlier work by Gancarz et al. (1999) using CO₂ plasma treatment gave a substantial decrease in the water contact
angle (88° down to 36°) but did not confirm the results obtained by Wavhal and Fisher (2005). Protein filtration results have also been improved in terms of more selective retention and less adhesion to the membrane surface as a result of the work by Gancarz et al. (1999) but again not as profoundly as in the work by Wavhal and Fisher (2005).

Low-temperature plasma treatment of PSU membranes followed by grafting of polyethylene glycol (PEG) onto the membrane surface was attempted by Song et al. (2000). A reduction in the contact angle and an increase in the membrane hydrophilicity were observed.

2.6.6 Modification of other polymeric membranes

Polyacrylonitrile membranes were treated using air plasma at different energies and the subsequent changes in the membrane functionality were investigated by Bryjak et al. (1996). Dextran solutions were used in order to estimate changes in the pore size distribution. For this type of membrane it was found that low-energy plasma (below 60 W) caused polymer etching and thus an increase in the pore diameter. In contrast, higher-energy plasma above 60 W up to 180 W did not have an effect on the pore diameter. Furthermore, contact angle measurements were used in order to support the hypothesis of a change in the surface energies due to plasma treatment. It was concluded that the surface energy increases when plasma power up to 60 W is used, beyond this point a gradual decline was observed. Similar behaviour was observed regarding the surface polarity which increased up to 60 W plasma power but beyond this energy level a reduction occurred (Bryjak et al., 1996).

Polypropylene and other polymer-based membranes were subjected to oxygen-, n-butane- and nitrogen-based plasma treatment by Johansson et al. (1998). As in most of the other cases, the resulting membrane was less prone to fouling and electrostatic interactions gained more importance. Polypropylene UF membranes were plasma treated with AA and ALY (Kang et al., 2001). In contrast to the work by Wavhal and Fisher (2002 and 2003), the hydrophilic properties of the membrane were not permanently sustained and difficulties such as pore blockage with increased plasma treatment time and damage to the membrane surface due to excessive power were observed. As expected, protein fouling was limited and the water flux was improved as a result of the plasma modification.
On a commercial scale, modified nylon MF membranes (N6,6 Posidyne® membranes, Pall corporation) with permanent positive charge are available. In literature, adsorption of yellow dye onto these membranes has been tested at pH 5.1 (the membrane has a pI of 7.6) and it was reported that the extent of adsorption increased with solution concentration (Jan and Raghavan, 1994).

### 2.6.7 Common effects during membrane modification

Nabe *et al.* (1997) compared surface-modified membranes to conventional ones and showed that the pure water flux for unmodified membranes was highest and a reduction in water flux was observed for all the chemically-modified membranes. However, an exception was the chemically-modified membrane with the lowest surface energy which had the highest pure water flux and was easiest to clean.

Water flux measurements also become important as a means of characterising unmodified and plasma-modified membranes. Non-polymerising gases such as CO₂ used during plasma surface modification, for instance, can cause a decline or an increase in water flux of the modified membrane depending on the plasma treatment time as demonstrated in Figure 2.15.

![Figure 2.15. Water flux dependence on CO₂ plasma treatment time for PSU membranes, adapted from Gascarz et al. (1999).](image_url)
The decrease in water flux at two minutes treatment time may seem rather obscure but according to Gancarz et al. (1999) this is due to possible deposition of degraded particles during the polymersation process which may take place at the same time as ablation. After six minutes treatment time ablation becomes more prominent, therefore a subsequent increase in water flux is observed because at this stage the pore size will have increased further so that potential adsorption of degraded membrane skin inside the pores becomes less significant. In a different study, Gancarz et al. (1999) investigated the graft polymerisation of AA onto PSU membranes. A high grafting yield resulted in significant water flux decline (as shown in Figure 2.16) so that the membrane lost its UF properties.

![Figure 2.16. Effect of grafting yield on PSU membrane permeability, adapted from Gancarz et al. (1999).](image)

The measurements conducted by this group also showed that modified membranes stored in air changed their wettability with time and hydrophilicity was reduced but was still high compared to conventional membranes as illustrated in Figure 2.18(a) and Figure 2.18(b).
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Figure 2.17. Surface tension dependence on plasma treatment time. (a) immediately after treatment; (b) after 24 h storage in air (Gancarz et al., 1999).

The small decrease in polarity observed after storage in air indicates the decreased wettability, which, however, was still relatively high. The authors also found a dependence of the contact angle measured with water solutions on pH.

2.7 Ultrafiltration devices

Over the years several devices have been designed for UF testing. There are a number of references available which discuss the types and operation of various membrane devices in greater detail (Baker, 2004; Cheryan, 1998 and Porter, 1990). In a laboratory environment most studies are carried out using cell type devices, which may either be operated in dead-end mode with or without a stirrer or in crossflow mode (see Figure 2.18).

Figure 2.18. Dead end vs. crossflow operation.
Chapter 2 - Literature Review

Other devices, more frequently encountered in industrial environments, include the plate and frame, the hollow-fibre, tubular and spiral wound. More recently, experiments with devices incorporating rotating disks or vibrating mechanisms were conducted (Jaffrin et al., 2004). Developments of novel membrane modules were reviewed by Charcosset (2006). At the industrial scale, some form of crossflow operation is used in virtually every application stressing the importance of the crossflow mode (Marshall et al., 1996). Some authors also refer to crossflow filtration as tangential flow filtration (TFF) (e.g. Ebersold and Zydney, 2004b; Almecija et al. 2007).

Hydrodynamic conditions can also influence the UF process, hence the choice of membrane module is quite important. Even though it is recognised that industrial applications are carried out using the crossflow arrangement, few authors have attempted to relate data from experiments with stirred cell and crossflow apparatuses. Mosqueda-Jimenez et al. (2004b) compared results for the filtration of test river water from different test units including dead-end and crossflow cells. Dead-end cells were found to perform very differently from continuous cells and the authors recommended that a continuous cell should be used when the thorough evaluation of a particular membrane is desired. The authors chose to maintain a constant crossflow velocity of 0.2 m/s in the different test cells as their basis of comparison, and provided a mathematical solution to calculate the crossflow velocity in a circular cell where fluid flow is non-uniform. Schipolowski et al. (2006) pointed out that test cell results can vary considerably depending on their design geometry and problems are often encountered when scale-up to larger units is desired. Their work focussed on how scale-up may be improved using existing devices and they concluded that the use of a larger membrane sample size does not seem to greatly improve reliability, but found the testing of a few samples from the same sheet can help to reduce the overall error. More recently Zydney and Xenopoulos (2007) investigated the influence of device configuration on dextran rejection for a stirred cell and a tangential flow cell (Millipore Minitan S unit), where, in the latter device, the membrane is situated on a gasket below which the flow is partitioned into nine different channels. Their study concluded that stirred cells provide a more accurate representation of the impact of concentration polarsation and better data reproducibility than the Minitan device. This is likely to be attributable to the special design of the tangential flow cell. Their general conclusion
was that the simpler design of the stirred cell makes it better suited to dextran retention tests.

2.8 Conclusions

This review has contextualised the different phenomena that influence UF such as solute size, pH, ionic strength, membrane charge and hydrodynamic conditions and also highlighted the potential benefits of membrane surface-modification by plasma to improve UF processes. An introduction to fouling phenomena, one of the main limitations to further commercial application of UF membrane processes, has been given.

Low-temperature plasma treatment holds promise as a way to alter the membrane hydrophilicity and to incorporate functional groups on the membrane surface that may allow selective separation of proteins. However, at present limited data is available on the filtration performance of such membranes.

A substantial body of literature is available pertaining to protein filtration using conventional membranes, fouling and cleaning of membranes. However, as can be seen from this literature review, research concerning the effectiveness of surface-modified membranes modified by plasma to reduce fouling and to exploit surface charge effects is currently limited. Furthermore, little work has been done to-date relating results obtained from different membrane test cells. As indicated in the introduction, this research work has several aims one of which is to evaluate the separation performance of plasma-modified UF membranes. Other aims include the study of solution pH and ionic strength effects on protein filtration and the impact of such parameters when different filtration devices are used.
Chapter 3 - Modelling of the Ultrafiltration Process

Most models concerned with protein ultrafiltration (UF) or microfiltration (MF) are related to flux decline and fouling. Tracey and Davis (1994), for instance, divided their theory of flux decline into two steps: Firstly, adsorption or deposition of proteins at the pore entry and membrane occurs followed by a build-up of a solute layer adjacent to the membrane surface as a result of agglomerate formation or general aggregation of more molecules at the surface. Choi et al. (2000) used a resistance-in-series model to predict permeate flux of bovine serum albumin (BSA) during MF using a stirred cell. In their study, the authors looked at BSA filtration in the presence and in absence of microspheres. They found that an increase in the BSA concentration and an increase in the stirrer speed only resulted in permeate flux increase in the presence of the microspheres. The authors hypothesised that this was due to agglomeration of microspheres onto which BSA had adsorbed, hence effectively creating larger diameter particles which are known to give rise to increased flux.

This chapter examines several protein filtration models to assess their usefulness in the current work. Based on the considerations presented the resistance-in-series model was employed in Chapters 6 and 7 in order to quantify the extent of fouling. In the same chapters further work was conducted using the same model basis to take into account concentration polarisation and charge effects. More detailed derivations of some of the equations presented in this chapter are provided in Appendix C.

3.1 Ultrafiltration models

Several theoretical models have emerged that attempt to simulate the various phenomena occurring during UF processes (Bhattacharjee et al., 1996; Le and Howell, 1984; Porter, 1972; Pradanos et al., 1995; Suki et al., 1986; Vincent Vela et al., 2007; Wijmans et al., 1984; Wijmans et al., 1985; Zydney, 1997) The most common include the stagnant film, resistance-in-series and gel polarisation models which attempt to predict permeate flux decline.
There are a variety of factors which can impact on flux in UF applications adding to the complexity of some of the more explicit models. Such factors include the membrane MWCO, feed concentration, solute size, diffusion coefficient, solute charge, membrane charge, solution pH, solution ionic strength and hydrodynamics (crossflow rate/stirrer speed).

The modelling of UF processes in terms of solute rejection and permeate flux in particular can therefore not be universally applied and is dependent on system conditions.

3.1.1 Rejection coefficients

The most widely applied terms in UF relate to rejection or transmission of the solute, although many alternatives exist the terms are essentially equivalent in that they can be related to each other (the choice of terminology employed in this thesis is provided here). The observed membrane rejection, \( R_o \), may be stated as:

\[
R_o = 1 - \frac{C_p}{C_f}
\]  

(3.1)

where \( C_p \) is the concentration in the permeate and \( C_f \) the feed or bulk concentration. This observed rejection does not account for the formation of a concentration polarisation layer. If such polarisation is to be considered then the feed or bulk concentration, \( C_f \), has to be replaced by \( C_M \) which represents the solute concentration at the membrane surface

\[
R_n = 1 - \frac{C_p}{C_M}
\]  

(3.2)

giving the "true" rejection coefficient, \( R_n \). The concentration at the membrane surface is difficult to find experimentally but a mathematical model can be developed to determine the "true" rejection coefficient (see Section 3.1.3).

It is also noteworthy that in the literature, instead of rejection, sieving coefficients (referring to transmission) are frequently utilised so that:
where $S_o$ is the observed sieving coefficient. This is important because using the sieving instead of the rejection coefficient will result in a slightly different equation when the observed and true coefficients are related to the concentration polarisation model (see also Section 3.1.2 and Section 7.3).

3.1.2 Selection of Ultrafiltration models

The literature provides a wide range of models applicable to UF. Some of the more common models and selected alternative models which attempt to explicitly incorporate additional effects such as charge are presented. Subsequently, models used to interpret experimental results obtained in the present work are discussed in greater detail. This is supported by additional reasoning for the choice of models. Moreover, a model incorporating membrane charge and therefore suited to the interpretation of rejection data obtained with plasma-modified membranes is presented. This model is developed in further detail in Chapter 7.

Table 3.1 provides an overview of different UF models which were considered for modelling purposes. However, only models which were useful in the interpretation of the experimental data were considered in greater detail. These models are further discussed in this section and also in the analysis in Chapters 6 and 7.
### Chapter 3 - Modelling of the Ultrafiltration Process

#### Table 3.1. Ultrafiltration models considered.

<table>
<thead>
<tr>
<th>Model name</th>
<th>Key equation(s)</th>
<th>Description/Comment</th>
</tr>
</thead>
</table>
| Collodial interaction model<sup>a</sup>         | \[ u \frac{\partial C}{\partial x} + v \frac{\partial C}{\partial y} = \frac{\partial}{\partial y} \left[ D_w(C) \frac{\partial C}{\partial y} \right] \]  
\[ \frac{d^2 \psi}{dr^2} + \frac{d \psi}{r \ dr} = \frac{2n_0 \ e}{\varepsilon_o \varepsilon_r} \sinh \left( \frac{e \ v \ e}{k_B T} \right) \] | Combines convection-diffusion, osmotic pressure and charge interaction effects into one model.  
Limited as it assumes a dependence on zeta potential  
Considers charge interaction influences by particle size and surface charge  
Primarily used to interpret the behaviour of more concentrated feeds. |
| Convection-diffusion-electrophoretic migration model<sup>b</sup> | \[ \frac{C_w}{C_b} = \exp \left( \frac{Jd}{D} \right) \exp \left( -\frac{\mu \psi +}{D} \right) \] | Extension of the film model by inclusion of electrokinetic effects  
Attempts to account for some form of charge interaction, but limited to repulsive charge interactions only. |
| Combined film and charge interaction model<sup>c</sup> | \[ S_o = \frac{S_o}{(1-S_o) \exp \left( \frac{J}{k} \right) + S_o} \]  
\[ \phi = (1 - \lambda)^2 \exp \left( -\frac{\psi_g}{k_B T} \right) \] | Extension of the film model, but extended to also consider charge interactions of solutes with the pore wall  
Relates solute rejection and membrane charge and should ideally 'predict' solute rejection for a given system |
| Film model<sup>d</sup> | \[ J = \frac{D}{\delta} \ln \frac{C_x}{C_b} \] | Developed to account for concentration polarisation effects  
Allows the calculation of concentration at the membrane wall and thus true rejection coefficient |
| Gel layer model<sup>e</sup> | \[ J = k \ln \frac{C_g}{C_b} \] | Extension of the film model which deals with the formation of a highly concentrated layer  
Calculates an additional layer of resistance at the membrane surface if higher pressures are applied |
### Model name | Key equation(s) | Description/Comment
---|---|---
**Mechanistic/Aggregation model** | \( J = \frac{\Delta P}{\eta} \left( R_m + \sum_{i=1}^{k} (\delta_x / P_x) \right) \) | Considers double layer interactions between solute molecules and accounts for flocculation effects. The model is unable to account for the electrokinetic properties such as pH and ionic strength. Assumess protein aggregation in the concentrated layer at the membrane surface thereby increasing resistance. Used to predict long-term flux decline.

**Osmotic pressure model** | \( J = \frac{1}{\mu} \left( \frac{\Delta P - \Delta \pi_m}{R_m} \right) \) | Similar to the film and resistance models but also considers osmotic pressure effects. Such effects are more of a concern in UF if the feed concentration is high or also if the fouling/gel layer has a very high concentration.

**Resistance-in-series model** | \( J = \frac{\Delta P}{\mu(R_m + R_i)} \) | Attributes flux decline to different resistance layers. Distinguishes and quantifies factors such as membrane resistance, fouling resistance and 'cake' resistance (if applicable).

**Shear controlled model** | \( J = J_0 \left( \frac{\tau_w}{\tau_{sw}} \right)^n a e^{-\frac{\mu}{\eta} \tau_{sw}} \) | The major resistance is assumed to be due to a foulant layer. Limited in that constants are essentially fitted to filtration data. Postulates a flux decline due to a concentration increase counteracted by shear at the wall.

---

* (Bowen *et al*., 1996)
* (Rabiller-Baudry *et al*., 2000)
* (Burns and Zydney, 1999)
* (Blatt *et al*., 1970)
* (Porter, 1972)
* (Suki *et al*., 1986)
* (Pradanos *et al*., 1995)
* (Fane and Fell, 1987)
* (Sayed Razavi and Harris, 1996)
Chapter 3 - Modelling of the Ultrafiltration Process

Nomenclature for Table 3.1

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a$</td>
<td>Constant in shear controlled model</td>
</tr>
<tr>
<td>$b$</td>
<td>Constant in shear controlled model</td>
</tr>
<tr>
<td>$C$</td>
<td>Concentration (kg/m³)</td>
</tr>
<tr>
<td>$C_b$</td>
<td>Concentration of solute in the bulk (kg/m³)</td>
</tr>
<tr>
<td>$C_g$</td>
<td>Solute concentration at the gel layer surface (kg/m³)</td>
</tr>
<tr>
<td>$C_w$</td>
<td>Concentration at the membrane wall (kg/m³)</td>
</tr>
<tr>
<td>$d$</td>
<td>Thickness of the polarsation layer (m)</td>
</tr>
<tr>
<td>$D$</td>
<td>Diffusion coefficient/diffusivity (m²/s or cm²/s)</td>
</tr>
<tr>
<td>$D_m(C)$</td>
<td>Mutual diffusion coefficient (m²/s)</td>
</tr>
<tr>
<td>$e$</td>
<td>Elementary charge (1.6x10⁻¹⁹)(C)</td>
</tr>
<tr>
<td>$J$</td>
<td>Permeate flux (m³/m² s or L/m² h)</td>
</tr>
<tr>
<td>$k$</td>
<td>Mass transfer coefficient (m/s)</td>
</tr>
<tr>
<td>$J_i$</td>
<td>Initial permeate flux (L/m² h)</td>
</tr>
<tr>
<td>$k_B$</td>
<td>Boltzmann constant (1.38x10⁻²³) (J/K)</td>
</tr>
<tr>
<td>$n$</td>
<td>Power number (-)</td>
</tr>
<tr>
<td>$n_s$</td>
<td>Number of ions per unit volume in the bulk electrolyte (1/m³)</td>
</tr>
<tr>
<td>$\Delta P$</td>
<td>Applied pressure or transmembrane pressure drop (Pa)</td>
</tr>
<tr>
<td>$P_x$</td>
<td>Hydraulic permeability (m²)</td>
</tr>
<tr>
<td>$r$</td>
<td>Radial coordinate (m)</td>
</tr>
<tr>
<td>$R_m$</td>
<td>Membrane resistance (1/m)</td>
</tr>
<tr>
<td>$R_t$</td>
<td>Total resistance (1/m)</td>
</tr>
<tr>
<td>$S_x$</td>
<td>Actual sieving coefficient (-)</td>
</tr>
<tr>
<td>$S_o$</td>
<td>Observed sieving coefficient (-)</td>
</tr>
<tr>
<td>$T$</td>
<td>Temperature (K)</td>
</tr>
<tr>
<td>$u$</td>
<td>Tangential velocity (m/s)</td>
</tr>
<tr>
<td>$v$</td>
<td>Transverse velocity (m/s)</td>
</tr>
<tr>
<td>$W$</td>
<td>Concentration (wt%)</td>
</tr>
<tr>
<td>$x$</td>
<td>Longitudinal coordinate (m)</td>
</tr>
<tr>
<td>$y$</td>
<td>Transverse coordinate (m)</td>
</tr>
<tr>
<td>$z$</td>
<td>Valency (-)</td>
</tr>
<tr>
<td>$\Delta \pi_m$</td>
<td>Membrane osmotic pressure difference (-)</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Boundary layer thickness (m)</td>
</tr>
<tr>
<td>$\delta_t$</td>
<td>Layer thickness (m)</td>
</tr>
<tr>
<td>$\varepsilon_r$</td>
<td>Dielectric constant (-)</td>
</tr>
<tr>
<td>$\varepsilon_0$</td>
<td>Permittivity of free vacuum (C/V m)</td>
</tr>
<tr>
<td>$\phi$</td>
<td>Solute partitioning coefficient (-)</td>
</tr>
<tr>
<td>$\eta$</td>
<td>Dynamic viscosity (Pa s)</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>Solute radius to pore size radius ratio (-)</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Solution viscosity (Pa s)</td>
</tr>
<tr>
<td>$\mu_a$</td>
<td>Electrophoretic mobility (m²/V s)</td>
</tr>
<tr>
<td>$\tau_w$</td>
<td>Shear stress at the wall (Pa)</td>
</tr>
<tr>
<td>$\tau_{wm}$</td>
<td>Initial wall shear stress (Pa)</td>
</tr>
<tr>
<td>$\psi$</td>
<td>Electrostatic potential (V)</td>
</tr>
<tr>
<td>$\psi_E$</td>
<td>Electrostatic energy of interaction (J)</td>
</tr>
<tr>
<td>$\psi_z$</td>
<td>Zeta-potential of the charged surface (V)</td>
</tr>
</tbody>
</table>
In order to model the experimental results obtained in this work the resistance-in-series model was considered appropriate to quantify the extent of fouling at varying pH and ionic strength. A form of this model was thus applied to model data for unmodified as well as plasma-modified membranes. The majority of the models do not explicitly consider any charge on the membrane surface. A model which is able to account for solute interactions with plasma-modified membranes would be of great interest and the closest attempt was made by Zydney and co-workers (Burns and Zydney, 1999; Burns and Zydney, 2001; Mehta and Zydney, 2006; Menon and Zydney, 1999; Mochizuki and Zydney, 1992). Their model is based on theoretical developments of electrostatic double-layer interactions for spherical colloids in cylindrical pores by Smith and Deen (1980). Thus, in addition to the resistance-in-series model, a different form of the combined film and charge interaction model listed in Table 3.1 was developed and applied in Chapter 7. For completeness, it is noted that inertial lift models exist and have been used by some researchers to interpret flux behaviour during membrane filtration. However, inertial phenomena become unimportant and are overtaken by electrokinetic effects for colloids and molecules in the sub-micron range relevant to UF (McDonogh et al., 1989).

The resistance-in-series model is based on a form of Darcy's law as shown in equation (3.4):

\[ J_s = \frac{Q}{A_m} = \frac{\Delta P}{\mu R_I} \]  

(3.4)

where \( J_s \) is the flux, \( Q \) the flow rate, \( A_m \) the membrane area, \( \Delta P \) the applied pressure gradient, \( \mu \) the liquid viscosity and \( R_I \) the total resistance to flow. The total resistance is commonly divided into three resistances (Fane and Fell, 1987), the resistance of the membrane, \( R_m \), the solute or ‘cake’ resistance, \( R_c \), and the resistance due to fouling, \( R_f \). Strictly speaking ‘cake’ resistance is only applicable to the formation of a thick layer in the presence of particles and liquid whereas in UF most applications deal with molecules and colloids, thus such a resistance is better referred to as solute resistance, \( R_s \).
Van den Berg (1988) sub-divided these resistances further into the resistance due to concentration polarisation, $R_{cp}$, the gel layer resistance, $R_g$, the resistance due to pore blocking, $R_p$, and that due to adsorption, $R_a$. Addition of the latter two can be expressed as the resistance due to fouling, $R_f$, and any solute layer resistance, $R_s$, may be viewed as a combination of the concentration polarisation layer, $R_{cp}$, and also any gel layer, $R_g$, if applicable. The possible resistances to solvent transport are shown in Figure 3.1.

$$R_t = R_m + R_a + R_f$$

Figure 3.1. Possible resistances to solvent transport.

Note that all resistances mentioned here, apart from the membrane resistance, $R_m$, are time-dependent and can change with filtration time. The membrane resistance, $R_m$, is the resistance of the clean membrane which can be obtained from clean water permeation data as shown in Figure 3.2.
The membrane resistance is most commonly taken to be equal to the membrane thickness, $L_m$, divided by the hydraulic membrane permeability, $k_p$. It can be obtained from the slope of a plot of clean water flux versus pressure. The definition of permeability is subject to confusion in the area of membrane science. Most commonly, the hydraulic permeability of pressure-driven membrane processes is reported as (Boyd and Zydney, 1998; Cheryan, 1998, Costa and de Pinho, 2005; Mulder, 1991):

$$L_p = \frac{J}{\Delta P}$$  \hspace{1cm} (3.6)

where $J$ is the solvent flux. The hydraulic membrane permeability in equation (3.6) is simply evaluated by dividing the pure water flux by the transmembrane pressure (TMP) and mostly reported in $L/m^2 h$ bar (Some authors will also include the solution viscosity in the numerator so that the membrane permeability becomes a measure of distance). The true permeability, $k_p$, generally used in filtration science is reported in $m^2$ and can be obtained from the slope of a flux versus pressure plot (see Figure 3.2) as given by Darcy's law (3.7):

$$J_s = \frac{Q}{A_m} = \frac{k_p \Delta P}{\mu L_m}$$  \hspace{1cm} (3.7)
Chapter 3 - Modelling of the Ultrafiltration Process

In this thesis, in order to avoid confusion, when equation (3.6) is used the term permeance is used.

The widely used stagnant film model was first developed by Blatt et al. (1970) and later reviewed by several authors (e.g. Fane and Fell, 1987; Suki et al., 1986; Zydney, 1997). The film model, sometimes also referred to as the boundary layer film model or the convection-diffusion model, assumes the existence of a thin, unmixed layer between the membrane surface and the fluid boundary layer (Baker, 2004). During pressure-driven membrane filtration, solute is transferred to the membrane surface by convective flow and a concentration gradient is formed at the membrane due to solute rejection. The concentration gradient results in back-diffusion of solute into the bulk solution and was illustrated in the literature review (see Figure 2.3). It is mathematically expressed as follows:

\[ J_f C_f - D \frac{dc}{dx} = J_r C_r \]  

(3.8)

where \( D \) is the diffusion coefficient. Applying the boundary conditions shown in Figure 2.3, equation (3.8) can be integrated to give (see Appendix C, part b).

\[ J_f = \frac{D}{\delta_b} \ln \frac{C_M - C_f}{C_f - C_p} \]  

(3.9)

where \( \frac{D}{\delta_b} = k_m \) represents the mass transfer coefficient which can be determined using dimensionless analysis analogous to heat transfer

\[ Sh = \frac{k_m d_h}{D} = X \frac{Re \cdot Sc}{Re \cdot Sc} \left( \frac{d_h}{L} \right)^x \]  

(3.10)

where \( d_h \) is the hydraulic diameter (refer to Section 4.7.2 and Appendix C, part a) and \( L \) is the axial membrane length. The dimensionless numbers are the Sherwood, Reynolds and Schmidt number, respectively (see Table 3.2).
Chapter 3 - Modelling of the Ultrafiltration Process

Table 3.2. Dimensionless numbers.

<table>
<thead>
<tr>
<th>Dimensionless number</th>
<th>Equation</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sherwood</td>
<td>( k_m d_b / D )</td>
<td>( Sh )</td>
</tr>
<tr>
<td>Reynolds</td>
<td>( \rho u d_b / \mu )</td>
<td>( Re )</td>
</tr>
<tr>
<td>Schmidt</td>
<td>( \mu / \rho D )</td>
<td>( Sc )</td>
</tr>
</tbody>
</table>

The constants \( \alpha, \beta \) and \( \chi \) are dependent on the flow regime and the membrane module. The diffusivity coefficient, \( D \), is the coefficient of proportionality between molecular flux and the concentration gradient that can be determined using theoretical correlations or measured diffusivity data, if available. An approach to determine the diffusivity coefficient and find \( C_M \) in order to solve equation (3.9) is developed in Section 3.1.3. If the gel polarisation model is applicable then equation (3.9) may still be used, but then \( C_M \) is replaced with the concentration at the gel, \( C_G \), and the thickness of the boundary layer, \( \delta_b \), then also includes the gel layer thickness (see also Figure 3.3).

Other classical models are based on osmotic pressure and gel formation. The former is mostly applicable to aqueous solutions with low molecular weight solutes whereas the latter is more suited to modelling of solutons with high molecular weight solutes (Choi et al., 2003). The gel layer model is an extension of the film model (see also Figure 2.3 and Table 3.1) for concentration polarisation where, in addition to the concentration profile, the formation of a dense molecule layer at the membrane surface is assumed, as shown in Figure 3.3. This phenomenon generally occurs at a TMP beyond which the flux does not increase further.
3.1.3 Stagnant film model extension

Equation (3.9) can be expressed in a different form by including the apparent/observed (equation (3.1)) and the true/intrinsic (equation (3.2)) rejection coefficients (Cheryan, 1998). Once the observed rejection and flux data are known, the mass transfer coefficient can be calculated using a correlation of the form shown in equation (3.10) so that the true rejection can be calculated from equation (3.11):

\[
\ln \left( \frac{1 - R_o}{R_p} \right) = \ln \left( \frac{1 - R_p}{R_p} \right) + \frac{J_s}{k_m}
\]  

(3.11)

Further detail of the mathematical steps required to obtain equation (3.11) are shown in Appendix C, part b. The diffusion coefficient, \(D\), was determined from the correlation developed by Young et al. (1980):

\[
D = 8.34 \times 10^{-8} \left( \frac{T}{\mu(M_w)^{1/2}} \right)
\]  

(3.12)
where \( T \) is the absolute temperature, \( \mu \) the viscosity (in cP) and \( M_w \) the molecular weight. The calculated diffusion coefficients for BSA and lysozyme (LYZ) at 22°C and reported diffusivity data at 20°C are shown in Table 3.3.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Calculated diffusivity* (m(^2)/s)</th>
<th>Reported diffusivity* (m(^2)/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>6.08x10(^{-11})</td>
<td>6.10x10(^{-11})</td>
</tr>
<tr>
<td>LYZ</td>
<td>1.00x10(^{-10})</td>
<td>1.13x10(^{-10})</td>
</tr>
</tbody>
</table>

*Equation by Young et al  
*(Chen et al., 2007) and (Muller et al., 2003)

Table 3.3 shows that the calculated diffusion coefficients are in agreement with those reported in the literature, although the data for BSA matches more closely than that for LYZ. Table 3.3 also shows that diffusivity increases with a decrease in particle size. For the purpose of modelling the calculated diffusivity coefficients were employed.

### 3.2 Conclusions

Classical UF models have been described and in addition a range of models specifically taking into account factors such as aggregation or solute charge were presented. It was found that classical models such as the film model and resistance-in-series model provide a useful starting point to interpret protein filtration results. Some of the process models described provide the theoretical framework to conduct a more informed discussion of the experimental results in later chapters.

It was also desirable to determine a model which explicitly accounts for charge effects, of which few are available. The foundation for a suitable model relating rejection to charge interaction is provided in this chapter and further developed in Chapter 7.
Chapter 4 – Materials, Methods and Experimental Apparatus

In this section the method development for all experimental work is described together with the techniques and methods of operation for the experimental equipment used for characterisation and membrane filtration. The results of the characterisation and filtration experiments performed are shown in Chapters 5 to 7. Supporting information concerning the crossflow apparatus design and more detailed information regarding the UV/Vis characterisation method are provided in the Appendices.

4.1 Dextran

Dextran is a type of high molecular weight polysaccharide which is a polymer composed of monosaccharide units joined by α- or β-glycosidic linkages with the loss of a molecule of water (McIlroy, 1967). Dextrans are well suited to rejection characterisation experiments due to their globular shape and relatively low tendency to bind to the membrane surface. The dextran standards used in the membrane molecular weight cut-off (MWCO) analysis were of narrow polydispersity and obtained from PSS Polymer Service; values closer to 1 indicate a narrower molecular weight distribution (Table 4.1). In addition to the various dextrans, glucose, with a molecular weight of 180 Da, was also employed in order to test membrane rejection characteristics for a very low molecular weight substance. Data for all the dextrans used in this study are shown in Table 4.1.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Molecular weight (Da)</th>
<th>Mass average molecular mass (M&lt;sub&gt;w&lt;/sub&gt;)</th>
<th>Number average molecular mass (M&lt;sub&gt;n&lt;/sub&gt;)</th>
<th>Polydispersity (M&lt;sub&gt;w&lt;/sub&gt;/M&lt;sub&gt;n&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextran1</td>
<td>1,000</td>
<td>1,320</td>
<td>1,080</td>
<td>1.22</td>
</tr>
<tr>
<td>Dextran5</td>
<td>5,000</td>
<td>5,200</td>
<td>3,300</td>
<td>1.58</td>
</tr>
<tr>
<td>Dextran12</td>
<td>12,000</td>
<td>11,600</td>
<td>8,100</td>
<td>1.43</td>
</tr>
<tr>
<td>Dextran25</td>
<td>25,000</td>
<td>23,800</td>
<td>18,300</td>
<td>1.30</td>
</tr>
<tr>
<td>Dextran50</td>
<td>50,000</td>
<td>48,600</td>
<td>35,600</td>
<td>1.37</td>
</tr>
<tr>
<td>Dextran150</td>
<td>150,000</td>
<td>148,000</td>
<td>100,000</td>
<td>1.48</td>
</tr>
<tr>
<td>Dextran670</td>
<td>670,000</td>
<td>668,000</td>
<td>333,000</td>
<td>2.01</td>
</tr>
</tbody>
</table>

When used in the MWCO experiments, standard solutions of known concentration were filtered and the feed and permeate analysed via High-performance liquid chromatography (HPLC). A typical chromatogram for a range of dextran standards showing their elution times is provided in Appendix F, part a, where the largest standard
by molecular weight elutes first. For the purpose of analysis calibration curves for several standards with different concentrations were prepared and analysed using the refractive index (RI) detector (Section 4 6.1) of the HPLC unit (see Appendix D, Parts a to c). It is noteworthy that the gradients of RI versus concentration for the different standards were very similar. This not only confirms the repeatability of the analysis, but also demonstrates that dextran analysis via RI is concentration dependent only and independent of the dextran size.

4.2 Proteins

Proteins, as used in the filtration experiments, are amphoteric, that is, they carry both basic and acidic functional groups. It is these weakly acidic and basic side-chains which cause the charge on a protein, hence making it dependent on solution pH. The shape of proteins is generally ellipsoidal but can be considered globular or having another shape dependent on solution environment conditions (Saksena and Zydney, 1997). At its isoelectric point (pI) a protein has an overall net charge of zero and therefore electrical charge interactions should be minimal. Further, the hydrodynamic radius is important as the protein is likely to exhibit a larger radius in its hydrated form. Table 4 2 provides useful information about the two proteins bovine serum albumin (BSA) and lysozyme (LYZ) used in the experimental studies of this thesis The Stokes radii were obtained from Blake et al (1965) and Axelsson (1978), respectively. It must also be noted that the pI of a protein can be subject to slight shifts depending on the ionic strength and buffer solution (Lucas et al, 1998).

It is important to note that the Stokes radius (sometimes also referred to as the hydrodynamic radius), \( r_s \), includes solvent and shape effects implying that the actual protein radius can differ in size in different solution environmental conditions, a fact that needs to be considered when relating protein size to the pore radii in a membrane

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular weight (g/mol)</th>
<th>Stokes radius (nm)</th>
<th>No. of amino acids</th>
<th>Isoelectric point (pI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>66,430</td>
<td>3.5</td>
<td>607</td>
<td>4.7 - 4.9</td>
</tr>
<tr>
<td>LYZ</td>
<td>14,700</td>
<td>1.7</td>
<td>129</td>
<td>11.0</td>
</tr>
</tbody>
</table>

Table 4 2. Protein information.
Chapter 4 - Materials, Methods and Experimental Apparatus

The radii for BSA and LYZ have also been reported as 3.5 nm and 166 nm, respectively (Carter and Ho, 1994, Friedli, 1996) On this basis BSA and LYZ occupy a spherical volume of 180 and 19 nm³, respectively, which compares to volumes of 212 and 29 nm³, respectively, in their hydrated states. Such information becomes very useful when considering the thickness of a single water layer which is reported to be approximately 0.28 nm (Cantor and Schimmel, 1980). The increase in the radius of BSA is about 0.2 nm and that of LYZ about 0.24 nm suggesting that approximately one single water layer surrounds each protein. Opong and Zydney (1991), for example, determined a projected protein radius for BSA based on pore size distribution data through a membrane as 4.3 nm. The reported Stokes radius of LYZ is sometimes larger than that given in Table 4.2, e.g. the LYZ Stokes radius was reported as 1.83 nm by Rabiller-Baudry et al (2000), hence it is important to know which radius is being referred to.

4.2.1 Bovine serum albumin

BSA, which is composed of a single polypeptide chain, is known as a reasonably stable protein with a molecular weight (MW) of 66,430 Da belonging to a group of proteins soluble in water. BSA is commonly obtained from bovine blood, as a by-product of the beef industry, hence providing a low cost protein for extensive study in laboratories. It is a protein known to bind to a large number of specific anions and cations, as reported by Menon and Zydney (1999) The same authors also state the effective radius of the BSA sphere as 3.45 nm. When estimating whether a protein will transmit through a pore of a given size, information about its charge must be considered as the protein radius can be significantly enlarged when the protein charge is high. It is also worth noting that BSA has been reported to partially unfold between 40°C and 50°C, thereby exposing non-polar residues on the surface and facilitating protein-protein interactions (Peters and Sjöhholm, 1977). The structural stability of proteins is very important in relation to protein adsorption. BSA is a ‘soft’ protein and therefore has low internal structure stability (Chen et al, 2007). Soft proteins tend to adsorb on all surfaces irrespective of electrostatic interactions (Bos et al, 1994)

4.2.2 Lysozyme

LYZ is an enzyme with a MW of 14,700 Da generally obtained from egg white. The protein is structurally hard which means it is difficult to change its structure. Such
proteins tend to adsorb less on hydrophilic surfaces but more on hydrophobic ones, due to electrostatic interactions (Bos et al., 1994). LYZ is one of the few enzymes which easily crystallises and as a result one must consider that the classical tetragonal form of this protein becomes unstable at temperatures above ~30°C and is transformed into orthorhombic form (Osserman et al., 1974)

### 4.3 Buffer solutions

Protein filtration experiments were conducted using buffer solutions in order to maintain a reasonably stable pH. For low pH experiments an acetate buffer was used, for intermediate pH values a phosphate buffer and for high pH values a glycine buffer. Sodium azide (0.02%) was added to each buffer solution in order to prevent bacterial growth and reverse-osmosis treated water was used throughout. All pH measurements were carried out at room temperature using a Jenway 3310 pH meter.

Acetic acid was available in a highly concentrated (17.4 M), water-free form, and required dilution prior to use. One litre of 0.1 M acetate buffer requires 982.3 mL of acetic acid and 17.7 mL of sodium acetate in order to obtain a buffer with pH 3. Phosphate buffer solutions of pH 4.9, 6.0, 7.0 and 8.4, respectively, were prepared by varying the ratios of mono- and di-sodium phosphate Molarities of 20 mM and 100 mM were prepared. Experiments carried out with lysozyme at pH 11.0 were conducted in a glycine buffer. The buffer was prepared using aminoethanoic acid, sodium chloride and sodium hydroxide solution.

When a pH shift of a given buffer was desired, instead of adding hydrochloric acid or sodium hydroxide, a new buffer using the conjugate acid and base (which in turn determine the ionic strength) was prepared in order to obtain the desired pH at the desired ionic strength. This prevents the risk of a change in ionic strength as a result of the pH shift (Beynon and Easterby, 1996). The solution pH of a protein buffer was always re-measured to ensure no unexpected pH shifts took place.

### 4.4 Membranes

Polymeric membranes are widely used in ultrafiltration (UF) applications. The membranes are generally of asymmetric construction, characterised by a thin, active
layer (typically 0.5 μm thick) supported by a macroporous backing layer. The typical mean pore size of UF membranes is 1 to 100 nm (Bowen and Sharif, 1998; Desai, 2000). With asymmetric membranes, the separation is brought about by the top layer whilst the backing layer is characterised by its tortuous path and serves only to provide mechanical strength to the top layer. The work presented in this thesis was conducted using polyethersulphone (PES) membranes where the chemical structure of PES is shown in Figure 4.1.

![Chemical structure of polyethersulphone.](image)

It is noteworthy that PES membranes are widely applied in industrial scale crossflow (CF) applications because of their robustness and inherent performance properties, i.e. they are generally hydrophilic and can be operated over a wide pH range (Meyeroltmanns, 2007). All membranes used in this research are commercially available (Manufacturer data sheets are attached in Appendix G for both Millipore and Microdyn-Nadir membranes). PES membranes from two different manufacturers, Millipore Corporation and Microdyn-Nadir GmbH, were used as it was considered useful to have results from membranes prepared of the same material, but which still may exhibit different properties as a result of the production method used. Hoenich et al. (1996), for instance, conducted a clinical study using three polysulphone membranes from different manufacturers and they found them to be different because different alloying polymers were used during manufacture.

Nadir membranes were found to be more robust than Millipore membranes when exposed to low-temperature plasma treatment. Finally, nylon microfiltration (MF) membranes are also briefly described as these were used to pre-filter protein buffer solutions. Throughout this thesis PES membranes not subjected to plasma-modification are referred to as unmodified membranes. However, they were always prepared in a consistent manner (see Section 4.11) prior to any experiments and thus were not ‘untreated’.
4.4.1 Millipore membranes

All preliminary research work was conducted using Biomax PES membranes as manufactured by Millipore Corporation, USA. These membranes are asymmetric membranes with an active top layer and a microporous support. According to the manufacturer the membranes are hydrophilic, can be used up to temperatures of 50°C and their microstructure renders them particularly suitable for biological applications and protein filtration. Membranes were available with MWCO's of 10, 30, 50, 100 and 300 kDa. Since the membranes are supplied with a coating including glycerine to prevent them from drying it is necessary to follow a pretreatment regime. Further, when a membrane was to be stored over longer time periods then sodium azide was added to prevent bacterial growth and the membrane was kept in a 10% ethanol/water mixture and refrigerated, as recommended by the manufacturer. Zeta potential measurements by the manufacturer show Biomax membranes to be negatively charged above pH 3.

It is important to note that Biomax membranes are not solely made of PES but they are modified during manufacture using an undisclosed method in order to reduce non-specific protein binding. As a result of this modification the retention profile or MWCO is claimed to be tighter than that of conventional PES membranes. Scanning electron micrographs (SEMs) of Biomax membranes are presented in Section 5.6.1

4.4.2 Nadir membranes

PES membranes manufactured by Microdyn-Nadir GmbH, Germany, are asymmetric membranes with a thin, active layer at the top surface. It is only the active layer that is made of PES and the inner support layer is actually made of polypropylene (PP) surrounded by a polyethylene (PE) support. According to the manufacturer these membranes are permanently hydrophilic and have a high chemical resistance. In addition, they can be used over a wide pH range and are temperature resistant up to 95°C. Moreover, according to zeta potential data provided by the supplier these membranes carry a small negative charge.

The data sheet supplied states that prior to use a membrane should be rinsed in water for at least one hour and cleaned with a chemical agent such as sodium hydroxide to ensure the removal of any wetting agents. In addition, it is recommended to flush the membrane with water at a pressure twice as high as the intended operating pressure in...
order to reduce the impact of any compaction. Based on these instructions a pretreatment method was developed (see Section 4.11) and Nadir membranes were stored refrigerated, in deionised water (including 0.2% (w/v) of sodium azide) in order to prevent any bacterial growth. Nadir membranes were preferentially used for most of this research (see also Section 7.1) Scanning electron micrographs of Nadir membranes are presented in Section 5.6.2. Membranes were available with MWCO's of 10, 20, 30 and 50 kDa, although the majority of the research was conducted using 50 kDa membranes.

4.4.3 Whatman membrane

The Whatman MF membranes are 47 mm diameter Nylon filters with a mean pore size of 0.45 μm that were used as part of a pre-filtration step when preparing protein and buffer solutions. This pre-filtration was conducted under vacuum and allowed for the removal of any loosely formed aggregates or any other impurities from the feed.

4.5 Cleaning methodology

It was noted in the literature review (Chapter 2) that an appropriate membrane cleaning regime can have noticeable effects on membrane life time and the effective re-use of a previously fouled membrane. Depending on the type of fouling encountered, different cleaning agents were chosen and guidelines from the manufacturer followed to prevent adverse affects to the membrane material. The cleaning of PES membranes is important because of cost. Most industrial UF applications have a cleaning-in-place methodology incorporated in their filtration process so that a given membrane can be used several times. For this research it was also important to have a workable cleaning regime, because only a limited number of plasma-modified membranes could be produced and re-use of the same membrane in different experiments was occasionally required.

4.5.1 Removal of dextran fouling

Although dextran standards are considered as neutral molecules it is possible, particularly at high TMP, that they cause membrane fouling. Experimental work showed that the permeation rate of water was noticeably reduced after dextran filtration indicating that some form of fouling may have taken place. Thus, a sequence of experiments was designed to assess the likely effectiveness of using sodium hydroxide to remove dextran that was previously deposited during a filtration experiment or
deposited in another manner. The manufacturers' recommendation was to use 0.1 M NaOH. As this molarity did not seem very effective during initial cleaning experiments, sodium hydroxide solutions of different concentrations (0.1 M, 0.5 M and 1.0 M) were prepared in 50 mL flasks. A 0.1\% solution of 5,000 Da dextrans was prepared by dissolving 0.05 g in 50 mL of deionised water. A sample of each concentration of sodium hydroxide was placed in a beaker with a magnetic stirrer and 10 mL of the dextran solution was added to it. The concentration change with time was measured by size exclusion chromatography (SEC). Prior to the injection of 50 \mu L of a sample into the SEC apparatus the pH was adjusted to neutral using hydrochloric acid.

4.5.2 Removal of protein fouling

According to Millipore, Biomax membranes, fouled by protein, are effectively cleaned by placing them in 0.1 M NaOH solution for 30 minutes followed by a subsequent water rinse. This cleaning procedure may subsequently be followed by cleaning with hypochlorite, if considered necessary. Cleaning of protein-fouled Nadir membranes was also conducted with sodium hydroxide.

4.5.3 Cleaning effectiveness

Experiments were conducted to determine the effectiveness of NaOH as a cleaning agent when attempting to remove dextran from the surface of, and from within, a membrane. 50 mL, 0.1\% 5 kDa dextran standard solutions were prepared to which 10 mL NaOH solutions at concentrations of 0.1 M, 0.5 M and 1 M NaOH were added. Samples were taken from each of the three mixtures at fixed times of intervals of 1 min., 5 mins, 10 mins., 30 mins., 1 h, 2 hs., and 8 hs. The pure dextran solution and the mixtures were analysed by HPLC using the SEC column with a sampling time of 35 minutes and reverse osmosis-treated water as the eluent.

4.6 Analytical methods

Protein analysis was generally conducted using a UV/Vis spectrophotometer (in order to avoid any potential electrostatic interaction issues which can arise in SEC) whereas the concentration of dextran standards was evaluated using the RI detector of the HPLC instrument.
4.6.1 High performance liquid chromatography

In the work reported in this thesis, HPLC was used to analyse dextran rejection data from PES membranes. The available HPLC unit (Agilent 1100) was equipped with a RI and a diode array detector (DAD). The DAD detector is similar to a UV/Vis spectrophotometer (see also Section 4.6.2) and allows the capture of light over the entire wavelength of a spectrometer, including UV scanning, i.e. several wavelengths may be monitored simultaneously. A PL aquagel-OH 30 8 μm (Polymer Laboratories) SEC column was employed for the dextran standard analysis. According to the manufacturer this column is ‘packed with macroporous copolymer beads with an extremely hydrophilic polyhydroxyl functionality’, which provides a ‘neutral’ surface that avoids adverse adsorption effects. The column may be operated in the pH range of 2 to 10. Neutral polymer analysis may simply be conducted with water as the eluent. Dextrans cannot be detected in the UV/Vis range, hence for the purpose of their analysis only the RI detector was used.

The following methodology was employed for the analysis of the dextran standards:

1. Fresh deionised water was pre-filtered through a 0.45 μm nylon filter and degassed prior to its use as the eluent; sodium azide was added if analysis over several days was required.
2. The solvent lines and pump were primed with eluent by purging.
3. After the column was fitted, water was eluted through the column for at least 60 minutes at 0.5 mL/mm to ensure a stable baseline and flushing of the column with at least one column volume.
4. The operational protocol was to conduct the analysis at 0.5 mL/min (corresponded to ~20 bar column pressure), with a sample volume of either 50 or 100 μL at 35°C giving one sample 45 minutes elution time; less than 30 minutes were sufficient for the smallest molecules to leave the column.
5. The temperature stability was monitored during experiments as the RI detector is highly temperature sensitive.
6. Subsequent to an experiment the column was flushed with the eluent only, removed for storage and fitted with end caps to prevent the column from drying out.
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7. Periodically, column preservation was carried out by flushing the column according to the manufacturers' guidelines.

Actual samples collected from the dextran UF experiments were transferred into 2 mL chromatography sample vials (Supelco, Sigma-Aldrich) using plastic pipettes. These samples were then placed into an automatic sampling unit (Hewlett Packard) of the HPLC from which the required sample was injected into the column. If the sample vials had to be kept for additional analysis at a later stage they were stored in a fridge.

4.6.2 UV/Vis spectroscopy for protein analysis

UV/Vis spectroscopy is a technique based on the absorption of light in the visible (400 to 700 nm) and ultraviolet (1 to 400 nm) region of the electromagnetic spectrum. The Lambda 12 (Perkin Elmer) UV/Vis spectrometer employed for this research is a so-called double-beam device where the light beam is split in order to monitor a reference and the sample simultaneously. The sample solutions produced in the experimental work were analysed using quartz cuvettes suitable for work in the UV region. Typical wavelength scan data for both BSA (1.0 g/L) and LYZ (1.0 g/L) are shown in Appendix F, parts b and c.

It is worth noting that spectrophotometers may be limited in their usefulness above certain concentrations. This is important in the context of the choice of feed concentration employed in the filtration studies. Cooper (2004) recommends to choose samples in such a way as to not exceed absorbance values of \( A = 1.5 \) to obtain a reliable answer. In the case of LYZ, if the starting concentration is higher than 0.6 g/L, this can be an issue (see Appendix D, part d), hence all experiments were conducted with feed concentrations of 0.5 g/L. Protein concentration measurements in solution can be difficult and often an error of ±5% has to be considered. The variation in UV/Vis samples taken in this research was no greater than ±4%.

Generally, the experimental evaluation of a calibration curve will be more accurate but it is also possible to determine an approximate absorbance value for a given protein at a particular wavelength using a calculation method. Such an approach provides an additional check on the magnitude of absorbance values. For detailed reasoning behind
such an approach and relevant calibration curves for BSA and LYZ the reader is referred to Appendix D, part d

4.7 Crossflow filtration apparatus

The majority of industrial UF processes are conducted with some form of CF apparatus. To accommodate the available membranes a new CF apparatus was designed and constructed. The design process is discussed in detail here together with aspects of commissioning and pump choice.

4.7.1 General considerations

During the design process several options for CF were considered including specialised arrangements where the inlet flow is arranged in a vortex creating fashion and the use of two oppositely running feed and outlet streams in order to create additional mixing. However, in light of the interest in comparative experiments between a CF and a stirred cell (SC) device it was decided to design a system containing a planar membrane where the flow channel is rectangular. Such an arrangement does not overcomplicate the calculation of the flow and shear profile.

At the initial stage a peristaltic pump was considered because it will add little heat to the system and it is unlikely to have any detrimental effects on the protein solutions to be used. However, further research and practical testing suggested that the frequent pulsing of a peristaltic pump may cause undesirable pressure fluctuations, particularly for fully developed flow. Hence, additional research was conducted looking at alternative pumps (Section 4.7.4).

Since all the membranes sourced from Millipore were circular, it was considered beneficial to have the ability to use circular membranes in the CF apparatus as well (Nadir membranes are supplied in sheet form and can thus be cut to the desired size and shape). Therefore, the CF module was designed in such a manner that a circular membrane could be fitted within the rectangular channel. The mounting holder is shown in Figure 4.2.
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Figure 4.2. Plan and side view of the circular membrane holder – part of the crossflow module design (refer to Appendix B for further details of the membrane module).

The actual width of the flow channel in the membrane module was 30 mm and thus a membrane area of 30x30 mm was available for filtration. It was mentioned in Section 2.2.2 that high shear rates are generally desirable and a flow channel of 2 mm height was chosen, which also improved the available CF velocity. Some 'thin-channel' designs are reported to have channel heights of 1 mm or less (Cheryan, 1998), however, it is important to note that such designs are typically limited to the laminar flow regime in order to minimise power consumption (Bird et al., 2001). At the experimental pressure conditions employed in this study the CF velocity in the module was about 0.5 m/s which is within the commonly used CF velocity range of 0.4 to 1.5 m/s. Materials of construction, particularly those in contact with the test solution, were chosen to prevent any solution contamination. Hence, the membrane module, piping and other materials were made of stainless steel or inert polymer with the added benefit that cleaning agents such as NaOH with high alkaline pH would not damage the material either.
4.7.2 Entrance flows

One of the aims of the CF module design was to allow for well developed flow before the solution reached the membrane as entry effects can have a significant influence. Particularly in laminar flow, a substantial entry length is required for the flow profile to fully develop. Fully developed flow can be based on different assumptions (Hughes and Brighton, 1999). On the basis of pressure drop the gradient is fully developed after three to four diameters whereas the mean velocity does not fully develop until 30 to 60 diameters downstream of the entrance. If the flow profile is turbulent the entry length, $L_e$, required is given by equation (4.1) (Perry and Green, 1997):

$$L_e / d_h = 40$$  \hspace{2cm} (4.1)

The hydraulic diameter, $d_h$, is determined from equation (4.2) using the rectangular channel dimensions of 0.03 m (width) and 0.002 m (height), respectively, and defined as four times the cross-sectional area, $A_c$, available for flow divided by the wetted perimeter, $P_w$:

$$d_h = \frac{4A_c}{P_w} = \frac{4ab}{2(a+b)} = \frac{2ab}{a+b}$$  \hspace{2cm} (4.2)

where $a$ is the width and $b$ the height of the channel. The hydraulic diameter for the CF apparatus was 0.00375 m (Appendix C, part a) and the entry length required for turbulent flow to fully develop was 15 cm. For fully developed laminar flow, the required entry length can be longer, depending on the CF velocity and determined using an empirical equation (Cheryan, 1998):

$$L_e = Bd_h \text{Re}$$  \hspace{2cm} (4.3)

where Re is the Reynolds number and $B$ a constant, typically 0.029. Figure 4.3 shows a graph of entry length vs. Reynolds number and CF velocity according to equation (4.3) which, assuming the transition from laminar to turbulent flow commences at a Reynolds number greater than 2,100 and a worst case scenario, that an entry length of 25 cm is required, for laminar flow to fully develop. Based on these findings the total flow channel length was designed as 39.6 cm, with the membrane holder positioned towards
the end of the channel. This allowed for a flow profile to develop, even at laminar Reynolds numbers close to the transitional regime.

![Graph](image_url)

**Figure 4.3.** Entry length vs. Reynolds number and crossflow velocity.

### 4.7.3 Crossflow module dimensions

Based on the entrance length calculations and knowing that exit length requirements are substantially shorter (Perry and Green, 1997), the dimensions of the module constructed were as shown in Table 4.3.

<table>
<thead>
<tr>
<th>Description</th>
<th>Length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total module length</td>
<td>45</td>
</tr>
<tr>
<td>Total internal channel length</td>
<td>39.6</td>
</tr>
<tr>
<td>Centre of inlet flow to start of the membrane</td>
<td>24.9</td>
</tr>
<tr>
<td>Membrane length</td>
<td>3</td>
</tr>
<tr>
<td>Membrane width</td>
<td>3</td>
</tr>
<tr>
<td>Membrane end to centre of outlet flow</td>
<td>10.0</td>
</tr>
</tbody>
</table>
More detailed dimensions of the CF module can be found in the design drawing in Appendix B.

The individual components of the specially designed CF module including the module base (A), the module top (B) including a rubber seal (C) and the membrane holder components (D and E) are shown in Figure 4.4. A pressure transducer (F) and a ball valve (G) are also shown.

The assembled CF module including the fitted membrane holder situated in an appropriate holding frame is shown in Appendix B.

Figure 4.4. Crossflow module – Individual components.

4.7.4 Pump selection

The selection of a suitable pump was made more difficult due to the potential influence of pulsation and shearing. A scan of the literature showed that other researchers rarely work with centrifugal pumps when using protein solutions. It was also obvious that no
standard has been established which deems a particular pump type to be superior over any other. Fernandez et al. (2005) used a centrifugal pump in an UF system with CF velocities up to 7 m/s. Li et al. (2005) operated at very low CF velocities (0.12 cm/s) and utilised a peristaltic pump. Chan et al. (2002) employed a peristaltic pump, at 0.15 m/s CF velocity and a pressure of 0.5 bar(g). Gan (2001) used an air driven double diaphragm pump in a microfiltration system.

The concern with peristaltic pumps is their tendency for strong pulsation. Two other considerations were a gear pump or diaphragm pump. The speed of a gear pump is easily controlled whilst other advantages include its gentle flow control and its ability to make very subtle changes to the pumping rate. Unfortunately, the cost for the latter pump is substantial so that a financially more viable alternative had to be considered. Initial experiments were conducted using a centrifugal pump, however, this proved unsatisfactory. In this context it is worth pointing out that improper impeller design is more likely to cause protein denaturation than high shear rates (Cheryan, 1998).

Ultimately, a 24V diaphragm pump, resistant to mild acids and bases, with a maximum operating pressure of 1 bar(g) and a temperature operation window of 7 to 54°C was chosen and purchased from Jabsco (Model 31800). Diaphragm pumps can also cause pulsing, but generally less so than peristaltic pumps. In order to ensure as little pulsing as possible prior to fluid entry into the CF module, the pipe diameter at the pump outlet was chosen as four times larger than at the inlet to the CF module, thereby substantially reducing pulsation effects.

4.7.5 Crossflow system design

The final design of the CF system, shown in Figure 4.5, consisted of a diaphragm pump (A), two pressure transducers (B and C), ball valves (D to H), needle valves (I and J) and a 1.5 litre capacity feed tank (K). Valve E allows recycling of the feed prior to the fluid entering the membrane module. Thus, the CF apparatus can be operated at a lower pressure via by-passing some of the feed. Valve F was generally left fully open but it can, for instance, be used in its fully closed position to run a pump cleaning cycle. Valves D and H were fitted to allow air venting from the retentate stream and feed tank. Needle valve J can be used to control the TMP but it was mostly operated fully open and the flow rate and pressure were controlled via the power supply to the pump. Valve
G and I were used to take permeate and retentate samples, respectively. The pressure transducers were monitored electronically and could be fitted at points B, C and L depending on which pressure was of interest.

The typical retentate flow rate during the protein filtration experiments was 1.5 L/min and after taking a permeate sample (for which approximately 3 to 4 mL volume) were required, the sample was re-inserted into the feed tank. Moreover, when a permeate sample was taken a feed sample was also taken. By taking these measures any permeate lost and feed concentration changes were minimised and thus considered negligible.

The diaphragm pump could only be operated up to a maximum delivery pressure of 1 bar(g). For the purpose of this study, however, it was desired to operate at low pressure to avoid extensive concentration polarisation and gel layer formation. Moreover, it is known that operating at high TMPs can increase membrane fouling (Gesan et al., 1993), thus this choice of pump was considered adequate. As mentioned previously, stainless steel was chosen as the material of construction of the membrane module to minimise...
contamination of the feed and to allow operation and cleaning over the entire pH range. When taking into account the system volume and considering the cost of the test materials such as proteins and dextrans a feed volume of 1 litre was considered appropriate and the feed tank was designed accordingly. The retentate line reached into the feed vessel and was situated approximately 3 cm above the tank bottom in order to minimise foaming and vortex formation. Foaming at the gas-liquid interface is more likely to be a cause for protein denaturation than high shear rates (Lee and Choo, 1989).

The pressure in the CF system was controlled by the flow rate or the retentate valve; the pump speed was adjusted by changing the power supply voltage. The pressure was recorded with two calibrated pressure transducers connected to an electronic switch box whilst the pressure was read from a digital display.

4.8 Stirred cell apparatus

In order to conduct the filtration experiments described in Chapter 6 and Chapter 7 two different membrane modules were employed. UF experiments, particularly in research laboratories, are frequently conducted using dead-end or pseudo CF filtration cells (i.e. stirred cells). In industrial applications CF filtration systems are preferred because they allow for continuous processing at large scale. It is not only sensible to conduct laboratory experiments with a CF unit from a scale-up point of view, but also as it is thought that there are difficulties in simulating realistic processing conditions with SC units. Later on in this work it will become clear how a CF unit can yield different experimental results compared to SC data even if an attempt is made to match experimental conditions.

A Millipore SC (model XFUF04701) was used, unless stated otherwise, in order to conduct the SC experiments with 47 mm diameter membranes giving an effective filtration area of 15 cm². A photograph of the SC unit is shown in Figure 4.6 whilst the individual components of the SC are summarised in Table 4.4. The cell (G) is constructed from stainless steel and borosilicate glass (F). The maximum feed volume was 50 mL with a total capacity of 75 mL whilst the minimum required stirred volume is 2.5 mL. A larger Millipore SC (model XFUF07601) was also available, which is
suited to larger diameter membranes (76 mm), has a maximum feed volume of 300 mL and an effective filtration area of 40 cm$^2$.

![Millipore stirred cell](image)

**Figure 4.6. Millipore stirred cell.**

**Table 4.4. Main components of the Millipore stirred cell.**

<table>
<thead>
<tr>
<th>Label</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>316L stainless steel top plate and PTFE stirring bar</td>
</tr>
<tr>
<td>B</td>
<td>Knobs</td>
</tr>
<tr>
<td>C</td>
<td>Fluoroelastomer O-rings</td>
</tr>
<tr>
<td>D</td>
<td>Clamps (used as glass holder)</td>
</tr>
<tr>
<td>E</td>
<td>Pressure relief cap</td>
</tr>
<tr>
<td>F</td>
<td>Borosilicate glass cylinder</td>
</tr>
<tr>
<td>G</td>
<td>Stainless steel base (in-house design)</td>
</tr>
</tbody>
</table>

Some of the materials of construction in the SC, such as the fluorocarbon O-rings, are sensitive to pH, thus most experiments should be carried out within pH 2.0 to pH 12.0 (a range of pH values from pH 3 to pH 11 was chosen for the experimental studies in this thesis). The SC can be operated up to pressures of 6 bar(g), however, most experiments were operated at lower TMP and within the range of 0.25 to 1 bar(g).
A schematic of the complete SC set-up is shown in Figure 4.7. Pressure in the cell was provided by a regulated nitrogen gas supply (A), since the use of compressed air could induce undesirable pH shifts. For each experiment, the magnetic stirrer speed (B) was fixed at the desired revolutions per minute (rpm). The nitrogen supply was connected to a ball valve (C) and a pressure regulator (D) as well as a pressure gauge (E) in order to allow for controlled pressure adjustments. Permeate samples were taken at suitable time intervals, based on the solute type and the MWCO of the membrane (A membrane with a higher MWCO – in most cases – resulted in higher permeate flux and required more frequent sampling).

![Figure 4.7. Stirred cell apparatus.](image-url)
Table 4.5 shows some of the notable advantages and disadvantages of a SC apparatus.

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low feed volume requirement</td>
<td>Unsteady state</td>
</tr>
<tr>
<td>Easy and quick to use</td>
<td>Non-uniform shear field at the membrane surface</td>
</tr>
<tr>
<td>Low cost</td>
<td>Varying shear along the impeller</td>
</tr>
<tr>
<td></td>
<td>Less suitable for scale-up</td>
</tr>
<tr>
<td></td>
<td>Batch process</td>
</tr>
<tr>
<td></td>
<td>Varying concentration on the feed side</td>
</tr>
</tbody>
</table>

During initial experimental work with the XFUF04701 SC it was found that the design of the base was poor as the membrane sits directly on top of a shallow base, below which permeate can only pass through a single small hole. A new base was designed that incorporated a uniformly permeable sinter plate with sufficient space below for liquid to rapidly exit the cell (see Figure 4.8).

![Figure 4.8. Millipore original base vs. in-house design.](image-url)
4.9 Plasma generator and membrane modification

Part of the research programme investigated the development of a plasma-modified membrane which offered the potential to selectively reject components present in the feed. Low-temperature plasma modification combined with the grafting of a monomer onto the surface of a polysulphone (PSU) membrane has previously been attempted (Zhan et al., 2004). These authors successfully grafted a positively charged monomer onto a membrane surface of negative charge which resulted in greater electrostatic repulsion when the filtration of the positively charged protein LYZ was attempted. Conversely, if a negatively charged monomer is grafted onto the membrane surface so the membrane negativity is increased thereby promoting electrostatic repulsion of negatively charged proteins such as BSA. This is an example of the importance of surface charge which indicates the potential to modify a membrane surface in such a manner as to exploit surface charge effects for specific purposes.

4.9.1 Plasma modification apparatus

A typical plasma modification unit consists of a vacuum chamber in which an upper and lower electrode is located. The vacuum chamber is connected to a radio frequency generator, a gas supply and a vacuum pump. A schematic of the radio frequency plasma generator used to modify the membranes for the present study is shown in Figure 4.9.

![Diagram of Plasma Modification Apparatus](image)

**Figure 4.9. Plasma modification apparatus (situated at Wroclaw University of Technology, Poland).**

The plasma modification was conducted in collaboration with the Department of Chemistry at Wroclaw University of Technology, Poland. PES membranes were
modified either by applying argon plasma only, or by exposing the membrane to argon plasma followed by dipping of the plasma-exposed membrane into acrylic acid (AA) solution.

The modification procedure was always conducted in the same manner and involved the following steps:

1. Soak the unmodified membrane in 0.1 M NaOH for 30 minutes prior to washing and rinsing with reverse-osmosis treated water and vacuum drying
2. Attach the dried membrane to the lower electrode and adjust the distance between the two electrodes to 5 cm
3. Introduce the inert argon gas into the chamber
4. Switch on the radio frequency generator to apply plasma treatment for a given time period (1 mm.) and intensity (200 W) using inert argon gas under a fixed pressure of 14 Torr (18.6 mbar) thereby generating a plasma
5. Store the plasma-modified membrane in air (if AA dipping was desired place the membrane into 25% AA solution for 2 days after 5 minutes in open air)
6. After two days of dipping the membrane was rinsed with distilled water and dried in open air.

Prior to any protein filtration experiments and water flux measurements at Loughborough the membranes were ‘wetted’ in 0.1 M NaOH for 30 minutes and stored in ultrapure water for 24 h prior to their first use.

4.9.2 The modification process

During the plasma grafting method free radicals are created on the membrane surface and exposure of these radicals to air leads to chemical reactions with atmospheric oxygen. As a result functional groups are formed including peroxides which can in turn produce a graft reaction of some vinyl monomer. An illustrative example for a similar process (i.e. using a PSU membrane) is shown in Figure 4.10
The plasma modification for this research was conducted both with and without dipping in AA solution in order to observe any differences between a membrane modified by the plasma only and one onto which charge groups were attached as well. Low-temperature plasma is comparable to UV-modification in the sense that surface modification generally only alters the top layer of the membrane, confined to the first several tens of nanometers, and the porous support layer remains unaffected (Stevens et al., 1998; Zhan et al., 2004). Wavhal and Fisher (2002) claimed that they conducted the modification in such a manner that the entire membrane structure was modified. Whilst this ensures permanency of the degree of hydrophilicity of the modified membrane it raises the concern whether structural weakness of the membrane may occur causing, for instance, extensive membrane swelling in contact with water.

4.10 Characterisation methods

In this section the experimental methodology employed for membrane characterisation experiments is provided. These include Molecular weight cut-off (MWCO), contact angle, membrane swelling and streaming potential measurements, and the evaluation of scanning electron micrographs.

4.10.1 Molecular weight-cut off methodology

In order to conduct initial MWCO work similar testing conditions to those set out by Cheryan (1998) were adopted. The testing conditions, in what is referred to as the retention test specification, are duplicated in Table 4.6 alongside the conditions employed for this research. It is advisable to maximise the stirrer speed as agitation helps to minimise concentration polarisation which is a necessary step in order to obtain a membrane rejection coefficient as close as possible to the true value.
Table 4.6. Retention test specification, adapted from Cheryan (1998).

<table>
<thead>
<tr>
<th>Test parameter</th>
<th>Value (Cheryan)</th>
<th>Value (used in this research)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transmembrane pressure</td>
<td>1 bar (g)</td>
<td>0.25 bar (g)</td>
</tr>
<tr>
<td>Temperature</td>
<td>Ambient (~ 25°C)</td>
<td>Ambient (~ 22°C)</td>
</tr>
<tr>
<td>Stirling rate</td>
<td>2,400 rpm</td>
<td>2,400 rpm</td>
</tr>
<tr>
<td>Solute concentration</td>
<td>0.1 % (w/w)</td>
<td>0.04% (w/w)</td>
</tr>
<tr>
<td>Solvent/Buffer system</td>
<td>Reverse osmosis-treated water</td>
<td>Reverse osmosis-treated water</td>
</tr>
<tr>
<td>Ratio of test solution volume to membrane surface area</td>
<td>50 mL/15 cm² filtration area</td>
<td>50 mL/15 cm² filtration area</td>
</tr>
<tr>
<td>Permeate-to-feed ratio</td>
<td>5 mL/45 mL (where possible)</td>
<td>5 mL/45 mL (where possible)</td>
</tr>
</tbody>
</table>

*0.04% was established as a reasonable starting concentration to use as it provided good resolution using the RI detector of the HPLC equipment.

The initial MWCO work was carried out using the SC described in Section 4.8. Note also that all characterisation work was carried out using the smaller SC, i.e. membranes with 47 mm diameter, unless stated otherwise. In the retention test specifications for this research the solute concentration was reduced to 0.04% w/w or 0.4 g/L, to be more cost effective, and because the same starting concentration was used when determining the MWCO using the CF apparatus. A calibration curve of the various dextrans showed that a starting concentration of 0.4 g/L was sufficient to allow for the detection of small amounts of dextran in the permeate using HPLC (Detection limit: ~ 0.01 g/L or ~ 8,000 nano Refractive Index Units (nRIU)). Calibration curves for 1 kDa, 5 kDa and 150 kDa dextran standards can be found in Appendix D, parts a to c. Table 4.7 shows the retention test conditions employed when determining the MWCO using the CF apparatus.

Prior to the first permeate measurement, the first 5 mL of permeate was recycled in order to remove any dead volume below the membrane. The sample for HPLC analysis was then taken from the next 5 mL of sample obtained from the filtration process thereby allowing consistent, repeatable sampling.
Table 4.7. Retention test specification – crossflow apparatus.

<table>
<thead>
<tr>
<th>Retention test/MWCO determination</th>
<th>Crossflow apparatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transmembrane pressure</td>
<td>0.25 bar(g)</td>
</tr>
<tr>
<td>Temperature</td>
<td>Ambient (~ 22°C)</td>
</tr>
<tr>
<td>Crossflow velocity</td>
<td>~ 0.45 m/s</td>
</tr>
<tr>
<td>Solute concentration</td>
<td>0.04% (w/w)</td>
</tr>
<tr>
<td>Solvent/Buffer system</td>
<td>Reverse osmosis treated water</td>
</tr>
<tr>
<td>Ratio of volume of test solution to membrane surface area</td>
<td>1000 mL/7.1 cm² filtration area</td>
</tr>
<tr>
<td>Permeate-to-feed ratio</td>
<td>10 mL/1000 mL</td>
</tr>
</tbody>
</table>

It should also be noted that the different size dextrans were individually transmitted and this was done using the larger solutes first followed by the smaller ones. By doing so the tendency for fouling should be lower, because the larger solutes are unable to enter the membrane pores. In a recent publication this approach was confirmed to be sensible as the extent of pore accessibility for dextrans of different sizes was reported to contribute to fouling as well (Susanto et al., 2007).

In order to confirm the repeatability of the MWCO experiments using the CF apparatus a 150 kDa dextran solution was continuously circulated over a two hour period. Flux measurements and rejection data were collected during this experiment and flux was found to be consistent after the first few minutes of filtration.

4.10.2 Contact angle measurements

The measurement of the contact angle is considered a classical method to determine whether the active surface of a membrane has hydrophilic or hydrophobic character. Static contact angle measurements of both unmodified and plasma-modified PES membranes were carried out with a KSV Instruments CAM 100 (Contact Angle Meter) at Lappeenranta University of Technology, Finland. The optical contact method utilised is also referred to as the sessile drop method that will be referred to later in the text. Reverse osmosis-treated water was used as the test solvent and the contact angle analysis was carried out with a high speed camera configured so that 400 frames per second could be recorded. For each contact angle measurement at least 20 drops were analysed in contact with the membrane surface. For each test new membranes were washed in order to remove any wetting agents from the manufacturing process and
subsequently dried in sealed glass containers. When ready for analysis, the membranes were cut and the samples mounted onto clean plates. The water droplets were placed onto the membrane surface with a specially designed micro-syringe which was stabilised in the syringe holder in a fixed position (Figure 4.11). The syringe allowed a 3 µL drop to be placed on the membrane with a minimum of kinetic effects. All measurements were carried out at room temperature 25°C ±1°C taking pictures of the droplet on the membrane surface according to the specification set earlier. The actual contact angle was then measured using the CAM 100 Software which allows computerised fitting of the tangent line to the picture allowing consistent, repeatable contact angle data to be obtained. The actual drop angle was determined via the software which uses the Young-Laplace equation; for each measurement a left, a right and a mean contact angle were computed (see Section 5.7).

Some contact angle measurements were also performed at Loughborough using a different set-up in order to compare the results obtained in Finland. In addition, it was of interest to observe any differences between washed and dried membranes and those in the ‘as manufactured’ state (i.e. still including any wetting agents) The membrane was placed in a hermetically closed and insulated chamber which allowed strict control of the environment. A high precision 10 µL Hamilton syringe (Hamilton GB Ltd., UK) was used to place 3 µL droplets of water onto the membrane. A mechanical manipulator enabled the placement of the droplet on the substrate whilst minimising the kinetic

Figure 4.11. KSV Instruments 100 Contact angle instrument (Lappeenranta University of Technology, Finland).
impact. Experiments were carried out at 25°C ±1°C. Using the sessile drop method the spreading process was captured at the observed equilibrium of the droplet with the membrane surface, this method was repeated with new droplets at least five times. Video imaging software 'ScionImage' was used to analyse the left and right contact angle of the droplets on the surface from which a mean contact angle was calculated.

4.10.3 Swelling experiments

One purpose of the swelling measurements was to identify whether the use of NaOH causes a change of dimensions in the membrane structure. If so, this could in turn result in flux changes which would be important during cleaning and for experiments conducted subsequent to membrane contact with NaOH. For each analysis five samples of a flat sheet PES membrane were cut to allow an averaged result to be obtained. A two centimetre square, dry sample (the membrane sample area only needs to be slightly larger than the contact area of the instrument) was positioned within a flat-bottomed dish on top of which a 10 mm diameter spacer and the measurement probe were placed. The Millimark instrument (Mahr, Millimark S1840) is known as a column amplifier for inductive probes. The probe/gauge combination has a resolution of 0.1 μm. 10 mL of either pure water or NaOH (0.1 M or 0.5 M) solution were added to the dish which resulted in complete immersion of the sample and the onset of swelling. One minute was allowed prior to taking a measure of displacement, as it had been established in a more extensive study by Tarleton et al. (2006) that equilibrium will be reached for most solvents at that point. A schematic of the test apparatus is shown in Figure 4.12.
The initial development of this technique, and more detail of the experimental method, is described elsewhere (Tarleton *et al.*, 2005).

### 4.10.4 Streaming potential apparatus

I organised a research exchange with Lappeenranta University of Technology, Finland, where I conducted ‘through the pore’ streaming potential measurements for unmodified and plasma-modified membranes. The apparatus allows simultaneous streaming potential and flux measurements and essentially comprises a membrane module through which electrolyte is flowed and a data acquisition system which allowed the measurement of the voltage generated.

A schematic diagram of the apparatus is shown in Figure 4.13. The module was made of polycarbonate with electrode plugs made from polyvinylchloride. The flat sheet membrane to be tested (10.4 cm$^2$ surface area) was placed on top of the PE, porous support mounted within the module and sealed into place using two O-rings (Figure 4.14). A small opening at the top and bottom of the module allowed the 2 mm diameter Ag/AgCl electrodes to be placed in close contact with either side of the membrane. These electrodes could only be safely operated up to pH 7 as the surface tends to dissolve in a more alkaline environment, thereby potentially damaging the electrodes and resulting in unstable pH values above 8. Circulation of the process fluid could be
carefully controlled via a gear pump and the system was also fitted with two pressure transducers from which the TMP could be determined. The pressure was monitored using pressure transducers and, as can be seen from the diagram, temperature sensors were installed at four different locations in the set-up (Figure 4.13). All permeate samples were weighed on an electronic balance that was directly linked to the computer. The ADDA14 interface card allowed the conversion of amplified measurement signals into binary code by conversion of analog-to-digital processing the information using a PC. A QuickBasic 4.5 computer programme which had been previously developed was used to retrieve all necessary data via the ADDA 14 interface card. Subsequent transfer of the data into Microsoft Excel allowed the zeta potential versus pH data to be plotted.

Figure 4.13. Streaming potential apparatus.
An experimental methodology was developed to conduct the streaming potential measurements. Each membrane was washed and stored overnight in reverse osmosis-treated water to remove any loose residue from the membrane surface. The silver electrodes were prepared in the following manner:

- Cleaning of the electrodes in 25% ammonia solution followed by further cleaning in 50% nitric acid solution
- Preparation of the electrodes in 0.1 M HCl in order to coat the electrodes with chloride by anodic deposition of the chloride on silver (the current density was 0.2 mA/cm²)
- Both electrodes were placed together in 0.1 M HCl solution for at least one hour.

Since both electrodes are prepared separately the potential difference between them may not be as low as desired, therefore, the last step ensures that the asymmetrical potential between the two electrodes does not exceed 1 mV. Periodically, the above outlined cleaning and preparation procedure has to be repeated in order to prevent too much polarisation of the electrodes. This is particularly true if high flow rates are used, as under such conditions the chloride layer wears off more easily. Once the membrane module has been tightly fitted onto the apparatus all electrodes, temperature and pH sensors were fitted. The solution pH in the feed tank was continuously monitored throughout each experiment.
Prior to each experiment 2 L of 0.001 M electrolyte (KCl) solution was prepared. A low electrolyte strength was chosen because the Helmholtz-Smoluchowski equation, used to determine the membrane zeta potential, is limited to low electrolyte strength (10^{-3} M) (Saxena and Shahi, 2007). The streaming potential apparatus was rinsed using ultrapure water, checking the membrane module for any leaks and the conductivity was monitored. A water bath was used to maintain the experimental temperature at 25°C ±1°C. Water was circulated at a pressure twice as high as the highest experimental pressure for at least 30 minutes to reduce the impact of any membrane compaction effects during the subsequent measurements which were conducted from 1 bar(g) to 0.2 bar(g) in 0.2 bar(g) steps. After the apparatus was thoroughly rinsed and stabilised it was drained of water and one litre of electrolyte solution was used to rinse the apparatus and increase the conductivity in the system. The software parameters were set with a standard deviation allowance no greater than 0.01 and a measurement time of at least 3 minutes was allowed per pH value corresponding to approximately 13 to 15 measurements from which an average at the given pH was computed. The streaming potential was first measured by reducing the solution pH from a starting pH, e.g. 5.8 down to about pH 3.0, using HCl of different molarity. Subsequently, the system was drained and rinsed with fresh electrolyte solution and new electrolyte solution was placed into the feed tank. Subsequent measurements were performed by increasing the pH using an alkali such as KOH. For each experimental run 7 measurements were conducted in the pH range from 3 to 7. Note that measurements below pH 3 were avoided because too many free hydrogen ions in solution were found to disturb the conductivity measurements. After an experiment had been completed, the raw data was transferred to disk for later analysis and the apparatus was flushed with water and dismantled. Further processing of the data and determination of the hydraulic membrane permeability (permeance) and the zeta potential was then conducted using a specifically developed Excel template.

4.10.5 Field emission scanning electron microscopy

Field emission scanning electron microscopy (FEGSEM) was used to obtain information about the cross-section and thickness of Millipore and Nadir PES membranes. All non-conductive membrane samples were coated with a gold layer using the sputter coating technique. The micrographs for this study were obtained with a FEGSEM apparatus (Leo -1530 VP) using an accelerating voltage of 20 keV. Typically,
accelerating voltages of 15 to 20 kV are recommended for analysis of a non-conductive specimen (Gabriel, 1985). The membrane samples were mounted onto a substrate which can be secured in the FEGSEM apparatus. If a cross-sectional image of the membrane was required the membrane was freeze-fractured using liquid nitrogen.

4.11 Membrane handling prior to filtration experiments

It is evident from the literature review that membrane cleaning has an important impact on membrane filtration processes. Furthermore, a standardised method to prepare the UF membrane prior to their use is sensible. Membrane manufacturers are known to use wetting agents such as glycerine in order to prevent their membranes from drying out. Sodium azide may also be present as a preservative. Thus, a methodology was developed to pretreat the Nadir and Millipore membranes. It is worth noting that such pretreatment methods should not necessarily be generalised because membranes from different manufacturers vary in their morphology even if they are notionally made of the same polymer. Such variations are evident in that differences in the surface chemistry and the overall surface roughness exist (Kim et al., 1990; Kim et al., 1992). UF membranes tend to have a shiny and a rougher side where the shinier side indicates the active layer which should face upwards during filtration experiments. During cleaning in a beaker, however, this shiny side should, therefore, face downward with respect to water to effectively pretreat the membrane.

Millipore membranes were always pretreated according to the following procedure:

- 2 h storage in de-ionised water changing the water at least three times
- Pre-compaction at a pressure at least twice as high as the desired filtration pressure.

Nadir membranes were pretreated in the following manner:

- 30 minute storage in 0.1 M NaOH
- 24 h storage in de-ionised water changing the water at least twice
- Pre-compaction with water at a pressure twice as high as the desired filtration pressure for at least 30 minutes.
Storage of the membranes from the different manufacturers also varied slightly and the manufacturers’ recommendations were followed. Millipore membranes were immersed in a 10% ethanol/water mixture and refrigerated. Nadir membranes were stored in deionised water only and refrigerated. 0.2% sodium azide was added to each storage solution in order to prevent any bacterial growth.

According to the manufacturer Millipore membranes with MWCO < 100,000 may be operated up to 4.6 bar(g) pressure (Membranes > 100,000 MWCO should not be operated above 0.7 bar(a) pressure). Nadir membranes are generally operated up to a maximum of 4 bar(g) and PES membranes from either manufacturer can be operated over the entire pH regime due to the high chemical stability of PES.

4.12 Protein filtration experiments

The majority of experiments were carried out using Nadir membranes. Millipore membranes can be sensitive to phosphoric acid therefore the use of phosphate buffer with these membranes entails the risk of experimental difficulties. In addition, water flux data for Nadir membranes was more consistent than that obtained with Millipore membranes Nadir membranes were also found to be physically more stable than Millipore membranes when exposed to the plasma modification process (Section 5.1.1).

4.12.1 Stirred cell operation

In Section 4.8 the SC apparatus was introduced. An illustration of the different parts required for the SC assembly was given in Figure 4.6. During assembly of the SC the membrane to be tested was placed with the shiny side (filtration side) facing upwards on top of the sinter plate (shown in Figure 4.8), on top of which an O-ring seal was placed. The other O-ring seal was fitted on top of the glass (see Figure 4.6). Pressure was applied from the nitrogen gas cylinder (which had been set via a calibrated pressure controller) and monitored continuously on a pressure gauge. Permeate flux was inferred from successive volume measurements taken with a graduated cylinder and a stopwatch. Feed and permeate samples of 3 to 4 mL were taken at fixed time intervals and analysed using the UV/Vis spectrophotometer. SC protein filtration experiments with new membranes were conducted over 30 minutes. When modified membranes were used the
experiment was conducted over several hours due to the substantially reduced permeate flux (see Section 5.3.5).

### 4.12.2 Crossflow operation

Membranes were placed in the module such that the shiny side faced the flow channel of the CF module (see also Section 4.7). The membrane holder (Figure 4.2) was firmly tightened and 1 L of water was subsequently introduced into the feed tank and pressure gradually raised to 1 bar(g) in order to test for any leaks. Subsequently, the membrane was compacted at 0.5 bar(g) and permeate flux measurements were taken at this pressure and at the experimental pressure of 0.25 bar(g) which allowed clean water flux and membrane permeability data to be determined. The permeate flux measurements were conducted using either a graduated cylinder and a stopwatch or an electronic balance with a beaker. During experiments with protein solutions feed and permeate samples of 3 to 4 mL were taken at fixed time intervals and analysed using the UV/Vis spectrophotometer. Permeate flux measurements were conducted periodically throughout the duration of each experiment. CF protein filtration experiments with new membranes were conducted over a minimum of two hours.

### 4.12.3 Experimental matrix – Chapter 6

Protein filtrations using unmodified membranes, pretreated according to the method described in Section 4.11, were conducted at two different ionic strengths (20 mM and 100 mM) and at several different solution pH values. Both BSA and LYZ filtrations were conducted at pH 4.9, 6.0, 7.0 and 8.4. One advantage of operating at these pH values when using BSA is that it does not undergo any significant conformational changes over this pH range (Rezwan et al., 2004). Additional experiments (at both ionic strengths) were conducted with LYZ with both the CF and SC at pH 11.0, the isoelectric point of LYZ. The membrane MWCO of 50,000 Da was chosen to ensure that some of the BSA protein would still be able to transmit through the membrane. LYZ is expected to transmit to a great extent using 50 kDa membranes. Manufacturers generally recommend the use of membranes with a MWCO as low as 10,000 Da in order to fully retain BSA protein. Detailed explanation of why the 50 kDa cut-off was chosen is provided in Sections 5.1.1 and 5.1.2. All protein filtration experiments were conducted using buffer solutions as described earlier (Section 4.3). The TMP in both SC and CF experiments was 25 kPa ±0.1. At this TMP the CF velocity in the CF apparatus was
~0.42 m/s (refer to Section 6.2.1). All experiments with unmodified membranes were carried out at 22±2°C using a new, pretreated Nadir membrane. Experiments with BSA at a higher feed concentration (1.0 g/L) were also carried out in order to investigate concentration effects on permeate flux and rejection (refer to Section 6.3).

4.12.4 Experimental matrix – Chapter 7
The results presented in Chapter 7 were obtained using plasma-modified and unmodified 50 kDa Nadir PES membranes. The filtration experiments were conducted at a TMP of 50 kPa ±0.1 using the SC apparatus. The feed concentration was always 0.5 g/L for all protein solutions. In order to demonstrate any potential charge-interactions the ionic strength had to be low, therefore all experiments were conducted at 20 mM ionic strength. Experiments were conducted at pH 3.2, pH 4.9 and pH 8.4 for BSA and at pH 11.0 for LYZ. All experiments were carried out at 22±2°C.

4.13 Conclusions
In this chapter an overview of the methodologies employed during the experimental work has been provided. The use of PES membranes from two different manufacturers and how membrane cleaning can be important both after solute rejection and protein filtration experiments was discussed. Several apparatus were described including the SC and CF apparatus of which the latter was purpose-built to more closely resemble industrial-scale flow conditions. Other apparatus included the HPLC and UV/Vis employed for analysis purposes and the plasma generator which was used to modify the surface of PES membranes. A range of characterisation methods (MWCO, contact angle, swelling, streaming potential, SEM) were developed and the necessary equipment was described. Initial experiments and information from the membrane manufacturers also led to the development of a membrane pretreatment methodology. Finally, the chapter was completed by discussing the set-up of the main protein filtration experiments. In Chapter 5 results obtained from characterisation experiments are provided.
Chapter 5 — Membrane Characterisation

In this chapter the results from membrane characterisation experiments are shown. These include molecular weight cut-off (MWCO) data obtained both with the stirred cell (SC) as well as the crossflow (CF) apparatus in addition to measurements of water permeability. Scanning electron microscopy (SEM) and contact angle measurements were used to gain insight into the structure and surface properties of the membrane. X-ray photoelectron spectroscopy was carried out with unmodified and plasma-modified membranes and streaming potential measurements were taken in order to obtain surface charge information. Relevant additional calculations and experimental data supporting this chapter are provided in the Appendices and referred to when appropriate.

5.1 Molecular weight cut-off

Ultrafiltration (UF) membranes are generally characterised by the molecular weight (MW) of the solute to be filtered. Membrane manufacturers use the MWCO/nMWCO, the (nominal) molecular weight cut-off, sometimes also referred to as the NMWL (nominal molecular weight limit), as a measure of the retention capabilities of the membrane. By definition, the MWCO of a membrane corresponds to the molecular weight of the probe that is 90% or more retained by the membrane. The MWCO has also been expressed as the upper molecular weight limit of transport above which less than 10% transport through the membrane occurs (Cooper and Van der Veer, 1979). Thus, by definition, if the membrane has a given MWCO then 90% of the solute molecules of the same MW as the cut-off of the membrane should be rejected. In reality, this is not necessarily the case, because the MW of a given solute may translate into an effective solute size that is different to that of the probe molecule (refer to Section 5.2.1). Moreover, experimental MWCO data for a given membrane may differ from that quoted by the manufacturer because of variations in testing conditions including solute concentration, feed flow and hydrodynamic conditions, the use of different standards for testing (including polyethylene glycol (PEG), dextran and certain proteins), or also variations during the membrane production process. In this study, the majority of MWCO measurements were conducted using the SC although the rejection profile, due to hydrodynamic and mass transfer effects, can be expected to vary in a CF configuration as discussed and demonstrated in Section 5.1.3.
In order to characterise an UF membrane in terms of its MWCO it is recommended to measure the permeability of between five to eight standards (commonly dextran, as used in the current work, or PEG) through the membrane under controlled conditions (Cheryan, 1998) whereby transmembrane pressure (TMP), stirring rate/crossflow velocity, solute concentration and temperature are fixed. Sometimes, other solutes such as glucose are also used in addition to dextran or PEG in order to extend the size range covered in the characterisation study. Hence, a few MWCO measurements were also carried out using glucose which has a MW of 180 Da. According to Cheryan (1998) it is also advisable to standardise the volume of test solution to membrane surface area, the permeate-to-feed ratio, and the pretreatment of the membrane. The experimental methodology employed for MWCO measurements with the SC and CF device were described in Section 4.10.1.

5.1.1 Retention curves for unmodified Millipore membranes

Initial research was conducted with unmodified Millipore membranes and their MWCO was evaluated at a TMP of 1 bar(g) (instead of 0.25 bar(g) used for the Nadir MWCO tests). MWCO data for Millipore membranes with 10, 30 and 50 kDa cut-off were obtained using the SC. Figure 5.1 demonstrates that the actual rejection of the membrane was always lower than that expected from the manufacturer MWCO. It is interesting to note that it is only the 10 kDa membrane which seems to be able to fully retain molecules with a MW of 70 kDa; Millipore recommends the use of 10 kDa membranes if full retention of bovine serum albumin (BSA) (~MW 67,000) is desired.

This data illustrates – purely on a size basis - that, although theoretically a cut-off of 50 kDa should retain most of BSA as a solute, this can only be achieved if a membrane with a substantially lower cut-off is chosen. This may not only be due to the variations in MWCO testing methods but also due to the fact that solutes preferentially transfer through the larger pores of a membrane (Cleveland et al., 2002).
Dextran retention tests were also tested for their repeatability, whereby small differences may also be attributed to variability in the membrane sheets. Figure 5.2 shows retention data for a 30 kDa Millipore membrane (47 mm diameter) taken from the same batch (Lot: K4JN7665) as that shown in Figure 5.1. Note that in addition to the dextran standards, a 180 Dalton glucose solution was able to completely transmit through the membrane, as expected. Moreover, the characterisation was also repeated using the larger diameter stirred cell (76 mm, see Section 4.8) to further assess repeatability of the method. The two different membrane sizes were employed in order to demonstrate that the rejection profiles are independent of the surface area of the membrane and also in order to demonstrate that possible variations between membrane sheets can exist. One must be aware that the MWCO method is limited by such variations between membrane sheets, variations in the membrane pore size distribution and also by potential variations introduced by way of preparing and transmitting the dextran solutions. Still, the method provides a useful indication of the expected rejection performance although it is necessary to take into account the error which can be introduced as a result of the mentioned variations.
Ideally, a comparison of the rejection profiles for the tested 30 kDa membranes should be very similar and this can be seen in Figure 5.2. The rejection obtained from the membranes of different diameter is similar. The deviation in rejection between the 47 mm diameter membranes was slightly higher. However, a direct comparison of the rejection values obtained from HPLC analysis yields a variation of no more than ±5%, but mostly ±2% at each data point suggesting that the characterisation technique is robust within the limitations mentioned above. It thus has to be pointed out that such variations have a noticeable effect on the reported rejection at 90% (as seen from the dashed lines in Figure 5.2).

![Figure 5.2. MWCO comparison of unmodified 30 kDa Millipore membranes (SC apparatus).](image)

On this basis, the rejection at 90% was 53 kDa for membrane A and 61 kDa for membrane B. This finding demonstrates two aspects. Firstly, an experimental error of a few percent has to be considered at each data point and variations in the membrane pore structure are to be expected, even for membranes from the same lot. Secondly, the MWCO for these 30 kDa membranes is, in fact, much higher under the chosen experimental conditions than would be expected based on the manufacturers' definition. Such factors have to be accounted for when comparing MWCO data for SC and CF filtration (Section 5.1.3). Refer also to the manufacturer data sheets (see Appendix G).
5.1.2 Retention curves for unmodified Nadir membranes

The MWCO of unmodified 50 kDa Nadir membranes was evaluated in the SC device at 0 25 bar(g) TMP and a stirrer speed of 2,400 rpm. The two membranes were cut from the same sheet in order to observe any potential variability. For both membranes the rejection obtained at 90% is in agreement with the MWCO definition as can be seen in Figure 5.3. Hence, in comparison to the MWCO results obtained with the Millipore membranes, the Nadir membranes showed better repeatability. Moreover, the characterisation method seems repeatable with only one noticeable deviation observed when transmitting the 5 kDa dextran standard. This difference does not have to be associated with any variations in the experimental method but it could be due to a noticeable difference in the pore size distribution (PSD) of the membrane cut from the same sheet.

![MWCO comparison of unmodified 50 kDa Millipore membranes (SC apparatus).](image)

At 90% rejection the MWCO corresponded to 60 kDa (Membrane A) and 52 kDa (Membrane B), respectively.
5.1.3 Stirred cell vs. crossflow MWCO data

Dextran retention for unmodified Nadir membranes was also determined using the CF apparatus (described in Section 4.7) under comparable conditions to the SC (refer to Section 6.2 for details). This was done in order to observe any variations between the two filtration modules. It would not be unreasonable to expect some differences between the MWCO profiles obtained from the two apparatus as the hydrodynamic configurations between the two modules are somewhat different. Whilst both filtration systems have drag components acting perpendicular and parallel to the membrane surface one key difference is that in the crossflow apparatus proteins exiting the device in the retentate stream are recycled back into the feed resulting in continuous regeneration of the concentration polarisation boundary layer at the device inlet. Thus, it is expected to see different degrees of concentration polarisation in the two devices coupled with different mass transfer rates.

A plot of the observed retention for a 50 kDa unmodified membrane using both the SC and CF device is shown in Figure 5.4. Whilst it is not surprising that the solute rejection in the CF device was different to that observed with the SC given the prior reasoning, the most noticeable deviations occurred over the lower MW range (up to 25 kDa). This may have to do with the fact that larger solutes will be mainly retained at the membrane surface, irrespective of which device is used. In such instances, rejection should be similar, although differences in flux may be observed as shown in Figure 5.5. Lower MW solutes, however, will transmit through the membrane according to the forces acting on them. Thus, since hydrodynamic drag and convective forces are different between the two devices due to differing mass transfer rates this is reflected in the solute rejection profile of those standards which are able to transmit through the membrane.

With reference to Figure 5.4, in the case of the SC the rejection correlates well with the manufacturer quoted MWCO, whereas for the CF apparatus the MWCO is closer to 75 kDa. Although it has been demonstrated earlier, that MWCO measurements for the same membrane type can occur due to variations in the testing method and/or the membrane structure, the difference between the measured SC and CF MWCO is considered too substantial to be simply due to experimental error or membrane variability. Even when membrane A from Figure 5.3 (corresponding MWCO was 60
kDa instead of 52 kDa) is compared to the MWCO obtained with the CF apparatus, the deviation is still 15 kDa. This demonstrates that with a CF apparatus it is most likely that a higher MWCO will be obtained compared to a SC device. Interestingly, the higher cut-off obtained with the CF apparatus agrees more closely with the general rule to select a membrane MWCO far lower than the size of the solute to be rejected; it is commonly understood to choose a membrane with a cut-off more than two-fold smaller than the solute to be rejected to obtain near 100% rejection. The previous observations can be of considerable importance in selecting the appropriate membrane for a given application. The finding suggests that it will be inappropriate to assume the MWCO obtained from a SC will allow the user to choose the most suitable membrane for an application which is to be conducted with an apparatus with a different flow configuration.

![Figure 5.4. MWCO comparison unmodified 50 kDa Nadir membranes (SC and CF apparatus).](image)

In order to support these findings a direct comparison of solute rejection data obtained with a 10 kDa membrane using the two devices was also conducted. Thereby it was possible to see whether similar results could be obtained using a membrane with a different cut-off (see Figure 5.6). Once again it can be seen that dextran solute rejection was lower with the CF apparatus. It is worth noting that in both cases (SC and CF retention test using a 10 kDa membrane) the MWCO was always higher than would be expected from the MWCO definition. Further discussion of the different findings that
were made for the SC and CF experiments can be found in the Journal paper publication, see Appendix G.

![Graph showing dextran flux vs. molecular weight for SC and CF filtration.](image1)

**Figure 5.5.** Dextran flux vs. molecular weight for SC and CF filtration.

![Graph showing MWCO comparison between SC and CF for unmodified 10 kDa Nadir membranes.](image2)

**Figure 5.6.** MWCO comparison between SC and CF for unmodified 10 kDa Nadir membranes.
5.1.4 Retention curves for plasma-modified membranes

The MWCO of an acrylic acid (AA) modified Millipore membrane with an untreated rating of 50 kDa MWCO was determined using the retention test and the SC at 1 bar(g). In this case, the modification process appeared to be significantly lower than the MWCO and hence the PSD of the membrane (as shown in Figure 5.7). The profile is compared with profiles for unmodified 10 kDa and 50 kDa Millipore membranes. The graph implies that the plasma-modified Millipore membrane now has a much smaller MWCO, lower than that of an unmodified 10 kDa membrane. Since dextrans are neutral solutes charge effects are expected to be absent, hence this finding suggests a reduction of the membrane PSD due to polymer deposition. Interestingly, a corresponding observation was not made after plasma modification of Nadir membranes as shown in Figure 5.8. The discrepancy is likely to be due to differences in membrane morphology between the membranes from the two different manufacturers, or possibly due to a difference in the application of the plasma modification process and potentially also the higher pressure that was used during the retention test. Testing of this particular modified membrane was carried out before the methodology to conduct retention tests at 0.25 bar(g) had been established. At 1 bar(g) the likelihood for dextran fouling to occur is higher (Susanto et al., 2007).

![Figure 5.7. MWCO of surface modified (AA) 50 kDa Millipore membrane (SC).](image)
Rejection curves for plasma-modified Nadir membranes were obtained for membranes exposed to plasma only and plasma- and AA-modified Nadir membranes (see also Section 4.9.2). In Figure 5.8 the rejection curve for the AA-modified membrane is directly compared with data for the plasma only modified membrane and an unmodified 50 kDa Nadir membrane. It can be observed that in the higher MW region the dextran solute rejection was higher with the unmodified 50 kDa membrane. In the lower MW region, however, rejection was greater with the modified membrane suggesting that the modification process may have caused closure of at least some of the smallest membrane pores. The vertical dashed lines also show that at 90% rejection, the dextran solute retention has actually decreased which suggests an increase in the size of the larger pores as a result of the modification process.

![Figure 5.8. MWCO comparison of unmodified and plasma-modified 50 kDa Nadir membranes (SC).](image)

Comparison of the plasma modified only and the AA-modified Nadir membranes shows that in the higher MW rejection range the membrane treated by plasma only shows more rejection, however, in the lower MW range rejection was less with membranes modified by plasma only. One possible explanation is the additional deposition of material taking
place during AA dipping which creates additional membrane resistance and/or blockage of some of the smaller pores.

It is unclear just from the MWCO data whether a charge was placed on the membrane surface as a result of the AA dipping and thus the MWCO data has to be interpreted in context with protein filtration and streaming potential data (see Section 5.9.1 and Chapter 7).

5.2 Interpretation of Molecular weight cut-off data

The Molecular weight cut-off (MWCO) is not as convenient a measure as pore size is, for instance, with microfiltration (MF) membranes. It is therefore warranted to attempt a more meaningful interpretation of MWCO data by relating it more closely to molecular size rather than MW. Moreover, it is then possible to relate the solute size of dextrans to that of the proteins used in the filtration studies.

5.2.1 Stokes radius

The MW of a solute such as dextran can be related to its actual spherical size. In order to do so, simple correlations are available to determine the Stokes radius of a given solute. Stokes radii of dextrans and the radii of the proteins to be separated can be determined and related in order to obtain an idea whether the separation can be solely attributed to size or if other physicochemical effects also have to be considered. According to the literature, bovine serum albumin (BSA), for instance, has a Stokes radius of 3.45 nm (Menon and Zydney, 1999). If the Stokes radius of an equivalent dextran is known then one may conclude that BSA behaves similar to such a neutral dextran under the same operating conditions, particularly at its isoelectric point (pI) assuming proteins and dextrans are of comparable shape. The Stokes radius (assuming a spherical molecule) may be determined using some form of a Mark-Kuhn-Hawink type equation (Cherkasov, 2005):

\[ r_s = k(M_w)^y \]  \hspace{1cm} (5.1)

where \( r_s \) is the minimal Stokes radius, \( M_w \) the MW of the molecule in question and \( k \) and \( y \) are constants. Table 5.1 illustrates data provided by the dextran supplier PSS.
(Polymer Standards Services, Germany) from which the Stokes radius according to Hemmelder (1999) was calculated. Equation (5.1), with \( k = 0.33 \) and \( \gamma = 0.463 \), as employed by Hemmelder and first introduced in this form by Granath and Kvist (1967) was preferentially used.

### Table 5.1. Dextran data from manufacturer and calculated Stokes radii.

<table>
<thead>
<tr>
<th>Dextran - Polymer Standards Service (Polydispersion, molecular weight &amp; Stokes radii)</th>
<th>Description</th>
<th>Symbol</th>
<th>Unit</th>
<th>Dextran 1</th>
<th>Dextran 5</th>
<th>Dextran 12</th>
<th>Dextran 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>g/mol</td>
<td>1,000</td>
<td>5,000</td>
<td>12,000</td>
<td>25,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mass average molecular mass</td>
<td>( M_w )</td>
<td>5,200</td>
<td>11,600</td>
<td>23,600</td>
<td>50,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number average molecular mass</td>
<td>( M_n )</td>
<td>3,300</td>
<td>8,100</td>
<td>18,300</td>
<td>150,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polydispersity</td>
<td>( M_w/M_n )</td>
<td>1.58</td>
<td>1.43</td>
<td>1.30</td>
<td>2.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecular weight correction for polydispersity</td>
<td>( M = (M_wM_n)^{1/2} )</td>
<td>1,194</td>
<td>4,142</td>
<td>9,693</td>
<td>20,670</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified Stokes' radius (0.33 x ( M^{0.463} )) ( \times 10^3 )</td>
<td>( \text{nm} )</td>
<td>8.77</td>
<td>15.81</td>
<td>23.13</td>
<td>33.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dextran - Polymer Standards Service (Polydispersion, molecular weight &amp; Stokes radii)</th>
<th>Description</th>
<th>Symbol</th>
<th>Unit</th>
<th>Dextran 50</th>
<th>Dextran 150</th>
<th>Dextran 670</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>g/mol</td>
<td>50,000</td>
<td>150,000</td>
<td>670,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mass average molecular mass</td>
<td>( M_w )</td>
<td>68,000</td>
<td>144,000</td>
<td>666,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number average molecular mass</td>
<td>( M_n )</td>
<td>35,000</td>
<td>100,000</td>
<td>333,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polydispersity</td>
<td>( M_w/M_n )</td>
<td>1.48</td>
<td>2.01</td>
<td>2.01</td>
<td>2.01</td>
<td></td>
</tr>
<tr>
<td>Molecular weight correction for polydispersity</td>
<td>( M = (M_wM_n)^{1/2} )</td>
<td>41,585</td>
<td>121,655</td>
<td>471,640</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified Stokes' radius (0.33 x ( M^{0.463} )) ( \times 10^3 )</td>
<td>( \text{nm} )</td>
<td>45.41</td>
<td>74.63</td>
<td>139.76</td>
<td>139.76</td>
<td></td>
</tr>
</tbody>
</table>

Hemmelder (1999)

Various authors used equation (5.1), but employed different values for the constants \( k \) and \( \gamma \), hence, there can be noticeable differences in the calculated Stokes radii. Such deviations are shown in Table 5.2. In the current work, the equation used by Hemmelder (1999) was used to determine the Stokes radii of dextrans, because it takes into account a MW correction for the polydispersity of the sample. The Stokes radii of dextrans are used in applying pore flow models to MWCO data in Section 5.2.2. Comparing all four approaches it is noticeable that two overestimate the dextran radius (Causerand et al., 2002, Oliver and Decn, 1994) and the equation used by Cherkasov (2005) greatly underestimates the dextran radius, if the approach by Hemmelder is assumed to be correct.
Table 5.2. Comparison of dextran radii using various forms of equation (5.1).

| Dextran standards - deviations in Stokes radii due to the use of different values for constants k and y. |
|----------------------------------|----------------------------------|---------|---------|---------|---------|
| Description | Symbol | Unit | Dextran 1 | Dextran 5 | Dextran 12 | Dextran 25 |
| Mass average molecular mass | \( M_a \) (g/mol) | 1.320 | 5.200 | 11.500 | 23.600 |
| Molecular weight correction for polydispersity | \( \frac{M}{(M_w^2 + M_m^2)^{1/2}} \) | 1.194 | 4.142 | 9.683 | 20.670 |
| Causserand et al. * | \( a = 0.33 \) \( (M_w^2 + M_m^2)^{1/2} \) | \( a \) (radius) | 0.90 | 1.69 | 2.44 | 3.40 |
| Oliver and Deen # | \( r = 0.448(M_w^2)^{1/3} \) | \( r \) (radius) | 1.04 | 1.88 | 2.68 | 3.66 |
| Cherkasov | \( r = 0.49(M_w^2)^{1/3} \) | \( r \) (radius) | 0.75 | 1.27 | 1.72 | 2.26 |
| Modified Stokes' radius \( (0.33 x M_w^2)^{1/3} \) | | (Å) | 0.77 | 1.56 | 2.33 | 3.30 |

* Causserand et al. (2002)
# Oliver and Deen (1994)
Cherkasov (2005)
Hemmelder (1999)

A log-plot of the Stokes radii vs. MW determined with the different forms of equation (5.1) shows that dextrans of the greatest interest (5 kDa to 50 kDa) have radii from less than 2 to about 5 nm (Figure 5.9).

![Figure 5.9. Log-scale dextran Stokes radii vs. molecular weight.](image)

Using equation (5.1) in the form of equation (5.2) allows the MW of the dextran corresponding to the radius of protein molecules to be determined (Causserand et al., 2002)
Chapter 5 – Membrane Characterisation

\[ M_w = e^{\ln\left(\frac{r_s}{0.33}\right) / 0.46} \]  

The equation was selected as it provides a value for the solute radius, \( r_s \), closest to the one determined when polydispersity is taken into account (i.e., as done in the approach by Hemmelder (1999)). On this basis, a radius of 3.45 nm of BSA corresponds to a dextran molecular weight \( M_w \) of 24,535 or 25 kDa. The BSA molecule can therefore, at least purely based on size, be assumed to be closely related to a 25 kDa dextran. This is important in selecting a membrane with an appropriate MWCO. A choice of membrane based on the Stokes radius seems more appropriate than simply on a molecular weight basis. In the case of BSA (MW 66,430) one would assume that a 50 kDa cut-off membrane will reject most of the protein, whereas the Stokes radius, in fact, suggests a membrane with a cut-off of at least 25 kDa or lower would be more appropriate (ignoring other physicochemical effects that may impact upon separation). In other words, considering the Stokes radius of BSA, one would expect this protein to be similarly transmitted (or rejected) through a membrane with a given MWCO as a 25 kDa dextran would.

If the same approach is also taken for LYZ, which is reported to have a Stokes radius of 1.83 nm (Rabiller-Baudry et al., 2000), one can immediately see that predictions of LYZ protein rejection on a molecular weight basis (MW 14,700) can also be misleading. Using the reported LYZ Stokes radius in equation (5.2) gives a corresponding MW of 6,183, therefore LYZ corresponds more closely to a 5 kDa dextran. Again, this suggests that for LYZ to be completely retained by the membrane on a size-basis (neglecting other effects) a membrane cut-off at least a third of the protein MW would have to be chosen.

This analysis confirms the general rule which is to select a membrane cut-off much lower than the protein MW in order for the protein to be fully rejected by the membrane.
5.2.2 Pore models

In this section, two models, Ferry's and the Steric Hindrance Pore (SHP) model are introduced as a means to determine an equivalent membrane pore size for a given solute size. Such models are useful in relating rejection data to different pore sizes and it will be demonstrated that the two pore models give very similar equivalent pore sizes. Knowledge of the Stokes radii of dextran solutes, as calculated in Section 5.2.1, can be used with pore models to allow the relationship between solute rejection and membrane pore size to be evaluated. The two pore models discussed below are commonly used when the pore size of the membrane and the Stokes radii of the solute(s) used in retention tests are known. With this information the rejection profile of a given membrane can be predicted. In the current work the models are reversed, that is existing data from MWCO retention tests and knowledge of the Stokes radii are used in order to determine the pore size of the membrane for a given solute rejection value. Hence, the models can only predict the approximate size of a pore relative to a solute of a fixed size. However, this approach allows one to obtain an idea of the membrane pore size at 90% rejection which is of interest in MWCO determinations.

It has been shown earlier that the membrane MWCO is not always the most convenient characterisation tool as it can be difficult to directly relate the solute size to the membrane cut-off. However, several researchers have attempted to relate solute diameter, \( d_s \), to the actual pore diameter, \( d_{mp} \), of an UF membrane (Ferry, 1936; Mehta and Zydney, 2005; Zydney et al., 1994; Van der Bruggen et al., 1999). Ferry’s model is one of the early attempts that assumes an equivalent uniform pore size, but is obviously a gross assumption as most membranes, particularly asymmetric membranes, will inherently have a PSD. Nevertheless, the model provides a simple and useful approach to obtain an estimate of the average pore size of a membrane for which rejection data has been obtained. Ferry’s model defines a reflection coefficient \( \sigma \) which can be directly related to the membrane rejection coefficient if boundary layer effects on the membrane are eliminated by agitation (Cifra and Bleha, 2005) such that:

\[
R_o = 1 - \frac{C_p}{C_b} = \sigma = 1 - 2(1 - \eta)^2 - (1 - \eta)^4
\]
where $\eta$ is the solute to pore diameter ratio ($\eta = d_s/d_{mp}$). The model is limited in that it does not explicitly consider any electrical charges of the dispersed molecules or the membrane and some solutes which penetrate the pore may not transmit through the membrane into the filtrate, which may lead to a slight underestimation of the pore size (Ferry, 1936).

The steric hindrance pore (SHP) model includes a wall correction parameter, $H_F$, and a steric hindrance parameter, $S_F$. The reflection coefficient is expressed as:

$$\sigma = 1 - H_F S_F$$  \hspace{1cm} (5.4)

with

$$H_F = 1 + \left(\frac{16}{9}\right) \eta^2$$  \hspace{1cm} (5.5)

and

$$S_F = (1 - \eta)^2 \left[2 - (1 - \eta)^2\right]$$  \hspace{1cm} (5.6)

By way of example, both Ferry's and the SHP model were applied to dextran rejection data of a 50 kDa Nadir membrane using the Stokes radii of dextrans as determined by Hemmelder's (1999) approach (refer to Table 5.1 earlier). In order to solve for the membrane pore size using the known rejection data and solute molecule size the mathematical software Maple 10.0 was employed. Because of the exponents present in both equation (5.3) and (5.6) solving for the pore size results in more than one solution. The “solve” function in Maple was used to obtain these solutions and subsequently constraints were employed, that is $0 < \eta < 1$. The first constraint is based on the fact that the solute to pore size ratio tends to zero when the solute size tends to zero. The second constraint considers the fact that the pore size has to be larger than the solute size for any transfer to take place and if the solute size is equal or greater than the pore size then 100% rejection should occur. A sample solution for both Ferry’s and the SHP models to determine the pore diameter of the membrane at 90% solute rejection is shown in Appendix C, part d. The relationship between the solute size and membrane pore size based on 50 kDa MWCO dextran rejection data is shown in Figure 5.10.
Generally, for solute transmission to take place the membrane pore size has to be larger than the solute size which is reflected in the size difference between the membrane pore and the solute. As the solute size increases from a low value the difference between membrane pore size and solute size should decrease which can be observed from Figure 5.10. It is also noticeable from Figure 5.10 that the pore sizes obtained are slightly larger with Ferry's model than with the SHP model. Generally, it can be inferred that the pores of a 50 kDa membrane range from at least ~4.4 nm in diameter up to ~28 nm. This is interesting, because if it is recalled that BSA has a 3.45 nm Stokes radius (6.9 nm diameter), it is not surprising that some of the BSA transmits through a 50 kDa membrane (as shown later on in Chapter 6). If only the MWCO of the membrane (50 kDa) and the MW of BSA (66.4 kDa) are considered, one would expect 100% rejection of BSA. However, Figure 5.10 clearly shows that (calculated) membrane pore sizes are mostly larger than the diameter of BSA.

It is also useful to apply the two models to rejection data obtained for an unmodified 10 kDa membrane, because – on a MWCO basis – such a membrane is expected to reject 100% of a BSA protein. In Figure 5.11 the two models are applied to 10 kDa rejection data which show that the smallest pore is greater than 4.4 nm when using Ferry’s model and greater than 4.1 nm when using the SHP model.
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Figure 5.11. Pore models applied to solute rejection data of a 10 kDa Nadir membrane.

At 90% rejection, the pore diameter for each model determined by interpolation, was 7.3 nm using Ferry’s model and 7.2 nm using the SHP model. Therefore, at 90% rejection (definition of MWCO) the membrane pore is still slightly larger than the diameter of BSA (6.9 nm) using either model. Hence, it is not completely surprising that even with a 10 kDa membrane 100% rejection of BSA is not achieved, as will be demonstrated in Section 6.3.

5.2.3 Further discussion

In order to fully appreciate the meaning of MWCO data obtained from dextran solute rejection it is important to be aware of recent literature. Firstly, MWCO characterisation should always be conducted at a fixed pressure, because, although dextrans are neutral solutes, they result in different rejection data at different TMP’s; as experimentally demonstrated by Le and Howell (1984). It is for this reason that, both MWCO experiments with the SC and the CF apparatus, were conducted at 0.25 bar(g) instead of 1 bar(g) as a low TMP helps to minimise any pore blockage by dextrans which would lead to membrane fouling. Moreover, it was desirable to match the MWCO experimental pressure with that employed during the majority of protein filtration experiments (see Chapter 6). It has, however, been suggested that using varying TMP for solute rejection measurements (using PEG) does not cause much alteration in the
observed MWCO, at least for the pressure range (380 to 520 kPa) studied by Cleveland et al (2002). In contrast, Cheng and Huang (2002) indicated in their study of dextran solute rejection in a dead-end UF cell that pressure does have an influence on the dextran rejection. Zaidi and Kumar (2004) showed a pressure dependence of dextran rejection as well (using a SC device), attributing the effect to the extent of any concentration polarisation layer formed. Moreover, a concentration dependence on the thickness of the concentration polarisation layer was found.

Although dextrans are considered as neutral solutes, Susanto and Ulbricht (2005) demonstrated that dextrans can adsorb onto a membrane and cause fouling. Their study was conducted using polyethersulfone (PES) and cellulose membranes suggesting that this phenomenon could also be an issue in the current work. Other research with fluorescent dextrans has indicated a change in membrane retention due to the presence of dextrans (Mulherkar and van Rels, 2004). In recent work experiments were performed in an attempt to quantify dextran fouling and comment upon the underlying mechanisms (Susanto et al., 2007). However, both of the aforementioned studies were carried out using a dead-end SC at relatively low stirring speed which raises the question of whether dextran fouling would not be less of an issue at higher stirring speeds and/or if a CF device had been used. Not surprisingly, Susanto et al. (2007) reported much lower permeate flux rates for dextran solutions compared with water. They attributed this to concentration polarisation. Moreover, a dextran concentration of 1 g/L was used in their study, which, whilst being a common feed concentration, is relatively high and a lower starting concentration would be expected to show less fouling. In fact, when trying to demonstrate dextran adsorption the concentration used by Susanto et al. (2007) was as high as 10 g/L, not directly resembling common dextran solute rejection testing conditions. Possible mechanisms for dextran adsorption onto PES membranes were found to include hydrogen bonding between free hydroxyl groups from the dextran and oxygen atoms from the SO₂ groups of the PES membrane, and adsorption due to displacement of water at a hydrophobic membrane surface. The latter mechanism could certainly be an issue with the PES membranes used in this study, as contact angle measurements indicated a tendency toward hydrophobicity (refer to Section 5.7.1)
5.3 Flux performance

5.3.1 Water flux

Water flux measurements are, in principle, relatively easy to conduct and can provide useful membrane characterisation data. The flux of ultrapure water is often used to determine the initial hydraulic resistance of a membrane prior to its use in the actual application (see also Section 6.4.6). Commonly, water flux is also re-evaluated after membrane filtration in order to determine the degree of reduction in flux due to fouling. Caution is advised when using water flux information only, because the unique properties of water such as a high dielectric constant and its polarity can also impact on its flux so that misleading conclusions may be drawn if water flux data are related to protein flux data (Israelachvili, 1992). Moreover, water permeability may not only be influenced by the pore size but also by the surface tension, \( \gamma \), of the material, hence contact angle measurements are often used to obtain additional information about the characteristics of UF membranes.

For a given membrane, the extent of membrane fouling can be quantified by comparison of the water permeate flux rate of the virgin, \( J_w \), and the fouled, \( J_p \), membrane. The water flux equation can simply be stated as:

\[
J_w = \frac{Q_w}{A_m \Delta t}
\]  

(5.7)

where \( Q_w \) is the volume of water permeated, \( A_m \) the membrane area in m\(^2\) and \( \Delta t \) the sampling time.

The percentage flux reduction (FR) is estimated by equation (5.8) as used by Ehsani et al. (1997):

\[
FR = \frac{J_w - J_p}{J_w} \times 100
\]  

(5.8)

Water flux measurements were used to characterise both unmodified and plasma-modified membranes.
5.3.2 Water flux data for Millipore membranes

Water flux generally increases linearly with pressure. However, water flux can change significantly with time if no prior membrane compaction was conducted; it is therefore advisable to flush a new membrane with water at a pressure higher than the TMP to be used during the actual application. This scenario was illustrated with an experiment using the 47 mm diameter SC and an unmodified 30 kDa Millipore membrane (Figure 5.12). The error bars represent the standard deviation between three sets of experiments.

![Figure 5.12. Water flux vs. time for an unmodified 30 kDa Millipore membrane at 1 bar(g) TMP (no prior compaction).](image)

For the Millipore membrane it takes approximately 50 minutes permeation time before a reasonably stable water flux can be obtained. The flux reported time averaged with variations around ±1%, but no more than ±2%. Figure 5.12 also illustrates why such wetting and/or membrane compaction effects cannot be neglected, because using this membrane after 10 minutes filtration of, for instance, a protein solution would likely lead to incorrect conclusions about protein permeate flux and/or other factors such as potential fouling and concentration polarisation effects. A plot of water flux over a 60 minute period for unmodified 30 kDa Millipore membranes using the SC with and without pre-compaction demonstrated that a membrane subjected to the pretreatment
procedure (Section 4.11) exhibits more consistent and stable water flux from the beginning of permeation (Figure 5.13). The experiments in Figure 5.13 were duplicated and an error of a mean standard deviation is shown.

![Graph showing water flux comparison](image)

**Figure 5.13. Water flux comparison of a pretreated and a virgin 30 kDa Millipore membrane.**

### 5.3.3 Water flux data for Nadir membranes

Microdyn-Nadir GmbH provides an indication of the clean water flux rates to be expected for their UF membranes (Table 5.3)

<table>
<thead>
<tr>
<th>Membrane-reference</th>
<th>MWCO (kDa)</th>
<th>Water flux (L/m²h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UP010</td>
<td>10</td>
<td>&gt; 150</td>
</tr>
<tr>
<td>UH030</td>
<td>30</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>UH050</td>
<td>50</td>
<td>&gt; 250</td>
</tr>
</tbody>
</table>

**Test conditions**: 3 bar, 20 °C, 700 rpm (SC)
It seems unusual that the flux rate tested at the same pressure for 30 kDa membranes should be lower than the flux rate tested for 10 kDa membranes, however, according to the manufacturer this has to do with differences used in the casting process employed during the manufacture of the 10 and 30 kDa membranes.

An example of clean water flux data for an unmodified Nadir 50 kDa membrane using the SC apparatus at different TMPs up to 1.5 bar(g) is shown in Figure 5.14. Also shown are data for a similar test conducted with the CF apparatus at several pressures up to 1 bar. The test was repeated for both increasing and decreasing TMPs in order to quantify any possible hysteresis effects (which were negligible). However, it must be noted that the TMP at a given data point can vary by ±0.01 bar. At exactly the same TMP, flux variations were less than ±1%. By comparing water fluxes it can be observed that generally the flux was higher in the CF apparatus than in the SC apparatus. Noting that the pretreatment methodology described in Section 4.11 was applied in each case, Figure 5.15 shows permeation data for 16 membranes tested in both the SC and CF apparatus and it is clear that the flux was always higher in the CF apparatus. The error bars show the standard deviation for each set of 16 membranes. Moreover, the flux variation error in the CF apparatus was slightly lower with ±5% variation compared to ±7% variation with the SC apparatus. An average flux rate was determined from three sets of data for each membrane. Figure 5.15 clearly shows that variations in clean water flux from membrane sample to membrane sample exist, even when these samples originated from the same sheet.
Chapter 5 – Membrane Characterisation

Figure 5.14. Clean water flux vs. TMP through a 50 kDa membrane using the SC and CF apparatus.

Figure 5.15. Clean water flux data for 16 Nadir 50 kDa membranes at 0.25 bar(g) (SC and CF).

Finally, water flux data using the CF apparatus is shown for pretreated, unmodified Nadir membranes with a respective MWCO of 10 and 50 kDa (Figure 5.16). The graph demonstrates that clean water flux for a 10 kDa membrane is more than two-fold lower.
than that for a 50 kDa membrane. Moreover, it also demonstrates that following pre-compaction a stable water flux rate is established rapidly.

![Graph showing water flux data for unmodified 50 and 10 kDa Nadir membranes at 25 kPa transmembrane pressure (CF).](image)

**Figure 5.16.** Clean water flux data for unmodified 50 and 10 kDa Nadir membranes at 25 kPa transmembrane pressure (CF).

### 5.3.4 Water flux for plasma-modified Nadir membranes

Water flux data for a plasma-modified membrane can provide an initial indication of any potential changes in membrane properties that may have occurred as a result of the modification. A water flux value notably different from the original membrane water flux would suggest a substantial change in the membrane properties. Using the SC, water flux data was obtained for 50 kDa (original rating) modified membranes subjected to plasma only and for modified membranes which had also been acrylic acid (AA) treated. The water flux for the modified and AA-treated membrane was also measured in the CF apparatus. Figure 5.17 demonstrates that the water flux of modified membranes is an order of magnitude lower than that of an unmodified 50 kDa membrane (see Figure 5.16). This implies that the MWCO of the membrane has become “tighter”, the membrane resistance has substantially increased and/or the membrane became hydrophobic. MWCO data for unmodified and modified 50 kDa membranes (Figure 5.8) suggest, although some change in the MWCO can be observed, that the
drastic reduction in water flux is not solely due to a change in MWCO. In order to be considered responsible for the substantially reduced water flux, the MWCO would have to be altered and not allow the transmission of any proteins such as BSA, but this was not the case (refer to Chapter 7). Thus, a reduction in flux due to added membrane resistance and/or a more hydrophobic membrane must also be considered (see Section 5.7.1)

Figure 5.17. Clean water flux for 50 kDa plasma-modified Nadir membranes (25 kPa, SC and CF).

Figure 5.17 also demonstrates that the flux in the CF apparatus was higher compared to that in the SC apparatus, as had been previously observed for unmodified membranes.

Water flux measurements provide a convenient means for initial comparison between unmodified and modified membranes. A previous study by Gancarz et al. (1999) using polysulphone membranes also showed cases where the water flux of the modified membrane was reduced after plasma modification. In that particular instance, however, this was due to the non-polymerising gas (CO₂) which can cause a decline or an increase in water flux of the modified membrane depending on the plasma treatment time (as shown earlier in Figure 2.15). The decrease in water flux seen at two minutes treatment time may seem rather obscure but it was attributed to the possibility of deposition of degraded particles during the polymerisation process. At longer treatment
times ablation becomes more prominent therefore a subsequent increase in water flux is observed because, at this stage, the pore size will have increased further so that potential adsorption of degraded membrane skin inside the pores becomes less significant. However, the membranes employed in this thesis were treated using argon plasma for 60 s only at an intensity of 200 W. Under such conditions a degradation of the membrane structure resulting in smaller pores would not be expected.

5.3.5 Electrolyte flux data for Nadir membranes

It was mentioned in the literature review (Section 2.4.5) that electroviscous effects generally play only a role if the membrane is significantly charged. If a membrane is charged, a polar liquid, such as water, will not only be oriented in a particular direction at the interface, but it will also yield a different flux rate compared to an electrolyte solution as double-layer interactions will be stronger compared to salt solutions due to the shielding effect; a high ionic strength causes the electrical double layer to be smaller and the charge around a molecule tends to become suppressed. Thus, a salt solution such as KCl or NaCl with a low or intermediate ionic strength can be passed through a membrane and compared to water flux data in order to obtain an indication of any charge interactions. This is also of interest when conducting streaming potential measurements as such measurements are generally conducted using KCl or NaCl solutions and the apparatus used for this study also allowed parallel salt solution flux measurements. The fact that a difference in water and electrolyte flux should be observed for a charged membrane also implies that for an uncharged membrane no difference may be seen. This is because salts are sufficiently small in size allowing them to fully transmit through an UF membrane.

Electrolyte (at low salt concentration) and water flux data were obtained for a 50 kDa unmodified Nadir membrane using the CF apparatus at 0.25 bar(g) TMP. When comparing electrolyte and water flux measurements care must be taken not to employ a high salt concentration as osmotic pressure effects can become important which may lead to erroneous conclusions about the existence of charge effects. It is evident from Figure 5.18 that there is no significant difference between water and salt solution flux when using an unmodified 50 kDa Nadir membrane, as was expected. Moreover, the flux variation error is no greater than \( \pm 4 \text{ L/m}^2\text{h} \) corresponding to approximately \( \pm 3.5\% \) variation. It was observed that it can take a few minutes until a steady filtrate flux is
established, even when the membrane was compacted beforehand. The result from Figure 5.18 also implies that the unmodified 50 kDa Nadir membrane carries no significant charge which was indeed confirmed by streaming potential measurements (Section 5.9.1).

\[\begin{array}{|c|c|c|c|c|c|c|c|}
\hline
\text{Time (mins)} & 0 & 10 & 20 & 30 & 40 & 50 & 60 \\
\hline
\text{Flux (L/m}^2\text{h}) & 100 & 100 & 100 & 100 & 100 & 100 & 100 \\
\hline
\end{array}\]

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{flux_vs_time.png}
\caption{Flux vs. time for water and an electrolyte solution with an unmodified 50 kDa Nadir membrane (CF).}
\end{figure}

In order to further assess the impact of any electroviscous effects 0.1 M KCl solution was permeated through unmodified and plasma-modified 50 kDa Nadir membranes using the SC at 0.25 bar(g). Figure 5.19 shows that it was not possible to observe a significant effect suggesting that electroviscous effects are not an issue with the modified membranes. In other words, it can be concluded that the significantly reduced permeate flux brought about by the surface modification masks any potential electroviscous effect or alternatively the membrane does not carry much charge.

The error bars indicate that flux varies with time and if the higher flux rate at the beginning of filtration is included in the calculation the variation is of the order of ±6% or 0.2 L/m$^2$h. It is also immediately obvious that permeate flux was substantially reduced as a result of the surface-modification. Moreover, the result suggests that the plasma-modified membrane is likely to carry less of a charge than would be expected. These aspects will be discussed in more detail in Chapter 7. The results also support
contact angle (Section 5.7.1) and streaming potential (Section 5.9.1) data which also demonstrate that the charge on the modified membranes is limited.

![Graph](image)

Figure 5.19. Flux vs. time for water and an electrolyte solution with a modified 50 kDa Nadir membrane (SC).

5.4 Membrane cleaning experiments

Membrane cleaning was studied early on in this research (as some membranes where only available in limited supply) and initial work was conducted using 50 kDa Millipore membranes. 0.1% dextran solutions were transmitted through the 47 mm diameter membranes using the SC. Despite the claims from Millipore that 0.1 M NaOH alone is suitable for most cleaning applications, the initial pure water flux could not be restored by this method as illustrated by measuring the water flux of virgin, fouled and cleaned membranes. Figure 5.20 shows typical results where flux with the dextran solution (1 g/L) was substantially lower than the clean water flux and that the water flux obtained after soaking in NaOH was only marginally improved. The substantially lower flux suggests that dextran fouling occurs with the Biomax membranes at 1 bar(g) TMP pressure.

The experimental methodology used to conduct this and other cleaning experiments was outlined in Section 4.5.3 and the most important results are summarised in Table 5.4. The results show that NaOH is able to break down the dextrans 4-fold or more,
depending on the NaOH concentration. The data show little improvement in the cleaning effectiveness when 0.5 M and 1.0 M NaOH were used, but the latter two seem to be at least slightly more effective at breaking down dextran into smaller components than the 0.1 M NaOH solution.

![Graph](image)

**Figure 5.20.** Different flux measurements through a 10 kDa Millipore membrane at 1 bar(g) TMP.

It can be seen from Table 5.4 that after 8 hours of contact the refractive index measurement reduces more than 8-fold when using 0.5 M NaOH or higher. Column retention times fluctuated by ±0.1 minute which was generally observed to be the achievable accuracy. The results also imply that the reaction is kinetically fast, because most of the reduction occurs within the first minute of contact between dextran and NaOH. However, even though these data show that dextran can be broken down into smaller components, thereby helping to clean a membrane, it does not provide a direct estimate of the efficiency of cleaning within the membrane structure. This is also why some researchers conduct membrane cleaning not only by soaking in solution but also by placing the membrane into an ultrasonic bath to provide movement. What the results do suggest is that any possible dextran accumulation at the membrane surface should be removed by soaking the membrane in NaOH solution followed by subsequent flushing with water under pressure. This does, however, not guarantee the removal of dextrans located within the membrane pore structure.
Table 5.4. Hydrolisation reaction data for various sodium hydroxide molarities and 5 kDa dextran.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Reaction time/ Sampling time</th>
<th>Column retention time (minutes)</th>
<th>Refractive index reading (nRIU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 kDa dextran, pure sample</td>
<td>-</td>
<td>15.3</td>
<td>877,000</td>
</tr>
<tr>
<td>0.1 M NaOH + dextran</td>
<td>1 min</td>
<td>15 2</td>
<td>276,000</td>
</tr>
<tr>
<td>0.1 M NaOH + dextran</td>
<td>1 hour</td>
<td>15 1</td>
<td>170,000</td>
</tr>
<tr>
<td>0.1 M NaOH + dextran</td>
<td>8 hours</td>
<td>15.2</td>
<td>155,000</td>
</tr>
<tr>
<td>0.5 M NaOH + dextran</td>
<td>1 min</td>
<td>15 1</td>
<td>146,000</td>
</tr>
<tr>
<td>0.5 M NaOH + dextran</td>
<td>1 hour</td>
<td>15.3</td>
<td>139,000</td>
</tr>
<tr>
<td>0.5 M NaOH + dextran</td>
<td>8 hours</td>
<td>15 2</td>
<td>108,000</td>
</tr>
<tr>
<td>1.0 M NaOH + dextran</td>
<td>1 min</td>
<td>15.2</td>
<td>118,000</td>
</tr>
<tr>
<td>1.0 M NaOH + dextran</td>
<td>1 hour</td>
<td>15.1</td>
<td>108,000</td>
</tr>
<tr>
<td>1.0 M NaOH + dextran</td>
<td>8 hours</td>
<td>15 1</td>
<td>104,000</td>
</tr>
</tbody>
</table>

It can also be seen from Table 5.4 that it was possible to reduce the dextran concentration by nearly 90% (using 1.0 M NaOH). The use of 0.1 M NaOH, as suggested by the manufacturer, resulted in a nearly 4-fold reduction in dextran concentration within the first minute of contact. This suggests that purely on a hydrolisation basis NaOH should work well as a cleaning agent. Nevertheless, NaOH cleaning of PES membranes is not always effective which is likely to be due to a lack of penetration of NaOH into the membrane structure, particularly if the membrane is simply soaked in the cleaning solution. As a result of these findings any membrane cleaning carried out in this research was conducted using 0.5 M NaOH and permeation of the cleaning agent at low pressure was also conducted instead of simply soaking the membrane in solution.

Plasma-modified membranes were more difficult to clean because of their low permeation rates. Soaking of the membrane in NaOH could be carried out in the same manner, but if NaOH was to be transmitted through the membrane under pressure extremely long permeation times were required. In general, new membranes were employed for each experiment. If re-use of a previously employed membrane was required the cleaning effectiveness using NaOH was subsequently tested by comparing the water permeation rate of the membrane before filtration and after cleaning.
5.5 Membrane swelling

Many polymeric materials will swell in a solvent. Swelling data only reflect the regions in the polymer where the solvent has chosen to reside, i.e. only if the solvent is homogenously distributed in the polymer can a solubility parameter be found representative of the whole polymer (Hansen, 2000) Swelling is of particular interest with respect to the cleaning steps taken in this research as will be demonstrated. Cleaning with NaOH is common in UF applications where the objective is to clean polysaccharides or proteins by the mechanism of hydrolysis, as discussed in Section 5.4. In fact, as is the case with Microdyn-Nadir GmbH, some membrane manufacturers recommend NaOH cleaning as part of a pretreatment procedure. Membrane swelling is likely to impact upon flux and solute rejection. Moreover, it was observed that subsequent to NaOH cleaning clean water flux rates were temporarily elevated.

Swelling experiments were carried out using water, 0.1M and 0.5 M NaOH solutions with a Millimar instrument as detailed in Section 4.10.3 Table 5.5 shows swelling data for 50 kDa Nadir PES membranes in contact with demineralised water and NaOH

<table>
<thead>
<tr>
<th>Test number</th>
<th>Deionised water swelling (µm)</th>
<th>0.1 M NaOH swelling (µm)</th>
<th>0.5 M NaOH swelling (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.8</td>
<td>2.1</td>
<td>1.6</td>
</tr>
<tr>
<td>2</td>
<td>0.6</td>
<td>3.0</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>4.1</td>
<td>2.1</td>
</tr>
<tr>
<td>4</td>
<td>0.7</td>
<td>2.0</td>
<td>1.9</td>
</tr>
<tr>
<td>5</td>
<td>0.9</td>
<td>3.2</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Average swelling: 0.8 (0.2)* 2.9 (0.9) 1.7 (0.3)

* Standard deviation in brackets

Water does swell the membrane by approximately 0.8 µm on average. The swelling in relation to the total membrane thickness increases by approximately 0.45% only. In the case of both 0.1 M and 0.5 M NaOH, the swelling data suggest that NaOH does swell the membrane more than water. The results indicate that after the membrane was in contact with NaOH, it is likely to exhibit different flux and solute rejection characteristics. In terms of membrane cleaning, water flux measured immediately after
NaOH cleaning will be temporarily elevated, hence it is advisable to permeate water through the membrane for a reasonable time period (i.e. at least 30 minutes) prior to conducting any further filtration experiments.

Notably, the extent of swelling seemed to be larger for the 0.1 M solution compared to the more concentrated 0.5 M solution. This is a very interesting finding, because it implies that using a higher concentration of solvent does not necessarily mean membrane swelling will be higher. In relation to membrane cleaning, this finding is in agreement with the work by Bartlett et al. (1995) who experimentally determined that using a more concentrated NaOH solution does not necessarily improve the membrane cleaning process.

Flux and rejection of a polymeric membrane can be significantly influenced by swelling (Tarleton et al., 2005). This is also illustrated in Figure 5.21 and Figure 5.22 where water flux is measured at 1 bar(g) through a 10 kDa Millipore membrane prior to and after 5 kDa dextran filtration using the SC, followed by several cleaning steps including the use of NaOH. The water flux obtained for a virgin membrane tended to reduce until a steady-state value was reached, probably because of membrane compaction effects. Figure 5.21 demonstrates a flux reduction for a dextran solution transmitted at 1 bar(g) TMP being most likely due to concentration polarisation effects and possibly also membrane fouling. Subsequent flushing of the membrane with water increased the flux but more of an improvement was observed after the membrane had been treated with 0.1 M NaOH. Sodium hydroxide as a cleaning agent should help to clean the membrane not only due interaction with the solute by hydrolysis but also because membrane swelling results in temporary opening of the pore structure. However, this also means that water has to be flushed through the membrane long enough after NaOH treatment in order to ensure that NaOH actually helped to clean the membrane and that flux was not solely elevated due to the swelling effect. The flux data shown in Figure 5.21 also implies that even though dextrans are neutral solutes they have caused some membrane fouling at this pressure, because the initial water flux could not be fully restored; the results are in agreement with the findings of Susanto and Ulbricht (2005) (see also Sections 5.2.3 and 5.4).
It can also be seen from Figure 5.21 that water flux after NaOH cleaning was highest showing that NaOH cleaning is more effective than simply rinsing with water. It was also noticed experimentally that once copious amounts of water were transmitted through the membrane to remove NaOH membrane swelling reduced.

As mentioned earlier, less swelling was observed when the membrane was placed in 0.5 M NaOH compared to 0.1 M NaOH. On this basis it is reasonable to expect a lower water flux value after 0.5 M NaOH cleaning compared to 0.1 M NaOH cleaning which is indeed what was observed with 10 kDa Millipore membranes (Figure 5.22). It is therefore advisable to be careful about the choice of concentration of the cleaning agent as the result implies that a higher concentration does not always yield better cleaning results. In fact, Bird (2006) recommends not to exceed 0.5 wt% NaOH concentration when cleaning protein foulant.
5.6 Scanning electron micrographs

The main purpose for using FEGSEM in this research was to examine both the surface and cross-section of the PES membrane, visualise the active and support layer(s) and obtain a measure of the total thickness of the membrane. Unfortunately, the resolution of conventional SEM was insufficient to yield any useful data concerning the pore size distribution (PSD) of the membrane.

5.6.1 Scanning electron micrographs – Millipore membrane

Figure 5.23 shows the bottom support layer of the two-layer 10 kDa unmodified Millipore PES membrane which reveals its fibrous structure.
A cross-sectional view of the top active layer of the membrane (Figure 5.24) reveals a very dense structure at the top with a progressively more open structure through the depth; the total thickness of the top layer was ~100 μm.
The total membrane thickness (i.e. two layers together) was measured as 250 µm. For completeness, an image of the surface of a 10 kDa membrane is shown in Figure 5.25 from which pores of a few nanometre in diameter and a few impurities can be visualised.

![Image of a 10 kDa Millipore PES membrane.](image)

Figure 5.25. Top view SEM of a 10 kDa Millipore PES membrane.

5.6.2 Scanning electron micrographs – Nadir membranes

A range of SEM images were obtained for the Nadir membranes. The image of the membrane cross-section shown in Figure 5.26 revealed the total membrane thickness to be about 175 µm, which is in agreement with manufacturer information who state a thickness of between 170 and 180 µm.
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Figure 5.26. Cross-section SEM view of a 50 kDa Nadir membrane.

Figure 5.27. Upper part cross-section SEM view of a 50 kDa Nadir membrane.

The active layer of a membrane, responsible for the actual separation, is thin compared to the porous support on which it rests. Figure 5.27 shows the active layer of a 50 kDa Nadir membrane to be approximately 300 nm thick. Hence, the active layer typically makes up less than 0.2% of the total membrane thickness.
A top view of a 50 kDa Nadir membrane with a resolution of 200 nm provides an image where some of the larger pores of the membrane become visible and can be estimated to be approximately of the order of 5 to 20 nm. This is also in agreement with the estimates of the mean pore size made from solute rejection data in Section 5.2.2 earlier.

Figure 5.28. SEM top-view of a 50 kDa Nadir membrane.

A sample view of scanning electron micrographs of the surface of an unmodified 50 kDa membrane is shown in Figure 5.29 and that of a modified 50 kDa membrane in Figure 5.30. The images of both unmodified and 50 kDa plasma-modified membranes were found not to show a significant visible difference. One may argue that the surface looks slightly denser in the case of the plasma-modified membrane, but it would not necessarily be possible to tell from the image if the membrane was modified or not. The same argument applies to a membrane with a smaller MWCO. A SEM of a 10 kDa membrane could not be visually distinguished from a SEM of a 50 kDa membrane.
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Figure 5.29. 50 kDa Nadir membrane – unmodified, top-view SEM.

Figure 5.30. 50 kDa Nadir membrane – plasma-modified, top-view SEM.
5.6.3 Comparison with other Scanning electron micrograph studies

It is also of interest to look at conventional and plasma-treated micrographs found in the literature in comparison to the images obtained from the current research. Gancarz et al. (1999) analysed polysulphone (PSU) membranes treated with CO₂ plasma for 2 and 6 minutes duration, respectively, using SEMs showing the membrane surface and cross-section (Figure 5.31).

Whilst it is very difficult to observe any difference between the two membranes, one may argue that the top-view of the membrane surface for the unmodified membrane differs from the image of the plasma-modified membrane, but only marginally. However, the authors concluded that membrane damage becomes obvious from the SEM which they attributed to visible cracks in the surface. It is worthwhile noting that cracks in the surface are sometimes due to the gold coating which is often used as a pre-treatment prior to SEM image processing. It is worth pointing out that overexposure to plasma by prolonged treatment time has also been reported to result in membrane damage by other authors (Johansson and Masuoka, 1999).

![Figure 5.31](image)

Figure 5.31. (a) Unmodified PSU membrane (2 mins.) – surface and cross-section, (b) modified PSU membrane (6 mins.) – surface and cross-section (Gancarz et al., 1999).
5.7 Contact angle measurements

The static contact angle, \( \theta \), of a sessile drop on a solid substrate was measured in this work, as shown in Figure 5.32. The angle is measured when the three phases (gas, liquid, solid) are in their natural equilibrium and the three phase line is stationary.

![Figure 5.32. Static contact angle \( \theta \).](image)

If \( \theta \) is greater than 90° the liquid tends to form droplets on the surface, below an angle of 90° the liquid tends to spread out over the surface. As a guideline, a membrane is said to become more hydrophilic and exhibit increased water flux rates if the contact angle can be reduced from about 70° down to about 40° or below. In addition, a reduced contact angle is considered as a method to prevent or at least reduce the fouling mechanism of pore plugging (Kim and Fane, 1995).

5.7.1 Results and discussion

Table 5.6 shows contact angle measurements for unmodified Nadir membranes (10, 20, 30 and 50 kDa) and an unmodified Millipore (50 kDa) membrane for which the experimental set-up was shown elsewhere (Section 4.10.2). For each membrane, an average contact angle on the left and right side of the drop in contact with the membrane and a mean contact angle were determined. The variation in contact angle was no higher than \( \pm 3° \) and the standard deviation for each data set including more detailed measurement results are provided in Appendix E. These contact angle measurements were conducted at Lappeenranta University of Technology, Finland.
### Table 5.6. Contact angle – unmodified membranes (Lappeenranta University of Technology).

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Left contact angle</th>
<th>Right contact angle</th>
<th>Mean contact angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nadir PES 10 kDa</td>
<td>88°</td>
<td>89°</td>
<td>88°</td>
</tr>
<tr>
<td>Nadir PES 20 kDa</td>
<td>85°</td>
<td>87°</td>
<td>86°</td>
</tr>
<tr>
<td>Nadir PES 30 kDa</td>
<td>83°</td>
<td>83°</td>
<td>83°</td>
</tr>
<tr>
<td>Nadir PES 50 kDa</td>
<td>81°</td>
<td>82°</td>
<td>81°</td>
</tr>
<tr>
<td>Millipore PES 50 kDa</td>
<td>93°</td>
<td>94°</td>
<td>94°</td>
</tr>
</tbody>
</table>

Table 5.6 shows that despite the manufacturers’ claim to have produced hydrophilic membranes the contact angle is always above 80° for these PES membranes and for Millipore it is even above 90°, hence in the latter case the surface is said to be non-wetting. Notably, the difference in MWCO for the Nadir PES membranes seems to have little influence on the contact angle as it remains between 80° to 90° in all cases. Hence, the membrane surface is wetted, but only very slightly so that it is questionable whether the membrane surface of the unmodified membranes is really hydrophilic, particularly when considering that membranes with significant hydrophilicity exhibit contact angles of 60° or less.

It is worth noting that the washing and drying process applied prior to the contact angle measurements may affect the results. Research work at the University of Exeter has shown that for a range of polymeric membranes which were washed and dried were found to have higher contact angles than those membranes which were simply washed and otherwise analysed directly from the manufacturer (Wakeman and Tarleton, 1992). Moreover, the contact angle was found to increase more dramatically if the drying time was prolonged. However, if the membranes are analysed without washing off any of the chemical agents from the manufacturer, the contact angle data are not really representative of experimental conditions, because wetting agents and other impurities potentially present are still remaining on top of the membrane surface.

The contact angle of 50 kDa plasma-modified membranes was also analysed in Finland using the same procedure. The contact angles with water for a Nadir membrane exposed to for a treatment time of 60 s and a plasma intensity of 200 W and a Nadir membrane exposed to 200 W plasma intensity at 60 s treatment followed by AA dipping were measured (Table 5.7). The results suggest that there is little difference between the two.
Moreover, both membranes actually became more hydrophobic which is contrary to what was expected. However, these findings are further supported by the streaming potential measurement results (through-pore) shown in Section 5.9. The increase in contact angle suggests that it was not possible for the acrylic acid groups to easily attach to the membrane surface, possibly due to prior contact of the modified membrane with air before the AA dipping. Alternatively, it may be possible that polyacrylic acid formation did not occur in the expected configuration and the groups sticking out from the surface are not oriented in a manner where the membrane can become more hydrophilic and charged.

Contact angle measurements of 50 kDa unmodified PES membranes were repeated at Loughborough in order to confirm the results from Finland and also investigate differences in contact angle between membranes taken directly from the manufacturer (i.e. including any wetting agents) and those which were washed and dried prior to analysis.

The data shown in Table 5.8 clearly demonstrate that in presence of wetting agents the membrane is more hydrophilic, as had been previously observed in the study at Exeter University. However, under industrial operating conditions any wetting agents will have been removed so that it is more sensible to measure the contact angle of the washed membranes and it is also advisable to measure the contact angle as soon as possible, once dried (industrial membranes are rarely dried). Prolonged drying time and extended contact with air could cause the membrane contact angle to increase even further which
was indeed observed in the separate study mentioned earlier (Wakeman and Tarleton, 1992).

Typical ranges of contact angles obtained with water (i.e. between the advancing and receding contact angle) of various polymer films are tabulated in Table 5.9 with the information obtained from various sources (Pham et al., 1999; Tian et al., 2007; Tretinnikov and Ikada, 1994). From this table it can be seen that a PES film, the material of interest to this thesis, can be reasonably hydrophilic (tending towards an angle of approximately 62°). Susanto et al. (2007), for example, studied the contact angle of non-porous PES films using the sessile drop method with water and the contact angle of the PES film was found to be 75.9 ± 2°.

<table>
<thead>
<tr>
<th>Polymer type</th>
<th>Average contact angle range with water (θ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polytetrafluoroethylene (PTFE)</td>
<td>97-127°</td>
</tr>
<tr>
<td>Polyethylene (PE)</td>
<td>82-110°</td>
</tr>
<tr>
<td>Polypropylene (PP)</td>
<td>72-98°</td>
</tr>
<tr>
<td>Polyether urethane (PU)</td>
<td>59-92°</td>
</tr>
<tr>
<td>Polyvinyl alcohol (PVA)</td>
<td>22-59°</td>
</tr>
<tr>
<td>Polyethylene terephthalate (PET)</td>
<td>50-82°</td>
</tr>
<tr>
<td>Nylon</td>
<td>53-81°</td>
</tr>
<tr>
<td>Cellulose</td>
<td>24-32°</td>
</tr>
<tr>
<td>Polysulphone (PSU)</td>
<td>72-78°</td>
</tr>
<tr>
<td>Polyethersulphone (PES)</td>
<td>62-80°</td>
</tr>
</tbody>
</table>

Gancarz et al. (1999) measured the water contact angle on conventional and plasma-modified PSU membranes and found a sharp decrease in the contact angle after a few minutes of plasma treatment suggesting that the membrane became more hydrophilic. In this context, it was interesting to note that, in this thesis, a comparison of contact angle of conventional, unmodified PES membranes with those which were plasma-modified showed the opposite effect, i.e. the contact angle became more hydrophobic.

Literature suggests that for a typical PES polymer film the average contact angle lies between 60° to 70°. Therefore, a permanently hydrophilic PES membrane should
ideally have a contact angle of about 60° or even less. The Millipore and Nadir PES membranes employed in this study are both reported to be hydrophilic by the manufacturer, but as shown in Table 5.6 earlier, unfortunately neither of the two membranes has a contact angle of 60° or lower (under the conditions measured)

5.8 X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) can be used to quantify the elemental composition of the first 1 to 10 nm of a surface. In the present study, it was of interest to compare the surface of an unmodified and a plasma-modified membrane with each other in terms of their chemical composition. XPS analysis was carried out by the Institute of Polymer Technology and Materials Engineering at Loughborough University.

In order to conduct this experiment each membrane was contacted with a 0.1 M silver nitrate solution for a minimum of 5 hours. The membrane and the silver nitrate solution were allowed to equilibrate followed by rinsing with and then soaking in distilled water in order to remove any adherent solution. Finally, after soaking the silver nitrate equilibrated membranes in distilled water they were dried overnight in a vacuum oven at a maximum temperature of 50°C to prevent modification of the polymer structure of the membrane. It is expected from this treatment that the silver ions (Ag⁺) exchange with carboxylate groups which are expected to be present on the surface of the modified membrane.

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Ag</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nadir - unmodified (50 kDa)</td>
<td>0.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Nadir - modified (50 kDa)</td>
<td>0.6</td>
<td>1.5</td>
</tr>
</tbody>
</table>

NOTE The elemental composition is shown in atom %

The elemental analysis shows a change in silver and sulphur concentration between an unmodified and plasma-modified membrane (Table 5.10). The values are given in atom percentage per 100 atoms. The reduction in the sulphur concentration and the presence of Ag indicates that some ion exchange groups were present at the membrane surface.
5.9 Streaming potential measurements

In collaboration with the Department of Chemical Technology at Lappeenranta University of Technology, Finland, streaming potential measurements of unmodified and plasma-modified Nadir PES membranes were conducted. In this thesis the streaming potential technique is used to examine whether surface-charge groups are present on the surface of the plasma-modified Nadir membranes and also to determine how zeta potential data compare to those obtained from original, unmodified Nadir membranes. Carboxylic groups are expected on top of the modified membrane surface as a result of the AA dipping step subsequent to the plasma modification. Weakly acidic groups such as carboxylic groups have higher zeta potentials as the pH becomes more alkaline because the charge groups will then eventually fully dissociate. With carboxylate on the surface a membrane PI around pH 4.0 is expected.

Streaming potential measurements can be conducted along the surface and through the pore. For the former experiment two pieces of membrane are required which were larger in size than the modified membranes available for this study. Therefore, streaming potential measurements were conducted through the pore only. However, as stated in the literature review (Section 24.1) any charge at or within the pore openings has a crucial impact on the filtration performance of a given membrane and should therefore provide sufficient insight.

5.9.1 Measurement results

The measurements were conducted using the apparatus described in Section 4.10.4. Since the apparatus allowed simultaneous streaming potential and flux measurements to be conducted, it was also possible to obtain permeability data from the slope of a graph of flux versus pressure. As pointed out in Section 3.1.2, the average hydraulic membrane permeability or permeance (in L/m²h bar) of the membrane can be determined from such data which are tabulated in Table 5.11.

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Average permeance (L/m²h bar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nadir 50 kDa – unmodified</td>
<td>464</td>
</tr>
<tr>
<td>Nadir 50 kDa – modified and no AA</td>
<td>29</td>
</tr>
<tr>
<td>Nadir 50 kDa – modified and AA dipping</td>
<td>52</td>
</tr>
</tbody>
</table>
The permeance data clearly show that the flux through the modified membranes is substantially lower than the flux through unmodified membranes. This is in agreement with the contact angle data shown in Section 5.7, where the modified membranes showed a larger contact angle than the unmodified membranes. When a membrane is hydrophobic it is classed as non-wetting, i.e. exhibiting a contact angle greater than 90°. A hydrophobic membrane is often associated with lower permeate flux and higher membrane fouling rates. This is because hydrophobic surfaces can promote adsorption onto the surface and within the pore structure which, in most cases, will lead to a higher membrane resistance. Moreover, due to the water-repellent nature of the surface it will be easier for air to get trapped between the solvent (water) and the membrane surface thereby influencing the surface tension of the membrane.

Streaming potential data for unmodified and plasma-modified Nadir membranes with 50 kDa MWCO are shown in Figure 5.33. As mentioned previously, some modified membranes were exposed for one minute to plasma at 200 W and others were modified in the same manner but with additional dipping into AA subsequent to the plasma modification. Firstly, it should be pointed out that the zeta potential data obtained for the unmodified Nadir membranes is in agreement with data provided by the manufacturer who has also shown that the membrane zeta potential is just below zero milliVolts. Whilst streaming potential measurements are a relative technique and a direct comparison to other zeta potential data of other membranes tested under different conditions should only be conducted very carefully, it is reasonable to assume that a zeta potential as low as ± 2 mV represents minimal charge. Zeta potential of charged particles are often of the order of 15 to 30 mV and charged membranes most commonly show a variation in zeta potential from a positive to a negative potential with a change in pH of at least 10 mV. Figure 5.33 also shows that, apparently, the original unmodified 50 kDa Nadir membrane does not have a pI in the range studied, whereas upon surface-modification of such membranes a membrane pI can be found at approximately pH 4.3.
The electrical potential for the membranes tested here is only ±2 mV which translates into a low membrane charge. It is of interest to highlight how the zeta potential values shown in Figure 5.33 compare to the zeta potential measured for a non-porous PES film (Susanto et al., 2007). The zeta potential of such a film was determined using streaming potential measurements (0.001 M KCl electrolyte solution) and the potential ranged from about approximately -10 mV to -30 mV for a surface measurement. It can therefore be concluded that the membranes in the current research had relatively little charge when measured by the 'through pore' method.

What can be seen from the zeta potential graph (Figure 5.33) is that its potential changed after the plasma modification. The graph also indicates that the modified membrane has a pH around pH 4.3 which was not seen for the unmodified membrane. Little change can be observed between the membrane exposed to one minute plasma treatment only and the membrane which was subsequently dipped into AA, suggesting that possibly not much polyacrylic acid chain formation on the membrane surface took place. This is also evident from the permeance data (Table 5.11) where there is no significant difference to be seen between the two types of modified membranes, although the flux for the membrane dipped into AA was marginally higher. The zeta

Figure 5.33. Zeta potential of unmodified and plasma-modified 50 kDa Nadir membranes.
potential of some plasma modified membranes has been reported to reduce with treatment time (Zhan et al., 2004). Thus, it is possible that a prolonged plasma treatment leads to a loss of the desirable increased surface charge. However, this should normally not have been the case for these membranes as the exposure time was only 60 s and a reduced zeta potential after plasma treatment was reported to occur after 120 s of plasma exposure. Since the total plasma input is dependent on the power input and radiation time it may be possible that a plasma intensity of 200 W was too high, yielding only a small number of active sites available to react with AA. Finally, lengthy exposure to air prior to AA dipping can also cause a reduced tendency for the monomers to attach to the membrane surface.

5.9.2 Discussion of theory

Membrane surfaces in contact with an aqueous electrolyte solution generally acquire an electric surface charge. The concept of the electrical double layer applies which comprises of a diffuse part as a result of mobile ions in solution and a compact inner part containing immobile surface charges (Behrens and Borkovec, 1999). If an electrolyte solution is made to flow between two electrodes a potential difference can be measured, known as the streaming potential. Hence, as already pointed out in the literature review, the streaming potential is obtained when a mechanical force is applied to force liquid movement across a stationary solid phase (Elimelech et al., 1994). The streaming potential measurement technique allows one to estimate the surface charge density of a given membrane. Streaming potential data are processed using the Helmholtz-Smoluchowski equation providing relative values of the zeta potential (Equation (2.1)). It is important to be aware that this equation provides only relative, and not true, values of zeta potentials when attempting comparisons with other membranes (Nystrom et al., 1994). For this reason Nystrom et al. (1994) conducted a study analysing possible correction factors to this equation in order to find more accurate zeta potentials values. In that study it was found that such corrections did not lead to any noticeable improvements, implying that the Helmholtz-Smoluchowski equation may be used as it stands. However, one must be aware that under conditions where the pores are small (as in UF), the dielectric constant of water in the pores may differ to that in the bulk which is a factor not corrected for (Pihlajamaki, 2007).
5.9.3 Application of the surface charge density model

When the zeta potential of a membrane is known its charge density can be determined. Knowledge of the charge density can help in interpreting any electrostatic interactions expected between a charged membrane and a charged solute. Burns and Zyden (1999) presented a graph of surface charge density versus solution pH for a clean Millipore membrane with a 100 kDa MWCO and a BSA-adsorbed membrane. The data showed that the surface charge density had increased after adsorption of BSA. Such an approach can also be applied to the streaming potential data for the unmodified and modified membranes used in this study in order to determine how the charge density in the membrane changes with pH at a given electrolyte concentration. The equations required are the Debye length (equation (2.3)), the Helmholtz-Smoluchowski equation (2.1) and the equation for the membrane surface charge density, $\sigma_m$, applicable to symmetrical electrolytes (Burns and Zyden, 1999; Hunter, 1981):

$$\sigma_m = 4C_0 F \lambda_D \sinh \left( \frac{F \zeta}{2 R_g T} \right)$$

(5.9)

where $C_0$ is the bulk electrolyte concentration, $F$ the Faraday constant and $R_g$ the ideal gas constant.

For ideal filtration conditions one should choose a pH where the charge density of the membrane and also of the solute molecules is highest and where the two are of like charge, because this should help to increase retention and reduce fouling (Nystrom et al., 1994). The streaming potential technique allows one to detect charge density variations on the membrane with pH and help to make an informed choice of the appropriate membrane. The calculated change in charge density (according to equation (5.9)) with pH is shown in Figure 5.34.
Figure 5.34. Surface charge density variation of the membrane with pH.

It can be observed from this result that the charge density of the membrane is quite low, confirming its relatively low charge. It is also noticeable that towards pH 3, i.e. in the acidic region, the modified membrane has a higher charge density compared to other pH values so that it may be possible to observe an effect if filtration is conducted at this low pH (a relevant experiment was conducted and is demonstrated in Chapter 7).

5.10 Conclusions

In this chapter various characterisation techniques were discussed. The MWCO analysis technique was used to determine the typical rejection curve for the membranes of interest. The rejection of dextran at 90% was always higher with the CF apparatus compared to the SC apparatus. This puts into question whether the definition of MWCO, usually obtained with SC data, can simply be transferred as a means of membrane selection when other filtration devices with different flow configuration are used. In addition, a separate means of characterising the membrane by relating the MWCO to an average pore size can be useful. For this reason a method to determine an equivalent pore size at a given solute radius was developed. This allowed one to estimate a range of typical pore sizes in relation to dextran solutes and proteins.
Water flux data was obtained for a range of membranes. Most notably, analysis of water flux data for plasma-modified membranes and unmodified membranes showed flux rates to be substantially lower for the former. Contact angle and streaming potential measurements were also conducted implying that the plasma-modified membranes became more hydrophobic and carry relatively little charge. The unmodified and plasma-modified membranes are distinguishable in terms of their contact angle and also the surface charge data, although the charge on the modified membranes was unfortunately not as substantial as desired.

In addition to the above membrane swelling experiments were conducted showing that cleaning agents such as NaOH temporarily swell the membrane which has to be taken into account when conducting cleaning cycles. Scanning electron micrographs were also taken as they provide useful information about the total membrane thickness. However, the use of such images to quantify membrane fouling or obtain pore size data is limited.
Chapter 6 – Filtration with Unmodified Membranes

The purpose of this chapter is to establish an understanding of protein flux and rejection data for unmodified membranes. The effect of operating variables such as the influence of pH and ionic strength on protein filtration using two different filtration apparatus was studied. In the systematic study bovine serum albumin (BSA) and lysozyme (LYZ) were studied at several pH and two ionic strengths (20 mM and 100 mM, respectively) employing both the stirred cell (SC) and the crossflow (CF) apparatus. NaTr membranes, having a molecular weight cut-off (MWCO) of 50 kDa, were used in all experiments (see Section 4.12). Additional experiments were conducted with LYZ at pH 11.0 with both apparatus and at both ionic strengths. The experimental results are discussed and contextualised with reference to existing data in the literature. In addition, an attempt has been made to compare data between the two different apparatus. Important properties of the feed solution and additional experiments with unmodified membranes of different MWCO are also discussed. Calculations of the shear stress at different impeller speeds relevant to this chapter are provided in Appendix C, part c.

6.1 Feed solution properties

Certain properties of the feed solution including solute charge and solution viscosity can be important during protein filtration. If, for instance, the membrane and the solute are of like charge, charge repulsive effects are mostly dominant. Such data relevant to the interpretation of the results are presented here.

6.1.1 Protein charge

The concentration of surface charge around proteins is dictated by the solution pH and, as shown in Table 4.2 earlier, bovine serum albumin (BSA) has an isoelectric point (pI) around pH 4.9 and lysozyme (LYZ) has a pI around pH 11.0. It is important to note that the location of the charge concentration will differ between proteins; and in the case of BSA and LYZ, the former carries a noticeably higher number of chargeable amino acid residues, as illustrated in Figure 6.1 (Rezwan et al., 2005). According to this graph over the pH range relevant to this chapter (pH 4.9 to pH 11.0, see Section 4.12.3) the protein charge is similar at pH 5 for both proteins and negative for BSA thereafter. The overall charge on LYZ remains positive until approximately pH 9.0. Notably, these charge...
values were theoretically evaluated yielding a BSA pI of 5.5 and a LYZ pI of 10.0 (different from the conventionally accepted, experimentally obtained pI of ~5.0 for BSA and ~11.0 for LYZ). The theoretical pI has a relative error of 10% for BSA and 13% for LYZ nevertheless Figure 6.1 provides a useful indication of the significance of charge for the two proteins. Generally, it can be concluded that, at certain pH levels, BSA should show more of a charge effect than LYZ (provided charge interaction with the membrane exists, see Chapter 7).

![Figure 6.1. Total protein charge for BSA and LYZ, adapted from Rezwan et al. (2005).](image)

Mukai et al. (1997) also reported zeta potential and surface charge density data for BSA. The zeta potential varied by ±40 mV, depending on the solution pH and the surface charge density ranged from approximately -0.05 to 0.05 C/m². It can be noted that the charge density of the protein is much tighter than that of the membrane determined from streaming potential measurements (refer to Figure 5.34). Palacio et al. (1999) also conducted zeta potential measurements for a BSA solution and found the zeta potential to be similar to that reported by Mukai et al. (1997) with a pI at pH 5.0.

### 6.1.2 Solution viscosity

Solution viscosity influences a range of parameters including the diffusion coefficient of the protein and it is generally accepted that macromolecular solutions are more viscous.
than the pure solvent. Although it must be remembered that the feed concentrations used in these studies are very dilute (0.5 g/L), this does not mean, however, that in the vicinity of the membrane surface viscosity is not higher. It is nevertheless useful to know the starting viscosity of a typical protein solution employed in these studies, therefore the viscosity of both bovine serum albumin (BSA) and lysozyme (LYZ) solutions was determined using a standard Ostwald viscometer over the temperature range from 15°C to 40°C. As shown in Figure 6.2, at 20°C, the viscosity of water is $1.002 \times 10^{-3}$ Pa.s and the average viscosities of BSA and LYZ were determined as $1.037 \times 10^{-3}$ Pa.s and $1.038 \times 10^{-3}$ Pa.s suggesting that at a feed concentration of 0.5 g/L viscosity differences are negligible.

![Figure 6.2. Viscosity of protein solutions and water versus temperature.](image)

**6.2 Crossflow versus stirred cell filtration**

In both SC and CF filtration, pH and ionic strength effects are reported to have a noticeable influence on filtration performance during UF of amphoteric macrosolutes (Fane et al., 1983a; Fane et al., 1983b; McDonogh et al., 1989). No attempt has
previously been made to dissect any differences such effects may have when analysing both SC and CF filtration under similar conditions, although it is widely known that many laboratory studies are carried out with SC systems in contrast to industrial applications which mostly use CF systems. Thus, an attempt is made to compare CF vs. SC filtration on the basis of shear by emulating similar shear conditions (Section 6.2.2) in order to establish reasonable hydrodynamic similarity between the two devices.

A comparison based on liquid mixing alone, i.e. in terms of Reynolds numbers is inappropriate. This is because the rotational Reynolds number (used for SC applications) and the conventional Reynolds number for a tube or duct (used for CF applications) are based on different scales (Laminar flow up to $Re < 10$ for the rotational Reynolds number and laminar flow up to $Re < 2,100$ for the conventional Reynolds number). Moreover, Cheryan (1998) states that several equipment designers think that shear rate rather than Reynolds number needs to be maximised in order to achieve low fouling filtration, further supporting a shear based approach. Ideally, similar shear rates in both devices should result in a comparable momentum boundary layer thickness. The thickness of the concentration boundary layer may still differ which would be evident from differences in the mass transfer coefficients obtained with the two devices for a given solute. Finally, to ensure that a reasonable comparison between the two devices is justified, as many parameters as possible were kept identical during the experiments including pressure, temperature, feed concentration, pH and ionic strength. Note also that the transmembrane pressure (TMP) of the CF device was calibrated, that is the TMP was not simply evaluated by adding the inlet and outlet pressures and dividing by 2. The TMP was actually evaluated using a pressure transducer placed below the membrane whilst testing different inlet and outlet pressures (i.e. flow rates).

In industry, it is generally understood that CF operation results in higher efficiencies of filtration than dead-end or SC filtration (Lpnižki, 2007). It is therefore of interest to conduct comparable studies with the two operational modes under similar conditions in order to compare and contrast this statement.
6.2.1 Reynolds number

The flow conditions in any membrane application are of great importance as surface shear is known to reduce membrane fouling. Knowledge of the Reynolds number and therefore the flow regime in which one operates can help to understand fouling and concentration polarization phenomena under given conditions. The conventional Reynolds number, \( \text{Re} \), is defined as:

\[
\text{Re} = \frac{\rho ud}{\mu}
\]  

(6.1)

where \( \rho \) is the density, \( u \) the velocity, \( d \) the diameter, and \( \mu \) the liquid viscosity. In order to calculate the Reynolds number in the CF module the diameter, \( d \), has to be replaced with the hydraulic diameter, \( d_h \) (Section 4.7.2). The membrane module of the CF apparatus has a channel width, \( a \), of 0.03 m and a duct height, \( b \), of 0.002 m. The hydraulic diameter, \( d_h \), is therefore 3.75\times10^{-1} \text{ m} \) (see also Appendix C - part a).

If the Reynolds number in the SC system is to be determined, the equation for the rotational Reynolds number, \( \text{Re}_r \), applies:

\[
\text{Re}_r = \frac{D_{imp} \omega \rho}{\mu}
\]

(6.2)

where \( D_{imp} \) is the impeller diameter and \( \omega \) the angular velocity.

6.2.2 Shear stress model

It was the intention to maintain similar shear conditions at the membrane surface both in the SC and CF apparatus. Therefore, a force balance across the CF membrane module was carried out, as illustrated in Figure 6.3, yielding:

\[
ab \Delta P_m = 2 \tau M_l (a + b)
\]

(6.3)

where \( \Delta P_m \) is the pressure drop across the membrane length, \( \tau \) the shear stress and \( M_l \) the membrane length. Rearrangement of equation (6.3) and inclusion of the hydraulic diameter (equation (4.2)) gives:
At the chosen transmembrane pressure (TMP) of 25 kPa (Section 4.12.3) the pressure drop across the membrane length is about 1100 Pa from which a shear stress of 34 Pa was calculated.

The shear stress in the CF module has to be related to that in the SC. The calculation of the shear stress in the SC is more difficult and requires some assumptions to be made. The shear model is based on a flat blade impeller (as present in the Millipore SC). The flow field underneath the impeller was divided into an inner and an outer region, where the two are divided by the critical radius, $r_c$, at which the maximum shear is experienced (Figure 6.4).
Following the approach of Kosvintsev et al. (2005), who compared oil droplet formation in a Weissenberg rheometer and a SC, the critical radius was determined from:

\[ r_c = \frac{D_{\text{imp}}}{2} \cdot 1.23 \left( 0.57 + 0.35 \frac{D_{\text{imp}}}{W_{\text{SC}}} \right) \left( \frac{h}{W_{\text{SC}}} \right)^{0.056} n_b^{0.116} \frac{\text{Re}_r}{1000 + 1.43 \text{Re}_r} \]  

(6.5)

where \( W_{\text{SC}} \) is the inner glass width of the SC, \( h \) the blade height and \( n_b \) the number of stirrer blades. For the SC apparatus \( D_{\text{imp}} = 3.8 \text{ cm}, W_{\text{SC}} = 4.2 \text{ cm} \) (inner cylinder width), \( h = 0.9 \text{ cm}, \) and \( n_b = 2. \) The shear stress below the critical radius (i.e., the inner region) is calculated from equation (6.6) whereas the shear stress at the critical radius and beyond (i.e., the outer region) is calculated from equation (6.7):

\[ \tau = 0.825 \mu \omega \frac{1}{\delta_b} \quad r < r_c \]  

(6.6)

\[ \tau = 0.825 \mu \omega \left( \frac{r_c}{r} \right)^{0.6} \frac{1}{\delta_b} \quad r > r_c \]  

(6.7)
where $r$ is the radial position along the impeller at which the shear stress is determined and $\delta_b$ the boundary layer thickness. The thickness of the boundary layer is determined from the Landau-Lifshitz equation (Landau and Lifshitz, 1959):

$$\delta_b = \frac{D_{mp}}{\sqrt{Re_r}} = \frac{\mu}{\sqrt{\rho \omega}}$$  \hspace{1cm} (6.8)

The boundary layer calculated here and relevant to the shear stress is the momentum boundary layer (which is generally always larger than the concentration boundary layer). The impeller critical radius for the Millipore SC was calculated as 1.47 cm at the forced impeller speed of 2,400 rpm. Table 6.1 illustrates how different impeller speeds affect the location of the critical radius along the impeller and shows the corresponding rotational Reynolds numbers. In order to determine $Re_r$, the revolutions per minute (rpm) of the impeller in the SC were measured using a Photo-Tachometer (Model TM-3011). At the chosen impeller speed of 2,400 rpm the rotational Reynolds number was $\sim 57,000$.

Table 6.1. Critical radius and rotational Reynolds number at different rotational speeds.

<table>
<thead>
<tr>
<th>Rotational speed (rpm)</th>
<th>Angular velocity (rad/s)</th>
<th>$Re_r$</th>
<th>Critical radius (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>11</td>
<td>2407</td>
<td>1.15</td>
</tr>
<tr>
<td>500</td>
<td>52</td>
<td>12033</td>
<td>1.40</td>
</tr>
<tr>
<td>1400</td>
<td>147</td>
<td>33693</td>
<td>1.46</td>
</tr>
<tr>
<td>2200</td>
<td>230</td>
<td>52947</td>
<td>1.47</td>
</tr>
<tr>
<td>2400</td>
<td>251</td>
<td>57760</td>
<td>1.47</td>
</tr>
<tr>
<td>2700</td>
<td>283</td>
<td>64980</td>
<td>1.47</td>
</tr>
</tbody>
</table>

The data clearly show that beyond a certain rotational speed, the location of the critical radius reaches a maximum, which, in this SC system, corresponds to 1.47 cm. That is, for the given impeller, a single blade is 1.9 cm long therefore 0.43 cm of the impeller comprises the inner region and 1.47 cm the outer region. In order to calculate an average shear stress the area under the impeller needs to be considered as depicted in Figure 6.4. The diagram also shows the shear stress at the critical radius was 48 Pa, which corresponds to the maximum shear obtained at 2,400 rpm at that location along the impeller (see also Appendix C, part c). The representative overall shear stress was determined by integration of equations (6.6) and (6.7):
where \( r_0 \) is the radius at point zero, i.e. the centre of the impeller and \( r_f \) is the final radius, i.e. at the tip of the impeller. The integration result was also confirmed by using Simpson's rule; upon its usage an average shear of 29 Pa was obtained. To match the 34 kPa shear stress in the SC, using equations (6.9) and (6.10), an impeller speed of 2,700 rpm is required which is beyond the maximum achievable speed in the SC without causing deep vortex formation. Therefore, the closest match between the SC and CF apparatus without detrimental effects on the process variables was chosen for comparison purposes, i.e. 2,400 rpm in the SC, yielding an average shear stress of 29 Pa. For the two given membrane modules a trade-off had to be found to match the conditions as closely as possible. A transmembrane pressure of 25 kPa, determining the crossflow velocity in the CF apparatus, was the lowest practicable pressure to be used to conduct the experiments. Moreover, it can be deduced from Table 6.1 that the maximum achievable critical radius and thus shear rate is obtained at about 2,200 rpm. Increasing the rpm does increase the shear but not the critical radius. Therefore, an unavoidable error of approximately ~15% is introduced when compared to the shear stress in the CF apparatus.

6.3 General experimental trends

In this section general trends observed from protein filtration of BSA and LYZ carried out as part of this research work are highlighted in terms of permeate flux, feed concentration and MWCO. It was also of interest to observe the impact of a change in feed concentration on filtration. In addition, protein flux data for the CF and SC devices and membranes of different MWCO can help in interpreting filtration phenomena.

The flux of BSA at 0.25 bar(g) and pH 7 obtained with unmodified 10 kDa and 50 kDa Nadir membranes using the CF apparatus was reported at 20 mM ionic strength.
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Figure 6.5 shows permeate flux data for BSA (1 g/L feed concentration, 20 mM, pH 7) for 2 hours filtration time:

It can be seen, that in both cases, a stable flux was reached after approximately 30 minutes of filtration which suggests that an equilibrium flux was reached at that point. It can also be inferred from the graph, because of the initial, more rapid flux decline, that membrane m-pore fouling takes place until a quasi equilibrium is reached. If such a noticeable flux decline is seen it is generally understood that a subsequent, less drastic decline in the filtration rate is due to solute accumulation at the membrane surface, a concept applicable to both ultrafiltration (UF) and microfiltration (MF) (Tarleton and Wakeman, 1993). The permeate flux was also substantially lower with the 10 kDa membrane compared to the 50 kDa membrane and is due to the lower hydraulic permeability of the 10 kDa membrane compared to that of the 50 kDa membrane.

It is also of interest to compare the CF filtration of BSA at higher (1 g/L) and lower (0.5 g/L) feed concentrations (using a 50 kDa membrane at pH 7). It was found that permeate flux is noticeably lower for the higher feed concentration, as demonstrated in Figure 6.6.
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Figure 6.6. BSA filtrate flux for 0.5 and 1.0 g/L feed concentrations with a 50 kDa Nadir membrane (0.25 bar TMP, 20 mM).

The lower flux is a result of the higher bulk density at higher concentration creating an additional resistance to flow. This can also be confirmed by applying the film model (see equation (3.9)) from which can be deduced, all other factors being equal, that the flux rate decreases as the bulk concentration increases.

Figure 6.7. Protein rejection of BSA with unmodified 10 and 50 kDa Nadir membranes (0.25 bar TMP and 20 mM).
Rejection data for both 10 kDa and 50 kDa MWCO filtration with 1 g/L and 50 kDa BSA filtration with 0.5 g/L feed concentration were obtained. It is expected, that BSA will be completely rejected, or at least to a greater extent, for 10 kDa membranes compared to the larger cut-off 50 kDa membranes (see Figure 6.7). Interestingly, although rejection for the 10 kDa membrane was higher, a small quantity of BSA molecules was still able to transmit through the membrane. This suggests that it is difficult to retain 100% of solute purely on a size basis, unless the chosen membrane has a MWCO several orders of magnitude smaller than the protein molecular weight (MW).

Since the permeate flux was lower at the higher feed concentration during the 50 kDa MWCO experiments shown in Figure 6.6 the solute concentration at the membrane also increased thereby reducing the flow through pores large enough to transmit solute than is the case for the less concentrated solution. As a result solute rejection will eventually increase. Figure 6.8 indeed shows higher solute rejection for the higher feed concentration.

![Figure 6.8. BSA rejection with a 50 kDa membrane for two different feed concentrations.](image-url)
6.4 Protein filtration results and discussion

In this section, filtration experiments with BSA and LYZ protein solutions using both the CF and SC apparatus are discussed. Since both of these proteins can become unstable above 30°C, all experiments were carried out below 25°C (see Section 4.2). New, unmodified and pretreated (but not plasma-modified) membranes were used for each experiment (as mentioned in Section 4.12.3). Membrane sample variability effects were minimized by measuring the water flux using an identical methodology. If the water flux variation deviated by more than 7% from a previously obtained mean water flux then another membrane was used in order to maintain a near constant average membrane hydraulic resistance prior to any experiment. For the purpose of any modelling osmotic pressure effects were assumed to be negligible in all experiments due to the relatively low protein concentration used (0.5 g/L). The osmotic pressure of macromolecules is mostly determined from virial expansions. However, for the purpose of demonstrating that the osmotic pressure can be neglected at low feed concentrations (irrespective of the higher solute concentration which, in some cases, will occur at the membrane surface) the osmotic pressure, $\Pi$, was determined from the following relationship (Cheryan, 1998):

$$\Pi = \frac{c}{M_w} \frac{R_g T}{V}$$

(6.11)

where $c$ is the feed concentration, $M_w$ the molecular weight of the solute, $V$ the solution volume (1 litre for the CF and 50 mL for the SC), $R_g$ the ideal gas constant and $T$ the temperature. The osmotic pressure values for both BSA and LYZ are tabulated for a range of concentrations, highlighting the feed concentration relevant to this work in italics (Table 6.2).
Table 6.2 demonstrates that osmotic pressure effects can become quite important for low molecular weight solutes at concentrations of 10 g/L and higher. However, the higher osmotic pressure for LYZ of ~0.09 kPa at a feed concentration of 0.5 g/L does not have a significant impact on a TMP of 25 kPa. Moreover, Saksena and Zydney (1997) demonstrated in their work that osmotic pressures for BSA really only become relevant at concentrations of 100 g/L and higher beyond which the osmotic pressure rises exponentially. Even though the concentration at the membrane surface is higher than in the bulk it does not reach levels where osmotic pressure effects would sufficiently affect the TMP for it to be of concern under the experimental conditions employed. In addition, Blatt et al. (1970) clearly state that for solutes with molecular diameters in excess of 1 nm osmotic pressure effects are usually absent because for high molecular weight solutes the osmotic pressure is very small in comparison to the applied pressure (as evident from Table 6.2).

The results shown in Sections 6.4.1 to 6.4.4 were obtained at 30 minutes filtration time. This corresponds to the maximum filtration time for the SC and the corresponding permeate flux and rejection data from the CF experiments. The same filtration time was chosen so that a comparison could be made knowing that filtration parameters such as pressure, feed concentration, pH, ionic strength and temperature were also equal, and shear rates were similar as explained in Section 6.2.2. Error bars are not shown in the
graphs as any flux variations were at most ±2% whilst variations in rejection were at most ±1%. The results shown are typical of those obtained.

6.4.1 Crossflow filtration of bovine serum albumin

BSA filtration (0.5 g/L) at 0.25 bar(g) was carried out in the CF apparatus for pH values of 4.9, 6.0, 7.0 and 8.4 at two different ionic strengths (20 mM and 100 mM) using a 50 kDa Nadir membrane. The CF velocity in the CF apparatus was dictated via the inlet valve. The cell area was 6x10⁻⁵ m², the volumetric flow rate was 2.4 – 2.7x10⁻³ m³/s at a corresponding TMP of 25 kPa ± 0.01 and an inlet pressure of 35 kPa ± 0.01. Dividing the volumetric flow rate by the cell area gives a CF velocity of 0.40 to 0.45 m/s corresponding to a Reynolds number of approximately 1,450 to 1,700. Hence, under these conditions, the flow regime in the CF apparatus was laminar. As mentioned in Section 4.7.2 it was intended to ensure fully developed laminar flow in the CF apparatus. The hydraulic diameter of the channel was approximately 1% of the channel length, thus it is reasonable to assume fully developed laminar flow (McDonogh et al., 1989)

Protein rejection and permeate flux data are shown in Figure 6.9. It can be seen that BSA was not 100% rejected with the 50 kDa membrane when using the CF apparatus. It is also noticeable that there is a relatively small difference in observed rejection with a change in ionic strength, although the rejection was somewhat higher at the higher ionic strength. In line with this observation, permeate flux was lower at the higher ionic strength. Permeate flux, particularly at the lower ionic strength, shows a gentle increase away from the pI of BSA (pH 4.9), i.e. with an increase of negative charge around the protein.
6.4.2 Crossflow filtration of lysozyme

LYZ filtration (0.5 g/L) was carried out in the CF apparatus for pH values of 4.9, 6.0, 7.0, 8.4, and 11.0 (its pI) and at two different ionic strengths (20 mM and 100 mM) using a 50 kDa Nadir membrane. Protein rejection and permeate flux data are shown in Figure 6.10. It can be seen from the graph that most of the LYZ is transmitted through the 50 kDa membrane, as would be expected. In contrast to BSA filtration, rejection was lower at the higher ionic strength. In line with this observation, permeate flux was also higher when the rejection was lower. Changes in pH seemed to have an influence on permeate flux reflected in a slight tendency for the flux to decrease away from the protein pI (pH 11.0). Moreover, the LYZ protein carried a positive charge at all pH values shown (whereas BSA was negatively charged at all pH values, except at pH 4.9, its pI).
6.4.3 Stirred cell filtration of bovine serum albumin

The experimental conditions in this section were identical to those of the CF BSA experiments shown in Section 6.4.1. Protein rejection and permeate flux data are shown in Figure 6.11. It is apparent that in case of the SC the ionic strength has a stronger influence on rejection than was observed with the CF apparatus. Rejection was higher at the higher ionic strength. It is also noticeable that the observed rejection was as low as 70% at the lower ionic strength, although, on a MWCO basis, neglecting other effects, most of the BSA should be rejected (MWCO 50 kDa vs. a protein MW of 66.4 kDa). Permeate flux was higher when the rejection was lower (20 mM ionic strength). A pH change away from the protein pI resulted in a higher permeate flux, which was observed for both ionic strengths.
6.4.4 Stirred cell filtration of lysozyme

LYZ filtration (0.5 g/L) at 0.25 bar(g) was carried out in the SC apparatus for otherwise identical conditions noted for LYZ in Section 6.4.2. Protein rejection and permeate flux data are shown in Figure 6.12. The filtration of LYZ with the SC resulted in lower rejection at the higher ionic strength, just as was observed with the CF apparatus. The effect of the change in ionic strength was again more pronounced for the SC result, than for the CF result. The influence of pH on permeate flux seems to go through a maximum around pH 7.0. Permeate flux was markedly higher at the higher ionic strength.
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Figure 6.12. LYZ stirred cell filtration at 25 kPa.

The key observations of SC and CF filtration are summarised in Table 6.3 which shows the higher and lower permeate flux ranges obtained over the pH range studied. It also shows the observed rejection range and the influence of ionic strength on both permeate flux and rejection.

Table 6.3. Overview of protein filtration results using 50 kDa unmodified membranes.

<table>
<thead>
<tr>
<th></th>
<th>Stirred cell</th>
<th></th>
<th>Crossflow</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BSA</td>
<td>LYZ</td>
<td>BSA</td>
<td>LYZ</td>
</tr>
<tr>
<td>Flux (UL)* L/m^2h</td>
<td>90</td>
<td>80</td>
<td>85</td>
<td>100</td>
</tr>
<tr>
<td>Flux (LL)* L/m^2h</td>
<td>60</td>
<td>55</td>
<td>55</td>
<td>70</td>
</tr>
<tr>
<td>Rejection range</td>
<td>~65 to 90%</td>
<td>~20 to 50%</td>
<td>~80 to 100%</td>
<td>~5 to 25%</td>
</tr>
<tr>
<td>Ionic strength effect on flux</td>
<td>20 mM &gt; 100 mM &gt; 20 mM &gt; 100 mM &gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ionic strength effect on rejection</td>
<td>100 mM &gt; 20 mM &gt; 100 mM &gt; 20 mM &gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*UL: Upper Limit  
*LL: Lower Limit  
*subtle effect

Permeate flux rates were comparable for BSA filtration between the two filtration devices. For LYZ, however, permeate flux rates were somewhat higher with the CF apparatus compared to the SC apparatus. When the protein MW was higher than the membrane MWCO, rejection was higher with the CF apparatus compared to the SC apparatus. In contrast, when the protein MW was lower, rejection was also lower with
the CF apparatus than with the SC apparatus. For both the CF and SC device the impact of ionic strength had on permeate flux and rejection followed the same pattern. However, the impact of ionic strength was more obvious with the SC apparatus.

Moreover, when the protein (BSA) had a higher MW compared to the MWCO of the membrane, permeate flux was higher at the lower ionic strength and rejection was correspondingly lower. When the protein (LYZ) had a lower MW compared to the MWCO, permeate flux was higher at the higher ionic strength and rejection correspondingly was lower. Hence, whichever ionic strength gave the higher permeate flux also resulted in lower rejection. This suggests that in case of higher solute rejection a larger resistance to flow is created leading to a lower permeate flux rate. Generally, as is known from the film theory, as the concentration at the membrane surface increases so does the amount of back-diffusion into the bulk. This is mostly accompanied by an increase in solute rejection, but not always, as charge effects also have to be considered and at low back-diffusion rates (high back-diffusion occurs at high concentrations at the membrane surface which can be brought about by the application of high pressures) it is occasionally easier for more of the solutes to penetrate the membrane now that they are located near the entry to the pore rather than in the bulk.

6.4.5 Ionic strength effect

The data obtained suggest that ionic strength effects have an important influence on the filtration properties of protein solutions. It should therefore be possible to utilise ionic strength in order to create favourable separation conditions as a high salt concentration in the feed influences the size of the protein by shielding its charge (Nyström et al., 1998). This can result in transmission of macromolecules at pH values far away from their pI, where they would not otherwise transmit. Hence, the ionic strength can be used to alter the hydrodynamic size of the macromolecules in solution. Interestingly, under the given experimental conditions, the extent of this effect seems to be more pronounced when using the SC apparatus. This would imply that the mass transfer coefficients for the two systems were not identical and mass transfer was probably higher in case of the SC experiments making the ionic strength effect more obvious. In order to tailor a specific filtration outcome by use of ionic strength effects, ideally the mass transfer coefficient in a given system should be maximised and the impact of the TMP will also have to be considered.
It is also of interest to put ionic strength into context in terms of its relation to the Debye length. As noted in Section 2.4.4, the Debye length varies with ionic strength. At very high ionic strength (> 200 mM) the Debye length remains short. However, at intermediate (50 to 200 mM) and low ionic strength (< 50 mM) the increase in the Debye length becomes more significant and noticeable, as illustrated in Figure 6.13. It becomes clear from the graph that ionic strength directly influences molecule charge. In addition, diffusivity is known to increase as the ionic strength increases (McDonogh et al., 1989).

Two specific Debye lengths were calculated using equation (2.3) and are highlighted in this graph, namely those corresponding to the ionic strengths employed in the protein filtration experiments. A Debye length of 2.15 nm corresponds to 20 mM and 0.98 nm corresponds to 100 mM ionic strength. This clearly shows that, in this case, a five-fold increase in the ionic strength results in a more than 50% reduction in the Debye screening length explaining why solutes at higher ionic strength can approach each other more readily. In other words, if the ionic strength is low and the Debye screening length significant, the charge around the protein causes repulsive interactions with
surrounding proteins of the same charge. Moreover, the hydrodynamic size of the protein will be increased due to the added Debye length and the diffusion coefficient will be lower. Therefore, at a low ionic strength, the solute molecules are further apart from each other preventing closer packing of the solutes. If this observation is contextualised with the BSA filtration results shown in Figure 6.9 and Figure 6.11 permeate flux was indeed higher at the lower ionic strength and rejection was lower than at the higher ionic strength, as expected. However, if the same reasoning is applied to the LYZ filtration results shown in Figure 6.10 and Figure 6.12, the theory does not hold. In this case, permeate flux was lower at the lower ionic strength and rejection was correspondingly higher. The influence of ionic strength must therefore be discussed in relation to the MWCO, the pore size distribution of the membrane and any charge of the membrane and solute.

As indicated in Section 4.4.2, the Nadir membrane carries a small negative charge. However, streaming potential data showed that this charge is small enough to be deemed as almost negligible (see Section 5.9.1). On this basis, it may be assumed that charge interactions between the membrane and the solutes are negligible and only solute-solute charge interactions and the membrane pore size are important in interpreting filtration results for BSA and LYZ under these conditions. Low ionic strength seems to have a different influence on permeate flux and rejection for different proteins which can only be explained by first discussing what happens at raised ionic strength. At a high ionic strength the Debye length is shortened and a charge-shielding effect takes place (Hiemenz, 1977; Shaw, 1992). However, the effect has a different impact on the filtration of BSA than it has on LYZ filtration. BSA (~3.45 nm) has a Stokes radius almost twice the size of LYZ (~1.83 nm) (refer to Section 5.21). Hence, purely on a size basis, more of the BSA is expected to be rejected by the 50 kDa membrane. Moreover, the majority of the BSA molecules were expected to be retained as they are also expected to be larger than most membrane pores.

At a high ionic strength, the charge around the BSA molecules becomes shielded, allowing them to approach each other more closely and form a closely packed deposit at the membrane surface resulting in a higher surface concentration that ultimately leads to higher resistance to flow and higher rejection. It is important to point out that when reference is made to a deposit or secondary layer this is not referring to a ‘cake’ or ‘gel
layer'. Gel layer formation at the membrane surface is a phenomenon which only occurs at very high applied pressures and/or high feed concentrations. Thus, in this work, when a deposit at the membrane surface is referred to it is meant to describe the existence of a more concentrated region of solutes at the membrane surface which causes increased back-diffusion and provides a noticeable barrier to solvent (water) transport.

In the case of LYZ, although the charge around the protein becomes shielded in the same manner as BSA, the solutes are small in relation to the average membrane pore. BSA and LYZ have diameters of 6.9 and 3.66 nm, respectively, based on their Stokes radii. Using the pore flow models shown in Figure 5.10 it can be seen that the pore sizes for a 50 kDa membrane range from approximately 4 to 16 nm demonstrating that the majority of pores are actually larger than the protein solutes. Moreover, although the charge-shielding theoretically enables LYZ solutes to become more closely packed, their effective size is now even smaller so that it is easier for them to transmit through the membrane. Although the LYZ molecules now have the ability to move closer together, they are unlikely to form a deposit on the membrane because they are sufficiently small to transmit easily. This concurs with the calculation shown in Section 6.4.10 which demonstrates that, at the same concentration, BSA molecules are in fact already slightly closer to each other than LYZ molecules are.

The above reasoning is also illustrated schematically for the low and high ionic strength case at an example pH of 7.0. Figure 6.14 helps to illustrate why more of BSA is rejected at a higher ionic strength but more LYZ is transmitted at a higher ionic strength compared to a lower one.
At low ionic strengths protein-protein interactions also seem to contribute more to the lower rejection than would be expected on a molecular size basis (Millesime et al., 1995).

6.4.6 Influence of pH

The filtration results obtained with both the SC and CF apparatus show that, for the given experimental conditions, pH had less of an effect than ionic strength. At its pI, BSA carries a net neutral charge and is therefore expected to be more compact. In essence, a solute at its pI will have similar characteristics as if the ionic strength is high, because of the absence of significant charge. Hence, it is not surprising that permeate flux was lowest at the pI of BSA for both SC and CF filtration. Li et al. (2005) made a similar observation during CF filtration of BSA at the same concentration as used here, but with an applied pressure of 140 kPa using a 35 kDa polysulphone membrane. They also observed a lower permeate flux at pH 4.9 compared to pH 6.9, which they attributed to a more compact deposit on the membrane surface. Fane et al. (1983a) argued that protein molecules are most compact at their pI, therefore this would lead to the formation of a deposit layer which is least permeable. A plot of BSA flux versus filtration time shown for the CF apparatus (Figure 6.15) indeed shows a lower flux at
the protein pI and it can be seen that the most drastic flux decline occurs within the initial 20 to 30 minutes. The lower flux rate for BSA at pH 4.9 is likely due to solute aggregation compared to the negative charge case at pH 6.9 where solute-solute repulsion effects are present.

![Figure 6.15. Flux of BSA crossflow filtration at 20 mM ionic strength and two pH values.](image)

A recent study of BSA filtration through charged MF membranes at varying pH revealed that pH only had an influence on permeate flux over approximately the first two hours of filtration, thereafter a steady permeate flux was reached, independent of the solution pH (Ouammou et al., 2007).

A MF study of BSA filtration through ceramic membranes also showed the highest transmission of the protein at its pI and permeate flux was at its lowest at this pH (De la Casa et al., 2007). This is an agreement with the findings made for BSA at 20 mM ionic strength in this study. It is also of interest to note that during particle filtration in MF contrasting observations have been made. Šmídová et al. (2004) studied the influence of pH and ionic strength on model dispersions and found the particles to aggregate around the pI which did not result in a more compact layer, but to the contrary, in a filter cake with higher porosity through which permeate flux increased.

Corresponding data for BSA was plotted for SC filtration. It is immediately seen from Figure 6.16 that the flux fluctuates more widely than in case of the CF apparatus. The
permeate flux, however, was again higher at the pH away from the pI, as expected. That is, the proteins were able to aggregate at neutral pH whereas at the higher pH the greater Debye length prevented the solutes from becoming more closely packed.

![Figure 6.16. Flux of BSA stirred cell filtration at 20 mM ionic strength and two pH values.](image)

In terms of rejection, however, the least amount of BSA protein was rejected at the pI (Figure 6.9 and Figure 6.11). This would be expected bearing in mind that the pI also coincides with minimum viscosity, therefore promoting transmission. Saksena and Zydney (1994) also found BSA rejection to be lowest at the pI which they attributed to the absence of long-range electrostatic repulsive forces thereby allowing more molecules to accumulate at the surface and hence also transmit. Moreover, the effective protein size is smaller at neutral net charge in comparison to its charged state further aiding the transmission. This is a general observation which has been reported by a range of authors (Burns and Zydney, 1997; Gan, 2001; Howell et al., 1999; Sudareva et al., 1992; Van Eijndhoven et al., 1995).

In summary, Figure 6.15 and Figure 6.16 support the theory of greater protein fouling at the pI due to a more hydrophobic, compact structure at that pH (Salgin et al., 2006). Water flux measurements after the protein filtration experiments (without conducting membrane cleaning) showed that the initial hydraulic permeation rate could not be restored suggesting that internal fouling may have occurred. Moreover, Matthiasson (1983) found protein adsorption to be at its maximum at the pI. It is therefore to be
expected to observe more fouling at the pI compared to a pH environment in which electrostatic repulsive forces prevail. The extent of fouling can be estimated by use of the resistance-in-series-model (see also Section 3 1.2):

\[
J = \frac{\Delta P}{\mu(R_m + R_f)}
\]  

(6.12)

For the given experiments, the resistance to mass transport is composed of the hydraulic resistance and the fouling resistance term. The hydraulic resistance of the membrane, \(R_m\), was obtained from pure water flux data measured prior to the protein filtration experiments. The resistance due to fouling, \(R_f\), was obtained by re-measuring the water flux after protein filtration and then subtracting the hydraulic resistance from the result. Any cake resistance, \(R_c\), was incorporated into the fouling resistance term, because the fouling data were not severe suggesting that it is unlikely for a solute or gel layer to have formed, particularly under the given pressure conditions. Commonly, such layers only form when operating beyond the limiting pressure, i.e. at a pressure sufficiently high so that a further pressure increase does not result in flux improvement or when the feed concentration is higher (at least 2% by weight or more).

Table 6.4 illustrates the magnitude of the resistance terms of interest for BSA filtration at the two ionic strengths tested (20 and 100 mM), both with the CF and SC apparatus. For this protein, the fouling resistance term was indeed highest in both CF and SC filtration at the protein pI (at both ionic strengths). This also agrees with a recent MF study where a minimum flux for BSA was reported at its pI using two types of charged membranes. The reduced flux was attributed to a greater tendency to form aggregates which could result in coverage of the majority of the membrane pores (Ouammou et al., 2007). It was also interesting to note that fouling decreased with an increase in pH, suggesting that increasing electrostatic repulsive interactions between the solute molecules also help to reduce membrane fouling. At the higher ionic strength, likely due to the charge-shielding effect, no trend, where fouling reduces when pH increases away from the protein pI, was observed. For both CF and SC, fouling was lowest at pH 7.0 and higher again at pH 8.4, suggesting that the deliberate change of pH to reduce fouling is not useful in high ionic strength environments.
### Table 6.4. Influence of solution chemistry on BSA fouling of Nadir membranes.

<table>
<thead>
<tr>
<th>Apparatus</th>
<th>Solution pH</th>
<th>Hydraulic resistance, $R_m (10^{11} \text{ m}^{-1} \cdot \text{m})$ (20 mM)</th>
<th>Fouling resistance, $R_f (10^{11} \text{ m}^{-1} \cdot \text{m})$ (20 mM)</th>
<th>Hydraulic resistance, $R_m (10^{11} \text{ m}^{-1} \cdot \text{m})$ (100 mM)</th>
<th>Fouling resistance, $R_f (10^{11} \text{ m}^{-1} \cdot \text{m})$ (100 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF</td>
<td>4.9</td>
<td>8.8</td>
<td>4.6</td>
<td>8.3</td>
<td>4.0</td>
</tr>
<tr>
<td>CF</td>
<td>6.0</td>
<td>8.7</td>
<td>1.4</td>
<td>8.3</td>
<td>2.2</td>
</tr>
<tr>
<td>CF</td>
<td>7.0</td>
<td>8.5</td>
<td>0.8</td>
<td>8.5</td>
<td>1.9</td>
</tr>
<tr>
<td>CF</td>
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<td>0.6</td>
<td>8.6</td>
<td>3.2</td>
</tr>
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<td>SC</td>
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<td>4.9</td>
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<td>SC</td>
<td>6.0</td>
<td>11.0</td>
<td>1.8</td>
<td>11.0</td>
<td>2.2</td>
</tr>
<tr>
<td>SC</td>
<td>7.0</td>
<td>11.0</td>
<td>1.2</td>
<td>12.0</td>
<td>1.9</td>
</tr>
<tr>
<td>SC</td>
<td>8.4</td>
<td>11.0</td>
<td>0.8</td>
<td>11.0</td>
<td>2.9</td>
</tr>
</tbody>
</table>

In the case of LYZ the pI is 11.0, hence the protein is positively charged at all pH values, except at its pI. Table 6.5 demonstrates that in case of LYZ in both SC and CF filtration at 20 mM ionic strength, a trend for fouling to increase as the pH moves closer to the protein pI can be observed. It can therefore be concluded, if charges on the membrane are negligible, in low ionic strength environments it is best to choose a solution pH away from the protein pI. In higher ionic strength environments fouling control via pH does not work well, because the charges around the protein are shielded. This is reflected in the more scattered fouling resistance data at 100 mM ionic strength and its apparent independence of pH for both SC and CF filtration. It is also noticeable that the extent of fouling, at a high ionic strength, was not necessarily highest at the protein pI.

It is also of interest to determine if fouling is generally higher at the higher ionic strength, due to the charge-shielding effect. A comparison of the extent of fouling for BSA at the lower and higher ionic strength indeed demonstrates that fouling was higher at 100 mM ionic strength, apart from the pI, where it was similar. However, if the same comparison is conducted for LYZ fouling data the result is different. In case of LYZ, fouling was mostly higher at the lower ionic strength, in agreement with the higher rejection which was observed at the lower ionic strength during LYZ filtration (Figure 6.10 and Figure 6.12).
Chapter 6 - Filtration with Unmodified Membranes

Table 6.5. Influence of solution chemistry on LYZ fouling of Nadir membranes.

<table>
<thead>
<tr>
<th>Apparatus</th>
<th>Solution pH</th>
<th>Hydraulic resistance, $R_m$ (10$^{11}$ m$^{-1}$) (20 mM)</th>
<th>Fouling resistance, $R_f$ (10$^{11}$ m$^{-1}$) (20 mM)</th>
<th>Hydraulic resistance, $R_m$ (10$^{11}$ m$^{-1}$) (100 mM)</th>
<th>Fouling resistance, $R_f$ (10$^{11}$ m$^{-1}$) (100 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF</td>
<td>4.9</td>
<td>8.8</td>
<td>2.2</td>
<td>9.4</td>
<td>3.0</td>
</tr>
<tr>
<td>CF</td>
<td>6.0</td>
<td>8.7</td>
<td>3.0</td>
<td>8.8</td>
<td>2.3</td>
</tr>
<tr>
<td>CF</td>
<td>7.0</td>
<td>8.5</td>
<td>3.0</td>
<td>9.3</td>
<td>1.5</td>
</tr>
<tr>
<td>CF</td>
<td>8.4</td>
<td>9.2</td>
<td>4.3</td>
<td>8.2</td>
<td>2.9</td>
</tr>
<tr>
<td>CF</td>
<td>11.0</td>
<td>8.5</td>
<td>4.4</td>
<td>8.5</td>
<td>2.5</td>
</tr>
<tr>
<td>SC</td>
<td>4.9</td>
<td>12.0</td>
<td>2.3</td>
<td>12.0</td>
<td>3.8</td>
</tr>
<tr>
<td>SC</td>
<td>6.0</td>
<td>12.0</td>
<td>3.0</td>
<td>11.0</td>
<td>2.8</td>
</tr>
<tr>
<td>SC</td>
<td>7.0</td>
<td>12.0</td>
<td>3.3</td>
<td>11.0</td>
<td>2.0</td>
</tr>
<tr>
<td>SC</td>
<td>8.4</td>
<td>12.0</td>
<td>4.5</td>
<td>12.0</td>
<td>4.6</td>
</tr>
<tr>
<td>SC</td>
<td>11.0</td>
<td>12.0</td>
<td>5.2</td>
<td>12.0</td>
<td>4.1</td>
</tr>
</tbody>
</table>

This finding illustrates the need to relate the membrane MWCO to the solute size before ionic strength effects can be interpreted correctly and used in such a way as to reduce protein fouling. Earlier (Figure 6.14) a hypothesis was developed as to how ionic strength influences the rejection of LYZ and BSA, respectively. The fouling resistance data obtained here are in agreement with this reasoning. Since BSA has a softer protein structure than LYZ (Section 4.2.1) one might expect BSA to cause more membrane fouling, because of its higher potential to adsorb at the membrane surface and in its pores. However, the properties of the membrane also have to be considered and contact angle data (Section 5.7.1) showed that the 50 kDa PES membranes used here are only moderately hydrophilic, hence LYZ fouling due to adsorption can still pose a problem as well. Since the fouling data shown in Table 6.4 and Table 6.5 represent the extent of fouling as a result of several factors a conclusion about greater fouling of one of the proteins due to adsorption cannot be drawn.

6.4.7 Mass transfer correlations

It is of great interest to compare the filtration results in terms of their mass transfer characteristics because, in UF, these directly impact on flux and rejection. The comparison of shear alone can only take account of momentum transfer effects. With the information given in Chapter 3 the Schmidt number, $Sc$ (see Table 3.2), can be determined and the rotational Reynolds number calculated (Section 6.2.1). Thereafter, the mass transfer coefficient, $k_m$, applicable to the SC apparatus can be determined from equation (6.13), a form of the Sherwood, $Sh$, correlations (Mehta and Zydney, 2006):
where $r_e$ is the cell radius and $D$ the diffusion coefficient. The mass transfer coefficient, $k_m$, is a function of device hydrodynamics including shear rate and module geometry and also solution properties such as viscosity and the diffusion coefficient (Zydney and Kuriyel, 2000). The mass transfer coefficients obtained for LYZ and BSA at different impeller rotations are tabulated in Table 6.6 with the frequency relevant to this work shown in italics.

<table>
<thead>
<tr>
<th>Frequency (rpm)</th>
<th>Mass transfer coefficient (BSA), $k_m (10^4 \text{ m/s})$</th>
<th>Mass transfer coefficient (LYZ), $k_m (10^4 \text{ m/s})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>2.31</td>
<td>3.24</td>
</tr>
<tr>
<td>300</td>
<td>4.31</td>
<td>6.04</td>
</tr>
<tr>
<td>500</td>
<td>5.76</td>
<td>8.07</td>
</tr>
<tr>
<td>1000</td>
<td>8.54</td>
<td>1.20</td>
</tr>
<tr>
<td>1500</td>
<td>1.07</td>
<td>1.50</td>
</tr>
<tr>
<td>2000</td>
<td>1.26</td>
<td>1.77</td>
</tr>
<tr>
<td>2300</td>
<td>1.37</td>
<td>1.92</td>
</tr>
<tr>
<td>2400*</td>
<td>1.40</td>
<td>1.96</td>
</tr>
<tr>
<td>2500</td>
<td>1.44</td>
<td>2.01</td>
</tr>
</tbody>
</table>

*Values shown in italics were used in the calculations of this thesis.

In order to determine the mass transfer coefficients for the two proteins in the crossflow apparatus correlations are available which are dependent on the flow regime. In Section 6.4.1 it was demonstrated that the flow regime in the CF apparatus under the conditions employed was laminar. For such circumstances the Graetz-Leveque relationship originating from convective heat transfer under laminar flow conditions provides a correlation to determine $k_m$ (Blatt et al., 1970; Porter, 1990; Rushton et al., 1996) of which the general form is

$$k_m = 0.816 \left( \frac{\nu \alpha}{L_c D^2} \right)^{\frac{1}{3}}$$

(6.14)
where \( \gamma_m \) is the fluid shear rate at the membrane surface and \( L_c \) the channel length over the membrane. The fluid shear rate for a rectangular channel can be obtained from:

\[
\gamma_m = \frac{6u}{b}
\]

where \( u \) is the crossflow velocity and \( b \) the channel height. The resulting mass transfer coefficients for both BSA and LYZ for crossflow velocities ranging from 0.4 to 0.45 m/s are tabulated in Table 6.7.

<table>
<thead>
<tr>
<th>Crossflow velocity (m/s)</th>
<th>Mass transfer coefficient (BSA), ( k_m ) ( \times 10^6 ) m/s</th>
<th>Mass transfer coefficient (LYZ), ( k_m ) ( \times 10^6 ) m/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>4.31</td>
<td>6.01</td>
</tr>
<tr>
<td>0.41</td>
<td>4.35</td>
<td>6.06</td>
</tr>
<tr>
<td>0.42</td>
<td>4.39</td>
<td>6.11</td>
</tr>
<tr>
<td>0.43</td>
<td>4.42</td>
<td>6.16</td>
</tr>
<tr>
<td>0.44</td>
<td>4.45</td>
<td>6.21</td>
</tr>
<tr>
<td>0.45</td>
<td>4.49</td>
<td>6.25</td>
</tr>
</tbody>
</table>

It must be stressed that the comparison of the mass transfer coefficients for these two systems must be done cautiously, because strictly speaking equation (6.14) is based on UF systems where there was a genuine gel layer present at the membrane surface which is not the case under the chosen experimental conditions.

Comparing the mass transfer coefficients between BSA and LYZ demonstrates that mass transfer is generally higher for smaller molecules. The data shown in Table 6.6 and Table 6.7 help to interpret the filtration results and can explain the limitations of the shear comparison model employed. Since the mass transfer coefficient can be expressed as a ratio of the diffusion coefficient and the boundary layer any existing differences in the boundary layer between the two systems will be apparent at experimental conditions where the diffusion coefficients are equal. During both BSA and LYZ filtration the mass transfer coefficient was one order of magnitude higher during SC filtration. This difference can be attributed to the variation in the concentration boundary layer thickness for both systems. The results suggest that the greater turbulence in the SC
allowed for the mass transfer coefficient to be greater. With the information obtained, it will be possible to estimate under what conditions the mass transfer coefficients and thus the concentration boundary layer can be kept the same which may be of great use in future comparative work. Under the present conditions a stirrer speed of approximately 300 rpm would allow one to match the mass transfer coefficients in both systems. However, it must be stressed that matching mass transfer coefficients for the two systems would result in differing shear stresses in the two systems. In other words, at least for the present experimental apparatus, one of the two parameters can be matched, but not both. Moreover, it must be emphasised that the mass transfer coefficient is under the influence of several factors including the boundary layer thickness and the diffusion coefficient which in turn is influenced by ionic strength, solution pH and temperature.

According to Table 6.6 and Table 6.7 concentration polarisation effects are likely to be higher during CF filtration because of the lower mass transfer coefficient obtained compared to the SC filtration. This is confirmed in the succeeding section by calculating the true rejection coefficient by use of the film model.

6.4.8 True rejection coefficient

The true rejection of the membrane (see also Chapter 3) takes into account the concentration at the membrane surface, \( C_m \), and can demonstrate the presence of a concentration boundary layer at the membrane surface. The true rejection can be calculated by rearranging equation (311) to give (see Appendix C, part b):

\[
R_t = \frac{R_o}{1 - R_o^* + R_o} \exp \left( \frac{J_s}{k_m} \right)
\]

where \( R_t \) is the true rejection coefficient, \( R_o \) the observed rejection coefficient and \( J_s \) the solute flux. The true rejection coefficients were calculated at 25 kPa TMP and reported in Table 6.8 alongside the observed rejection coefficients for BSA and LYZ for both SC and CF filtration. As anticipated from the previous remarks concerning the mass transfer coefficient the true rejection was always higher in the case of CF filtration. This
implies that concentration polarisation effects were more pronounced in the CF apparatus

Table 6.8. Membrane observed and true rejection for conventional 50 kDa Nadir membranes (20 mM).

<table>
<thead>
<tr>
<th>Filtration apparatus</th>
<th>pH</th>
<th>Observed rejection, $R_o$ (-) BSA</th>
<th>True rejection, $R_o$ (-) BSA</th>
<th>Observed rejection, $R_o$ (-) LYZ</th>
<th>True rejection, $R_o$ (-) LYZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>11.0</td>
<td>0</td>
<td>0</td>
<td>0.31</td>
<td>0.50</td>
</tr>
<tr>
<td>SC</td>
<td>8.4</td>
<td>0.76</td>
<td>0.95</td>
<td>0.32</td>
<td>0.53</td>
</tr>
<tr>
<td>SC</td>
<td>7.0</td>
<td>0.71</td>
<td>0.92</td>
<td>0.34</td>
<td>0.57</td>
</tr>
<tr>
<td>SC</td>
<td>6.0</td>
<td>0.70</td>
<td>0.92</td>
<td>0.36</td>
<td>0.58</td>
</tr>
<tr>
<td>SC</td>
<td>4.9</td>
<td>0.70</td>
<td>0.90</td>
<td>0.48</td>
<td>0.69</td>
</tr>
<tr>
<td>CF</td>
<td>11.0</td>
<td>N/A</td>
<td>N/A</td>
<td>0.08</td>
<td>0.79</td>
</tr>
<tr>
<td>CF</td>
<td>8.4</td>
<td>0.93</td>
<td>1.00</td>
<td>0.09</td>
<td>0.81</td>
</tr>
<tr>
<td>CF</td>
<td>7.0</td>
<td>0.91</td>
<td>1.00</td>
<td>0.10</td>
<td>0.84</td>
</tr>
<tr>
<td>CF</td>
<td>6.0</td>
<td>0.89</td>
<td>1.00</td>
<td>0.11</td>
<td>0.83</td>
</tr>
<tr>
<td>CF</td>
<td>4.9</td>
<td>0.84</td>
<td>1.00</td>
<td>0.21</td>
<td>0.89</td>
</tr>
</tbody>
</table>

The data in Table 6.8 is presented for a fixed mass transfer coefficient based on the diffusion coefficients for BSA and LYZ, respectively (for the lower ionic strength case, 20 mM). The impact of the ionic strength on the diffusion coefficient should ideally be accounted for. A proposed method to do so is given in Section 6.4.9.

6.4.9 Ionic strength impact on mass transfer and true rejection coefficient

Instead of using a correlation to determine the diffusion coefficient of a typical protein based on its molecular weight the Stokes-Einstein equation can be used to evaluate the diffusion coefficient (Mochizuki and Zydney, 1992):

$$D = \frac{k_BT}{6\pi\mu r_s}$$

(6.17)

where $k_B$ is the Boltzmann constant, $T$ the temperature, $\mu$ the viscosity and $r_s$ the solute radius. It is now possible to include the Debye length into this equation by adding it onto the solute radius. Thereby, it is possible to account for the difference the ionic...
strength magnitude will make to the diffusion coefficient and therefore the mass transfer coefficient. The calculated mass transfer coefficients were then used in equation (6.16) to determine the influence of ionic strength on the true rejection coefficient for which the results are shown in Table 6.9 and Table 6.10.

Table 6.9. Ionic strength influence on true rejection of 50 kDa Nadir membranes (20 mM).

<table>
<thead>
<tr>
<th>Filtration apparatus</th>
<th>pH</th>
<th>Observed rejection, ( R_e ) BSA</th>
<th>True rejection, ( R_e ) BSA</th>
<th>Observed rejection, ( R_e ) LYZ</th>
<th>True rejection, ( R_e ) LYZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>11.0</td>
<td>0</td>
<td>0.00</td>
<td>0.31</td>
<td>0.59</td>
</tr>
<tr>
<td>SC</td>
<td>8.4</td>
<td>0.76</td>
<td>0.97</td>
<td>0.32</td>
<td>0.64</td>
</tr>
<tr>
<td>SC</td>
<td>7.0</td>
<td>0.71</td>
<td>0.95</td>
<td>0.34</td>
<td>0.68</td>
</tr>
<tr>
<td>SC</td>
<td>6.0</td>
<td>0.70</td>
<td>0.95</td>
<td>0.36</td>
<td>0.68</td>
</tr>
<tr>
<td>SC</td>
<td>4.9</td>
<td>0.70</td>
<td>0.94</td>
<td>0.48</td>
<td>0.77</td>
</tr>
<tr>
<td>CF</td>
<td>11.0</td>
<td>N/A</td>
<td>N/A</td>
<td>0.08</td>
<td>0.96</td>
</tr>
<tr>
<td>CF</td>
<td>8.4</td>
<td>0.93</td>
<td>1.00</td>
<td>0.09</td>
<td>0.96</td>
</tr>
<tr>
<td>CF</td>
<td>7.0</td>
<td>0.91</td>
<td>1.00</td>
<td>0.10</td>
<td>0.97</td>
</tr>
<tr>
<td>CF</td>
<td>6.0</td>
<td>0.89</td>
<td>1.00</td>
<td>0.11</td>
<td>0.97</td>
</tr>
<tr>
<td>CF</td>
<td>4.9</td>
<td>0.84</td>
<td>1.00</td>
<td>0.21</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Firstly, when comparing these results to the 20 mM ionic strength case (where the diffusion coefficient was determined without taking into account the ionic strength, see Table 6.8), it can be seen that the true rejection coefficient was very similar for the stirred cell system (a slightly higher true rejection was calculated when ionic strength was considered) and the true rejection was 100% in both cases for the CF apparatus. When the 20 mM and 100 mM ionic strength impact on the true rejection coefficient are compared for the BSA case, it can be seen that, again, there is no difference for the CF case. This implies that due to the lower mass transfer coefficient in the CF system there was a considerable amount of concentration polarisation present during BSA filtration. It is also interesting to see that at pH 4.9 the true rejection was higher at the higher ionic strength implying that BSA aggregation occurs due to the charge-shielding effect. Looking at the LYZ data in Table 6.8 and Table 6.9 demonstrates that taking into account the ionic strength also leads to a larger true rejection coefficient. This is of relevance because the simpler approach which ignores the ionic strength effect would
underestimate the total size of the protein and therefore its rejection and interaction characteristics.

<table>
<thead>
<tr>
<th>Filtration apparatus</th>
<th>pH</th>
<th>Observed rejection, $R_o$ (-) BSA</th>
<th>True rejection, $R_o$ (-) BSA</th>
<th>Observed rejection, $R_o$ (-) LYZ</th>
<th>True rejection, $R_o$ (-) LYZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>11.0</td>
<td>0</td>
<td>0.00</td>
<td>0.26</td>
<td>0.51</td>
</tr>
<tr>
<td>SC</td>
<td>8.4</td>
<td>0.84</td>
<td>0.97</td>
<td>0.23</td>
<td>0.50</td>
</tr>
<tr>
<td>SC</td>
<td>7.0</td>
<td>0.92</td>
<td>0.98</td>
<td>0.24</td>
<td>0.54</td>
</tr>
<tr>
<td>SC</td>
<td>6.0</td>
<td>0.87</td>
<td>0.97</td>
<td>0.23</td>
<td>0.52</td>
</tr>
<tr>
<td>SC</td>
<td>4.9</td>
<td>0.92</td>
<td>0.98</td>
<td>0.32</td>
<td>0.61</td>
</tr>
<tr>
<td>CF</td>
<td>11.0</td>
<td>N/A</td>
<td>N/A</td>
<td>0.05</td>
<td>0.89</td>
</tr>
<tr>
<td>CF</td>
<td>8.4</td>
<td>0.99</td>
<td>1.00</td>
<td>0.06</td>
<td>0.93</td>
</tr>
<tr>
<td>CF</td>
<td>7.0</td>
<td>0.93</td>
<td>1.00</td>
<td>0.07</td>
<td>0.94</td>
</tr>
<tr>
<td>CF</td>
<td>6.0</td>
<td>0.91</td>
<td>1.00</td>
<td>0.08</td>
<td>0.91</td>
</tr>
<tr>
<td>CF</td>
<td>4.9</td>
<td>0.94</td>
<td>1.00</td>
<td>0.16</td>
<td>0.93</td>
</tr>
</tbody>
</table>

It is also interesting to note that, although the observed rejection was low during CF filtration of LYZ, the true rejection coefficient is actually considerably high which is again due to the lower mass transfer coefficient obtained in the CF system implying concentration polarization effects. It can also be seen from Table 6.9 and Table 6.10 that during CF filtration LYZ rejection was actually higher at the lower ionic strength. As mentioned before, this is because LYZ will have a larger hydrodynamic diameter in its hydrated state at a low ionic strength, whereas its effective size will be smaller at the higher ionic strength. When comparing the LYZ data for both CF and SC filtration and also referring back to Figure 6.10 and Figure 6.12, it becomes clear that the transmission is actually higher in the CF system due to higher concentration polarization which allows more solutes to be near the membrane surface and transmit. This is, of course, different for BSA where the solutes are mostly larger than the average membrane pores and therefore the increased concentration polarization does not aid the transmission and rather promotes solute aggregation and back diffusion.
6.4.10 Protein chemistry

There are certain properties of proteins which can be strongly affected by the solution environment, hence a brief discussion of their important properties is provided here. Proteins are biological macromolecules consisting of chains of amino acids linked together to form a biopolymer. They have a complex structure where their globular shape is formed due to three-dimensional arrangement of secondary structure elements whilst the primary structure contains the sequence of amino acids in the polypeptide chain (Cooper, 2004). In addition, tertiary and quaternary structures contribute to the globular shape of a protein. This is important because pure protein samples usually contain proteins in a folded condition (specific arrangement of tertiary and quaternary structure). Folded proteins are considered unstable and can denature, i.e. unfold without difficulty thereby loosing their tertiary and quaternary structure and most importantly tend to become stickier. In other words, in the denatured state, which may be caused by a change in temperature or pH, the unfolded proteins tend to either aggregate or stick to surfaces (Cooper, 2004). Hence, adsorption of proteins may increase if a protein has denatured during the filtration process and as a result of the unfolding the protein will also change its shape. It has been demonstrated that protein adsorption can play a critical role in UF and moreover solution pH and ionic strength were also shown to influence the degree of adsorption (Fane et al., 1983b).

In Table 6.11 useful information about protein solutions with a feed concentration of 0.5 g/L and a feed volume of 50 mL is provided. This includes the total number of molecules in the feed for a given protein, the average distance between the molecules in solution, the surface area of each protein and the total area occupied by the proteins in solution. Such data can be of use when considering charge interactions and fouling effects.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Total no. of molecules in feed</th>
<th>Average distance between molecules (nm)</th>
<th>Surface area of a single protein (nm²)</th>
<th>Surface area of all proteins in solution (nm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>2.27x10¹⁷</td>
<td>13</td>
<td>154</td>
<td>3.5x10¹⁹</td>
</tr>
<tr>
<td>LYZ</td>
<td>1.02x10¹⁸</td>
<td>8</td>
<td>45</td>
<td>4.65x10¹⁹</td>
</tr>
</tbody>
</table>
The number of molecules in the feed is obtained by dividing the feed mass by the molecular mass of the protein and multiplying with Avogadro’s number (6.022×10^{23} \text{ mol}^{-1}). Subsequently, dividing the feed concentration by the number of molecules in the feed and taking the cube root provides an estimate of the average distance between proteins assuming each protein occupies approximately the volume of a cube. The surface area of a protein can be estimated by assuming a spherical protein shape. It can be deduced from Table 6.11 that BSA molecules occupy a larger volume than LYZ molecules. BSA molecules are also further spaced out than LYZ molecules as shown in Table 6.11. BSA molecules (~7.0 nm diameter) are, on average, 13 nm apart and LYZ molecules (~3.7 nm diameter) are 8 nm apart. It is also noticeable that the surface area of BSA is 3 times larger than that of LYZ, although the Stokes radius of BSA is only twice that of LYZ. Thus, charge interactions may be expected to be more prominent with BSA compared to LYZ (as previously indicated in Figure 6.1). However, the number of molecules in solution is larger for LYZ than for BSA, thus the LYZ solution has actually more surface area available.

6.4.11 Relating MWCO and protein rejection

To an extent, it is possible to relate the MWCO data of dextrans of a given size to that of proteins of a similar size (as indicated in Section 5.2). However, as has been mentioned on several occasions in this thesis, such a correlation can only be made if a comparison is made purely on a size basis and other effects such as the solution environment conditions are neglected. In Section 5.2 it was shown that BSA, with a Stokes radius of 3.45 nm closely corresponds to a 25 kDa dextran and LYZ, with a Stokes radius of 1.83 nm is more closely related to a 5 kDa dextran. With this information, purely on a size basis, MWCO data can be used to estimate the expected rejection for a given protein. For this purpose Figure 5.4 is re-plotted as Figure 6.17:
Chapter 6 - Filtration with Unmodified Membranes

On a MWCO basis, a 25 kDa dextran is expected to be approximately 70% rejected when the SC is used and even less when the CF apparatus is used. A 5 kDa dextran is expected to be about 30% rejected with the SC and about 10% rejected with the CF. (This is also in agreement with the pore size estimate in relation to the solute size shown in Figure 5.10) In addition, if this information is compared to actual protein rejection data tabulated in Table 6.3 it can be seen that the rejection expected from MWCO data corresponds closely to the actual rejection in the case of BSA for SC filtration, and in the case of LYZ for both SC and CF filtration. However, in the case of CF filtration of BSA, the protein should only be rejected about 50%, but the observed rejection is in fact 90% or higher. One possible cause for this occurrence may be the presence of high shear rates in this crossflow application which can result in aggregation of proteins near the membrane surface (Chen et al., 2007) Moreover, it has been demonstrated earlier that concentration polarisation was, in fact, higher in the CF apparatus due to lower mass transfer explaining why the rejection was so high.

The different effects that MWCO was seen to have on protein filtration, depending on the solute size and apparatus used, indicates that care must be taken in how MWCO data are employed to predict protein rejection. Firstly, a noticeable difference is seen between SC and CF filtration. This demonstrates that a suitable choice of membrane
MWCO is likely to be different for SC and CF applications. This is important as it suggests that the use of SC data from laboratory experiments as a basis for selection in CF applications is inappropriate. Secondly, the definition of the MWCO and its merit in choosing an adequate membrane for a given application is put into question. A MWCO of 50 kDa should result in 90% rejection of a similar sized protein and thus in even greater rejection of a protein with a higher MW than the cut-off, such as BSA. This definition holds for CF of BSA, where indeed rejection of the protein was between 90 to 100%. This was the case because of concentration polarization. For all other filtration experiments a comparison of the Stokes radius and an equivalent size dextran would have provided a better prediction of the observed rejection.

6.4.12 Flux and rejection data for unmodified membranes at different TMP

Filtration experiments were carried out with the SC at pH 4.9 and 8.4 (for BSA) and pH 11.0 (for LYZ) at 50 kPa TMP in order to directly compare corresponding experimental data from plasma-modified membranes (see Chapter 7). It is also of interest to compare the corresponding results for unmodified membranes at 25 kPa with those obtained at 50 kPa TMP. All these experiments were conducted at a fixed ionic strength of 20 mM.

The data in Figure 6.18 were obtained at 30 minutes filtration time. Filled symbols represent filtration data at 50 kPa and ‘open’ symbols represent filtration data at 25 kPa. For LYZ, at its pI, flux was higher at the higher TMP. However, rejection was not correspondingly lower. LYZ rejection was higher at the higher TMP which may be attributable to membrane fouling.

The influence of the solution pH on the fouling resistance at 20 mM ionic strength and 50 kDa TMP was tabulated using the resistance-in-series model in Table 6.12 (see also Section 6.4.6). Indeed, a comparison of the fouling resistances reported in Table 6.5 and Table 6.12, respectively, show that LYZ fouling was higher at the higher TMP. Although permeate flux may be higher as a result of an increased applied pressure, protein rejection does not necessarily reduce and may, in fact, increase.
Figure 6.18. Comparison of BSA and LYZ filtration at two different TMP using unmodified membranes.

Table 6.12. Influence of solution chemistry on the fouling resistance of unmodified membranes at 50 kPa TMP.

<table>
<thead>
<tr>
<th>Protein</th>
<th>pH</th>
<th>Buffer</th>
<th>Hydraulic resistance, $R_m$ ($10^{12} \text{ m}^{-1}$)</th>
<th>Fouling resistance, $R_f$ ($10^{12} \text{ m}^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>4.9</td>
<td>Phosphate buffer</td>
<td>1.2</td>
<td>0.7</td>
</tr>
<tr>
<td>BSA</td>
<td>8.4</td>
<td>Phosphate buffer</td>
<td>1.2</td>
<td>0.5</td>
</tr>
<tr>
<td>LYZ</td>
<td>11.0</td>
<td>Glycine buffer</td>
<td>1.2</td>
<td>1.2</td>
</tr>
</tbody>
</table>

In the case of BSA, protein rejection was similar at 25 and 50 kPa at its pI which should also be reflected in the fouling resistance data. A comparison between the values reported for BSA filtration at pH 4.9 (see Table 6.5 and Table 6.12) shows that the difference in fouling resistance is relatively small. Again, as in the case of LYZ, permeate flux was higher at the higher TMP. At pH 8.4 the rejection of BSA was higher at the lower TMP suggesting that the higher TMP causes more of the protein to transmit. Permeate flux, however, was similar at both TMP and the flux rate was even slightly higher at the lower TMP. Such a reduction in flux at a higher TMP may be due to protein fouling. The fouling resistance at 50 kPa was $4 \times 10^{11} \text{ m}^{-1}$ compared to $7.5 \times 10^{10} \text{ m}^{-1}$ at 25 kPa which supports this hypothesis.
6.4.13 Further discussion

According to Salgin (2007), during protein filtration with PES membranes using a CF system, an increase in the ionic strength results in a lower membrane resistance and reduced fouling as a result of the suppression of charge effects. For the data presented here this was true for LYZ but not for BSA filtration. Moreover, in contrast to Salgin, Falbish et al. (1998) showed an increase in membrane resistance with an increase in the ionic strength in their study of colloidal suspensions. The findings of this thesis suggest that it is not possible to generalise that a higher ionic strength will result in either reduced or increased resistance to flow, because it will also depend on the solute to pore size ratio and the interaction of the solute with the membrane. In Section 5.2.2 an average pore size at 90% rejection was calculated for a 50 kDa membrane. At 90% rejection, it gave a pore diameter of 11.7 nm (Ferry’s model) and 10.7 nm (SHP model), respectively. Thus, it is possible to calculate a solute to pore size ratio at this rejection for BSA (6.9 nm) and LYZ (3.66 nm) as shown in Table 6.13. On this basis, at 90% rejection a single protein molecule is always smaller than the size of the corresponding pore. In Section 5.2.2, it was shown that for a 10 kDa membrane the pore diameter obtained at 90% solute rejection is similar to the size of BSA so that the solute to pore size ratio is closer to 1. Generally, a ratio closer to one should also result in higher rejection, although rejection is also dependent on the magnitude of the feed concentration.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Protein diameter (nm)</th>
<th>Solute to pore size ratio (Ferry’s model*)</th>
<th>Solute to pore size ratio (SHP model*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>6.9</td>
<td>0.59</td>
<td>0.64</td>
</tr>
<tr>
<td>LYZ</td>
<td>3.66</td>
<td>0.31</td>
<td>0.34</td>
</tr>
</tbody>
</table>

* For details on the pore models refer to Section 5.2.2

Both Elimelech et al. (1994) and Costa and de Pinho (2005) studied the filtration of humic substances at different ionic strengths and in their studies the effect of pH was mostly negligible. However, it is of interest to note that in this work, at least in the case of BSA filtration, flux tended to increase away from the protein pI at the lower ionic strength. A UF study by Kim and Fane (1995) also showed higher permeate flux data at low ionic strength and away from the pI. At a pH above the pI of BSA the protein is negatively charged and for charged proteins the solubility (their stability) increases.
whereas the affinity for the membrane material decreases (Nyström, 1989), hence supporting the finding of an observed increase in rejection with an increase in pH (Figure 6.9 and Figure 6.11).

It has been reported that pore blocking (i.e. more likely with the larger BSA) contributes more significantly to an increase in membrane resistance than pore narrowing (when solutes adsorb within the pore structure) (Belfort et al., 1994). However, such an effect could not be observed for the data obtained in this thesis where, in fact, the membrane resistance was mostly higher for LYZ filtration, irrespective of the filtration apparatus used. This observation is also supported by the findings of other authors (Tarleton and Wakeman, 1993) who found smaller solutes to cause more membrane fouling than larger ones, resulting in an increased resistance to filtration. An increase in resistance caused by the smallest solutes in solution was also found by Tarabara et al. (2004). These authors also stated that this type of fouling resulted in the most severe flux decline.

Crossflow BSA filtration with a 50 kDa PES membrane (Figure 6.15) showed a substantial permeate flux decline within the initial 20 to 30 minutes whereas thereafter a steadier flux was reached. It is of interest to relate this finding to a study by Salgin (2007) who filtered BSA with a 10 kDa PES membrane at different pH and ionic strength at 20 kPa. Her study showed a severe flux decline within the first 5 to 10 minutes and a steady flux thereafter. It has been shown that a higher TMP causes steady state flux to be established more rapidly, although that particular study was conducted for particle systems (Tarleton and Wakeman, 1994). However, one might expect a steady-state flux to be established more quickly at the higher pressure (25 kPa) used in the current work. Yet, this is only true if the membrane MWCO used in the comparison is also the same. As mentioned, Salgin employed a 10 kDa membrane whereas the current work was conducted with a 50 kDa membrane. The smaller MWCO membrane is likely to reject almost 100% of the albumin (as was also the case with the 10 kDa membrane used in this study (Figure 6.7)) whereas the higher MWCO membrane will – depending on the solution pH – transmit a larger amount of BSA, explaining why it takes longer to form a solute layer at the membrane surface when the membrane cut-off is larger.
It was concluded by Nyström et al. (1998) that the addition of salt will promote the transmission of charged proteins for most solutions. However, this statement seems to be too simplistic if the solute to pore size ratio is not considered as well. The results presented here demonstrate that the ionic strength increase only promoted solute transmission if the solute size (in terms of MW) was significantly smaller than the membrane cut-off.

The higher flux rate mostly observed with CF filtration compared to SC filtration is probably attributable to the difference in hydrodynamics between the two systems. In CF, the liquid flow is parallel to the membrane surface, whereas in SC, liquid flow is downward towards the membrane and solute accumulation is only reduced as a result of continuous stirring of the feed. Generally, liquid flow in parallel to the membrane surface, as in CF, is considered to lead to less solute build-up and hence reduced resistance at the membrane thereby yielding higher permeate flux rates (Cheryan, 1998). Another possibility for this occurrence may be the resistance to flow provided by the base design below the membrane, as the SC base somewhat differs from the CF base, although they are both supported by a sinter plate.

As discussed, agglomeration or aggregation of proteins is considered to be more prominent when the charge on a molecule is low thereby allowing molecules to more closely approach each other. The formation of aggregates can, however, occur as a result of increased ionic strength. Contrary to this belief, Higuchi et al. (2004) discovered, using flow cytometry, a technique based on light scattering, that upon addition of NaCl the effective size of protein aggregates was actually reduced. Hence, care must be taken in drawing conclusions from ionic strength data without also paying close attention to the experimental conditions employed.

### 6.5 Conclusions

The filtration of BSA and LYZ at various pH and two ionic strengths was studied using a 50 kDa unmodified PES membrane and, in some cases, also using different TMPs. Additional experiments were conducted using smaller MWCO 10 kDa PES membranes. These studies were carried out under similar conditions using a SC and CF apparatus. An approach was developed to incorporate shear similarity into such a comparison. It
was found that SC and CF filtration yielded noticeably different results suggesting that care must be taken when scaling laboratory data obtained with an SC apparatus to CF applications, more widely used in industry. Generally, pH effects seemed to be less influential using both apparatus, than sometimes reported in the literature. The reduced influence of pH observed in these studies may quite possibly be due to the fact that the original PES membrane carries very little charge, as evident from streaming potential measurements. As expected, pH effects are more noticeable at lower ionic strength, because a high ionic strength results in charge-shielding thereby masking charge-interactions between proteins. Ionic strength effects, although less pronounced with the CF apparatus, gave a more noticeable effect suggesting that an appropriate choice of ionic strength can yield pre-determined filtration results. The less pronounced ionic strength effect seen during CF filtration was attributed to the lower mass transfer coefficient which was evident compared to the SC filtration experiments. Moreover, ionic strength effects have to be considered in conjunction with the solute to pore size ratio, because a solute much larger than the average membrane pore will be rejected to a greater extent at higher ionic strengths whereas a solute smaller than the average pore will be rejected less at higher ionic strengths.

At the lower ionic strength, protein fouling data determined with the resistance-in-series model showed lower membrane fouling with the CF apparatus compared to the SC apparatus, at least at the lower ionic strength. At the higher ionic strength membrane fouling was similar for both devices. Despite the benefit of lower fouling during CF filtration, concentration polarsation effects were actually higher. This was evident from the lower mass transfer coefficients which suggest that an increase in TMP would be required to increase the mass transfer rate in the CF apparatus. Alternatively, the channel length could be shortened but this would have the drawback of losing a fully developed flow profile.
Chapter 7 – Filtration with Plasma-Modified Membranes

In this chapter experimental work using plasma-modified membranes (produced in collaboration with Wroclaw University of Technology, Poland) is discussed. The results are further interpreted by application of filtration models. These membranes were characterised in the same way as the unmodified membranes in order to highlight any differences between the two membranes (see Chapter 5). Specific experiments were conducted with protein solutions in order to determine the filtration performance of these membranes and to compare these with results from conventional, unmodified membranes. All experiments referred to in this chapter were conducted with Nadir polyethersulphone (PES) membranes. The plasma modification process employed to obtain the plasma-modified membranes discussed here was described in Section 4.9. The necessary derivations to arrive at some of the equations employed in this chapter are provided in Appendix C.

7.1 Introduction

The literature review (Chapter 2) clearly showed that whilst ultrafiltration (UF) is principally a size-based separation process, other effects, particularly those influencing charge interaction such as pH and ionic strength, can be of great importance. This has not only been demonstrated experimentally by other researchers but is also supported by theoretical work, for instance, Bowen and Sharif (1998) concluded that electrostatic effects are very important when developing a model to quantify colloidal interaction effects on rejected particles larger than the pore size of the membrane. They also stated that the development of membranes of high surface potential should receive more attention. For these reasons, an attempt was made to study plasma-modified membranes with the intent to relate the membrane properties and their filtration performance to those of unmodified membranes.

During initial surface-modification studies Millipore membranes were found to be more prone to damage during plasma modification than Nadir membranes. In an attempt to modify Millipore membranes without pre-washing, the top layer of the membrane was found to be destroyed in that it started to peel away from the backing layer. In contrast, low-temperature plasma modification of Nadir membranes did not result in any visible
damage to the membrane, regardless of any prior pretreatment. Due to their apparent superior physical stability Nadir membranes were plasma-modified and used in the experiments demonstrated in this chapter. A possible reason for the more ‘stable’ properties of the Nadir membranes may be their graduated support structure in comparison to the distinct fibrous backing layer of the Millipore membranes (see also Section 5.6).

The characterisation work of the plasma-modified membranes has demonstrated that these membranes became hydrophobic and the streaming potential data suggest that the membranes carry little charge. Due to the increase in hydrophobicity an increase in fouling might be expected for the plasma-modified membranes. It must also be noted that for the present membranes it was not possible to take streaming potential measurements along the surface which could reveal a slightly different charge data compared to the measurement through the pore. It has been discussed at various occasions in this thesis that charge effects can be important in membrane filtration applications which warrants further study of this aspect.

7.1.1 Electrophoretic mobility and effective charge

Electrophoretic mobility data for BSA and LYZ are readily available in the literature. It can be used to obtain information on the number of effective charges on a protein molecule, $Z$, at different pH by using equation (7.1) (Boehme and Scheler, 2007):

$$Z = \frac{\mu_{el} k_B T}{e D}$$

where $\mu_{el}$ is the electrophoretic mobility, $k_B$ the Boltzmann constant, $T$ the temperature, $e$ the elementary charge and $D$ the protein diffusivity. Electrophoretic mobility data for BSA was sourced from Boehme and Scheler (2007) and data for LYZ was sourced from Kim et al. (2006). Electrophoretic mobility and the number of effective charges on a protein molecule for the pH values of interest are tabulated in Table 7.1. It can be observed that the number of protein charges is lowest at the pl, which is expected. Moreover, the data provides a reasonable estimate of the charge on the protein with pH.
Chapter 7 - Filtration with Plasma-Modified Membranes

Table 7.1. Electrophoretic mobility and effective protein charges.

<table>
<thead>
<tr>
<th>pH</th>
<th>BSA $\mu_{e}$ ($m^2/V.s$)</th>
<th>LYZ $\mu_{e}$ ($m^2/V.s$)</th>
<th>BSA $Z$ (-)</th>
<th>LYZ $Z$ (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2</td>
<td>$1.28 \times 10^{-8}$</td>
<td>-</td>
<td>5.4</td>
<td>-</td>
</tr>
<tr>
<td>4.9</td>
<td>$-6.86 \times 10^{-10}$</td>
<td>$1.10 \times 10^{-8}$</td>
<td>-0.3</td>
<td>2.8</td>
</tr>
<tr>
<td>6.0</td>
<td>$-9.44 \times 10^{-9}$</td>
<td>$1.05 \times 10^{-8}$</td>
<td>-4.0</td>
<td>2.7</td>
</tr>
<tr>
<td>7.0</td>
<td>$-1.63 \times 10^{-8}$</td>
<td>$1.00 \times 10^{-8}$</td>
<td>-6.8</td>
<td>2.5</td>
</tr>
<tr>
<td>8.4</td>
<td>$-1.96 \times 10^{-8}$</td>
<td>$7.81 \times 10^{-9}$</td>
<td>-8.2</td>
<td>2.0</td>
</tr>
<tr>
<td>11.0</td>
<td>-</td>
<td>$3.75 \times 10^{-9}$</td>
<td>-</td>
<td>1.0</td>
</tr>
</tbody>
</table>

7.2 Results and discussion

The experimental results shown in this chapter are based on the experimental matrix described in Section 4.12.4. Filtration experiments with bovine serum albumin (BSA) and lysozyme (LYZ) using plasma-modified membranes were conducted at a low ionic strength (20 mM), because it is of interest to demonstrate any potential charge effects (which would otherwise be masked at a higher ionic strength). It was demonstrated in Figure 6.13 that the Debye length is short at high ionic strength which translates into the electrical double layer (EDL) extending only a short distance from the pore wall so that most of the pore remains electrically neutral. In contrast, at a low ionic strength a greater fraction of the pore area will be occupied by the EDL making charge interactions more prevalent (Saksena and Zydney, 1995). It is known from the zeta potential and streaming potential measurement results that limited effects of charge are to be expected, nevertheless the experimental pH values were chosen in such a way as to maximise any potential charge interaction. The pH corresponding to the protein isoelectric point (pI) was also tested. BSA was used for most experiments as it carries a higher charge compared to LYZ (see Figure 6.1).

For each experiment, an original 50 kDa cut-off membrane was either modified by plasma only (at 200 W for 1 minute) or modified by plasma followed by subsequent dipping into AA. In preliminary work, it was found that permeate flux with plasma-modified membranes was substantially lower than for unmodified 50 kDa membranes (see also Section 5.3.4). Therefore, all experiments with modified membranes were carried out at 50 kPa (0.5 bar(g)) and a fixed concentration of 0.5 g/L. Duplicated
experiments with unmodified 50 kDa MWCO membranes at 50 kPa were already presented in Chapter 6. All experiments with the modified membranes were conducted using the stirred cell (SC) due to ease of operation and the lower concentration polarisation tendency as demonstrated in Chapter 6.

**7.2.1 Comparison of unmodified and plasma-modified membranes**

In this section flux and rejection data versus time are shown at pH 3.2, 4.9 and 8.4 for unmodified 50 kDa and plasma-modified 50 kDa membranes dipped into AA. Note also that permeate and rejection data are only shown from 20 minutes filtration time onwards because of the very low filtration rate obtained with the plasma-modified membranes.

The data at pH 8.4 in Figure 7.1 demonstrate that permeate flux for modified membranes has reduced dramatically. Rejection was also noticeably higher with the modified membrane compared to the unmodified ones. Although no streaming potential measurements could be taken at pH 8.4 (see Section 5.9.1), in general, the negative zeta potential on a membrane or a molecule tends to increase away from the pI so that it is reasonable to assume this is the case at pH 8.4. The streaming potential data showed that the membrane pI was around pH 4 and the pI of BSA is around pH 5, thus, in the presence of any charge on the membrane surface, one will expect charge repulsion between the membrane and the protein at pH 8.4. Certainly, one would expect solute-solute repulsion thereby allowing the BSA molecules to flow more freely than, for instance, at the protein pI. It is known from Figure 5.8 that the MWCO difference between an unmodified and a plasma-modified membrane dipped into AA was not vastly different. However, that said, in the range of 25 to 150 kDa the unmodified membrane showed a higher dextran rejection.

In Figure 7.1 the solute rejection is higher for the plasma-modified membrane than for the unmodified membrane which therefore stands in contrast to the MWCO data (see Figure 5.8). The result suggests that, in fact, charge repulsion may have been responsible for the observed increased rejection in the case of the plasma-modified membrane. This is, however, not in agreement with Figure 5.34 which showed a higher charge density for the unmodified membrane compared to the modified membrane at pH 7. Hence, if it is assumed that this is still the case at pH 8.4 rejection should be higher with the unmodified membrane. Therefore, a possible scenario explaining the
higher rejection with the plasma-modified membrane is due to membrane fouling. If membrane fouling is indeed more pronounced in the case of the plasma-modified membrane this would also lead to an increase in rejection. Since the plasma-modified membrane is hydrophobic membrane fouling is likely to be more of an issue than for the mildly hydrophilic, unmodified membrane. A further discussion of this hypothesis is conducted in Section 7.2.3.

If Figure 7.1 is related to the SC filtration data obtained at 25 kPa (see Figure 6.11) it is observed that the rejection is lower at the higher applied pressure. Generally, one would expect transmission to be higher at a higher transmembrane pressure (TMP) provided that fouling effects are not overly prevalent.

Figure 7.2 shows BSA filtration at 50 kPa at the pI of this protein using unmodified and AA plasma-modified membranes. Interestingly, the extent of rejection was very similar for both membranes being in agreement with rejection behaviour in the absence of charge effects. At the pI, the protein carries a balanced charge and is hence considered as neutral. Thus, if the MWCO is comparable, no noticeable difference in rejection is expected between an unmodified and a plasma-modified membrane. Permeate flux,
however, was again much lower with the modified membrane suggesting that factors such as the membrane hydrophobicity play a key role.

Figure 7.2. BSA (pH 4.9, 20 mM) flux and rejection vs. time (unmodified and AA-modified membranes).

In Figure 7.3 flux and rejection data versus time are shown for unmodified and AA-modified 50 kDa Nadir membranes. The charge density of the AA-modified membrane showed an increase at low pH (see Figure 5.34). The positive charge density value suggests that at pH 3.2 the membrane will be mildly positively charged. Therefore, BSA filtration was conducted at this pH. Note that BSA instead of LYZ was chosen, because the former has a higher charge density (Rezwan et al., 2005). The experiment was carried out with 0.5 g/L of BSA prepared in a 20 mM sodium acetate buffer.
At these conditions, streaming potential and zeta potential data indicated that both the membrane and BSA carry a positive charge. However, at that pH a charge on the membrane could not be due to carboxylate groups (if present) as they would be undissociated at this pH. Generally, for like charge, repulsion interaction effects can be anticipated which should result in higher protein rejection. Indeed, rejection was noticeably higher with the modified membrane. Permeate flux was again much lower for the modified membrane and it was lowest at this pH in comparison to the other pH values studied with modified membranes. If charge repulsion between the BSA protein and the membrane dominates one would expect membrane fouling to be lower compared to a pH where such effects are not present. According to Figure 6.1 and data provided by Mukai et al. (1997) BSA will be electrostatically repulsed by the membrane at pH 3.2. Moreover, the zeta potential increases away from the protein pI where the DLVO theory postulates that the electrostatic repulsive interaction is proportional to the square of the surface potential (the zeta potential of the protein) (Hiemenz, 1977). Hence, in theory, any repulsive charge interactions between the membrane and the protein should be high under these conditions if the membrane surface carries a notable charge.
Experiments were conducted using LYZ at its pI in order to observe whether the AA-modified membrane has a noticeable impact on the retention behaviour of this smaller protein. Figure 7.4 shows that rejection is higher for the AA-modified membrane compared to the unmodified 50 kDa membrane. Permeate flux was again significantly lower for the modified membrane. At pH 11.0 the protein is expected to be uncharged, thus it is likely that the difference in rejection between the unmodified and modified membrane was brought about on a size basis only.

When comparing the data obtained for BSA and LYZ at their respective pI it can be observed that in each case rejection was higher with the plasma-modified membrane (compared to the unmodified membrane). There seemed to be more of a difference in rejection between the unmodified and plasma-modified membrane in the case of LYZ. If this is related to Figure 5.8, which was replotted as Figure 7.5 for easier referencing, it can be observed that the difference in cut-off at the relevant MW range was also higher in the case of LYZ. At the respective pI, LYZ flux was higher than BSA flux which can be due to the smaller molecule size of LYZ and possibly less fouling in the case of LYZ transmission. Figure 7.5 also shows that the MWCO data are in agreement with the reasoning that rejection of LYZ is due to size only, i.e. the plasma-modified
membrane showed a higher dextran rejection than the unmodified membrane in the relevant MW range. In Figure 7.5 the dashed lines indicate the molecular weight (MW) of BSA and LYZ, respectively. In addition, the arrows shown indicate the MW which BSA and LYZ would correspond to purely on a globular size basis (using the Stokes radius approach as discussed in Section 5.2.1).

![Figure 7.5. MWCO of unmodified and plasma-modified 50 kDa Nadir membranes.](image)

If this information is further put into context, LYZ was rejected to a greater extent by the modified membrane. The MWCO data shows that regardless of using the protein MW (~14.7 kDa) or the corresponding molecular size determined from the Stokes radius (~6.1 kDa) as the deciding factor, in either case, the MWCO rejection with the unmodified 50 kDa membrane was lower than that of the modified membrane (in the region below a MW of 25 kDa).

### 7.2.2 Comparison of plasma-modified membranes

At pH 8.4 BSA filtrations were conducted using membranes modified by (i) plasma only and (ii) plasma followed by AA dipping. Figure 7.6 shows that permeate flux is lower for AA modified membranes. It can also be observed that rejection was somewhat higher with the membrane modified by plasma only which is perhaps surprising given...
that the permeate flux was also higher. However, if this result is related to the MWCO data for both of these modified membranes (see Figure 7.5) it can be seen that the membrane modified by plasma only showed a higher rejection in the relevant MW range. This is not only true if the protein MW (~66 kDa) is considered but also if the Stokes radius of BSA is used to determine the corresponding MW (~25 kDa) of BSA.

When the modified and the AA-modified membrane data for filtrate flux are compared, the filtrate flux is lower for the latter. A possible explanation for this flux difference may be due to additional chemical groups likely to be present on the AA-modified membrane. Whilst the membrane characterisation data showed that the changes to the modified membranes were limited, X-ray photoelectron spectroscopy and streaming potential measurements did show a change in the membrane characteristics, so that any chemical groups formed as a result of the AA dipping could potentially stick out from the membrane surface or be loosely spread over the membrane surface as illustrated in Figure 7.7.
Chapter 7 - Filtration with Plasma-Modified Membranes

Figure 7.7. Possible physical layout of plasma-modified membranes with AA dipping.

Generally, it can be inferred from the plasma-modified membrane flux data that the resistance to flow has dramatically increased for the modified membrane but the selective characteristics of the membrane and those pores relevant to filtration have been largely unaffected by the surface-modification. It can be hypothesised that as a result of the surface-modification closure of some of the smaller pores of the membrane occurred thereby reducing the area available for permeate flow. However, solutes preferentially transmit through the larger pores (Cleveland et al., 2002), thus rejection should not be greatly affected by such a change in the membrane structure, a flux reduction, however, would be expected. Moreover, it had been mentioned in Section 2.6 that desirable characteristics such as increased hydrophilicity of plasma-modified membranes can deteriorate with storage time. This is an area which would benefit from future work.

A desirable result of the plasma modification conditions used for these experiments was that seemingly little surface etching occurred, as evident by the similar MWCO data for unmodified and modified membranes (see Figure 7.5). In fact, the cut-off of the modified membranes at 90% rejection was slightly higher than that for the unmodified membranes suggesting that ablation may have taken place resulting in enlargement of some of the pores. An inappropriate choice of plasma generating power and treatment time can affect the membrane pore size and as can be seen from the results for these modified membranes it was possible to mostly retain the pore size characteristics of the original 50 kDa membrane in terms of MWCO (a more or less similar rejection profile was obtained for the modified membranes). On that basis, the higher protein rejection observed with the plasma-modified membranes implies that charge effects therefore, may have contributed to protein capture. The hydraulic resistance of the membrane, however, changed dramatically as evident from the much reduced permeate flux (refer
to Figure 7.6 and Section 7.2.3. This occurrence may be explained as follows: The membrane was rendered more hydrophobic rather than hydrophilic by the modification process as was demonstrated by contact angle measurements in Section 5.7.1.

In order to ensure that the hydrophilicity/hydrophobicity problem was not simply due to the choice of solvent an experiment was conducted in which the membrane was contacted with isopropanol alcohol (IPA). The idea was to help 'wet' the membrane and possibly obtain an improved filtration flux rate thereafter. As can be seen from Figure 7.8 water flux and BSA protein flux (at pH 8.4 and 20 mM ionic strength) were measured after soaking the modified membrane in 0.5 M NaOH solution and in 40% IPA solution. It can be seen that wetting the membrane with alcohol did not improve the subsequent water and protein flux. This also supports the contact angle measurement data (see Table 5.7) which suggested that the plasma-modified membranes became hydrophobic as a result of the modification.

Zeta potential data obtained from streaming potential measurements for unmodified and plasma-modified membranes showed that the plasma-modification caused a change in the zeta potential of the membrane (see Section 5.9). This stands in contrast to a study by Zhan et al. (2004) who did not observe a change in zeta potential between an unmodified, hydrophilic polysulphone membrane and an argon plasma-modified membrane. They concluded that a change in surface charge can only be brought about by surface grafting of charged groups. In this work, Figure 5.33 demonstrated that argon plasma alone seemed to have an effect on the membrane characteristics, although the change was very small. Additional dipping into AA, in this case, did not seem to be much different from the membrane modified by plasma only, although a small positive charge was seen towards pH 3 for the AA-modified membrane.
The small difference between argon plasma-modification only and argon plasma-modification followed by AA dipping is likely to be due to the short lifetime of the free radicals on the modified membrane surface. That is, most probably the time delay between exposure to air of the plasma-modified membranes and the actual dipping into AA solution was likely too great to show a more noticeable effect.

7.2.3 Fouling resistance analysis

The influence of solution chemistry on the fouling resistance term of the membrane was quantified using the resistance-in-series model (equation (6.12)) as previously shown in Section 6.4.6. The relevant data for the range of plasma-modified membranes used in this chapter and modified in the same manner (200 W, 1 min, AA), but tested at different pH conditions (all 20 mM ionic strength) using the SC apparatus are shown in Table 7.2. In addition, resistance terms obtained with BSA at pH 8.4 for a plasma-modified membrane not subjected to AA dipping are shown.
Chapter 7 - Filtration with Plasma-Modified Membranes

Table 7.2. Influence of solution chemistry on the fouling resistance of plasma-modified membranes.

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Solution chemistry</th>
<th>Buffer</th>
<th>Hydraulic resistance, $R_h$ ($10^{13} \text{ m}^{-1}$)</th>
<th>Fouling resistance, $R_f$ ($10^{13} \text{ m}^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>modified+AA</td>
<td>BSA, pH 8.4</td>
<td>Phosphate buffer</td>
<td>1.7</td>
<td>1.0</td>
</tr>
<tr>
<td>modified+no AA</td>
<td>BSA, pH 8.4</td>
<td>Phosphate buffer</td>
<td>1.9</td>
<td>0.8</td>
</tr>
<tr>
<td>modified+AA</td>
<td>BSA, pH 4.9</td>
<td>Phosphate buffer</td>
<td>1.6</td>
<td>1.5</td>
</tr>
<tr>
<td>modified+AA</td>
<td>BSA, pH 3.2</td>
<td>Acetate buffer</td>
<td>2.1</td>
<td>9.9</td>
</tr>
<tr>
<td>modified+AA</td>
<td>LYZ, pH 11.0</td>
<td>Glycine buffer</td>
<td>1.7</td>
<td>0.6</td>
</tr>
</tbody>
</table>

The fouling resistance shown in Table 7.2 was exceptionally high at pH 3.2 which confirms the hypothesis that although BSA was positively charged, the membrane charge was too small (refer to Figure 5.33 and Figure 5.34) and/or charge interaction was prevented by the lack of dissociation of the potential groups present on the membrane surface. Although the desired result was obtained, i.e. protein rejection was highest at pH 3.2, it was unlikely to be due to charge repulsion effects which demonstrates that the earlier hypothesis, where the higher rejection may be due to charge interactions between the solute and the membrane which carry the same charge, does not hold. This is because, if higher rejection was purely due to charge repulsion effects then less fouling and a better permeate flux than observed (see Figure 7.3) would be expected. The implication is that under the modification conditions used, it was not possible to obtain a large number of charge groups on the plasma-modified membrane surface. Thus, the hypothesis made earlier that the high rate of fouling could be attributable to the hydrophobic properties of the membrane, as determined from contact angle measurements (see Table 5.7), is appropriate. Hydrophobic surfaces are known to be more susceptible to membrane fouling than hydrophilic ones (Chan et al., 2004; Teng et al., 2006). Moreover, protein molecules were reported to unfold in the presence of hydrophobic membranes thereby increasing their tendency to foul (Marshall et al., 1993).

Such a conclusion is further supported by the experimental attempt to pre-wet the membrane using IPA which had been unsuccessful. Indeed, it was also noted in Section 2.4.2 that even when the protein and membrane are of like charge, as was theoretically the case in the experiment at pH 3.2, serious flux decline can occur (Van der Meeren et al., 2004). It is also known from the discussion in Section 2.4.3 that solutes which do not aggregate (due to charge repulsion at low ionic strengths) can cause significant flux...
decline due to pore blocking. This phenomenon poses an added risk for the plasma-modified membranes studied here as their cut-off data show (see Figure 7.5) that – on a solute size basis only – a notable amount of BSA should be able to transmit through the membrane and thus potentially result in membrane pore blocking.

Moreover, a study by Arai and Norde (1990) illustrated that adsorption of protein occurs on a hydrophobic surface irrespective of the protein charge, in contrast to hydrophilic surfaces where adsorption only occurs if the solute is electrostatically attracted. The magnitude of the fouling resistance shown in Table 7.2 is seen to decrease with increasing pH. If the contribution of fouling due to adsorption is considered it has been reported by Ingham et al. (1980) that, as the pH decreases, protein adsorption tends to increase irrespective of the applied pressure, being in agreement with the data presented.

7.3 Filtration modelling of charged membranes

According to the streaming potential results (Section 5.9) the modified membranes used in the current study carried little charge. Nevertheless, it was attempted to use the experimentally obtained rejection data to build a model which accounts for charge effects as this also allows one to estimate the impact of charge for these membranes and what the potential benefits, if any, would be for highly charged membranes. The basis of the model employed here is a combination of the model for a spherical colloid in a cylindrical pore (Smith and Deen, 1980), the film model (Blatt et al., 1970) and model development work by the research group of Zydney (Burns and Zydney, 1999, Burns and Zydney, 2001; Mehta and Zydney, 2006; Shao and Zydney, 2004).

Several assumptions were made in order to apply the model including a constant liquid density and viscosity and assuming those parameters to be equal to the pure solvent (Bowen and Williams, 2001; Hansen et al., 1998). The diffusion coefficient of the solute is concentration dependent but a constant value was assumed for modelling purposes (Boulanouar et al., 1996). Whilst the diffusion coefficient is also dependent on pH and ionic strength it is not unreasonable to assume a constant value, because typical macromolecular diffusion coefficients are of the order of $10^{-11}$ m$^2$/s so that a small
change in that value is not going to make a substantial difference to the overall model result.

7.3.1 Modelling of rejection, pore size and charge

In order to relate the electrostatic potential energy (which provides insight into the extent of charge effects) and experimentally obtained rejection data, the true rejection of the membrane and a membrane pore size had to be calculated. To do so, the concentration polarisation model (Section 3.1.3) and the two pore size models (Section 5.2.2) discussed previously, were employed. In section 6.4.8 it has been shown that equation (3.11) can be re-arranged to give:

\[ R_p = \frac{R_o}{1 - R_o + R_o \exp \left( \frac{J}{J_k} \right)} \]  

(7.2)

where \( R_p \) is the "true" rejection coefficient, \( R_o \) the observed rejection coefficient and \( J \), the solute flux. In the case of the plasma-modified membranes the observed and true rejection coefficients were found to be nearly identical. In contrast, for the unmodified membranes the true rejection was always higher than the observed rejection (see Table 7.3).

Table 7.3. Surface concentration, observed and true rejection for unmodified and modified membranes.

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Protein</th>
<th>pH</th>
<th>Observed rejection, ( R_o ) (-)</th>
<th>True rejection, ( R_p ) (-)</th>
<th>Concentration at the membrane, ( C_M ) (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unmodified</td>
<td>BSA</td>
<td>8.4</td>
<td>0.52</td>
<td>0.86</td>
<td>1.69</td>
</tr>
<tr>
<td>Unmodified</td>
<td>BSA</td>
<td>4.9</td>
<td>0.63</td>
<td>0.89</td>
<td>1.75</td>
</tr>
<tr>
<td>Unmodified</td>
<td>BSA</td>
<td>3.2</td>
<td>0.72</td>
<td>0.94</td>
<td>2.24</td>
</tr>
<tr>
<td>Unmodified</td>
<td>LYZ</td>
<td>11.0</td>
<td>0.38</td>
<td>0.63</td>
<td>0.84</td>
</tr>
<tr>
<td>Modified, no AA</td>
<td>BSA</td>
<td>8.4</td>
<td>0.82</td>
<td>0.84</td>
<td>0.56</td>
</tr>
<tr>
<td>Modified, AA</td>
<td>BSA</td>
<td>8.4</td>
<td>0.73</td>
<td>0.74</td>
<td>0.53</td>
</tr>
<tr>
<td>Modified, AA</td>
<td>BSA</td>
<td>4.9</td>
<td>0.67</td>
<td>0.69</td>
<td>0.53</td>
</tr>
<tr>
<td>Modified, AA</td>
<td>BSA</td>
<td>3.2</td>
<td>0.89</td>
<td>0.89</td>
<td>0.51</td>
</tr>
<tr>
<td>Modified, AA</td>
<td>LYZ</td>
<td>11.0</td>
<td>0.48</td>
<td>0.50</td>
<td>0.52</td>
</tr>
</tbody>
</table>

The data suggest that in the case of the modified membranes concentration polarisation was hardly present. This is also reflected in the low concentration values at the
membrane surface, \( C_M \), for the plasma-modified membranes. The reduced flux rate can therefore not be due to a polarisation layer. Since water flux rates for modified membranes were also much lower than those for unmodified membranes and MWCO measurements of both types of membranes did not show a significant difference, the flux reduction must be due to other influences. Generally, lower overall flux rates will help to limit concentration polarisation effects. A possible scenario is that the membrane hydrophobicity reduces flux rates and additionally also promotes fouling. In order to substantiate this hypothesis an experiment was conducted using unmodified membranes dried over a 12h period followed by a subsequent water flux analysis (the PES membranes employed in this work are not to be dried so that their favourable wetting properties can be maintained). However, for the purpose of modifying the membrane surface it is unavoidable to dry the membranes (unless surface-modification is conducted in the presence of wetting agents present on the membrane from the manufacturing process). It is of interest to see if the water flux of unmodified membranes actually deteriorates after drying as this would further support the theory that noticeably lower flux rates are obtained when the membrane is hydrophobic. In Figure 7.9 water flux rates for 50 kDa Nadir membranes at 0.5 bar(g) TMP are shown for different conditions.

1) Test 1 – Membrane pretreated and wetted in the manner outlined in Section 4.11 (wetting agent removed, membrane always kept wet thereafter)
2) Test 2 – Membrane was dried for 12 h after the removal of any wetting agents
3) Test 3 – Membrane was dried for 12 h and also stored in 40% IPA prior to measuring the water flux.

The findings from Figure 7.9 illustrate that drying a membrane alters the permeation properties. It is evident that the water flux reduction is substantial suggesting that the plasma-modified membranes may exhibit such low permeation rates not because of the modification process but because it was necessary to dry the membranes.
Wetting the membranes with alcohol after drying did only marginally improve the flux rate. This is an important finding and suggests room for future work, for instance, a polymeric membrane could be sourced which can be modified without requiring any pre-treatment steps.

A UF model including charge effects is of great interest because such a model would allow one to account for pH and ionic strength effects on filtration and potentially relate membrane charge to protein rejection. Hence, an attempt was made to develop a simplified model which can account for such effects. From streaming potential data, the charge density of the membrane at a given pH can be determined (see Section 5.9.3). Moreover, to quantify the charge on proteins the rejection electrostatic interaction effects on the partitioning coefficient, $\phi$, of a charged spherical solute in a cylindrical pore can be evaluated (Smith and Deen, 1980)

$$\phi = (1 - \lambda)^2 \exp\left( -\frac{\Delta W_1}{k_B T} \right)$$

(7.3)
where $\lambda$ is the ratio of the solute radius to pore radius and $\Delta W$, the solvation energy barrier (Bowen and Welfoot, 2002) which is sometimes also referred to as the energy of interaction, $\psi_E$ (Pujar and Zydney, 1998). The energy of interaction corresponds to the product of the dimensionless ion charge number, $z_n$ with the elementary charge, $e$, and the electrical potential, $\psi_0$. According to Burns and Zydney (1999), at low to moderate permeate flux rates, it is reasonable to assume $\phi=(1-R_p)$ and thus possible to relate charge effects to solute rejection. It is noteworthy that $(1-\lambda)^2$ in equation (7.3) accounts for steric exclusion within one solute radius of the pore wall whilst the exponential term accounts for the energy change due to the charged solute being placed inside the charged cylindrical pore (Shao and Zydney, 2004). The ratio of the solute radius to pore radius $\lambda = r_s/r_p$ can be found using the Stokes-Einstein equation introduced in Section 6.4.9 and solving for the solute radius, $r_s$ (Mochizuki and Zydney, 1992):

$$r_s = \frac{k_BT}{6\pi \eta D}$$

(7.4)

The diffusivity coefficient was evaluated using Young's equation (3.12) as demonstrated in Section 3 1 3. In order to evaluate the pore radius, $r_p$, both Ferry's and the Steric hindrance pore (SHP) model (described in Section 5 2 2) were employed. The pore radii obtained from the two models are similar but Ferry's model tends to give a slightly larger pore size. The protein solute radii evaluated with equation (7.4) gave a Stokes radius for BSA of 3.56 nm and a Stokes radius for LYZ of 2.15 nm which are both slightly larger than those reported in the literature. However, since proteins are known to be larger in their hydrated state in aqueous solution it seems reasonable to conduct the modelling with the larger, calculated radii.

The dimensionless electrostatic energy of interaction term, $E_i$, is the exponential term in equation (7.3):

$$E_i = \frac{\Delta W}{k_BT}$$

(7.5)
Low values of $E_i$ indicate limited charge interaction between proteins and also the membrane, whereas higher values imply more significant interaction. $E_i$ can be determined by rearranging equation (7.3) and replacing $\phi$ with $(1-R_p)$ to give:

$$E_i = \ln(1 - R_p) - \ln(1 - \lambda)^2$$  \hspace{1cm} (7.6)

The model equations were solved using Excel spreadsheets and the pore radii were calculated using Maple 10.0. For easier assimilation of the modelling procedure a block diagram illustrating the main steps taken to arrive at a solution for the dimensionless electrostatic energy of interaction is shown in Figure 7.10.

Figure 7.10. Block diagram of the main input and output data for the charge model.

The model was applied to averaged rejection and pore radii values for the results shown in Section 7.2.1. The different pore models gave very similar results, hence Figure 7.11 only shows the results for Ferry's model. For completeness, the SHP model results were included in Appendix F, part d.
Figure 7.11 shows the electrostatic interaction term and the true rejection coefficient versus the solution pH. A negative interaction indicates an attractive interaction between the membrane and the solute. The model suggests that such an interaction occurred in the case of BSA filtration using the plasma-modified membranes and also for LYZ filtration at pH 11.0 using the unmodified membranes. It is particularly noticeable that more interaction took place during LYZ filtration with the plasma-modified compared to the unmodified membrane. The interaction potential for BSA and LYZ, when using the plasma-modified membranes seems to be independent of the solution pH suggesting that charge effects were not significant. This is in agreement with the low surface charge density data shown for plasma-modified membranes in Figure 5.34. Typically, if charge interactions due to attractive forces are prevailing the electrostatic interaction term would be greater than -1 (Burns and Zydney, 1999). During BSA filtration using the unmodified membranes the interaction potential was positive indicating that both repulsive interactions caused by electrical double layer distortions and interactions with the pore boundary were important, although these forces were not very strong since the interaction term was not greater than 1. It is also noticeable that the interactions were greatest at pH 3.2 where the protein is positively charged suggesting that interactions at this pH were not due to repulsive interactions but electrostatic and steric interactions between the charged protein and the pore boundary. The data presented is a further
indication that the plasma-modified membranes carry little charge, which is also confirmed when the results are considered in context with permeate flux and fouling resistance data. In addition, at pH 3.2, where the electrostatic interaction potential was highest for the given experimental conditions, the surface charge density was still relatively low (-8x10^{-4} C/m²). Typically, charged membranes have charge densities of the order of 1 to 3x10^{-3} C/m² as reported by Rao and Zydney (2006). The surface charge density for a PES membrane, in particular, was reported as -5.0x10^{-3} C/m² by Pujar and Zydney (1994). The permeate flux was actually lowest at pH 3.2 (as evident from Figure 7.3) in comparison to BSA filtration at other pH values. In context with the fouling resistance data shown in Table 7.2, this suggests that membrane fouling was highest at this pH, which would not be expected if the filtration was governed by charge repulsion between the solute and the membrane. The model calculations suggest that any interactions were highest at low and high pH. Finally, the rejection data show that the true rejection coefficient was higher when using the unmodified membranes, i.e. concentration polarisation was lower when using the plasma-modified membranes.

7.3.2 Further application of the model

If the assumption to relate the true rejection coefficient to the solute partitioning coefficient is correct, it will be possible to predict the expected rejection for a given membrane if the dimensionless electrostatic interaction energy term is known. It is thus of interest to ‘reverse’ the model using typical charge density values for charged membranes as quoted earlier. The membrane surface charge can be related to the dimensionless electrostatic energy of interaction by the following equation:

\[ E_i = \frac{\Delta W_i}{k_BT} = \frac{Z\sigma_m}{r_pFI} \]  

(7.7)

where Z is the number of effective charges, \( \sigma_m \) the membrane charge density, F the Faraday constant and I the ionic strength. In Table 7.1 typical numbers of effective charges for the proteins of interest at varying pH were reported. If a typical value for the membrane pore size, \( r_p \), is assumed (e.g. 6 nm in this example) for each case the electrostatic interaction term can be calculated if a typical surface charge density (-5x10^{-3} C/m²) for charged PES membranes is used. Equation (7.7) can then be related
to equation (7.6) in order to predict the true rejection coefficient. This approach is illustrated in Table 7.4 for both BSA and LYZ using a range of pH values.

<table>
<thead>
<tr>
<th>Protein</th>
<th>pH</th>
<th>Z</th>
<th>$E_i$</th>
<th>$r_s$</th>
<th>$R_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>3.2</td>
<td>5.4</td>
<td>2332</td>
<td>3.56</td>
<td>0.71</td>
</tr>
<tr>
<td>BSA</td>
<td>4.9</td>
<td>-0.3</td>
<td>-0.130</td>
<td>3.56</td>
<td>0.85</td>
</tr>
<tr>
<td>BSA</td>
<td>8.4</td>
<td>-8.2</td>
<td>-3.541</td>
<td>3.56</td>
<td>0.99</td>
</tr>
<tr>
<td>LYZ</td>
<td>11.0</td>
<td>1.0</td>
<td>0.432</td>
<td>2.15</td>
<td>0.37</td>
</tr>
</tbody>
</table>

The data demonstrates that, as expected, the rejection for LYZ is lower than that for BSA because of the lower solute size of LYZ. The rejection of BSA is highest when the membrane and protein are of like charge and fairly high at the pI of the protein. At pH 8.4 where the protein and the membrane are both negatively charged the rejection was found to be essentially complete demonstrating the potential merit to be gained from a highly charged membrane. Whilst the model is not expected to accurately reflect the rejection, as it does not account for fouling and other important effects, it provides a useful initial estimate of what the expected rejection behaviour for a membrane with a given charge might be.

### 7.3.3 Further discussion of results

It is immediately obvious that water flux and protein flux data obtained with the plasma-modified membranes used in this research were substantially lower than the fluxes obtained with unmodified membranes. The MWCO data (Figure 7.5) show that the membrane cut-off did become smaller, i.e. rejection increased, in the lower MW region below 25 kDa and particularly below 10 kDa. One may therefore conclude that the plasma modification process causes some of the pores to become blocked and/or increases their resistance to solvent transfer. In the larger MW region, the data suggest that the majority of the pores largely retained their original size. If one regards a selection of small pores as A, and a selection of large pores as B, it is possible that the application of plasma causes pores A to be blocked hence limiting solvent flow to a smaller number of larger pores B. The larger pores are still available for solute-solvent transport and are sufficiently large to permit the transmission of a protein such as BSA.
When permeate flux is low, rejection is not necessarily high because at low flux rates fouling effects are typically reduced. Moreover, concentration polarisation is generally less of a problem so that the concentration in the bulk should correspond closely to the concentration at the membrane surface, i.e. \( R_o = R_n \), which was indeed found (refer to Table 7.3). Permeate flux may be low for various reasons, for instance, if the pore size is small, if the active layer is thick or protein fouling is high. Low permeate flux due to a thick active layer, for instance, was reported by Schipolowski et al. (2006).

Figure 7.11 demonstrated that whilst the type of electrostatic interaction observed for unmodified and modified membranes was different, the magnitude of their respective interaction was not actually that great. The surface charge density data for the membrane pores showed that (except at pH 3.2) the difference in charge density between the unmodified and modified membrane was not very large. In such cases any contribution from direct charge-charge repulsion between the protein and the membrane is relatively unimportant (Van Eijndhoven et al., 1995).

### 7.4 Conclusions

In this chapter filtration data obtained with unmodified and plasma-modified membranes were reported. Plasma-modified membranes (with and without AA dipping) were found to exhibit similar MWCO data to the original, unmodified 50 kDa membrane in the higher MW region but not in the lower MW region. Permeate flux was found to be greatly reduced as a result of the plasma-modification. While no definite conclusions can be drawn from these findings, some inferences can be made. A reduction in the number of smaller pores available for solute/solvent transport is likely due to a reduction in size of the smaller pores. An additional reason for the dramatic flux decline is most likely the change of the membrane characteristic from hydrophilic to hydrophobic as indicated by contact angle measurements. Moreover, it has been demonstrated that drying of the membranes has a dramatic effect on the flux rate also providing an explanation for the reduced flux rates observed for plasma-modified membranes.

Protein rejection was generally higher with the plasma-modified membranes compared to the unmodified ones, which is generally desirable. The highest rejection was
observed at pH 3.2, although the charge potential of the membrane was likely negligible. This is supported by the fact that the membrane became hydrophobic and in the presence of carboxylic charge groups one would expect the membrane to become more hydrophilic thereby helping to reduce membrane fouling, which was not the case. Plasma-modification of membranes does provide a method which results in a change in the membrane surface properties. The changes under these conditions resulted in membrane properties where concentration polarsation could be reduced, but flux rates were very low, thus the use of these membranes is a trade off between increased rejection and low flux rates. Further work would benefit from sourcing membranes which can be modified directly from the manufacturing process.

The stagnant film model applied in this chapter demonstrated that, unlike with unmodified membranes, the observed and true rejection coefficient were nearly identical, i.e. concentration polarsation was prevented with the modified membranes as a result of the low permeate flux rates. A further attempt to model the data obtained with unmodified and plasma-modified membranes allowed a relation between protein rejection and charge interaction data to be established. The model suggested that little charge was present on the plasma-modified membranes. A predictive model was also provided to show the extent of interaction and true rejection that could be expected for a membrane carrying a significant surface charge.
Chapter 8 - Overall Conclusions and Recommendations

In this chapter the main conclusions for the thesis are drawn. The key findings from the study of ionic strength and pH using two different membrane device configurations are summarised. Moreover, the main conclusions from the experiments with both unmodified and plasma-modified membranes are highlighted. Suggestions are also made for possible future research, which may prove of interest based on the results presented in this work.

8.1 Conclusions

The topics and research discussed in this thesis demonstrate that to-date membrane fouling is still the key problem in ultrafiltration (UF) membrane processes. Whilst UF is a molecular size-based separation process, mounting evidence exists that various other parameters influence the separation at the colloidal size range including hydrodynamics, pH and ionic strength. This thesis emphasises, whilst it has been known for some time that pH and ionic strength can influence protein filtration in particular, their degree of influence is also dependent on the membrane properties. In this work, where membrane charge was subtle, it is really the influence of ionic strength that is more dramatic than that of the pH. The influence of pH becomes more noticeable if the membrane used also carries a significant charge because solute-membrane electrostatic interactions become more prominent. Moreover, irrespective of any membrane charge, the influence of pH is only noticeable at low ionic strength because of the absence of charge-shielding. In context of the ionic strength it was also found that the effect it has will differ depending on the relationship between the solute size and the membrane pore. If the ionic strength is high and the solute much smaller than the average pore size, increased transmission will be observed. However, at a high ionic strength in the presence of solutes which are of similar or larger size than the membrane pore the charge-shielding can lead to more solute aggregation thereby reducing the transmission rate even further.
It was also highlighted in this research that many laboratory studies are routinely carried out using stirred cell (SC) devices. In contrast, industrial applications are mostly conducted using some form of a crossflow (CF) device. Therefore, a novel crossflow apparatus was designed in order to enable a comparison study of two such devices. The CF apparatus accommodated for fully developed laminar flow and it allowed for the recirculation of the liquid and better control of variables such as pressure, temperature and flow rate as compared to the SC device. The use of both CF and SC devices showed that the SC is an important device for experiments which require ease of operation and it can yield results relatively quickly. In addition, the SC can be useful where only small volumes of sample are available. It can also be more cost-effective than a CF device.

Based on the CF design a method was developed which can be used to compare and match the shear stress experienced in the CF apparatus with that of the SC. It was demonstrated that shear is an important parameter in ultrafiltration which is sometimes overlooked because the general tendency is to solely focus on the Reynolds number or other parameters such as the transmembrane pressure (TMP) employed. In this work, a relatively low TMP of 25 kPa was used for the majority of the experiments which made it possible to operate both apparatus at the same, selected, TMP. In addition, at such a pressure the extent of membrane fouling generally does not have a detrimental effect on the process.

The protein filtration studies using bovine serum albumin (BSA) and lysozyme (LYZ) as model proteins in the two apparatus showed that differences in permeation and filtration outcomes exist between the two devices, even if it is attempted to maintain comparable conditions. Differences were observed for factors such as permeate flux rates and mass transfer coefficients. Moreover, the CF apparatus was also used to conduct molecular weight cut off (MWCO) measurements which could be compared and contrasted with those obtained from stirred cell experiments. The MWCO measurements were found to differ for the two devices as well. The measurement using the CF apparatus usually gave a higher MWCO. These findings suggest that a prudent approach is necessary when laboratory data is to be transferred to industrial scale applications. The results also imply that there is significant difficulty in simply using SC data as a basis for scale-up to a CF device, because the resulting flux and rejection as well as MWCO data may not turn out as expected.
As a result of this research it is thus proposed to take into account shear at the membrane surface when using SC data as a basis for further design. Generally, high turbulence and high shear rates are desirable in order to minimise concentration polarisation, but any effort to maximise shear has to be balanced with any potential detrimental effect on the solutes used.

The filtration results for both unmodified and plasma-modified membranes were also interpreted using common filtration models. The application of such models has demonstrated that mass transfer was generally higher in the SC under the conditions employed. Concentration polarisation effects were therefore found to be greater during CF applications. This finding implies that increased turbulence can reduce concentration polarisation whereas shear alone is unable to achieve this effect. Protein fouling, however, was mostly higher during SC filtration. Therefore, it is important to know what the objective of a given filtration process is so that operating conditions can be chosen in such a manner as to select the most appropriate conditions. Fouling, for instance, could be reduced by working away from the protein isoelectric point and/or by operating at a low ionic strength. At a high ionic strength, due to charge-shielding effects, the influence of pH was masked so that operation at a pH away from the protein pI did not provide a benefit in terms of fouling reduction. The application of the resistance-in-series model also demonstrated that not much difference in the extent of fouling could be observed at a higher ionic strength.

As part of the characterisation studies scanning electron micrographs (SEMs) and swelling measurements of the membranes were taken. It was found that the SEMs could be used to provide information about the membrane thickness but they were of little use regarding the quantification of membrane fouling or an estimate of the average pore size. Instead, it was found more useful to develop a method which relates the MWCO data to the Stokes radius thereby allowing an estimate of a typical pore size at a given rejection. Swelling measurements showed that cleaning agents such as NaOH temporarily swell the membrane which has to be considered when re-use of cleaned membranes is required. Moreover, it was established that it is necessary to subject the membranes to a consistent pretreatment methodology. Such an approach will prevent the risk of misinterpreting a given filtration result which may actually be influenced by
variations in the membrane properties (even if sourced from the same sheet) and/or membrane compaction.

The capability of low-temperature plasma surface-modification applied to the polyethersulfone (PES) membranes used in this work was also studied. These newly obtained membranes and the original PES membranes were characterised in terms of MWCO, flux, contact angle and streaming potential. It was demonstrated that the plasma-modified membranes had substantially lower permeate flux rates than unmodified membranes. The MWCO, however, was not vastly different in the higher molecular weight range suggesting that the changes in the lower molecular weight range where due to loss of some of the smaller pores as a result of the modification. Contact angle data revealed that the membranes became more hydrophobic. Hydrophobic membranes are often under the influence of secondary effects such as air entrapment or more extensive protein adsorption which can also lead to lower permeate flux rates. In this work, the hydrophobic nature of the plasma-modified membranes was partly attributed to the need for drying of the membranes prior to the plasma application (membrane permeate flux was found to reduce after drying). Streaming potential data showed that both the unmodified and the plasma-modified membranes carried little charge. This was further supported by the application of a charge model which predicted little electrostatic interaction for these membranes. The model was also used to relate the true rejection coefficient to electrostatic interaction which enabled a demonstration of how charge interaction increases when the membrane carries a higher charge density.

The present research work has contributed towards a better understanding of the influence of device-dependent hydrodynamics and the influence of charge during UF of proteins with both unmodified and plasma-modified PES membranes.

8.2 Future work

There are several aspects to this work from which useful further studies could be carried out.

It was mentioned in Section 5.2.3 that dextran standards, frequently used for membrane characterisation, were reported to foul PES membranes, at least under the conditions
tested. Those conditions were manifested in the operation of a dead-end SC at a relatively low stirrer speed (300 rpm). It would be interesting to repeat such a study at higher stirring speeds and also using the CF device. Based on the findings for dextran rejection using the CF apparatus and knowing that the flow profile in the latter apparatus will differ from that in a SC it may be of interest to determine if dextran fouling would occur under the proposed conditions and/or what the extent of fouling might be.

A large percentage of the cost in UF processes is due to the need for membrane replacement as a result of fouling or inadequate cleaning effectiveness. It is hence recommended to carry out further studies using low-temperature plasma treatment but using different polymeric membranes and different co-polymers in order to find a modification-scenario where a membrane can be created which becomes more hydrophilic and can retain high permeate flux rates. To do so it would be beneficial to source a polymer material that can be modified directly from the manufactured state. It may also be possible to achieve further improvements in the membrane characteristics if a systematic study is carried out where a given membrane material is modified over a range of intensities and treatment times in order to determine the optimal modification parameters.

In terms of a comparison of filtration devices the present study could be extended to comparison tests at different conditions. The mass transfer coefficients for the two systems could be kept identical, although this would mean that shear rates are different, at least for the present system. The comparative study between SC and CF devices could also benefit from a study using suitably plasma-modified membranes in order to explicitly look at charge interactions between the membrane and the protein(s) and any differences which may be observed due to the difference in device configuration. If such studies were to give quite different results this would suggest that conclusions from many laboratory studies (using SC devices) will be inappropriate when used as a design basis for the important CF configuration widely represented in industrial applications.

Also, it must be stressed that the current research is limited as it has only been applied to two different single protein solutions. It would therefore be of interest to study whether similar findings could be made using other model protein solutions and to test
how transferable the methodologies developed in this thesis are. Eventually, since real systems are always made up of multi-component mixtures it would be of interest to extent this work to binary and multi-component mixtures. Such studies would have to account for secondary effects such as protein-protein interactions in the bulk and at the membrane surface which were out of the scope of this work.
References


References


References


References


221
References


222
References


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References


Appendices

A. List of publications and presentations

Posters


Presentations

Publications
Extended abstract.
B. Crossflow apparatus - Design

a) Technical design drawing of the crossflow module.
b) Assembled membrane module and membrane holder situated in framework
C. Supporting calculations

a) Calculation of the hydraulic membrane diameter

\[ d_h = \frac{4 \times 30 \times 2}{2(30 + 2)} = 3.75 \text{ mm} \]  

(b) Stagnant film model derivations

Application of the boundary conditions of the concentration polarisation phenomenon described by the film model yields:

\[ J = \int \frac{dc}{c - C_p} = \frac{J_s}{D} \int_0^{\delta_h} dx \]  

which can be integrated to give:

\[ J_s = \frac{D}{\delta_h} \ln \frac{C_M - C_p}{C_f - C_p} \]  

Equation (A.3) can be re-arranged in order to determine the concentration at the membrane surface, \( C_M \):

\[ C_M = C_p + (C_f - C_p) \times \frac{J_s}{J_{w}} \]  

A combination of equations (3.1), (3.2) and (A.3) can be re-arranged in such a manner as to give equation (3.11). In order to arrive at this conclusion, equations (3.1) and (3.2) are expressed as follows.

\[ C_f = \frac{C_p}{(1 - R)} \quad \text{and} \quad C_M = \frac{C_p}{(1 - R_n)} \]  

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Substituting into equation (A.3) with \( k_m = D/\delta_b \) gives

\[
J_s = k_m \ln \left( \frac{C_p - C_p}{1 - R_o C_p} \right)
\]  

(A 6)

Dividing equation (A.6) by \( C_p \) and further rearrangement yields.

\[
J_s = k_m \ln \left( \frac{R_o}{1 - R_o} \right)
\]  

(A 7)

which then can be expressed as equation (A.8), i.e. another form of equation (3.11):

\[
\ln \left( \frac{R_o}{1 - R_o} \right) - \ln \left( \frac{R_o}{1 - R_o} \right) = \frac{J_s}{k_m}
\]  

(A.8)

Changing signs for each term on the left-hand side and inverting the numerator and denominator of equation (A.8) gives the form of equation (3.11). Solving equation (3.11) or equation (A.8) for \( R_o \) leads to equation (7.2) via the following steps.

Rearranging equation (A.8) and converting the logarithm into an exponential term yields equation (A.9):

\[
\left( \frac{R_o}{1 - R_o} \right) = \left( \frac{R_o}{1 - R_o} \right) e^{J_s/k_m}
\]  

(A.9)

After rearrangement equation (A.9) becomes:

\[
R_o (1 - R_o + R_o e^{J_s/k_m}) = R_o e^{J_s/k_m}
\]  

(A.10)
which then can be solved to give equation (7.2) and which can also be written as equation (A.11)

\[
R_\nu = \frac{\frac{1}{\lambda}}{R_s e^{i\omega}} \frac{1}{1 - \frac{1}{\lambda} R_s + R_s e^{i\omega}}
\]  

(A.11)
c) Shear stress calculation along the stirred cell impeller for different frequencies

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<th>RPM</th>
<th>Radius cm</th>
<th>( \tau ) Pa</th>
<th>RPM</th>
<th>Radius cm</th>
<th>( \tau ) Pa</th>
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</tr>
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<td>181.65</td>
<td></td>
<td>1.30</td>
<td>67.14</td>
</tr>
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d) Sample Maple solution using Ferry’s and the SHP pore model

Ferry’s model applied to 90% rejection of a 50 kDa dextran.

\[
> \text{solve}\left\{0.9 = 1 - 2 \left(1 - \frac{9.08}{d}\right)^2 - \left(1 - \frac{9.08}{d}\right)^4\right\}, \{d\};
\]

\[
[[d = 7.436970571], [d = 11.65488070], [d = 2.978212296 + 4.262912053 I], [d = 2.978212296 - 4.262912053 I]]
\]

SHP model applied to 90% rejection of a 50 kDa dextran.

\[
> \text{solve}\left\{0.9 = 1 - \left(\left(1 + \frac{16}{9} \cdot \left(1 - \frac{9.08}{d}\right)^2\right) \cdot \left(1 - \frac{9.08}{d}\right)^2 \cdot \left(1 - \frac{9.08}{d}\right)^2\right)\right\}, \{d\};
\]

\[
[[d = 3.767182991], [d = 7.458811133], [d = 11.60164224], [d = 5.679766598 + 4.394602610 I], [d = 5.679766598 - 4.394602610 I]]
\]
D. Calculated and experimental calibration curves

a) Dextran calibration (1 kDa) – in the range of 0.01 g/L to 2 g/L

The maximum error for a given data point (of which three measurements were averaged) was ±5%.
b) Dextran calibration (5 kDa) – in the range of 0.1 g/L to 2.0 g/L

\[ y = 870560x \]
\[ R^2 = 0.9995 \]

\[ 0 \quad 0.2 \quad 0.4 \quad 0.6 \quad 0.8 \quad 1.0 \quad 1.2 \quad 1.4 \quad 1.6 \quad 1.8 \quad 2.0 \]
\[ \text{Concentration (g/L)} \]

\[ 0 \quad 200,000 \quad 400,000 \quad 600,000 \quad 800,000 \quad 1,000,000 \quad 1,200,000 \quad 1,400,000 \quad 1,600,000 \quad 1,800,000 \quad 2,000,000 \]
\[ \text{Refractive Index (nRIU)} \]

-- Linear (5 kDa calibration)

---

c) Dextran calibration (150 kDa) – in the range of 0.005 g/L to 1.0 g/L

\[ y = 871152x \]
\[ R^2 = 0.9999 \]

\[ 0 \quad 0.2 \quad 0.4 \quad 0.6 \quad 0.8 \quad 1 \]
\[ \text{Concentration (g/L)} \]

\[ 0 \quad 100,000 \quad 200,000 \quad 300,000 \quad 400,000 \quad 500,000 \quad 600,000 \quad 700,000 \quad 800,000 \quad 900,000 \quad 1,000,000 \]
\[ \text{Refractive Index (nRIU)} \]

-- Linear (Dextran 150 kDa)
d) UV/Vis absorbance data for common proteins

It was mentioned in Section 4.6.2 that protein absorbance data can be determined using either UV/Vis spectroscopy or by use of a calculation approach. The calculation is slightly less accurate, because absorbance of proteins can differ in different solvents, and thus can only provide an estimate. However, the absorbance calculation provides an additional means to check if experimentally obtained calibration curves are reasonable.

According to the Beer-Lambert law the absorbance of light can be determined if the concentration of the absorbing sample, the path length for which the light travels through the sample and the molar extinction or absorption coefficient are known. The law can be expressed in the form of

\[ A = \varepsilon c m l \]  

(A.12)

where \( A \) is absorbance, \( \varepsilon \) the molar extinction coefficient, \( c m \) the molar sample concentration and \( l \) the sample thickness or path length. The extinction coefficient is based on the chromophores in a molecule, i.e. the part of the molecule which can be detected in the UV range (1 to 400 nm). In a protein certain amino acids are classed as chromophores, hence if the amino acid composition of a protein is known then the expected absorbance of the entire protein can be estimated (Gill and Vonhuppel, 1989). Typically, the extinction coefficient is determined at a wavelength of 280 nm for most proteins. Table A.1 lists relevant amino acids, Tryptophan (Trp), Tyrosine (Tyr), Cysteine (Cys) and their molar extinction coefficients for the proteins used in this research.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>BSA</th>
<th>LYZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trp</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Tyr</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>Cys</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>( \varepsilon ) (280 nm)</td>
<td>42,925</td>
<td>37,970</td>
</tr>
</tbody>
</table>

Based on the data in Table A.1, extracted from Pace et al. (1995), the molar extinction coefficient at 280 nm can be determined using equation (A.13):
$\varepsilon(280) = 5,500(#Trp) + 2,490(#Tyr) + 125(#Cys)$ \quad (A.13)

Once the extinction coefficient and the relative molecular mass (RMM) of the protein is known the expected absorbance can be determined using the Beer-Lambert law. Note that slight variations of equation (A.13) exist, but after extensive studies of the average $\varepsilon$-values of eighty proteins obtained from different methods Pace et al. (1995) recommended to use the above equation. Absorbance values at varying concentrations for BSA (RMM 66,330) and LYZ (RMM 14,700) are provided in Table A.2.

<table>
<thead>
<tr>
<th>BSA (g/L)</th>
<th>Absorbance (Abs units)</th>
<th>LYZ (g/L)</th>
<th>Absorbance (Abs units)</th>
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<tbody>
<tr>
<td>0.05</td>
<td>0.016</td>
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<td>0.065</td>
</tr>
<tr>
<td>0.075</td>
<td>0.032</td>
<td>0.075</td>
<td>0.194</td>
</tr>
<tr>
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<td>0.065</td>
<td>0.1</td>
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<td>0.2</td>
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<tr>
<td>1.0</td>
<td>0.647</td>
<td>1.0</td>
<td>2.583</td>
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</table>
e) UV/Vis BSA calibration (0.025 – 1 g/L)

The maximum error in any absorbance reading was ±3%. The absorbance obtained at 0.5 g/L from this curve differs less than 3% from the calculated value (Table A.2).

f) UV/Vis LYZ calibration (0.025 – 1 g/L)

The maximum error in any absorbance reading was ±4%. The absorbance obtained at 0.5 g/L from this curve differs 10% from the calculated value demonstrating that it is best to work with experimental calibration curves (Table A.2).
## E. Characterisation data

### a) Contact angle data – unmodified membranes

*(Lappeenranta University of Technology)*

#### Static contact angle

<table>
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<td>Drop number</td>
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<tr>
<td>Left contact angle</td>
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<tr>
<td>Right contact angle</td>
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<tr>
<td>Mean contact angle</td>
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<td>Drop number</td>
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<tr>
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<td>88.86</td>
</tr>
<tr>
<td>Right contact angle</td>
<td>87.53</td>
</tr>
<tr>
<td>Mean contact angle</td>
<td>88.2</td>
</tr>
<tr>
<td>Overall average contact angle</td>
<td>89 °</td>
</tr>
<tr>
<td>Contact angle variation (°)</td>
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<tr>
<td>Standard deviation (°)</td>
<td>1.4 °</td>
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<table>
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<th>Membrane: Nadir PES 20 kDa</th>
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<tbody>
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<tr>
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</tr>
<tr>
<td>Mean contact angle</td>
</tr>
<tr>
<td>Drop number</td>
</tr>
<tr>
<td>Left contact angle</td>
</tr>
<tr>
<td>Right contact angle</td>
</tr>
<tr>
<td>Mean contact angle</td>
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<tr>
<td>Overall average contact angle</td>
</tr>
<tr>
<td>Contact angle variation (°)</td>
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<tr>
<td>Standard deviation (°)</td>
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<table>
<thead>
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<th>Membrane: Nadir PES 30 kDa</th>
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<tbody>
<tr>
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</tr>
<tr>
<td>Mean contact angle</td>
</tr>
<tr>
<td>Drop number</td>
</tr>
<tr>
<td>Left contact angle</td>
</tr>
<tr>
<td>Right contact angle</td>
</tr>
<tr>
<td>Mean contact angle</td>
</tr>
<tr>
<td>Overall average contact angle</td>
</tr>
<tr>
<td>Contact angle variation (°)</td>
</tr>
<tr>
<td>Standard deviation (°)</td>
</tr>
</tbody>
</table>
### Static contact angle

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Naflor PES 50 kDa</th>
<th>Surface status: untreated</th>
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</thead>
<tbody>
<tr>
<td>Drop number</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Left contact angle</td>
<td>80.13</td>
<td>79.34</td>
</tr>
<tr>
<td>Right contact angle</td>
<td>82.33</td>
<td>82.06</td>
</tr>
<tr>
<td>Mean contact angle</td>
<td>81.48</td>
<td>80.70</td>
</tr>
<tr>
<td>Overall average contact angle</td>
<td>81.0</td>
<td>Contact angle variation (±)</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>1.3</td>
<td></td>
</tr>
</tbody>
</table>

### Static contact angle

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Milpore PES 50 kDa</th>
<th>Surface status: untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drop number</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Left contact angle</td>
<td>93.08</td>
<td>92.93</td>
</tr>
<tr>
<td>Right contact angle</td>
<td>91.07</td>
<td>91.39</td>
</tr>
<tr>
<td>Mean contact angle</td>
<td>92.07</td>
<td>91.95</td>
</tr>
<tr>
<td>Overall average contact angle</td>
<td>94.0</td>
<td>Contact angle variation (±)</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>1.3</td>
<td></td>
</tr>
</tbody>
</table>

b) Contact angle data – plasma-modified membranes

(Lappeenranta University of Technology)
c) Contact angle data – Water cleaned and direct from manufacturer

(Loughborough University)

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Water-washed and dried</th>
<th>untreated,</th>
<th>untreated, including wetting agent from manufacture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left contact angle</td>
<td>79° 81° 80° 81° 80°</td>
<td>81° 80° 82° 81° 80°</td>
<td>49° 52° 50° 51° 50°</td>
</tr>
<tr>
<td>Right contact angle</td>
<td>77° 80° 80° 81° 80°</td>
<td>81° 80° 81° 80° 80°</td>
<td>49° 51° 49° 52° 52°</td>
</tr>
<tr>
<td>Mean contact angle</td>
<td>78° 80° 80° 81° 80°</td>
<td>81° 81° 81° 81° 80°</td>
<td>49° 51° 49° 52° 52°</td>
</tr>
</tbody>
</table>

Average left CA: 81° ± 2°
Average right CA: 80° ± 2°
Average mean CA: 81° ± 2°
Contact angle variation: 2° ± 1°
Standard deviation: 1° ± 1°
F. Supporting Figures

a) Dextran standards chromatogram

b) BSA UV wavelength scan
Appendices

c) LYZ UV wavelength scan

![UV wavelength scan graph]

Protein detection wavelength = 280 nm

UV Wavelength (nm)

Absorbance (Abs unit)

0 0.5 1 1.5 2 2.5 3

200 220 240 260 280 300 320 340 360 380

25 15 5

d) Charge interaction and true rejection versus pH (Steric hindrance pore model, see Section 7.3.1 and Figure 7.11 for further details)

![Charge interaction and true rejection graph]

Electrostatic energy of interaction, $\Delta W/\Delta q_1$ vs. pH

$\text{pH}$

$-1 -0.8 -0.6 -0.4 -0.2 0 0.2 0.4 0.6 0.8 1$

$-1 -0.8 -0.6 -0.4 -0.2 0 0.2 0.4 0.6 0.8 1$

modified, AA - SHP model - BSA ▲ unmodified - SHP model - BSA ● unmodified - SHP model - LYZ

□ modified, AA - SHP model - LYZ × modified, AA - rejection + unmodified - rejection
Appendices

G. Membrane Manufacturer Data Sheets and Journal Paper publication
Biomax™ Membranes

- The membrane of choice for fast processing, and exceptional chemical resistance

Biomax membranes are the membranes of choice for applications requiring high flux, low to moderate protein binding, and harsh chemical cleaning and/or sanitization.

Typical Applications
- Concentration, buffer exchange, and depyrogenation of protein solutions containing biomolecules such as albumin, IgG, IgM, monoclonal antibodies, hormones, and growth factors.
- Harvest, clarification, and concentration of vaccines.

The more open average pore size permitted by the void-free structure of the Biomax membrane results in higher fluxes with maximum retention.

Conventional UF membranes cast with macrovoids have tighter average pore sizes and must operate with reduced flux to keep retention high.

The high flux and high retention properties of the Biomax membrane result in faster processing speeds with higher yields, which means shortened processing times and a bioprocessing system that can be smaller and more compact.

Biomax membranes are composed of polyethersulfone and are resistant to harsh chemicals used in cleaning, biological decontamination, and sanitization. The polyethersulfone Biomax membrane has been modified to reduce non-specific protein binding compared to conventional polyethersulfone membranes.

Advantages of Choosing Biomax Membranes
- Void-free structure results in high flux, excellent retention and higher yields.
- Composite polyethersulfone membrane provides a stable hydraulic environment, resulting in excellent mechanical strength and integrity.
- Polyethersulfone membrane has superior resistance to harsh cleaning chemicals with no degradation of processing performance through multiple cleaning cycles.
- Biomax membranes are available in a wide range of molecular weight cut-offs to meet all of your application needs.
Tight Retention Profile

The retention profile of Biomax 10 kDa membrane is much sharper than that of a conventional 10 kDa membrane, translating into improved protein retention in your process stream (Figure 1).

![Figure 1: Protein retention of Biomax membrane versus conventional Polyethersulfone UF membrane](image)

Improved Integrity

The void-free structure of the Biomax membrane significantly reduces the incidence of microdefects, resulting in improved membrane integrity (Figure 2).

![Figure 2: Integrity testing of Biomax membranes versus conventional Polyethersulfone UF membranes](image)

Improved Process Yields

You can decrease the size of the system and improve your yield, thereby reducing your overall processing costs (Table 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Biomax 10</th>
<th>Conventional Polyethersulfone (10 kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention (%)</td>
<td>99.95</td>
<td>99.9</td>
</tr>
<tr>
<td>Flux (lmh)</td>
<td>118.0</td>
<td>80.0</td>
</tr>
<tr>
<td>Recirculation rate (lpm)</td>
<td>4.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Pipe diameter (inches)</td>
<td>1.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Hold-up volume (liters)</td>
<td>8.4</td>
<td>20.8</td>
</tr>
<tr>
<td>Yield improvement (%)</td>
<td>2 - 3</td>
<td></td>
</tr>
</tbody>
</table>

Table 1
Superior Flux
At working concentrations of protein, Biomax membranes have higher flux for a given protein retention than conventional polyethersulfone UF membranes. In this example, Biomax 10 membrane demonstrates a 40% improvement in process flux over a conventional 10 kDa polyethersulfone membrane using 10% BSA (Figure 3).

Superior Chemical Resistance
Results in Excellent Cleanability
After 380 hours in 1 N NaOH at 50 °C, Biomax membranes show consistently better retention and integrity than competitive polyethersulfone UF membranes. Caustic cleaning regimens have no effect on the ability of the Biomax membrane to retain proteins and do not adversely affect integrity (Figure 4).

A rigorous cleaning regimen of caustic plus hypochlorite restores normalized water permeability (NWP) to near initial values following sequential process runs (Figure 5).

Results
After 100 hours in 600 ppm chlorine, the Biomax 10 membrane showed no appreciable change in air integrity or BSA retention (Table 2).

<table>
<thead>
<tr>
<th></th>
<th>Sample A</th>
<th>Sample B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air integrity (sccm) prior to exposure</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>BSA retention % prior to exposure</td>
<td>99.97</td>
<td>99.97</td>
</tr>
<tr>
<td>Air integrity (sccm) after exposure</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>BSA retention % after exposure</td>
<td>99.97</td>
<td>99.97</td>
</tr>
</tbody>
</table>

Table 2
To Place an Order or Receive Technical Assistance
For additional information call your nearest Millipore office
In the U S and Canada, call toll-free 1-800-MILLIPORE (1-800-645-5476)
In the U S , Canada and Puerto Rico, fax orders to 1-800-MILLIFX (1-800-645-5439)
Internet www.millipore.com
Tech Service www.millipore.com/techservice

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Biomax Membrane Dextran Rejection

Figure 7 UF membrane Dextran retention profile

Specifications

Membrane
Composite polyethersulfone with void-free structure, compatible with solutions having a pH between 1 and 14

Relative protein binding
Low to moderate, for use with protein solutions containing more than 0.1 mg/mL of protein

Biomax Applications

<table>
<thead>
<tr>
<th>Biomax Type</th>
<th>Membrane Code</th>
<th>NMWL* [kDa]</th>
<th>Typical Application</th>
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<tbody>
<tr>
<td>Biomax 5</td>
<td>PBCC</td>
<td>5</td>
<td>Growth factors, hormones</td>
</tr>
<tr>
<td>Biomax 8</td>
<td>PBFC</td>
<td>8</td>
<td>Growth factors, hormones</td>
</tr>
<tr>
<td>Biomax 10</td>
<td>PBGC</td>
<td>10</td>
<td>Albumin, hemoglobin</td>
</tr>
<tr>
<td>Biomax 30</td>
<td>PBTK</td>
<td>30</td>
<td>Enzymes</td>
</tr>
<tr>
<td>Biomax 35</td>
<td>PBQK</td>
<td>50</td>
<td>IgGs</td>
</tr>
<tr>
<td>Biomax 100</td>
<td>PBHK</td>
<td>100</td>
<td>Small viruses, antigens</td>
</tr>
<tr>
<td>Biomax 300</td>
<td>PBMK</td>
<td>300</td>
<td>IgGs, large viruses</td>
</tr>
<tr>
<td>Biomax 500</td>
<td>PBWK</td>
<td>500</td>
<td>Large viruses, collods, particulates</td>
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<tr>
<td>Biomax 1000</td>
<td>PBXK</td>
<td>1000</td>
<td>Large viruses, cells, collods, particulates</td>
</tr>
</tbody>
</table>

* NMWL = Nominal Molecular Weight Limit

Product Usage
Biomax membranes are found in Pellicon® XL devices and Pellicon 2 Cassettes from Millipore
NADIR® Datenblatt
- Ultrafiltrationsmembranen -
H-Serie

Membran
Membranmaterial

Membrandaten
Nominelle Trenngrenze kDa
Wasserfluss I / (m²h)
Rückhalt %
Testmedium

<table>
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<th>UH004</th>
<th>UH030</th>
<th>UH050</th>
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<td>Membranmaterial</td>
<td>PESH</td>
<td>PESH</td>
<td>PESH</td>
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<tr>
<td>Nominelle Trenngrenze</td>
<td>&gt; 4</td>
<td>30</td>
<td>50</td>
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<td>Wasserfluss</td>
<td>&gt; 20</td>
<td>&gt; 100</td>
<td>&gt; 250</td>
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<tr>
<td>Rückhalt</td>
<td>92 – 97</td>
<td>82 – 88</td>
<td>75 – 83</td>
</tr>
<tr>
<td>Testmedium</td>
<td>Dextran T10 (1%)</td>
<td>PVP K30 (2%)</td>
<td>PVP K30 (2%)</td>
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</tbody>
</table>

Zulassige Betriebsbedingungen
pH-Bereich
Temperaturbereich °C

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<th>UH030</th>
<th>UH050</th>
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<td>0 – 14</td>
<td>0 – 14</td>
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<tr>
<td>pH-Bereich</td>
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</tr>
<tr>
<td>Temperaturbereich</td>
<td>5 – 95</td>
<td>5 – 95</td>
<td>5 – 95</td>
</tr>
</tbody>
</table>

Testbedingungen 3 bar, 20 °C, Rührzelle (700 U/min)
Evaluation and comparison of protein ultrafiltration test results: Dead-end stirred cell compared with a cross-flow system

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Advanced Separations Technologies Group, Department of Chemical Engineering, Loughborough University, Loughborough, Leicestershire LE11 3TU, UK

Received 9 June 2007, received in revised form 15 January 2008, accepted 18 January 2008

Abstract
Dead-end stirred cell devices are commonly used in laboratories to characterise ultrafiltration membranes and their separation behaviour in addition to protein separation data from such systems are used for process scale-up. Such devices are operated under conditions that are inherently different from those used during the continuous or semi-continuous processing of industrial feed streams. The work presented in this paper compares the rejection behaviour of single protein solutions in a dead-end stirred cell (SC) device with that for a cross-flow system (CF). The effect of ionic strength (20 and 100 mM) and solution pH (4, 6, 7, 8 4 and 11) on protein filtration (bovine serum albumin (BSA) and lysozyme (LYZ) from buffered aqueous solutions) behaviour has been investigated using polyethersulfone (PES) membranes with a manufacturer-specified molecular weight cut-off (MWCO) of 50 kDa. PES membranes were characterised in terms of dextran MWCO using both the SC and the CF systems. The mode of operation resulted in significant observed differences in the resulting dextran solute rejection curves for the two systems. The observed rejection $R_{des}$ values for a series of dextran standards were consistently found to be lower for the CF system compared with the SC unit suggesting higher wall concentrations ($C_w$) due to concentration polarisation effects in the CF unit. Protein ultrafiltration studies with the 50 kDa PES membranes highlighted important differences in observed protein rejection behaviour despite operation of the two systems at the same transmembrane pressures (25 kPa). Solution pH was found to have little effect on the rejection of both BSA and LYZ. The solute rejection was found to be more sensitive to ionic strength effects for the SC device both during BSA and LYZ filtration. Convective mass transfer coefficients and hence the true rejection coefficients $R_{des}$ were calculated for both systems using the stagnant film model to understand the influence of hydrodynamic effects on the ultrafiltration behaviour of the two systems. The magnitude of the membrane Peclet number (Pe) provides a means of comparing hydrodynamic conditions for the two systems and thereby allows differences in observed solute rejection to be better understood.

Keywords: Stagnant film model, Ultrafiltration, Cross-flow, Stirred-cell, Proteins

I. Introduction
Ultrafiltration finds wide application in diverse industries such as the pharmaceutical, bio-medical and food industries where separation is predominantly controlled on the basis of molecular size differences although solution pH, ionic strength and system hydrodynamics have been acknowledged to play a role in the separation of proteins [1–6]. Saksena and Zydney [5] demonstrated the importance of electrostatic interactions on protein filtration when attempting to separate bovine serum albumin (BSA) from immunoglobulin (IgG) using an Amicon stirred ultrafiltration cell. The authors [5] carefully selected solution pH and ionic strength thereby creating the possibility to transport more of the larger IgG through a 100 kDa polymeric membrane compared to the smaller albumin molecule, a phenomenon termed ‘reversed selectivity’. In a later study, Eijndhoven et al. [6] also demonstrated that it is, in fact, possible to separate molecules of a similar size with ultrafiltration thereby challenging the general understanding that ultrafiltration is only suited to the separation of molecules having at least two to three orders of magnitude difference in size. The same authors [6] also pointed out that the degree of separation can be improved with a cross-flow device (as compared to a stirred cell) which attributed to a higher bulk mass transfer rate. Evidence from previous studies suggests that solution ionic strength influences the extent of charge interaction in terms of protein–protein and protein–membrane interactions. Several researchers have demonstrated the influence of ionic strength...
on permeate flux. Solution pH has been shown to have less of an effect at higher ionic strengths due to charge shielding effects [6–9]. Whilst sufficient evidence exists in the literature to demonstrate the importance of electrostatic interactions [1,4,9,10] there still seem to be differing observations concerning permeate flux and rejection at and around the pI of the protein.

A review of recent literature found little published work comparing ultrafiltration measurements made at the laboratory-scale (usually, researchers seem to employ a dead-end stirred cell apparatus) and how such results correlate with those from process-scale measurements (typically cross-flow semicontinuous/continuous systems) [11–13]. This is surprising, given that hydrodynamic conditions (such as wall shear rates and convective mass transfer coefficients facilitating rejected solute back-mixing, etc.) can have a noticeable impact on ultrafiltration and such conditions are known to vary with membrane configuration [14]. It is particularly noticeable that the experimental basis for comparison of various membrane modules in terms of the system hydrodynamics (Reynolds number, membrane surface shear, etc.) and general operating conditions such as feed concentration, pH, etc. have received little attention. It is necessary to provide more experimental details of such parameters if an informative comparison between two different membrane configurations is to be made. Tansel et al. [13], for example, compared ion permeability data from nanofiltration experiments for both dead-end (using a stirred cell) and cross-flow modes but did not describe the basic conditions of their comparison. This makes it difficult to draw conclusions from their findings. More recently Zydney and Xenopoulos [15] pointed out that the effects of device configuration and operational parameters, particularly for dextran characterization experiments, are still poorly understood. They compared solute rejection data for a stirred cell and a cross-flow system and stated that the stirred cell provides more accurate dextran rejection data which they attributed to better flux uniformity and the reduced influence of concentration polarization due to higher convective mass transfer rates in the stirred cell system.

In the current work single protein ultrafiltration with BSA and lysozyme (LYZ) was studied using a Millipore stirred cell (XFUF4701) and a specially designed cross-flow device. Each protein was studied at four different pH values (4.9, 6.0, 7.1, 8.4) and two different ionic strengths (20 mM and 100 mM). Due to the high isoelectric point of LYZ (pH 11.0), lysozyme ultrafiltration at pH 11.0 was also conducted at both ionic strengths using the stirred cell. The ultrafiltration data are discussed in terms of the effects of solution pH and ionic strength as well as effects of convective mass transfer in the stirred cell and cross-flow devices. These were found to show marked differences in the observed ultrafiltration results under commonly controlled experimental conditions such as the transmembrane pressure.

### Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tbody>
<tr>
<td>a</td>
<td>channel width (m)</td>
</tr>
<tr>
<td>b</td>
<td>channel height (m)</td>
</tr>
<tr>
<td>C</td>
<td>protein concentration (g/L)</td>
</tr>
<tr>
<td>D</td>
<td>diameter (m) or diffusion coefficient (m²/s)</td>
</tr>
<tr>
<td>e</td>
<td>elementary charge, 1.602 × 10⁻¹⁹ (C)</td>
</tr>
<tr>
<td>h</td>
<td>blade height (cm)</td>
</tr>
<tr>
<td>I</td>
<td>ionic strength (mol/L)</td>
</tr>
<tr>
<td>J</td>
<td>flux rate (m/s)</td>
</tr>
<tr>
<td>k</td>
<td>Boltzmann constant, 1.38 × 10⁻²³ (J/K) or mass transfer coefficient (m/s)</td>
</tr>
<tr>
<td>L</td>
<td>membrane length (m)</td>
</tr>
<tr>
<td>n</td>
<td>constant</td>
</tr>
<tr>
<td>MW</td>
<td>molecular weight (g/mol or kg/mol)</td>
</tr>
<tr>
<td>N</td>
<td>impeller speed (rev/s) or Avogadro’s number (1/mol)</td>
</tr>
<tr>
<td>ΔP</td>
<td>transmembrane pressure (Pa)</td>
</tr>
<tr>
<td>R</td>
<td>rejection (—)</td>
</tr>
<tr>
<td>r</td>
<td>radius (m or Å)</td>
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<tr>
<td>Re</td>
<td>Reynolds number</td>
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<tr>
<td>Sc</td>
<td>Schmidt number</td>
</tr>
<tr>
<td>Sh</td>
<td>Sherwood number</td>
</tr>
<tr>
<td>W</td>
<td>cell width (cm)</td>
</tr>
<tr>
<td>T</td>
<td>temperature (K)</td>
</tr>
<tr>
<td>u</td>
<td>velocity (m/s)</td>
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### Greek letters

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<td>boundary layer thickness (m or mm)</td>
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<tr>
<td>ε₀</td>
<td>permittivity of free space (J/m/C²)</td>
</tr>
<tr>
<td>εᵣ</td>
<td>dielectric constant (—)</td>
</tr>
<tr>
<td>γ</td>
<td>fluid shear rate (1/s)</td>
</tr>
<tr>
<td>λ</td>
<td>Debye length (nm)</td>
</tr>
<tr>
<td>μ</td>
<td>viscosity (kg/m s, Poise or centiPoise)</td>
</tr>
<tr>
<td>ρ</td>
<td>density (kg/m³ or g/mL)</td>
</tr>
<tr>
<td>τ</td>
<td>shear stress (N/m²)</td>
</tr>
<tr>
<td>ω</td>
<td>angular velocity (rad/s)</td>
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</tbody>
</table>

### Indices

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
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<td>a</td>
<td>actual</td>
</tr>
<tr>
<td>A</td>
<td>Avogadro</td>
</tr>
<tr>
<td>b</td>
<td>blades</td>
</tr>
<tr>
<td>B</td>
<td>Boltzmann</td>
</tr>
<tr>
<td>c</td>
<td>critical or channel</td>
</tr>
<tr>
<td>cf</td>
<td>cross-flow</td>
</tr>
<tr>
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<td>Dcbye</td>
</tr>
<tr>
<td>f</td>
<td>feed</td>
</tr>
<tr>
<td>h</td>
<td>hydraulic</td>
</tr>
<tr>
<td>i</td>
<td>impeller</td>
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<td>I</td>
<td>Landau-Lifshitz</td>
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<tr>
<td>m</td>
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<td>M</td>
<td>membrane</td>
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<td>o</td>
<td>observed</td>
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<tr>
<td>p</td>
<td>permeate</td>
</tr>
<tr>
<td>s</td>
<td>solute</td>
</tr>
<tr>
<td>sc</td>
<td>stirred cell</td>
</tr>
</tbody>
</table>

### Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>t</td>
<td>tank</td>
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<tr>
<td>tru</td>
<td>true</td>
</tr>
</tbody>
</table>

### Notes

- The observed ultracentrifugation...
2. Experimental

2.1 Materials

Polyethersulfone (FES) membranes with MWCO of 50 kDa were supplied by Microdyn-Nadir GmbH, Germany. These membranes are described by the manufacturer as hydrophilic, with strong chemical stability and carry a minimal negative charge over the pH range studied (4.9–11.0). Lysozyme (MW 14.3 kDa, 11.0 pI) and Bovine Serum Albumin (MW 66.4 kDa, 4.7–4.9 pI) were purchased from Sigma–Aldrich (UK) Ltd. FES membranes with a 50 kDa cut-off were chosen deliberately since it was desirable for some of the BSA to potentially transmit through the membrane. In order to carry out MWCO studies, dextran standards with narrow polydispersity and molecular weights of 1, 5, 12, 25, 50, and 150 kDa were obtained from Polymer Standards Services GmbH, Germany.

2.2 Preparation

For all experiments deionised water with a resistivity of at least 18.2 MΩ cm was used. All membranes were pretreated prior to their first use according to a set procedure, a methodology which was developed through experience from previous experiments with a range of different FES membranes. This included storage of the membranes for 24 h in deionised water followed by 30 min storage in 0.1 M NaOH. Subsequently, the membranes were flushed with water at 50 kPa transmembrane pressure for at least 60 min in order to remove any residual agents from the manufacture. Permeation at this elevated pressure also helped to prevent any membrane compaction effects when conducting subsequent water flux and solute flux measurements at 25 kPa. Water flux measurements were made for each membrane sample over 1 h period. Single protein solutions for BSA and LYZ were prepared in phosphate buffer with 20 and 100 mM ionic strength, respectively. LYZ solution was also prepared at pH 11.0 for stirred cell experiments in a glycine buffer (pKₐ closer to the protein pI). All buffer solutions were pre-filtered to remove any potential aggregates and undissolved proteins from the solution using 0.45 μm nylon membranes (Whatman plc, UK). The concentrations of both BSA and LYZ were determined using a UV/vis spectrophotometer (Lambda 12, PerkinElmer) at 280 nm and evaluated against predetermined calibration curves.

2.3 Membrane filtration apparatus

Two membrane configurations were employed. The Millipore stirred cell (Model XUF04701) had a diameter of 47 mm and an effective membrane area of 15 cm². The cell was operated with a stirrer speed of 2400 rpm as determined using a phototachometer (Model TM-3011). The feed volume was fixed at 50 mL. This device was operated by pressurising the head space of the stirred cell using nitrogen from a gas cylinder as shown in Fig. 1.

The cross-flow apparatus incorporated a flat sheet membrane placed within the rectangular flow channel (see Fig. 2). The module was designed in a manner that allowed the fluid flow profile to develop inside the channel before the feed reached the membrane. The cross-flow apparatus had a feed volume of 1 L and the membrane area was 71 cm². The apparatus, constructed from stainless steel, comprised of a feed tank from which the feed was supplied to the membrane module via a diaphragm pump (Jabsco, Model 31800). The retentate was recycled continuously to the feed tank whilst permeate was either sampled and/or returned to the feed tank. The system was equipped with calibrated pressure transducers (upstream, at the entrance and downstream, at the exit of the membrane module) linked to a digital pressure display to monitor the pressure drop across the membrane module. The pressure gauges for the stirred cell and the cross-flow module were calibrated. All experiments were conducted at a temperature of 22°C (±2°C).

Each experiment was performed with a new, clean membrane sample, pretreated in the manner mentioned earlier. Protein filtration experiments were conducted for 30 min using the stirred

![Fig 1 Schematic diagram of the Stirred cell apparatus](image1)

![Fig 2 Schematic diagram of the cross-flow apparatus](image2)
cell and for at least 2 h using the cross-flow apparatus; samples were taken at regular intervals. Protein filtration with the stirred cell device was conducted over a shorter time period because of system limitations such as the restricted feed volume. All filtrate samples were corrected for hold-up volume and sampled accordingly. In case of the stirred cell, the feed sample was obtained immediately after taking the filtrate sample by opening the stirred cell. The feed sample of the cross-flow apparatus could be readily obtained from the retentate line.

2.4 Membrane characterisation

The molecular weight cut-off (MWCO), defined as the membrane cut-off at which 90% of the molecule with the same molecular weight as that specified by the membrane manufacturer is retained, was evaluated using dextran standards with narrow polydispersity. As mentioned previously, the standards ranged from 1 to 150 kDa relative molecular mass.

3. Theory

3.1 Determination of Reynolds number for the SC and the CF units

The Reynolds number in the cross-flow module (Re<sub>CF</sub>) was determined in the same manner as for fluid flow in a duct since the flow channel is rectangular, whereas in the case of the stirred cell, the flow regime is determined from the rotational Reynolds number (Re<sub>rot</sub>) as given by Eqs. (1) and (2), respectively

\[
Re_{\text{CF}} = \frac{\rho u D_h}{\mu} \quad (1)
\]

\[
Re_{\text{rot}} = \frac{\rho \omega r^2}{\mu} \quad (2)
\]

where \( \rho \) is the fluid density, \( u \) is the cross-flow velocity (maintained at 0.2 m/s), \( D_h \) the hydraulic diameter, \( \mu \) the dynamic viscosity, \( \omega \) the angular velocity and \( r \) is the radius of the stirred cell.

3.2 Determination of shear stress

The difficulty in making a direct comparison between a stirred cell and a cross-flow module arises from the need to match hydrodynamic conditions in each system. Ideally, not only the shear at the membrane surface ought to be the same but all other parameters (e.g., the solvent velocity normal to the membrane surface) also need to be equal. In this work, the transmembrane pressure, temperature, feed concentration, pH and ionic strength were all maintained at fixed levels during the experiments with the cross-flow and stirred cell devices.

It has previously been shown impracticable to compare the rotational Reynolds number in the stirred cell (laminar flow up to \( Re < 10 \)) with the Reynolds number for the cross-flow module (laminar flow up to \( Re \approx 2100 \)) [16]. In other words, a comparison of the two apparatus on the basis of liquid mixing and flow profile is inadequate. Instead, an attempt was made to select operating conditions in a manner that allowed similar shear stress conditions to be maintained at the membrane surface. A force balance across the cross-flow membrane module depicted in Fig. 3 gives

\[
ab \Delta P = 2\pi L (a + b)
\]

where \( a \) is the channel width, \( b \) the channel height, \( L \) the membrane length, \( \Delta P \) the pressure drop across the membrane length and \( \tau \) the shear stress. Solving for the shear stress \( \tau \) and including the hydraulic diameter of the membrane channel yields

\[
\tau = \frac{\Delta PD_h}{4L} \quad (4)
\]

The pressure drop across the membrane length (based on a constant \( \Delta P \) across the entire membrane module) is calculated as 110 Pa and including the module dimensions a wall shear stress of 34 Pa was determined for the cross-flow apparatus (using Eq (4)).

Determination of the shear stress in the stirred cell device is more difficult. To enable calculations the stirrer in the Millipore cell was assumed to resemble a flat blade paddle impeller and the flow-field was subdivided into an inner region (where the shear stress increases up to the critical radius of the impeller) and an outer region beyond which the shear stress decreases again (see Fig 4), such a procedure has previously been used to compare oil droplet formation in a Weissenberg rheometer and a stirred

---

**Fig. 3** Force balance across the membrane in the cross-flow module \((a=30 \text{ mm}, \quad b=2 \text{ mm}, \quad L=30 \text{ mm})\)

**Fig. 4** Shear stress variation along the impeller length.
The correlations (given below) are for the flow-field approximated as a rigid-body rotation above a stationary surface (Bodewadt flow). The maximum shear stress is experienced at the critical radius \( r_c \) of the impeller which is given by:

\[
r_c = \frac{D_t}{2} \left( 0.57 + 0.35 \left( \frac{D_t}{D_i} \right) \right) \times \left( \frac{h}{D_t} \right)^{0.036} \frac{R_e}{n_b^{0.116}} \frac{R_e}{1000 + 1.43 R_e}
\]

(5)

where \( D_t \) is the stirred cell diameter, \( h \) is the blade height, \( n_b \) corresponds to the number of stirrer blades and \( R_e \), the impeller Reynolds number as defined by Eq (2) except that the length-scale used in the equation is the impeller diameter rather than the tank diameter. For the Millipore stirred cell \( D_t = 3.8 \, \text{cm} \), \( D_i = 4.2 \, \text{cm} \), \( h = 0.9 \, \text{cm} \), and \( n_b = 2 \). The shear stress at the critical radius was calculated to be 48 Pa using Eq (5) whereas the shear stress below and above the critical radius was calculated using Eqs. (6) and (7), as defined by Kosvintsev et al. [17], such that

\[
\tau = 0.825 \mu \omega \frac{1}{\delta} \quad r < r_c
\]

(6)

\[
\tau = 0.825 \mu \omega r \left( \frac{r_c}{r} \right) ^{0.6} \frac{1}{\delta} \quad r > r_c
\]

(7)

and the thickness of the momentum boundary layer (\( \delta \)) is obtained from the relationship between the momentum boundary layer (\( \delta \)) and the concentration boundary layer (\( \delta_c \)) giving as [18] :

\[
\delta = C^{0.33} \delta_c
\]

(8a)

The concentration boundary layer (\( \delta_c \)) here was evaluated using the Landau-Lifshitz equation [19].

\[
\delta_c = \frac{D_t}{S e^{1/3} \sqrt{R_e}}
\]

(8b)

Combining (8a) and (8b) gives the momentum boundary layer thickness (\( \delta \)) used in Eqs (6) and (7):

\[
\delta = \sqrt{\frac{\mu}{\rho \omega}}
\]

(8c)

The critical radius for the impeller in the Millipore cell was determined as 1.47 cm for the forced impeller speed of 2400 rpm which corresponds to the maximum speed that can be achieved with the stirrer ensuring that the depth of the vortex created by the impeller does not become too large. Moreover, an increase in the stirrer speed beyond this level does not further impact on the position of the critical radius. An average shear stress across the impeller was obtained by integration of Eqs (6) and (7). A stirrer speed of 2700 rpm results in an average shear stress of 34 Pa, i.e., equal to that in the cross-flow module, but limited by no further increase in the critical radius. However, with the experimental conditions used it was only possible to achieve a maximum stirrer speed of 2400 rpm, i.e., \( \tau = 29 \, \text{Pa} \). This lower rpm corresponds to an error in the average shear stress of circa 15%, but it represents the closest match that could be obtained whilst still maintaining the values of other process variables.

### 3.3 Determination of mass transfer coefficients

During ultrafiltration, the retained solute concentration close to the membrane wall increases with respect to the bulk concentration. Thus, facilitation of back diffusion of solute is important in determining the solute rejection and the limiting permeate flux for a given process. Under ideal comparison conditions, in addition to the other parameters, the convective mass transfer coefficients should also be identical in the two filtration systems in question. The mass transfer coefficient is the ratio of the diffusion coefficient of the solute to the thickness of the concentration boundary layer (\( \delta_c \)). The diffusion coefficients, \( D_s \) for BSA and LYZ can be determined from the Stokes–Einstein equation, this also allows one to also take account of the influence of ionic strength (e.g., by evaluating the Debye layer thickness as a function of ionic strength, discussed below). The effective size of BSA was found by Puja and Zydney [20] to vary linearly with the Debye length (i.e., inversely with the square root of the solution ionic strength). The magnitude of the Debye length \( \lambda_D \), a measure of the extent of the charge layer around a molecule in an electrolyte solution, can be calculated using Eq. (9):

\[
\lambda_D = \sqrt{\frac{e_\text{eff} e_k T}{2 N_A k_B T}}
\]

(9)

where \( e_\text{eff} \) is the permittivity of free space, \( e_k \) the dielectric constant, \( k_B \) the Boltzmann constant, \( N_A \) Avogadro's number, \( e \) the elementary charge and \( T \) the ionic strength of the electrolyte. From Eq. (9) the Debye length \( \lambda_D \) for the two ionic strengths employed in this work was 2.15 nm (20 mM) and 0.96 nm (100 mM), respectively. The influence of pH on the number of charges at the protein surface has not been considered here. To incorporate the effect of the Debye length, it is added to the protein radius and an effective hydrodynamic radius of the protein is obtained, which includes the thickness of the charged double layer around the protein. Therefore, using the Stokes–Einstein equation [21], different diffusion coefficients may be calculated based on the ionic strength difference.
was obtained from the following Sherwood, Sh, correlation [22]
\[
\frac{k_m n}{D_e} = Sh = 0.27 Re_t^{0.567} Sc^{0.33}
\]
(11)
where \(n\) is the radius of the stirred cell. The mass transfer coefficient in the cross-flow system can be obtained from the Gniets-Leveque relationship [23] which is applicable to laminar flows in channels.
\[
k_m = 0.816 \left( \frac{y}{L_e} \right)^{1/3}
\]
(12a)

An alternative equation Eq. (12b) [23] that is similar in form to that for the SC Eq. (11) also gave similar orders of magnitude mass transfer coefficients for the CF system.
\[
\left( \frac{k_m D_h}{D_e} \right) = Sh = 0.04 Re^{0.75} Sc^{0.33}
\]
(12b)

In Eq. (12a), \(y\) is the fluid shear rate at the membrane surface and \(L_e\) the channel length. Thus, the mass transfer coefficient and hence the limiting solvent flux would be expected to vary as the cube root of the wall shear rate as shown by Blatt [23]. The fluid shear rate for a rectangular channel can be obtained from
\[
y = \frac{6u}{b}
\]
(13)

where \(b\) is the channel height. The calculated wall shear rate \(y_w\) for a cross-flow velocity of 0.42 m/s corresponds to 1260 s\(^{-1}\). Using Newton’s law of viscosity, wall shear stress is only 1.3 Pa. The difference between the calculated and the measured significant pressure drop due to entry and exit effects resulting in higher measured values of the pressure drop and hence wall shear stress values.

The membrane Peclet number \((Pe_m = k_m D_h / D_e)\) provides a comparison of the relative importance of diffusion and convection. In the present study evaluation of the \(Pe_m\) for the SC and CF has been undertaken.

4. Results and discussion

4.1. Membrane characterisation

It is generally accepted that the structure and crosslinking of asymmetric, polymeric membranes can vary from lot-to-lot. Moreover, it is also not uncommon to find slight variations in the membrane pore structure for membranes cut from the same sheet. For this work 32 samples of PES membranes were characterised in terms of their water flux at a constant operating transmembrane pressure of 25 kPa in the stirred cell and cross-flow apparatus (16 samples were studied in each setup). Two observations were made: (1) the water flux was higher in the cross-flow apparatus compared to the stirred cell and (2) the water flux variation around the mean was slightly higher in the stirred cell system compared to the cross-flow system. As can be seen in Fig. 5 the average flux of pure water for 16 different membrane samples was approximately 100 L/m\(^2\) h for the cross-flow system compared with 80 L/m\(^2\) h for the stirred cell device. The higher flux in the cross-flow system is due to the lower hydraulic resistance of the membrane supporting base plate design (base plate permeability without membrane, \(L_{sc,CF} = 1.5 \times 10^{-11} \text{ m} \) compared with the base plate permeability for the SC system \(L_{sc,SC} = 1.9 \times 10^{-12} \text{ m} \) ). The overall solvent permeability for the 50 kDa membrane was for the CF \((L_{p,CF} = 1.1 \times 10^{-12} \text{ m} \) ) and for the SC \((L_{p,SC} = 8.9 \times 10^{-13} \text{ m} \) ) respectively. Fluctuations in the solvent (water) flux were slightly higher for the stirred cell (up to ±7%) compared with data for the cross-flow apparatus (up to ±5%).

Ultrafiltration membranes are typically characterised by the nominal molecular weight cut-off (MWCO) defined as the molecular weight of a solute (usually low polydispersity dextrans or polyethylene glycols) that has a rejection coefficient of 90% Dextran solute rejection measurements (using a range of dextran molecular weights) were used to characterise the solute rejection behaviour of 50 kDa PES membranes. Data were obtained for both the stirred cell and the cross-flow apparatus at a constant transmembrane pressure of 25 kPa. It is immediately obvious from the data in Fig 6, that dextran rejection with the cross-flow system was generally lower than for the stirred cell device. This result may be explained in terms of concentration polarsation effects (see calculated values of mass transfer coefficients for the dextrans in Table 1). Accumulation of the completely or partially rejected dextran solutes close to the membrane surface results in a higher wall concentration \((C_w\) of the solute for the CF system. For the cross-flow system, tangential flow of the fluid results in a continuous regen-

![Fig 5 Flux variation for the 50kDa polyethersulfone membranes (TMP 25kPa)](image)

![Fig 6 Dextran rejection comparison between the stirred cell and the cross-flow module (for a 50kDa membrane, TMP 25kPa)](image)
eration of any concentration polarisation at the device inlet. The observed lower rejection of dextrans using the cross-flow apparatus suggests that concentration polarisation effects were higher in comparison to the stirred cell. Calculation of the true rejection coefficients (based on the calculated solute wall concentration) using the stagnant film model for the protein filtration results supports this hypothesis. Permeate flux data for dextran standard solutions were found to be higher for the cross-flow module compared to the stirred cell system, permeate flux was approximately 20 L/m² h higher for the cross-flow apparatus compared to the stirred cell (similar to water flux data reported in Fig. 5).

It is interesting to note that the manufacturer quoted MWCO for the PES membranes (50 kDa) correlates well with dextran solute rejection data obtained using the stirred cell device (see Fig. 6). However, the MWCO value based on dextran rejection measurements with the cross-flow apparatus would put the value close to 75 kDa. This is of some importance in selecting membranes to separate proteins or to retain them (e.g. to swap buffers in biotechnological applications) For dextrans and proteins, an equivalent hard sphere radius, \( r \) (estimated using the Stoke's–Einstein equation) results in simple correlations for the dextran (\( \bar{A} \)) and protein radius (nm) respectively as a function of the protein molecular weight (MW):

For dextrans, the following correlation by Granath and Kviast [24] allows calculation of the dextran radius

\[
r = 0.33(MW)^{0.46}
\]

(14)

where the radius (\( r, \bar{A} \)) and the molecular weight (MW, g/mol). For proteins, the correlation by Squire [25] may be used:

\[
r = 0.794(MW)^{1/3}
\]

(15)

where the radius (\( r, \) nm) and the molecular weight (MW, kg/mol).

Table 1 provides Stoke's radii for the calibration dextrans, BSA and LYZ used in the present study. Comparison of the Stoke's radii for a 50 kDa dextran (\( r = 4.8 \) nm, using the correlation above) would suggest that a 50 kDa MWCO PES membrane would not be well suited to reject a protein such as BSA (MW 66 4 kDa and \( r = 3.2 \) nm) The MWCO value of 75 kDa obtained from the dextran solute rejection measurements using the cross-flow system suggests 90% rejection of solutes with an equivalent Stoke's radius of 5.8 nm (determined using Eq (14)). Under the operating conditions used to take the solute rejection data, the rejection of any concentration polarisation at the device inlet. The observed lower rejection of dextrans using the cross-flow apparatus suggests that concentration polarisation effects were higher in comparison to the stirred cell. Calculation of the true rejection coefficients (based on the calculated solute wall concentration) using the stagnant film model for the protein filtration results supports this hypothesis. Permeate flux data for dextran standard solutions were found to be higher for the cross-flow module compared to the stirred cell system, permeate flux was approximately 20 L/m² h higher for the cross-flow apparatus compared to the stirred cell (similar to water flux data reported in Fig. 5).

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<table>
<thead>
<tr>
<th>Solute</th>
<th>Dxt1</th>
<th>Dxt5</th>
<th>Dxt12</th>
<th>Dxt25</th>
<th>Dxt50</th>
<th>Dxt150</th>
<th>LYZ</th>
<th>BSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW (kDa)</td>
<td>1</td>
<td>5</td>
<td>12</td>
<td>25</td>
<td>50</td>
<td>150</td>
<td>143</td>
<td>664</td>
</tr>
<tr>
<td>Stokes radius (nm)</td>
<td>0.8</td>
<td>1.7</td>
<td>2.5</td>
<td>3.5</td>
<td>4.8</td>
<td>7.9</td>
<td>1.9</td>
<td>3.2</td>
</tr>
<tr>
<td>( D \times 10^{11} ) (m²/s)</td>
<td>27.3</td>
<td>12.8</td>
<td>8.8</td>
<td>6</td>
<td>4</td>
<td>2.2</td>
<td>5.3 (20 mM)</td>
<td>4 (20 mM)</td>
</tr>
<tr>
<td>SC, ( k_m \times 10^6 )</td>
<td>39</td>
<td>23</td>
<td>18</td>
<td>14</td>
<td>11</td>
<td>7</td>
<td>13 (20 mM)</td>
<td>11 (20 mM)</td>
</tr>
<tr>
<td>( 1/m^2 )</td>
<td>3.6</td>
<td>2.8</td>
<td>2.1</td>
<td>1.6</td>
<td>1.1</td>
<td>4 (20 mM)</td>
<td>5.1 (100 mM)</td>
<td>4 (100 mM)</td>
</tr>
</tbody>
</table>

4.2 Protein filtration results

All filtration experiments were carried out with a protein concentration of 0.5 g/L. In the cross-flow module the cross-flow velocity was 0.42 m/s and the flow profile was laminar (corresponding Reynolds number of 1560, determined by Eq (1)). The rotational Reynolds number in the stirred cell (determined by Eq (2)) at a stirrer speed of 2400 rpm was 57,000 where the flow is turbulent. Whilst liquid mixing is different under these conditions, the shear stress experienced at the membrane surface was calculated to be similar to that experienced in the cross-flow system, i.e. in the range of 29–34 Pa. In Figs 7–10 both flux and protein observed rejection data versus pH are shown for two different ionic strengths (20 and 100 mM). The maximum filtration
Flux and rejection vs pH for BSA with the cross-flow module. Feed concentration 0.5 g/L and pH of BSA = ~4.9 (for a 50 kDa membrane, TMP 25 kPa)

The permeate flux for the 50 kDa PES membrane to be greater than 85% and almost invariant to the measurements obtained at the low (20 mM) and the high (100 mM) ionic strength values. BSA rejection for both the cross-flow and the stirred cell systems were found to be pH invariant. However, the stirred cell system displayed greater transmission of BSA compared with the cross-flow system. Additionally, BSA rejection was found to be higher at the high ionic strength (100 mM) compared with BSA rejection data obtained at 20 mM. These effects were not seen for the cross-flow system. Additionally, the permeate flux for the SC was slightly higher than for the CF system. During water permeability measurements, the CF system had a higher solvent permeability (almost 25% higher).

The observed rejection of LYZ was found to be higher for the lower ionic strength data (mainly for the data obtained using the stirred cell system). LYZ rejection was found to be mainly pH invariant except at pH 5 when a marked increase in LYZ rejection was noted. The effect of pH was more pronounced at need lower ionic strength (20 mM) but still apparent for data recorded at the higher ionic strength (100 mM). This was found to be the case for data obtained using both the stirred cell and the cross-flow systems; however, the effects were more noticeable during LYZ filtration using the SC.

The permeate flux was found to be higher for the CF (~25%) compared with SC system, mimicking water permeability results reported earlier.

In order to better explain the filtration results, the data were also analyzed from a quantitative viewpoint using the film model for concentration polarization [23]. The model is based on a mass balance near the membrane wall and its integration over the concentration boundary layer (δc) and yields the well-known equation:

\[ J_s = \frac{D_s}{\delta_c} \ln \left( \frac{C_w - C_p}{C_b - C_p} \right) \]

where \( J_s \) is the solute flux, \( C_w \) the concentration at the membrane surface, \( C_b \) the bulk concentration and \( C_p \) the concentration in the permeate. This equation is often rewritten in terms of the observed \( (S_o = C_p/C_b) \) and the true or actual sieving coefficient \( (S_a = C_p/C_w) \) [26]

\[ S_a = \frac{S_o}{(1 - S_o) \exp(J_s/k) + S_o} \]

In this work, the equation was expressed in terms of rejection coefficients, i.e., the actual or true rejection coefficient \( (R_t = 1 - C_p/C_b) \) was evaluated from the data for the observed rejection coefficient \( (R_{obs} = 1 - C_p/C_b) \) using Eq (18):

\[ R_t = \frac{R_{obs}}{(1 - R_{obs} / \exp(J_s/k_m)) + R_{obs}} \]
Table 2
Summary of protein ultrafiltration results comparing data for stirred cell and cross-flow mode of operation

<table>
<thead>
<tr>
<th></th>
<th>BSA</th>
<th>LYZ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SC(^a)</td>
<td>CF(^b)</td>
</tr>
<tr>
<td>Flux (L/hr/(m^2))</td>
<td>60-90</td>
<td>55-85</td>
</tr>
<tr>
<td>Influence of pH on flux(^c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(20) mM</td>
<td>30% increase in flux as the pH increased from 5 to 8.5</td>
<td>15% increase in flux as the pH increased from 5 to 8.5</td>
</tr>
<tr>
<td>(100) mM</td>
<td>~20% increase in flux as the pH increased from 5 to 8.5</td>
<td>~20% increase in flux as the pH increased from 5 to 8.5</td>
</tr>
<tr>
<td>Influence of Ionic strength (mM) on flux(^c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(20) &gt; (100)</td>
<td>(20) &gt; (100)</td>
<td>(100) &gt; (20)</td>
</tr>
<tr>
<td>Influence of pH on protein rejection(^c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(20) mM</td>
<td>Invariant, ~10% change (increase) over the pH range 5-8.5</td>
<td>Invariant, ~10% change (increase) over the pH range 5-8.5</td>
</tr>
<tr>
<td>(100) mM</td>
<td>Invariant, ~5% change (decrease) over the pH range 5-8.5</td>
<td>Invariant, ~5% change (increase) over the pH range 5-8.5</td>
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<tr>
<td>Influence of Ionic strength (mM) on protein rejection(^c)</td>
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<tr>
<td>(100) &gt; (20)</td>
<td>Almost invariant</td>
<td>(20) &gt; (20)</td>
</tr>
<tr>
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<td>(20) &gt; 85%</td>
<td>(20) 30-60%</td>
</tr>
<tr>
<td>(100) &gt; 85%</td>
<td>(100) &gt; 95%</td>
<td>(100) 20-30%</td>
</tr>
</tbody>
</table>

\(^a\) LB Lower bound  
\(^b\) UB Upper bound  
\(^c\) Stirred cell  
\(^d\) Cross-flow cell

rejection at 20 mM and 85-90% rejection for measurements taken at 100 mM. The MWCO of the 50 kDa PES membrane (refer to Fig. 6, data for the SC) based on the dextran measurements obtained with the stirred cell suggests the cut-off for the membrane is ~50 kDa (Stoke's radius 4.8 nm). Thus, the BSA rejection is higher than expected based on the dextran solute rejection data, provided the comparison is carried out using the Stoke's radius size comparison. Based on the relative molecular mass of BSA (~66 kDa) and the calculated Stoke's radius of 3.2 nm alone, use of a membrane with a MWCO of 50 kDa should result in BSA rejection of around 40% (for the stirred cell). The rejection is higher than this and suggests that due to a high surface charge density on the BSA molecule (~3 charges at pH 7) and low ionic strength (20 mM) electrostatic effects result in a larger effective protein size resulting in a higher observed solute rejection. The larger size results in steric exclusion from pores of similar and smaller size thereby reducing overall transmission. At higher ionic strength, charge shielding may result in a reduction in the overall hydrodynamic size however, this effect is more complicated due to the following: (1) smaller solute size may allow greater access to the membrane pores and may result in higher transmission, however (ii) smaller size also results in faster back diffusion into the bulk of the retentate fluid, thereby reducing the solute concentration at the wall and hence lower overall solute transport in the permeate. The calculated \( P_{\text{em}} \) for the SC system was ~2 (20 mM) and ~1.5 (100 mM). Higher \( P_{\text{em}} \) lead to higher solute wall concentrations and lower solute rejections (observed here).

The calculation of the Debye length for the two different ionic strengths results in the 'modified' Stoke's radius (i.e. including the Debye length) value of ~4.2 nm (100 mM) or ~5.4 nm (20 mM). The calculated true rejection coefficients using Eq. (18) are presented in Table 3. The higher (almost complete rejection) values of the true rejection coefficients are a consequence of the higher calculated solute concentration at the membrane wall due to the phenomenon of concentration polarisation.

Fig. 8 shows flux and rejection data for BSA filtration at both 20 and 100 mM ionic strengths with the cross-flow cell. The data in Fig. 8 show BSA rejection ranging from 85 to 98% (rejection was found to be pH and ionic strength invariant). The MWCO of the 50 kDa PES membrane (refer to Fig. 6, CF data) based on the dextran measurements obtained with the cross-flow cell suggests the cut-off for the membrane is ~75 kDa (Stoke's radius 5.8 nm). Thus, the BSA rejection is again considerably higher than expected based on the dextran solute rejection data provided the comparison is carried out on the basis of steric hindrance using the Stoke's radius for comparison. The expected BSA rejection should be around 40% for the cross-flow cell. The permeate flux measurements were found to be similar to those obtained for the stirred cell suggesting accumulation of
solute at the membrane surface resulting in increased hydraulic resistance to permeate flow. The permeate flux was found to be moderately higher (70-80 L/m² h) at the lower ionic strength compared with (55-70 L/m² h) for data measured at 100 mM. The calculated true rejection coefficient values are shown in Table 3 and suggest near complete rejection of BSA. The calculated $P_{em}$ for the CF system was ~7 (20 mM) and ~5 (100 mM). The high values of $P_{em}$ suggest the possibility of a secondary membrane forming at the surface. The reduction in solvent flux (compared with the water permeability values) is evidence in support of this hypothesis. The higher wall concentration may result in a higher local viscosity of the protein solution excluding solvent flow through smaller pores. This may be the reason behind the higher observed BSA rejection values for the CF system.

4.4 Protein filtration of lysozyme

Lysozyme has a MW of 14.3 kDa and a Stoke’s radius of 1.9 nm. On the basis of steric hindrance alone, LYZ rejection behaviour would be similar to a dextran of size ~7 kDa (~15% rejection). LYZ is also positively charged over the pH range studied (except at its isoelectric point ~pH 11) and the membrane carries a moderate negative surface charge at the pH values examined.

Fig. 9 shows flux and rejection data obtained with the stirred cell device for LYZ filtration at both 20 and 100 mM ionic strengths. The data show LYZ rejection ranging from 50% (pH 5) to ~30% (pH 6-11) for LYZ in 20 mM ionic strength solution. At 100 mM ionic strength, LYZ rejection was comparatively lower, ranging from ~30% (pH 5) to ~25% (pH 6-11). Thus rejection was pH invariant over the pH range 6-11 but increased at pH 5 (at both ionic strengths). The rejection values are higher than would be expected based on the Stoke’s radius of LYZ and suggests a larger effective radius with a dependency on the solution ionic strength (Debye length). The increased rejection at pH 5 is difficult to explain. The authors did not find in literature detailed charge distribution on LYZ as a function of pH. However, the membrane carries a moderately negative charge at pH 5 (from streaming potential measurements) and the LYZ molecule is expected to carry a large net positive charge as the pH is shifted away from the protein’s $pI$. Hence, some LYZ adsorption onto the membrane surface may be expected which could possibly lead to “self-rejection” of positively charged LYZ in the solution bulk by positively charged LYZ adsorbed at the membrane surface. Ghosh and Cu [8] observed greater self-rejection of LYZ at a pH far away from the $pI$. Muller et al [27] also reported that LYZ forms a positively charged layer at the membrane surface below its $pI$. The permeate flux was found to be lower for the LYZ experiments compared with for the BSA experiments. The permeate flux was found to be higher at the higher ionic strength, between 60 and 75 L/m² h for the 100 mM solution and between 55 and 65 L/m² h for the 20 mM solution. The evaluated true rejection coefficient values ($P_{em}$) are presented in Table 3. Between pH 6 and 11, $R_r$ is around 0.5 (lyng between the observed rejection values of dxt12 and dxt25 in Fig. 6) at pH5. The $R_r$ is around 0.6.

The values of the evaluated mass transfer coefficients for LYZ filtration with the stirred cell are shown in Table 1. Charge shielding at higher solution ionic strength (100 mM) results in higher convective mass transfer coefficients. However, the smaller solute size may result in greater accessibility of smaller membrane pores (not accessible to the solute at 20 mM) and this results in greater protein transmission of LYZ at the higher ionic strength. The $P_{em}$ for both sets of experiments is around 1.2 so the influence of ionic strength on size exclusion is better represented due to comparison on a like for like basis.

Fig. 10 shows flux and rejection data obtained with the cross-flow device for LYZ filtration at both 20 and 100 mM ionic strengths. The data in Fig. 10 show LYZ rejection ranging from 22% (pH 5) to ~10% (pH 6-11) for data recorded for LYZ in the 20 mM ionic strength solution. At 100 mM ionic strength, LYZ rejection was slightly lower, ranging from ~15% (pH 5) to ~6% (pH 6-11). Thus observed rejections appear to be pH invariant over the pH range 6-11 but increased moderately at pH5 (thus effect is seen at both ionic strengths). Permeate flux was found to be higher at the high ionic strength, between 80 and 100 L/m² h for the 100 mM solution and between 75 and 80 L/m² h for the 20 mM solution. The $P_{em}$ is ~5.5 (20 mM) and ~5 (100 mM) and this results in higher solute concentration at the wall thereby increasing the transmission of LYZ.

Comparing the LYZ ultrafiltration data for the stirred cell device (Fig. 9) with the cross-flow device (Fig. 10) show effects attributed to the type of system used for the study. LYZ rejection was observed to be notably higher for the stirred cell (low values of $P_{em}$) compared with the cross-flow device (high val-

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**Table 3**

<table>
<thead>
<tr>
<th>Filtration apparatus</th>
<th>pH</th>
<th>True rejection, $R_r$ (-) BSA 20 mM</th>
<th>True rejection, $R_r$ (-) BSA 100 mM</th>
<th>True rejection, $R_r$ (-) LYZ 20 mM</th>
<th>True rejection, $R_r$ (-) LYZ 100 mM</th>
</tr>
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<tbody>
<tr>
<td>SC</td>
<td>11.0</td>
<td>N/A</td>
<td>0.09</td>
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<td>0.51</td>
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<td>SC</td>
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<td>0.97</td>
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<td>0.50</td>
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<tr>
<td>SC</td>
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<td>0.95</td>
<td>0.98</td>
<td>0.68</td>
<td>0.54</td>
</tr>
<tr>
<td>SC</td>
<td>6.0</td>
<td>0.95</td>
<td>0.97</td>
<td>0.68</td>
<td>0.52</td>
</tr>
<tr>
<td>SC</td>
<td>4.9</td>
<td>0.94</td>
<td>0.98</td>
<td>0.77</td>
<td>0.61</td>
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<tr>
<td>CF</td>
<td>8.4</td>
<td>1.00</td>
<td>1.00</td>
<td>0.96</td>
<td>0.93</td>
</tr>
<tr>
<td>CF</td>
<td>7.0</td>
<td>1.00</td>
<td>1.00</td>
<td>0.97</td>
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<tr>
<td>CF</td>
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<td>1.00</td>
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</tr>
<tr>
<td>CF</td>
<td>4.9</td>
<td>1.00</td>
<td>1.00</td>
<td>0.98</td>
<td>0.93</td>
</tr>
</tbody>
</table>
ues of $P_{e,m}$). The rejection data for the cross-flow system were dominated by concentration polarization effects. For the stirred cell system effects of ionic strength on protein rejection become apparent. The observed rejections in the stirred cell were always higher due to the higher values of the convective mass transfer coefficients compared to the laminar flow device. It is known that concentration polarization can lead to a higher protein transmission due to increased wall concentration of the partially retained solutes close to the membrane surface. This may also adversely affect the solvent flux over time due to protein fouling.

As in the case of BSA filtration pH did not seem to have a significant effect on LYZ rejection at the higher ionic strength. Only at pH 4.9 was a slight increase in rejection observed. At the higher ionic strength, charge-shielding of the proteins results in reduction in the protein effective size [27]. In addition, less self-rejection of LYZ is anticipated in a solution environment where the protein charge is shielded. It is therefore not surprising that LYZ permeate flux behaves differently to the flux observed for BSA. Thus permeate flux is higher at the higher ionic strength.

5. Conclusions

Differences in the operating dynamics of a stirred cell and a cross-flow cell manifest in differences in observed solute rejection behaviour. Characterisation of a 50 kDa PES membrane using conventional dextran solute rejection data demonstrated a higher observed membrane cut-off of 75 kDa equivalent dextran size, when the membrane was characterised using the cross-flow system compared with 50 kDa when the membrane was characterised using the stirred cell device. This is found to be due to differences in the $P_{e,m}$ for the two systems. For the laminar flow system (CF), poor back-mixing of partially or completely rejected solute at the surface of the membrane results in a higher local solute concentration at the membrane wall resulting in lower observed solute rejection.

The dextran rejection data does not provide a good indication of the observed protein rejection behaviour due to the protein hydrodynamic size being a function of the solution properties (especially ionic strength). Incorporating the effect of solution properties on the protein hydrodynamic size and accounting for the hydrodynamic conditions (magnitude of $P_{e,m}$) within the filtration system helps in understanding the effect of operating conditions on the observed solute rejection values.

Under the conditions studied, concentration polarisation was more pronounced in the cross-flow device compared to the stirred cell which is significant if scale-up from a stirred cell to an industrial tangential flow device is desired. In this study, the magnitude of the wall shear stress was supposed to be similar for the two devices. In reality, this was found not to be the case with the wall shear stress for the SC ~30 Pa compared with CF ~1 Pa. This resulted in significantly different mixing conditions within the two systems. Future work will look at the comparison of the two systems by keeping the membrane Peclet number constant.

Characterisation of the ultrafiltration membrane on the basis of dextran rejection behaviour and use for protein separations requires an appreciation of the influence of the solution environment such as ionic strength and pH that influence the rejection/transmission of proteins by changing the effective size of the solute. The influence of hydrodynamic conditions on concentration polarization can be more easily quantified using the stirred-cell system. Thus, the use of dead-end stirred cells provides the means to evaluate the sensitivity of the process to changes in operating conditions. This will be evaluated in greater detail in future studies.

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