Membrane Fouling during Microfiltration of Protein Solutions

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Abstract

Membrane fouling during cross-flow microfiltration (CFMF) of proteins is complex depending upon feed properties, operating conditions and membrane properties. Four different protein solutions (reconstituted whey protein, BSA, lactoferrin and ferritin) with a range of physicochemical properties were investigated at a variety of permeate fluxes under different solution conditions to elucidate fouling mechanisms during constant flux CFMF.

MF fouling usually occurs in three stages: i) adsorption ii) pore fouling (pore plugging or deposition near the pore entrance) and iii) formation of a surface layer. The importance of step (ii) depends upon whether a protein is completely or partially permeable through the membrane.

BSA probably fouled internally first by pore plugging followed by formation of a surface layer once all the pores were plugged. Prefiltration and the presence of SDS reduced fouling but did not prevent it, suggesting that aggregates present in the initial feed as well as those formed during MF contribute to pore plugging and so lead to severe fouling.

Fouling resistance curves for lactoferrin indicate an initial phase of slow fouling by plugging or deposition of aggregates. Mathematical modelling suggested that fouling was particularly severe at the pore entrance. As flux was increased, lactoferrin formed a concentration-induced surface layer.

Ferritin formed a concentration-induced gel layer even at relatively low fluxes (≥91 $L/m^2.h$) when the wall concentration of protein reached the "gel concentration". The gel layer was highly reversible to changes in hydrodynamic conditions such as cross-flow velocity and transmembrane pressure. Fouling was more severe with reconstituted whey than with fresh whey due to the presence of protein aggregates in the reconstituted whey.

The role of the physicochemical properties of proteins in aggregation and probable fouling mechanisms during CFMF are discussed. Protein-protein interactions under the influence of shear particularly at higher fluxes lead to aggregation and subsequent fouling.

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1. Introduction

Membrane separation processes have found a wide range of applications in the food and biotechnological industries. Ultrafiltration (UF) is extensively employed to concentrate milk, whey proteins, clarify juices, wines and process antibiotics. Recent progress in cross-flow microfiltration (CFMF) has opened up new possible applications such as separation of individual proteins, casein standardisation of cheese milk, defatting and clarification of whey in the production of high quality whey protein concentrate (WPC), cell recovery from fermentation broths and sterile filtration of heat labile protein solutions etc.

Membrane fouling is a major problem retarding further application of CFMF. Membrane fouling has been extensively studied. Most of these studies are pertinent to fouling of polymeric UF and microfiltration (MF) membranes in stirred/unstirred batch cells using constant pressure operation. In UF, fouling occurs predominantly on the membrane surface where the dynamic membrane controls membrane behaviour. In MF, severe pore plugging by protein occurs, in spite of the pores being an order of magnitude larger than the protein. In both the above processes, fouling results in a decline in flux with time and change in selectivity of the membrane. The increase in retention is an advantage in UF applications but is a disadvantage in some MF applications that require high protein transmission. Cross-flow devices are generally preferred due to reduction in concentration polarisation and surface fouling compared to dead-end devices.

There is growing interest in the use of ceramic membranes for MF. There are three major advantages of using ceramic membranes: 1) they are resistant to chemical cleaning; 2) they can be steam sterilized and backflushed; and 3) they have long membrane life. With the advent of ceramic membranes and their application in the industry, there is a need to study microfiltration fouling on ceramic membranes. As membrane material influences the fouling process, it is not reasonable to apply the results obtained for polymeric membranes to ceramic membranes. It is suggested in the literature that MF operation at constant flux is better than operation at constant pressure because the former avoids high permeate fluxes in the first few minutes.

Very few studies have been conducted in cross-flow mode under constant permeate flux conditions using ceramic membranes.

Recent fundamental studies indicated that fouling of MF membranes is influenced by 1) membrane properties 2) operating conditions and 3) properties of feed material. Severe membrane fouling has been reported in microfiltration experiments with bovine serum albumin (BSA) even when the pore size was much larger than the protein (Bowen & Hughes, 1990; Franken *et al.*, 1990; Bowen & Gan, 1991; Jonsson *et al.*, 1992a; Kelly *et al.*, 1993; Kelly & Zydney, 1994, 1995; Tracey & Davis, 1994; Jonsson *et al.*, 1996; Mueller & Davis, 1996; Herrero *et al.*, 1997). The reason for the severe fouling appears to be the presence of protein aggregates in the feed. However, Hlavacek and Bouchet (1993) showed that prefiltered BSA solutions can still foul the membrane.

Kelly and Zydney (1995) showed that initial fouling was caused by the convective deposition of protein aggregates on to the membrane surface. Jonsson *et al.* (1996) and Herrero *et al.* (1997) have proposed two consequent steps in fouling, surface blocking and cake formation during BSA filtration. In almost all these cases, fouling occurs predominantly on the membrane surface by protein aggregates. On the other hand, Franken *et al.* (1990), Bowen and Gan (1991, 1992) and Jonsson *et al.* (1992a) have suggested that shear within the membrane pores causes the protein to deposit. Tracey & Davis (1994) and Mueller & Davis (1996) during fouling studies using BSA on 0.2 μm MF membrane hypothesised that protein molecules or aggregates deposit at the pore walls or mouths. Bowen and Gan (1993) and Marshall *et al.* (1997) have suggested that fouling during MF of protein solutions is most likely due to the interaction of protein with the pore geometry at the pore entrance. There is considerable debate whether fouling is initiated on the membrane surface or within the pores or at the pore entrance.

Although the above studies gave some understanding of the possible mechanisms of protein fouling, there are still conflicting views about the underlying principles that govern protein fouling during MF.

The present study was undertaken as an extension of Marshall (1994) PhD thesis work which investigated two aspects of MF fouling, one in which the feed contained proteins considerably larger than the membrane pores (casein micelles) and the other, in which the protein (β-lactoglobulin) was much smaller than the pores. It was demonstrated that a casein "gel layer" formed on the membrane surface causing severe fouling during MF of skim milk on a 0.1μm polysulfone membrane (Marshall *et al.*, 1996). During MF of β-lactoglobulin on 50 and 100 nm zirconium oxide membranes at high fluxes, protein-protein interactions at or near the pore led to pore narrowing and the eventual retention of the protein by the membrane (Marshall *et al.*, 1997). The work stressed the importance of feed properties on fouling and recommended further work using a range of proteins of different molecular weights to investigate the effect of physicochemical properties of proteins on fouling mechanisms.

The objective of the present work was to investigate the fouling behaviour of protein solutions containing whey proteins or bovine serum albumin or bovine lactoferrin or bovine ferritin on ceramic membranes during CFMF under constant permeate flux conditions. The effects of protein size in solution and pretreatments such as prefiltration, addition of calcium and addition of a protein dissociating agent (sodium dodecyl sulfate, SDS) on fouling mechanisms were investigated. In particular, the effect of permeate flux and protein solution environment on fouling behaviour were investigated.

2. Literature review: fouling by proteins during microfiltration

2.1. Introduction

Microfiltration membrane fouling has been a major problem in the process industry. Even though the pore sizes in MF membranes are generally over an order of magnitude larger than the characteristic size of the protein, there is considerable experimental evidence that severe fouling occurs and proteins play a critical role in MF fouling. A number of fouling mechanisms may arise depending upon operational variables, feed and membrane properties. Membrane fouling in general has been extensively studied and has been reviewed by Fane and Fell (1987), Nilsson (1990), Marshall *et al.*(1993) and Belfort *et al.* (1994). However, there is currently little information available on the fouling behaviour of proteins on ceramic microfiltration membranes under constant flux and cross-flow conditions. Proteins are complex molecules and a greater understanding of their conformation, stability and interactions in different membrane environments and under conditions of shear is crucial to understand and control fouling in these processes. The present literature review builds on the review of Marshall *et al.* (1993) and focuses on more recent work mainly related to membrane fouling during MF of protein solutions.

It is common to find various terms in the fouling literature used by different authors depending on their background, as there is no universally accepted terminology. Fouling is the deposition of solute particles both on the membrane surface and/or in its pores leading to a change in the membrane performance (Gekas, 1988). For the purpose of this review, a broad categorisation is made under the terms, adsorption, deposition, aggregation and pore fouling (which includes pore plugging and pore narrowing). The term adsorption is irreversible attachment of a macromolecule due to interfacial forces and is limited to a monolayer thickness. This is different from deposition which is also irreversible, but occurs by a variety of mechanisms in addition to adsorption. Protein deposition includes the protein adsorbed under static conditions plus additional protein strongly associated with the membrane during filtration. Aggregation is a term used to refer to a variety of protein-protein interactions. The term pore plugging is the physical attachment of a macromolecule at the pore opening or within the pore and is considered synonymous with pore blocking /sealing /blinding /clogging. However, the term pore bridging means that

the pore is plugged by more than one molecule by some sort of aggregation or coalescence of protein. Pore narrowing or pore constriction is a process by which internal pores of the membrane will have reduced pore diameter due to initial adsorption and subsequent deposition of protein on the pore wall.

Bowen and Hughes (1990) investigated the deposition of BSA on aluminium oxide MF membranes. They suggested that deposition occurred in two phases. The first phase of rapid deposition was the result of monolayer adsorption on to the membrane surface and the BSA was very strongly bound. The second less rapid phase was the building up of relatively weakly bound multilayers of protein. They concluded that the level of protein deposited increased if permeate flux increased possibly due to increased collisions within the pores resulting in greater interaction. Clark *et al.* (1991) during constant pressure filtration of BSA on a 100 nm alumina membrane hypothesised that the fouling was due to adsorption related pore plugging, but did not differentiate fouling by adsorption and pore plugging. Protein deposits have also been identified within the membrane structure in MF. Attia *et al.* (1991b) showed that casein both formed a layer on the surface of the membrane and penetrated the membrane structure to deposit within the pores during MF using 0.8 μm membranes. In most MF processes, transport of protein through the membrane is required (Bowen & Gan, 1993). Interactions of proteins with the internal pore structure of the membrane can have a substantial influence on the effectiveness of such separations. It has been shown that effectiveness of selective protein filtration can be dramatically altered by appropriately controlling electrostatic interactions through changes in pH and ionic strength (Saksena & Zydney, 1994).

Belfort *et al.* (1994) presented different possibilities of fouling mechanisms in MF (Fig. 2.1) depending on the protein to pore size ratio. They defined particle diameter d and pore diameter d_p to illustrate possible fouling mechanisms. When $d << d_p$, the particles could enter most pores and could conceivably close smaller pores thereby reducing the open cross-sectional area for flow.

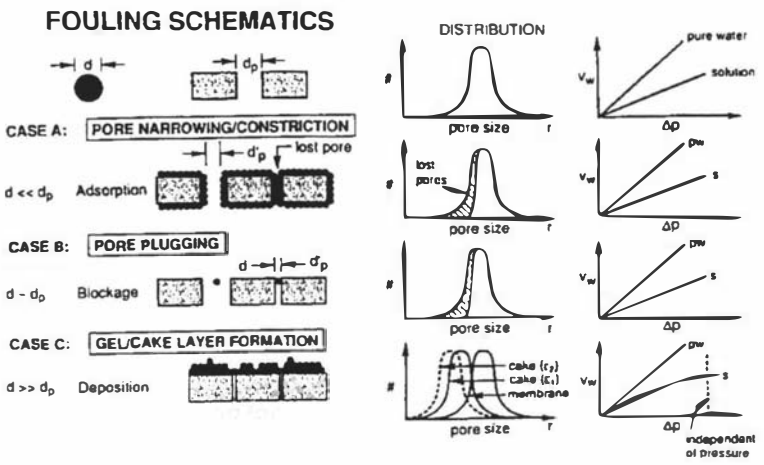

Figure 2.1. Possible fouling mechanisms during membrane filtration (Belfort *et al.*, 1994).

This is shown schematically as a loss of pores from the pore size distribution and a decrease in the slope of the flux. A similar effect occurs when $d \approx d_p$ and some pores are lost because of pore plugging. For the case when $d >> d_p$, the particles are unable to enter most pores and hence deposit on the face of the membrane resulting in cake/gel build up. Assuming the pore size distribution of the membrane remains the same as for a clean membrane, the shape of the pore size distribution of the gel/cake will most likely change with time and transmembrane pressure (TMP). Compaction, rearrangement and deposition of smaller particles in the pores of the gel/cake could explain this. In this case, the slope of the flux versus TMP curve will decrease with increasing pressure. This is often observed experimentally and has been attributed to increased deposit thickness (Blatt *et al.*, 1970) and /or osmotic effects (Jonsson, 1984).

Mueller and Davis (1996) presented data that showed occurrence of internal and external fouling during MF of BSA under constant pressure conditions (Fig. 2.2). They represented fouling by total resistance which they calculated using $R_{total} = \Delta P/\mu.J$. The slope of the resistance curves was used to interpret whether fouling was internal or external.

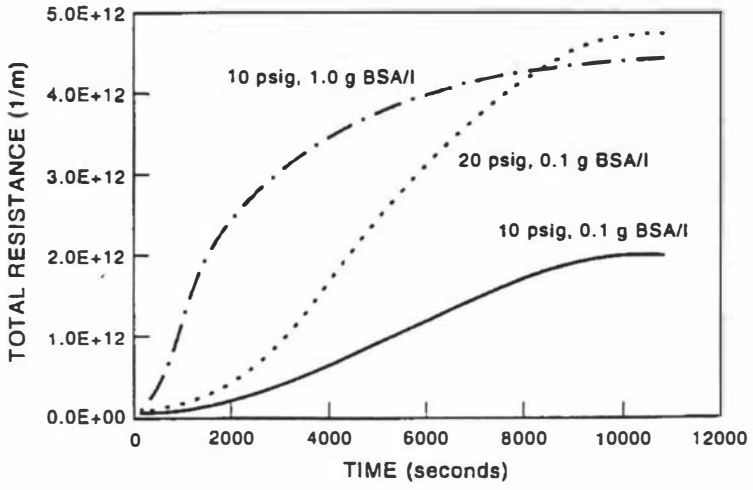

Figure 2.2. Fouling resistance curves at various feed concentrations and under different TMP's for 0.2 μm polycarbonate membrane (Mueller & Davis, 1996).

More recently, Kelly & Zydney (1997) studied fouling behaviour of a range of proteins with different physicochemical characteristics to determine the relationship between protein structure and fouling behaviour using a stirred cell. The typical flux decline and rate of flux decline curves are shown in Fig. 2.3. Flux declined rapidly for all the proteins when filtered through a 0.22 μm polyvinylidene fluoride (PVDF) membrane under constant pressure conditions. A nitrogen pressurised acrylic solution reservoir was used to avoid flow associated aggregation. They proposed that fouling under the conditions studied occurred by two distinct mechanisms: deposition of large protein aggregates and chemical attachment of native proteins to the growing deposit.

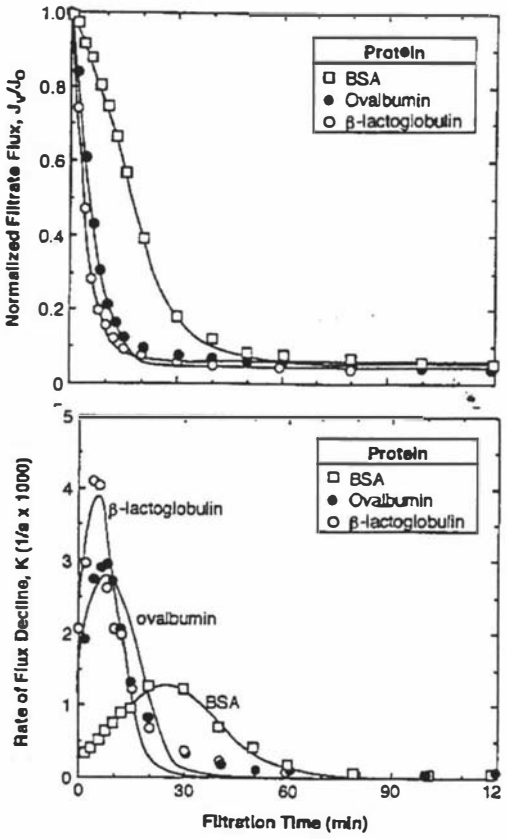

Figure 2.3. Permeate flux loss (top panel) and rate of flux decline (bottom panel) during MF of BSA, ovalbumin and β-lactoglobulin through 0.22 μm PVDF membrane at a constant pressure of 14 kPa and 600 rpm. Solid lines are model lines (Kelly & Zydney, 1997).

They proposed a model that considered surface charge interactions and drag force due to convective flux. In this case fouling occurs predominantly in the form of a surface layer.

Recently Marshall *et al.* (1997) have used the relationship between fouling resistance and protein transmission to elucidate fouling behaviour of β-lactoglobulin (Fig. 2.4). They plotted fouling resistance data from all the experiments performed at different constant permeate fluxes against corresponding protein transmission values. They all fall close to a single curve that represents a decrease in protein transmission continuously with an increase in fouling resistance. They developed a mathematical model which showed that protein deposition occurs predominantly near the pore entrance.

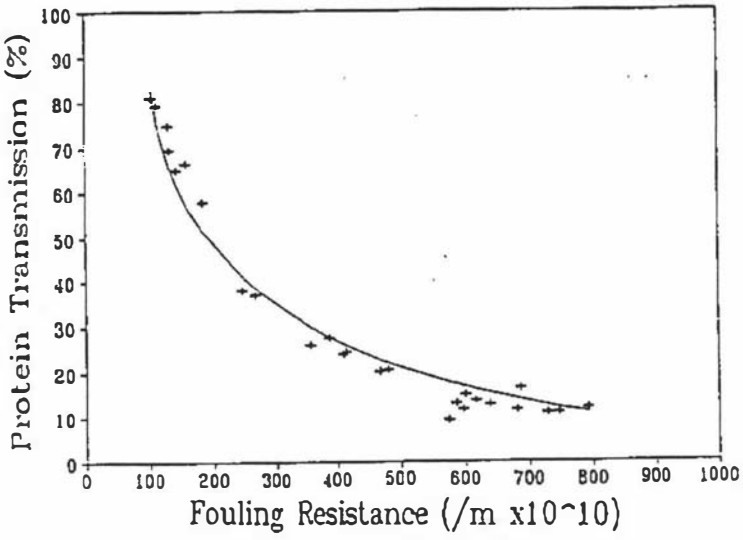

Figure 2.4. Protein transmission versus fouling resistance for a 50 nm membrane during MF of β-lactoglobulin. The line is a model prediction, details of which are given in the text (Marshall *et al.*, 1997).

Fouling which results in a decline in permeate flux and poor selectivity is due to a variety of mechanisms. The flux decline is caused by several phenomena in or near the membrane. In general, the flux decline is caused by a decreased driving force and/or an increased resistance. The resistance during a filtration process can be grouped into R_a, R_p, R_{cp}, R_g and the resistance of the membrane itself R_m (Fig. 2.5). Pores can become plugged by the solute (R_p) and adsorption of the solute on to the walls of the pores and /or the membrane surface (R_a). R_{cp} is caused by a phenomenon known as 'concentration polarisation'(CP) which is accumulation of retained molecules near the membrane surface. There has been, however, a considerable debate over how CP reduces the permeate flux. One argument is that the concentrated layer near the membrane offers resistance to permeate flow and the other argument is that the osmotic pressure ($\Delta\Pi$) induced by the accumulation of macromolecular solutes at the membrane surface effectively decreases the transmembrane pressure and hence the permeate flux.

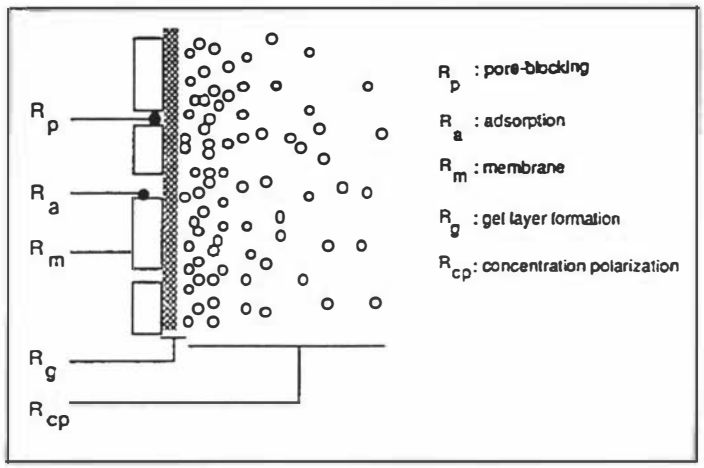

Figure 2.5. Possible resistances to permeate flux in membrane filtration (Van den Berg, 1988).

When the concentration at the membrane interface reaches a certain level, depending on the type of feed, the concentrated solution may change into a "gel layer" with a resistance R_g. In this review, experimental evidence on each phenomenon causing flux decline will be presented separately and then the most important factors that influence MF fouling by proteins will be discussed.

2.2. Protein adsorption

Adsorption is a thermodynamically spontaneous process and is probably the first step in the fouling process. It is well known that proteins adsorb at liquid-solid interfaces and the amount of protein adsorbed depends on the membrane surface characteristics (hydrophobic, hydrophilic and surface charge), the protein type and the solution properties (pH, ionic strength and concentration). In static studies, Matthiasson (1983) on polymeric membranes and Clark *et al.* (1991) on an alumina membrane have found that BSA adsorption increased to a saturation value with increasing solution concentration and the adsorption was much greater near the protein isoelectric point of pH 4.9. Bowen and Gan (1991) during MF of BSA using 0.22 μm capillary pore aluminium oxide membranes found that protein adsorption corresponded to approximately a monolayer. However, the loss of permeability due to adsorption was substantially less than the decrease in the rate of permeation during the filtration of protein solution. Bowen and Gan (1993) using polysulfone MF membranes under dynamic conditions, found that the membranes adsorbed only ~3/4

of a monolayer of the enzyme yeast alcohol dehydrogenase (YADH). The observed decrease in permeation rate was due neither to concentration polarisation nor to protein adsorption alone. However it was quantified using the standard blocking filtration law which describes a decrease in pore volume due to deposition of protein in the interior structure of the membrane. Persson *et al.* (1993a) using β-lactoglobulin on four different polymeric MF membranes also found that protein adsorption corresponded to a monolayer on and in the pores of the membrane.

Protein adsorption was found to be slightly reduced on hydrophilic membranes using BSA (Bowen & Gan, 1991) and β-lactoglobulin (Persson *et al.*, 1993a). Akhtar *et al.* (1995) treated PVDF and CA membranes with a surface grafting of methacryloyloxyethyl phosphorylcholine. They showed that membranes with this coating that inhibited protein binding improved the initial flux in both the membranes investigated, but proved to be more effective for PVDF membranes in reducing an 86% flux decline to 10% than for the CA membrane in reducing a 48% flux decline to 17% during MF of BSA. This improvement in membrane performance was concomitant with a reduction in protein fouling on the surface and within the matrix of the coated membranes as assessed by transmission electron microscopy. For a typical MF membrane (with a pore diameter of 0.2-1.0 μm), the flux reduction caused by monolayer adsorption of a protein such as BSA is only 2-12 % (Belfort *et al.*, 1994). However, a larger percent reduction in flux would be expected to occur upon adsorption of very large macromolecules or aggregates.

At least in MF, due to relatively larger pores, flux decline due to fouling can be attributed in a small part to protein adsorption of a monolayer, but the major contribution is from protein deposition under dynamic conditions. However adsorption of proteins on the membrane plays an important part in the initial stages of fouling particularly in the case of internal fouling which is a more dominant form of fouling with MF membranes.

To conclude, protein adsorption at the protein-membrane interface is almost inevitable and is influenced by a number of parameters. However it can be minimised by suitable membrane surface modification techniques or choosing membranes that are very low adsorbers-mainly used in processing of high value proteins. At least in MF though, adsorption alone may not affect flux substantially but it can play an important role in the subsequent fouling.

2.3. Concentration polarisation

The convective transport of solute towards a membrane in UF and reverse osmosis (RO) causes it to accumulate at the membrane surface in a concentrated boundary layer leading to a lowering of flux relative to that of pure solvent. In UF & RO, concentration polarisation builds up quickly (Chudacek & Fane, 1984; Fane, 1986; Aimar *et al.,* 1991) and is a function of the hydrodynamics in the membrane system. It is independent of the properties of the membrane if retention is 100%. It is important to distinguish between concentration polarisation and fouling. Fouling is the coupling of deposited material to the membrane through the intermediate step of CP, which first causes an accumulation or increase in concentration on the membrane surface (Marshall *et al.,* 1993). The loss of flux due to CP is reversible *i.e.* upon flushing of the membrane with water, the portion of the flux loss due to CP is restored and where fouling is insignificant, the flux returns to its original value (Marshall & Daufin, 1995). On the other hand, loss of flux due to fouling is generally irreversible *i.e.* upon flushing with water, the portion of flux loss due to fouling is not restored until after the membrane has been chemically cleaned.

The contribution of CP to fouling is dominant in UF whereas its effect is relatively less in MF. In situations where there is retention of macromolecules either initially or later in the process due to pore fouling, CP can play an important role even in MF, controlling flux and protein transmission.

Both of these effects should be negligible for the filtration of protein solutions through the clean MF membranes since these large pore membranes are essentially non retentive to most proteins. However these phenomena can be important for fouled membranes due to protein deposits (Opong & Zydney, 1991; Mochizuki & Zydney, 1992). Osmotic pressure may become important for a fouled membrane even in MF.

The accumulation of solute near the membrane surface is generally described by the film model. The film model assumes that the concentration boundary layer resides within a thin laminar film at the membrane surface and that all mass transfer takes place by Fickian diffusion perpendicular to the membrane. The film is dynamic in nature. Some investigators (for *e.g.* Nakao *et al.,* 1979; Isaacson *et al.,* 1980; Jonsson, 1984) have used the osmotic model to explain the flux decline. The osmotic model assumes that resistance to flow resides within the solute accumulated and is caused by osmotic pressure. Unfortunately these models when applied to MF of colloid particles underpredict the flux observed. The reason for this "flux paradox" (Romero & Davis 1988, 1990) at least for colloids appears to be due to increased back-transport caused by hydrodynamic effects like 1) shear induced diffusion and 2) lateral migration due to inertial lift (See references in Belfort *et al.* (1994) for more details) in addition to diffusion. Alternative models such as shear induced diffusion and lateral migration models have been proposed to describe the transport of particles away from the membrane. Romero and Davis (1988, 1990) consider this issue in more detail. Romaro & Davis (1988) and Lojkine *et al.* (1992) reviewed the various models that have been proposed to explain this behaviour. Recently a unified model combining gel and osmotic models has been proposed to predict flux in UF (Bhattacharjee *et al.,* 1996). However, application of these models to macromolecular solutions is questionable as flux decline in MF is predominantly due to internal fouling.

2.4. Formation of a dynamic membrane/surface layer

Several researchers have reported formation of a dynamic layer by proteins during RO and UF (Lim *et al.,* 1971; Glover & Brooker, 1974; Lee & Merson, 1975, 1976a; Kim *et al.,* 1992; Marshall *et al.*, 1996). This is generally expected as the protein size is larger than the pore size of the membranes used in their studies. Surprisingly, a dynamic layer was reported even during MF for example, using skim milk on a 0.2 μm membrane (Attia *et al.,* 1991a). Casein micelles formed a surface layer as they are of similar size to the membrane pores. Surface layer formation was observed even during MF of proteins which are much smaller than the pore size mainly during the later stages of the processing due to complete loss of internal pore area (Jonsson *et al.*, 1996; Marshall *et al.*, 1997). Generally in MF once a surface layer has formed, it reduces the protein transmissions to a level that would be determined by the porosity of the deposited surface layer.

Interestingly in some cases using non-protein feed streams, a dynamic membrane had a positive influence on the membrane performance, for example, Cakl and Mikulášek (1995) during MF of latex particles of broad size distribution using alumina membranes showed that an adequate secondary membrane can improve flux by protecting the membrane from pore plugging and therefore contributes to a higher and more stable flux (also see section 2.9). Al-Malack and Anderson (1996) showed that the dynamic membrane formed on the primary membrane can be used as an anti fouling technique. They formed a dynamic membrane using potassium permanganate ($KMnO_4$) with particle sizes less than the pore size of the primary membrane. Pore narrowing, pore bridging by the particle and precipitation on the membrane surface were reported to be the mechanisms of dynamic membrane formation. Improved flux and turbidity were observed with increased concentration of $KMnO_4$ when this dynamic membrane was used to treat a secondary effluent from waste water. Kuberkar and Davis (1998) reported that a secondary membrane formed by yeast and BSA mixture improved BSA transmission compared to when BSA alone was microfiltered on a 0.07 μm cellulose acetate membrane.

2.5. Pore fouling or protein deposition

2.5.1. Fouling within the membrane structure

Pusch and Walch (1982) showed electron micrographs of the top surface of a 3 μm Nuclepore membrane after filtration of latex/styrene particles. Even though the particles were only one-tenth of the pore diameter, they interacted with the pore walls of the membrane, thus forming agglomerates that finally blocked the pores. A number of investigators have found protein adsorption within the membrane pores (Nakao *et al.*, 1988; Dejmek & Nilsson 1989; Hanemaaijer *et al.*, 1989; Bowen & Gan, 1991). Matsumoto *et al.* (1988) compared resistance due to internal and surface layer fouling with ovalbumin (~44 kDa) on ceramic membranes. With low velocities (laminar) and with the 0.2 μm and to a lesser extent the 0.8 μm membrane, surface layer fouling dominated. With larger pore sizes (1.5, 3 μm) pore plugging appeared to be the predominant fouling mechanism. With high velocities, for all four membranes, pore plugging was predominant. Bowen and Gan (1993) used a thin film composite polysulfone membrane during MF of YADH. They suggested that deposition of macromolecules inside the pore structure was an important mechanism for flux loss. To conclude, there is experimental evidence that protein deposits within the membrane pores as well as on the surface.

2.5.2. Fouling at the pore entrance

Visvanathan and Ben Aïm (1989) suggested that in the early stages of filtration, colloids can deposit on the membrane surface in between the pores and thus accumulate. Later, aggregates of colloids can form bridges over the pore openings, resulting in partial blocking of the pores. A smaller pore structure is then available for subsequent colloids to deposit. This bridging of aggregated colloids leads to the eventual formation of a film of colloids on the membrane surface. Bowen and Gan (1991) proposed that protein deposition occurs in the immediate vicinity of the pore entrance due to the high shear caused by the local permeate flows. Tracey and Davis (1994) during MF of BSA solutions using track-etched polycarbonate membranes found that the pore radii decreased on the feed side but not on the permeate side, indicating that the fouling layer is not evenly distributed throughout the pores, but instead concentrated near the pore entrances. Marshall *et al.* (1997) during MF of β-lactoglobulin showed that fouling occurred over a small part of the membrane

probably at the pore entrance. Kim *et al.* (1997) have also reported BSA fouling data that supports protein deposition at the pore entrance (also see section 2.7.3).

To summarise, a number of studies have hypothesised that protein deposition occurs near the entrance of the membrane pore.

2.6. Effect of feed properties

2.6.1. Protein size

Prádanos *et al.* (1996) filtered 5 different proteins (pepsin, BSA, lipase, γ-globulin and invertase) with molecular weights of 36, 67, 80, 150 and 270 kDa on 0.02 μm partially retentive Anopore membranes. They found that as the molecular weight increased, there was a decline in flux. However, invertase showed a strange behaviour; it was the biggest protein, but its flux decline curve was between those for lipase (80 kDa) and BSA (67 kDa). This behaviour was attributed to the ratio of molecular and mean pore sizes which is >1 for invertase and also it is the only protein studied that is totally retained by the membrane whereas others gave retentions below 70%. Under the same conditions of filtration, invertase appeared to have blocked very few pores reducing the flux only a small amount whereas lipase and γ-globulin could block a lot more pores and consequently reduce the permeate flux substantially. Güell and Davis (1996) found that BSA and lysozyme displayed only internal membrane fouling on 0.2 μm PS and PC membranes whereas ovalbumin (higher molecular weight) exhibited an initial phase in which internal fouling dominated followed by a surface layer on the same membranes indicating the effect of protein size on fouling behaviour.

2.6.2. Aggregation or state of the protein

Pouliot *et al.* (1994) during MF of whey proteins using alumina membranes found that fouling was strongly dependent upon the state of the proteins in the feed. They concluded that pH and ionic strength are the important solution properties that influence aggregation of the protein thereby affecting protein fouling. Bowen and Hall (1995) observed that the rate of filtration and transmission of YADH through an MF membrane depend on the molecular state (degree of aggregation of the enzyme in solution). Kelly *et al.* (1993) showed that BSA fouling was associated with the

deposition of trace quantities of aggregated and/or denatured BSA, with these fouling species serving as initiation sites for fouling during stirred cell filtration through 0.16 μm membranes. The fouling tendency of different BSA preparations was highly correlated with the concentration of BSA dimers and other high molecular weight species present in these BSA solutions. Gésan *et al.* (1993) found that during MF of pretreated whey using carbosep M14 membranes, proteins and calcium phosphate aggregates were responsible for fouling which increased according to the "complete blocking" filtration law. Maa and Hsu (1996a) reported that when zinc ions were added to induce aggregates, the fouling tendency of recombinant human growth hormone (rhGH) solutions increased with increasing amount and size of the aggregates, indicating that the aggregates present before filtration might be responsible for membrane fouling. In summary there is considerable experimental evidence that protein aggregates are responsible for severe fouling during MF.

2.6.3. Secondary aggregation

Suki *et al.* (1984) hypothesised that aggregation of protein BSA is possible at high TMP due to convective flow leading to supersaturation of membrane surface with protein. They proposed that accumulation of solute provides a reservoir of molecules on the membrane thus gradually fixing protein molecules due to aggregation and flocculation on the membrane surface. Later, Suki *et al.* (1986) attempted to model UF fouling by assuming that protein aggregated in the concentrated layer next to the membrane based on flocculation theory. Using data available for BSA, the flux was calculated as the aggregated layers built up. Increased flux decline at higher feed concentrations was predicted by the model. However the model does not allow for differences in the membrane properties.

Meireles *et al.* (1990) using light scattering analysis and turbidity measurements showed that BSA formed aggregates of large size during UF at 22°C. They showed that at a temperature of 15°C, the higher the gear pump speed, the heavier the denaturation which they interpreted using turbidity measurements. In a later publication, Meireles *et al.* (1991a) reported that the rate of protein denaturation increased with temperature, cross-flow velocity (CFV) and time of processing. They suggested that under high concentration polarisation, denaturation can occur in the

boundary layer if the wall concentration exceeds 40%. They attributed BSA fouling to partial denaturation leading to aggregation. Xu-Jiang *et al.* (1995) during dead end MF using two membranes in series hypothesised that at room temperature (22°C) and with a piston pump giving high shear conditions, BSA is susceptible to denaturation causing a rapid rise in pressure drop during constant rate filtration. They attributed the pressure drop to adsorption alone neglecting pressure drop due to pore plugging by aggregates. Blanpain *et al.* (1993) during MF of beer reported formation of local aggregates of macrosolutes over the pore openings leading to fouling.

Kim *et al.* (1992) concluded that aggregate formation during UF is exacerbated by conditions like membrane surface roughness and high convective flows close to the membrane surface. They found formation of aggregates with higher initial flux UF membranes, but not on the lower flux membranes. They attributed aggregation to conformational changes in the BSA molecules associated with the high shear rates that exist near the membrane surface. They hypothesised that these conformational changes exposed hydrophobic regions on the BSA molecules which then interacted to form large protein aggregates at the membrane surface.

Maa and Hsu (1996a) suggested that fouling was more attributed to the aggregation taking place in the filter pores during filtration (secondary aggregation) than to the aggregates present before filtration. Formation of BSA aggregates during actual stirred cell filtration was hypothesised by Kelly and Zydney (1995). Jonsson *et al.* (1996) hypothesised in-situ generation of BSA aggregates during pumping. It was suggested that high denaturation due to pumping leads to formation of aggregates that cannot penetrate into pores but form a cake.

The effect of operating conditions on protein aggregation during UF was investigated by Kim *et al.* (1993a). They hypothesised that high shear rate combined with high protein concentration enhanced protein aggregation. Hlavacek and Bouchet (1993) demonstrated that protein solutions initially free of aggregates can impair a MF process, if denaturation and aggregation conditions are present in the system. Protein aggregates can form by a variety of mechanisms. Persson and Gekas (1994) in their

review, suggested that aggregation of macromolecules is due to their flexible secondary and tertiary structure.

In conclusion, protein aggregates in the feed or those generated during processing appear to play an important role in the severe fouling observed with MF membranes.

2.6.4. pH and ionic strength

The influence of pH and ionic strength on protein fouling in UF was reported by Fane *et al.* (1983) and Suki *et al.* (1984). They showed that deposition was highest at the isoelectric pH (IEP) of the protein BSA and relatively low at pH away from the IEP. Addition of salt (NaCl) generally increased deposition. The explanation given is as follows: BSA molecules at the IEP (no salt) are in their most compact state and carry no net charge giving the least permeable deposit layers. Away from the IEP and in the absence of salt, the molecules expand and have significant net charge giving relatively permeable layers. Addition of salt at these pH extremes will shield charges causing molecular contraction. At the IEP, the effect would be different since the addition of salt causes anion binding, enlargement of the molecule and acquisition of charge with an expected increase in permeability.

Heinemann *et al.* (1988) studied the effect of salt on fouling and protein transmission during MF of whey protein solution through a 0.2 μm hydrophilic membrane. They found that addition of 100 mM NaCl decreased flux of protein solutions, the flux decrease being independent of pH. However steady state protein transmission was 30-35% higher and the degree of loss of transmission was less at pH values away from the IEP. Protein transmission of the salt-free solution was at a maximum around the IEP, whereas in the presence of salt, the steady state transmission was at a minimum. They considered interaction of charged molecules with the charges on the membrane surface to explain the results. Addition of salt seems to have reduced electrokinetic interaction between protein and membrane leading to a compressed double layer, less interaction and increased protein transmission. However reduced size of the protein apparently caused pore plugging and denser deposits reducing flux. They proposed that at the IEP, in the presence of salt, the "salting out" effect

enhances aggregation and the build up of denser deposits resulting in reduced flux and protein transmission.

Palecek *et al.* (1993) investigated the effect of ionic environment on the hydraulic permeability of the deposits during MF of BSA using a 0.16 μm Polyethersulfone (PES) membrane. They found that permeability of deposited layers of BSA was a minimum at the protein isoelectric pH and decreased with increasing solution ionic strength (IS) at pH both above and below the IEP. They also found that flux was a function of the electrolyte composition, with the flux for BSA in NaCl approximately 40% greater than that for BSA in a $CaCl_2$ solution of the same IS. This dependency on IS and ion valency was attributed to porosity in the deposit which was determined primarily by the balance between the compressive pressure associated with the filtrate flux and the electrostatic repulsion between the charged proteins within the deposit.

Palecek and Zydney (1994a) found that steady-state permeability for albumin, lysozyme, ribonuclease A, haemoglobin and immunoglobulin deposits was a minimum at the protein isoelectric pH and decreased with increasing salt concentration at pH values both above and below the IEP. In a later publication, Palecek and Zydney (1994b) suggested that flux decline during protein filtration through even large pore MF membranes is due to the formation of a protein deposit on the membrane with the deposited protein layer providing a large additional hydraulic resistance to the filtrate flow. The steady state flux through the different protein deposits was a minimum at the protein isoelectric point and appeared to increase almost linearly with the protein surface charge density. Pouliot *et al.* (1994) have also found significantly higher fouling at the protein IEP due to increased deposition and hydraulic resistance.

Balakrishnan and Agarwal (1996) used a vortex flow filter to study flux and transmission behaviour of lysozyme (~14 kD, pI: 10.6) ovalbumin (~44 kD, pI: 4.6) and myoglobin (~17 kD, pI: 6.8) using a 100 kD cut-off hydrophilic polyacrylonitrile membrane. For all these proteins, the flux increased linearly with increasing TMP up to 30 kPa and further increase in TMP resulted in a marginal nonlinear increase in flux. The fouling was minimal for ovalbumin but lysozyme and myoglobin showed a

small reduction (11.7 & 13.5%) in flux during protein filtration. The transmission however varied markedly for these three proteins. While ovalbumin and myoglobin exhibited protein transmissions in the range of 25-50 & 75-95% respectively, lysozyme exhibited an unexpectedly high transmission up to 120% implying concentration of protein in the permeate stream. They attributed this to the presence of attractive protein-membrane electrostatic interactions under the conditions used. The difference in protein transmission was attributed to the pH conditions of the solutions. The experiments were conducted at pH 6.8 which was below the pI of lysozyme. Consequently, lysozyme was positively charged at this pH and experienced overall attraction towards the hydrophilic membrane apparently leading to a high protein transmission of about 120%. On the other hand, ovalbumin (pI: 4.6) which is negatively charged at this pH was repelled by the membrane surface and displayed transmission markedly below 100% (25-55%). Myglobin which is a zwitterion at pH 6.8, followed a profile similar to that of ovalbumin, but with much higher transmissions (75-95%). This was apparently because ionic interactions between the neutral myoglobin molecules and the membrane surface were minimal, causing the myoglobin transport to occur mainly by molecular sieving.

To summarise, fouling is severe at protein IEP and becomes less severe both above and below IEP. Addition of salt generally increases severity of fouling at pH away from IEP, however at IEP, it decreases fouling.

2.6.5. Concentration

Concentration of the feed seems to determine the type of fouling mechanism. Tracey and Davis (1994) found that fouling by 0.01% BSA solutions was more severe than with 0.1% solutions on 0.2 μm polycarbonate membranes. It was hypothesised that protein aggregates that formed at 0.1% concentration are larger than aggregates formed at 0.01%, causing external fouling leading to formation of a porous cake, whereas smaller aggregates at low concentration are sufficient to enter the pores and cause internal fouling, reducing protein transmission below that obtained with external fouling. On the other hand, Güell and Davis (1996) using a 0.2 μm membrane reported that ovalbumin aggregates that formed at high concentration increased flux decline by pore plugging. Bowen and Hall (1995) using the analysis of

filtration data obtained from MF of YADH showed that the fouling mechanism changed from in-pore deposition to surface deposition as concentration was increased.

In summary, concentration of protein, which is linked to aggregate formation, can affect fouling by plugging the pores or forming a surface layer depending upon aggregate size. The porosity of the deposited layer of aggregates affects the flux and protein transmission.

2.6.6. Prefiltration

Kelly *et al.* (1993) demonstrated that prefiltration of BSA solutions prior to MF substantially reduced their fouling tendency with the degree of improvement increasing as the prefiltration was performed through smaller molecular weight cut-off membranes. Maa and Hsu (1996a) also observed reduction in fouling when recombinant human growth hormone (rhGH) was prefiltered. On the other hand Hlavacek and Bouchet (1993) found that prefiltered BSA solutions still fouled during MF. Jonsson *et al.* (1996) observed a significant delay in cake formation when BSA was prefiltered. In summary prefiltration seems to reduce the severity of fouling but does not eliminate it completely especially when secondary aggregation occurs.

2.6.7. Presence of minerals and surfactants

Vetier *et al.* (1988) during MF of milk on alumina membranes found that calcium and phosphorous salts increased fouling probably by allowing better adsorption of casein micelles on the alumina and by acting as intermicellar bonds in the deposit. The serum milk proteins were retained by the porous micellar deposit under both static and dynamic conditions resulting in progressive fouling of the dynamic micellar membrane, enabling milk to be processed with a mineral MF membrane. Scanning electron microscopy (SEM) studies revealed that fouling occurred in dynamic filtration which was similar to that described for static conditions except that the layer was much thinner with a minimum of 5 μm (thicknesses 5-10 times greater were observed in static experiments). The most important role of the calcium and phosphorous salts present in the soluble phase of milk is to act as a cement between micelles and alumina in fouling and between the micelles themselves. It was suggested that fouling was initiated by adsorption of a thin film of casein and salts, on

which were then deposited other micelles which may be connected by phosphocalcic bonds.

Maa and Hsu (1996b) observed improved filtration by the addition of sodium dodecyl sulfate (SDS), a surfactant. Elzo *et al.* (1996) found that the presence of triton X-100, a non-ionic surfactant, caused weaker adhesion forces between glass particles and a model membrane consisting of a cellulose diacetate film. They found easy removal of particles from the surface due to the presence of surfactant. The reduction in adhesion energy due to the presence of surfactant is explained by a number of factors. The hydrophobic tails of the triton are adsorbed on particle surfaces and the membrane surface is thus exposed to the hydrophilic head group of the surfactant. The presence of triton induced steric effects between surfaces and a substantial decrease in adhesion between particles and the membrane surface was observed.

2.7. Effect of membrane properties

2.7.1. Pore size

Higher permeate fluxes are expected with MF because of the relatively larger pore size. But Attia *et al.* (1991b) while processing skim milk on aluminium oxide MF membranes found higher permeate fluxes with a 0.2 μm membrane than a 0.8 μm membrane. Surprisingly several investigators have shown that though fluxes increased with increasing pore size, flux decline was greater as the pore size increased. Piot *et al.* (1984) considered the performance of Nuclepore membranes (0.01, 0.03, 0.05, 0.08, 0.1 μm) with cheese whey in an Amicon Cell. Fluxes increased until the 0.08 μm membranes, after which the flux declined. In addition, they found that retention was lowest for the 0.05 μm membrane, increasing as the pore size both decreased and increased. Meireles *et al.* (1991b) considered the performance of a range of polysulfone membranes with BSA, ovalbumin and α-lactalbumin. After 1 minute the protein retentions for BSA were 100, 85 and 20% for 10, 40 and 100 kD MWCO membranes respectively. However, protein retentions for the three membranes at steady state were 100, 100 and 98%. Changes in protein retention by fouling are more severe as the membrane pore size increases. In a number of cases an optimum pore size with respect to permeate flux has been identified below which the resistance of the membrane itself and the formation of a

surface fouling layer reduce the permeate flux and above which the increased rate of membrane fouling due to deposition within the pores causes lower long term fluxes. Jonsson *et al.* (1996) studied the effect of pore size on fouling. It was observed that surface blocking rate decreased with an increase in mean pore radii and a decrease in porosity whereas, the cake formation step decreased with an increase in mean pore radii. Kim *et al.* (1992), comparing the different membranes used in their study, found that there is a general relationship for the UF membranes between the amount of protein deposited and the flux decline. This observation was less apparent with the Nuclepore and Anopore MF membranes. Whilst the Anopore membrane had a lower flux decline in spite of its greater protein deposition, the Nuclepore membrane showed a tremendous flux loss (>90%) for a relatively small amount of protein deposition. The explanation advanced was the lower porosity of the Nuclepore membrane (~10%) compared to the Anopore membrane (~50%). Comparing 0.2 μm and 0.05 μm polycarbonate membranes during filtration of BSA solutions, Tracey & Davis (1994) hypothesised that the 0.2 μm membrane initially fouls internally as no significant protein rejection is occurring and yet the resistance is increasing; once the pores are constricted, fouling becomes external. However on the 0.05 μm membrane, cake layer build up occurs immediately on the surface of the membrane.

2.7.2. Pore morphology

Use of membranes with the matrix side facing the feed solution (YADH) gave much slower decreases in filtration flux, showing the importance of pore morphology in the optimisation of filtration rates (Bowen & Gan, 1993). However the result was not repeated when filtering industrial fermentation broth. The results were attributed to pressure-induced pore broadening of the polymeric membrane, whereas with fermentation broth, the high suspended solids present blocked the exposed matrix. Persson *et al.* (1993b) used liquid displacement porosity (LDP) to characterise four polymeric MF membranes. LDP showed a clear pore size distribution for all membranes analysed, with 10% of the pores having at least 90% of the permeability.

Mueller and Davis (1996) investigated effects of varying membrane morphology and surface chemistry on protein fouling mechanisms of MF membranes. A 0.2 μm track-etched polycarbonate (PC) membrane fouled internally, with external fouling

becoming the dominant means of fouling only at later times. A 0.2 μm cellulose acetate (CA) membrane showed only internal fouling, while 0.2 μm polysulfone (PS) and polyvinylidene fluoride (PVDF) membranes showed only external fouling. It is hypothesised that the low surface porosities and large thickness of the PS and PVDF membranes reduced permeate flux and allowed protein aggregates to quickly plug the few pores, leading to almost immediate external fouling (surface layer), while the higher surface porosities and small thickness of the PC and CA membranes yielded higher fluxes and caused extensive internal fouling before external fouling took over. They reported significant loss of protein transmission during external fouling. In part II of the study, on the effects of various surface modifications, results show that surface-modified polyethylene and polypropylene membranes have lower initial fluxes than the unmodified membranes. However, the hydrophilic modified membranes demonstrated comparable final fluxes and lower percent flux declines than the unmodified membranes. The azlactone modified membranes showed very low long-term fluxes and large decreases in permeate protein concentration due to efficient protein binding. Again, protein transmission remained constant or only slightly decreased during internal fouling, while a significant loss of protein transmission was observed during external fouling. Jonsson *et al.* (1991) suggested that membrane morphology rather than surface chemistry is more important in MF fouling as the primary cause of the flux decline is the deposition of protein aggregates (affected by morphology) rather than adsorption of protein molecules (affected by surface chemistry). Güell and Davis (1996) found that lower surface porosity membranes exhibited more rapid external fouling probably due to plugging of the few pores that contribute to the larger permeate flows.

In summary, pore fouling appears to have a significant influence on subsequent MF fouling. Membranes with large internal area may have increased interactions between proteins and membranes. These interactions sometimes lead to formation of protein aggregates and plugging of large pores resulting in dramatically lower fluxes even though initial fluxes are high.

2.7.3. Microscopy studies on fouling

Direct observation of fouled membranes using SEM, Atomic Force Microscopy (AFM) and Field Emission Scanning Electron Microscopy (FESEM) have been performed by several investigators to elucidate the fouling mechanisms. Kim *et al.* (1992) using FESEM observed surface deposition even on membranes that pass substantial amounts of proteins. There was no FESEM observable protein within the pores. Two different types of foulant deposits were observed on the membrane surface; fouling by multilayer (cake) coating and fouling by aggregates of proteins. They showed that protein aggregates were formed on a polysulfone membrane immediately (1 min). Growth of aggregated protein with time during UF of 0.1% BSA on polysulfone membranes was demonstrated. Hlavacek and Bouchet (1993) using SEM showed that the fouling during constant flux MF of BSA on a 0.2 μm Millipore membrane is mostly a surface deposit made up of protein aggregates. Kelly and Zydney (1994) have also showed that fouling was predominantly by aggregates on the surface layer. Tracey and Davis (1994) using SEM revealed that pore radii and pore density both decreased with filtration time. Bowen and Hall (1995) using AFM found large amounts of YADH deposited on a Cyclopore membrane surface with only a few pores visible uncovered by deposits. Maa and Hsu (1996a) using SEM analysis showed that 0.22 μm membranes when used to filter rhGH solutions under 0.35 bar TMP, were plugged to a great extent.

Kim *et al.* (1997) measured streaming potentials of virgin and fouled membranes to study fouling. They used sets of polycarbonate track etched membranes of two pore size ranges, smaller pore size (0.01, 0.03, 0.05 μm) and larger pore size (0.1 and 0.2 μm) to filter BSA. Comparison of apparent zeta potentials (calculated based on streaming potential measurements) of virgin and fouled membranes suggested that the measured potentials are determined by the protein properties. It appears that the protein adsorption completely modified the surface charge of the original membranes. They used fourier transform infrared (FTIR) spectroscopy to look at the extent of protein deposition. For the membranes with small pores, the BSA peak did not appear in the spectra but the BSA peaks were seen clearly with bigger pore size membranes. The amount of protein adsorbed on the smaller pore size membrane may be under the detection limit of the instrument due to their low porosity and small pore

size. For the membranes with larger pores however, the BSA peaks increased with increasing pore size due to the greater volume of BSA solution passed. Analysis of the FTIR spectra of the protein fouled 0.2 μm membrane performed on both sides of the membrane indicated that the feed side gave more intense BSA peaks than the permeate side suggesting that the adsorption is predominantly on the feed surface. The depth profiling of the fouled membrane also suggests that protein adsorption was mainly on the surface or near the pore entries rather than throughout the membrane pore. Surface properties of the fouled membranes mirror properties of the deposited protein. There is a lack of pore size effect on the zeta potentials of the protein fouled membrane suggesting again predominantly surface layer, which was confirmed by Electron microscopy. To simulate BSA fouling they filtered silver colloids of size 8 nm using a 0.2 μm membrane. FESEM pictures showed gradual pore bridging and eventual coverage by the silver colloids. They proposed that a similar mechanism is expected with BSA even though BSA molecules are quite different from silver colloids.

To summarise, all the above direct observations of MF membranes obtained after protein filtration indicate that fouling did not occur throughout the membrane structure but predominantly on the surface or near the pore entrance.

2.8. Effect of operating variables

2.8.1. Permeate flux/Transmembrane pressure (TMP)

Piot *et al.* (1986) obtained results when processing whole milk on a 1.8 μm ceramic membrane. The rate of flux decline decreased and the average permeate rates increased as the TMP was reduced from 10 to 0.8 to 0.65 bar. These results show that there is possibly an optimum TMP at which flux rate is a maximum for the given pore size. Optimum TMP decreased as the pore size increased. Increasing the TMP also resulted in lower water flux recovery after water flushing suggesting that more severe fouling had occurred (Persson *et al.*, 1993a). Van der Horst and Piersma (1988) during CFMF of skim milk found that for both 0.4 and 0.7 μm membranes, the flux decreased with increasing TMP and decreasing cross-flow velocity. Bowen and Gan (1991) when processing BSA with 0.2 μm Anopore capillary membranes found that increasing the TMP (0.14, 0.34, 0.69 and 1.38 bar) increased the initial permeate flux

but also increased the rate of flux decline. The final permeate fluxes were fairly similar. They suggested that the reason for continuous decline in flux was a shear induced deposition of protein molecules. Jonsson *et al.* (1992b) observed flux decline during MF of BSA solutions. They suggested that the flux decline could be related to denaturation of the BSA molecules at the pore entries because of the high shear forces. Bowen and Gan (1992) found that flux decline was more severe when operated at higher TMP. They demonstrated that decreased activity of YADH was because of enzyme interactions resulting from a shear induced deformation of the enzyme structure. Daufin and Merin (1995) reported quoting Piot *et al.* (1986) and Malmberg and Holm (1988) work that the TMP is a most critical parameter influencing flux decline in MF of milk . It was shown that increasing TMP by 0.15 bar doubled the flux but when TMP exceeded 0.4 bar or 0.65 bar, fouling occurred much faster. Frenander and Jönsson (1996) highlighted a critical TMP to minimise fouling during constant flux operation.

The importance of constant flux operation has been stressed to reduce fouling in MF. Sheehan *et al.* (1988) used permeate flow control in preference to permeate pressure control, and the average flux was found to increase by a factor of 2.5 during the recovery of an extracellular bacterial protease. They reported that, in order to reduce the fouling rate, it is important to keep TMP as low as possible while maintaining recirculation rate as high as possible. Marshall *et al.* (1996) during MF of skim milk using 0.1 μm polysulfone membrane demonstrated that severe fouling could be prevented by careful start up procedures and by control of permeate flux. However it was found that there was no benefit of using constant flux operation during UF. van Reis *et al.* (1997) while separating IgG-BSA and BSA monomer-oligomer mixtures suggested that operation in the pressure dependent regime of the filtrate flux curve and careful control of fluid dynamic start up conditions will minimise fouling and increase selectivity.

Mueller and Davis (1996) showed that increased transmembrane pressure resulted in more severe fouling of the polysulfone and PVDF membranes. Persson *et al.* (1993a) using four different polymeric MF membranes found that increased TMP resulted in an increased fouling layer for the whey protein. Irreversible fouling increased with

increasing TMP leading to poor flux recovery. They suggested that increased fouling was a combination of pore narrowing, pore plugging and cake layer build up (covering all the possible options).

Blanpain *et al.* (1993) have reported increase in adsorption at higher TMP's. Increase in TMP seems to increase driving force to the membrane enhancing protein concentration at the surface of the membrane and thus increasing the adsorption. Marshall *et al.* (1997) have reported severe fouling at higher permeate fluxes. They proposed that as the flux is increased the shear stress in the pore is increased causing an increase in the protein unfolding, deposition and fouling during MF of β-lactoglobulin.

Boyaval *et al.* (1996) studied the effect of TMP on permeate flux during MF of lactic acid bacteria on a 0.2 μm membrane. In this case the cells were totally rejected by the membrane due to their size compared to the largest pores of the membrane. Fouling was predominantly due to the formation of a reversible cake of microbial particles on the surface. At lower pressures, an increase in TMP (from 0.1 to 0.3 bar) induced a sharp increase of flux which reached 120 $L/m^2.h$ for a TMP of 0.3 bar. At TMP above this value, flux reached almost a stable value. At constant TMP below this value, flux was constant with time. This behaviour is similar to the classical pressure versus flux relationship observed in UF. This type of behaviour is generally observed when there is predominantly a surface layer on the MF membrane. They also studied the effect of transient operation conditions on fouling. They found that fast increase of TMP (5 min) resulted in severe fouling compared to increase of TMP over a period of 30 min. They suggested that in a fast transient regime, the convective forces are stronger and particles have less time and space to organise. The deposit composed of a less dense structure would then be capable of entrapment of medium molecules whereas the slow transient regime induced a more organised deposit.

A different observation was made by Cakl and Mikulášek (1995) while filtering PVC latex. They showed that increasing TMP did not always increase the flux. They reported that TMP increased flux linearly if the particles were bigger than the pores.

It was found that the flux increased linearly with TMP at low values up to a critical value, then the rate of increase decreased and finally flux become nearly independent of TMP at high values. It seems there is an optimum TMP below which the driving force is too low and above which increased fouling causes a reduction in flux. This is attributed to the formation of a filter cake with higher resistance once the deposited particles undergo compaction under higher TMPs.

Mc Donogh *et al.* (1995) used a radio isotope technique and electron diode array microscope (EDAM) to study CP during UF and MF of BSA and dextran blue (2000 kD) solutions on 0.01 μm pore polysulfone and 0.45 and 0.8 μm pore cellulose acetate membranes. The filtration cell was placed between the two lenses of the EDAM such that the region extending from the membrane surface into the flow channel was viewed parallel to the membrane surface and perpendicular to the direction of cross-flow. They showed that increase in TMP increased the solute concentration near the membrane, and also increased the extent or size of the polarised layer, the region where the concentration is higher than the bulk. The near wall concentration of dextran measured at a distance of 20 μm (from the membrane wall due to the equipment constraint) increased from about 20 g/l for the case of 0.5 bar to 115 g /l at 1 bar and to 140 g/l at 1.5 bar.

In summary, it appears that operation on the low TMP side of the maximum in the pressure dependent region is better for reducing fouling. Permeate fluxes are generally observed to first increase with increasing TMP and after a certain critical value, they would decrease with further increase in TMP. TMP is very critical to prevent severe fouling in MF processes. The TMP where the maximum flux occurs seems to decrease as the membrane pore size increases for the given type of feed. Low and uniform TMP appears to be crucial to minimise fouling during MF.

2.8.2. Shear

Nelson and Glatz (1985) reported that under fluid shear conditions, aggregation involves the following steps. Initially, aggregation occurs by collisions induced by the Brownian motion of the proteins. This results in the formation of 'primary aggregates'. Aggregation continues by this mechanism until the aggregates become

large enough for fluid motion to promote collisions, at which point, further aggregation is governed by shear induced collisions. Aggregation does not continue indefinitely; instead as the aggregates get bigger their growth rate decreases and eventually they reach a limiting size which depends on the intensity of the shear field. Jonsson *et al.* (1996) attributed fouling of membranes to a slight denaturation of proteins due to the high shear stresses present inside the pump. Comparative fouling studies by them with and without pumping indicated less severe fouling in the no-pumping case suggesting no aggregate formation in the absence of pumping. Maa and Hsu (1996b) reported some changes in protein conformation when recombinant human growth hormone (rhGH) was subjected to high shear rates ($> 10^5$ s^{-1}) in a homogeniser. Maiorella *et al.* (1991) reported that shear damages nearly 30% of animal cells by a lobe pump at a flow velocity of 3.5 m/s. Though there is no direct experimental evidence of protein denaturation by shear, it is hypothesised that shear at the membrane pore causes aggregation of protein leading to subsequent fouling in MF (Chandavarkar, 1990; Meireles *et al.*, 1991a). On the other hand a host of other investigators (Franken *et al.*, 1990; Bowen & Gan, 1991, 1992, 1993; Jonsson *et al.*, 1992a; Marshall *et al.*, 1997) hypothesised that shear at the membrane pore entrance caused proteins to deposit during MF. One thing is clear in MF, as the internal membrane area is significantly larger than that of UF membranes, interaction of protein with the membrane geometry can play an important role especially under high shear given that protein and membrane are both surface active.

High shear stress at the membrane wall is generally helpful in sweeping away any deposits on the membrane surface. A critical ratio of flux and shear stress (J/τ_w) that determines the balance between convective flow towards the membrane, the tangential erosion (Aubert *et al.*, 1993) and shear induced diffusion (Davis & Leighton, 1987; Belfort *et al.*, 1994) was proposed to minimise fouling in situations where fouling is predominantly by formation of a surface layer or cake. Boyaval *et al.* (1996) during MF of lactic acid bacteria found that fouling was faster and more severe when J/τ_w was greater than a critical value of 1.15 $l\ h^{-1}\ m^{-2}\ Pa^{-1}$. Le Berre and Daufin (1994) previously reported a similar concept and presented a critical value of 1 $L.h^{-1}.m^{-2}.Pa^{-1}$ for MF of skim milk and pretreated whey (Gésan *et al.*, 1995). It is not known why the value is so close with these two feeds tested.

To summarise, shear can cause protein to deposit at the pore entrance or enhance aggregation of protein leading to severe fouling in most cases.

2.8.3. Cross-flow velocity

Sheehan *et al.* (1988) observed that increasing recirculation rate from 60 to 100 litres/minute (corresponding to linear velocities of 0.6 to 1.0 $m.s^{-1}$) produced a roughly 50% increase in flux performance for the same TMP profile during cell separation. However Meireles *et al.* (1990) reported that increase in retentate velocity increased protein aggregation and subsequent fouling during BSA filtration on polymeric membranes. Bowen and Gan (1991) studied the effect of stirring during UF and MF of BSA solutions. They showed that stirring (500 rpm) had little effect on permeate flux when filtering using a 0.2 μm Anopore membrane, whereas using a UF membrane (MW cut-off of 10 kDa) in the same cell under comparable conditions, stirring gave a doubling in the permeation rate. In the case of the UF membrane, both CP and deposition on the front face of the membrane appeared to be important. Stirring had little effect in MF as there is no apparent accumulation of protein deposits on the front face of the membrane. On the other hand, Cakl and Mikulášek (1995) during filtration of acrylic copolymer latex particles on a 0.1 μm membrane showed that increasing CFV did not always increase the flux. In this case the membrane fouled internally. They found a power law relationship between flux and feed velocity in the case of PVC latex particles on a 0.2 μm membrane. In this case fouling was in the form of a cake. Mackley and Sherman (1993) during filtration of a polyethylene suspension found that increased CFV decreased filtrate flux. Increased CFV apparently formed an efficiently packed filter cake leading to low porosity. Tarleton and Wakeman (1993) citing Fischer *et al.* (1986) filtering calcium carbonate slurries reported that the size of the particles in the cake layer decreased as the CFV increased, leading to a reduction of the permeate flux even though the cake thickness was decreased. Li *et al.* (1996) reported MF of *E-Coli* using a ceramic membrane. *E-Coli* formed a surface cake as it is significantly larger than the membrane pore size (0.2 μm). Increasing CFV increased permeate flux.

In conclusion, CFV appears to affect fouling in situations where surface layer formation is the dominant mechanism of fouling. However, its effect was minimal

when fouling was predominantly by an internal pore fouling mechanism except in cases where increased CFV reduced particle size increasing the possibility of plugging.

2.9. Fouling mechanisms

The mechanisms by which fouling may occur are several. These depend on: 1) whether the given protein is smaller or larger than the average pore size of the membrane; 2) the type of protein-protein interactions; and 3) the protein-membrane interactions involved. A single type of mechanism appears to be rare as there is usually a size distribution in both the membrane pores and the macromolecules filtered. Simultaneous occurrence of more than one mechanism is most likely dictating flux and selectivity in MF. As a result, the use of existing fouling models is limited to the analysis of experimental data with quantitative predictions of flux under specific conditions rather than providing an understanding of the fouling phenomenon. A better understanding of the fouling mechanisms is essential if CFMF is to be exploited fully by minimising fouling.

First, fouling mechanisms during filtration of non-protein feeds are considered followed by fouling mechanisms involving proteins. Then the relevance of non-protein fouling to protein fouling will be discussed.

Pore bridging by colloids was reported by Visvanathan and Ben Aïm (1989) while filtering colloids of average diameter 12 nm through a 200 nm pore size membrane. They found that fouling at low applied pressure, was mainly due to a combination of the bridging of colloids over the pore openings and a concentration polarisation layer, which led to external membrane fouling, whereas internal fouling of the membrane played a relatively insignificant role. The bridging was presumably by colloidal aggregates that were formed on the membrane surface apparently due to deposition and clustering. Proteins in similar situations would probably give more severe fouling as protein-membrane and protein-protein interactions are expected to be far more significant.

Tarleton and Wakeman (1993) suggested that fouling by particulates appears to be caused by two independent mechanisms which occur simultaneously. The first accounts for initial sharp decline in permeate flux and is irreversible for all practical purposes and is due to the rapid deposition and capture of finer particles from suspension and their subsequent penetration into the pores of the membrane. The second mechanism is largely reversible and causes further particulate layer(s) to form above the membrane surface in the form of a cake.

Kim *et al.* (1993b) studied the effect of colloid size on the membrane pore size during filtration of dilute silver colloids of size 8.3±2.6 nm using 30, 100, 300 kD and 0.22 μm membranes. Silver particles formed a cake on the small pore size (30 kD) membrane. With the 100 and 300 kD membranes whose pore size was larger than the size of the foulant, severe fouling occurred due to fouling within the membrane pores. However with the 0.22 μm membrane, the retention of silver colloids was low and the small increase in resistance was perhaps due to the deposition of silver particles on the pore walls.

Cakl and Mikulášek (1995) identified two phases of flux decline during constant pressure MF of synthetic colloids. Acrylic copolymer (AC) latex & poly vinyl chloride (PVC) latex particles of average sizes 0.1 μm and 10 μm respectively were processed on 0.1 and 0.2 μm alumina based ceramic membranes respectively. First reversible concentration polarisation built up within the first minute. Then flux continued to decline for up to several hours due to polymer colloid deposition on the front surface of the membrane or within the membrane pores. They concluded that cake formation prevails if the particles are larger than the pores (PVC latex). Increasing feed velocity generally increased permeate flux. A different fouling behaviour occurred if the active layer pore size was very close to the diameter of the particles filtered (AC latex particles). Increasing the feed velocity had a limited effect. If the particles are sufficiently flexible, they can enter the pores and remain inside probably due to some interaction with the membrane material. When the feed velocity was sufficiently low (<1 m/s), the period of pore filling was short. The pores may be only slightly entered by particles which then begin instantly to bridge over the pore and form a very thin filter cake. This deposit can be taken as a secondary

membrane which protects the MF membrane against plugging by particles and therefore contributes to a higher and more stable flux. At higher velocities (>1 m/s), smaller particles can block the pores reducing flux.

Boyaval *et al.* (1996) during MF of *Lactobacillus helveticus* (rods of 1-5μm in length and 0.5 -1 μm in diameter) using 0.2 μm alumina membranes, found that fouling was due to a reasonably constant irreversible layer formation and to a dominant reversible cell cake under various permeate fluxes investigated. Dominant reversible cake was due to the total rejection of the cells by the membrane. This work suggests that when microbial cells of size much bigger than the membrane pore size are filtered, a constant amount of irreversible fouling predominantly by adsorption and a dominant reversible surface layer or cake occurs.

The effect of pore size on the type of fouling mechanism was reported by Dal-Cin *et al.* (1996). They found that membrane fouling was the result of varying combinations of adsorption, pore plugging and concentration polarisation or gel layer formation during UF of a pulp mill effluent containing particles of a broad size range 0.1 to ~37 μm using membranes of molecular weight cut-off (MWCO) range 300 Da to >500 kDa. They hypothesised that surface adsorption was likely for lower MWCO membranes and that the adsorbed layer was controlling membrane performance whereas adsorption seemed to have caused pore narrowing with higher MWCO membranes.

To summarise fouling by non protein materials, fouling within the membrane or as a cake layer occurs depending upon whether the particle size is larger or smaller than the pore size. Colloidal aggregates may form bridges over pore openings leading to pore constriction and formation of a cake. In some cases, formation of a surface layer (secondary membrane) can protect against pore plugging. With silver colloids, the fouling mechanism changes from deposition within the membrane pores to formation of a cake as the pore size is decreased. It appears that fouling by less interactive non-protein feeds is dominated by deposition and cake formation whereas with proteins additional factors like their interactions with the membrane and themselves play a major role. At least in theory, more severe fouling is expected with proteins

compared to less interactive non-protein feeds. Nevertheless, knowledge of non-protein fouling could form a basis for understanding protein fouling. The aggregation tendency of proteins is an important aspect that must be considered with protein fouling.

Meireles *et al.* (1990) suggested that in general, membrane fouling by proteins can be due to a combination of native protein adsorption and of aggregate deposition, the latter being sensitive to operating conditions, the former dependent on the concentration of the protein.

Adsorption and pore plugging played a significant role on 35 and 100 nm pore size alumina membranes to form a gel layer during filtration of BSA under constant pressure conditions (Clark *et al.,* 1991). They attributed gel layer formation to protein-protein interactions. van Reis *et al.* (1991) while separating proteins from mammalian cells using a 0.2 μm polypropylene membrane, hypothesised the following flux decline mechanisms: 1) adsorption; 2) denaturation due to protein-membrane interaction or the generation of air-liquid interfaces; and 3) retention by molecular sieving due to aggregates in the feed or aggregates produced during filtration itself.

Severe fouling by BSA on a range of membranes of various pore sizes was reported by Jonsson *et al.* (1992a). Adsorption of BSA had a major role on a 20 kD polysulfone membrane. However, with 0.2 and 1 μm pore size membranes when exposed to BSA under static conditions, adsorption alone caused a very small drop in flux. However, under low pressure filtration (0.1-0.5 bar), very severe fouling occurred showing that pore plugging dominated fouling with these membranes. Using SEM, they have demonstrated that fouling occurred on the front face of the membrane. It is interesting to note this behaviour with BSA as severe fouling has been observed with a range of pore sizes. Its fouling behaviour under constant pressure operation has been reported as a two step process (Jonsson *et al.,* 1996). It fouled internally first and later a surface layer formed. They suggested that BSA interacts with the membrane structure and finally forms a surface layer. It is not clear whether this behaviour is specific to BSA. Its fouling behaviour under constant flux

conditions will be interesting to investigate as low initial TMPs might avoid TMP induced protein-membrane interactions and subsequent fouling.

Larger aggregates can actually reduce the fouling effect if they are bigger than the pore size as reported by Tracey and Davis (1994). They used the lumped terms "external fouling" and "internal fouling" during MF. External fouling refers to fouling due to the accumulation of protein aggregates that do not enter the pores, whereas internal fouling refers to fouling within the internal pore structure of the membrane due to adsorption and deposition. They hypothesised that during MF of very dilute BSA solutions (0.01%) using a 0.2 μm polycarbonate membrane, fouling occurred at the mouths of the pores, slowly closing off pore entrance while allowing complete transmission of protein for a period of time. Eventually, the pores became so constricted that protein transmission decreased and a layer of rejected protein formed on the external membrane surface. However with 0.1% solutions of BSA on the same membrane, fouling allowed for nearly complete transmission of proteins for the entire length of the experiments. It was hypothesised that protein aggregates that form at 0.1% concentration are larger than aggregates formed at 0.01% concentration. The larger aggregates did not pass through the membrane and so the fouling was external. The rejected layer of larger protein aggregates was apparently packed loosely enough to allow protein monomers to pass through. For dilute protein solutions, it is proposed that aggregates are sufficiently small to enter the pore mouth and cause internal fouling eventually plugging the pores reducing protein transmission.

Similar results showing that larger aggregates can minimize fouling under certain conditions were also reported by Gésan *et al.* (1995). They demonstrated the benefits of a modified pretreatment method to minimise fouling for clarifying whey by MF. Longer MF operation and better whey clarification due to larger calcium phosphate particles and lower calcium and phosphate content in the aqueous phase were achieved as a result of modified pretreatment. Loosely structured deposits caused by larger complex-lipid-calcium phosphate particles on the membrane surface, increased the permeation rate of deposits enhancing MF fluxes.

Initiation of fouling by aggregates present in the feed seems to be the key factor in determining the fouling mechanism (Kelly & Zydney, 1995, 1997). The fouling behaviour of BSA, cysteinylated BSA, ovalbumin, lysozyme, pepsin, myglobin, α-lactalbumin & β-lactoglobulin on a 0.22 μm pore size PVDF membrane under constant pressure conditions was studied (Kelly & Zydney, 1997). Fouling was severe with β-lactoglobulin and ovalbumin and lighter with lysozyme while other proteins behaved in the intermediate range. Lysozyme was smallest of all in molecular weight. They reported two distinct mechanisms 1) deposition of large protein aggregates and 2) chemical attachment of native proteins to the growing deposit. The chemical attachment was found to occur via the formation of intermolecular disulfide linkages involving a free sulphydryl group in the native protein. Among the proteins investigated, β-lactoglobulin fouled severely although it has only one free sulphydryl group. They attributed the fouling to the reactivity of the sulphydryl groups rather than the number of groups. Ovalbumin also fouled severely even though it does not have any free sulphydryl group. They attributed the fouling behaviour of ovalbumin to aggregation by other mechanisms such as hydrophobic interactions.

Daufin and Merin (1995) attributed fouling during MF of pretreated whey at constant flux to pore blocking. This phenomenon exhibited itself as a slow increase of fouling resistance (R_f) with time, in which pores were progressively blocked until a point when the remaining active filtering layer must compensate for blocked pores by filtering larger volumes. The inevitable consequence was a fast fouling which was expressed in a sharp increase of R_f until the limiting value. As a consequence, a fast decrease in protein transmission was also observed. The fouling mechanism was described as a two step process and the fouling was composed of two layers: monomer proteins- as irreversible fouling on the membrane surface and some deeper in the membrane itself, and aggregates- as the reversible fouling layer which was the major contributor to the overall fouling.

Two steps in the fouling mechanism, surface blocking and cake formation were proposed by Jonsson *et al.* (1996) during MF of BSA. Using air permeation porometry, they studied the porous matrix formed as a deposited cake on the retentate

layer of the membrane. They found that when BSA solution was prefiltered, the surface blocking step had slower kinetics suggesting that prefiltration can only delay fouling but cannot prevent it.

Prádanos *et al.* (1996) when filtering proteins (pepsin, BSA, lipase, γ-globulin and invertase) with molecular weights of 36, 67, 80, 150, and 270 kDa respectively using a 0.02 μm Anopore membrane under constant pressure, found that initial particularly intense flux decline was due to external pore plugging followed by an internal deposition (partially retained proteins) or the formation of a cake (totally retained proteins). Flux decline can be divided into two successive steps separated by a narrow transition zone. When retention is appreciable a first step of pore plugging appears accounting for the major part of the permeate flux decline. The nature of the slower second step in fouling depends on the ratio of the protein size to the mean pore size. When this ratio is far over 1, the retained proteins form a cake on the top of the active side of the membrane. Under other conditions the inner surfaces of the unblocked pores are covered by adsorbed proteins leading to a standard blocking model.

Mueller and Davis (1996) during fouling studies using BSA on 0.2 μm MF membranes showed that internal fouling (caused by adsorption and deposition within the pores) of the membranes was followed by external fouling (the build up of a deposit layer on the membrane surface). Internal fouling was characterised by a concave up resistance versus time curve and nearly complete transmission of protein. During internal fouling it is hypothesised that protein molecules or aggregates attach to the pore walls or mouths so that flux is reduced due to the resulting pore blockage and constriction. External fouling is characterised by a concave down total resistance versus time curve and significant protein rejection.

Herrero *et al.* (1997) studied flux decline during dead-end MF of BSA solutions of different concentrations (1, 3, 5 and 10 g/L) through cyclopore track-etched polycarbonate membranes (nominal pore size 0.1 μm). They found a two step fouling process, a rapid initial internal blocking, strongly dependent on operation parameters, and a final stage of external blocking with lower sensitivity of the flux behaviour on

operation conditions. Pore size distribution studies on the protein adsorbed membrane showed that the initial internal pore blocking can be attributed to protein adsorption while the longer term fouling should be caused mainly by solute-solute interactions. Gan *et al.* (1997) during MF of beer on ceramic membranes found that enhanced surface hydrodynamics through flow pulsation had little impact on flux suggesting that pore blocking by in-depth adsorption/deposition was the dominant factor.

Palecek and Zydney (1994a) during MF of BSA, Immunoglobulins, hemoglobin, ribonuclease A and lysozyme on a 0.16 μm PES membrane found that flux decline was due to the formation of a protein deposit on the upper surface of the membrane. They found that the quasi-steady state fluxes at the individual protein IEP were essentially identical despite the large differences in molecular weights and physicochemical properties of these proteins. The flux decline was explained in terms of a simple physical model in which the protein deposit continued to grow and thus the flux continued to decline until the drag force on the proteins associated with the filtrate flow was no longer able to overcome the intermolecular repulsive interactions (predominantly electrostatic) between the protein in the bulk solution and those in the deposit. Pierre *et al.* (1994) reported pore plugging in larger pores by colloidal aggregates during MF of pretreated wheys using 0.14 μm carbosep membranes. Belfort *et al.* (1993) studied MF fouling using a rotating annular membrane filter module. They attributed fouling to pore narrowing and plugging during MF of BSA using a 0.45 μm hydrophilized polysulfone membrane. Marshall *et al.* (1996) compared fouling during UF and MF of skim milk. Severe fouling in MF was dominated by the formation of a hydrodynamically controlled surface layer of "casein" whereas in UF, fouling appeared to be by adsorption and multi layer deposition as indicated by high irreversible fouling resistance.

Marshall *et al.* (1997) during MF of β-lactoglobulin under constant flux conditions demonstrated that fouling occurred in the immediate vicinity of the pore entrance. They hypothesised that shear forces on the protein caused denaturation and aggregation leading to deposition at the pore entrance.

2.10. Summary of literature review

There is considerable experimental evidence in the literature that proteins (particularly BSA) which are much smaller (<10 nm) than the pore size can successfully plug the pores of 100-200 nm membranes. A summary of recent MF fouling studies using proteins is given in Table 2.1. There seem to be several arguments to explain severe fouling of MF membranes by proteins. The general consensus is that the protein must be aggregated, hence cannot permeate through the membrane. Aggregated protein plugs the membrane pore causing severe fouling. Aggregation may be caused by 1) shear at the pore entrance leading to conformational changes or partial denaturation of the protein and subsequent aggregation (Bowen & Gan, 1991) or 2) thiol-mediated reaction (Kelly & Zydney, 1994, 1997) or 3) some other form of protein-protein interaction (Bowen & Gan, 1991; Marshall *et al.,* 1997; Herrero *et al.*, 1997).

To summarise the possible fouling mechanisms from the current body of literature, protein adsorption is the first step in the fouling process, although its effect is small on MF membranes. Pore fouling is usually the second step. Pore fouling appears to be dominated by pore plugging most probably at the pore entrance by aggregates that are present in the feed or those produced during processing (Kelly & Zydney, 1994; Marshall *et al.,* 1997). Protein to pore size ratio seems to be an important factor determining this step. Surface layer formation or accumulation of protein aggregates on the membrane surface as a third step appears to follow once the MF membrane pores are completely plugged or covered by protein deposition. The surface layer could be in the form of a gel layer, if the protein size is much bigger than the pore size and if there are protein-protein interactions. The situation is exacerbated under high flux conditions apparently due to concentration-induced effects. A filter cake is formed if the interactions between the protein molecules are minimal. A surface layer may sometimes result from polymerisation or aggregation of solute on the membrane surface due to supersaturation of solute at higher fluxes. Generally with the smaller proteins, all these steps occur with time when fouling is severe. Pore fouling is generally a prerequisite for step 3) to occur as MF membranes are generally non retentive to most proteins. However, with larger proteins perhaps only steps 1 and 3 occur omitting step 2.

Two important factors that appear to influence MF fouling mechanisms are i) protein to pore size ratio and ii) effect of TMP or permeate flux.

Protein to pore size ratio is very important in determining the type of fouling mechanism. In the UF pore size range, adsorption and multilayer deposition is predominant whereas in MF, fouling mechanisms change from surface layer to internal membrane fouling (pore plugging to pore narrowing) as pore size is increased. Fouling is minimal under low TMP, and constant flux MF operation appears to be better than the constant pressure process to minimise fouling.

2.11. Need for further studies

The bulk of the previous studies on MF membrane fouling have been performed in stirred cells using constant TMP operation. It has been shown that MF operation in constant flux is better than constant TMP mode. Very few proteins have been studied in cross-flow, constant flux conditions. These are the conditions that are industrially relevant. The majority of the fouling studies were performed using BSA under controlled conditions. BSA fouling on MF membranes has been largely attributed to protein aggregation. Several investigators have hypothesised that BSA aggregates under shear. Also BSA has been reported to foul on a range of various pore size membranes. BSA is a relatively reactive protein macromolecule (Foster, 1977) and its fouling behaviour may not be typical of other proteins. It would be interesting to study whether other proteins also behave in similar way.

Though aggregation of protein was primarily linked to thiol-mediated interactions (Kelly & Zydney, 1994, 1997), the mechanism of aggregation for proteins that do not have free sulphydryl groups was not clear. It is also not clear whether aggregation under shear conditions is likely with other proteins. Actual mechanisms of protein aggregate formation under shear need further investigation using different proteins to develop broad strategies to prevent fouling. The main thrust of the present work is therefore to study a range of proteins under industrially relevant conditions, *i.e.* cross-flow and constant flux, and BSA was included in the present work as a link to so many other published studies.

To conclude, there are many factors contributing to MF membrane fouling, *e.g.* membrane surface properties (chemistry, morphology etc), operating conditions, solution environment and feed properties. There is a need for further studies to elucidate fouling mechanisms with a range of proteins. Hence, the effect of feed properties particularly physicochemical properties of proteins, the effect of flux/TMP and to some extent, the effect of solution environment on MF fouling under cross-flow, constant flux conditions were studied in the present work.

Table 2.1. Previous studies on MF fouling using proteins

Reference	Membrane pore size (μm)/ material	Device/operation mode/driving force	Proteins	Fouling behaviour	Key findings & remarks
Tracey & Davis (1994)	0.2 & 0.05 PC	Stir cell/constant pressure/air pressurisation	BSA	Internal (pore plugging & narrowing) and external (surface layer) fouling	Predominantly internal fouling with larger pore membrane and immediate external fouling with smaller pore membrane. With higher protein concentration on larger membrane, fouling was predominantly external giving stable protein transmission. Fouling at the mouth of the pore is hypothesised.
Palecek & Zydney (1994a, b)	0.16 PES	Stir cell/constant pressure/air pressurisation	BSA, immunoglobulins, hemoglobin, ribonuclease A & lysozyme	Protein deposition on the upper surface of the membrane	There was no apparent dependence of flux on protein molecular weight or on the specific physical and/or chemical characteristics of the proteins at their isoelectric pH. Deposition at solution pH away from the protein isoelectric point is influenced by protein surface charge density with flux increasing linearly with the square of the charge density.
Kelly & Zydney (1995)	0.22 PVDF	Stir cell/constant pressure/nitrogen pressurisation	BSA	Two stage fouling mechanism: convective deposition and surface layer formation via intermolecular disulfide linkages	Fouling is predominantly by aggregates on the surface of the membrane. Prefiltration reduced fouling. Fouling was essentially eliminated with prefiltered cysteinyl BSA. However unfiltered cysteinyl BSA still fouled.
Bowen & Hall (1995)	0.1, 0.2 & 0.8 PC	Unstir cell/constant pressure/nitrogen pressurisation	Enzyme yeast alcohol dehydrogenase (YADH)	Combination of pore plugging and surface deposition	In-pore plugging at low enzyme concentration and deposition on the front face of the membrane at high concentration were observed.
Jonsson *et al.* (1996)	0.2±0.1 PS, PP & 0.9±0.1 PES	Crossflow rig/constant pressure/pumping	BSA	Two consecutive steps: pore plugging at the surface and cake formation	Fouling is delayed/slower when the applied pressure is set up by a liquid column rather than pumping. Prefiltration delayed fouling. Flux recovery was low for the membranes fouled under pump pressures. Hypothesised that pumping caused slight denaturation of BSA which led to more severe fouling.

Mueller & Davis (1996)	0.2 PC, CA, PS, PVDF & 0.5 PE & PP for surface modification studies	Stir cell/constant pressure/air pressurisation	BSA	Two steps: internal (pore plugging or constriction) and external (build up of deposit layer on the membrane surface) fouling	Low surface porosity membranes fouled externally almost immediately whereas high surface porosity membranes showed a significant period of time for internal fouling to occur. Protein transmission stayed almost constant during internal fouling. Significant loss of protein transmission occurred during external fouling. Surface modifications to membranes improved long term fluxes. Increased TMP and feed concentration generally increased fouling resistance.
Prádanos *et al.* (1996)	0.02 Alumina	Crossflow/constant pressure/pumping	BSA, pepsin, lipase, γ-globulin, invertase	When the retention is appreciable, the first step of pore plugging occurs and the nature of second step depends on ratio of protein to mean pore size. When this is >1, the retained protein forms cake otherwise deposition on the unblocked pore walls	Formation of cake with totally retained proteins whereas with partially retained proteins, pore plugging and deposition of permeable proteins within the pores occurred. Increase in molecular weight of the protein generally increased fouling.
Marshall *et al.* (1996)	0.1 PS	Crossflow/ constant flux/pumping	skim milk	If the casein concentration at the wall is >ca.18%, casein gel layer formed on the membrane surface	MF fouling using skim milk is dominated by the formation of a hydrodynamically controlled surface layer rather than by protein adsorption as in UF. Constant flux MF operation was found to be beneficial.
Le Berre & Daufin (1996)	0.1 Zirconia	Crossflow/constant flux/pumping	skim milk	When ratio of flux to wall shear stress is less than a critical value, a cake of casein micelles was formed on the membrane surface	The importance of flux and wall shear stress is demonstrated to minimise surface layer formation by casein micelles.

Güell & Davis (1996)	0.2 PS, PC, PVDF & CA	Stir cell/constant pressure/air pressurisation	BSA, lysozyme, ovalbumin separately & their mixtures	Internal (adsorption & deposition on the pore walls and mouths) & external (cake layer on the surface) fouling	BSA and lysozyme fouled internally whereas ovalbumin showed an initial phase of internal fouling followed by external fouling. Fouling was apparently not related to the size of the individual protein but instead to the aggregates that each protein forms. For different binary mixtures, severe fouling was observed with protein mixtures containing ovalbumin. With ternary mixtures, rapid external fouling occurred particularly on lower surface porosity membranes.
Herrero *et al.* (1997)	0.1 PC	Dead end cell/constant pressure/pumping	BSA	Two successive steps: internal deposition followed by pore plugging & external surface fouling	Porometric studies reveal shift to narrow mean pores at the beginning of the fouling process followed by a decrease in porosity during external fouling.
Kim *et al.* (1997)	0.01, 0.03, 0.05, 0.1 & 0.2 PC	Unstir cell/constant pressure/pumping	BSA	Pore bridging and eventual coverage of the surface	Small pore membranes fouled by adsorption whereas larger membranes fouled by deposition at the pore entrance and eventual surface coverage.
Marshall *et al.* (1997)	0.1 Zirconia	Crossflow/constant flux/pumping	β-lactoglobulin	Protein deposition occurred near the pore entrance	Shear forces on the protein perhaps caused denaturation, aggregation and pore narrowing in the vicinity of the pore entrance.
Kelly & Zydney (1997)	0.22 PVDF	Stir cell/constant pressure/nitrogen pressurisation	BSA, cys-BSA, ovalbumin, lysozyme, β-lactoglobulin, pepsin & myoglobin	Two step mechanism: deposition of larger protein aggregates and chemical attachment of the native proteins to the growing deposit via intermolecular disulfide linkages involving free thiol groups in the native protein	Flux decline is independent of size of the protein, but dependent on the ability of the protein to form aggregates via disulfide linkages. The actual rate of intermolecular thiol-disulfide interchange reaction is dependent on the reactivity (not number) of the free sulfhydryls, as well as nature of the existing disulfide bonds and the steric accessibility of the reacting moieties. Myoglobin does not have any disulfide bonds or free thiol groups, hence its deposition is attributed to occur via hydrophobic interactions. Fouling is primarily by deposition of aggregates on the membrane surface. Internal fouling is not considered.

Table Nomenclature:

CA	Cellulose acetate	PP	Polypropylene
PC	Polycarbonate	PS	Polysulfone
PE	Polyethylene	PVDF	Polyvinylidene fluoride
PES	Polyethersulfone		

3.Theoretical and quantitative aspects of membrane processes

3.1. Introduction

Microfiltration has been known for quite a long time in its dead end mode. In its first application stage, the primary objective was to separate the majority of large particles from solution. Fouling was a major problem giving very low permeate fluxes in the dead end mode. More recently a lot of interest has been shown in CFMF in which feed flows transverse to the membrane surface reducing any surface layer, thus achieving relatively high fluxes compared to the dead end mode. However high fluxes have been sustained only over a short period of time due to concentration polarisation and membrane fouling leading to flux decline sometimes to a level lower than that obtained in UF. To exploit the full potential of CFMF technology, there is current research interest in investigating ways of minimising membrane fouling during CFMF.

3.2. Polarisation in filtration

In membrane processes involving filtration of proteins, permeate flux is always lower than pure water flux. The flux increases with increasing TMP up to a limiting value and then reaches an essentially constant value independent of pressure. The value which is known as the limiting flux can be increased by either increasing CFV or decreasing feed concentration or both (Michaels, 1968; Blatt *et al.,* 1970). The pressure independence of flux is generally explained by CP. There are several mathematical models to describe CP. The film model is the most common one in the literature to mathematically quantify the effect of concentration polarisation on flux decline in UF. When steady state is reached the solvent flux and the thickness of the boundary layer remain constant with time. At this steady state, the convection of the retained molecules to the membrane wall by the filtrate flux J is balanced by its diffusion away from the wall in response to the concentration gradient, (dC/dy) and by loss of solute to the permeate.

At the steady state the mass balance gives,

$$JC_b - \frac{D.dC}{dy} - JC_p = 0 \qquad \textbf{(3.1)}$$

where D is the diffusivity of the retained solute.

Integrating across the boundary layer thickness (δ),

we get an equation for the permeate flux

$$J = (\frac{D}{\delta}).\ln\frac{C_w - C_p}{C_b - C_p} \quad \textbf{(3.2)}$$

The quantity $(\frac{D}{\delta})$ is known as mass transfer coefficient (k) which is dependent on the solute properties and equipment type. C_w, C_b and C_p are the solute concentration at the membrane wall, in the bulk solution and in the permeate respectively. Any factors that reduce (δ) such as increasing turbulence, increasing shear rate by using increased cross-flow velocity or other turbulence promoters (*e.g.* thin channels) will increase the mass transfer coefficient thereby reducing CP. Increasing the diffusion coefficient (D) or reducing the viscosity μ by increasing the temperature will also increase the mass transfer coefficient. k and C_w are generally unknowns. k can be estimated using the Chilton-Colburn equation (Gekas & Hallström, 1987).

For the turbulent region,

$$k = 0.023.(\frac{\rho}{\mu})^{0.47}.D^{0.67}.\frac{u^{0.8}}{d^{0.2}} \quad \textbf{(3.3)}$$

where ρ is the permeate density and d is the retentate channel diameter.

Under turbulent conditions, the cross-flow velocity (u) has the most influence on the mass transfer coefficient and offers the most potential for minimising polarisation.

The film model has been found to predict the performance of protein UF reasonably well. However, when applied to the MF of small particles, permeate fluxes are predicted to be as much as two orders of magnitude lower than experimentally observed. The predicted fluxes are too low because the value of the diffusion coefficient, which is inversely proportional to the particle radius, is quite low and understates the movement of particles away from the membrane. See section 2.3 for more details.

3.3. CP models

Flux limitation by CP is generally described by either osmotic effects or hydrodynamic flow resistance offered by the boundary layer. The two most widely used models are the gel and osmotic models.

The gel model assumes the formation of a gel layer on the membrane surface offering an increased resistance to flow so that there is no further effect of TMP on permeate flux. The idea was first proposed by Michaels (1968) that the limiting flux is reached when the wall concentration reaches a physical maximum which is the gel concentration for the solute molecules under consideration. Further increases in TMP will only increase the thickness of the gel layer with no effect on permeate flux. Experiments in which the permeate flux becomes independent of the TMP lend support to the gel model. Although the gel model is useful in correlating filtration data, there are contradictory observations reported by various investigators. For example, permeate flux versus $\ln(C_b)$ plots are linear for a given solute, but when extrapolated to zero flux to get the gel concentration, the C_g intercept is not always constant and found to vary with bulk concentration and cross-flow velocity (Nakao *et al.*, 1979). Gel concentration is found to be not always equal to the realistic concentration at which a gel layer would form (Le & Howell, 1984). Also the mass transfer coefficient obtained from the slope of the plot does not necessarily agree with the value obtained by equation **(3.3)** (Nakao *et al.*, 1979). Furthermore, solutions of concentration C_g often display considerable fluidity and are below the solubility limit of the solute (Nakao *et al.*, 1979; Isaacson *et al.*, 1980; Jonsson, 1984).

The osmotic pressure difference that develops across a boundary layer reduces driving force at the membrane. At a typical feed concentration used in membrane processes, macromolecules have negligible osmotic pressure and so are generally ignored for flux calculations. However at the wall, the concentration of the solute may be high enough to make the osmotic pressure significant in comparison to the TMP even with high molecular weight solutes (Vilker *et al.*, 1984). The effect of osmotic pressure can be described as follows. The presence of solute molecules in a solution lowers its chemical potential relative to that of the permeate. A TMP greater

than the osmotic pressure is required for solvent to flow from the retentate to the permeate and the consequence of the osmotic pressure difference across the membrane is a reduction in the effective TMP driving force.

The osmotic pressure for an ideal, dilute solution can be found from van't Hoffs equation

$$\pi = \frac{R_g.T.C}{M} \qquad \textbf{(3.4)}$$

Where R_g is the gas constant, T is the absolute temperature, C is the component concentration and M is the molecular weight.

According to the van't Hoff equation of osmotic pressure, the smaller the component the greater is its contribution to the osmotic pressure of the liquid. Thus, the contribution from macromolecules like proteins should be small. However, several researchers have pointed out that the osmotic pressure of protein containing solutions increases exponentially rather than linearly with concentration. Thus when protein is concentrated near the membrane surface, the osmotic pressures may be comparable with typical TMPs and have significant impact on flux (Goldsmith, 1971; Vilker *et al.,* 1984; Jonsson 1984).

Wijmans *et al.* (1985) have shown that the increase in osmotic pressure from the bulk to the wall is equivalent to a hydraulic resistance in series with the membrane provided the solvent and solutes are in local thermodynamic equilibrium at all points within the boundary layer.

In summary both the models are similar mathematically and both essentially describe the reversible changes in TMP or membrane resistance typically seen in UF.

3.4. Membrane fouling models

3.4.1. Fouling resistance model

Membrane fouling can be described as a deposit formed on the membrane surface plus additional resistance due to internal pore fouling. A fouling resistance model using resistances in series can be derived from Darcy's law.

Accordingly, when a pure solvent like water is filtered through a membrane, the permeate flux (J) is proportional to the applied transmembrane pressure.

$$J = \frac{\Delta P_{TM}}{\mu_P R_m} \tag{3.5}$$

The effect of fouling is generally calculated as an extra resistance (R_f) in addition to the resistance of the membrane (R_m).

$$i.e.\ J = \frac{\Delta P_{TM}}{\mu_P (R_m + R_f)} \tag{3.6}$$

R_f is often broken up into reversible fouling (R_{rf}), which is the fouling removed by flushing with water, and irreversible fouling (R_{if}), which is removed by chemical cleaning. Writing Darcy's law in terms of total fouling resistance (R_f), we get

$$R_f = \frac{\Delta P_{TM}}{\mu_P . J} - R_m \tag{3.7}$$

where μ_P is the viscosity of the permeate.

The equation can be modified to account for the osmotic pressure of the solute (assuming an osmotic model).

$$R_f = \frac{(\Delta P_{TM} - \Delta\Pi)}{\mu_P . J} - R_m \tag{3.8}$$

3.4.2. Cake layer model

Deposition in the form of a cake layer on the membrane surface during dead-end or unstirred cell filtration can be written in the following form.

$$R_f = \alpha_f . \frac{V . C_b}{A_m} \tag{3.9}$$

where α_f is the intrinsic resistance of the cake and V is the volume of filtrate passed. The specific cake resistance is often estimated from the Carman-Kozeny equation for a packed bed of rigid particles. On the basis that a globular protein can be approximated by a sphere α_f is given by

$$\alpha_f = \frac{180.(1-\varepsilon)^2}{\rho_p . d_p^{\,2} . \varepsilon^3} \tag{3.10}$$

where ε is the porosity or void fraction and d_p is the mean diameter of particles being filtered.

Kimura and Nakao (1975), Chudacek & Fane (1984) and Aimar *et al.* (1988) have considered the effect of stirring on cake formation. Assuming that the removal of cake is constant and equal to the convective solute transport at steady state ($J_{ss}.C_b$) then the fouling resistance can be calculated from,

$$R_f = \alpha_f . C_b . (\frac{V}{A_m} - J_{ss}.t) \qquad \textbf{(3.11)}$$

3.4.3. Pore restriction model

Deposition within the pore is considered in this model. Fouling is assumed to be uniform throughout the membrane pore. An apparent membrane pore size due to fouling can be calculated from this type of model. Based on work by Ferry (1936) and Zeman (1983), the apparent membrane pore size can be calculated from the fouling resistance using the following equation:

$$d_{pore} = d_{pore_o} \left[\frac{Rm}{Rf + Rm} \right]^{0.25} \qquad \textbf{(3.12)}$$

where d_{pore_o} is the diameter of the membrane pore under unfouled conditions.

3.4.4. Protein deposition models

Bhattacharyya *et al.* (1979), Probstein *et al.* (1981), Suki *et al.* (1984) and Aimar *et al.* (1986) proposed mathematical models that describe protein deposition with respect to time of filtration. They can be written as first order kinetic equations either in terms of protein deposition or fouling resistance. The basic form of this equation is ;

$$\frac{dR_f}{dt} = k_d (R_f^* - R_f) \qquad \textbf{(3.13)}$$

where R_f* is the plateau membrane resistance and k_d is the rate constant,

So $R_f = R_f^*.(1-e^{-k_d t})$ **(3.14)**

3.5. Protein transmission calculations

Protein transmission was calculated using the following equation:

Protein Transmission (%) = $T_r = \frac{C_p}{C_r} x100$ **(3.15)**

where $\mathbf{C_r}$ and $\mathbf{C_p}$ are the protein concentrations in the retentate and permeate.

Protein transmission can also be related via solute rejection (R) to the ratio of the molecular diameter of the species and the pore diameter using a size exclusion model as proposed by Ferry (1936).

i.e. $1\text{-}T_r = R = [\lambda(\lambda\text{-}2)]^2$ for $\lambda < 1$ **(3.16)**

$= 1$ for $\lambda \geq 1$

where λ is the ratio of species diameter to pore diameter.

3.6. Pore blocking laws

Macromolecular fouling on the surface of an UF membrane is generally explained by CP. However during MF of protein solutions, proteins are much smaller than the pores and generally do not concentrate on the membrane surface. Hence pore blocking laws have been used to explain this type of fouling mechanism within the membrane pores.

A series of blocking laws that correlate filtration data (TMP or permeate flux) to time or permeate volume were given by Grace (1956), Hermia (1982) and Hlavacek & Bouchet (1993).

Thus, for constant rate (flux) filtration,

$$\frac{d(\Delta P)}{dV} = k(\Delta P)^n \quad \textbf{(3.17)}$$

Equation **(3.17)** states that rate of change of TMP with respect to volume filtered is proportional to the instantaneous filtration TMP raised to a power which is dependent

on the mode of filtration. The value of n defines whether the filtration is occurring by the standard blocking law (pore narrowing), the complete blocking law (pore plugging) or the intermediate law. The value of k for a particular mode of filtration depends on the system, the filter medium and the condition of the filtration. The values of n are 3/2 for standard blocking, 2 for complete blocking and 1 for intermediate blocking. By integrating equation (3.17) and substituting q.t for V, various blocking models are obtained.

3.6.1. Pore narrowing model (Standard blocking law)

In deriving the pore narrowing model it is assumed that pore volume decreases proportionally to the filtrate volume by particle deposition on the pore walls.

$$\frac{R_m}{R} = \frac{\Delta P_o}{\Delta P} = (1 - K_s.q.t)^2 \qquad \textbf{(3.18)}$$

where q is the filtration rate. K_s is a constant and is given by

$$K_s = \frac{M_s}{N.\pi.L.r_o^2} \qquad \textbf{(3.19)}$$

where M_s is the mass of protein deposited per unit volume of permeate, N is the number of pores, r_o is the pore radius and L is the pore length.

Plotting $\sqrt{(\frac{R_m}{R})}$ vs t from the fouling data would give a straight line if a pore narrowing model holds good.

3.6.2. Pore plugging model (Complete blocking law)

The physical mechanism of this model is that each solute particle coming into contact with the membrane plugs perfectly one pore and no super imposition of particles is possible. The reduction of active surface is thus proportional to the volume of filtrate.

$$\frac{R_m}{R} = \frac{\Delta P_o}{\Delta P} = (1 - K_p.t) \qquad \textbf{(3.20)}$$

where K_p is given by:

$$K_p = \frac{M_p.\pi.r^4.\Delta P}{8.\mu.L} \qquad (3.21)$$

where M_P is the proportion of the feed material that actually plugs the pores. In other words, M_p can be considered as a measure of "plugging tendency" for the feed material. Plotting $(\frac{Rm}{R})$ vs t would give a straight line if pore plugging is the fouling mechanism.

3.6.3. Intermediate law

The detailed theoretical background for the intermediate law is given by Hermia (1982).

$$\ln\left[\frac{R}{Rm}\right] = \ln\left[\frac{\Delta P}{\Delta P_o}\right] = K_i.q.t \qquad (3.22)$$

where $K_i = \frac{A_i}{A_o}$ (3.23)

where A_i is the area of the membrane blocked per unit volume of permeate. A_o is the original area of the membrane (Hermia, 1982).

Plotting $\ln\left[\frac{R}{Rm}\right]$ vs t would give a straight line if the intermediate law is valid.

In deriving these blocking laws, membrane surface is assumed to have pores of constant diameter and length. This is not necessarily true with most configurations of existing membranes except for capillary pore membranes.

3.7. Miscellaneous calculations

The shear stress at the membrane wall was calculated from:

$$\tau_w = \frac{de}{4} \times \frac{\Delta P_L}{L} \qquad (3.24)$$

where d_e is the equivalent diameter of the channel, ΔP_L is the retentate side pressure drop and L is the distance between pressure tappings. The ΔP_L quoted in this thesis

(used in the shear equation) was that measured during the first few minutes of filtration as this is considered as the most critical time for membrane fouling.

For the purpose of calculating the membrane resistance the viscosity was assumed to be the same as that of water. The following equation (Marshall, 1994) based on regression analysis of the viscosity of water in the range 10 to 60 °C was used to account for variations in the permeate viscosity (Pa.s) due to temperature.

$$\mu = \frac{2.428 - 0.4788.\ln(T)}{1000} \qquad \textbf{(3.25)}$$

Temperature is in °C

Diffusivity was estimated using the following equations:

1) Based on molecular weight of the protein as proposed by Young *et al.* (1980);

$$D = \left[\frac{1.45 \times 10^{-14}}{M}\right]^{1/3} \qquad \textbf{(3.26)}$$

Where M is molecular weight of the solute in daltons

2) Based on Stoke-Einstein relation;

$$D = \frac{k_b T}{6.\pi.\mu.r} \qquad \textbf{(3.27)}$$

where k_b is the Boltzmann constant and is 1.38 x 10^{-23} J/K, T is the absolute temperature K, μ is the viscosity Pa.s and r is the radius of the solute m.

Fouling rate, which is rate of increase in fouling resistance over a period, is calculated using following equation:

$$\text{Fouling rate} = \frac{1}{R}.\frac{dR}{dt}, \text{ Units in } s^{-1} \qquad \textbf{(3.28)}$$

4. Materials and methods

4.1. Membrane rig and preparation

Experiments were conducted on a computer-controlled cross-flow membrane rig developed by Marshall (1994) but using a tubular membrane module rather than a flat sheet one. A flow diagram of the CFMF rig is shown in Fig. 4.1. A centrifugal pump (Model CRN 2, 0.055kW by Grundfoss Pumps NZ Ltd, Palmerston North) was used to circulate feed from a jacketed balance tank through a membrane module and back to the balance tank. Control of cross-flow velocity was obtained by varying the pump speed with a frequency controller. In most of the experiments except with ferritin, permeate was passed through a UV cell (Pharmacia, Sweden) installed on line to measure protein concentration. The UV cell was calibrated with data from a bench spectrophotometer (Beckman DU 7500, Beckman Instruments NZ Ltd, Auckland). A Membralox^{TM} 1T1-70 module capable of supporting zirconium oxide membranes (Société des Céramiques Techniques, Tarbes, France) with nominal pore sizes of 20, 50, 100 and 800 nm was used in the experiments. The diameter and length of the membrane tubes were 7 and 250 mm respectively. The membrane area was about 50 cm^2. A temperature controller (Model LTD 20 by Grant Instruments Ltd, Cambridge, UK, range -30 to 100°C) was used in the experiments. The temperature in all experiments was 25±1°C. Permeate was recycled to maintain constant feed concentration.

The rig was capable of operating either in constant pressure or constant flux mode. Key process parameters were accurately controlled (Marshall *et al.,* 1996). Operation of MF in constant flux mode rather than constant pressure mode was found to be beneficial as it minimises fouling (Sheehan *et al.,* 1988 & Marshall *et al.,* 1996). Constant flux operation generally involves starting filtration with a small TMP, usually <10 kPa, and increasing it as the membrane fouls. This was achieved with a small permeate control valve in the permeate line (Fig 4.2).

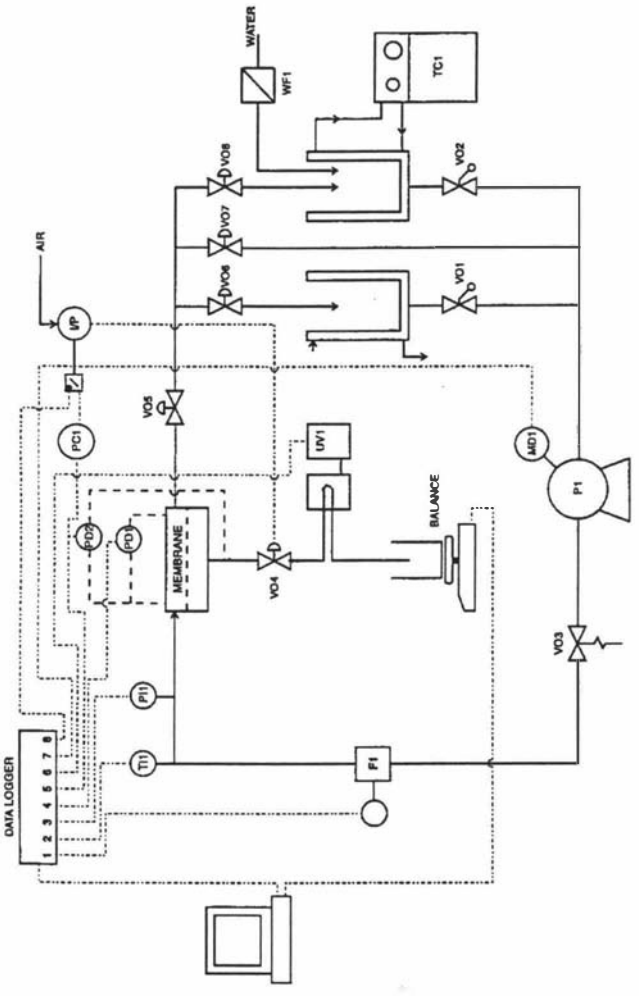

Fig. 4.1. Flow diagram of the cross-flow microfiltration rig. V01 and V02 were 25 mm butterfly valves. V03 was a pressure relief valve. V05-V08 inclusive were Saunders 15 mm manual diaphragm control valves. TC1 was a temperature controller (Marshall *et al.*, 1996).

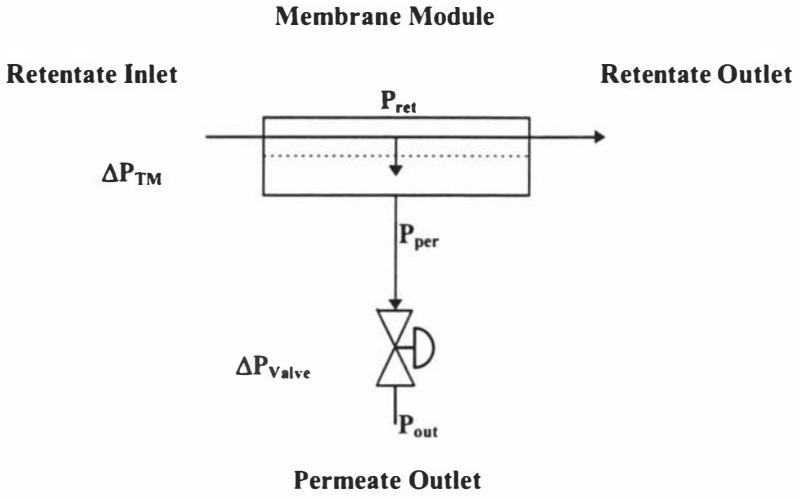

Fig. 4.2. Schematic diagram of membrane module and pressure drops.

For constant flux operation of the rig, the permeate flow was controlled by a computer based PID controller. Permeate flow was measured using an electronic balance linked directly to the computer. The measured permeate flow was computed as the average difference in weight recorded over ten consecutive 2 s intervals. In a typical constant flux run the controller was required to open the permeate valve as ΔP_{TM} increased with fouling. All the experiments were started in constant flux mode. However in some runs, when fouling was severe and if TMP was > 200 kPa, then the controller was switched to constant pressure mode as the rig could not be operated at more than 200 kPa. For constant pressure operation of the rig, ΔP_{TM} was controlled by a PID controller operating the permeate valve. The retentate pressure was set at a suitable value using a manual valve. Opening the permeate valve increased the permeate flow and decreased the permeate side pressure, increasing the ΔP_{TM}. In the course of a typical constant pressure run, the permeate valve was slowly closed as the permeate flow decreased with membrane fouling.

Before commencing an experiment, the membrane was flushed for about 8-10 minutes with 50°C water. A small quantity of NaCl (typically to give a conductivity of 0.5 mS) was added to water to obtain a satisfactory flow meter performance. After flushing, the membrane was then cleaned with 0.4% HNO_3 for 30 min at 50°C. The

membrane was then flushed for 8-10 minutes with 25°C water to remove the cleaning chemicals in the plant and left circulating on water (typically with a permeate flux of 110 L/m^2.h). The conductivity of the water was adjusted by the addition of NaCl to match that of the feed solution. The clean membrane resistance was then assessed by measuring the transmembrane pressure for at least 5 permeate fluxes (typically 76, 112, 223, 338 and 450 L/m^2.h). A linear regression was performed on these data, the slope of the line allowing the calculation of the clean membrane resistance.

At the completion of a run, the membrane was flushed with 90-100 L of 25°C water of conductivity 5 mS for 10-15 min. The permeate flux was maintained at the same value as during protein processing. When the permeate controller was in constant pressure operation, the flux was allowed to increase back to the initial flux set point on product and then operation was switched back to constant flux and this flux maintained until the end of flushing. After flushing, water was circulated for 30 min before the water flux was measured. Reversible fouling resistance (resistance that was removed by water flushing) and irreversible fouling resistance (resistance that was removed by chemical cleaning) were evaluated from these measurements. The membrane was then cleaned with a 1.4% solution of Ultrasil-25 for 40-50 min at 50°C. This cleaning regime enabled reuse of membrane by successfully restoring the clean membrane resistance. All the process parameters were logged onto a computer and converted into a spreadsheet file for analysis.

4.2. Feed preparation

4.2.1 Whey protein solutions

A 0.5% (w/v) whey protein solution was prepared by mixing whey protein concentrate (WPC) powder and demineralised water using a magnetic stirrer for at least 30 min at ambient temperature (20-25°C). The composition of the WPC powder (ALACEN 421, supplied by the New Zealand Dairy Board) was: 56.5% protein (TN x 6.38), 4.3% fat, 31.2% lactose, 4% ash and 4% moisture. The conductivity of this WPC solution was 0.5 mS. If NaCl was the only salt present this would correspond to an ionic strength of 0.005 and this value is assumed in the experiments. In some

experiments, NaCl was added after the 30 min mixing to give a ten-fold increase in conductivity (*i.e.* ionic strength about 0.05M). After mixing, the protein solution was allowed to stand for at least 30 min before processing. The temperature of the solution was adjusted to 25°C using a water bath. The pH of the reconstituted WPC solution was 6.78-6.90.

4.2.2 Fresh cheese whey

Fresh cheese whey was obtained from the New Zealand Dairy Research Institute pilot plant on the day of the trial. The temperature of the solution was adjusted to 25°C using a water bath. The pH of the cheese whey was in the range of 6.33-6.34; conductivity was 5.71-5.72 mS.

4.2.3. Pure protein solutions

A 0.25% w/v pure protein solution was prepared by mixing protein powder and demineralised water using a magnetic stirrer for at least 30 min at ambient temperature (25°C). NaCl was added after mixing to achieve the required ionic strength. After mixing, the protein solution was allowed to stand for at least 30 minutes before processing. If necessary the temperature of the solution was adjusted to 25°C by placing the beaker in hot water (35-45°C). The composition and specific procedures used for preparing each protein solution were as follows:

Bovine serum albumin (BSA)

The BSA powder (cold ethanol precipitated, 98-99%, A3350, Lot 36H0417) was obtained from Sigma Chemical Co, St Louis, MO, USA. In some experiments, calcium chloride (8 mM) or sodium dodecyl sulfate (1.4 g/g of protein) or N-ethylmaleimide (NEM) of concentrations 0.04 and 1 mM was added first and then NaCl was added to make up the required ionic strength of 0.051-0.054 M. The pH of the BSA solutions was 6.80-6.82. In some experiments, prefiltration of the feed was performed using a polysulfone membrane (PM 500; Koch Membrane Systems Inc., Wilmington, USA). Cysteinyl BSA (Sigma, A-0161, Lot 96F9375) lyophilized,

essentially salt free having a sulphydryl content of 0.01 mol/mol BSA was used in some runs.

Bovine lactoferrin

Bovine lactoferrin (BLf) was obtained from Tatua Co-operative Dairy Company Limited, Tatuanui, New Zealand. The details of manufacture are not available. The composition of the powder was: protein (TN x 6.38) 98.2%; moisture 1.2%; ash 0.6%; iron saturation 13.2%; purity 93.5%.

Bovine ferritin

Bovine ferritin was obtained from New Zealand Pharmaceuticals Limited, Linton, Palmerston North. The details of manufacture are not available. Composition: total protein 80.5%; moisture 1.5 %; ash 18 % (mostly iron).

All the chemicals, SDS, NaCl, NEM, except calcium dichloride (Merck.p.a.grade) used in the experiments were of analytical grade obtained from BDH Chemicals, Poole, England.

4.3. Plant operation

4.3.1 Whey protein solutions and fresh cheese whey

At start up the water in the plant was removed by flushing the plant with 5 L of reconstituted whey protein solution. A further 5 L was added to the balance tank and used in the experiment. Since the hold up volume of the recirculation loop was approximately 1 L, this procedure minimised the dilution of feed. The protein concentration of the final feed solution was in the range 0.47-0.50%.

4.3.2 Pure protein solutions

As pure protein solutions were expensive, a slightly different start up procedure was followed to minimise the quantity of the feed required. Four litres of prepared product was added to the balance tank and used in the experiment by switching from the water balance tank to the tank containing the feed solution. Since water in the

circulation loop was added to the protein solution, this procedure caused 15-20% dilution of feed. The protein concentration of the feed solution was therefore in the range 0.19-0.2%.

Experiments were performed at various permeate fluxes. In some experiments with ferritin and lactoferrin, a reduced constant permeate flux operation was reintroduced after onset of severe fouling and about 60 minutes of constant pressure operation. The operating temperature and cross-flow velocity were 25°C and 3 m. s^{-1} respectively unless otherwise stated.

4.4. Measurements and analysis.

The ionic strength (assessed by measuring conductivity) and pH of the feed were measured at regular intervals throughout each run. Samples of the feed (typically raw, 5, 60 and 120 minutes) and permeate (typically 30, 60, 90, 120 minutes) were collected in each run and the protein content was determined by measurement of absorbance using a spectrophotometer at 280 nm. Samples were diluted to ensure that the measured absorbance was <1.0. The protein content in some permeate and feed samples was also determined by the Kjeldahl method (Tecator Kjeltec, Auto 1030 Analyser, Tecator NZ Ltd, Auckland). Duplicates of samples were analysed. The following equations were established by measuring absorbances and Kjeldahl nitrogens for a range of standard protein samples and performing regression analysis. With whey protein solutions, UV cell data were considered to be indicative of trends only as the whey was cloudy.

For whey protein solutions $C_{protein}$ = 0.2769 x Absorbance - 0.00275 (1)

For BSA solutions $C_{protein}$ = 0.15385 x Absorbance - 0.00024 (2)

For Blf solutions $C_{protein}$ = 0.08126 x Absorbance - 0.000132 (3)

For Ferritin solutions $C_{protein}$ = 0.10526 x Absorbance - 0.000122 (4)

5. Microfiltration fouling by reconstituted whey protein solutions

5.1. Introduction

Whey is the largest volume dairy stream processed by membranes. It contains valuable proteins that have high nutritional value and excellent functional properties which make them valuable ingredients in the food industry. Table 5.1 summarises characteristics of the constituents of whey. Considerable progress has been made in whey processing technology to produce a range of whey derived products such as whey protein concentrates (WPC), whey protein isolates (WPI) etc. However, membrane fouling has been a limiting factor in whey processing increasing both operating and capital costs. Membrane fouling by whey has been studied previously (Lim *et al.*, 1971; Hayes *et al.*, 1974; Lee & Merson, 1975, 1976a, b; Taddei *et al.*, 1988; Daufin *et al.*, 1991, 1992, 1993, 1994; Gésan *et al.*, 1993, 1994). The general consensus is that fouling by whey is a complex process involving interaction of both minerals and proteins with the membrane materials during processing. The mechanism of fouling is not completely understood perhaps due to the complex physicochemical nature of whey.

Table 5.1. Some characteristics of proteins in whey*

Protein	% of whey proteins	Molecular weight (Daltons)	IEP
β-lactoglobulin	55-65	18 400	5.35-5.49
α-lactalbumin	15-25	14 200	4.2-4.5
Immunoglobulins	10-15	80 000-900 000	5.5-8.3
BSA	5-6	66 300	5.1
Proteose-peptone	10-20	4000-80 000	5.1-6.0
β-Caseins	1-2	24 000	4.7
Minor proteins	<0.5	30 000-100 000	-

* Data from Marshall & Harper (1988)

The present study was undertaken to investigate whey fouling on a range of pore size membranes. Reconstituted whey protein solutions were used since they provided a more consistent material than fresh whey. CFMF experiments were conducted with

20, 100 and 800 nm pore size ceramic membranes which approximate respectively a UF membrane, a "tight" MF membrane and an "open" MF membrane. Fouling resistance and protein transmission were measured as a function of pore size, permeate flux and ionic strength to provide insights into the principal fouling mechanisms for each membrane.

5.2. Results

5.2.1. Overview of experiments

Most of the experiments were performed using reconstituted whey protein solution on 20, 100 and 800 nm membranes. Two experiments were performed using fresh cheese whey on an 800 nm membrane. On a 100 nm membrane, two sets of experiments were performed, one at an ionic strength of 0.005M and the other at 0.05M *i.e.* ten-fold increase in ionic strength obtained by adding NaCl to WPC solution.

5.2.2. Effect of the permeate flux

The initial membrane resistances of the 20, 100 and 800 nm pore size membranes were 110-114 x 10^{10} /m, 10-13 x 10^{10} /m and 1.8-2.2 x 10^{10} /m, respectively. Table 5.2 summarises the runs performed. All runs were conducted in duplicate and reproducibility was very good. Initial experiments were performed on a 100 nm pore size membrane at an ionic strength of 0.05 M. Under these conditions, fouling was minimal at a flux of 50 L/m^2.h but severe at 75 and 100 L/m^2.h (Fig. 5.1). At 75 and 100 L/m^2.h, the operation was changed to constant pressure mode when severe fouling occurred. Protein transmission as measured by a UV cell at 50 L/m^2.h was almost constant at about 65-67% (Fig. 5.2). Initially, the apparent transmission of protein was zero but increased rapidly to a maximum value as the water initially in the permeate chamber was removed. There was a time lag of 4-5 minutes for the permeate to pass through the UV cell system. After severe fouling the transmission of protein was <20% (Fig. 5.2 & Table 5.2).

With the 800 nm membrane, fouling was severe at 100 and 200 L/m^2.h, minimal at 50 L/m^2.h and intermediate at 75 L/m^2.h (Fig. 5.3). When severe fouling occurred, the operation was switched to constant pressure mode.

Table 5.2. Summary of final protein transmissions and fouling resistances for all the reconstituted WPC and fresh cheese whey runs.

Membrane pore size nm	Ionic strength M	Flux L/m^2.h	Protein Transm -ission[a] %	Final Resistance			
				Total /m x 10^{10}	Membrane %	Reversible %	Irreversible %
Feed- Reconstituted cheese WPC							
20	0.05	50	10	2380	4.86	60.70	34.44
20	0.05	50	11	2400	4.60	62.08	33.32
100	0.005	50	44	81.8	13.4	61.2	25.4
100	0.005	50	42	87.4	14.0	60.8	25.2
100	0.005	75	40	94.2	12.8	61.0	26.1
100	0.005	75	41	97.5	12.3	63.0	27.4
100	0.005	100	18	579.0	2.2	79.2	18.5
100	0.005	100	16	591.6	1.8	78.6	19.6
100	0.05	50	65	59.4	15.1	65.5	19.4
100	0.05	50	67	61.0	17.9	61.1	21.0
100	0.05	75	18	1124.7	0.9	67.6	31.4
100	0.05	75	19	1143.5	1.0	67.9	31.0
100	0.05	100	17	1196.2	0.9	72.8	26.3
100	0.05	100	16	1211.3	1.0	69.8	29.3
800	0.05	50	86	25.8	7.9	45.0	47.1
800	0.05	50	84	22.4	9.3	41.5	49.2
800	0.05	75	83	542.0	0.4	96.6	3.0
800	0.05	75	82	438.7	0.5	96.5	3.1
800	0.05	100	81	984.3	0.2	97.8	2.0
800	0.05	100	80	681.8*	0.3	96.6	3.1
800	0.05	200	80	977.9	0.2	96.7	3.1
800	0.05	200	79	925.5	0.3	97.2	2.5
Feed-Fresh Cheese Whey							
800		200	97	297.0	0.6	71.0	28.3
800		200	98	372.9	0.5	77.4	22.1

a Protein transmission at 120 min.

* Experiment in which flux was reduced to 50 L/m^2.h at 60 min.

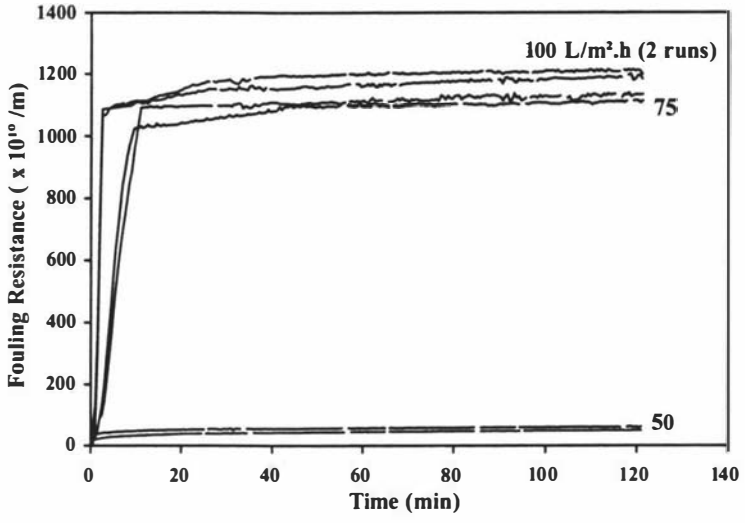

Figure 5.1. Fouling resistance at various permeate fluxes during MF of 0.5% reconstituted WPC solution on a 100 nm membrane. Ionic strength, 0.05 M; pH, 6.78-6.90.

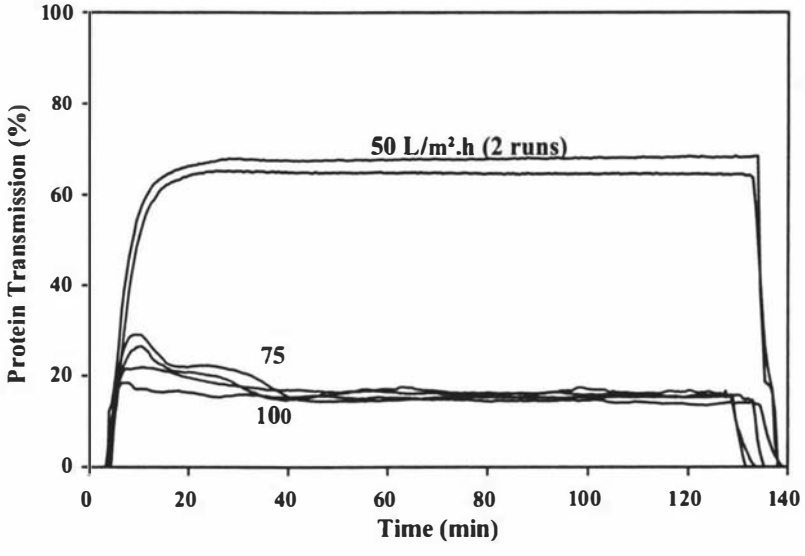

Figure 5.2. Protein transmission (UV Cell) at various permeate fluxes during MF of 0.5% reconstituted WPC solution on a 100 nm membrane. Ionic strength, 0.05 M; pH, 6.78-6.90.

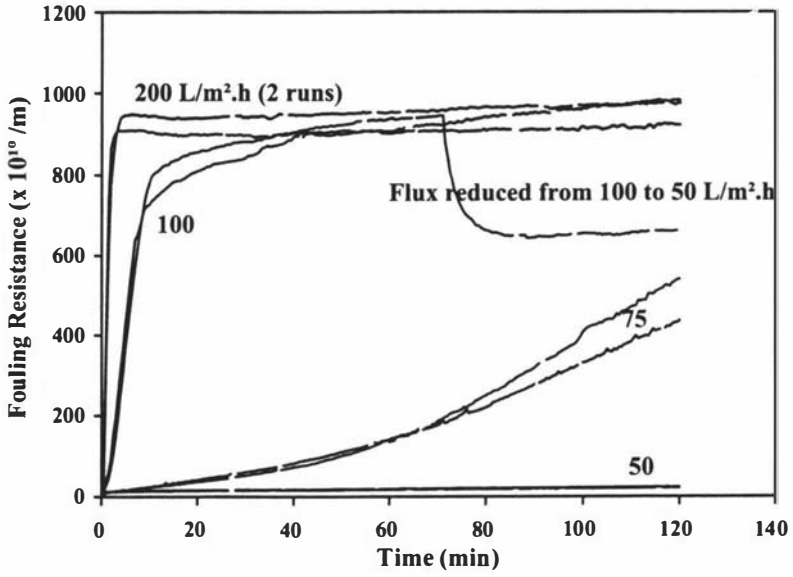

Figure 5.3. Fouling resistance at various permeate fluxes during MF of 0.5% reconstituted WPC solution on a 800 nm membrane. Ionic strength, 0.05 M; pH, 6.78-6.90.

In one experiment at 100 L/m^2.h, the flux was reduced to 50 L/m^2.h after one hour when severe fouling had already occurred. This resulted in a reduction in the fouling resistance, initially rapidly, to about 75% of that with a constant transmembrane pressure of 200 kPa.

The resistance did not drop to the resistance obtained when a flux of 50 L/m^2.h was set throughout the run, indicating that severe fouling was only partially reversible by the changes in operating conditions. Protein transmissions were almost constant with time and varied little with permeate flux or fouling on the 800 nm pore size membrane (Fig. 5.4 & Table 5.2). They were also uniformly high (>79%) in spite of severe fouling. These results indicate that fouling is severe at higher permeate fluxes both on 100 and 800 nm pore size membranes. With the 100 nm membrane, increased fouling resistance corresponded to decreased protein transmission. However, protein transmission did not decrease with increased fouling resistance on the 800 nm membrane.

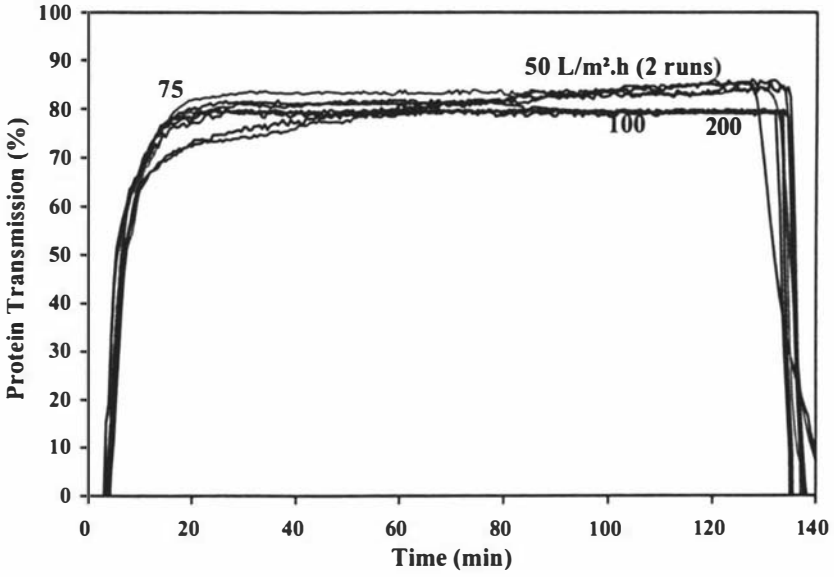

Figure 5.4. Protein transmission (UV Cell) at various permeate fluxes during MF of 0.5% reconstituted WPC solution on a 800 nm membrane. Ionic strength, 0.05 M; pH, 6.78-6.90.

5.2.3. Effect of ionic strength

Experiments were also performed with the 100 nm membrane at an ionic strength of about 0.005 M (no added salt). At 50 and 75 L/m^2.h, fouling was minimal and the increase in transmembrane pressure was very slow (Fig. 5.5). At 100 L/m^2.h, fouling increased slowly with time and after 120 min the control system had still not switched to constant pressure operation.

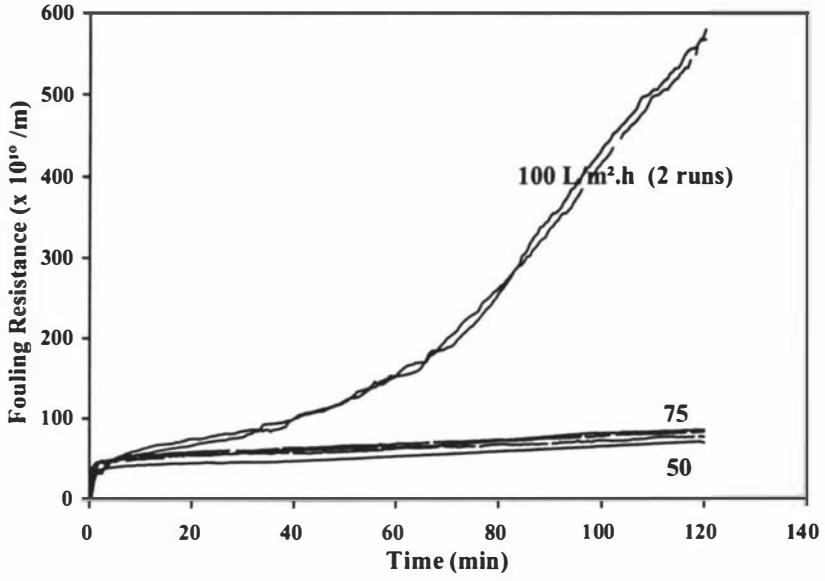

Figure 5.5. Fouling resistance at various permeate fluxes during MF of 0.5% reconstituted WPC solution on a 100 nm membrane. Ionic strength, 0.005 M; pH, 6.78-6.90.

Fouling had no significant effect on the protein transmission within the first 30 min because of light fouling during this period (Fig. 5.6). However, protein transmission decreased steadily after 30 min for the 100 L/m^2.h runs corresponding to the gradual increase in fouling resistance. After severe fouling, the transmission of protein was <20% (Fig. 5.6 & Table 5.2). Comparing these results with Fig. 5.1, it is clear that fouling is less severe at the lower ionic strength. Under conditions where severe fouling was prevented the protein transmission was about 43% and 66% with solution ionic strengths of 0.005 M and 0.05 M respectively. Fouling was less but protein transmission was lower at low ionic strength.

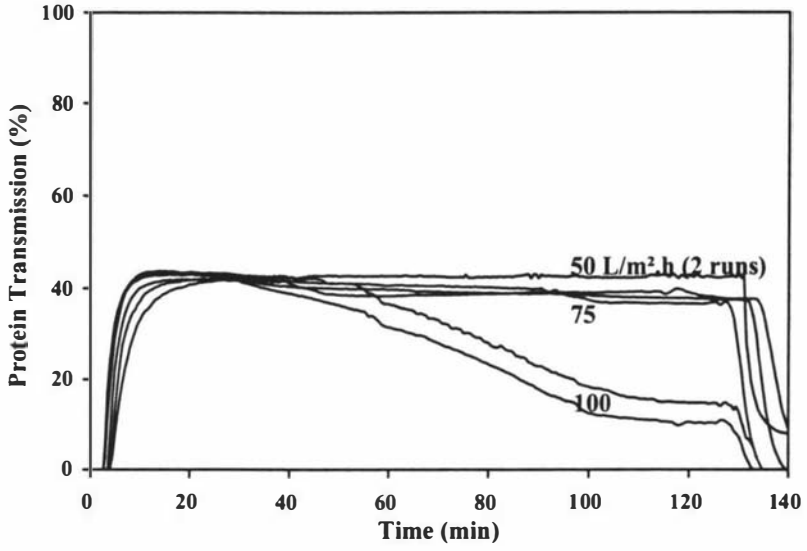

Figure 5.6. Protein transmission (UV Cell) at various permeate fluxes during MF of 0.5% reconstituted WPC solution on a 100 nm membrane. Ionic strength, 0.005 M; pH, 6.78-6.90.

5.2.4. Nature of the feed material

Relatively low protein transmissions of only about 66 and 85% were obtained on unfouled 100 and 800 nm membranes respectively despite the protein molecular size (about 5-15 nm) being much smaller than the membrane pore size. One possibility is that the reconstituted WPC feed contained large protein aggregates which were retained by the membrane. To investigate the possibility of aggregated protein, feed samples were centrifuged (16, 300 *g*, 15 min) and supernatant samples analysed for protein content. Sediments were observed in the centrifuge tubes. Supernatant protein content was 0.41% (Kjeldahl N x 6.38) compared to 0.49-0.5% in the feed. Feed samples analysed by a Malvern Mastersizer indicated a d(0.5) of the suspended particles of 0.38 μm. The suspended particles constitute nearly 20% of the total protein in the feed.

Four runs were performed at 200 L/m^2.h on the 800 nm membrane to compare the severity of fouling in experiments with fresh cheese whey and reconstituted WPC (Fig. 5.7). Fouling was light with fresh cheese whey giving protein transmission of 97-98% (Table 5.2).

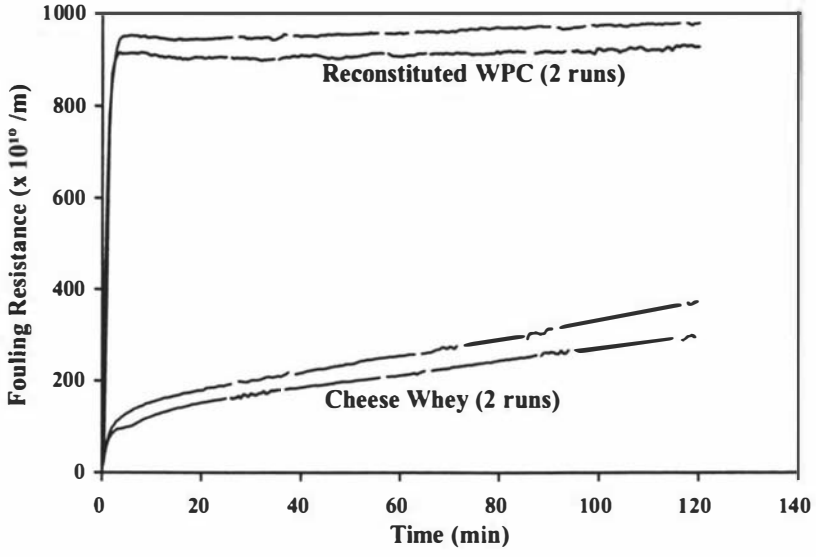

Figure 5.7. Comparison of fouling of fresh cheese whey and reconstituted WPC solution at 200 L/m^2.h on a 800 nm membrane. Ionic strength, ~0.05 M; pH 6.3-6.4 for cheese whey, 6.78-6.90 for reconstituted WPC solution.

Fouling was much more severe and protein transmission was lower (Table 5.2) with reconstituted WPC. This suggests that much of the fouling by reconstituted WPC was caused by the aggregated protein particles since fresh whey would not contain such particles.

5.2.5. Effect of the membrane pore size

Two runs were performed on the 20 nm pore size membrane at 50 L/m^2.h. The fouling was severe and transmembrane pressure increased to more than 200 kPa within 18-24 minutes. The controller was switched to constant pressure mode (Fig. 5.8). Protein transmission under these conditions did not vary much with time and was about 10% (Table 5.2). At 50 L/m^2.h, fouling was much more severe with the 20 nm membrane than the 100 and 800 nm membranes. Protein transmissions at 50 L/m^2.h decreased in the order of decreasing membrane pore size. At 100 L/m^2.h, fouling resistance was slightly lower with the 800 nm membrane than with the 100 nm membrane (Table 5.2).

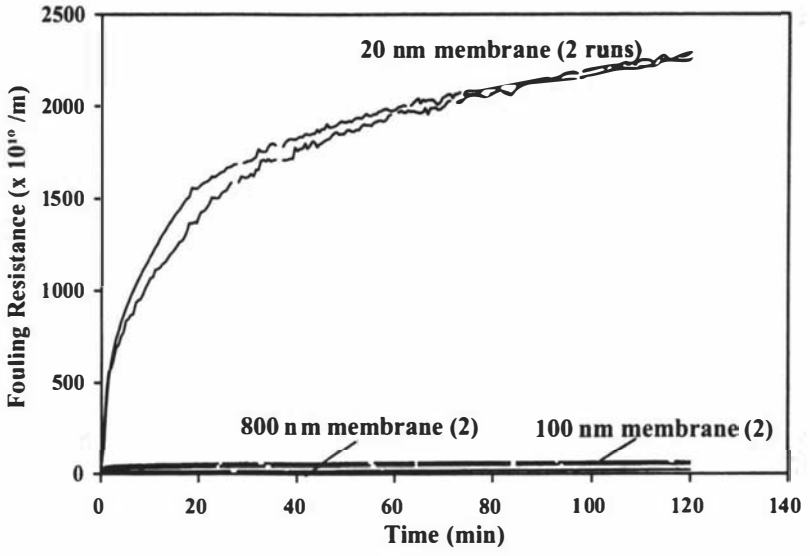

Figure 5.8. Effect of membrane pore size on fouling resistance during MF of 0.5% reconstituted WPC solution at a flux of 50 L/m^2.h. Ionic strength, 0.05 M; pH, 6.78-6.90.

On the 800 nm membrane, light fouling at 50 and 75 L/m^2.h fluxes and severe fouling within 10 minutes both at 100 and 200 L/m^2.h occurred suggesting a "break point" between 75 and 100 L/m^2.h for severe fouling to occur whereas, the break point on the 100 nm membrane appeared to be between 50 and 75 L/m^2.h (Figs. 5.3 & 5.1).

5.2.6. Reversible versus Irreversible fouling resistance

Under all experimental conditions used except 50 L/m^2.h on the 800 nm pore size membrane more than 60% of the fouling resistance was reversible (Table 5.2). High reversible fouling indicates surface layer formation by the deposited material. Irreversible fouling is perhaps caused by protein adsorption and was the highest percentage of total fouling at 50 L/m^2.h on the 800 nm pore size membrane where total fouling was very low.

Comparing cheese whey and reconstituted WPC solution, most of the resistance associated with both wheys was reversible but the irreversible fouling was much higher with cheese whey (Table 5.2). This may be because of a higher content of minerals and unaggregated protein in the cheese whey.

5.2.7. Relationship between protein transmission and fouling resistance

The protein transmission data obtained on each membrane at each ionic strength with reconstituted whey and cheese whey solutions was plotted against the corresponding fouling resistance (Fig. 5.9). There was a variable effect of fouling resistance on protein transmission depending upon the membrane pore size and ionic strength. With reconstituted whey, there was a large decline in protein transmission with an increase in fouling resistance on the 100 nm membrane whereas the decline was very low with the 800 nm membrane. However, it is interesting to note that increase in fouling resistance did not affect protein transmission with cheese whey. It was surprising to see relatively low protein transmissions with reconstituted whey particularly with both 100 and 800 nm membranes, even when the fouling resistance was low. All these membranes are expected to permeate whey proteins such as β-lactoglobulin, α-lactalbumin, BSA and Immunoglobulins. One possible reason for such low protein transmissions is that part of the protein was aggregated. On the 100 nm membrane, at the same resistance, protein transmission was higher at higher ionic strength, suggesting that a decrease in the size of the protein at higher ionic strength may have resulted in higher protein transmission.

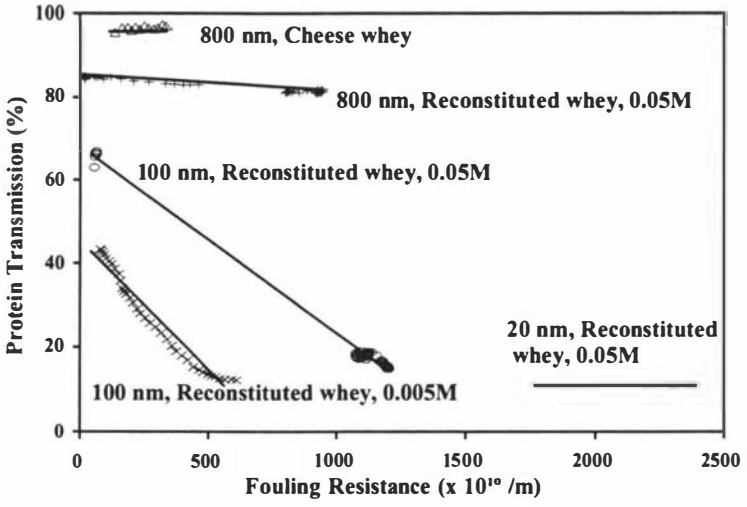

Figure 5.9. Effect of pore size and ionic strength on the relationship between protein transmission and fouling resistance for various trials. 20 nm membrane, reconstituted WPC, ionic strength, 0.05 M(-); 100 nm membrane, reconstituted WPC, ionic strength, 0.005 M(x), 0.05 M(O); 800 nm membrane, reconstituted WPC, ionic strength, 0.05 M(+); cheese whey, ionic strength ~ 0.05 M(Δ). All the lines are best fit lines.

5.3. Discussion

To summarise results reported in the previous section, the retention of protein increased in the order of decreasing pore size causing severe fouling as the pore size decreased. Permeate flux had a dramatic effect on fouling both on the 100 and 800 nm membranes. Increase in ionic strength led to severe fouling although protein transmissions were improved when fouling was light. It is useful to discuss the influence of experimental variables such as permeate flux and ionic strength on fouling before elucidating probable fouling mechanisms.

5.3.1. Fouling on the 100 and 800 nm membranes

5.3.1.1. Influence of permeate flux

Fouling was highly dependent upon permeate flux on both the 100 and 800 nm pore membranes. Increased fouling at higher permeate flux was probably due to both increased deposition within the membrane pores (due to increased collisions or some form of shear induced denaturation) (Bowen & Hughes, 1990; Bowen & Gan, 1991; Jonsson *et al.,* 1992a) and retention of protein on the membrane surface. Increase in flux has been shown to increase the deposition of β-lactoglobulin in the presence of calcium probably due to shear induced protein-protein interactions in the membrane pores (Marshall *et al.*, 1997). Alternatively Kelly *et al.* (1993) showed that aggregates severely fouled an MF membrane leading to surface layer formation. Increasing the flux might have increased the driving force towards the membrane wall leading to the deposition of retained protein aggregates either through pore plugging or surface layer formation. Severe fouling seems to initiate when wall concentration of retained aggregates is increased to a critical value at a flux depending upon membrane pore size and ionic strength of the feed.

5.3.1.2. Impact of ionic strength

The increased transmission of an unfouled membrane with increased ionic strength suggests that the average size of the protein has been reduced. This is supported by a decrease in apparent pore size reduction (Table 5.3) (calculated using equation 3.12). Several researchers (*e.g.* Muller *et al.,* 1973; Hayes *et al.,* 1974; Turker & Hubble, 1987; Clark *et al.,* 1991; Palecek *et al.,* 1993; Palecek & Zydney, 1994b) have reported the influence of ionic strength on protein deposits and permeate flux.

Table 5.3. Apparent pore size reduction in trials with low fouling on the 100 and 800 nm membranes performed at various fluxes.

Flux ($L/m^2.h$)	Clean membrane resistance ($x\ 10^{10}/m$)	Irreversible fouling resistance ($x\ 10^{10}/m$)	Apparent reduction in pore diameter (nm)
100 nm membrane, Ionic strength; 0.005 M			
50	11.0	20.7	23
50	12.2	22.1	23
75	12.1	24.6	24
75	12.0	26.7	25
100 nm membrane, Ionic strength; 0.05 M			
50	11.2	14.4	19
50	10.9	12.8	18
800 nm membrane, Ionic strength; 0.05 M			
50	2.0	12.1	38
50	2.1	11.0	37
75	2.2	16.4	42
75	2.1	13.5	40

Fane *et al.* (1983) suggested that at the isoelectric point (IEP) in the presence of ions, anion binding leads to an increase in the size of the protein thus increasing the porosity of the deposited layer. Away from the IEP, added salts tend to reduce the electrostatic repulsions by shielding charges causing molecular contraction and thereby decreasing permeability. Palecek *et al.* (1993) showed that addition of NaCl at concentrations 0.02, 0.15 and 0.5M increased flux decline during MF of BSA at a pH of 7.4. Heinemann *et al.* (1988) also found that addition of 0.1M NaCl during MF of whey protein solution through 0.2 μm MF membranes decreased flux at pH values away from the IEP. They suggested that the presence of salt compressed the double layer on the membrane leading to less interactions with the membrane and also formation of denser deposits due to reduced protein size.

The effect of salt in the protein-membrane environment may be explained by 1) protein-protein interactions and 2) electrical double layer near the membrane surface *(i.e.* interaction of charged molecules with the charges on the membrane surface). Addition of salt causes ionic shielding weakening protein-protein interactions (Fane *et al.,* 1983; Suki *et al.*, 1984; Palecek *et al.*, 1993) and protein-membrane interactions (Pouliot *et al.*, 1994; Balakrishnan & Agarwal, 1996). The solution pH in the present

study was 6.78-6.90 which is away from the IEP of whey proteins (~5.2). Reduced electrostatic repulsions by the addition of NaCl may have led to reduced size of the protein giving improved transmission under light fouling conditions. However when fouling was severe, a compact layer may have formed giving very low protein transmission (Suki *et al.*, 1984). Smaller size of the protein in the deposition layer would give higher resistance. This is consistent with the observed fouling resistance and protein transmission data for the feed containing higher ionic strength (0.05M).

5.3.2. Probable fouling mechanisms

5.3.2.1. Fouling on the 20 nm membrane

It is known that protein adsorption is generally the first step in membrane fouling. Monolayers of protein form on the membrane surface when it is brought in contact with whey protein containing solutions (Brink *et al.*, 1987). There was a 'jump' in fouling resistance to about 500 x 10^{10} /m (Fig. 5.8) as soon as the protein containing feed was introduced in the plant. This "instant" increase in fouling resistance may be due to inner pore effects during the passage of protein through the membrane or to surface layer effects. Adsorption alone would have caused pore narrowing or constriction on this membrane. Deposition on the top of the first adsorbed layer and eventual bridging of the pore entrances would occur and a complete surface layer would build up (Hallström *et al.*, 1989). The increase in fouling resistance right from the start, even at relatively low flux (50 L/m^2.h) on this membrane supports the immediate formation of a surface layer perhaps by the retained bulk of the protein and aggregates.

The shape of the resistance curve for the 20 nm membrane was different indicating that the principal fouling mechanism for this membrane may be different from the 100 and 800 nm membranes. The final fouling resistance was much higher with the 20 nm membrane compared with the 100 and 800 nm membranes at 50 L/m^2.h (Fig. 5.8). There was more than a 20 fold increase in resistance of the membrane because of fouling and about 60% of the fouling resistance was reversible (Table 5.2) further supporting the formation of a surface layer. Overall, the 20 nm pore membrane behaved like a typical UF membrane. A similar type of fouling behaviour; adsorption and multilayer deposition of protein leading to a surface layer on UF membranes has

been reported previously (Howell & Velicangil, 1980; Suki *et al.*, 1984; Fane & Fell, 1987; Marshall *et al.*, 1993). The 20 nm membrane acquired properties closely linked to the deposited surface layer as shown by nearly constant protein transmission of about 10% throughout the experiment. It is interesting to note that once the deposit layer is formed, protein transmission is not significantly affected by further increases in fouling resistance with time (Fig. 5.9).

To summarise fouling behaviour on the 20 nm membrane, adsorption and multilayer deposition by the retained protein lead to formation of a surface layer reducing protein transmission almost immediately.

5.3.2.2. Fouling on the 100 nm membrane

First, the experiments in which there was no added NaCl (IS: 0.005 M) will be considered.

There was an increase in fouling resistance to about 30-40 x 10^{10}/m as soon as the feed was introduced in the plant (Fig. 5.5). This may be due to some form of dynamic fouling. Adsorption occurred in all the experiments as indicated by a small increase in resistance at low fluxes. Protein transmission was only about 43% even under light fouling conditions suggesting that some of the protein, probably aggregates are retained initiating deposition on the membrane surface leading to formation of a "cake" of aggregates. This is supported by high reversibility of the fouling layer (Table 5.2). Most WPC powders exhibit 30-50% denaturation (Morr, 1985) and as a result the corresponding solubility of the WPC is limited to 77-80%, the most insoluble material consists of both lactose and aggregated protein (Morr *et al.*, 1973). The present study showed that about 20% of the protein was insoluble. The remaining 80% soluble protein would permeate the membrane and aggregate cake initially. However with time, some of the permeable protein may be trapped in the interstices of the aggregate cake and "infill" the cake further tightening it. This may have resulted in retention of smaller proteins. A much tighter fouling layer results due to increased interaction of protein when flux is increased to 100 L/m^2.h. This is consistent with the observation of low final protein transmission (16-18%). A similar fouling mechanism; formation of a lattice network by micro-organisms and protein

complexes and filling the interstices by permeable whey proteins such as β-lactoglobulin during UF of cottage cheese whey was previously reported by Lee and Merson (1976a).

Therefore, it is proposed that fouling occurs by the following steps:

1) adsorption

2) formation of a cake of aggregates

3) "infilling" the aggregate cake with smaller proteins leading to a "tighter surface layer"

The formation of a tighter surface layer in step 3 may have caused retention of smaller proteins. Protein transmission decreased with increase in fouling resistance (Fig. 5.6 & 5.9) supporting the above hypothesis. The final protein transmissions seem to depend on the permeability of the deposited cake. The increased fouling resistance at higher permeate flux was mostly reversible further supporting the formation of a surface layer/cake layer.

A similar fouling mechanism is proposed for the higher ionic strength (0.05M) solutions. However, it is important to consider the effects of electrostatic repulsions discussed in section 5.3.1.2. It is likely that an increase in ionic strength of the feed alters the size distribution of the protein *i.e.* % of monomers and aggregates in the solution, leading to an early formation of a surface layer at ≥ 75 $L/m^2.h$ (Figs. 5.1 & 5.5).

The porosity of the fouling layer affects both permeate flux and protein transmission when a surface layer builds up on the membrane surface. The most important factors that may affect the final protein transmission through the fouling layer are; the relative proportion of monomer and aggregates that contribute to the formation of a deposition layer, size and packing density of the fouling layer and infill of the aggregate fouling layer by smaller aggregates or monomers. With larger aggregates, the porosity of the surface layer will be high giving a better flux and higher protein transmission for monomers whereas a surface layer of low porosity is possible with smaller aggregates, giving relatively low protein transmissions. However, if the fouling layer is made up

mainly of protein monomers, a tighter and more organised fouling layer would occur resulting in very low protein transmissions. Conditions that change the size of protein molecules in the feed, such as an increase in ionic strength have a dramatic influence on permeate flux and protein transmission, as observed from the experimental data.

5.3.2.3. Fouling on the 800 nm membrane

The apparent reduction in pore size increased from 18-19 to 37-38 nm as pore size of the membrane increased from 100 to 800 nm (Table 5.3). This is probably due to more internal area available for the protein to interact with the membrane structure and adsorb.

Fouling resistance at low flux (50 $L/m^2.h$) was due to both reversible and irreversible fouling supporting the idea that fouling may be internal (pore narrowing or pore plugging) as well as external (surface layer). To narrow down the actual mechanism, *e.g.* pore narrowing or plugging, different blocking laws (described in section 3.6) were applied to the resistance data. None of the laws showed straight lines (not shown here) suggesting that perhaps more than one mechanism is active simultaneously. Also similar analysis for 75 $L/m^2.h$ data did not show straight lines. Cake filtration law could not be applied to the fouling data due to a lack of required constants such as specific cake resistance, porosity or void fraction for whey protein aggregate cake.

Therefore a qualitative analysis of fouling data was made to propose probable fouling mechanisms on 800 nm membrane. Smaller soluble proteins might pass freely through the membrane apart from initial adsorption. Larger aggregates (probably 20% insoluble protein of average size 0.38 μm) could then form a surface layer that has a porosity large enough to allow passage of smaller proteins. A small amount of pore constriction may have occurred causing a small reduction in the transmission of smaller proteins. The fouling is probably limited to the cake of aggregated material, as infilling by monomers would not have tightened the surface layer. Monomers permeate easily through the cake and primary membrane (800 nm pore size) as the latter is much more open than the 100 nm membrane. Protein transmissions were consistently high on the 800 nm membrane (>79%) at all the fluxes supporting the

idea that all the soluble protein is able to permeate the membrane even in its fouled state. A complete surface layer formation probably occurs only at higher fluxes where the convection of aggregates is high. This is supported by high reversibility of fouling resistance at high permeate fluxes (>50 $L/m^2.h$).

5.3.2.4. Comparison of fouling by fresh whey and reconstituted whey

Interestingly fresh cheese whey gave less fouling than reconstituted whey. Fouling resistance was almost 60% less than that observed with reconstituted whey protein solutions. With fresh whey, increased fouling did not affect protein transmission, whereas it affected transmission slightly in the case of reconstituted whey protein solutions probably due to the presence of aggregated protein in the feed. Large aggregates in the reconstituted whey were retained by all the membranes investigated and played a major role in determining the type of fouling mechanism. From the protein transmission data, it is evident that almost all the soluble protein in the feed (0.41%) is passing through the 800 nm pore size membrane whereas the aggregated protein seems to be retained, although this did not significantly affect the transmission of protein monomers.

5.3.2.5. Comparison of fouling behaviour on 100 and 800 nm membranes

The results on the 800 nm membrane suggest a similar fouling mechanism to that of the 100 nm membrane, except that fouling is limited to a porous cake of aggregates and also it was possible to operate at higher fluxes on the 800 nm membrane without affecting protein transmission significantly. Fouling on the 800 nm membrane is essentially limited to formation of a cake of aggregated material. The smaller proteins easily permeate the cake and pores. The fouling layer did not have much impact on protein transmission even when severe fouling occurred with the 800 nm membrane. However with the 100 nm membrane, the smaller proteins would "infill" the cake forming a tighter fouling layer that reduced transmission of protein monomers significantly (16-19%). Thus the fouling mechanism appears to change from formation of a predominantly aggregate fouling layer on the 800 nm membrane to formation of a fouling layer of both aggregates and monomers on the 100 nm membrane. Under severe fouling conditions, fouling resistance has a dramatic effect on protein transmission for the 100 nm membrane.

5.4. Conclusions

Fouling was highly dependent upon the membrane pore size, permeate flux and ionic strength of the feed during MF of reconstituted whey protein solutions. It was possible to prevent severe fouling and have high protein transmission when operating at low fluxes (50 $L/m^2.h$) on the 100 nm membrane. Though fouling was significant at higher fluxes (>50 $L/m^2.h$) on the 800 nm pore size membrane, protein transmission was largely unaffected by increased fouling resistance. However fouling was severe even at 50 $L/m^2.h$ on the 20 nm pore size membrane.

It is proposed that fouling by reconstituted whey protein solutions occurs by at least three steps:

1) adsorption 2) formation of a cake of aggregates and 3) infilling with smaller proteins leading to a "tighter surface layer".

All the three steps are likely to occur immediately when the feed is introduced to the 20 nm membrane. Step 3 may not occur with the 800 nm membrane whereas it is an important step reducing protein transmission significantly on the 100 nm membrane. Increase in ionic strength exacerbates fouling via step 3 causing early formation of a surface layer. Increased convection of aggregates led to severe fouling at higher fluxes on 100 and 800 nm membranes whereas on the 20 nm membrane, adsorption and multilayer deposition by the retained protein dominated. Severe fouling was predominantly reversible *i.e.* it could be removed by water on all the membranes supporting the concept that fouling is predominantly by formation of a surface layer.

Fouling was more severe with reconstituted WPC solution than with fresh cheese whey. Protein aggregates in reconstituted whey played an important role in causing severe fouling.

Increase in ionic strength of the feed has a dramatic effect on the fouling behaviour of the protein solutions.

6. Microfiltration fouling by bovine serum albumin solutions

6.1. Introduction

The experiments with reconstituted whey protein solutions showed that larger aggregates retained by the membrane at higher permeate fluxes form a surface layer increasing fouling resistance and reducing protein transmission. Whey protein solutions are a complex mixture of different proteins which vary considerably in their physicochemical characteristics. It is difficult to understand the protein-protein interactions and their effect on membrane fouling using a mixture like a whey protein solution. Hence further fouling studies were focussed on pure protein solutions in order to investigate whether pure proteins freely permeable through the membrane, also caused membrane fouling. The first protein chosen was bovine serum albumin (BSA) as it is a well characterised protein.

There have been a number of MF fouling studies using BSA. Previous literature on fouling of MF membranes by BSA is given in the introduction and literature review chapters. The general consensus is that severe fouling occurs even though the size of the protein is much smaller than the membrane pore size. Protein aggregation has been regarded as the main reason for this observed behaviour. The majority of the studies reported in the literature were performed in constant pressure mode under non-pumping conditions to avoid shear associated problems with the protein. There is a suggestion that pumping causes protein denaturation and then adsorption of protein leads to severe fouling (Meireles *et al.*, 1991a; Xu-Jiang *et al.*, 1995) although the mechanisms involved in these processes are not clear. MF under pumping has been reported to cause severe fouling with BSA in comparison to using a liquid column which caused much less fouling (Jonsson *et al.*, 1996). Although previous studies gave some fundamental understanding of the fouling mechanisms, it is not appropriate to elucidate the actual fouling mechanisms without considering shear associated interactions, particularly with surface active macromolecules like proteins. It is suggested that constant flux operation is better than constant pressure operation particularly for MF as the former avoids initial high TMP resulting in minimal fouling (Marshall *et al.*, 1996; van Reis *et al.*, 1997). CFMF fouling studies on BSA under shear or actual pumping, particularly in constant flux operation are limited.

The present study was undertaken with a specific objective of investigating the effect of permeate flux and protein chemical environment on membrane fouling during CFMF of BSA solutions under constant permeate flux conditions.

Preliminary experiments were performed on 50 and 100 nm membranes to investigate the effect of flux, addition of calcium and membrane pore size on fouling. As both the membranes were observed to foul by similar mechanisms, investigations were then focussed on the 100 nm membrane. A summary of experiments on 50 and 100 nm membranes and key experimental data are given in Table 6.1.

6.2. Results

6.2.1. Effect of the permeate flux

Experiments were performed at a range of permeate fluxes. On the 100 nm membrane, fouling resistance increased dramatically when flux was increased ≥ 300 $L/m^2.h$ (Fig. 6.1). Fouling was prevented at 50 and 200 $L/m^2.h$ and protein transmissions of about 90-95% were obtained (Fig. 6.2). Fouling was light at 300 $L/m^2.h$ reducing protein transmissions to about 70%. Severe fouling occurred when permeate flux was increased to 400 $L/m^2.h$ reducing protein transmissions to about 20% after 2 h. Protein transmissions were high during the first 30 minutes at all fluxes and increased slightly as permeate flux was increased perhaps due to increased concentration of protein in the boundary layer at higher fluxes. There appeared to be a break point between 300 and 400 $L/m^2.h$ for severe fouling to occur under the conditions used in the experiments. Protein transmissions as measured by a UV cell decreased as the fouling resistance increased (Fig 6.3). Initially, the apparent transmission of protein was zero, but increased rapidly to a maximum value as the water initially in the permeate chamber was removed.

Table 6.1. Summary of protein transmissions and fouling resistances on 50 and 100 nm pore size membranes during microfiltration of 0.2% BSA solutions. pH: 6.80-6.82, IS: ~0.05 M.

Membrane pore size nm	Flux $L.m^{-2}.h^{-1}$	Protein Transmission[a] %	Total final resistance			
			Membrane	Reversible	Irreversible	
			/m x 10^{10}	%	%	%
50	50	92.09	148.8	19.95	20.96	59.07
50	50	92.59	138.00	22.17	15.00	62.82
50	200	68.99	252.1	13.01	37.68	49.31
50	200	67.37	265.70	12.27	39.55	48.17
50	200*	65.68	222.20	13.23	25.56	61.21
50	200*	66.41	220.9	13.45	23.04	63.51
50	300	28.36	323.7	10.10	38.12	51.77
50	300	26.52	332.80	9.74	38.85	51.41
50	300*	12.5	385.5	7.78	42.12	50.09
50	300*	11.01	417.90	7.01	44.19	48.79
50	400	17.10	413.50	7.32	42.87	49.79
50	400	19.46	399.6	7.86	41.96	50.17
100	50	92.09	23.04	47.48	8.68	43.83
100	50	92.74	25.08	46.29	12.75	40.94
100	200	89.90	40.07	29.27	30.69	40.02
100	200	89.08	33.99	32.53	25.30	42.15
100	200*	85.91	46.91	20.69	36.51	42.78
100	200*	88.7	49.00	20.61	35.91	43.46
100	300	84.7	107.71	10.10	54.83	35.06
100	300	73.7	169.5	7.31	60.53	32.15
100	300*	12.42	397.06	2.81	64.73	32.45
100	300*	11.95	433.03	2.52	65.93	31.54
100	400	23.48	325.68	3.37	59.39	37.22
100	400	22.30	333.78	3.02	59.67	37.30
100	400*	7.43	432.59	2.79	64.77	32.42
100	400*	8.21	431.54	2.63	64.71	32.65
100	400@	34.83	282.9	3.60	89.62	6.76
100	400@	36.75	290.4	3.43	89.88	6.67
100	400#	72.76	243.5	4.20	63.17	32.61
100	400#	71.68	246.9	4.18	66.17	29.63
100	400$	30.61	294.6	3.68	60.10	36.23
100	400$	31.04	299.9	3.73	61.80	34.50
20	50	7.30	1156	9.59	17.82	72.58
20	50	6.80	1110	9.55	9.25	81.21

a Protein transmission at 120 min.

* Experiments using BSA solutions containing 8 mM Calcium.

@ Experiments using BSA solutions containing 1.4 g SDS/g of protein

Experiments using prefiltered BSA solutions; $ Experiments using cysteinyl BSA solutions

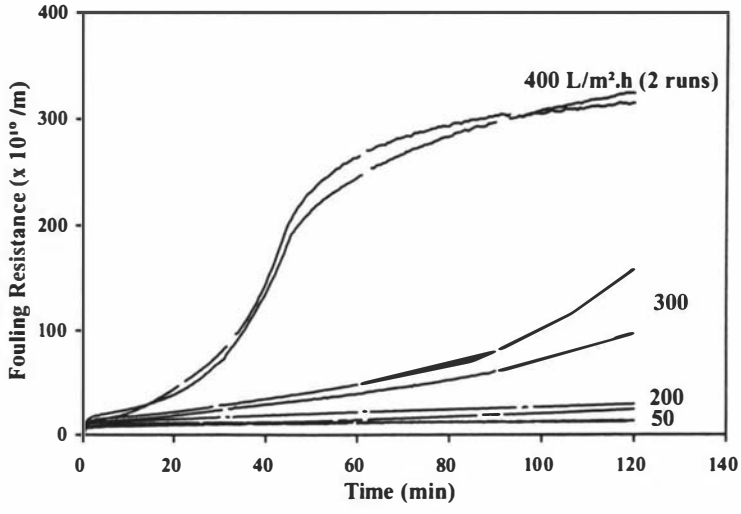

Figure 6.1. Fouling resistance at various permeate fluxes during MF of 0.2% bovine serum albumin solution in the absence of calcium on a 100 nm membrane. The ionic strength of the solutions was 0.052-0.054 and the pH from 6.80-6.82.

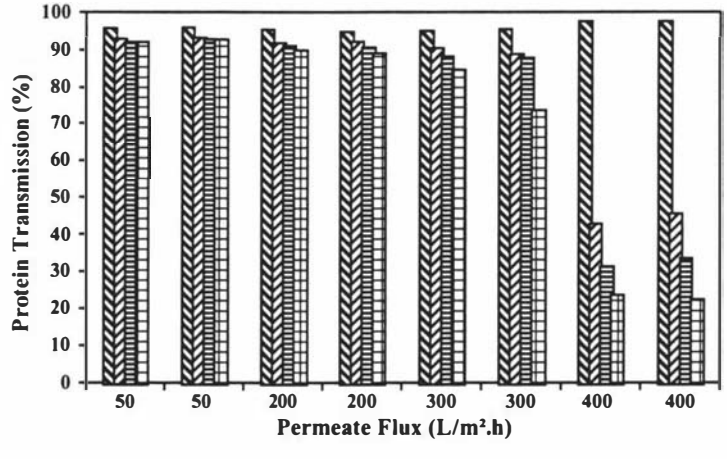

Figure 6.2. Protein transmission at various permeate fluxes during MF of 0.2% bovine serum albumin solution in the absence of calcium on a 100 nm membrane. The ionic strength of the solution was 0.052-0.054 and the pH from 6.80-6.82. For each permeate flux the 4 bars represent values measured at 30, 60, 90 & 120 min respectively.

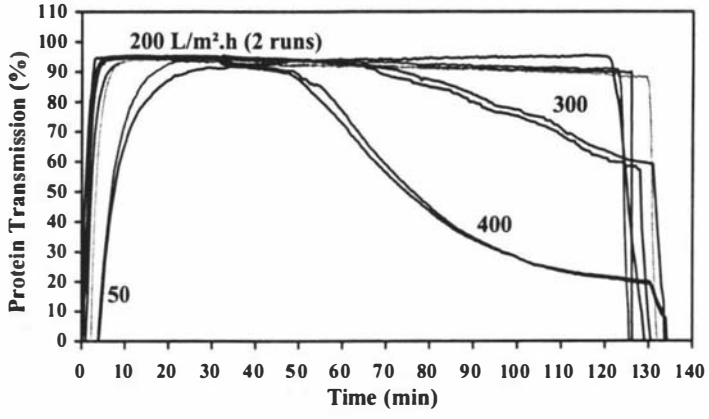

Figure 6.3. Protein transmission (as measured by a UV-cell) at various permeate fluxes during MF of 0.2% bovine serum albumin solution in the absence of calcium on a 100 nm membrane. The ionic strength of the solution was 0.052-0.054 and the pH from 6.80-6.82.

There was a time lag of 4-5 minutes for the permeate to pass through the UV cell system at 50 L/m^2.h. The increase in fouling resistance due to the increase in permeate flux was both reversible and irreversible (Fig. 6.4).

Similarly, on a 50 nm membrane, permeate flux had a dramatic effect on fouling (Fig. 6.5). Fouling was prevented at 50 L/m^2.h allowing protein transmissions of about 90-93% (Fig. 6.6). Severe fouling occurred at fluxes >200 L/m^2.h reducing protein transmissions to about 15% after 2 h at 400 L/m^2.h. Protein transmissions (UV cell) dropped continuously with time at fluxes > 50 L/m^2.h (Fig. 6.7). The increase in permeate flux increased both irreversible and reversible resistances (Fig. 6.8).

An initial increase of about 50-100 x 10^{10}/m and 10-12 x 10^{10}/m in fouling resistance for 50 nm and 100 nm membranes respectively was observed as soon as the feed was introduced due to the presence of protein in the feed solution (Figs. 6.5 & 6.1).

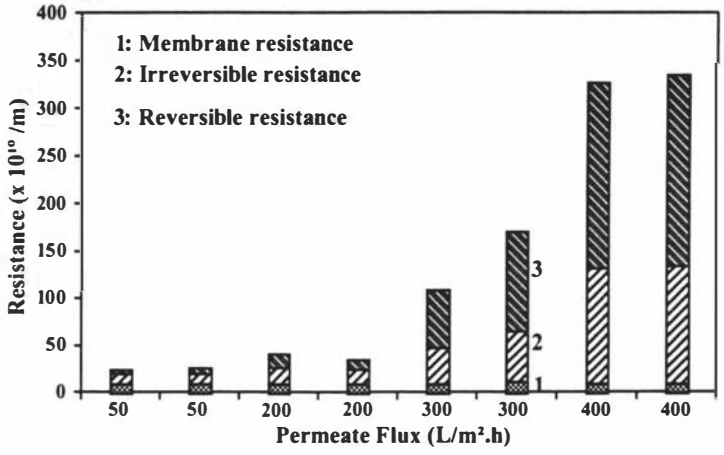

Figure 6.4. Irreversible and reversible fouling resistance at various permeate fluxes during MF of 0.2% bovine serum albumin solution in the absence of calcium on a 100 nm membrane. The ionic strength of the solutions was 0.052-0.054 and the pH from 6.80-6.82.

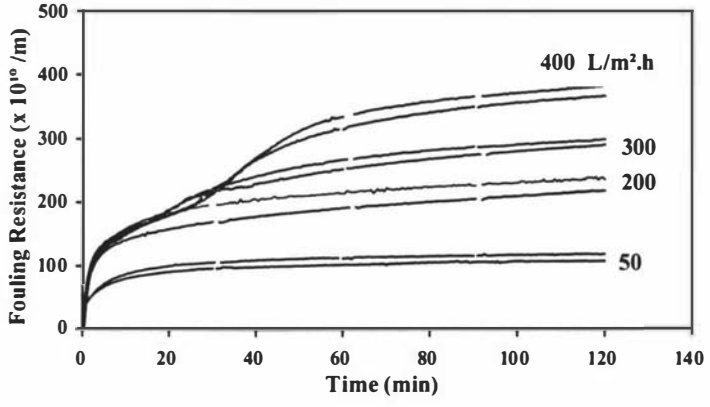

Figure 6.5. Fouling resistance at various permeate fluxes during MF of 0.2% bovine serum albumin solution in the absence of calcium on a 50 nm membrane. The ionic strength of the solutions was 0.051-0.056 and the pH from 6.80-6.82.

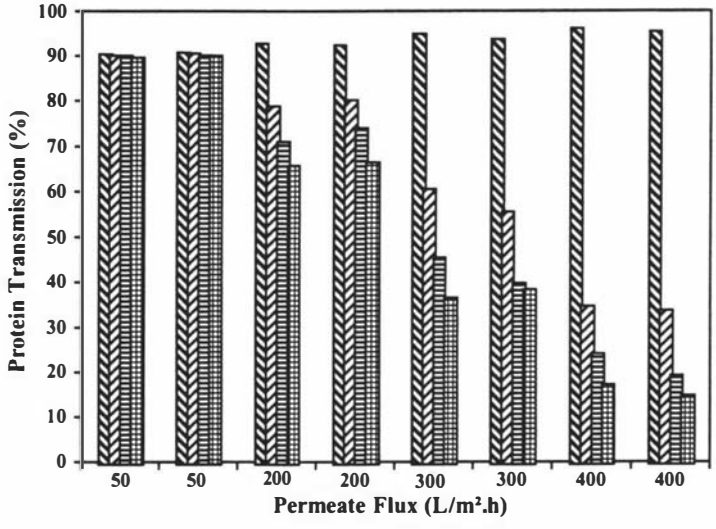

Figure 6.6. Protein transmission at various permeate fluxes during MF of 0.2% bovine serum albumin solution in the absence of calcium on a 50 nm membrane. The ionic strength of the solution was 0.051-0.056 and the pH from 6.80-6.82. For each permeate flux the 4 bars represent values measured at 30, 60, 90 & 120 min respectively.

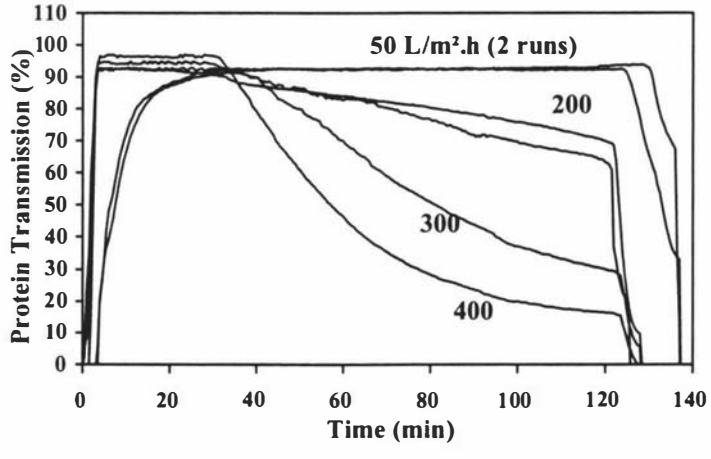

Figure 6.7. Protein transmission (as measured by a UV-cell) at various permeate fluxes during MF of 0.2% bovine serum albumin solution in the absence of calcium on a 50 nm membrane. The ionic strength of the solution was 0.051-0.056 and the pH from 6.80-6.82.

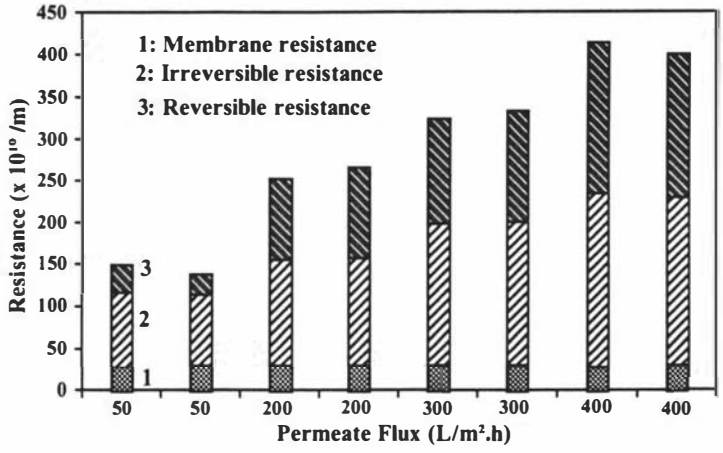

Figure 6.8. Irreversible and reversible fouling resistance at various permeate fluxes during MF of 0.2% bovine serum albumin solution in the absence of calcium on a 50 nm membrane. The ionic strength of the solutions was 0.051-0.056 and the pH from 6.80-6.82.

The fouling resistance then continuously increased with time at varying rate depending upon the flux. The rate of increase in fouling resistance increased with permeate flux. Protein transmissions on both the membranes stayed at about 90-95% during the first 30 minutes irrespective of permeate flux indicating that almost all the protein was passing through the membranes initially.

6.2.2. Effect of calcium addition

Experiments were also performed using BSA in the presence of calcium. The ionic strength was almost the same (~0.05M) in both the cases, but was achieved by either addition of NaCl alone or 8 mM calcium ($CaCl_2$) and NaCl.

On the 100 nm membrane, the presence of calcium in solution exacerbated severe fouling, particularly at permeate fluxes > 200 L/m^2.h (Fig. 6.9). Severe fouling occurred at 300 and 400 L/m^2.h reducing protein transmissions after 2 h to about 12 and 8%, respectively (Fig. 6.10).

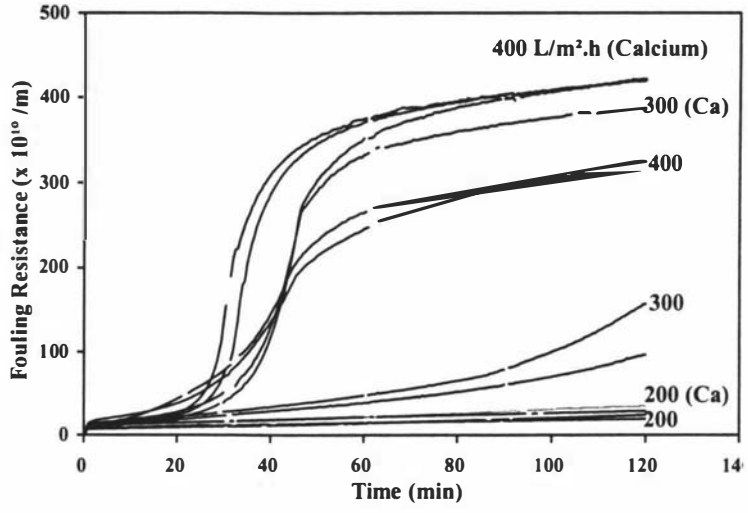

Figure 6.9. Fouling resistance at various permeate fluxes during MF of 0.2% bovine serum albumin solution in the presence of calcium on a 100 nm membrane. The ionic strength of the solutions was 0.054-0.055; the calcium content was 8.0 to 8.1 mmol/L and the pH from 6.80-6.82. Results without calcium are also shown for comparison.

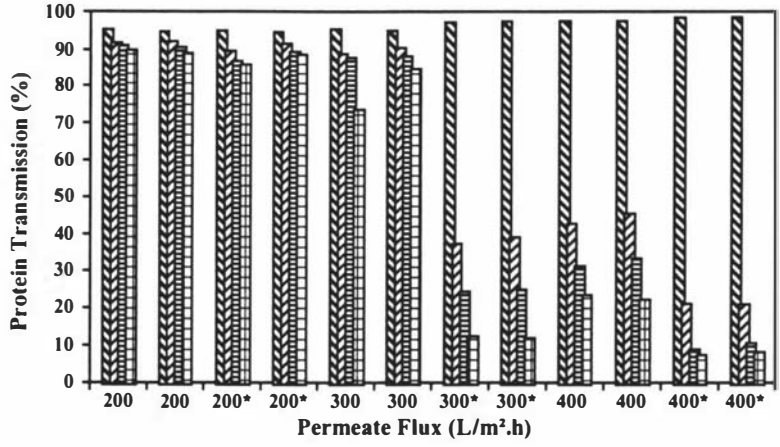

Figure 6.10. Protein transmission at various permeate fluxes during MF of 0.2% bovine serum albumin solution in the presence of calcium on a 100 nm membrane. The ionic strength of the solution was 0.054-0.055; the calcium content was 8.0 to 8.1 mmol/L and the pH from 6.80-6.82. For each permeate flux the 4 bars represent values measured at 30, 60, 90 and 120 min respectively. The calcium runs are denoted by (*).

Severe fouling was prevented at 200 L/m^2.h giving protein transmissions of about 85-95%. Similar trends can be seen in data obtained from the UV cell (Fig. 6.11). The presence of calcium appeared initially to give a slight "depression" in the fouling resistance curve. However more rapid and severe fouling occurred with time. The initial protein transmission was observed to be higher in the presence of calcium but lower for the severely fouled membranes. These observations suggest that in the presence of calcium, any aggregates are of smaller size and increase in size with time particularly at higher fluxes. The presence of calcium increased both irreversible and reversible fouling resistance (Fig. 6.12).

On a 50 nm membrane, the presence of calcium again exacerbated severe fouling at fluxes particularly > 200 L/m^2.h (Fig. 6.13). Protein transmissions reduced to about 12% after 2 h (Figs. 6.14 & 6.15). At 200 L/m^2.h, calcium addition gave lower fouling resistance than the control on this membrane. The presence of calcium increased both irreversible and reversible fouling resistance at 300 L/m^2.h (Fig. 6.16). Calcium appeared to enhance aggregation of protein leading to severe fouling. To see any presence of aggregated protein, calcium containing BSA samples were ultracentrifuged (150,000 g) for 60 minutes. Sediments, probably aggregated protein, were observed. Protein content analysis of supernatant and total sample by UV spectrophotometer showed that 0.021% of the total protein is in the form of sediment compared to the control which had 0.013% under the same conditions of ultracentrifugation.

As both 50 and 100 nm membranes appeared to behave similarly (see section 6.2.7), further investigations on fouling were focused on a 100 nm pore membrane.

6.2.3. Effect of prefiltration and SDS addition

Experiments with varying permeate flux revealed that protein transmissions were much lower than expected for the monomeric protein BSA on the 50 or 100 nm membranes used in the investigation. Intuitively it was hypothesised that aggregates in the feed or hydrophobic aggregates that formed during MF itself may be responsible for severe fouling.

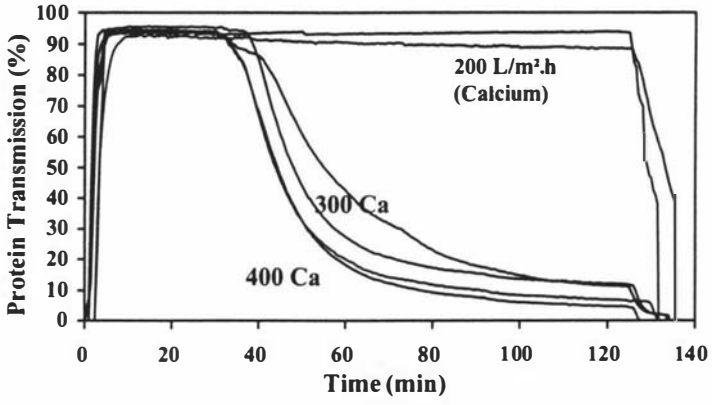

Figure 6.11. Protein transmission (as measured by a UV-cell) at various permeate fluxes during MF of 0.2% bovine serum albumin solution in the presence of calcium on a 100 nm membrane. The ionic strength of the solution was 0.054 to 0.055; the calcium content was 8.0 to 8.1 mmol/L and the pH from 6.80 to 6.82.

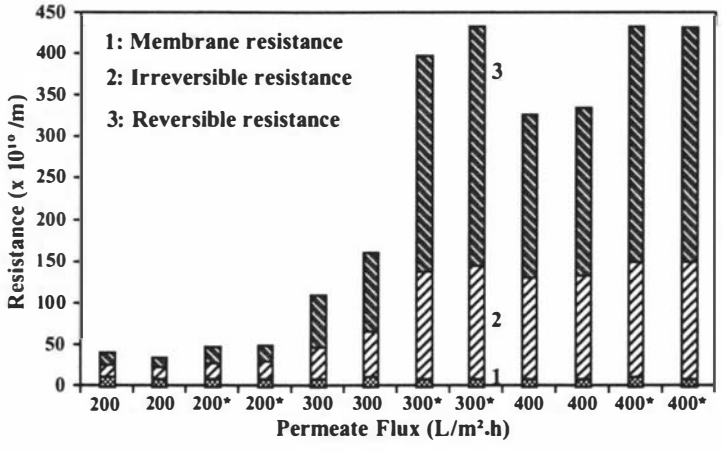

Figure 6.12. Irreversible and reversible fouling resistance at various permeate fluxes during MF of 0.2% bovine serum albumin solution in the presence of calcium on a 100 nm membrane. The ionic strength of the solutions was 0.054 to 0.055; the calcium content was 8.0 to 8.1 mmol/L and the pH from 6.80 to 6.82. The calcium runs are denoted by (*).

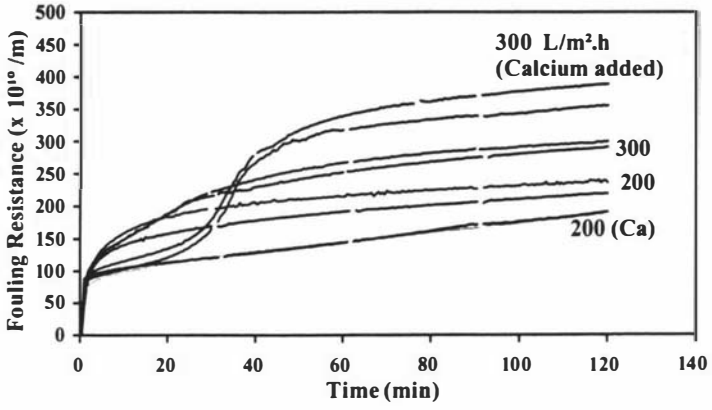

Figure 6.13. Fouling resistance at various permeate fluxes during MF of 0.2% bovine serum albumin solution in the presence of calcium on a 50 nm membrane. The ionic strength of the solutions was 0.048 to 0.052; the calcium content was 8.0 to 8.1 mmol/L and the pH from 6.80 to 6.82. Results without calcium are also shown for comparison.

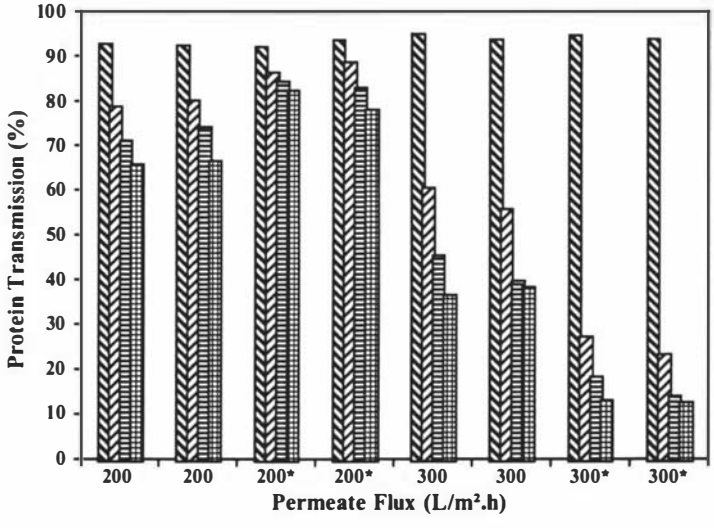

Figure 6.14. Protein transmission at various permeate fluxes during MF of 0.2% bovine serum albumin solution in the presence of calcium on a 50 nm membrane. The ionic strength of the solution was 0.048 to 0.052; the calcium content was 8.0 to 8.1 mmol/L and the pH from 6.80 to 6.82. For each permeate flux the 4 bars represent values measured at 30, 60, 90 & 120 min respectively. The calcium runs are denoted by (*).

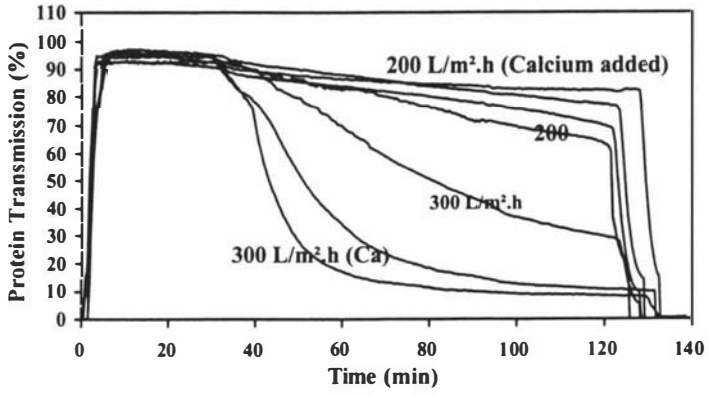

Figure 6.15. Protein transmission (as measured by a UV-cell) at various permeate fluxes during MF of 0.2% bovine serum albumin solution in the presence of calcium on a 50 nm membrane. The ionic strength of the solution was 0.048 to 0.052; the calcium content was 8.0 to 8.1 mmol/L and the pH from 6.80 to 6.82. Results without calcium are also shown for comparison.

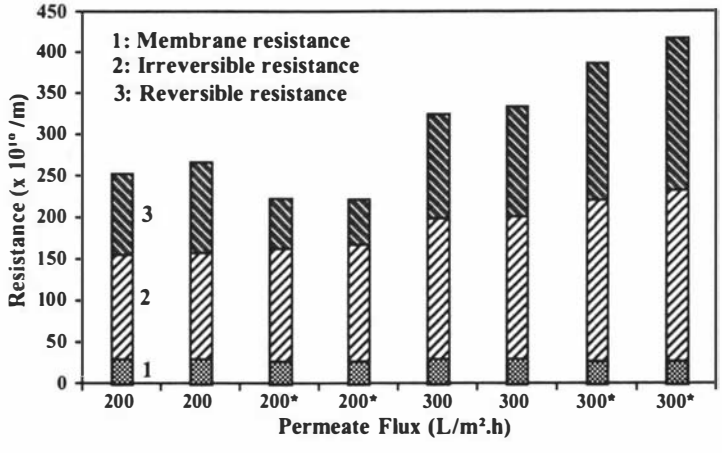

Figure 6.16. Irreversible and reversible fouling resistance at various permeate fluxes during MF of 0.2% bovine serum albumin solution in the presence of calcium on a 50 nm membrane. The ionic strength of the solutions was 0.048 to 0.052; the calcium content was 8.0 to 8.1 mmol/L and the pH from 6.80 to 6.82. The calcium runs are denoted by (*).

To investigate this in more depth, a systematic study was conducted to determine the effects of 1) prefiltering the feed before MF and 2) addition of sodium dodecyl sulfate (SDS), a surface active agent, to the feed. Prefiltration of BSA solution was performed using a polysulfone membrane (PM 500; Koch Membrane Systems Inc., Wilmington, USA) to remove any aggregates present in the feed. Prefiltration reduced fouling and improved protein transmission to about 70% compared to 20% with the control (Figs. 6.17 & 6.18) but did not eliminate fouling completely. There was a slight increase in protein transmission during the first 30 minutes compared to the control.

The presence of SDS (1.4g/g of protein *i.e.* ~0.3%) in solution also reduced fouling and increased protein transmission slightly, particularly during the first hour, perhaps due to a smaller number of aggregates that may have otherwise deposited within the pores (Fig. 6.18). It is interesting to note that irreversible fouling resistance was significantly lower in the presence of SDS (Fig. 6.19).

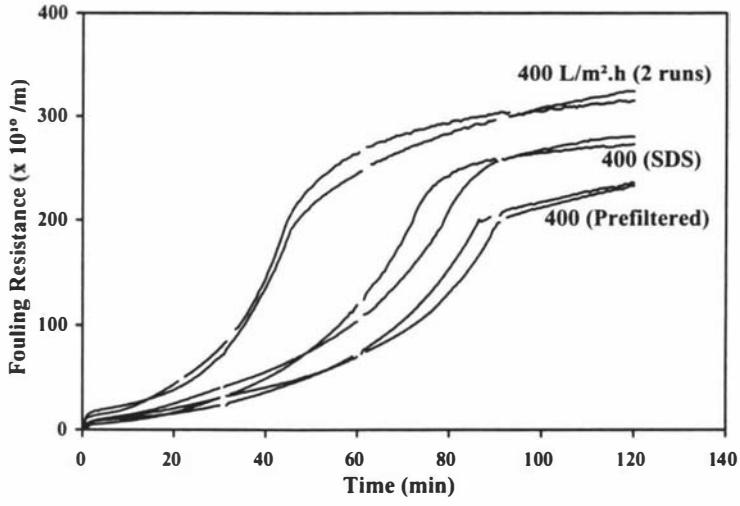

Figure 6.17. Effect of prefiltration and presence of SDS solution on the fouling resistance during MF of 0.2% bovine serum albumin solution on a 100 nm membrane. The ionic strength of the solutions was 0.051 to 0.054 and the pH from 6.80 to 6.82.

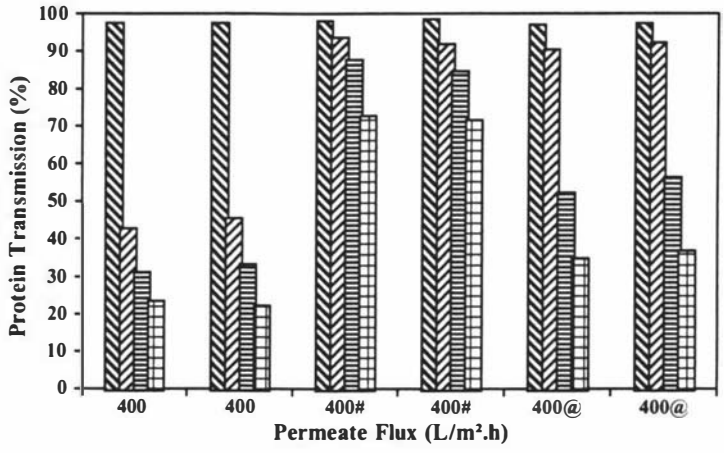

Figure 6.18. Effect of prefiltration (#) and presence of SDS solution (@) on the protein transmission during MF of 0.2% bovine serum albumin solution on a 100 nm membrane. The ionic strength of the solution was 0.051 to 0.054 and the pH from 6.80 to 6.82. For each permeate flux the 4 bars represent values measured at 30, 60, 90 & 120 min respectively.

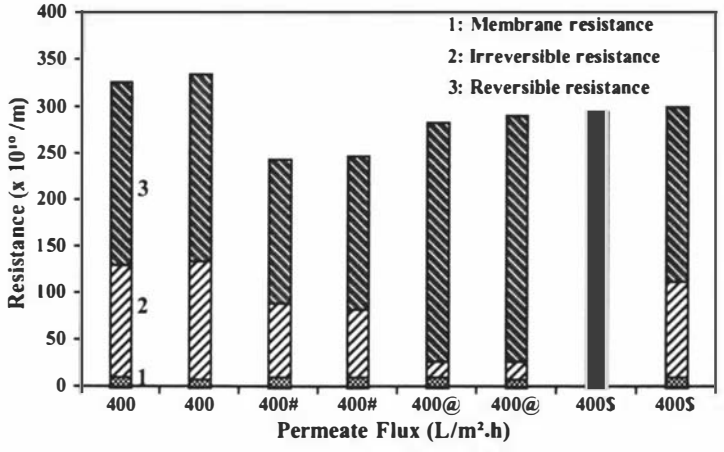

Figure 6.19. Effect of prefiltration (#), presence of SDS solution (@) and cysteinyl BSA ($) on the irreversible and reversible fouling resistance during MF of 0.2% bovine serum albumin solution in the absence of calcium on a 100 nm membrane. The ionic strength of the solutions was 0.051 to 0.054 and the pH from 6.80 to 6.82.

6.2.4. Effect of sulphydryl blocking agent

Having seen that aggregates in the feed and those produced perhaps by the exposure of hydrophobic groups during MF contribute to severe fouling, the possible reasons for aggregation of protein were explored. The possibility of thiol-mediated aggregation of BSA was investigated by conducting experiments with BSA in which the free thiol group was blocked. BSA possesses one free thiol group that can interact with disulfide bond on another BSA molecule to form disulfide-linked aggregates. A sulphydryl blocking agent, N-ethylmaleimide (NEM), was added to the feed to investigate whether or not thiol blocking by NEM will reduce thiol-mediated aggregation thereby, potentially reducing fouling. Two concentrations of NEM were tried. Initially, 1 mM and then 0.04 mM (equivalent to 1:1 molar ratio of NEM required to block free thiol group in BSA) of NEM were used. Fouling was severe at 1 mM concentration (Fig. 6.20). Fouling was not reduced at 0.04 mM concentration, in fact it was slightly higher than the control. Protein transmissions at 1 mM concentration were lower than the control (Fig. 6.21). There appears to be a critical concentration at which NEM effectively blocks free thiol group in BSA. As problems were encountered 1) in the optimisation of NEM to BSA ratio to effectively block the free thiol group and 2) in the control of possible non specific aggregation due to NEM, further experiments were not performed using NEM in BSA solutions.

6.2.5. Effect of cysteinyl blocked BSA

Further experiments were performed using Cysteinyl BSA (A-0161, Sigma Chemical Co) which has been reported to have less than 0.02 M free sulphydryl per mole of BSA. Fouling did occur severely but slightly less than the control at 400 $L/m^2.h$ (Fig 6.22) giving slightly improved protein transmissions particularly after 30 min (Fig. 6.23). The fouling was both due to irreversible and reversible fouling resistance (Fig. 6.19).

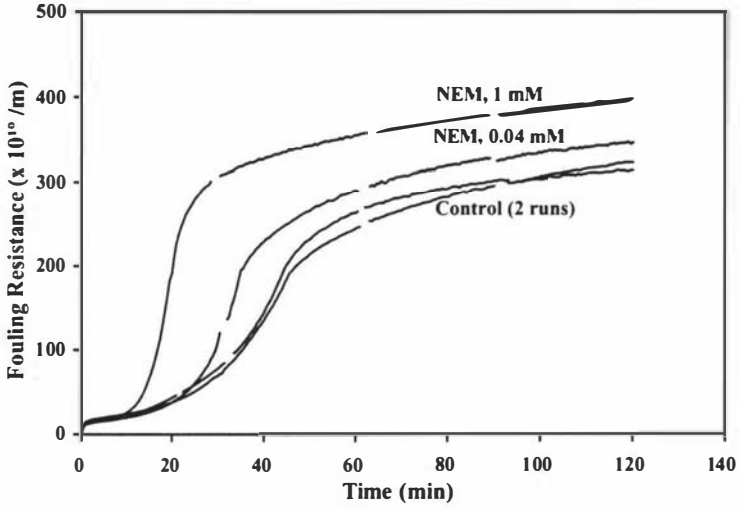

Figure 6.20. Effect of NEM on the fouling resistance during MF of 0.2% bovine serum albumin solution on a 100 nm membrane. The ionic strength of the solutions was 0.050 to 0.053 and the pH from 6.80 to 6.82.

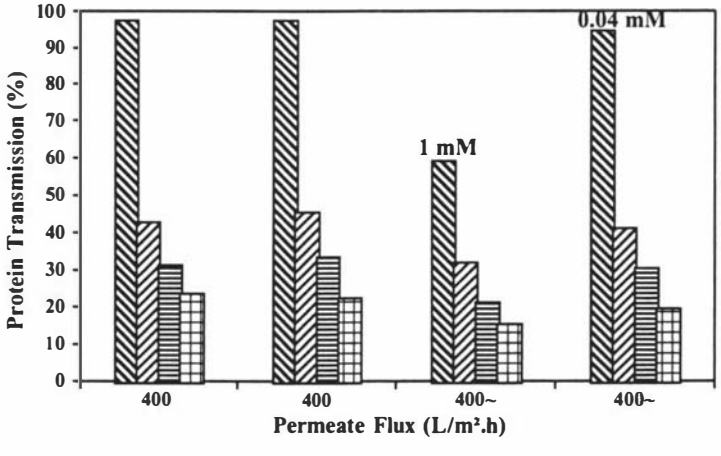

Figure 6.21. Effect of NEM on the protein transmission during MF of 0.2% bovine serum albumin solution on a 100 nm membrane. The ionic strength of the solution was 0.050 to 0.053 and the pH from 6.80 to 6.82. For each permeate flux the 4 bars represent values measured at 30, 60, 90 & 120 min respectively. The NEM runs are denoted by (~).

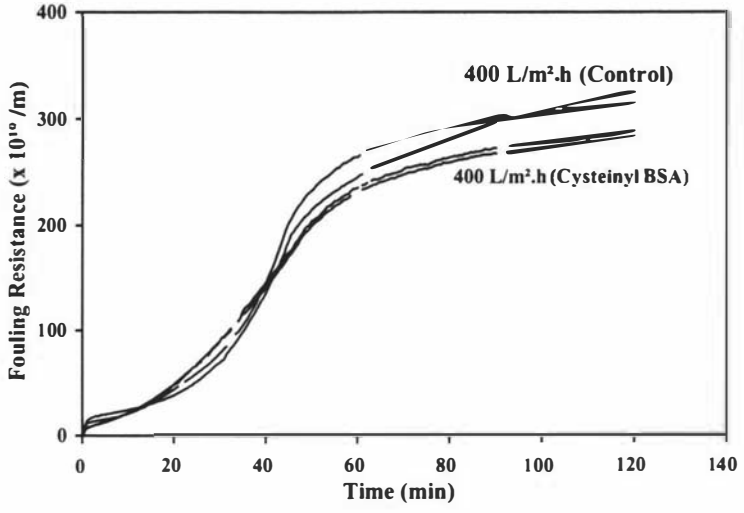

Figure 6.22. Effect of the cysteinyl BSA on the fouling resistance during MF of 0.2% bovine serum albumin solution on a 100 nm membrane. The ionic strength of the solutions was 0.052 to 0.054 and the pH from 6.80 to 6.82.

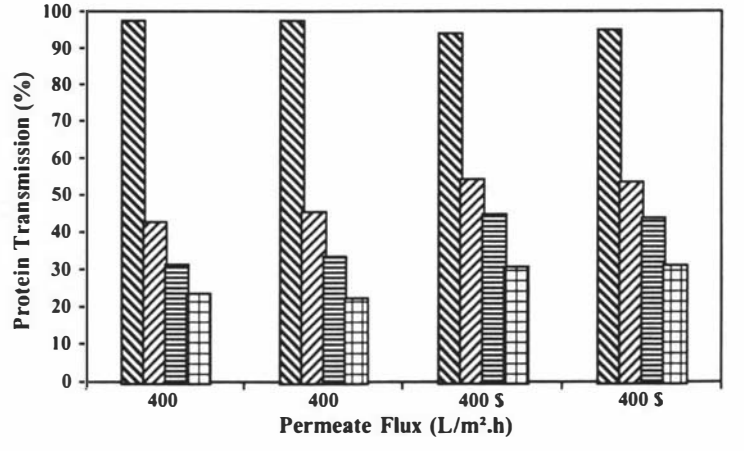

Figure 6.23 Effect of the cysteinyl BSA on the protein transmission during MF of 0.2% bovine serum albumin solution on a 100 nm membrane. The ionic strength of the solutions was 0.052 to 0.054 and the pH from 6.80 to 6.82. For each permeate flux the 4 bars represent values measured at 30, 60, 90 & 120 min respectively. The cysteinyl runs are denoted by ($).

6.2.6. Fouling resistance versus protein transmission

On the 100 nm membrane there was a general relationship between protein transmission (measured by UV spectrophotometer & UV cell) and fouling resistance under different solution conditions used in the experiments (Figs. 6.24 & 6.25). Varying the permeate flux did not appear to affect the relationship between fouling resistance and protein transmission. A best fit line was drawn to represent the data (in Fig. 6.24). The line shows an almost constant protein transmission up to a fouling resistance of about 100 x 10^{10} /m and then a continuous decrease of protein transmission with an increase in fouling resistance (Fig. 6.24). During the first 30 minutes, when fouling was light, the addition of calcium improved protein transmission slightly. The details of the Zeman and Marshall models are presented in the discussion section. A similar relationship was observed for the 50 nm membrane (Fig. 6.26). Few high protein transmission data points were obtained in the presence of calcium which increased protein transmission when fouling was light. The best fit lines for the data on this membrane also showed a similar trend.

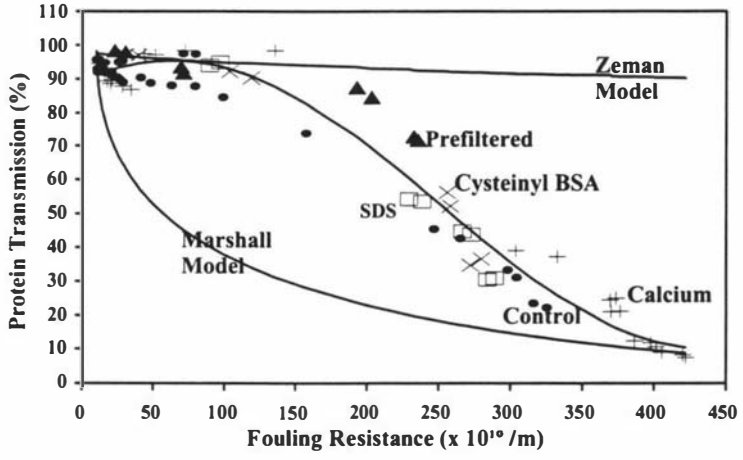

Figure. 6.24. Effect of calcium, SDS, prefiltration and cysteinyl BSA on the relationship between fouling resistance and protein transmission (as measured by a UV spectrophotometer) for a 100 nm membrane. See text for explanation of model and fitted lines. The legends are calcium (+), SDS (□), prefiltered BSA (▲), cysteinyl BSA (X), and Control (•)

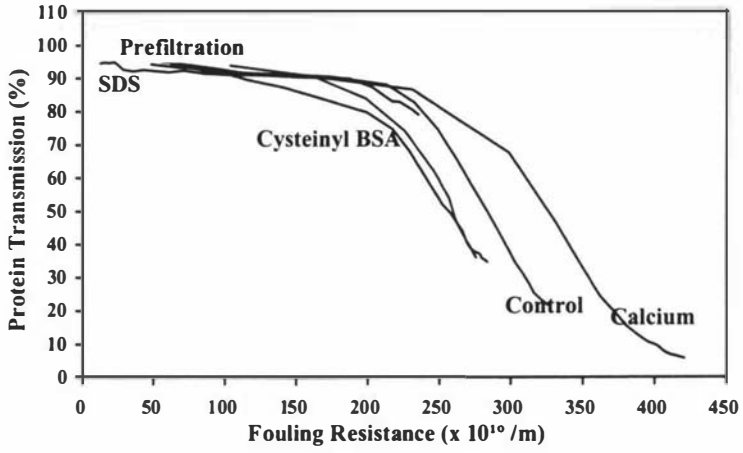

Figure. 6.25. Effect of calcium, SDS, prefiltration and cysteinyl BSA on the relationship between fouling resistance and protein transmission (as measured by a UV cell) for a 100 nm membrane.

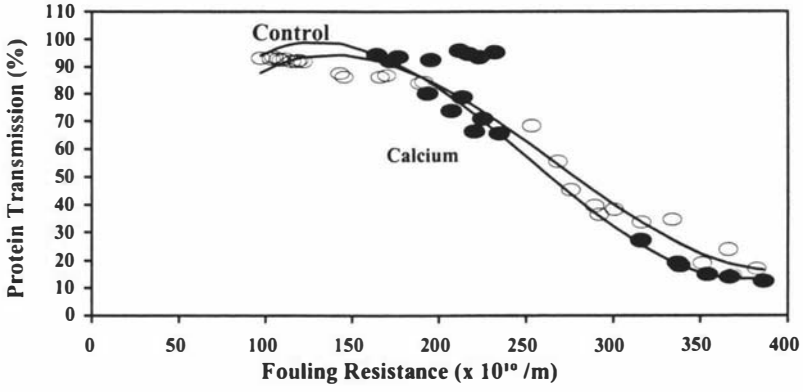

Figure. 6.26. Effect of calcium on the relationship between fouling resistance and protein transmission (as measured by a UV spectrophotometer) on a 50 nm membrane. See text for explanation of fitted lines. Calcium and control runs are denoted by (•) and (○) respectively.

6.2.7. Effect of the membrane pore size

Two experiments were performed on a 20 nm membrane to investigate the impact of fouling on a relatively small pore size membrane. Severe fouling occurred even at a permeate flux of 50 $L/m^2.h$ on the 20 nm pore membrane reducing protein transmissions to about 6-8% (Figs. 6.27 & 6.28). The increase in fouling resistance was dramatic on this membrane compared to the 50 and 100 nm pore membranes at the same flux.

Fouling was light both on 50 and 100 nm membranes at a permeate flux of 50 $L/m^2.h$ (Figs. 6.1 & 6.5). Protein transmissions > 90% were obtained (Figs. 6.2 & 6.6). Protein transmissions during the first 30 min were slightly higher for the 100 nm membrane reflecting the larger pore size of the membrane. At 400 $L/m^2.h$, where severe fouling occurred on both the membranes, fouling resistance curves look similar in shape except that the 50 nm membrane had a higher "jump" in the initial fouling resistance due to the relatively tighter pore size (Figs. 6.1 & 6.5). Protein transmission dropped from 93% to 15% for 50 nm and 95% to 22% for the 100 nm pore size membranes respectively (Figs. 6.2 & 6.6) again reflecting the initial pore size difference of the two membranes. UV protein transmissions started decreasing from about 30 minutes (Figs. 6.3 & 6.7). At 300 $L/m^2.h$ permeate flux, in the presence of calcium, severe fouling occurred on both the membranes (Fig. 6.9 & 6.13). The shapes of the curves were similar except for the 50 nm membrane which had a high initial jump in fouling resistance. Protein transmission finally reduced to about 12% after 2 h (Figs. 6.10 & 6.14 and also 6.11 & 6.15).

Comparing types of reversible and irreversible fouling resistances, irreversible fouling resistance increased as pore size decreased (Figs. 6.4 & 6.8). Severe fouling caused by the increase in permeate flux resulted in slightly more reversible fouling resistance on the 100 nm membrane whereas there was more irreversible resistance on the 50 nm membrane.

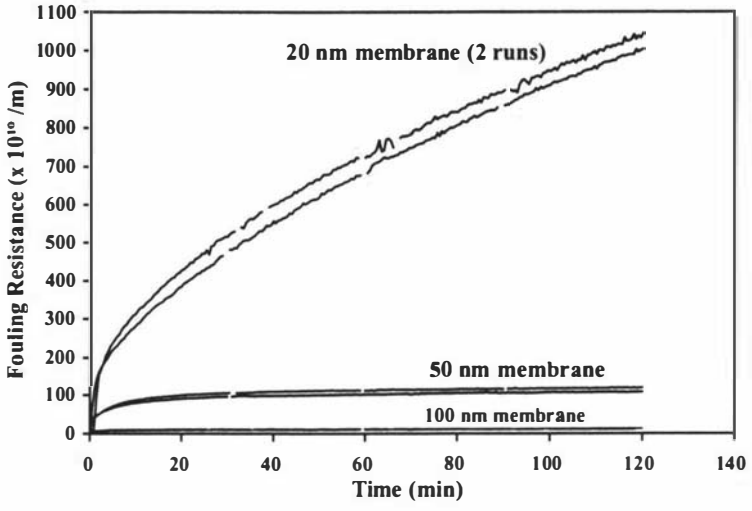

Figure 6.27. Effect of the pore size on the fouling resistance during the MF of 0.2% bovine serum albumin solution at an initial flux of 50 L/ m^2.h. No calcium was added in these trials. For the 20 nm membrane the IS was 0.049-0.051, the pH 6.79-6.81; for the 50 nm membrane the IS was 0.051-0.053, the pH 6.80-6.82; for the 100 nm membrane the IS was 0.052-0.053, the pH 6.80-6.82.

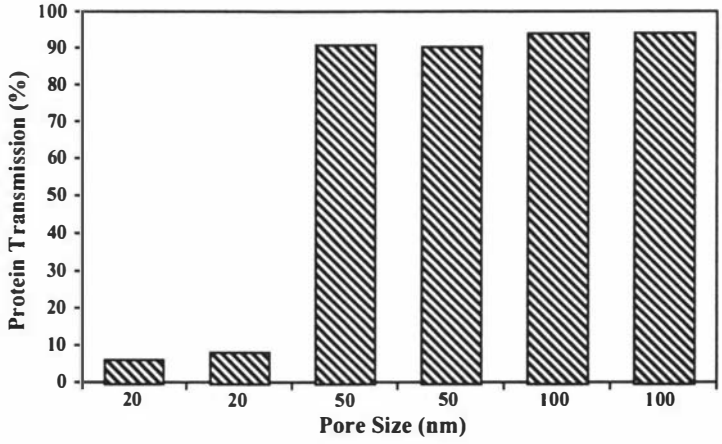

Figure 6.28. Effect of the pore size on protein transmission (at 2 hours) during the MF of 0.2% bovine serum albumin solution at an initial flux of 50 L/ m^2.h. No calcium was added in these trials. For the 20 nm membrane the IS was 0.049-0.051, the pH 6.79-6.81; for the 50 nm membrane the IS was 0.051-0.053, the pH 6.80-6.82; for the 100 nm membrane the IS was 0.052-0.053, the pH 6.80-6.82.

The relationship between protein transmission and fouling resistance was similar for the 100 and 50 nm membranes (Fig. 6.29) confirming that both the membranes are fouled by a similar fouling mechanism. In general, protein transmission stayed almost constant for fouling resistances up to 100 x 10^{10} /m for the 100 nm membrane and 200 x 10^{10} /m for the 50 nm membrane and decreased continuously after that before it finally reached a protein transmission of about 10%.

The initiation of fouling is somewhat different by way of delay for the two membranes because of the difference in their initial membrane pore size. However after about 60 minutes (corresponding to a fouling resistance of about 300 x 10^{10} /m), the fouling resistance curves were similar in shape and trend. The 100 nm membrane had a time lag before increase in the fouling resistance, because of the larger initial pore size and lower clean membrane resistance. Fouling was prevented and protein transmissions of about 90-95% were obtained up to permeate flux of 50 L/m^2.h on both the membranes.

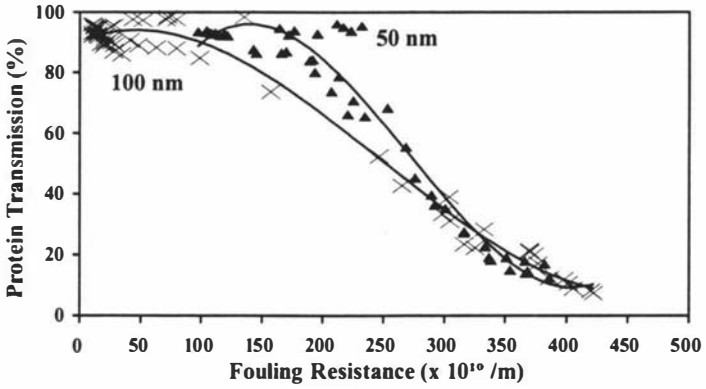

Figure 6.29. Effect of the pore size on the relationship between protein transmission (UV spectrophotometer) and fouling resistance during the MF of 0.2% bovine serum albumin solution both in the presence and absence of calcium. Calcium concentration was 8.0-8.1 mM, and pH 6.80-6.82. For the 50 nm membrane the IS was 0.052-0.054, for the 100 nm membrane the IS was 0.052-0.053. Calcium and control runs are denoted by (▲) and (X) respectively. The best fitted lines for the data are shown.

6.3. Discussion

With the 50 and 100 nm membranes, the fouling behaviour was highly dependent on the permeate flux and presence of calcium. Severe fouling that reduced the protein transmission from ~ 90% to 10% occurred under conditions of calcium presence and high permeate fluxes (200 + $L/m^2.h$). The effect of calcium was not solely due to ionic strength since trials with the same ionic strength achieved by addition of NaCl only did not invoke severe fouling at fluxes where calcium caused severe fouling. Calcium appeared to play a specific role in severe fouling at higher fluxes.

6.3.1. Effect of the permeate flux

It is hypothesised that as the flux is increased the shear stress in the pore is increased, causing an increase in protein unfolding, deposition and fouling as observed in the experiments. Shear stress exerted on the protein near the pore wall as it enters the pore "stretches" or reversibly unfolds the protein as suggested by Franken *et al.* (1990), Bowen & Gan (1992), Jonsson *et al.* (1992a) and Marshall *et al.* (1997). As a result, the hydrophobic and thiol groups may be exposed enhancing intermolecular protein-protein interactions leading to formation of protein aggregates. These aggregates may cause pore narrowing or may block the pores depending upon the ratio of aggregate size to pore size. After the pores are completely covered with protein aggregates, a surface layer would form on the membrane with further deposition of protein. A similar type of mechanism, flux-induced aggregation and subsequent fouling was reported by Kim *et al.* (1992).

Another possible explanation is increase in protein deposition due to increase in TMP at higher fluxes. At the high permeate fluxes, to combat increase in resistance to permeate flow due to fouling, TMP was increased rapidly by the permeate controller to maintain constant flux. Increased TMP is likely to increase adsorption and deposition due to increased rate of interaction of protein with the membrane geometry. Blanpain *et al.* (1993) reported that at a TMP of 100 kPa, kinetics of build up of the internal adsorbed protein layer were faster than at 10 kPa. Therefore, it is proposed that high TMP enhances protein adsorption probably due to protein denaturation by high wall shear stress in the pores. The proposed increase in adsorption and deposition by aggregates at higher fluxes is supported by an increase

in both reversible and irreversible fouling resistance (Table 6.1) at higher fluxes. High reversible resistance normally suggests loosely bound surface layer whereas irreversible resistance is usually due to adsorption.

6.3.2. Role of calcium in protein-protein interactions

On both 100 and 50 nm membranes, at fluxes above 200 $L/m^2.h$, the presence of calcium increased the fouling resistance reducing protein transmission to about 10%. The presence of calcium in the feed may change protein-protein interactions or the nature of the protein-membrane interactions, possibly affecting the degree of protein adsorption or the nature of the deposited layer.

Addition of calcium at 200 $L/m^2.h$ increased apparent pore size reduction from 18-19 to 24-25 nm for the 100 nm membrane and from 16 to 18 nm for the 50 nm membrane (Tables 6.2 & 6.3). Aggregated protein due to calcium may have deposited within the pore initially decreasing apparent pore size. The apparent pore size reduction on the 50 nm membrane was slightly lower. It may be that the impact on this membrane is less because of the smaller pore size. Once the pore is narrowed sufficiently, a surface layer starts building up reducing the protein transmission to a very low level as observed in the experiments with the calcium containing feed and at higher permeate fluxes. It is known that calcium affects the degree of protein deposition on the membranes (Hayes *et al.,* 1974; Marshall & Daufin 1995) although it is not clear by what mechanism. Calcium appeared to have a more specific interaction with BSA probably leading to aggregation of protein. Sediment observed in the calcium containing samples was evidence of aggregated protein.

When fouling occurs, membrane surface properties are completely masked by the properties of the deposited protein. So any further deposition occurs by protein-protein interactions rather than protein-membrane interactions. Several researchers have studied protein-protein interactions and their role in aggregation and gelation of whey proteins. Denaturation and gelation studies on whey proteins particularly BSA are reviewed to see any analogies related to the role of calcium in protein-protein interactions and subsequent aggregation.

Table 6.2. Apparent pore size reduction in trials on the 100 and 50 nm membranes performed at 200 L/m^2.h in the absence of calcium.

Membrane Pore Size (nm)	Clean membrane resistance (x 10^{10}/m)	Irreversible fouling resistance (x 10^{10}/m)	Apparent reduction in pore size (nm)
50	32.8	124.3	16
50	32.6	128.0	16
100	11.7	16.0	18
100	11.1	14.3	19

Table 6.3. Apparent pore size reduction in trials on the 100 and 50 nm membranes performed at 200 L/m^2.h in the presence of calcium.

Membrane Pore Size (nm)	Clean membrane resistance (x 10^{10}/m)	Irreversible fouling resistance (x 10^{10}/m)	Apparent reduction in pore size (nm)
50	29.4	136.0	18
50	29.7	140.3	18
100	9.7	20.1	24
100	10.1	21.3	25

BSA is a large globular protein consisting of 580 amino acid residues with 17 interchain disulfide bonds and one free thiol group at residue 34 (Kinsella & Whitehead, 1989). The protein consists of approximately 54% α-helix and 40% β-structure (β-sheets and β-turns) and contains three domains specific for metal ion binding, lipid binding and nucleotide binding (Peters & Reed, 1977).

Thermally-induced gelation is a complex process; the initial phase involves heat induced conformational changes in the protein with unfolding of some polypeptide segments followed by a subsequent phase of protein-protein interactions resulting in polymerisation (aggregation) and formation of a network structure (Ferry, 1948; Bernal & Jelen, 1985). BSA is susceptible to thermally induced denaturation which is largely determined by temperature, pH and other conditions, such as presence of other components *e.g.* calcium. During thermally-induced gelation of BSA, functional groups engaged in the native state become available for intermolecular interactions

(Damodaran, 1994). Exposure of hydrophobic groups results in hydrophobic interactions which are necessary in the aggregation and cross-linking of the gel network. Buried sulphydryl groups initiate disulfide-sulphydryl interchange reactions which contribute to cross-linking (Shimada & Cheftel, 1989).

Boye *et al.* (1996) studied interactions involved in gelation of BSA. The addition of $CaCl_2$ was found to be more effective in increasing strength of BSA gels than the addition of NaCl (Matsudomi *et al.*, 1991). The calcium ions were reported to engage in the formation of calcium bridges between negatively charged groups on adjacent unfolded protein molecules thereby strengthening the gel structure (Mulvihill & Kinsella, 1988; Kinsella & Whitehead, 1989; Kuhn & Foegeding, 1991; Lupano *et al.*, 1992; Barbut & Foegeding, 1993; Smith & Rose, 1994). Several studies showed aggregation and precipitation of heated whey proteins and β-lactoglobulin (β-Lg) by calcium ions (Zittle *et al.*, 1957; Varunasatian *et al.*, 1983; Patocka & Jelen, 1991). Intermolecular calcium bridges during β-Lg aggregation were reported by Jeyarajah & Allen (1994) and Li *et al.* (1994).

Marshall (1994) suggested that a calcium bridge is the most likely mechanism for the protein-protein interaction to block the 50-100 nm pores during MF of β-Lg. Van Camp *et al.* (1997) studied the effect of calcium on the high pressure (400 MPa for 30 minutes) induced aggregation of whey proteins. An increase in calcium concentration (0 to 7 mM) reduced the overall solubility of whey proteins after pressurisation at pH 6 and 7 but not at 5. They showed that the amount of native β-Lg was reduced significantly after pressurisation compared to α-lactalbumin (α-La). The amount of native BSA after pressurisation could not be quantified due to the small amount of this protein in whey and also limitations of the technique.

Zhu & Damodaran (1994) studied the effects of Ca^{2+} and Mg^{2+} ions on aggregation of whey proteins and the effect on foaming properties. Calcium ions induced formation of stable colloidal aggregates in whey proteins. In the presence of about 0.04 M salt, the extent of protein aggregation was greater in $CaCl_2$ than in $MgCl_2$ solution. The aggregation behaviour was attributed to specific binding of Ca^{2+} and Mg^{2+} ions to individual whey proteins. Binding of divalent cations seems to cross-link the protein

via ionic bridges leading to polymerisation. Zhu & Damodaran (1994) reported time dependent aggregation of whey protein by calcium ions. The extent of whey protein aggregation was dependent upon the presence of calcium (de Wit, 1981). Calcium seems to have an additional effect beyond charge neutralisation (Varunasatian *et al.*, 1983; Mulvihill & Kinsella, 1988; Foegeding *et al.*, 1992; Xiong, 1992; Xiong *et al.*, 1993). Calcium is capable of forming an ion bridge between two adjacent carboxyl groups from different peptide chains because it is divalent (Xiong, 1992). Also Gault and Fauquant (1992) suggested that calcium was more efficient than sodium in inducing gelation because it was able to form intermolecular calcium bridges.

From the forgoing discussion it is likely that calcium most probably forms an ion bridge enhancing protein-protein interactions leading to aggregation. Based on protein-protein interactions, calcium in solution and the presence of high shear at high permeate flux, it is proposed that the ionic calcium enhanced the aggregation of BSA resulting in more severe fouling compared to experiments in the absence of calcium.

6.3.3. Effect of shear and protein-protein interactions

At fluxes above 300 $L/m^2.h$, severe fouling occurred reducing protein transmission to about 20% on both 100 and 50 nm membranes. Considering the size of the BSA molecule, 7 nm in its monomeric form in solution (Gésan *et al.*, 1993; Bowen & Williams, 1996), compared to the pore size of the membranes (50 and 100 nm) in these experiments, it is surprising to observe severe fouling and lower protein transmission at higher fluxes. When fouling was light, the apparent pore size reduction calculated using equation (3.12) was in the range of 14-15 nm (Table 6.4). This is reasonably in agreement with the expectation that adsorption will cause a narrowing of the pores by the equivalent of two times the diameter of the protein. Initially the membrane pore size was reduced by 14-15 nm probably by monolayer adsorption of protein. Thus further protein passing through a 100 or 50 nm membrane would essentially pass through a 85-86 or 35-36 nm pore respectively with its internal surface covered by adsorbed protein.

Table 6.4. Apparent pore size reduction in trials on the 100 and 50 nm membranes performed at 50 L/m^2.h in the absence of calcium.

Membrane Pore Size (nm)	Clean membrane resistance (x 10^{10}/m)	Irreversible fouling resistance (x 10^{10}/m)	Apparent reduction in pore size (nm)
50	29.7	87.9	15
50	30.6	80.7	14
100	11.0	10.1	15
100	11.6	10.3	15

Protein adsorption to a membrane is generally irreversible and can result in disruption of the protein structure and denaturation of the protein (Lundström, 1985) thereby exposing active groups and different binding sites for protein on the membrane surface. This may help in further attachment of protein on the existing deposit probably via ionic, hydrophobic and disulfide interactions between the protein in the bulk and in the deposit. To block a 50 or 100 nm pore, 6-7 or 12-14 protein molecules would be required, respectively, if they stack directly on top of each other. This suggests that if BSA molecules exist as monomers, all the protein molecules must pass through the pores even after initial monolayer adsorption. This clearly indicates that protein-protein interactions must predominate rather than protein-membrane interactions. Also after initial protein adsorption, the membrane surface properties may have little impact on fouling behaviour.

Marshall (1994), while reviewing previous work related to shear effects on proteins, suggested that protein may be "conditioned" *i.e.* shear may unfold the protein making it ready for subsequent protein-protein interactions and deposition. It is proposed that thiol-disulfide and hydrophobic interactions are predominant in BSA aggregation under shear.

6.3.3.1. Disulfide interactions

Unfolding is a prerequisite for disulfide-linked aggregation as the free thiol group is usually buried inside the protein structure. Unfolding due to shear may lead to formation of some disulfide-linked aggregates, even though the temperature is relatively low (25°C) in the present experiments. Monahan *et al.* (1995) studied

thiol/disulfide interchange reactions during gelation of whey proteins. They reported that at pH 9 and 11, significant SH-SH oxidation to S-S occurred even at room temperature (22°C). They estimated the degree of unfolding of whey proteins by measuring the exposure of hydrophobic amino acid residues. They reported that at pH 9 and 11 extensive irreversible unfolding of the protein molecules had occurred at room temperature.

BSA due to its higher disulfide bond content compared to β-Lg and α-La engages in polymerisation reactions readily during heating (Monahan *et al.*, 1995). BSA gelation occurred even at 22°C under a pH of 11 though BSA molecules have strongly repulsive charges at this pH; extensive unfolding facilitates sufficient protein interactions including disulfide bond formation.

There are different possible interactions between BSA molecules. BSA possesses 35 cysteine residues, 34 of which are covalently linked to form 17 intramolecular disulfide bonds with the remaining cysteine residue present as a free thiol near the amino terminus of the molecule (Carter & Ho, 1994). Torchinsky (1981) has provided an overview of the chemistry of these sulfur containing amino acids. In general, two distinct pathways exist for the formation of thiol-mediated BSA aggregates: thiol oxidation and the thiol-disulfide interchange reaction. Thiol oxidation is catalysed in the presence of metal ions. This may result in the formation of dimers increasing the fouling tendency of the BSA molecules. Thiol-disulfide interchange reactions involve nucleophilic attack by an ionised thiol group or mercaptide ion on an existing disulfide bond leading to possible aggregation. Unfolding of the protein changes the protein conformation to expose groups that are buried in the native protein structure, thus resulting in an increased activity of such groups. Unfolding may also enable interchange reactions such as disulfide interchange to occur as a part of aggregation. Kelly & Zydney (1994) have reported this type of disulfide interchange aggregation of BSA during microfiltration experiments at 25°C.

MF of thiol blocked BSA (cysteinyl BSA having no free thiol group) compared to normal BSA in the present studies showed slightly reduced fouling suggesting some

contribution of disulfide-mediated aggregation. Kelly & Zydney (1994) showed that it is possible to prevent fouling with prefiltered cysteinyl BSA under non-pumping conditions demonstrating the role of disulfide-linked aggregates in fouling. However, they showed that unfiltered cysteinyl BSA did foul. This supports the hypothesis that aggregates present in the feed initially or produced under pumping contribute to the fouling as observed in the experiments. In the present work, cysteinyl BSA was not prefiltered, so perhaps aggregates present in the feed and those produced during actual filtration would have caused fouling, even though the effect of disulfide mediated aggregation would have been minimal. Similarly using NEM, which is a sulfhydryl blocking agent, Lee & Merson (1976b) showed that the rate and extent of flux decline during UF of cottage cheese whey could be reduced, further supporting the concept that disulfide interactions are important in BSA aggregation.

6.3.3.2. Hydrophobic interactions

Evidence of hydrophobic interactions contributing to protein deposition on membranes exists in the literature. Sheldon *et al.* (1991) showed that the tertiary structure of BSA was affected on adsorption to a hydrophobic polysulfone membrane. They observed a long and filamentous structure instead of a globular structure. Under similar conditions, BSA structure was not affected on deposition to a hydrophilic regenerated cellulose membrane. It was hypothesised that on adsorption to a hydrophobic material, BSA unfolded exposing hydrophobic sites. Their work showed the importance of membrane material on hydrophobic interactions of BSA. Le & Howell (1983) during UF of ovalbumin concluded that further deposition after the initial adsorption occurred via hydrophobic and intermolecular disulfide linkages between the adsorbed protein and the protein in the highly concentrated layer immediately adjacent to the membrane. Hydrophobic interactions are generally reversible whereas disulfide interactions are not (Morr & Josephson, 1968). Boye *et al.* (1996) during gelation studies reported that denaturation of BSA resulted in the loss of α-helical structure and formation of ordered non-native β-sheet structure associated with aggregation. The β-sheet content of native BSA is relatively low compared to other proteins and a decrease in α-helical structure and concomitant increase in β-sheets is observed on heating (Clark *et al.*, 1981; Byler & Purcell, 1989). This supports the observation of fouling in the form of β-sheets reported

during MF of whey using a 0.4 μm membrane (Lee & Merson, 1975). It is most probable that BSA unfolds exposing hydrophobic groups forming β-sheets on the surface of the membrane on adsorption.

SDS is known to dissociate hydrophobic aggregates in protein solutions. SDS may have dispersed hydrophobically linked aggregates present in the feed as well as those produced during MF giving reduced fouling as observed with SDS containing BSA solutions in the present investigation (Fig. 6.17). Fouling was however, not completely eliminated. This is consistent with minimal aggregation of BSA observed on heating in the presence of SDS (Boye *et al.*, 1996).

To summarise, protein unfolding is a first step in any aggregation/gelation process. Shear within the membrane pore at high fluxes may unfold the protein structure exposing reactive groups to enable protein-protein interactions. When protein is unfolded, protein aggregation is enhanced by a number of protein-protein interactions depending upon the physico-chemical state of the solution, the presence of other components or minerals and any treatment given to the solution. Hydrophobic interactions can occur as a first step in the aggregation process and are generally reversible. Disulfide interchange reactions occur in the presence of metal ions or in the presence of protein with a free thiol group and are usually irreversible.

Based on these protein-protein interactions in solution and the presence of high shear at high permeate flux, it is proposed that the conformational changes in the native BSA expose hydrophobic groups and also may provide access to previously inaccessible thiol groups. The partial reversibility of the fouling layer indicates aggregate fouling at higher fluxes is most probably due to both hydrophobic and thiol-mediated interactions that lead to aggregate formation and deposition.

6.3.4. Proposed fouling mechanisms

Under any operating conditions, there are three major contributors to fouling. 1) protein adsorption to the membrane 2) deposition of protein within the pores or at the pore entrance 3) surface layer formation on the membrane. Adsorption occurred in all the experiments as indicated by the slight increase in fouling resistance under light

fouling conditions (50 L/m^2.h) on both 50 and 100 nm membranes. The adsorbed layer was equal to about a monolayer of protein and did not appear to affect protein transmission on both of these membranes. However, it affected protein transmission on the 20 nm membrane as monolayer adsorption alone was sufficient to constrict pores, leading to an immediate surface layer. The surface layer reduced protein transmissions to about 10% right from the start on this membrane.

The possibility of pore fouling by narrowing or plugging was investigated by applying pore blocking laws (See section 3.6 and also Grace, 1956, Hermia, 1982, Hlavack & Bouchet, 1993 for details) to the experimental data on the 100 nm membrane. Plotting Ro/R versus time will give a straight line if the pore plugging mechanism occurs; plotting the square root of Ro/R versus time will be a straight line if it is pore narrowing mechanism and plotting the natural log of R/Ro versus time will be a straight line if the intermediate blocking law applies. Each of these plots for trials at 50, 300 and 400 L/m^2.h fluxes in the absence of calcium are shown in Figs. 6.30 to 6.32 for clarity. Data for the experiments in the presence of calcium were plotted in Figs. 6.33 to 6.35. Similar plots were obtained for the 50 nm membrane (not shown here).

None of the models show straight lines during the first few minutes probably reflecting dynamic resistance due to the presence of protein and the formation of an adsorbed layer. At a flux of 300 L/m^2.h in the absence of calcium, the data may fit a pore narrowing model (standard blocking law), but overall, these graphs do not explain the observed relationship between protein transmission and fouling resistance. A unique/single unmodified blocking law is unlikely to represent the fouling mechanism as shown by Iritani *et al.* (1995) and Marshall *et al.* (1997). It is possible that more than one mechanism may occur simultaneously to foul the membrane. These mechanisms are likely to compete depending upon the ratio of protein to pore size. Having seen that none of the unmodified pore blocking laws explain the observed fouling behaviour, the relationship between fouling resistance and protein transmissions (Fig. 6.24) was analysed to explore possible fouling mechanisms.

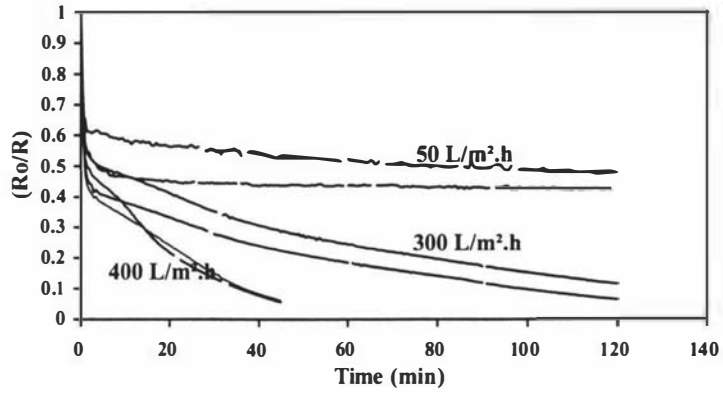

Figure 6.30. Investigation of pore plugging mechanism (complete blocking law) in the MF of bovine serum albumin in the absence of calcium on a 100 nm membrane.

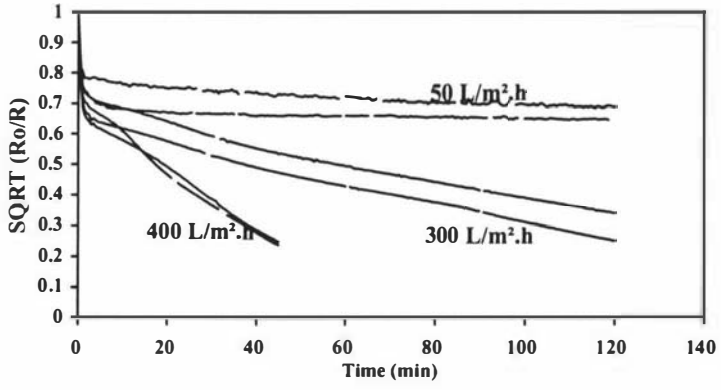

Figure 6.31. Investigation of pore narrowing mechanism (standard blocking law) in the MF of bovine serum albumin in the absence of calcium on a 100 nm membrane.

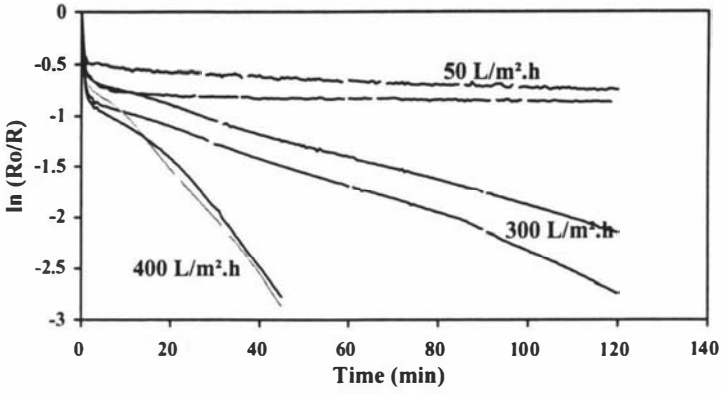

Figure 6.32. Investigation of intermediate blocking law in the MF of bovine serum albumin in the absence of calcium on a 100 nm membrane.

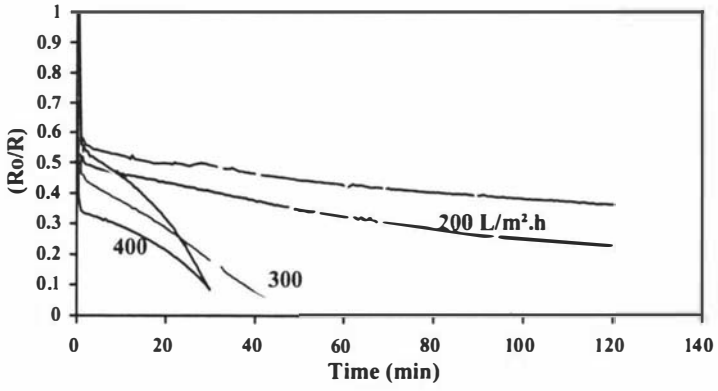

Figure 6.33. Investigation of pore plugging mechanism (complete blocking law) in the MF of bovine serum albumin in the presence of calcium on a 100 nm membrane.

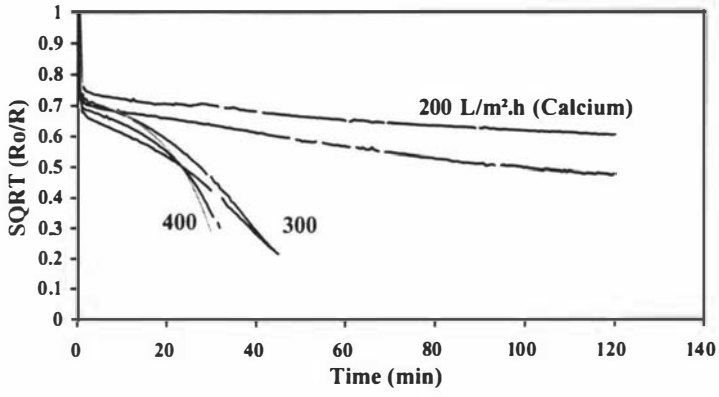

Figure 6.34. Investigation of pore narrowing mechanism (standard blocking law) in the MF of bovine serum albumin in the presence of calcium on a 100 nm membrane.

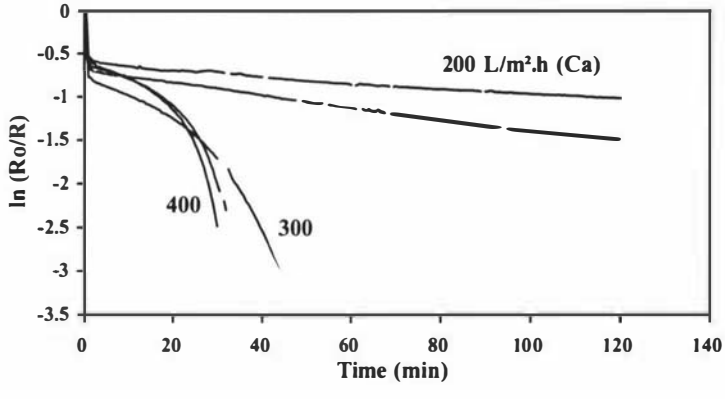

Figure 6.35. Investigation of intermediate blocking law in the MF of bovine serum albumin in the presence of calcium on a 100 nm membrane.

The apparent pore size reduction was determined from the fouling resistance data using an equation by Zeman (3.12) and then protein transmissions were calculated using the Ferry equation (3.16). The Zeman model line in Fig. 6.24 shows the predicted transmission/resistance relationship given an initial pore size of 100 nm, a protein diameter of 7 nm and assuming a pore narrowing mechanism. The model did not come anywhere near to predicting the observed behaviour.

Recently Marshall *et al.* (1997) developed a mathematical model that fitted the relationship between protein transmission and fouling resistance during MF of β-Lg. The model qualitatively showed that fouling by β-Lg occurs in a small section of pore length near the pore entrance. The model was applied to the BSA data obtained in the present investigation (Fig. 6.24). Again the model predictions did not come anywhere near the observed relationship between protein transmission and fouling resistance suggesting that deposition by BSA may not be in a small section of the pore. Having seen that the above models do not adequately explain the fouling behaviour, a qualitative analysis of the relationship between protein transmission and resistance was made to hypothesise probable fouling mechanisms.

Referring to figure 6.24, protein transmission remained almost constant up to a fouling resistance of about 100 x 10^{10} /m and decreased continuously with time after that. There appear to be two stages in MF fouling by BSA. The first stage perhaps consists of the formation of a very open, porous fouling layer so that BSA transmission is not affected. The second stage corresponds to a fouling resistance beyond 100 x 10^{10}/m. During this stage, protein transmission reduced from 90 to about 10% suggesting formation of a tighter fouling layer. It is likely that the open porous fouling layer in the first stage is formed by protein aggregates *i.e.* giving a porous structure between them. The second stage, a tighter fouling layer, may have formed by fouling at the monomer level filling the gaps in the porous layer formed in the first stage. Now the questions arise as to how and where fouling occurs, *i.e.* mechanism and possible location. These questions are open to debate.

Based on fouling resistance and protein transmission behaviour, a two step fouling mechanism by BSA is therefore proposed. The following two options appear feasible to explain the fouling mechanism and location of fouling.

Two options for the proposed two step fouling mechanism by BSA

1: It is proposed that the first stage consists of simultaneous plugging of larger pores by aggregates present in the feed and pore narrowing by monomers. Protein transmission data during the first 30 minutes support pore plugging as it does not usually affect protein transmission. Pore plugging is also supported by a reduction in fouling when aggregates were removed by prefiltration or aggregates were dissociated by the presence of SDS. The presence of calcium which enhanced aggregation exacerbated fouling further supporting the concept that pore plugging is the initial fouling mechanism. The second step involves the formation of aggregates during microfiltration because of high shear, *i.e.* high permeate flux, or calcium or both leading to further pore fouling and consequent surface layer formation. Surface layer formation would be dependent upon the size and concentration of protein aggregates in the boundary layer and their ability to plug the pores. The speed of the second step would be influenced by the state of the protein in solution and by permeate flux. This type of two step mechanism agrees with the hypothesis proposed by Jonsson *et al.* (1996) for BSA fouling during MF. Mueller and Davis (1996) made similar observations during MF of BSA. Internal fouling did not affect protein transmission until the occurrence of external fouling (a surface layer) which reduced protein transmission drastically. To exactly identify the nature or dominance of each mechanism (pore plugging or pore narrowing) in the first step, the pore size distribution of the membrane and the protein aggregate/monomer size distribution of the feed would be required. Plugging or deposition does not seem to occur mainly near the pore entrance as the BSA data does not fit the Marshall model. It would be interesting to investigate the effect of CFV on the initiation of fouling. If pore plugging is predominant, then CFV should not affect the initiation of fouling.

2: Alternatively, the first step may involve accumulation of aggregates on the membrane surface due to convective flow which depends on flux. These aggregates may form sheets on the membrane surface and act as nuclei for further deposition and

growth of a fouling layer as a consequent second step. Lee and Merson (1975) using SEM demonstrated that BSA forms sheets on the membrane surface during MF of whey on a 0.4 μm membrane. The second step may finally cover up the membrane surface completely. Reduction in fouling due to the presence of SDS or with prefiltered BSA strongly supports the role of aggregates in the first step. Similarly more severe fouling observed in the presence of calcium supports the concept that the initial fouling is by aggregates. This type of two step mechanism of BSA fouling during MF is consistent with the dual mode of fouling proposed by Kelly & Zydney (1995, 1997). With this mechanism protein transmission would not be affected until the membrane surface was fully covered by a protein deposit (possibly until a fouling resistance of 100 x 10^{10}/m and 200 x 10^{10}/m on 100 and 50 nm membranes respectively). Once the membrane surface was fully covered by a deposited layer, protein transmission would be influenced by the porosity of the surface layer. The second step of surface layer formation is likely to be caused by either high flux or presence of calcium in solution.

Final protein transmissions of about 20% in the absence of calcium and 10% in the presence of calcium (Table 6.1) in all the cases of severe fouling on these membranes suggest that the steady state protein transmission is reasonably constant irrespective of the initial pore size of the membrane. The steady state protein transmission is influenced by the porosity of the deposited layer but not the actual membrane pore size. The porosity of the surface layer in the presence of calcium appears to be low leading to a low final protein transmission. The author prefers option 2 as it explains influence of flux on fouling or flux-induced fouling behaviour of BSA better.

6.4 Conclusions

1) BSA is susceptible to flux-induced fouling. Permeate flux increased fouling dramatically both on 100 and 50 nm membranes probably due to shear induced denaturation of proteins leading to exposure of hydrophobic and thiol groups and subsequent protein aggregation. It is proposed that the conformational changes in the native BSA provide access to previously inaccessible intramolecular disulfide linkages leading to aggregation. It was not possible to prevent fouling on the 20 nm

membrane as protein adsorption alone was sufficient to cause pore narrowing leading to immediate surface layer formation.

2) The presence of calcium exacerbates severe fouling. Calcium played a major role at higher fluxes enhancing aggregation of protein and subsequent fouling. Ionic calcium may have enhanced BSA aggregation.

3) Prefiltration of the BSA solution reduced fouling and improved protein transmissions but did not eliminate fouling completely. Protein aggregates both in the feed and formed during MF appear to cause pore narrowing or may have blocked the pores depending upon the ratio of aggregate size to pore size.

4) The presence of SDS in solution again reduced fouling but did not eliminate it. SDS, an ionic detergent that is known to have protein dissociating properties by binding to hydrophobic groups, may have broken up aggregates reducing fouling and increasing protein transmission.

5) Two possible options of two step fouling mechanisms are proposed during MF of BSA. i) The initial fouling step is pore plugging. Once the pores are sufficiently plugged, the second step is surface layer formation reducing protein transmission drastically. ii) Convective transport of aggregates leads to accumulation on the membrane surface forming sheets which act as nuclei for further deposition. As membrane pores are completely covered, a surface layer forms reducing protein transmission.

The final protein transmissions are essentially controlled by the porosity of the deposited layer in both options.

7. Microfiltration fouling by bovine lactoferrin solutions

7.1. Introduction

Lactoferrin has been isolated from bovine whey using different chromatographic methods, for example, hydrophobic interaction chromatography (Yoshida, 1989), ion exchange chromatography (Yoshida & Xiuyun, 1991a & b) and affinity chromatography (Chen & Wang, 1991; Grasselli & Cascone, 1996). Membrane filtration in combination with chromatography or more recently membrane adsorbers which immobilize the exchange functional groups on a microporous membrane were reported to isolate lactoferrin from bovine milk or whey (Smithers *et al.*, 1996; Chiu & Etzel, 1997). Microfiltration (MF) in particular offers excellent potential when combined with adsorbers due to very high processing flow rates, selectivity and ease of scale up. MF was used in the processing of bovine lactoferrin from whey (Burling, 1990, Chen & Wang, 1991). It was of interest to see the fouling behaviour of bovine lactoferrin on a 100 nm zirconium oxide membrane. Hence experiments were performed to investigate MF fouling using bovine lactoferrin as a model protein. Bovine lactoferrin is an iron-binding glycoprotein. It consists of a single polypeptide chain having a molecular weight of about 80,000 daltons and two iron-binding sites per molecule (Lönnerdal & Iyer, 1995). Its isoelectric pH is 8.0±0.2 (Abe *et al.*, 1991). Bovine lactoferrin was chosen as it is a basic protein and also different from BSA in molecular weight and surface charge under the conditions used in the present investigation.

7.2. Results

Initial experiments were performed at a range of permeate fluxes to investigate the influence of permeate flux on fouling (Table 7.1). Subsequent experiments were performed to study the effect of solution environment *i.e.* addition of calcium, SDS. Finally, the effect of cross-flow velocity on fouling was investigated.

Table 7.1. Summary of protein transmissions and fouling resistances on a 100 nm pore size membrane during microfiltration of 0.2% lactoferrin solutions. pH: 6.80-6.82, IS: ~0.05 M.

Flux	Protein	Total final resistance			
			Membrane	Reversible	Irreversible
$L.m^{-2}.h^{-1}$	Transmission[a] %	$/m \times 10^{10}$	%	%	%
50	88.72	49.80	20.98	12.15	66.87
50	90.34	51.70	20.17	11.59	68.24
100	37.44	231.00	4.70	55.69	39.61
100	39.21	201.30	5.47	50.64	43.89
200	20.88	698.00	1.48	57.95	40.57
200	19.25	730.00	1.49	61.63	36.88
100*	42.33	261.80	4.41	24.74	70.85
100*	45.60	240.00	4.23	28.70	67.07
200*	16.70	792.00	1.29	47.13	51.58
200*	17.27	771.50	1.30	48.73	49.97
200@	83.60	43.50	23.45	36.03	40.52
200@	81.64	40.50	24.94	37.29	37.77
300@	4.47	378.00	2.74	42.77	54.49
300@	3.06	365.00	2.84	43.97	53.19
200#	2.79	410.10	2.42	88.41	9.17
200#	2.43	399.20	2.33	89.13	8.54

a Protein Transmission at 120 min.
* Experiments using solutions containing 8 mmol/L Calcium.
@ Experiments using solutions containing 0.1% SDS.
\# Experiments in which SDS was added after 60 minutes.

7.2.1. Effect of the permeate flux

Permeate flux had a dramatic effect on fouling (Fig. 7.1). At a permeate flux of 50 $L/m^2.h$, fouling resistance was low, increasing very little with time. Protein transmissions of about 90% were obtained under these conditions (Fig. 7.2). At 100 $L/m^2.h$, although fouling was not severe, protein transmissions reduced to about 30-35%. Severe fouling occurred when the permeate flux was increased to 200 $L/m^2.h$ reducing protein transmission to about 20%. Protein transmissions of about 35-45% were obtained after 30 minutes at this flux as fouling occurred rapidly. The protein transmission trends from UV cell measurements are shown in Fig. 7.3.

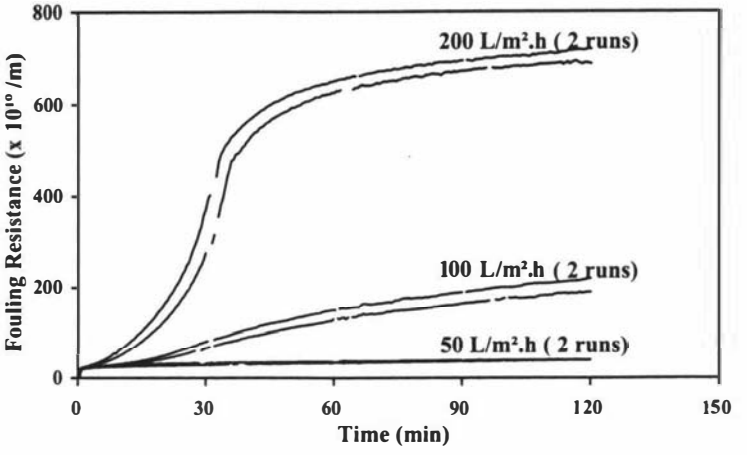

Figure 7.1. Fouling resistance during MF of a 0.2% bovine lactoferrin solution on a 100 nm membrane. The ionic strength of the solutions was 0.051 to 0.053 and the pH from 6.80 to 6.82.

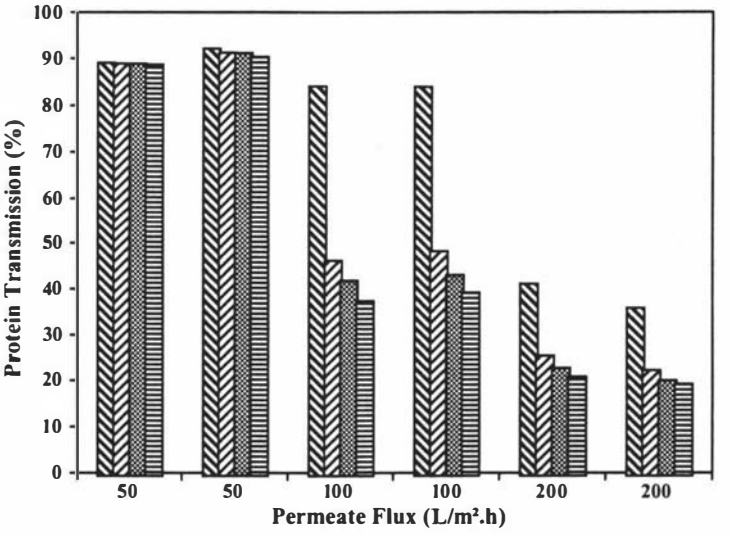

Figure 7.2. Protein transmission during MF of a 0.2% bovine lactoferrin solution on a 100 nm membrane. The ionic strength of the solution was 0.051 to 0.053 and the pH from 6.80 to 6.82. For each permeate flux the 4 bars represent values measured at 30, 60, 90 & 120 min respectively.

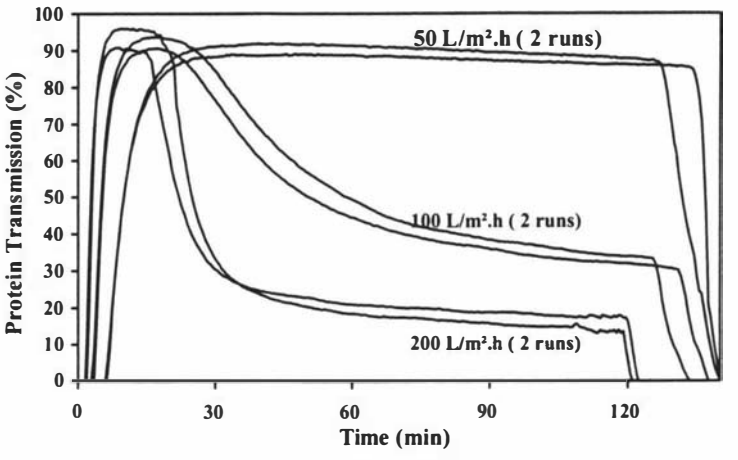

Figure 7.3. Protein transmission (UV cell data) during MF of a 0.2% bovine lactoferrin solution on a 100 nm membrane. The ionic strength of the solution was 0.051 to 0.053 and the pH from 6.80 to 6.82.

The apparent protein transmission was zero initially, but it increased rapidly to a maximum value as the water initially in the permeate chamber was removed. It is interesting to note that protein transmission was about 90% for about 15-25 minutes at 100 and 200 L/m^2.h fluxes before it started decreasing rapidly. It is surprising to see severe fouling and lower protein transmissions at fluxes ≥ 100 L/m^2.h even when protein size is more than 10 times smaller than the average pore size of the membrane.

Protein transmissions were only about 90% even at 50 L/m^2.h where fouling was light suggesting the presence of aggregated protein that may have been retained by the membrane. To examine this, feed samples were centrifuged at 48, 200 *g* for 30 min. Sediments were observed in the centrifuge tubes, confirming the presence of a small amount of aggregated protein which was probably retained right from the start of filtration.

7.2.2. Effect of calcium

Among the minerals that are present in whey, calcium has been implicated as a major factor that can influence the protein solution environment causing fouling (Marshall & Daufin, 1995). Calcium in solution has been found to have a significant effect, particularly at higher fluxes during MF of β-lactoglobulin (Marshall, 1994) and BSA (see section 6.3.2). To investigate whether it had any effect on lactoferrin a few experiments were conducted with 8 mM calcium added to the feed.

The presence of calcium in the feed increased the level of fouling after 120 min slightly particularly at 200 L/m^2.h (Fig. 7.4). The protein transmissions were usually almost the same as those obtained in the absence of calcium (Fig. 7.5). However after 30 minutes, there was a slight increase in protein transmission in the presence of calcium. However when severe fouling occurred, the calcium did not appear to change protein transmission significantly.

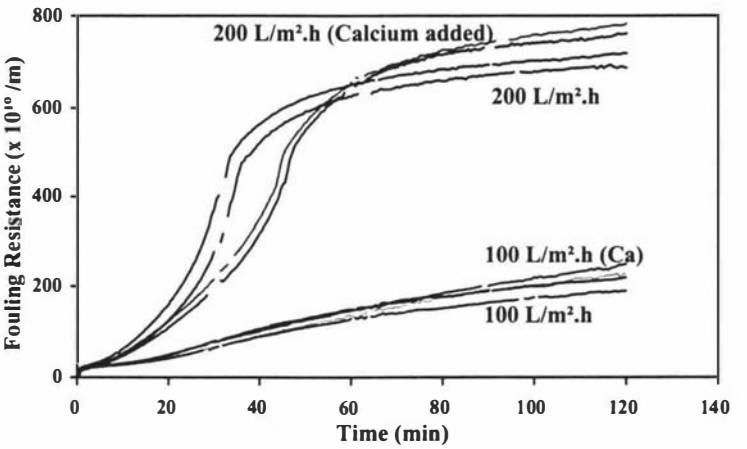

Figure 7.4. Fouling resistance during MF of a 0.2% bovine lactoferrin solution on a 100 nm membrane at 100 and 200 L/m^2.h. The ionic strength of the solution was 0.051 to 0.053 for the trials without calcium; 0.053 to 0.055 for the trials with calcium and pH 6.80 to 6.82. The calcium content was 8.0-8.3 mmol/L.

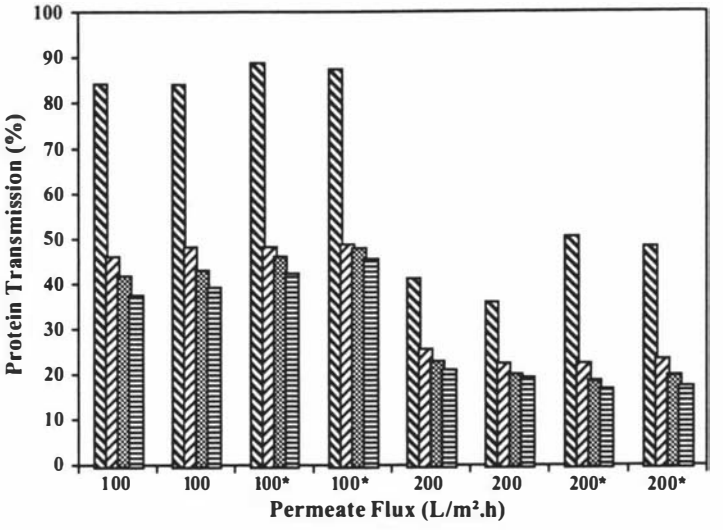

Figure 7.5. Protein transmission during MF of a 0.2% bovine lactoferrin solution on a 100 nm membrane at 100 and 200 L/m^2.h. Experiments with added calcium (8.0-8.3 mmol/L) in the feed are indicated by (*). The ionic strength of the solution was 0.051 to 0.053 for the trials without calcium; 0.053 to 0.055 for the trials with calcium and pH 6.80 to 6.82.

Interestingly, at 200 L/m^2.h flux, the shape of the fouling resistance curve was slightly different with calcium present, suggesting a different type of fouling mechanism of initiation and build up of fouling deposits. The final fouling resistance in the presence of calcium was slightly higher than the control (absence of calcium). But overall, the effect of calcium on lactoferrin fouling appears to be minimal. The experiments were performed at pH 6.80-6.82 which is below the IEP of lactoferrin (*i.e.* ~ pH 8). The above observation is reasonable as little Ca^{2+} ion binding and aggregation are generally expected at the pH used in the present study.

7.2.3. Effect of permeate flux on irreversible and reversible fouling resistance

Both in the absence and presence of calcium, increase in permeate flux increased the reversible fouling resistance (Table 7.1). This may be due to increased volume of protein interacting with the membrane leading to multilayer deposition which may be reversible. Comparing reversible and irreversible fouling resistances in the absence and presence of calcium, there was a slight increase in irreversible fouling resistance in the presence of calcium.

7.2.4. Effect of SDS on fouling

Solution environment has been reported to have a significant effect on fouling during protein filtration (Palecek & Zydney, 1994a, b; Saksena & Zydney, 1994; Marshall & Daufin, 1995). SDS was added in the feed (0.1% SDS) and experiments were performed to investigate any effect it may have on protein fouling.

It is important to note that two concentrations of SDS *i.e.* 0.1% and ~0.3% (1.4 g SDS/g of protein) are reported in this thesis. Initially, a concentration of 0.1% SDS was tried with lactoferrin. Later it was found from the literature that 1.4 g SDS/g of protein is generally required to bind the protein completely (Reynolds & Tanford, 1970; Jones, 1992). Subsequently, a concentration of 1.4g SDS/g of protein was used with BSA and ferritin solutions (see sections 6.2.3 & 8.2.2).

In the presence of SDS, fouling was dramatically reduced even at a permeate flux of 200 L/m^2.h (Fig. 7.6) and protein transmissions of about 85-95% were obtained (Fig. 7.7). Fouling did not occur even at a flux of 300 L/m^2.h until about 60-70 minutes after which fouling resistance increased quickly and the controller was shifted to constant pressure mode as the TMP was > 200 kPa. However, transmissions at 300 L/m^2.h and at 60, 90 and 120 minutes were much lower than the 200 L/m^2.h control although the fouling resistance in comparison was much lower.

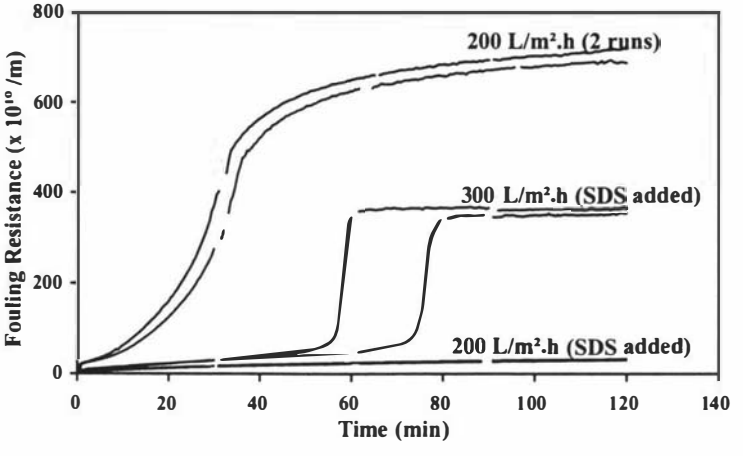

Figure 7.6. Fouling resistance during MF of a 0.2% bovine lactoferrin solution in the presence and absence of 0.1% SDS on a 100 nm membrane. The ionic strength of the solutions was 0.053 to 0.054 and the pH from 6.80 to 6.82.

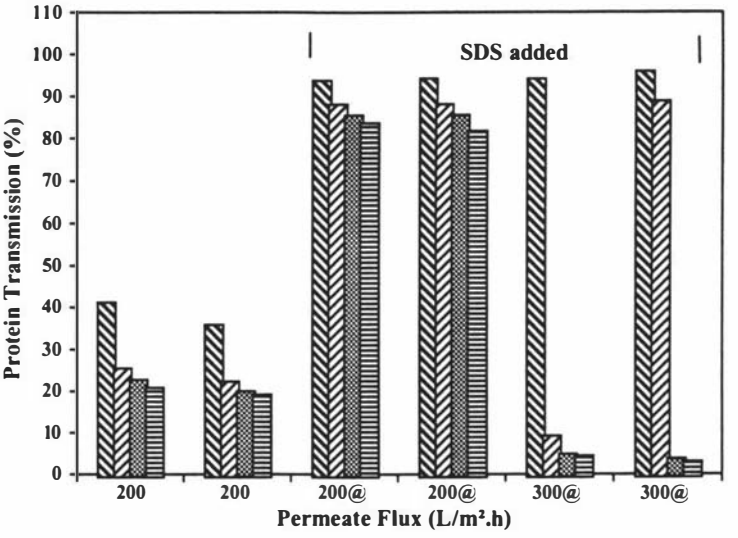

Figure 7.7. Protein transmission during MF of a 0.2% bovine lactoferrin solution in the presence and absence of 0.1% SDS on a 100 nm membrane. Experiments with added SDS in the feed are indicated by (@). The ionic strength of the solution was 0.053 to 0.054 and the pH from 6.80 to 6.82. For each permeate flux the 4 bars represent values measured at 30, 60, 90 & 120 min respectively

To further investigate the effect of SDS on fouling, 0.1 % SDS solution was prepared separately and added half way during an experiment in which severe fouling had already occurred. Fouling resistance dropped to about 60% as soon as the SDS solution was added to the feed (Fig. 7.8). The controller continued to be in constant pressure mode although the fouling resistance dropped. The fouling resistance remained almost constant from then. Protein transmissions at 90 and 120 minutes were much lower than those obtained at 200 $L/m^2.h$ without SDS in solution (Fig. 7.9). The UV cell protein transmission data is shown in Fig. 7.10. Despite the decrease in fouling resistance, protein transmission did not increase but actually dropped indicating that SDS somehow influenced protein-protein or protein-membrane interactions reducing fouling resistance but not restoring protein transmission.

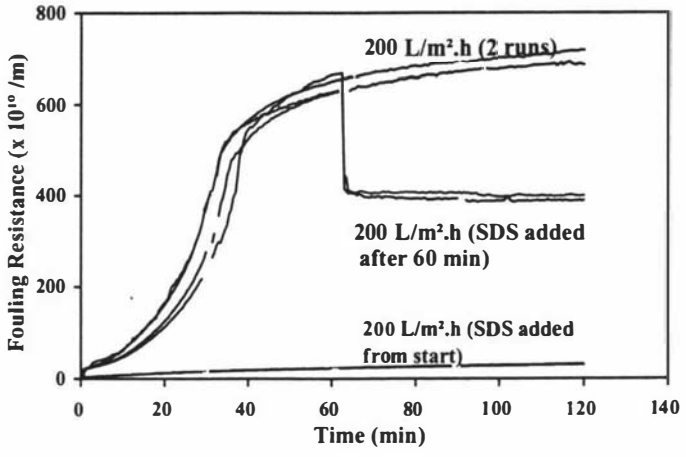

Figure 7.8. Fouling resistance during MF of a 0.2% bovine lactoferrin solution on a 100 nm membrane at 200 L/m^2.h. The ionic strength of the solution was 0.051 to 0.053 for the trials without SDS; 0.053 to 0.054 for the trials with SDS and the pH 6.80 to 6.82.

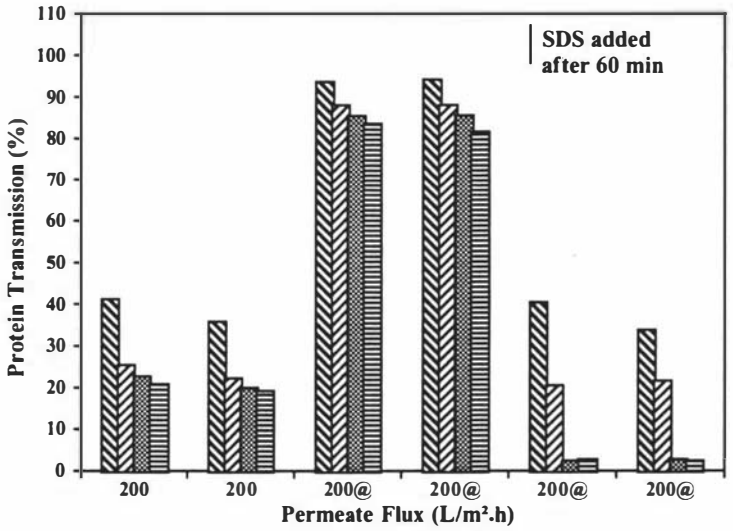

Figure 7.9. Protein transmission during MF of a 0.2% bovine lactoferrin solution on a 100 nm membrane at 200 L/m^2.h. Experiments with added SDS in the feed are indicated by (@). The ionic strength of the solution was 0.051 to 0.053 for the trials without SDS; 0.053 to 0.054 for the trials with SDS and pH 6.80 to 6.82.

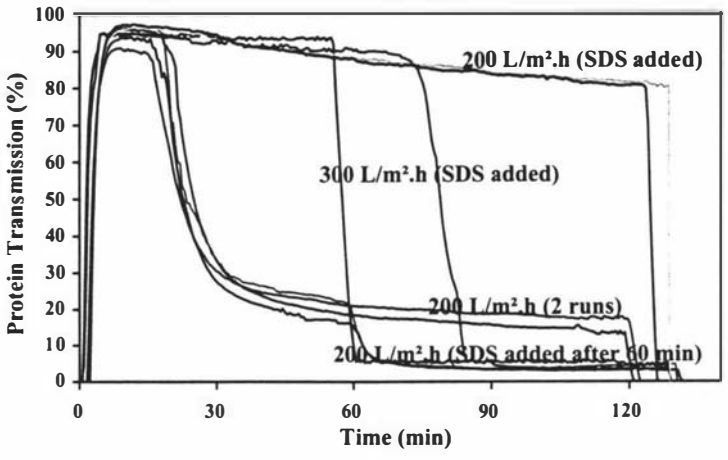

Figure 7.10. Protein transmission (UV cell) during MF of a 0.2% bovine lactoferrin solution on a 100 nm membrane. The ionic strength of the solution was 0.051 to 0.053 for the trials without SDS; 0.053 to 0.054 for the trials with SDS and pH 6.80 to 6.82.

In another experiment, SDS solution without protein was used to investigate the fouling behaviour of SDS alone at 200 L/m^2.h. There was no fouling observed and the clean membrane resistance stayed constant even after 30 minutes of operation on SDS solution (data not shown). Protein solution alone was then introduced as usual *i.e.* after about 30 minutes of pretreatment of the membrane with SDS at 200 L/m^2.h. This procedure resulted in a final concentration of about 0.03% SDS in solution. There was no significant increase in fouling resistance similar to the fouling resistance curve that was obtained when SDS and protein were present from the beginning (Fig. 7.6). This experiment again suggested that SDS has interacted with the protein environment resulting in minimal fouling.

The permeate flux data was plotted against time for all the experiments during which final TMP was 200 kPa and the controller was changed to constant pressure mode (Fig. 7.11).

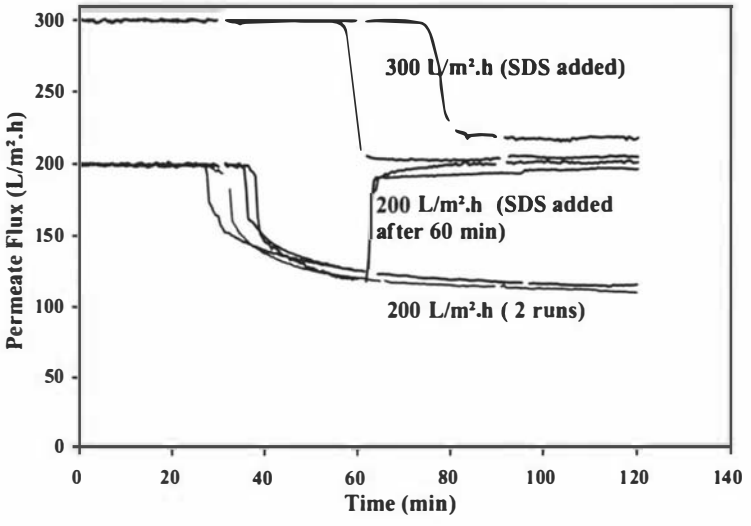

Figure 7.11. Flux decline during MF of a 0.2% bovine lactoferrin solution on a 100 nm membrane. The ionic strength of the solution was 0.051 to 0.053 for the trials without SDS; 0.053 to 0.054 for the trials with SDS and pH 6.80 to 6.82.

In the absence of SDS, it is interesting to see that the flux in the run which started at 200 L/m^2.h declined after about 30 minutes (the point of change over to constant TMP), achieving a steady state value of about 110 L/m^2.h. Interestingly, in the presence of SDS the steady state flux was about 205-220 L/m^2.h as seen in the 300 L/m^2.h trial which achieved a steady state and in the 200 L/m^2.h trial when SDS was added after 60 min. Permeate flux was almost restored to 200 L/m^2.h as soon as SDS was added to the feed half way during a trial at 200 L/m^2.h. This suggested the possibility of increasing steady state flux during protein filtration using SDS.

Looking at the type of reversible and irreversible resistances, most of the resistance remaining after SDS addition at 60 minutes was reversible (Table 7.1). However, if the SDS was present in solution right from the start, fouling resistance was both reversible and irreversible.

There was a sudden increase in fouling resistance as soon as feed was introduced in the plant, perhaps by some form of 'dynamic fouling resistance' due to the presence of protein in the system. Comparing the low fouling cases of 50 L/m^2.h control and 200 L/m^2.h with SDS in solution, dynamic fouling resistance was about 4-6 x 10^{10} /m at 200 L/m^2.h, compared to 22-23 x 10^{10} /m at 50 L/m^2.h though the driving force was 4 times higher (Fig. 7.12) suggesting that adsorption may have been low or size of the protein is reduced in the presence of SDS. The protein transmissions were almost the same in both the cases (Fig. 7.2 & 7.7) except that initial protein transmissions were slightly higher in the presence of SDS and also decreased somewhat with time.

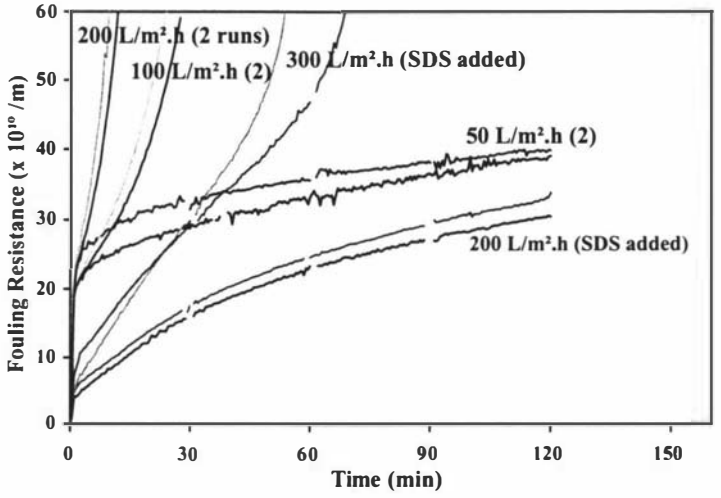

Figure 7.12. Fouling resistance curves at expanded scale for different fluxes with and without SDS in solution during MF of a 0.2% bovine lactoferrin solution on a 100 nm membrane. The ionic strength of the solution was 0.051 to 0.053 for the trials without SDS; 0.053 to 0.054 for the trials with SDS and pH 6.80 to 6.82.

7.2.5. Effect of fouling resistance on protein transmission

It is reasonable to expect that increase in fouling resistance causes a decrease in protein transmission. To analyse this relationship, all the protein transmission data (measured by UV spectrophotometer) from trials with calcium, SDS and control (no calcium and no SDS) were plotted against fouling resistance measured at an equivalent time in each of the trials (Fig. 7.13).

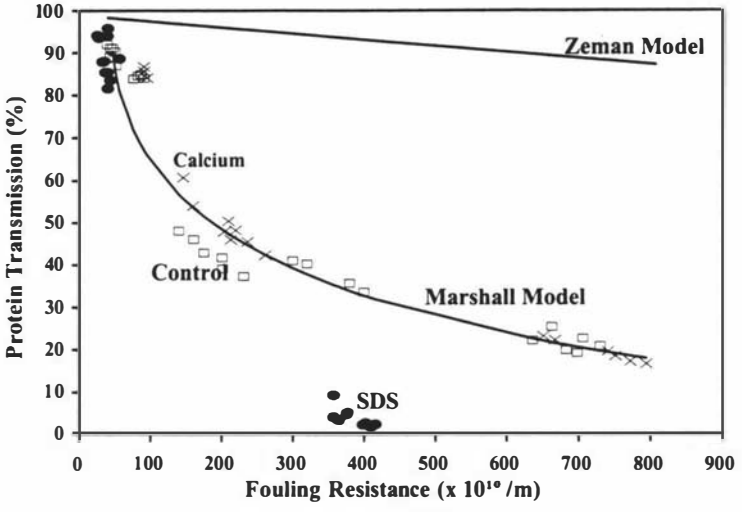

Figure 7.13. Protein transmission versus the fouling resistance for a 100 nm membrane: (□) data collected from trials without calcium; (X) trials with calcium; (●) trials with SDS. Details of Zeman and Marshall models are given in the discussion section.

The plot suggested that, both in the presence and absence of calcium, the relationship was similar. With calcium present, protein transmissions were slightly higher than the control during the initial stages. When fouling was light, SDS apparently improved protein transmission. However under severe fouling conditions in the presence of SDS, increase in fouling resistance reduced protein transmission drastically to less than that expected for a non-SDS experiment.

7.2.6. Effect of the cross-flow velocity (CFV)

Increasing CFV has generally been found to decrease membrane fouling. This is due to a reduction in concentration polarisation which is common in UF due to retention of proteins on the membrane surface. However, its effect on MF fouling is not expected to be significant as long as fouling is within the pores (Bowen & Hughes, 1990). Marshall (1994) showed that CFV did not have a significant effect on fouling during MF of β-lactoglobulin. In the present investigation using lactoferrin, protein transmissions were only about 90% for a short time and reduced thereafter when severe fouling occurred. It was thought that the small amount of retained aggregates may have initiated fouling by deposition on the membrane surface rather than within

the pores. If that was the case, CFV would be expected to have an effect on the initial fouling with this protein.

Two sets of experiments were performed at 200 $L/m^2.h$ to see the effect of CFV on the initial fouling as well as its effect on the fouling resistance after severe fouling had occurred. In one set, constant flux experiments were started at either 2 m/s or 4 m/s (usual value 3 m/s) and switched to constant pressure mode when severe fouling occurred and then after about 90 minutes of the experiment, the CFV was changed to 4 m/s and 2 m/s respectively. CFV seems to have no significant effect on the initiation of fouling (Figs. 7.14 & 7.15). However, changing CFV had a small effect, once the severe fouling had occurred and the controller had been switched to constant pressure mode.

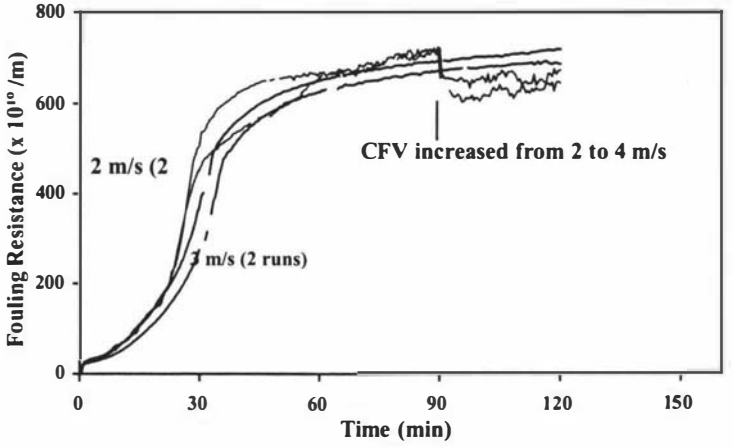

Figure 7.14. Fouling resistance during MF of bovine lactoferrin on a 100 nm membrane. Cross-flow velocity was increased from 2 m/s to 4 m/s at 90 minutes.

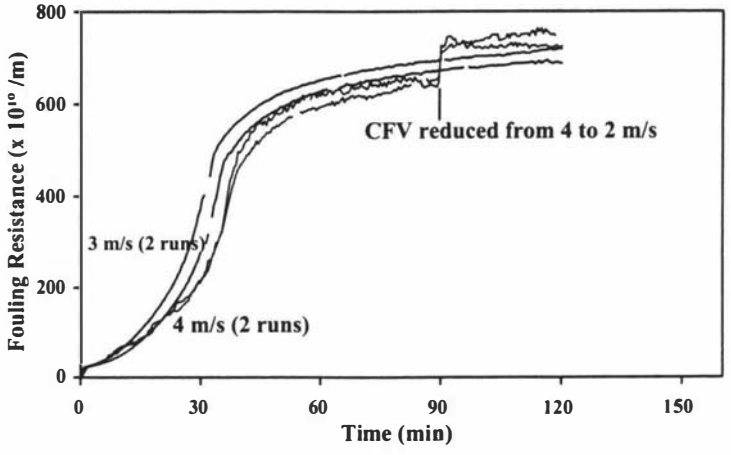

Figure 7.15. Fouling resistance during MF of bovine lactoferrin on a 100 nm membrane. Cross-flow velocity was reduced from 4 m/s to 2 m/s at 90 minutes.

In another set of experiments, CFV was reduced to 1 m/s after severe fouling had occurred at 3 m/s and switched to constant pressure mode and again CFV was changed back to 3 m/s to determine its effect on protein transmission. Reducing CFV from 3 to 1 m/s increased fouling resistance dramatically (Fig. 7.16). This is probably due to an increase in concentration polarisation or an increase in thickness of the fouling layer on the membrane. The fouling resistance decreased to the usual level when CFV was increased back to 3 m/s. There was a small change in protein transmission due to change in CFV once severe fouling had occurred (Fig. 7.17).

Fouling appears to be not initiated by deposition of aggregates on the membrane surface. Overall, when severe fouling occurred and the permeate controller was switched to constant pressure mode, change in CFV affected protein transmissions relatively little at all these velocities suggesting that CFV had an effect only on fouling resistance but not on protein transmission. It is possible that protein transmission is controlled by a thin layer of deposits at the pore entrance and the deposition layer is apparently not affected by changes in CFV.

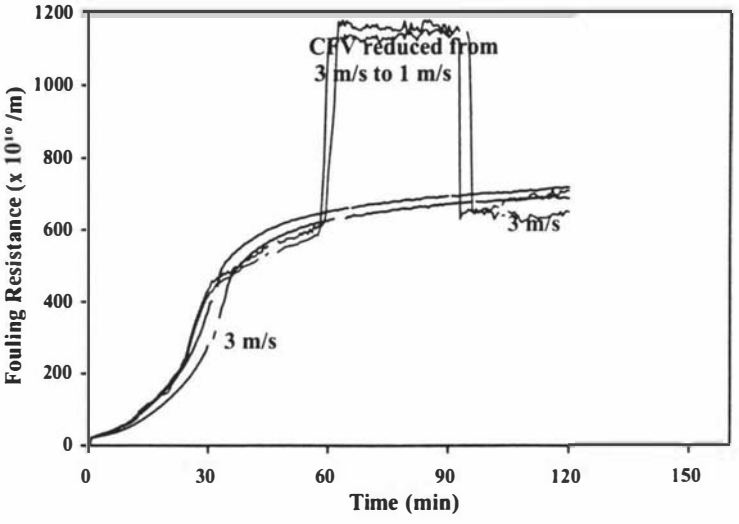

Figure 7.16. Fouling resistance during MF of a 0.2% bovine lactoferrin solution on a 100 nm membrane. Cross-flow velocity was changed from 3 m/s to 1 m/s between 60 and 90 minutes.

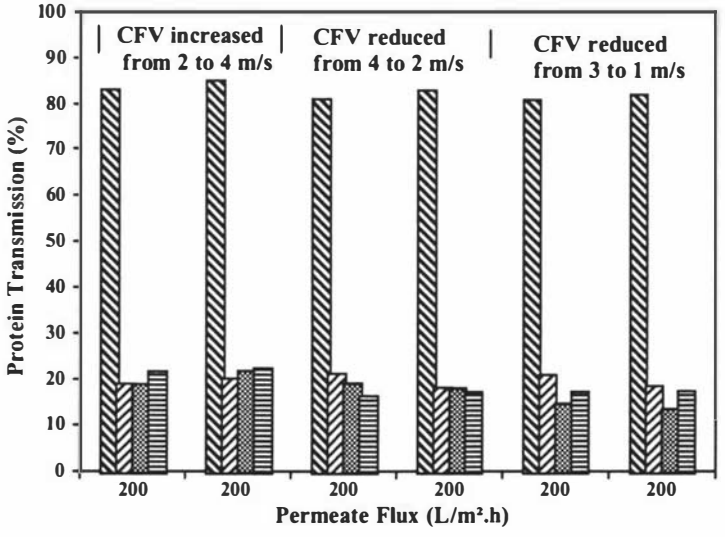

Figure 7.17. Protein transmissions at different cross-flow velocities during MF of a 0.2% bovine lactoferrin solution on a 100 nm membrane.

7.3. Discussion

7.3.1. Probable fouling mechanisms

Based on the experimental data, it can be conceived that fouling occurred by the following steps either in sequence or simultaneously 1) protein adsorption initiates both on the membrane and within the pores as soon as feed comes in contact with the membrane 2) protein deposits at the pore entrance or within the pores leading to loss of permeable pores and 3) surface layer formation.

7.3.1.1. Protein adsorption

Protein adsorption on to a membrane surface is well known. Fouling resistance increased even at low fluxes (50 $L/m^2.h$) indicating that adsorption had occurred. The trials at 50 $L/m^2.h$ without SDS and 200 $L/m^2.h$ with SDS resulted in minimal fouling. Apparent pore size reductions in these trials were calculated using equation (3.12) and presented in Table 7.2.

Considering the size of lactoferrin, about 7 nm (calculated based on molecular weight using an equation by Squire, 1981) in its monomeric form, apparent pore size reduction of about 30 nm suggests adsorption of about two layers of protein within the pores. However, adsorption did not seem to significantly affect the protein transmission at lower fluxes when fouling was light. At higher fluxes, other fouling mechanisms in addition to protein adsorption may have played a role reducing protein transmission drastically.

Comparison of the apparent reduction in pore size of the low fouling cases of 50 $L/m^2.h$ control and 200 $L/m^2.h$ with SDS in solution (Table 7.2) suggests that SDS probably reduced the size of aggregates or reduced adsorption, which resulted in a relatively small reduction in apparent pore size.

Table 7.2. Apparent pore size reduction in trials performed at various fluxes and conditions on the 100 nm membrane.

Permeate flux ($L/m^2.h$)	Clean membrane resistance ($x\ 10^{10}/m$)	Irreversible fouling resistance ($x\ 10^{10}/m$)	Apparent reduction in pore size (nm)
50	10.5	33.3	30
50	10.9	35.3	30
100	10.9	91.5	43
100	11.0	88.4	42
200@	10.2	17.6	22
200@	10.1	15.3	21
200#	9.9	37.6	32
200#	10.1	34.1	32

@ Trials with SDS presence
# Trials with SDS added after 60 minutes

Thus solution environment which affects the state of protein in solution has a significant effect on adsorption at the membrane-protein interface as observed.

7.3.1.2. Protein deposition

Deposition on to the adsorbed protein layer is likely to occur both on the membrane surface and within the pores. It is proposed that protein deposition could occur by the following mechanisms.

1) A small number of retained aggregates accumulate on the membrane surface and act as nuclei for growth of deposits (Kelly *et al.*, 1993); further deposition probably by multilayers of protein with time finally leads to a surface layer covering the membrane pores completely. In this case, as protein deposition is predominantly initiated by the aggregates on the membrane surface, CFV should affect initiation of protein deposition at least during the early stages. However changes in CFV did not affect the initiation of fouling discarding this hypothesis.

2) Pore fouling occurs by aggregates plugging the pores or depositing at the pore entrance or within the pores. Smaller protein molecules or monomers may deposit on the pore walls narrowing the pores. There is likely to be a competition between pore plugging and pore narrowing depending upon the ratio of protein/aggregate to pore size. A surface layer would have formed immediately after complete pore fouling. In this case fouling initiates predominantly within the pores followed by surface layer formation. CFV should not affect fouling at least during the initial stages as protein deposition is initiated within the pore. As observed from the experiments, CFV had little effect on the initiation of fouling. However after severe fouling occurred, CFV affected the surface layer. This supports the hypothesis that the deposition may have been initiated by aggregates via some form of pore fouling mechanism that led to the formation of a surface layer.

It is proposed that 'in-pore' fouling by way of pore plugging is a major contributor to the fouling and a surface layer forms after complete plugging of the pores leading to increased retention. To investigate this hypothesis, the constant flux portions of the fouling resistance versus time curves of trials with calcium, SDS and varying fluxes were considered so that constant flux blocking laws could be applied to the data. Theoretical details of the blocking laws have been given by Grace (1956), Hermia (1982) and Hlavacek and Bouchet (1993). The assumptions in these models are 1) pores consists of long straight circular ducts, all of the same size and shape and 2) membrane pressure drop can be described by the Hagen-Poiseuille equation. Pore fouling models considered are 1) pore plugging (complete blocking law) where it is assumed that if particles sit in a pore, the pore is completely blocked (2) pore narrowing (standard blocking law) where it is assumed that small particles progressively deposit on the pore walls causing a reduction in the pore diameter and 3) an intermediate blocking law (refer Hermia, 1982). Plotting the square root of Ro/R versus time will be a straight line if pore narrowing is the mechanism (Eqn. 3.18), plotting Ro/R versus time will give a straight line if the pore plugging mechanism is occurring (Eqn.3.20); and plotting natural log of (R/Ro) versus time will be a straight line if the intermediate blocking law applies (Eqn. 3.22). The plots are shown in Figs. 7.18-7.20 for trials at 50, 100 and 200 $L/m^2.h$. The models were also tested for other trials with SDS and calcium (not shown here).

None of the graphs showed straight lines in the first 30 min of operation. This represents the establishment of a dynamic resistance due to the presence of protein near the membrane and resistance due to the adsorption of the protein. Even after the first 30 minutes, none of the other blocking laws or fluxes gave straight lines except the intermediate blocking law which gave reasonably straight lines at 200 $L/m^2.h$. However, it does not explain the decline in protein transmission obtained in these experiments. It appears that more than one fouling mechanism is in action. It is also possible that there is a combination of fouling mechanisms depending up on the ratio of protein/aggregate to pore size. A number of other questions arise 1) what is causing or leading to surface layer formation 2) the size of the aggregates that form surface layer and 3) the location of deposit, at the pore entrance or within the pore.

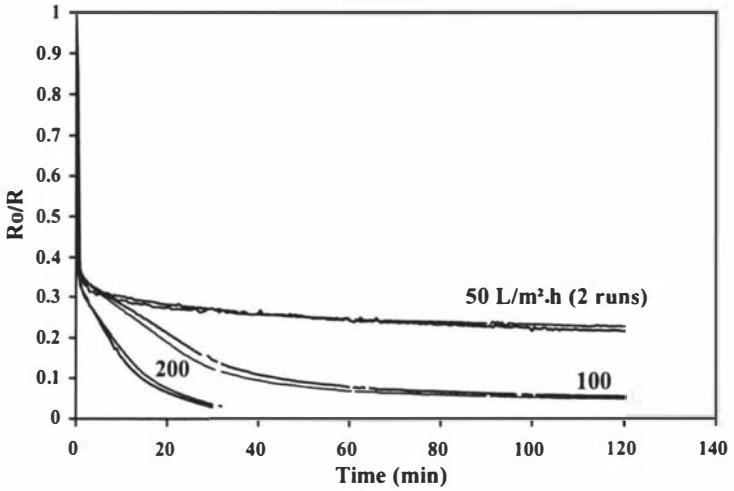

Figure 7.18. Investigation of pore plugging mechanism (complete blocking law) in the MF of bovine lactoferrin on a 100 nm membrane.

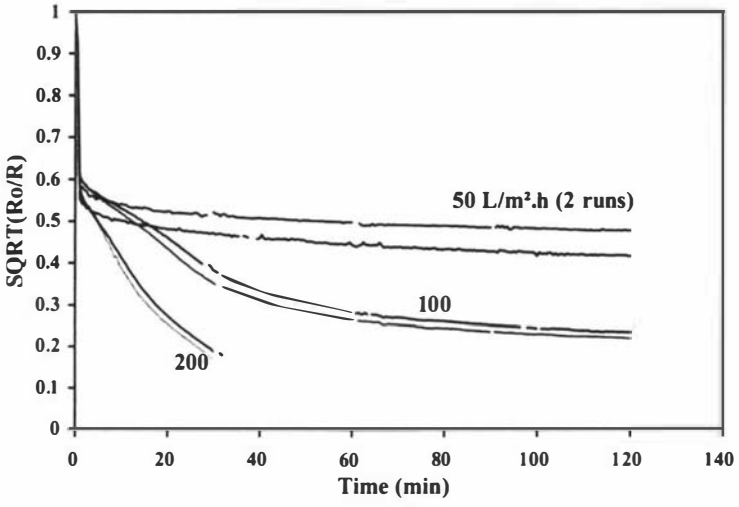

Figure 7.19. Investigation of pore narrowing mechanism (standard blocking law) in the MF of bovine lactoferrin on a 100 nm membrane.

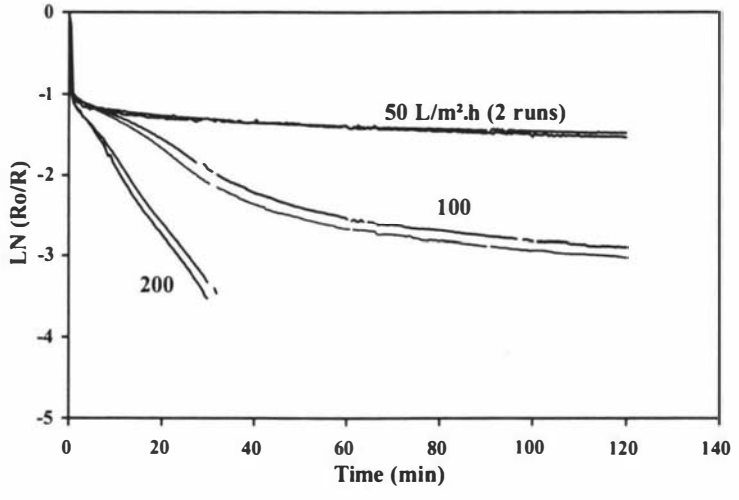

Figure 7.20. Investigation of intermediate blocking law in the MF of bovine lactoferrin on a 100 nm membrane.

7.3.1.3. Marshall model

Alternatively, a model given by Marshall *et al.* (1997) was also considered. Having seen that none of the single pore blocking laws represent the fouling behaviour observed, the possibility of protein deposition at or near the pore entrance was examined using a mathematical model developed by Marshall *et al.* (1997). The model assumes that protein deposition occurs along a small part of the membrane particularly at the pore entrance. Initially the model developed by Zeman (1983) was considered and the apparent pore size of the membrane was determined from the fouling resistance data using equation 3.12. Protein transmission or retention is then calculated based on apparent pore size using Ferry equation (3.16). The model line in Fig. 7.13 shows the predicted transmission/resistance relationship assuming a protein size of 7 nm passing through a 100 nm membrane and assuming a pore narrowing mechanism. The observed protein transmissions were lower than the predicted values suggesting that a pore narrowing mechanism is unlikely.

The next approach was to consider the Marshall *et al.* (1997) model which suggests that protein deposition occurs in the vicinity of the pore entrance. From the fouling resistance curves at various fluxes in the absence of SDS, it can be seen that the initial jump in resistance was 20-23 x 10^{10}/m as soon as the product was introduced. The average total fouling resistance when severe fouling did not occur was 40 x 10^{10}/m. This includes the initial jump. Subtracting 21.5 from this gives a resistance of 18.5 x 10^{10}/m caused by protein adsorption assuming that the initial jump has not contributed to adsorption. However, when fouling was light (50 L/m^2.h) the irreversible resistance was in the range of 33.3-35.3 x 10^{10}/m (Table 7.2) suggesting that some of the initial jump has actually contributed towards adsorption. Hence the average irreversible resistance was considered to cause adsorption and this corresponded to an apparent reduction of 30 nm in pore size. Hence the adsorbed membrane pore size was taken as 70 nm. The average clean membrane resistance was 10.75 x 10^{10}/m. The model fitted reasonably well when the value for the part membrane resistance corresponded to 0.95% of the total pore length or assuming a total pore length of 10 μm, a fouling length of 95 nm which is of the same order as the initial pore size of the membrane. The physical interpretation of the Marshall *et al.* (1997) model is that protein deposition essentially occurs at the pore entrance

restricting the pore opening and this is followed by subsequent formation of a surface layer. This model explains the observed relationship between protein transmission and fouling resistance (see Fig. 7.13) suggesting that fouling occurs in a small part of the membrane, most likely at the pore entrance. Once this happens, protein transmission reduces drastically.

7.3.1.4. Surface layer

A surface layer of protein deposits may have formed after pore fouling and changed protein transmission by reducing the porosity of the deposited layer. This step depends on the concentration of protein/aggregates in the boundary layer. Once severe fouling occurred, the deposited layer on the membrane surface reduced protein transmission to about 20%.

Overall the protein transmission versus resistance data (Fig. 7.13) indicated that protein transmission dropped continuously as fouling resistance increased. This is reasonable as deposition at the pore entrance initially contributed to severe fouling increasing fouling resistance. The continuous reduction in protein transmission appears to be due to the formation of a surface layer that has a decreasing pore size and increasing thickness with time. Any amount of increase in fouling resistance after severe fouling occurred is essentially due to contributions from the increased thickness of the fouling layer. Once the surface layer had formed, CFV affected marginally (by way of scouring) the surface deposit leading to a reduction in fouling resistance but not in protein transmission. This suggested that protein transmission was largely determined by the porosity of the surface layer rather than the depth. All these three steps of fouling occur very quickly causing severe fouling as the permeate flux is increased. This is consistent with the fact that as the flux is increased, the number of proteins interacting with the membrane also increased leading to severe fouling.

7.3.2. Effect of permeate flux and concentration induced fouling by aggregates

At 50 L/m^2.h, adsorption and to some extent, perhaps pore plugging by aggregates were insufficient to increase fouling resistance significantly resulting in almost a constant protein transmission. It is also possible that back diffusion may keep aggregate concentration at the membrane low enough to prevent severe fouling. At 100 L/m^2.h, the slight increase in resistance was probably due to the deposition of aggregates which was sufficient to somewhat decrease protein transmission. At 200 L/m^2.h, an increased flux of aggregates probably caused concentration induced multilayer deposition on the membrane surface further reducing protein transmission.

The aggregates present in the feed do not seem to be a major problem under low flux conditions (50 L/m^2.h). Why then does increase in flux cause severe fouling? To explore whether initial deposition by pore plugging was influenced by the flux, the average initial fouling rate over the first 30 minutes was calculated using equation 3.28 and plotted against the mass of protein convected to the membrane surface ($J.C_b$). The increase in average fouling rate was not linear but dramatic beyond a convective mass flux of protein (0.055 g/m^2.s) corresponding to 100 L/m^2.h (Fig. 7.21), suggesting that initial fouling in this system was not governed by convection of existing aggregates towards the membrane surface. Initial fouling rate will be linear with convective mass flux if the pore fouling is purely by aggregates convected to the pores. Some other mechanism of fouling seems to initiate when flux is increased beyond 100 L/m^2.h. One possibility is that aggregates are created at higher fluxes and add to the deposit. At higher fluxes, perhaps aggregation is induced due to increased shear particularly at the membrane pores (Bowen & Gan, 1991, Marshall *et al.*, 1997). Flux-induced aggregation and fouling during UF was reported by Suki *et al.* (1984).

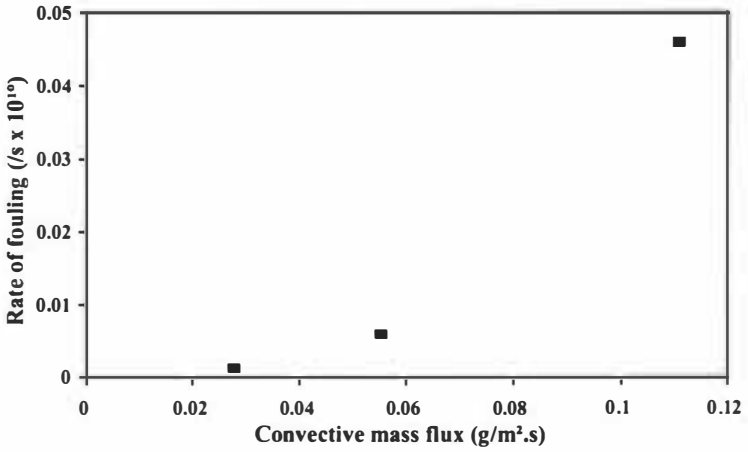

Figure 7.21. Average rate of fouling and convective mass flux during the first 30 minutes of MF of bovine lactoferrin on a 100 nm membrane.

Also at higher fluxes, initial high TMP's probably enhanced protein-protein and protein-membrane interactions particularly at the pore entrance perhaps unfolding hydrophobic groups of proteins leading to increased aggregation. The probability of deposition by the increased number of aggregates is greater under these conditions leading to severe fouling.

7.3.3. SDS-Protein interactions

Fouling data at different permeate fluxes and with SDS in solution indicated that fouling was highly dependent on the permeate flux and solution environment of the protein. SDS appeared to have affected protein-protein and protein-membrane interactions changing the deposition of protein or the nature of the deposited layer. It is suggested that the state of the protein in the adsorbed layer formed in the presence of SDS is different from that formed without SDS and in some way reduced fouling resistance. Experiments with SDS both from the start and added halfway through indicated that SDS may have broken up aggregates in the feed or protein deposits on or inside the membrane pore. It is also possible that SDS would displace the adsorbed protein layer or reduce adsorption as SDS is known to be better surfactant than proteins and adsorbs at interfaces in preference to proteins.

It is useful to discuss SDS-protein interactions here before any major conclusions are drawn from the results. It is known that SDS is a surface active agent which disrupts hydrophobic bonds. SDS is an amphiphile possessing hydrophilic and hydrophobic parts. One of the characteristic properties of an amphiphile is that they tend to assemble at interfaces. The formation of large aggregates or micelles, is another characteristic property of amphiphilic substances. They have a critical micellar concentration (CMC), where they associate to form large aggregates (micelles). The critical micelle concentration of SDS is 8.3×10^{-3} M in pure water (Mukerjee & Mysels, 1971). The present study was performed at a concentration of 0.1% (w/v) which is below the CMC. The presence of SDS in protein solutions results in the formation of SDS-protein complexes primarily due to hydrophobic interactions (Reynolds & Tanford, 1970). It is important to consider the size and shape of these SDS-protein complexes. The complexes between SDS and proteins are reported to be rod-like with the SDS molecules as a shell around the protein (Reynolds & Tanford, 1970).

Wahlgren & Arnebrant (1991) have reported a reduction in protein adsorption by β-lactoglobulin and lysozyme due to the presence of SDS on four different surfaces. They observed desorption of protein using ellipsometry as soon as SDS was added to the solution. Swaminathan *et al.* (1980) and Fane *et al.* (1985) have found that pretreatment of membranes with surfactant (amphiphile) gave a significant enhancement in flux using proteins. Fane *et al.* (1985) found that surfactant pretreatment prior to BSA ultrafiltration enhanced flux typically 20%. They suggested that surfactant reduces surface rugosity and increases hydrophilicity by coating the membrane. These effects apparently reduced polarisation around pores and reduced the availability of hydrophobic sites for adhesion. Wahlgren & Arnebrant (1991) using insitu ellipsometry showed that under certain conditions, the presence of surfactant completely prevented protein adsorption. They also found that the amounts of proteins adsorbed were below or in the range of monolayer adsorption.

From the flux versus time graphs (Fig. 7.11), in the absence of SDS, starting at 200 $L/m^2.h$, flux stayed constant for 25-37 minutes and then declined to a steady state value of 110 $L/m^2.h$ when severe fouling occurred and the permeate controller was switched to constant pressure mode. It appears that 110 $L/m^2.h$ is the limiting/final steady state flux in the absence of SDS above which severe fouling occurred. The final steady state was increased to about 210 $L/m^2.h$ in the presence of SDS. Though the fouling resistance has been reduced considerably due to the addition of SDS solution half way through the runs, protein transmissions did not increase, but instead dropped to <10 %. This is contrary to the general observation that an increase in fouling resistance reduces protein transmission. Flux has almost been restored to the set value due to reduced fouling resistance. However, protein transmission dropped. There are two possible explanations. 1) Perhaps the SDS interacted with the fouling layer on the membrane surface and apparently reduced the thickness of the fouling layer by breaking and removing part or all of the aggregates in the surface layer on the membrane, but still leaving a layer of tightly packed protein 2) The process of breaking aggregates in the surface layer may have caused compaction by reorientation or reorganisation of the protein molecules in the surface layer so that its porosity may have reduced resulting in protein transmissions lower than the control. When SDS was present right from the start, when severe fouling occurred (at 300 $L/m^2.h$), protein transmissions were much lower than those observed at 200 $L/m^2.h$ without SDS, despite the lower fouling resistance. This is probably due to the formation of a surface layer by smaller unaggregated protein leading to a less porous cake on the membrane surface. This is supported by the observation of a sharp reduction in protein transmission at higher fouling resistances in the presence of SDS (Fig. 7.13).

7.3.4. Summary of the proposed fouling mechanisms

Lactoferrin appears to form a concentration-induced fouling layer at the pore entrances perhaps largely composed of aggregates rather than the monomer protein. Increase in the flux probably resulted in flux-induced aggregation, causing increased plugging at pore entrances leading to surface layer formation. Once formed, CFV affected flux and fouling resistance, but not protein transmission suggesting that fouling occurred by aggregates plugging at the pore entrance and formation of a

surface layer that largely controlled final protein transmission (20%). However, in the presence of SDS, aggregates may have been broken making it possible to operate without severe fouling at a higher flux (200 $L/m^2.h$). However when fouling was severe, smaller aggregated protein formed a tighter layer reducing protein transmission drastically (2-8%). The Marshall *et al.* (1997) model when applied to lactoferrin on a 100 nm pore size membrane, agrees reasonably well with experimental data suggesting that a thin layer of fouling forms in the vicinity of the membrane pore. SDS reduced or broke up protein aggregates reducing the thickness of the deposited layer but also reducing the apparent pore size of the layer.

7.4. Conclusions

1) Fouling was highly dependent on the permeate flux and the state of the protein in the feed.

2) There are three consequent steps in MF fouling by lactoferrin, i) adsorption ii) pore plugging and iii) surface layer formation. CFV did not have a significant effect on the initiation of fouling which suggests that the initiation of fouling is probably by pore fouling. However once a surface layer had formed, CFV had a small effect on fouling resistance but not on protein transmission which was about 20% without SDS and 2-8% with SDS in solution.

3) The presence of a surface active agent like SDS reduced adsorption and aggregation of protein minimising fouling. It was possible to operate at a higher permeate flux without fouling and with improved protein transmissions using SDS in protein solution under certain conditions. SDS apparently reduced the size of the protein aggregates thereby slowing down the fouling process. However, when severe fouling occurred with these smaller aggregates/monomer proteins, protein transmissions were reduced drastically probably due to the formation of a surface layer of low pore size.

8. Microfiltration fouling by bovine ferritin solutions

8.1 Introduction

Most of the fouling studies on microfiltration membranes have been limited to proteins such as BSA (Bowen & Gan, 1991; Tracey & Davis, 1994; Kelly & Zydney, 1995; Mueller & Davis, 1996; Jonsson *et al.*, 1996; Herrero *et al.*, 1997), lysozyme (Palecek & Zydney, 1994a & b; Güell & Davis, 1996; Kelly & Zydney, 1997) and β-lactoglobulin (Marshall *et al.*, 1997; Kelly & Zydney, 1997) etc., and the author is not aware of any microfiltration fouling studies reported on relatively large proteins, like ferritin. Hence further fouling studies were conducted to investigate whether higher molecular weight proteins like ferritin behaved differently from other proteins during MF.

Ferritin consists of a protein moiety, apoferritin, with a molecular weight of about 465 kD and a micellar complex of iron hydroxide phosphate (Aisen & Listowsky, 1980; Cetinkaya *et al.*, 1985; Massover, 1993). As demonstrated by electron microscopy, the iron hydroxide micelles form the core of the ferritin molecule. The iron content of ferritin varies, depending on source and method of preparation, but averages about 20% of dry weight. Ferritin has 24 equivalent protein subunits (MW of 18-19.5 kD each) arranged symmetrically around the iron core (Loehr, 1989). The iron core measures about 6 nm in diameter in a dried preparation, or 7.5 nm when wet and is surrounded by a shell of apoferritin. The isoelectric pH of ferritin varies from 4.5 to 5.5 depending upon the source. Ultracentrifugation, X-ray data and electron microscopy have indicated that the apoferritin shell of the ferritin molecule is close to spherical, with an outer diameter of 12.2 nm and an inner diameter of 7.5 nm when hydrated (Loehr, 1989).

8.2 Results

8.2.1. Effect of the permeate flux

Microfiltration at various constant permeate fluxes was performed to see any flux-induced effect on fouling by ferritin. There was a dramatic increase in fouling resistance when the permeate flux was increased to 100 $L/m^2.h$ or above (Fig. 8.1). Although the fouling resistances at 50 and 75 $L/m^2.h$ were relatively low, the protein transmissions were only about 17-25% and 4-12% respectively (Fig. 8.2). At 100 and 200 $L/m^2.h$, severe fouling occurred and the permeate controller was shifted to constant pressure mode when the TMP reached 200 kPa. After the permeate flux controller had been switched to constant pressure mode, the resistance did not increase with time (*i.e.* permeate flux attained a limiting value) suggesting lack of further fouling as the driving force for fouling was removed. Protein transmissions of about 3% were obtained when operated at these fluxes. There appears to be a critical flux between 75 and 100 $L/m^2.h$ for severe fouling to occur. At higher fluxes ($\geq$ 100 $L/m^2.h$) retained protein may have formed a surface layer leading to severe fouling.

To see whether changes in hydrodynamic conditions affect the surface layer, two experiments were performed starting at a constant flux of 200 $L/m^2.h$. Severe fouling occurred as found previously and the controller was switched to constant pressure mode within minutes. After about 60 minutes of operation in constant pressure mode, the permeate flux was reduced to 50 $L/m^2.h$ and constant flux operation reintroduced. The fouling resistance decreased rapidly to close to that of runs at 50 $L/m^2.h$ (Fig. 8.3). However protein transmission increased only slightly (Fig. 8.4). When the hydrodynamic conditions were changed *i.e.* under a constant TMP operation when the flux was reduced, the surface layer was probably sheared off or removed until a new dynamic equilibrium that governs protein deposition on the surface by convection and back diffusion at the membrane wall was reached. The virtually complete reversibility of the fouling resistance suggested that the surface layer was probably formed largely by retained protein molecules.

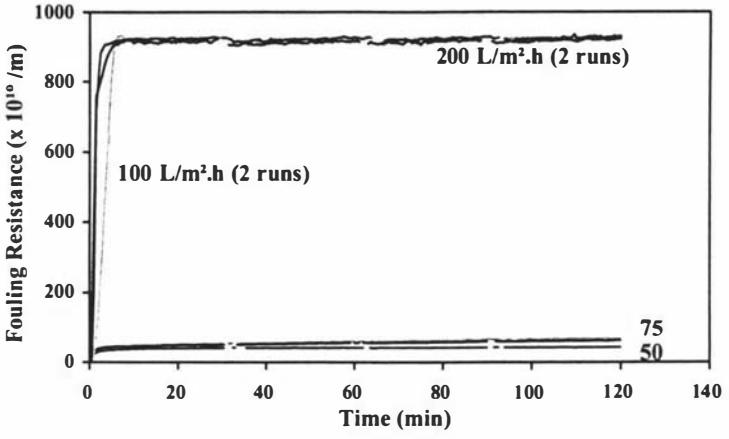

Figure 8.1. Fouling resistance during MF of a 0.2% bovine ferritin solution on a 100 nm membrane. The ionic strength of the solution was 0.05 to 0.052 and the pH: 6.80-6.82.

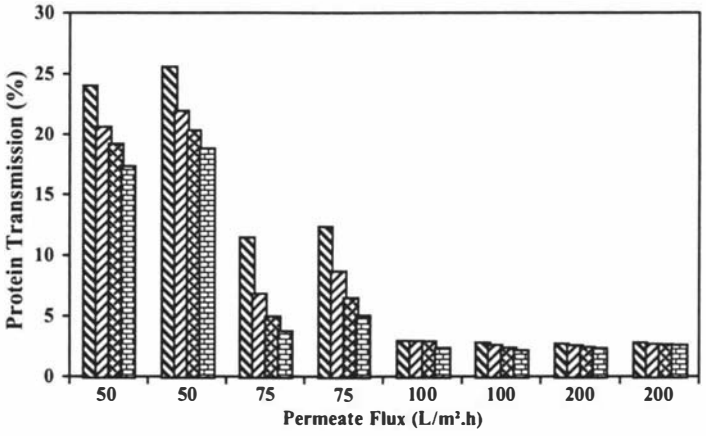

Figure 8.2. Protein transmission during MF of a 0.2% bovine ferritin solution on a 100 nm membrane. The ionic strength of the solution was 0.05 to 0.052 and the pH: 6.80-6.82. For each permeate flux the 4 bars represent values measured at 30, 60, 90 & 120 min respectively.

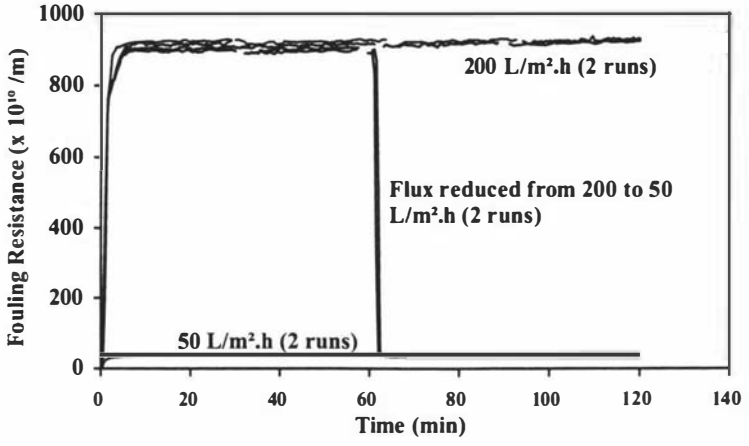

Figure 8.3. Fouling resistance during MF of a 0.2% bovine ferritin solution on a 100 nm membrane when permeate flux was reduced from 200 to 50 L/m^2.h at 60 minutes. The ionic strength of the solution was 0.05 to 0.053 and the pH 6.80 to 6.82.

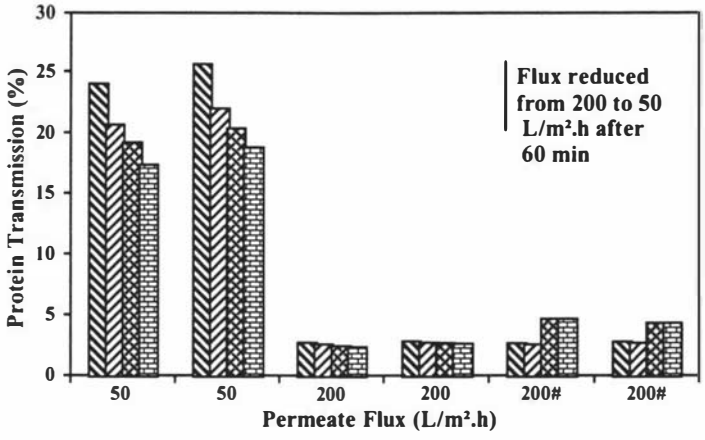

Figure 8.4. Protein transmission during MF of a 0.2% bovine ferritin solution on a 100 nm membrane when permeate flux was reduced from 200 to 50 L/m^2.h at 60 minutes (denoted by #). The ionic strength of the solution was 0.05 to 0.053 and the pH 6.80 to 6.82.

Similar flux related fouling was observed in the presence of SDS (1.4 g/g of protein) (Fig. 8.5). However, interestingly fouling was minimal at 100 L/m^2.h giving protein transmissions of about 60-90% (Fig. 8.6) compared to severe fouling at the same flux without SDS. These transmissions are about 3 to 4 times higher than those obtained even under low fouling conditions without SDS.

Further experiments were performed using feed containing SDS or calcium to investigate the effect of solution environment on protein deposition.

8.2.2. Effect of SDS

In the presence of SDS at 200 L/m^2.h, fouling resistance was much lower than runs with no SDS, but protein transmission increased by only a small amount (cf, Figs. 8.1-8.2 and 8.5-8.6).

Experiments were conducted at 200 L/m^2.h in which SDS solution was added after 60 minutes. There was a sudden drop in fouling resistance as soon as SDS was added (Fig. 8.7). There was a reduction of about 40% in fouling resistance and the experiment continued in constant pressure mode. The fouling resistance then increased slightly and stabilised towards the end of the experiment. Protein transmission increased slightly at 90 and 120 minutes probably due to the reduction in fouling resistance (Fig. 8.8). The quick drop in fouling resistance on the introduction of SDS indicated removal of at least some of a loosely bound surface layer of protein that may have formed on the membrane.

Steady state permeate fluxes under constant pressure operation are shown in Fig. 8.9. In the absence of calcium and SDS, constant flux experiments starting at 100 and 200 L/m^2.h attained a steady state flux of 91 L/m^2.h suggesting this is a critical flux to prevent fouling. This is further confirmed by the fact that constant flux operation at 50 and 75 L/m^2.h was possible without decline in flux (not shown in Fig. 8.9 for clarity). In the presence of SDS, the critical flux increased to 140-150 L/m^2.h. When SDS was added at 60 minutes during a 200 L/m^2.h run, the flux which had declined to about 91 L/m^2.h recovered to about 140 L/m^2.h and then started declining before it finally stabilised at about 130 L/m^2.h.

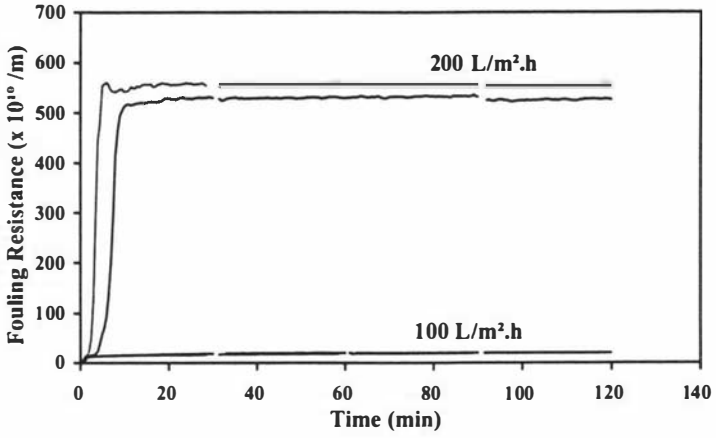

Figure 8.5. Fouling resistance during MF of a 0.2% bovine ferritin solution in the presence of SDS on a 100 nm membrane. The ionic strength of the solution was 0.051 to 0.053 and the pH: 6.80-6.82.

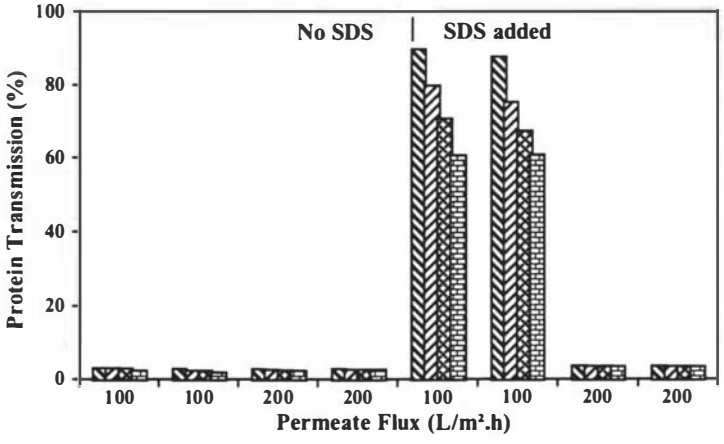

Figure 8.6. Protein transmission during MF of a 0.2% bovine ferritin solution in the presence of SDS on a 100 nm membrane. The ionic strength of the solution was 0.051 to 0.053 and the pH: 6.80-6.82. For each permeate flux the 4 bars represent values measured at 30, 60, 90 & 120 min respectively.

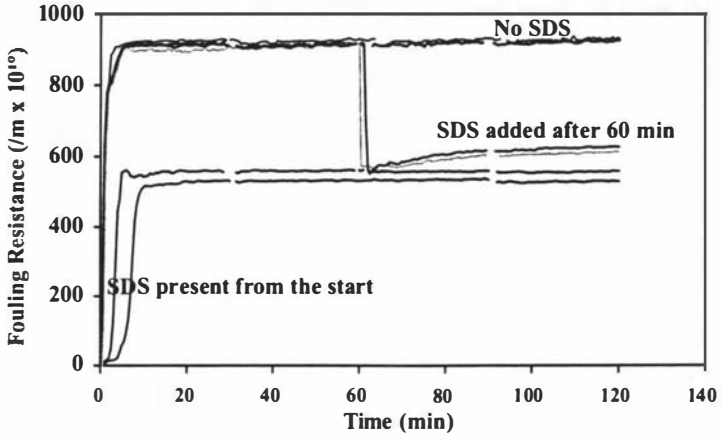

Figure 8.7. Change in fouling resistance due to the addition of SDS at about 60 minutes during MF of a 0.2% bovine ferritin solution on a 100 nm membrane at 200 L/m^2.h. The ionic strength of the solution was 0.05 to 0.052 and the pH 6.80 to 6.82 for the trials without SDS and 0.049 to 0.052 and pH 6.80 to 6.82 for the trials with SDS.

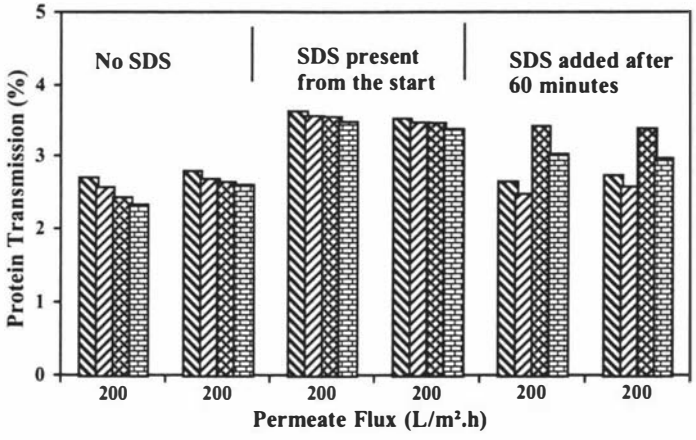

Figure 8.8. Change in protein transmission due to the addition of SDS at about 60 minutes during MF of a 0.2% bovine ferritin solution on a 100 nm membrane at 200 L/m^2.h. The ionic strength of the solution was 0.05 to 0.052 and the pH 6.80 to 6.82 for the trials without SDS and 0.049 to 0.052 and pH 6.80 to 6.82 for the trials with SDS.

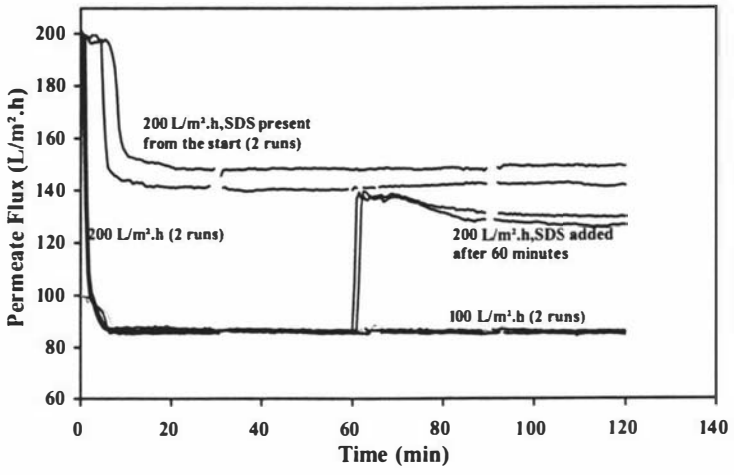

Figure 8.9. Flux decline during MF of a 0.2% bovine ferritin solution on a 100 nm membrane. The ionic strength of the solution was 0.051 to 0.053 and the pH 6.80 to 6.82 for the trials without SDS and 0.049 to 0.052 and pH 6.80 to 6.82 for the trials with SDS.

Looking at the initiation of fouling, the increase in dynamic resistance (*i.e.* the increase in resistance when the feed containing protein was introduced in the plant) with SDS present was low indicating potentially lower adsorption on to the membrane whereas at permeate fluxes of 50 and 75 $L/m^2.h$ in the absence of SDS, the so called dynamic resistance was observed to be higher (Fig. 8.10). The difference may perhaps also be due to the smaller size of protein aggregates in the presence of SDS.

8.2.3. Effect of calcium

In the presence of calcium, severe fouling occurred even at 75 $L/m^2.h$ compared to the control (Fig. 8.11) reducing protein transmissions to about 2% (Fig. 8.12). Calcium in solution appears to have a specific interaction with ferritin leading to a change in fouling mechanism as solutions with the same ionic strength (control) achieved by the addition of NaCl did not cause severe fouling.

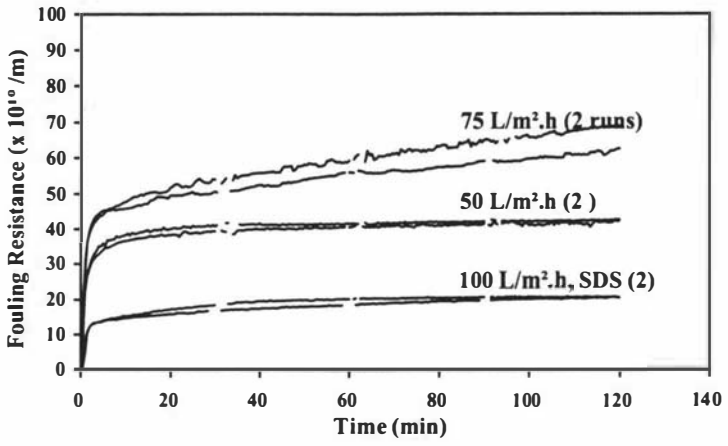

Figure 8.10. Selected resistance curves for different fluxes with and without SDS in solution during MF of a 0.2% bovine ferritin solution on a 100 nm membrane. The ionic strength of the solution was 0.049 to 0.052 and the pH 6.80 to 6.82 for the trials without SDS and 0.051 to 0.053 and pH 6.80 to 6.82 for the trials with SDS.

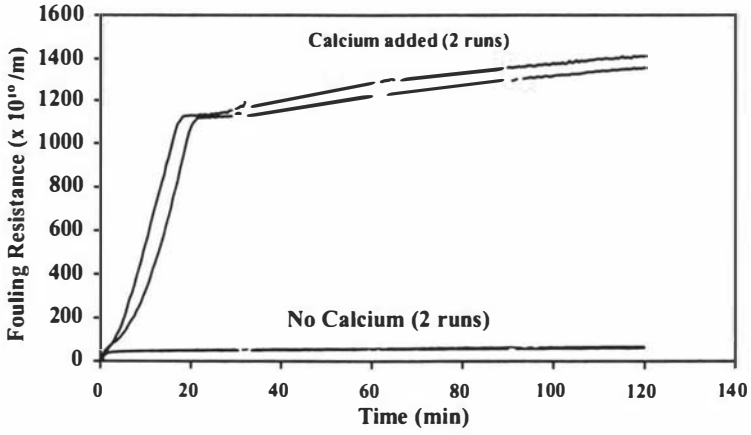

Figure 8.11. Fouling resistance during MF of a 0.2% bovine ferritin solution in the presence of calcium at 75 L/m^2.h on a 100 nm membrane. The ionic strength of the solution was 0.049 to 0.051 for the trials without calcium and 0.05 to 0.052 for the trials with calcium; the calcium content was 8.0 to 8.1 mmol/L and the pH: 6.80-6.82.

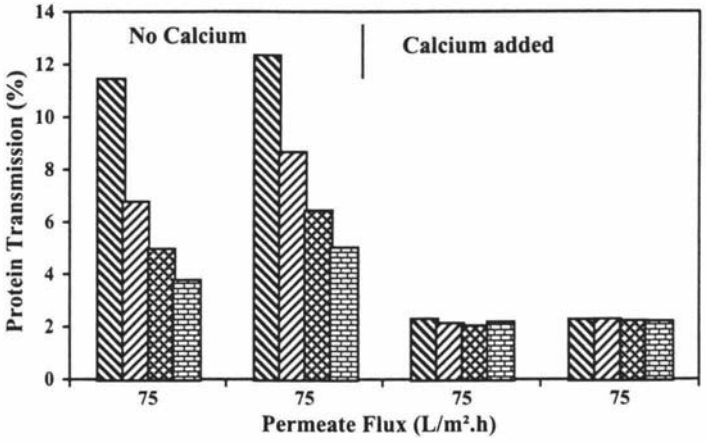

Figure 8.12. Protein transmission during MF of a 0.2% bovine ferritin solution in the presence of calcium at 75 L/m^2.h on a 100 nm membrane. The ionic strength of the solution was 0.049 to 0.051 for the trials without calcium and 0.05 to 0.052 for the trials with calcium, the calcium content was 8.0 to 8.1 mmol/L and the pH: 6.80-6.82. For each permeate flux the 4 bars represent values measured at 30, 60, 90 & 120 min respectively.

8.2.4. Effect of fouling resistance on protein transmission

Protein transmissions from all the experiments were plotted against corresponding fouling resistance data (Fig. 8.13). Increase in fouling resistance caused a dramatic and rapid decrease in protein transmission in the range of <100 x 10^{10} /m and then stayed almost constant with higher fouling resistance. The presence of SDS gave higher protein transmissions at lower fouling resistances. Protein transmissions were lower in the presence of calcium due to severe fouling.

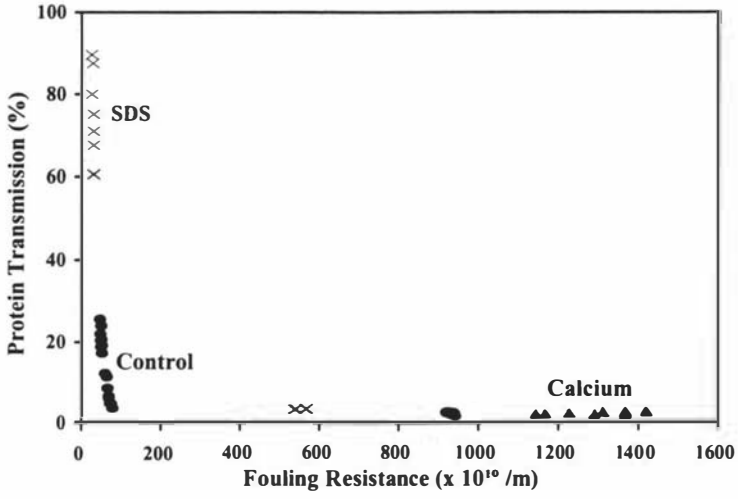

Figure 8.13. Fouling resistance versus protein transmission for a 100 nm membrane: (x) data from trials with SDS in solution; (▲) trials with calcium; (●) trials without SDS or calcium (control).

8.2.5. Effect of permeate flux on the reversibility of fouling resistance

Most of the extra fouling resistance associated with increase in permeate flux both in the presence and absence of calcium was reversible *i.e.* removed by water flushing (Table 8.1). A similar trend was observed in the presence of SDS (Table 8.1). When SDS was added half way through the run (60 min), most of the resistance remaining after SDS addition was also reversible (Table 8.1). When permeate flux was reduced from 200 to 50 $L/m^2.h$, most of the resistance was removed due to the change in hydrodynamic balance and about 50% of the remaining resistance was also reversible (Table 8.1).

Table 8.1. Summary of protein transmissions and fouling resistances on a 100 nm pore size membrane during microfiltration of 0.2% bovine ferritin solutions. pH: 6.80-6.82, IS: ~0.05 M.

Flux $L.m^{-2}.h^{-1}$	Protein Transmission[a] %	Total final resistance			
			Membrane	Reversible	Irreversible
		/m x 10^{10}	%	%	%
50	17.32	53.70	19.27	36.45	44.28
50	18.79	51.00	20.64	36.32	43.04
75	3.74	79.30	12.71	50.82	36.47
75	5.01	73.00	13.73	49.74	36.53
100	2.35	933.00	1.11	97.33	1.56
100	2.15	942.10	1.07	97.43	1.50
200	2.33	933.5	1.11	97.59	1.30
200	2.6	968.00	1.07	97.18	1.75
75*	2.17	1421.00	0.69	98.43	0.88
75*	2.20	1369.00	0.80	98.37	0.83
100@	60.56	31.00	32.48	31.71	35.81
100@	60.74	32.00	31.72	31.25	37.03
200@	3.47	566.00	1.85	95.39	2.76
200@	3.37	539.30	1.84	94.88	3.28
200#	3.03	635.00	1.54	95.02	3.44
200#	2.97	621.00	1.63	95.47	2.90
200$	4.66	49.00	20.00	55.06	24.94
200$	4.30	45.80	21.51	51.51	26.99

a Protein Transmission at 120 min.
* Experiments using solutions containing 8 mmol/L Calcium.
@ Experiments using solutions containing 1.4 g SDS/g of protein
# Experiments in which SDS was added after 60 minutes
$ Experiments in which flux was reduced from 200 to 50 $L/m^2.h$ at 60 minutes

8.2.6. Effect of cross-flow velocity and transmembrane pressure on the reversibility of the fouling layer

Two experiments were performed starting at 100 $L/m^2.h$ constant flux and at a CFV of 3 m/s. Severe fouling occurred as shown previously. The permeate controller was switched to constant pressure mode and then CFV was varied to 1, 4, 2 and back to 3 m/s (Fig. 8.14). Repeated experiments gave consistent data. At the end of about 2 h of experiment, the effect of TMP on permeate flux was investigated by operating the rig at different constant TMP's.

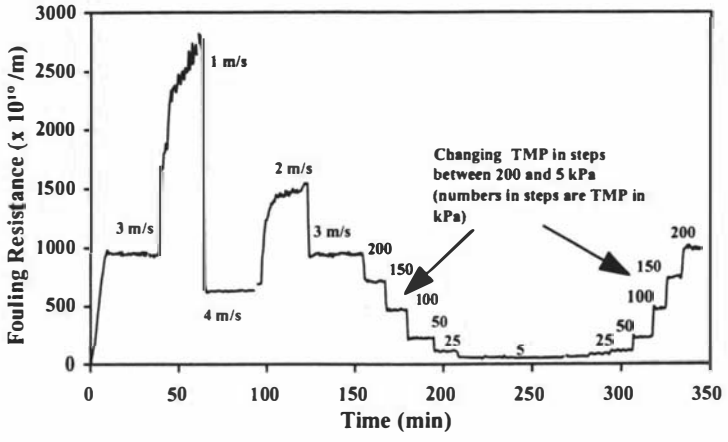

Figure 8.14. Fouling resistance under different cross-flow velocities and TMPs during MF of a 0.2% bovine ferritin solution on a 100 nm membrane. The experiment was started at a constant flux of 100 L/m^2.h and changed to constant pressure mode when severe fouling occurred. The ionic strength of the solution was 0.049 to 0.052 and the pH 6.80 to 6.82.

The TMP was decreased from 200 to 50 kPa in steps of 50 kPa and from 50 to 5 kPa in steps of 25 or 5 kPa with time. The TMP was then increased back to 200 kPa in steps.

TMP had no effect on permeate flux down to a value of about 20 kPa (Fig. 8.15) *i.e.* permeate flux was independent of TMP in the region of 200 to 20 kPa. Repeated experiments gave the same results. Average values of permeate flux and TMPs were considered to determine the relationship between them below 20 kPa. There was almost a linear relationship between TMP and flux suggesting a pressure dependent region of 0 to 20 kPa. A detailed plot of TMP versus flux below a TMP of 100 kPa is shown in Fig. 8.16. The final TMPs in the experiments where fouling was light are shown in Table 8.2. It suggests that as long as the rig is operated at <20 kPa *i.e.* about 16 kPa, fouling is minimal suggesting a critical TMP of about 16 kPa to prevent severe fouling during MF of ferritin.

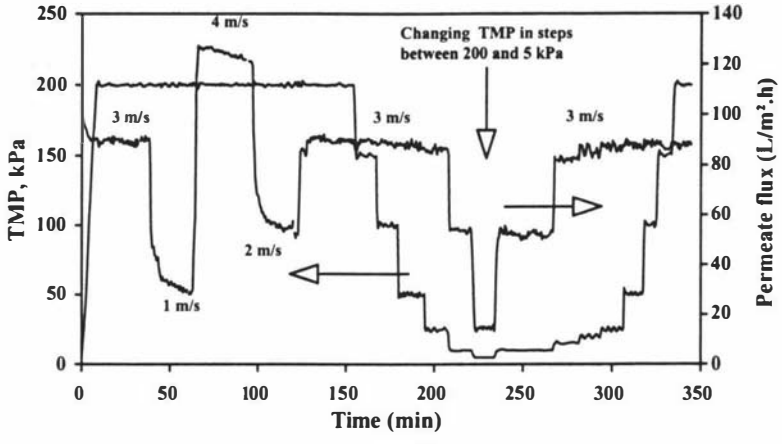

Figure 8.15. Flux decline at different cross-flow velocities and TMPs during constant pressure MF of a 0.2% bovine ferritin solution on a 100 nm membrane. The experiments were started at a constant flux of 100 L/m^2.h and changed to constant pressure mode when severe fouling occurred. The ionic strength of the solution was 0.049 to 0.052 and the pH 6.80 to 6.82.

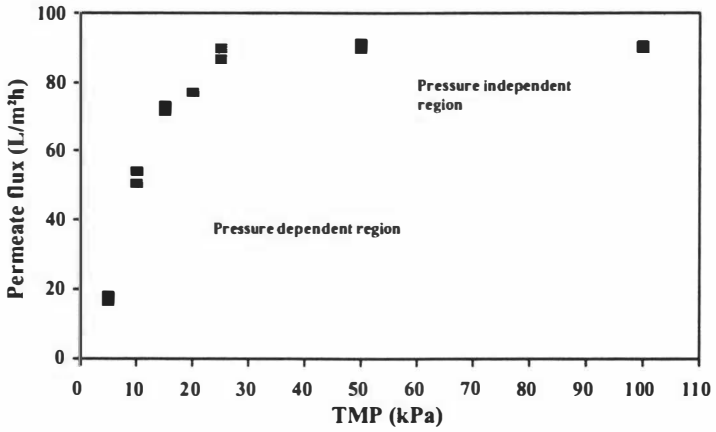

Figure 8.16. The relationship between transmembrane pressure and permeate flux during constant pressure MF of a 0.2% bovine ferritin solution on a 100 nm membrane. The experiment was started at a constant flux of 100 L/m^2.h and changed to constant pressure mode when severe fouling occurred. The cross-flow velocity was 3 m/s, ionic strength of the solution; 0.049 to 0.052 and the pH 6.80 to 6.82.

Table 8.2. Final TMP's in experiments where fouling was minimal

Permeate flux ($L/m^2.h$)	Maximum TMP reached (kPa)
50	6.5
50	7.5
75	14.5
75	15.5

CFV had a significant effect on the fouling resistance of the fouled membrane and steady state permeate flux (Figs. 8.14 & 8.15). The slopes of the fouling resistance curves show that protein deposition rate was much faster when CFV was <3 m/s. Though CFV had a significant effect on fouling resistance, its effect on protein transmission was small (Fig. 8.17).

A plot of steady state permeate flux versus CFV is shown in Fig. 8.18. The velocities studied were all in the turbulent region with Reynolds number ranging from 6, 900 to 27, 600. By regression analysis the relationship between CFV and permeate flux (J) is established as;

$J = f(CFV)^n$

The value of n was varied till $r^2 \approx 1$. A value of n=1 suited best. This value of n is close to the value reported by Cheryan (1986) and Singh and Cheryan (1997) for modules operating in turbulent flow.

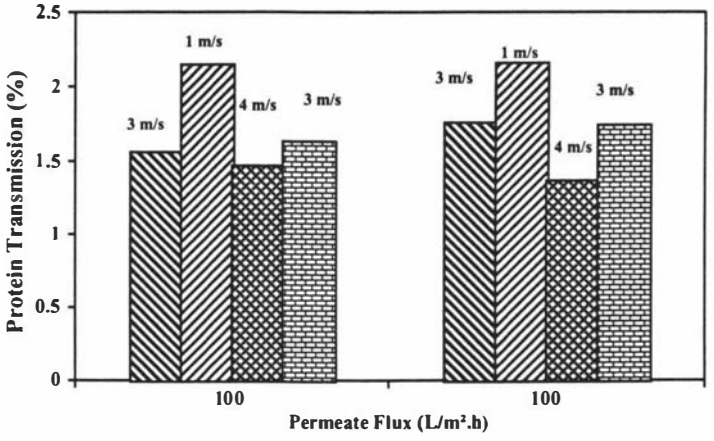

Figure 8.17. Protein transmission under different cross-flow velocities during constant pressure MF of a 0.2% bovine ferritin solution on a 100 nm membrane. The experiment was started at a constant flux of 100 L/m^2.h and changed to constant pressure mode when severe fouling occurred. The ionic strength of the solution was 0.049 to 0.052 and the pH 6.80 to 6.82.

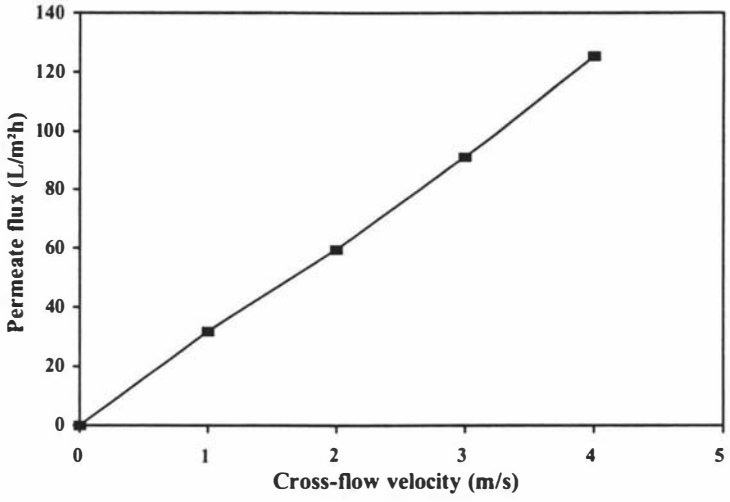

Figure 8.18. The relationship between cross-flow velocity and permeate flux during constant pressure MF of a 0.2% bovine ferritin solution on a 100 nm membrane at 200 kPa. The experiment was started at a constant flux of 100 L/m^2.h and changed to constant pressure mode when severe fouling occurred. The ionic strength of the solution; 0.049 to 0.052 and the pH 6.80 to 6.82.

8.3. Discussion

8.3.1. Probable fouling mechanisms

It is proposed that at least the following steps are involved in MF fouling by ferritin 1) adsorption and 2) "gel layer" formation.

8.3.1.1. Adsorption

Firstly, to see the effect of protein adsorption alone on fouling, it is worth considering experiments in which fouling was light. At 50 $L/m^2.h$ the fouling resistance was small and remained almost constant with time indicating that fouling was mostly due to adsorption. Constant fouling resistance may be due to equilibrium protein adsorption (Matthiasson, 1983). Apparent pore size reduction in these trials calculated using equation (3.12) was 25-26 nm (Table 8.3). Considering the size of ferritin, 12-13 nm (Loehr, 1989) in solution, this is approximately twice the diameter of the ferritin suggesting that protein adsorbed approximately equivalent to a monolayer. In static studies using proteins, more than a monolayer of adsorbed protein was reported (Matthiasson, 1983; Vetier *et al.,* 1986). In cross-flow studies, Bowen & Gan (1991) and Persson *et al.* (1993a) have reported about a monolayer of protein adsorption. At 75 $L/m^2.h$, there was a slight increase in fouling resistance with time (Fig. 8.10), indicating that probably a small amount of deposition occurred over the monolayer of adsorbed protein giving a pore size reduction of 28-29 nm. At higher fluxes multilayer deposition of protein would have occurred increasing fouling resistance dramatically (Bowen & Hughes, 1990; Kim *et al.*, 1992; Jonsson *et al.*, 1996). However, in the presence of SDS at 100 $L/m^2.h$ where fouling resistance was much lower than at 50 $L/m^2.h$, with no SDS, the apparent pore size reduction was only 17-18 nm perhaps due to a reduced size of ferritin or reduced adsorption of protein in the presence of SDS (Wahlgren & Arnebrant, 1991).

Table 8.3. Apparent pore size reduction in trials performed at various fluxes and conditions on the 100 nm membrane.

Permeate flux ($L/m^2.h$)	Clean membrane resistance ($x\ 10^{10}/m$)	Irreversible fouling resistance ($x\ 10^{10}/m$)	Apparent reduction in pore size (nm)
50	10.4	23.8	26
50	10.5	22.0	25
75	10.1	28.9	29
75	10.0	26.7	28
100@	10.1	11.1	17
100@	10.2	11.9	18
200 #	9.8	14.22	20
200#	9.9	15.36	21

@ Trials with SDS present

Trials in which flux was reduced from 200 to 50 $L/m^2.h$ after 60 minutes

Some pore plugging by the retained protein may have occurred initially along with adsorption (Prádanos *et al.*, 1996). An increase in flux might have increased both the rate of pore plugging and the immediate protein retention at the membrane wall leading to formation of a surface layer.

8.3.1.2. Gel layer formation

Evidence of gel layer formation

It is surprising to observe protein transmissions of only about 17-25% even under light fouling conditions (at <75 $L/m^2.h$), when the average size of the protein in its monomeric form (~12 nm) is much smaller than the pore size of the membrane (100 nm). One possibility is that some of the protein is aggregated. Aggregated protein retained on the membrane surface may have formed a surface layer. When flux is increased to above some critical value (>75 $L/m^2.h$), the concentration of protein at the membrane wall increases until a limiting concentration is reached when further concentration cannot take place because of the limited permeability of the polarised macromolecular layer.

When the limiting concentration at the wall (C_{wall}) reaches gel concentration (C_g) particularly at fluxes >75 $L/m^2.h$, further build up of the protein leads to thickening of

the gel layer. The gel layer reduces flux to the point where the reduced convective forward transport of the protein is balanced by diffusive back transport from the concentrated gel layer into the bulk solution. This state of gel formation and limiting flux is achieved within minutes of the start of the filtration process. At this stage, the most common way of increasing flux is using operating variables that can enhance back transport of concentrated species from the membrane, such as increased cross-flow velocity to enhance shear rates at the membrane surface or increased process temperatures to enhance diffusivity. Increasing CFV indeed decreased fouling resistance as found from the experiments. This is probably due to reduced thickness of the fouling layer. The reversibility of the fouling layer and its dependence on the CFV is consistent with gel layer theory in membrane processing.

Any increase in TMP can increase the convective driving force but does not enhance back transport and results in the build up of a thicker layer of retained species. The limiting flux value would remain the same. However at TMP below the gel polarisation region, pressure generally has a positive effect to increase the flux as found from the experiments (Fig. 8.16). It is clear from the present investigation (at a CFV of 3 m/s) that any increase in TMP beyond 20 kPa does not increase permeate flux, suggesting a pressure independent region beyond this critical TMP. Critical TMP is a function of CFV (Samuelsson *et al.*, 1997). Any attempt to increase the flux by operating beyond this TMP will not be beneficial unless CFV is increased. Instead, it is better to operate below or at this TMP to minimise fouling.

8.3.2. Application of gel model to predict permeate flux

The influence of CFV on limiting flux and observation of a pressure independent region in the TMP versus flux plot support the mechanism of gel layer formation. The hypothesis of gel layer formation is also supported by the reversibility of the fouling layer on flushing. Pore plugging and adsorption are not usually reversible. Further to prove that fouling is predominantly by gel layer formation, the following calculations based on film theory were performed. A set of experimental values were considered to calculate a gel concentration first assuming a protein radius. The fluxes at other CFV's were then predicted using the gel model and compared to experimental values.

The average feed and permeate concentrations under severe fouling conditions were 0.2% and 0.03% respectively. At a CFV of 3 m/s, process temperature of 25°C, the steady state or limiting flux (J) was about 91 $L/m^2.h$ in the absence of calcium and SDS, as found from the experiments. The value of the diffusion coefficient (D) is very important to estimate the mass transfer coefficient (k). D value for bovine ferritin was not available in the literature; however, values for Horse spleen apoferritin (MW: 469.9 kDa) were quoted as 3.99×10^{-11} m^2/s by Walters *et al.* (1984) and 3.61×10^{-11} m^2/s by Smith (1970).

Assuming the diameter of the ferritin molecule in monomeric form as 12 nm, D was calculated (using Stokes-Einstein's relationship (Equation 3.27)) as 3.64×10^{-11} m^2/s which agrees with literature value for Horse spleen apoferritin (Table 8.4). The mass transfer coefficient at a CFV of 3 m/s was calculated using equation 3.3 (Table 8.4). The exponent of the velocity term in the equation was modified to 1.0 as found from the experiments instead of the 0.8 generally cited in theory for turbulent flow.

The membrane wall concentration was estimated using equation 3.2 (Table 8.4). The estimated wall concentration of 1.32% seems unreasonable given the fact that the gel concentration for proteins and nucleic acids is generally 10-30% (Blatt *et al.*, 1970; Cheryan, 1986). At a particular CFV, the wall concentration is highly dependent upon the assumed diameter of the protein (which alters the calculated diffusivity value) and upon other errors associated with the estimation of diffusivity. It was thought that the assumption of ferritin as a monomer of size 12 nm diameter in solution was unlikely. A few large aggregates present in the ferritin sample used may have actually formed the gel layer. Hence, the radius of the protein aggregate was increased in the range of 6-25 nm so that the calculated values of D and C_w fall in the range cited in the literature (Table 8.5).

Table 8.4. Calculation of concentration of protein at the wall (C_w)

Calculation of Diffusion Coefficient (Equation (3.27))	
k_b=1.38 x 10^{-23}	J/K
T = 298	K
μ = 0.001	Pa.s
r = 6 nm *i.e.* 6 x 10^{-9} m	
D = 3.64 x 10^{-11}	m^2/s
Calculation of Mass Transfer Coefficient (Equation (3.3))	
ρ = 1000	kg/m^3
μ = 0.001	Pa.s
u = 3	m/s
d = 0.0069	m
k = 1.25 x 10^{-5}	m/s
Calculation of Wall Concentration (Equation (3.2))	
C_p = 0.03	%
C_b = 0.2	%
J = 91 $L/m^2.h$ = 2.53 x 10^{-5} m/s	
C_w = 1.32	%

Table 8.5. Effect of protein size on diffusivity and wall concentration

r (nm)	D x 10^{-11} (m^2/s)	C_{wall} (%)
6	3.6361595	1.32
8	2.7271196	2.00
10	2.1816957	2.96
14	1.5583541	6.10
16	1.3635598	8.45
17	1.2833500	9.94
18	1.2120532	11.64
20	1.0908479	15.84
24	0.9090399	28.52
25	0.8726780	32.87

The estimated diffusivity values ranged from 3.64 to 0.87 x 10^{-11} m^2/s. The wall concentrations ranged from 1.32% to 32.87%. An assumed protein radius of 17-24 nm gave a wall concentration of 10-29% which agrees with the gel concentration for

proteins (Blatt *et al.*, 1970; Cheryan, 1986). However, estimated diffusivity values do not agree with literature values which are based on a monomeric size for the protein.

The limiting flux values at various CFV's were predicted using the gel model:

$J_u = k_u . Ln(C_g/C_b)$

J_u is flux at a CFV, u m/s

k_u is mass transfer coefficient corresponding to a CFV of u m/s

Taking C_b as 0.2% and considering any value of protein radius and using calculated D and C_g, permeate fluxes were predicted and compared to the experimental values (Table 8.6 & Fig. 8.19). Interestingly the predictions are the same for any chosen value of protein radius supporting the idea that limiting fluxes are essentially dependent upon CFV when the gel layer is formed. This is consistent with the gel model when feed temperature, bulk concentration and diffusivity are constant, the only variable that can be changed according to the gel theory is CFV.

The prediction of the model over the range of CFV's is very good considering the assumptions made in the model. Physical properties of protein solutions were assumed constant across the concentration boundary layer and also protein is assumed to be aggregated and of uniform size.

A more rigorous treatment of the gel model is obviously possible by considering the effect of boundary layer concentration of protein on the physical properties. A particle size distribution (67% monomers; 26% dimers; 7% larger aggregates) in ferritin solutions was observed when samples were run using a superose 6 HPLC column in the present work (data not shown). It is crucial to verify the size distribution of ferritin in solution and also C_g of ferritin by doing experiments at various bulk concentrations and determining limiting flux values. The calculations however do show that the gel model can be used to explain the observed fouling behaviour of ferritin. The actual figures are not considered to be highly accurate.

Table 8.6. Limiting fluxes at various CFV's

u	j-experimental	J by gel model
m/s	$L/m^2.h$	$L/m^2.h$
1	32.0	30.3
2	59.5	60.7
3	91.0	91.0
4	125.5	123.3

There is currently debate for (Blatt *et al.*, 1970) and against (Nakao *et al.*, 1979; Isaacson *et al.*, 1980; Fane *et al.*, 1981; Jonsson, 1984) the gel theory. In UF gel layer limitation is most likely with high molecular weight solutes (> 100 kD) whereas osmotic pressure limitation is expected when using small solutes (10-100 kD) as concluded by Wijmans *et al.* (1984) and Vilker *et al.* (1984). Gel layer formation seems to be reasonable here given that the molecular weight of the ferritin is 465 kD.

It appears that as long as MF is operated below a critical permeate flux, where wall concentration does not exceed gel concentration, severe fouling can be prevented. Most of the resistance due to the fouling layer is reversible by water flushing and also highly dependent upon the hydrodynamic conditions indicating that the gel layer is not strongly bound to the membrane.

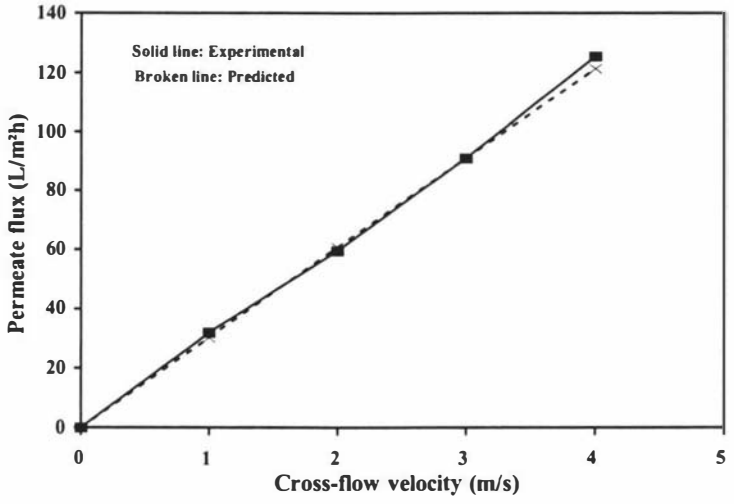

Figure 8.19. Comparison of predicted permeate flux and experimental values under different cross-flow velocities during MF of a 0.2% bovine ferritin solution on a 100 nm membrane. The ionic strength of the solution; 0.049 to 0.052 and the pH 6.80 to 6.82.

However, changes in the hydrodynamic or operating conditions only changed the thickness of the gel layer but not the protein transmission. This is probably due to the formation of a gel layer in the membrane matrix and this part of the gel layer was probably not reversible to changes in permeate flux (hydrodynamic conditions).

Decreasing TMP did not cause any decrease in permeate flux down to 20 kPa suggesting that the gel layer was already consolidated and hence pressure had no effect further confirming the proposed theory of a concentration induced gel layer. However under 20 kPa, the convective flux was so low that rate of removal of deposits on the membrane surface by shear was high resulting in reduced thickness of the gel layer and a pressure dependent flux regime. The observed pressure dependent and independent regions (Fig. 8.16) again suggest that a gel layer occurred on the surface of the membrane at fluxes where wall concentration reached gel concentration. This is consistent with gel theory (Michaels, 1968; Blatt *et al.*, 1970; Porter, 1972; Cheryan, 1986).

Overall, adsorption of monomers should not constrict or narrow the pores of a 100 nm membrane very much, hence adsorption alone cannot explain the reduction in protein transmission even under light fouling. Aggregated protein may have plugged the pores leading to the formation of a surface layer. It is hypothesised that initially protein adsorbs with simultaneous pore plugging resulting in immediate formation of a surface layer by the retained aggregates. At higher fluxes, due to increased collisions and a different diffusion balance, further deposition by the retained protein over the monolayer of adsorbed protein occurred perhaps leading to a concentration-induced gel layer on the surface of the membrane. Though the gel layer appeared mostly reversible, irreversible fouling near the pore or within the pore that occurred initially, reduced protein transmission.

8.3.3. Effect of solution environment on fouling mechanisms

Solution environment seems to play an important role in fouling apparently by altering the size of the protein molecules or aggregates. Change in aggregate size may cause either pore plugging or affect the porosity of the deposited layer on the membrane. The presence of calcium made fouling severe at 75 $L/m^2.h$ probably by

enhancing aggregation via specific interaction with ferritin. Whey protein aggregation and consequent fouling was attributed to the presence of calcium in solution (Rao *et al.*, 1994; also see section 6.3.2).

However in the presence of SDS, 'dynamic fouling' and total fouling resistance at 100 $L/m^2.h$ were significantly lower than those obtained at 50 and 75 $L/m^2.h$ without SDS (Fig. 8.10) perhaps due to a reduction in size of the protein/aggregate. SDS increased protein transmission when fouling was light, supporting the idea that aggregate/protein size in solution may have been reduced by SDS. Fouling was prevented in the presence of SDS at 100 $L/m^2.h$ further supporting the concept that aggregates may have reduced in size causing minimal fouling. This suggests the possibility of increasing critical flux in the presence of SDS perhaps due to reduced size of ferritin and increased diffusivity. However, at a higher flux (200 $L/m^2.h$), even in the presence of SDS, severe fouling occurred reducing protein transmission. Increased convective transport of the protein may have enhanced protein-membrane interactions in the presence of SDS forming a compact or tight protein layer on the membrane. Smaller protein/aggregates may have filled in the surface layer further tightening the porosity of the layer resulting in low protein transmission.

The subunit structure of the ferritin molecule is remarkably stable (Massover, 1993). So it is unlikely that SDS breaks up the protein into individual subunits in solution. However SDS may have dissociated larger aggregates (see section 6.3.2) or reduced adsorption. SDS also appears to have a desorption effect on the fouling layer (Wahlgren & Arnebrant, 1991). When SDS was added half way through, its effect was mostly on the surface of the membrane probably reducing the thickness of the fouling layer but not affecting protein transmission. When severe fouling occurred, the final protein transmission in all the solution environments investigated was almost the same indicating that the porosity of the gel layer was reasonably constant (Fig. 8.13). Thus it is clear from the fouling data obtained in the presence of SDS and calcium, that any conditions that increase or reduce the degree of protein aggregation in the feed have a dramatic effect on fouling and protein transmission.

8.3.4. Summary of fouling behaviour by ferritin

To summarise the fouling behaviour of ferritin on a 100 nm pore size membrane, flux had a dramatic effect on the severity of MF fouling by ferritin. With low fluxes, although fouling was minimal, protein transmissions were a maximum of only about 25%, perhaps due to the presence of aggregates in the feed. With higher permeate fluxes (>75 $L/m^2.h$), severe fouling occurred within minutes reducing protein transmissions drastically. Flushing with water removed almost all of the fouling resistance suggesting that fouling was predominantly by surface layer formation. There appears to be a break point in the permeate flux above which severe fouling occurred. This corresponded to 91 $L/m^2.h$ flux where wall concentration was estimated to be in the range of 10-29%. However at 100 $L/m^2.h$ where wall concentration was >10-29%, a gel layer formed. It is evident that if the protein concentration at the wall reaches gel concentration, severe fouling in the form of a gel layer occurs.

The gel layer formed was highly reversible and was influenced by changes in hydrodynamic conditions such as CFV and TMP. High reversibility of the fouling resistance is clear evidence that fouling was predominantly by a concentration-induced reversible gel layer. When severe fouling occurred, the porosity of the gel layer determined final protein transmissions. It appears that any solution conditions that increase or decrease aggregation have a major effect on fouling of MF membranes by ferritin.

8.4. Conclusions

1. A flux related fouling/break point corresponding to an estimated protein wall concentration of 10-29% is observed with ferritin.
2. Fouling is predominantly by a concentration-induced gel layer with adsorption and a small amount of pore plugging initially.
3. Fouling is highly reversible on water flushing, but the protein transmission is not.
4. SDS increased critical flux and increased protein transmission. SDS probably reduced protein size enhancing diffusivity leading to minimal fouling at low fluxes.
5. Increasing CFV was effective at improving the flux, but not protein transmission.
6. Operating at a TMP of 16 kPa or under is beneficial to minimise fouling at a bulk protein concentration used in these experiments.

9. MF fouling by different protein solutions - a general discussion

9.1. Introduction

Previous studies on MF membrane fouling using proteins present different views on the possible fouling mechanisms:

Typically in MF, when the protein size is much smaller than the pore size of the membrane, the following different fouling mechanisms have been reported (See Table 2.1).

1. Convective deposition of larger protein aggregates on the membrane surface serving as nucleation sites. Continued deposition of bulk protein (non aggregated) occurs via the formation of intermolecular disulfide-linkages between existing protein deposit and native protein (Kelly *et al.*, 1993; Kelly & Zydney, 1995, 1997). Fouling in this case occurs predominantly on the membrane surface.

2. Pore plugging by aggregates is the initial mechanism followed by surface layer formation once the pores are completely covered (Jonsson *et al.*, 1996; Mueller & Davis, 1996).

3. Deposition near the pore entrance (Bowen & Gan, 1991; Tracey & Davis, 1994; Marshall *et al.*, 1997; Kim *et al.*, 1997) or within the pores (Franken *et al.*, 1990; Bowen & Gan 1991, 1992; Jonsson *et al.*, 1992a; Herrero *et al.*, 1997) slowly constricting the pores and leading to subsequent surface layer formation.

On the other hand, when the protein is slightly larger than or equal to the membrane pore size, a dynamic membrane or gel layer on the membrane surface has been observed (Vetier *et al.*, 1988; Attia *et al.*, 1991a & b; Marshall *et al.*, 1996). This dynamic layer restricts the passage of smaller protein molecules (Meireles *et al.*, 1991b; Güell & Davis, 1996).

Although there are conflicting theories on the protein fouling behaviour during MF in general, at least with BSA, there is some agreement on the fouling behaviour reported in the literature. A two step mechanism of pore fouling, pore plugging or deposition on the pore walls and consequent surface layer formation is commonly reported (Table 2.1). The key parameters that determine fouling mechanisms in

these systems appear to be protein to pore size ratio and transmembrane pressure (TMP).

The following fouling steps during MF of protein solutions may be proposed:

1) Protein adsorption on to membranes is generally inevitable, however its effect during MF is minimal as MF membrane pores are much larger than the size of the protein.

2) Pore plugging is predominant if protein is of similar or slightly smaller size than the pore size. Though plugging by monomers is rare, plugging by aggregates is more common. Aggregates formed during processing may also plug the pores (Chandavarkar, 1990; Meireles *et al.,* 1991a; Marshall, 1994). The ratio of protein aggregate size to pore size appears to be a significant factor for the plugging to occur (Marshall *et al.,* 1993).

3) Subsequent surface layer formation is probably caused by any of the following:

i) Concentration-induced gel layer, if the ratio of protein/protein aggregate size to pore size is >1 and if there are interactions involved in the feed material (Vetier *et al.,* 1988; Attia *et al.,* 1991a & b; Marshall *et al.,* 1996).

ii) Filter cake formation, perhaps with less interacting feed material (Madaeni & Fane, 1996).

iii) Some form of polymerisation of protein on the membrane surface (Lee & Merson, 1976a; Le & Howell, 1983; Suki *et al.,* 1984; Kelly *et al.,* 1993).

The above general overview of fouling mechanisms may not be valid for all proteins on all membranes. Physicochemical properties of the feed determine the type and stages involved in fouling mechanisms (Marshall *et al.,* 1993; Kelly & Zydney, 1997). With smaller proteins, steps 1 to 3 occur while with larger proteins perhaps only steps 1 and 3 occur if the ratio of protein to pore size >1.

Most of the studies cited in the literature were performed using a single protein solution *i.e.* BSA (Table 2.1). It is difficult to extrapolate based on these results as BSA has unique physicochemical properties (Foster, 1977). Studies using multiple proteins through partially retentive membranes (Prádanos *et al.,* 1996) and totally permeable proteins (Kelly & Zydney, 1997) gave a different picture of fouling by

proteins and their effect on underlying fouling phenomena. Most of the previous studies were performed using stirred cells without pumping thereby avoiding shear induced denaturation (Meireles *et al.*, 1991a). Kelly & Zydney (1997) compared fouling behaviour of a variety of proteins in stirred cells using nitrogen pressurisation. They reported that fouling occurred by two distinct mechanisms (see literature review). They attributed aggregation primarily to intermolecular disulfide bonds. It was not clear how this chemical attachment occurred with proteins that do not have a free thiol group. They attributed this to hydrophobic interactions.

In this chapter the fouling behaviour of four different feed materials, one containing a mixture of whey proteins and others containing three different single proteins, is compared. The effect of protein size, solution properties and permeate flux on the underlying fouling mechanisms is discussed.

9.2. Comparative results

Fouling data obtained during MF experiments with the four different feed solutions, BSA, lactoferrin, ferritin and whey protein is considered in this section. At a permeate flux of 50 $L/m^2.h$, BSA and whey protein solutions showed the lowest and highest fouling resistances respectively whereas ferritin and lactoferrin showed intermediate values (Fig. 9.1). Overall at this permeate flux, all the four protein solutions investigated showed minimal fouling giving protein transmissions of about 95%, 90%, 17-25 % and 65-67% for BSA, lactoferrin, ferritin and whey protein solutions respectively (Fig. 9.2). Low protein transmissions particularly for ferritin and whey protein solutions suggest that a proportion of the protein is probably aggregated in the initial feed. The dynamic fouling resistance (initial increase in fouling resistance as soon as the protein containing feed was introduced in the plant) was about 8-9 x 10^{10} /m, 20-23 x 10^{10} /m, 30-35 x 10^{10}/m and 37-40 for BSA, lactoferrin, ferritin and whey protein solutions respectively suggesting different sizes of protein/aggregates in solution. Comparison of the dynamic resistances indicates that the order of dynamic fouling resistances is in the same order as the molecular weight of the protein or aggregate. The fouling resistance curves for BSA and ferritin indicate that deposition rates are almost constant with time whereas for lactoferrin and whey protein solutions, resistance curves showed a slightly increasing

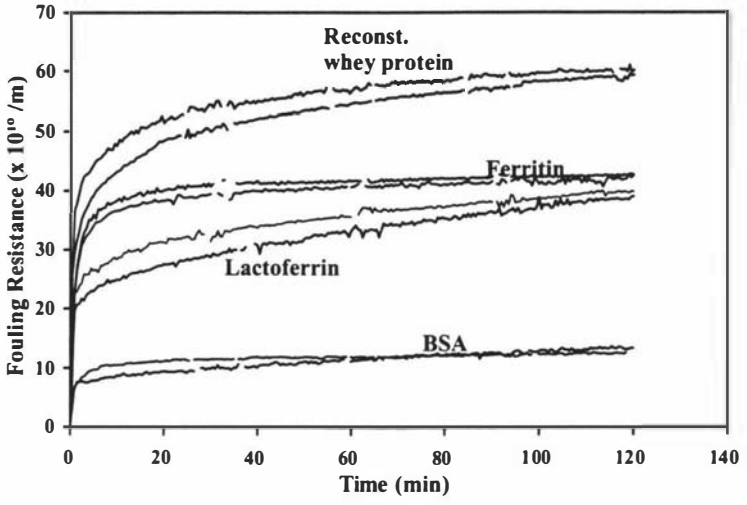

Figure 9.1. Comparison of fouling resistance at 50 L/m^2.h during MF of BSA, lactoferrin, ferritin and whey protein solutions on a 100 nm membrane. Fouling was minimal with all these proteins at this flux. pH: 6.78-6.89, IS ~0.05 M.

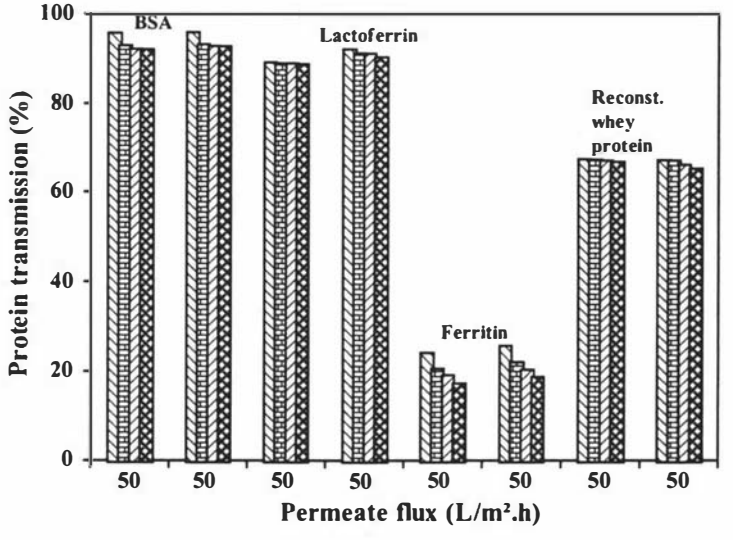

Figure 9.2. Comparison of protein transmission at 50 L/m^2.h during MF of BSA, lactoferrin, ferritin and whey protein solutions on a 100 nm membrane. Fouling was minimal with all these proteins at this flux. pH: 6.78-6.89, IS ~0.05 M.

fouling resistance suggesting continued deposition via a different mechanism. It seems at least with BSA and ferritin at 50 L/m^2.h, fouling resistance increased very little indicating minimal deposition of protein with time.

The difference in fouling behaviour can also be seen by comparing severity of fouling by ferritin and lactoferrin and minimal fouling by BSA at a permeate flux of 200 L/m^2.h (Fig. 9.3). Experiments using whey protein solutions were not performed at this flux as severe fouling occurred even at 75 L/m^2.h. For lactoferrin and ferritin, fouling resistance increased significantly during the first 30 minutes of filtration followed by a less rapid increase for lactoferrin or almost a constant value for ferritin. Protein transmissions were low and decreased further with time particularly for lactoferrin (Fig. 9.4). The fouling resistance for ferritin increased rapidly to about 900 x 10^{10}/m within minutes whereas for lactoferrin, the increase in fouling resistance to about 500 x 10^{10}/m occurred in about 34-37 minutes, further suggesting that the fouling kinetics are different with these proteins. Looking at the initial rise in fouling resistance at this flux more closely, ferritin had a rapid increase in fouling resistance within minutes, whereas lactoferrin and BSA had a relatively small increase (Fig. 9.5) further showing the effect of protein size/molecular weight on fouling.

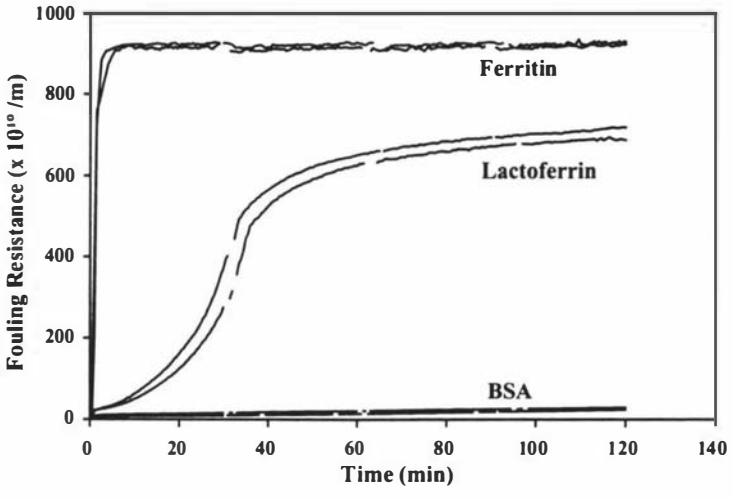

Figure 9.3. Comparison of fouling resistance at 200 L/m^2.h during MF of BSA, lactoferrin and ferritin on a 100 nm membrane. Severe fouling with lactoferrin and ferritin but minimal fouling with BSA was observed at this flux. pH: 6.78-6.89, IS ~0.05 M.

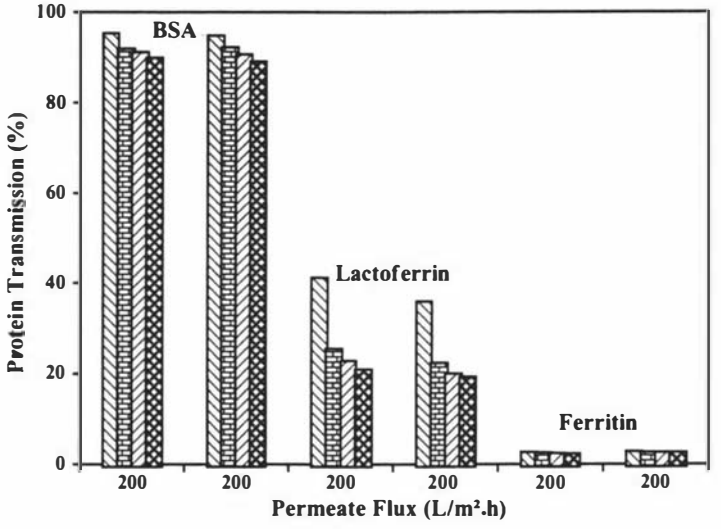

Figure 9.4. Comparison of protein transmission at 200 L/m^2.h during MF of BSA, lactoferrin and ferritin on a 100 nm membrane. Severe fouling with lactoferrin and ferritin but minimal fouling with BSA was observed at this flux. pH: 6.78-6.89, IS ~0.05 M.

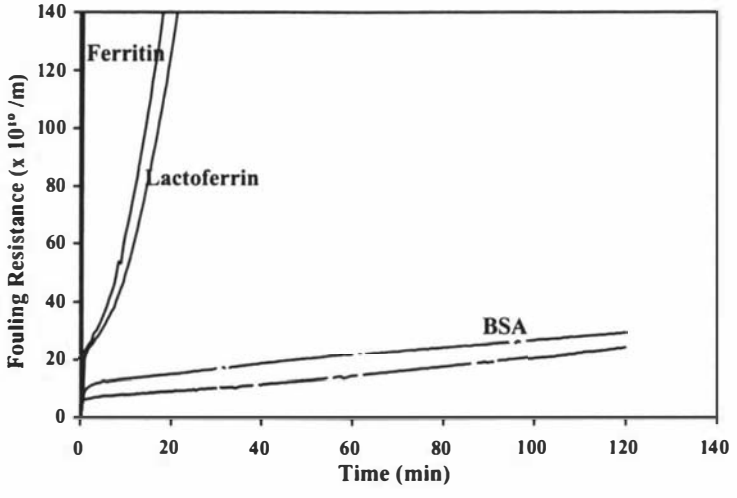

Figure 9.5. Expanded scale for initial fouling resistance at 200 L/m^2.h during MF of BSA, lactoferrin and ferritin on a 100 nm membrane. Severe fouling with lactoferrin and ferritin but minimal fouling with BSA was observed at this flux. pH: 6.78-6.89, IS ~0.05 M.

Comparing fouling behaviour under conditions where severe fouling occurred with all these proteins, they all have different final fouling resistances and different rates of increase in fouling resistance (Fig. 9.6). When severe fouling occurred at different constant fluxes, TMP exceeded 200 kPa and the permeate controller was shifted to constant pressure mode at different times ranging from 2-3, 9-10, 34-37 and 46-47 minutes for ferritin, whey protein, lactoferrin and BSA solutions respectively further confirming the different fouling behaviour by these proteins. Ferritin and whey protein solutions gave almost flat fouling resistance lines under constant pressure mode compared to BSA and lactoferrin which showed a small amount of further increase in fouling resistance. Protein transmissions reduced drastically, and were particularly low for ferritin (Fig. 9.7). However, BSA gave high protein transmissions and an increase in fouling resistance during the first 30 minutes even at a permeate flux of 400 L/m^2.h. The effect of increasing permeate flux is dramatic on all the proteins particularly for large proteins like ferritin and whey proteins.

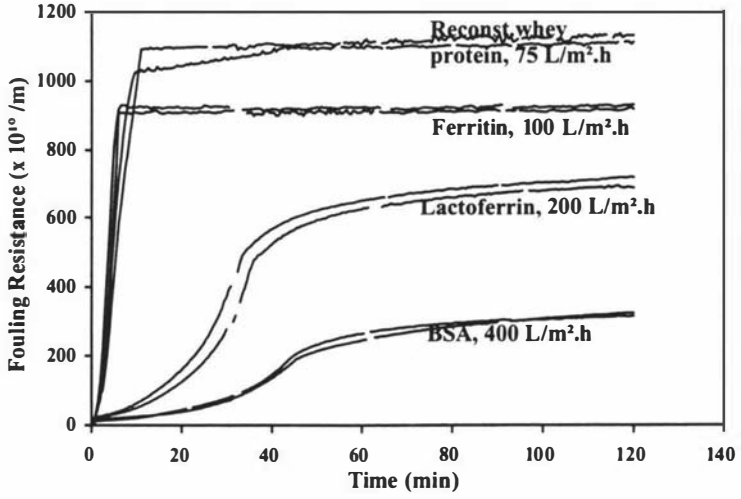

Figure 9.6. Comparison of fouling resistance when severe fouling occurred during MF of BSA, lactoferrin, ferritin and whey protein solutions on a 100 nm membrane. Severe fouling occurred with all these proteins at different fluxes. pH: 6.78-6.89, IS ~0.05 M.

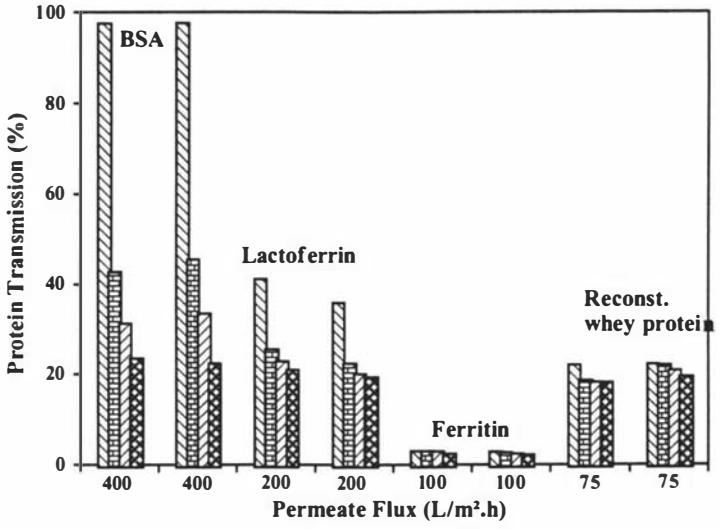

Figure 9.7. Comparison of protein transmission when severe fouling occurred during MF of BSA, lactoferrin, ferritin and whey protein solutions on a 100 nm membrane. Severe fouling occurred with all these proteins at different fluxes. pH: 6.78-6.89, IS ~0.05 M.

Severe fouling occurred when flux was increased from 50 L/m^2.h to 75, 100, 200 and 400 L/m^2.h respectively for whey protein solutions, ferritin, lactoferrin and BSA indicating the effect of protein or aggregate size on severity of fouling which is consistent with the increase in molecular weight of the protein or aggregates. The apparent critical fluxes to prevent fouling were 50, 75, 100 and 300 L/m^2.h for whey protein solution, ferritin, lactoferrin and BSA respectively. This suggested that the permeate flux at which MF can be operated without severe fouling is dependent on the protein size and perhaps also on the aggregation tendency of the protein.

In terms of reversibility of the fouling layer by these proteins, ferritin had almost all of the fouling resistance reversible on flushing with water suggesting formation of a gel layer (see section 8.3.1.2) whereas for BSA, lactoferrin and whey protein, fouling resistances were partly reversible and partly irreversible (Fig. 9.8). A slightly higher proportion (about 67%) of the fouling resistance by whey protein solutions was reversible.

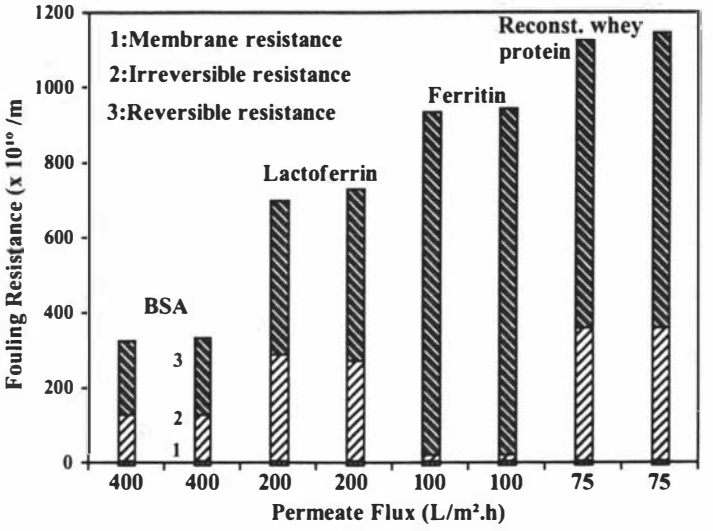

Figure 9.8. Comparison of reversible and irreversible fouling resistance when severe fouling occurred during MF of BSA, lactoferrin, ferritin and whey protein solutions on a 100 nm membrane. Severe fouling occurred with all these proteins at different fluxes. pH: 6.78-6.89, IS ~0.05 M.

The protein transmission data for all these proteins obtained at different fluxes was plotted against corresponding fouling resistance. For a given protein, all these values fall roughly on a single curve that indicates a decrease in protein transmission with increase in fouling resistance (Fig. 9.9). Best fit lines are shown in this figure. β-lactoglobulin data from Marshall *et al.,* 1997 is included to compare its fouling behaviour with the proteins studied in the present work. Data for whey protein solution could not be fitted to a curve because of limited data points under similar solution conditions. All these proteins were microfiltered under similar experimental conditions: pH; 6.78-6.89, ionic strength; ~0.05 M, temperature; 25°C, protein concentration; 0.2% except for whey protein solution which was ~0.5% concentration.

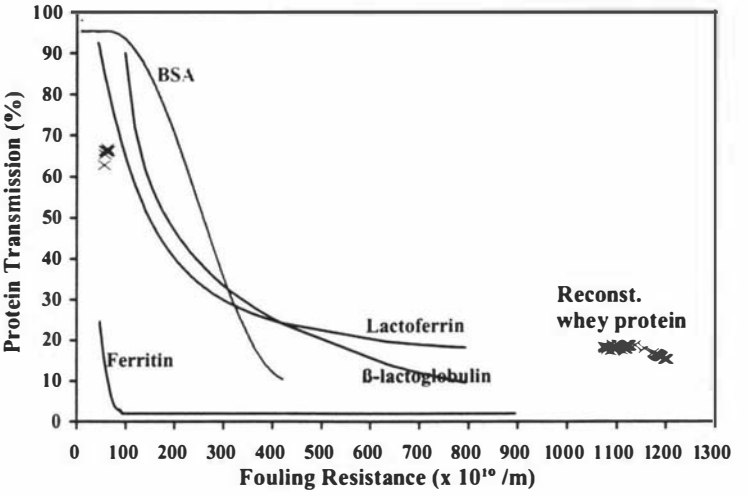

Figure 9.9. The relationship between protein transmission and fouling resistance during MF of BSA, lactoferrin, ferritin, whey protein solutions and β-lactoglobulin. Data is fitted to a smooth line for each protein. The data for β-lactoglobulin is taken from Marshall *et al.* (1997). Crosses represent data for reconstituted whey protein.

9.3. Discussion

9.3.1. Role of physicochemical properties of proteins in aggregation and fouling

Physicochemical properties of the proteins play a significant role in fouling as seen from the fouling data. Protein size and aggregation tendency in particular are very important to determine the type of fouling mechanism. It appears from the resistance curves obtained for different proteins that the size of the protein/aggregate in solution is largest for whey protein solution and smallest for BSA. This is reasonable as size of the protein in solution is in the same order as molecular weight of the protein/aggregate. The apparent reduction in pore size due to protein adsorption is calculated using equation 3.12 and compared (Table 9.1). BSA and ferritin showed an apparent reduction in pore size corresponding to monolayer adsorption (Table 9.2) whereas lactoferrin showed protein adsorption to an extent of two layers of protein. Whey protein solutions showed an apparent reduction in pore size corresponding to an adsorbed protein layer of about 9 nm thickness.

Table 9.1. Apparent pore size reduction in trials performed using BSA, lactoferrin, ferritin and whey protein solutions at 50 L/m^2.h on a 100 nm membrane.

Protein	Clean membrane resistance (x 10^{10}/m)	Irreversible fouling resistance (x 10^{10}/m)	Apparent reduction in pore size (nm)
BSA	11.0	10.1	15
	11.6	10.3	15
Lactoferrin	10.5	33.3	30
	10.9	35.3	30
Ferritin	10.4	23.8	26
	10.5	22.0	25
Whey protein solution	11.2	14.4	19
	10.9	12.8	18

Table 9.2. Some physicochemical properties of proteins investigated in the present study*

Protein	Source	MW kDa	-S-S-	-SH	IEP	Approximate diameter in solution, nm
BSA	Bovine serum	67	17	1	4.8	6-7
Lactoferrin	Bovine milk	80	19	0	~8.0	7-8
Ferritin	Bovine organs	465	0	0	4.5-5.5	12-13
Whey protein solution	Bovine milk	-	-	-	~5.2	<10

* Data from Kinsella & Whitehead (1989); Crichton & Wauters (1987)

The four protein solutions investigated have different protein transmissions initially even under light fouling conditions. Ideally the size of all these proteins should be <15 nm if they are not aggregated and complete protein transmission is therefore expected. However lower protein transmissions observed even under minimal fouling conditions, suggest that the protein is aggregated initially at least in the case of lactoferrin, ferritin and whey protein solutions. A relatively higher fouling resistance obtained with whey protein solutions supports the idea that the protein is

aggregated. Aggregation of protein has been hypothesised by several investigators. When the protein is aggregated, it is appropriate to say that the size of the aggregates determines the fouling resistance. Then the question arises: what are the possible mechanisms of protein aggregation and how can protein aggregation be prevented ? Proteins are macromolecules with a tendency to aggregate depending upon solution conditions like pH and ionic strength. The presence of ions like calcium or surface active agents like SDS in solution are also important in determining the extent of aggregation. Also during manufacture of protein powders, heat treatment and drying are the processing steps which promote aggregation and some of these dried aggregates do not always dissociate into monomers on rehydration.

Aggregation of protein can arise from a range of intermolecular and intramolecular interactions (Persson & Gekas, 1994). van der Waals interactions, hydrogen bonds, coulombic interactions and hydrophobic interactions are the most common intermolecular bonds whereas disulfide bridges are the most common intramolecular bonds influencing aggregation of macromolecules in solution. Native protein can denature on membrane surfaces because of hydrophobic, electrostatic and van der Waals interactions and hydrogen bonding between a protein and the membrane surface (Norde, 1986). Energy transfer is necessary to disturb the folded state and transform the molecule into an unfolded moiety leading to increased protein-protein interactions. Energy input such as an increase in temperature, radiation, shear is required to disturb the native state and transfer protein into an unfolded state or partially denatured state.

During MF, shear acting on proteins may change their secondary and tertiary structures. Once the protein structure is unfolded, hydrophobic interactions are highly likely and readily result in aggregation leading to increased adsorption on to the membrane surface. Bowen and Gan (1991) hypothesised shear-induced aggregation leading to greater loss of flux at high applied pressures during MF of BSA. Franken *et al.* (1990) using circular dichroism studies showed that small changes occur in the secondary structure of the protein during filtration. Some of the factors that affect protein aggregation are 1. structure and stability of protein under

different solution conditions 2. shear in the system and its effect on protein structure during actual filtration 3. effect of heat treatment/temperature.

Protein aggregation may arise from (i) partial denaturation of protein during several processing steps used in protein manufacture (Kelly *et al.*, 1993; Kelly & Zydney, 1997) (ii) pumping in MF (perhaps due to protein-protein interactions) and (iii) protein-membrane interactions. Proteins may partially denature due to shear exposing the internal tertiary structure (Maa & Hsu, 1996a). Previously inaccessible sites may get exposed leading to rearrangement and aggregation of proteins (Kelly *et al.*, 1993). The size of these protein aggregates rather than protein monomer size appears to be more important in determining the type of fouling mechanism. To analyse the possible mechanisms of aggregation with these proteins, their physicochemical characteristics are considered (Table 9.2). All these proteins except ferritin have disulfide bonds in their structure. BSA has one free thiol (Cys 34) and 17 disulfide bonds which hold the protein in a multiloop structure (Walstra & Jenness, 1984). Even prefiltered BSA solutions fouled badly suggesting that aggregation of protein during MF contributes to fouling (see section 6.3.3 in BSA chapter). How and by what mechanism? It is proposed that shear exposes hydrophobic and thiol groups in the protein structure leading to aggregation. Reduction of fouling in the presence of SDS supports the involvement of hydrophobic interaction in fouling (see section 6.3.3). SDS apparently reduced aggregate size resulting in minimal fouling at fluxes where severe fouling otherwise occurred. Another possibility is thiol-mediated disulfide interchange reactions (Kelly & Zydney, 1994). However, thiol-mediated interactions may not always be possible at low temperatures like 25°C used in these studies, unless some metal chelators or other proteins that can interchange free thiol groups are present in solution. A significant part of the fouling resistance with all these proteins except ferritin is irreversible on flushing with water suggesting that the deposits are possibly bound by disulfide interactions as these are usually not reversible.

9.3.2. Fouling behaviour of BSA, lactoferrin, ferritin and whey protein solutions

Fouling resistance has a significant effect on protein transmission depending on the physicochemical characteristics of the particular protein (Fig. 9.9). The gradual increase in fouling resistance during MF of BSA suggests that internal fouling by pore plugging or pore narrowing was dominant. However protein transmission data supports pore plugging as protein transmissions remained almost constant during the initial period of fouling. BSA had a range of fouling resistance up to 100 x 10^{10}/m where the protein transmission was not significantly affected by fouling indicating that predominantly pore plugging occurred during this period. If pore plugging occurred in its pure form, then protein transmission should not decrease with fouling. Pore plugging reduces flux due to complete loss of some pores. However, the remaining pores are still open and permeate protein molecules allowing constant protein transmission. When nearly all the pores are plugged, protein gets rejected leading to formation of a surface layer. Protein transmissions started decreasing continuously with increasing fouling resistance after this initial period of fouling. At a fouling resistance of about 425 x 10^{10} /m and above, the protein transmission was almost constant at about 12%. Once the surface layer is formed, the porosity of the deposited layer dictates the protein transmission and flux. This type of effect of fouling on protein transmission was also observed by Mueller & Davis (1996) during MF of BSA.

A somewhat different fouling behaviour is observed with lactoferrin as an increase in fouling resistance has affected protein transmission right from the beginning which suggests that a small percentage of retained aggregates may have plugged the pores (Prádanos *et al.*, 1996) giving relatively high fouling resistance compared to BSA in the same period. The final protein transmissions at high fouling resistance are about 20%. Lactoferrin and β-lactoglobulin seem to have somewhat similar fouling behaviour except that final protein transmission in the case of β-lactoglobulin reached about 12%. With ferritin, initial protein transmissions are much lower (about 25%) even under light fouling conditions and reduced to about 3% quickly when severe fouling occurred. The final protein transmission remained almost constant at this value even at higher fouling resistances. Severe fouling in the form of surface layer formation by aggregated protein seems to occur even at relatively

low fluxes (75 $L/m^2.h$) with whey protein solutions giving a final protein transmission of 16-19% when the wall concentration of aggregates is increased to a critical value.

9.3.3. Effect of fouling on steady state protein transmissions

It is interesting to note that all these proteins have different steady state protein transmissions under severe fouling conditions. This suggests that they are of different size and structure leading to a difference in porosity of the protein deposits. The difference in final fouling resistance also suggests that thickness of the surface layer is different. When the surface layer builds up, all pores are covered reducing permeate flux and the deposited layer sieves the protein solution. This clearly depends on layer porosity. There is a suggestion that the dynamic layer formed on the membrane changes the selectivity of the intrinsic membrane (Marshall *et al.*, 1993). Final protein transmission is almost certainly the permeation of protein monomer through the deposited surface layer of aggregates of various sizes. The critical factors in final protein transmission are therefore likely to be: % of monomer in the feed, size and packing density of aggregates and infill of the aggregate layer by other smaller aggregates or monomers. Ideally with larger aggregates, the porosity of the surface layer will be relatively high giving a better flux and higher protein transmission for monomers. With smaller aggregates, a much tighter surface layer may form giving relatively low protein transmissions. In the case of a deposit containing a narrow size distribution of protein monomers, a tighter and more organised deposit layer is possible giving very low protein transmissions.

Final protein transmission largely depends on the porosity of the deposited layer whereas fouling resistance and flux depend on the thickness of the fouling layer as well. It is interesting to speculate on the size effects on porosity and protein transmission. Polydispersity of ferritin in solutions has been reported (Harrison & Gregory, 1965; also in the present work, see section 8.3.2). It is possible that effective tightening of the surface layer of large aggregates (oligomers etc) by filling the pores with monomers has reduced protein transmission drastically for ferritin. β-lactoglobulin is in the form of dimers (Marshall, 1994). These dimers would be similar in size to BSA giving similar steady state protein transmissions. The size of

lactoferrin aggregates in solution is apparently larger than BSA aggregates forming a porous layer and giving slightly higher protein transmissions. The larger size of the whey protein aggregates also resulted in a relatively porous layer giving final protein transmissions in the similar range.

9.3.4. Comparative fouling mechanisms

The differences in protein transmissions and fouling resistance during the first 30 minutes with all these proteins suggest that they have different fouling mechanisms at least initially. Lactoferrin and BSA have started off with predominantly internal fouling, probably by pore plugging or deposition near the pore entrance, whereas with ferritin, initial retention of about 75% may have initiated surface layer formation right from the start of the filtration. Pore plugging by the retained protein and surface layer formation may possibly occur simultaneously in the case of lactoferrin, ferritin and whey protein solution as there are initial protein retentions with these proteins, whereas pore plugging is dominant with BSA as there is no significant initial retention with this protein.

With lactoferrin and whey protein solutions, retention of about 10% and 33-35% of protein respectively no doubt serves to initiate deposition or plugging of the pores near the membrane surface followed by surface layer formation. With whey protein, when flux increased above a critical value, the retained protein aggregates at the membrane wall increased to a critical wall concentration leading to severe fouling in the form of a surface layer. Unfortunately it is difficult to apply the film model to quantitatively estimate the wall concentration because of limited data and also the complex nature of the feed. With 10% aggregates retained for lactoferrin, fouling is still predominantly by an internal mechanism whereas with whey protein solutions, 33-35% of retained protein leads to surface layer formation. It appears that as the amount of initial retention of protein increases, the chances of formation of a surface layer relatively quickly also increases as seen from lactoferrin, whey protein solution and ferritin fouling results. Any amount of retention of protein initiates fouling by pore plugging (Prádanos *et al.,* 1996) and/or "sticking" nucleation sites for further deposition (Kelly *et al.,* 1993; Kelly & Zydney, 1995) on the membrane surface.

However, with totally permeable proteins like BSA, fouling initiates by deposition within the membrane pores. The lack of CFV effect on initiation of fouling during MF of β-lactoglobulin (Marshall, 1994), BSA (Bowen & Hughes, 1990) and lactoferrin in the present work gave further support to the idea that almost totally permeable proteins like BSA and lactoferrin fouled internally by pore plugging or pore deposition at least initially.

Further deposition in the form of a surface layer during MF of BSA and lactoferrin probably occurred via multiple mechanisms as the fouling resistance was both reversible and irreversible.

One possible explanation is that the irreversible resistance was due to adsorption and deposition within the pores whereas reversible resistance was due to the accumulation of retained protein after complete pore fouling. Fouling resistance observed with ferritin was almost completely reversible suggesting that fouling was predominantly by surface layer formation. The deposition of whey proteins also appears to be largely by surface layer formation as about 67% of the fouling resistance at higher fluxes was reversible.

Another possible explanation for the reversible/irreversible fouling mix was that surface layer formation during MF of BSA and lactoferrin occurred via hydrophobic and disulfide interactions. Hydrophobic interactions are usually reversible whereas disulfide interactions are not (Morr & Josephson, 1968). The surface layer formation observed with ferritin was almost completely reversible suggesting that the deposition mechanism was predominantly by hydrophobic interactions. The deposition mechanism for whey protein solution also appears to be mostly by hydrophobic interactions as about 67% of the fouling resistance was reversible.

For proteins that initially permeate the membrane, fouling appears to occur by deposition within the pore or near the pore entrance followed by formation of a surface layer. If the retention is very high initially as in ferritin and whey protein solutions, the retained protein may initially plug the pores and/or initiate deposition by retained protein on the membrane surface followed by an immediate surface layer

formation. On the other hand, lactoferrin and BSA have a slower pore fouling phase followed by surface layer formation. As pores are plugged or constricted with time, further retention or protein deposition occurs. This leads to an increase in fouling resistance with time as a result of increase in thickness of the surface layer/cake. With lactoferrin, surface layer formation occurs more quickly than with BSA suggesting that the bigger size of lactoferrin and also the 10% retained aggregates right from the start contributes to the formation of a surface layer. Fouling generally increased with increase in molecular weight or size of the protein in solution. Prádanos *et al.* (1996) also observed severe fouling as molecular weight of the protein increased. It appears that larger proteins like ferritin when partially retained by a membrane form a gel layer even at fluxes where flux-induced fouling is minimal with totally permeable proteins like BSA.

The physical mechanism of MF fouling by proteins largely depends on whether the protein is partially permeable or totally permeable at the start of the filtration. For totally permeable proteins, the fouling mechanism is that adsorption and pore plugging or narrowing occur simultaneously. As the pores are lost, the remaining pores have to permeate more solution as the operation is under constant flux mode. Pore narrowing results in a continuous decrease in protein transmission whereas pore plugging gives almost constant protein transmission as the unplugged pores still permeate the protein. Complete pore plugging or constriction leads to retention and subsequent surface layer formation. Pore fouling (pore plugging and narrowing) appears to occur predominantly at the pore entrance due to increased shear at the mouths of the pores leading to partial denaturation, aggregation and deposition.

With partially permeable proteins, nucleation of surface deposits by the retained protein and some pore plugging/deposition at the pore entrance occur simultaneously. When the retained protein increases to a sufficiently high wall concentration, perhaps due to increased flux, protein transmissions decrease quickly leading to further retention and subsequent surface layer formation. Whether the surface layer is a gel layer or a cake is determined by the properties of the protein and type of protein-protein interactions.

9.3.5. Effect of solution environment on the fouling behaviour of proteins

The presence of SDS reduced fouling dramatically on those proteins with high levels of aggregates. The effect of calcium, on the other hand, exacerbated fouling particularly at higher fluxes, though its effect is minimal at low fluxes with lactoferrin. Thus any solution conditions that enhance or decrease aggregation have a major impact on the fouling behaviour. In situations where aggregation is enhanced for example, the presence of ions like calcium can actually cause severe fouling by aggregates plugging the pores. On the other hand, if aggregates can be dissociated by SDS addition or removed by prefiltration, a reduction in fouling occurs.

9.4. Conclusions

MF fouling by proteins is complex and depends on a variety of factors like operating conditions, membrane and feed properties. One of the most important factors is properties of the feed. Fouling behaviour is specific to protein physicochemical characteristics and also subject to solution environment as this influences protein aggregation. Protein size and aggregation behaviour determine the type of fouling mechanism. MF fouling by proteins depends on whether the protein is larger or smaller than the pore size of the membrane.

Membrane fouling during MF of proteins occurs by at least three different steps, if the protein is smaller than the pore size of the membrane.

The first step is adsorption of protein both on the membrane surface and within the pores leading to a reduction in apparent pore size. The adsorbed layer is equivalent to at least a monolayer of protein and is dependent on the characteristics of the protein and solution environment.

Pore fouling in the form of either pore plugging or deposition near the pore entrance occurs as a second step. This step depends on the protein to pore size ratio. Protein size depends on the molecular weight and aggregation tendency of the protein. The solution environment *e.g.* the presence of calcium or SDS, changes aggregate size

thereby affecting this step. Protein aggregates in the feed and those produced during MF can contribute to this mechanism.

As a final step, once the pores are completely plugged or constricted, a surface layer forms due to retained protein. The mechanism of further deposition after the surface layer formation is highly specific to the protein tertiary and secondary structure and the relative accessibility and reactivity of amino acid groups.

If the protein/aggregate size is larger than the pore size, the above steps may not occur but a concentration-induced gel layer or surface layer may immediately form on the membrane surface depending upon the types of protein-protein interactions.

Experimental data obtained during MF of BSA indicate that fouling steps 1 to 3 occur *i.e.* 1) adsorption 2) pore plugging via aggregates present in the feed as well as those formed during MF and 3) surface layer formation perhaps via hydrophobic and thiol mediated interactions.

Bovine lactoferrin also fouled by these steps 1-3. But the predominant mechanism was formation of a concentration-induced fouling layer by aggregates rather than the bulk protein and initially some pore plugging by aggregates. Fouling occurred near the pore entrance perhaps by deposition of aggregates leading to pore constriction. SDS reduced the deposition and extent of constriction probably by reducing the size or number of aggregates. SDS might also have minimised adsorption of protein leading to low fouling and improved protein transmissions at lower fluxes. However at higher fluxes a concentration-induced surface layer formed probably due to some form of change in conformation of protein or dissociation of protein aggregates into monomers forming a thin layer of compact/tighter protein deposits.

Data on ferritin showed that fouling essentially occurred via gel layer formation. The presence of SDS in the feed delayed fouling. However, when fouling was severe, especially at higher fluxes, the gel layer appeared to have penetrated into the membrane matrix reducing protein transmissions drastically.

Whey protein solutions fouled predominantly by aggregates forming a surface layer at relatively low fluxes (75 $L/m^2.h$).

10. Recommendations for further research

10.1. MF fouling by protein solutions

Probable fouling mechanisms during CFMF of different proteins were proposed in the present work. A distribution of particle sizes in ferritin solutions was observed in this study. It is important to measure aggregate size distributions in this and other protein solutions used in the study to more accurately determine the dominant fouling mechanisms.

Further research is required to determine whether deposition of further protein on to existing protein deposits occurs via disulfide, hydrophobic, ionic interactions or H-bonds so that s. Flushing studies using different chemicals (*e.g.* SDS, Urea, NEM, Mercaptoethanol) to remove deposits will be interesting and useful to elucidate likely mechanisms of protein deposition.

Obviously complete understanding of MF fouling during processing of complex streams such as whey is a long way off. Studies using binary and ternary mixtures of proteins to understand fouling mechanisms and the contribution/dominance of each of the proteins would be a useful step in this direction.

It would be interesting to investigate the effect of cross-flow velocity on BSA fouling and protein transmission to identify whether fouling initiates on the membrane surface or within the pores. Cross-flow velocity and protein transmission data is needed to elucidate whether protein transmission is controlled by protein deposition within the pores or deposition on the membrane surface.

A more rigorous treatment of gel theory is possible on the fouling data obtained for ferritin if the diffusivity and gel concentration data are available. Further work is required to determine the gel concentration of ferritin by conducting experiments at various bulk concentrations and determining limiting flux values.

10.2. General

There have been several hypotheses on shear causing protein denaturation and deposition (*e.g.* Franken *et al.,* 1990; Bowen & Gan, 1991; Meireles *et al.,* 1991a and Jonsson *et al.,* 1996), but there is no direct experimental evidence that shear causes denaturation of proteins. Separate studies are required in this area apart from fouling studies.

Further research is required to determine the actual mechanism of aggregate formation of different proteins under shear so as to develop strategies to avoid formation of aggregates during MF itself. Further studies are also required to understand rates of aggregation under shear and in different solution environments. Further studies are useful to correlate directly the extent of protein aggregation and severity of MF fouling. This could be performed by heat treating protein solutions initially to achieve different degrees of aggregation and then filtering or ultracentrifuging them, as well as performing MF fouling studies with them.

11. Nomenclature

Symbol	Description	Units
A_i	Area of blocked membrane (Eq. 3.23)	m^2
A_m	Membrane area	m^2
A_o	Original membrane area (Eq. 3.23)	m^2
C	Concentration	%
CA	Cellulose Acetate	
C_b	Bulk or feed concentration	%
CFV	Cross-flow velocity	m/s
C_g	Gel concentration	%
C_p	Permeate concentration	%
C_r	Retentate concentration	%
C_w	Membrane wall concentration	%
D	Diffusion coefficient	m^2/s
d	Diameter	m
d_p	Pore diameter	m
IEP	Isoelectric point	
J	Permeate flux	$L/m^2.h$
J_{ss}	Steady state permeate flux (Eq. 3.11)	$L/m^2.h$
k	Mass transfer coefficient	m/s
k_b	Boltzmann constant	J/K
k_d	Rate constant	/s
K_i	Constant in intermediate blocking law	$/m^3$
K_p	Constant in complete blocking law	/s
K_s	Constant in standard blocking law	$/m^3$
L	Length	m
M	Molecular weight	daltons
ΔP	Pressure difference	Pa
ΔP_L	Longitudinal pressure drop	kPa

Symbol	Description	Units
ΔP_o	Original pressure difference	kPa
ΔP_{TM}	Transmembrane pressure	kPa
PC	Polycarbonate	
PES	Polyethersulfone	
PS	Polysulfone	
PVDF	Polyvinylidene fluoride	
R	Resistance	/m x 10^{10}
R_g	Gas constant	J/mole.K
R_m	Membrane resistance	/m x 10^{10}
R_{rf}	Reversible fouling resistance	/m x 10^{10}
R_{total}	Membrane resistance plus fouling resistance	/m x 10^{10}
R_f^*	Plateau Fouling resistance (Eq. 3.13)	/m x 10^{10}
T	Temperature	°C or K
t	Time	s or min or h
TMP	Transmembrane pressure	kPa
T_r	Transmission	%
u	Cross-flow velocity	m/s
μ	Dynamic viscosity	Pa.s
μ_p	Permeate viscosity	Pa.s
$\Delta\Pi$	Osmotic pressure	kPa
ρ	Density	kg/m^3
ρ_p	Density	kg/m^3
τ	Shear stress	Pa
ε	Porosity or void fraction	
λ	Ratio of particle to pore diameter	

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Addenda

Clarification of term "fouling": On page 4 para.2, line 3, the definition of fouling from Gekas (1988) was given. By this definition, concentration polarisation (CP) is not fouling, since it does not cause any deposition. However, CP does contribute to fouling since it produces a high protein concentration near the membrane surface making deposition more likely. There is still debate in the literature about the definitions of various types of fouling *e.g.* reversible versus irreversible.

Clarification of term "gel layer": The term" gel layer" is widely used in the literature on membrane fouling. However by normal chemical and rheological definitions of a gel, it is highly unlikely that a gel actually forms at the membrane surface. The term has therefore been the subject of a lot of debate. In the membrane literature, the term is probably being used less frequently now because it is not strictly correct scientifically.

Errata

p. 74. Figure 5.9. The two straight lines for reconstituted whey on the 100 nm membrane should be deleted.

p.96, para. 2, After first sentence, add "SDS has been shown to reduce aggregation of BSA during gelation studies (Boye *et al.*, 1996)".

p.105, Figure 6.29 legend. Last sentence. Instead of "calcium and control runs", it should read as "50 and 100 nm runs".

p.136, Figure. 7.14 legend. After first sentence, add "Two runs were performed at a constant CFV of 3 m/s and two runs at first 2 m/s and then 4 m/s".

p. 185, Figure 9.8. y-axis should read as resistance instead of fouling resistance.

p.197, para.2, line 3, delete "so that s".

p. 200, line 25, ρ_p is particle density instead of density.